Manaaki Whenua
Landcare Research

Host range testing for Chilean flame creeper beetle *Blaptea elguetai*

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Host range testing for Chilean flame creeper beetle *Blaptea elguetai*

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Summary

Project and client

- The host range of the Chilean flame creeper beetle, *Blaptea elguetai*, was assessed for the National Biocontrol Collective and MPI as part of the Multi-weed Biocontrol grant S3F20095.

Objectives

- To determine the suitability of the Chilean flame creeper beetle, *Blaptea elguetai*, as a candidate biocontrol agent for Chilean flame creeper, *Tropaeolum speciosum*.

Methods

- We conducted adult no-choice feeding tests, and larval no-choice feeding and development tests in the invertebrate containment facility at Lincoln, New Zealand.
- We conducted surveys in the native range of Chile to look for beetle attack on non-target hosts within the same genus as the target weed (the genus *Tropaeolum*), and on non-target hosts in the genus *Brassica* which shares phylogenetic links with *Tropaeolum*, and which includes numerous economically important crops.
- We also searched the grey literature in Chile for evidence of association between the beetle and *Brassica* crops.

Results

- *Blaptea elguetai* completed development on all species in the genus *Tropaeolum* that were tested; development on these other species was mostly comparable to that on *T. speciosum*.
- One individual completed development on each of pak choi 'Dark Dragon', Chinese cabbage, and moringa.
- Chinese cabbage and moringa were determined to be at very low risk of becoming field hosts of the beetle.
- Additional testing of pak choi 'Dark Dragon' in no-choice larval feeding and development tests confirmed this host is highly unlikely to be a field host of *B. elguetai*.
- Field surveys in Chile found no evidence of attack by the beetle on other *Tropaeolum* species and no evidence of attack on unsprayed *Brassica* crops and wild plants growing near *T. speciosum*.
- There was no mention of a leaf beetle as a pest of *Brassica* in Chile in the grey literature we reviewed from this country.

Conclusions

- Based on the evidence from laboratory testing we conclude that *B. elguetai* appears to be host specific to the genus *Tropaeolum*. However, other species of *Tropaeolum* that are present in New Zealand are unlikely to be attacked due to seasonal asynchrony in the presence of foliage.
- *Blaptea elguetai* is a safe candidate biocontrol agent for *T. speciosum*.

Recommendation

- We recommend proceeding with the regulatory path to apply for the introduction of *B. elguetai* as a biocontrol agent for *Tropaeolum speciosum* in New Zealand.

1 Introduction

This report outlines the history of the biological control programme against Chilean flame creeper, *Tropaeolum speciosum* Poepp. & Endl., in New Zealand and presents the results of research to determine the host range of the Chilean flame creeper beetle, *Blaptea elguetai* Petitpierre, and hence the direct risk it would pose to valued plants if introduced to New Zealand.

2 The biological control programme against *T. speciosum*

Tropaeolum speciosum is a climbing vine which has become an important environmental weed in New Zealand in Southland, Otago and Canterbury. It is also present in the lower parts of the North Island but only considered a minor weed there. A biological control programme was initiated in 2021, following the serendipitous discovery of a leaf beetle *B. elguetai* (Coleoptera: Chrysomelidae) on *T. speciosum* in Chile in November 2019.

2.1 Suitability of *T. speciosum* as a target for biological control

A feasibility study (Harman 2006) recommended *T. speciosum* as a suitable target for biological control because of its vigorous climbing growth, its ability to reproduce both vegetatively and by seeds that get dispersed by birds, the difficulty of control by chemical and mechanical methods, and the lack of closely-related species in the indigenous New Zealand flora. A serendipitous discovery of candidate biocontrol agent, the beetle *Blaptea elguetai* Petitpierre in Chile in 2019, elevated the priority status of *T. speciosum* as a target for biocontrol. A routine survey in New Zealand in 2021 found 63 species of herbivorous insects and 36 species of primary pathogens associated with *T. speciosum*, with none of them specialist to Chilean flame creeper and all producing no more than minimal damage (Probst et al. 2022).

2.2 Biology and ecology of the leaf beetle *B. elguetai*

Taxonomy

Order	Coleoptera
Family	Chrysomelidae
Subfamily	Chrysomelinae
Genus	*Blaptea*
Species	*Blaptea elguetai* Petitpierre

Blaptea elguetai was described in 2011 as a sister species to *Blaptea balyi* Weise (Petitpierre 2011). Up to that point the genus *Blaptea* was considered a monotypic genus (Blackwelder 1945), with its only species, *B. balyi*, reported from Colombia, Chile and Brazil (Daccordi 1982). The disjunct distribution of *B. balyi*, with no records over the more than 3,000 km between the known records from central-southern Colombia and central Chile, sparked renewed interest in *B. balyi*. Subsequently, the specimens from central Chile were described as an allopatric sister species, with distinct morphological differences in both

males (in the aedeagus) and females (in the spermatheca) (Petitpierre 2011). It is not known if the two sister species *B. balyi* and *B. elguetai* feed on botanically close host plants (Petitpierre 2011).

Blaptea elguetai was collected in central Chile from *Tropaeolum brachyceras* Hook. et Arn. (Petitpierre & Elgueta, 2012).

2.3 Description

Adult *B. elguetai* are around 5 mm long with distinct metallic brown or blue elytra.

2.4 Distribution

The genus *Blaptea* has a Neotropical distribution that includes Chile, Colombia and Brazil (Jerez and Pizarro-Araya 2020). *Blaptea elguetai* was previously identified as *B. balyi*, a species native to Colombia, but is now considered a separate species with its known distribution restricted to Chile (Petitpierre 2011).

2.5 Life cycle

There is no description of the biology of *B. elguetai* in the literature. Our knowledge on the beetle's life cycle comes from our own observations working with the beetle in Chile and in containment in New Zealand.

Both adults and larvae feed on the foliage of *T. speciosum* and will feed on flowers when they are available. Eggs are deposited on surfaces in the vicinity of *T. speciosum* – either on nearby plants or in the leaf litter. In the laboratory, eggs were deposited on filter paper as well as on plant material in petri dishes. Field-collected adults in Chile started laying eggs in October at the lower-altitude site and in November at the higher altitude site. Eggs take 3-4 days to hatch. Larvae take around 40 days to go through five larval instars. Prior to pupation the larva builds a nest-like pupal case between two layers of leaf/filter paper. The prepupal and pupal stages take around two weeks, after which the adults emerge.

In the field, eggs were only found in spring, indicating the beetle has one generation per year. It is likely that the beetle goes into diapause during winter, when *T. speciosum* dies back. In the laboratory, we have been able to keep the beetle continuously and reproducing under mild temperatures, confirming that winter diapause is not obligatory and that multiple generations per year can be produced under warm conditions and if suitable host foliage was available.

2.6 Predicted impact in New Zealand

In the native range in Chile, *T. speciosum* is an uncommon plant.

This status is partly due to habitat loss from human activities such as construction and forestry disturbances. However, where *T. speciosum* is present, it appears to be attacked

by *B. elguetai* (H. Norambuena, pers. obs.). Attacked plants are of much smaller stature compared to plants in New Zealand.

3 Methods

3.1 Beetle populations

Reproductive *B. elguetai* beetles were collected from two source populations in Chile: One from Vilcún (38°38.952'S, 72°12.714'W, Alt: 340 m.a.s.l., 45 adults) and one from Cherquenco (38°42.009'S, 71°58.564'W, Alt: 587 m.a.s.l., 20 adults). The two populations were maintained separately and tested separately. At the time of arrival in New Zealand the beetles from Vilcún had already deposited 350 eggs and the beetles from the higher altitude site of Cherquenco had deposited 100 eggs. The beetles readily deposit eggs on any available substrate and on plants growing in the vicinity of the host plant, *T. speciosum*. The beetles were held under temperatures of 22°C–24°C : 8°C–10°C (Day:Night) and 14:10 h (L:D) for rearing and testing.

