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Assessing the fundamental host-range of *Leptinotarsa texana* Schaeffer as an essential precursor to biological control risk analysis

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ABSTRACT

The leaf beetle *Leptinotarsa texana* Schaeffer (Coleoptera: Chrysomelidae) was introduced to South Africa from the USA to control silverleaf nightshade *Solanum elaeagnifolium* Cav. (Solanales: Solanaceae). Subsequent post-release studies in South Africa found the beetle to be an effective, host-specific biocontrol agent of *S. elaeagnifolium*. *Leptinotarsa texana* has potential for biological control of *S. elaeagnifolium* in other countries where the weed adversely impacts agricultural production, including Australia. However, *L. texana* can only be introduced to Australia if risk analysis demonstrates that the agent poses a negligible or very low risk to the environment and economy. We initiated an assessment of the agent's possible impact in Australia by prioritising non-target species, and conducting quarantine laboratory experiments on 49 Australian native species and economically important plant species and cultivars. We observed feeding damage greater than 50% leaf area removed on plants of 12 Australian *Solanum* spp. and two crop species (potato *Solanum tuberosum* L. and eggplant *Solanum melongena* L.). *Leptinotarsa texana* successfully developed from first instar larva to adult on 15 Australian *Solanum* spp. and two crop species (a single eggplant cultivar and four potato cultivars). When given a choice of *S. elaeagnifolium* and 10 Australian *Solanum* spp. in a large cage experiment, *L. texana* oviposited on *S. elaeagnifolium* and three of the non-target Australian *Solanum* spp. We consider possible reasons why potato was not found to be within the beetle's fundamental host-range in previous host-specificity experiments, and argue there are important differences between potato cultivars that have implications for future host-specificity testing. We outline priorities for further risk analysis, and criteria for assessing the feasibility of these approaches in different contexts.

1. Introduction

Exotic arthropods proposed for classical biological control of weeds pose risks to non-target species that must be assessed prior to introduction. It has been asserted that non-target plants most at risk of direct impacts are those most closely related to the target weed (Briese & Walker, 2002; Hsiao, 1974; Wapshere, 1974), because they are more likely to stimulate feeding and oviposition behavior of arthropods that utilise the target (Wapshere, 1989). *Host-specificity testing* attempts to elicit an arthropod's feeding and oviposition behavior by exposing it to closely related and other non-target plants in controlled experiments either under quarantine, usually within cages in a quarantine laboratory, or in the agent's native or introduced range (Marohasy, 1998). Host-specificity testing conducted in a laboratory does not replicate field conditions but can inform risk analysis by describing which native species or valued exotic plants are within the *fundamental host-range* of the agent. The fundamental host-range *"…includes all the plant species that an insect is capable of accepting and/or utilizing"* (van Klinken, 2000). Fundamental host-range can be described for the entire life-history of an arthropod, or for aspects of the life-history where the arthropod and host interact, for example, the larval stage of an insect (van Klinken, 2000). Describing the fundamental host-range is an important step in many biological control agent risk assessments because there is a reduced risk of off-target damage to plants outside the fundamental host-range (van Klinken, 2000). Risk assessment can then focus on plants within the fundamental host-range to predict the *realized host-range*

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(also called *field host specificity*); the range of hosts utilized under field conditions (van Klinken, 2000).

For targets with many closely-related species, it is not practical to implement host-specificity testing for all closely-related plants, even at the genus level. In these cases, non-target species for host-specificity testing must be prioritised based on criteria such as phylogenetic affinity to the target, biogeographic overlap and ecological similarity (Briese and Walker, 2002; Wapshere, 1974). Deciding which cultivated plant varieties (cultivars) to include on the host specificity test plant list (the list of plant species prioritised for testing) is similarly important because some crop and ornamental species have many cultivars, and cultivars within a species complex may vary in their genetic make-up and susceptibility to insect damage (Douglas, 2018; Pelletier et al., 2011).

This challenge of selecting a relevant and feasible list of plants for host-specificity testing applies to the biological control of silverleaf nightshade *Solanum elaeagnifolium* Cav. (Solanaceae) in Australia. *Solanum elaeagnifolium* is a deep-rooted perennial herb native to northern Mexico, south western USA and South America (Knapp et al., 2017) that is now invasive in many parts of the world including Australia, South Africa, North Africa and southern Europe (AWC, 2012; Brunel, 2011; EPPO, 2007; Knapp et al., 2017; Mekki, 2007). All parts of the plant are toxic to livestock, and its extensive root system depletes soil moisture and nutrients, and outcompetes pasture and crops (Boukhris-Bouhachem et al., 2007; Brunel, 2011; EPPO, 2007; Green et al., 2017; Mekki, 2007). *Solanum elaeagnifolium* can indirectly impact reproduction of co-flowering native species in Greece (Tscheulin and Petanidou, 2013), but its impact on native vegetation in Australia is not known.

Management of *S. elaeagnifolium* in Australia relies on chemical application to control small infestations or slow the spread of larger infestations. However, options for reducing the extent and impacts of large infestations are limited because herbicides are expensive, require repeated application, or have off-target effects on crop and pasture species (AWC, 2012). Without effective control methods for large infestations, the economic and environmental impacts of *S. elaeagnifolium* continue to accrue (Kwong, 2006; Stanton et al., 2009). Biological control is therefore proposed as an additional tool to help manage *S. elaeagnifolium* in Australia (SCA, 1986), despite the challenge of testing a large number of Australian native and economically important *Solanum* species.

The North American leaf beetle *Leptinotarsa texana* Schaeffer (Coleoptera: Chrysomelidae) has considerable potential for biological control of *S. elaeagnifolium* in Australia. *Leptinotarsa texana* was introduced to South Africa in the 1990s, where it is reported to be an effective and host-specific biocontrol agent of *S. elaeagnifolium* (Olckers et al., 1999; Olckers and Hulley, 1994; Olckers et al., 1995). Plants from 37 native and exotic species were selected for host-specificity testing in South Africa (Olckers et al., 1995). Eggplant *Solanum melongena* L. and five native South African species were found to be within the fundamental host-range of *L. texana*. Importantly, two major crop species in the genus *Solanum*, potato *Solanum tuberosum* L. and tomato *Solanum lycopersicum* L., were considered not at risk. Plants within the fundamental host-range became the focus of further risk assessment, resulting in approval to introduce *L. texana* to South Africa (Olckers and Hulley, 1994).

The main objective of this research was to determine which non-target species could be at risk if *L. texana* were to be released in Australia. Firstly, we identified non-target species in Australia and prioritized plant taxa considered to be most at risk (Briese and Walker, 2002; Wapshere, 1974), ensuring broad representation across the genus *Solanum*. Secondly, we presented *L. texana* to the non-target plants considered most at risk in quarantine laboratory experiments. We measured feeding and development from first instar through to adulthood under no-choice conditions, and oviposition by adults provided with a choice of target and non-target plants. Plant taxa capable of supporting feeding and development of *L. texana* larvae, or feeding and oviposition by adults, were determined to be within the insect's fundamental host-range and recommended for further risk assessment.

2. Materials and methods

2.1. Insect culture

Leptinotarsa texana adults (n = 152) were imported into quarantine in Australia from South Africa on 14 April 2016 (Permit and quarantine registration details in Supplementary Material, Table S1). South African populations of *L. texana* are believed to have originated from Texas, USA (Helmuth Zimmermann pers. comm.). Prior to shipment, beetles were reared for at least one generation on *S. elaeagnifolium* in laboratory cages at Rhodes University, South Africa (−33.310122, 26.518409) to reduce the risk of importing contaminants such as parasitoids. On arrival, *L. texana* adults were placed onto either *S. elaeagnifolium* plants in pots or bouquets of foliage inside fine gauze insect cages (400 × 400 × 400 mm) in a controlled environment room (25 °C, 16 h light:8 h dark). New pots or bouquets were added to cages as required. Eggs were collected into Petri dishes containing moistened filter paper using a fine camel hair brush two to three times each week. Petri dishes were then sealed with parafilm and incubated at 25 °C, 16 h light:8 h dark until egg hatch. Newly emerged larvae were transferred either to a larval rearing cage, or to plants used in no-choice experiments, eliminating the potential effect of natal host on larval host preference (Izzo et al., 2014). Pupation occurred in potting mix or in a substrate of washed sand and sphagnum peat moss in some rearing cages.

