The host range of *Freudeita cf. cupripennis*, a biological control agent for moth plant, *Araujia hortorum*

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Summary

- The host range of the moth plant beetle was determined in laboratory tests. Moth plant beetles were sourced from Melilla, Uruguay.
- Experiments conducted in the Landcare Research containment facility at Auckland, New Zealand tested 10 plant species related to moth plant in the family Apocynaceae.
- Newly-emerged adult beetles fed heavily on the foliage of moth plant controls and on *Oxypetalum caeruleum*. The foliage of other test plants was not significantly affected.
- Females lay eggs at the base of plants on which they feed. It is unlikely that females would lay eggs on any species other than moth plant and *O. caeruleum*
- Newly-hatched larvae placed on the surface of the soil of potted plants of *O. caeruleum* and
 moth plant controls colonised the roots and substantially completed development. Larvae
 placed on the soil surface over the roots of the other plant species tested did not survive,
 except for two atypical larvae on the roots of *Hoya carnosa*, which might be contaminants.
 The risk of larval development on non-target plants other than *O. caeruleum* is insignificant.
- Oxypetalum caeruleum is closely related to moth plant in the sub-tribe Oxypetalinae. Tests indicate that it is a fundamental host of the moth plant beetle population from Uruguay and could be at risk in New Zealand.
- There is no evidence that plants belonging to the Apocynaceae but outside the sub-tribe Oxypetalinae could be used by *F. cf. cupripennis*, and that plants outside the family will not be hosts for this insect in New Zealand.
- Moth plant beetles introduced to New Zealand will be sourced from Melilla, Uruguay only
- Release of a population of F. cf. cupripennis from La Plata, Argentina was approved by EPA in 2011 under the name Colaspis argentinensis. it was never released from containment. The predicted host range of the Melilla population is identical to that of the La Plata population previously approved for release.

Introduction

This report outlines the history of the biological control programme against moth plant in New Zealand and presents the results of research to determine the host range of *Freudeita cf. cupripennis* (Lefèvre), and hence the direct risk it would pose to valued plants if introduced to New Zealand.

The biological control programme against moth plant

Moth plant, *Araujia hortorum* Fournier, is an increasingly important environmental weed in northern regions of New Zealand. A biological control programme was initiated in 2000 to mitigate the effects of the weed and to reduce the rate of spread, because conventional control methods such as herbicide application and physical removal are expensive, damaging to non-target plants, and often impractical.

Approval to release the beetle *Colaspis argentinensis* was granted by EPA in 2011 (APP201039). The approval could not be exercised within the statutory 5-year period and lapsed in 2016. Subsequent research shows that the beetle approved for release should be called *F. cf. cupripennis*.

Suitability of moth plant as a target for biological control

The programme began with an assessment of the prospects for biological control as a management tool (Winks and Fowler, 2000). A survey was conducted to determine what resident insects and diseases were present on moth plant in New Zealand, and to assess their role in moth plant ecology. Fifty-three invertebrate species were collected and 46 were identified. No specialist moth plant natural enemies were found, and any damage that could be attributed to invertebrate herbivory was minimal (Winks et al., 2004). The polyphagous sap-feeding passionvine hopper, *Scolypopa australis*, was the only abundant invertebrate found on moth plant during the survey. The oleander aphid, *Aphis nerii*, occasionally produced 'outbreaks' that led temporarily to very large populations.

Overall none of the herbivore niches on moth plant were well utilised in New Zealand (Winks et al. 2004). Most disease symptoms observed were superficial, non-systemic, minor leaf spots that did not impact severely on the aggressive vegetative growth or flower/fruit/seed production (Winks et al., 2004; Waipara et al., 2006). It was concluded that moth plant was not subject to any significant biotic pressures, and there were no existing relationships that could influence a biological control programme.

Selection of potential control agents

The programme takes two approaches to management of moth plant. Landcare Research is seeking to:

- 1. limit the survival and/or biomass (pest status) of moth plant by the introduction of a range of invertebrates and diseases to attack the roots and foliage.
- 2. limit the rate at which moth plant populations establish and grow by introducing invertebrates that reduce seed production by attacking reproductive structures such as fruits.