3.2 Test plants list

The centrifugal phylogenetic method (Wapshere 1974) has long been used to determine the host range of a potential biological control agent. The method works by sequentially testing plant taxa most closely related to the target weed followed by increasingly distantly-related taxa until the host range has been circumscribed. This approach is supported by recent advances in molecular techniques. Specialist phytophagous insects show a strong phylogenetic conservatism of host associations (see Briese 1996; Briese & Walker 2002). This pattern of strong phylogenetic conservatism in diet suggests the non-target plants of greatest risk are those closely related to known hosts (Futuyma et al. 2000), and this has been validated by reviews of non-target attack by insect (Pemberton 2000; Briese & Walker 2002; Louda et al. 2003; Paynter et al. 2004) and fungal (Barton (née Fröhlich) 2004) weed biological control agents.

Chilean flame creeper (*T. speciosum*) belongs to the family Tropaeolaceae which previously comprised 3 genera: *Tropaeolum*, *Magallana* (2 species) and *Tropheastrum* (1 species) based on morphology (Sparre & Andersson 1991) but it has since been reduced to the genus *Tropaeolum* by molecular analyses (Andersson & Andersson 2000). *Tropaeolum* was originally placed in the order Geraniales before being moved to the order Brassicales (Andersson & Andersson 2000).

The family Tropaeolaceae contains around 90 species of herbaceous flowering plants from South and Central America. The genus *Tropaeolum* is divided into two sections: section *Chilensia* with around 22 species located mainly in Chile and section *Tropaeolum* which is spread across the American tropics (Hershkovitz et al. 2006).

Tropeaolum section *Chilensia* is subdivided into 5 subsections: *Chymocarpus*, *Magallana*, *Tropheastrum*, *Chilensia* and *Graciles* (Watson & Flores 2010).

There are no indigenous *Tropaeolum* species in New Zealand. However, two other species have naturalised in addition to *T. speciosum* (see Breitwieser et al. 2010-2020): *T. pentaphyllum* (ladies' legs) and *T. majus* (garden nasturtium). *Tropaeolum speciosum* and *T. pentaphyllum* are in the section *Chilensia*, subsection *Chymocarpus*, while *T. majus* belongs to the section *Tropaeolum*.

In New Zealand, seeds of nasturtiums, *T. majus* and *T. minus*, are still sold. There are occasional reports of other *Tropaeolum* species grown in private New Zealand gardens, including *T. tricolor*, *T. tuberosum*, *T. azureum* and *T. brachyceras*.

Test plants for this project were selected from plant families in the order Brassicales represented in New Zealand by indigenous species and/or by exotic species grown commercially. Those families and genera/species within were selected based on the phylogeny represented in Figure 1 and are listed in Table 1.

The species of *Tropaeolum* in the section *Chilensia* are climbers. The target host, *T. speciosum* is dormant in winter and develops foliage and flowers in spring and summer, whereas all the other climber species of *Tropaeolum* present in New Zealand are summer dormant, having foliage in winter. We grew the test plants in opposite season conditions in glasshouses to ensure availability of foliage of all species simultaneously for testing.

Similarly, test plant species that normally grow in tropical climates such as *Moringa* and *Carica* had to be grown in hot glasshouse conditions to be able to be included in the tests.

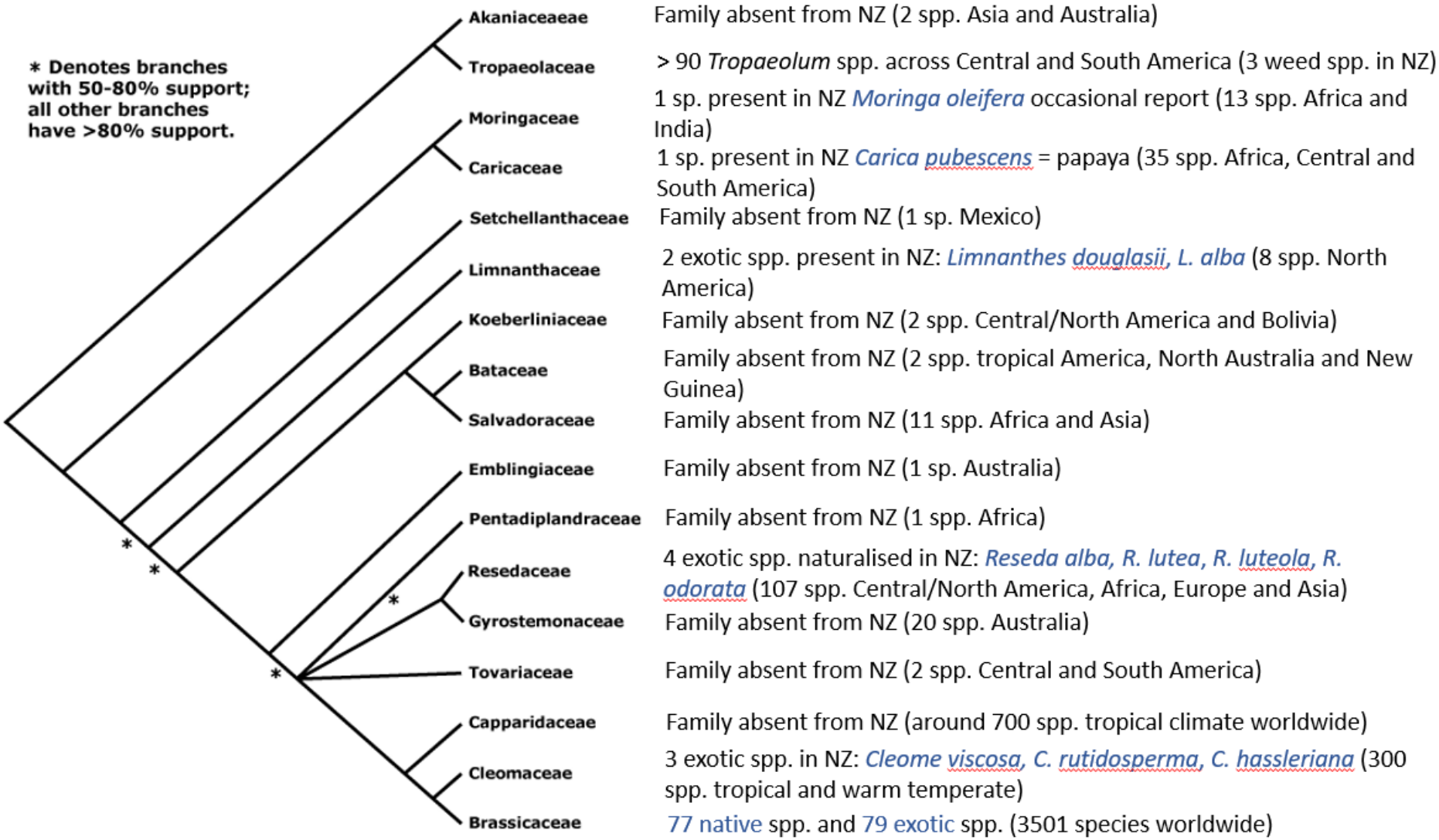

Figure 1. Phylogeny of the order Brassicales. Genera represented in New Zealand by either indigenous species or exotic species are in blue.