2.2. Identification of Leptinotarsa texana

Adult *L. texana* were identified by morphological features and differentiated from other species, including the significant crop pest *Leptinotarsa decemlineata* (Say), using online diagnostic resources (PaDIL, 2019; SPHDS, 2013). We also conducted genetic tests for the potential presence of cryptic species in the imported beetles to avoid any confounding results of the host specificity testing. Larvae raised from our imported beetles were DNA barcoded (Hebert et al., 2003) using methods (Gopurenko et al., 2013) modified here (Supplementary Material, Method S2), and compared to available DNA barcodes of five *Leptinotarsa* species (*L. decemlineata* [N = 31], *L. haldemani* (Rogers) [N = 3], *L. juncta* (Germer) [N = 2], *L. lineolata* (Stål) [N = 1], *L. texana* [N = 2] (listed in Supplementary Material, Table S3).

2.3. Test plant selection

In the first instance, the selection of plants for the test list followed the principles of the centrifugal phylogenetic method and its refinements (Briese and Walker, 2002; Wapshere, 1974) with an emphasis on phylogenetic affinities, especially within the genus *Solanum* in Australia. However, relationships within *Solanum*, and between *Solanum* and other genera, are uncertain. For example, it is unclear how *S. elaeagnifolium* is related to the 163 Australian species in the Leptostemonum Clade - there are differing interpretations of available evidence and this knowledge is still developing. Subgroups of Leptostemonum based on morphological and micro-morphological criteria (Bean, 2004; Whalen, 1984) are only partly supported by more recent molecular phylogenetic studies (Aubriot et al., 2016; Levin et al., 2006; Särkinen et al., 2013; Vorontsova et al., 2013) (Supplementary material, Table S4). Furthermore, these global or regional studies generally include relatively few Australian species (a maximum of 18 of the 163 currently recognized species) and can be used only as a partial guide. We therefore adapted the existing scheme of groupings in *Solanum* based on morphological features and compared those to recently published results from DNA sequencing. We diversified our sampling, rather than restrict it to species considered most closely related to *S. elaeagnifolium*,

to better account for taxonomic and phylogenetic uncertainty (sensu lato Cabrera et al., 2017). This approach generated a large (and impractical) list of plant species potentially at risk, which was therefore prioritized for testing using the following criteria:

1. Biogeographic overlap and ecological similarity (Briese and Walker, 2002).
2. Conservation status; *Solanum karsense* Symon was included as it was the only native species listed nationally for conservation status as vulnerable (Threatened Species Scientific Committee, 2010).
3. Economic importance as a food crop; Solanaceae (including at least three species of *Solanum*) are of exceptional significance as food plants, are grown on an extensive scale commercially and are of major economic importance.
4. Native species commercially grown as part of an emerging native food crop (known as "bush tucker") industry.
5. Australian *Solanum* species important in Aboriginal culture.
6. Bridging hosts; *Solanum torvum* Sw., an exotic weed species, was included as a potential host (Cuda et al., 2002) that could facilitate the movement of *L. texana* from *S. elaeagnifolium* infested areas to eggplant growing regions of northern Queensland.

Subjective rankings of high, medium and low priority were applied based on these criteria (Supplementary material, Table S4). The prioritized list was continually reviewed to ensure that all recognized informal taxonomic groups within *Solanum* were represented for the geographical extent of *S. elaeagnifolium* and predicted extent of *L. texana* (Senaratne et al., 2008), as were all known clades recognized by available molecular phylogenetic studies. If propagating material of preferred species was not obtained, material of closely related substitute species was collected. When important crop cultivars responded differently as hosts, related or similar cultivars were added to the list.

2.4. Sourcing and propagating test plants

Most Australian *Solanum* spp. tested were propagated from seed collected in the field (to maximize genetic variability), or from field-collected cuttings where seed proved unavailable (Supplementary material, Table S4). Three field trips were undertaken between April 2016 and July 2017, each timed and planned (in terms of geographic area) to optimize the number and subgroup/clade coverage of species as well as the chance of obtaining fresh, viable seed. Seeds and cuttings were also obtained opportunistically during other travel and from colleagues. Ornamental and crop cultivars were obtained from industry contacts or purchased from commercial nurseries as seed, tubers (certified seed potato) or young potted plants.

Solanum elaeagnifolium plants used for rearing and experiments were grown in a glasshouse or screenhouse from seed (sourced from various locations in the states of Victoria, South Australia and New South Wales) or root fragments.

2.5. Plant identification, vouchering and documentation

Propagation material of all wild-collected species was supported by voucher specimens lodged mostly in the State Herbarium of South Australia; for material collected in other states duplicates were collected for deposition in the principal herbarium of the state where they were collected. Plant material was identified by LH, a Solanaceae specialist. In the case of plants and propagules from other sources, vouchers were usually available and deposited in a registered herbarium. In a few cases, specimens were made from propagated plants for deposition in the State Herbarium of South Australia.

2.6. No-choice experiment with larvae

We applied a conservative no-choice test design to minimize the possibility of false-negative results on the plants tested (Marohasy, 1998). Newly emerged (1–2 day old) larvae were exposed to an individual potted test plant (a *S. elaeagnifolium* plant or non-target plant) in replicated no-choice experiments. Five, six or ten newly emerged larvae (depending on availability) were collected from egg incubation dishes and confined on individual plants using a fine gauze sleeve. Test plants were set up only when the availability of healthy test plants and sufficient numbers of first instar larvae coincided. At least one *S. elaeagnifolium* control was set up on each day that non-target species were tested to compare *L. texana* survival on a known host. All no-choice experiments with larvae were conducted in a controlled environment room (25 °C, 16 h light:8 h dark) where they remained for at least six weeks to allow time for feeding and complete development (Olckers et al., 1995). Plants were watered regularly into saucers to minimize disturbance to insects.

Foliar damage on each plant was assessed two to three times each week and scored as 0 = no damage; T = trace damage or almost no damage (~0.1%); 1 = 0.1–10% leaf area consumed; 2 = 10–50% leaf area consumed; 3 = > 50% leaf area consumed.

In addition to host suitability, foliar damage estimates were potentially influenced by plant leaf area, the number of larvae placed on each plant and plant health (especially impacts of greenhouse pests). For example, two equally acceptable non-target plant species could have different damage scores if a plant with small narrow leaves (which could be consumed quickly resulting in a high damage score) was compared to another plant species with large broad leaves (which would take longer or require more larvae to consume, potentially resulting in a lower damage score). Plants were therefore pruned when necessary to approximately equalize plant leaf area. We also attempted to apply equal numbers of larvae to each plant, but numbers varied occasionally depending on the availability of newly-emerged larvae.

Development of larvae was noted if observed, and the number of adults emerged on each plant was counted after six weeks.

2.7. Choice experiments with adults

We conducted choice experiments with adults using two experimental designs, (1) choice minus target in small cages, and (2) choice (with target) in a large walk-in cage (Sheppard et al., 2005).

2.7.1. Small-cage choice minus target experiments with adults

2.7.1.1. Experiment 1: Australian Solanum spp.. Individual potted plants of four different non-target Australian *Solanum* spp. were randomly placed in a 400 × 400 × 400 mm fine gauze cage (four plants per cage). Six newly emerged adult beetles (i.e. emerged in the previous 1–3 days) that were not exposed to host material were collected into a Petri dish with a lid. Mating pairs of adults were selected if observed, otherwise adults were selected according to size (three large and three small adults) as genitalia are difficult to observe in live *L. texana* (Olckers et al. (1995) found that females are larger than males). The Petri dish containing three pairs of adults was placed in the center of the cage and the lid removed. Three pairs of adults were similarly collected and placed into a cage containing a single *S. elaeagnifolium* plant (target). The number of eggs on each plant, feeding damage, and location of adults was assessed two to three times each week for six weeks. Cages were held in a controlled environment room (25 °C, 16 h light:8 h dark).

2.7.1.2. Experiment 2: horticultural Solanum spp.. A separate experiment was conducted on four potted non-target horticultural species using the method described above.

2.7.2. Large-cage choice experiment with adults

Ten potted Australian *Solanum* spp. that supported feeding and development of larvae in no-choice experiments and a single *S. elaeagnifolium* plant were placed equidistant in a 2 m diameter circle in

a large insect-proof tent (3 m × 3 m × 1.8 m) in a quarantine glasshouse. Eight mating pairs of adults, emerged in the previous week and exposed only to *S. elaeagnifolium*, were collected in a Petri dish and placed in the center of the cage. Plants were rotated one position clockwise two to three times each week. The number of eggs on each plant, feeding damage, and location of adults was recorded two to three times each week for eight weeks. Eggs were collected from target and non-target plants on six separate days and incubated in Petri dishes, under conditions described above, and percentage egg hatch was recorded.

