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# Technical Note

## Laboratory tests to determine if an Australian wasp, *Eadya daenerys*, is suitable for biological control of the Eucalyptus tortoise beetle, *Paropsis charybdis*

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**Summary:** We conducted extensive laboratory host-range tests with female *Eadya daenerys* against the target pest *Paropsis charybdis* and also against nine other closely-related beetle species found in New Zealand. These allow us to predict how the Australian parasitoid might behave in New Zealand, and the possible consequences and potential risks it poses to other non-target beetle species.

### Introduction

The Australian Eucalyptus tortoise beetle, *Paropsis charybdis*, is a major pest within New Zealand gum plantations. Present for over 100 years (Withers & Peters 2017), the pest has caused damage, defoliation, and sometimes death, to many gum trees throughout the country (Bain & Kay 1989). *Paropsis charybdis* finds some species of gum trees to be particularly palatable, especially *Eucalyptus nitens* (shining gum), a species grown mainly for wood and pulp and paper making (Murphy & Kay 2000), and other *Eucalyptus* species being grown for ground durable-wood and lumber production (Lin et al. 2017). The pest is responsible for economic losses within the entire forest products industry.

To manage the pest population of *Paropsis charybdis*, chemical control with aerial spraying of insecticides occurs in up to a quarter of large plantations annually. However, the costs associated with aerial spraying are prohibitive for many growers and a major barrier to increasing eucalypt plantations (Withers et al. 2013). Other undesirable outcomes, such as environmental and ecological harm and risking FSC certification could also result from long-term use of chemical insecticides. An alternative approach to insecticides is using classical biological control.

Biological control, known also as biocontrol, exploits a naturally occurring process in which a natural enemy of a target pest is introduced to an area from which it is absent, to give long-term control of the



Eucalyptus tortoise beetle larvae, the target pest



*Eadya daenerys*, proposed biological control agent

target pest. In the case of *Paropsis charybdis*, four potential agents have been introduced already in previous decades. Only two of these have been helpful, and a more effective control is needed. We were particularly interested in targeting the spring larval life stage, which currently goes largely unchecked. A promising agent that does target the feeding larval life stage is the native Australian parasitoid *Eadya daenerys* (Hymenoptera: Braconidae). (This was investigated under the name *Eadya paropsisidis* but a name change has now been advised).

To understand how the Australian parasitoid might behave in New Zealand, and the possible consequences and potential risks on other non-target beetle species, we conducted extensive laboratory host-range tests with female *Eadya daenerys*. Previously, this species has only ever been reared from eucalypt-feeding *Paropsis* and *Paropsisterna* tortoise beetles in Tasmania (Rice & Allen 2009). Tests were conducted against *Paropsis charybdis* and also against other closely-related beetle species found in New Zealand that *Eadya daenerys* may never have contacted before.

## Selection of closely related beetles

Beetles were selected for host range testing based on how closely related they were to the Eucalyptus tortoise beetle, *P. charybdis*, as relatedness is the best predictor of risk. *Paropsis charybdis* is a leaf beetle in the family Chrysomelidae. More specifically, tortoise beetles belong to the subfamily Chrysomelinae and within that, the tribe Chrysomelini (Leschen & Reid 2004).

After careful consideration, our laboratory tests were confined to the species listed in Table 1 (Withers et al. 2015). The two most closely-related beetle species to *P. charybdis* in New Zealand are *Trachymela sloanei* and *Dicranosterna semipunctata*. These were used in tests to usefully inform and help to delimit the host range of *Eadya daenerys*. Both species are pests that have invaded New Zealand from Australia in recent decades, with the former feeding on *Eucalyptus* species and the latter feeding on *Acacia* trees, particularly blackwood.