Surveys of plants, pathogens and insects were conducted in the native range of moth plant in the 2004-2006 seasons to locate and identify potential agents for classical biocontrol. In all, 48 plant populations were examined (Waipara et al., 2006). A range of disease-forming organisms and 23 invertebrate species were collected during this survey (Carpintero 2006, 2009; Waipara et al., 2006). Waipara et al. (2006) discuss the merits of the recorded diseases as biological control agents. Carpintero (2006 and personal communication) listed 46 insect species that had been recorded attacking moth plant and related species and suggested that 13 insect species were worthy of further research. Landcare Research staff analysed which had the best characteristics to be control agents in New Zealand and selected four. *Freudeita cf. cupripennis* (as *Colaspis argentinensis*) was

considered one of these (Q. Paynter, Landcare Research, personal communication). A further survey was conducted in Uruguay in 2018. A population of *Freudeita cf. cupripennis* was found in Melilla, near Montevideo and was returned to New Zealand for assessment in containment.

Biology and ecology of Freudeita cf. cupripennis

Taxonomy

Order Coleoptera

Superfamily Chrysomeloidea

Family Chrysomelidae

Subfamily Eumolpinae

Genus Freudeita

Species cf. cupripennis (Lefèvre)

The term *cf.* (an abbreviation of 'confer') is commonly placed between the generic name and the specific name to describe specimens for which identification is yet to be confirmed because of practical difficulties, such as lack of taxonomic revision and specialists, the condition of the specimens, too few available specimens, or lack of access to type specimens. In this case, *F. cf. cupripennis* indicates that the population of beetles belongs to the genus *Freudeita*, and is believed to be *F. cupripennis*, but definitive identification requires additional confirmation.

The genus *Freudeita* is distributed from Costa Rica south to northern Argentina and contains 21 species, three of which are divided into subspecies, with several species and forms sympatric with moth plant in Brazil, Paraguay, Uruguay and Argentina. Morphological differences between the similar species *F. cupripennis* and *F. cuprinula* have been documented, and several characteristics are variable between the species. Between these two species there are three subspecies and one aberration (in Leschen, 2018).

Dr R. Leschen (Manaaki Whenua Landcare Research, pers. comm.) has examined specimens from the Melilla (Uruguay) population of moth plant beetle and considers that these specimens are drawn from a single population of generally similar size, morphology, and colour range. He has referred these specimens to *F. cf. cupripennis* (Leschen, pers. comm.). This is the population imported into containment in New Zealand and used in the host-range tests reported in section 3.1.4. **If this application is approved, these will be the parents of any moth plant beetles released.**

In 2010/11 moth plant beetles were collected from sites near Buenos Aires and La Plata and were identified in Argentina incorrectly (in Leschen pers. comm.) as *Colaspis argentinensis*. The host range of this population was determined, and release of this agent for the biocontrol of moth plant was approved by EPA in 2011 (https://epa.govt.nz/database-search/hsno-application-register/view/APP201039). This approval was not exercised and lapsed in 2016. Dr Leschen has subsequently examined a limited number of specimens from this population and states that these are *F. cf. cupripennis* and not *C. argentinensis*. Like the Melilla population, he considers the specimens examined to belong to a single population.

Dr Leschen states that there is variation between specimens sourced from the populations in Argentina and Uruguay, and in specimens held in other collections (Leschen, pers. comm.). He

suggests that without a full taxonomic revision or genetic study it is difficult to determine if the *cupripennis* complex represents a group of related species or a widespread, panmictic and highly variable species. The nominal species (and subspecies) form a grade, and each taxonomic name (and series of consistently identified specimens) may represent morphological extremes among the phenotypic range of the entire *cupripennis* complex.

As a result, Dr Leschen cannot yet state categorically that the two populations of moth plant beetles are the same species, or to settle on *F. cupripennis* as the name of the Melilla population. The 2011 and 2018 collection sites are approximately 250 kilometres apart and separated by the Rio de la Platte. However, moth plant is common on the river bank in both countries (H. Gourlay, MWLR, pers. comm.), and it is unlikely that the two beetle populations are geographically isolated.

The applicant intends to introduce beetles from the Melilla population only. Tests to determine the host range of this population are reported here.

