final report

Project code:	B.WBC.0080 SLN Biocontrol Final Report
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Date published:	30 April 2018

PUBLISHED BY
Meat and Livestock Australia Limited
Locked Bag 1961
NORTH SYDNEY NSW 2059

Silverleaf nightshade biological control
RnD4Profit-14-01-040

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Plain English Summary

Silverleaf nightshade (SLN) is an introduced perennial weed with a deep root system that is very hard to kill. It reduces crop and pasture yields for farmers in the wheat-sheep zone of Australia. In 1992 South Africa released a beetle (SLN leaf beetle – SLNLB) from North America to eat SLN. It was very successful, and so this project aimed to test whether it was suitable for release in Australia.

A team of seven scientists based in Adelaide (PIRSA and DEW), Melbourne (DEDJTR) and Wagga Wagga (NSWDPI) joined forces to work on the project. It was funded by the Australian Government, MLA, PIRSA and SAGIT. SLNLB was imported into Melbourne under quarantine laboratory conditions and was offered a wide range of native plants and crops closely-related to SLN.

Unfortunately, the SLNLB fed on 15 native plants as well as eggplant. In late 2017 it also attacked a group of related potato varieties, something not recorded by the South African researchers. The research team immediately ruled out SLNLB as suitable for release in Australia. Although not successful in releasing the beetle, the project undertook thorough and detailed processes to ensure that integrity in the testing procedures was followed. This project provided a significant amount of new information, and a large seed collection, that will be invaluable for future biological control projects against SLN.

Executive summary

Background. Silverleaf nightshade (*Solanum elaeagnifolium* Cav.; SLN) is a deep-rooted invasive perennial weed that reduces productivity and profitability across the wheat-sheep agricultural zone of Australia. It infests over one million hectares in Australia, and costs farmers $70 million every year, and biological control is the most likely long-term solution. In 1992 the silverleaf nightshade leaf beetle (*Leptinotarsa texana*: SLNLB), native to southern USA, was released as a biological control agent against SLN in South Africa. It has been a spectacular and continuing success - defoliating vast infestations and reducing SLN density severely, with no field reports of off-target damage. This project aimed to import the SLNLB, under quarantine, and assess its suitability as a biological control agent for SLN in Australia. The research team was led by PIRSA and comprised seven scientists: John Heap, Laurie Haegi and Jane Prider from SA (project leader, taxonomic/phylogenetic expertise and plant collection/propagation); Greg Lefoe from Vic (host specificity testing and risk analysis); and Hanwen Wu, David Gopurenko and Xiaocheng Zhu from NSW (molecular biology).

Three appendices, detailing 1) the research project Agreement, 2) details of research and results, and 3) draft research papers, accompany this report. The following sections summarise the research, results and implications for biological control of silverleaf nightshade in Australia.

Where does SLN come from? SLN is native to both North and South America. It is also an introduced weed in many countries. Information on its origins is useful to identify likely sources for biocontrol agents. DNA from 488 SLN plants from all over the world was analysed to identify 41 different types of SLN. The DNA evidence strongly suggests that SLN in Australia originated in North America. SLN in Australia, South Africa and the Mediterranean show moderate genetic diversity and all had a moderately common type that is only observed in Oklahoma and Kansas. This suggests that SLN introductions to Australia, South Africa and the Mediterranean were mostly from the central US states, and that co-evolved biological control agents from North America may be best suited for use as biocontrol agents for SLN in Australia. Potential agents may also occur in South America.

Establishment of a SLNLB breeding colony in Australia. A total of 152 SLNLB adults were imported from South Africa in April 2016, with import licences from the Departments of Agriculture and Water Resources and the Environment and Energy. They were reared for at least one generation prior to shipment to reduce the risk of importing other pests and diseases. The colony was successfully maintained through successive generations for the duration of the project. DNA from 94 larval specimens from the breeding colony described above was tested to verify their identity. Two slightly different types of SLNLB were found, but all beetles were classified as SLNLB. Importantly, DNA from the beetles differed greatly from SLNLB's relative and potato pest, the Colarado potato beetle.

Consultation, communication and evaluation. Crops in the Solanaceae family are most at risk from biocontrol agents for SLN. Technical information on research activities and risk management was provided to the vegetable peak body, AusVeg. Direct industry consultation was undertaken with eggplant growers in Qld and SA. A number of native Australian *Solanum* plants also have importance to Australian Aboriginal culture, and to the growing Australian Bush Foods industry. Several *Solanum* species are important food plants or have cultural, medicinal, mythological or psychoactive properties. A visit was made to one of Australia's most important Bush Foods enterprises (Outback Fresh, Reedy Creek, SA) in March, 2017. A list of five significant *Solanum* species was obtained, and these were factored into specificity testing lists. Numerous conference, media, and community presentations were delivered during the course of the project.

Australian native *Solanum* plants – family tree studies using DNA. In Australia there are numerous native and introduced *Solanum* species, and many have cultural, pharmaceutical, culinary and conservation importance. There is also a significant presence of non-Australian species, as major food crops (e.g. potato, tomato, eggplant), as garden ornamentals and as naturalised weeds. These plants need to be tested to determine whether SLNLB will attack them. Construction of a robust and safe host specificity test list relies on very good and detailed family tree information. This research analsysed four separate short sections of DNA from native Australian *Solanum* species. A total of 341 samples, taken from 162 *Solanum* species, were included in DNA analyses. These samples covered almost 90% of the known Australian species. Samples were obtained from dried plants in Australian herbaria or from collection trips for this project. It generated new information that, used in conjunction with traditional classification based on morphological features, will be very useful to all subsequent SLN biocontrol projects. In summary, the vastly improved picture of relationships among the Australian spiny Solanums not only provides re-assurance for the basis of sampling for host specificity testing in the current project, but also provides greater confidence and reliability in selecting species in any future proposals, even in the face of any potential expansion of the occurrence of silverleaf nightshade in Australia in the meantime.

Host plant specificity testing list for SLNLB. Weeds within the genus *Solanum* have unusually large numbers of close relatives in Australia – both cultivated crops and native Australian species. This greatly increases the resources required to conduct host specificity testing. A comprehensive list of potential host plants from the Solanaceae family was constructed by Laurie Haegi, concentrating on species in *Solanum* – the group containing SLN. Plants on the list were given High, Medium, or Low priority. High priority species were choosen to give good representative coverage of the Solanaceae family. Medium and Low priority species were collected while collecting High priority species. Three extensive field collection trips were taken - one to northern SA, and two to NSW – to collect seed and cuttings. Seed and cuttings from SA, NT, WA and Qld were also obtained opportunistically during unrelated travel, and through colleagues. Horticultural varieties were obtained from commercial nurseries. All, but one, species collected were able to be propagated. A total of 654 test plants were sent to Agriculture Victoria's AgriBio. These included 44 species from the *Solanum* group, and 5 other species from the Solanaceae family. Seed of six other species was collected and stored. Duplicate seed collections from this research are held at the AgriBio facility in Melbourne, with a back-up collection at the SA Seed Conservation Centre in the Botanic Gardens and State Herbarium of SA. This collection of propagation material of wild species is a major output of the project. The new collections are enhanced by fully-labelled voucher specimens lodged in State Herbaria.

Research was also conducted into methods to germinate seeds of Australian *Solanum* plants, to improve the supply of test plants. Seed coat scratching, water leaching and the chemical stimulants gibberellic acid and smoke water were tested on 30 *Solanum* species. Several species had high total germination without any treatment at 25 °C in the dark. Five species had a 20 - 100% increase in germination in gibberellic acid treatments compared to untreated controls. Smoke water significantly increased the germination of three of the tested species by 27 – 66 %. A further 3 species were significantly stimulated by both gibberellic acid and smoke water. The addition of stimulants enabled the germination of several *Solanum* species under the same temperature and light conditions, simplifying seed propagation methods.

Host specificity testing.

An important component of biological control risk analysis is assessment of an agents' host-range. Host-range experiments are typically conducted in a quarantine laboratory, where non-target plants are exposed to the insect in replicated cage experiments. The host-range expressed in the laboratory is termed the *fundamental host range*. The fundamental host range encompasses the full range of plants an insect agent is capable of utilising. The range of hosts actually utilised by the agent under field conditions is termed the *realised host range* (also referred to as the agent's *field host specificity*), and may be a subset of the fundamental host range. A host specific agent can express a broader fundamental host range if important behavioural or chemical cues are absent or disrupted in the confines of small laboratory cages. False positive results (attack in the laboratory that does not occur in the field) may complicate risk analysis, because Australian regulators rely heavily on the results of host-range experiments conducted in quarantine laboratories (Department of Agriculture and Water Resources, n.d.). In these cases, agents are not likely to be approved for introduction unless additional evidence is produced to support field host specificity.

This research was undertaken to identify 1) what native and economically important plants could be at risk of off-target damage in Australia, 2) the likely nature and extent of off-target damage should it occur, 3) whether additional research is required to predict the actual field host-range, and 4) whether further research on SLNLB is warranted. Host specificity experiments tested 28 native species, and 16 cultivars of various crop and ornamental species. SLNLB damaged plants in no-choice and choice experiments. Feeding damage greater than 50% leaf area removed occurred on plants of 12 native Australian *Solanum* species, and two crop species (potato and eggplant). SLNLB successfully developed from a small grub to adult beetles on 15 Australian *Solanum* species and two crop species (a single eggplant cultivar and four potato cultivars). When given a choice of plants in a large cage experiment SLNLB laid eggs on SLN and three native Australian *Solanum* species. These results expand the known fundamental host-range of SLNLB to include some Australian *Solanum* species, and certain cultivars of potato. Damage to potato was not anticipated, as potato had been previously tested in South Africa. However, potato cultivars tested here may not have been available when the research was conducted in South Africa. SLNLB feeding damage to eggplant was anticipated because it previously fed on eggplant in laboratory experiments in South Africa, even though eggplant is not known to be a host in North America where SLNLB occurs naturally. A field experiment conducted by this project in Texas (USA) with a wild population of SLNLB demonstrated that, despite our laboratory results, eggplant is not used as a host in the field.

Based on the results of these host-range experiments, SLNLB is considered to be an unacceptable risk to the Australian environment and economy, and would not be approved for release. Additional field experiments with susceptible potato cultivars are currently underway in Texas.

Abandonment of *Leptinotarsa texana* and future prospects. As described above, host range laboratory test results disqualified SLNLB from release in Australia. Although extremely disappointed, the SLN research team were pleased to have identified the potential problem. The size of the potato industry in Australia neccesarily sets the risk bar very high. However, there are two very significant new opportunities:

1. To draft suggested changes to the Australian host-specificity testing protocols to include systematic study of the breeding lineages for major related crops. In future, biocontrol projects

could select related crop test varieties (e.g. within potato, tomato, eggplant, chilli and capsicum, for SLN) based on varieties that represent all major breeding lineages.

2. The next opportunity is a long shot, but with potentially very high impact. The discovery of feeding on several potato varieties could lead to the identification of protective or discouraging compounds for SLNLB that are missing in susceptible varieties. This might allow breeders to increase the production of these protective molecules in other potato varieties (increased expression via multiple copies of the gene/genes responsible; or GMO) to the extent that it could deter feeding by the closely-related potato pest, the Colorado potato beetle. A similar approach might be followed if var. "Nadine" is found to have an attractant molecule(s). Thus it is *possible* that our discovery could lead to advances against the world's worst potato insect pest.

Future work

The SLN research team has collected a large seed collection and a substantial store of data, information and knowledge on SLN. It will use these resources to complete a draft Application to Release, but will not submit it. There are six scientific papers in preparation that document our research, results, and implications.

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1 Project rationale

Silverleaf nightshade (*Solanum elaeagnifolium* Cav.) is a deep-rooted invasive perennial weed that reduces productivity and profitability across the wheat-sheep agricultural zone of Australia. It infests over one million hectares in Australia, and costs farmers $70 million every year, and biological control is the most likely long-term solution. In 1992 the silverleaf nightshade leaf beetle (*Leptinotarsa texana*), native to southern USA, was released in South Africa. It has been a spectacular and continuing success - defoliating vast infestations and reducing silverleaf nightshade density severely, with no field reports of off-target damage. This project aimed to import *Leptinotarsa texana*, under quarantine, to assess its suitability for Australia. Contingent on favourable assessment results, it planned to seek approval for release of the agent in Australia.

Previous biological weed control projects have improved agricultural production and benefited the Australian economy, providing an average 23:1 return on investment. This project (B.WBC.0080 SLN Biocontrol) is a component of a national program that aimed to deliver benefit by hastening control of six national priority agricultural weeds (parkinsonia, parthenium, blackberry, silverleaf nightshade, cylindropuntia, and gorse) across northern and southern Australia by generating and delivering eight biocontrol agents to producers.

2 Project objectives

2.1 Overview

This project assessed whether releasing the silverleaf nightshade leaf beetle (*Leptinotarsa texana)* is an acceptable risk to economic, ecological and social values in Australia. In doing so, it progressed biological control of silverleaf nightshade towards future improvements in weed management for producers. The silverleaf nightshade leaf beetle was imported and tested against a range of closely related Australian native and commercially important non-target species. The project also investigated novel approaches to biological control risk analysis, and prepared an Application to Release the agent. The Application for Release will not now be submitted, but will form the basis for future applications for SLN agents. Genetic diversity of SLN and the beetle were also investigated.

In meeting these objectives, the project addressed the following outputs:

(*Note: rearranged from original to be in time sequence*).

Output (8a) Obtained approvals for importation of beetle.

Output (8b) Developed SLN plant cultures, sourced SLN shoot material and confirmed sequencing protocols.

Output (e) Developed a detailed plan for specificity testing and propagule collection, using the centrifugal phylogenetic method to prioritise native and commercially important Solanaceae occurring in locations where the ranges of silverleaf nightshade and potential non-target species overlap. Over 30 species/cultivars were collected for host specificity testing, covering over 30 locations.

Output (d) Imported a colony of *Leptinotarsa texana* into quarantine and refined rearing methodologies to maximise colony development.

Output (8c) Undertook host specificity testing of plant species collected.

Output (f) Completed DNA sequencing of SLN material from Australia and overseas.

Output (h) Initiated Australian Government Import Risk Analysis procedures to seek formal approval for release of SLN agent. This has been completed, but not submitted.

Output (g) Prepared a plan for next steps in the biological control of SLN. This included abandoning plans to release *Leptinotarsa texana* in Australia, and ensuring that maximum support was provided to the Round 2 SLN project looking at alternative agents.

Output (i) Prepared six scientific papers on the project research.

2.2 Specific objectives from contract agreement

By Sept. 1 2018:

1. Have assessed whether the release of silverleaf nightshade leaf beetle (SLNLB) is an acceptable risk to economic, ecological and social values in Australia.
2. Import and test (SLNLB) against a range of closely related (to silverleaf nightshade) Australian native and commercially-important non-target species.
3. Investigate novel approaches to biological control risk analysis.
4. Prepare an application to release the agent (pending a favourable risk analysis) to be submitted to Commonwealth regulatory authorities responsible for the decision to release biological control agents in Australia.
5. Investigate genetic diversity of silverleaf nightshade and (SLNLB).
6. Provide the best evidence-based on-farm best practice recommendations to integrate biocontrol into production systems. (Note: If such recommendations are largely based on observations/experience/reflections/intuitions this will be noted).”

Note: More details of these objectives are given in the Milestone sections below.

2.2.1 Changes to objectives

After the beetle was observed attacking some potato varieties in late 2017 plans to seek permission for release in Australia were abandoned. A new emphasis was placed on the documenting the nature and scope of potato susceptibility to the beetle. This information has broader implications for all future biocontrol programs in Australia. These experiments are on-going, and at the time of writing crucial field experiments are being conducted in Texas (USA) by Greg Lefoe to assess the field susceptibility of eggplant and potato.

3 Method and project locations

3.1 Overview of methods and locations

In contrast to most of the projects within the Round 1 biocontrol program, the SLN project did not undertake an agent distribution and release campaign, or extensive stakeholder consultations, so the geographic aspects of the project are restricted to sample collection, stakeholder consultation and research facility locations. All of the activities listed below have applicability to the entire wheat/sheep agricultural zone of Australia (Fig. 1).

Figure 1. The Australian wheat-sheep zone. This zone coincides with SLN distribution, and is where the research in this project is applicable to (https://industryinfomercialdomesticlamb.weebly.com/).

The project comprised a number of research components with a wide geographic spread:

1. Project coordination and management. Based at PIRSA, Waite Campus, Adelaide, SA. Main team members: John Heap.
2. Obtain Australian import permits and establish a quarantine breeding colony of *Leptinotarsa texana*. The beetles were sourced from colleagues in South Africa, and the colony was established at AgriBio, La Trobe University, Melbourne, Vic. Main team members: Greg Lefoe and colleagues.
3. Construct a host specificity testing list, obtain test plants and collect propagation material, establish a propagation pipeline for supplying test plants to AgriBio. These components were based in Adelaide, but extensive field collection expeditions were made to northern SA and most parts of NSW. Propagation material was also collected by team members and colleagues in WA, Qld and NT. Main team members: Laurie Haegi, John Heap and Jane Prider.
4. Undertake molecular biology research to elucidate the likely geographic origin of SLN in Australia, and the phylogenetic relationships amongst native Australian *Solanum* species. This researchwas based at the Wagga Wagga Agricultural Institute (NSW DPI). Samples were obtained from many overseas countries and state herbaria throught Australia. Main team members: Hanwen Wu, David Gopurenko, Xiaocheng Zhu and Laurie Haegi.
5. Host specificity testing. Testing performed under quarantine at AgriBio, La Trobe University, Melbourne, Vic. Main team members: Greg Lefoe and colleagues.
6. Industry and community liaison: Predominantly for the vegetable and Bush Foods industries. These efforts were ceased when it became clear that release would not be possible. The consultation took place on the Northern Adelaide Plains and Reedy Creek (SA), and in far north Qld. Main team memebers: John Heap and Greg Lefoe.
7. Prepare draft research paper manuscripts for publication in journals. These papers are in preparation in Adelaide, Wagga Wagga, and Melbourne. Main team members: All.

