



Manaaki Whenua  
Landcare Research

# **Invertebrates and fungi associated with Chilean flame creeper, *Tropaeolum speciosum* Poepp. & Endl., in New Zealand**

Prepared for: Ministry for Primary Industries and the National Biocontrol Collective

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# **Invertebrates and fungi associated with Chilean flame creeper, *Tropaeolum speciosum* Poepp. & Endl., in New Zealand**

*Contract Report: LC4109*

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# Summary

## Project and Client

- A survey of the invertebrate fauna and fungal pathogens associated with Chilean flame creeper, *Tropaeolum speciosum* Poepp. & Endl., in New Zealand was carried out during the autumn of 2021 and the summer of 2022 by Manaaki Whenua – Landcare Research for the National Biocontrol Collective and the Ministry for Primary Industries contract number S3F-20095.

## Objectives

- To survey the invertebrate fauna and plant disease symptoms associated with Chilean flame creeper, *Tropaeolum speciosum*, in New Zealand and to identify the herbivores (and their associated predators and parasitoids) and plant pathogens present.

## Methods

- The invertebrate fauna and plant disease symptoms associated with Chilean flame creeper were surveyed at ten New Zealand sites, from Manawatu-Whanganui to Southland, during autumn 2021 and summer 2022. One site was surveyed twice.
- Invertebrates were collected, reared and identified.
- Disease symptoms were photographed and recorded.
- Fungi were isolated from surface-disinfected symptomatic material.
- DNA sequences of pure fungal cultures were analysed.
- Literature was reviewed for information on life histories of the identified fungal cultures isolated from Chilean flame creeper tissues.

## Results

- A total of 63 invertebrate species, or groups of related taxonomic units, were recorded from Chilean flame creeper during the surveys.
- None of the herbivores recorded were Chilean flame creeper specialists.
- Very few plant disease symptoms were observed on Chilean flame creeper. These were mainly leaf spots, leaf yellowing and leaf reddening. No damage on rhizomes was observed.
- A total of 76 pure fungal cultures were recovered from symptomatic tissues comprising 34 species from 18 genera. Two additional isolates were identified to the order Pleosporales and to the family Xylariaceae.
- The predominant genera isolated were *Colletotrichum*, *Diaporthe* and *Xylaria* from leaf yellowing, leaf reddening and leaf spots. *Colletotrichum* and *Diaporthe* species are common plant pathogens causing fruit and stem rots, leaf spots and foliar blight on a wide range of plant species while *Xylaria* species are found as endophytes.

- None of the primary<sup>1</sup> plant pathogens found to be associated with disease symptoms of Chilean flame creeper are host-specific, and none had any major impact.
- The remaining species identified were considered either saprophytes, endophytes or secondary<sup>2</sup> pathogens.

### **Conclusions**

- No specialised Chilean flame creeper-feeding invertebrates were collected during these surveys.
- None of the primary pathogens found on Chilean flame creeper in the surveys are host specific and none had any major damage on this plant.

### **Recommendations**

- A leaf beetle was identified in a preliminary survey in Chile as a potential candidate for biological control against Chilean flame creeper
- Given that no specialised Chilean flame creeper-feeding invertebrates and no specialised pathogenic fungi have been recorded on Chilean flame creeper in New Zealand, we recommend that the invertebrate species found in Chile should be investigated for its use in a classical biological control programme for Chilean flame creeper.

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<sup>1</sup> A primary pathogen can cause disease on a healthy host.

<sup>2</sup> A secondary pathogen needs a wounded or weakened host to cause disease.



## 1 Introduction

A survey of the invertebrate fauna and fungi associated with Chilean flame creeper, *Tropaeolum speciosum* Poepp. & Endl., in New Zealand was carried out during the autumn of 2021 and the summer of 2022. This work was carried out by Manaaki Whenua – Landcare Research for the National Biocontrol Collective and the Ministry of Primary Industries contract number S3F-20095.

## 2 Background

Chilean flame creeper, *Tropaeolum speciosum*, is a climbing perennial vine from the family Tropaeolaceae. It forms slender stems that can climb 4 m or higher, through or over other plants to access sunlight (Fig. 1A; Rix 2010). The thin green leaves are peltate, with five to six unequal, 10–35 x 5–16 mm elliptic to obovate lobes (Fig. 2; Rix 2010; NZPCN 2021) that die off over winter. Petioles are pilose and slender, 35–60 mm long, coiling around other plants for support. Chilean flame creeper produces four- to six-lobed stipules, 10–18 mm long. In the upper part of stems, solitary, deep scarlet, 150 mm diameter, tubular flowers develop from leaf axils on 70–150 mm long pedicels (Fig. 1B). The calyx has five green lobes that turn purplish-red and accrescent when fruiting, with lobes recurving and a 250–300 mm spur that is red and swollen in the basal half, and green, slender and curved in the apical half, becoming purplish-red when fruiting (Fig. 1C). Each flower has five petals notched at the apex, the upper pair is cuneate, 12–16 x 5 mm, while the lower three petals are rectangular, 12 x 13 mm, with a narrow, 6-mm long claw (Fig. 1B). In New Zealand, Chilean flame creeper flowers between November and April. Each flower produces one to three blue, 6–7 mm long fleshy fruits (Figure 1C). Seeds are dispersed by birds feeding on these fruits. The plant reproduces vegetatively through its white, fleshy, 4 mm diameter, stoloniferous rhizomes (Rix 2010) that resprout in spring.



**Figure 1. Chilean flame creeper. A. Chilean flame creeper smothering vegetation, B. flower, C. fruits.**



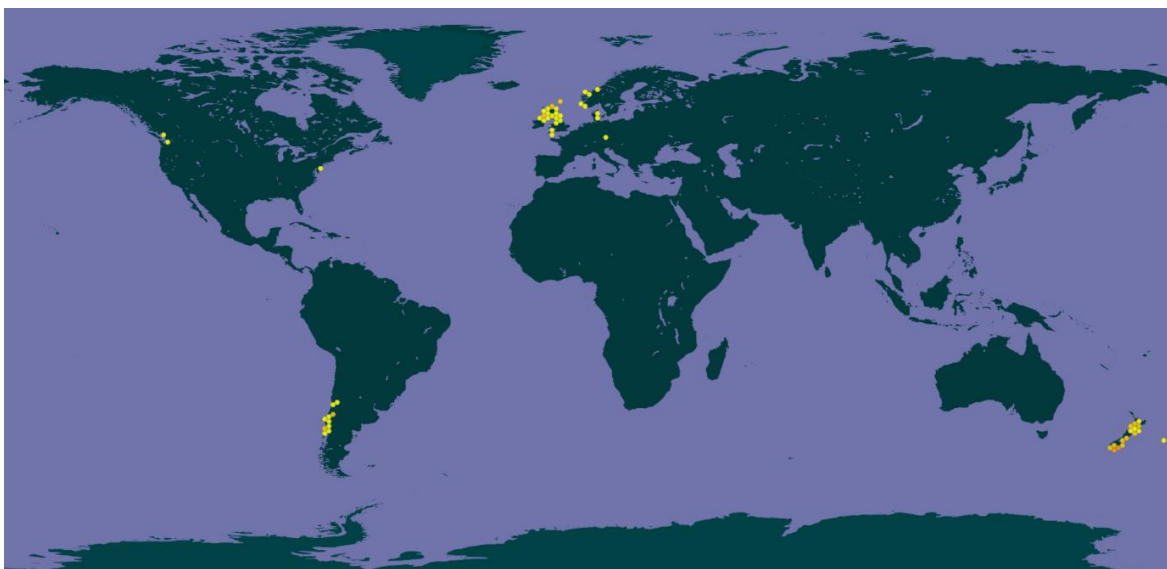
Plate 686 *Tropaeolum speciosum*

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Figure 2. *Tropaeolum speciosum* (Rix 2010).

