





## final report

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# Innovative management of silverleaf nightshade (SLN) and prairie ground cherry (PGC)

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#### Abstract

*Silverleaf nightshade* (SLN) and prairie ground cherry (PGC) are typical examples of intractable, deep-rooted, summer perennial weeds which significantly impact on livestock productivity and health. McLaren *et al.* (2004) estimated that average total farm impact of SLN was \$1730 per year in direct control costs and \$7786 in lost production. Few options other than high rate of expensive residual herbicides, that affect the establishment of following pastures and crops, were available to reduce their spread and impact. This project considerably broadened herbicide control options and identified potential new non-chemical tactics. Current research has demonstrated the importance of controlling perennial weeds through management practices that target both the seedbank and the rootbank. An effective dual spray program for controlling SLN and PGC was developed, with an early herbicide application to early flowering plants in late Spring/early summer to target seedbank, followed by a late herbicide application in late March/early April to target rootbank. This dual spray program can effectively reduce stem emergence by up to 97% in the following year.

Competitive pastures and winter cover crops also provided effective weed suppression, inhibiting the growth of SLN and PGC by 84 - 97%. Research on *Eucalyptus* allelopathy has demonstrated its potent bioactivity on SLN, providing the basis for the development of a new bioherbicide. Limited host specificity testing of the chrysomelid beetle has indicated opportunity for biocontrol of PGC.

A best management package based on the above chemical and non-chemical options has been developed. This BMP can potentially generate \$30/ha profit to growers by cost-effective integrated weed management. Continued research and extension is required to delivery the BMP to growers to reduce weed management costs, improve perennial pasture productivity, and increase capacity of growers to manage perennial weeds.

### **Executive summary**

Summer perennial weeds are a major cost to animal production in SE Australian mixed farming systems. Predicted climate change towards warmer, moister summers is expected to increase the spread and impact of summer weeds on pasture systems. *Silverleaf nightshade* (SLN) and prairie ground cherry (PGC) are typical examples of intractable, deep-rooted, summer perennial weeds which significantly impact on livestock productivity and health. SLN and PGC have both been identified as priority weeds of perennial pasture and pasture/cropping zones (Grice 2002) and their spread, persistence and intractable nature are attributable to the presence of both a seedbank and a rootbank.

SLN and PGC infest 140,000 and 29,000 hectares respectively in SE Australia, with the potential to infest 398 and 410 million hectares respectively in Australia (Kwong 2006) and reduce the productivity of hundreds of farms, with the potential to reduce the productivity of thousands of farms in the future. The economic impact of these weeds comprises direct control costs, production loss (yield and hay value), reduced land value, and environmental degradation. In addition, farmers often discontinue cropping in SLN-infested paddocks due to the competitive effect of this weed.

The lack of effective control tactics makes management of SLN and PGC extremely difficult, with management limited to a few unreliable and expensive residual herbicides. A 2003 survey of 229 growers across SE Australia (McLaren, unpublished) identified that 84% of growers needed more information on *silverleaf nightshade* for effective control. The impact of the weed has been described as "*This is silverleaf nightmare*" and farmers suggest that they need a package of actions that can be used to develop successful management plans.

The key outputs from this research project were:

- More reliable herbicide recommendations: A single herbicide application, either early or late, does not stop regrowth from both the rootbank and the seedbank of these perennial weeds in the following season. Additionally, the current single herbicide recommendation does not eliminate seed production if the application timing is too late. These two limitations mean that perennial weed populations persist. Newly-developed "Dual Spray and BMP" from the previous project cost-effectively reduce the weed population.
- Improved knowledge of weed biology and ecology to underpin a BMP strategy: Extensive data on seed production, seed dormancy, germination requirements and seed persistence were collected. Burial studies indicate that seedbanks may persist for five years or more. Root fragments of 10 mm in length can be viable, demonstrating the impact of cultivation on SLN spread. This highlights the importance of separate control tactics to manage both the seedbank and the rootbank.
- Competitive pastures/cover crops identified: Both lucerne and phalaris were competitive against SLN and PGC in glasshouse trials, suppressing the growth of SLN and PGC by 76-93% and 93-97%, respectively. Additionally, maintaining C4 grass pasture biomass reduced SLN biomass by 34-68% in both glasshouse and field studies. Winter and summer cover crops (cereals) reduced early SLN stem emergence by 60-95%. These results indicate the role of competitive pastures/crops in managing weed populations.
- Understanding of eucalyptus allelopathy on SLN: Preliminary studies showed that aqueous extracts from *Eucalyptus spathulata, E. brockwayi, E. dundasii and E. salubris* were phytotoxic to SLN and PGC germination, depending on the species and concentration. Essential oils, soluble

essential oils and steam extracts were phytotoxic to the germination and seedling growth of SLN and PGC. More than 40 compounds were identified in the essential oils which could contribute to the phytotoxicity observed. These outputs provide the basis for the development of a new bioherbicide.

- Recommendation for biological control: A survey of pathogens found in SLN populations did not find any agents suitable as a biocontrol agent for SLN. However, opportunistically two insects were discovered, an eggfruit caterpillar feeding on SLN berries and a chrysomelid beetle feeding on PGC leaves. Limited host specificity testing of the chrysomelid beetle has indicated opportunity for biocontrol.
- Recommended best management practices (BMP) for land managers:
  - Effective long-term management of SLN and PGC requires at least 2-3 years of Dual Spray to significantly reduce weed density by depleting both the seedbank and the rootbank. The Dual-spray can be carried out in annual grass pasture phase or cereal cropping phase or fallow phase. Mechanical control can replace the first spray.
  - First spray or mechanical control in late Spring or early Summer to control seed set
  - Second spray in Autumn to control the rootbank
  - Competitive winter cereal crops or annual grass pastures to further reduce weed population re-establishing via the seedbank or remnant rootbank. Cereals and grass pasture can be sown after the second spray. Broadleaved crops or pasture species are sensitive to the residual chemicals used in the second spray.
  - Repeat the above three steps for three seasons
  - Assess stem numbers; if < 1 stem/10m<sup>2</sup>, spot spray as appropriate
  - Broadleaved pastures or crops can be introduced after one year of application of the last second spray
  - Incorporate summer active pasture or crops as appropriate
  - Monitor the area for five years for new seedlings. Assess stem numbers; if < 1 stem/10m<sup>2</sup>, spot spray as appropriate

This integrated approach targeting both the seedbank and the rootbank needs to be demonstrated and validated to land managers, advisors and weed officers in a broader range of environments across southern NSW.

- Knowledge gaps identified for further improvement in the management of these weeds:
  - Greater understanding of the phenology of the weeds to understand the relative contributions made by the seedbank and the rootbank to population dynamics, together with the environmental cues that stimulate emergence
  - Improved herbicide uptake and translocation to target the rootbank
  - The interaction between competitive pastures or crops and herbicides

With the predicted saving of \$30/ha in direct control cost by implementing the best-bet IWM strategy, the livestock industry will potentially reduce management costs by \$1.52 million per annum based the current infestation levels of SLN and PGC and an adoption rate of 50%. Development and demonstration studies are required to show the combined and additive benefits of new chemical and non-chemical control tactics over a number of seasons for the control of perennial weeds in southern Australia, leading to less herbicides in the environment, improved perennial pasture productivity, and increased capacity of growers to manage perennial weeds.

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## Background

Weeds of agriculture are estimated to cost the Australian economy over \$4 billion annually. SLN is currently classified as a noxious weed state-wide in South Australia and Victoria, and in one third of the local control authority regions of New South Wales. SLN and PGC have both been identified as priority weeds of perennial pasture and pasture/cropping zones (Grice 2002). Both weeds are highly invasive, with invasiveness scores of over 0.66 out of a maximum potential score of 1. Their spread, persistence and intractable nature are attributable to the presence of both a seedbank and a rootbank. The seedbank is estimated to last for at least six years, and the extensive root system is thought to persist for longer, resulting in a need for long term management to reduce or eliminate a population once established.

Emergence of the weeds occurs under favourable conditions in spring, either as seedlings or as new stems from the existing rootbank. Flowering may commence from November and continue through to late summer. Berries typically start forming from December onwards. Both weeds are dormant over winter, with die back of aerial growth commencing in mid to late autumn.

SLN and PGC infest 140,000 and 29,000 hectares respectively in SE Australia, with the potential to infest 398 and 410 million hectares respectively in Australia, with nearly 95% of the infested areas affecting pasture lands (Kwong 2006). These weeds currently reduce the productivity of hundreds of farms, with the potential to reduce the productivity of thousands of farms in the future. The economic impact of these weeds comprises direct control costs, production loss (yield and hay value), reduced land value, and environmental degradation. A survey of 254 land managers in SE Australia estimated that average total farm impact of SLN was \$1730 per year in direct control costs and \$7786 in lost production (McLaren et al. 2004). SLN can cause cereal yield reduction of up to 70% (Heap and Carter 1999) due to the depletion of soil moisture and nutrients during the previous summer as well as in-crop competition. The yield loss is highest in regions of dry sandy soils and seasons with low rainfall.

Deep-rooted summer weeds compete directly with summer-growing pastures, reducing production, forage value and carrying capacity. Annual winter pastures are also affected by delayed emergence and lower production and quality. In SA alone, SLN has been estimated to cost producers more than \$10 million per year. The weeds can reduce management options, such as the use of land and sale of hay. The greatest economic effect of SLN in eastern Australia is the reduction of land values of both infested and nearby properties, with the potential to reduce land value by 25%. Abandonment of farm properties has also been reported overseas as a result of heavy SLN infestation (Parsons 1973). In addition, farmers often discontinue cropping in SLN-infested paddocks due to the competitive effect of this weed. Not only is SLN a major agricultural weed, it has the potential to become an important environmental weed by its means of distribution and competitive nature (Parsons and Cuthbertson 2001) with heavy infestations potentially leading to biodiversity loss.

The lack of effective control tactics makes management of SLN and PGC extremely difficult, with management limited to a few unreliable and expensive residual herbicides (Kidston *et al.*2007, Ensbay 2009). A 2003 survey of 229 growers across SE Australia (McLaren, unpublished), identified that 84% growers needed more information on *silverleaf nightshade* for effective control. This is extremely frustrating and demoralising for farmers.

## 1 Project objectives

This project will develop and evaluate effective management tactics for *Silverleaf nightshade* and Prairie ground cherry - weeds of significance to the gazing industries.

By 31 October, 2009 outputs of Research activities will include:

Objective 1 —	effective use of competitive perennial pastures (legume and non-legume) in combination with forage conservation and targeted herbicide use will be determined and recommendations made available for producers and advisors
Objective 2 —	herbicide efficacy will be increased with better timing in relation to weed size, weed phenology and time of day, and new application technology and recommendations will be made available for producers and advisors
Objective 3 —	factors affecting herbicide translocation in SLN and PGC will be determined and this information will be integrated into development of herbicide application recommendations
Objective 4 —	allelochemicals for control of SLN and PGC will be identified and evaluated in glasshouse and field trials and selectivity determined and published
Objective 5 —	indigenous pathogens will be identified and evaluated as potential bioherbicides and formulations and selectivity determined and published. This biological control component has been replaced by Objective 5B "Biology and Ecology Study" (Factors affecting germination and emergence of SLN and PGC will be identified. Seed persistence and other biological data will be collected for better understanding of the target weeds
Objective 6 — Objective 7 —	drafted 3 scientific publications for peer review journals, and 3 conference papers 4 student projects completed on SLN & PGC, co-supported by the Graham Centre
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## 2 Methodology

Effective long term control of these intractable weeds will require development and implementation of an integrated management program where combinations of strategies are employed to weaken and overcome the longevity and vigour of the weeds. This project has investigated a range of control options to determine the relative level of control that may be contributed by each single component of an integrated package. Further study was also undertaken to obtain information regards the biology and ecology of the weeds to determine the importance of controlling the seedbank and the rootbank, as well as defining the length of time required to achieve long term control.

The control options considered in this project covered investigation of pastures or crops to provide competition against the weeds, investigation of methods to improve herbicide efficacy, investigation for naturally occurring pathogens that may provide biocontrol against the weeds, and investigation of novel compounds from eucalypts that may have herbicidal activity against the weeds. Once the most effective treatments within each control options have been identified, these can be incorporated together into an integrated strategy where synergistic effects may be obtained.

Unless otherwise stated, homogeneity of variance was determined using Barlett's Test. Data were analysed with GENSTAT software using analysis of variance and Fisher's Protected LSD at 5% level of significance used to separate treatment means.

#### 2.1 Pastures

#### 2.1.1 Proof-of-concept glasshouse experiments

In the first experiment, the competitive effect of lucerne and phalaris was assessed under greenhouse conditions. Three replicates were established in spring 2007 in a randomised block design using large pasture competition pots containing three segments of either SLN or PGC roots together with a pasture of either lucerne or phalaris. Control pots with root fragments were also established with no pasture. Pots were maintained for two years, with biomass estimates, height/spread of SLN/PGC, seed set collected each summer. The containers were dismantled in autumn 2009 and the root systems of the pastures and the weeds carefully recovered, dried and weighed.

In the second experiment, the competitive effect of a range of pasture species was assessed under glasshouse conditions, with the aim to determine if there is any difference in the competitiveness of the species and identify the most competitive pasture species for further field evaluation. Three replicates of nine pasture species were evaluated in a randomised block design for competitiveness commencing in spring 2008, including Bambatsi Panic (*Panicum coloratum* var. *makarikariense*), Currie Cocksfoot (*Dactylis glomerata* cv. Currie), Digit Grass (*Digitaria eriantha* spp. *eriantha*), Lucerne (*Medicago sativa* cv. Aurora), Rhodes Grass (*Chloris gayana* cv. Katambora), Sirolan Phalaris (*Phalaris aquatica* cv. Sirolan), Finger Grass (*Digitaria milanjiana* cv. Strickland), Biserrula (*Biserrula pelecinus* cv. Casbah) and Chicory (*Chicorium intybus* cv. Puna). Each pot contained a single 10cm long fragment of SLN or PGC together with four pasture plants. Aerial growth of pasture and weeds was harvested and recorded as dry matter production twice during summer to simulate pasture utilisation, and at the end of summer the final shoot and root dry weights were recorded.

#### 2.1.2 Field experiments

Field experiments were established to assess the competitiveness of a range of pasture species against both weeds under field conditions across a range of environments. Field sites were selected in July 2006 at Wellington, Ganmain and Narrandera on SLN populations and a site selected in June 2007 at Tocumwal on a PGC population. Eight pastures were examined at each site in a complete randomised design with three replicates. Poor seasonal conditions required the sites to be resown, with only the Wellington site being able to be established by the 2008/009 season. Pasture biomass and composition data were collected quarterly to determine the competitive ability of the different pastures under field conditions.

In a second field experiment, the competitive effect of winter active crops or pastures and their stubble residue against SLN was examined. Four crop species and a fallow control were used in a complete randomised design with three replicates. Cereal rye, wheat, saia oats and annual ryegrass were sown in May 2008 at Narrandera. Biomass was either left standing or was mown in November to determine if the architecture of the stubble load influenced SLN emergence and growth. SLN density and berry production were recorded twice during the summer.

#### 2.2 Herbicides

#### 2.2.1 Glasshouse experiments

The effect of SLN stage of growth and the use of adjuvants was examined to determine whether there is an optimum time of spray application and whether adjuvants influence herbicide efficacy. An Honours student was recruited to conduct a two factorial experiment, established in a split plot design (application timing as main plots) with four replicates.

Herbicde treatments of 1080g ai./ha glyphosate, 400 g a.i./ha fluroxypyr or 225 g a.i./ha picloram+ 900 g a.i./ha 2,4-D amine were applied at vegetative and flowering growth stages, either alone or with 19.2 g a.i./ha oxyfluorfen, 1 L/ha Uptake paraffinic oil surfactant or 1 L/ha Hasten vegetable oil surfactant as an adjuvant. Efficacy was measured in terms of within season control of existing biomass 14, 28, 56 and 84 days after treatment, and between season control measured as stem emergence the following season and rhizome weight determined in October 2008.

#### 2.2.2 Field experiments

Field experiments were conducted to determine herbicide efficacy could be improved through the use of new herbicide combinations or timings. Field sites were established in 2006 at Culcairn and Leeton for SLN and Tarcutta for PGC, and a second PGC site at Tocumwal in 2007, with 19 herbicides and an untreated control included at each site in a randomised complete design with three replicates. Treatments were applied in the 2006/07 season based on current registered herbicides and current reported practice, together with several new options based on knowledge gained from research work reported in the literature (Table 3.1). The treatments applied to the Tocumwal PGC site in 2007/08 were modified to allow Hasten (0.5 L/ha) and Sprinta (0.2 L/100L) adjuvants to be compared to Uptake spray oil (1.0 L/ha) within several herbicide treatments (Table 3.2).

The 2007/08 SLN were slightly modified based on knowledge gained from glasshouse and field experiments conducted to date. The Tordon and Grazon Extra treatments were modified from a single application mid season to include an additional late season application of the same herbicide.

Data mortality and reproductive stage were collected four to six weeks after each herbicide application to determine the effectiveness of the herbicide treatments for within season control, as well as emergence at the start of each season to determine the effectiveness of the herbicide treatments for between season control. In addition, the effect of herbicide application on seed production and viability was assessed. Individual flowers and berries were tagged at the first herbicide application for the Glyphosate, Starane, Tordon, 2,4-D amine, and Grazon Extra treatments. Berries were collected after six weeks, numbers of seeds per berry counted and seed viability determined using a tetrazolium assay.