4 Results

4.1 Overview

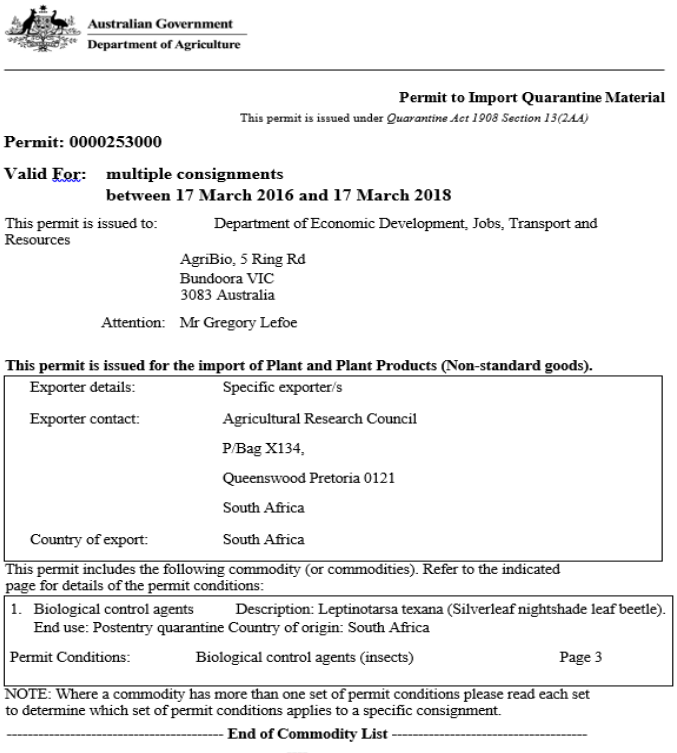
Please note: Research publications, communications and stakeholder liaison activities are covered in separate sections below. More details of results are provided in the Appendix to this report (separate file).

Results presented in this section below fall under a number of component research headings:

1) Overview
2) Obtaining Australian import permits.
3) Establishing a quarantine breeding colony of *Leptinotarsa texana*.
4) Constructing a host specificity testing list and obtaining plants.
5) Propagation pipeline for supplying test plants to AgriBio.
6) Molecular biology research.
7) Host specificity testing.

4.2 Obtaining Australian import permits

Two Australian Government permits were required to import live *Leptinotarsa texana* beetles into quarantine in Australia. Greg Lefoe submitted applications to, and obtained permits from, both the Department of Agriculture and the Department of the Environment (Figs. 2 and 3). It should be noted that applications were required to be very detailed (not presented), and each permit came with detailed and strict quarantine requirements (not presented). Further details may be obtained from Greg Lefoe.



Australian Government
Department of Agriculture

Permit to Import Quarantine Material

This permit is issued under *Quarantine Act 1908 Section 13(2AA)*

Permit: 0000253000

Valid For: multiple consignments
between 17 March 2016 and 17 March 2018

This permit is issued to: Department of Economic Development, Jobs, Transport and Resources
AgriBio, 5 Ring Rd
Bundoora VIC
3083 Australia

Attention: Mr Gregory Lefoe

This permit is issued for the import of Plant and Plant Products (Non-standard goods).

Exporter details:	Specific exporter/s
Exporter contact:	Agricultural Research Council
	P/Bag X134,
	Queenswood Pretoria 0121
	South Africa
Country of export:	South Africa

This permit includes the following commodity (or commodities). Refer to the indicated page for details of the permit conditions:

1. Biological control agents Description: Leptinotarsa texana (Silverleaf nightshade leaf beetle).
End use: Postentry quarantine Country of origin: South Africa

Permit Conditions: Biological control agents (insects) Page 3

NOTE: Where a commodity has more than one set of permit conditions please read each set to determine which set of permit conditions applies to a specific consignment.

---------- **End of Commodity List** ----------

Figure 2. Permit to import Leptinotarsa texana beetles into Australia – Dept. of Agriculture.

Australian Government
Department of the Environment

Type of document: NON-CITES
Export
Re-export
Import
Other: Testing Permit

Original - Valid for Multiple Consignments
1. Multiple Consignment Authority No: PWS2016-AU-000050
2. Valid Until: 12/7/2016

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3. Importer (Name, Address, and Country)

DEPARTMENT OF ECONOMIC DEVELOPMENT JOBS TRANSPORT AND RESOURCES
AGRIBIO, 5 RING ROAD
BUNDOORA VIC 3196
AUSTRALIA

4. Exporter (Name, Address, and Country)

Ms Hildegard Klein
AGRICULTURAL RESEARCH COUNCIL
PLANT PROTECTION RESEARCH INSTITUTE
P/BAG X134, QUEENSWOOD
PRETORIA 0121
SOUTH AFRICA

5. Conditions
- This permit is not transferable
- If for live animals, this permit or certificate is valid only if the transport conditions comply with the IATA Live Animals Regulations; if for live plants, with the IATA Perishable Cargo Regulations
- For special conditions specific to this permit also refer below

5a. Purpose: Not Required

6. Name, Address and Country of Issuing Authority

Wildlife Trade and
Biosecurity Branch
GPO BOX 787
CANBERRA ACT 2601

7/8. Scientific & Common Name (Genus and Species of Animal or Plant)	9. Description	10. Source	11. Quantity and Unit
1 *Leptinotarsa texana* Satanaboa Leaf Beetle, Texan Potato Beetle	Live insect	(C)	2000 NO
12. Country of Origin: XX			

Special Conditions

Under Part 13A of the EPBC Act all Commonwealth and State/Territory requirements in relation to this specimen(s) must be met.

Specimens are approved for import into an approved quarantine containment facility only.

The importer must ensure that on arrival in Australia, the animal(s) and/or eggs are transported directly to the secure, approved quarantine containment facility.

The importer must ensure that the facility maintains work practices and adequate security to prevent the escape or theft of any animal(s) or reproductive material.

The importer must obtain written approval from the Issuing Authority prior to the transfer or release of any live specimens (or progeny) from the importer's approved facilities.

The importer must provide to the Issuing Authority, a report on the results of the research. The report should be supplied to the Issuing Authority within 12 months of the permit issue date, or with an application for another permit for the same proposed activity, whichever is the earlier.

Within two weeks of each import authorised under this multiple consignment authority a completed Testing Import Notification Form must be forwarded to the Issuing Authority.

For Exports, a photocopy of the Multiple Consignment Authority should accompany the original Specimen Export Record which details the specific items in each consignment

13. Authority for Permit/Certificate

Issued By: Andrew Murrell

Issue Date: 12/01/2016

Issued under s303GD of the Environment Protection and Biodiversity Conservation Act 1999

Signature

Official seal

008

Australian Government
Department of the Environment

Figure 3. Permit to import Leptinotarsa texana beetles into Australia – Dept. of the Environment.

4.3 Establishing a quarantine breeding colony of *Leptinotarsa texana*

A total of 152 live *Leptinotarsa texana* adults were imported from South Africa on 14 April 2016, following granting of two Federal import licences (see above). Prior to shipment, beetles were reared for at least one generation on *Solanum elaeagnifolium* in laboratory cages at Rhodes University, South Africa, to reduce the risk of importing contaminants such as hyper-parasitoids and to meet permit conditions.

On arrival in Australia, *L. texana* adults were transported under quarantine to the AgriBio insect quarantine laboratory (V2276) where they were unpacked, processed and checked for contaminants and abnormalities (Fig. 4). Live *L. texana* adults were transferred to insect cages (400 x 400 x 400 mm) in a controlled environment room (H.032; set at 25 °C, 16 hrs light:8 hrs dark). A potted silverleaf nightshade plant was placed in each cage as a food source. Silverleaf nightshade used for rearing and subsequent experiments were grown at AgriBio from seed (sourced from various locations in Victoria, South Australia and New South Wales) or root fragments (collected from Calivil, Victoria). The colony was successufully maintained for the duration of the project, and is still active at the time of writing.

Figure 4. Processing *Leptinortarsa texana* shortly after entry into the insect quarantine laboratory at AgriBio (left) and examining adults for external contaminants and abnormalities (right).

4.4 Constructing a host specificity testing list and obtaining plants

4.4.1 Host specificity collecting list

Many species of Solanaceae are of great economic and cultural importance. Food plants include potato, tomato, eggplant (all species of *Solanum*) and Chilli and Bell Peppers (species of *Capsicum*). Solanaceae includes plants of stimulant or pharmacological value, including some in commercial production, for example Tobacco, *Datura*, *Duboisia* and *Hyoscyamus*. In addition, there is a large group of native Australian *Solanum* species that are potentially at risk from released biological control agents, and some of these have special significance to Aboriginal culture.

A large amount of taxonomic expertise, research, discussion and deliberation contributed to construction of a well-balanced and representative host specificity testing list for Solanaceae in Australia. This list will be directly transferable for use in future SLN biocontrol projects. We have

been fortunate to have the expert input of Laurie Haegi to prepare and curate the list. Full details of the methods used to derive the list are given in Appendix 2.

The list considered currently accepted phylogenetic relationships, based on morphological taxonomy, and limited available molecular phylogenetic data, to choose representative species that covered the spectrum of subgenera and clade groupings within Australian Solanaceae, concentrating on *Solanum* species. Plant species on the list were divided into three categories – High, Medium, and Low priority. Efforts were directed at obtaining and testing the High priority species because this category was designed to give good diagnostic coverage of Australian Solanaceae. Where possible, Medium and Low priority species were collected while collecting High priority species. The major clades underpinning the list construction is given in Table 1. It can be seen from this table that the most important subgeneric group for Australia is the Leptostemonum clade, which contains the Australian spiny *Solanum* species.

Table 1. Major clades of *Solanum* in Australia – Native and Adventive species

Major Clade (after Saerkinen et al 2013)	Native species	Adventive species	Totals
Archeasolanum	7	-	7
Brevantherum	-	3	3
Cyphomandra	-	1	1
Dulcamaroid	-	2	2
Geminata	-	2	2
Leptostemonum	162	1	163
Morelloid	1	9	10
Potatoe	-	6	6
Wendlandii	-	1	1
Totals	170	25	195

Taking into account recently described new species our overall list of Solanaceae for this study numbers 214 species including the target *Solanum elaeagnifolium* (SLN). This number is made up of 195 species of *Solanum* and 19 species of other Solanaceae. Propagating material of 55 species, covering High priority and several Medium priority species has been secured. All, but one, have been successfully propagated (by seed, or by cuttings) to produce plants for host specificity trials. Of the 54 species for which plants are held, 36 are native Australian species of *Solanum*, 5 are naturalised weedy species of *Solanum*, 5 are cultivated species of *Solanum*, 5 are native Solanaceae other than *Solanum* (representing 5 separate genera) and 3 are cultivated Solanaceae other than *Solanum.*

The final list of species tested taking into account all of the criteria (Appendix 2) is presented below in two parts: firstly, all the species of the genus *Solanum* tested and secondly, species from other genera in the Solanaceae grown for testing (Tables 2 and 3). Table 4 provides explanatory information for interpreting the tables. The only native species (*Solanum karsense*) listed nationally for conservation status, as vulnerable, is included. Within S*olanum*, all recognised informal taxonomic groups are represented for the geographical extent of SLN, as are all the known clades recognised by available molecular phylogenetic work. In a small number of cases attempts to obtain propagating material of preferred species have not been successful but in all cases material of closely related substitute species has been successfully collected. A robust test list has therefore

been achieved, meeting criteria designed to provide comprehensive and representative taxonomic coverage. This allows the risk posed by the beetle to be robustly assessed.

Table 2. Species list for host-specificity testing: Part 1, *Solanum* spp.

Scientific Name (not grown for testing*)	Occurrence	Adventive = A Native = N	Reason for priority
Solanum amblymerum	N, Q	N	Lepto.27B
Solanum aridicola	NT, SA	N	Lepto.27Z; Indig.
Solanum aviculare	*W, *S, Q, N,V	N	Archae.
Solanum betaceum	*Q, *N, cult. all States	Tamarillo	Cypho.
Solanum brownii	N	N	Lepto.27B
Solanum campanulatum	N	N	Lepto.25Z; OW(v)
Solanum capsicoides	Q, S	A	Lepto.23Z
Solanum centrale	W, NT, S, Q, cult.	N, food crop	Lepto.27B; Cult; Indig.
Solanum chenopodinum	NT, S, Q, N	N	Lepto.13Z; (vi)
Solanum chippendalei	NT, Q, W, cult.	N, food crop	Lepto.28Z; Cult; Indig.
Solanum chrysotrichum	*N, *Q	A	Lepto.14Z
Solanum cinereum	*S, Q, N, A, *V	N	Lepto.27B; OW(v)
Solanum cleistogamum	W, NT, S, Q, N	N, food crop	Lepto.27Z; OW(iii); Indig.
Solanum coactiliferum	W, NT, S, N, V	N	Lepto.27C: Indig.
Solanum ditrichum	Q, N	N	Lepto.25Z
Solanum elaeagnifolium	*W, *S, *Q, *N, *V	A (Target)	Lepto.27C
Solanum eremophilum	S, N	N	Lepto.25Z
Solanum esuriale	W, NT, S, Q, N, V	N	Lepto.27C; Indig.
Solanum ferocissimum	W, NT, S, Q, N	N	Lepto13Z; OW(vi)
Solanum hapalum	Q, N	N	Lepto.09A
Solanum hoplopetalum	W, *W , *S	N	Lepto.25Z
Solanum inaequilaterum	Q, N	N	Lepto.13Z
Solanum jucundum	Q, N	N	Lepto.27B
Solanum karsense	S, N	N	Lepto. 27C
Solanum laciniatum	*W, S, N, V, T	N	Archae.
Solanum lacunarium	S, Q, N, V	N	Lepto. 25Z
Solanum lasiophyllum	W, NT, S	N	Lepto. 27D
Solanum lithophilum	N, NT, Q, S, W, cult.	N, food crop	Lepto. 27Z; Cult; Indig.
Solanum lycopersicum	*W,*NT,*S,*Q,*N,*A, cult.	A,Tomato	Potato; Cult.
Solanum mauritianum	*N, *Q, *S, *V, cult.	A, Garden orn.	Brevanth., Hort.
Solanum melongena	Cult. all states	A, Eggplant	Lepto.28Z, Cult.
Solanum mitchellianum	Q, N	N	Lepto.13A
*Solanum muricatum**	Q, N	Pepino crop	Potato, Cult.
Solanum nigrum	*W,*NT,*S,*Q,*N,*A,	A	Morell.
Solanum nummularium	W	N	Lepto.27C; OW(iv)
*Solanum oldfieldii**	W	N	Lepto.27C
Solanum oligacanthum	S, Q, N	N	Lepto.27C
Solanum opacum	S, Q, N, V, T	N	Morell.
Solanum orbiculatum	W, NT, S	N	Lepto.27C; Indig.
Solanum parvifolium	Q, N	N	Lepto.13Z
Solanum petrophilum	W, S, N	N	Lepto.25Z; OW(v)
Solanum pseudocapsicum	*W, *S, *Q, *N, *Tas	N	Geminata
Solanum quadriloculatum	W, NT, S, Q, N	N	Lepto.27Z
Solanum quitoense	[S, Q, N]	Minor food	Lepto.22Z, Cult.
Solanum stelligerum	Q, N	N	Lepto.13Z; OW(vi)
*Solanum stupefactum**	Q	N	Lepto.29Z
Solanum sturtianum	N, NT, Q, S, W	N	Lepto.27C
*Solanum torvum**	*NT, *Q	A	Lepto.14Z
Solanum tuberosum	*W, *A, cult.	A, Potato	Potato, Cult.
Solanum vicinum	Q, N	N	Lepto.25Z

(See Table 4 for explanatory notes)

A major output from this project was a collection of propagation material of all wild species, supported by fully labelled voucher specimens lodged for the most part in the State Herbarium of South Australia; for material collected in other states duplicates were collected and will be deposited in the principal herbarium of the state where they were collected. Plant material has been identified by the team's taxonomist, a Solanaceae specialist.

Table 3. Species list for host-specificity testing: Part 2, Solanaceae species (excluding *Solanum*). See Table 4 for explanatory notes, etc.

Scientific Name (not grown for testing*)	Occurrence	Native = N	Reason for priority
Capsicum annuum	Widely cult.	Chilli crop	Cultivated
Capsicum frutescens	Widely cult.	Chilli crop	Cultivated
Cyphanthera albicans	N	N	Other Solanaceae
Datura leichhardtii	W, NT, S, Q, N	?N	Other Solanaceae
Duboisia myoporoides	Q, N	N, Pharm. crop	Cultivated, Indig. value
Lycium australe	W, S, N, V	N	Other Solanaceae
*Nicotiana tabacum**	Cult. crop	Tobacco	Cultivated
Nicotiana velutina	W, NT, S, Q, N, V	N	Indig. value

Table 4. Explanatory notes, definitions, symbols and abbreviations in Tables 2 and 3.

Column heading	Notes
Occurrence	Distribution is given by Australian States and Territories; an asterisk* indicates a naturalised (adventive) occurrence; ? = questionably native or naturalised; State-based information generally not available for species in cultivation.
Adventive, Native, Cultivation information	The term adventive is used interchangeably with "naturalised". This may apply to the whole species (where non-Australian species have been introduced and become weedy), but may also apply where self-sustaining populations of species native to one region have been introduced elsewhere (e.g. *Solanum cinereum*, native to Qld, NSW & ACT but naturalised in SA & Vic). Some non-native species are only found in cultivation; some native species are now cultivated on a commercial basis. Information is provided on whether the cultivated species is of major commercial importance, e.g. as a food plant or as an ornamental, or whether grown as a garden ornamental generally or on a limited basis.
Reason for priority	The major subgrouping within the genus Solanum. SLN (*Solanum elaeagnifolium*) falls within the *Leptostemonum* clade ("Lepto"), as with most native Australian species. Priorities include coverage of subgroups in this clade (see Table 2); the other main clades as follows *Archaesolanum* ("Archae"), a small number of characteristically Australasian species), the Morelloids ("Morell"), with several weedy species and one native species and the Brevantherum ("Brevanth"), Cyphomandra ("Cypho"), Dulcamaroid ("Dulcam"), Geminata, Potato and Wendlandii "Wendl") clades, each represented by a small number of naturalised and cultivated species; overlap in geographical occurrence with SLN; coverage of Old World clades recognised to date; important species in cultivation ("Cult.") and species of importance in Australian Aboriginal culture ("Indig.").

Table 5. Information relating to species of *Solanum* of importance to the Aboriginal people in the Central Australian Groups Alyawarr, Anmatyerr, Eastern Arrernte, Western Arrernte, Pintupi, Pitjanjatjara and Warlpiri (after Latz 1995) and their propagation for host-specificity testing.

