Systematic cultivar selection for weed biological control risk assessment

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Abstract

1. Context and need for the work

Classical biological control, using specialised natural enemies (biocontrol agents), is important for long-term, sustainable management of invasive species such as weeds. To be acceptable for introduction, new biocontrol agents must not damage crops, native plants or other non-target species. Host-specificity experiments inform risk assessment of new biocontrol agents by prioritising and testing non-target plant species. However, it was recently highlighted that current approaches may be inadequate for assessing risks to crop and ornamental species because susceptibility to damage can vary between cultivars of the same species.

2. Approach and methods

We reviewed and documented current cultivar selection practice published in prominent biological control journals and government documents.

To address perceived gaps, we elicited expert opinion through brain-storming and, combined with our assessment of best practice, developed a decision support tool comprising a process chart and criteria for prioritising cultivars for biological control host-specificity testing. We applied the decision tool to an important and complex host-specificity testing case study.

3. Main results

In our review, most papers either did not mention cultivars of the crop or ornamental species being tested, or they provided incomplete descriptions of cultivars without explaining omissions. In most cases, if cultivars were listed then the criteria used to select cultivars were not described, were incorrectly applied or inconsistently applied. Only one of 29 papers fully described the method for selecting and prioritising cultivars and reported the results for each cultivar tested.

By applying our criteria and process chart to a case study we demonstrated how current gaps in cultivar selection practices can be addressed. From the thousands of potato cultivars grown world-wide, we selected a short-list of cultivars that is feasible to test, and which can be scrutinized and updated. We demonstrated how selections could be made through a collaborative and transparent process involving key stakeholders and risk bearers.

4. Synthesis and applications;

The decision tool has broad application in weed biological control risk assessment. We demonstrated that the decision tool is easy to use, can account for uncertainty, is adaptable to different species, and is suitable for both small and large cultivar groups irrespective of complexity. We argue that our approach, if adopted, will result in more transparent, defensible and reproducible cultivar selection practices leading to greater confidence in biological control risk assessments.

1. Introduction

Classical biological control is the use of specialised natural enemies to manage invasive species in an invaded range. These natural enemies, called biocontrol agents, may be insects or other invertebrates, or pathogens. Classical biological control can be an effective and sustainable method of control once biocontrol agents are permanently established. Well-documented examples of successful biological control include the management of Cassava mealybug *Phenacoccus manihoti* (Mat.-Ferr.) in tropical Africa (Zeddies *et al.* 2001), winter moth *Operophtera brumata* (L.) in North America (Roland & Embree 2003), and rush skeletonweed *Chondrilla juncea* (L.) in Australia. However, it is critical to assess new introductions to minimise the risks of off-target damage that impact social, environmental and economic values. Deleterious impacts of biological control, such as parasitism of North American Saturniidae by the Gypsy moth biological control agent *Compsilura concinnata* (Boettner, Elkinton & Boettner 2000), have occurred when the risks of introduction were not adequately considered by decision makers. Consequently, considerable research effort has been directed to classical biological control risk analysis in recent decades, and countries importing biocontrol agents impose strict pre-introduction regulatory regimes (FAO 2005; Barratt *et al.* 2010).

An integral component of classical biological control risk analysis is to estimate the potential host-range of new biocontrol agents (van Klinken 2000). Host-range estimation relies heavily on the results of experiments, conducted in quarantine laboratories, that expose the agent to target and non-target species (Marohasy 1998; Sheppard, van Klinken & Heard 2005). Other evidence from the native range, literature, and experts can be considered (Sheppard, van Klinken & Heard 2005; Schaffner, Smith & Cristofaro 2018). For weed biological control,

the aim is to select and test the range of plant species that would allow detection of off-target damage, if the damage is to occur (i.e. avoiding Type II errors). This involves selecting plant taxa largely based on phylogenetic relatedness to the target weed, but may include other criteria that consider the ecology and biogeography of non-target species (Wapshere 1974; Briese & Walker 2002; Briese 2006). The list of plant species selected for testing is known as the *host-specificity test plant list* (Sheppard, van Klinken & Heard 2005).

When the test plant list includes crop or ornamental plant species, there is a need to assess the risk to cultivated varieties (cultivars) of the species being tested, to ensure the species as a whole is not at risk, or is at low risk, of off-target damage. Cultivars are defined as "*an assemblage of plants that (a) has been selected for a particular character or combination of characters, and (b) remains distinct, uniform, and stable in these characters when propagated by appropriate means*"(Brickell *et al.* 2016). In biological control, multiple cultivars of a non-target species are often tested because susceptibility to arthropod or pathogen damage can vary between cultivars (Balagawi, Drew & Clarke 2013). This was recently demonstrated in laboratory testing of a proposed biocontrol agent for silverleaf nightshade *Solanum elaeagnifolium*, where experiments highlighted differences in the susceptibility of eight potato cultivars (Lefoe *et al.* 2020).

This highlights an important issue for biological control testing, because there are more than 4000 potato cultivars world-wide, as well as new cultivars in development (ECPD 2019). Previous laboratory testing in South Africa had not highlighted there was a risk to potato, but with so many cultivars there is a greater risk of a Type II error. In these cases, it is clearly not possible, or even necessary, to expose every available cultivar to a proposed biocontrol agent. Rather, certain cultivars are selected for testing, and the experimental results for those cultivars, along with other evidence, are used to estimate the risk to the non-target species in the area of introduction. There is a trade-off then between testing a sufficiently representative

99 sample of cultivars to detect cultivar differences if they are present and ensuring the test list is
100 not so large that testing becomes impractical or superfluous.