2.8. Statistical analysis

We considered the no-choice experiment with larvae to be a blocked design, with date of larval application to plants corresponding to blocks and the unit of analysis being a single replicate of an individual taxon. We analyzed adult emergence data using a general logistic model, with binomial errors and estimated overdispersion parameter, with additive terms (on the logistic scale) for date of larval application and plant taxa. To avoid under-estimation of the overdispersion parameter, the overdispersion parameter estimate and residual degrees of freedom were obtained from a model of the same form, but which excluded data from all plant taxa with no observed adult emergence. Although the final analysis used the overdispersion parameter and residual degrees of freedom from this restricted data set, we used all data to fit the general logistic model.

For the purpose of statistical analysis, we divided taxa into nine functional groups, namely i) target *S. elaeagnifolium*, ii) Australian *Solanum*, iii) other Australian Solanaceae, cultivars of iv) potato, v) *Capsicum*, vi) eggplant, vii) tamarillo, viii) tomato and finally ix) exotic Solanaceae. Preliminary examination of the data indicated that no adults emerged on taxa in the groups 'other Australian Solanaceae', 'exotic Solanaceae', '*Capsicum*' or 'tomato'. Also, only one cultivar was tested from each of the groups 'eggplant' and 'tamarillo'. We therefore decided to divide the plant taxa into effects for i) taxa groups, ii) species of Australian *Solanum*, iii) cultivars of potato and iv) other species/cultivar/accession effects within taxa groups. This combined fourth term jointly examined for i) differences between other Australian Solanaceae, ii) differences between exotic Solanaceae, iii) differences between *Capsicum* cultivars, iv) differences between tomato cultivars and v) accession differences of *Solanum cleistogamum*. The resultant Analysis of Deviance (an analog of Analysis of Variance for logistic models) is presented in Table 1.

We presented adult emergence results as odds ratios of the probability of emergence for taxa or taxa groups compared to *S. elaeagnifolium*. We obtained 95% confidence intervals for the odds ratios by using a t distribution for the differences between the estimates of the taxon (or taxa group) and *S. elaeagnifolium* on the logistic scale, and then using an exponential transformation on the derived confidence limits. We conducted our analysis using the generalized linear facilities of Genstat 18 (VSN International, 2015).

We presented feeding damage scores for the no-choice experiment with larvae as dot plots in order to display the entire dataset. Choice experiments with adults were stopped early (before adequate replication, see Section 3.4). We report our observations but limit our inferences to avoid Type II errors (i.e. failing to detect a genuine effect of, or preference for, non-target species).

3. Results

3.1. Genetic analysis of imported beetle progeny

Two novel DNA barcode haplotypes were evident among sequences of 91 early instar beetles bred from the imported quarantine population and reported at GenBank under accessions MK288007 and MK288008. The two haplotype sequences marginally differed from each other (< 0.456%), and each is closest in genetic match (> 99.848% sequence similarity) to GenBank accessions of *L. texana* sampled from its type region in Texas, USA. DNA barcodes of four other *Leptinotarsa* species differed from the novel haplotypes by 5.649–16.565% sequence difference, including the economically important pest *L. decemlineata* (Colorado potato beetle) which differed by > 12.459%. Maximum Likelihood phylogeny relationships among the two haplotypes and all comparable sequences (N = 43) of five *Leptinotarsa* species are shown in Fig. 1, where the two novel haplotypes each have closest genetic relationships to intraspecific variants of *L. texana*, and distant relationships to other *Leptinotarsa* species.

3.2. Test plant selection, identification, vouchering and documentation

Our initial list of Solanaceae, including the target *S. elaeagnifolium*, comprised 212 species of *Solanum* and 20 species of other Solanaceae (Supplementary material, Table S4). A final test list of 59 species (including *S. elaeagnifolium*) consisted of all high and some medium priority species. From this list we obtained and cultivated 53 species, including four cultivars of tomato *S. lycopersicum*, eight cultivars of potato *S. tuberosum* and four cultivars of *Capsicum annuum* L. (giving a total of 63 accessions in cultivation). Of these, we tested 36 species: 31 species of *Solanum* and five species of other Solanaceae, or a total of 49 accessions including cultivars (Table 2). We planned to include Australian populations of *S. torvum* in host-specificity tests, as this species was reported to be within the fundamental host-range of *L. texana* (Cuda et al. 2002). However, a consequence of the expanded fundamental host-range of *L. texana* to include Potato and Archaesolanum (Section 3.3) was that research into *L. texana* was terminated before *S. torvum* was grown and tested.

3.3. No-choice experiment with larvae

Leptinotarsa texana larvae utilized some non-target species in no-

Table 1
Analysis of deviance of probability of development to adult of each larva. P values less than 0.05 are in bold.

Term	Degrees of freedom	Mean deviance	F ratio	P value
Date of larvae placement	35	5.88	2.24	**0.0016**
Taxa group[a]	8	43.52	16.55	**3.4×10^{-14}**
Species of Australian *Solanum* (adjusted for cultivar of potato)	22	7.07	2.69	**0.00069**
Cultivar of potato (adjusted for species of Australian *Solanum*)	7	11.18	4.25	**0.00049**
Other species/cultivar/accession effects within taxa groups[b]	11	0.76	0.29	0.99
Residual deviance[c]	80	2.63		

[a] Nine taxa groups i) target *S. elaeagnifolium*, ii) Australian *Solanum*, iii) other Australian Solanaceae, cultivars of iv) potato, v) *Capsicum*, vi) eggplant, vii) tamarillo, viii) tomato and ix) exotic Solanaceae.

[b] Three species of other Australian Solanaceae; four cultivars of *Capsicum*, four cultivars of tomato; three species of exotic Solanaceae; two accessions of *Solanum cleistogamum*.

[c] Calculated using only those cultivars/accessions that had some emergence to avoid systematic underestimation of residual deviance.

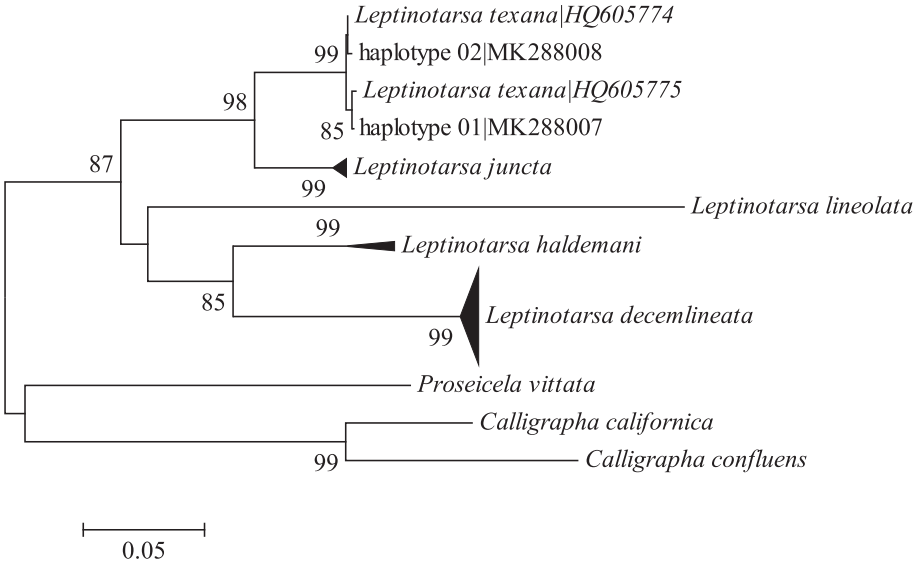

Fig. 1. Phylogenetic relationships among haplotypes (01 & 02) from quarantine beetle larvae (progeny of imported beetles) (N = 91) and DNA barcode sequences of *Leptinotarsa* species (refer Supplementary material: Table S3 for associated GenBank sequence accessions). Phylogeny reconstructed by Maximum Likelihood (ML), refer Supplementary material: Method S2. Bootstrap clade supports > 70% as indicated (N = 100 replicates). Terminal clades collapsed for *L. decemlineata*, *L. haldemani* and *L. juncta*. ML tree outgroup rooted using (Chrysomelinae: Doryphorini) leaf beetles: *Proseicela vittata* (Fabricius), *Calligrapha confluens* Schaeffer and *Calligrapha californica* Linell. Scale bar indicates the number of substitutions per site (GTR + G + I model adjusted).

choice experiments (Tables 1 and 2, Fig. 2). Feeding damage greater than 50% leaf area removed was recorded on plants of twelve Australian *Solanum* spp. and two crop species (potato *S. tuberosum* and eggplant *S. melongena*) (Fig. 2). *Leptinotarsa texana* successfully developed from first instar larva to adult on seventeen Australian *Solanum* spp., including the native species *S. karsense* listed nationally for conservation status as vulnerable (Threatened Species Scientific Committee, 2010), and the crop species *S. tuberosum* and *S. melongena* (Table 2). After observing feeding and development on the *S. tuberosum* cultivar 'Nadine' we preferentially selected cultivars related to 'Nadine' and conducted additional no-choice experiments. We found cultivars related to 'Nadine' also supported *L. texana* feeding and development in no-choice laboratory experiments (Table 2, Fig. 2).