Species	Occurrence in Central Australia	Part used	Food Rating	Other uses	Used by	Plants grown for testing
S. aridicola (as *S.* sp. aff. *ellipticum*)	Throughout	Fruit	Less important	None recorded	All groups	Yes
S. centrale	Throughout	Fruit	Staple	Mythology	All groups	Yes
S. chippendalei	North-west half	Fruit	Staple	Mythology	All excl. Arrernte	Yes
S. cleistogamum	Throughout	Fruit	Important	Mythology	All groups	Yes
S. coactiliferum	Southern two-thirds	Fruit	Less important	Mythology	Arrernte, Pintupi, Pitjanjatjara	Yes
S. diversiflorum	North-west tenth	Fruit	Important	Mythology	Pintupi	No
S.esuriale	Eastern quarter	Fruit	Less important	None recorded	Alyawarr, Anmatyerr	Yes
S. gilesii	North-west tenth	Fruit	Less important	None recorded	Pintupi	No
S. lithophilum (as *S. ellipticum*)	Throughout	Fruit	Staple to important	Mythology	All groups	Yes
S. orbiculatum	Western half	Fruit	Use doubtful	None recorded	Warlpiri	Yes

4.4.2 Obtaining plants for testing

For the most part plants were produced by propagating seed collected in the field (to maximise genetic variability) but also from field-collected cuttings where seed proved unavailable. This material was supplemented by donated propagating material and plants of horticultural varieties obtained from commercial nurseries, as follows:

- Three extensive field trips were undertaken, each timed and planned (in terms of geographic area) to optimise the number and subgroup / clade coverage of species as well as the chance of obtaining fresh, viable seed or, as a fall-back, material for vegetative propagation.
- Field trips were conducted in northern South Australia (1 week), April 2016 – seed of 11 species collected and successfully propagated); western, central and eastern New South Wales (2 weeks), December 2016 - seed of 9 species collected and successfully propagated); North-western, central northern and north-eastern New South Wales (2 weeks), July 2017 – seed of 14 species and cuttings of 10 species collected and successfully propagated).
- In addition, 39 accessions of seed and cuttings from South Australia, the Northern Territory, Western Australia and Queensland were obtained opportunistically during related and unrelated travel and through colleagues; where these added to species coverage they were propagated to produce plants for testing.
- Plants and propagules of commercial food crop cultivars (see section on host specificity testing) were obtained through industry contacts.
- Plants of species grown as food plants and in ornamental horticulture were also obtained from retail nurseries.

Propagation material of all wild-collected species is supported by fully labelled voucher specimens lodged for the most part in the State Herbarium of South Australia; for material collected in other states duplicates were collected and will be deposited in the principal herbarium of the state where they were collected. Plant material has been identified by the team's taxonomist, a Solanaceae specialist.

Figure 5. A collage of some of the variety amongst native Australian *Solanum* species collected for specificity testing with *Leptinotarsa texana*.

4.5 Propagation pipeline for supplying test plants to AgriBio

Groups of test plants were sequencially propagated in Adelaide, until large enough for use in experiments, then shipped to the Agribio quarantine facilities in Melbourne. The majority of plants propagated for host specificity testing were grown from seed collected from plants in their naturalised or native range in Australia (Figs. 5 and 6). Some species were propagated from cutting material when fruits were not present at the time of collection. Seeds were generally removed from dried fruits. Fruits were dried by placing in a sealed container with silica gel beads. Most fruits dried within four weeks if picked fresh. There were 80 seed collections made from 42 species; 38 *Solanum* species and four other Solanaceae species (Table 6). These seed collections have been divided and sent as duplicate collections, one to Greg Lefoe (AgriBio, La Trobe University Campus) and the other to State Herbarium of SA.

The viability of several suspect (small, light or discoloured) seed collections was assessed by dissecting ten randomly selected seeds to determine if there was an intact embryo and subsequently soaking the dissected seeds in tetrazolium solution. This was to identify species for which

recollection was required. Seeds of Solanaceae are typically physiologically dormant on maturity (Commander et al. 2008). Specific light and temperature cues must be met before seed germinates. As these cues are difficult to determine for a large number of species, germination stimulants can be used to over-ride any specific germination requirements. In some *Solanum* species, germination has been increased by damaging the seed coat by scarifying or leaching, or soaking seeds to remove inhibitors present on the seed coat (Ahmed et al. 2006, Commander et al. 2008). Scarifying was used for species that did not germinate using stimulants (*S. coactiliferum, S. oligacanthum, Datura leichhhardtii*).

For most species propagated in this project, gibberellic acid (GA_3) was a successful germination stimulant. A few species responded more favourably to smoke water solutions. For many *Solanum* species seeds had reached 50% of final germination after 4 days, indicating that *Solanum* species are able to quickly respond to ephemeral rainfall events. Experimental results from this project have been prepared as research paper, documenting the effect of a range of stimulants on a many species of Solanaceae (see Appendix 3).

A total of 654 plants were supplied from Adelaide to Agribio in Melbourne for host specificity testing. These represented 64 collections; 44 *Solanum* species and 5 other Solanaceae species (Table 7). In addition, seed of six species was supplied in petri dishes with stimulant added.

Table 6. Duplicate seed collections held at Agribio (Melbourne) and at the State Herbarium of SA in March 2018.

Species	Collection no.	Collection date	Seed number	Lots
Cyphanthera albicans ssp. *notabilis*	LH3030	Dec 2016	175 seeds	2
Datura leichhardtii	LH2980	May 2016	80 seeds	2
Duboisia myoporoides	LH3043	Dec 2016	140 seeds	2
Nicotiana velutina	LH2985	May 2016	2500 seeds	2
Solanum aridicola	LH3017	Sep 2016	24 seeds	1
Solanum aviculare	JP03	Feb 2017	860 seeds	2
Solanum aviculare	JP04	March 2017	2000 seeds	2
Solanum aviculare	JP08	April 2017	760 seeds	2
Solanum brownii	LH3042	Dec 2016	230 seeds	2
Solanum campanulatum	LH3041	Dec 2016	1100 seeds	2
Solanum capsicoides	LH3069	July 2017	490 seeds	2
Solanum chenopodinum	LH3016	Sep 2016	65 seeds	2
Solanum chippendalei	MGQ1	Aug 2017	12 seeds	1
Solanum chrysotrichum	LH3067	July 2017	7000 seeds	2
Solanum cinereum	HWW01	Aug 2017	250 seeds	2
Solanum cinereum	LH3031	Dec 2016	5380 seeds	2
Solanum cleistogamum	LH3022	Dec 2016	210 seeds	2
Solanum cleistogamum	LH3033	Dec 2016	610 seeds	2
Solanum cleistogamum	LH3053A	July 2017	230 seeds	2
Solanum cleistogamum	LH3054	July 2017	590 seeds	2
Solanum cleistogamum	TB06	April 2016	150 seeds	2
Solanum coactiferum	TB07	Feb 2017	6000 seeds	2
Solanum coactiliferum	BS1137-200	Sep 2017	590 seeds	2
Solanum coactiliferum	BS1137-529	Sep 2017	150 seeds	2

Species	Collection no.	Collection date	Seed number	Lots
Solanum coactiliferum	JH04	March 2017	2000 seeds	2
Solanum coactiliferum	JP02	Feb 2017	1500 seeds	2
Solanum coactiliferum	LH2997	May 2016	140 seeds	2
Solanum eremophilum	LH3028	July 2017	16 seeds	1
Solanum esuriale	JH03	March 2017	760 seeds	2
Solanum esuriale	JP01	Feb 2017	300 seeds	1
Solanum esuriale	JP12	June 2017	100 seeds	2
Solanum esuriale	LH2987	May 2016	190 seeds	2
Solanum ferocissium	LH3034	July 2017	95 seeds	1
Solanum hoplopetalum	DEM8624	Jan 2017	550 seeds	2
Solanum hoplopetalum	DEM8628	Jan 2017	1880 seeds	2
Solanum inaequilaterum	LH3076	July 2017	230 seeds	2
Solanum jucundum	LH3032	July 2017	77 seeds	1
Solanum karsense	LH3023	Dec 2016	220 seeds	2
Solanum laciniatum	LH3044	Feb 2017	seeds	2
Solanum lacunarium	JP11	June 2017	12 seeds	1
Solanum lacunarium	LH2991	May 2016	37 seeds	1
Solanum lasiophyllum	LH3014	Sep 2016	3150 seeds	2
Solanum lithophilum	BS1097-904		35 seeds	1
Solanum lithophilum	JH02	Oct 2016	550 seeds	2
Solanum lithophilum	LH2982	May 2016	5 seeds	1
Solanum lithophilum	LH2994	May 2016	40 seeds	1
Solanum lithophilum	LH3049	May 2017	44 seeds	1
Solanum lithophilum	LH3052A	July 2017	375 seeds	2
Solanum mauritianum	LH3072	July 2017	5370 seeds	2
Solanum mitchellianum	LH3060	July 2017	6 seeds	1
Solanum nigrum	JP16	July 2017	280 seeds	2
Solanum nigrum	JP17	July 2017	1450 seeds	2
Solanum nigrum	LH3081	July 2017	110 seeds	2
Solanum nummularium	DEM8620	Jan 2017	270 seeds	2
Solanum oligacanthum	JP10	June 2017	610 seeds	2
Solanum oligacanthum	LH2992	May 2016	80 seeds	2
Solanum opacum	LH3063	July 2017	121 seeds	1
Solanum opacum	LH3080	July 2017	580 seeds	2
Solanum orbiculatum ssp. orbiculatum	BS1137-191	Sep 2017	250 seeds	2
Solanum orbiculatum ssp. orbiculatum	BS1137-466	Sep 2017	830 seeds	2
Solanum orbiculatum ssp. orbiculatum	CJB7220	Oct 2016	86 seeds	1
Solanum parvifolium	LH3029	Dec 2016	62 seeds	1
Solanum parvifolium	LH3059	July 2017	14 seeds	1
Solanum petrophilum	JH08	Aug 2017	109 seeds	1
Solanum petrophilum	JH09	Oct 2017	180 seeds	2
Solanum petrophilum	LH2995	May 2016	410 seeds	2
Solanum plicatile	DEM8621	Jan 2017	1390 seeds	2
Solanum pseudocapsicum	LH3061	July 2017	790 seeds	2
Solanum quadriloculatum	JP09	June 2017	3270 seeds	2

Species	Collection no.	Collection date	Seed number	Lots
Solanum quadriloculatum	JP13	June 2017	1040 seeds	2
Solanum quadriloculatum	JP14	June 2017	9400 seeds	2
Solanum quadriloculatum	LH2976	May 2016	530 seeds	2
Solanum quadriloculatum	LH3048	May 2017	750 seeds	2
Solanum quitoense	FDS01	July 2017	131 seeds	1
Solanum simile	JP15	Sep 2017	1160 seeds	2
Solanum sturtianum	JH01	Oct 2016	260 seeds	2
Solanum sturtianum	JP06	May 2017	1020 seeds	2
Solanum sturtianum	JP07	May 2017	1890 seeds	2
Solanum sturtianum	LH2979	May 2016	58 seeds	1
Solanum torvum	RA001	Feb 2018	2050 seeds	2

Table 7. Plants supplied to AgriBio for host specificity testing.

Species	Collection number	Number of plants
Cyphanthera albicans ssp. *notabilis*	LH3030	7
Datura leichhardtii	LH2980	4
Duboisia myoporoides	LH3043	4
Lycium australe	LH3082	10
Nicotiana velutina	LH2985	7
S. acanthodapis	LH3070	5
S. amblymerum	LH3064	4
S. amblymerum	LH3058	11
S. aridicola	LH3017	26
S. aviculare	JP03	12
S. betaceum	BUN01	4
S. brownii	LH3042	18
S. campanulatum	LH3041	29
S. capsicoides	LH3069	6
S. centrale	LH3015	3
S. chenopodinum	LH3016	29
S. chenopodinum	JH05	5
S. chippendalei	MGQ1	4
S. chrysotrichum	LH3067	2
S. cinereum	LH3031	18
S. cinereum	LH3065	1
S. cleistogamum	LH3033	28
S. cleistogamum	TB06	9
S. cleistogamum	LH3022	2
S. coactiliferum	JH04	7
S. coactiliferum	LH2979	3
S. coactiliferum	LH2997	4
S. ditrichum	LH3062	13
S. eremophilum	LH3027	8
S. esuriale	JH03	12
S. esuriale	LH2987	1
S. ferocissimum	LH3034	20
S. hapalum	LH3074	17
S. hoplopetalum	DEM8628	6

Species	Collection number	Number of plants
S. inaequilaterum	LH3073	12
S. inaequilaterum	LH3076??	6
S. jucundum	LH3032	30
S. karsense	LH3023	17 + seed
S. laciniatum	LH3044	12
S. lacunarium	JP11	3
S. lacunarium	LH2991	12 + seed
S. lasiophyllum	LH3014	42
S. lithophilum	BS1097-904	23
S. lithophilum	LH2994	14 + seed
S. lithophilum	JH02	seed
S. mauritianum	LH3072	8
S. mitchellianum	LH3060	6
S. nigrum	LH3081	6
S. nummularium	DEM8620	18
S. oligacanthum	LH2992	3
S. oligacanthum	JP10	3
S. opacum	LH3080	6
S. orbiculatum ssp. *orbiculatum*	BS1137-191	5
S. parvifolium	LH3059	1
S. parvifolium	LH3029	7
S. petrophilum	LH2995	20 + seed
S. plicatile	DEM8621	2
S. pseudocapsicum	LH3061	12
S. quadriloculatum	JP14	15
S. quadriloculatum	LH2976	2 + seed
S. simile	JP15	3
S. stelligerum	LH3066	4
S. sturtianum	LH2979	32
S. vicinum	LH3078	3
TOTAL		654

Figure 6. Glasshouse propagation in Adelaide, prior to host specificity testing at AgriBio, La Trobe University, Melbourne

4.6 Molecular biology research

There were three molecular biology (DNA analysis) components within the SLN biocontrol project. The first investigated the geographic origins of SLN introduced to Australia, and analysed SLN DNA from around the world to compare it to the DNA of Australian SLN. The second component analysed the DNA of native Australian *Solanum* species to construct a phylogenetic model based on molecular evidence, so that a representative subset could be chosen for host specificity testing with greater confidence. A third component examined the DNA of *Leptinotarsa texana* beetles imported to establish our Australian research colony, to confirm that it was true to species.

4.6.1 Origin of Australian SLN

Note: This research is described in detail in a draft research paper in Appendix 3 to this report.

Silverleaf nightshade has a native distribution in North and South America. Though contentious, the species is proposed to have originated in the southern portion of North America where a greater diversity of arthropod herbivores are specialized to use the plant as a host. SLN is an economically-important introduced weed in a variety of northern and southern hemisphere countries (including Australia, South Africa and the Mediterranean region. Knowledge of the origins and genetic diversity of introduced SLN in Australia is useful for choosing overseas locations to look for potential biocontrol agents.

Two sections of chloroplast DNA were anlaysed from 155 SLN specimens collected from its native range, and 333 specimens from the three major regions to which it has spread (Tables 8 and 9). A total of 41 haplotypes (identified genetic types) were identified in the sample and the relationships between them is shown in Figure 7. The ancestral haplotype in the network was dominant in North America, but less common in many of the introduced populations, and absent from South America. All haplotypes found in South America were unique to that continent, and most were related as a clade minimally separated by > 0.337 % sequence difference from the most recent common ancestor (MRCA) and all other SLN haplotypes. Presence of an additional widespread haplotype, unique to South America but derived from the MRCA in North America, is supportive of prior genetic analyses of the species which suggested there have been historical episodes of secondary entry of the species into South America from the North American continent.

These results are supportive of a North American provenance for SLN populations in the Mediterranean region, South Africa and Australia. The data also show that introduced SLN in Australia, the Mediterranean region and South Africa contain a moderately diverse array of chloroplast DNA lineages derived from a North American provenance. There is no evidence that any of the South American haplotypes have been introduced to other countries.

Our results have implications for understanding the major pathways involved in the invasive ecology of this species, and for choice of putative agents proposed for biocontrol of the weed. The introduced regional populations of SLN in Australia, South Africa and the Mediterranean show moderately high levels of genetic diversity, marginally less than that of native American populations. Australia and the Mediterranean share much of their diversity (Table 9). Surprisingly most introduced populations share a moderately high frequency haplotype that is only observed in the Americas in two central US states (Oklahoma and Kansas) at low frequency. This suggests that initial SLN introductions to Australia, South Africa and the Mediterranean, in the early to mid -20th C, were commonly derived from the central US

states. These introduced populations also display mixed levels of genetic relatedness and have an exclusively North American source. It may be concluded from this genetic evidence that co-evolved agents from the North American continent are best suited for use as biocontrol agents for SLN. The potential use of biocontrol agents from South America should not be discounted, as there exists the possibility that they have evolved host relationships with the aforementioned historically recent North American SLN lineage present and widespread in South America.

Table 8. Summary of genetic diversity measures estimated from chloroplast DNA gene sequences from native and introduced SLN populations. Constituent Mediterranean countries are indicated in parentheses. Numbers of specimens sequenced (*N*), haplotypes (*K*), private haplotypes – mutations not part of the haplotype definition (K_{priv}); proportion of specimens with private haplotypes ($K_{priv\%}$). Haplotype (*h*) and nucleotide (π) diversity estimates as per Nei (1987). Diversity estimates included indels (insertions or deletions of DNA) recoded as single mutation events as described by Pearce (2006).

Source	region	*N*	*K*	K_{priv}	$K_{priv\%}$	*h*	π
native							
	North America	107	18	11	0.252	0.818	0.0015
	South America	48	8	8	1.000	0.820	0.0024
introduced							
	Australia	185	20	8	0.065	0.712	0.0015
	Mediterranean	128	13	2	0.023	0.777	0.0018
	(France)	20	2	0	0	0.256	0.0004
	(Greece)	42	9	2	0	0.774	0.0020
	(Israel)	16	5	0	0	0.767	0.0016
	(Morocco)	20	7	0	0	0.863	0.0015
	(Tunisia)	30	2	0	0	0.287	0.0006
	South Africa	20	3	0	0	0.574	0.0009

Table 9. Pairwise estimates of population genetic structure between populations of *Solanum elaeagnifolium*. Estimates of F_{ST} (haplotype frequency only; lower diagonal) and Φ_{ST} (incorporating nucleotide differences; upper diagonal) structure. All estimates significant at P < 0.01 unless indicated ^{NS}.