101 There is some guidance available on the selection of plant species for host-specificity test
102 lists (Wapshere 1974; Kelch & McClay 2004; Briese 2006). We expected that methods used
103 to select cultivars for host-specificity testing would be similarly described in biological
104 control literature. However, there is an apparent lack of comprehensive criteria directly
105 applicable to prioritizing cultivars within a test plant species (Lefoe *et al.* 2020). Without
106 such guidance there is potential for cultivar selection practices to be inconsistently applied,
107 and not able to be scrutinised. We reasoned a standardized framework would make cultivar
108 selection practice more transparent and reproducible and provide a stronger basis for
109 justifying test lists used in biological control risk assessment.

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2. Materials and Method

2.1 Review of current practice

We assessed current cultivar selection methods by reviewing articles published in three peer-reviewed international biological control journals prominent in the weed biological control community; (1) *Biological Control*, (2) *Biocontrol* and (3) *Biocontrol Science and Technology*. For each journal the search terms "cultivar" or "variety" or "host-specificity" or "host-range" were applied for the period January 2015 to June 2019. We briefly reviewed the abstract and methods for all papers returned from the searches [n=1335] to determine if they described host-specificity testing of weed biocontrol agents. If they did, the full paper was downloaded for further scrutiny [n=53]. We read each downloaded paper to determine if plant species containing multiple cultivars were listed. If listed, then detail of the cultivar selection method described in the paper (if any) was recorded and tabulated [n=29].

In addition, we assessed test list selection guidelines and risk assessment reports published by biological control regulatory authorities in Australia, New Zealand and USA during the same period.

2.2 Developing a decision support tool

A team of seven co-authors were selected to provide decision analytical expertise, and substantive expertise in the areas of invasive species management, classical biological control and plant breeding.

All members of the team participated in a guided workshop that commenced with a brainstorming exercise (Clemen & Reilly 2001) aimed at documenting the decision problem

and any criteria that should be considered for prioritising and selecting cultivars. We agreed on definitions for key terms and assessed criteria, short-listing those we thought were important for selecting and prioritising cultivars. We considered different structuring tools before ordering short-listed criteria in a rudimentary flowchart.

2.2.1 Definitions

Cultivars are named and regulated under the International Code of Nomenclature for Cultivated Plants (the Cultivated Plant Code) (Brickell *et al.* 2016) as set out by the International Union of Biological Sciences (IUBS) International Commission for the Nomenclature of Cultivated Plants, or in some cases under the International Code of Nomenclature for algae, fungi, and plants (Turland *et al.* 2018).

We expanded the definition of *susceptibility* developed by Wiseman (1994), who described a susceptible cultivar as "*one on which average* [for the crop] *or more than average damage is inflicted by an insect pest species*", to include other herbivorous arthropods and plant pathogens.

2.2.2 Structuring tools

We developed a flowchart from information gathered in our workshop to graphically represent the sequence of events for selecting cultivars for host-specificity testing. We used the free online version of Lucidchart (2020) to build, test and update the flowchart through several iterations. Each event in the flowchart was described with text enclosed within a standard symbol (representing a decision, process or document/database) and linked by lines and arrows (Harris 1999). Detailed descriptions of each event or decision were documented for further reference. We tested each draft version of the flowchart by applying case studies of varying complexity.

158 *2.3 Applying the decision support tool*

159 We retrospectively applied the decision support tool to the selection of potato cultivars for
160 host-specificity testing of the North American leaf beetle *Leptinotarsa texana* in Australian
161 quarantine (Lefoe *et al.* 2020).

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163 **3. Results**

164 *3.1 Review of current practice*

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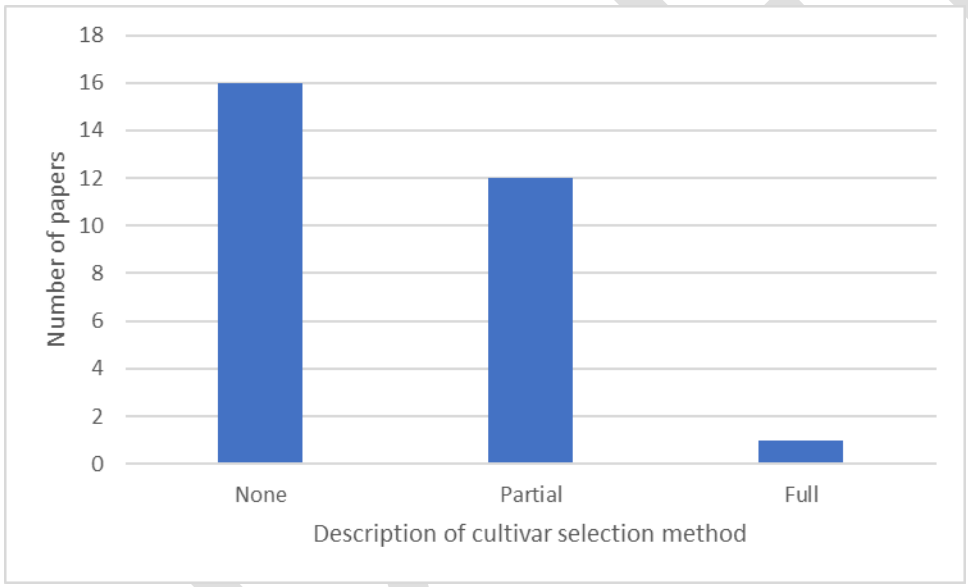

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167 Figure 1. Cultivar selection methods described in 29 papers that listed at least one test plant
168 species known to comprise multiple cultivars (as published in three biological control
169 journals from January 2015 - June 2019). None = no description of cultivars; Partial =
170 cultivars were listed for some test plant species but not others, and/or the method for selecting
171 cultivars was not fully described; Full = cultivars and selection method described.