Treatment	Active Ingredients
1 - Control	untreated control
2 - Glyphosate	1080g a.i./ha glyphosate
3 - Glyphosate / Glyphosate	1080g a.i./ha glyphosate followed by 1080g a.i./ha glyphosate (late)
4 - Glyphosate / 2,4-D amine	1080g a.i./ha glyphosate followed by 937.5g a.i./ha 2,4-D amine (late)
5 - 2,4-D amine	937.5g a.i./ha 2,4-D amine
6 - Glyphosate + 2,4-D amine	937.5g a.i./ha 2,4-D amine + 1080g a.i./ha glyphosate
7 - Dicamba	2000g a.i./ha dicamba
8 - Starane	200g a.i./ha fluroxypyr
9 - Starane + 2,4-D amine	200g a.i./ha fluroxypyr + 937.5g a.i./ha 2,4-D amine
10 - Hotshot	15g a.i./ha Aminopyralid + 210g a.i./ha fluroxypyr
11 - Tordon 75D	900g a.i./ha 2,4-D + 225g a.i./ha picloram
12 - Grazon Extra	900g a.i./ha triclopyr + 300g a.i./ha picloram + 24g a.i./ha aminopyralid
13 - Amitrole	500g a.i./ha amitrole
14 - Atrazine	2000g a.i./ha atrazine
15 - Atrazine + Spray.Seed	2000g a.i./ha atrazine + 324g a.i./ha paraquat + 276g a.i./ha diquat
16 - Glyphosate + Goal	1080g a.i./ha glyphosate + 19.2g a.i./ha oxyfluorfen
17 - Glyphosate + Ally	1080g a.i./ha glyphosate + 9g a.i./ha metsulfuron methyl
18 - Glyphosate + Ally + Goal	1080g a.i./ha glyphosate + 9g a.i./ha metsulfuron methyl + 19.2g a.i./ha oxyfluorfen
19 - Glyphosate + OnDuty	1080g a.i./ha glyphosate + 21g a.i./ha imazapic + 7g a.i./ha imazapyr
20 - Tordon 75D + Ally	900g a.i./ha 2,4-D + 225g a.i./ha picloram + 9g a.i./ha metsulfuron methyl

Table 3.1. Herbicide treatments applied to SLN sites 2006/07.

Treatment	Active Ingredients
1 - control	untreated control
2 - Glyphosate control	1080g a.i./ha glyphosate
3 - Glyphosate x 2	1080g a.i./ha glyphosate + 1080g a.i./ha glyphosate
4 - Glyphosate / 2,4-D amine	1080g a.i./ha glyphosate + 937.5g a.i./ha 2,4-D amine
5 - 2,4-D amine	937.5g a.i./ha 2,4-D amine
6 - Glyphosate + 2,4-D amine	937.5g a.i./ha 2,4-D amine + 1080g a.i./ha glyphosate
7 - Glyphosate + Ally (Sprinta)	1080g a.i./ha glyphosate + 9g a.i./ha metsulfuron methyl
8 - Starane (Uptake)	200g a.i./ha fluroxypyr
9 - Tordon + Ally (Hasten)	900g a.i./ha 2,4-D + 225g a.i./ha picloram + 9g a.i./ha metsulfuron methyl
10 - HotShot (Uptake)	15g a.i./ha Aminopyralid + 210g a.i./ha fluroxypyr
11 - Tordon 75D	900g a.i./ha 2,4-D + 225g a.i./ha picloram
12 - Grazon Xtra	900g a.i./ha triclopyr + 300g a.i./ha picloram + 24g a.i./ha aminopyralid
13 - Amitrole	500g a.i./ha amitrole
14 - Glyphosate + Ally (Hasten)	1080g a.i./ha glyphosate + 9g a.i./ha metsulfuron methyl
15 - Tordon + Ally (Sprinta)	900g a.i./ha 2,4-D + 225g a.i./ha picloram + 9g a.i./ha metsulfuron methyl
16 - Glyphosate + Goal	1080g a.i./ha glyphosate + 19.2g a.i./ha oxyfluorfen
17 - Glyphosate + Ally (Uptake)	1080g a.i./ha glyphosate + 9g a.i./ha metsulfuron methyl
18 - Glyphosate + Ally + Goal	1080g a.i./ha glyphosate + 9g a.i./ha metsulfuron methyl + 19.2g a.i./ha oxyfluorfen
19 - Glyphosate + OnDuty	1080g a.i./ha glyphosate + 21g a.i./ha imazapic + 7g a.i./ha imazapyr
20 - Tordon + Ally (Uptake)	900g a.i./ha 2,4-D + 225g a.i./ha picloram + 9g a.i./ha metsulfuron methyl

#### Table 3.2. Herbicide treatments applied to PGC in 2007/08.

#### 2.2.3 Herbicide translocation

A preliminary study was conducted in the glasshouse in April 2009 to investigate the impact of growth stage (early vegetative and flowering/berry stage) and adjuvant ( $\pm$  Uptake) on herbicide translocation. The "Impact of upper and lower leaf surface application on herbicide translocation and uptake" and the "Impact of preconditioning of herbicide stress on translocation" were also studied. C<sup>14</sup> labelled fluroxypyr from Dow Agrosciences was used.

Plant materials were harvested and separated into roots, stems, treated leaf and leaves, and analysed for radioactivity through biological combustion and liquid scintillation spectrometry at the University of Adelaide in March 2010.

#### 2.3 Allelopathy

#### 2.3.1 Laboratory experiments

A site established at Ungarie contained saplings of four Eucalypt species of interest, *E. brockwayii, E. dundasii, E. salubris* and *E. spathulata.* Approximately two kilograms of leaves were collected

from these species as well as *E. melliodora* as a control species. Steam distillation was used to produce essential oils, soluble essential oils and stream extracts for bioassays and chemical analyses.

Gas chromatography and mass spectrometry was used to determine the chemical composition of the three distillation products derived for each of the Eucalypt species. All laboratory procedures were replicated three times.

The bioactivity of extract fractions were applied at 0, 25, 50, 75 and 100% concentrations to SLN and PGC seeds in petri dish assays to determine the impact on germination and growth rates using three replicates in a complete randomised design. Preliminary investigations of essential oils on seedlings were conducted using SLN and PGC seedlings in the glasshouse to determine herbicidal activity of foliar application of oil applied at different rates using two replicates.

The phytotoxic specificity of the extracts was examined across a range of broadleaf and grass weed species (wild radish (WR), annual ryegrass (ARG), barley grass (BAR), wild oat (WO), barley brome grass (BRO) and barnyard grass (BYG)) following the same protocols as used for SLN and PGC.

#### 2.3.2 Field experiments

Prior to this project, a nursery site had been established at Ungarie with 25 trees of each of the four Eucalypt species of interest planted in blocks, providing a single replicate. In January 2008 and 2009, SLN stems within 0.5 of the trunk of each tree were recorded, together with the height of the eucalypt tree. The *E. brockwayii* and *E. dundasii* had been replanted due to effects of grazing, therefore these species were smaller than *E. salubris* and *E. spathulata*.

#### 2.4 Biology and Ecology

#### 2.4.1 Pathogens (Biological control)

Screening of naturally occurring pathogens for activity against SLN and PGC was part of the original research program. The objective was to assess pathogens for potential for development into a new biocontrol agent.

Leaf, stem and root samples were collected in a random survey from SLN and PGC plants in the field showing symptoms of presence of pathogens. Leaf and stem tissue samples were cultured on potato dextrose agar (PDA) or nutrient agar (NA) and root tissue samples were spiral plated onto NA. Pure pathogen colonies from 47 samples were sub-sampled and plated onto fresh plates of PDA or NA, respectively, and incubated for at least two days at 25 C.

SLN seedlings were inoculated by placing an agar plate containing a pure pathogen colony 7 cm deep in the pot, with two replicates per pathogen. Seedlings were maintained in the glasshouse and regularly monitored for signs of pathological symptoms consistent with field records from the original sampling.

No seedlings showed any pathological symptoms after six months, despite the root systems having penetrated through the agar plates. If seedlings had showed symptoms, leaf material was to be collected where similar responses are observed in both replicates. Cultures were to be prepared following the previous protocols to determine if the observed pathology was a result of infection by the isolate used.

#### 2.4.2 Seed production and viability

SLN and PGC are both native to South and Central America and arrived in Australia as a result of seed contamination of grain or fodder. An understanding of the seed production capacity of both weeds is important to inform land managers of the need to ensure berry production is minimised as much as possible, and to ensure that management practices are implemented o reduce movement of seed away from the site of infestation via livestock, machinery, grain or fodder.

Samples of SLN and PGC plants were collected in autumn 2008 at two sites each for SLN and PGC to estimate the seed production capacity for weed population dynamics modelling. Ten plants were randomly chosen at each site and plant size recorded, together with number of berries on the plant. Ten berries were randomly harvested from each plant for laboratory analysis to collect berry diameter and weight and seeds count per berry.

#### 2.4.3 Seedbank dynamics

To successfully eradicate a SLN or PGC population, it is necessary to understand how long seed may persist in the soil seedbank and to therefore monitor the infestation site for a period of time beyond the last year of seed production to control any new seedlings that might emerge.

Little data are available regarding seedbank dynamics of SLN or PGC. As both species are capable of producing large numbers of seeds per plant, the longevity of seeds in the soil needs to be understood when considering management plans.

Packets measuring 10 x 10 cm were formed from quarantine mesh and contained either 50 seeds or four intact pods together with a small quantity of sieved soil. Packets were placed at four depths (0, 2.5, 5 and 10 cm) at two sites and were recovered after four time periods (3, 6, 12 and 24 months) in a randomised complete block design with three replicates per site. Recovered seeds were counted and tested for germination and viability. Germination and viability of recovered seeds was compared to that of seed stored in the laboratory. Weather stations were installed at both sites to collect meteorological data and soil temperature and moisture data at the burial depths.

#### 2.4.4 Germination requirements

Understanding the requirements or limitations that influence the germination of SLN and PGC seeds is critical to understanding what environmental conditions are required for germination to occur and what soil types are more conducive to germination occurring. Knowledge of when and where seedlings are more likely to occur can allow land managers to be more strategic with monitoring programs for control of seedlings. The following experiments assessed the influence of temperature, osmotic stress, salinity and pH on germination of SLN and PGC seed.

Unless stated otherwise, the following protocol was used to determine germination level. Fifty seeds were placed on Whatman No.2 filter paper moistened with 4 ml of solution in a 9 cm petri dish. Petri dishes were sealed with parafilm and incubated for 21 days under a fluctuating 30/15 C cycle with a 12 hr photoperiod coinciding with the higher temperature. Germinated seeds were recorded every three or four days. Three replicates were conducted for each treatment, using a split plot design (temperate regime as main plot) when different temperature regimes were one of the factor being examined, or a complete randomised design in all other experiments.

The effect of osmotic stress was studied for fixed (10, 20 and 30 C) and fluctuating (10/25, 15/30 C) temperature regimes. Solutions with osmotic pressures of 0, 0.03, 0.06, 0.12, 0.24, 0.48, and 0.96

MPa were prepared by dissolving polyethylene glycol (PEG) 8000 in distilled water as described by Michel (1983). In the case of fluctuating temperature regimes, PEG 8000 quantities were determined from the average for the two temperatures.

The effect of salinity was studied by incubating seeds in solutions containing 0, 10, 20, 40, 80, 160 and 320 mM solutions of sodium chloride (NaCl) (Chauhan *et al* 2006).

The effect of pH was studied by incubating seeds in buffered solutions of pH 4 to 10 prepared according to the method described by Chen et al (2009). The seeds were exposed to aqueous HCl solutions of pH = 4, 5 and, 6 or NaOH solutions of pH = 8, 9 and 10, respectively, in comparison with deionized water (pH = 7).

#### 2.4.5 Asexual reproduction

SLN and PGC are perennial weeds with extensive root systems that produce new growth each season. Fragmentation of the root system via cultivation could potentially increase the population density if the small fragments are capable of regeneration. Additionally, any viable fragments that adhere to machinery could be a potential source of new infestations elsewhere. An understanding of the asexual reproductive capacity of both species is necessary for land managers to develop strategies to minimise the impact of these two weeds.

*Silverleaf nightshade* root samples were collected in autumn of 2006 and 2007 from near Narrandera, NSW. A randomised complete block design with three replicates was used with two factors, root fragment length (1, 2.5, 5 and 10 cm) and root burial depth (2.5, 5 and 10 cm). The uppermost 10 cm was used from each root sample, and was cut into one, two, four or ten fragments.

Pots were maintained in a glasshouse and watered regularly. Shoot emergence was recorded weekly for each root fragment. Pots were maintained for six months to simulate a normal growing season before aerial growth was harvested at ground level and maximum shoot height and total fresh weight recorded. Root fragments were exhumed, washed of excess soil and blotted dry before root length and fresh weight were recorded. Root fragments that had lost weight during the experiment were classified as dead. Mortality for each pot was determined as the percentage of dead root segments in the pot.

#### 2.5 Economic modelling

To evaluate the economics of adopting a new approach based on outcomes from work reported from this project, preliminary economic modelling was undertaken for three separate scenarios. The first scenario assumed no control was undertaken targeting SLN. The second scenario assumed control was undertaken using current practice representing the operations recommended or undertaken by a weeds control officer, namely one application of 2 L/ha glyphosate at flowering/early berry set. The third scenario is based on the strategy of using two herbicide applications per season to sequentially target the seedbank and the rootbank, respectively.

In all scenarios, herbicide costs were based on common retail prices published by NSW DPI, or actual costs from a rural supplier if the product was not covered in the NSW DPI list. Variable costs to apply the herbicide (machinery, labour and time) were based on NSW DPI Guide to tractor and implement costs for a small (76-95 HP) tractor. The impact of SLN on economic returns was taken from case studies, with 40% decrease in returns assumed for a SLN population density of 10 stems/m<sup>2</sup>, declining linearly with SLN density to zero. A nominal inflation rate on 1% p.a. was included over the ten years of the model.

All modelling assumed an initial SLN stem density of 10 stems/m<sup>2</sup>, present as a uniform population across the entire area to be treated. It was also assumed that there would be no benefit to be derived from use of spray technology such as WeedSeeker until the average weed population was less than 3 stems/m<sup>2</sup>, therefore the whole area was sprayed at SLN densities above this level.

## 3 Results and discussion

The knowledge gained from each of the areas of investigation conducted during this research provides the foundation for the development of Best Management Practice guidelines (Figure 4.0). These guidelines will empower individual producers and land managers to develop an IWM strategy for deep rooted perennial weeds that can be integrated into their current practices. A successful IWM plan does not require major operational changes to be made or involve large additional or ongoing financial commitments. Strategic use of existing resources and capital will provide improved management capacity of these weeds.

SLN and PGC populations can regenerate or spread from both the seedbank and the rootbank. Traditional weed control tactics developed for annual weeds do not provide adequate control of the rootbank of these perennial weeds. Control of the aerial growth present during summer may not reduce the number of new stems emerging from the rootbank the following spring.

Seedlings of both species can germinate across a range of soil salinity and pH conditions, suggesting that producers and land managers need to be vigilant in monitoring all areas for the occurrence of new infestations. Monitoring for seedlings should be undertaken when soil temperatures are above 15 C and after rainfall events occur that raise soil moisture levels to near field capacity, as both weeds require warm, moist conditions for seed germination. SLN seedlings are commonly found in summer when there is an extended period of moist conditions for 5-7 days.

Control of seedlings is easier than control of established plants which have an extensive root system, therefore new infestations should be managed before the plants are able to establish. Good stock, grain and machinery hygiene will minimise the risks of importation or movement of viable seed or root fragments to new areas previously not infested with these weeds.

Use of crops or pastures to reduce soil moisture levels during spring and early summer will assist in delaying the emergence of seedlings or new stems. Maintenance of pasture dry matter biomass above 1.5t/ha over summer will reduce growth and seed production of the weeds

Herbicides and mechanical control (slashing) can be used at flowering to minimise viable seed set. Control of the rootbank is more effective when herbicides are applied during the vegetative stage when translocation of resources within the plant to the roots is greatest.

The components of a Best Management Practice as outlined above are discussed in more detail in the following sections.

Figure 4.0	. Best mana	gement praction	ce guidelines	for SLN ar	nd PGC control.
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	IWM Options	Purpose
	Monitor your land for new infestations	Management planning
Spring	<ul> <li>Maintain ground cover in areas where weeds occur using:</li> <li>good winter crop agronomy to maximise ground cover and soil moisture utilisation during spring/early summer</li> <li>good winter-active pasture agronomy to maximise biomass, ground cover and soil moisture utilisation during spring/early summer</li> <li>good summer-active pasture agronomy to maximise biomass, ground cover and soil moisture utilisation during spring/early summer</li> </ul>	Provide competition to emerging stems and seedlings
	Minimise soil disturbance to reduce seedling germination Spot spraying seedlings or new stems with glyphosate, 2,4-D amine or fluroxypyr Boom spraying seedlings or new stems with glyphosate, 2,4-D amine or fluroxypyr Chipping seedlings or digging out new stems	Control eedlings and new stems
Summer	Spot spraying at flowering with glyphosate, 2,4-D amine or fluroxypyr Boom spraying at flowering with glyphosate, 2,4-D amine or fluroxypyr Monitor seedling emergence and spray as required Slashing at flowering Chipping isolated plants at any time prior to berry formation	Prevent viable seed set (seedbank control)
	Minimise grazing once berries have started to form Avoid making hay or silage from pastures once berries have formed Hold livestock in a quarantine area for 14 days before moving them to a new field if they have been grazing where berries are present	Prevent seed spread
	Retain standing biomass from crop stubble for as long as possible Maintain pasture dry matter biomass above 1.5t/ha where possible	Provide competition
	Monitor your land and note where infestations are/were present for attention next season	Management planning
Autumn	Use residual herbicides as directed Use a herbicide containing picloram in autumn before plants die back to maximise root control. <i>Be aware of plant back periods when using</i> <i>this technique</i> Dig out new or isolated plants before they become established	Control root stock (rootbank control)
	Minimise cultivation to reduce the number of intact berries being buried Remove or destroy as many berries as possible	Reduce the soil seedbank (seedbank control)
	Hold livestock in a quarantine area for 14 days before moving them to a new field if they have been grazing where SLN berries are present	Prevent seed spread
Winter	Minimise cultivation in infested areas Clean down implements to minimise movement of root stock	Reduce root spread
	Plan future land use to maximise potential competition during the next season(s)	Provide competition

#### 3.1 Pastures

#### 3.1.1 Proof-of-concept glasshouse experiments

In the absence of pasture competition, both weeds formed large root biomass, with roots being present at and across the bottom of the 600mm deep containers, with PGC producing higher root biomass (Table 4.1). The total stem weight of PGC was reduced due to the unexpected impact from insect (*Lema bilineata*) damage.

Both lucerne and phalaris were highly competitive on the stem and root growth of SLN and PGC. Lucerne suppressed SLN stem growth by 87% and root growth by 91%, while phalaris suppressed SLN stem growth by 83% and root growth by 27%. Similarly, lucerne suppressed PGC stem growth by 59% and root growth by 100%, while phalaris suppressed PGC stem growth by 83% and root growth by 67%. These results indicate that lucerne is more competitive than phalaris in reducing the biomass and root production for both weed species. While phalaris produced larger root biomass, it was less effective at reducing root production in either weed species.