	North America	South America	Australia	Mediterranean	South Africa
North America		0.602	0.116	0.051	0.167
South America	0.181		0.635	0.600	0.584
Australia	0.177	0.242		0.045	0.090
Mediterranean	0.133	0.204	0.009^{NS}		0.147
South Africa	0.230	0.286	0.107	0.104	

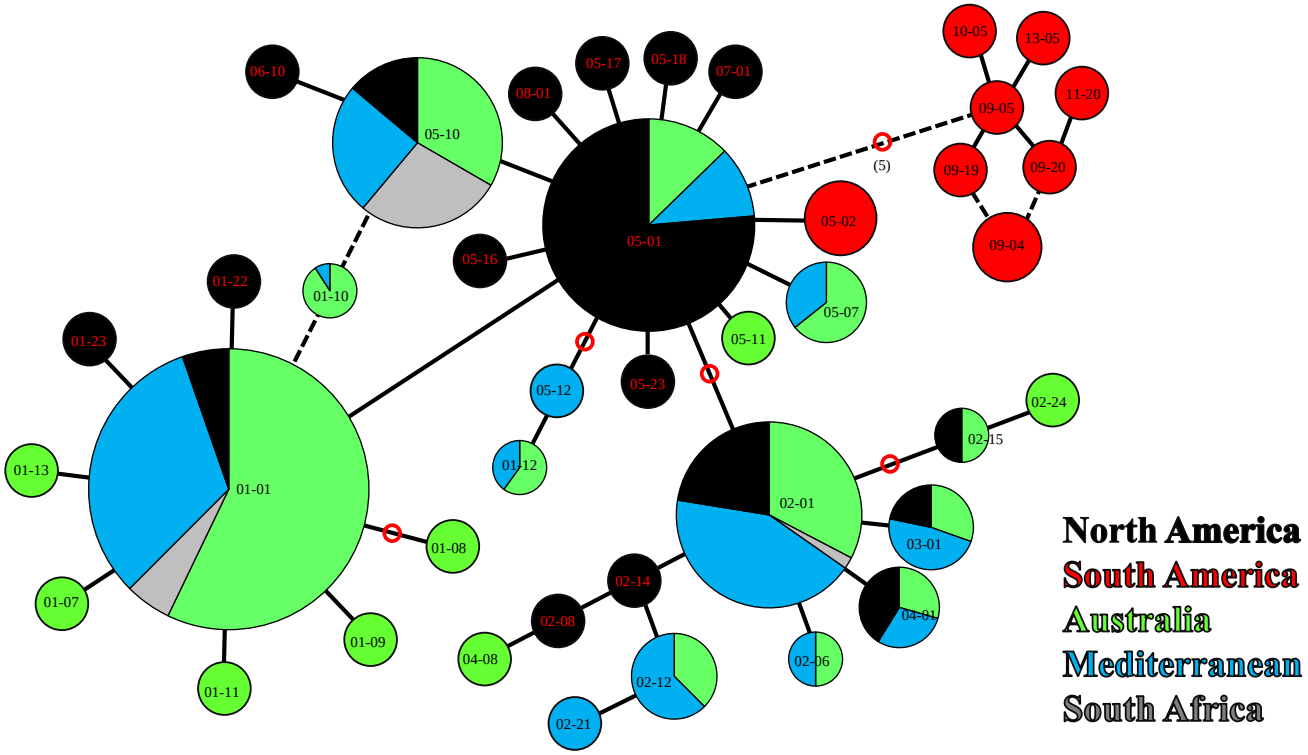

Figure 7. Network of genetic relationships among 41 silverleaf nightshade haplotypes with different chloroplast DNA sequences, each represented by coloured circles. Internal labels indicate the name given to the haplotype. Haplotypes are color coded according to their proportional representation in geographic regions; haplotype circle sizes are approximately representative of their frequency found in the sample. Connections between haplotypes represent a single mutation difference; presence of additional mutations indicated by small open red circles (and numbered if more than two mutations). Dashed connections indicate unresolved relationships.

4.6.2 Phylogeny of Australian *Solanum* species

Note: This research is described in detail in a draft research paper in the Appendix 3 to this report.

This project provided DNA data based on sequences from several genes. Data was obtained from a broad variety of *Solanum* species native to Australia, with a focus on species in the *Leptostemonum* clade, so that phylogenetic relationships among Australian species could be reconstructed. This provides a means to establish a more sound understanding of the relationships among taxa within the Australian species of *Solanum*, as well as their broader relationships to other sections in the clade, including SLN. The knowledge obtained from this research is essential for understanding *Solanum* host specificity for proposed biocontrol agents for SLN, and as a means of predicting potential novel host shifts by the agents to non-target endemic *Solanum*.

Host specificity testing was an important cornerstone of this project. It was vital to ensure that a well-choosen representative group of test species be assembled to reduce the risk of off-target damage to native Australian *Solanum* species. A robust and safe testing list relies on very good and detailed phylogenetic information at the species level. Although this information was available for Australia, it was mostly based on traditional macro- and micro-morphological evidence. Of the c.177 native Australian *Solanum* species recorded, fewer than 20 have been included in phylogenetic analyses using molecular biology. These DNA analyses from our project provided a second data set to compare to, and validate, morphological evidence. This section of research analysed phylogenitic relationships between Australian *Solanum* species using molecular techniques (DNA sequences) and advanced computer algorithms, to strengthen the phylogenetic model used to select species for the list. David Gopurenko, Xiaocheng Zhu and Hanwen Wu (NSW DPI, Wagga Wagga) collaborated with Laurie Haegi (State Herbarium of SA) to produce a large and important body of work that now underpins our understanding of *Solanum* species in Australia. This is almost all new and fundamental information that will also be very useful for future SLN biocontrol projects.

A strategic approach to species sampling was used, taking into account published sequence data and morphological taxonomic knowledge to optimise species coverage and to target good quality material. The principal sources of material were from herbarium specimens (< 30 years old) and vouchered samples newly collected in the field for our project. A total of 341 specimens, representative of 162 Solanaceae species, were included in DNA analyses. Most specimens were obtained from existing collections available at the State Herbarium of SA (N= 187). Additional samples were obtained from the Sydney Herbarium (N=16), the Queensland Herbarium (N=72), accessions (N=14) from overseas contributors, and recent sampling efforts (N=52) during 2016-2017 (vouchered at the Adelaide Herbarium).

Four genetic loci (DNA regions) are reported to contain levels of phylogenetic variability appropriate to understanding the molecular systematics of Solanaceae (Levin et al 2006; Saerkinen et al 2013; Aubriot et al 2016) these were used for genetic analyses. The nuclear (nr) DNA targets were named *waxy* and *ITS*, and the chloroplast (cp) DNA targets were named *matK* and *trnL-trnF*. PCR products and high quality sequences were obtained from N = 328, 315 and 277 specimens at *matK*, *trnL-trnF* and *waxy* gene regions, representing 154, 153 and 141 species, respectively. In contrast, PCR success and sequence quality of the nuclear ribosomal ITS region was highly variable among species and replicates, resulting in sequence availability for less than 41.7% of specimens. An analyses of the suggested phylogeny trees is presented in Figures 8A –8C. Please note that this research is the subject of a draft scientific paper manuscript (Appendix 3) and the authors are currently interrogating the data to finalise their conclusions.

Explanatory notes for Figures 8A to 8C. Black triangles: length represents the amount of genetic diversity (i.e. longer triangles have higher diversity); height represents the number of species comprising the triangle cluster; node number (e.g. 78) denotes the statistical strength of the classification (<50% regarded as insignificant). Scale bar measures the relative genetic distance within the diagrams.

Figure 8 (A, B, C). Phylogenetic relationships among the Leptostemonum clade ("spiny solanums") from Australia, and SLN and other Solanaceae groups. Statistically-significant (>50%) differences between clades are indicated at the nodes. Trees reporting collapsed (condensed) phylogenetic relationships among the Leptostemonum clade and other taxa (Fig. 8A); Collapsed (condensed) clades of the major groups within the target spiny solanum sub-generic groups as proposed by Whalen (1984) (Fig. 8B); Uncollapsed (full detail) tree showing genetic relationships among all specimen tips (Fig. 8C). Please note that the detail in Figure 8C can been viewed by using the page zoom function in electronic formats, however it is illegible in paper copies of this report. Please refer to the published version of the paper (expected in late 2018) for details printed on paper.

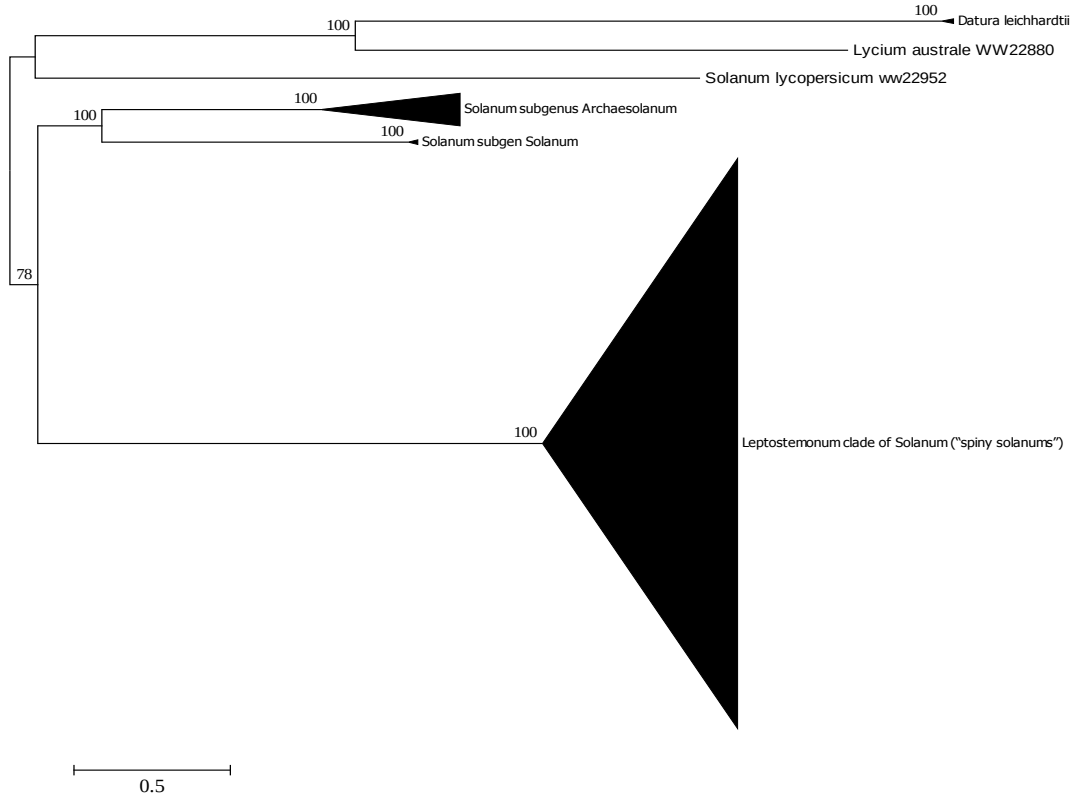

Figure 8A

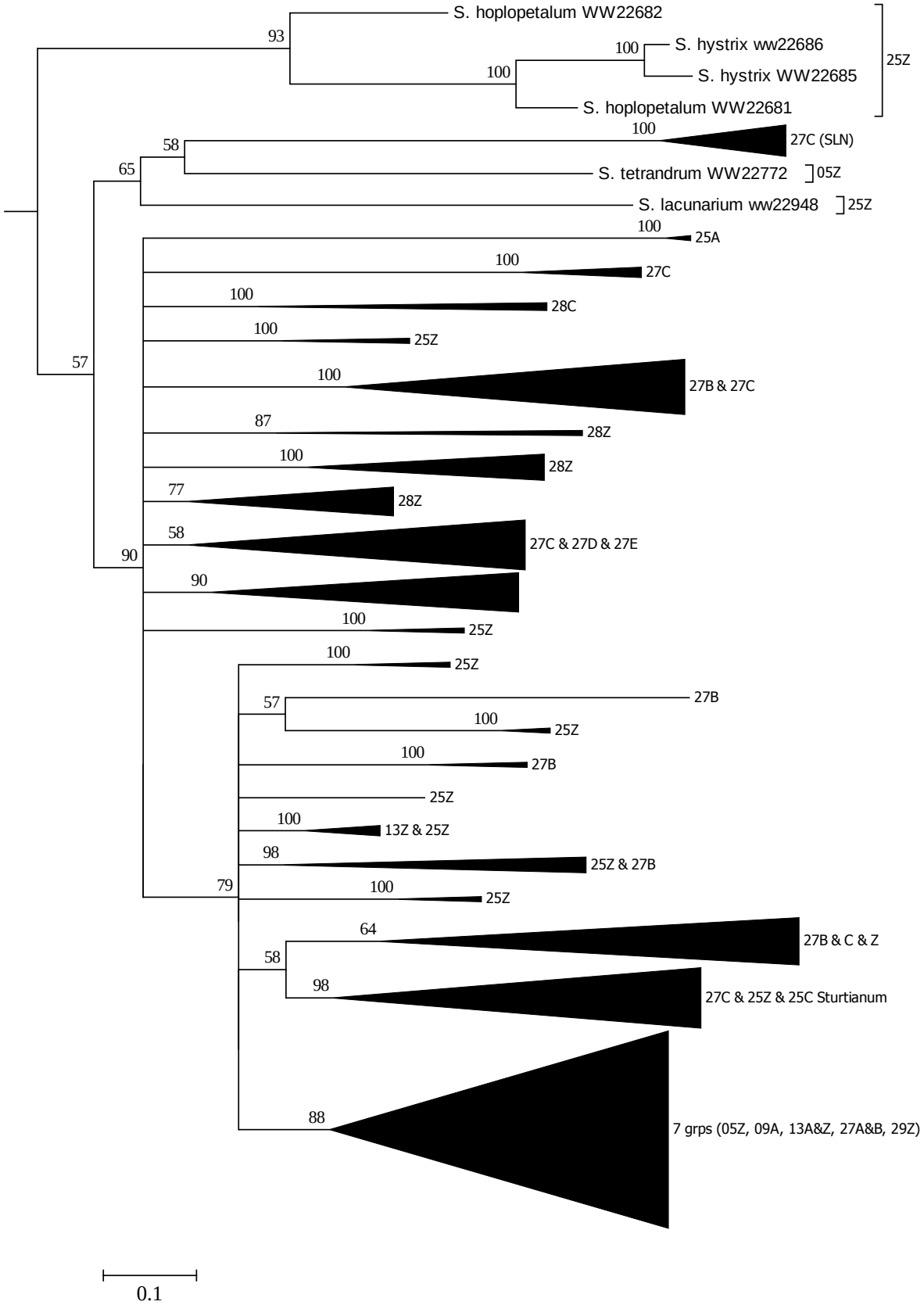
S. hoplopetalum WW22682
S. hystrix ww22686
S. hystrix WW22685
S. hoplopetalum WW22681
25Z
27C (SLN)
S. tetrandrum WW22772
05Z
S. lacunarium ww22948
25Z
25A
27C
28C
25Z
27B & 27C
28Z
28Z
28Z
27C & 27D & 27E
25Z
25Z
27B
25Z
27B
25Z
13Z & 25Z
25Z & 27B
25Z
27B & C & Z
27C & 25Z & 25C Sturtianum
7 grps (05Z, 09A, 13A&Z, 27A&B, 29Z)
93
100
100
58
65
100
100
100
100
57
100
87
100
77
58
90
90
100
100
57
100
100
100
98
79
100
64
58
98
88
0.1

Figure 8B

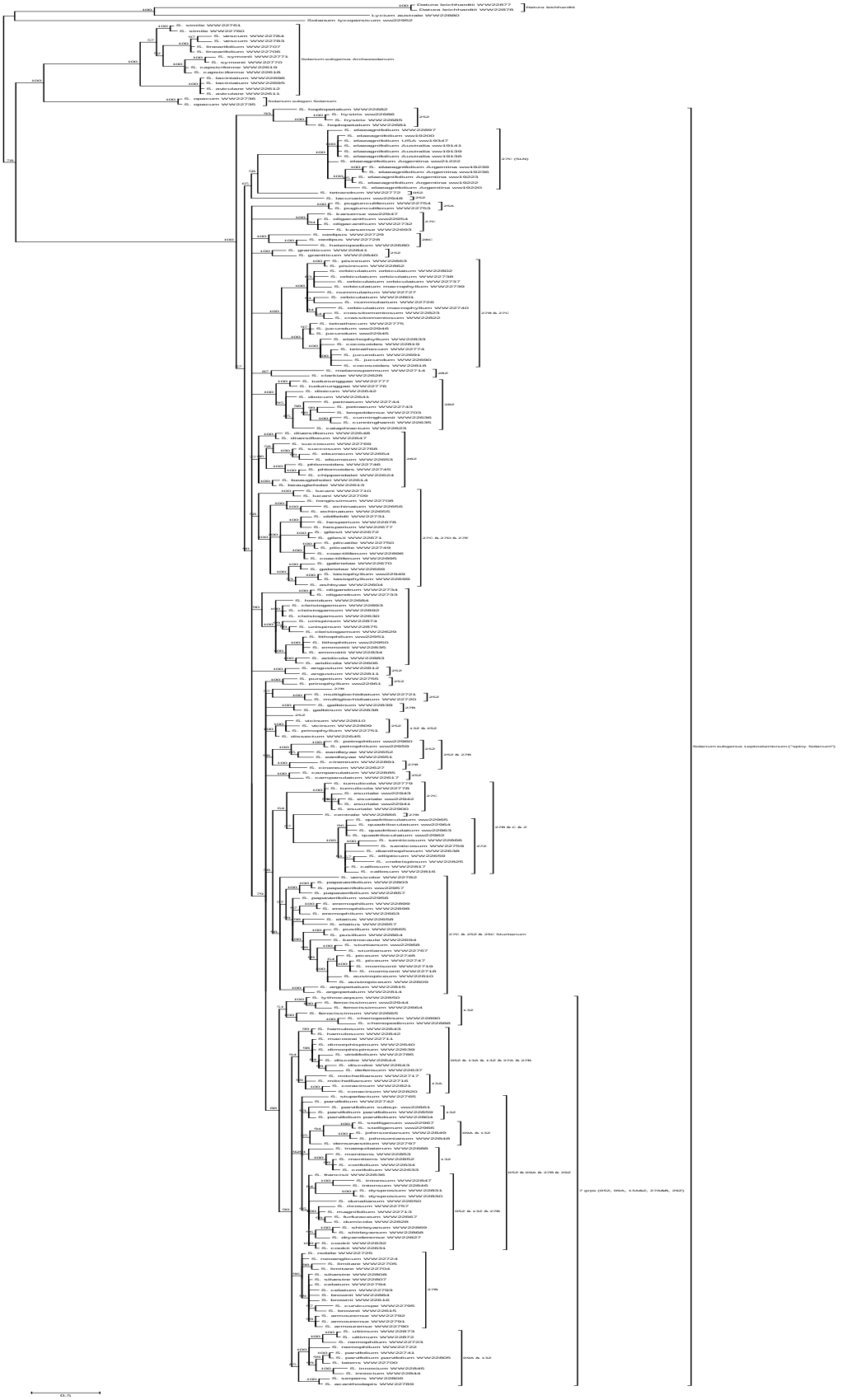

Figure 8C

These results build significantly on previous global surveys providing an initial assessment of relationships within the broad group of *Solanum* species, which includes SLN, in the Leptostemonum clade or 'spiny solanums'. Those earlier studies have to date included less than 15% of the Australian species in the group while our study, covering almost 90% of the known Australian species, both reinforces some former phylogenetic conclusions, though with greater reliability, and suggests some significant departures from previous understanding.