Distribution

The extent of the native range in South America is uncertain, but it is present in Buenos Aires and La Plata regions, and is commonly found near Montevideo, Uruguay. The winter temperatures in these regions resemble those of northern New Zealand. *Freudeita cf. cupripennis* is expected to establish wherever *A. hortorum* is abundant in New Zealand

		Temperature (°C)		
	Latitude	Summer mean maximum	Winter mean maximum	Annual average temperature
Buenos Aires	34° 34′	28.5	14.9	16.6
La Plata, Argentina	34° 58′	28.9	14.9	
Montevideo, Uruguay	34° 74′	26.6	18.3	19.3
Auckland	37° 01′	23.0	14.0	15.1

Table 1. Comparative climates

Adult *Freudeita cf. cupripennis* are common on *Araujia hortorum* in Buenos Aires province in summer (D. Carpintero pers. comm.) but the ecology of this beetle in its native range is poorly known. Adults feed on moth plant stems, chewing the green bark material, but appear to do little damage. Eggs are laid on or in the ground around the base of the stem, and hatching larvae burrow down to feed on or in the roots. Plant deaths have been observed in Argentina that have been attributed to attack by natural enemies, of which *Freudeita cf. cupripennis* is one of the most common. Adults appear to be relatively long lived, and observations in containment in New Zealand suggest that lifetime fecundity may reach several hundred eggs over 2-4 months. Development

from egg to adult takes 30-80 days in the laboratory, depending on the temperature, and from this it might be expected that 2-3 generations could be completed each year in New Zealand.

Predicted impact in New Zealand

Little is known about the effect of root feeding by *Freudeita cf. cupripennis* on the fitness of moth plant in South America. However, closely related species of *Colaspis* with root feeding larvae are known to be significant pests. For example, *Colaspis brunnea* (grape colaspis) is a significant polyphagous pest of maize and soybeans in the US

(http://bulletin.ipm.illinois.edu/pastpest/articles/200311b.html). Colaspis pini Barber is a pest of plantation forests. Unlike Freudeita cf. cupripennis these species have a wide host range. Beetles of this genus can build populations large enough to adversely affect their host plants.

Methods

Origin of the beetle population that was tested

The beetles used in 2018 tests were collected from the Melilla area north west of Montevideo, Uruguay. Populations were shipped to New Zealand and maintained in the Landcare Research containment facility at Tamaki, Auckland. Host range experiments were conducted in containment.

Test plant selection

Freudeita cf. cupripennis has not been observed or recorded attacking species other than moth plant in Argentina or Uruguay, and was expected to be host specific. Moth plant belongs to the family Apocynaceae. There are only three native species in New Zealand, all belonging to the genus Parsonsia within the sub-family Apocynoideae. There are 12 further non-native species recorded in the New Zealand flora, though many of these are only encountered casually and are not naturalised. Other species (such as hoya) are grown as indoor ornamentals and are not at significant risk from biological control agents.

Ten species representing 6 tribes (3 sub-families) of this plant family were selected for testing (Figure 1). This was considered adequate coverage to delimit the host range of the insect in New Zealand (e.g. Briese 2002). *Vinca major* was added to the test plant list in 2018.

There are only three native plant species in this family (*Parsonsia* spp.), and these are commonly known as native jasmines. These species belong to a different sub-family from moth plant (Table 1), and so are only distantly related to the weed

(http://nzflora.landcareresearch.co.nz/default.aspx?selected=NameDetails&TabNum=0&NameId=F D22DF4B-68CF-426E-9C74-F59688C52F60). Parsonsia praeruptis is restricted to ultramafic soils of the North Cape. This is far outside the current range of moth plant, and far north of the boundary at which Northland Regional Council intends to contain moth plant (Hill, 2018). Freudeita cf. cupripennis is unlikely to ever encounter this species. Various forms of P. capsularis occur throughout the country. It appears to have similar growth form, appearance, habit and distribution as Parsonsia heterophylla. P. heterophylla and P. capsularis were considered to adequately represent this genus in tests.

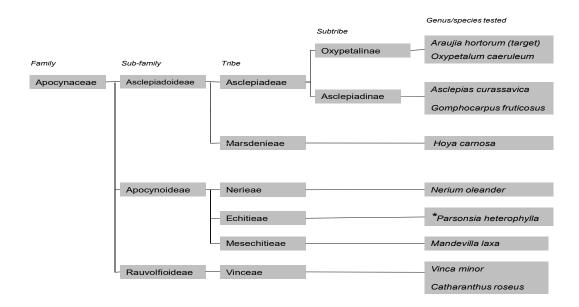


Figure 1. Taxonomic relatedness of selected plants in the family Apocynaceae in New Zealand. * indicates the one native genus in the family in New Zealand.

