Selecting potential non-target species for host range testing of *Eadya paropsidis*

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Abstract Classical biological control is proposed for *Paropsis charybdis* (Coleoptera: Chrysomelidae: Chrysomelinae), a eucalypt pest established in New Zealand. The Australian solitary larval endoparasitoid *Eadya paropsidis* (Hymenoptera: Braconidae) is under investigation. A potential non-target species list was compiled for host range testing. There are no endemic species of paropsines in the New Zealand fauna, only invasive pest beetles. The most closely related endemic beetles to the paropsines are Chrysomelinae in the genera *Allocharis*, *Aphilon*, *Cacmomolpus*, *Chalcolampa* and *Cyrtonogetus*. Little is known about these species. New Zealand has also introduced 12 beneficial chrysomelid weed biological control agents, which include Chrysomelinae and their sister group the Galerucinae. One endemic beetle, six beneficial beetles and two pest beetles are listed as the highest priority species for host specificity testing.

Keywords biological control, host specificity testing, Chrysomelidae, paropsine.

INTRODUCTION

Host range testing prior to introducing a classical biological control agent provides an estimate of the risk of negative impacts on non-target species Barratt et al. (1999). Phylogeny is a valuable starting point for predicting and assessing the field host range of a parasitoid (Hoddle 2004), but other criteria such as ecological similarities are also very important (Kuhlmann et al. 2006). Kuhlmann et al. (2006) proposed developing an initial list of all potential non-target species based on phylogenetic affinities, ecological similarity to the target, and socioeconomic considerations. This list is then filtered by spatial, temporal and biological attributes such as size that might make a species effectively inaccessible to the proposed

biological control agent, and secondly by the feasibility of obtaining laboratory colonies of the non-target species for testing. During host range testing, any new information gathered (such as attack by the proposed agent on one of the non-target species) may alter the type or extent of testing required, and potentially reduce or increase the final list of non-target species that are actually screened (Kuhlmann et al. 2006). This approach will be closely followed for obtaining a list of non-targets for host range testing of *Eadya paropsidis* Huddleston & Short (Hymenoptera: Braconidae).

Paropsis charybdis Stål is a eucalypt defoliator from Australia that has been present in New

Zealand (NZ) since 1916 (Bain & Kay 1989) and continues to be the most significant pest of eucalypts throughout the country. In particular *Eucalyptus nitens* (Deane et Maiden) Maiden plantations from Southland to the central North Island can be heavily defoliated, and numerous other *Eucalyptus* species in warmer regions are also highly palatable to the pest. The cost of managing *P. charybdis* (Withers et al. 2013) is a risk for new forest plantations being developed for timber, pulp and paper. Egg parasitoids of *P. charybdis* have proven inadequate for population suppression (Mansfield et al. 2011). Tasmania was selected as the source area for a renewed search for natural enemies of *P. charybdis*, because this species outbreaks occasionally there (De Little 1989). Also Tasmania is known to be a good climatic match to plantation forest areas of NZ (Murphy 2006). Three years of field and laboratory research identified the most promising agent to target first generation larvae of *P. charybdis* as the parasitoid, *E. paropsidis* (Withers et al. 2012).

Eadya paropsidis is a solitary larval koinobiont parasitoid specific to *Paropsis* and *Paropsisterna* species (Coleoptera: Chrysomelidae: Chrysomelinae) in Australia (Rice 2005a). It is a medium sized (ca 10 mm) black wasp with a bright orange head. *Eadya paropsidis* oviposits small (0.16 mm), hydropic eggs directly into the haemocoel of its hosts, and can attack all larval instars (Rice 2005b). Eggs of *E. paropsidis* hatch in around 5 days at 22°C (Rice 2005a). Developmental rates of *E. paropsidis* from egg insertion in the host to pupation in *Paropsisterna agricola* (Chapuis) have been determined over a range of temperatures and average 21 days at 20°C (Rice & Allen 2009). *Eadya paropsidis* emerges from the host's prepupal stage, spins a silk cocoon, and then undergoes an obligate pupal diapause for around 10 months until the following summer (Rice 2005a). Information (Rice 2005b) collected from one field site over 2 years suggests *E. paropsidis* is univoltine in Tasmania, with adults present in November and December. Recent field collections reveal it is widely distributed from sea level (near Hobart) to 600 m (near Cradle Mountain)