Importantly, the current results confirm that the vast majority of Australian spiny Solanums are grouped together within a clade quite separate from silverleaf nightshade. This contradicts long-held views, based on morphological evidence, that suggested a close relationship between silverleaf nightshade and several Australian species.

These results add weight to evidence that suggests that groupings based on observable morphological characteristics sometimes do not reflect true relationships among *Solanum* species. These evolutionary relationships can only be understood using molecular biology, and understanding them is crucial to ensuring that host-specificity testing lists encompass a representative range of species. This has implications for sampling across the Australian native spiny solanums for host specificity testing, suggesting that species be choosen using a revised sub-group structure. A small number of morphologically-based monophyletic groups are supported by our data and therefore remain useful for sampling.

Perhaps the most far-reaching outcome of our study is the clear demonstration that the Australian spiny Solanums are paraphyletic with respect to silverleaf nightshade, with a group of two distinctive Australian species emerging - the *S. hystrix* / *S. hoplopetalum* clade. How these species relate to the other major groups of spiny Solanums from the New World, Old World, South-east Asia and Oceania respectively, will await further studies.

Within the broader project, these studies were of necessity being conducted concurrently with assessment of the biological control agent. The results were therefore not available to guide species sampling and now provide a retrospective view. The carefully-planned and rigorous approach taken in constructing a test list across the Australian spiny Solanums has proved to be robust in the light of the new knowledge from our molecular phylogenetic studies. That confirmation will prove of great value in providing sampling confidence for any future proposals for the introduction of biological control agents for silverleaf nightshade.

Of the 25 clades (excluding SLN) recognised in our study, 15 are represented among the species grown for testing. This includes the outlying *S. hystrix* / *S. hoplopetalum* clade. In the event, species falling into the remaining ten clades were all rated low priority and not included in testing: all of the species concerned are well separated geographically and climatically from actual and potential areas of occurrence of silverleaf nightshade.

In summary, the vastly improved picture of relationships among the Australian spiny Solanums not only provides re-assurance for the basis of sampling for host specificity testing in the current project, but also provides greater confidence and reliability in selecting species in any future proposals, even in the face of any potential expansion of the occurrence of silverleaf nightshade in Australia in the meantime.

4.6.3 Confirming the identity of *Leptinotarsa texana* imported for this project

Introduction

Note: This research is described in detail in a draft research paper in Appendix 3 to this report.

L. texana beetles used in quarantine host specificity testing in Australia were bred in captivity and were originally derived from a naturalised source of the beetle released for biocontrol purposes in South Africa (Lefoe et al. 2016). It was important to test for the presence of cryptic species diversity in our imported beetles which, if present, had the capacity to confound results of the host specificity testing. DNA barcoding (Hebert et al. 2003) of samples from 94 larvae was used to ensure that the progeny of our imported beetles were taxonomically accurate for *L. texana.* High quality mitochondrial DNA barcodes of 91 specimens were available for comparison to all pre-existing DNA barcodes for six *Leptinotarsa* spp (*L. decemlineata* [N=31], *L. haldemani* [N=1], *L. juncta* [N=2], *L. lineolata* [N=1], *L. texana* [N=2]). Two DNA barcode haplotypes were identified among the 91 specimens and these two haplotypes had their closest genetic match (> 99.3 % sequence similarity) to published sequences for *L. texana* sourced from its native range. DNA barcodes of other species, including the economically important pest Colorado potato beetle (*L. decemlineata*), differed from the query specimens by 5.65 – 16.50 %. These results support the contention that the beetles brought to Australia were taxonomically accurate to *L. texana*. DNA barcodes for a potentially closely related species, *L. defecta,* were not available for comparison in the current study, but their availability for future analysis is recommended given the close taxonomic and biogeographical association of this species to *L. texana*, and their shared introduction as silverleaf nightshade biocontrol agents in South Africa.

Neighbor-joining sequence distance (%) relationships between the query specimens (N=91) and all publicly-available and comparable target gene sequences (N=41) from five *Leptinotarsa* species at BOLD and or GenBank are shown in Figure 9.

All sequences from the 91 specimens were clustered in a shallow clade, suggesting low genetic variability. Their closest genetic matches (< 0.61 % sequence difference) were to two taxonomically vouchered *L. texana* specimens (HQ605774 & HQ605775) sampled from Weslaco, Texas, USA. Available *Leptinotarsa* species were each genetically monophyletic (Fig. 10). Maximum intraspecific variation among *L. texana* (0.61 %) was an order of magnitude lower than the minimum distance (5.7 %) to its nearest genetic neighbour species (*L. juncta*) and > 12% to all other available *Leptinotarsa* species, including *L. decemlineata*.

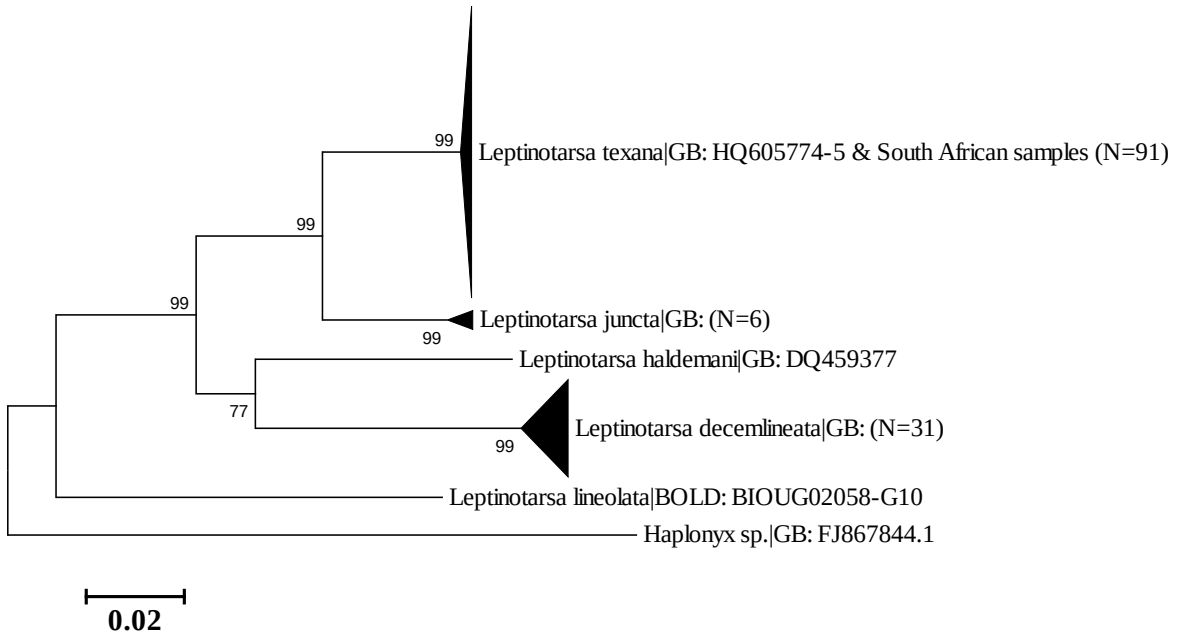

Figure 9. Genetic distance tree based on target mitochondrial sequences obtained from query beetle larvae (N=91) bred from naturalised South African *L. texana*, and DNA sequences of additional *Leptinotarsa* spp. available at GenBank and BOLD sequence repositories. Species clades are collapsed (condensed) into triangles, and are representative of interspecific variation. Scale bar equals 2% sequence difference (equivalent to one mutation). Numbers (> 70) indicating statistical significance for clades are indicated at the nodes. Refer to Figure 10 for details of the *L. texana* intra-species clades.

Explanatory notes for Figures 9. Black triangles: length represents the amount of genetic diversity (i.e. longer triangles have higher diversity); height represents the number of species comprising the triangle cluster; node number (e.g. 77) denotes the statistical strength of the classification (<50% regarded as insignificant). Scale bar measures the relative genetic distance within the diagrams.

The results suggest that the original agent sent to South Africa was genetically diverse, albeit at very low levels of population genetic difference. This data will be useful for future diagnostics and population diversity assessment of *L. texana*.

Given that both *L. texana* and *L. defecta* were introduced from Texas USA to South Africa as biological agents for the control of silverleaf nightshade (Olckers, et al. 1999), it would be prudent to examine mitochondrial and nuclear DNA barcodes from *L. defecta* for direct genetic comparison to *L. texana.* This would determine the extent to which the two species could be identified by this method, and also as a means to test for possible genetic introgression between the species where they occur together.

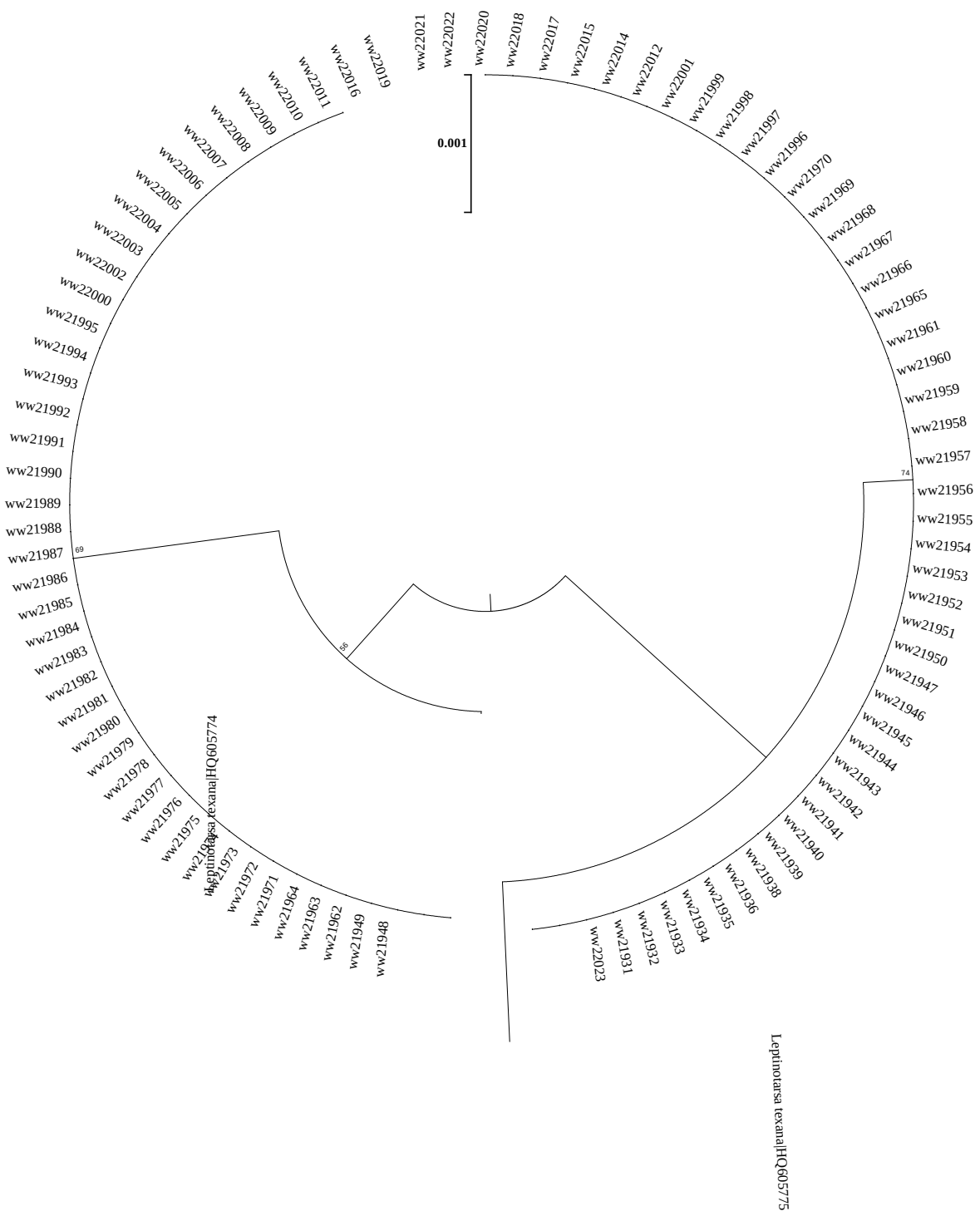

Figure 10. Circular genetic distance tree based on target mitochondrial gene sequences obtained from query beetles (N=91) and voucher *Leptinotarsa texana* sequences (GenBank accessions: HQ605774 & HQ6057750) from Texas USA. Scale bar = 0.1% sequence difference (equivalent to one mutation). Please note that the detail in Figure 10 can been viewed better by using the page zoom function in electronic formats, however it may be illegible in paper copies of this report. Please refer to the published version of the paper (expected in late 2018) for details printed on paper.

4.7 Host specificity testing

4.7.1 Introduction

An important component of biological control risk analysis is assessment of an agents' host-range. Host-range experiments are typically conducted in a quarantine laboratory, where non-target plants are exposed to the insect in replicated cage experiments. The host-range expressed in the laboratory is termed the *fundamental host range*. The fundamental host range encompasses the full range of plants an insect agent is capable of utilising. The range of hosts actually utilised by the agent under field conditions is termed the *realised host range* (also referred to as the agent's *field host specificity*), and may be a subset of the fundamental host range. A host specific agent can express a broader fundamental host range if important behavioural or chemical cues are absent or disrupted in the confines of small laboratory cages. False positive results (attack in the laboratory that does not occur in the field) may complicate risk analysis, because Australian regulators rely heavily on the results of host-range experiments conducted in quarantine laboratories (Department of Agriculture and Water Resources, n.d.). In these cases, agents are not likely to be approved for introduction unless additional evidence is produced to support field host specificity.

In host-range experiments conducted in Agriculture Victoria's insect quarantine laboratory, *L. texana* developed on eggplant, potato and fifteen Australian native plant species when confined in benchtop laboratory cages. This result was anticipated to some extent, because previous research in South Africa showed that off-target damage to eggplant and some *Solanum* species native to South Africa could occur in laboratory cages (Olckers et al., 1995). South African researchers attempted to simulate field conditions by using large walk-in cages in a glasshouse, but off-target damage still occurred. Despite these laboratory results, there is no record of *L. texana* feeding on eggplant or potato crops in North America or South Africa (where *L. texana* was subsequently introduced).

To determine if the Australian laboratory results for non-target species could represent false positives, eggplant (April 2017) and potato (planned for August 2018) plants were exposed to *L. texana* in open field experiments in the insects' native range of Texas, USA. The aim of the open field experiments is to assess the realised host-range of the insect by allowing *L. texana* to express its natural host-seeking and host-acceptance behaviour (Clement and Cristofaro, 1995). Data obtained in replicated open field experiments in the insects' native range could make an important contribution to assessing the level of risk to two economically important crop species.

Challenges in this risk analysis include the large number and importance of *Solanum* spp. closely related to the target weed (including crops and native species), and relatedness of the proposed agent to a well-known potato pest *Leptinotarsa decemlineata*.

The aims, therefore, were to identify 1) what native and economically important plants could be at risk of off-target damage in Australia, 2) the likely nature and extent of off-target damage should it occur, 3) whether additional research is required to predict the realised host-range, and 4) whether further research on the agent is warranted.

4.7.2 Host specificity testing in glasshouse cages

Materials and methods

There are many ways in which biological control agents can be tested for host specificity. Different laboratory conditions can produce different results, even with the same agent and the same test plant species. Laboratory results often differ from those obserbed in open field experiments. In "no-choice" experiments the agent is offered only one plant species to feed on, or perish. In "choice" experiments two or more species of plant are offered at the same time, allowing the agent to chose

its preferred host. In small enclosures there is an increased chance that the beetle will stumble onto a plant without making a directed choice. “Large tent” experiments are aimed at minimising this effect, and when using them it is more likely that airborne molecules from host plants will guide host selection. Open field experiments provide “true” host specifity results, but are often not feasible due to quarantine constraints.

No-choice experiments with larvae

Newly emerged larvae were exposed to individual potted test plants (SLN or non-target plant). Newly emerged larvae were collected from egg incubation dishes and confined on individual plants using a fine gauze sleeve (Fig. 11). SLN was tested, as a standard control, on each day that non-target species were tested (Fig. 12) to record *L. texana* survival on a known host. Test plants with larvae were housed in a controlled environment room (H.032; set at 25 °C, 16 hrs light:8 hrs dark) where they remained for at least six weeks to allow time for feeding and development.

Foliar damage on each plant was assessed two to three times each week and scored as: 0 = no damage; T = trace damage or almost no damage (~ 0.1%); 1 = 0.1-10% leaf area consumed; 2 =10-50% leaf area consumed; 3 = >50% leaf area consumed (Fig. 13). Development of larvae was noted if observed, and the number of adults emerged on each plant was counted after six weeks.

Figure 11. Close-up of a fine gauze sleeve used to confine larvae to no-choice test plants (left). Zipper access facilitated inspection and assessment with minimal disturbance to insects.

Figure 12. Potted silverleaf nightshade and tomato *Solanum lycopersicum* plants being prepared for a no-choice experiment (below).

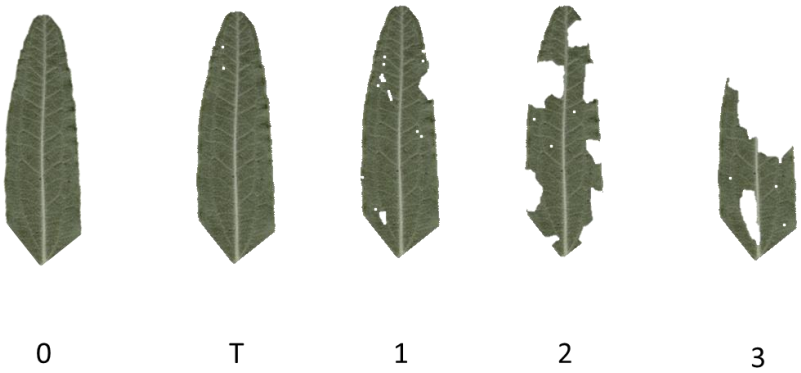

Figure 13. Visual guide to scoring foliar damage to test plants.

Small-cage choice minus target experiment with adults

Four non-target Australian *Solanum* spp. were placed in a fine gauze cage and three pairs of newly-emerged adult beetles were added. Three pairs of adults were similarly added to a cage with a single SLN plant. Number of eggs on each plant, feeding damage, and location of adults was assessed two to three times each week for six weeks. A separate experiment was conducted on four non-target horticultural species using the same method. Cages were placed into a controlled environment room (H.032; set at 25 °C, 16 hrs light:8 hrs dark).