3.3 No-choice larval feeding and development

Host range testing began as soon as the beetles arrived in containment November 2022. Most of the testing was completed in January 2023. Additional replicates were run for one test species, pak choi 'Dark Dragon' in August–September 2023. We used F1 (i.e. first generation) larvae from eggs deposited by the beetles imported from Chile. For each replicate, two neonate, unfed larvae, 0–2 days old, were transferred onto a whole excised leaf of the test plant in a Petri dish lined with moist filter paper. Dishes were checked every 2–3 days and the following parameters recorded: Number of individuals alive and their instar; number of individuals dead and their instar; level of feeding in the following categories: 0, trace (<1%), 1%-5%, 6%-10%, 11%-20%, 21%-50%, >50%; and presence of frass. At the time of the second check (4-5 days from start), if larvae on test plants were still alive but had not fed at all on the plant material in the dish, those replicates were terminated, and the larvae added to the rearing colony. If any feeding occurred on a test plant species, that replicate was continued alongside one replicate of the host plant, *T. speciosum*, as control, until the larvae on the test plant either died or completed development. Leaf material was replenished as necessary, and the filter paper moistened.

We conducted at least five replicates per species per source population for most test plant species. For species with multiple cultivars, we tested three cultivars at two replicates per cultivar per source population (six replicates per source population). These were the species *B. oleracea* (cultivars broccoli, cauliflower, savoy cabbage) and *B. rapa* (cultivars Chinese cabbage, turnip, pak choi 'HonTsai Tai'). Table 1 lists the number of replicates per test plant species/variety/cultivar per source population.

Following analysis of the first set of host range tests, we repeated testing of *Brassica chinensis*, (pak choi 'Dark Dragon') for which the Relative Performance Index (RPI) score (Paynter et al. 2015) of 0.57 in the first batch of tests (see Section 4, Results and discussion) indicated that this test species may be at risk of experiencing spillover attack[1]. We wanted to increase replication for this species to increase certainty around these results. Similarly to the original tests, each replicate consisted of two neonate, unfed, 0-2 days old larvae. However, in this test, we monitored all larvae until they died or had completed development to adults. We ran an additional 32 replicates per beetle population with *B. chinensis* and 15 replicates per beetle population with *T. speciosum* as controls. Excessive moisture in the Petri dishes caused high mortality on both hosts, and no larvae completed development on either. We therefore ran another set of 10 replicates per beetle population on both *B. chinensis* and *T. speciosum* in slightly drier conditions. Under the drier conditions 11 larvae on *T. speciosum* successfully completed development to adults. All 84 replicates with *B. chinensis* and 50 replicates with *T. speciosum* were used in the analysis.

[1] The Relative Performance Index calculates a combined score for the survival and reproductive fitness of a candidate agent performing on a non-target host plant species comparted to its performance on the target weed in the testing environment in containment. Scores below 0.21 have been shown with high confidence to be of negligible risk of translating to any non-target attack in the field. Scores above 0.33 have been shown to be at risk of translating to potential spillover non-target attack in the field. No full utilisation of plants was shown for scores below 0.57.

Table 1. List of test species and the number of replicates from each of the two beetle populations (Vilcún and Cherquenco)

Family	Section	Genus	Species	Common name/cultivar/variety	Indigenous / exotic / crop	comments	No. replicates Vilcún / Cherquenco (No. larvae)	No. replicates Vilcún / Cherquenco. One adult per replicate.
Tropaeolaceae	*Chilensia*	*Tropaeolum*	*T. speciosum* Poepp. & Endl.	Chilean flame creeper	Exotic	Target weed	12 (24) / 12 (23)	
	Chilensia	*Tropaeolum*	*T. azureum* L.		Exotic		9 (18) / 5 (10)	1 / 1
	Chilensia	*Tropaeolum*	*T. brachyceras* Hook. & Arn.		Exotic		5 (10) / 5 (10)	2 / 1
	Chilensia	*Tropaeolum*	*T. pentaphyllum* Lam.	Ladies' legs	Exotic	Naturalised	5 (10) / 5 (9)	1 / 2
	Chilensia	*Tropaeolum*	*T. tricolor* Sweet		Exotic		5 (10) / 5 (7)	4 / 1
	Chilensia	*Tropaeolum*	*T. tuberosum* Ruiz & Pav.	Mashua	Exotic	Edible tuber	5 (10) / 5 (8)	2 / 1
	Tropaeolum	*Tropaeolum*	*T. majus* L.	Garden nasturtium	Exotic	Naturalised	5 (10) / 5 (10)	2 / 1
	Tropaeolum	*Tropaeolum*	*T. minus* L	Dwarf nasturtium 'Bloody Mary'	Exotic		5 (10) / 5 (10)	2 / 1
Moringaceae		*Moringa*	*M. oleifera* Lam.,	Moringa	Exotic. Minor crop		5 (10) / 5 (10)	3 / 3
Caricaceae		*Carica*	*C. pubescens* Lenné & K. Koch	Mountain papaya	Exotic		5 (10) / 5 (9)	3 / 3
		Carica	*C. papaya* L.	Papaya	Exotic. Minor crop		5 (10) / 5 (9)	3 / 3
Limnanthaceae		*Limnanthes*	*L. douglasii* R.Br.		Exotic		5 (10) / 5 (10)	2 / 3
Resedaceae		*Reseda*	*R. alba* L		Exotic		5 (10) / 5 (10)	2 / 1
		Reseda	*R. odorata* L		Exotic		5 (10) / 5 (10)	2 / 1
Cleomaceae		*Cleome*	*C. spinosa* Jacq.		Exotic		5 (10) / 5 (8)	2 / 3
Brassicaceae	*Brassica*	*Brassica*	*B. chinensis* L.	Pak choi 'Dark Dragon'	Exotic. Crop		5 (10) / 5 (10)	5 / 6
	Brassica	*Brassica*	*B. rapa* L.	Chinese cabbage	Exotic. Crop		2 (4) / 2 (4)	2 / 1
				Turnip 'Snowball'	Exotic. Crop		2 (4) / 2 (4)	2 / 1
				Pak choi 'Hon Tsai Tai'	Exotic. Crop		2 (4) / 2 (4)	2 / 1
	Brassica	*Brassica*	*B. juncea* Zotov	Mustard 'Gai choy'	Exotic. Crop		5 (10) / 5 (10)	2 / 3
	Brassica	*Brassica*	*B. oleracea* L.	Cabbage 'Vertus savoy'	Exotic. Crop		2 (4) / 2 (4)	1 / 2
				Cauliflower 'All year round'	Exotic. Crop		2 (4) / 2 (4)	2 / 1
				Broccoli 'de Cicco'	Exotic. Crop		2 (4) / 2 (4)	2 / 1
	Lepidium	*Lepidium*	*L. sativum* L.	Cress 'Moss Curled'	Exotic. Crop		5 (10) / 5 (10)	2 / 4
	Lepidium	*Lepidium*	*L. solandri* Kirk		Indigenous		6 (12) / 5 (10)	2 / 3
	Lepidium	*Lepidium*	*L. banksii* Kirk		Indigenous		5 (10) / 5 (10)	3 / 2
	Lobularia	*Lobularia*	*L. maritima* (L.) Desv.		Exotic		6 (12) / 8 (16)	4 / 1
	Cardamine	*Cardamine*	*C. grandiscapa* Heenan		Indigenous		5 (10) / 8 (16)	2 / 3
	Pachycladon	*Pachycladon*	*P. fastigiatum* (Hook.f.) Heenan & A.D. Mitch		Indigenous		5 (10) / 5 (10)	2 / 3
	Rorippa	*Rorippa*	*R. palustris* (L.) Besser		Indigenous		5 (10) / 5 (10)	3 / 2

