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## Results of the first year of a SFF study to locate a spring-active parasitoid in Tasmania for potential biological control of *Paropsis charybdis*

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# EXECUTIVE SUMMARY

Despite considerable efforts the eucalyptus tortoise beetle *Paropsis charybdis* continues to defoliate *Eucalyptus nitens* plantations throughout New Zealand, preventing expansion of this forest resource, or requiring constant management through aerial insecticide application.

This reports on an SFF research project named Contract 12-039 “Scoping Biological Control for Eucalyptus Tortoise Beetle Larvae”. This report summarises the 2012 Fieldwork conducted in Tasmania by the project team and presents the data and analyses in more depth than its equivalent FFR report number DS-064.

A parasitoid wasp of the spring-time larval stage of the eucalyptus leaf beetle *Paropsisterna agricola* (Chapuis) (Chrysomelidae) in Tasmania is being investigated as a potential biological control agent for New Zealand. The potential agent *Eadya paropsidis* Huddleston and Short (Braconidae) was caught as adults on the wing from *E. nitens* plantations in northern Tasmania in December 2012 and brought into the laboratory in Hobart for behavioural testing. Both sequential no-choice and two-choice testing methods examined the response of individual field-caught females towards *P. agricola* and *P. charybdis* larvae. Females behaved significantly more positively in attacking *P. agricola* larvae than in attacking *P. charybdis* larvae, but both species were attacked and *E. paropsidis* reared out from them. This preference for attacking *P. agricola* may just be a result of the prior field experience they had unavoidably had.

Sentinel larval field trials were conducted by seeding groups of laboratory reared *P. charybdis* larvae out into *E. nitens* foliage in five separate locations in Tasmania, as well as field collections made of wild *P. charybdis* larvae. Reared from these collections were larvae of natural enemies including both Tachinid flies and *E. paropsidis*, specimens of which have been sent overseas for identification, while the remaining have been placed into over-wintering conditions in the laboratory. The results suggest this spring-active parasitoid *E. paropsidis* has good potential as a biological control agent for *P. charybdis* in New Zealand. This potential agent will be further evaluated in year 2 of the SFF study.

# Results of the first year of a SFF study to locate a spring-active parasitoid in Tasmania for potential biological control of *Paropsis charybdis*

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8 August 2013

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# Introduction

Paropsine beetles (Coleoptera: Chrysomelidae) are extremely diverse and abundant in their native Australian range but they rarely cause substantial damage in natural and undisturbed forest. They have emerged as significant eucalypt defoliators only since the expansion of managed plantation forestry, particularly when host trees are planted outside their native range. In New Zealand since 1916, *Paropsis charybdis* Stål effectively prevented the commercial establishment of the highly favoured pulp species *Eucalyptus nitens* (Deane & Maiden) Maiden for a long time until the introduction of the egg parasitoid *Enoggera nassaui* (Girault) (Hymenoptera: Pteromalidae) (Bain and Kay, 1989).

*Paropsis charybdis* is bivoltine in New Zealand. The first generation of eggs are laid in spring from October onwards and those laid early often escape any natural enemies (Murphy, 1998). After appearing in November *E. nassaui* can control the latter portion of first generation eggs and the second generation of eggs laid in summer time are controlled by *E. nassaui* as well as by a self-introduced primary egg parasitoid (*Neopolycystus insectifurax* Girault) which was first found here in 2000 (Jones and Withers, 2003). However since then the biological control has been disrupted by the arrival of the hyperparasitoid of *E. nassaui*, *Baeoanusia albifunicle* Girault (Encyrtidae) (Mansfield et al., 2011). This difference in control between beetle generations was attributed to a mismatch between the climate requirements of *E. nassaui*, which was obtained from a frost-free area of Western Australia, and the conditions experienced in the central North Island of New Zealand (Murphy and Kay, 2000). To improve performance against the first *P. charybdis* generation, another biotype of *E. nassaui* was imported from a cooler climate (Tasmania) and established in the central North Island in 2000 (Murphy and Kay, 2000; Murphy and Kay, 2004). There is not yet any evidence that this Tasmanian biotype has been able to exert any better control of the first pest generation in New Zealand (Withers et al., 2011).

