Comparing traditional methods of test species selection with the PRONTI tool for host-range testing of *Eadya daenerys* (Braconidae)

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Abstract A computer-based tool (PRONTI; Priority Ranking Of Non-Target Invertebrates) has been developed to assist the selection of invertebrate species for risk-assessment testing with entomophagous biological control agents (BCAs). PRONTI was used to produce a prioritised list of taxa for host-range testing with the braconid parasitoid *Eadya daenerys*, a potential BCA for the eucalypt pest, *Paropsis charybdis*. The resulting list was compared with a list developed using traditional species selection methods. Seven of the nine species on the traditional list were in the PRONTI top 20. The remaining two species (*Agasicles hygrophila* and *Cassida rubiginosa*) may not have been selected if the PRONTI tool had been used. These two species were on the traditional list because they are in the same family as the target and are considered valuable BCAs. Alternative BCAs were prioritised by PRONTI. The other 13 taxa prioritised by PRONTI were not on the traditional list: the taxa are little-known natives in the target subfamily or sister subfamily but larvae are presumed to be root-feeders, whereas target larvae are leaf-feeders. PRONTI can support the traditional approach by providing transparent evidence to support the selection (or rejection) of non-target species for host-range testing.

Keywords host-range testing, biosafety, host-testing list, phylogeny, risk assessment.

INTRODUCTION

Biological control agents (BCA) proposed for release in New Zealand must first be assessed for any risks they may pose to the receiving ecosystem, particularly to non-target species that could be used as alternative hosts or prey by entomophagous BCAs (Barratt & Moeed 2005). Therefore, selecting the non-target species to test is a very important task given the large number of native and valued introduced invertebrate species in New Zealand. Traditional non-target species selection methods have been outlined by the Food and Agriculture Organization and reviewed by Kuhlmann et al. (2006) to provide

biological control researchers with a sound approach to follow. In general, phylogeny plays a strong part in the species selection process (Hoddle 2004). In addition to phylogeny, species that are valued, such as beneficial BCAs and invertebrates (endemic or exotic) with cultural or aesthetic significance, are also recommended to be included in test species lists (Kuhlmann et al. 2006). Often this approach leads to a very large initial list that, for practical reasons, must be “filtered” by eliminating species that have disparate spatial, temporal and/or morphological characteristics from the target species. However, there is always room for interpretation when

assessing the phylogenetic and ecological affinities between the target host (invading pest) and each potential non-target. For instance, published phylogenies can change over time with taxonomic revisions, such as occurred with *Senecio* species (Compositae) endemic to New Zealand (Paynter et al. 2004), and non-targets can be overlooked as valued, such as occurred with fiddlewood (*Citharexylum spinosum* L.; Verbenaceae) in Australia (Manners et al. 2010).

This traditional approach for drawing up a host testing list was followed in 2015 when New Zealand *Eucalyptus* (Myrtaceae) growers expressed the need for a BCA to target the first generation of the eucalyptus tortoise beetle pest, *Paropsis charybdis* Stål (Coleoptera: Chrysomelidae) (Withers et al. 2015). The candidate BCA was the solitary larval parasitoid *Eadya paropsidis* Huddleston and Short (Hymenoptera: Braconidae). This species has since been split into three species, following intensive molecular and field research in Tasmania (Ridenbaugh et al. 2018). The agent introduced into containment and evaluated in New Zealand is *Eadya daenerys* Ridenbaugh 2018 (Peixoto et al. 2018).

In 2015, a non-target species list was compiled for host range testing with *E. daenerys* using the traditional approach for entomophagous BCAs (Kuhlmann et al. 2006); herein referred to as the “traditional list”. Initially, a list of the closest relatives to the target paropsine chrysomelid was made. No endemic species of paropsines occur in the New Zealand fauna so the closest related endemic species to the paropsines from the Chrysomelinae subfamily were considered, particularly those in the genera *Allocharis*, *Aphilon*, *Caccommolpus*, *Chalcolampra* and *Cyrtonotus* (Coleoptera: Chrysomelidae) of which there are 40 species (Reid 2006). Species in the Galerucinae were also considered because they have been shown to be the phylogenetic sister group to the Chrysomelinae, and New Zealand has approximately 100 endemic galerucine species (Reid 2014). New Zealand also has a large number of valued introduced weed BCAs, and all 13 chrysomelids on this list were