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173 Most papers we reviewed either did not mention cultivars of the crop or ornamental species
174 being tested, or they provided incomplete descriptions of cultivars without explaining
175 omissions (Figure 1). In most cases, if cultivars were listed then the criteria used to select

cultivars were not described, were incorrectly applied or inconsistently applied. Results for individual cultivars were therefore absent or incomplete in most cases. Only one of 29 papers fully described the method for selecting and prioritising cultivars and reported the results for each cultivar tested.

We documented the following cultivar selection methods from papers that provided a description:

- Preferential selection based on perceived or known susceptibility of the cultivar to insect attack (Goolsby *et al.* 2017),
- Cultivar availability (named as one of two criteria for species selection, along with relatedness-to-target; we have assumed availability was the primary criterion for cultivar selection as relatedness-to-target is only relevant at species level) (Jones & Wheeler 2017),
- Biogeographic overlap and availability (relatedness was also included as a criterion but it is not applicable here; see previous) (Mphephu, Olckers & Simelane 2017)
- Economic importance in the area of introduction according to expert opinion (McConnachie & McKay 2015; McConnachie 2015b; McConnachie 2015a; Mersie *et al.* 2019),
- Widespread cultivation in the area of introduction according to expert opinion (McConnachie 2015b; McConnachie 2015a; McConnachie & McKay 2016).

3.2 Developing a decision support tool

We propose an ordered set of steps for building and refining a list of cultivars for testing (Figure 2). Key considerations include 1) the global context, 2) the area of introduction, 3)

any previous testing, 4) an exposure analysis, 5) phylogenetic information, and 6) a sampling strategy.

3.2.1 Global context

Documenting a global list of cultivars is possible for some test plant species. A global cultivar list may comprise a single database, or a collection of databases or publications compiled to represent the range of cultivars grown in different regions or globally e.g. (Brickell *et al.* 2016; ECPD 2019) While biological control scientists cannot account for every future importation or cultivar under development, cultivars present or previously introduced into the receiving environment or proposed for introduction (as identified through industry consultation), are identified and documented. For example, the Australian sugar industry conducts overseas field trials to pre-screen sugarcane cultivars for resistance to major biosecurity threats (ref from PBRI proceedings). These cultivars may never be released in Australia, but they are available for rapid introduction, release and industry adoption in the event of an incursion by the pest or pathogen of concern. It is therefore important to consult with industry to identify these cultivars and include them in host-specificity testing where possible.

3.2.2 Area of introduction

The initial cultivar list for the proposed area of introduction comprises a subset of the global list. It contains those cultivars present or selected for the area of introduction, which may be a nation, but could also include neighbouring countries "*with similar fauna, flora and climate and hence similar concerns about the introduction of biological control agents*"(IPPC 1996).

3.2.3 Previous testing

It is important to identify previous host-specificity testing conducted on cultivars that are grown in the area of introduction to both inform risk assessment and avoid unnecessary duplication of research effort. However, it is essential that previous research be examined and critically evaluated. Cultivars that have previously been tested in local or overseas research may not require re-testing, provided that:

(1) the cultivar was named according to an accepted naming scheme, such as the Cultivated Plant Code (Brickell *et al.* 2016), and

(2) the method for testing and results for the cultivar are published or available, and testing was of a standard acceptable to regulatory authorities in the proposed area of introduction.

Even if re-testing is not required, documenting previous research will help to inform subsequent risk assessment.

3.2.4 Exposure analysis

Exposure analysis attempts to identify which cultivars would be exposed to risk. Generally, exposure of plants (or suitable life-stages or parts of the plant) to agents in the area of introduction can occur:

a. Spatially – the cultivar's range overlaps with the predicted range of the agent and co-occurs with the target weed, or the agent is likely to spread to areas where the cultivar grows through natural dispersal or an identified pathway.

b. Temporally – susceptible stages of the cultivar (for example, fruit for fruit- and seed-feeders) are available at the time the agent is searching for a food source or oviposition site.

c. Ecologically – the cultivar is grown in habitat suitable for the agent.

Cultivars exposed to risk are retained at this stage, and the nature and extent of exposure is documented for subsequent risk analysis.

Cultivars that are not exposed to risk do not require testing. These cultivars, and the rationale for not testing, should nevertheless be documented as part of the broader risk assessment, as it provides useful information to decision makers and stake-holders. Exceptions may occur where there is evidence of future change that would expose these cultivars to risk. These exceptions could include predicted range expansion of the target weed, predicted effects of climate change on the target weed or agent (where there is evidence that this could occur), or anticipated shifts in the location of crops. Protected cropping in greenhouses, or chemical protection (e.g. of eggplant from *L. texana*; (Olckers & Hulley 1994)) may also be sufficient to prevent exposure.

3.2.5 Phylogenetic information

At this stage it is useful to compile phylogenetic information on the non-target species and its cultivars. For some non-target species, comprehensive phylogenetic information on cultivars is available from journals and online databases; in other cases, phylogenies may be incomplete or not available. Documenting available phylogenetic information assists in devising an appropriate sampling strategy (see 3.2.6) for test lists that still have too many cultivars to test.