A native moderately-sized beetle, *Allocharis* near *tarsalis* (det. R. Leschen, Landcare Research, pers. comm 2018), active in spring feeding on the leaves of the subalpine shrub *Veronica* (*Hebe*) *albicans*, was collected from Kahurangi National Park in the vicinity of Mt. Peel (C. Wardhaugh, unpub. data, 2018). This was the only endemic species able to be located for host-range testing from the approximately 40 species of Chrysomelinae believed to exist in New Zealand. This species, being of moderate size and active in the spring made it very relevant to test, as most the

other species are substantially smaller with some suspected to be nocturnal. Little is known about the other species and they are classified as “naturally uncommon”. Sufficient larvae of *Allocharis* were collected with iwi and DOC permission, and transported safely back to the laboratory to enable the full suite of host testing experiments with *Eadya*.

*Chrysolina*, the tutsan leaf beetle, and *Gonioctena olivacea*, the broom leaf beetle, which both belong to the same beetle subfamily as the pest, were introduced to New Zealand as biological control agents for weed suppression (Hayes 2007). The former beetle was approved for release to biologically control tutsan, a serious weed in the North Island, but has not yet established in the field. The latter beetle has established as a biological control agent against scotch broom, a serious weed of roadside, productive and conservation lands in New Zealand. These two beetle species were included in our laboratory tests to ensure that *Eadya daenerys* would not harm them and therefore reduce the effectiveness of their weed suppression ability.

Two species of weed biocontrol agents of the subfamily Galerucinae were also used in testing, the heather beetle (*Lochmaea suturalis*) and the alligator weed leaf beetle (*Agasicles hygrophila*). These beetles are much smaller in body size than the tortoise beetles, but unlike our native Galerucines, some have leaf-feeding larvae that are active in spring. The heather beetle is established in the central North Island against the conservation weed heather (Ericaceae), and the alligator weed leaf beetle (*Agasicles hygrophila*) is a biocontrol agent for the semi-aquatic rooted alligator weed in Northland and the Bay of Plenty.

To ensure robust testing, we also tested two more species of more distantly-related leaf beetles that are also weed biocontrol agents with active, springtime, leaf feeding larvae. We chose to test the external feeding tradescantia leaf beetle (*Neolema ogloblini*) from the subfamily Criocerinae. It is established although not yet abundant, on tradescantia weed in some areas of the North Island. There are two more beneficial beetles in this subfamily that feed on tradescantia weed, but those larvae feed hidden inside the leaf and tip of the plant so we did not test them as we believe *Eadya daenerys* would not find them. We also tested the larvae of the green thistle leaf beetle (*Cassida rubiginosa*) established on pasture thistles, *Cirsium arvense*. It is in the subfamily Cassidinae. Interestingly, both these species of larvae carry a shield of frass (beetle poo) on their backs, called a faecal shield. We thought it possible the black colour of this shield may cause the larvae to be visually attractive to the *Eadya* wasps,

**Table 1.** The status of all the beetles (Coleoptera) used in the testing of *Eadya daenerys*. The darker the shading, the more closely related the beetle species is to the target pest *P. charybdis*.

Non-target beetles									
Target pest	small tortoise beetle	blackwood tortoise beetle	veronica leaf beetle	tutsan leaf beetle	broom leaf beetle	heather beetle	alligator weed leaf beetle	green thistle leaf beetle	tridascantia beetle
Family	Chrysomelidae	Chrysomelidae	Chrysomelidae	Chrysomelidae	Chrysomelidae	Chrysomelidae	Chrysomelidae	Chrysomelidae	Chrysomelidae
Sub-family	Chrysomelinae	Chrysomelinae	Chrysomelinae	Chrysomelinae	Chrysomelinae	Galerucinae	Galerucinae	Cassidinae	Citocerinae
Tribe	Chrysomelini	Chrysomelini	Phyllochroarini	Gonioctenini	Gonioctenini	Luperini	<b>Aitcini</b>	<b>Cassidini</b>	
Genus	<i>Trachymela</i>	<i>Dicranosterna</i>	<i>Allocharis</i>	<i>Chrysolina</i>	<i>Gonioctena</i>	<i>Lochmaea</i>	<i>Agasicles</i>	<i>Cassida</i>	<i>Neolema</i>
Species	<i>sloanei</i>	<i>semipunctata</i>	near <i>tarsalis</i>	<i>abchasica</i>	<i>olivacea</i>	<i>suturalis</i>	<i>hygrophila</i>	<i>rubiginosa</i>	<i>ogloblini</i>
									