Chilean flame creeper is native to Chile where it grows in humid areas from the Bío Bío to Los Lagos regions and is described as 'rare' according to the Chileflora website (Belov 2005–2012). It grows along the coast of the Valdivian rain forest (up to 1000 m) where this plant is mainly associated with the native bamboo *Chusquea quila*, on the edges of *Nothofagus* forest (Rix 2010). Replacement of native vegetation with *Pinus radiata* plantations has contributed to the scarcity of this plant in its native range.

It has been introduced to Scotland where it is known as 'Scottish flame flower' and has been described as 'famously doing well'. It can be purchased online, in nurseries and garden centres across the UK where it has been awarded the Royal Horticultural Society Award of Garden Merit and in Ireland (Ballyroberts Gardens 2021; Plants of Distinction 2021). A few sightings have been recorded in northern Europe including Norway and Denmark and in northern America (Fig. 3; GBIF 2021). It is only known to be a weed in New Zealand where it was reported to become naturalised in 1958 (Webb et al. 1988).



**Figure 3. Global distribution of Chilean flame creeper (GBIF 2021).**

Chilean flame creeper grows best in shady hummus rich, well-drained soils; however, it can survive in a wide range of conditions, including warm to cold temperatures, salt, wind, various soil types and damp to dry environments (Otago Regional Council 2019a; Gardenia 2021). It is found in remnant stands of forests, scrubs, forest clearings, shrubland, roadside and wasteland (NZPCN 2021; Weedbusters 2021).

In New Zealand, Chilean flame creeper is an unwanted organism under the Biosecurity Act (1993) and a National Plant Pest Accord Species which prevents its propagation, sale and distribution throughout the country. It is included in the Regional Pest Management Plans in four regions with the following status:

- 'Site-led control' in Otago (Otago Regional Council 2019b)
- 'Sustained control' in Auckland (Auckland Council 2021)

- 'Exclusion' in Waikato where all plants have been eradicated from historic sites (Waikato Regional Council 2019) and in Bay of Plenty (Bay of Plenty Regional Council 2021)

Originally an escape from gardens in New Zealand, Chilean flame creeper has been recorded from Stewart Island to Waikato although Chilean flame creeper historic sites in Waikato have been eradicated (Fig. 4). In the South Island, Chilean flame creeper is mainly invasive in Southland where the plant suppresses and replaces native species by shading and smothering plants. It is dispersed by birds that can scatter seeds in remote areas, making new infestations undetectable, and by root fragments from garden waste that can resprout (Auckland Council 2021). Only isolated populations are present in the North Island.



**Figure 4. Distribution of Chilean flame creeper in New Zealand (GBIF 2021).**

Plants can be controlled physically by cutting vines at waist height, letting the upper stems die off or pulling them out and digging up the roots (Auckland Council 2021). The plant material needs to be disposed of at a refuse transfer station. Several chemical treatments have been trialled; however, good control was only achieved at the expense of surrounding native vegetation and losses were deemed unacceptable (James et al. 2012). Although no fully effective herbicide treatment is available for Chilean flame creeper, the

following treatments have been recommended by councils (Otago Regional Council 2019a, Auckland Council 2021, Environment Southland 2021; Weedbuster 2021):

- Cut vines at waist height and spray vines on the ground with glyphosate (20 mL/L) with a penetrant, Organic Interceptor (100 mL/L) or Grazon (6 mL/L) from spring to summer.
- Cut vines and paste base of stems with a herbicide gel such as Tordon BK containing glyphosate, metsulfuron-methyl or a combination of picloram and triclopyr all year.

Treatments need to be followed up 6-monthly to manage re-growths from rhizomes.

Biological control could offer many advantages over current control methods for Chilean flame creeper. Use of host-specific biocontrol agents would reduce herbicide impacts on desirable flora, and biological control also offers continuous action and self-dispersal that current control methods do not offer. Biological control is an attractive option for a target weed that is spreading and where no effective control option is available such as Chilean flame creeper. In 2019, a potential biocontrol agent, a leaf beetle, was found at different locations in Chile (Norambuena 2020; Probst 2020). Larvae and adults feed on leaves of Chilean flame creeper, completely defoliating plants. The insect has been identified as *Blaptea elguetai* Petitpierre (Coleoptera: Chrysomelidae) and our collaborator, Dr Hernán Norambuena, in Chile is studying the biology of the beetle in preparation for testing its host specificity (H. Norambuena, entomologist, Temuco, Chile, pers. comm.).

This report describes the results of a survey of the invertebrate fauna and fungal pathogens associated with Chilean flame creeper in New Zealand.

### **3 Objectives**

To survey the invertebrate fauna and plant disease symptoms associated with Chilean flame creeper (*Tropaeolum speciosum*) in New Zealand and identify the herbivores (and their associated predators and parasitoids) and plant pathogens present.

The main aims of the survey were to:

- 1 determine whether any specialist Chilean flame creeper invertebrates or pathogens are already present in New Zealand.
- 2 determine whether any generalist invertebrate herbivores or plant pathogens are exerting a significant adverse impact on Chilean flame creeper in New Zealand.
- 3 record the invertebrate parasitoids and predators associated with the herbivorous invertebrates on Chilean flame creeper in New Zealand.

Such information is useful at the early stages of a biological control programme to avoid wasting resources on importing something that is already present in NZ. Also, knowing whether a candidate invertebrate biocontrol agent has similar 'analogue' herbivores already utilising the weed in New Zealand helps to predict whether natural enemies of the analogue (e.g. predators and parasitoids) could potentially interfere with the efficacy of biocontrol agents released in New Zealand (Paynter et al. 2010).

## 4 Methods

### 4.1 Invertebrates

The invertebrate fauna of Chilean flame creeper was surveyed at ten New Zealand sites from Raetihi in the North Island to Lake George in the South Island, between May 2021 and January 2022 (Fig. 5 and Appendix 1). The surveys took place in summer to allow enough time to observe herbivory activity on plants and in autumn to allow disease expression.