3.4. Choice experiments with adults

Leptinotarsa texana's expanded fundamental host-range (Section 3.3) meant further, lengthy cage experiments were unlikely to produce evidence to support introduction to Australia. Choice experiments were therefore stopped early, before they were adequately replicated. Despite this limitation, we observed feeding and oviposition on non-target species in both small-cage and large-cage choice experiments. We report these observations but limit our inferences from these data.

3.4.1. Small-cage choice minus target experiment with adults

3.4.1.1. Experiment 1: Australian Solanum spp.. *Leptinotarsa texana* was observed on all plants and on cage walls. Eggs were laid on the target *S. elaeagnifolium* and the Australian native *Solanum chenopodinum* F. Muell., while feeding damage was recorded on the target and each of the four Australian *Solanum* species (Table 3).

3.4.1.2. Experiment 2: Horticultural Solanum spp.. Feeding and egg-laying was recorded on *S. melongena* 'Black Beauty'. Eggs were observed on *C. annuum* 'California Wonder' on one day but were missing at the next assessment (9 days later) and were possibly cannibalized by adults (a behavior we observed in rearing cages). No larvae or additional feeding damage were subsequently recorded on *C. annuum* 'California Wonder'. Adults were mostly observed on *S. melongena* 'Black Beauty' or cage walls. No eggs were recorded on the target *S. elaeagnifolium;* possibly because no females were introduced to the cage, or because rapid defoliation of the target led to egg-dumping on cage walls (observed only).

3.4.2. Large-cage choice experiment with adults

Leptinotarsa texana fed and oviposited on *S. elaeagnifolium* and three non-target Australian plants that were previously shown to support larval development; *Solanum petrophilum* F. Muell., *S. cleistogamum* Symon and *S. aridicola* A. R. Bean (Table 4). Egg-laying and summed adult observations over a period of eight weeks were highest on *S. petrophilum* and *S. cleistogamum.* No adults were observed on *S. cleistogamum* during a six-week period between initial feeding and subsequent egg-laying, suggesting females didn't simply settle and remain on the first plant encountered (data not shown). Larvae emerged from eggs deposited on the three non-target species (Table 4). Feeding damage, but no egg-laying, was recorded on the nationally-listed vulnerable species *S. karsense*.

4. Discussion

Solanum weeds are difficult targets for classical biological control in Australia because of the large number and importance of closely related plants that could be at risk of off-target damage (Bean, 2004; Levin et al., 2006; Purdie et al., 1982; Symon, 1981). The centrifugal phylogenetic method, and later refinements of the method, provide guidance on risk-based selection of plant species for biological control host-specificity testing (Briese and Walker, 2002; Wapshere, 1974). However, a challenge when selecting non-target species to test for this study was the considerable uncertainty surrounding phylogenetic relationships. We compiled a host-specificity test plant list representing major clades, morphologically-based groupings and clade groupings based on molecular phylogenetic studies within Australian *Solanum*. To this we added major crop species, certain weedy species (such as potential bridging species) and some representatives of other genera.

Using quarantine no-choice experiments with larvae and choice experiments with adults we attempted to circumscribe the fundamental host-range of *L. texana,* thereby reducing the number of plant species requiring further risk assessment (Wapshere, 1989). While restricted to the genus *Solanum*, our results expanded the known fundamental host-range of *L. texana* to include kangaroo apple *Solanum aviculare* G. Forst. in the major clade Archaesolanum and five cultivars of potato *S. tuberosum* in the major clade Potato (Särkinen et al., 2013; Symon, 1994). Also included were 16 Australian *Solanum* spp. from the major Leptostemonum clade (to which *S. elaeagnifolium* also belongs) (Levin et al., 2006). Feeding and development on eggplant and at least some Australian Leptostemonum was anticipated because *L. texana* was shown to utilize eggplant and five African Leptostemonum species in no-choice experiments in South Africa (Olckers et al., 1995). In contrast, we did

Table 2

Leptinotarsa texana adult emergence on *Solanum elaeagnifolium* and non-target plants after six weeks under no-choice conditions (neonate larvae applied). Odds ratio between 0 and 1 indicates the probability of emergence is less for the taxon than *S. elaeagnifolium*; a result of 1 indicates that the probability of emergence is the same for the taxon and *S. elaeagnifolium*, and a result between 1 and infinity indicates the probability of emergence is greater for the taxon than for *S. elaeagnifolium*.

Major clade	Group	ID	Species and cultivar (if applicable)	Number of reps	Number of larvae applied	Number adults emerged	Percent adult emergence	Odds ratio of adult emergence compared to target	95% confidence interval of odds ratio	
									Lower limit	Upper limit
Target										
Leptostemonum	27C	Sel	*Solanum elaeagnifolium*	45	294	188	64			
Australian Solanum										
Archaesolanum		Sav	*Solanum aviculare*	4	40	28	70	3.93	0.84	18.3
Leptostemonum	27C	Sco	*Solanum coactiliferum*	3	15	13	87	2.11	0.073	61.1
		Ses	*Solanum esuriale*	1	5	1	20	0.0037	0.0000	1.73
		Ska	*Solanum karsense*	4	24	13	54	0.24	0.029	2.05
		Snu	*Solanum nummularium*	2	10	0	0	0	No emergence[c]	
		Sol	*Solanum oligacanthum*	1	5	4	80	0.06	0.00001	27.6
		Sst	*Solanum sturtianum*	3	16	8	50	0.066	0.0015	2.95
	27B	Sam	*Solanum amblymerum*	4	20	5	25	0.28	0.025	3.18
		Sbr	*Solanum brownii*	3	18	15	83	1.18	0.079	17.6
		Sce	*Solanum centrale*	1	5	0	0	0	No emergence[a]	
		Sci	*Solanum cinereum*	4	21	15	71	0.57	0.044	7.41
		Sju	*Solanum jucundum*	3	18	3	17	0.036	0.0024	0.54
	27D	Sla	*Solanum lasiophyllum*	4	20	0	0	1.00	Zero emergence from target[b]	
	27Z	Sar	*Solanum aridicola*	4	20	2	10	0.11	0.0047	2.60
		Scl	*Solanum cleistogamum*	7	35	21	60	1.50	0.15	15.3
		Sli	*Solanum lithophilum*	4	20	14	70	∞	Zero emergence from target[b]	
	13Z	Sch	*Solanum chenopodinum*	4	20	2	10	∞	Zero emergence from target[b]	
		Sfe	*Solanum ferocissimum*	4	18	16	89	0.51	0.063	4.19
		Sse	*Solanum stelligerum*	3	15	–	–	Excluded from analysis[e]		
	25Z	Sca	*Solanum campanulatum*	3	15	0	0	0	No emergence[a]	
		Sdi	*Solanum ditrichum*	1	5	0	0	0	No emergence[c]	
		Slc	*Solanum lacunarium*	4	23	18	78	1.15	0.076	17.6
		Spe	*Solanum petrophilum*	4	20	11	55	0.0000	Full emergence from target[d]	
	28Z	Scp	*Solanum chippendalei*	1	6	0	0	0	No emergence[a]	
Eggplant										
		BB	*Solanum melongena* 'Black Beauty'	8	60	44	73	1.00	0.24	4.15
Tamarillo										
Cyphomandra		EO	*Solanum betaceum* 'Ecuador Orange'	4	23	0	0	0	No emergence[a]	
Tomato										
Potato		GL	*Solanum lycopersicum* 'Grosse Lisse'	4	40	0	0	0	No emergence[a]	
		RC	*Solanum lycopersicum* 'Red Cherry'	4	20	0	0			
		RV	*Solanum lycopersicum* 'Roma VF'	4	20	0	0			
		TT	*Solanum lycopersicum* 'Tiny Tom'	4	20	0	0			

(*continued on next page*)

Table 2 (*continued*)