considered. This resulted in an initial list of 153 potential non-target beetles. To reduce the list to a manageable number, the list was filtered using a number of ecological attributes (Kuhlmann et al. 2006). As *E. daenerys* has only one generation per year present only during springtime (November–December), all species without spring-active, leaf-feeding larvae were excluded and the remaining endemic beetles with an adult body length greater than approximately 5-mm long (based on the minimum size of the smallest known host, *Paropsisterna agricola* (Chapuis) were prioritised. This process resulted in a list of ten non-target species to try to locate for testing with *E. daenerys* (Table 1).

The aim of the current research was to compare the non-target species list for *E. daenerys* produced using the traditional method with the list produced by a new computer-based tool known as PRONTI (Priority Ranking Of Non-Target Invertebrates; (Todd et al. 2015)). The PRONTI tool consists of two parts: a Microsoft® Access 2013 database (called the Eco Invertebase); and a mathematical model that uses the information in Eco Invertebase to produce a prioritised list of species for testing with the proposed BCA. Eco Invertebase is used to collate information: taxonomy; food web; biomass; ecology; anthropocentric value and testability (i.e. the ability to rear and test the species in captivity) on the invertebrate taxa found in New Zealand. It also provides data on the proposed BCA and the potential interaction between the BCA and each taxon. The model combines this information (using the selection criteria outlined in Kuhlmann et al. (2006)) and converts the data into a score by which the taxa can be ranked for host range testing with the proposed BCA. PRONTI has been actively tested for its ability to improve the selection of invertebrate species for risk assessment of entomophagous BCA with two BCAs previously released in New Zealand. These were *Microctonus aethiopoides* Loan (Hymenoptera: Braconidae) released to control *Sitona discoideus* Gyllenhal (Coleoptera: Curculionidae), and *Cotesia urabae* Austin & Allen (Hymenoptera: Braconidae) released to

Table 1 The “traditional list” of non-target species for host testing with *Eadya daenerys*. Species are listed in order of phylogenetic relatedness to the target. Status is E = Exotic self-introduced pest, N = Native, I = Introduced beneficial biocontrol agent (BCA). Exposed larvae = larvae are known to feed externally on the leaf surface. Ranks are included for clarity, and the two native genera *Chalcolampra* and *Allocharis* are listed separately, whereas in the original list they were considered so similar that they were ranked equally. Recreated from Table 2, page 184 of Withers et al. (2015).

Rank	Species and Status	Subfamily	Host	Primary reason for inclusion
1	<i>Trachymela sloanei</i> E	Chrysomelinae	<i>Eucalyptus</i> spp.	Same subfamily Same habitat
2	<i>Dicranosterna semipunctata</i> E	Chrysomelinae	<i>Acacia melanoxylon</i>	Same subfamily Adjacent habitat
3	<i>Chalcolampra</i> sp. N	Chrysomelinae	<i>Olearia colensoi</i>	Same subfamily
4	<i>Allocharis</i> sp. N	Chrysomelinae	uncertain	Same subfamily
5	<i>Gonioctena olivacea</i> I	Chrysomelinae	<i>Cytisus scoparius</i>	Same subfamily, BCA
6	<i>Chrysolina abchasica</i> I	Chrysomelinae	<i>Hypericum androsaemum</i>	Same subfamily, BCA
7	<i>Lochmaea suturalis</i> I	Galerucinae	<i>Calluna vulgaris</i>	Sister subfamily, exposed larvae, BCA
8	<i>Agasicles hygrophila</i> I	Galerucinae	<i>Alternanthera philoxeroides</i>	Sister subfamily, exposed larvae, BCA
9	<i>Neolema ogloblini</i> I	Criocerinae	<i>Tradescantia fluminensis</i>	Same family, exposed larvae, BCA
10	<i>Cassida rubiginosa</i> I	Cassidinae	<i>Cirsium arvense</i>	Same family, exposed larvae, BCA

control *Uraba lugens* Walker (Lepidoptera: Nolidae) (Barratt et al. 2016; Todd et al. 2016). The aim of the current study was to test the PRONTI tool prior to completing an actual host-range assessment for a BCA that was proposed for release in New Zealand. Of particular interest were whether or not: (a) PRONTI would prioritise different or additional at-risk non-target species that had not been identified using the traditional approach; and (b) if the tool provided information that may have resulted in the selection of different species from those on the traditional list (Table 1).