3.2.6 Sampling strategy

Cultivars can be further shortlisted by sampling from one or more published phylogenies (if available; see 3.2.5) and applying additional sub-criteria, or by sampling using relevant sub-criteria alone in cases where a phylogeny is not described.

Sample using phylogeny

An adequately described phylogeny should illustrate cultivar relationships by grouping closely-related cultivars, based on genetic distance, into clusters (ref). Sampling can then represent the range of genotypes or lineages present by selecting cultivars from different clusters (see case study for an example). The aim is to ensure no important lineages are overlooked in developing the test list, while at the same time avoiding over-representation of one or a few lineages without justification for doing so.

Sample using sub-criteria

Briese (2006) advocated the use of secondary criteria to refine species-level test lists, and secondary- or sub-criteria that are relevant to cultivar selection can also be applied here. We propose applying the following (or additional) sub-criteria in conjunction with or instead of phylogenetic sampling, or in addition to phylogenetic sampling where phylogenies are incomplete:

a. Cultivar type

   i. Does the cultivar type or breeding approach provide evidence of genetic variability or suggest how broadly or narrowly sampling should occur?

   ii. Does cultivar type suggest how consistently characteristics of interest (e.g. leaf morphology, flowering time etc) will be expressed in the area of introduction?

b. Economic or amenity importance.

Importance implies some measure of value that could be interpreted in different ways and will be different for each country. Cultivar *importance* should therefore be carefully defined in each instance. For example, importance of a crop or ornamental species cultivar could be defined as area under cultivation, market share or segment, export value, cultural or heritage significance, or amenity (ref?). If cultivar importance is not defined, then the rationale for including or excluding cultivars from host-specificity testing cannot be scrutinized. When using this criterion to sample from a phylogenetic tree:

i. Prioritising the most important cultivars within a cluster is justifiable provided sampling is not otherwise compromised (sensu lato Sheppard, van Klinken & Heard 2005).

ii. In some cases, it may even be prudent to sample more heavily from clusters that contain a disproportionately large number of important cultivars in the area of introduction.

In any case "importance" must be defined, and evidence to support the claim of importance documented.

c. Availability of cultivar for testing.

i. Can the cultivar be sourced and grown to the appropriate life-stage for testing (ie. flowering, fruiting)?

d. Known or potential susceptibility to other pests and diseases.

i. Is the cultivar known to be susceptible to pests?

ii. Is the level of resistance, compared to other cultivars, uncertain?

e. Rootstocks

Is there evidence of an effect of rootstock on the part/s of the plant damaged by the agent? There may be more than one rootstock available for a single cultivar, or a variety of

rootstocks available for a cultivar group. In these cases the effect of each rootstock, or each combination of rootstock and scion should be considered.

f. Other possible sub-criteria:
    i. protection from insect attack,
    ii. plant chemistry or morphology (e.g. leaf trichome type or density) similar to target,
    iii. same breeding program or probable lineage,
    iv. phylogenetic distance of non-target species from target (i.e. there may be an argument to test fewer cultivars of distantly related non-target test plants, in which case representatives of very broad cultivar groupings could be selected),
    v. not previously exposed to the agent in the field (i.e. cultivars not previously exposed to the agent in the field may be a higher priority for testing than cultivars that co-occur with the agent in the native or an introduced range without evidence of attack).

3.2.7 How many cultivars?

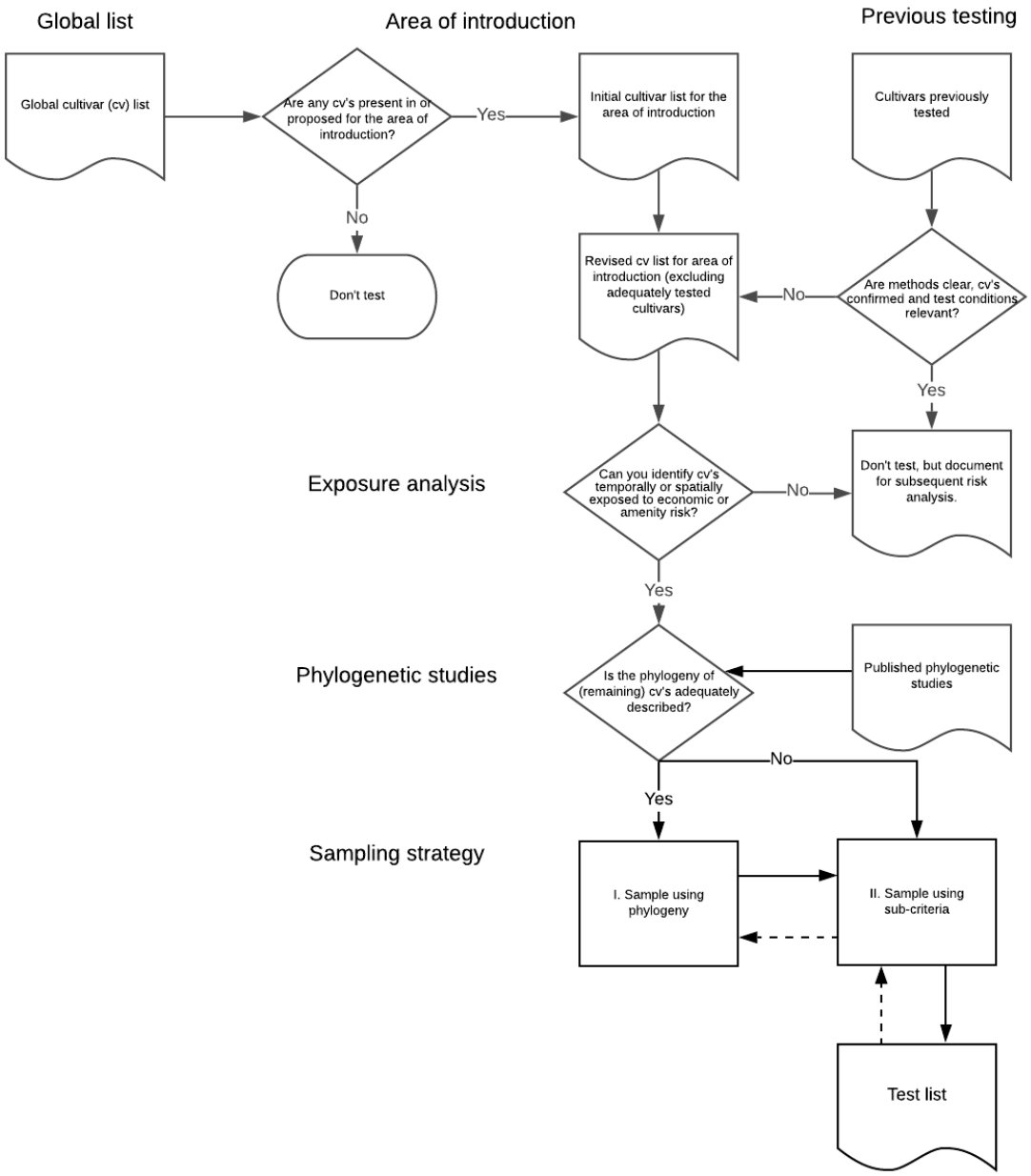
We recommend selecting a reasoned and diverse sample of cultivars that is still feasible to test. In doing so, we reject arguments (often implicit) that it is always better to test more cultivars or as many cultivars as possible. Conversely, we also argue against testing a few "important" cultivars without justification.