Large-cage choice experiment with adults

Ten non-target Australian *Solanum* spp. in pots and a single SLN plant were placed equidistant in a 2m diameter circle in a large insect tent (3m x 3m x 1.8m) in a quarantine glasshouse. Eight pairs of adults, emerged in the previous week and exposed only to *Solanum elaeagnifolium*, were collected in a petri dish and placed in the centre of the cage. Number of eggs on each plant, feeding damage, and location of adults was assessed two to three times each week for eight weeks.

Continuation experiments

Adult *L. texana* emerged from no-choice experimental test plants were collected and confined on a new plant of the same non-target species (dependant on the availability of non-target plants). Survival, feeding damage and number of eggs laid was recorded over more than one generation.

Results

No-choice experiment with larvae

Table 10. Larval feeding damage and adult emergence on *Solanum elaeagnifolium* and non-target plants after six weeks (neonate larvae applied). Damage rating 0 = no damage; T = trace damage or almost no damage (~ 0.1%); 1 = 0.1-10% leaf area consumed; 2 = 10-50% leaf area consumed; 3 = >50% leaf area consumed, Fig. 11, above).

Subgenus	Group	Species and cultivar (if applicable)	Number of replicates	Number of larvae applied	Number adults emerged	Percent adult emergence	Max damage score
Archaeosolanum	n/a	*Solanum aviculare*	4	40	28	70	3
Leptostemonum	27C	*Solanum elaeagnifolium*	45	294	188	64	3
		Solanum coactiliferum	3	15	13	87	2
		Solanum esuriale	1	5	1	20	2
		Solanum karsense	4	24	13	54	3
		Solanum nummularium	2	10	0	0	2
		Solanum oligacanthum	1	5	4	80	2
		Solanum sturtianum	3	16	8	50	3
	27B	*Solanum amblymerum*	4	20	5	25	3
		Solanum brownii	3	18	15	83	3
		Solanum centrale	1	5	0	0	T
		Solanum cinereum	4	21	15	71	3
		Solanum jucundum	3	18	3	17	2
	27D	*Solanum lasiophyllum*	4	20	0	0	1
	27Z	*Solanum aridicola*	4	20	2	10	2
		Solanum cleistogamum	7	35	-	-	3
		Solanum lithophilum	4	20	14	70	3
	13Z	*Solanum chenopodinum*	4	20	-	-	3
		Solanum ferocissimum	4	18	16	89	3
		Solanum stelligerum	3	15	-	-	2
	25Z	*Solanum campanulatum*	3	15	0	0	T
		Solanum ditrichum	1	5	0	0	0

		Solanum lacunarium	4	23	18	78	3
		Solanum petrophilum	4	20	11	55	3
	28Z	*Solanum chippendalei*	1	6	0	0	0
	n/a	*Solanum melongena* "Black Beauty"	8	60	44	73	3
n/a	*n*/a	Solanum *betaceum* "Ecuador Orange"	4	23	0	0	0
		Solanum *lycopersicum* "Grosse Lisse"	4	40	0	0	0
		Solanum *lycopersicum* "Red Cherry"	4	20	0	0	0
		Solanum *lycopersicum* "Roma VF"	4	20	0	0	0
		Solanum *lycopersicum* "Tiny Tom"	4	20	0	0	0
		Solanum *mauritianum*	4	20	0	0	T
		Solanum *pseudocapsicum*	2	10	0	0	2
		Solanum *tuberosum* "Argos"	5	25	11	44	3
		Solanum *tuberosum* "Daisy"	5	25	3	12	3
		Solanum *tuberosum* "Desiree"	4	20	0	0	1
		Solanum *tuberosum* "Nadine"	6	30	21	70	3
		Solanum *tuberosum* "Pontiac"	4	20	4	20	2
		Solanum *tuberosum* "Russett Burbank"	4	20	0	0	1
		Solanum *tuberosum* "Sebago"	4	20	0	0	0
		Solanum *tuberosum* "Valor"	6	30	15	50	3
		Capsicum *annuum* "California Wonder"	4	20	0	0	0
		Capsicum *annuum* "Cayenne"	4	20	0	0	T
		Capsicum *annuum* "Hot Thai Bird's Eye"	3	15	0	0	T
		Capsicum *annuum* "Jalapeno"	4	20	0	0	0
		Cypanthera *albicans* subsp. *notabilis*	3	18	0	0	2
		Datura *leichhardtii*	3	17	0	0	T
		Nicotiana *velutina*	1	5	0	0	0
		Petunia *x atkinsiana* "Grandiflora"	4	20	0	0	0

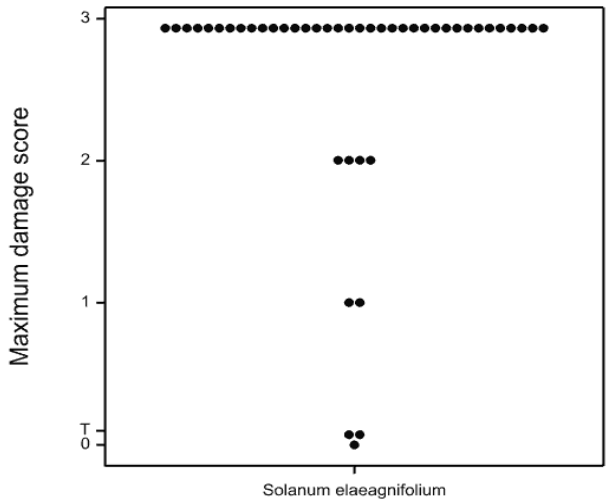

Figure 14. Larval feeding damage to *Solanum elaeagnifolium* (Subgenus Leptostemonum, Group 27C) in no-choice experiments. Damage rating 0 = no damage; T = trace damage or almost no damage (~ 0.1%); 1 = 0.1-10% leaf area consumed; 2 = 10-50% leaf area consumed; 3 = >50% leaf area consumed.

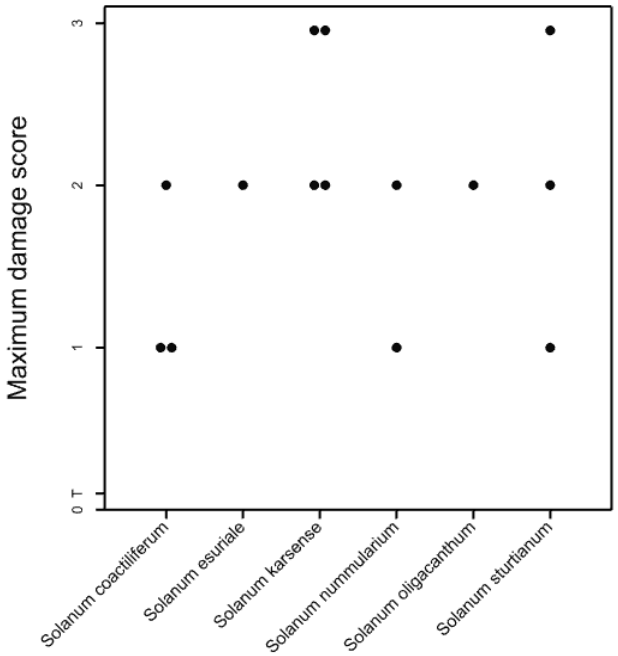

Figure 15. Larval feeding damage to selected Australian *Solanum* spp. (Subgenus Leptostemonum, Group 27C) in no-choice experiments. Damage rating 0 = no damage; T = trace damage or almost no damage (~ 0.1%); 1 = 0.1-10% leaf area consumed; 2 = 10-50% leaf area consumed; 3 = >50% leaf area consumed.

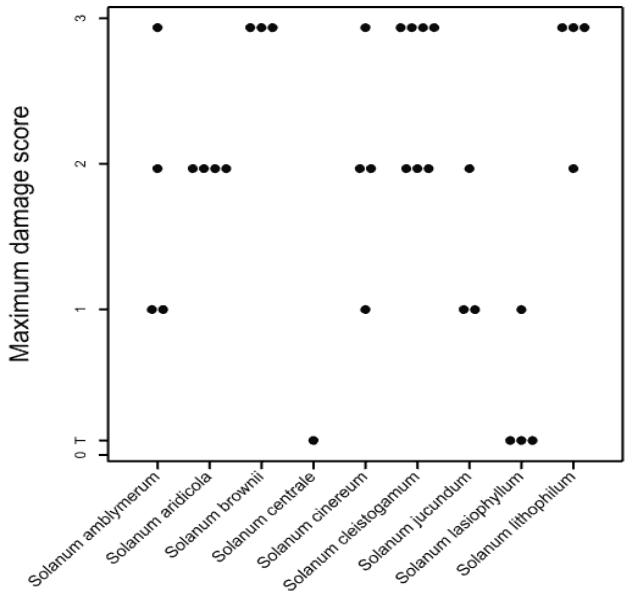

Figure 16. Larval feeding damage to selected Australian *Solanum* spp. (Subgenus Leptostemonum, Groups 27B, 27D and 27Z) in no-choice experiments. Damage rating 0 = no damage; T = trace damage or almost no damage (~ 0.1%); 1 = 0.1-10% leaf area consumed; 2 = 10-50% leaf area consumed; 3 = >50% leaf area consumed.

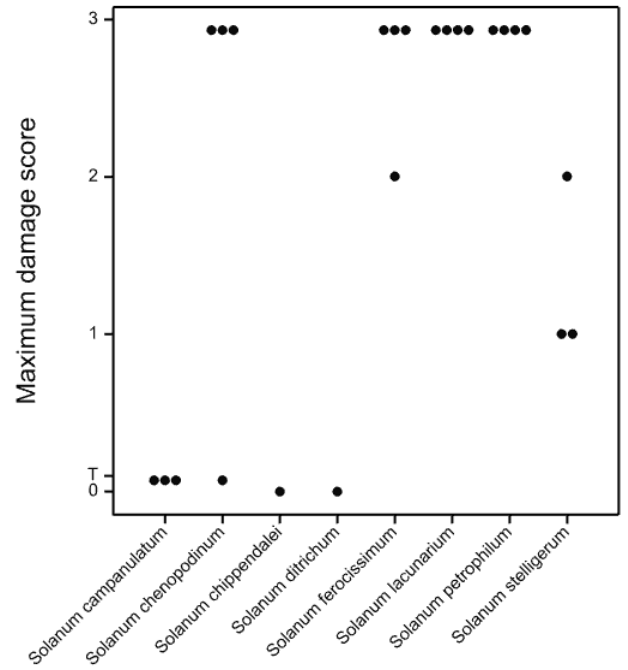

Figure 17. Larval feeding damage to selected Australian *Solanum* spp. (Subgenus Leptostemonum, Groups 13Z, 25Z and 28Z) in no-choice experiments. Damage rating 0 = no damage; T = trace damage or almost no damage (~ 0.1%); 1 = 0.1-10% leaf area consumed; 2 = 10-50% leaf area consumed; 3 = >50% leaf area consumed.

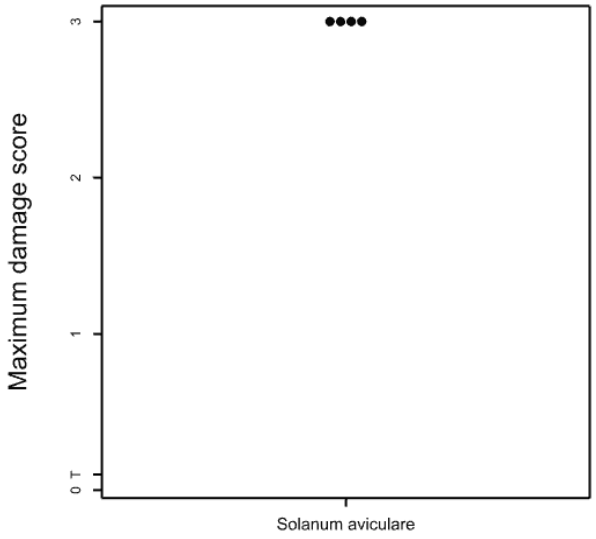

Figure 18. Larval feeding damage to the native *Solanum aviculare* (Subgenus Archaesolanum) in a no-choice experiment. Damage rating 0 = no damage; T = trace damage or almost no damage (~ 0.1%); 1 = 0.1-10% leaf area consumed; 2 = 10-50% leaf area consumed; 3 = >50% leaf area consumed.

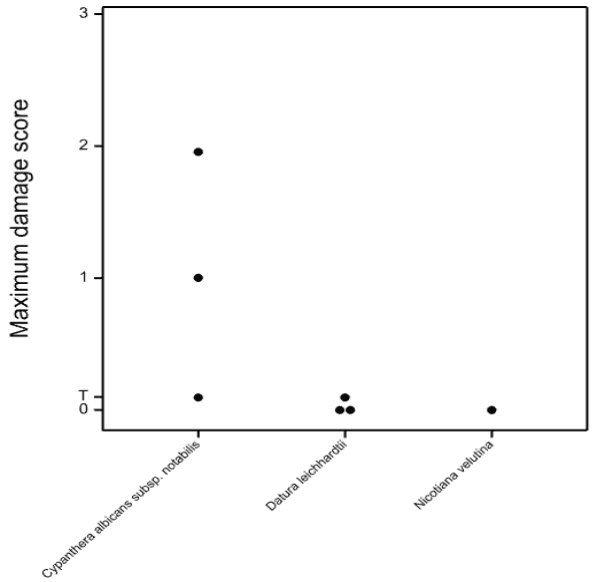

Figure 19. Larval feeding damage to three Australian Solanaceae (L to R: Tribes Anthocercideae, Datureae and Nicotianeae respectively) in no-choice experiments. Damage rating 0 = no damage; T = trace damage or almost no damage (~ 0.1%); 1 = 0.1-10% leaf area consumed; 2 = 10-50% leaf area consumed; 3 = >50% leaf area consumed.

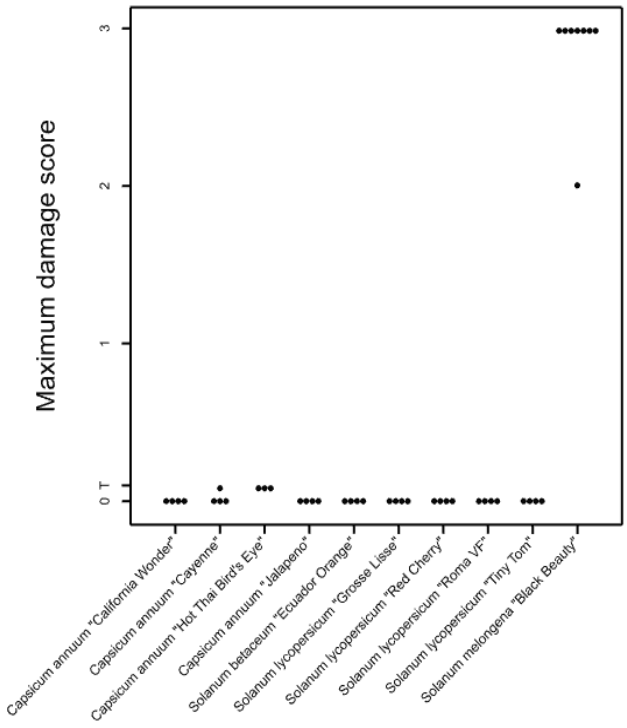

Figure 20. Larval feeding damage to ten Solaneae crop cultivars (excluding potato) in no-choice experiments. Damage rating 0 = no damage; T = trace damage or almost no damage (~ 0.1%); 1 = 0.1-10% leaf area consumed; 2 = 10-50% leaf area consumed; 3 = >50% leaf area consumed.

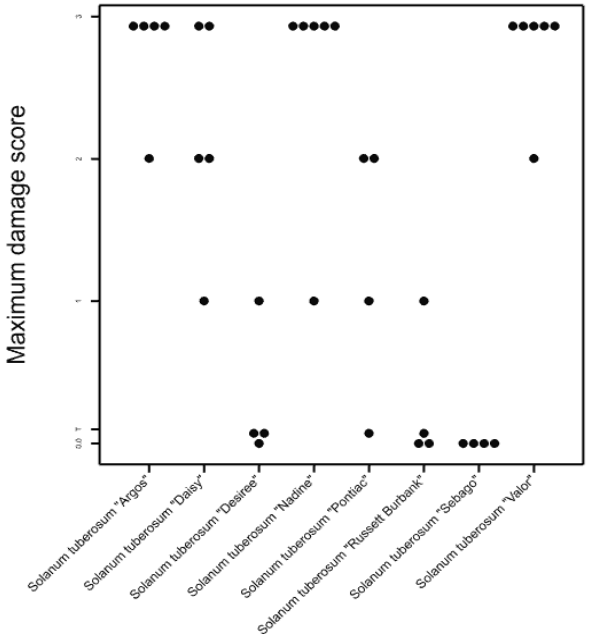

Figure 21. Larval feeding damage to eight potato *Solanum tuberosum* cultivars in no-choice experiments. Damage rating 0 = no damage; T = trace damage or almost no damage (~ 0.1%); 1 = 0.1-10% leaf area consumed; 2 = 10-50% leaf area consumed; 3 = >50% leaf area consumed.

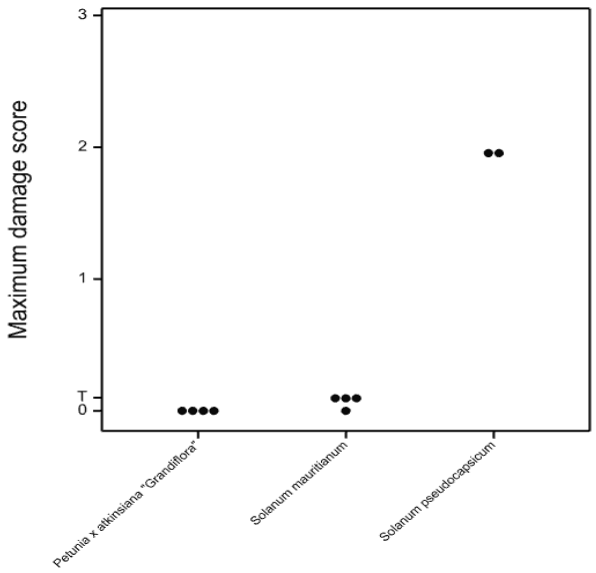

Figure 22. Larval feeding damage to three exotic Solanaceae in no-choice experiments. Damage rating 0 = no damage; T = trace damage or almost no damage (~ 0.1%); 1 = 0.1-10% leaf area consumed; 2 = 10-50% leaf area consumed; 3 = >50% leaf area consumed.

Small-cage choice experiment with adults

Analysis due for completion June 2018.