MATERIALS AND METHODS

The candidate BCA: *Eadya daenerys*

The Eco Invertebase was populated with the following relevant biological and ecological information about the proposed BCA, *E. daenerys*. *Eadya daenerys* is a solitary larval endoparasitoid (Rice 2005) and is now known to be most commonly associated with a small number of species within the genera *Paropsis* and *Paropsisterna* (Ps.) (formerly *Chrysophtharta*), together known as paropsines (Peixoto et al. 2018). *Eadya daenerys* is patchily distributed (G. Allen and S. Quarrell pers. comm.) in Tasmania but has been repeatedly collected throughout the state since 2011, from sea level to 600 m, so is considered abundant. Adults have been collected during the months of November to January with a peak in December. Adults can fly, and larval

E. daenerys use its host for only limited dispersal (the infested host larva drops to the ground to pupate). *Eadya daenerys* can infest all larval instars of its paropsine hosts, which range in adult size from 8.2 – 11.5 mm long, *Ps. agricola* to *P. charybdis*, respectively (de Little 1979; Nahrung & Allen 2004). All *Eadya daenerys* hosts are restricted to feeding on *Eucalyptus* spp., and more than one species of host can co-occur in its main habitat of mixed native Australian forests as well as commercial *Eucalyptus* spp. plantations in Tasmania.

Model criteria and weightings

Ideally, the PRONTI tool would be used to rank all invertebrate species that could be exposed to the BCA if it were released in New Zealand. In the case of *E. daenerys*, that would be all invertebrates found in or near *Eucalyptus* spp., which are very widespread in parks, gardens and plantations throughout New Zealand. Since such a list would be unreasonably large, the following ten categories were used to select a set of 127 invertebrate taxa more likely to be at risk from *E. daenerys* if it were released in New Zealand, and data on each were entered into the Eco Invertebase. This list included most of the 153 taxa considered using the traditional approach. However, where very little information was available for individual species within a genus (e.g., the ten species of *Allocharis*), the data were combined into a single entry for the genus. Although PRONTI can deal with some data gaps (see below), combining all available data into a single entry for the genus increased the certainty with which these taxa could be ranked by PRONTI.

1. Native Coleoptera most closely related to *P. charybdis*: we selected 16 of the 17 taxa in this category: 12 Chrysomelinae and Galerucinae taxa, plus four Chrysomelidae from other subfamilies from this category (the genus *Arnemus* was excluded because of the lack of available data).
2. Native Coleoptera in the same environment as *P. charybdis*: we selected ten of the 38 native coleopteran taxa known to feed on, or

in association with, *Eucalyptus* spp., including some predatory beetles.

3. Valued Chrysomelidae: we included ten of the 11 established chrysomelid weed BCAs in New Zealand.
4. Other valued, non-native Coleoptera in the same environment as *P. charybdis*: four of the six coleopteran BCA or non-native natural enemies found in association with *Eucalyptus* spp. were included.
5. New Zealand Euphorinae that could be at risk of hybridisation with *E. daenerys*: we included ten of the 21 taxa in this category: five native species, three BCAs, and two other non-native Euphorinae.
6. Invertebrate natural enemies of *P. charybdis*: all four known predators, the two known parasitoids, plus three spider species found in association with *Eucalyptus* spp. were selected.
7. Pests of *Eucalyptus* spp. that could be released from competition following control of *P. charybdis*: we included 42 of 75 taxa in this category, including three non-native chrysomelids, 11 other Coleoptera, and 28 non-coleopteran eucalypt leaf-feeders.
8. Chrysomelid pests of other forestry trees that could be released from competition following control of *P. charybdis*: only *Dicranosterna semipunctata* on *Acacia melanoxylon* (Fabaceae) fits this category.
9. Coleopteran hosts of Euphorinae in New Zealand: we included 21 taxa in this category because they were already in the Eco Invertebase, including 11 weevil hosts of *Microctonus* spp. (Hymenoptera: Braconidae).
10. Chrysomelid pests of non-forestry plants that could be controlled by *E. daenerys*: all four non-native paropsine pests in this category were included.