With the market projections for sustainably grown *E. nitens* continuing to increase we undertook a fresh look at biological control prospects available to us for targeting the first generation of *P. charybdis*. The braconid wasp *Eadya paropsidis* Huddleston and Short (Hymenoptera: Braconidae: Euphorinae), was the obvious first choice for consideration, being univoltine, and responsible for high percentages of first generation parasitism of *Paropsisterna agricola* (Chapuis) in *E. nitens* plantations in Tasmania (Rice, 2005a; Rice, 2005b). Our first priority was to establish whether *E. paropsidis* would be effective against *P. charybdis* and be physiologically compatible.

A preliminary study undertaken in 2011 confirmed that *P. charybdis* was indeed a highly suitable physiological host for *E. paropsidis* (Withers, 2012). Additional research is now being undertaken under a SFF research project namely Contract 12-039 “Scoping Biological Control for Eucalyptus Tortoise Beetle Larvae”. This report summarises the outcome of the first year of research undertaken by the project team and also under sub-contract to entomologists at the University of Tasmania (TIA) in 2012-13.

# Materials and Methods

## *Insects*

*Eadya paropsidis* were caught as adults of unknown age on the wing from *E. nitens* plantations at Moina forest, Mersey District, northern Tasmania on 30 November and 6 December 2012. This forest had been sprayed approximately 24 months prior with alpha-cypermethrin to control leaf beetles. Adult female wasps were returned in chilled boxes to the laboratory within glass vials with mesh inserts in their lids, and provisioned with a male and a drop of liquid honey. They were maintained in an $18^0$ C temperature controlled cabinet for a maximum of 7 days before being used in experiments.

Control larvae of *Chrysophtharta agricola* (Chapuis) were obtained as eggs laid on juvenile foliage of *E. nitens* from Moina forest, and maintained in the laboratory on cut juvenile leaves of *E. nitens*.

The *Paropsis charybdis* colony was initiated from adults collected in 2011 from Hobart, Tasmania off *Eucalyptus ovata* Labill. and *Eucalyptus viminalis* Labill., and maintained in a cage with *E. viminalis* branches in a $20^0$C laboratory 16:8 L:D. Egg laying commenced in late November and as egg batches hatched, larvae were maintained on adult flush foliage of *E. nitens* before being used in experiments.

## *Behavioural Observations*

- Experimental arenas were large glass petri dishes measuring 240 mm diameter x 45mm high. Each contained a sprig of *E. nitens* foliage was placed, either juvenile foliage (bearing *C. agricola*) or adult flush foliage (bearing *P. charybdis*). A drop of honey was smeared onto the flat glass lid of the dish. Experiments were conducted at bench height under both fluorescent and natural lighting within a laboratory at ambient (20-23ºC) temperature in Hobart, Tasmania.
- The methods chosen for testing the hypothesis of the behavioural preference of *E. paropsidis* consisted of one female parasitoid observed at a time with either eight target or eight non-target host larvae in experimental arenas using a cross-over study of A-C or C-A sequence. A total of sixteen replicates were conducted.
- Parasitoids were introduced onto either the target host (eight larval *C. agricola*) for 10 mins (A), then almost immediately moved on to the test host (*P. charybdis*) in the C arena and observed for 10 minutes, or visa versa (hence cross-over type study). Behavioural observations began once the parasitoid first encountered (antennating) the first larva in the first arena, and began immediately upon entering the second arena. To eliminate parasitoids that were not in a physiological state suitable for testing, those that did not show interest in any hosts within 30 minutes were excluded and trialled again the next day.
- Behavioural observations consisted of total frequencies of the number of times parasitoids attempted to attack larvae “attacks” and proportion of the time spent actively searching on the foliage, versus being off the leaf. Also any attacks on frass (faecal pellets) were recorded. Larval probing behaviour consisted of the parasitoid stabbing forwards with its ovipositor. As it was not always possible to tell if the ovipositor had been successfully inserted for long enough (approx.. one second, (Rice, 2005a) for an egg to pass into the larva, resulting in a successful attack, probing and attacking were both counted together. Each female parasitoid was tested only once for behavioural observations using the cross-over A-C or C-A sequence, however those that were still alive were used on subsequent days in the two-choice assays (described below).