	Pasture		Weed	
Treatment	Stem Wt (g)	Root Wt (g)	Stem Wt (g)	Root Wt (g)
control	0.0	0.0	249.8	182.3
Lucerne	852.8	449.9	33.7	16.4
Phalaris	454.5	629.7	41.8	133.1
control	0.0	0.0	264.6	294.5
Lucerne	1282.3	416.6	108.1	0.0
Phalaris	477.8	1642.9	45.0	95.8
	406.6	832.4	104.7	162.7
	Treatment control Lucerne Phalaris control Lucerne Phalaris	PastureTreatmentStem Wt (g)control0.0Lucerne852.8Phalaris454.5control0.0Lucerne1282.3Phalaris477.8406.6	Pasture           Treatment         Stem Wt (g)         Root Wt (g)           control         0.0         0.0           Lucerne         852.8         449.9           Phalaris         454.5         629.7           control         0.0         0.0           Lucerne         1282.3         416.6           Phalaris         477.8         1642.9           406.6         832.4	Pasture         Weed           Treatment         Stem Wt (g)         Root Wt (g)         Stem Wt (g)           control         0.0         0.0         249.8           Lucerne         852.8         449.9         33.7           Phalaris         454.5         629.7         41.8           control         0.0         0.0         264.6           Lucerne         1282.3         416.6         108.1           Phalaris         477.8         1642.9         45.0           406.6         832.4         104.7

Table 4.1. Pasture suppression of two summer weeds under o	controlled environment
conditions.	

When a range of pasture species were compared in the glasshouse, all species significantly reduced the stem biomass of SLN by 94-99% and root biomass by 69-90%, when compared to the control (Table 4.2). *Digitaria* and *Chloris* species provided the largest biomass production under glasshouse conditions and also the greatest reduction in SLN vigour.

The experiment was conducted within an enclosed glasshouse where insect pollination was not possible. Therefore, no data were able to be collected on the influence of pasture competition on berry production. In the absence of competition, the SLN stems flowered during the season, but no berries were set as pollination did not occur. When competition was present, the SLN stems were less robust and produced few flowers.

The range of pasture species examined all significantly reduced the SLN vigour under glasshouse conditions. It is speculated that the use of pasture competition will reduce SLN and PGC production in the field as long as a pasture sward appropriate to the environmental conditions is utilised and adequate ground cover is maintained. Good pasture maintenance and agronomy can be used as effective tool within an integrated management plan.

Pasture		Stem weigh	Stem weight (g) Root weight (		nt (g)	(g) Total Weight (g)	
		SLN	PGC	SLN	PGC	SLN	PGC
Control		14.33	0.00	10.00	0.00	24.33	0.00
Currie	Dactylis	0.73	0.20	3.13	0.00	3.86	0.20
cocksfoot	<i>glomerata</i> cv. Currie						
Bambatsi	Panicum	0.87	0.35	2.23	0.27	3.10	0.62
Panic	coloratum var. makarikariense						
Biserrula	Biserrula pelecinus cy	0.80	1.91	1.77	0.33	2.57	2.24
	Casbah						
Sirolan	Phalaris	0.44	0.10	2.13	0.17	2.57	0.27
Phalaris	aquatica cv.						
	Sirolan		0.04	4 70	o <b>77</b>	0.00	4.40
Chicory	Chicorium	0.66	0.34	1.70	0.77	2.36	1.10
	Puna						
Lucerne	Medicago sativa	0.62	0.30	1.53	0.23	2.15	0.53
	cv. Aurora						
Strickland	Digitaria	0.26	0.05	1.67	0.17	1.93	0.22
Finger Grass	milanjiana	0.00	0.00	0.07	0.00	4.00	0.40
Digit Grass	Digitaria oriantha son	0.26	0.26	0.97	0.23	1.23	0.49
	eriantha						
Katambora	Chloris gayana	0.18	0.00	1.00	0.07	1.18	0.07
Rhodes							
grass							
LSD (0.05)		1.72		2.10		2.99	

## Table 4.2. *Silverleaf nightshade* and prairie ground cherry suppression by pasture under glasshouse conditions.

#### 3.1.2 Field experiments

#### Impact of competitive pastures

Similar to glasshouse findings, *Chloris, Digitaria* and *Panicum* grass pastures were competitive against SLN in field experiments at Wellington during the first year of establishment (Tables 4.3 and 4.4). The chicory and phalaris were not competitive due to poor establishment. Long term control or suppression of the SLN will need to be determined from continued monitoring of the site. SLN stem density fell rapidly once competition was present (Fig. 4.1), with the greatest decline in stem numbers occurring when up to 3 t/ha of pasture biomass was present. The decline in SLN stem density when pasture biomass was above 3 t/ha was lower, suggesting that biomass in excess of 3 t/ha could be grazed or conserved for fodder as there is only a small additional weed control benefit to be derived.

Ideally, pasture biomass needs to be managed for another season to further investigate the usefulness of these species to control SLN as well as provide information of the impact of fodder conservation. Reducing the percentage contribution of SLN to the total pasture biomass through the

use of competitive pastures will improve the overall forage value, as dry matter intake and body weight gain in goats decreases when SLN contributes more than 25% of the daily dry matter intake (Mellado *et al.* 2008).

Pasture	Total	Pasture category			
	(t/ha)	Pasture	B/leaf	Grasses	SLN
Annual pasture (clover)	2.03	0.00	0.76	0.84	0.43
Lucerne (cv. Aurora)	1.51	0.69	0.29	0.26	0.27
Digit grass	2.65	1.65	0.36	0.36	0.28
Katambora rhodes grass	6.55	6.26	0.15	0.00	0.14
Phalaris (cv. Sirolan)	1.49	0.36	0.34	0.39	0.39
Chicory	1.41	0.55	0.24	0.09	0.53
Bambatsi panic	3.22	2.21	0.52	0.26	0.22
Strickland finger grass	2.70	1.92	0.39	0.13	0.26
French millet	5.66	4.86	0.39	0.00	0.41
Sorghum (cv. stargrazer)	2.15	0.92	0.71	0.35	0.17
LSD (0.05)	1.41	1.50	n.s.	0.43	n.s.

 Table 4.3. Summer pasture biomass at Wellington.



Pasture	Total	Pasture category			Percent	
	(t/ha)	Pasture	B/leaf	Grasses	SLN	Green
Annual pasture (clover)	1.48	0.00	0.29	1.19	0.00	18.0
Lucerne (cv. Aurora)	1.57	0.94	0.24	0.39	0.00	48.7
Digit grass	3.00	3.00	0.00	0.00	0.00	36.7
Katambora rhodes grass	4.00	4.00	0.00	0.00	0.00	25.3
Phalaris (cv. Sirolan)	1.42	0.00	0.34	1.07	0.00	20.0
Chicory	1.28	0.15	0.48	0.65	0.00	21.3
Bambatsi panic	3.59	3.48	0.10	0.00	0.01	27.3
Strickland finger grass	2.73	2.51	0.22	0.00	0.00	42.7
French millet	3.00	2.00	0.74	0.24	0.03	24.7
Sorghum (cv. stargrazer)	2.10	0.80	0.37	0.92	0.00	24.7
LSD (0.05)	0.77	0.72	n.s.	0.55	n.s.	n.s.

#### Table 4.4. Autumn pasture biomass and percentage green at Wellington.

#### Impact of winter cover crops

The presence of biomass generated from winter cover crops can reduce emergence and productivity of SLN. Although crop growth was limited due to a dry spring, the presence of any crop stubble during spring reduced early emergence of SLN (Table 4.5), with less emergence occurring with increasing stubble burden.

However, delayed emergence of SLN stems overcame the influence of stubble effects by the end of summer. However, the average stem weight and number of berries produced for the season were higher where more SLN had emerged early in the season. This indicates that maintaining good winter crop agronomy and creating sufficient crop biomass can delay SLN emergence and reduce the vigour and productivity of later emerging SLN stems.

Treatment	Crop biomass (t/ha)	SLN Density (Nov)	SLN Density (Mar)	SLN height (mm)	SLN biomass (t/ha)	SLN berries/stem
Oats (cv. Saia)	1.4	0.2	7.7	185.0	0.3	0.1
Control	0.0	4.6	5.8	176.7	0.5	2.7
Cereal rye	2.0	0.2	5.9	137.2	0.3	0.3
Wheat (cv. Diamondbird)	1.6	0.7	5.6	171.1	0.3	0.3
Annual ryegrass	0.3	1.2	4.4	157.2	0.4	1.6
_LSD (0.05)	0.2	1.7	n.s.	n.s.	n.s.	1.3

#### Table 4.5. Effect of winter crop stubble on SLN emergence and berry production.

SLN populations can be manipulated by use of ground cover, present either as an actively growing pasture or as stubble. The presence of 2-3 t/ha of active pasture can halve the number of stems present in mid to late summer. Stem emergence can be delayed by the presence of ground cover, shortening the growing period of SLN and potentially reducing the seed production levels during the season. Additionally, when stem emergence can be delayed, land managers may be presented with a more uniform weed population, allowing for more timely application of herbicides for controlling seed set.

The results obtained from field and glasshouse experiments conducted in this project suggest that both these weeds are poorly competitive. Maintenance of ground cover via crops, crop residue or pastures will assist in the suppression of emergence of these weeds, as well as plant vigour and seed production attained during the season.

This field site will be used as an established pasture site for experiments to be conducted under a new project (B.Wee.040). In March 2010, half of each plot was treated with 600 g a.i./ha tricloypr, 200 g a.i./ha picloram and 16 g a.i./ha aminopyralid in a spray volume of 100L/ha containing 1% v/v Uptake spray oil. Ongoing field research will be investigating the usefulness of applying herbicides for rootbank control within a perennial pasture situation. Observations at the time of spray application indicated a range of SLN density of 0.7-0.8 stems/m<sup>2</sup> within the digit grass and Strickland finger grass plots (pasture biomass ~10-11 t/ha), 2.4-2.7 stems/m<sup>2</sup> within the panic and Rhodes grass pastures (~6-7 t/ha), through to 5.3 stems/m<sup>2</sup> within the annual pasture treatment. Although lucern only had an average of 2.3 t/ha pasture biomass, It also suppressed the stem emergence (an average of 1.8 stems/m<sup>2</sup>), suggesting belowground root competition is also important in suppressing SLN stem emergence. Autumn biomass and composition data are next due to be collected in May, 2010.

#### 3.2 Herbicides

#### 3.2.1 Glasshouse experiments

Twelve weeks after treatment, there was no significant difference in within-season mortality as a result of time of application (vegetative or flowering), therefore data for control in-season has been combined for presentation (Table 4.6). The rate of picloram used resulted in 100% mortality, preventing any conclusions to be drawn regards adjuvants. Efficacy of 2 L/ha glyphosate was significantly improved (P<0.01) by the use of 1 L/100L of Uptake spray oil compared to the other adjuvants and the untreated control. Fluroxypyr efficacy appears to have been best with the use of Uptake spray oil, however the high mortality rates in all treatments resulted in no significant differences being detected.

Trea	tment	Application	Application % control		Final root	weight (g)
		Rate	within season	next season	vegetative *	flowering *
1	Control	-	0	3.75	4.53	8.26
2	Uptake	1L/100L	0	3.5	4.39	7.28
3	Hasten	1L/100L	4.4	3.25	4.54	6.26
4	oxyfluorfen	80mL/ha	12.5	2.25	3.98	8.4
5	Glyphosate (Roundup CT)	2L/ha	28.8	2.87	4.11	5.15
6	Glyphosate (Roundup CT) + Uptake	2L/ha + 1L/100L	58.7	2.37	1.79	6.59
7	Glyphosate (Roundup CT) + Hasten	2L/ha + 1L/100L	18.7	4.25	3.14	5.72
8	Glyphosate (Roundup CT) + oxyfluorfen	2L/ha + 80mL/ha	29.4	5.12	2.33	7.69
9	fluroxypyr	1L/ha	78.7	3.5	2.93	4.48
10	fluroxypyr + Uptake	1L/ha + 1L/100L	93.7	0.5	0.05	2.51
11	fluroxypyr + Hasten	1L/ha + 1L/100L	89.4	1.87	2.02	3.06
12	fluroxypyr + oxyfluorfen	1L/ha + 80mL/ha	87.7	1.62	0.25	9.39
13	picloram + 2,4-D amine	4L/ha	100	0	0	0
14	picloram + 2,4-D amine + Uptake	4L/ha + 1L/100L	100	0	0	0
15	picloram + 2,4-D amine + Hasten	4L/ha + 1L/100L	100	0	0	0.27
16	picloram + 2,4-D amine + oxyfluorfen	4L/ha + 80mL/ha	100	0	0	0
	l.s.d. (0.05)		19.49	1.95	3.	.28

#### Table 4.6 Effect of herbicide and adjuvant on SLN control within and between seasons.

\* - refers to stage of growth when herbicide applied in first season

Stem emergence between seasons was significantly influenced (P<0.01) by the previous herbicide treatment. No stems emerged from any of the picloram treatments. Significantly less stems emerged after Uptake had been used with fluroxpyr compared to fluroxypyr alone, while the use of either of the other adjuvants resulted in an intermediary reduction is stems. Similarly, efficacy of glyphosate was significantly improved with the addition of Uptake spray oil adjuvant compared to glyphosate alone or mixed with either oxyfluorfen or Hasten. This work focused on rootbank control, and did not report impact on seed production as the plants were maintained in a relatively insect free facility, resulting in very few flowers being pollinated and berries being formed.

When the plants were harvested six weeks after the first stems emerged, there was significant effect of previous treatment and application timing on root mass. Compared to the untreated controls, glyphosate or fluroxypyr applied at the vegetative stage resulted in significantly less root mass than when herbicides were applied at the flowering stage in the previous season. Very few root fragments were recovered from any of the picloram treatments, suggesting that the rate of herbicide active ingredient applied was sufficient to cause complete root mortality, leading to near complete decay over winter. Across both application timings, glyphosate treated plants produced less root mass than the control plants, although there was no significant difference caused by the adjuvants. Significantly less root mass was present in treatments where Uptake spray oil had been used with fluroxypyr compared to fluroxypyr alone or mixed with oxyfluorfen.

These results indicate that within season control of SLN aerial growth is influenced more by herbicide and adjuvant choice than time of application, with herbicide efficacy generally improved by the use of Uptake crop oil.

However, the level of aerial growth control achieved within season may not correspond to regrowth the following season. When herbicides are applied at the vegetative stage compared to the flowering stage, there is a greater impact on the SLN root system and subsequent vigour the next season. This is in line with observations made by Greenfield (2003), who found that radio-labelled glyphosate was more readily transported to the roots when SLN is in a vegetative state rather than in a reproductive state.

Horsenettle (*S. carolinense*) is a related solanaceous weed which is a major pest in the United States of America due to its extensive root system (Whaley and VanGessel 2002a). Herbicides are typically applied at flowering, but a single application does not provide adequate long term control. The use of glyphosate applied in autumn can reduce horsenettle emergence the following season by 90% (Whaley and Vangessel 2002a), similar to the findings for SLN and PGC in this project. This would suggest that the implementation of an autumn herbicide regime would be a management tactic that would be applied to a range of perennial weeds. However, it would be critical that the autumn herbicide application occurs before senescence to achieve the greatest control (Whaley and Vangessel 2002b).

#### 3.2.2 Field experiments

SLN densities at the commencement of the 2008/09 summer at the Leeton site are significantly different (P<0.01) as a result of two seasons of herbicide experiments (Table 4.7). Amitrole, glyphosate+imazapyr, atrazine + paraquat/diquat, 2,4-D amine alone or applied late after a glyphosate treatment and glyphosate+oxyflourfen+ally have not consistently decreased SLN stem density compared to the untreated control. Two applications of either picloram or triclopyr+picloram+aminopyralid, or a single application of fluroxypyr+aminopyralid have provided the greatest decrease in SLN density.

Results from the Culcairn field site are less conclusive (P=0.07), although some similar trends are evident. Single application of 2,4-D amine, amitrole or atrazine + paraquat/diquat also failed to consistently reduce SLN density compared to the untreated control. Two applications of picloram or triclopyr+picloram+aminopyralid again were among the best treatments. Fluroxypyr, either alone or mixed with 2,4-D amine, has reduced SLN density compared to the untreated control. Annual ground cover (estimated 2-3t/ha across the site) accumulated at the Culcairn site prior to winter annual weed control which may have contributed to the lower stem emergence observed, whereas the Leeton site had minimal ground cover between seasons.

Atrazine alone provided contrasting results between the two sites. It is speculated that differences in soil type and general ground cover contributed to the observed differences. Similar levels of control as observed at Culcairn have also been noted at a District Agronomist's demonstration site near Ungarie. However, as the level of control achievable is not consistent it would difficult to recommend this treatment.

Timing of application is important, with consistently better control being observed when a second herbicide is application is made late in the season (Plate 4.1). The improved control may be as result of timing of application in relation to plant phenology, or as result of double the amount of active ingredient being applied. Further research is required to resolve this issue.

Seed set control as a result of herbicide application is more effective when herbicides are applied at the flowering growth stage (Table 4.8). At the flowering stage, the majority of flowers were aborted after the herbicide application, whereas when herbicides were applied at the early berry stage viable seed production was approximately halved by fluroxypyr, glyphosate and 2,4-D amine and reduced by 74-85% when picloram based herbicides were used.

However, the two herbicides that provided the best control of viable seed production at the early berry stage are also the most expensive to apply (\$95-100/ha) than the other treatments such as 2,4\_D amine (\$15/ha) or glyphosate (\$20/ha). The less expensive herbicides (2,4-D, glyphosate and fluroxypyr) achieved excellent seedset control (94-99%) when applied at the flowering stage, indicating that an economically sound management plan for SLN should focus on applying the less expensive herbicides at flowering, followed by late season use of picloram herbicides to target the rootbank.

Table 4.7 S	<i>ilverleaf nightshade</i> sten	n density (stems/m <sup>2</sup> )	) following two s	seasons of he	rbicide
application.					

