Plant & Food RESEARCH
RANGAHAU AHUMĀRA KAI

www.plantandfood.com

Risks to non-target species from the potential biological control agent *Tamarixia triozae,* proposed for use against *Bactericera cockerelli* in New Zealand: A summary of host-range testing

Gardner-Gee R

16 July 2012

A report prepared for

Horticulture New Zealand
Ref: SFF09-143 (Sustainable TPP), Milestone 7

Robin Gardner-Gee

Plant & Food Research, Mt Albert Research Centre, Auckland

PFR SPTS No. 7296

PUBLICATION DATA

Gardner-Gee R. 2012. Risks to non-target species from the potential biological control agent *Tamarixia triozae,* proposed for use against *Bactericera cockerelli* in New Zealand: A summary of host-range testing. A report prepared for: Horticulture New Zealand (SFF09-143 (Sustainable TPP), Milestone 7), Plant & Food Research Client Report No. 48662. SPTS No. 7296.

This report has been prepared by The New Zealand Institute for Plant & Food Research Limited (Plant & Food Research), which has its Head Office at 120 Mt Albert Rd, Mt Albert, Auckland.

This report has been approved by:

Robin Gardner-Gee
Scientist, Applied Entomology Group
Date: 16 July 2012

John Charles (acting for Louise Malone)
Science Group Leader, Applied Entomology
Date: 25 July 2012

Contents

Executive summary i

1 Introduction 1
1.1 Description of the target pest and potential biological control agents 1
1.2 Description of the proposed biological control agent 1
1.3 Description of psyllid fauna in New Zealand 4
1.4 Psyllid species in New Zealand of value as biological control agents or of cultural or conservation value 5
1.5 Aims of the study 5

2 Methods 6
2.1 Development of species list for host range testing 6
2.2 Source of psyllids used in host range tests 7
2.3 General description and justification of host range tests 8
2.4 Data analysis 11

3 Results 11
3.1 Oviposition tests 11
3.2 Follow-up tests 12
3.3 Emergence tests 13
3.4 First-generation performance tests 13

4 Discussion 14
4.1 Interpretation of host range tests 14
4.2 Extrapolation of test results to impacts in the field 15
4.3 Extrapolation of results to psyllid species not tested 17

5 Recommendations for future work 18

6 Summary 18

7 Acknowledgements 19

8 References 19

Executive summary

Risks to non-target species from the potential biological control agent *Tamarixia triozae,* proposed for use against *Bactericera cockerelli* in New Zealand: A summary of host-range testing.

Gardner-Gee R, 16 July 2012, PFR SPTS No. 7296PFR SPTS No. 7296

Bactericera cockerelli is a North American pest species known in New Zealand as the tomato/potato psyllid (TPP). First reported in New Zealand in 2006, it has now become a major pest on both greenhouse and outdoor solanaceous crops in New Zealand. Searches conducted between 2006 and 2008 failed to identify any natural enemies within New Zealand that were likely to control TPP on tomatoes, so in 2009 a North American parasitoid, *Tamarixia triozae,* was imported into quarantine facilities at the Mt Albert Research Centre, Auckland, for assessment as a biological control agent. Host-range testing has been carried out to evaluate the potential for *T. triozae* to impact negatively on non-target psyllid species in New Zealand. *Tamarixia triozae* did not oviposit on six of the eight non-target psyllid species it was exposed to in no-choice screening tests. *Tamarixia triozae* did oviposit on two native psyllid species, *Trioza curta* and *Trioza panacis*. However, the oviposition rate on both was lower than the oviposition rate on the target pest TPP. In addition, no *T. triozae* adults emerged from parasitized *T. curta*, suggesting that *T. triozae* would not be able to maintain itself over time in situations where *T. curta* was the only host available. *Tamarixia triozae* did emerge from parasitized *T. panacis* nymphs but the first generation female parasitoids from *T. panacis* had reduced ability to produce further offspring compared with parasitoids that emerged from their usual host (TPP). Testing indicates that *T. triozae* will attempt to use novel species as hosts, and that *T. triozae* is capable of developing in at least some of these novel species. Consequently species such as *T. panacis* (and possibly other native psyllid species) could act as alternative hosts to TPP for *T. triozae.* The impacts of this non-target parasitism are unlikely to be severe for widespread and abundant psyllid species, but rare species may be vulnerable.