3.4 No-choice adult feeding and oviposition

Adult females of *B. elguetai* lay eggs on vegetation adjacent to *T. speciosum* in the natural habitat in the native range, and larvae crawl to the host plant upon hatching. We therefore determined that oviposition under choice conditions would not provide an indication of the host specificity of the beetle. Adult testing was not considered essential to determining host specificity since the larvae are the selective life stage. Nevertheless, we tested adult feeding, and recorded oviposition when it took place in the test dishes. We used the adults from Chile (F0), which had fed on *T. speciosum* before testing. Each adult was used multiple times because of the small number of adults available. Adults were placed individually in a Petri dish containing an excised whole leaf of a test species on moist filter paper and checked every 2–3 days for up to 4–5 days. The following parameters were recorded: Number of individuals alive; number of individuals dead; level of feeding in the following categories: 0, trace (<1%), 1%–5%, 6%–10%, 11%–20%, 21%–50%, >50%; presence of eggs, and their location (on the leaf/dish/filter paper); and presence of frass. At the end of 4–5 days on the test plant, each beetle was transferred to *T. speciosum* to confirm feeding on the target host. Once feeding on the target was confirmed, the adult was transferred to a different test species for another 4-5 days. Each adult was tested in such a sequence on several test plant species, but never exposed to the same test species more than once. The final test for each individual was confirmation of feeding on *T. speciosum*.

3.5 Analysis

No-choice Larval feeding and development

The response variables 'midpoint of feeding category' and 'Proportion of replicates with at least one larva surviving to day 4 or 5', were analysed using generalised linear regression models with binomial distribution. Each source population was analysed separately to begin with, but since the patterns they displayed were identical, the data from both populations was combined into a single analysis for each response variable. The explanatory variable was plant species. Since feeding patterns on the different varieties of *Brassica* species with multiple varieties were similar within species, these hosts were analysed at the species level (not at the variety level). For example, broccoli, cauliflower and savoy cabbage were all analysed as one entity: *Brassica oleracea*. Least Significant Differences (LSDs) are presented.

The results from the two tests (feeding and survival) were applied to the formula to calculate the Relative Performance Index (RPI) score (Paynter et al. 2015) and the corresponding risk of each plant species being a field host of *B. elguetai*.

The calculated RPI scores showed borderline results for *B. chinensis*, and additional tests were conducted (see Section 3.3 No-choice larval feeding and development) Three response variables were analysed using generalised linear regression models with binomial distribution. These were: proportion of replicates with at least one larva surviving to: i) pre-pupal stage; ii) pupal stage; iii) adult stage. Here too, each source population was analysed separately to begin with, but since the patterns they displayed were identical, the

data from both populations was combined into a single analysis for each response variable. The explanatory variable was plant species.

Adult feeding and oviposition – no-choice

Descriptive results are presented for adult feeding and oviposition tests. No statistical analysis was performed for this data set.

3.6 Surveys of endemic climbers in the genus *Tropaeolum* in Chile

To test our hypothesis of seasonal separation as a mechanism for escape of the winter climbers in the genus *Tropaeolum* from attack by *B. elguetai* we surveyed species with winter foliage in the native range for signs of herbivory by the beetle. The survey was conducted during winter–spring (August–September) of 2022, when *T. speciosum* is still dormant and the other climbing species are in full foliage and bloom. The surveys were carried out in 5 regions and 14 provinces of central and northern Chile, where 5 endemic *Tropaeolum* species were recorded previously: *T. austropurpureum, T. azureum T. brachyceras, T. hookerianum,* and *T. tricolor*. Plants were inspected for the leaf beetle at field sites. A minimum of two plants of each species present at each site were searched for immatures and adult beetles or evidence of its damage to the foliage. Plants were then beaten over a beating tray (white cloth, 80 × 80 cm) to dislodge any arthropods. In addition, a soil/litter sample of about 0.5 kg extracted from underneath the plant was spread out on the beating tray and searched for beetles.

3.7 Focused surveys on *Brassica* crops in Chile

Following the development of *B. elguetai* on *B. chinensis* in the initial testing we searched for evidence (or lack thereof) of any interactions between the beetle and *Brassica* crops in the native range in Chile, via i) a survey of the grey literature in Spanish; ii) a field survey of unsprayed *Brassica* crops near sites with *T. speciosum* and *B. elguetai*.

Survey of the grey literature

Archives of Chile's National Museum of Natural History and the Chilean National Library were searched for entomological and agricultural literature (including monographs, annals, journals, books, bulletins, periodicals, magazines, and reports for farmers). The search focused on mentions of insects on plants in the family Brassicaceae in Chile. Search terms included the following list.

Search term in Spanish	Translation to English
• Repollo	• Cabbage
• Raps	• Rapeseed
• *Blaptea*	• *Blaptea*
• *B. elguetai*	• *B. elguetai*
• *Blaptea balyi*	• *Blaptea balyi*
• *Metastyla balyi*	• *Metastyla balyi*
• Chrysomelidae	• Chrysomelidae
• Chrysomelids	• Chrysomelids
• Plagas	• Pests
• Plagas de las cruciferas	• Pests of crucifers
• Plagas del raps	• Pests of rapeseed
• Plagas del repollo	• Pests of cabbage
• Brassicaceae	• Brassicaceae
• Hortalizas	• Vegetables
• Col	• Col (Short for Brassicaceae)
• Coliflor	• Cauliflower
• Brocoli	• Broccoli
• Khale	• Kale
• Yuyo	• Field mustard[1]
• Chrysomelianos	• Chrysomelianos

[1]Yuyo, field mustard, is one of only few plants present in Chile mentioned in records from the Spanish conquest of Chile.

Professionals in the rapeseed (*B. napus*) crop production/pest management were contacted regarding their personal archives and personal experience/observations. Finally, websites of agricultural services were searched.

Field surveys

Brassica crops in Chile were surveyed during the spring and summer of 2023–24 season (October 2023 to January 2024). The key criteria for site selection for the survey were that the *Brassica* crop was unsprayed, and that it grew in proximity to sites with *T. speciosum* and *B. elguetai*. The main crop fitting those criteria was rapeseed (*B. napus*). Small lots of other *Brassica* crops were also surveyed: cabbage, cauliflower, kale, arugula, and radish. Wild plants of *B. campestris* (yuyo, field mustard) that were found at the *T. speciosum* location where *B. elguetai* was discovered in 2019 and at one small-cropping site were also surveyed.

At the large rapeseed crop fields, the survey consisted of 200 sweep net sweeps and visual inspection of 200 rapeseed plants along the field margins. In the smaller crop fields, the survey consisted of 50 net sweeps and visual inspection of 50 plants. Any arthropods present at each site were noted.

4 Results and discussion

Preliminary analyses of the two source populations (Vilcún and Cherquenco) separately showed that there were no differences between the populations. Accordingly, we present our analysis for the combined dataset of the two populations.

4.1 Larval feeding

Beetle larvae fed on all species of *Tropaeolum*. The proportion of leaf consumed of most *Tropaeolum* species in the section *Chilensia* (the climbers) was as high or higher (but not significantly different) than of the target species *T. speciosum* (Table 2, Figure 2). The proportion of leaf consumed of *T. pentaphyllum* was significantly lower than of *T. speciosum*.