Nine sites were surveyed once each, and one site (Dunedin Botanic Gardens) was surveyed twice, making a total of 11 'collecting events'. Five collecting events were done during autumn and six during summer. At each site, ten collection locations were selected, except for site 6 where only one plant was present. Depending on the number of Chilean flame creeper plants present at each site, plants with sufficient biomass were sampled every 5–10 m. A collecting tray, 80 × 80 cm, was placed under climbing stems of selected plants, and the foliage above the tray was hit five times with a stick. Most invertebrates that fell onto the tray were collected with an aspirator and preserved in 95% alcohol. Caterpillars (Lepidoptera) were collected live, and placed, along with Chilean flame creeper foliage, into ventilated containers to rear through to adult for identification. Any parasitoids emerging from the caterpillars were identified. The ten plants per site or their closest accessible neighbour were dug up and their roots cut open lengthwise in search for root feeders.

A rapid visual inspection of foliage (generally less than a minute for each of the ten collection locations per site) was made for signs of invertebrates such as gall-formers, leaf miners, scale insects and stem/shoot borers. Invertebrates found during the visual inspections were collected live, along with the plant material they were on, for identification. At each site, a visual estimate was made of the amount of herbivore-related damage, and the likely cause of the damage was noted (e.g. adult beetles, caterpillars).

The invertebrates collected were identified to species or genus level where feasible. However, some invertebrates were placed into higher-level taxonomic units (e.g. order or family). They were then ranked on a scale of abundance according to the total number of individuals collected, and the number of collecting events at which they were present. Invertebrates were classed as rare, occasional, common or abundant according to the definitions below:

|                    |  |
|--------------------|--|
| <b>rare:</b>       | fewer than 5 individuals collected in total                                  |
| <b>occasional:</b> | 5–15 individuals collected in total, <b>or</b> present at fewer than 5 sites |
| <b>common:</b>     | 16–99 individuals collected <b>and</b> present at 5 or more sites            |
| <b>abundant:</b>   | 100+ individuals collected <b>and</b> present at 7 or more sites             |

## 4.2 Plant pathogens

Plant pathogens associated with Chilean flame creeper were surveyed at the same ten New Zealand sites and the same times as for invertebrates (Fig. 5 and Appendix 1). At each site, plants were inspected for signs of pathogen damage and/or presence of either diseased leaves, rhizomes or fruits. Samples of symptomatic tissues were placed in paper bags, and then placed into labelled zip-lock bags, and kept cool in transit, then held at 4–10°C until processing. Collected material was usually processed within five days of collection.

In the laboratory, disease symptoms were recorded and photographed. A dissecting microscope was used to search necrotic areas for fungal reproductive structures especially, conidia and spores. Symptomatic tissues were surface disinfected to remove any fungi that were present on the surface of plant material but have not penetrated plant tissues, removing a number of saprophytes. This process was achieved by immersion in 70% ethanol for 30 s, 2% sodium hypochlorite for 1 min, and 70% ethanol again for 30 s, followed by two rinses in sterile reverse osmosis water. The tissue fragments were air-dried, placed on potato dextrose agar (PDA; Difco Labs, Detroit, MI, USA) amended with 0.02% streptomycin (Sigma, St Louis, MI, USA), contained in 9-cm Petri dishes. Plates were sealed with parafilm, and incubated under white light (12-hour photoperiod) at temperatures of  $18 \pm 2^\circ\text{C}$ . This method allowed fungi present in symptomatic tissue fragments to grow onto agar.

Fungal colonies that grew out of the tissue fragments were transferred to fresh PDA plates. Each identified isolate was given a unique number. Isolates that produced spores were identified to species level where possible. Pure mycelial isolates had their nuclear ribosomal internal transcribed spacer (ITS) region sequenced, using a standard polymerase chain reaction (PCR) protocol with the fungal-specific primers ITS1F and ITS4 (White et al. 1990; Gardes & Bruns 1993). The ITS region is the most widely sequenced DNA region in molecular ecology of fungi and is used as a universal fungal barcode sequence. All PCR products were sequenced in both directions using standard protocols established in the EcoGene® laboratory; and DNA sequences were assembled using Geneious v 10.2.6 (<http://www.geneious.com>; Kearse et al. 2012). Sequences were subjected to a GenBank BLASTn search (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>; Altschul et al. 1990) to determine the closest match to organisms whose sequences have already been deposited in this databank. Species identifications were confirmed using spore or cultural morphology combined with the sequence data where possible.

Taxonomic literature and fungal systematists were consulted to determine which of the identified fungi were likely to be causing the damage, and which of the fungi were associative saprotrophs, and/or endophytic organisms.