For further information please contact:

Robin Gardner-Gee
The New Zealand Institute for Plant & Food Research Ltd
Plant & Food Research Mt Albert
Private Bag 92 169
Victoria Street West
Auckland 1142
NEW ZEALAND
Tel: +64-9-926-3518
Fax: +64-9-925 7001
Email: robin.gardner-gee@plantandfood.co.nz

1 Introduction

1.1 Description of the target pest and potential biological control agents

The tomato/potato psyllid (*Bactericera cockerelli* (Sulk) Hemiptera: Triozidae) is a North American pest that was first reported in New Zealand in 2006 (Teulon et al. 2009). This psyllid has been found to vector the bacterial pathogen *Candidatus* Liberibacter solanacearum (Liefting et al. 2009) and has now become a major pest on both greenhouse and outdoor solanaceous crops in New Zealand (Teulon et al. 2009). The presence of the tomato/potato psyllid (TPP) has disrupted existing integrated pest management (IPM) programmes, and effective biological control agents (BCAs) are urgently required to restore IPM in solanaceous crops. Since 2006 Crop & Food Research (now Plant & Food Research) has searched intensively for potential BCAs, both within New Zealand and overseas. This research effort is summarised below.

In December and January 2006–07 Crop & Food Research conducted searches around Auckland for natural enemies of the common native psyllid *Trioza vitreoradiata* which feeds on a widespread native tree (karo; *Pittosporum crassifolium)* (Workman et al. 2006; Pedley & Workman 2007). Seven natural enemies (six predators and one parasitoid) of *T. vitreoradiata* were found to be common at the 10 sites surveyed in Auckland. Laboratory trials indicated that all the predators readily fed on TPP but the parasitoid did not attack TPP. The three most promising predators (two lady bird beetle species and a lacewing species) were then used in a small-scale glasshouse trial to investigate their ability to control TPP infestations on capsicum and tomato plants. All three predators were effective at reducing the number of psyllids on capsicum but only the lacewing (*Drepanacra binocular*) appeared to have potential as a BCA on tomato, as the two ladybird species trialled avoided going onto the tomato plants.

In 2007 Crop & Food Research undertook a literature review (for Horticulture New Zealand) evaluating natural enemies already occurring in New Zealand and those present overseas that could be useful for covered crops in New Zealand (Workman & Davidson 2007). The review found that, in addition to the natural enemies already in New Zealand, four overseas species had potential to provide effective biological control of TPP. These were a predatory mirid (*Dicyphus hesperus*), two predatory green lacewings (*Chrysoperla carnea* and *C. rufilabris*) and a parasitic wasp (*Tamarixia triozae*).

Following this review, Crop & Food Research undertook a glasshouse trial to determine the ability of five natural enemies (that already occur in New Zealand) to adapt to greenhouse conditions and control TPP in covered crops of capsicums and tomatoes (Workman 2008). The greenhouse trial results were similar to the earlier small-scale trials, as once again the natural enemies reduced TPP on capsicums, but failed to achieve any control of TPP on tomatoes. Consequently, research effort focused on overseas species that have potential to control TPP on tomatoes. In 2008 a Horticulture NZ application was approved to import 10 arthropod species (*Eretmocerus mundus, Delphastus catalinae, Dicyphus hesperus, Macrolophus caliginosus, Amblyseius swirskii, Amblyseius degenerans, Typhlodromips montdorensis, Orius laevigatus, Tamarixia triozae,* and *Chrysoperla carnea*) into containment. These species are used against a range of greenhouse pests overseas and it was intended that at least some of the species would be imported into containment for host specificity tests and evaluation as biological control agents for the New Zealand greenhouse industry. Of these species, *Tamarixia triozae* was selected as the most promising BCA for TPP on tomatoes and was imported into quarantine on 27 February 2009.

1.2 Description of the proposed biological control agent

1.2.1 *Tamarixia triozae* (Burks 1943) (Hymenoptera: Eulophidae)

In 1939 parasitism was observed on TPP on uncultivated hosts in North America and attributed to a hymenopteran parasitoid in the *Tetrastichus* genus (Eulophidae) (Romney 1939). The species was described 1943 by Burks as *T. triozae* and has subsequently transferred to *Tamarixia* by Boucek (1988). *Tamarixia* species are primary parasitoids of Psylloidea (La Salle 1994), although reports of non-psyllid hosts do exist (Zuparko et al. 2011). *Tamaraxia triozae* is

a small wasp (0.7–1.05 mm in length) that is an ectoparasitoid of psyllids. Adult female *T. triozae* typically lay a single egg on the ventral surface of the host and the larva develops as an external parasitoid beneath the body of the host. It is widespread in North America (Arizona, California, Colorado, Idaho, Kansas, Montana, Nebraska, New Mexico, Texas, Washington) and more recently has been recorded from Mexico (Lomeli-Flores & Bueno 2002; Zuparko et al. 2011).