- Two-choice assays were carried out in the same arenas (all glassware had been cleaned and oven baked overnight at 90◦C between replicates and treatments to remove any chemical contaminants) using the same female parasitoids. One leaf of each foliage type bearing 8 approximately second instar larvae as above were both put into the dish at the same time approximately 10cm apart from each other. The orientation of the leaf in relation to the laboratory was changed between each observation. Assays were run for a maximum period of 25 minutes. Females were tested in a random order, then alternated between which leaf the female was encouraged to first alight upon. First leaf contact could never been totally controlled. Therefore first contact was assessed as a grouping variable in analyses (see below).
- Identical behavioural observations were recorded as described above, but in all cases the timing and type of leaf landed upon or larva contacted was recorded as either A (agricola) or C (charybdis).
- After the completion of the observations target and non-target larvae were transferred to 200 ml plastic containers into which holes for air movement were made. They were then reared in a 20 deg C, 16:8 L:D Contherm incubator for up to three weeks after completion of experiments. Fresh foliage was supplied as required, and all larvae were monitored twice weekly for either premature mortality, successful pupation, or emergence of an *E. paropsidis* parasitoid larva.

## *Data Analysis*

We applied linear mixed-effects models using restricted maximum likelihood estimation (R-package nlme) to analyse the residence time and the rate of larval attacks (number of larval attacks standardised by residence time). The fixed term of the model comprised 'host identity' and, in case of the crossover study, we also incorporated the host 'exposure sequence' and the interaction between these two explanatory variables. We used graphical model validation tools (residual plots and quantile-quantile plots) to check the model assumptions of homoscedasticity and normality. Variance heterogeneity occurred and was modelled using a constant variance function with 'host identity' as grouping variable. The significance of the fixed model terms was assessed via backwards selection using Akaike's Information Criterion (AIC) and likelihood ratio tests (Zuur et al., 2009). Because of the total lack of attacks on frass of *P. charybdis* (zero response), we applied a one-sample t-test to assess the significance of the attack rate (number of frass attacks standardised by residence time) on *P. agricola* frass.

## *Sentinel Larval Studies*

Sentinel larval trials were conducted at the following sites: Ellendate, Moina, Runnymeade, Pangarinda, and The Lea, Tasmania.

On each tree alongside a track, a branch of approximately 1 cm diameter was selected and tied down firmly to a stake in the ground, to prevent wind-thrash. The stake and the branch closer to the main stem were smothered in Tanglefoot™. Then branch foliage was clipped back to approximately 0.33m$^2$ of foliage. All insects and spiders that were located on that foliage were carefully removed, in three separate inspections. When confident that the foliage was insect and natural-enemy free, as many laboratory-reared larvae of either *P. charybdis,* (and at three sites also *P. agricola)* were released onto each branch with minimal disturbance, by either transferring with a brush, or stapling and tying the foliage on which they had been feeding, onto the cleared branch so the larvae could transfer easily to new foliage. Larvae were left for 72 hours.

Exactly 72 hours later we returned and carefully removed all larvae from each branch, putting them into plastic aerated containers, one for each replicate, and returning them to

the laboratory in chilly-bins. Each replicate was split up so that no more than 20 larvae were put into each container, and larvae were fed on *E. nitens* foliage as required and reared to pupation within a Contherm chamber set at 20ºC and 16:8 L:D cycle.

## *Field Collections of Larvae*

In field sites where larvae of *P. charybdis* (and a closely related *P. tasmanica*) were located, groups of larvae were brought back to the laboratory and reared to pupation. A subsample of the *E. paropsidis* reared from these field collections will be sent overseas for molecular and taxonomic identification. All remaining *E. paropsidis* (n=24) and Tachinidae (n=60) larvae that successfully pupated in the laboratory were transferred to a range of laboratory artificial over-wintering conditions, the results of which will be reported on in the next internal report.

Collections were made from natural populations at the following sites: The Lea, Runnymede, and Pangarinda, Tasmania.