The 127 taxa were then ranked by the model that sits within the PRONTI tool by applying five selection criteria to the data in the Eco Invertebase. The criteria were: (1) the potential hazard (H) posed by *E. daenerys* to each non-

target taxon; (2) the potential degree of exposure (E) of each non-target to *E. daenerys*; (3) the hypothetical ecological impact (R & S) that may result from the exposure of the non-target to the hazard posed by *E. daenerys*; (4) the estimated

economic, social and cultural value (V) of each non-target; and (5) the assessed ability to source each non-target and to conduct tests (T), Table 2. To enable the model to do this, each datum in the Eco Invertebase was automatically assigned a

Table 2 Details of the changes to scores or weights given to questions used to inform each of the criteria used in the PRONTI test with *Eadya daenerys*. The questions listed are only part of the data used to obtain each criterion score (i.e. these changes only affected part of each score). NTS = non-target species; BCA = biological control agent.

Criterion	Question	Reason for change
Hazard (H)	What is the phylogenetic separation between target and NTS?	<i>E. daenerys</i> poses the greatest risk to NTS closely related to the target, so this attribute was given the highest weight in the calculation of H by multiplying all scores by 2. This was also done in the test of PRONTI with <i>C. urabae</i> (Todd et al. 2016).
Exposure (E)	Does the NTS occur in same community or habitat as the target?	These attributes were given the highest weight (multiplied by 2) in the calculation of E when PRONTI was tested with <i>M. aethiopoides</i> because of the risk posed to NTS in the same habitat as the target. This was a lower risk for NTS from <i>E. daenerys</i> because this agent is not known to attack unrelated species in the same habitat in Australia, so the multiplier was removed.
Resilience (R)	Five questions on NTS mobility, abundance, host range, dispersal and phenological overlap	Reducing the weight of attributes used to calculate E also reduced the maximum risk score (i.e., $H \times E$). In Equation 1, the NTS risk score is modified by the R score (i.e. the ability of the NTS to mitigate the risk). If the maximum risk score is reduced, the maximum resilience score must also be reduced so that it doesn't have too much weight in the model. Consequently, the scores for each of these attributes were reduced in this test of PRONTI by dividing them by 2.
Status (S)	Is the NTS rare or are there many documented foodweb links?	The S score indicates how "important" each NTS is to the ecosystem (i.e., NTS with large biomass and many links to the foodweb are more "important"). In previous tests of PRONTI, rare NTS were assigned a biomass of 0.001 per m ² ; however, this was found to reduce the NTS status score unfairly. Thus, in this test, rare NTS were assigned a biomass of 0.01 per m ² . Also, NTS that have been well studied may have many known foodweb links and obtain a much higher status score than other NTS. Consequently, foodweb scores were all divided by 2 to reduce their weight in the calculation of S.
Value (V)	Is the non-target endemic or rare?	Rare or endemic NTS often obtain a low S score because of their predicted low biomass and few known foodweb links. However, these species are highly valued, so questions on rarity and endemism were multiplied by 3 in the calculation of V in this test of PRONTI.
Testability (T)	Can the non-target be collected, reared, or does an existing protocol for rearing exist?	Criteria S, V and T were designed to be approximately equal because each was considered an equally important criterion for selecting NTS. Examination of these scores in this test showed that the average T score was higher than the average S and V scores. In previous tests of PRONTI, the attributes used to measure T were multiplied by 2, so these multipliers were removed in this test of PRONTI to bring T in line with S and V.

score, ranging from 0 to 10, with 10 assigned to those attributes that were most informative to the selection criteria. For example, species known to feed on eucalypt leaves were assigned a score of 10 for criterion 2 (exposure), while species feeding on other parts of eucalypt trees, or on other plant species, were assigned lower scores. A number of different pieces of data in the Eco Invertebase were used to inform each of the five criteria. Where data were “unknown” for a taxon, the middle score of five was always assigned, and this was then used to calculate the amount of uncertainty in each species’ ranking (see Todd et al. (2015) for more details).