Fifteen non-native species belonging to 7 genera of this family are of varying commercial significance to the horticultural industry in New Zealand as ornamentals and house plants. None have major economic significance. *Oxypetalum caeruleum* (also known as tweedia) and three *Asclepias* species are closely related to moth plant (same tribe) and are minor ornamentals (Hill, 2018). Foliage of these species can be harvested to feed starving monarch butterfly larvae (*Danaus plexippus*). *Asclepias curassavica* and *Oxypetalum caeruleum* were selected for testing.

Swan plants (*Gomphocarpus fruticosus* and *G. physocarpus*) are the common hosts of monarch butterflies (http://www.monarch.org.nz/monarch). *G. fruticosus* was included in the host-range tests. This is the commonest species grown as swan plant and the species usually sold by nurseries.

At least seven species of hoya are cultivated in New Zealand (Winks and Fowler 2000). Hoya carnosa was selected as a test plant to represent this genus. Mandevilla laxa and Stephanotis floribunda are well-known ornamental species and belong to the tribe Mesechitieae. Mandevilla was selected to represent these genera in tests. Vinca minor and Nerium oleander were selected for testing but Catharanthus roseus was not sourced (Figure 1). All but two of the tribes of the family Apocynaceae growing in New Zealand (Winks & Fowler, 2000) were represented in tests. The tribe Ceropegiae is represented in New Zealand by a diverse range of indoor plants. As there are no outdoor representatives that would be exposed to the agent, and because of the clear use patterns revealed during testing, it was not felt necessary to test species from this tribe. The tribe Alyxieae is represented in the New Zealand flora by the non-native Alyxia ruscifolia, which is encountered casually. This tribe belongs to the same sub-family as the Vinceae and was adequately represented in tests by this tribe.

Freshly emerged, unmated, adult beetles were collected from rearing cages and confined on a shoot of control or test plant foliage that had been placed in a plastic specimen tube containing water and then placed in a plastic container (one adult/shoot per container). Soil was placed in the bottom of the containers in case beetles laid eggs, although extensive oviposition data was not expected. This is because the beetles were not sexed (as it is not easy to determine the sex of living beetles), so assuming a 50:50 sex ratio only half of the beetles would have been females. Moreover, many insects undergo a preoviposition period, which can be long for long-lived beetles and extended in individuals that have not mated (Danks, 2013).

Beetles were left for 7-8 days after which the presence of feeding damage on the leaves was investigated and it was noted if the beetle was alive or dead.

The following test plants were included in testing, with replicates set up between 16 October and 21 October 2018: *Araujia hortorum* (8 replicates); *Oxypetalum caeruleum* (7 replicates); *Asclepias curassavica* (8 replicates); *Gomphocarpus fruticosus* (8 replicates); *Hoya carnosa* (8 replicates); *Mandevilla laxa* (8 replicates); *Nerium oleander* (6 replicates); *Parsonsia capsularis* (8 replicates); *Parsonsia heterophylla* (8 replicates); *Vinca major* (5 replicates); *Vinca minor* (5 replicates).

Survival of larvae on potted plants

Adult *Freudeita cupripennis* beetles were reared in plastic boxes containing sprays of fresh food plant (changed every 2-3 days) and c. 2 cm of soil as a substrate for oviposition. The soil was checked every 2-3 days for the presence of bright yellow egg batches. Any egg batches found were removed using a small paint brush and incubated in Petri dishes containing moist filter paper. Freshly emerged first instar larvae were placed onto the soil near to the stems of potted test plants. Generally, each plant was inoculated with 10 larvae, which previous rearing had shown should result in high survival on *Araujia hortorum* plants of the size used in the test. However, replicates 2-5 of *Oxypetalum caeruleum* were smaller plants, so only 5 larvae were used to prevent possible starvation due to a lack of food. Replicate 4 of *Parsonsia heterophylla* was inoculated with only 7 larvae, due to a shortage of larvae.

The following test plants were included in testing, with replicates set up between 20 September and 26 October 2018: *Araujia hortorum* (5 replicates); *Oxypetalum caeruleum* (5 replicates); *Asclepias curassavica* (5 replicates); *Gomphocarpus fruticosus* (5 replicates); *Hoya carnosa* (5 replicates); *Mandevilla laxa* (5 replicates); *Nerium oleander* (4 replicates); *Parsonsia capsularis* (5 replicates); *Parsonsia heterophylla* (5 replicates); *Vinca major* (5 replicates); *Vinca minor* (5 replicates).