Status	Pest	Pest	NZ Native	Beneficial	Beneficial	Beneficial	Beneficial	Beneficial	Beneficial
Size	5 x 8 mm	7 x 9 mm	3 x 7 mm	4 x 5.5 mm	3 x 4.5 mm	2.5 x 5.5 mm	2 x 5 mm	3 x 6 mm	2 x 5 mm

since wild *Eadya* appear visually attracted to the young black larvae of their host in Tasmania.

To be at risk of exposure to *Eadya daenerys*, larvae of non-target beetles need to feed during the daytime externally on the leaves of their host plants for at least a portion of their lifecycle. They would also need to do this in springtime (November-December). These criteria ruled out testing a number of non-target beetles including native Galerucinae (considered the “sister group” to Chrysomelinae) that are all thought to have only root-feeding larvae, and ruled out the St John’s Wort leaf beetles (beneficial biocontrol agents) as their larvae do feed on leaves but at other times of the year and are not present in spring.

We tested nine non-target species in total, always using *P. charybdis* as our control pest species for comparison. All tests were conducted with *Eadya daenerys* assuming the role of a natural enemy.

## The *Eadya daenerys* wasp

*Eadya daenerys* is a promising parasitoid because it attacks paropsine beetles from Australia. Rather than attacking the eggs of a pest (we already have two egg parasitoids in New Zealand that effect some control of *P. charybdis* populations (Mansfield et al. 2011)), it attacks the larval life stage in spring. Such larval attacks have been observed repeatedly and studied in depth in eucalyptus plantations in Tasmania (Peixoto et al. 2018).

*Eadya* has a one-year life-cycle, with adults present only in November and December (Rice 2005). At this time, in Tasmania, there is an abundance of young larvae of *Paropsisterna agricola* which the *Eadya daenerys* feed upon. This host is not available in New Zealand but *P. charybdis* is. The role of the adult is to lay an egg in the beetle larva. That larva then eats the inside out of its host. When it has reached maturity it pops out of the larva, leaving just a skin behind. It spins a silken cocoon in the soil where it hibernates for the rest of the year before emerging in the following spring as an adult to locate a mate and reproduce itself.

## No-choice physiological host range tests

No-choice tests (van Driesche and Murray, 2004) are considered to be the most thorough type of test that will reveal any possible harm to non-targets. This is because they force the parasitoid to make contact with non-target larvae by giving them no choice of anything else, and by holding the species together for a very long time without the parasitoid being able to escape.

For each of our no-choice tests against target and non-target beetle species, one female *Eadya*

*daenerys* was introduced to a 500ml plastic cage, with honey and water provided, and a sprig of foliage on which 8 larvae of the non-target beetle were feeding. They were then left undisturbed together for exactly 24 hours, after which time the parasitoid was removed and the larvae were reared to a beetle pupa stage. Any larvae dying prematurely were frozen then dissected to look for evidence of internal parasitism that might indicate that they had been attacked or stung by the parasitoid during the test. We are certain this long duration will have created maximum possible motivation for the parasitoid to attack the non-target larvae present.

With the target pest, our results indicated that when not attacked by *Eadya daenerys*, *P. charybdis* had a survival success to pupation of 79% (depending on disease incidence in the colony, it ranged from 66% to 95% survival). This is called a negative control. But when attacked purposefully by *Eadya daenerys* (one sting observed into each larva) the survival rate to pupation of *P. charybdis* dropped to less than 10%. This is called a positive control. This demonstrates the effectiveness of the parasitoid at causing additional mortality to the pest, and is why we hope it will be safe to release in New Zealand.