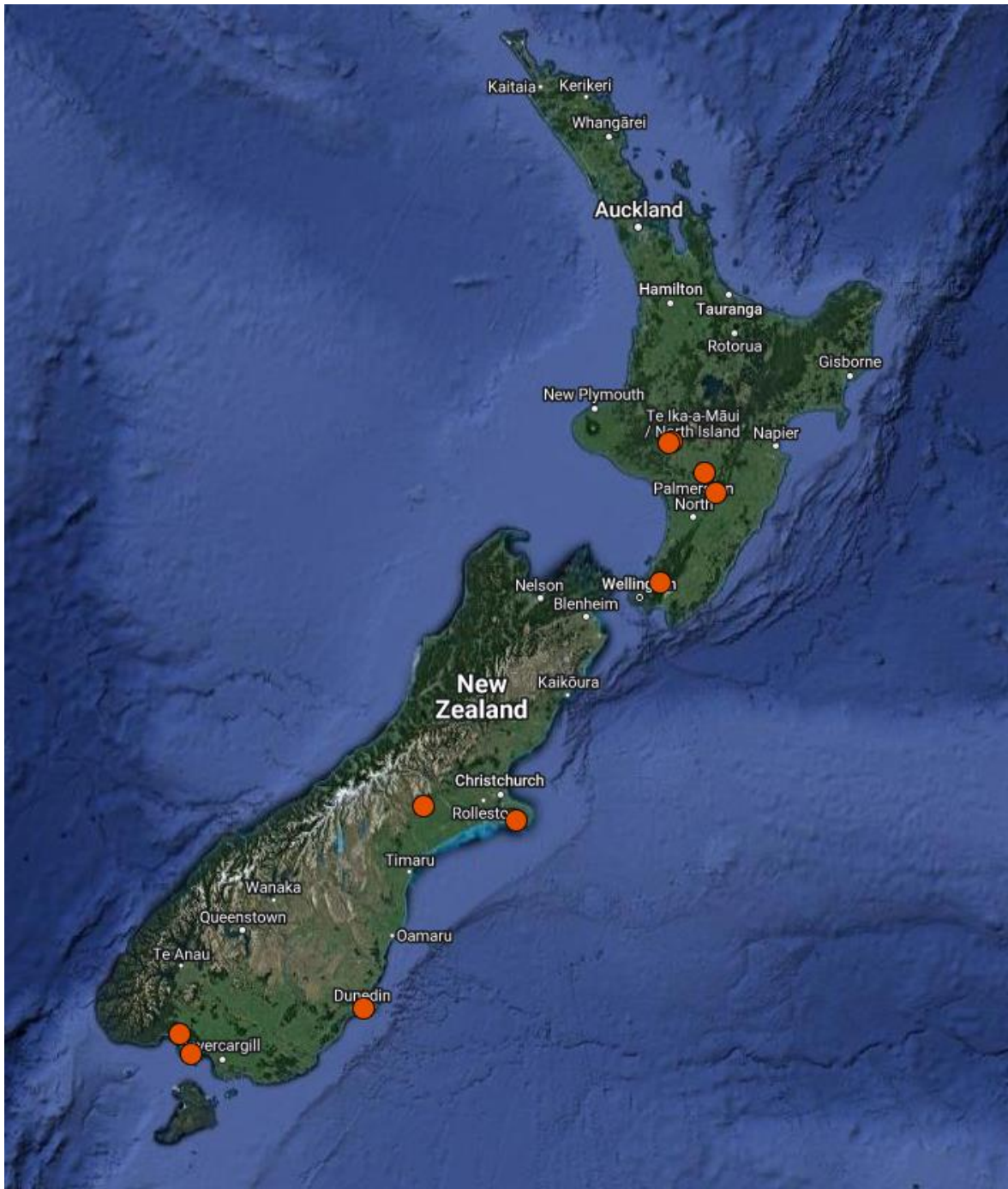


Figure 5. Map of the survey sites showing the extent of geographic spread.



## 5 Results

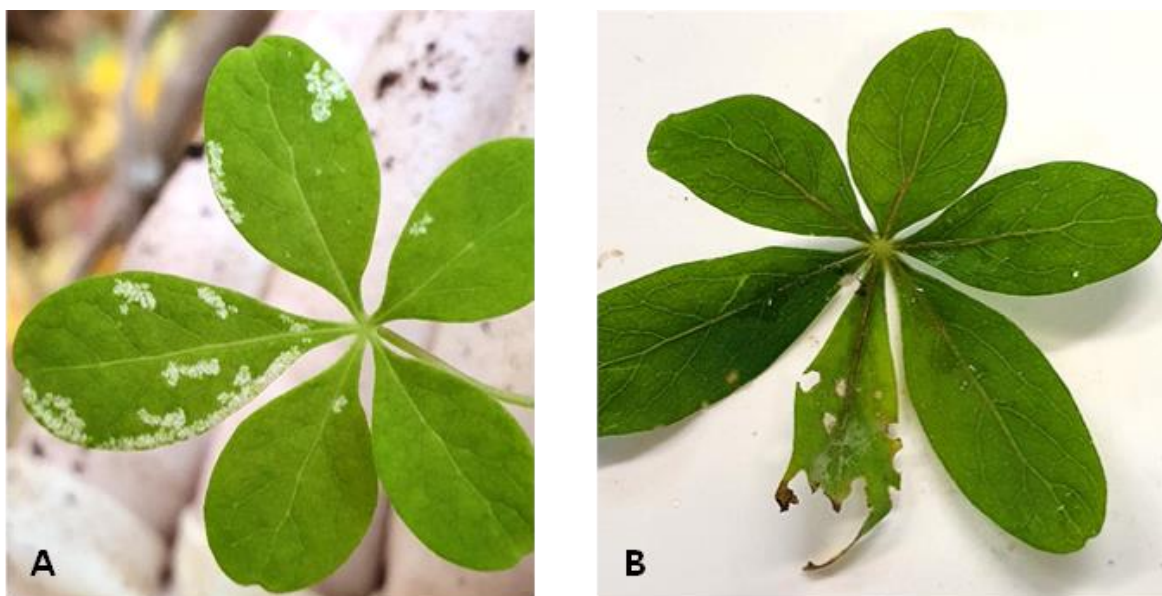
### 5.1 Invertebrates

A total of 63 invertebrate species or taxonomic units were recorded during the *Tropaeolum speciosum* surveys, roughly classified as herbivores (23, including sap feeders), predators (5), parasitoids (4) or saprophytes/fungivores (20). Three were classified as omnivorous and eight species feeding mode was unknown (Appendix 2). Surprisingly, no invertebrates were found on Chilean flame creeper at three sites: one in Upper Hutt and two in Raetihi.

#### 5.1.1 Herbivores

A total of 26 herbivorous (including omnivorous) invertebrate species and taxonomic units (where identification to species level was not feasible) were recorded from Chilean flame creeper during the surveys. Four species or taxonomic units were classified as 'Occasional' and 19 as 'Rare' (Appendix 2). No species or taxonomic units were categorised as 'Abundant' or 'Common'.

Damage caused by herbivorous species was considered minimal and the overall amount of foliage consumed by herbivores was estimated to be less than 1%. There were no signs of gall-formers, leaf miners or scale insects. Even minor notching, sucking or chewing damage, as well as damage to flowers was rare (Fig. 6).



**Figure 6. Feeding damage on Chilean flame creeper. A. sucking damage, B. chewing damage.**

The most common herbivore was the green planthopper *Siphanta acuta* (Hemiptera: Flatidae), with 10 individuals recorded across three sites. This exotic species originates from Australia. It is a polyphagous species that feeds on a wide variety of plant species. Eight other sap-feeding species, or groups of taxonomically related sap-feeding species,

were collected from Chilean flame creeper during the surveys. Two of these were classified as 'Occasional' and seven were 'Rare' (Appendix 2). Of these eight sap-feeders, five are polyphagous and three (unidentified hemipteran, unidentified aphididae, and unidentified meridae) are unknown.

Three herbivorous Lepidopteran species or taxonomic units were collected from Chilean flame creeper during the survey, but damage attributed to lepidopterans was minimal. These species are either host-specific to native plants (e.g. *Pasiphila* sp.) or polyphagous (e.g. *Ctenopseustis* sp.). These invertebrates were collected as adults, indicating that their presence on Chilean flame creeper was transient and therefore they were unlikely to cause damage to Chilean flame creeper. All three Lepidopteran species collected during the survey were classed as 'Rare'.

Ten species or taxonomic units of herbivorous adult beetles were collected during the survey but no foliage damage that could be attributed to beetles was observed. Eight of these herbivorous beetles are polyphagous and the remaining two (*Psilacnaeia* sp. and *Peristoreus* sp.) are unknown. Only one – *Eucolaspis* sp. – was classified as 'Occasional', with the rest classified as 'Rare'. Most beetle species observed are likely to be transient on Chilean flame creeper. The potential biocontrol agent for Chilean flame creeper – the leaf-feeding chrysomelid beetle *Blaptea elguetai* – was not found during the survey.

The three omnivorous species identified included a sun fly (*Allophylopsis scutellata*), the invasive German wasp *Vespula germanica* and a species of cave weta (*Pleiopectron* sp.).

### **5.1.2 Parasitoids**

Parasitoids and predators were recorded to help identify factors that might inhibit introduced biological control agents. The candidate biocontrol agent currently being considered for introduction into New Zealand is a Coleopteran, so Coleopteran parasitoids are of particular interest. However, no parasitoids of Coleoptera were found during the survey. A total of four parasitoids were collected and identified: a leafroller fly that is a parasitoid of Lepidoptera; a Braconidae wasp that is a parasitoid of Diptera; and two Ichneumonidae wasps that are parasitoids of other Hymenoptera (Appendix 2). All parasitoids recorded during the survey were collected directly from the plants by beating.