1.2.2 Efficacy of agent

Early field observations in the USA suggested *T. triozae* was unlikely to achieve control of TPP in outdoor crops because of poor synchronization between the psyllid and the parasitoid, high parasitoid pupal mortality and patchy establishment within crops (Pletsch 1947; Johnson 1971). Parasitism rates of TPP by *T. triozae* were below 20% in southern California fields (Butler & Trumble 2012). However, surveys of unsprayed pepper crops in Oaxaca, Mexico, have found that *T. triozae* can achieve over 80% parasitism of TPP (Bravo & Lopez 2007; cited in Luna Cruz 2010). In addition, laboratory studies point to the potential of *T. triozae* as a BCA in some crops, as these studies indicate that the life cycle of *T. triozae* is almost half the time of its host on peppers (P. Workman unpublished data; Rojas et al. 2009; Rojas 2010) and that *T. triozae* causes host death through feeding as well as through parasitism (P. Workman unpublished data; Rojas et al. 2009; Vega 2010). Liu et al. (2012) suggest that releases of commercially reared *T. triozae* into outdoor crops late in the growing season could be feasible, providing some level of non-chemical control at a time when transmission of Liberibacter is less damaging to crop yields.

In New Zealand most fresh pepper and tomato crops are grown under cover, rather than outdoors, hence the potential use of *T. triozae* within glasshouses is of interest. Overseas, inundative releases of *T. triozae* have been undertaken within glasshouses, with anecdotal reports of successful control, and *T. triozae* is sporadically available from commercial insectaries. For example, *T. triozae* was commercially produced in Canada, until demand for it lessened due to a decline in psyllid populations, at which time the parasitoid became uneconomic to produce (D. Gillespie, pers. comm.). In Canada, *T. triozae* reportedly provided effective control of psyllids on capsicum greenhouse crops, provided the pest was detected early and sufficient numbers of the parasitic wasp were released (Elmhirst 2005). *Tamarixia triozae* is the only biological control agent that has been commercially reared specifically for use against TPP in glasshouses.

1.2.3 Source of agent

Tamarixia triozae was imported by Plant & Food Research into containment at the quarantine facility at Mt Albert Research Centre (MARC), Auckland, on 27 February 2009. Approval to import *T. triozae* was obtained under the HSNO Act, 1996 and HSNO Order, 1998 (ERMA Approval Code: NOC002530-39) and the Biosecurity Act, 1993 (MAF Biosecurity, Permit to Import Live Animals: 2008035896). Specimens were imported from Koppert Mexico (S.A.de C.V. Av. Del marquee # 38-1, Parque Industrial Bernardo Quintana (3rd Etapa), Municipico El Marques, 76246 Queretaro, Mexico). On the arrival of *T. triozae* into the Plant & Food Research quarantine facility, samples of 10 females and 10 males were placed in 95% ethanol and deposited with the New Zealand Arthropod Collection (NZAC), Landcare Research, Auckland, New Zealand. Further samples of 10 females and 10 males were sent to Dr Ian Scott, Plant & Food Research, Lincoln, for molecular analysis. A sample of 100 *T. triozae* (bred from the initial imported stock) was supplied to Dr Louise Malone, Plant & Food Research, Auckland, in April 2009 for standard examination for internal pathogens. There were no signs of any viruses, fungi, or other pathogens in any of the smears that were made from the insects. Since importation, *T. triozae* have been maintained in containment on TPP nymphs reared in non-quarantine glasshouse colonies at MARC.

1.2.4 Hosts in the native range of agent

Tamarixia triozae is a widespread parasitoid, occurring in many arid or semi-arid regions through North America and also in Mexico (La Salle 1994; Lomeli-Flores & Bueno 2002). It attacks psyllids from a number of families (Zuparko et al. 2011). At the species level, psyllids typically exhibit very narrow host-plant ranges (Burckhardt 1994). However, some hosts used by *T. triozae* are notable exceptions; TPP, for example, can complete development on at least 40 host-plant species (Wallis 1955). In addition, the range of psyllid hosts used by *T. triozae* means

that the parasitoid is found in association with a wide range of plants with varying growth forms (Table 1).

TPP is the most well-studied of the species parasitized by *T. triozae*. TPP nymphs typically feed on the undersides of leaves of their host-plant and seldom move. Other psyllid species attacked by *T. triozae* feed on flower buds (e.g. *Calophya californica*), or on woody branches (e.g. *Calophya nigrella*) (Jensen 1957). Many of the species parasitized by *T. triozae* have free-living nymphs but *Euphalerus vermiculosus* nymphs form a waxy cell that completely surrounds them (Jensen 1957).

Table 1: Psyllid hosts of the parasitoid *Tamarixia triozae* and the host-plants used by the psyllid species (data from Wallis 1955; Jensen 1957; Zuparko et al. 2011; Ouvrard 2012).