			Leeton			Culcairn	
	Active ingredients	Dec-06	Dec-08	% control	Dec-06	Dec-08	% control
1	Untreated control	12.83	15.33	(-19%)	8.3	6	(28%)
2	glyphosate	12	7.5	(38%)	14.3	2	(86%)
3	Glyphosate, glyphosate (late) *	11.83	9.17	(22%)	10.2	3	(71%)
4	glyphosate, 2,4-D amine (late) *	16.33	12	(27%)	15.2	2.5	(84%)
5	2,4-D amine	11.83	13.5	(-14%)	6.5	5.33	(18%)
6	2,4-D amine + glyphosate	12.17	5	(59%)	6.5	7.33	(-13%)
7	dicamba	11.17	7.83	(30%)	4.3	3.83	(11%)
8	Fluroxypyr	9.17	7.5	(18%)	4.3	1.67	(61%)
9	Fluroxypyr+2,4-D amine	7.67	7.33	(4%)	4	2.5	(38%)
10	Aminopyralid+fluroxypyr	4.17	4.5	(-8%)	8.8	2.67	(70%)
11	picloram + 2,4-D #	9.5	4.83	(49%)	6.7	0.83	(88%)
12	triclopyr + picloram + aminopyralid #	9	3.83	(57%)	6.3	1.33	(79%)
13	amitrole	9.33	11.17	(-20%)	9	8	(11%)
14	atrazine	3.67	8.5	(-132%)	4.5	0.83	(82%)
15	atrazine+paraquat/diquat	7.5	10.67	(-42%)	8.2	6.17	(25%)
16	glyphosate+oxyfluorfen	7.17	8.83	(-23%)	10	4.67	(53%)
17	glyphosate+mesulfuron methyl	6.83	8.5	(-24%)	5	4	(20%)
18	glyphosate+mesulfuron methyl+oxyfluorfen	8.83	14	(-59%)	7	3.67	(48%)
19	glyphosate+imazapyr	9.83	13	(-32%)	5	2.5	(50%)
20	picloram + 2,4-D+mesulfuron methyl	14.17	7.83	(45%)	3.7	1.17	(68%)
	l.s.d.	n.s.	5.971		n.s.	4.665	

\* - two herbicide applications per season; # - two applications in 2007/08 only

When herbicides are applied at flowering, all flowers were aborted when picloram based products were used, with some berries being formed for the other three herbicides used (Table 4.8). Where berries were formed, the viability of the seed was significantly reduced compared to the untreated control, resulting in greater than 94% reduction in viable seed production when SLN is sprayed at flowering. Herbicides were less effective at reducing seed numbers per berry when applied once berries had formed. The viability of the seeds in berries was by all herbicides, with the total viable seed production halved by the use of 2,4-D amine or glyphosate, or reduced by three quarters when picloram based herbicides were used.

Treatment	Seeds per berry	Viability (%)	Viable Seeds
Flowering			
1 - control	57.2	77.3	44.3
5 - 2,4-D amine	9.3	5.0	0.5
3 - glyphosate	16.3	15.7	2.6
8 - fluroxypyr	2.3	16.7	0.4
11 - picloram + 2,4-D amine	0.0	0.0	0.0
12 -triclopyr + picloram + aminopyralid	0.0	0.0	0.0
LSD (0.05)	29.8	34.6	26.8
Early Berry			
1 - control	84.2	99.0	83.4
5 - 2,4-D amine	55.1	75.6	41.7
3 - glyphosate	63.8	66.0	42.1
8 - fluroxypyr	73.5	65.7	48.3
11 - picloram + 2,4-D amine	64.5	33.7	21.7
12 - triclopyr + picloram + aminopyralid	34.0	36.0	12.2
LSD (0.05)	n.s.	n.s.	n.s.

Table 4.8.	Effect of herbicides or	n SLN seed viabilitv	when applied at two	arowth stages
				g. e n n e tagee

PGC densities at the end of the 2008 summer at the Tocumwal site are significantly different (P<0.05) as a result of one season of herbicide treatments (Table 4.9). Similar to SLN, the amitrole, glyphosate+imazapyr, 2,4-D amine alone or applied late after a glyphosate treatment and glyphosate + goal + ally treatments failed to decrease population density compared to the untreated control. Picloram, triclopyr+picloram+aminopyralid and glyphosate (alone or mixed individually with 2,4-D amine, metsulfuron methyl or oxyflourfen) provided significantly better control. triclopyr+picloram+aminopyralid and picloram were the most effective treatments within season. Data collected at the commencement of the 2008/09 season was inconclusive (P>0.05), mainly due to the low level of plants emerged. Three of the four treatments where herbicides were applied at the end of the season (picloram, triclopyr+picloram+aminopyralid and glyphosate) resulted in the greatest decrease in PGC emergence. The comparison of adjuvants was inconclusive due to the low level of emergence.

Several important outcomes have been identified relating to use of herbicides to manage SLN and PGC. While herbicides have been used to control these weeds in the past (Donaldson 1984, Cuthbertson *et al.* 1976, Lemerle 1982), long term control or eradication has been difficult to achieve in the field. This research identifies that separate applications are needed to effectively target seed set and control of the rootbank. Seed set control is best undertaken when the stems are flowering, whereas best control of the rootbank is achieved by applying herbicide in early autumn. The addition of suitable adjuvants (eg., Uptake or Hasten crop oils) can improve herbicide efficacy. A range of herbicides are useful for controlling seed set, while picloram based products are most effective for rootbank control. Autumn application of glyphosate can provide similar levels of rootbank control for PGC.

Adoption of strategic herbicide application timings has the potential to deplete the rootbank, enabling herbicides to be used more efficiently. However, the picloram based products are typically five times more expensive to use than traditional herbicide choices (\$95-100 and \$15-20, respectively), even at the rates examined in these field experiments.

Active ingredients		Mar-07	Apr-08
1 Untreated control		23.5	6.83
2 glyphosate		22.7	2.67
3 Glyphosate, glyphosate (late) *		17.7	2.17
4 glyphosate, 2,4-D amine (late) *		21.3	1.67
5 2,4-D amine		18.3	4.5
6 2,4-D amine + glyphosate		20.7	1.17
7 glyphosate+mesulfuron methyl (S	sprinta)	25.8	2.33
8 fluroxypyr		9.2	4
9 picloram + 2,4-D amine+mesulfu	on methyl (Hasten)	33.7	0.83
10 aminopyralid+fluroxypyr		30.5	3.5
11 picloram + 2,4-D #		15.7	1.17
12 triclopyr + picloram + aminopyrali	d #	17.5	0.17
13 amitrole		11	4.17
14 glyphosate+mesulfuron methyl (H	lasten)	14	4
15 picloram + 2,4-D amine+mesulfu	on methyl (Sprinta)	24.5	3.67
16 Glyphosate + oxyfluorfen		18	2
17 glyphosate+mesulfuron methyl (L	lptake)	9	5.17
18 glyphosate+mesulfuron methyl+o	xyfluorfen	27.5	7.33
19 glyphosate+imazapyr		18	5.33
20 picloram + 2,4-D amine+mesulfu	on methyl (Uptake)	19.7	1.5
lo d			4 106
I.S.U.		п.s.	4.120

#### Table 4.9. Prairie ground cherry density following one season of herbicide application.

\* - two herbicide applications per season; # - two applications in 2007/08 only

#### Innovative Management of SLN and PGC



Plate 4.1. Herbicide control of s*ilverleaf nightshade* regrowth the following season (a) untreated control, (b) two applications of 3 L/ha Grazon Extra  $^{\circ}$  in one season.

#### 3.2.3 Herbicide translocation

The first translocation experiment was to investigate three factors (age of roots, plant growth stages and adjuvants) on herbicide absorption and translocation. For the plants regenerated from the one-year old roots (root fragments buried in October 2008), addition of Uptake oil only slightly increased absorption by 3% or 6% when <sup>14</sup>C-fluroxypyr was applied at the early vegetative (EARLY) or at the flowering/early berry (LATE) stage in April 2009, respectively. However, the Uptake oil increased the total translocation by 356% and 49% when <sup>14</sup>C-fluroxypyr was applied at EARLY and LATE stages, respectively (Table 4.10).

For the plants regenerated from the two-year old roots (root fragments buried in October 2007 to establish a larger root system, a mimic to field situations), addition of Uptake also had only slight increase in absorption by 11% and 3.5% when <sup>14</sup>C-fluroxypyr was applied at EARLY and LATE stages, respectively. However, the Uptake decreased the total translocation by 12% and 36% when <sup>14</sup>C-fluroxypyr was applied at EARLY and LATE stages, respectively. This negative impact of Uptake on the translocation needs to be further confirmed using SLN plants with a large root system.

SLN growth stages had no effects on absorption, however, fluroxypyr applied LATE resulted in more than three times increases in total translocation within the plants regenerated either from one- or two-year old roots, highlighting the importance in application timing in relation to SLN growth stages.

On average, about 63% of the applied fluroxypyr was in the treated leaf (not translocated), and only 12%, 6% and 2% translocated to untreated leaves, stems and roots. The low levels of translocation into the roots (0.2-3.7%) represent a significant challenge for effective control of this perennial weed.

Growth Stage	Uptake oils	Absorption	Translocation	Treated- leaf	Other Leaves	Stem	Root
Plants regenerated from 1-year old roots							
Early vegetative	+	85.2	19.4	65.7	11.9	6.3	1.2
Flower/early berry	+	87.9	49.6	38.3	32.4	15.3	1.9
Early vegetative	-	82.6	4.3	78.3	2.8	1.2	0.2
Flower/early berry	-	82.9	33.3	49.6	26.4	5.4	1.4
Plants regenerated f	rom 2-yea	ar old roots					
Early vegetative	+	86.4	5.9	80.5	3.5	0.4	1.9
Flower/early berry	+	81.1	15.4	65.7	2.9	10.6	2.0
Early vegetative	-	77.6	6.7	70.9	2.3	0.7	3.6
Flower/early berry	-	78.3	24.2	54.1	12.0	8.4	3.7

## Table 4.10. Impact of growth stage and adjuvants on herbicide translocation (% of total herbicide applied).

The second translocation experiment was to investigate the impact of upper and lower leaf surface application on translocation and uptake using pot plants regenerated from 2-year old roots. Herbicide application via upper surface had higher 8.7% absorption than the lower surface application, while the lower surface application had a 103% increase in translocation (Table 4.11). The results coincide with anecdotal comments that herbicide application to the lower leaf surface appears more effective than application to the upper leaf surface. Our results showed that trichomes were denser on the lower leaf surface than on the upper surface (Plate 4.2). The significance of the trichome in relative to herbicide uptake and translocation is yet to be determined.

	neide applied	<u>/</u>				
	Absorption	Translocation	Treated leaf	Other leaves	Stem	Root
Upper surface	86.4	5.9	80.5	3.5	0.4	1.9
Lower surface	79.5	11.9	67.5	8.4	1.1	2.5

Table 4.11. Impact of upper and lower surface on herbicide transloc	ation
(% of total herbicide applied).	



Lower leaf surface

Upper leaf surface

Plate 4.2. Differences in trichome density between the lower and upper leaf surfaces (Photos: Dr G Burrows).

The third translocation experiment was to investigate the impact of preconditioning of herbicide stress on translocation using pot plants regenerated from 2-year old roots. The three treatments had similar levels of absorption (about 88%), while the "Whole plant pre-spray leaf with fluroxypyr" treatment had the lowest herbicide translocation into the other leaves, stem and roots. The results suggest that herbicide preconditioning on SLN plants could have negative impact on herbicide translocation.

	Absorption	Translocation	Treated leaf	Other leaves	Stem	Root
No pre-sprayed control Plant pre-sprayed with fluroxypyr and left one leaf covered by aluminium for C14	87.2	29.5	57.8	13.7	11.4	4.3
spotting	89.0	28.4	60.7	13.8	11.0	3.6
Whole plant pre-spray with fluroxypyr	86.9	17.9	69.0	7.8	7.6	2.6

## Table 4.12. Impact of preconditioning of herbicide stress on translocation (% of total herbicide applied).

#### 3.3 Allelopathy

#### 3.3.1 Laboratory experiments

#### Bioassay with the Steam Extract.

During the distillable process, large quantities of steam extracts were collected (about 900 ml per 400-500 g of leaf materials). The bioactivities of steam extracts were tested using 0, 25, 50, 75 and 100%. Results showed that eucalyptus species differed in their bioactivities on germination, with *E. Spatulata* the most phytotoxic and the *E. Melliodura* (as a control species) the least phytotoxic (Fig 4.3). In addition, steam extracts were inhibitory to SLN germination than PGC.

#### Bioassay with the Essential Oils:

The bioactivities of essential oils were tested using 0, 10, 30, 90, 270  $\mu$ /petri dish. Results showed that essential oils significantly inhibited the germination of SLN and PGC, depending on the concentrations. PGC was more sensitive than SLN when exposed to essential oils (Fig 4.4). At the concentration of 10  $\mu$ /petri dish, PGC germination was inhibited by 64-95%.

#### Bioassay with the Soluble Essential Oils:

Soluble essential oils were produced in large quantifies during the distillable process. About 100 ml was collected per 400-500 g of leaf materials. The bioactivities of soluble essential oils were tested using 0, 25, 50, 75 and 100%. Results showed that both SLN and PGC had similar responses to the soluble essential oils (Fig 4.5). *E. Spatulata* was the most phytotoxic, inhibiting the germination of SLN by 76% and PGC by 83% at the concentration of 25%.

Extracts from *E. spathulata* was consistently more phytotoxic to SLN and PGC than extracts from the other eucalypt species examined. The soluble oil fraction from the distillation provided the largest difference between eucalypt species, suggesting that the compounds of interest occur in the highest concentrations in this fraction. Further work needs to be completed to analyse the chemical composition of the various fractions to identify the compounds that are linked to the phytotoxic effect in order to develop a novel herbicide for weed control.



#### Figure 4.3. Bioactivity of steam extracts on the germination of SLN and PGC.



#### Figure 4.4. Bioactivity of essential oils on the germination of SLN and PGC.





#### Figure 4.5. Bioactivity of soluble essential oils on the germination of SLN and PGC.



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Concentrations of essential oils (%)

Glasshouse bioassay: Spraying Essential Oils on SLN/PGC seedlings:

The bioactivities of essential oils were tested on seedling growth of SLN and PGC using 0, 5, 10, 20 and 40%. Tween 80 (0.05%) was used as an inert medium to dilute the essential oils for the required concentrations. Each seedling was sprayed with a volume of 50 µL. Results showed that essential oils were phytotoxic to the seedling growth of SLN and PGC (Fig 4.6). The four suspected eucalyptus species were more inhibitory than the commercially purchased essential oils. Essential oils at higher concentrations can cause the complete seedling death (Fig 4.7).

Silverleaf Nightshade Prairie Ground Cherry 100 100 – E. Salubris E. Spatulata Growth inhibition (%) Growth inhibition (%) E. Brockwayii 80 80 E. Dundasii Commercial oil 60 60 E. Salubris 40 40 E. Spatulata E. Brockwayii 20 20 E. Dundasii Commercial oil 0 0 0 10 20 40 0 5 10 20 40 Concentrations of essential oils (%)

Figure 4.6. Bioactivity of essential oils on seedling growth of SLN and PGC.

### Figure 4.7. Bioactivity of essential oils on seedling growth of PGC.

PGC seedlings prior to the application of essential oils



PGC seedlings after the application of essential oils



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Chemical analysis of essential oils:

The strong inhibitory effects of eucalyptus allelopathy on the germination and seedling growth of SLN and PGC have warranted the further investigation of bioactive chemicals in essential oils. The gas chromatography and mass spectrometry (GC/MS) at the Environmental and Analytical Laboratories of Charles Sturt University was used to determine the chemical profiles of essential oils. A typical chemical profile of essential oils is illustrated in Fig 4.8. Different eucalyptus species differed significantly in the yield and composition of essential oils. The GC/MS data is currently being analysed. Overall, more than 40 compounds were identified in essential oils, with predominant compounds being  $\alpha$ -pinene, 1,8-cineole, trans-pinocarveol, limonene and allo-aromadendrene. The essential oils contained 22-47%  $\alpha$ -pinene and 22-63% 1,8-cineole.

### Figure 4.8. Characteristic chromatogram of essential oils by GC/MS analysis.



Phytotoxic specificity screening of E. spathulata extracts:

The most phytotoxic extracts were derived from *E. spathulata*, therefore data from this species is presented here.

The essential oil from *E. spathulata* showed strong germination inhibition on all weeds tested (Fig 4.9), with four species fully inhibited at the lowest concentration rate. Annual ryegrass and wild radish germination was inhibited by 75-79% at the lowest concentration, but both were fully inhibited at the highest concentration.

Germination of all weeds examined was significantly reduced by *E. spathulata* soluble oil extract (Fig 4.10). Brome grass was fully inhibited at the lowest concentration, while the germination of all weeds was fully inhibited at a concentration of 75%. Annual ryegrass and barnyard grass were less affected by the lower concentrations than were the other weed species examined.

The steam extract of *E. spathulata* inhibited germination (Fig 4.11), however the response was less than that observed for the essential oil and the soluble oil distillation fractions. Germination was inhibited by more than 60% for all weed species when treated with 100% steam extract.

The three distillation fractions obtained from *E. spathulata* leaves exhibit phytotoxic activity on a range of weed species, although the level of inhibition differed between the distillation fractions. The active compounds associated with the phytotoxic effects are yet to be isolated, however these resuklts indiucate there is potential to develop a non-selective herbicide based on active ingredients contained within *E. spathulata* essential oils or soluble oils.







Figure 4.10. Bioactivity of *E. spathulata* soluble oil extract on weed seed germination.

Figure 4.11. Bioactivity of *E. spathulata* steam extract on weed seed germination.



Key messages: The phytotoxic effects and the bioactive chemicals of these four *Eucalyptus* species identified in this study may explain the SLN suppression observed in the field within the *Eucalyptus* dripline. Further research is needed to isolate and identify key active compounds for the development of any potential new bioherbicides. 3.3.2 <u>s</u> Field experiments

*E. brockwayi* and *E. dundasii* are the least mature seedlings but a trend is already evident for less SLN stems to be present as tree height increases for both species (Fig 4.12). Underneath the more mature *E. spathulata* and *E. salubris* trees, SLN stem numbers has been greatly decreased under the taller trees. Seedlings of *E. melliodora* have been planted at the site to allow direct comparison of SLN stem numbers in the field between the four eucalypt species of interest and a common eucalypt species not known for providing SLN control to provide field evidence of eucalyptus species impact.





### 3.4 Biology and ecology

### 3.4.1 Pathogens (Biocontrol)

A decision was made to drop this research component. Any future research focus should be directed toward isolation of pathogens from SLN roots of suspected SLN plants. The isolated root pathogens might have greater chance of surviving and attacking the extensive root systems of SLN when compared to the leaf pathogens. In the pilot study, screening of 47 isolates yielded no promising results. An alternative to this method is to prepare fungal spore solution and spray it onto the leaves. However, these in-depth studies will require much more resources than originally planned and would need to be funded as a separate project.

Several studies have evaluated potential insect and nematode biocontrol agents for SLN (Goeden 1971, Northam and Orr 1982, Wapshere 1988, Hoffmann et al. 1998), however no agents have been identified to date that would be suitable for release in southern Australia where the weeds occur (Kwong 2006). Potential exists to conduct further research into Lema bilineata, which has been observed feeding on PGC to determine if there is scope for this naturally occurring beetle to be cultivated as a biocontrol agent for control of PGC.