### **5.1.3 Predators**

Six species or taxonomic units of predatory invertebrates were recorded on Chilean flame creeper during this survey. Only one – the coccinellid *Adoxellus picinus* – was categorized as 'Occasional'. The remaining five predatory species or taxonomic units (snout mites, spiders, the Tasmanian lacewing *Micromus tasmaniae*, the brown shield bug *Cermatulus nasalis*, and the carabid beetle *Amarotypus edwardsii*) were categorised as 'Rare' (Appendix 2).

### 5.1.4 Saprophytic and fungivorous invertebrates

Seven species/taxonomic units of saprophytic and 13 of fungivorous invertebrates were recorded during this survey (Appendix 2), and they would most probably have been associated with decaying material and not damaging living plant material.

## 5.2 Plant pathogens

Very few symptoms caused by pathogens on Chilean flame creeper were observed in the field. These symptoms consisted of leaf spots, leaf reddening and leaf yellowing (Fig. 7).

From a total of 76 fungal pure cultures recovered from symptomatic tissues, 34 species were identified from 18 genera (Appendix 3). One isolate was identified to the family Xylariaceae and one isolate was identified to the order Pleosporales. The predominant genera isolated were *Colletotrichum*, *Diaporthe* and *Xylaria*. Only species belonging to the genera *Colletotrichum*, *Diaporthe*, *Alternaria*, *Nigrospora* and *Didymella* were considered as potential pathogens; the remaining species identified were saprophytes, endophytes or secondary pathogens.



**Figure 7. Chilean flame creeper pathogen symptoms. A. leaf yellowing, B. leaf reddening.**

*Colletotrichum* spp. are responsible for anthracnose foliar blight and fruit and stem rots on over 3000 plant species (Liang et al. 2018). *Colletotrichum* species were isolated from most sites and are likely responsible for leaf yellowing, leaf spots and leaf reddening. Four different *Colletotrichum* species isolated in this survey are known pathogens on a wide range of hosts: *Colletotrichum salicis* has been isolated from woody hosts including *Salix*, *Malus*, *Pyrus*, *Populus*, *Pyrus* and *Araucaria* (UniProt 2022); *C. boninense* causes anthracnose predominantly on species from the families Amaryllidaceae, Orchidaceae, Proteaceae and Solanaceae (Damm et al. 2012); *C. godetiae* has been isolated from fruit, leaf and stem diseases from hosts such as *Fragaria*, *Malus* and *Prunus* (Damm et al. 2012) and *C. destructivum* is a pathogen of forage and grain legumes and causes anthracnose of many plant species (Damm et al. 2014). The fifth species, *Colletotrichum cigarro*, has been

isolated from leaves of New Zealand native trees and is considered as an endophyte (Weir et al. 2012).

*Diaporthe* species have been described as plant pathogens, endophytes, and saprophytes (CABI 2021), causing root and fruit rots, dieback, stem cankers, leaf spots, leaf and pod blights, and seed decay on a wide range of hosts including vegetables, fruit crops, and forest plants (Guarnaccia et al. 2018). Eight *Diaporthe* species have been isolated from Chilean flame creeper; however, only half the isolates were identified to species level. *Diaporthe eres* causes diseases in forest plants and fruit crops including *Prunus persica*, *Vitis vinifera* and *Vaccinium corymbosum*; however, it has also been described as an endophyte in *Citrus* and *Ulmus* (CABI 2021). *Diaporthe rudis* has been associated with twig blight and dieback of *Vaccinium corymbosum* (Hilário et al. 2020), cane and leaf spot of *Vitis vinifera* (Guarnaccia et al. 2018), and fruit rot (KC & Rasmussen 2019). *Diaporthe phaseolorum* is responsible for stem canker, pod and stem blight, leaf, petiole and stem lesions on a wide range of hosts including legumes (Punithalingam & Holliday 1972). *Diaporthe nothofagi* has been described as an endophyte on *Nothofagus* (Anonymous 2022). These species were isolated from leaf yellowing, leaf spots, and leaf reddening at seven sites and are likely to have a wide host range.

Species from the genus *Nigrospora* can be pathogens, endophytes, and saprophytes of various hosts. *Nigrospora oryzae* had been reported to cause leaf spots, stem blight, and foliar blight on a wide range of plants (Hao et al. 2020). It was recovered from leaf reddening only at one site.

*Alternaria* species usually target leaves (Abbasi et al. 2018; Chen et al. 2018; Garibaldi et al. 2018; Moccellini et al. 2018; Garibaldi et al. 2020; Matic et al. 2020) as spores are dispersed by wind or water splashes but they can also lead to stem lesions, fruit lesions, damping off, and collar rot (Laemmlen 2001). They are more commonly found attacking tissues that are stressed, senescent or wounded (Laemmlen 2001). However, *Alternaria* species are also considered to be part of fungal endophytic communities associated with different plant species (Zhang et al. 2014; Varanda et al. 2016; Hamzah et al. 2018). *Alternaria alternata* and *A. rosae* were isolated from reddening leaves and yellowing leaves, respectively and are known to have a wide host range.

*Didymella* species are pathogens of many important crops and can cause stem blight, leaf spots, stem and fruit rots. A *Didymella* species was isolated from yellowing leaves once in our survey.

## 6 Conclusions

### 6.1 Herbivores

A range of native and introduced invertebrates are associated with Chilean flame creeper in New Zealand but no specialised Chilean flame creeper-feeding invertebrates were recorded in the current survey. Most herbivorous invertebrates recorded in our survey occurred in small numbers and the damage they caused to Chilean flame creeper was considered minimal.

Damage to Chilean flame creeper that could be attributed to Coleopterans was minimal. The candidate biocontrol agent currently being considered for introduction into New Zealand is a Coleopteran. The leaf-feeding chrysomelid beetle (*Blaptea elguetai*) larvae feed on leaves and is unlikely to meet any significant competition from other Coleopterans in New Zealand. Furthermore, we found no parasitoids of Coleopterans during the survey.

None of the herbivorous invertebrates identified associated with Chilean flame creeper can be recommended for further testing as biological controls as they are polyphagous and the damage they cause to Chilean flame creeper is minimal.

### 6.2 Pathogens

None of the plant pathogen associated with disease symptoms of Chilean flame creeper are host specific nor had any major impact. Very few disease symptoms were observed in the surveys, and these only affected leaves. No damage was observed on rhizomes, stems, flowers or fruits.

None of the main primary pathogens identified associated with Chilean flame creeper can be recommended for further testing as biological controls for Chilean flame creeper as they have a very broad host-range, including valued horticultural species and overall their combined impact observed in the field was minimal.