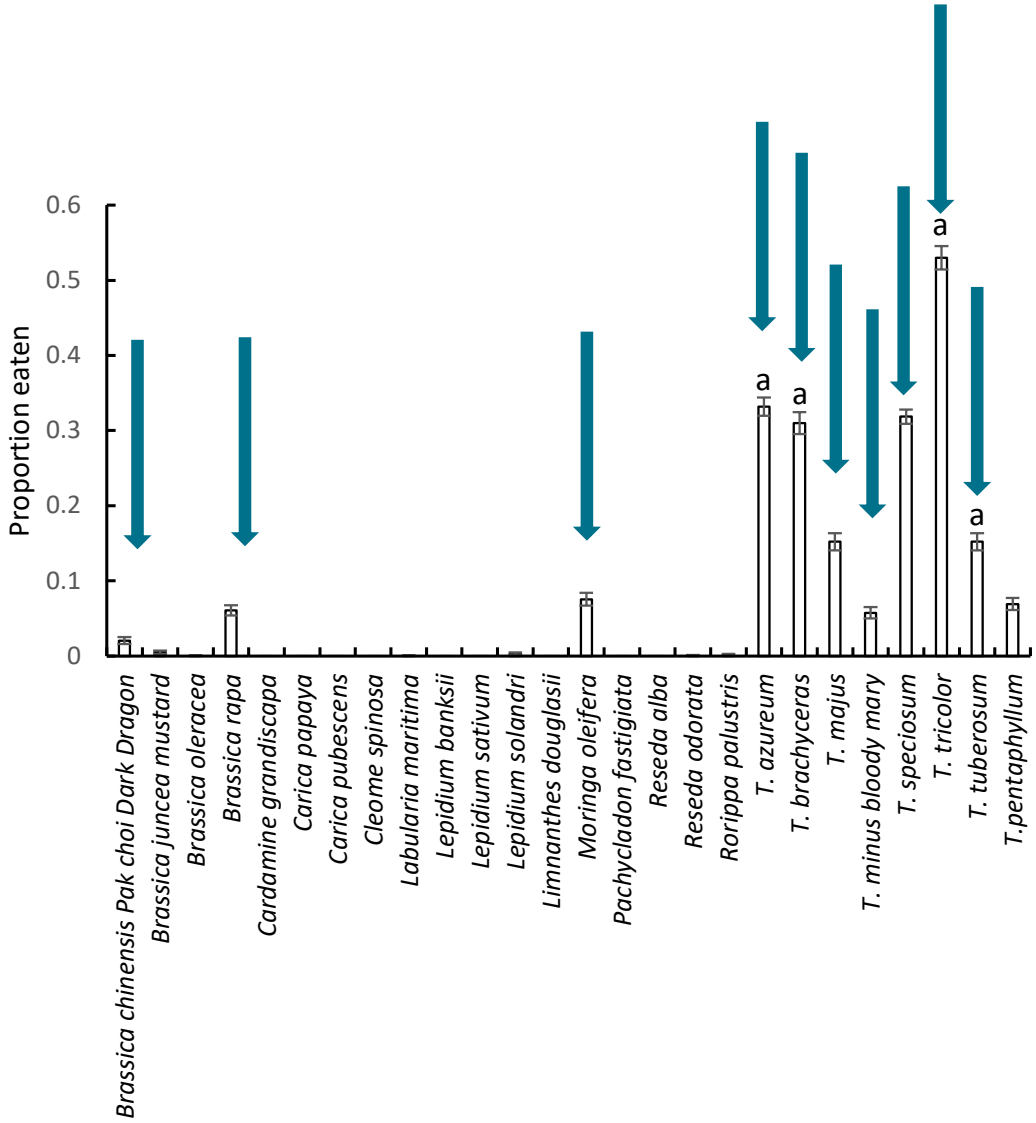

Figure 2. Proportion of leaf eaten per replicate per host species. The blue arrows indicate species confirmed as fundamental hosts for full development. Error bars represent ±SEM. Letters represent species with proportion feeding not significantly different to feeding on *T. speciosum*.

It is interesting to note that *T. speciosum* secreted droplets of thick fluid from its severed leaf edges in response to larval feeding (Figure 3), which the other *Tropaeolum* species did not. It is possible that the summer-growing *T. speciosum* exhibits chemical defence against herbivory that the summer-dormant congenerics may not require due to their lesser risk of exposure to the beetle. This may explain the apparently higher feeding on some of the summer-dormant congenerics in the testing environment.

Figure 3. Leaf of *Tropaeolum speciosum* with two *Blaptea elguetai* larvae and feeding notches. Yellow arrows pointing at droplets of thick fluid exudates.

Feeding on the *Tropaeolum* species in the section *Tropaeolum* (the nasturtiums) was less severe than on the climbing congenerics. The proportion of nasturtiums leaf consumed was significantly lower than of *T. speciosum* (Table 2, Figure 2).

There was trace-level feeding on three exotic species: *Brassica juncea*, *B. oleracea* and *Reseda odorata*, as well as on three indigenous species: *Labularia marittima*, *Lepidium solandri* and *Rorippa palustris*. In addition, there was higher than trace feeding on three exotic species: *Brassica chinensis*, *B. rapa* and *Moringa oleifera*.

Table 2. Midpoint of percentage feeding range categories as proportion (binomial distribution). Species listed in alphabetic order.

Plant species	Prediction[1]	SEM[2]	Diff. cf. *T. speciosum*[3]	LSD[4]	Significant difference vs *T. speciosum* (0=No; 1=Yes)
Brassica chinensis	0.0205	0.004481	0.29804	0.13058	1
Brassica juncea mustard	0.005	0.00223	0.31354	0.08856	1
Brassica oleracea	0.00042	0.000589	0.31812	0.08856	1
Brassica rapa	0.06083	0.0069	0.25771	0.08856	1
Cardamine grandiscapa	0	0.000036	0.31854	0.08856	1
Carica papaya	0	0.000041	0.31854	0.24668	1
Carica pubescens	0	0.000041	0.31854	0.08856	1
Cleome spinosa	0	0.000041	0.31854	0.08856	1
Labularia maritima	0.00036	0.000505	0.31818	0.08856	1
Lepidium banksii	0	0.000043	0.31854	0.08856	1
Lepidium sativum	0	0.000041	0.31854	0.08856	1
Lepidium solandri	0.00292	0.001557	0.31562	0.08856	1
Limnanthes douglasii	0	0.000041	0.31854	0.08856	1
Moringa oleifera	0.0755	0.008355	0.24304	0.08856	1
Pachycladon fastigiatum	0	0.000041	0.31854	0.08856	1
Reseda alba	0	0.000041	0.31854	0.08856	1
Reseda odorata	0.0005	0.000707	0.31804	0.08856	1
Rorippa palustris	0.0015	0.001224	0.31704	0.13058	1
Tropaeolum azureum	0.33167	0.012156	-0.01313	0.08856	0
Tropaeolum brachyceras	0.31	0.014625	0.00854	0.08856	0
Tropaeolum majus	0.152	0.011353	0.16654	0.08856	1
Tropaeolum minus	0.0575	0.007362	0.26104	0.08856	1
Tropaeolum speciosum	0.31854	0.00951	Ref sp.[5]	0.08856	Ref sp.
Tropaeolum tricolor	0.53	0.015783	-0.21146	0.08856	0
Tropaeolum tuberosum	0.152	0.011353	0.16654	0.18045	0
Tropaeolum pentaphyllum	0.069	0.008015	0.24954	0.15903	1

[1]Model prediction for midpoint of percentage feeding range

[2]Standard error of mean

[3]Difference between predicted midpoint of percentage feeding on *T. speciosum* and the predicted midpoint of percentage feeding on the test species.

[4]Least significant difference

[5] *T. speciosum* being the reference species is not compared to itself.