# Results and Discussion

## *Behavioural Observations*

The residence time of *E. paropsidis* on leaves presenting *P. agricola* was between 3 and 8 times longer compared to leaves with *P. charybdis*, depending on the laboratory method of host exposure given (Table 1 and 2, Figs. 1 and 4). In the crossover no-choice study, the rate of larval attacks differed significantly between the two Paropsine larval host species (Table 1). *P. agricola* was on average 2.7 times more often attacked than *P. charybdis* (Fig. 2). Given simultaneous two host choice exposure, this significant difference in larval attack rate per residence time disappeared, although a slight preference for attacking *P. agricola* more quickly than *P. charybdis* remained (Table 2, Fig. 5). Interestingly, frass derived from *P. charybdis* completely failed to attract the parasitoid wasp to oviposit, whereas *P. agricola* frass stimulated on average one mis-directed attack per a two-minute period spent on the juvenile leaf type, irrespective of the laboratory method of host exposure given (no-choice crossover study: $t = 4.695$, $P < 0.001$; two choice study: $t = 5.395$, $P < 0.001$; Figs. 3 and 6).

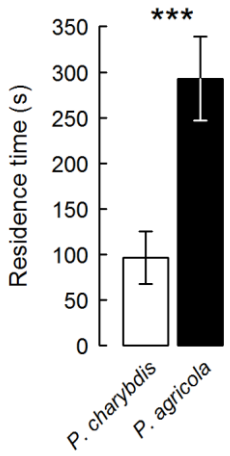


**Figure 1.** Average Total Residence time on the leaf type bearing the paropsine larvae either *P. charybdis* or *P. agricola* in cross-over no-choice study

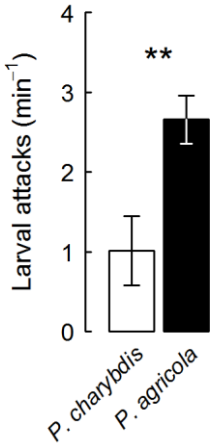


**Figure 2.** Mean number of larval attacks per minute on either *P. charybdis* or *P. agricola* in cross-over no-choice study

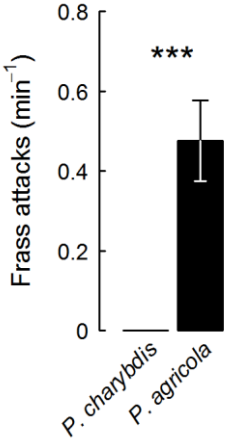


**Figure 3.** Mean number of frass pellets attacked per minute on either *P. charybdis* or *P. agricola*-bearing leaves in cross-over no-choice study. One sample t-test result for frass attack rate t = 4.6952, df = 16, p-value = 0.0002432

**Table 1** Results from model comparison procedures using the linear mixed-effects models for residence time and larval attack in the no-choice crossover study (backwards model selection based on Akaike's Information Criterion (AIC) and likelihood ratios tests).

| Dropped term | AIC | *df* | *L* | *P* | |
|---|---|---|---|---|---|
| ***Residence time*** | | | | | |
| None | 447.81 | | | | |
| Host × sequence | 445.85 | 1 | 0.043 | 0.835 | |
| Sequence | 444.37 | 1 | 0.520 | 0.471 | |
| Host | 456.01 | 1 | 12.162 | <0.001 | *** |
| ***Larval attack*** | | | | | |
| None | -136.62 | | | | |
| Host × sequence | -138.61 | 1 | 0.004 | 0.946 | |
| Sequence | -139.51 | 1 | 1.108 | 0.293 | |
| Host | -131.39 | 1 | 9.227 | 0.002 | ** |

## *Two-choice host experiment*

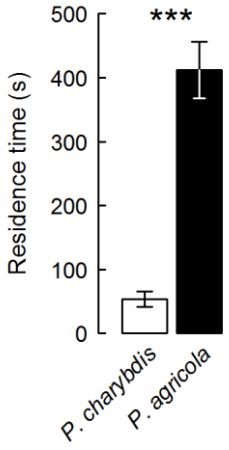


**Figure 4.** Average Total Residence time on the leaf type bearing the paropsine larvae either *P. charybdis* or *P. agricola* in two-choice study