With each test of PRONTI, the attributes of the agent, the characteristics of the agent’s hosts, and the context into which the agent would be released were examined, and changes were made where the scores or the weights given to certain attributes were more or less relevant to a selection criterion. For example, where an agent was only known to attack species that were closely related, the data representing that relationship were given a higher hazard weighting, whereas for agents known to attack species in a particular feeding niche, these data were given the highest weighting. Changes made to scores and weights for *E. daenerys* are given in Table 2.

To produce the prioritised list of species, several questions and therefore several scores were combined to provide a total score for each criterion. The scores for each criterion were then combined using the following equation:

$$\text{PRONTI score} = [(H \times E)/R] \times (S+V+T)$$

Equation 1

Where H = hazard (criterion 1); E = exposure (criterion 2); R = resilience (ability of the species to mitigate the risk; criterion 3); S = the status of the species in the receiving environment (criterion 3); V = the value of the species (criterion 4); and T = the ‘testability’ of the species (criterion 5). More details of scores, weights and criteria and how they are applied and calculated in the PRONTI tool can be found in Todd et al. (2015).

RESULTS AND DISCUSSION

A number of diagnostic tools were used to check the model was working correctly and that data had been entered consistently. Firstly, ‘dummy’ high, low and medium risk species were used to check that the scores were being applied appropriately in the model and were being ranked as expected (Todd et al. 2015; Barratt et al. 2016). Secondly, the scores obtained by the non-target species for each of the five criteria were examined to ensure the data gaps (i.e. all the unknown attributes assigned a score of five) were not having undue influence on the scores for any of the criteria, or on the final PRONTI scores. These attributes may be assigned higher or lower scores when known, so it is important to ensure that taxa with many unknown attributes do not obtain the highest or lowest score for any criterion. The top 25 non-target species ranked using the PRONTI tool are provided in Table 3, along with the rank assigned using the traditional method for test species selection.

The top 20 taxa on the list produced by the PRONTI tool (herein referred to as the “PRONTI list”) are different to those on the traditional list (Table 1), although the lists have seven taxa in common. Deciding which taxa to test from the PRONTI list requires interrogation of the data in the table: the PRONTI tool was developed as a decision-support system such that the information in the list should be used to support decisions on the taxa to undergo risk assessment with the BCA. For instance, it may be decided that taxa with low uncertainty in their ranking (e.g. less than 50%), and where the hazard is high (e.g. a hazard score greater than 75) should be selected, and only one species from each genus should be included in the test species list. Using these criteria, the following ten Chrysomelidae could be selected from the PRONTI list for testing with *E. daenerys*: *Gonioctena olivacea*, *Trachymela* spp., *Chaetocnema* spp., *Chrysolina abchasica*, *Aphilon* spp., *Pleuraltica cyanea*, *Allocharis* spp., *Longitarsus jacobaeae*, *Alema* spp., *Chalcolampra speculifera*. Whichever species are selected, the data in the table can be used to justify those decisions in any application to release the BCA submitted to the New Zealand

Table 3 Top 25 non-target species listed by PRONTI for host testing of *Eadya daenerys*. Species are ranked by their PRONTI scores rounded to the closest whole number. Origin is E = Exotic self-introduced pest, N = Native, I = Introduced beneficial biocontrol agent (BCA). Subfamily is given for Chrysomelidae. % uncertainty indicates the percentage of data used to calculate the PRONTI score that was unknown for each species.