(G.R. Allen, unpublished data). The peak adult stage in December coincides with the peak early instar larval stage of many paropsines, including the abundant *P. agricola* (Rice 2005b). In 2014 *Eadya paropsidis* was imported into containment in NZ for rearing so that host range testing could begin. This paper outlines the process for developing a list of non-target species for host range testing.

METHODS

An analysis of the NZ coleopteran fauna and published phylogenies was conducted to establish which species of Chrysomelidae present in NZ had the closest taxonomic affinities to *P. charybdis*. To reduce all these potential non-target species down to a testing list, the "filters" of spatial, temporal and biological attributes were then applied. Spatially, those species present in the same or overlapping habitats to *P. charybdis* can be identified, but any non-target species cannot be ruled out at this stage because there is no information on the propensity for *E. paropsidis* to search for hosts in other habitats. Furthermore, *P. charybdis* has a nationwide distribution in NZ, wherever eucalypts are grown, which includes trees on the margin of other habitats. Temporally, species can be filtered down to those with larvae present when the adult parasitoid *E. paropsidis* will be active. The target pest *P. charybdis* is broadly bivoltine in NZ. Larvae are present from November to December, and February to March (Bain & Kay 1989; Murphy & Kay 2000; Jones & Withers 2003), with overlapping adult generations.

Rice (2005b) documented adult *E. paropsidis* activity in the field from adult emergence traps, malaise traps and parasitised larvae collections and found adults to be active from early December to early February but peaking in December. Adult *E. paropsidis* collected in the field live for a maximum of 24 days in the laboratory (Rice 2005a). Based on climate matching the phenology of *E. paropsidis* is expected to be identical in both countries. In summary, only non-target species with larvae present in early to mid summer are under likelihood of attack.

The final filter to be applied to predict risk to non-target species is biological attributes. Size is

the most obvious. *Eadya paropsidis* has been reared successfully in the laboratory on a wide range of host larval sizes, with oviposition into hosts ranging in size from first instar *P. agricola* (ca 0.5 mg) through to final instar *P. charybdis* (ca 120 mg) (G.R. Allen, unpublished data). *Eadya paropsidis* prefers early instars of *P. agricola* to parasitise and reaches on average around 26–36 mg pupal weight in this host (G.R. Allen unpublished data; Rice 2005b). Minimum viable host size limits have not yet been ascertained so non-target species cannot be ruled out at this stage based on larval size. The only biological attributes required for non-target larvae are that they have a leaf feeding mode (*E. paropsidis* searches on eucalypt leaves for host larvae), and are exposed during the day when *E. paropsidis* is active.

Potential non-target species present in NZ are taken through these filters to produce a revised list of species for host range testing against *E. paropsidis*.

RESULTS AND DISCUSSION

Categories for consideration of non-target species

The categories used for selecting an initial test list include ecological similarities, phylogenetic relatedness and safeguarding of beneficial insects (Kuhlmann et al. 2006).

The ecological similarity category includes beetles with external leaf-feeding larvae that feed on *Eucalyptus* spp. in NZ, and includes several pest paropsine species: *Trachymela catenata* (Chapuis) and *Trachymela sloanei* (Blackburn) (Murray et al. 2010), and *Paropsisterna beata* (Newman) (Kean et al. 2015) (currently under an eradication campaign). Also present is the Australian gum tree weevil *Gonipterus platensis* Marelli (formerly *G. scutellatus*). It feeds on the same host species as *P. charybdis* and the exposed larvae are leaf feeders in late spring (Nuttall 1989), but being a curculionid it may be too distantly related to paropsines to warrant further consideration (Kuhlmann et al. 2006). No native beetles with leaf-feeding larvae feed on *Eucalyptus* or other Myrtaceae.