Large-cage choice experiment with adults

Table 11. Number of eggs, maximum damage score and sum of adults observed on *Solanum elaeagnifolium* and ten non-target Australian *Solanum* spp. in an 8-week period in a large cage (3m x 3m x 1.8m) choice experiment. Damage rating 0 = no damage; T = trace damage or almost no damage (~ 0.1%); 1 = 0.1-10% leaf area consumed; 2 = 10-50% leaf area consumed; 3 = >50% leaf area consumed.

Subgenus	Group	Species	Total number of eggs	Maximum damage score	Sum of adult observations
Leptostemonum	13Z	*Solanum ferocissium*	0	1	22
	25Z	*Solanum petrophilum*	269	3	108
	25Z	*Solanum lacunarium*	0	1	0
	27B	*Solanum brownii*	0	2	15
	27C	*Solanum sturtianum*	0	1	4
	27C	*Solanum elaeagnifolium*	179	2	10
	27C	*Solanum karsense*	0	2	11
	27Z	*Solanum cleistogamum*	182	2	22
	27Z	*Solanum aridicola*	22	3	19
	27Z	*Solanum lithophillum*	0	1	5
Archaesolanum		*Solanum aviculare*	0	1	0

Continuation experiments

In progress. Expected completion June 2018.

Discussion

Leptinotarsa texana utilised non-target species in no-choice and choice experiments (Figs. 23 to 26). Feeding damage greater than 50% leaf area removed occurred on plants of twelve Australian *Solanum* spp. and two crop species (potato *S. tuberosum* and eggplant *S. melongena*) (Table 10; Figures 14-22). *Leptinotarsa texana* successfully developed from first instar larva to adult on fifteen Australian *Solanum* spp. and two crop species (comprising a single eggplant cultivar and four potato cultivars) (Table 10). When given a choice of plants in a large cage experiment, *L. texana* oviposited on *Solanum elaeagnifolium* and three non-target Australian *Solanum* spp. (Table 11). These results expand the known fundamental host-range of *L. texana* to include Australian *Solanum*, and certain cultivars of potato *Solanum tuberosum*. Off-target damage to potato was not anticipated, as potato had been previously tested in South Africa (Olckers et al., 1995). However, potato cultivars tested here may not have been available when research was conducted in South Africa.

Leptinotarsa texana feeding damage to eggplant *Solanum melongena* was anticipated. *Leptinotarsa texana* utilised eggplant in laboratory host-range experiments in South Africa, even though eggplant is not known to be a host in North America (Olckers et al., 1995). Feeding and development of *L. texana* also occurred on five native African *Solanum* spp. in South African laboratory experiments (Olckers et al., 1995). South African researchers proposed that the artificial conditions in the laboratory study disrupted normal host-seeking and host-acceptance behaviour of this insect. The researchers argued that the results of laboratory host-range experiments on eggplant were not reliable, and should be disregarded. They also asserted that the impact on native species would be negligible, however it is not clear whether ecological modelling informed their assertion (Olckers et al., 1995). Nevertheless, South African regulatory authorities eventually accepted their argument. The final risk analysis seemingly placed greater weight on native range studies, expert opinion, and stakeholder consultation, and less weight on laboratory host-range experiments. The onus, therefore, was on biological control scientists to consider what additional evidence could support introduction, and whether evidence gathering was feasible.

While Australian regulators consider native range studies and other evidence types, they rely primarily on the results of host-range experiments conducted in quarantine laboratories (Department of Agriculture and Water Resources, n.d.). Based on the results of host-range experiments presented here, *L. texana* would be viewed as representing an unacceptable risk to the Australian environment and economy, and would not be approved for release.

Figure 23. Greg Lefoe (Agriculture Victoria) and Dr Libby Rumpff (PhD Supervisor - University of Melbourne) assessing no-choice experiments. No-choice experiments confine insects to individual test plants, and provide data on the range of species at risk (i.e. those within the insect's fundamental host-range). Potato var. "Nadine" was defoliated under these conditions.

Figure 24. University of Melbourne Science & Technology interns Nina Guo and Tripti Chawla help to assess a large-cage choice experiment. Large-cage or tent experiments provide a larger area for insects to exhibit host-selection behaviours. Ten native *Solanum* spp. and *S. elaeagnifolium* (SLN) were presented to *L. texana* adults in this 3m x 3m arena.

Figure 25. Leader of the host specificity and risk analysis research - Greg Lefoe with some experiment cages.

Figure 26. Defoliation of potato *Solanum tuberosum* "Nadine" and emergence of adult *Leptinotarsa texana* in a no-choice quarantine laboratory experiment.

4.7.3 Field host specificity testing in Texas, USA - 2017 and 2018

Materials and methods, April 2017

A field was selected at the USDA Weslaco Research Station, Weslaco, south-east Texas, where silverleaf nightshade and *L. texana* occur together naturally (Weslaco Research Station South Field, latitude, longitude: 26.134753, -97.955085). The field was ploughed and set aside for 12 months before the experiment, allowing silverleaf nightshade to grow at densities of up to 90% ground cover in some parts of the field.

Due to the uncertain distribution and density of *L. texana* at the site, three experimental designs were implemented in different parts of the field (Fig. 27):

1. Completely randomised design with removal of silverleaf nightshade from twenty 1m x 1m plots (except for tagged experimental plants). Each plot therefore contained either a single silverleaf nightshade plant or an eggplant *Solanum melongena*.
2. Randomised block design with removal of silverleaf nightshade from twenty 2m x 2m plots (except for tagged experimental plants). Each plot therefore contained either a single silverleaf nightshade plant or an eggplant.
3. Completely randomised design with sixteen 2m x 2m plots without removal of silverleaf nightshade from plots. Each plot therefore contained naturally occurring silverleaf nightshade plus either a single eggplant or a labelled silverleaf nightshade plant.

Eggplants were grown from seed in an organic glasshouse at the University of Texas Rio Grande Valley campus, and transported to a screenhouse at the Weslaco Research Station prior to planting. Wire guards were constructed onsite and placed over eggplants to prevent jackrabbits feeding on plants (Fig. 28). Shade cloth was placed on top of each wire guard to prevent eggplant leaves being scorched. Plants were hand-watered each morning between 8am-10am. Each plant was assessed at least twice each day (late-morning to midday, and mid-late afternoon) for up to ten days. For each plant, the number of eggs, larvae and adults, and the length (mm) of each larva was estimated. The percent leaf area removed by *L. texana* feeding was also estimated.

Figure 27. South field (ploughed area) at USDA's Weslaco Research Farm, Texas, USA, with experimental designs overlayed. A single eggplant planted into each purple plot and a single SLN was selected and labelled in each white plot.

Figure 28. Wire jackrabbit guard protecting an eggplant (above left) and monitoring plants in Experiment 1 (1m x 1m completely randomised experiment) (above right), April 2017.

Preliminary results and Discussion

A native range field experiment conducted in Texas, USA in 2017 demonstrated that, despite laboratory results, eggplant is not utilised as a host in the field. The experiment will be repeated in 2018, and will include at least one of the susceptible potato cultivars as it is important to determine 1) whether certain potato cultivars are more susceptible to attack than previously thought, or 2) if off-target attack only occurs under laboratory conditions. It is not considered feasible to include Australian *Solanum* spp. in USA field trials at this time.

As agreed with PIRSA and MLA, further field experiments in Texas on potato and eggplant are underway in 2018. From a preliminary analysis of 2017 data there was a clear preference for silverleaf nightshade in experiment 1, where *L. texana* density was greatest. Data analysis will be completed when 2018 eggplant and potato field data is obtained and collated.

5 Discussion

5.1 Overview

Leptinotarsa texana was not found to be a suitable biological control agent for silverleaf nightshade, because of unfavourable host specificity testing results. It caused unacceptable damage to 12 native Australian *Solanum* species, and a number of potato and eggplant varieties. The damage to the eggplants was expected, because this had been observed by scientists in South Africa, but the damage to potato varieties was completely unexpected. The South African researchers reported no damage to potato, although it is unlikely that they had access to the var. “Nadine” breeding lineage. The South Africans also recorded some damage to some of their native *Solanum* species, so similar results for native Australian *Solanum* species was unfortunate, but not a great surprise. The adverse findings demonstrated that existing protocols can detect potential problems under quarantine, and have also provided an opportunity for some valuable follow-up research that should have broad application to future biological control projects.

There were a number of valuable outputs generated by this project, which will also be invaluable for future biocontrol projects aimed at weeds in Solanaceae.

The first was a comprehensive DNA study of Australian *Solanum* species, and results have been used to construct a new phylogenetic model. This model will allow future representative host specificity testing lists to be constructed with increased confidence and safety. Another study examined a global silverleaf nightshade DNA survey and analysis to confirm for the first time that the origin of silverleaf nightshade is in southern USA, and that South American populations are derivative of the

USA populations. This information will be used to choose the most suitable locations from which agents are sourced.

Another major legacy output is the creation of a native Australian *Solanum* species location database and physical propagation methodology and collection. Physically locating *Solanum* populations around Australia required a significant effort, as did propagating the material (seeds and transplants) and collecting fresh seed. The remaining plant collection has been relocated to AgriBio in Melbourne, and the comprehensive seed collection has been divided into two duplicates – one has been sent to AgriBio in Melbourne, and the other is housed at the South Australian Seed Conservation Centre, Botanic Gardens and State Herbarium.

Another major spin-off from this project will be the insights and publications generated by Greg Lefoe's PhD studies into risk analyses and strategies in biocontrol projects. In addition to new information and knowledge, valuable scientific capacity has been developed in this field.

The disqualification of *Leptinotarsa texana* as a biocontrol candidate for release was extremely disappointing for the research team, but an analysis has shown that c. 90% of our work will remain useful and will greatly assist similar future projects. Our propagation collections have already assisted the Round 2 biocontrol projects on African boxthorn and silverleaf nightshade.

5.2 Key messages and lessons learned

5.2.1 Key messages

1) *Leptinotarsa texana* is unsuitable for release in Australia because it damaged a number of potato varieties and 12 species of native *Solanum* in quarantine feeding tests.
2) Silverleaf nightshade continues to spread, and its' impact on Australian agriculture is increasing.
3) Available management options for reducing the rootbank in large, established infestations of silverleaf nightshade remain very limited, and often ineffectual.
4) Biological control is still the best hope for managing silverleaf nightshade in Australia. Further exploration and experimentation with potential biocontrol agents for silverleaf nightshade is of great importance.
5) Weeds within the genus *Solanum* have unusually large numbers of close relatives in Australia – both cultivated crops and native Australian species. This greatly increases the resources required to conduct host specificity testing.
6) Although unsuccessful with *Leptinotarsa texana*, this project produced some critical information, knowledge and networks that will be essential for future agent assessments.
7) A study of outputs shows that around 90% of the activities of this project will contribute to future biocontrol projects.
8) Future biocontrol projects should adopt host-specificity testing protocols to include a systematic study of the breeding lineages for major related crops. In future, SLN biocontrol projects should select related crop varieties (e.g. within potato, tomato, eggplant, chilli and capsicum) that represent all major breeding lineages.
9) The discovery that *Leptinotarsa texana* attacked a number of potato varieties could potentially lead to advances in control of the world's worst potato insect pest, Colorado potato beetle.

5.2.2 Lesson learned

1. Crop host specificity testing list.

The unexpected potential threat from *L. texana* to the Australian potato industry was identified by host-specificity protocols. This indicates that the current protocols are capable of finding major potential problems. However, future SLN biocontrol projects should select related crop varieties (e.g. within potato, tomato, eggplant, chilli and capsicum) that represent all major breeding lineages in a more systematic way, to minimise the chance that an important breeding lineage will be unrepresented.

This systematic approach is already used to choose representative related Australian native plants, based on phylogenetic models. A similar approach for crop plants might involve an initial screening of several varieties to detect any major feeding. If there are no problems with the initial screening, then additional resources could be used to examine of published breeding lineages, and major breeding companies should also be given an opportunity to submit advanced or important new lineages.

2. Molecular biology (DNA) research is very valuable.

Host specificity test lists are a critical component of a safe biocontrol project. Constructed well, they minimise the risk of unexpected off-target damage to an acceptable level. The list, however, can only be representative and robust if a good phylogenetic model exists. Until recently, these models have relied on micro and macro physical taxonomic features to elucidate relationships within plants closely related to the target weed.

This study has demonstrated the value of molecular biology techniques to compare DNA sequences between different plants. In most cases this evidence has confirmed existing models, but in a few cases the new evidence may change the list of plants chosen. In addition, DNA studies to determine the most likely origins of Australian SLN (central USA) has provided strong guidance for the most likely places to source co-evolved, effective agents.

3. Industry consultation at an early stage is very important.

In cases where important plants are potentially at risk from putative biocontrol agents (in our case, eggplant), it is important to engage with stakeholders near the start of the project to inform them of what is being done, and to give them confidence that due process and diligence is being observed.
In this project we travelled to far north Qld to liaise with vegetable growers, their advisors, and eggplant growers in particular. The approach, in person, was very well received and stakeholders told us that they rarely felt that they were consulted properly in many cases. The consultation worked well, with industry leaders aware of the situation and able to communicate with and reassure any growers with concerns.

5.3 Recommendations

The following recommendations are made to MLA and other prospective research funders:

1. No attempt should be made to release *Leptinotarsa texana*, as it represents an unacceptable risk to the Australian environment (e.g. 12 native *Solanum* species) and economy (e.g. potato damage).
2. Continue to support field surveys in South America (AgriFutures-led RR&D4P Round 2 project) that aim to identify other prospective biological control agents for silverleaf nightshade. New associations of agents and targets (e.g. *Cactoblastis* on prickly pear) have produced good results in the past.

3. Use the plant locality information, seed/plant collections and new understanding of phylogenetic relationships, based on *Solanum* molecular biology (DNA) data, to assist with future SLN biocontrol projects.
4. Promote more representative crop host testing lists, taking a systematic approach to account for breeding lineages.
5. Consider the commercial opportunity (with third party partners) to exploit our potato feeding results to potentially reduce the impact of Colorado potato beetle (*Leptinotarsa decemlineata*) in North America.
6. Cease further preparation of an application to release *L. texana* in Australia, but complete those sections of the draft application that address the target weed (and could therefore be incorporated into a future application for a different agent).
7. Complete research into the predicted field host-specificity of *L. texana*, especially as it relates to one or more of the potato cultivars "Nadine", "Valor", "Daisy", "Pontiac" and "Argos". Research to include field experiments in Texas, USA.

6 Project Achievements

6.1 Sub-project level achievements

In most cases the specific experimental results are given in detail in "Section 4 Results" (above), and in attached Appendices, so this section will deal with the broader aspects of the work, to avoid repetition.

6.1.1 Report against Project Outputs and KPIs

Note: Detailed reports on communications and collaborations are given in Sections 7 and 8 below.

Output 8(a) Obtain approvals for importation of beetle.

KPI 1.12 Report on permits for importation of beetle, development of SLN culture and sequencing protocol (Outputs 8(a) and 8(b)).

Two Australian Government permits, required to import live *Leptinotarsa texana* beetles into quarantine in Australia, were obtained from the Department of Agriculture and the Department of the Environment in early 2016 (see 4.2 above and Appendices for detail). Further details may also be obtained from Greg Lefoe.

Output 8(b) Develop SLN plant cultures, source SLN shoot material and confirm sequencing protocol.

KPI 1.12 Report on permits for importation of beetle, development of SLN culture and sequencing protocol (Outputs 8(a) and 8(b))

Silverleaf nightshade plants were successfully established at AgriBio, Melbourne, in early 2016 and the cultures are still active at the time of writing. The cultures were based on propagating material from SA and Vic, and have provided an adequate supply of shoot material throughout the duration of the project. Silverleaf nightshade DNA sequencing protocols were successfully developed and tested at the Wagga Wagga Agricultural Institute (NSW DPI). DNA sequences from two linked intergenic spacer regions in chloroplast DNA (cpDNA) were obtained from 155 specimens native to the Americas, and 333 specimens from the three major introduced regions.

Output 8(c) Develop a detailed plan for specificity testing and propagule collection, using the centrifugal phylogenetic method to prioritise native and commercially important Solanaceae occurring in locations where the ranges of silverleaf nightshade and potential non-target species overlap. At least 30 species/cultivars will be collected for host specificity testing, covering at least 30 locations.

KP1 2.9 Update on plant material collection (Output 8(c)) and importation of Leptinotarsa texana (Output 8(d)). Provide the detailed plan for specificity testing and propagule collection, including locations (Output 8(e)).

Laurie Haegi led the construction of the host specificity testing list. It was based on existing phylogenetic models for Solanaceae, and in particular *Solanum* in Australia, and was confirmed and fine-tuned using our molecular studies of DNA of native Australian *Solanum* species. The test list included 214 Solanaceae species including the target, *Solanum elaeagnifolium*, divided into High, Medium or Low priority. This comprised 195 species of *Solanum* and 19 species of other Solanaceae. Propagating material of 55 species, covering High priority and several Medium priority species has been secured. All, but one, have been successfully propagated (by seed, or by cuttings) to produce plants for host specificity trials. Of the 54 species for which plants are held, 36 are native Australian species of *Solanum*, 5 are naturalised weedy species of *Solanum*, 5 are cultivated species of *Solanum*, 5 are native Solanaceae other than *Solanum* (representing 5 separate genera) and 3 are cultivated Solanaceae other than *Solanum*. The plants were collected from over 50 separate locations (see 4.4 above and Appendices for details).

Output 8(d) Import colony of Leptinotarsa texana into quarantine and refine rearing methodologies to maximise colony development.

KP1 2.9 Update on plant material collection (Output 8(c)) and importation of Leptinotarsa texana (Output 8(d)). Provide the detailed plan for specificity testing and propagule collection, including locations (Output 8(e)).

KP1 3.13 Report on finalisation of host specificity testing protocol and testing in quarantine (Output (8(c)) and Leptinotarsa texana rearing methodologies (Output 8(d)).

A total of 152 live *Leptinotarsa texana* adults were imported from South Africa on 14 April 2016, following granting of two Federal import licences (see above). Prior to shipment, beetles were reared for at least one generation on *Solanum elaeagnifolium* in laboratory cages at Rhodes University, South Africa, to reduce the risk of importing contaminants such as hyper-parasitoids and to meet permit conditions. On arrival in Australia, *L. texana* adults were transported under quarantine to the AgriBio insect quarantine laboratory (V2276) where they were unpacked, processed and checked for contaminants and abnormalities. Live *L. texana* adults were transferred to insect cages in a controlled environment room (H.032; set at 25 °C, 16 hrs light:8 hrs dark) and rearing protocols were fine-tuned. The colony was successufully maintained for the duration of the project, and is still active at the time of writing (see 4.3 above and Appendices for details).