Rank from PRONTI	Species and Origin	Subfamily or Family	Hazard score	Exposure score	Resilience score	Status score	Value score	Testability score	PRONTI score	% uncertainty	Rank in Table 1
1	<i>Gonioctena olivacea</i> I	Chrysomelinae	84	27	32	3	50	42	6660	13	5
2	<i>Trachymela catenata</i> E	Chrysomelinae	90	35	47	7	37	52	6481	17	-
3	<i>Chaetocnema</i> spp. N	Galerucinae	78	29	41	22	63	31	6388	36	-
4	<i>Trachymela sloanei</i> E	Chrysomelinae	90	34	51	41	17	48	6371	17	1
5	<i>Chrysolina abchasica</i> I	Chrysomelinae	84	23	40	31	45	45	5913	26	6
6	<i>Eucolaspis brunnea</i> N	Eumolpinae	65	29	49	50	53	49	5819	14	-
7	<i>Cyrtonegetus crassus</i> N	Chrysomelinae	85	22	40	27	68	20	5352	57	-
8	<i>Aphilon</i> spp. N	Chrysomelinae	84	23	43	25	68	26	5270	45	-
9	<i>Pleuraltica cyanea</i> N	Galerucinae	78	27	41	26	45	28	5131	31	-
10	<i>Allocharis</i> spp. N	Chrysomelinae	84	23	44	28	68	20	5094	45	4
11	<i>Longitarsus jacobaeae</i> I	Galerucinae	78	27	48	24	30	62	5089	18	-
12	<i>Allastena</i> spp. N	Galerucinae	77	22	45	24	78	31	5020	66	-
13	<i>Bryobates</i> spp. N	Galerucinae	77	22	45	24	78	31	5020	66	-
14	<i>Alema</i> spp. N	Galerucinae	78	26	48	4	78	36	4973	45	-
15	<i>Chrysolina hyperici</i> I	Chrysomelinae	84	27	48	24	25	52	4805	11	-
16	<i>Chalcolampra speculifera</i> N	Chrysomelinae	85	22	43	4	83	24	4803	47	3
17	<i>Dicranosterna semipunctata</i> E	Chrysomelinae	84	29	52	33	12	56	4739	19	2
18	<i>Phyllotreta undulata</i> N	Galerucinae	78	27	47	27	28	45	4416	32	-
19	<i>Lochmaea suturalis</i> I	Galerucinae	78	27	42	35	17	36	4386	11	7

Table 3 continued

Rank from PRONTI	Species and Origin	Subfamily or Family	Hazard score	Exposure score	Resilience score	Status score	Value score	Testability score	PRONTI score	% uncertainty	Rank in Table 1
20	<i>Trachytetra</i> spp. N	Galerucinae	78	24	49	27	48	36	4258	40	-
21	<i>Bruchidius villosus</i> I	Bruchinae	61	24	36	20	30	55	4231	11	-
22	<i>Aporocera melanocephala</i> E	Cryptocephalinae	63	33	38	31	10	36	4198	26	-
23	<i>Atrichatus ochraceus</i> N	Eumolpinae	65	22	43	32	63	31	4157	57	-
24	<i>Oemona hirta</i> E	Cerambycidae	44	29	47	59	56	36	4134	18	-
25	<i>Chrysolina quadrigemina</i> I	Chrysomelinae	68	27	44	31	20	49	4131	19	-

Environmental Protection Authority (EPA).

There were many similarities between the two lists, including four of the species on the traditional list also selected from the PRONTI list using the criteria given above, plus both lists include a species of *Trachymela*. It is perhaps more interesting to focus on the differences between the two lists.

The ten genera or species ranked highest from the PRONTI list above, are all Chrysomelinae or Galerucinae (Table 3), whereas the traditional list (Table 1), included two species from other subfamilies: *Neolema ogloblini* and *Cassida rubiginosa*. Unfortunately, *N. ogloblini* was accidentally excluded from the set of 127 taxa ranked by PRONTI, so it is not possible to examine that species further here. *Cassida rubiginosa* was on the traditional list because it is a useful BCA for Californian thistles, *Cirsium* spp. (Compositae), in areas of the Bay of Plenty and Wairarapa (M. Cripps, AgResearch pers. comm.). Also, this species met the ecological criteria for phenology overlap, with leaf-feeding larvae, a moderate body size, and potential habitat overlap where infested weeds might grow beneath or adjacent to eucalypt plantations. *Cassida rubiginosa* was ranked lower by PRONTI, primarily because of its more distant relationship to the target. If host range testing shows that *C. rubiginosa* is attacked by *E. daenerys* then improvements will need to be made to the PRONTI tool.