Ecological similarity can also include insects associated with plant species found in directly adjacent habitats. Australian *Acacia* species are often planted mixed with *Eucalyptus* in

NZ. Beetles that feed on *Acacia* in NZ include *Peltoschema* sp. (Kuschel 1990; Reid 2006) and *Dicranosterna semipunctata* (Chapuis). The former has a much smaller body size than *P. charybdis*, whereas the latter has a similar body size and phenology to *P. charybdis*, and the first generation larvae feed on new phyllodes in December (Murray & Withers 2011).

The phylogenetic relatedness of the target species to other Chrysomelidae in NZ, is an important consideration for selecting non-target species for testing. The introduced beetle *P. charybdis*, belongs to the genus *Paropsis*, with approximately 70 species, 68 in Australia and 2 in New Guinea (Reid 2006). This is one of 11 closely related genera known in Australia as paropsines (Reid 2006; Jurado-Rivera et al. 2009). There are no native NZ paropsines, apart from the introduced pests already mentioned. The paropsines are a clade of genera in the chrysomelid subfamily Chrysomelinae, which includes more than 120 genera and 4000 species worldwide. Larvae of Chrysomelinae feed on leaves or rarely flowers. New Zealand has approximately 40 native species of Chrysomelinae in five genera: *Allocharis*, *Aphilon*, *Caccommolpus*, *Chalcolampra* and *Cyrtonegetus* (Reid 2006). These genera form a separate clade or clades from paropsines and are most closely related to genera in Australia, New Caledonia and South America (Reid & Smith 2004; Reid 2006; Jurado-Rivera et al. 2009; Reid et al. 2009).

New Zealand also has Chrysomelinae that have socioeconomic importance as biological control agents against exotic weeds (Hayes 2007) (Table 1). Within this list are three species of *Chrysolina*, and one species of *Gonioctena*, all related to paropsines and with similar adults and larvae to *P. charybdis*.

Furthermore several phylogenetic analyses have shown that the sister group to Chrysomelinae is Galerucinae (Reid 2014a). This is a huge subfamily with more than 11,000 species, but less than 100 in NZ. Most species have hidden larvae that feed in stems, leaf-mines or roots, and are therefore very unlikely to be available to *E. paropsidis*, but a few species have free-living leaf-feeding larvae.

None of the latter are native to NZ (Reid 2014b), but two species are introduced biological control agents (*Lochmaea suturalis* (Thompson) and *Agasicles hygrophila* Selman & Vogt) on heather and alligator weed, respectively (Hayes 2007). Based on the phylogenetic and biological affinities it is therefore suggested that these two above-named weed biological control agents belonging to the Galerucinae should be tested against *E. paropsidis* (Table 1).

The other native Chrysomelidae in NZ are genera in the Cryptocephalinae and Eumolpinae, neither of which are closely related to Chrysomelinae (Reid 2014a). The other introduced Chrysomelidae in NZ are species of Bruchinae (pest species), Cassidinae (1 potential pest, 1 biological control agent) and Criocerinae (4 biological control agents). Although these are phylogenetically distant from Chrysomelinae, Cassidinae and Criocerinae do contain species with external leaf-feeding larvae, for example, biological control agents from the genera *Cassida*, *Lema* and *Neolema* (Table 1). Because they are ecologically similar, and Chrysomelidae, a single species is included from each subfamily on the *E. paropsidis* non-target testing list. Preference will be given to those most easily collected, with the highest potential for spatial and temporal overlap with *P. charybdis*. Which representatives to choose will be considered below.