Major clade	Group	ID	Species and cultivar (if applicable)	Number of reps	Number of larvae applied	Number adults emerged	Percent adult emergence	Odds ratio of adult emergence compared to target	95% confidence interval of odds ratio	
									Lower limit	Upper limit
Potato										
		A	*Solanum tuberosum* 'Argos'	5	25	11	44	3.89	0.38	40.3
		Da	*Solanum tuberosum* 'Daisy'	5	25	3	12	0.34	0.027	4.44
		De	*Solanum tuberosum* 'Desiree'	4	20	0	0	0	No emergence[a]	
		N	*Solanum tuberosum* 'Nadine'	6	30	21	70	1.71	0.25	11.70
		P	*Solanum tuberosum* 'Pontiac'	4	20	4	20	0.075	0.0042	1.35
		RB	*Solanum tuberosum* 'Russett Burbank'	4	20	0	0	0	No emergence[a]	
		S	*Solanum tuberosum* 'Sebago'	4	20	0	0	0	No emergence[a]	
		V	*Solanum tuberosum* 'Valor'	6	30	15	50	2.70	0.34	21.1
Capsicum										
n/a		CW	*Capsicum annuum* 'California Wonder'	4	20	0	0	0	No emergence[a]	
		Ca	*Capsicum annuum* 'Cayenne'	4	20	0	0			
		BE	*Capsicum annuum* 'Hot Thai Bird's Eye'	3	15	0	0			
		Ja	*Capsicum annuum* 'Jalapeno'	4	20	0	0			
Exotic Solanaceae										
Geminata		Sps	*Solanum pseudocapsicum*	2	10	0	0	0	No emergence[a]	
Brevantherum		Sma	*Solanum mauritianum*	4	20	0	0			
n/a		Pxa	*Petunia* × *atkinsiana* 'Grandiflora'	4	20	0	0			
Other Australian Solanaceae										
		Cal	*Cypanthera albicans* subsp. *notabilis*	3	18	0	0	0	No emergence[a]	
		Dle	*Datura leichhardtii*	3	17	0	0			
		Nve	*Nicotiana velutina*	1	5	0	0			

[a] On or includes day(s) of strong emergence on *S. elaeagnifolium*.
[b] For 6/02/2017, 14 of 20 *S. lithophilum*, 0 of 20 *S. lasiophyllum*, 2 of 20 *S. chenopodinum* and 0 of 5 *S. elaeagnifolium* larvae emerged as adults.
[c] For 20/10/2017, 0 of 5 *S. ditrichum*, 0 of 10 *S. nummularium*, 5 of 5 *S. amblymerum* and 1 of 5 *S. elaeagnifolium* larvae emerged as adults.
[d] Despite best estimate of odds ratio being equal to 0, 11 out of 20 *S. petrophilum* larvae emerged as adults. Estimate was 0 because 5 out of 5 larvae applied to *S. elaeagnifolium* on 8/02/2017 emerged as adults.
[e] Adult emergence data missing for *Solanum stelligerum*.

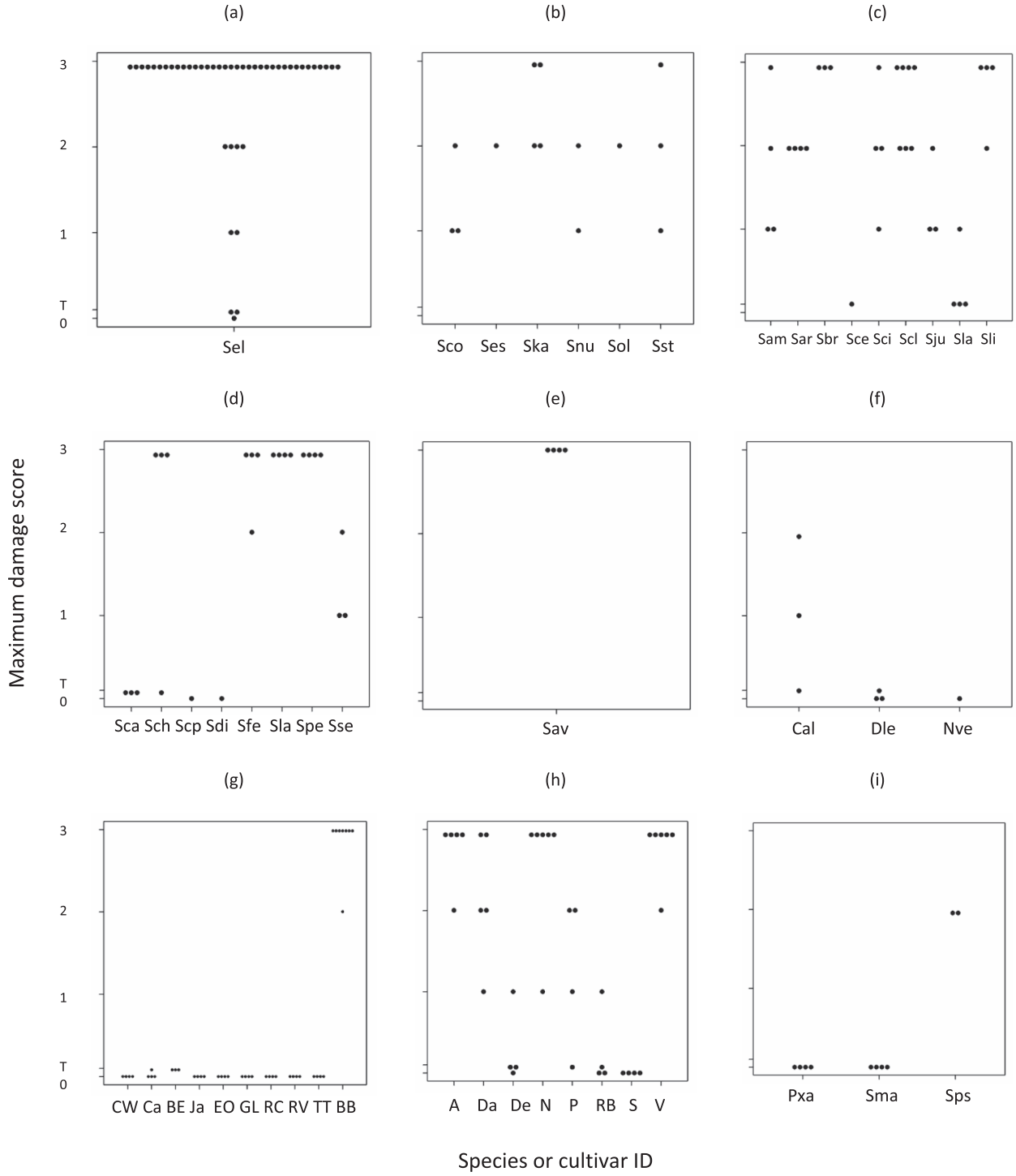

Fig. 2. Larval feeding damage to whole plants in no-choice experiments, with each dot representing an individual plant (a) *Solanum elaeagnifolium* (Subgenus Leptostemonum, Group 27C); (b)-(d) Australian *Solanum* spp. (Subgenus Leptostemonum) in Groups (b) 27C; (c) 27B, 27D and 27Z; and (d) 13Z, 25Z and 28Z; (e) *Solanum aviculare* G. Forster (Subgenus Archaesolanum); (f) other Australian Solanaceae (Tribes Anthocercideae, Datureae and Nicotianeae); (g) Solanaceae crop cultivars (excluding potato); (h) potato *Solanum tuberosum* cultivars; and (i) exotic Solanaceae. Damage rating 0 = no damage; T = trace damage or almost no damage (~0.1%); 1 = 0.1–10% leaf area consumed; 2 = 10–50% leaf area consumed; 3 = > 50% leaf area consumed. Species and cultivar ID's follow Table 1.

not anticipate feeding and development on potato because *L. texana* larvae and adults either did not feed on potato or initiated some feeding but no development on potato in the South African host-specificity testing (Olckers et al., 1995).

Consequently, we have expanded but not fully circumscribed the fundamental host range of *L. texana*. We recorded feeding and development on both economically important agricultural species (potato) and indigenous species that are both culturally and ecologically significant (e.g. *S. cleistogamum* and *S. karsense*). We discuss possible explanations for our observations on potato and the implications of these results for future selection, prioritization and reporting of cultivars used in host-specificity testing. Finally, we propose additional research to assess the risk of introducing *L. texana* to Australia or to other jurisdictions invaded by *S. elaeagnifolium*.

Table 3
Number of *Leptinotarsa texana* eggs, maximum damage score and sum of adult observations on *Solanum elaeagnifolium* (bold) and selected non-target *Solanum* spp. in a 6-week period in a small-cage (400 mm × 400 mm × 400 mm) choice minus target experiment. Each plant represents a replicate. Damage rating 0 = no damage; T = trace damage or almost no damage (~0.1%); 1 = 0.1–10% leaf area consumed; 2 = 10–50% leaf area consumed; 3 = > 50% leaf area consumed.