Of the four Galerucinae selected from the PRONTI list, three are native taxa (i.e. *Chaetocnema* spp., *P. cyanea*, and *Alema* spp.) and one is a weed BCA (*L. jacobaeae*, the ragwort flea beetle). Both Galerucinae on the traditional list are introduced weed BCA: *Agasicles hygrophila* is an important BCA of alligator weed in warmer climates such as Northland, Auckland and the Bay of Plenty (Stewart et al. 1999), and *Lochmaea suturalis* was introduced to control heather in central North Island. Both species have phenology overlap with the target, with leaf-feeding larvae, and there is potential habitat overlap should infested alligator weed or heather be found growing amongst eucalypt plantations. Both species are lower on the PRONTI list

because they are not common or well distributed nationwide. From the PRONTI list, *L. jacobaeae* could arguably have been selected as a test species: it is a BCA of ragwort in the Galerucinae sub family, and although the larvae feed on the roots, they emerge from eggs on the foliage so could be at-risk from parasitism before moving below ground. This species has lower resilience, with only one generation per year in some parts of the country (Gourlay et al. 2005). The three native galerucine taxa could also be alternative test species, although little is known about the larval feeding habits of these species, with larvae never having been collected it is presumed they feed on roots. Similarly, little is known about the endemic chrysomeline genus *Aphilon*, which was ranked highly by PRONTI but not included on the traditional list because of the difficulty of collecting and testing this species, and their small size. Host range tests with *E. daenerys* and the two native Chrysomelinae on the traditional list (i.e., *Chalcolampra* sp. and *Allocharis* sp.) may indicate whether further tests with other native Chrysomelinae are needed.

PRONTI ranked some other species highly that had not been included on the traditional list for a number of reasons. *Eucolaspis brunnea* was ranked highly on the PRONTI list because it is a chrysomelid, it has many links to the foodweb (they are generalist plant feeders), it is native and easy to collect and test. Although the adults are known to feed on eucalypt leaves, the larvae are root-feeders and so they were excluded from the traditional list.

The differences between the traditional list (Table 1) and the PRONTI list (Table 3) are primarily because the traditional list uses filters (e.g. phenology or testability) that remove taxa completely from the list, whereas PRONTI keeps all species on the list, but ranks those with low testability or mismatching phenology further down the list. There is no requirement to select the top 'x' species from the PRONTI list. The value of PRONTI is that the model considers a wider ecological range of non-target risks than the traditional method currently does (Todd et al. 2015). The PRONTI list (Table 3) included

nine endemic New Zealand galerucines in the top 20 that were not on the traditional list because the larvae were all thought to be root-feeders. This prompted a re-examination of the scarce literature and collections with much more care, and a reconsideration of whether or not any endemic species may be external leaf-feeders after all. No additional evidence for external leaf-feeding larvae was uncovered and *Lochmaea suturalis* and *Agasicles hygrophila* remained the only representatives of the Galerucinae on the final test-species list. However, the value of investigating NTS with less available data, and therefore higher uncertainty in their ranking, is obvious. This is encouraged by the EPA.

CONCLUSION

Compared with the traditional method of test-species selection for biological control risk assessment, PRONTI adds value by delivering a more consistently-applied, transparent evidence-based ranking of each non-target species. Also, it provides for the examination of how the rankings and test species selection have been achieved. Consequently, researchers are able to quantify their thoughts around why a species should, or should not, be considered for testing against a particular BCA, and examine those carrying higher uncertainty. PRONTI will become easier and faster to use as more species are added to the Eco Invertebase, and is available for use with other entomophagous BCAs under consideration for release in New Zealand.

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