Biology and phenology of NZ Chrysomelidae considered for host testing

There is little information about the NZ native species of Chrysomelinae. *Allocharis robusta* Broun has been recorded on *Veronica* (Hudson 1934 as *Hebe* sp. cited in Spiller & Wise (1982) (Plantaginaceae) and one other species has been collected from *Olearia* (Compositae) (New Zealand Arthropod Collection, R.A.B. Leschen, Landcare Research, unpublished data). The apical plate of larval *Allocharis* may indicate that these are nocturnal larvae that burrow into stems for shelter during the day (Reid 2014b). Larvae and adults of *Allocharis marginata* Sharp have been reported as feeding on *Veronica salicifolia* (Plantaginaceae) along riverbanks (Jolivet & Hawkeswood 1995). *Aphilon* species are small and have been recorded

on mosses and liverworts (Bryophyta) in native bush, the adults being nocturnal (Kuschel 1990). Little is known of *Cyrtonogetus*, but it is suspected to have nocturnal larvae, while *Chalcolampra speculifera* Sharp may have diurnal larvae, but nothing is known of its native habitat or hosts. Australian species of *Chalcolampra* have been recorded from *Parahebe* (Plantaginaceae) and *Prostanthera* (Lamiaceae) (Reid 1993).

Selecting the species to test by applying the temporal and biological filters

The number of phylogenetically closely related species identified above was reduced by applying the filters of temporal and biological similarity to the target. Beetles in the sub-family Chrysomelinae or the closely related Galerucinae are identified at highest risk.

Phenology of the larval stages, in particular presence on leaves from late spring (November) to summer (January), is a significant limiting factor. On that basis both *Chrysolina* species on *Hypericum* are excluded as they never have larvae in early to mid-summer. Larvae of these species feed through the colder months from autumn until spring (Hayes 2007), and would be impossible to either obtain or test when *E. paropsidis* adults are present.

Larval habitat filters exclude *Neolema abbreviata* (Lacordaire) and *Lema basicostata* Monrós as the larvae feed predominantly within host stems (Hayes 2007). *Longitarsus jacobaeae* (Waterhouse) and *Bruchidius villosus* (F.) are excluded on the basis of larvae feeding fully protected within roots or seeds, respectively.

Despite its distribution in an aquatic habitat, *A. hygrophila* is not excluded, as it has external summer-active larvae and the host plant can invade terrestrial habitats (Stewart et al. 1999).

As noted above, the list is completed by choice of one of each of the Cassidinae and Criocerinae, these being *Cassida rubiginosa* Müller and *Neolema ogloblini* (Monrós), both of which feed on plants that are common in the vicinity of eucalypts. Both have larvae that are readily available for rearing.

Among the endemic Chrysomelinae, the species are selected for testing on the basis of ease of collection, ease of rearing and size. It is proposed that either *Allocharis* or *Chalcolampra*

Table 1 Beneficial weed biological control agents in the family Chrysomelidae in New Zealand, in decreasing order of phylogenetic similarity to Paropsini (Hayes 2007).