Initially, the plan was to sleeve plants and wait for adults to emerge to quantify development through to adult on the test plants. However, on 27 November 2018 it was noticed that the first replicate of *Oxypetalum caeruleum* was dying and examination of the roots indicated they had been severely damaged by *Freudeita cupripennis* larval feeding. As there was a risk that tests might give a 'false negative' due to larvae being unable to complete development due to starvation, it was decided that it would be better to sift through the roots and soil of this plant and all other species in rep 1 to quantify any larvae and pupae present, rather than wait to see if any adults developed. It proved relatively easy to locate larvae and pupae, so we did the same for replicates 2-5 (sequentially, so that all replicates were sampled c. 9 weeks after inoculation).

The data were analysed using Genstat (VSN International Ltd) by selecting Generalized Linear Models, binomial errors and a logit link. The denominator array (binomial totals) contained the

number of first instar larvae and the response variable was the number of larvae and pupae counted after 9 weeks. The model fitted was "Test plant species", treated as a factor corresponding to the plant species used in the host-range testing.

Results

Feeding of adult beetles on test plant foliage

Overall, most ($^{\sim}75\%$) of beetles were still alive at the end of each test: All the beetles feeding on *Araujia hortorum* survived, however, there was no significant effect of treatment (plant species) on beetle survival ($\times^2 = 1.31 \text{ d.f.} = 10$, P = 0.219).

Only three beetles laid eggs and there was no significant effect of treatment (plant species) on oviposition ($x^2 = 1.17$ d.f. = 10, P = 0.304): as discussed in the Methods, the duration of the test (determined by how long plant material remained fresh) may have been too short for extensive oviposition to have occurred. It is also possible that the sex ratio was skewed towards males, as freshly emerged insects were used and male insects tend to emerge before females (Wiklund and Fagerström, 1977).

There was extensive feeding damage to multiple leaves on *Araujia hortorum* and *Oxypetalum caeruleum* in all replicates. Minor, barely discernible, nibbling was recorded on *Asclepias curassavica* (1 replicate only); *Nerium oleander* (1 replicate only); *Parsonsia capsularis* (1 replicate only); and *Parsonsia heterophylla* (1 replicate only). No feeding damage at all was recorded on other test plant species.

There was a highly significant effect of test plant on the proportion of beetles that fed ($x^2 = 6.37$ d.f. = 10, P < 0.001).

Based on these results, it is likely that foliage of *Araujia hortorum* and the closely-related *Oxypetalum caeruleum* will be attractive to adult *F. cupripennis* beetles in the field New Zealand. It is unlikely that the other plant species detected will be sufficiently attractive to arrest food-seeking beetles.

Survival of larvae on potted plants

Final instar larvae and/or pupae were present in all replicates on *Araujia hortorum* and *Oxypetalum caeruleum* and mean survival was >45% on both species.

Two larvae were found on one *Hoya carnosa* plant (mean survival 4%), which appeared healthy, although slightly different in colour to those on *Araujia hortorum* and *Oxypetalum caeruleum*. The slightly different appearance could be a result of feeding in a different food plant, although it is also possible that the soil was contaminated with larvae of other beetle species (e.g. bronze beetle *Eucolaspis* sp.).

No larvae or pupae were found on any other test plant.

There was a highly significant effect of test plant on the proportion of larvae that were still alive ($x^2 = 16.28 \text{ d.f.} = 10$, P < 0.001).

Based on these results, it is likely that only *Araujia hortorum* and the closely-related *Oxypetalum caeruleum* (both subtribe Oxypetalinae) could support extensive development of *F. cupripennis* larvae in New Zealand.

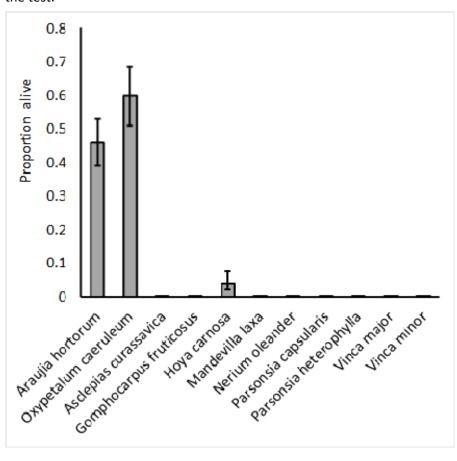


Figure 1. The proportion of first instar larvae that were alive (as older larvae or pupae) at the end of the test.