Species and cultivar (if applicable)	Number of plants tested	Total number of eggs	Maximum damage score	Sum of adult observations
Experiment 1: Native non-targets				
Solanum chenopodinum	2	10	3	13
Solanum lasiophyllum	2	0	2	16
Solanum lithophilum	2	0	2	9
Solanum cinereum	2	0	3	20
Solanum elaeagnifolium	**1**	**21**	**3**	**12**
Experiment 2: Horticultural non-targets				
Capsicum annuum 'California Wonder'	1	33[a]	1[b]	0
Capsicum annuum 'Cayenne'	1	0	0	2
Solanum lycopersicum 'Tiny Tom'	1	0	0	1
Solanum melongena 'Black Beauty'	1	35	3	16
Solanum elaeagnifolium	**1**	**0**[c]	**3**	**9**

[a] no eggs or larvae were evident nine days later.
[b] feeding damage < 1%.
[c] plant defoliated.

4.1. Development on potato in no-choice experiments

An important question that arose from this study was: why did *L. texana* larvae feed and develop on potato in our experiments, but not in host-range experiments conducted in South Africa? There are at least four plausible explanations:

1. Previous experiments by Olckers et al. (1995) generated false negative results,
2. The beetles we imported were incorrectly identified as *L. texana*,
3. The beetles we imported were derived from an *L. texana* population with a different fundamental host-range to those previously tested,
4. There are important differences between the potato cultivars tested.

We rule out the first three explanations and argue there were important differences between potato cultivars. Firstly, Olckers et al. (1995) applied *L. texana* larvae and adults to potato in no-choice (starvation) experiments; an experimental approach unlikely to generate false negatives on the plants that were tested (Marohasy, 1998). We assume adequate replication, but the number of plants of each cultivar tested by Olckers et al. (1995) was not reported. Ghebremariam (2017) cautioned that insects may prefer glasshouse-grown *Solanum* spp. to field grown plants, potentially confounding the results of no-choice experiments, but identified potato as a notable exception.

Secondly, we rule out the possibility that we incorrectly identified *L. texana* because specimens from our culture were identified by morphological examination and molecular analysis (Fig. 1), and our culture was imported from South Africa (the known potato pest *L. decemlineata* is not recorded in Africa (CABI, 2018; EPPO, 2019; Grobbelaar, 2016)). Thirdly, our quarantine culture was derived from the same *L. texana* population as that tested by Olckers et al. (1995) (Helmuth Zimmermann, pers. comm.). Therefore, the most likely explanation is that the potato cultivars tested here and by Olckers et al. (1995) varied in their susceptibility to *L. texana* larvae under no-choice conditions.

It is known that insects can exhibit preferences for, and variable performance on, cultivars of the same species (Balagawi et al., 2013), and we observed differences in the cultivars we tested (Tables 1–3). Adults emerged on five of the eight potato cultivars in our no-choice experiments with larvae. Two of the five cultivars had low adult emergence (12% and 20%), but on three cultivars > 40% of larvae developed to adulthood (Table 2). Furthermore, this higher level of feeding and development was on a group of closely-related cultivars; 'Argos', 'Nadine' and 'Valor'. These three cultivars originated from the same potato breeding program and two have a common parent, while all three have the South American *S. vernei* Bitter & Wittm. in their background (ECPD, 2019). Olckers et al. (1995) tested three potato cultivars (named 'BP1', 'Up-to-Date' and 'Van der Plank') (Helmuth Zimmermann, pers. comm.) in their *L. texana* no-choice experiments, all of which fall outside the 'Nadine' lineage. While potato is classified as *S. tuberosum*, the taxonomy of cultivated potatoes has been investigated a number of times and different studies have recognized between one and 21 species (Huamán and Spooner, 2002). Modern potato cultivars are the products of intensive breeding using *S. tuberosum* and 15 other members of section *Petota* (Ovchinnikova et al., 2011; Plaisted and Hoopes, 1989; Ross, 1986) and are hybrids. Caution is therefore recommended when extrapolating results from one or a few cultivars to a

Table 4
Number of eggs and percent egg hatch, maximum damage score and sum of adult observations on *Solanum elaeagnifolium* (bold) and ten non-target Australian *Solanum* spp. in an 8-week period in a large cage (3 m × 3 m × 1.8 m) choice experiment. Damage rating 0 = no damage; T = trace damage or almost no damage (~0.1%); 1 = 0.1–10% leaf area consumed; 2 = 10–50% leaf area consumed; 3 = > 50% leaf area consumed.

Major clade	Species	Total number of eggs	% egg hatch	Maximum damage score	Sum of adult observations
Leptostemonum	*Solanum ferocissium*	0		1	22
	Solanum petrophilum	269	77	3	108
	Solanum lacunarium	0		1	0
	Solanum brownii	0		2	15
	Solanum sturtianum	0		1	4
	Solanum elaeagnifolium	**179**	**70**	**2**	**10**
	Solanum karsense	0		2	11
	Solanum cleistogamum	182	81	2	22
	Solanum aridicola	22	91	3	19
	Solanum lithophilum	0		1	5
Archaesolanum	*Solanum aviculare*	0		1	0

large crop or ornamental species complex. This is especially important if the extrapolation is cited as the principal evidence that the non-target species is outside the fundamental host-range, and therefore not at risk or at low risk of off-target damage.

Clearly, whenever a crop or ornamental species is selected for host-specificity testing the complexity and importance of cultivars within the species must be carefully assessed. We argue that in all cases i) the method for selecting and prioritizing cultivars should be described in sufficient detail to justify the final cultivar list, ii) the cultivar or cultivars selected for host-specificity testing should be named according to the Cultivated Plant Code (Brickell et al., 2016), and iii) host-specificity testing data for individual cultivars should be reported or accessible.

4.2. Risk to Australian native Solanum

Interpretation of the choice experiments with adults was hampered by lack of replication. Nevertheless, of 11 Australian non-target species exposed to *L. texana* in a large-cage choice test, three species were accepted for oviposition. In the case of *S. petrophilum* and *S. cleistogamum*, all life-stages of *L. texana* were supported under choice and no-choice conditions, specifically 1) feeding and development by larvae in no-choice experiments, and 2) feeding and oviposition by adults in choice experiments. Given these results, it is possible that *L. texana* could persist on *S. petrophilum* and *S. cleistogamum* growing near *S. elaeagnifolium* infestations. *Solanum chenopodinum* also supported larval development under no-choice conditions, as well as feeding and oviposition by adults in the absence of the target weed. Continuation studies (Day, 1999) could measure the viability of *L. texana* populations on these non-target species over more than one generation.

Despite *L. texana* larvae feeding and developing on the nationally-listed vulnerable species *S. karsense* in a no-choice feeding experiment (Table 2, Fig. 2), adult females did not oviposit on *S. karsense* in the large-cage choice test (Table 4). However, we can't rule out the possibility that *S. karsense* is a lower ranked host for oviposition since the large cage experiment comprised only one replicate, due to time and space constraints. Repeating the experiment with removal of higher ranked hosts (Marohasy, 1998) may determine the relative acceptability of *S. karsense* for oviposition. If *S. karsense* is not utilized for oviposition, there still remains the risk of "spill-over" damage caused by larvae and adults dispersing from defoliated *S. elaeagnifolium* infestations. Sheppard et al. (2005) defined spill-over as "agents damaging but not developing on nontargets". *Solanum karsense* occurs in south western New South Wales and just across the border into South Australia where its range is contiguous with *S. elaeagnifolium* infestations (ALA, 2019). Climate modelling predicted *S. elaeagnifolium* could occupy most areas where *S. karsense* grows (Wilson et al., 2011); areas also predicted to be suitable for *L. texana* (Senaratne et al., 2008). Further assessment of the likelihood and magnitude of spill-over, and the consequences for individuals and populations of *S. karsense* and other native *Solanum* spp. identified as being at risk (Table 2, Fig. 2), would be required for a full risk analysis.