However, when phylogenetic information is available it is appropriate to specify how many cultivars were selected from each cluster and how they were selected. Options include selecting a fixed number from each cluster based on expert judgment of what is feasible, which can be subject to feedback and adjustment. Alternately, more samples may be taken

from large or disproportionately important clusters. There may be some element of random sampling (possibly proportionate sampling) from clusters, however this could result in the selection of unimportant or uncommon cultivars. An intermediate approach could be to remove/filter out cultivars that don't meet the selected sub-criteria, and then sample cultivars randomly and proportionately from those remaining in each cluster. The sampling approach adopted may depend on the suspected variability between cultivars.

3.2.8 Incorporating new information and feedback

Stakeholder analysis and input occurs throughout the development of the test list, and feedback can be incorporated at any stage of the selection process. Specific feedback loops during sampling allow the test list to be scrutinized and further refined; for example, surrogates can be selected based on stakeholder input, or for cultivars that are not available or difficult to source. We can also preferentially add cultivars from clusters where higher levels of feeding and development are detected (Lefoe *et al.* 2020).



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356 Figure 2. Process chart representing steps to select plant cultivars (cv) for host-specificity

357 testing.

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3.3 Applying the decision support tool:

Silverleaf nightshade *Solanum elaeagnifolium* is a widespread invasive plant that is toxic to livestock and depletes soil moisture and nutrients (EPPO 2007; Mekki 2007; Brunel 2011). Control of *S. elaeagnifolium* can be difficult due to the plants extensive root system, the need for repeated herbicide application, and the potential for off-target herbicide damage to pasture and crops (AWC 2012).

The North American leaf beetle *Leptinotarsa texana* was introduced to South Africa in the 1990's and is an effective biocontrol agent of *S. eleaegnifolium* in that country (Olckers *et al.* 1999). *Leptinotarsa texana* has also been proposed for introduction to Australia (SCA 1986). Lefoe *et al.* (2020) developed a host-specificity test list to assess the risk of introducing *L. texana* to Australia. Their test list comprised 59 Australian native and economically important plant species including the valuable crop potato *Solanum tuberosum*. We retrospectively applied our decision tool to the selection of potato cultivars for testing *L. texana* in Australia.

3.3.1 Selecting potato cultivars

From a global list containing thousands of cultivars we narrowed our selection to less than two hundred cultivars that we found were grown in Australia (the actual number grown in Australia may be greater). We compared the distribution of *Solanum elaeagnifolium* and *S. tuberosum* in Australia (Figure 3) and the predicted distribution of *L. texana* (Senaratne, Palmer & Sutherst 2008), and determined that most or all cultivars could be exposed to risk. We identified and noted three cultivars that were previously tested (Olckers, Zimmermann & Hoffmann 1995). Caruana *et al.* (2019) characterized the genetic relationships between 169 potato cultivars grown in Australia, and we used their study to inform our sampling. We visually divided the phylogenetic tree developed by Caruana *et al.* (2019) into major clusters,

and selected three criteria to sub-sample from each identified cluster, namely (1) cultivar type, (2) economic importance, and (3) availability of cultivar for testing. We used the categories of Caruana *et al.* (2019) to account for the three different usages or market segments they identified e.g. French fry, crisping and fresh, ensuring each of these usages were represented in the test list. We reviewed online resources from the four largest potato producing States, South Australia, Tasmania, Victoria and Western Australia (Wilson 2010), to identify economically important cultivars not captured by Caruana *et al.* (2019) and added those to our list. This process produced a list of 16 cultivars, of which 15 require testing (Table 1).