Discussion

Approval to introduce *F.* cf. *cupripennis* was granted by EPA in 2011 under the name *Colaspis* argentinensis. The application for approval to release included an assessment of the potential risk to non-target plants in New Zealand, based on the results of host-range tests using beetles sourced from La Plata, Argentina. The application stated that the host range appeared to be restricted to the sub-tribe Oxypetalinae which, in New Zealand, might well include the garden ornamental tweedia (*O. caeruleum*). No other non-target plants were considered at significant risk. The same conclusion can be drawn from the current tests which used beetles from a *F.* cf. *cupripennis* population found at Melilla, Uruguay.

Female beetles typically walk down the plant on which they feed to lay eggs in the soil around the crown of the plant and then return to feed in the foliage. This behaviour occurs repeatedly (H. Gourlay, Z. McGrath, Landcare Research, unpubl. obs.). With one exception, damage to the leaves

of the non-target plants tested was trivial. However, adult beetles fed heavily on *O. caeruleum* foliage in laboratory tests. If this behaviour occurred in the field, then eggs would likely be laid on *O. caeruleum* roots. If a plant cannot attract adult beetles and elicit feeding then it is unlikely to be exposed to *F. cf. cupripennis* eggs or larvae, unless it is in very close proximity to infested *A. hortorum* or *O. caeruleum* plants. There was no significant feeding on other test plants so it is unlikely that *F. cf. cupripennis* could colonise any plants outside the sub-tribe Oxypetalinae, the sub-tribe of moth plant and tweedia.

It was expected that the ability of newly-hatched larvae to feed, survive and develop would be the critical factor governing host plant suitability. This proved to be the case as, with the possible exception of hoya, the beetle could not complete development on plant species outside the tribe Oxypetalinae. Only *O. caeruleum* roots provided the conditions for normal larval development. Had the test continued, it is likely that this plant would have supported full development from egg to adult. It must be considered a fundamental host of the moth plant beetle.

Freudeita cf. cupripennis poses negligible risk to the three native Parsonsia species. Adults did not feed consistently on the foliage of *P. heterophylla* or *P. capsularis* and would have no reason to dwell on this plant. Larvae placed on the roots did not survive. The pattern of host plant use revealed in tests provides no reason to suggest that the third Parsonsia species in New Zealand would be any more susceptible. As there are no other native species in this family, the risk to other native non-target plants is negligible.

Swan plant, *Gomphocarpus* spp., is an important amenity plant in gardens, and is the main host of the monarch butterfly. Adults did not feed on swan plant foliage and larvae placed on the roots did not survive. As with *P. heterophylla*, these tests give no indication that swan plant is a potential host for *Freudeita cf. cupripennis*, and the risk of this insect causing even minor damage to swan plant in the field is insignificant. *Asclepias curassavica* is a relatively rare ornamental but its foliage can also support the development of larvae of the monarch butterfly. There was no significant damage to foliage by adults and no larvae were detected at the conclusion of tests.

Apart from incidental oviposition, there was no evidence that *Freudeita cf. cupripennis* could colonise *Mandevilla laxa*, *Hoya carnosa*, or *Nerium oleander*, or cause significant damage to these ornamental species. Two larvae were found in the soil/roots of one *H. carnosa* plant at the conclusion of the larval survival test. The larvae may yet prove to be extraneous to the experiment, but even if these prove to be *F. cf cupripennis* beetles, the risk to the health of hoya plants is nevertheless very low. Adult beetles could not feed on the foliage. It is therefore unlikely that this plant would be exposed to significant numbers of eggs or hatching larvae, especially as it is largely restricted to indoor ornamental use in New Zealand.

Conclusions

Centrifugal testing centred on the phylogenetic relatedness of test plants remains the best method for predicting the host range of potential biological control agents for weeds (Briese 2002). In this case, the plants initially selected for testing all belonged to the family Apocynaceae, but the pattern of host-plant use within the family is sufficiently clear to predict that plants outside the family will not be hosts for this insect in New Zealand.

Together, the host-range experiments provide compelling evidence that the host range of *Freudeita* cf. cupripennis is narrow within the family Apocynaceae. However, beetles fed on, and laid eggs on

Oxypetalum caeruleum (sometimes called tweedia), and larvae successfully developed in the roots of this host. Results prove that this plant is an adequate laboratory host for *Freudeita cf. cupripennis* sourced from Melilla, as it was for beetles sourced from Argentina. The application will take a conservative approach and assume that *O. caeruleum* will be a field host of moth plant beetle in New Zealand and could be at risk of significant attack. Further, the results suggest that any other species within this sub-tribe that are resident in New Zealand would be equally susceptible to attack. At this point, no such species are known.

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