4.3. Prospects for Leptinotarsa texana as a biological control agent in Australia

Our findings have shown that there is potential for *L. texana* to utilize economically, ecologically and culturally important species. However, these are trials conducted under laboratory conditions, with a highly risk averse experimental strategy (i.e. no choice experiments and choice experiments in cages). Other evidence from the field suggests that at least the economically important species may not be impacted (John Goolsby, pers. comm.). While potato and other non-target species are not necessarily within the realized host-range of *L. texana*, the expanded fundamental host-range identifies risks that must be addressed if *L. texana* is to be considered for introduction. Results from South Africa indicate field testing in the native or introduced range of *L. texana* may be warranted for the species. However, whilst it may be feasible to conduct open field experiments with selected cultivars of eggplant and potato, it is probably not feasible to conduct the same experiments with Australian *Solanum* spp. because transplanted non-indigenous plants could escape to become weeds (Clement and Cristofaro, 1995). To address concerns with non-target *Solanum* spp., other lines of evidence that might be considered include behavioral studies in quarantine using olfactometers or wind tunnels (Park et al., 2018; Sutton et al., 2017), or structured elicitation and quantification of expert opinion (Hemming et al., 2018; van Klinken et al., 2016). Collecting extra information with further experimentation could be informative to some decision makers, but whether further information would result in a (positive) decision in Australia about introduction is questionable, because it is unclear whether the perceived risks to potato and native *Solanum* can be adequately addressed for the Australian context. Careful consideration of the risk attitude of stakeholders and decision makers, the resources required for these studies, the likelihood of obtaining useful data that demonstrates an acceptable level of risk to non-target species, and the costs to Australia's economic and biodiversity values of not introducing biological control is required before further testing is done. Irrespective of whether further investment is allocated to *L. texana* testing, the poor prospects for controlling *S. elaeagnifolium* with herbicides highlight that the economic and ecological risks of not continuing to invest in biological control options for *S. elaeagnifolium* should not be ignored.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocontrol.2019.104165.

References

ALA, 2019. Distribution of *Solanum karsense* and *S. elaeagnifolium*. Retrieved 20 May 2019, from Atlas of Living Australia https://spatial.ala.org.au/?q=lsid:http:%2F%2Fid.biodiversity.org.au%2Fnode%2Fapni%2F2892552.

Aubriot, X., Singh, P., Knapp, S., 2016. Tropical Asian species show that the Old World clade of 'spiny solanums'(*Solanum* subgenus *Leptostemonum pro parte*: Solanaceae) is not monophyletic. Bot. J. Linn. Soc. 181 (2), 199–223. https://doi.org/10.1111/boj.12412.

AWC, 2012. Weeds of National Significance: Silverleaf Nightshade (*Solanum elaeagnifolium*) Strategic Plan. Australian Weeds Committee, Canberra.

Balagawi, S., Drew, R.A.I., Clarke, A.R., 2013. Simultaneous tests of the preference-performance and phylogenetic conservatism hypotheses: is either theory useful? Arthropod-Plant Interactions 7 (3), 299–313. https://doi.org/10.1007/s11829-012-9244-x.

Bean, A.R., 2004. The taxonomy and ecology of *Solanum* subg. *Leptostemonum* (Dunal) Bitter (Solanaceae) in Queensland and far north-eastern New South Wales, Australia. Austrobaileya 6 (4), 639–816.

Boukhris-Bouhachem, S., Hullé, M., Rouzé-Jouan, J., Glais, L., Kerlan, C., 2007. *Solanum elaeagnifolium*, a potential source of Potato virus Y (PVY) propagation. EPPO Bull. 37 (1), 125–128. https://doi.org/10.1111/j.1365-2338.2007.01095.x.

Brickell, C.D., Alexander, C., Cubey, J.J., David, J.C., Hoffman, M.H.A., Leslie, A.C. (Eds.), 2016. International Code of Nomenclature for Cultivated Plants, ninth ed. International Society for Horticultural Science, Belgium.

Briese, D.T., Walker, A., 2002. A new perspective on the selection of test plants for

evaluating the host-specificity of weed biological control agents: the case of *Deuterocampta quadrijuga*, a potential insect control agent of *Heliotropium amplexicaule*. Biol. Control 25 (3), 273–287. https://doi.org/10.1016/S1049-9644(02)00111-1.

Brunel, S., 2011. Pest risk analysis for *Solanum elaeagnifolium* and international management measures proposed. EPPO Bull. 41 (2), 232–242. https://doi.org/10.1111/j.1365-2338.2011.02457.x.

CABI, 2018. *Leptinotarsa decemlineata* (Colorado potato beetle). From CAB International, viewed 3 December 2018 http://www.cabi.org/isc/datasheet/30380#20127201272.

Cabrera Walsh, G., Maestro, M., 2017. Assessing the specificity of a herbivore on a plant of uncertain phylogenetic placing: *Listronotus elongatus* a herbivore of *Hydrocotyle ranunculoides*. Biocontrol 62 (2), 269–279. https://doi.org/10.1007/s10526-017-9785-0.

Clement, S.L., Cristofaro, M., 1995. Open-field tests in host-specificity determination of insects for biological control of weeds. Biocontrol Sci. Tech. 5 (4), 395–406. https://doi.org/10.1080/09583159550039594.

Cuda, J.P., Parker, P.E., Coon, B.R., Vasquez, F.E., Harrison, J.M., 2002. Evaluation of exotic *Solanum* spp. (Solanales: Solanaceae) in Florida as host plants for the leaf beetles *Leptinotarsa defecta* and *L. texana* (Coleoptera: Chrysomelidae). Florida Entomol. (4), 599–610. https://doi.org/10.1653/0015-4040(2002)085[0599:EOESSS]2.0.CO;2.

Day, M.D., 1999. Continuation trials: their use in assessing the host range of a potential biological control agent. In: Withers, T.M., Barton Browne, L., Stanley, J. (Eds.), Host Specificity Testing in Australasia: Towards Improved Assays for Biological Control. CRC for Tropical Pest Management, Brisbane, Australia, pp. 11–19.

Douglas, A.E., 2018. Strategies for enhanced crop resistance to insect pests. Annu. Rev. Plant Biol. 69 (1), 637–660. https://doi.org/10.1146/annurev-arplant-042817-040248.

ECPD, 2019. European Cultivated Potato Database. Retrieved 10 December 2018, from Science and Advice for Scottish Agriculture http://www.europotato.org/.

EPPO, 2007. *Solanum elaeagnifolium*. EPPO Bull. 37, 236–245. https://doi.org/10.1111/j.1365-2338.2007.01112.x.

EPPO, 2019. *Leptinotarsa decemlineata*. Retrieved 22 August 2019, from EPPO Global Database https://gd.eppo.int/taxon/LPTNDE/distribution.

Ghebremariam, T., 2017. Suitability of Quarantine Evaluations to Determine the Host Plant Range of Insect Herbivores in the Field: Focus on *Solanum* (Solanaceae) species. University of Pretoria PhD.

Gopurenko, D., Fletcher, M., Löcker, H., Mitchell, A., 2013. Morphological and DNA barcode species identifications of leafhoppers, planthoppers and treehoppers (Hemiptera: Auchenorrhyncha) at Barrow Island. Rec. Western Aust. Museum Supplement 83, 253–285. https://doi.org/10.18195/issn.0313-122x.83.2013.253-285.

Green, J.D., Murray, D.S., Verhalen, L.M., 2017. Full-season interference of silverleaf nightshade (*Solanum elaeagnifolium*) with cotton (*Gossypium hirsutum*). Weed Sci. 35 (6), 813–818. https://doi.org/10.1017/S004317450007939X.

Grobbelaar, E., 2016. Enquiry to South African National Collection of Insects (SANC) concerning the presence/absence of *Leptinotarsa decemlineata* (Say) in South Africa. Letter. Plant Protection Research Institute. Agricultural Research Council.

Hebert, P.D.N., Cywinska, A., Ball, S.L., deWaard, J.R., 2003. Biological identifications through DNA barcodes. Proc. R. Soc. Lond. B Biol. Sci. 270 (1512), 313–321. https://doi.org/10.1098/rspb.2002.2218.

Hemming, V., Burgman, M.A., Hanea, A.M., McBride, M.F., Wintle, B.C., 2018. A practical guide to structured expert elicitation using the IDEA protocol. Methods Ecol. Evol. 9 (1), 169–180. https://doi.org/10.1111/2041-210X.12857.

Hsiao, T.H., 1974. Chemical influence on feeding behavior of *Leptinotarsa* beetles. In: Barton Browne, L. (Ed.), Experimental Analysis of Insect Behaviour. Springer, Berlin, Heidelberg, pp. 237–248.

Huamán, Z., Spooner, D.M., 2002. Reclassification of landrace populations of cultivated potatoes (*Solanum* sect. *Petota*). Am. J. Bot. 89 (6), 947–965. https://doi.org/10.3732/ajb.89.6.947.

Izzo, V., Mercer, N., Armstrong, J., Chen, Y., 2014. Variation in host usage among geographic populations of *Leptinotarsa decemlineata*, the Colorado potato beetle. J. Pest. Sci. 87. https://doi.org/10.1007/s10340-014-0578-2.