Figure 3. Main potato growing regions in Australia (ref or create my own map) (above left) and *S. elaeagnifolium* (ALA 2020a) distribution (above right).

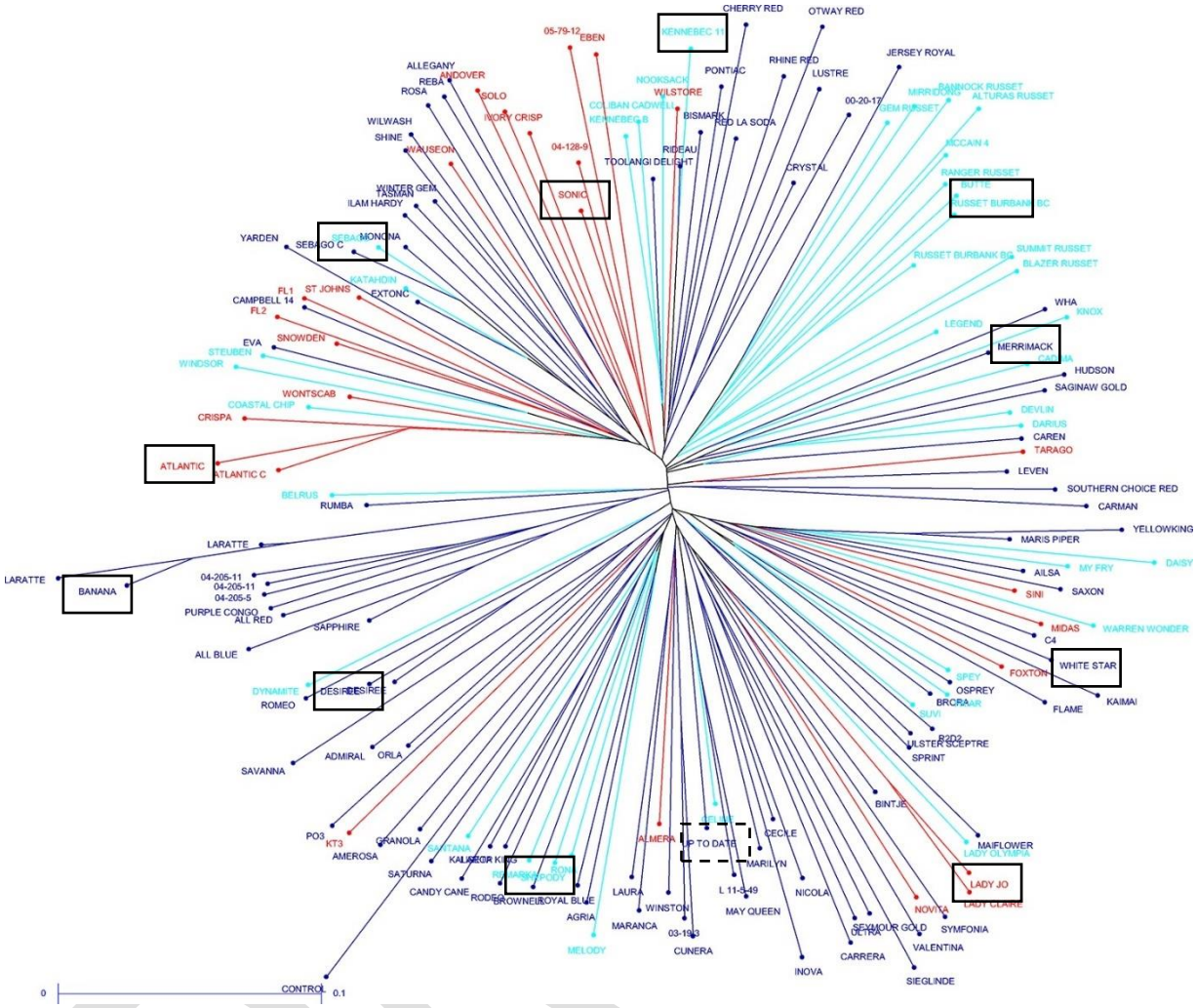

Figure 4. Unrooted neighbour joining dendrogram of the genetic dissimilarity between 169 potato cultivars available in Australia (Caruana *et al.* 2019) (CC BY). Dendrogram colour coding used here by Caruana *et al.* (2019) indicates usage type (light blue = French fry; red = crisping; dark blue = fresh). Rectangles overlayed onto the original image indicate a previously tested cultivar (- - -) (Olckers, Zimmermann & Hoffmann 1995) (Helmuth Zimmermann, pers. comm.) and additional cultivars selected for testing (——).

427 **Table 1. Assessment of potato cultivars for inclusion on a host-specificity test list for *L. texana* in Australia**