### 3.4.2 Seed production and viability

SLN plants tended to produce fewer berries and fewer seeds per berry across the sites surveyed (Table 4.13). Assuming a light SLN or PGC infestation of 1 plant/m<sup>2</sup>, this would equate to seed production levels of 23.8 million and 45.9 million seeds per hectare, respectively.

### Table 4.13. Berry and seed production levels for SLN and PGC.

	SLN	PGC
Berries per plant	59.4 (± 13.4)	75.8 (± 13.3)
Berry diameter (mm)	10.0 (± 0.5)	12.5 (± 0.6)
Berry weight (g)	0.4 (± 0.1)	0.6 (± 0.4)
Seeds per berry	40.1 (± 8.1)	60.6 (± 10.1)
Average seeds per plant	2381	4591

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### 3.4.3 Seedbank dynamics

There were no significant differences between sites (P>0.05), therefore data were combined across sites for analysis. After 24 months, the number of bare SLN seeds still viable had decreased significantly (P<0.01) compared to seed stored in the laboratory (Fig 4.13). Seed left of the soil surface was less viable than seed that was buried at any depth in the soil profile, while seed buried in an intact seed pod retained a higher level of viability (Fig 4.14) at all burial depths.

### Figure 4.13. Effect of burial depth and duration on survival of bare silverleaf nightshade seed.



Figure 4.14. Effect of burial depth and duration on survival of *silverleaf nightshade* seed buried in intact berries.



Seed recovered from buried pods was more viable than seed buried alone, suggesting that the pods afford some protection whilst intact. Seed pods appear more likely to remain intact on the soil

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surface, or at shallow depths, although a total of only three pods that appeared nearly intact were recovered after 24 months. This suggests that the benefit or protection derived from the seed pod may not persist indefinitely, but rather delay the seed decay process in the order of 12-24 months. Nearly all seed recovered from seed pods readily germinated in the germination assay, suggesting that the pod no longer afforded protection.

After 24 months in the field at any depth in the soil profile, the number of bare PGC seeds still viable had decreased significantly (P<0.01) compared to seed stored in the laboratory (Fig 4.15). Seed that had been buried in an intact seed pod had the lowest level of viability (Fig 4.16) when left on the soil surface and was significantly lower (P<0.01) than seed from pods stored in the laboratory. Viability of seed from pods buried at all depths in the soil profile were comparable, and were all slightly lower than for seed stored in pods in the laboratory.

The dried berry appears to afford PGC seed protection when buried, and seed viability is less affected at depth, irrespective of whether the seed was in an intact berry or not. Seed persists for a shorter period of time if the berries and seeds are left of the soil surface, presumably as they are subjected to greater fluctuations in temperature and moisture.

These results indicate that cultivation could contribute to the enforced burial of seeds into the soil, resulting in prolonged persistence of SLN and PGC. Berries present on stems could be grazed by livestock or adhere to the hooves or coat of livestock, providing opportunity for seed to be dispersed. SLN seed ingested by livestock can remain in the intestine and be viable if excreted up to 14 days after ingestion (Heap and Honan 1993), providing opportunity for seed to be dispersed and potentially incorporated into the soil seedbank in a previously weed free field.

Linear regression suggests that SLN seedbank of bare seed could potentially be depleted within four years through natural processes, while exponential decay of the seedbank could take ten to twelve years (Table 4.14). However, seed buried in intact pods appear to derive additional protection for the first one or two seasons, potentially delaying the decay process of the seedbank by that length of time. The PGC seedbank at shallow depths couple also be depleted within four years, but similar to SLN the dried PGC fruit may increase the survival of PGC seed by several years.

Further data needs to be collected to allow a better understanding of the nature of the decay curve to refine estimates on seedbank survival. Seed was exhumed on 30 April, 2010 after burial for 36 months. Germination and viability assays will be conducted in May, 2010 to add to existing knowledge on seedbank dynamics. The results will be reported in the new project.

	Depth	а	b	r <sup>2</sup>	90% decline	99% decline
Linear regre	ession					
SLN seed	0	0.86	-0.02	0.85	2.94	3.28
	2.5	0.80	-0.02	0.67	2.85	3.21
	5	0.88	-0.02	0.88	3.28	3.66
	10	0.99	-0.02	0.90	3.09	3.40
PGC seed	0	0.89	-0.03	0.90	1.89	2.11
	2.5	0.77	-0.03	0.60	2.20	2.49
	5	0.87	-0.02	0.77	3.03	3.39
	10	0.97	-0.02	0.99	3.25	3.58
Exponentia	l rearession	1				
, SLN seed	õ	0.87	-0.03	0.92	5.21	10.75
	2.5	0.78	-0.03	0.76	5.12	10.86
	5	0.89	-0.03	0.94	5.98	12.29
	10	1.05	-0.04	0.87	4.93	9.75
PGC seed	0	0.99	-0.06	0.99	3.03	6.07
	2.5	0.71	-0.05	0.64	3.52	7.65
	5	0.86	-0.03	0.86	5.60	11.60
	10	1.00	-0.03	0.99	5.76	11.52

Table 4.14. Time in years for selected levels of decline of SLN and PGC seedbanks using linear and exponential regressions.

Linear regression determined using the equation y = a.x + b

Exponential regression determined using the equation  $y = a e^{bx}$ 



Figure 4.15. Effect of burial depth and duration on survival of bare prairie ground cherry seed.

Figure 4.16. Effect of burial depth and duration on survival of prairie ground cherry seed buried in intact berries.



### 3.4.4 Germination requirements

DOC

Germination of both species was reduced under constant temperature regimes, with the best germination at 30 C (Table 4.15). PGC germinated more readily than SLN under constant temperatures. Germination levels of both species increased when exposed to diurnal temperature fluctuations, with germination best when the minimum temperature was 15 C.

PGC is able to germinate under drier conditions than SLN, with germination levels under fluctuating temperature regimes not significantly decreasing until the osmotic potential exceeded -0.48MPa. SLN germination was inhibited when osmotic potential exceeded -0.24 MPa, suggesting that SLN requires soils to be at or near field capacity before germination is likely to occur.

PGC is more tolerant of the presence of salt than SLN, with germination significantly declining once the sodium chloride concentration reached 240 mM (Table 4.16). SLN germination levels were more than halved once sodium chloride levels exceeded 20 mM.

PGC germination does not significantly alter in response to the pH level of the environment (Table 4.17), whereas SLN germination increases with increasing pH. Significant levels of SLN germination occurs even in an acidic environment, therefore while SLN may be less suited to acidic soil conditions, it has the potential to invade all soil types.

Potential	Tempera	ature (°C)					
(MPa)	10	20	30	40	10/25	15/25	15/30
0	0%	0%	3%	0%	19%	87%	41%
-0.03	0%	1%	5%	1%	15%	77%	20%
-0.06	0%	0%	1%	3%	22%	59%	39%
-0.12	0%	0%	1%	1%	13%	49%	24%
-0.24	0%	0%	2%	0%	7%	52%	9%
-0.48	0%	0%	0%	0%	1%	17%	3%
-0.96	0%	0%	0%	0%	0%	0%	1%

Table 4.15.	Influence of temperature and osmotic stress on the germination of SLN and PGC.
SLN	

FGC							
Potential	Tempera	ature (°C)					
(MPa)	10	20	30	40	10/25	15/25	15/30
0	0%	12%	52%	10%	63%	72%	81%
-0.03	0%	11%	41%	23%	75%	81%	83%
-0.06	0%	8%	39%	16%	77%	81%	77%
-0.12	0%	2%	46%	9%	70%	76%	78%
-0.24	0%	1%	30%	22%	71%	77%	77%
-0.48	0%	0%	13%	13%	53%	45%	72%
-0.96	0%	0%	0%	1%	3%	3%	8%

Salt (mM)	SLN germination (%)	PGC germination (%)
0	69%	85%
10	46%	86%
20	15%	81%
40	32%	75%
80	13%	76%
160	2%	63%
240	0%	9%
320	0%	0%

Table 4.16.	Influence of sodium chloride (NaCl) concentration on germination of SLN a	and
PGC.		

### Table 4.17. Influence of pH on germination of SLN and PGC.

Acidity (pH)	SLN germination (%)	PGC germination (%)
4	61%	79%
5	50%	76%
6	57%	75%
7	76%	79%
8	76%	81%
9	83%	77%
10	81%	78%

### Table 4.18. Influence of photoperiod and temperature on germination of SLN and PGC.

	Temperature (°C)	SLN germination (%)	PGC germination (%)
Light	10/25	19%	63%
Dark	10/25	23%	43%
Light	15/30	41%	81%
Dark	15/30	27%	70%

Both SLN and PGC exhibited a higher level of germination when there was a photoperiod included in the diurnal cycle, except for SLN at the lower temperature regime where light did not have a positive effect on germination level (Table 4.18). These results suggest that exposure to light through the impact of cultivation may increase the germination rates of these weeds, highlighting the advantage of minimising tillage practices in spring and autumn when there are suitable temperature and soil moisture conditions to reduce germination of both species.

These two weed species are capable of germinating across arrange of soil saline and acid conditions, enabling them to colonise a wide range of new areas. PGC is capable of germinating on lower soil moisture condition than SLN, however both species exhibit the ability to germinate in response to a range of soil moisture availability combined with the presence of a diurnal temperature fluctuation.

These two weeds require warm, moist soils for seed germination to be initiated. Under ideal conditions can take 1-2 weeks for the radicle to emerge from the seed, suggesting that the favourable conditions may need to persist for some time for a seedling to successfully be formed

and emerge above ground. Both weeds are not affected by low salinity levels, while PGC is more tolerant of medium saline conditions. As germination of neither weed is influenced by pH, these weeds are capable of establishing on a range of soil types, as long as there is good soil moisture and temperature conditions at the time of establishment.

### 3.4.5 Asexual reproduction

Burial depth had a significant effect on the number of emerged shoots (P<0.05). Where emergence occurred, segment lengths less than 10 cm produced the most number of shoots per segment when buried at 5 cm depth (Table 4.19). Root segments 10 cm long produced more than one shoot each, with the most shoots produced from 2.5 cm burial depth.

## Table 4.19. The effect of root segment length and burial depth on number of shoots produced per root segment length.

Root length	Burial depth (c	m)		
(cm)	2.5	5	10	
1	-	0.4 <sup>a,b</sup>	-	
2.5	0.5 <sup>a,b</sup>	1.1 <sup>b,c</sup>	0.2 <sup>a</sup>	
5	0.5 <sup>a,b</sup>	1.0 <sup>a,b,c</sup>	0.3 <sup>a,b</sup>	
10	2.8 <sup>e</sup>	2.2 <sup>d,e</sup>	1.7 <sup>c,d</sup>	
LSD (P<0.05)		0.8		

\* Different letters represent significant difference according to Fisher's protected LSD (P<0.05)

Root segment mortality was significantly affected by segment length (P<0.05). No root mortality was observed for 10 cm segments at any depth (Table 4.20). Root mortality significantly increased with decreasing segment length, with 80% mortality observed for 1 cm root segments (P<0.05). Burial depth influenced mortality of 2.5 and 5 cm root segments, with higher mortality observed at 10 cm depth.

Root length	Burial depth (c	m)	10	
(cm)	2.5	5		
1	72 a,b	75 a,b	80 a	
2.5	46 b,c	46 b,c	67 a,b	
5	8 d	33 c,d	58 a,b,c	
10	0 d	0 d	0 d	
LSD (P<0.05)		33		

Table 4.20. T	he effect of ro	ot segment	t length and b	urial depth on	percent mortality.
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\* Different letters represent significant difference according to Fisher's protected LSD (P<0.05)

*Silverleaf nightshade* roots of 10 cm in length readily produce one or more shoots, irrespective of burial depth. The optimal depth for regeneration of SLN from shorter root segments was 5 cm, with segments as short as 1 cm capable of producing shoots. Shallower burial approximately halved the number of shoots produced. Similarly, Boyd and Murray (1982) reported that 1-3 shoots are produced from 5 and 10 cm long root segments respectively when buried at 8 cm depth in the field, with significantly more shoots produced from 15 and 20 cm long root segments.

One centimetre segments did not produce any shoots at shallow depths, possibly due to the more extreme fluctuations in temperature and moisture levels. Burial of 1 cm length to 10 cm also prevented regeneration. Richardson and McKenzie (1981) reported that 0.5 and 1.0 cm root lengths produce 0 and 0.2 shoots per segment respectively when buried at 2 cm in pots in a glasshouse.

Asexual reproduction has been recorded as more important than sexual reproduction in similar perennial weeds such as polymeria take-all (*Polymeria longifolia* Lindley) (Johnson and Sindel 2005) and smooth ground cherry (*Physalis virginiana*) (Abdullahi and Cavers 1997). Seed and root segments are dispersal mechanisms for SLN (Wapshere 1988, Richardson and McKenzie 1981) and PGC (Faulkner and Young 2006), but the relative importance is not fully understood. Seed can be dispersed by livestock by attachment to fibre or via ingestion, mechanically by attachment to vehicles or machinery and naturally via wind or water movement. Root segments may be dispersed as a contaminant of agricultural produce or attached to machinery (Gmira *et al.* 1998, Wapshere 1988). Successful long-term control of SLN, PGC and other perennial weeds, will require the development of integrated management packages that provide focus on control of the rootbank as well as the seedbank.

Key message: Similar to the previously reported data for PGC, SLN is also capable of regenerating from root fragments as short as 1 cm. In terms of management of SLN or PGC infestations, it is critical that cultivation is avoided as much as possible to reduce fragmentation of the rootbank. Where implements are used that impact on the rootbank, machinery should be thoroughly inspected prior to being moving away from an area where either of these weeds occur.

### 3.5 Project evaluation

Modelling was undertaken to determine the long term economic benefits for adopting strategic herbicide tactics. A major constraint in developing the model was the absence of accurate and comprehensive data on the impact of various SLN densities on production within individual production systems. Estimates were derived based on published case studies and feedback from landholders.

Initial modelling has indicated that SLN stems decline very slowly under the current management practice of reliance on one or two mid-season applications of glyphosate (Figure 4.2), and that the population can increase over time if no control measures are taken. The proposed best-best herbicide strategy of glyphosate or 2,4-D amine at flowering to limit seed set followed by picloram in autumn for rootbank control could potentially deplete the rootbank during the first couple of seasons. Although the initial cost with the best-bet was higher and associated profit was initially lower than the do-nothing or current practice, over the projected 10-year period this best-bet regime has the highest returns. On average per year based on **a dense SLN population**, assuming a gross margin of 137\$/ha for a Prime Lamb enterprise in North East VIC, the 'Best-bet' has a direct control cost (herbicide, lost production, labour and machinery) of \$31ha and generates a net profit of \$113/ha, while the "Current Practice" costs growers \$60/ha with a profit of \$83/ha per annum. In the long-term, the best-bet can potentially generate an additional \$30/ha profit to growers in control costs. As 90% of SLN infestations are medium, scattered and rare (McLaren, unpublished), our model shows that 'Best-bet' on **a sparse SLN population** will generate an additional profit of \$47/ha when compared to the "Current Practice".

Figure 4.2. Predicted economic impact of SLN management strategies over 10 years. (expenses include direct control and lost production costs and the profits include both improved crop and livestock production).







As perennial weeds are not reliant upon annual germination events, their management requires implementation of long-term strategies that include tactics to control both the seedbank and the rootbank. Herbicide applications need to be timed to effectively target seed production and the root system, with consideration given to outcomes on a long term basis rather than just within season.

### 3.6 Synthesis to derive best management

Synthesising these results enables best management practices to be developed. Table 4.0 describes seasonal actions for management of these weeds. These tactics need to be implemented for at leats 4 seasons and in concert with crop pasture rotations.

Recommended best management practices (BMP) for land managers:

### For dense weed populations (>10 stems/m2)

- Effective long-term management of SLN and PGC requires at least 2-3 years of Dual Spray to significantly reduce weed density by depleting both the seedbank and the rootbank. Mechanical control can replace the first spray. The Dual-spray can be carried out in annual grass pasture phase or cereal cropping phase or fallow phase.
- First spray or mechanical control in late Spring or early Summer (after crop harvest) to control seed set
- Second spray in Autumn to control the rootbank
- Competitive winter cereal crops or annual grass pastures to further reduce weed population reestablishing via the seedbank or remnant rootbank. (Cereals and grass pasture can be sown after the second spray. Broadleaved crops or pasture species are sensitive to the residual chemicals used in the second spray).
- Repeat the above three steps for three seasons
- Broadleaved pastures or crops can be introduced after one year of application of the last second spray
- Assess stem numbers; if < 1 stem/10m2, spot spray as appropriate
- Incorporate summer active pasture or crops as appropriate
- Monitor the area for five years for new seedlings

### For light weed populations (<5 stems/m2)

- Effective long-term management of SLN and PGC requires at least 1-2 years of Dual Spray mentioned above
- Competitive winter cereal crops or annual grass pastures to further reduce weed population reestablishing via the seedbank or remnant rootbank

### 4 Success in achieving objectives

Seven main objectives of innovative management of SLN and PGC as part of this project have been completed. The successes achieved within each of these objectives are presented below.

**Objective 1** — effective use of competitive perennial pastures (legume and non-legume) in combination with forage conservation and targeted herbicide use will be determined.

Glasshouse studies verified that a range of pasture species, both leguminous and non-leguminous are capable of providing suppression of SLN and PGC under suitable conditions. Data obtained from one field site established under adverse conditions reinforced the findings that a range of perennial pastures can be a useful management tool.

Maintenance of stubble loads or ground cover during spring and early summer can delay the emergence of SLN. This can decrease seed production levels at the end of the season.

The critical output from this research is that suitable summer active perennial pastures should be chosen to suit the environment, with the view of maintaining 1-3 t/ha active growth over summer.

**Objective 2** — herbicide efficacy will be increased with better timing in relation to weed size, weed phenology and time of day, and new application technology.

This research has identified that herbicides applications need to be strategically applied to target either the seedbank of the rootbank. Herbicide uptake and impact on the rootstock is greater when applied while the plants are in a vegetative growth stage as compared to the reproductive growth stage. The largest reduction in stem emergence the following season is obtained when picloram based herbicides are applied in autumn after the plants would normally have completed flowering and berry set. Use of all registered herbicides at flowering can provide over 95% viable seed set control, while application during berry formation can provide 42-85% control of viable seed set. Use of crop oil spray adjuvants can improve herbicide efficacy, presumably as they assist in alleviating the effects of potentially adverse spray application conditions.