For the non-target beetles, about two-thirds of the beetle larvae that we took out of each 24-hour no-choice tests successfully survived to pupate, seemingly unaffected by *Eadya daenerys*, with an average survival of 5.4 out of 8 (Table 2). The best rearing survival was achieved on the non-targets: the native veronica leaf beetle *Allocharis* (90%), the broom leaf beetle *Gonioctena* (85%) and tradescantia leaf beetle *Neolema* (85%). The worst rearing success to pupation of just 40% was achieved on the tutsan leaf beetle *Chrysolina*, a problem inherent to the species and shared with Landcare Research from whom we obtained the colony.

Apart from the target host *P. charybdis*, *Eadya daenerys* parasitoids only completed development, and emerged as a fully developed adult wasp from one non-target species, the small tortoise beetle pest *Trachymela sloanei* (Table 2). Three of the five emergent parasitoid larvae from *T. sloanei* spun cocoons, with one cocoon producing one tiny adult *Eadya daenerys* after overwintering. This confirms *Trachymela* as a host for *Eadya* (= development to adult within a species).

The only other evidence of attempted parasitism by *Eadya daenerys* parasitoids was discovered by dissecting dead non-target larvae, or by killing and dissecting prepupae (mature larvae) that had failed to pupate. Internal parasitism was found in four non-target species, all from the subfamily Chrysomelinae: the blackwood tortoise beetle pest *Dicranosterna*, the

**Table 2.** Outcomes of the no-choice physiological host range tests of *Eadya daenerys* to non-target beetles subsequently reared to pupation.

Species	No. Reps	Total larvae reared	No. became beetle pupae	Beetle survival (%)	No. died but contain'd <i>Eadya</i>	No. died unknown causes	No. <i>Eadya</i> emerged from
Positive control <i>P. charybdis</i> 2015	4	32	3	9.4	0	18	11
Positive control <i>P. charybdis</i> 2016	26	205	14	7.0	0	108	63
<i>Trachymela</i>	5	40	23	57.5	0	7	5
<i>Dicranosterna</i>	16	128	80	62.5	2	46	0
<i>Allocharis</i>	10	80	72	90.0	6*	2	0
<i>Chrysolina</i>	14	112	45	40.2	2	65	0
<i>Gonioctena</i>	12	96	82	85.4	5	9	0
<i>Lochmaea</i>	11	92	69	75.0	0	23	0
<i>Agasicles</i>	14	112	62	55.4	0	50	0
<i>Cassida</i>	16	128	77	60.2	0	51	0
<i>Neolema</i>	15	120	102	85.0	0	18	0

\*These larvae failed to pupate but had not died so were killed by the researchers and discovered at that point to contain *Eadya* larvae

native veronica leaf beetle *Allocharis*, the tutsan leaf beetle *Chrysolina* and the broom leaf beetle *Gonioctena* (Table 2). Interestingly, six *Allocharis* larvae had not died and appeared quite normal, but had remained stuck in the pre-pupal stage (non-feeding mature larvae) for longer than they should have, and failed to pupate. Twenty days after they should have pupated we became suspicious, and killed these for dissection, which is how we discovered the presence of parasitoid larvae inside them. This is referred to as being an “unsuitable physiological host” (= unable to complete development all the way to adult within a species).

### Behavioural preference tests

The behaviour of individual *Eadya* parasitoids was also observed, in experimental arenas that were large clean glass petri dishes measuring 140 mm diameter x 20 mm high, and under two test conditions: either sequential no-choice tests or two-choice tests.

For the sequential no-choice tests, one female *Eadya* parasitoid was observed for 10 minutes with either eight target or eight non-target, host larvae settled onto a piece of leaf or sprig of host foliage, using an A-B or B-A sequence representing whether the target larvae were presented to the parasitoid first (A) and the non-target larvae presented second (B), or vice-versa.

For the two-choice tests, one female parasitoid was observed for 25 minutes with two sprigs of foliage present in the arena; at the same time, one sprig of *E. nitens* bearing eight larvae of the target larvae *P.*

*charybdis*, plus one of the non-target foliage bearing eight of the non-target larvae (A+B) appropriate to whichever species was being tested. Time recording began when the female *Eadya* contacted a host plant. All times spent on the plants were recorded, along with any interactions with larvae, such as attempted or successful stings (attacks), and probing of frass or other objects.