## 7 Recommendations

In light of our conclusions that:

- 1 no specialised Chilean flame creeper-feeding invertebrates and no specialised pathogenic fungi have been recorded on Chilean flame creeper in New Zealand and
- 2 the candidate biocontrol agent, the leaf beetle *Blaptea elguetai*, is unlikely to meet with significant levels of competition from other herbivorous species currently in New Zealand

we recommend that a classical biological control programme for Chilean flame creeper should proceed.

## 8 Acknowledgements

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## Appendix 1 – Chilean flame creeper survey (New Zealand 2021-2022) site details

| Site number | Site name               | Collection date | GPS coordinates (NZTM) |
|-------------|-------------------------|-----------------|------------------------|
| 1           | Lake George             | 17/05/2021      | E1204994 N4854039      |
| 2           | Dunedin Botanic Gardens | 18/05/2021      | E1407529 N4918603      |
| 3           | Pohangina               | 25/05/2021      | E1852410 N5562941      |
| 4           | Mangaweka               | 25/05/2021      | E1838150 N5589448      |
| 5           | Stavely camp            | 24/06/2021      | E1474314 N5166832      |
| 6           | Gillespies              | 10/01/2022      | E1776531 N5449003      |
| 7           | Raetihi                 | 11/01/2022      | E1796522 N5632666      |
| 8           | MRS                     | 12/01/2022      | E1792076 N5629195      |
| 9           | Tuatapere               | 17/01/2022      | E1189447 N4878024      |
| 10          | Wainui                  | 21/01/2022      | E1590179 N5150409      |

## Appendix 2 – Invertebrates associated with Chilean flame creeper, *Tropaeolum speciosum*, at ten New Zealand sites (2021-2022)

| Taxon                              | Common name            | Feeding mode | Abundance category <sup>1</sup><br>(number of individuals) | Collection sites <sup>2</sup> |
|------------------------------------|------------------------|--------------|--|-------------------------------|
| <b>Phylum Arthropoda</b>           |                        |              |  |                               |
| <b>Class Crustacea</b>             |                        |              |  |                               |
| <b>Isopoda</b>                     | <b>Slaters</b>         |              |  |                               |
| Unidentified Isopoda               |                        | Saprophytic  | Occasional (6)   | 2b, 9, 10                     |
| <b>Class Diplopoda</b>             |                        |              |  |                               |
| <b>Polydesmida</b>                 | <b>Millipedes</b>      |              |  |                               |
| Unidentified Polydesmida           |                        | Saprophytic  | Rare (1)   | 10                            |
| <b>Class Arachnida</b>             |                        |              |  |                               |
| <b>Acarina</b>                     | <b>Mites and ticks</b> |              |  |                               |
| Oribatida                          | Oribatid mites         |              |  |                               |
| Unidentified Oribatida             |                        | Saprophytic  | Rare (2)   | 10                            |
| Trombidiformes                     |                        |              |  |                               |
| Bdellidae                          | Snout mites            |              |  |                               |
| Unidentified Bdellidae             |                        | Predatory    | Rare (3)   | 1, 5, 9                       |
| <b>Araneida</b>                    | <b>Spiders</b>         |              |  |                               |
| Unidentified Araneida              |                        | Predatory    | Rare (4)   | 3, 4, 10                      |
| <b>Class Insecta</b>               |                        |              |  |                               |
| <b>Insects</b>                     |                        |              |  |                               |
| <b>Coleoptera</b>                  | <b>Beetles</b>         |              |  |                               |
| Anthribidae                        | Fungus weevils         |              |  |                               |
| <i>Liromus pardalis</i> (Pascoe)   |                        | Fungivorous  | Rare (1)   | 9                             |
| <i>Sharpus brouni</i> (Sharp)      |                        | Fungivorous  | Rare (1)   | 9                             |
| <i>Sharpus</i> sp.                 |                        | Fungivorous  | Rare (1)   | 9                             |
| Carabidae                          | Ground beetles         |              |  |                               |
| <i>Amarotypus edwardsii</i> Bates  |                        | Predatory    | Rare (3)   | 9                             |
| Cerambycidae                       | Longhorn beetles       |              |  |                               |
| <i>Psilacnaeia</i> sp.             |                        | Herbivorous  | Rare (2)   | 2b, 3                         |
| <i>Xylotoles</i> sp.               |                        | Herbivorous  | Rare (4)   | 9                             |
| <i>Xylotoles griseus</i> Fabricius | Fig longhorn           | Herbivorous  | Rare (3)   | 10                            |
| Chrysomelidae                      |                        |              |  |                               |
| <i>Eucolaspis antennata</i> Shaw   |                        | Herbivorous  | Rare (1)   | 2b                            |
| <i>Eucolaspis</i> sp.              |                        | Herbivorous  | Occasional (6)   | 10                            |
| Clambidae                          | Minute beetles         |              |  |                               |
| <i>Clambus</i> sp.                 |                        | Fungivorous  | Rare (1)   | 9                             |

| <b>Taxon</b>                             | <b>Common name</b>     | <b>Feeding mode</b>         | <b>Abundance category<sup>1</sup><br/>(number of individuals)</b> | <b>Collection sites<sup>2</sup></b> |
|--|------------------------|-----------------------------|---|-------------------------------------|
| Coccinellidae                            | Ladybirds              |                             |   |                                     |
| <i>Adoxellus picinus</i> Weise           |                        | Predatory                   | Occasional (11)   | 9                                   |
| Cryptophagidae                           | Cryptic beetles        |                             |   |                                     |
| <i>Atomaria lewisi</i> Reitter           | Silken fungus beetle   | Fungivorous                 | Rare (4)  | 1, 5                                |
| <i>Paratomaria crowsoni</i> Leschen      |                        | Pollen/ fungus feeder       | Rare (3)  | 2b, 9                               |
| Curculionidae                            | Weevils                |                             |   |                                     |
| <i>Catoptes</i> sp.                      |                        | Herbivorous                 | Rare (2)  | 9                                   |
| <i>Chalepistes compressus</i> (Broun)    | Compressed weevil      | Herbivorous                 | Rare (1)  | 10                                  |
| <i>Peristoreus</i> sp.                   |                        | Herbivorous                 | Rare (2)  | 2b, 9                               |
| <i>Praolepra infusca</i> Broun           |                        | Herbivorous                 | Rare (1)  | 10                                  |
| Latridiidae                              | Mildew beetles         |                             |   |                                     |
| <i>Cartodere bifasciata</i> (Reitter)    |                        | Fungivorous                 | Rare (4)  | 3, 5, 9                             |
| <i>Corticicara meridianus</i> Johnson    |                        | Fungivorous                 | Occasional (5)  | 2b                                  |
| <i>Corticicara</i> sp. 1                 |                        | Fungivorous                 | Rare (2)  | 2b                                  |
| <i>Corticicara</i> sp. 2                 |                        | Fungivorous                 | Rare (2)  | 2b                                  |
| Melandryidae                             | False darkling beetles |                             |   |                                     |
| <i>Hylobia</i> sp.                       |                        | Fungivorous                 | Rare (2)  | 9                                   |
| Oedemeridae                              | False blister beetles  |                             |   |                                     |
| <i>Thelyphassa nemoralis</i> (Braun)     |                        | Fungivorous                 | Rare (2)  | 9, 10                               |
| Scarabaeidae                             | Scarab beetles         |                             |   |                                     |
| <i>Pyronota festiva</i> (Fabricius)      | Green chafer beetle    | Herbivorous                 | Rare (2)  | 10                                  |
| Scirtidae                                | Marsh beetles          |                             |   |                                     |
| <i>Contacyphon</i> sp.                   |                        | Saprophytic                 | Rare (3)  | 9                                   |
| Zopheridae                               | Ironclad beetles       |                             |   |                                     |
| <i>Tarphiomimus wollastoni</i> Sharp     |                        | Fungivorous                 | Rare (1)  | 9                                   |
| Unidentified Coleoptera                  |                        | Unknown                     | Rare (1)  | 5                                   |
| <b>Diptera</b>                           | <b>Flies</b>           |                             |   |                                     |
| Heleomyzidae                             | Sun flies              |                             |   |                                     |
| <i>Allophylopsis scutellata</i> (Hutton) |                        | Omnivorous                  | Rare (1)  | 4                                   |
| Lauxaniidae                              | Acalyptrate flies      |                             |   |                                     |
| <i>Sapromyza</i> sp.                     |                        | Saprophytic                 | Rare (1)  | 10                                  |
| Tachinidae                               | Parasitoid flies       |                             |   |                                     |
| <i>Trigonospila brevifacies</i> (Hardy)  | Leafroller fly         | Parasitoid (of lepidoptera) | Rare (1)  | 3                                   |
| Unidentified Diptera                     |                        | Unknown                     | Occasional (8)  | 2b, 9                               |
| <b>Hemiptera</b>                         | <b>Bugs</b>            |                             |   |                                     |