Section/node	Assessment	References
Global context Global cultivar list	Potato *Solanum tuberosum* is a globally important crop. Estimates of the number of cultivars worldwide range from to .	ref
Area of introduction Are any cultivars present in or proposed for the area of introduction?	Yes, many cultivars are grown in Australia. Potato is Australia's largest vegetable commodity by volume and total value.	Caruana *et al.* (2019)
Initial cultivar list for the area of introduction	A complete cultivar list for Australia was not found. However, one study sampled 181 potato cultivars grown in Australia, and to that list we added additional cultivars gleaned from scientific journals, industry seed catalogues (refs), industry experts including TS, and from growers and seed company representatives. This process greatly reduced the number of cultivars at risk (from thousands worldwide to hundreds in Australia) but there were still too many to feasibly test.	Caruana *et al.* (2019),
Previous testing Cultivars previously tested	*L. texana* did not utilise three potato cultivars tested in South Africa; 'Up-To-Date', 'Van Der Plank' and 'BP-1'.	Olckers et al 1995
Are methods clear, cv's confirmed, and results relevant?	Yes. Potato cultivars 'Up-To-Date', 'Van Der Plank' and 'BP-1' were exposed to first instar *L. texana* larvae and adults in replicated, no-choice feeding experiments. The number of plants tested of each cultivar was not published but we assume adequate replication. Some feeding was observed (not quantified but assumed to be negligible), but no development of larvae was recorded.	Olckers et al 1995
Don't test but document for subsequent risk analysis	We assessed 'Up-To-Date', 'Van Der Plank' and 'BP-1' as non-susceptible and removed these cultivars from the draft test list.	Olckers et al 1995
Exposure analysis Can you identify cv's temporally or spatially exposed to economic or amenity risk?	Yes. The distribution of *S. elaeagnifolium* in Australia largely overlaps or is contiguous with areas of potato production, and potatoes are grown throughout the period when *L. texana* would be active. Potato crops, including most or all cultivars, would therefore be likely to be exposed to *L. texana* both spatially and temporally if the agent were introduced to Australia.	ALA (2020b)
Phylogenetic analysis Is the phylogeny of remaining cv's adequately described?	Yes. A large study produced a phylogenetic tree illustrating the relationships between 169 potato cultivars available in Australia.	Caruana *et al.* (2019)

Sampling strategy Sample using phylogeny	Major groups or clusters of closely related cultivars were identified for sampling.	Caruana *et al*. (2019);
Sample using sub-criteria	We selected three criteria relevant to the Australian context to inform sub-sampling from the published phylogeny: **1. Cultivar type** We anticipated variability between potato cultivars because various traits, such as resistance to potato pests and diseases, have been introduced through interspecific hybridization. For example, resistance to potato cyst nematode *Globodera rostochiensis* was acquired in most new European potato *S. tuberosum* cultivars from *S. vernei*. Resistance to late blight *Phytophthora infestans* was also acquired through interspecific hybridization; in this case with *S. demissum*. Furthermore, potato is … (TS). We therefore prioritized sampling across clusters to better capture the genetic diversity of potato, rather than concentrating sampling (intentionally or not) from a few large or otherwise important groupings. **2. Economic importance** Potato has an estimated value of .. in Australia, and is grown in … South Australia, Tasmania and Victoria are the largest potato producing States (ref). Data on the economic importance of individual cultivars in Australia was difficult to obtain since the market is usually segmented according to usage (fresh or processing) or colour. South Australian and Victorian government factsheets highlight six cultivars as economically important; Sebago, Red Pontiac, Coliban, Russet Burbank, Desiree and Atlantic. Tasmanian production is dominated by two cultivars grown for the processing sector, Russet Burbank and Ranger Russet. A New South Wales government fact sheet reported the main cultivars grown in the State, by usage type, as Coliban and Sebago (fresh white-skinned), Desiree and Red Pontiac (fresh red-skinned), Atlantic (crisping) and Shepody (French fry). Data for Western Australia showed that the cultivar Nadine accounted for more than half the production in that State in 2013-14. We preferentially sampled these cultivars from the clusters illustrated in the partial phylogeny of Caruana *et al*. (2019), or added them to our list if they were not included in that study. We accounted for market segment by ensuring that each of the usage types listed by Caruana *et al*. (2019) (crisping, fresh and French fry) were represented in our final list in order to meet stakeholder expectations without compromising the scientific rationale for inclusion. **3. Availability of cultivar for testing** To determine cultivar availability, we examined certified seed potato catalogues, and contacted growers, home garden suppliers and representatives of industry peak bodies.	Wade (2010); AAC (2014); Brown, Caligari and Campos (2014); Elders (2018); Caruana *et al*. (2019); SPV (2019); freshlogic (n.d.)
Draft Test list	1. Up-to-date (already tested) 2. Desiree 3. Atlantic 4. Sonic 5. Shepody 6. Banana 7. Sebago 8. Kennebec 9. Russet Burbank[1] 10. White Star 11. Merrimack 12. Lady Jo 13. Red Pontiac 14. Coliban 15. Nadine 16. Ranger Russet[1]	Figure 4

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4. Discussion

A well-researched and documented *host-specificity test plant list* is a critical component of biological control risk assessment because it directs research effort to the plants considered most at risk of off-target impacts (Briese & Walker 2002; Briese 2003; Sheppard, van Klinken & Heard 2005). For crop and ornamental test plants, it is important that potential variability between cultivars is explicitly considered, especially for species that have many cultivars. A possible consequence of inadequate cultivar selection is Type II error; the failure to detect susceptible cultivars of important crop or ornamental plants. Such a consequence would not only increase the risk of off-target damage in cases where Type II error occurred but could damage the reputation of classical biological control as a safe and sustainable method of control for widespread weeds. Both outcomes are to be avoided, even more so at a time when there are ever increasing demands for reduced pesticide inputs into food and fibre production and the environment (Lamichhane *et al.* 2016). We argue that systematic selection and reporting of cultivars used in host-specificity testing will reduce the likelihood of Type II errors, without the need for excessive and unnecessary testing that could render classical biological control unfeasible.