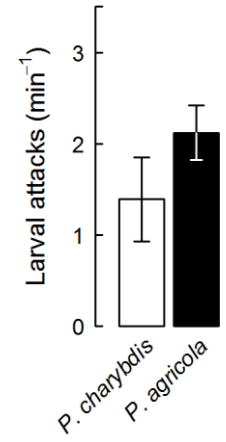


**Figure 5.** Mean number of larval attacks per minute on either *P. charybdis* or *P. agricola* in two-choice study

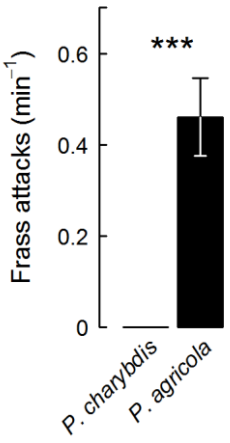


**Figure 6.** Mean number of frass pellets attacked per minute on either *P. charybdis* or *P. agricola*-bearing leaves in two-choice study. One sample t-test result for frass attack rate (two choice study):t = 5.3951, df = 20, p-value = 2.791e-05

**Table 2** Results from the linear mixed-effects models for residence time and larval attack in the two host choice experiment.

| Parameter | *Estimate* | *SE* | *t* | *P* | |
|---|---|---|---|---|---|
| ***Residence time*** | | | | | |
| Intercept | 412.29 | 44.32 | 9.30 | <0.001 | *** |
| Host | -358.57 | 46.07 | -7.78 | <0.001 | *** |
| ***Larval attack*** | | | | | |
| Intercept | 2.12 | 0.30 | 7.15 | <0.001 | *** |
| Host | -0.73 | 0.54 | -1.33 | 0.192 | |

All larvae that had been suspected of being attacked by *E. paropsidis* in the no-choice or two-choice assays were separately reared to pupation in the laboratory. Both paropsine species were hosts for *E. paropsidis*, but a lower number of both species of beetle larvae were parasitized by *E. paropsidis* in the two-choice tests, than remained unparasitised. The opposite was the case for *P. charybdis* larvae in the no-choice tests (Table 3).

**Table 3:** Total survival of beetle larvae or *E. paropsidis* larvae to pupation according to experiment.

| | **n beetle larvae reared from no-choice** | **n beetle larvae reared from two-choice** | **n *E. paropsidis* reared from no-choice** | **n *E. paropsidis* reared from two-choice** |
|---|---|---|---|---|
| *P. charybdis* | 18 | 27 | 6 | 11 |
| *P. agricola* | 29 | 74 | 36 | 48 |

## *Sentinel Larval Studies*

After undertaking sentinel larval trials with *P. charybdis* (and in three of the sites compared directly against control larvae of *P. agricola)*, each exposure for 72 hours in the following field locations (Table 4) we returned these larvae to the laboratory for identification and rearing. We are now able to confirm that *E. paropsidis* attacks the spring generation of *P. charybdis* in the wild in Tasmania. Furthermore the infestation rate was over 3% at four of the five sites where these sentinel larval trials were conducted. At three of the sites a greater percentage of larvae were infested with *E. paropsidis* than Tachinids. Only at the Moina site did Tachinid flies infest a far greater proportion of *P. charybdis* larvae (almost one third!) than did *E. paropsidis* (Table 4).

**Table 4:** Percentage of recollected larvae that were confirmed to be parasitized by rearing out either *E. paropsidis* or Tachinidae following 72 hours in the field in sentinel larval trials, December 2013. The sample size recorded is the total number of larvae seeded into the field.

| | **% beetle larvae infested with *E. paropsidis*** | **% beetle larvae infested with Tachinidae** | **Total n larvae** |
|---|---|---|---|
| **Ellendale:** | | | |
| *P. charybdis* | 5.0 | 4.1 | 188 |
| *P. agricola* | 6.0 | 9.6 | 194 |
| **Moina:** | | | |
| *P. charybdis* | 3.1 | 29.0 | 287 |
| *P. agricola* | 12.9 | 20.9 | 219 |
| **Runnymede:** | | | |
| *P. charybdis* | 6.0 | 0 | 150 |
| **Pangarinda:** | | | |
| *P. charybdis* | 6.25 | 0 | 150 |
| **The Lea:** | | | |
| *P. charybdis* | 0 | 0 | 150 |

## *Field Collections of Larvae*

From locating field populations of *P. charybdis* and returning these larvae to the laboratory for identification and rearing we are now able to confirm that *E. paropsidis* attacks the spring generation of *P. charybdis* in the wild in Tasmania. We were able to confirm this in three of the four wild populations located (Table 5). The site where sentinel larvae were NOT attacked (The Lea, in Table 4), we later located a wild population of *P. charybdis* on a different tree, and these HAD been attacked by *E. paropsidis* (Table 5), specimens of which have been sent overseas for identification. This means *E. paropsidis* was found to be active in every site except for one (Kingston turn-off) in which we located wild populations or undertook sentinel larval trials with *P. charybdis* in December 2012.