A "Dual Spray" program was developed. It is recommended that a mid season herbicide application be made at flowering of these weeds for the purposes of reducing viable seed set, with a second herbicide application made in autumn for the purposes of controlling the rootstock.

**Objective 3** — Factors affecting herbicide translocation in SLN and PGC will be determined and this information will be integrated into development of herbicide application recommendations;

Preliminary herbicide translocation studies showed that use of the Uptake oil increased the total translocation by 356% and 49% when <sup>14</sup>C-fluroxypyr was applied at early vegetative (EARLY) and at flowering/early berry (LATE) stages to plants regenerated from one-year old root, respectively. However, the Uptake decreased the total translocation by 12% and 36% when <sup>14</sup>C-fluroxypyr was applied at EARLY and LATE stages to the plants regenerated from the two-year old roots, respectively.

SLN growth stages had remarkable impact on translocation. Fluroxypyr applied LATE resulted in more than three times increases in total translocation within the plants regenerated either from one-

or two-year old roots, highlighting the importance in application timing in relation to SLN growth stages.

On average, about 63% of the applied fluroxypyr was in the treated leaf (not translocated), and only 12%, 6% and 2% translocated to untreated leaves, stems and roots. The limited herbicide translocated into the roots (0.2-3.7%) represents a significant challenge for the effective control of this perennial weed. Further research is needed to improve control efficacy by identifying suitable wetting agents, optimum application timing for improved translocation into the rootbank.

**Objective 4** — allelochemicals for control of SLN and PGC will be identified and evaluated in glasshouse and field trials and selectivity determined and published;

Four putatively allelopathic eucalyptus species and one control eucalyptus species were evaluated in field and glasshouse trials, with one species (*E. spathulata*) consistently suppressing both SLN and PGC. The chemical profiles of extracts from the five species have been determined for the purposes of identifying components that may contribute to the observed allelopathic effects.

Specificity testing of the extracts from *E. spathulata* against other weed species indicates that the compounds have phytotoxic activity against germination of six common grass and broadleaf weeds species. The compounds, if identified, may provide the basis for development of a novel herbicide active ingredient with activity against a range of weed species.

**Objective 5** — indigenous pathogens will be identified and evaluated as potential bioherbicides and formulations and selectivity determined and published.

Pathogen screening was conducted at the start of the project with screening of cultured pathogens undertaken in the glasshouse. Results were inconclusive and this aspect of the research project was replaced with **Objective 5B**.

**Objective 5B** — "Biology and Ecology Study" (Factors affecting germination and emergence of SLN and PGC will be identified. Seed persistence and other biological data will be collected for better understanding of the target weeds.

Bare seed of both species may persist to up to 4 -6 years, with PGC seed less persistent than SLN, particularly at shallower depths. The presence of an intact berry can increase longevity on the soil seedbank, although the additional protection afforded by the berry appears to last less than two years.

Generally, PGC seed is capable of germinating over a wide range of soil moisture contents, soil salinity levels and soil acidity levels, suggesting that this species has a greater potential to establish from seed than SLN. With higher tolerance to variations in environmental conditions, PGC has the potential to expand more rapidly than SLN.

With the potential for SLN and PGC to produce in the order of 2,500 and 4,500 seeds per plant, respectively, a moderate infestation of four plants per metre will result in over 10,000 seeds being produced per season. After two years, the soil seedbank can potentially still contain 4,000 viable seeds, highlighting the need to annually minimise seed production to deplete the soil seedbank and the risk of reinfestation via new seedlings.

Objective 6 - drafted 3 scientific publications for peer review journals, and 3 conference papers;

#### Scientific publications

- Stanton R, Heap J, Carter R and Wu H (2009). Solanum Biology of silverleaf nightshade (Solanum elaeagnifolium). In The biology of Australian weeds Volume 3, F.D. Panetta (ed.), pp. 274-293, R.G. and F.J. Richardson. Melbourne, Australia.
- Zhang J, An M, Wu H, Stanton R and Lemerle D. (2010). Eucalyptus essential oils: chemistry and bioactivity. *Allelopathy Journal* 25(2), 313-330.
- Stevens M, Stanton R, Wu H, Sampson B, Weir T, Reid C and Mo J (2010). Detection of *Lema bilineata* Germar (Coleoptera: chrysomelidae) in Australia. *General and Applied Entomology* (accepted).
- Stanton R, Wu H and Lemerle D (2010). Herbicide control of silverleaf nightshade (*Solanum elaeagnifolium*) in Australia. Weed Technology, (submitted).
- Rex Stanton, Hanwen Wu and Deirdre Lemerle. (2010). Root regenerative ability of silverleaf nightshade (*Solanum elaeagnifolium* Cav.). Plant Protection Quarterly (submitted).

#### Conference papers

- Stanton R, Wu H and Lemerle D (2010). Herbicide control of summer active perennial weeds in southern Australia. *The 17<sup>th</sup> Australian Weeds Conference*, to be held in Christchurch 26-30 September, New Zealand.
- Stanton R, Wu H and Lemerle D (2009) silverleaf nightshade silverleaf nightmare or just a bad memory?. Proceedings of 15th Biennial NSW Weeds Conference, Narrabri, Australia.
- Stanton R, Wu H, An, M and Lemerle D (2009) silverleaf nightshade new approaches to an old problem. *Proceedings of the 1<sup>st</sup> South Australian Weeds Conference*, Adelaide, Australia.
- Stanton R, Wu H, An, M and Lemerle D (2008) Evaluation of potential natural herbicides for *Physalis* viscosa control. *Proceedings of the* 5<sup>th</sup> *International Weed Science Congress*, Vancouver, Canada.
- Stanton R, Wu H, An M and Lemerle D (2008) Home among the gum trees not necessarily so for *silverleaf nightshade. Proceedings of the 16<sup>th</sup> Australian Weeds Conference*, Cairns, Australia. pp 330-332.
- Stanton R, Wu H, Dear B and Lemerle D (2008) Managing perennial summer weeds with competitive pastures. *Proceedings of the 16<sup>th</sup> Australian Weeds Conference*, Cairns, Australia. p 503.
- Stanton R. Wu H and Lemerle L (2007) Regeneration of *silverleaf nightshade* root segments from various depths. *Proceedings of 14th Biennial NSW Weeds Conference,* Wollongong, Australia.

**Objective 7** – 4 student projects completed on SLN & PGC, co-supported by the Graham Centre.

The project team were not able to recruit or retain sufficient students to complete the four student projects initially proposed. One student was recruited and completed their research experiments, but withdrew prior to completing their thesis. The research data was analysed by the project team and some complementary work conducted. This work has been detailed within the herbicide section of this report.

The project team has been successful in recruiting a PhD candidate to undertake research into the genetic and morphological variation within SLN. This student is funded outside the current project and has currently been employed for less than six months. Research findings will become available in due course.

# 5 Impact on meat and livestock industry – now & in five years time

The outputs from this research project will impact on the meat and livestock industry by providing the industry with new confidence to deal with SLN and PGC, both previously regarded as intractable weeds.

Adoption of pasture management strategies in line with the recommended use of biomass to suppress these weeds will start to benefit the industry in the short term, not only with suppression of these two perennial weeds, but also for suppression and management of other annual and perennial weeds, therefore leading to reduced need for herbicides for pasture maintenance and reduced soil erosion risks through the maintenance of a minimum level of ground cover. The introduction of perennial components into the pasture system may also lead to more a uniform feedbase throughout the year.

Adoption of the recommended herbicide strategies may increase immediate financial costs to producers within the meat and livestock industry, however the improved level of control of the rootbank of these perennial weeds will enable producers to substantially reduce their herbicide costs in five years time.

Implementation of extension and advisory programs now will provide producers and consultants operating within the meat and livestock industry with a broader knowledge base of the biology and control of these two perennial weeds and arm them with tactics that can be applied to these weeds, and potentially other difficult to control perennial weeds, to achieve long term management of infestations.

To date, the research has been evaluated in the field in southern New South Wales. However, the principles underpinning the recommendations arising from this research project are sufficiently robust to allow the concepts to be transferred to other regions within Australia with only minor adjustments required.

Model analyses of the impact of the recommended herbicide strategies upon financial returns conducted for wool and prime lamb enterprises indicate that significant long term financial benefits to the meat and livestock industry can be derived by implementing the outputs from this research project (Table 6.1).

Investment in extension programs to ensure that the potential outcomes are achieved via adoption of new management practices would be required to realise the full benefits. This project has identified key guidelines that can be incorporated into a successful management strategy, and these need to be developed and adopted as best management practice by advisors and producers.

	Wool enterprise			Prime Lamb enterprise		
Stocking rate (DSE/Grazed ha)	8.9			7.8		
Gross margin (\$/DSE)	\$13			\$16		
Gross margin (\$/ha)	\$106			\$137		
	<pre>\$ Lost Production</pre>	Chemical cost	Actual Profit	\$ Lost Production	Chemical cost	Actual Profit
No control	\$711.39	\$0.00	\$396.31	\$919.44	\$0.00	\$512.21
Current Practice	\$286.23	\$228.51	\$592.96	\$369.94	\$228.51	\$833.20
Best-best on a dense population (>10 stems/m <sup>2</sup> )	\$68.88	\$216.86	\$821.96	\$89.03	\$216.86	\$1,125.76
Best-best on a sparse population (<2.5 stems/m <sup>2</sup> )	\$51.35	\$62.53	\$993.82	\$66.36	\$62.53	\$1,302.75

# Table 6.1. Cost/benefit analysis of SLN management strategies (cumulative profit over ten years, \$/ha).

### 6 Conclusions and recommendations

Current management practices focus on limiting seed production through herbicide application during the early reproductive phases in mid-summer. These practices typify the conventional approaches that have been successfully applied to annual weeds that rely upon seedbanks for population survival. However, successful management programs for perennial weeds need to also include practices that reduce the rootbank.

The following recommendations provide components of an integrated management package that will address recruitment from both the seedbank and the rootbank:

- Seed set control should be commenced while plants are flowering to maximise the reduction in viable seed production. All registered and off-label permit herbicides can be effectively used for this purpose. Mechanical options may be an appropriate alternative to herbicides.
- Rootbank control using herbicides is more effective when herbicides are applied in autumn after the normal reproductive season. Picloram based herbicides are most effective for controlling SLN, while both picloram and glyphosate herbicides are effective at controlling PGC rootbanks when applied in autumn.
- Pastures can provide effective competition against perennial summer weeds, leading to a reduction in seed production and to reduced root vigour.
- Good crop agronomy for winter crops can provide competition during spring that can delay emergence of summer perennial weeds, leading to a shorter growing season ad reduced ability of the weeds to produce seed.
- Reducing tillage operations in areas infested by SLN and PGC will limit fragmentation of the root systems and subsequent vegetative recruitment, and also reduce the risk of burial of seed and berries within the soil profile where the seed may persist an be a source of reinfestation if management practices are adopted that allow the rootbank to be controlled.

Integrated management packages need to be tailored to suit each infestation. The use of residual herbicides has the inherent risk of impacting upon other components of the system. The residual picloram herbicides that have proven effective for controlling the rootbank of these perennial weeds will also reduce the capacity of producers to establish and maintain broadleaf pasture and crop species in the following winter and spring, therefore their use must be carefully planned to fit within the projected rotation of crops and pastures in each field. Herbicides for seed set control need to be selected that will not impact of perennial pasture production over summer, while the choice of autumn applied herbicides for rootbank control needs to be made with consideration given to the proposed winter pasture or cropping practices.

Research is needed to integrate the pasture and herbicide components to demonstrate the synergies able to be derived from combining the two management tactics. Participatory demonstration sites will provide an opportunity to demonstrate and quantify the economic and biological benefits of adopting IWM practices for these deep-rooted perennial weeds.

Management of the rootbank of these weeds is critical for achieving long term control of the weeds. This research has provided clear outputs for using herbicides more effectively for rootbank control, however the increased cost associated with using the most effective herbicides may inhibit adoption in some situations. These two weeds can occur in scattered or light infestations that can be either time consuming to individually spot-spray or uneconomic to treat with a broadacre boom application. The use of technology such as WeedSeeker provides a tool to enable scattered infestations to be treated with an effective herbicide in a cost and time efficient manner.

This project has demonstrated potential for extracts from eucalypts to be used to suppress both SLN and PGC, with extracts from *E. spathulata* showing the greatest potential for further research. Field observations have shown that SLN populations are significantly reduced in the presence of *E. spathulata*, while laboratory assays have shown that germination and growth of both SLN and PGC are reduced. Specificity assays on the extracts indicate that the soluble oil fraction and the essential oil fraction are phytotoxic against a range of broadleaf and grass weeds, suggesting that the active compounds, if isolated, could form the basis of a novel non-selective herbicide.

Limited information is currently available to landholders relating to the successful long term control of these two perennial weeds. It is highly recommended that the BMP information developed from this project be promptly deliver to growers to reduce weed management costs, improve perennial pasture productivity, and increase capacity of growers to manage perennial weeds.

Appendix 9.2 outlines the BMP information delivery structure and key areas for continued research into these weeds. The new project proposal captures the information and knowledge gained from this project and delivers this new understanding of management of summer active perennial weeds into measurable outcomes and benefits to the meat and livestock industry.

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### 8 Appendices

### 8.1 Scientific publications arising to date from the research

### ONE Book Chapters:

Stanton R, Heap J, Carter R and Wu H (2009). Solanum Biology of silverleaf nightshade (Solanum elaeagnifolium). In *The biology of Australian weeds* Volume 3, F.D. Panetta (ed.), pp. 274-293, R.G. and F.J. Richardson. Melbourne, Australia.

### FOUR Journal papers

- Zhang J, An M, Wu H, Stanton R and Lemerle D. (2010). Eucalyptus essential oils: chemistry and bioactivity. *Allelopathy Journal* 25(2), 313-330.
- Stevens M, Stanton R, Wu H, Sampson B, Weir T, Reid C and Mo J (2010). Detection of *Lema bilineata* Germar (Coleoptera: chrysomelidae) in Australia. *General and Applied Entomology* (accepted).
- Stanton R, Wu H and Lemerle D (2010). Herbicide control of silverleaf nightshade (*Solanum elaeagnifolium*) in Australia. Weed Technology, (submitted).
- Rex Stanton, Hanwen Wu and Deirdre Lemerle. (2010). Root regenerative ability of silverleaf nightshade (*Solanum elaeagnifolium* Cav.). Plant Protection Quarterly (submitted).

### SEVEN Conference papers

- Stanton R, Wu H and Lemerle D (2010). Herbicide control of summer active perennial weeds in southern Australia. *The 17<sup>th</sup> Australian Weeds Conference*, to be held in Christchurch 26-30 September, New Zealand.
- Stanton R, Wu H and Lemerle D (2009) silverleaf nightshade silverleaf nightmare or just a bad memory?. Proceedings of 15th Biennial NSW Weeds Conference, Narrabri, Australia.
- Stanton R, Wu H, An, M and Lemerle D (2009) silverleaf nightshade new approaches to an old problem. *Proceedings of the 1<sup>st</sup> South Australian Weeds Conference*, Adelaide, Australia.
- Stanton R, Wu H, An, M and Lemerle D (2008) Evaluation of potential natural herbicides for *Physalis viscosa* control. *Proceedings of the* 5<sup>th</sup> *International Weed Science Congress*, Vancouver, Canada.
- Stanton R, Wu H, An M and Lemerle D (2008) Home among the gum trees not necessarily so for *silverleaf nightshade. Proceedings of the 16<sup>th</sup> Australian Weeds Conference*, Cairns, Australia. pp 330-332.
- Stanton R, Wu H, Dear B and Lemerle D (2008) Managing perennial summer weeds with competitive pastures. *Proceedings of the 16<sup>th</sup> Australian Weeds Conference*, Cairns, Australia. p 503.
- Stanton R, Wu H and Lemerle L (2007) Regeneration of *silverleaf nightshade* root segments from various depths. *Proceedings of 14th Biennial NSW Weeds Conference,* Wollongong, Australia.

### Detection of Lema Bilineata Germar (Coleoptera: Chrysomelidae) In Australia

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### Summary

The South America chrysomelid *Lema bilineata* Germar is recorded for the first time in Australia. Adults and larvae were found feeding on Prairie Ground Cherry (*Physalis viscosa* L.) in an open greenhouse in Wagga Wagga in southern New South Wales during November 2008. The greenhouse population was eradicated immediately after it was recognised as a potential biosecurity threat, however further detections of *L. bilineata* were subsequently made in the Cootamundra area. *L. bilineata* feeds on a range of solanaceous plants including both crop species and weeds, and its potential impact on agriculture in south-eastern Australia is discussed.

### Eucalyptus essential oils: chemistry and bioactivity

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### ABSTRACT

The essential oils obtained from eucalyptus have many medicinal and commercial values. The oils have a wide range of bioactivities, such as antimicrobial, antiviral, fungicidal, insecticidal and herbicidal activities. A growing prospect of the commercial applications of essential oils has led to an intense research on their extraction, chemical composition, bioactivity as well as mode of actions. Eucalyptus species differ in their chemical composition. The bioactivity of essential oils is highly associated with the unique chemical composition in the oils. The novel biological functions of eucalyptus essential oils suggest that research need to be expanded to cover many other eucalyptus species to fully exploit their potential commercial benefits. In this review, recent progresses in the above research areas are summarized and future research prospects are discussed.

Keyword: Eucalyptus, essential oils, allelopathy, bioactivity, chemical composition.

### Herbicide Control of Silverleaf Nightshade (Solanum elaeagnifolium) in Australia

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### ABSTRACT

Silverleaf nightshade is a widespread deep-rooted summer-growing perennial weed considered as one of the worst weeds of crop and pasture systems. Effective long term control is difficult to obtain due to the extensive root system which is capable of propagating from lateral and tap roots. Field experiments were conducted at two locations in Southern Australia between 2006 and 2008 to examine a range of herbicides for improved control on silverleaf nightshade, both within and between seasons. Pyridine herbicides were the most effective herbicides for controlling growth within season (60-90%), with overall control using glyphosate based treatments generally reduced due to emergence of new stems after herbicides were applied. The pyridine herbicide treatment (triclopyr plus picloram plus aminopyralid) provided the best and consistent long-term control on stem emergence (60-72%), particularly if the herbicides were applied in autumn when silverleaf nightshade is translocating more resources into the root system.