| <b>Taxon</b>                          | <b>Common name</b>           | <b>Feeding mode</b>            | <b>Abundance category<sup>1</sup><br/>(number of individuals)</b> | <b>Collection sites<sup>2</sup></b> |
|---------------------------------------|------------------------------|--------------------------------|---|-------------------------------------|
| Acanthosomatidae                      | Shield bugs                  |                                |   |                                     |
| <i>Oncacontias vittatus</i> Fabricius | Forest shield bug            | Sap feeder                     | Rare (1)  | 9                                   |
| Aphididae                             | Aphids                       |                                |   |                                     |
| Unidentified Aphididae                |                              | Sap feeder                     | Rare (3)  | 2b, 9                               |
| Aphrophoridae                         | Spittle bugs                 |                                |   |                                     |
| <i>Philaenus spumarius</i> (Linnaeus) | Meadow spittlebug            | Sap feeder                     | Occasional (8)  | 2b, 3, 9                            |
| Flatidae                              | Planthoppers                 |                                |   |                                     |
| <i>Siphanta acuta</i> (Walker)        |                              | Sap feeder                     | Occasional (10)   | 2a, 2b, 9                           |
| Miridae                               | Mirid bugs                   |                                |   |                                     |
| <i>Stenotus binotatus</i> (Fabricius) | Two-spotted grass bug        | Sap feeder                     | Rare (1)  | 10                                  |
| Unidentified Miridae                  |                              | Sap feeder                     | Occasional (9)  | 2b, 4, 5, 9                         |
| Pentatomidae                          | Shield bugs                  |                                |   |                                     |
| <i>Cermatulus nasalis</i> (Westwood)  | Brown shield bug             | Predatory                      | Rare (1)  | 9                                   |
| <i>Nezara viridula</i> (Linnaeus)     | Green vegetable bug          | Sap feeder                     | Rare (1)  | 10                                  |
| Ricaniidae                            | Planthoppers                 |                                |   |                                     |
| <i>Scolytopa australis</i>            | Passion vine hopper          | Sap feeder                     | Rare (1)  | 4                                   |
| Unidentified Hemipteran               |                              | Sap feeder                     | Rare (3)  | 10                                  |
| <b>Hymenoptera</b>                    | <b>Bees, wasps, ants</b>     |                                |   |                                     |
| Braconidae                            | Parasitic wasps              |                                |   |                                     |
| <i>Aspilota parecur</i> Berry         |                              | Parasitoid<br>(of diptera)     | Rare (1)  | 9                                   |
| Unidentified Braconidae               |                              | Unknown                        | Rare (1)  | 1                                   |
| Chalcidoidea                          | Chalcid wasps                |                                |   |                                     |
| Unidentified Chalcidoidea             |                              | Unknown                        | Rare (1)  | 4                                   |
| Ichneumonidae                         | Darwin wasps                 |                                |   |                                     |
| <i>Aucklandella</i> sp.               |                              | Parasitoid (of<br>hymenoptera) | Rare (1)  | 9                                   |
| Unidentified Ichneumonidae            |                              | Parasitoid (of<br>hymenoptera) | Rare (1)  | 5                                   |
| Pteromalidae                          | Pteromalid wasps             |                                |   |                                     |
| Unidentified Pteromalidae             |                              | Unknown                        | Rare (1)  | 2b                                  |
| Vespididae                            | Vespid wasps                 |                                |   |                                     |
| <i>Vespa germanica</i> (Fabricius)    | German wasp                  | Omnivorous                     | Rare (1)  | 3                                   |
| Unidentified Hymenoptera              |                              | Unknown                        | Rare (1)  | 10                                  |
| <b>Lepidoptera</b>                    | <b>Moths and butterflies</b> |                                |   |                                     |
| Geometridae                           | Looper moths                 |                                |   |                                     |
| <i>Pasiphila</i> sp.                  |                              | Herbivorous                    | Rare (1)  | 9                                   |

| <b>Taxon</b>                       | <b>Common name</b>                  | <b>Feeding mode</b> | <b>Abundance category<sup>1</sup><br/>(number of individuals)</b> | <b>Collection sites<sup>2</sup></b> |
|------------------------------------|-------------------------------------|---------------------|---|-------------------------------------|
| Oecophoridae                       | Concealer moths                     |                     |   |                                     |
| <i>Tingena</i> sp.                 |                                     | Saprophytic         | Rare (2)  | 9                                   |
| Tineidae                           | Fungi moths                         |                     |   |                                     |
| <i>Opogona omoscopa</i> (Meyrick)  |                                     | Saprophytic         | Rare (1)  | 9                                   |
| Tortricidae                        | Leafroller moths                    |                     |   |                                     |
| <i>Ctenopseustis</i> sp.           |                                     | Herbivorous         | Rare (1)  | 2b                                  |
| Unidentified Lepidoptera           |                                     | Herbivorous         | Rare (1)  | 10                                  |
| Neuroptera                         | Lacewings                           |                     |   |                                     |
| Hemerobiidae                       | Brown lacewings                     |                     |   |                                     |
| <i>Micromus tasmaniae</i> (Walker) | Tasmanian lacewing                  | Predatory           | Rare (3)  | 10                                  |
| <b>Orthoptera</b>                  | <b>Grasshoppers, crickets, weta</b> |                     |   |                                     |
| Raphidoporidae                     | Cave weta                           |                     |   |                                     |
| <i>Pleioplectron</i> sp.           |                                     | Omnivorous          | Rare (2)  | 9                                   |
| Thysanoptera                       | Thrips                              |                     |   |                                     |
| Unidentified Thysanoptera          |                                     | Unknown             | Occasional (9)  | 5                                   |
| <b>Trichoptera</b>                 | <b>Caddisflies</b>                  |                     |   |                                     |
| Hydroptilidae                      | Micro-caddisflies                   |                     |   |                                     |
| Unidentified Hydroptilidae         |                                     | Unknown             | Rare (1)  | 2b                                  |