Output 8(e) Undertake host specificity testing of plant species collected.

KP1 3.13 Report on finalisation of host specificity testing protocol and testing in quarantine (Output (8(c)) and Leptinotarsa texana rearing methodologies (Output 8(d)).

KPI 4.11 Report on host specificity testing of at least half of the test species (Output 8(c)) and DNA sequencing of SLN material from Australia and overseas (Output 8(f)), and implications for host testing from understanding of the Australia SLN biotypes in relation to overseas biotypes (Output (8f)).

KPI 5.10 Report on results of host specificity testing of remaining test species (Output 8(c)).
Host-range experiments were conducted at AgriBio's insect quarantine laboratory (Melbourne) on 32 *Solanum* species and 5 other Solanaceae species. *Leptinotarsa texana* developed on eggplant, potato and 15 Australian native plant species when confined in benchtop laboratory cages. This result was anticipated to some extent, because previous research in South Africa showed that off-target damage could occur in laboratory cages. Feeding damage to eggplant was anticipated as it has been reported from laboratory tests in South Africa, even though eggplant crops are not known to be a host in North America. Off-target damage to potato was not anticipated, as potato had been previously tested without problem in South Africa. However, potato cultivars tested in these experiments may not have been available when research was conducted in South Africa. Based on the results of host-range experiments in this project, release of *Leptinotarsa texana* is an unacceptable risk to the Australian environment and economy (see 4.7 above and Appendices for details).

Output 8(f) Complete DNA sequencing of SLN material from Australia and overseas.

KPI 4.11 — Report on host specificity testing of at least half of the test species (Output 8(c)) and DNA sequencing of SLN material from Australia and overseas (Output 8(f)), and implications for host testing from understanding of the Australia SLN biotypes in relation to overseas biotypes (Output (8f)).
Sequencing of DNA for two linked intergenic spacer regions in chloroplast DNA (cpDNA) was completed for 155 SLN specimens from the Americas, and 333 specimens from the three major introduced regions. A total of 41 haplotypes were identified and the results give, for the first time, strong evidence for both the global origin of SLN (North America), and the origin of SLN introduced to Australia (central USA) (see 4.6.1. above and Appendices for details).

Output 8(g) Initiate Australian Government Import Risk Analysis procedures to seek formal approval for release of SLN agent.

Although no attempt will be made to release *Leptinotarsa texana* in Australia, the SLN research team still intends to complete a draft "Application for Release" but will not submit it. The team has an enormous store of knowledge and information that will be formally documented under the relevant headings required for an Application to Release. Generic headings that relate to all potential agents for SLN will be completed in detail, but sections relating specifically to *Leptinotarsa texana* will be given lower emphasis because future interest will be largely academic. At the time of writing an advanced draft has been prepared, with contributions from all team members, and will be completed before the end of the project.

Output 8(h) Prepare a plan for next steps in the biological control of SLN. This should include detailed planning for release of Leptinotarsa texana in Australia, if Outputs 8(a) to 8(g) indicate that the beetle may be a successful control agent for SLN.

KPI 5.11 Plan for next steps in biological control of SLN (as per Output 8(g)) completed and approved by the Department.

KPI 6.12 Report on the applications for release of Leptinotarsa texana submitted (Output 8(h)) including risk assessment procedures. Scientific papers prepared on the project's outcomes (Output 8(i)).

No plans will be prepared for the release of *Leptinotarsa texana* in Australia, however a "Round 2" biological control project (led by Greg Lefoe) will source and test alternative agents for SLN. This project has activity supported the new Round 2 project, and has gifted it a full collection of Solanaceae seed from the current project, in addition to all data, information and knowledge gained from the current project.

Output 8(i) Prepare scientific papers on the project research.

KPI 6.12- Report on the applications for release of Leptinotarsa texana submitted (Output 8(h)) including risk assessment procedures. Scientific papers prepared on the project's outcomes (Output 8(i)).

A list of publications (Table 12) is planned and many are currently in preparation. A number of advanced drafts are presented in the attached Appendix 2.

Table 12. Planned scientific papers and nominated authors.

Paper	Subject	Authors	Status (May, 2018)
1	Host specificity testing – species selection, laboratory testing, field experiments.	Greg Lefoe and Laurie Haegi	Most material assembled. Experiments in Texas conclude May 2018
2	DNA barcoding of *Leptinotarsa texana* beetles used for host specificity trials in Australia.	David Gopurenko and Hanwen Wu	Advanced draft prepared
3	Chloroplast DNA evidence for North American origins of the weed *Solanum elaeagnifolium* Cavanilles (Solanaceae:*Solanu Leptostemonum*) introduced to Australia, the Mediterranean region, and South Africa.	David Gopurenko, Xiaocheng Zhu and Hanwen Wu	Submitted for publication
4	Molecular phylogenetic analysis of Australian *Solanum* (Solanaceae).	Laurie Haegi, David Gopurenko, Xiaocheng Zhu and Hanwen Wu	Advanced draft prepared
5	Germination of some *Solanum* species.	Jane Prider and Shannon Robertson (student intern).	Advanced draft prepared
6	Molecular identification of *Solanum elaeagnifolium* in Australia using DNA barcoding, a solution for better management.	Xiaocheng Zhu, David Gopurenko, Laurie Haegi, Hanwen Wu	Submitted for publication

6.2 Contribution to project expectations

Note: The SLN biological control project differed from most in the Program, because it did not release or redistribute any agents. Neither did it undertake any extensive public consultation or extension activities. Therefore, our project is not applicable to some of the dot points below, and these points are denoted by NA.

a) Greatly increase the on-farm populations of 8 weed biocontrol agents - NA
b) Reduce weed competition and herbicide use across more than 25 million ha - NA
c) Reduce the densities of the six target weeds across northern and southern Australia
   Legacy contribution to "Round 2" SLN project, and future SLN biocontrol projects.
d) Increase long-term annual yield and reduce annual weed control costs
   Legacy contribution to "Round 2" SLN project, and future SLN biocontrol projects.
e) Improve agricultural natural resource management nationally
   Legacy contribution to "Round 2" SLN project, and future SLN biocontrol projects.
f) Inform producers of weed management options - NA, and
g) Establish a new collaborative national approach to weed biocontrol
   Legacy contribution to "Round 2" SLN project, and future SLN biocontrol projects.

6.3 Contribution to Rural Profit R&D programme objectives

As previously noted, this project will not seek to release a biological control agent.

The project has, however, been very successful in generating outputs that will leave a very valuable legacy for future biocontrol projects. Some of the knowledge and outputs are:

1) A robust and comprehensive host specificity testing list.
2) Localities for High priority test species, along with duplicate seed collections for each.
3) Confirmation of the global origin (North America) of SLN, along with the source (central USA) of SLN introduced to Australia.
4) A comprehensive study of the phylogeny of native Australian *Solanum* species, based on DNA analysis. This includes a complete DNA sample library of plants tested.
5) A group of scientific papers prepared for publication, covering all major aspects of the project.
6) A PhD on risk management for biological control in Australia, along with a very competent and experienced researcher for future projects (Greg Lefoe).
7) Propagation protocols for many native Australian *Solanum* species.
8) Data on the relationship between feeding on eggplant and potato in the laboratory, and attack in under field conditions.
9) Recognition of a need for more systematic consideration of test crop varieties, taking into account representation from all important breeding lineages.

7 Collaboration

A list of project collaborations is given below (Table 13), including their contributions, the likelihood of the collaboration continuing beyond the project.

Note: Ongoing collaboration (actual and anticipated) for the "Round 2" SLN biological control project is included.

Table 13. SLN biological control project collaborators.

Collaborators	Organisation	Contribution	Ongoing?	Contacts	Comments
Australian eggplant Industry - researchers and consultants in FNQ, and individual growers in Qld and SA.	AusVeg, Bowen/Gumlu Growers Association	Consultation and liaison to inform the vegetable industry of the project's plans and due diligence.	Yes	Jessica Lye jessica.lye@ausveg.com.au	Personal visits made to Northern Adelaide Plains and Bowen (Qld) region by John Heap and Greg Lefoe.
Australian Bush Foods Industry - Mike and Gayle Quarmby	Outback Pride Fresh, Kingston, SA.	Consultation and liaison to inform the bush foods industry of the project's plans and due diligence	?	Mike and Gayle Quarmby foodservice@outbackpridefresh.com.au	Australia's largest bush foods supplier and key representatives on peak body committee.
Professional Australian network	Various	Sourced and supplied seed of some test species. Provided information on Aboriginal cultural value of Australian *Solanum* spp.	Y	Various – ref SLN team members	Team members were able to access propagation material and information from around Australia, using pre-existing professional networks. These contributions were vital.
Australian Herbaria	Various, around Australia	Supplying DNA material for a range of native Australian Solanums.	Yes	Various – ref Laurie Haegi laurence.haegi@sa.gov.au	This project also deposited many duplicate specimens of *Solanum* species collected.
International SLN research community	Various, around the world	Collaborated in supplying SLN DNA material from around the world.	Yes	Various – ref Hanwen Wu hanwen.wu@dpi.nsw.gov.au	Including our South African colleagues who supplied the *Leptinotarsa texana* colony.
African boxthorn biological control project - Louise Morin	CSIRO	Sharing information on Solanaceae and host specificity testing lists. The SLN project also supplied some seed and plant material to the ABT project.	Yes	Louise Morin Louise.Morin@csiro.au	The SLN project also supplied ABT seed collections from several locations in SA.

Collaborators	Organisation	Contribution	Ongoing?	Contacts	Comments
Aboriginal communities - Hayden Bromley	Aboriginal Indigenous Cultural Awareness and Consultancy Services	Background and introduction into aboriginal communities for consultations on culturally-important Australian *Solanum* spp.	No	Haydyn Bromley haydyn@bookabee.com.au	John Heap attended an Aboriginal culture awareness course and later met face to face with the facilitator.
Dr John Goolsby,	USDA, Edinburg, Texas, USA	SLN research collaboration in Texas which reduced costs substantially, and ensured feasibility.	Yes	TBA – ref Greg Lefoe	The USDA has offered access to their research farm in 2018 for the second round of field experiments. Field equipment was stored on-site in preparation for further field experiments in 2018.
Dr Alex Racelis,	University of Texas, Edinburg, Texas, USA	SLN research collaboration in Texas which reduced costs substantially, and ensured feasibility.	Yes	TBA – ref Greg Lefoe	The University of Texas may also have a Masters students interested in conducting field studies of *L. texana.*
Dr Patrick Moran,	USDA, California, USA	SLN research collaboration in Texas which reduced costs substantially, and ensured feasibility.	Yes	TBA – ref Greg Lefoe	Assistance with aspects of field work in Texas.
Chetta Owens	US Army Corps of Engineers, Lewisville, Texas, USA	SLN research collaboration in Texas which reduced costs substantially, and ensured feasibility.	Yes	TBA – ref Greg Lefoe	Assistance with aspects of field work in Texas.

8 Extension and adoption activities

Note: The SLN biological control project differed from most in the Program, because it did not release or redistribute any agents. Neither did it undertake any extensive public consultation or extension activities. Most extension activities involved industry liaison, or public presentations on research. Therefore, our project is not applicable to some of the dot points below, and these points are denoted by NA.

A list of extension and educational activities delivered by team members is presented below.

Conference presentations

John Heap presented a silverleaf nightshade paper to the 20th Australasian Weeds Conference. "Silverleaf nightshade (*Solanum elaeagnifolium*) - field research on biology and management in South Australia". Perth, 12 September, 2016 (included discussion of SLN biological control).

Greg Lefoe presented "Biological control of silverleaf nightshade *Solanum elaeagnifolium* in Australia: a new hope?" to the International Organisation for Biological Control (IOBC) symposium at the Australia New Zealand Entomological Conference in Melbourne, 29 November 2016.

John Heap presented a paper on the biological control project to the 6th South Australian Weeds Conference (Adelaide). "Biocontrol of Silverleaf nightshade – trials and tribulations". 2 May, 2018.

Public meetings or workshops

Greg Lefoe presentation: "*An Australian in Weslaco*" to USDA staff at the USDA's Moorefield biological control laboratory, Edinburg, Texas, USA. April 2017.

John Heap presentation: "Silverleaf nightshade - a WoNS on the Northern Adelaide Plains" to constituents of the Adelaide-Mt. Lofty Region Natural Resources region, 5 May, 2017 (included discussion of SLN biological control).

John Heap presentation: "Biological Control of some Weeds of National Significance (WoNS)" to the Yackamoorundie Landcare Group (SA), 23 August, 2017 (included discussion of SLN biological control).

Greg Lefoe presentation: "*The CASE for introduction of a new weed biological control agent*" to University of Melbourne's School of BioSciences, 21 September, 2017.

Represented the project at the DoAWR biological control planning workshop in Canberra, 16 October, 2017.

Greg Lefoe presentation: "*New tools for biological control decision making*" at AgriBio Science Conference, 26 October, 2017.

John Heap presentation: "Silverleaf nightshade - a WoNS on the Northern Adelaide Plains" at Smithfield for Adelaide-Mt. Lofty Region Natural Resources region, 15 Feb, 2018 (included discussion of SLN biological control).

John Heap presentation: "Silverleaf nightshade and African rue". Farmers' meeting. Spalding, SA. 22 March, 2018 (included discussion of SLN biological control).

John Heap presentation: “Silverleaf nightshade”. Local farmers meeting, Mt. Pleasant, SA. 27 March, 2018 (included discussion of SLN biological control).

Industry liaison

Vegetable (particularly eggplant) industry. John Heap visited a major eggplant grower on the Northern Adelaide Plains on 29 July, 2016 to learn about eggplant production, and to inform him of the SLN project plans. On 22-23 November, 2016 John Heap and Greg Lefoe undertook an eggplant grower liaison exercise, this time with Australia’s largest producers in the Bowen-Gumlu horticultural region of North Qld. Key growers, consultants and government representatives were appraised of our project and intentions, and valuable industry information was collected for the project.

Bush foods. John Heap visited Australia’s largest bush foods supplier, Outback Pride Fresh, on 31 March, 2017, near Kingston, SA. Mike and Gayle Quarmby are key representatives on the peak body committee for the Australian Bush Foods Industry.

Media

The silverleaf nightshade biological control project story was published in MLA’s “Feedback” publication (Aug/Sept 2016 edition, pp 29-30).

9 Financial Statement

Government of South Australia
Primary Industries and Regions SA

Telephone No: 0428 833 119

29 May 2018

Invasive Species
Biosecurity SA
Primary Industries and Regions SA

PIRSA, GPO Box 1671
Adelaide SA 5001

P: 08 8429 0822
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E: john.heap@sa.gov.au

Mr Cameron Allan
Program Manager - Sustainable Feedbase Resources
Meat and Livestock Australia

Dear Mr Allan

MLA SLN biocontrol project financial acquittal

Please find below an approved and signed financial acquittal (Table 1) for the MLA project "Silverleaf nightshade biological control RnD4Profit-14-01-040" to 30 March 2018. As requested, forward estimates to 1 September 2018 have also been added.

Yours sincerely,

John Heap 29/5/2018

John Heap
SENIOR BIOSECURITY RESEARCH OFFICER – WEEDS

Table 1. Financial acquittal for the MLA project "Silverleaf nightshade biological control RnD4Profit-14-01-040". The May to Sept expected expenditure figures in blue are estimates.

MLA x SLN Biocontrol Financial Summary April 30 2018					
Item	**2015/2016**	**2016/2017**	**2017/2018 YTD**	2017/2018 May-Sept Forecast	**Total**
Income (MLA)					
Income	0	311810	219844	105304	636958
Expenditure					
Salaries and on-costs	0	65566	59722	16329	141617
Contractors DEDJTR	0	133250	122700	62950	318900
Contractors NSWDPI	0	83800	0	18700	102500
Contractors DEWNR/PIRSA	0	12880	0	18000	30880
Operating	0	16323	18767	0	35090
Total Expenditure	**0**	**311819**	**201189**	115979	628987
Balance	**0**	**-9**	**18655**	-10675	7971

PIRSA Financial Services approval

I, Marko Rac, certify that that the Financial Acquittal presented in Table 1 (above) is true and correct, and that I am authorised to approve this acquittal on behalf of PIRSA.

Signature: ..... Position: Senior Manager Financial Services

Date: 29.5.18

Objective ID: **A3597288**

9.1 Unexpended funds

It is estimated that there will approximately $8,000 unexpended at the end of the project. During the course of this project John Heap (PIRSA) and Hanwen Wu (NSW DPI) have been co-authoring a comprehensive draft Best Practice Management manual, incorporating all of the important current knowledge about SLN and its management in Australia. This manual is similar to those previously published for many other Weeds of National Significance (WoNS). The draft is at an advanced stage, and has been reviewed by a number of people. We are now at the stage of seeking about $10,000 funding for the final stage – graphic design and printing, and hope to publish in the second half of 2018.

We believe that using the unexpended funds to publish this manual would be both timely and worthwhile. The manual has been designed to be the major reference on SLN management for Australian farmers for at least 10 years.

9.2 Project partners

Not applicable.

9.3 Additional Funds

Please see 9.1 above.

10 Appendix

10.1 Project, media and communications material and intellectual property

A number of Appendices (Table A1) are associated with this report. The signed project Agreement is Appendix 1. Appendix 2 is a comprehensive and detailed project report. Draft scientific papers are presented in Appendix 3.

Table A1. Appendices for Final Report for B.WBC.008 SLN Final Report.

Appendix number	Appendix name	Appendix file title	Content
1	Agreement	SLN Contract B.WBC.0080.pdf	Signed project contract
2	Project detail	SLN Appendix 2 Detailed Final Report.docx	Project details
3	Paper drafts	SLN Appendix 3 Draft papers.docx	Prepared paper drafts

10.2 Equipment and assets

Not applicable.

10.3 Staffing levels

The staff members involved with the silverleaf nightshade biological control project are shown in Table A2.

Table A2. Staff involved with SLN biological control project.

Team member	Organisation	FTE	Position	Years
Dr John Heap	PIRSA	0.2* then 0.4	Research Scientist	2015/16*- 17/18
Dr Laurie Haegi	DEWNR	Contract	Research Scientist	2015/16 - 17/18
Dr Jane Prider	PIRSA	0.1	Technical Officer	2015/16 - 17/18
Mr Greg Lefoe	DEDJTR	0.5	Research Scientist	2015/16 - 17/18
Dr Hanwen Wu	NSWDPI	Contract	Research Scientist	2015/16 - 17/18
Dr David Gopurenko	NSWDPI	Contract	Research Scientist	2015/16 - 17/18
Dr Xiaocheng Zhu	NSWDPI	Contract	Research Scientist	2015/16 - 17/18

10.4 References

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