### Root regenerative ability of silverleaf nightshade (Solanum elaeagnifolium Cav.).

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### Abstract

Silverleaf nightshade is considered amongst the worst weeds of crop and pasture systems in Australia due to its extensive root system. Cultivation may exacerbate the problem due to the regenerative capacity of the root system. Glasshouse experiments were conducted to determine the importance of cultivation in the spread of silverleaf nightshade by investigating the regenerative abilities of various root fragment lengths (1, 2.5, 5 and 10 cm) buried at three soil depths of 2.5, 5 and 10 cm. Regeneration occurred from root fragments as short as 1 cm, with shoot production increasing with root fragment length. Optimum burial depth was 5 cm for 1- and 2.5-cm root fragments, while 5- and 10-cm root fragments were equally prolific at stem production from shallow depth. High levels of fragment mortality occurred in 1 cm fragments, with mortality levels significantly declining as fragment length increased. This research suggests that minimum tillage techniques should be encouraged on areas with silverleaf nightshade infestations. Implements should be thoroughly cleaned before leaving the infested area, as even short root fragments adhered to machinery are capable of starting a new infestation in a clean field.

### Herbicide control of summer active perennial weeds in southern Australia

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#### Summary

Perennial weeds are problematic to control due to the presence of both a seedbank and a rootbank. In addition, summer active perennial weeds generally require herbicides to be applied under hot, dry conditions which are not conducive to good herbicide uptake and translocation. Applying herbicides under more favourable conditions in late summer or early autmn may lead to increased herbicide efficacy.

Field experiments were conducted on silverleaf nightshade (*Solanum elaeagnifolium*) and prairie ground cherry (*Physalis viscosa*) investigating the effect of herbicide time of application upon rootbank dynamics. The rootbank was most effectively controlled when herbicides were applied in autumn prior to the weeds beginning senescence.

These results have significant implications for developing appropriate management packages for these weeds to achieve long term control. Similar approaches could be applied to other intractable perennial weeds.

Keywords Solanum, Physalis, herbicide,

### Silverleaf nightshade - silverleaf nightmare or just a bad memory?

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Deirdre Lemerle Director EH Graham Centre for Agricultural Innovation (an alliance between NSW DPI and Charles Sturt University)

### ABSTRACT

Control of *silverleaf nightshade* (SLN), a summer active deep-rooted perennial weed, has challenged land managers for decades. SLN infestations have been estimated to affect over 26 million hectares in NSW, or approximately one third of the state. SLN can reduce pasture production and subsequent winter crop production by as much as 20% and also reduce land values by a similar amount.

Land managers report SLN control as expensive or not effective. Traditional control approaches have centred upon herbicides, with recommended application timing targeting prevention of seed set. Control of seed production can be achieved with several herbicides, however these generally require several applications during summer and can result in land managers spending \$30/ha or more in control costs. Importantly, SLN can regenerate from rootstock and the current herbicide practices do not significantly reduce the SLN rootbank from season to season.

To achieve long term control of SLN, it is suggested that both the seedbank and the rootbank need to be managed through implementation of an integrated management package. Pastures and active spring biomass production can significantly reduce SLN density and vigour, which will reduce seed production and also decrease the amount of resources being returned to the rootbank. Alternative timing of herbicide application may assist with targeting rootbank control, therefore decreasing the potential for SLN populations to regenerate the following season from existing roots.

Options for SLN control that can be used as part of an integrated management plan are proposed. It is further suggested that land managers can tailor a management plan to suit their particular situation.

### Silverleaf nightshade - new approaches to an old problem

Rex Stanton, Hanwen Wu, Deirdre Lemerle

E H Graham Centre for Agricultural Innovation (NSW Department of Primary Industries and Charles Sturt University)

### Summary

*Silverleaf nightshade* (*Solanum elaeagnifolium* Cav.) is a widespread deep-rooted summer-growing perennial weed of cropping and pasture systems. The extensive root system of *silverleaf nightshade* competes both directly and indirectly with summer and winter pastures/crops, respectively, through depletion of soil moisture and other resources. Several innovative techniques are being investigated to broaden the control options for this weed.

A range of herbicides are currently used to manage *silverleaf nightshade* infestations. The cost of the most effective herbicide treatments can be prohibitive, particularly if long term control can not be achieved. Herbicide application techniques and timing are being evaluated to determine if herbicides can be used more efficiently.

Competitive pastures may reduce the vigour of *silverleaf nightshade* plants and over time assist with depleting stored energy reserves in the roots. This may enhance the effectiveness of other control techniques.

At least one new herbicide, Callisto<sup>™</sup>, has been developed from allelopathic compounds. Anecdotal reports suggested several Eucaplytus species can inhibit the growth of *silverleaf nightshade*. The nature and extent of this inhibition is being evaluated.

Classical biological control agents for *silverleaf nightshade* have been investigated previously, with several invertebrates identified as potential candidates. However, lack of host specificity precluded them as viable agents. Pathogens are being evaluated as part of a current project. A tobacco mild green mosaic virus that has high efficacy against tropical soda apple (*S. viarum*) is being evaluated as a potential bioherbicide for *silverleaf nightshade* control.

An integrated management package can be developed around these innovative techniques to assist with the long term control of *silverleaf nightshade*.

### Evaluation of potential natural herbicides for Physalis viscosa control

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### ABSTRACT

*Phyalis viscosa* (prairie ground cherry), native to North and South America, is a weed in South Africa, Chile, Argentina, Brazil, Uruguay and through the western United States. This species was first recorded in Australia in 1909 in Melbourne and considered naturalised by 1914. It is a declared noxious weed in New South Wales, Victoria and Western Australia. *P. viscosa* grows from seed or roots in spring and is relatively hardy, capable of withstanding drought, trampling and shading. Established populations can not be readily eradicated using current control techniques.

There is no evidence in the literature of potential phytochemical evaluation for *P. viscosa* control. *Eucalyptus* species have been widely examined for allelopathic potential, with a diverse range of bioactive substances identified from the leaves, bark and roots. The aim of this research is to evaluate the phytotoxic potential of *Eucalyptus* species on *P. viscosa*.

Leaves were collected from *E. salubris*, *E. spathulata*, *E. brockwayi*, *E. dundasii* and *E. melliodora*. Leaves of *Arctotheca calendula* (capeweed) were collected as a control. Leaf samples were dried at 40 °C for 72 hours and ground. Aqueous extracts were formed by incubating 10 g powder in 100 mL deionised water at 20 °C for 72 hours. Solution were filtered and centrifuged to remove particulate matter. Thirty *P. viscosa* seeds were placed on filter paper in Petri dishes and incubated for 14 days at 25/15 °C (8/16 hrs cycle) with four extract concentrations (0, 25, 50 and 100%) and germinated seeds counted.

A. calendula and E. melliodora extracts had the least impact on germination. All four remaining *Eucalyptus* species reduced germination to less than 2% at 100% extract concentration, with *E. spathulata* extract significantly inhibiting germination at all extract concentrations compared to *A. calendula* and *E. melliodora*. These preliminary results suggest that germination of *P. viscosa* can be inhibited by compounds present in aqueous extracts from several eucalyptus species.

The potential impact of the aqueous extracts on seedlings and young plants emerged from root stock will be critical in the evaluation of these compounds as herbicidal agents for controlling *P*. *viscosa* in the field. Should the impact observed on seed germination also occur in plants in the field there is the potential to develop a new herbicide, similar to the mesotrione herbicide developed from *Callistemon citrinus* for control of a range of broadleaf weeds. Further research is underway to determine the phytotoxic potential of *Eucalyptus* extracts on root stock regeneration of *P. viscosa* and to identify substances responsible for such inhibition.
#### Home among the gum trees - not necessarily so for silverleaf nightshade

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#### Summary

*Silverleaf nightshade* is a widespread weed in south eastern Australia. Despite many years of use of synthetic herbicides, populations of this deep rooted summer active weed continue to pose a problem, with more infestations occurring. As part of an innovative management strategy, allelopathic potential of eucalyptus species were evaluated.

Aqueous extracts were prepared from ground *E. spathulata*, *E. salubris*, *E. brockwayii* and *E. dundasii* leaves. Root and shoot growth of pre-germinated *silverleaf nightshade* seedlings was significantly decreased by aqueous extracts from all species.

Results suggest that compounds in the aqueous extract exhibit an allelopathic effect on *silverleaf nightshade* seedling development. Further research is being conducted to determine the effect of these compounds on mature *silverleaf nightshade* plants. Allelopathic control of *silverleaf nightshade* may allow development of innovative new management options such as bioherbicides or use of agroforesty for weed management.

#### Keywords

Silverleaf nightshade, allelopathy, eucalyptus

#### Managing perennial summer weeds with competitive pastures

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#### Summary

Prairie ground cherry (*Physalis viscosa*) and *silverleaf nightshade* (*Solanum elaeagnifolium*) were both introduced into Australia from central America in the early 20th century as contaminants of fodder and grain (Parsons and Cuthbertson 2001).

Both these species are deep rooted, summer active perennials that utilise resources over summer, therefore competing directly with pastures and indirectly with the subsequent winter crops. Herbicides are relied upon as the primary method of controlling these weeds, although few land managers have reported successfully eradicating wide-spread infestations.

Tideman (1960) reported that *silverleaf nightshade* did not appear to be greatly controlled by pastures containing either lucerne or phalaris. However, pastures is still worth consideration in an integrated management program, as Wapshere (1988) reported that *silverleaf nightshade* densities declined over three years in fields returned to pasture after a cropping phase. Moerkerk and Snell (2003) have suggested that competitive perennial pastures are a control option for prairie ground cherry.

This research aims to identify a range of summer pasture species that will suppress prairie ground cherry and *silverleaf nightshade* populations. Use of competitive pastures within an IWM program will have the benefits of increasing available summer pastures and decreasing reliance on herbicides for weed management.

Field sites have been selected throughout the current range of these two species in New South Wales to cover a diversity of climates, with a site at Wellington with summer rainfall and other sites near Ganmain, Narrandera and Tocumwal with winter dominant rainfall patterns.

Sub-tropical pasture grass species (Premier Digit grass, Katambora Rhodes grass, Bambatsi panic), phalaris, lucerne and chicory are being evaluated for control in summer rainfall areas, while biserrula, lucerne, cocksfoot and phalaris are being evaluated at the southern sites. All pasture species are sown individually with sub-clover, and also as a mixture. A control treatment of sub-clover is included at all sites.

#### Regeneration of silverleaf nightshade root segments from various depths

# Rex Stanton<sup>1</sup>, Hanwen Wu<sup>2</sup>, Deirdre Lemerle<sup>1</sup>

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#### ABSTRACT

*Silverleaf nightshade* (*Solanum elaeagnifolium* Cav.) is a widespread deep-rooted summer-growing perennial weed. It is considered amongst the worst weeds of cropping and pasture systems. The extensive root system of *silverleaf nightshade* competes both directly and indirectly with summer and winter pastures/crops, respectively, through depletion of soil moisture and other resources.

Silverleaf nightshade is capable of forming new plants from both seeds and root segments. It is believed that cultivation is a major factor contributing to the spread of *silverleaf nightshade*. To determine the importance of cultivation in the spread of *silverleaf nightshade*, a glasshouse experiment investigated the response of various lengths of *silverleaf nightshade* root when buried at several depths.

Reduced cultivation systems may decrease the spread of *silverleaf nightshade*. Results indicate that *silverleaf nightshade* can regenerate from root segments as short as 1 cm. Significantly more shoots are produced from 10 cm root segments at all depths compared to shorter root segments. No mortality was observed for 10 cm root segments, whereas mortality significantly increased as segment length decreased. However, despite the increased mortality of shorter segments, increasing fragment size did not reduce overall shoot density.

These findings have implications for how a paddock infested with *silverleaf nightshade* should be managed. Minimum tillage techniques should be used when any sowing operations are undertaken. It would also be crucial that all implements are thoroughly cleaned prior to moving machinery out of an infested field, as even short root segments should be considered viable and therefore capable of starting a new infestation in a clean field.

# 8.2 Proposed Stage 2 project. Best Management Practice for Summer Perennial Weeds of southern NSW

# FULL APPLICATION FORM

# PARTIES

#### **Research Organisation**

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## PROJECT

Project Title (Maximum of Ten Words)

Best Management Practice for Summer Perennial Weeds of southern NSW

# **Background of Research Work**

Summer perennial weeds are a major cost to animal production in SE Australian mixed farming systems. *Silverleaf nightshade* (SLN) and prairie ground cherry (PGC) are typical examples of intractable, deep-rooted, summer perennial weeds which significantly impact on livestock productivity and health. McLaren *et al.* (2004) estimated that average total farm impact of SLN was \$1730 per year in direct control costs and \$7786 in lost production. Few options other than high rate of expensive residual herbicides, that affect the establishment of following pastures and crops, were available to reduce their spread and impact. The previous MLA project considerably broadened herbicide control options and identified potential new non-chemical tactics. This includes best-bet strategies that can potentially generate \$30/ha profit to growers by cost-effective integrated weed management. The aims of this new project will be:

- The change of on-farm management of SLN and PGC
- Improved effectiveness of delivery by an innovative delivery process, coordinating the efforts of state and local government agencies and private sector contractors and advisors
- Further improved efficacy of interactions between herbicide uptake, translocation and timing of application, as well as synergies with competitive pastures

Outcomes will be reduced weed costs, less herbicides in the environment, improved perennial pasture productivity, and increased capacity of growers to manage perennial weeds leading to an enhanced performance against environmental, economic, social, and cultural outcomes. Best management practice (BMP) information will potentially be extended to other states depending on the success of this pilot study.

# **Project Description**

#### Background

# Previous project (2006-2009)

MLA commissioned a 3-year research project "Innovative management of *silverleaf nightshade* and prairie ground cherry" in 2006 at the E.H. Graham Centre for Agricultural Innovation (NSW DPI and CSU) to determine innovative approaches to reduce the impact of these two weeds on animal production systems in southern Australian grazing zones. The project covered a broad range of innovative research areas, including improved herbicide efficacy, identification of competitive pastures, biological control, eucalyptus allelopathy, and basic biological and ecological studies to identify potential weaknesses in the life-cycle of the two weeds.

The key outputs were:

- <u>More reliable herbicide recommendations</u>: A single herbicide application, either early or late, does not stop regrowth from both the rootbank and the seedbank of these perennial weeds in the following season. Additionally, the current single herbicide recommendation does not eliminate seed production if the application timing is too late. These two limitations mean that perennial weed populations persist. Newly-developed "Dual Spray and BMP" from the previous project cost-effectively reduce the weed population.
- Improved knowledge of weed biology and ecology to underpin a BMP strategy: Extensive data on seed production, seed dormancy, germination requirements and seed persistence were collected. Burial studies indicate that seedbanks may persist for five years or more. Root fragments of 10 mm in length can be viable, demonstrating the impact of cultivation on SLN

spread. This highlights the importance of separate control tactics to manage both the seedbank and the rootbank.

- <u>Competitive pastures/cover crops identified: Both lucerne and phalaris were competitive against SLN and PGC in glasshouse trials, suppressing the growth of SLN and PGC by 76-93% and 93-97%, respectively. Additionally, maintaining C4 grass pasture biomass reduced SLN biomass by 34-68% in both glasshouse and field studies. Winter and summer cover crops (cereals) reduced early SLN stem emergence by 60-95%. These results indicate the role of competitive pastures/crops in managing weed populations.
  </u>
- Understanding of eucalyptus allelopathy on SLN: Preliminary studies showed that aqueous extracts from Eucalyptus spathulata, E. brockwayi, E. dundasii and E. salubris were phytotoxic to SLN and PGC germination, depending on the species and concentration. Essential oils, soluble essential oils and steam extracts were phytotoxic to the germination and seedling growth of SLN and PGC. More than 40 compounds were identified in the essential oils which could contribute to the phytotoxicity observed. These outputs provide the basis for the development of a new bioherbicide.
- Recommendation for biological control: A survey of pathogens found in SLN populations did not find any agents suitable as a biocontrol agent for SLN. However, opportunistically two insects were discovered, an eggfruit caterpillar feeding on SLN berries and a chrysomelid beetle feeding on PGC leaves. Limited host specificity testing of the chrysomelid beetle has indicated opportunity for biocontrol.
- Recommended best management practices (BMP) for land managers:
  - Effective long-term management of SLN and PGC requires at least 2-3 years of Dual Spray to significantly reduce weed density by depleting both the seedbank and the rootbank. Mechanical control can replace the first spray.
  - First spray or mechanical control in late Spring or early Summer to control seed set.
  - Second spray in Autumn to control the rootbank.
  - Competitive winter cereal crops or grass pastures to further reduce weed population reestablishing via the seedbank or remnant rootbank.
  - Repeat the above three steps for three seasons.
  - Assess stem numbers; if < 1 stem/10m2, spot spray as appropriate.
  - Incorporate summer active pasture or crops as appropriate.
  - Monitor the area for five years for new seedlings.

This integrated approach targeting both the seedbank and the rootbank needs to be demonstrated and validated to land managers, advisors and weed officers in a broader range of environments across southern NSW.

Evaluation of BMP strategies - Under the current management practices SLN stems decline very slowly and the population increases over time in the absence of any control measures. The proposed BMP strategy of 2,4-D amine or glyphosate at flowering to limit seed set followed by picloram in autumn for rootbank control could potentially deplete the rootbank during the first three seasons. Higher initial cost with the BMP was offset by improved profit and reduced cost in later years. On average per year based on a dense SLN population, assuming a gross margin of 137\$/ha for a Prime Lamb enterprise in North East VIC, the BMP has a direct control cost (herbicide, lost production, labour and machinery) of \$31ha and generates a net profit of \$113/ha, while the typical current practice costs growers \$60/ha with a profit of \$83/ha per annum. In the long-term, the BMP can potentially generate an additional \$30/ha profit to growers in control costs. As 90% of SLN infestations are medium, scattered and rare (McLaren,

unpublished), our model shows that BMP on a sparse SLN population will generate an additional profit of \$47/ha when compared to the current practice.

#### **Objectives and Outcomes of Project**

By 31 December 2012, this project will deliver:

- 100 public, regulatory and private advisors and spray contractors to recommend BMP for SLN and PGC control to NSW growers
- More effective BMP delivery process endorsed by public and private partners
- 1000 growers with the knowledge and confidence to implement a long-term BMP
- 400 growers adopting the management packages, with potentially 20,000 ha under improved management
- New knowledge on the impact of integrated herbicide and pasture programs on weed population dynamics to achieve sustainable, long-term reduction or eradication of currently intractable perennial weed population
- A research and delivery protocol for managing other perennial weed species such as skeleton weed and African rue
- A bioeconomic decision tool for growers and advisors

The project consists of an extension program (60%) and a research component (40%). The extension program is conducted via collaborative extension efforts between cross-agency interactions, and working with growers groups. The research components on weed biology and ecology and on herbicide translocation will identify the weakest link in the weed life cycles to further improve control efficacy.