No female *Eadya* were tested repeatedly against the same non-target in the same type of test. We aimed to test 15 independent females in each test type against each non-target. Unfortunately, sometimes there were fewer than this due to either insufficient larvae or of live female *Eadya* during the testing stages. The actual number of replicates conducted ranged between 8 and 17 females for each type of test.

Analysis revealed the order of presentation (A-B vs B-A) had no significant effect on the likelihood or number of attacks by *Eadya* parasitoids. With the combined sequential test results, overall attack behaviour towards all species (target and non-targets combined) was not significantly different either between no-choice and two-choice test designs ( $p=0.065$ ).

A significant difference in *Eadya* attack behaviour towards *Paropsis* larvae compared to each of the paired non-target larvae occurred in all except for the paired *Paropsis-Trachymela* no-choice test. This can be seen in the box-and-whisker plots (Figure 1) which show a clear overlap of boxes only for the *Paropsis-Trachymela* pair and little or no overlap for the other species pairings. The median number of

attacks for the *Paropsis-Trachymela* pair was 2.5 for *Paropsis* and 1.5 for *Trachymela* while that for each other paired non-target larvae was identically zero attacks, while those towards *Paropsis* larvae ranged from two to 10 attacks.

In addition to examining the number of larval attacks, we developed a measure of excitation behaviour that the *Eadya daenerys* expressed towards the larvae present, while being in contact with that larva's host plant. We called this the 'attack rate towards larvae while on their plant' in a test:

*Attack rate on plant = Number of successful attacks observed / total time spent on that hosts' plant*

'Attack rate on plant' by *Eadya* towards *Paropsis* larvae was significantly greater ( $p < 0.05$ ) than 'attack rate on plant' towards non-target larvae for the majority of non-target species (Figure 2; as indicated by the lack of overlap of boxes in the box and whisker plots). The observations during assays suggested that the majority of the time *Eadya* was in contact with non-target plants bearing non-target larvae, she just sat and rested or undertook grooming behaviour. The only exceptions where a significant difference in behaviour did not occur (and boxes in Figure 2 overlap), were found for attack rate towards *Trachymela* in the no-choice test compared to *Paropsis* (both of which involved *Eucalyptus* leaves), but also towards *Allocharis* while on *Veronica* but only in the *Paropsis-Allocharis* two-choice test.

To determine the likelihood of attack on non-targets in the confines of these petri dishes, it can also be useful to understand what proportion of the *Eadya* females were responsible for the attack behaviour that is summarised above in Figures 1 and 2. It was more common for a female *Eadya* to attack a blackwood tortoise beetle *Dicranosterna* larva (6/17 attacked), than a tutsan leaf beetle *Chrysolina* larva (3/16 attacked), particularly under two-choice test conditions. However, the highest proportion of *Eadya* females exhibiting attacking behaviours towards non-target species was associated with *Trachymela* in the no-choice test (5/8 attacked). In the no-choice assays, on average only 8% of female *Eadya* attacked a non-target larvae compared to 100% attacking *Paropsis*. Within two-choice assays (which did not include *Trachymela*) the average was 15% of

*Eadya* females attacking a non-target, compared to 100% attacking *Paropsis*.