This problem of adequately selecting and reporting cultivars was previously highlighted by Lefoe *et al.* (2020) who recommended that if cultivars are selected for host-specificity testing, then *"i) the method for selecting and prioritising cultivars should be described in sufficient detail to justify the final cultivar list, …".* However, this recommendation assumed that criteria for selecting and prioritising cultivars were standardized, or at least widely known and accepted in biological control literature. We did not find comprehensive or consistent criteria directly applicable to prioritizing cultivars of a species on a test plant list. Rather, we found it was often difficult to discern from the literature whether cultivars were

considered during the development of host-specificity test lists, or why some cultivars were tested while others were not.

This lack of consistently applied and accepted cultivar selection practice represents a barrier to transparent and reproducible host-specificity testing. While the Centrifugal Phylogenetic Method (CPM) (Wapshere 1974) and its refinements (Kelch & McClay 2004; Briese 2006) provide guidance on the selection of plant species for host-specificity test lists, the method is not directly applicable to selecting cultivars. The CPM applies molecular phylogenetic information and certain secondary criteria to prioritize non-target species that are most closely related to the target weed. However, relatedness to the target is not informative when prioritizing cultivars within an already selected test plant species. The test plant species has, presumably, already been selected based on relatedness; what we are now interested in is infraspecific diversity or hybridisation and any possible implications for host-range testing.

In his summary of the Wapshere (1974) method, Briese (2006) recommended testing "*other forms of the same species*", but Wapshere (1974) and Briese (2006) referred only to other forms of the target, not other forms of non-target test plants (furthermore, plant 'form' is a separate botanical category to 'cultivar' and the two labels shouldn't be confused). The International Standard for Phytosanitary Measures (IPPC 2016) states that for pest risk analyses "*the taxonomic level at which hosts are considered should normally be the "species".* While it could be argued that systematic selection and testing of plant cultivars is not required for biological control agent risk analysis, the standard also states "*The use of higher or lower taxonomic levels should be justified by scientifically sound rationale*"(IPPC 2016). For biological control host-specificity testing there is a sound rationale for considering lower taxonomic levels where selection and breeding of the plant may have altered the susceptibility of cultivars to natural enemies.

Therefore, we propose defensible criteria and a flowchart to support decisions of which cultivars to prioritise for biological control agent host-specificity testing. Our case study on the large, complex and economically valuable crop species potato *Solanum tuberosum*, highlighted that which cultivars are tested is a more important question than how many cultivars are tested. The focus in our case study was on selecting representatives of the cultivars that would be exposed to risk in the area of introduction while also providing a sound and defensible rationale for the selections. Also important in the case study was the demonstration of a collaborative and transparent process that enabled a draft list to be readily prepared using current knowledge, but still allowed for further scrutiny and updating. We showed that the process need not be onerous, regardless of the size and complexity of the cultivar group.

Lefoe *et al.* (2020) also highlighted the dangers of extrapolating host-specificity testing of certain cultivars to conclude a test species was not at risk. For example, Withers, Olckers and Fowler (2002) argued that *Gargaphia decoris* posed a low risk to potato in New Zealand because potato was not attacked in previous research in South Africa (Olckers 2000). However, Olckers (2000) did not report which, or how many, potato cultivars were tested in South African research. It is therefore difficult to assess, in this case, whether previous research is informative for New Zealand. This does not means that potato would be at risk in the field, since other evidence can be used in decision making (ERMA 2009). But the results of previous host-specificity testing alone may be insufficient without accounting for potential cultivar differences, testing approaches and context.

Transferability of cultivar test lists

For large or complex cultivar groups the list can be duplicated or modified for the same or similar targets in the area of introduction. For example, the potato cultivar list for *L. texana* could be readily adapted to other prospective agents of *S. elaeagnifolium*, or for test lists of

related targets such as *Solanum viarum* (IPAC 2017). Test list development in one country can also inform similar work in other countries, as was the case in Vanuatu where testing of a congeneric agent and target is planned with the leaf beetle *Leptinotarsa undecemlineata* (Quentin Paynter pers. comm.). However, in each instance researchers must consider any differences that could influence the composition of the cultivar test list. For example, the life-cycle or predicted range of a new agent or target could lead to a different assessment of exposure to risk.

Apart from host-specificity test list development, an added benefit of the decision tool is its potential application to post-release impact assessment. Post-release studies are important to assess the impact of agents on target weeds, and to identify any unanticipated direct or indirect off-target impacts in the field. Off-target impacts may not manifest for many years, and it could be useful at that stage to reassess the test list to identify new cultivars introduced in the intervening years. For example, the potato cultivar 'Harmony' was not tested against *L. texana* in South Africa because the cultivar was not available at that time. A re-assessment of potato cultivars now would likely prioritise 'Harmony' for post-release field surveys given its close relationship to the (possibly) susceptible 'Nadine' cultivar (Lefoe *et al.* 2020). Such an assessment would result in more targeted post-release field surveys.

The decision support tool could also be applied to the selection of cultivars for screening susceptibility to pests, keeping in mind that the aims of biological control risk analysis are not identical to those for pest screening. Pest screening is broadly concerned with (i) assessing the potential for economically significant damage to the main cultivars (by area or value) that are grown in a defined area, or (ii) screening new cultivars. Biological control risk analysis uses the results of cultivar testing to infer the level of risk to the species as a whole. Nevertheless, the decision tool provides a blueprint for a similar approach in pest screening research.

Parker *et al.* (2016) called for "*more thorough reporting of methods, results, data…*" in order to promote transparency and scientific rigour in ecology and evolutionary biology. We have identified an example of under-reporting in classical biological control which, if not addressed, could impede progress in the sustainable management of invasive species. We therefore encourage the adoption of these or similar approaches to the selection of cultivars for weed biological control host-specificity testing.

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