**Table 5:** Percentage of wild-caught and collected *P. charybdis* larvae that were confirmed to be parasitized by rearing out either *E. paropsidis* or Tachinidae

| | **Number of beetle larvae infested with *E. paropsidis* of those collected** |
|---|---|
| **The Lea:** *P. charybdis* | 2 (from 7) |
| **Runnymeade:** *P. charybdis* | 3 (from 10) |
| **Pangarinda:** *P. charybdis* | 1 (from 4) |
| **Kingston turn-off:** *P. charybdis* | 0 (from 2) |

# Recommendations and Conclusions

The first year of the SFF project “Scoping Biological Control for Eucalyptus Tortoise Beetle Larvae” has been a great success. Vin Patel (TIA) succeeded in rearing a large and healthy laboratory colony of *P. charybdis*, our target for biological control in New Zealand. With these larvae we were able to carry out a number of laboratory experiments and sentinel larval trials in the field. The laboratory experiments have revealed that field-caught female *E. paropsidis*, which is the spring-active natural enemy we are most interested in, readily attacks *P. charybdis* in the laboratory. Despite females showing a greater propensity to search juvenile leaves infested with the field host *P. agricola*, and to attack frass pellets of *P. agricola*, in a two-choice situation once the data was corrected for leaf residence time, attack rates were not significantly different between *P. agricola* and *P. charybdis*.

Unlike *P. agricola* larvae which are gregarious on juvenile leaves, *P. charybdis* feed independently and disperse all over their branches of adult flush foliage, making host location arguably more difficult for *E. paropsidis* as they can locate only one at a time. Whereas the common field host *P. agricola* feed gregariously clustered all together on juvenile leaves, are slower to thrash out at the parasitoid and the parasitoid is more efficiently able to locate and attack clusters of these species of larvae, and can attack a number of them in quick succession before they disperse in reaction to the attack. Considering that all females caught from Moina had undoubtedly had field experience of *P. agricola* prior to the laboratory trials, and may also have been reared from that host in the field, this is a promising result. Furthermore attacked *P. charybdis* larvae reared right through showed similar infestation levels from *E. paropsidis* to the *P. agricola* larvae. This backs up the preliminary findings of Withers (2012). If this parasitoid wasp were introduced into New Zealand it is likely that if the rearing host was *P. charybdis* and field experience was limited to infestations of *P. charybdis*, that female *E. paropsidis* search and attack behaviour would not be a limiting factor to biological control success.

The careful field searches conducted by Dean Satchell in December 2012 resulted in additional information, that of wild populations of *P. charybdis* in Tasmania being readily attacked by *E. paropsidis.* This is very encouraging to the potential biological control project. Adding weight to this was the results of the sentinel larval trials, in which *E. paropsidis* infested a higher proportion of most larvae that had only been exposed to them in the field for 72 hours than did Tachinid flies in three out of four sites (Tachinidae are another common natural enemy of paropsine larvae in Tasmania, Rice 2005b). In fact *E. paropsidis* was found to be active in every site except for one (Kingston turn-off) in which we located wild populations or undertook sentinel larval trials with *P. charybdis* in December 2012.

The second year of planned research under this SFF-funded project will answer many more of the important questions that arise from this study, including confirming parasitoid identity, and establishing laboratory rearing methods.

## Acknowledgements

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Tasmanian Museum, for assisting us. Without Vin maintaining such a healthy laboratory colony of *P. charybdis* this research would not have been possible. Thanks to Forestry Tasmania for access to field sites. Special thanks to Southwood Exports Ltd for sending Hannah to assist us in Tasmania. Thanks to the families of all of those involved who rallied around in support of us to ensure this project work would be a success. Funding, both cash and in-kind, was provided by SFF (MPI), FFA, Scion (MBIE Core-Funding), FFR Diverse Species, Southwood Exports Ltd, CHH and the FOA.

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# Photos

*Image 1: Female* Eadya paropsidis *feeding on honey during a no-choice laboratory trial*

*Image 2: Vin Patel sets up a secured branch for attaching sentinel larvae at the Moina field site*