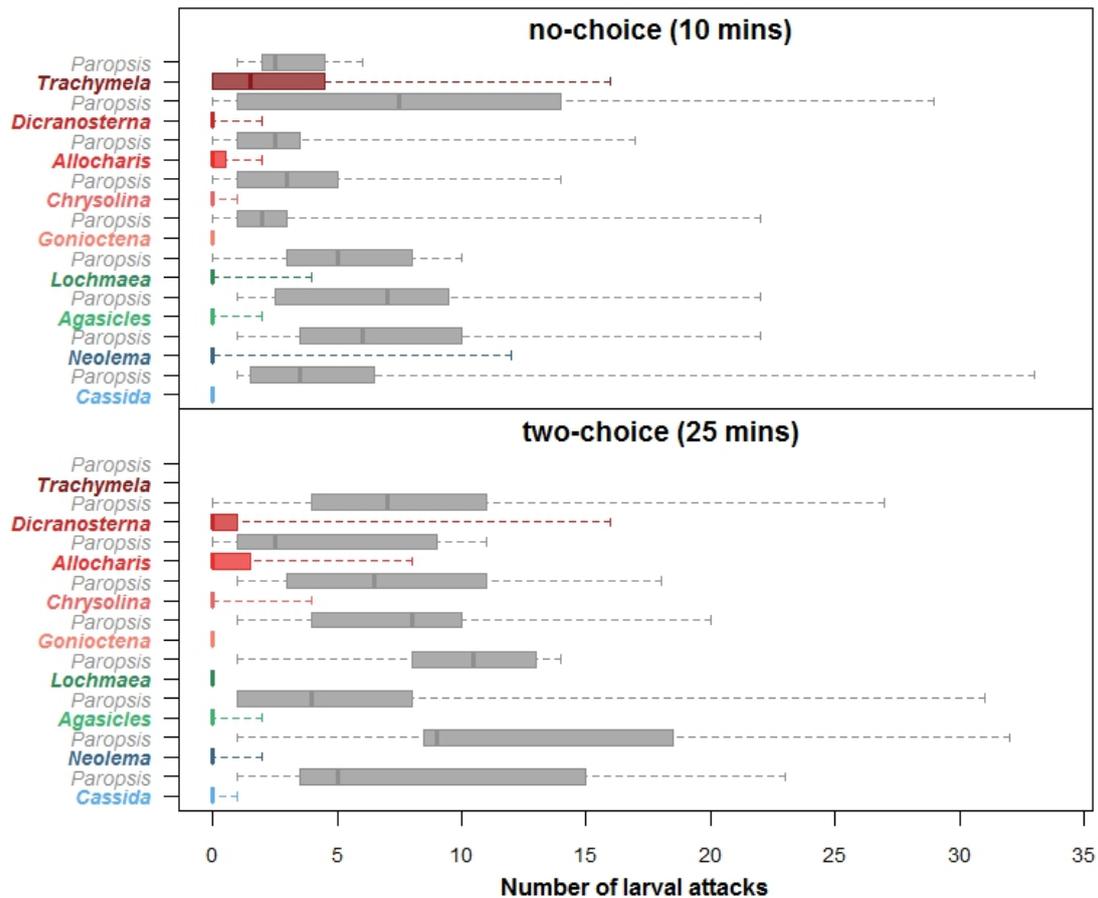
The on-rearing of target and non-target larvae in replicates, when attacks had been observed during behavioural observations, added a little more data to the physiological host range development data presented in Table 2. Observed attacks against tutsan leaf beetle *Chrysolina* resulted in 3/20 reared larvae being parasitized internally by *Eadya*. The same pattern as observed previously was repeated with native veronica leaf beetles *Allocharis* larvae; observed attacks resulted in another 5/19 larvae reaching pre-pupal stage but failing to pupate. These five larvae were dissected and three contained very small or encapsulated *Eadya* larvae, and one contained a well-developed *Eadya*.

## Conclusions

We have herein summarised the results of laboratory host specificity testing of *Eadya daenerys* female parasitoids against two pest paropsines beetles, one native species, and six beneficial biological control agents. All species tested had springtime-active, and external leaf-feeding larvae. Physiological development through to emergence of the parasitoid only occurred in the target *Paropsis*, and the pest *Trachymela sloanei* (at 12%).