The noxious weed officer networks such as Eastern Riverina Noxious Weeds Advisory Group (ERNWAG) and Western Riverina Noxious Weeds Advisory Group (WRNWAG) will also assist in the adoption process.

Newsletters and research updates will be directly distributed to growers, advisors and noxious weeds officers. An extensive contact list in NSW has been established during the previous project. A group of 100 advisors/Weeds Officers and 1,000 growers is to be targeted, with 400 growers expected to adopt the management packages. The priority will focus on advisors, including I&I NSW extension networks, noxious weeds officers, agricultural consultants and spraying contractors. They will play a key role in the effective extension of management information to growers. If each advisor can influence practices of at least 4 growers, then the multiplying power will ensure that 400 growers are influenced by the advisors.

# Method

1. Delivery of best management practices (60%)

1.1 Communication and Delivery (30%):

#### Establishment of a Consultative Committee

A consultative group of members consisting of researchers, extension officers, noxious weeds officers, agricultural advisors, spraying contractors, and growers will refine BMP packages and advocate and manage the implementation through their organisations.

#### Establish key delivery pathways

The Project Team has identified the following tiers of key delivery pathways:

#### A. <u>I&I NSW Extension Network contributing experienced extension personnel in the target zone.</u>

Personnel are purposely selected from SLN/PGC-infested areas, which include West Wyalong, Forbes/Parkes, Dubbo, Wellington, Griffith, Yanco and Albury. On average, each of the seven extension staff targeted will be responsible for motivating 4 advocate growers across differing localities, soil types and crop rotations. A total of 28 advocate growers are therefore expected to run demonstration sites (Fig. 1). An incentive mechanism will be introduced to motivate growers' participation.

The core responsibilities of the extension staff will include:

- Motivating at least 28 advocate growers to undertake detailed demonstration
- Motivating at least 100 growers to trial the BMP with less rigorous reporting
- Communicating the project information and activities to growers and advisors
- B. <u>I&I NSW Weeds Management Program and noxious weeds advisory groups:</u>

The core responsibilities of this group will be:

- Coordinating the implementation of the project through noxious weeds advisory groups
- Communicating the project information and activities to weeds officers and regulatory officers
- Motivating at least 10 advocate growers to undertake detailed demonstration
- Motivating at least 100 growers to trial the BMP with less rigorous reporting
- Motivating 30 weeds officers to implement the BMP
- <u>C.</u> <u>Growers groups</u>:

The project team has established a close link with a number of growers groups such as Conservation Agriculture & No-Till Farming Association (CANFA), FarmLink and Riverine Plains in NSW. Each of the 3 growers group will attract at least 4 advocate growers. Limited funding support will be provided to each grower group to ensure that they meet their core responsibilities.



Figure 1. Key delivery pathways and the projected numbers of advocate growers and weeds officers targeted by their respective consulting group.

# D. Agricultural advisors and spraying contractors:

The EH Graham Centre has an extensive stakeholder list of more than 500 entries. The Project Team will compile a list of accredited contract sprayers in the region. The agricultural advisors and spraying contractors will be promptly informed about the latest research update and BMP information.

The core responsibilities of this group will be:

- Motivating at least 10 advocate growers to undertake detailed demonstration
- Motivating at least 100 growers to trial the BMP with less rigorous reporting
- · Communicating the project information and activities to growers

# Project monitoring and evaluation

A broadbased monitoring and evaluation plan will be established. Surveys of field day participants over several seasons will identify the percentage adoption over time. More than 200 growers are to be targeted through extension activities such as field days, farm walks and focus group. Benchmarking surveys will be undertaken to determine the knowledge base, attitude and practices of growers at the commencement and the conclusion of the research.

#### Communication

A broadbased communication plan will be established, utilising the network of all collaborative partners. This also consists of interactive presentations at growers demonstration sites, field days, workshops, News Forums/BLOG, and also accessible via Webinars and You Tube.

The Project Team will collaborate with the successful IWM Manual Training Program developed by previous Weeds CRC and implemented by ICAN (Mr John Cameron) to incorporate BMP information for deep rooted perennial weeds into their training program. The collaboration with ICAN will further increase the coverage of BMP to agricultural advisors, resellers and growers.

Printed BMP information and research updates will be widely distributed to more than 1000 growers and 100 advisors. The management information will also be published in local media such as AgToday through the Land Newspaper as well as MLA's ProGrazier magazine. Three conference papers and three scientific journal papers will be produced to target wider a audience.

# Products

- Support management tools for best management practice, such as phenology charts, weed emergence guides or interactive and instructive spreadsheets, will provide growers with confidence to address their weed issues and develop long-term strategies.
- Project information, real-time data and management decisions will be readily accessible through both EH Graham Centre and I&I NSW websites.
- A bio-economic modelling tool for growers and advisors to compare the best-bet control strategies with their current control practices.
- 1.2 Advocate growers demonstration trials (20%):

It is anticipated to attract at least 65 advocate growers to practice recommended BMPs across a range of infestation levels, soil types and climatic regions in NSW (Fig. 1). Advocate grower participation in the project will include the implementation of on-farm demonstrations with a few key treatments jointly designed by the Project Team and the growers. Growers will be comparing the recommended control strategies with their current practices. These experiments will be undertaken by the growers under the guidance and coordination of the Project Team. A standardised recording protocol will be provided, including control efficacy, stem emergence, control cost and impact of weeds on pasture/crop production.

These advocate grower demonstration sites will provide additional information on seasonal and site effects on control efficacy. These demonstration sites, together with two long-term research sites are to encourage greater participation and rapid adoption of growers. Through these sites, it is expected to extend the BMP to other growers, noxious weeds officers and advisors via on-site field days.

1.3 Application of precision herbicide application technology (10%):

A survey has identified that 90% of SLN infestations are medium, scattered and rare (McLaren, unpublished). Smart technology (eg.,WeedSeeker) can be used to spray scattered weeds while minimising total herbicide and labour costs, allowing expensive but effective residual herbicides such as picloram to be used.

Six farms with sparse *silverleaf nightshade* infestations will be selected. Advocate growers will be asked to employ the WeedSeeker Technology to apply the herbicide program recommended by the Project Team. Current control practices will also be compared. Cost/benefit analysis will be conducted.

2. Research component (40%)

2.1 Long-term integration sites to validate the best management practices strategies (20%)

Two multi-year replicated field trials will be established with farming systems groups to evaluate and demonstrate the impact of BMP strategies on management of SLN and PGC, including one SLN and one PGC sites with FarmLink or Riverine Plains in southern NSW. Integration of BMP components consisting of herbicides and competitive pastures/cover crops will be validated. Data will be collected on perennial weed population dynamics, productivity and economics of imposed strategies.

2.2 Improved efficacy by fine-tuning herbicide applications (10%)

Current recommendations based on residual chemicals restricts crop and pasture options. There is a need to identify cost-effective non-residual herbicide combinations and best application timing for improved control.

The current recommendation of spraying at flowering/green berry stages is too late for total seedbank control. The previous project has identified that the early herbicide application with glyphosate, 24-D amine or fluroxypyr for seedbank control should be carried out at the flowering stage. However, the optimum spraying window for the early application needs to be defined. It is unclear if control efficacy can be further increased by earlier application prior to flowering, with regrowth controlled by the late season herbicide application.

Picloram based products (eg., Tordon 75D or Grazon Extra) were used in autumn as a late

application to target the rootbank. The current recommended rate of Tordon 75D for SLN boom spraying is 15L/ha. This was significantly reduced to 3-4L/ha and still achieved excellent control efficacy as part of a Dual Spray. The following areas should be tested to further improve control efficacy or to reduce control costs:

- Identifying an optimum spraying window for late application between late March and early May
- Reducing the rate of picloram based products, combined with the best application timing
- Exploring non-residual herbicide combinations

Glasshouse-based translocation studies will complement the above field research to identify the best timing and method of herbicide application for rootbank control, which is expected to result in either a 30% increase in herbicides translocation into the rootbank or increase control by 30%. This research will aim to answer the hypotheses that targeted herbicide application will provide increased perennial weed control, increased profitability and increased environmental benefits.

2.3. Bio-economic modelling to assist the adoption process (10%):

Current weed population models are developed to simulate population dynamics for annual weeds derived from seedbanks only, which are not applicable to the perennial weeds such as SLN and PGC which are derived from rootbanks as well as seedbanks. The bio-economic model developed by Dr Randall Jones (2006) will be modified to include the rootbank to simulate the population dynamics of these perennial weeds. The field data collected in the two long-term research sites and 65 advocate grower demonstration sites will be used to parameterise the model. The model will be a valuable decision support tool for growers and advisors to assess the long-term impact of BMP strategies on SLN/PGC population dynamics and farm economics, based on the history of cropping systems, weed management and herbicide uses.

The Project Team will work closely with Dr Tom Nordblom and Dr De Li Liu to determine the economic aspects of the long-term best management practices recognising population dynamics. Benefit-cost analysis will be performed to determine the potential return as a result of adopting the best management packages.

#### Interest (IP proportions)

MLA	46%
Research Organisation	54%

# Milestones

Milestone	Achievement criteria	Due date	
1	On signing	01/01/2010	
	Project planning meeting:		
	<ul> <li>BMP delivered to 100 public and private partners, noxious weeds advisory groups, local councils, spray contractors</li> </ul>		
	<ul> <li>Public and private partners actively promote the BMP to the end users</li> </ul>		
2	<ul> <li>Operational plan developed describing key research and delivery tasks and success measures for the first year</li> </ul>	31/05/2010	
	- Communication plan outlined to target the partner organisations		
	- Two multiple-year research sites selected and established in NSW		
	<ul> <li>Ten advocate growers more detailed on-farm demonstrations established</li> </ul>		
	Annual report received and accepted by MLA:		
	- Full financial statement at October 2010		
	- Progress and achievements against the operational plan		
	- Summary of analyses to date		
3	<ul> <li>Communication plan implemented a target of 500 advisors and growers</li> </ul>	30/11/2010	
	- One presentation to Noxious weeds advisory groups		
	- 400 participating growers trialling the BMP package		
	<ul> <li>45 advocate growers identified for more detailed on-farm demonstrations</li> </ul>		
	Report on success of operational plan success measures including:		
4	- Communication plan implemented		
	<ul> <li>Operational plan developed describing key research and delivery tasks and success measures for the second year</li> </ul>	31/05/2011	
	<ul> <li>Population dynamics model completed and available to advisors and growers for validation</li> </ul>		
	<ul> <li>A total of 65 advocate growers on-farm demonstrations established</li> </ul>		

5	Annual report received and accepted by MLA:	
	<ul> <li>Communication plan implemented to a target of 800 advisors and growers</li> </ul>	30/11/2011
	- Full financial statement at November 2011	
	- Progress and achievements against the operational plan	
	- Summary of analyses to date	
	- A total of 2 presentations to Noxious weeds advisory groups	
	Report on success of operational plan success measures including producers engaged and progress towards change targets	
6	Evaluation plan in place to demonstrate success at the project conclusion	31/05/2012
0	- Operational plan developed describing key research and delivery tasks and success measures for the third year.	
	- Benefit/cost analysis completed	
	- Herbicide translocation study completed	
	Final Report received and accepted by MLA:	
	- Full financial statement	
7	<ul> <li>Change on farm and the commitment of 400 producers to continue the implementation of BMP</li> </ul>	
	- Over 20,000 ha of SLN/PGC-infested areas under improved management 30/11/201:	
	<ul> <li>Communication plan implemented to a target of 1000 advisors and growers</li> </ul>	
	- A total of 3 presentations to noxious weeds advisory groups	
	- Three scientific papers and conference papers submitted	
	- Technical report completed and submitted to MLA	

# Project team

Name	Organisation	Skills	% time allocated	% Salary funded by MLA
Dr Rex Stanton CSU		Research Fellow	100	100
Casual Assistant	I&I NSW		10	100
Prof. Deirdre Lemerle	CSU/ I&I NSW	IWM	10	0
Dr Geoff Burrows	CSU	Plant Anatomy	5	0
Dr Hanwen Wu	I&I NSW	IWM	20	0
Dr Tom Nordblom	I&I NSW	Economics	10	0
Dr De Li Liu I&I NSW		Modelling	10	0
Dr Neil Coombes I&I NSW		Biometrics	5	0
Ms Birgitte Verbeek	I&I NSW	Weed Extension	5	0
Mr Bob Thompson	I&I NSW	DA-West Wyalong	10	0
Ms Karen Roberts	I&I NSW	DA-Parkes/Forbes	5	0
Mr Greg Brooke	I&I NSW	DA-Wellington	5	0
Ms Rachael Whitworth	I&I NSW	DA-Griffith	5	0
Ms Kathi Hertel I&I NSW		DA-Dubbo	5	0
Ms Janet Walker	I&I NSW	DA-Albury	5	0
Ms Mary-Anne Lattimore	I&I NSW	DA-Yanco	10	0

# Nominated Person(s)

Title/First	Dr Hanwen Wu
Name/Surname	E.H. Graham Centre for Agricultural Innovation. Industry & Investment
Mailing Address	NSW, Pine Gully Rd, Wagga Wagga, NSW 2650
Phone Number	02 6938 1602
Facsimile Number	02 69381 861
Email Address	hanwen.wu@industry.nsw.gov.au

Project Budget and Funding – Indicative Budget OR Cash Flow Budget (complete only one)

#### Indicative Budget

The Project budget is recorded as **GST exclusive**. MLA will pay GST, in addition to the budget, on presentation of a tax invoice from the Research Organisation.

	2009-10	2010-011	2011-12	TOTAL
	\$	\$	\$	2009-12
Salaries and on-costs	\$97,832	\$101,745	\$105,815	\$305,391
Travel, including I&I NSW extension personnel	\$8,400	\$8,400	\$8,400	\$25,200
Operating (NSW)	\$36,530	\$36,530	\$36,530	\$109,590
3 Grower groups (\$2000 each)	\$6,000	\$6,000	\$6,000	\$18,000
Incentive program for advocate growers and weeds officers	\$5,700	\$5,700	\$5,700	\$17,100
CSU/ NSW DII Service Levy	\$7,723	\$7,919	\$8,122	\$23,764
TOTAL BUDGET (excl. GST)	\$162,185	\$166,294	\$170,567	\$499,045

#### **Detailed Budget justification:**

#### Salaries and On-costs

Salaries and on-costs for one Research Fellow and one Casual Assistant (10%) are requested. The research fellow is to undertake the planning and execution of the experimentation and extension activities in consultation with the research team, collect and collate data and do preliminary data analysis, and co-author conference and scientific papers, technical publications, and media releases. The causal Assistant is required to meet seasonal peak demand and assist the management and measurement of all trial sites across NSW. The Assistant is also expected to assist all extension activities and workshops.

# Travel budget:

- Travel budget is allowed for Dr Rex Stanton or Dr Hanwen Wu to travel to grower participatory sites, including field days @ \$2000 pa.
- Qfleet for I&I NSW extension personnel to organise and attend workshops, field days, farm walks, project meetings and conferences, as well as meeting their responsibilities: \$800 x 8 = \$6,400 pa.

# **Operating Costs:**

- Hire of laptop computer, software and IT support @ \$1,100 pa.
- Two long-term research field sites in NSW: set up, maintain, and measure treatment responses at two sites. Funds required to establish and to maintain trials are estimated at \$2,000. Travel expenses to field sites that are distanced 150 and 500 km from Wagga, including 2 truck visits @ \$2/km (\$5,200) and 5 ute/sedan visits @ 58c/km (\$3,770), for an overall total of \$10,970 pa.
- Five growers' sites in NSW: set up, maintain, and measure treatment responses at five sites. Funds required to establish and to maintain trials are estimated at \$3,500. Travel to field sites that are distanced 100, 300 and 500 km from Wagga. Growers are expected to manage any sowing operations. Expenses include 8 ute/sedan visits @ 58c/km (\$6,960) for site monitoring and measurements, totalling \$10,960 pa.
- Herbicide uptake and translocation studies: examining application and translocation of <sup>14</sup>C-labelled starane, a product which shares a similar chemical structure to picloram (<sup>14</sup>C-labelled picloram is not available). Funds are required for consumables and instrument hire costs (biological combustion and liquid scintillation spectrometry at the University of Adelaide) @ \$5,000 pa.
- Field days to be held every year in conjunction with the Noxious Weeds Officers and farmer groups, \$3,000 pa.
- Publication cost estimates include costs of reprints of scientific papers (\$1,000), extension materials (\$1,000), giving a total of \$2,000 pa.
- Workshop cost estimates include costs of venue hire, handouts and catering: \$2,000 pa.
- General expenses for expendable items such as seed, herbicides, fertilisers, office, computer and laboratory consumables. An allowance is made for general equipment maintenance, including spray gear, sowing equipment, vehicles etc., valued @ \$2,000 pa.

#### CSU/I&I NSW Service levy (5%):

Five percent of the budget will be charged for services costs to cover library services, office space and general infrastructure and biometrical support.

#### Growers groups:

Financial assistance will be provided to 3 grower groups in monitoring 12 growers demonstration sites and meeting the specified responsibilities:  $2000 \times 3 = 6,000 \text{ pa}$ .

Advocate growers and noxious weeds officers:

Incentive program will be introduced to motivate the participation of growers and weeds officers:  $60 \times 95 = 5,700$  pa.

#### In-kind contribution:

In-kind contributions are calculated based on the % time commitment in the project from Prof. Lemerle (10% -IWM), Dr Burrows (5% - Plant anatomy), Dr Wu (20% - IWM), Dr Nordblom (10% - Weed Risk Model and Benefit/cost analysis), Dr Liu (10% - Weed modelling), Ms Birgitte Verbeek (5%, extension), Mr Bob Thompson (10%, extension), Ms Karen Roberts (5%, extension), Mr Greg Brooke (5%, extension), Ms Rachael Whitworth (5%, extension), Ms Kathi Hertel (5%, extension), Ms Janet Walker (5%, extension), Ms Mary-Anne Lattimore (10%, extension) and Dr Neil Coombes (5% - Statistical analysis).

#### In-kind contribution breakdown

		09-10	10-11	11-12	Total
CSU	Salary	\$11,577	\$12,040	\$12,521	\$36,138
	Non-salary	\$3,241	\$3,371	\$3,506	\$10,119
I&I NSW	Salary	\$116,416	\$121,213	\$126,349	\$363,978
	Non-salary	\$57,481	\$57,481	\$57,481	\$172,444

Total

\$582,678