4.2 Larval survival to Day 4/5

Survival of larvae to termination of the test on Day 4/5 on all species in the genus *Tropaeolum* was similar to or better than on *T. speciosum*, and not significantly different (Table 3, Figure 4).

Survival on all other test species outside the genus *Tropaeolum* was significantly lower than on *T. speciosum*.

Table 3. Survival of larvae to termination of testing on Day 4/5 (proportion of replicates in which at least one larva survived; binomial distribution). Species listed in alphabetic order.

Plant species	Prediction[1]	SEM[2]	Diff. cf. *T. speciosum*[3]	LSD[4]	Significant difference vs *T. speciosum* (0=No; 1=Yes)
Brassica chinensis	0.50	0.11	0.37	0.13	1
Brassica juncea mustard	0.15	0.08	0.72	0.09	1
Brassica oleracea	0.17	0.08	0.71	0.09	1
Brassica rapa	0.21	0.08	0.66	0.09	1
Cardamine grandiscapa	0.12	0.06	0.76	0.09	1
Carica papaya	0.21	0.09	0.66	0.25	1
Carica pubescens	0.11	0.07	0.77	0.09	1
Cleome spinosa	0.11	0.07	0.76	0.09	1
Labularia maritima	0.21	0.08	0.66	0.09	1
Lepidium banksii	0.11	0.07	0.76	0.09	1
Lepidium sativum	0.05	0.05	0.82	0.09	1
Lepidium solandri	0.08	0.06	0.79	0.09	1
Limnanthes douglasii	0.15	0.08	0.72	0.09	1
Moringa oleifera	0.15	0.08	0.72	0.09	1
Pachycladon fastigiatum	0.00	0.00	0.87	0.09	1
Reseda alba	0.10	0.07	0.77	0.09	1
Reseda odorata	0.20	0.09	0.67	0.09	1
Rorippa palustris	0.05	0.05	0.82	0.13	1
Tropaeolum azureum	1.00	0.00	-0.13	0.09	0
Tropaeolum brachyceras	0.95	0.05	-0.08	0.09	0
Tropaeolum majus	0.95	0.05	-0.08	0.09	0
Tropaeolum minus	0.85	0.08	0.02	0.09	0
Tropaeolum speciosum	0.87	0.05	Ref sp.[5]	0.09	Ref sp.
Tropaeolum tricolor	0.88	0.08	-0.01	0.09	0
Tropaeolum tuberosum	0.94	0.05	-0.07	0.18	0
Tropaeolum pentaphyllum	1.00	0.00	-0.13	0.16	0

[1]Model prediction for proportion of replicates with at least one larva alive at Day 4/5

[2]Standard error of mean

[3]Difference between predicted proportion of replicates with at least one larva alive at Day 4/5 on *T. speciosum* and the predicted proportion on the test species.

[4]Least significant difference

[5] *T. speciosum* being the reference species is not compared to itself.

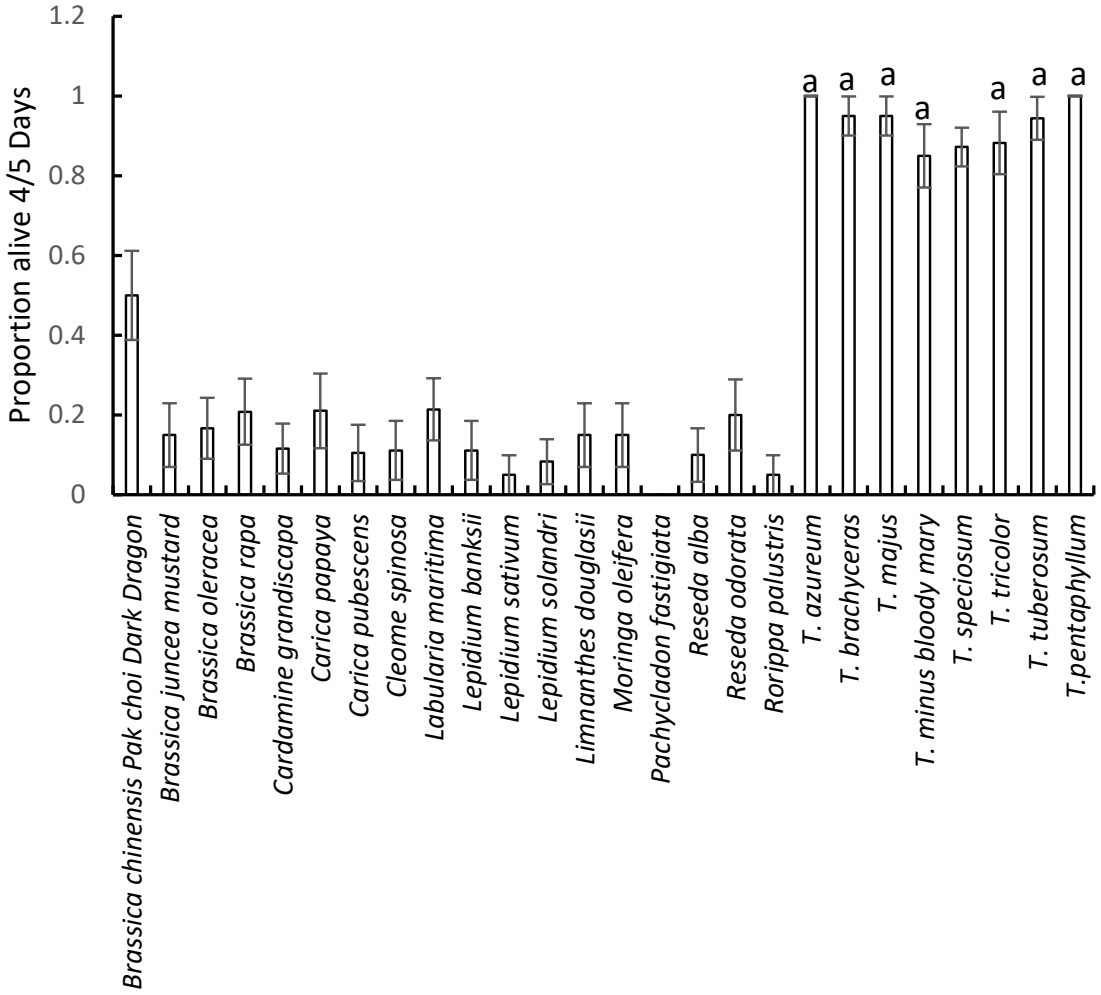

Figure 4. Proportion of replicates where at least one larva survived to termination on Day 4/5. Error bars represent ±SEM. Letters represent species with proportion of such replicates not significantly different to *T. speciosum*.

4.3 Relative performance score

All *Tropaeolum* species were confirmed to be within the fundamental host range[2] of *B. elguetai* and also at high likelihood of being field hosts, with Relative Performance Index (RPI) scores ranging from 0.97 to 1.15 (i.e. beetle performance on these hosts was almost as good as or slightly better relative to performance on *T. speciosum*). See Table 4.

We consider three species outside the genus *Tropaeolum* to be within the fundamental host range of *B. elguetai*, because one larva completed development to adult on each of those hosts. They are *Brassica chinensis* pak choi 'Dark Dragon' (RPI score = 0.57; Table 4),

[2] Fundamental host range of a natural enemy is defined as the sum of all species on which the agent can complete its life cycle. The fundamental host range can be broader than the ecological, or realised host range, which is the subset of the fundamental host range that can be used by the natural enemy under natural conditions.