### Appendix 3 – Fungal species isolated from symptomatic Chilean flame creeper tissues in New Zealand (2021-2022)

| Species recovered   | Symptoms associated     | Plant material isolated from | Collection sites | Comments                           |
|---|-------------------------|------------------------------|------------------|------------------------------------|
| <i>Aureobasidium pullulans</i> (De Bary)<br>G. Arnaud ex Cif., Ribaldi & Corte          | spots                   | leaves                       | 1                | endophyte                          |
| <i>Sphaerobolus</i> Tode sp.  | spots                   | leaves                       | 1                | saprophyte                         |
| <i>Diaporthe rudis</i> (Fr.) Nitschke<br>(= <i>Diaporthe viticola</i> Nitschke)         | spots,<br>yellowing     | leaves                       | 2a, 6            | endophyte/<br>saprophyte/ pathogen |
| <i>Diaporthe phaseolorum</i><br>(Cooke & Ellis) Sacc.                                   | yellowing               | leaves                       | 4, 6             | endophyte/<br>saprophyte/ pathogen |
| <i>Diaporthe eres</i> Nitschke  | reddening               | leaves                       | 8                | endophyte/<br>saprophyte/ pathogen |
| <i>Diaporthe nothofagi</i> Nitschke   | yellowing               | leaves                       | 7                | endophyte/<br>saprophyte/ pathogen |
| <i>Diaporthe</i> Fuckel sp. 1   | spots                   | leaves                       | 2a               | endophyte/<br>saprophyte/ pathogen |
| <i>Diaporthe</i> Fuckel sp. 2   | reddening               | leaves                       | 3, 7, 8          | endophyte/<br>saprophyte/ pathogen |
| <i>Diaporthe</i> Fuckel sp. 3   | yellowing               | leaves                       | 9                | endophyte/<br>saprophyte/ pathogen |
| <i>Diaporthe</i> Fuckel sp. 4   | yellowing               | leaves                       | 2b               | endophyte/<br>saprophyte/ pathogen |
| <i>Colletotrichum salicis</i> (Fuckel)<br>Damm, P.F. Cannon & Crous                     | spots                   | leaves                       | 2a, 6            | pathogen                           |
| <i>Colletotrichum boninense</i><br>Moriwaki, Toy. Sato & Tsukiboshi                     | yellowing,<br>reddening | leaves                       | 3, 4, 5, 7       | pathogen                           |
| <i>Colletotrichum godetiae</i> Neerg.   | reddening               | leaves                       | 5                | pathogen                           |
| <i>Colletotrichum cigarro</i><br>(B.S. Weir & P.R. Johnst.) A. Cabral<br>& P. Talhinhas | yellowing               | leaves                       | 6                | endophyte                          |
| <i>Colletotrichum destructivum</i><br>O'Gara  | yellowing               | leaves                       | 7, 8             | pathogen                           |
| <i>Colletotrichum karsti</i> You L. Yang,<br>Zuo Y. Liu, K.D. Hyde & L. Cai             | yellowing               | leaves                       | 9                | pathogen                           |
| <i>Cladosporium cladosporioides</i><br>(Fresen.) G.A. de Vries                          | spots,<br>yellowing     | leaves                       | 2a, 4, 6         | endophyte/ saprophyte              |
| <i>Cladosporium allcinum</i><br>(Fr.) Bensch, U. Braun & Crous                          | yellowing               | leaves                       | 5                | endophyte/ saprophyte              |
| <i>Botrytis cinerea</i> Pers.   | yellowing               | leaves                       | 2b               | grey mould                         |
| <i>Epicoccum nigrum</i> Link  | yellowing,<br>reddening | leaves                       | 2b, 3, 4, 5      | endophyte                          |
| <i>Nigrospora oryzae</i><br>(Berk. & Broome) Petch                                      | reddening               | leaves                       | 3                | pathogen                           |
| <i>Alternaria alternata</i> (Fr.) Keissl.   | reddening               | leaves                       | 3                | pathogen                           |



| <b>Species recovered</b>                                      | <b>Symptoms associated</b> | <b>Plant material isolated from</b> | <b>Collection sites</b> | <b>Comments</b>                    |
|---|----------------------------|-------------------------------------|-------------------------|------------------------------------|
| <i>Alternaria rosae</i><br>E.G. Simmons & C.F. Hill           | yellowing                  | leaves                              | 4                       | pathogen                           |
| <i>Boeremia exigua</i> (Desm.)<br>Aveskamp, Gruyter & Verkley | yellowing                  | leaves                              | 5, 6                    | endophyte/<br>weak pathogen        |
| <i>Xylaria apiculata</i> Cooke                                | yellowing                  | leaves                              | 6                       | endophyte                          |
| <i>Xylaria Hill</i> ex Schrank sp. 1                          | reddening,<br>yellowing    | leaves                              | 5, 9                    | endophyte                          |
| <i>Xylaria Hill</i> ex Schrank sp. 2                          | yellow<br>spots            | leaves                              | 6                       | endophyte                          |
| <i>Stylodothis puccinioides</i><br>(DC.) Arx & E. Müll.       | yellowing                  | leaves                              | 5                       | saprophyte                         |
| <i>Didymella</i> Sacc.  | yellowing                  | leaves                              | 6                       | pathogen                           |
| <i>Seimatosporium</i> Corda                                   | reddening                  | leaves                              | 8                       | saprophyte/ pathogen               |
| <i>Cladorrhinum</i> Sacc. & Marchal sp.                       | reddening                  | leaves                              | 7                       | saprophyte                         |
| <i>Biscogniauxia</i> Kuntze                                   | yellowing                  | leaves                              | 7                       | endophyte/ pathogen                |
| <i>Trichoderma atroviride</i> P. Karst.                       | reddening                  | leaves                              | 7                       | promote plant growth               |
| <i>Annulohyphoxylon</i><br>Y.M. Ju, J.D. Rogers & H.M. Hsieh  | yellowing                  | leaves                              | 10                      | powdery mildew                     |
| <i>Xylariaceae</i> Tul. & C. Tul.                             | yellowing                  | leaves                              | 7                       | saprophyte/ pathogen               |
| Pleosporales Luttr. ex M.E. Barr                              | reddening                  | leaves                              | 8                       | saprophyte/<br>endophyte/ pathogen |