Knapp, S., Sagona, E., Zapata Carbonell, A.K., Chiarini, F., 2017. A revision of the *Solanum elaeagnifolium* clade (Elaeagnifolium clade; subgenus *Leptostemonum*, Solanaceae). PhytoKeys 84, 1–104. https://doi.org/10.3897/phytokeys.84.12695.

Kwong, R., 2006. Feasibility of Biological Control of Solanaceous Weeds of Temperate Australia. Meat and Livestock Australia, North Sydney.

Levin, R.A., Nicole, R.M., Bohs, L., 2006. Phylogenetic relationships among the "Spiny Solanums" (*Solanum* Subgenus *Leptostemonum*, Solanaceae). Am. J. Bot. 93 (1), 157–169. https://doi.org/10.3732/ajb.93.1.157.

Marohasy, J., 1998. The design and interpretation of host-specificity tests for weed biological control with particular reference to insect behaviour. Biocontrol News Inf. 19, 13N–20N.

Mekki, M., 2007. Biology, distribution and impacts of silverleaf nightshade (*Solanum elaeagnifolium* Cav.). EPPO Bull. 37 (1), 114–118. https://doi.org/10.1111/j.1365-2338.2007.01094.x.

Olckers, T., Hulley, P.E., 1994. Resolving ambiguous results of host-specificity tests: the case of two *Leptinotarsa* species (Coleoptera: Chrysomelidae) for biological control of *Solanum elaeagnifolium* Cavanilles (Solanaceae) in South Africa. Afr. Entomol. 2 (2), 137–144.

Olckers, T., Zimmermann, H.G., Hoffmann, J.H., 1995. Interpreting ambiguous results of host-specificity tests in biological control of weeds: assessment of two *Leptinotarsa* species (Chrysomelidae) for the control of *Solanum elaeagnifolium* (Solanaceae) in South Africa. Biol. Control 5, 336–344. https://doi.org/10.1006/bcon.1995.1039.

Olckers, T., Hoffmann, J.H., Moran, V.C., Impson, F.A.C., Hill, M.P., 1999. The initiation of biological control programmes against *Solanum elaeagnifolium* Cavanilles and *S. sisymbriifolium* Lamarck (Solanaceae) in South Africa. Afr. Entomol. Memoir 1, 55–63.

Ovchinnikova, A., Krylova, E., Gavrilenko, T., Smekalova, T., Zhuk, M., Knapp, S., Spooner, D.M., 2011. Taxonomy of cultivated potatoes *(Solanum* section Petota: Solanaceae). Botanical J. Linn. Soc. 165 (2), 107–155. https://doi.org/10.1111/j.1095-8339.2010.01107.x.

PaDIL, 2019. Pests and Diseases Image Library. Retrieved 22 August 2019, from http://www.padil.gov.au.

Park, I., Eigenbrode, S.D., Cook, S.P., Harmon, B.L., Hinz, H.L., Schaffner, U., Schwarzlander, M., 2018. Examining olfactory and visual cues governing host-specificity of a weed biological control candidate species to refine pre-release risk assessment. Biocontrol 63, 377–389. https://doi.org/10.1007/s10526-018-9867-7.

Pelletier, Y., Horgan, F., Pompon, J., 2011. Potato resistance to insects. Am. J. Plant Sci. Biotechnol. 5 (1), 37–52.

Plaisted, R., Hoopes, R., 1989. The past record and future prospects for the use of exotic potato germplasm. Am. Potato J. 66 (10), 603–627. https://doi.org/10.1007/BF02853982.

Purdie, R., Symon, D., Haegi, L., 1982. Solanaceae. In: George, S. (Ed.), Flora of Australia. Bureau of Flora and Fauna, Canberra, pp. 208.

Ross, H., 1986. Potato breeding-problems and perspectives. Advances in Plant Breeding Vol. 13.

Särkinen, T., Bohs, L., Olmstead, R.G., Knapp, S., 2013. A phylogenetic framework for evolutionary study of the nightshades (Solanaceae): a dated 1000-tip tree. BMC Evol. Biol. 13 (1), 214. https://doi.org/10.1186/1471-2148-13-214.

SCA, 1986. Declaration of *Solanum elaeagnifolium* as a target for biological control. Standing Committee on Agriculture, Retrieved 22 August 2019, from http://weeds.ala.org.au/target.htm.

Senaratne, K.A.D.W., Palmer, W.A., Sutherst, R.W., 2008. Applications of CLIMEX modelling leading to improved biological control. Paper Presented at the 16th Australian Weeds Conference, Cairns, Queensland, Australia.

Sheppard, A.W., van Klinken, R.D., Heard, T.A., 2005. Scientific advances in the analysis of direct risks of weed biological control agents to nontarget plants. Biol. Control 35 (3), 215–226. https://doi.org/10.1016/j.biocontrol.2005.05.010.

SPHDS, 2013. National diagnostic protocol for Colorado potato beetle, *Leptinotarsa decemlineata*. Subcommittee on Plant Health Diagnostic Standards, Retrieved 22 August 2019, from http://www.plantbiosecuritydiagnostics.net.au/app/uploads/2018/11/NDP-22-Colorado-potato-beetle-Leptinotarsa-decemlineata-V1.2.pdf.

Stanton, R., Heap, J.W., Carter, R.W., Wu, H.W., 2009. *Solanum elaeagnifolium*. In: In: Panetta, F.B. (Ed.), The Biology of Australian Weeds Vol. 3. R. G & F. J. Richardson, Melbourne, pp. 274–293.

Sutton, G.F., Paterson, I.D., Compton, S.G., Paynter, Q., 2017. Predicting the risk of non-target damage to a close relative of a target weed using sequential no-choice tests, paired-choice tests and olfactory discrimination experiments. Biocontrol Sci. Tech. 27 (3), 364–377. https://doi.org/10.1080/09583157.2017.1291907.

Symon, D.E., 1981. A revision of the genus *Solanum* in Australia. J. Adelaide Botanic Gardens 4, 1–367.

Symon, D., 1994. Kangaroo apples: *Solanum* sect. *Archaesolanum*. Published by the author, Keswick, South Australia.

Threatened Species Scientific Committee, 2010. Advice from the Threatened Species Scientific Committee (the Committee) on the list of Threatened Species under the Environment Protection and Biodiversity Conservation Act 1999 (EPBC Act). Retrieved 22 August 2019, from http://www.environment.gov.au/biodiversity/threatened/species/pubs/7776-listing-advice.pdf.

Tscheulin, T., Petanidou, T., 2013. The presence of the invasive plant *Solanum elaeagnifolium* deters honeybees and increases pollen limitation in the native co-flowering species *Glaucium flavum*. Biol. Invasions 15 (2), 385–393. https://doi.org/10.1007/s10530-012-0293-y.

van Klinken, R.D., 2000. Host specificity testing: why we do it and how we can do it better. In: Paper presented at the Host-specificity testing of exotic arthropod biological control agents - the biological basis for improvement in safety., Morgantown, West Virginia.

van Klinken, R.D., Morin, L., Sheppard, A., Raghu, S., 2016. Experts know more than just facts: eliciting functional understanding to help prioritise weed biological control targets. Biol. Invasions 18 (10), 2853–2870. https://doi.org/10.1007/s10530-016-1175-5.

Vorontsova, M.S., Stern, S., Bohs, L., Knapp, S., 2013. African spiny *Solanum* (subgenus *Leptostemonum*, Solanaceae): a thorny phylogenetic tangle. Bot. J. Linn. Soc. 173 (2), 176–193. https://doi.org/10.1111/boj.12053.

VSN International, 2015. The guide to the Genstat Command Language (Release 18), Part 2 Statistics. VSN International, Hemel Hempstead, UK.

Wapshere, A.J., 1974. A strategy for evaluating the safety of organisms for biological weed control. Ann. Appl. Biol. 77 (2), 201–211. https://doi.org/10.1111/j.1744-7348.1974.tb06886.x.

Wapshere, A.J., 1989. A testing sequence for reducing rejection of potential biological control agents for weeds. Ann. Appl. Biol. 114 (3), 515–526. https://doi.org/10.1111/j.1744-7348.1989.tb03367.x.

Whalen, M.D., 1984. Conspectus of species groups in *Solanum* subgenus *Leptostemonum*. Gentes Herbarum 12, 179–282.

Wilson, P.D., Downey, P.O., Gallagher, R.V., O'Donell, J., Leishman, M.R., Hughes, L., 2011. Modelling climate suitability for exotic plants in Australia under future climate. Sydney, Australia: Macquarie University and New South Wales Office of Environment and Heritage.