B. rapa (RPI score = 0.24), and *Moringa oleifera* (RPI score = 0.17). The likelihood of these three species being field hosts is 24.4%, 5.6% and 3.9%, respectively. The time to complete development on these three hosts was 38, 39, and 39 days respectively, compared to 34 days on average (range 32 to 37 days) on *T. speciosum*. The individual that completed development on *B. chinensis* had deformed wings.

We determined that the likelihood of *B. rapa* and *M. oleifera* being field hosts is below the threshold of risk, but that the likelihood of *B. chinensis* pak choi 'Dark Dragon' being a field host is a borderline risk. We therefore conducted additional replicates with *B. chinensis* to further assess the risk to this crop (see Section 3.3.)

There was no significant difference between the two source populations. Therefore, the analysis for the two source populations combined is presented. On the target host plant, *T. speciosum*, 42%, 34% and 22% of the replicates (n = 50) had at least one larva reach the prepupal, pupal and adult stages, respectively. On *B. chinensis*, no replicate (of n = 84 replicates) had any larvae survive beyond the second larval instar, which was significantly lower compared to development on *T. speciosum* (Table 5). These differences confirm that *B. chinensis* is highly unlikely to be a field host of *B. elguetai*.

Table 4. The likelihood of each species to be a field host of *Blaptea elguetai* based on the Relative Performance Index (Paynter et al. 2015). Species are listed in decreasing difference compared to *Tropaeolum speciosum*. Index scores below 1 suggest the performance of *B. elguetai* on the test species is inferior to its performance on *T. speciosum*. A score below 0.21 suggests that minor non-target effects in testing remain artefacts of testing, posing no risk in the environment. A score above 0.33 suggests potential spillover effects. The species highlighted in green at the top part of the table are outside the fundamental host range of *B. elguetai*.

Plant species	Proportion eaten	SEM[1]	Diff. cf. *T. speciosum*[2]	LSD[3]	Significant difference vs *T. speciosum* (0=No; 1=Yes)	Proportion alive after 4/5 days	SEM	Diff cf. *T. speciosum*	LSD	Significant difference vs *T. speciosum* (0=No; 1=Yes)	Relative performance score	Approx. probability of being a field host	Fundamental host
Cardamine grandiscapa	0.00	0.00	0.32	0.09	1	0.12	0.06	0.76	0.09	1	0.13		N
Carica papaya	0.00	0.00	0.32	0.25	1	0.21	0.09	0.66	0.25	1	0.24		N
Carica pubescens	0.00	0.00	0.32	0.09	1	0.11	0.07	0.77	0.09	1	0.12		N
Cleome spinosa	0.00	0.00	0.32	0.09	1	0.11	0.07	0.76	0.09	1	0.13		N
Lepidium banksii	0.00	0.00	0.32	0.09	1	0.11	0.07	0.76	0.09	1	0.13		N
Lepidium sativum	0.00	0.00	0.32	0.09	1	0.05	0.05	0.82	0.09	1	0.06		N
Limnanthes douglasii	0.00	0.00	0.32	0.09	1	0.15	0.08	0.72	0.09	1	0.17		N
Pachycladon fastigiatum	0.00	0.00	0.32	0.09	1	0.00	0.00	0.87	0.09	1	0.00		N
Reseda alba	0.00	0.00	0.32	0.09	1	0.10	0.07	0.77	0.09	1	0.11		N
Labularia maritima	0.00	0.00	0.32	0.09	1	0.21	0.08	0.66	0.09	1	0.25		N

Plant species	Proportion eaten	SEM[1]	Diff. cf. *T. speciosum*[2]	LSD[3]	Significant difference vs *T. speciosum* (0=No; 1=Yes)	Proportion alive after 4/5 days	SEM	Diff cf. *T. speciosum*	LSD	Significant difference vs *T. speciosum* (0=No; 1=Yes)	Relative performance score	Approx. probability of being a field host	Fundamental host
Brassica oleracea	0.00	0.00	0.32	0.09	1	0.17	0.08	0.71	0.09	1	0.19		N
Reseda odorata	0.00	0.00	0.32	0.09	1	0.20	0.09	0.67	0.09	1	0.23		N
Rorippa palustris	0.00	0.00	0.32	0.13	1	0.05	0.05	0.82	0.13	1	0.06		N
Lepidium solandri	0.00	0.00	0.32	0.09	1	0.08	0.06	0.79	0.09	1	0.10		N
Brassica juncea mustard	0.01	0.00	0.31	0.09	1	0.15	0.08	0.72	0.09	1	0.17		N
Brassica chinensis pak choi 'Dark Dragon'	0.02	0.00	0.30	0.13	1	0.50	0.11	0.37	0.13	1	0.57	24.40%	Y
Tropaeolum minus	0.06	0.01	0.26	0.09	1	0.85	0.08	0.02	0.09	0	0.97	72.30%	Y
Brassica rapa	0.06	0.01	0.26	0.09	1	0.21	0.08	0.66	0.09	1	0.24	5.60%	Y
Tropaeolum pentaphyllum	0.07	0.01	0.25	0.16	1	1.00	0.00	-0.13	0.16	0	1.15	86.60%	Y
Moringa oleifera	0.08	0.01	0.24	0.09	1	0.15	0.08	0.72	0.09	1	0.17	3.90%	Y
Tropaeolum majus	0.15	0.01	0.17	0.09	1	0.95	0.05	-0.08	0.09	0	1.09	82.50%	Y

Plant species	Proportion eaten	SEM[1]	Diff. cf. *T. speciosum*[2]	LSD[3]	Significant difference vs *T. speciosum* (0=No; 1=Yes)	Proportion alive after 4/5 days	SEM	Diff cf. *T. speciosum*	LSD	Significant difference vs *T. speciosum* (0=No; 1=Yes)	Relative performance score	Approx. probability of being a field host	Fundamental host
Tropaeolum tuberosum	0.15	0.01	0.17	0.18	0	0.94	0.05	-0.07	0.18	0	1.08	81.80%	Y
Tropaeolum brachyceras	0.31	0.01	0.01	0.09	0	0.95	0.05	-0.08	0.09	0	1.09	82.50%	Y
Tropaeolum speciosum	0.32	0.01	Ref sp.[4]	0.09	Ref sp.	0.87	0.05	Ref sp.	0.09	Ref sp.	Ref sp.	Ref sp.	Ref sp.
Tropaeolum azureum	0.33	0.01	-0.01	0.09	0	1.00	0.00	-0.13	0.09	0	1.15	86.60%	Y
Tropaeolum tricolor	0.53	0.02	-0.21	0.09	0	0.88	0.08	-0.01	0.09	0	1.01	75.80%	Y

[1]Standard error of mean

[2]Difference between predicted proportion of replicates with at least one larva alive at Day 4/5 on *T. speciosum* and the predicted proportion on the test species.

[3]Least significant difference

[4] *T. speciosum* being the reference species is not compared to itself.

Table 5. Survival of larvae to different developmental stages on *Brassica chinensis* (*n* = 84 replicates) and on the target *Tropaeolum speciosum* (*n* = 50 replicates): (A) prepupal; (B) pupal; (C) adult. Data shows proportion of replicates in which at least one larva survived; binomial distribution.