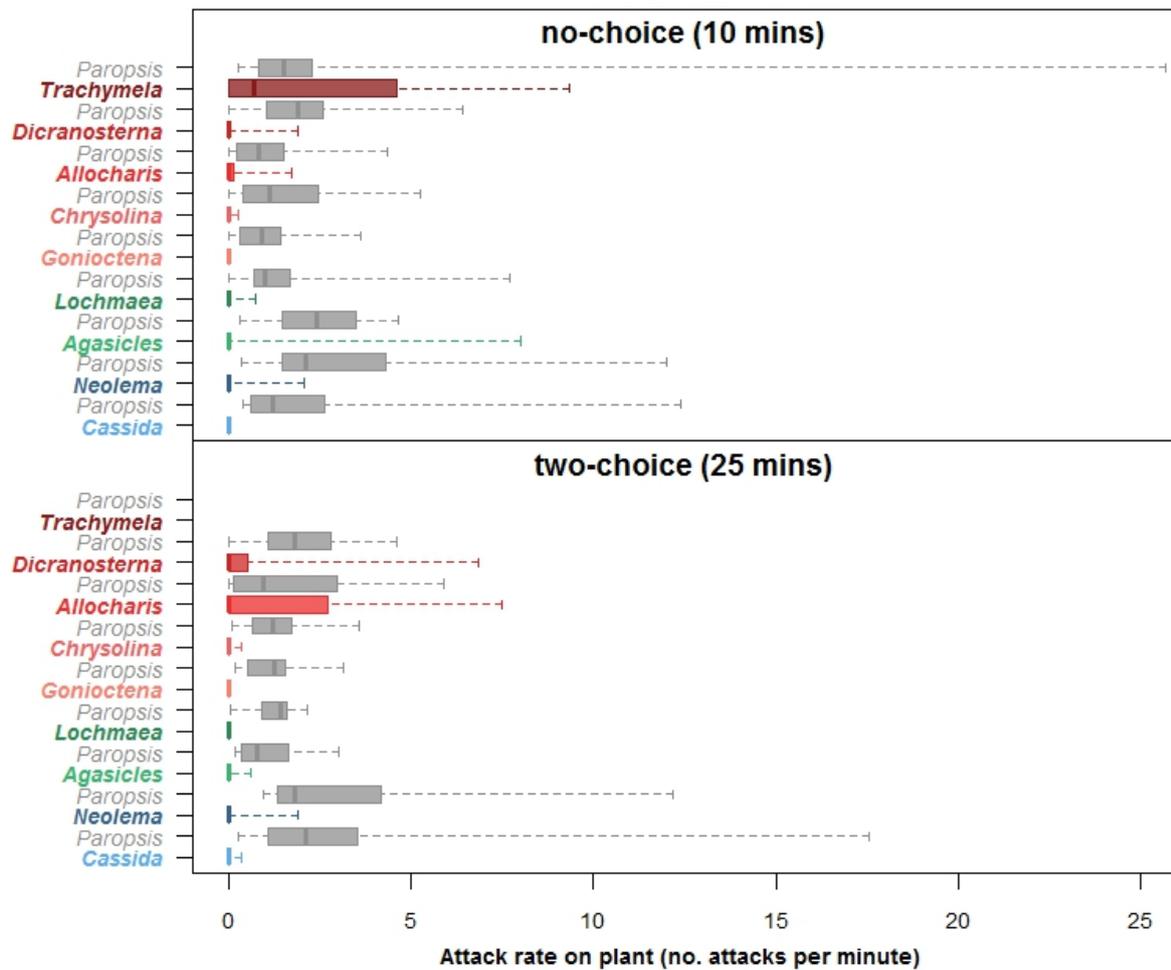
Incomplete physiological development by *Eadya daenerys* indicative of laying an egg, was discovered upon dissection at the level of 2-5% in another four non-target species that were all subfamily Chrysomelinae: in the pest *Dicranosterna*, in the native sub-alpine veronica leaf beetle *Allocharis*, in the tutsan leaf beetle *Chrysolina* and in the broom leaf beetle *Gonioctena*. *Eadya daenerys* will not form a self-sustaining population on any of these beetles.