Species	Target weed	Larval phenology	Feeding habitat
Subfamily			
<i>Chrysolina hyperici</i> Chrysomelinae	<i>Hypericum perforatum</i>	Autumn to spring	Exposed leaf feeding larvae
<i>Chrysolina quadrigemina</i> Chrysomelinae	<i>Hypericum perforatum</i>	Autumn to spring	Exposed leaf feeding larvae
<i>Chrysolina abchasica</i> ¹ Chrysomelinae	<i>Hypericum androsaemum</i>	Spring to summer	Exposed leaf feeding larvae
<i>Gonioctena olivacea</i> Chrysomelinae	<i>Cytisus scoparius</i>	Spring	Exposed leaf feeding larvae
<i>Lochmaea suturalis</i> Galerucinae	<i>Calluna vulgaris</i>	Early summer	Exposed leaf feeding larvae
<i>Agasicles hygrophila</i> Galerucinae	<i>Alternanthera philoxeroides</i>	Spring to summer	Exposed larvae feed on non-submerged leaves, aquatic habitat
<i>Longitarsus jacobaeae</i> Galerucinae	<i>Senecio jacobaea</i>	Winter or summer	Soil dwelling root-feeding larvae
<i>Cassida rubiginosa</i> Cassidinae	<i>Cirsium arvense</i>	Spring and summer	External leaf feeding, covered in own frass
<i>Neolema ogloblini</i> Criocerinae	<i>Tradescantia fluminensis</i>	Spring to summer	External leaf feeding, shade, larvae covered in own frass
<i>Neolema abbreviata</i> Criocerinae	<i>Tradescantia fluminensis</i>	Spring to summer	Internal feeding in stems, last instar may feed on leaves, shade
<i>Lema basicostata</i> Criocerinae	<i>Tradescantia fluminensis</i>	Spring to summer	Internal feeding larvae in stems
<i>Lema cyanella</i> Criocerinae	<i>Cirsium arvense</i>	Early summer	External leaf feeding, shade.
<i>Bruchidius villosus</i> Bruchinae	<i>Cystisus scoparius</i>	Spring to autumn	Internal seed feeding larvae

¹Currently in containment.

species be used as the endemic species for testing.

The pests *T. sloanei*, *T. catenata* and to a lesser extent, *D. semipunctata*, share phenology and habitat overlap to the target *P. charybdis* and are potential hosts. However, since resources are limited and these are pest species, they are a low priority as test species.

Based on the reasoning outlined above, the species listed in Table 2 are the candidates proposed for non-target testing. The initial results of host testing will further refine this list, with additional species being added to the testing list if required, based on interim results, as is recommended by Kuhlmann et al. (2006).

CONCLUSIONS

The non-target list presented in Table 2 prioritises non-target species within the same subfamily to *P. charybdis*, the Chrysomelinae, including a species of endemic NZ beetle, if it can be obtained and cultured, as well as weed biological control agents in the sister group Galerucinae. It is hypothesised that if any non-target hosts are identified during host range testing, they will be from the most closely related species. Of lower priority, but included in the non-target species list, are one weed biological control agent from each of the less closely related Criocerinae and Cassidinae. Finally two pest beetles sharing the same niche

Table 2 Proposed non-target species list for host testing of *Eadya paropsidis*. Species are listed in descending order of priority.

	Species Subfamily	Status	Host	Similarity to target
1	<i>Allocharis</i> or <i>Chalcolampra</i> sp. Chrysomelinae	Endemic	<i>Olearia</i> or <i>Veronica</i>	Same subfamily
2	<i>Chrysolina abchasica</i> Chrysomelinae	Exotic beneficial	<i>Hypericum</i> <i>androsaemum</i>	Same subfamily
3	<i>Gonioctena olivacea</i> Chrysomelinae	Exotic beneficial	<i>Cytisus scoparius</i>	Same subfamily
4	<i>Lochmaea suturalis</i> Galerucinae	Exotic beneficial	<i>Calluna vulgaris</i>	Closely related subfamily
5	<i>Agasicles hygrophila</i> Galerucinae	Exotic beneficial	<i>Alternanthera</i> <i>philoxeroides</i>	Closely related subfamily
6	<i>Neolema ogloblini</i> Criocerinae	Exotic beneficial	<i>Tradescantia</i> <i>fluminensis</i>	Same family as target
7	<i>Cassida rubiginosa</i> Cassidinae	Exotic beneficial	<i>Cirsium arvense</i>	Same family as target
8	<i>Trachymela sloanei</i> Chrysomelinae	Exotic pest	<i>Eucalyptus</i> spp.	Same subfamily, Same niche
9	<i>Dicranosterna</i> <i>semipunctata</i> Chrysomelinae	Exotic pest	<i>Acacia melanoxylon</i>	Same subfamily Adjacent habitat

and an adjacent habitat complete the list. The final testing may be influenced by results of the host range tests as these are conducted, as well as insect availability, phenology and resources.

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