	Plant species	Prediction	SEM[1]
A) Proportion of replicates where at least one larva reached the prepupal stage	*Brassica chinensis* pak choi 'Dark Dragon'	0.0000	0.00020
	Tropaeolum speciosum	0.4200	0.06980
B) Proportion of replicates where at least one larva successfully pupated	*Brassica chinensis* pak choi 'Dark Dragon'	0.0000	0.00020
	Tropaeolum speciosum	0.3400	0.06699
C) Proportion of replicates where at least one adult emerged	*Brassica chinensis* pak choi 'Dark Dragon'	0.0000	0.00020
	Tropaeolum speciosum	0.2200	0.05858

[1]Standard error of mean

4.4 Adult feeding and oviposition

Adult beetles fed and oviposited on all species in the genus *Tropaeolum* that were tested (Table 6). One adult out of three replicates on turnip (a variety of *Brassica rapa*) trace-fed on this host. One adult also trace-fed on Chinese cabbage (also a variety of *B. rapa*). No other feeding by adults took place on *B. rapa* (turnip, Chinese cabbage and pak choi 'Hon Tsai', n = 9 replicates in total). One adult nibbled on *Rorippa palustris* (out of n = 5 replicates). Feeding on *T. speciosum* occurred in all dishes after the non-target species was removed and replaced with *Tropaeolum*. Oviposition occurred in 1 dish out of 11 replicates with *Brassica chinensis* (pak choi 'Dark Dragon'), in 1 dish out of n = 3 replicates of turnip (one of the three varieties of *B. rapa*), and in 1 dish out of n = 6 replicates with *Carica papaya*. For test species in the genus *Tropaeolum* other than Chilean flame creeper, oviposition was recorded in 2 dishes out of n = 3 with *T. pentaphyllum*, and on 1 dish out of n = 3 replicates of each *T. brachyceras*, *T. majus* and *T. tuberosum*. Oviposition occurred in 28 out of the n = 126 replicates of post-test feeding on the target *T. speciosum*. We could not tell which of the tested individuals was a male and which was a female other than by having recorded certain individuals as having oviposited (and therefore known to be females from that point onwards).

4.5 Surveys of endemic climbers in the genus *Tropaeolum* in Chile

A total of 100 locations were surveyed and one or more species of endemic winter-climbing *Tropaeolum* were found at 73 of the sites. None of the five endemic species of winter-climber *Tropaeolum* found during the surveys had any signs of association with *B. elguetai* either on visual inspection or by beating and leaf litter inspections. The only signs of herbivory were minor damage by a leaf mining lepidopteran larva on *T. brachyceras*, *T. hookerianum* and *T. tricolor* leaves; major damage to the flowers of *T. austropurpureum* at one site; and similar damage to flowers of *T. brachyceras* at another site, where a weevil larva was found associated with the damage. The most common arthropods found on the plants during the survey were ants, pollinating beetles and flies, and spiders. The winter-climbing *Tropaeolum* species were found in northern and central Chile, whereas *T. speciosum* in association with *B. elguetai* has been recorded from southern Chile. The survey provides evidence that in northern and central Chile the endemic species of *Tropaeolum* that exhibit winter growth appear to be escaping attack by *B. elguetai*. However, it is interesting to note that the beetle has previously been associated with *T. brachyceras* (Petitpierre & Elgueta 2012).

Table 6. Adult feeding and oviposition. Species in alphabetic order.

Plant species	No. replicates	No. replicates where feeding occurred	No. replicates with oviposition in the dish	No. replicates with post-test feeding on *T. speciosum*	No. replicates with post-test oviposition in dish with *T. speciosum*
*Brassica chinensis p*ak choi 'Dark Dragon'	11	0	1	11	8
Brassica juncea mustard	5	0	0	5	0
Brassica oleracea[1]	9	0	0	9	1
Brassica rapa[2]	9	2	1	9	1
Cardamine grandiscapa	5	0	0	5	3
Carica papaya	6	0	1	6	1
Carica pubescens	6	0	0	6	1
Cleome spinosa	5	0	0	5	1
Labularia maritima	5	0	0	5	0
Lepidium banksii	4	0	0	4	0
Lepidium sativum	6	0	0	6	2
Lepidium solandri	5	0	0	5	0
Limnanthes douglasii	5	0	0	5	0
Moringa oleifera	6	0	0	6	1
Pachycladon fastigiatum	5	0	0	5	1
Reseda alba	3	0	0	3	1
Reseda odorata	3	0	0	3	0
Rorippa palustris	5	1	0	5	1
Tropaeolum azureum	2	2	0	2	0
Tropaeolum brachyceras	3	3	1	3	2
Tropaeolum majus	3	2	1	3	2
Tropaeolum minus	3	2	0	3	0
Tropaeolum tricolor	5	5	0	5	1
Tropaeolum tuberosum	3	3	1	3	0
Tropaeolum pentaphyllum	3	3	2	3	1

[1] *n* = 3 replicates of each of broccoli, cauliflower, and savoy cabbage

[2] *n* = 3 replicates of each of turnip, Chinese cabbage, and pak choi 'Hon Tsai'

4.6 Focused surveys on *Brassica* crops in Chile

Survey of the grey literature

The literature revealed that *Brassica* crops such as cabbage have been commonly grown in Chile since the 18th century, with the only mention of pests referring to white 'piojo' ('lice', a general term for a nuisance). There was no mention of beetles as pests of Brassicaceae, and specifically, no mention of *B. elguetai* – or the synonyms *B. balyi* and *Metastyla balyi*.

Similarly, conversations with entomologists, agronomists and rapeseed growers, and searches of agricultural services websites confirmed they have not encountered beetles matching the description of *B. elguetai* in association with these crops.

Field surveys

No leaf feeding beetles of any species were found in 13 rapeseed crops as well as in any of the ten other *Brassica* fields or wild field mustard plants at any of the sites.

The distance of the surveyed sites from beetle-infested *T. speciosum* ranged from 30 m to 25 km, averaging 5 km. Six sites were ≤1 km from beetle-infested *T. speciosum* sites, and all but two sites were ≤ 6.5 km from beetle-infested *T. speciosum* ones. These beetles are winged, and we can assume that they are capable of natural dispersal.

Other arthropods detected in the *Brassica* crops included honeybees, pests such as aphids, leaf miners, and the diamond back moth, and natural enemies such as parasitic wasps, hoverflies, and ladybird beetles.

5 Conclusions

While all species within the genus *Tropaeolum* can be field hosts of *B. elguetai*, the likelihood of field attack on the winter-climbing species is low under current climate conditions, since the beetle is expected to exhibit winter diapause in the regions where *T. speciosum* is a problem weed (i.e. Southland, Otago, Canterbury), allowing the winter-climbers to escape attack. This likely scenario is supported by findings in the native range, where winter-climbing species of *Tropaeolum* exhibited no signs of association with the beetle. This escape mechanism may break under a warming climate. However, it is also possible that under such a scenario *B. elguetai* may assist in keeping under check the *Tropaeolum* species that have already naturalised in New Zealand (*T. pentaphyllum* and *T. majus*) or those that may naturalise in the future.

Larval feeding tests confirmed that *B. elguetai* is restricted to the genus *Tropaeolum*. While adults were pre-fed on the target host, *T. speciosum*, their lack of feeding on hosts outside the genus *Tropaeolum* indicates that these hosts are not attractive to the beetle. Oviposition in nature occurs on non-host plants adjacent to *T. speciosum* and thus, oviposition in dishes with non-*Tropaeolum* hosts is in line with natural behaviour of the beetle and not considered relevant to host selection behaviour. Field surveys and grey

literature surveys from Chile further support our conclusion that *Brassica* species are highly unlikely to be field hosts for *B. elguetai*.

We conclude that *B. elguetai* is unable to form populations on plants outside the genus *Tropaeolum* and that even minor spillover attack on hosts outside the genus *Tropaeolum* is highly unlikely.

6 Recommendation

We recommend proceeding with the regulatory path to apply for the introduction of *B. elguetai* as a biocontrol agent for *Tropaeolum speciosum* in New Zealand.

7 Acknowledgements

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