Behavioural assays with female *Eadya daenerys* closely confined with larvae resulted in occasional observed attacks against almost all non-targets (but interestingly never against broom leaf beetle despite 3 replicates of the 24-hour no-choice tests resulting in internal parasitism). The stimulation to attack was highly significantly less towards all non-target species compared to *Paropsis* and leads us to conclude non-target attack was likely to be due to the confines of the testing environment.



**Figure 1.** Box and whisker plots representing total number of successful attacks counted by each *Eadya daenerys* in no-choice sequential and two-choice tests. The *P. charybdis* target larvae paired against each non-target larval species are shown in grey.

The vertical line within each box represents the median (i.e. middle value of the ordered data), the box represents the midspread (i.e. middle 50% of values), the whiskers represent the lower and upper data quartiles, and terminate at the minimum and maximum values respectively. Where no box is visible, just a line, it means that all the data points are sitting at or close to zero.



**Figure 2.** Box and whisker plots of *Eadya daenerys* ‘attack rate on plant’ behaviour directed against a beetle species in no-choice sequential and two-choice tests. The *P. charybdis* target larvae paired against each non-target larval species are shown in grey.

The vertical line within each box represents the median (i.e. middle value of the ordered data), the box represents the midspread (i.e. middle 50% of values), the whiskers represent the lower and upper data quartiles, and terminate at the minimum and maximum values respectively. Where no box is visible, just a line, it means that all the data points are sitting at or close to zero.

Only the other *Eucalyptus*-foliage feeding species, the small tortoise beetle pest *Trachymela* was an attractive non-target. The proportion of female *Eadya* exhibiting attraction to *Trachymela* larvae was relatively high. However, even this level of attack may not equate to them being suitable hosts, *Trachymela* larvae are nocturnal and hide during the day. The act of transferring them to leaves during the daytime disturbed them, causing them to run very rapidly around the petri dish perhaps as they sought out shelter. This made them a difficult target for *Eadya*, although they presented an attractive cue for oviposition when the *Eadya* were able to catch them.

The internal parasitism of the broom and tutsan leaf beetle larvae following exposure to *Eadya daenerys*, is likely to equate to minimal or nil impact in the field. This is because attraction to these larvae in behavioural assays was highly significantly less than that towards target larvae. It is likely that both broom and tutsan will be present in the same geographical areas of New Zealand as eucalypt trees and plantations. It is possible that, if *Eadya* establishes, it may overlap with these non-target beetle species, and could potentially encounter them if *Eadya daenerys* ever lands on these weeds to rest and groom. However, in our observations *Eadya daenerys* females were significantly more likely to actively search for paropsine larvae to attack when they were on eucalypt foliage, so we feel the risks of non-target attack occurring against broom and tutsan leaf beetles are low.

The internal parasitism of the veronica leaf beetle *Allocharis* may cause some concern. This beetle has only ever been collected between 1100 to 1300 m above sea level in Kahurangi National Park. There are no eucalypts growing in this national park, although they exist in the Motueka and Takaka river valleys approximately 50 km away. It is unknown whether *Eadya daenerys* will ever fly up into native subalpine forest habitats that are free of its host insects' plants (eucalypts). In Tasmania *Eadya daenerys* has been collected at 600 m above sea level at Moina, but that is the highest altitude at which *E. nitens* has been commercially planted there, and paropsine hosts were abundant at that site. We were unsuccessful at locating any other native sub-alpine beetle species that are medium sized and may have leaf-feeding larvae. They may be similar to *Allocharis* or may have internal feeding larvae such as the largest native Chrysomelinae beetle, *Chalcolampra speculifera*, whose larvae have been found sheltering within holes in the stem of *Olearia colensoi* (Wardle et al. 1971). Bearing all these uncertainties in mind, we consider there is minimal, although non-negligible, risk to native beetles in New Zealand from *Eadya daenerys*.

In summary, the data is consistent with field host relationship studies in Tasmania (Peixoto et al unpublished), and concludes *Eadya daenerys* is

unlikely to attack any species apart from pest paropsine (Chrysomelini) species feeding on *Eucalyptus*. Therefore we propose *Eadya* is safe to release in New Zealand.

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