Psyllid family	Psyllid species	Host-plant family	Host-plant species	Host-plant details
Calophyidae	*Calophya californica*	Anacardiaceae	*Rhus integrifolia* *Rhus ovata*	Genus members are typically shrubs and small trees growing 1–10 m tall
	Calophya nigrella	Anacardiaceae	*Rhus trilobata*	
	Calophya nigripennis	Anacardiaceae	*Rhus capallina*	
	Calophya triozomima	Anacardiaceae	*Rhus trilobata*	
Psyllidae	*Ceanothia ceanothi*	Rhamnaceae	*Ceanothus tomentosus*	Genus members are typically shrubs growing 0.5–3 m tall
	Euglyptoneura minuta	Rhamnaceae	*Ceanothus crassifolius*	
	Euphalerus vermiculosus	Rhamnaceae	*Ceanothus leucodermis*	
	Pexopsylla cercocarpi	Rosaceae	*Cercocarpus betuloides* *Cercocarpus ledifolius*	Genus members are deciduous shrubs or small trees, growing 3–6 m tall
Triozidae	*Bactericera cockerelli*	Solanaceae Convolvulaceae Lamiaceae	40 + host species including many solanaceous crop species such as potato, tomato and eggplant	Hosts include vines, shrubs and herbaceous plants
	Bactericera minuta	Salicaceae	*Salix exigua* *Salix lasiandra* *Salix lasiolepsis* *Salix longifolia*	Genus members are deciduous trees and shrubs, normally growing in moist soils
	Bactericera nigricornis	Asteraceae, Apiaceae Brassicaceae, Liliaceae, Solanaceae	Various including onion, potato and carrot	Various growth habits including herbaceous plants
	Trioza albifrons	Urticaceae	*Urtica* sp.	Genus members are typically annuals or perennial herbaceous plants
	Trioza beameri	Rhamnaceae	*Rhamnus californica*	Evergreen shrub growing to 2–5 m tall

1.3 Description of psyllid fauna in New Zealand

Psyllids belong to the superfamily Psylloidea, which is relatively well documented in New Zealand (Tuthill 1952; Dale 1985). Of the six families of Psylloidea, two families (Phacopteronidae and Carsidaridae) are not found in New Zealand and another two (Calophyidae and Homotomidae) are each represented by only a single adventive species (P. Dale, pers. comm.). Of the two remaining families, Psyllidae is dominated by adventives (34 species of which 23 are adventives) whereas Triozidae has 51 species, 50 of which are endemic and only one of which is adventive (P. Dale, pers. comm.). New Zealand psyllids feed on many native plant genera, including *Alseuosmia, Carmichaelia, Dacrydium, Discaria,*

Dodonaea, Fuchsia, Pseudopanax, and *Schefflera.* They are not associated with host deaths but some disfigure their hosts, causing pitting and yellow streaks to appear on distorted leaves. More than half of the New Zealand psyllids occur on small trees and shrubs of open country, with 17 of these open country species occurring in lowland-subalpine areas and another 25 more occurring from lowland to alpine areas (Dale 1985). Many are found on seral-stage plant hosts (e.g. *Kunzea ericoides*) (Dale 1985). Very few are found on large trees or in mature forests (Dale 1985). Most (57%) are widespread, occurring in all three main islands. Diversity increases towards the south, and one third of species do not occur in the North Island (Dale 1985). Overall the New Zealand psyllids are predominantly a cold-adapted shrub-land fauna, with a relatively small northern sub-tropical element (Dale 1985). Correspondingly, most are not active all year round. However, a few will oviposit all year round if suitable foliage is available (e.g. *Trioza curta, T. vitreoradiata, T. panacis* and *Ctenarytaina* species).

1.4 Psyllid species in New Zealand of value as biological control agents or of cultural or conservation value

No psyllid species are recorded as having specific cultural value in New Zealand, but there is one beneficial exotic psyllid species. The broom psyllid *Arytainilla spartiophila* (Psyllidae) has been introduced into New Zealand as a biological control agent for Scotch broom (*Cytisus scoparius*). The broom psyllid was imported from England by the DSIR in 1992, and released throughout New Zealand in the mid-1990s (Hayes 2005). It is now widespread and common through both the North and South Islands (Hayes 2005).

The 2001 publication "Conservation requirements of New Zealand's nationally threatened invertebrates" did not list any psyllids as threatened or in need of conservation attention (McGuinness 2001). However, in a more recent revision of the conservation status of New Zealand Hemiptera two psyllid species have been designated "Threatened : Nationally Critical", the highest category in current threat classification system (Stringer et al. 2012). The first of these is an undescribed species of *Anomalopsylla* (Psyllidae) that has only been collected from a single plant of *Olearia solandri* at Port Underwood, east of Picton, northern South Island. This single plant was last searched for psyllids in 1982 and at that time the plant was at risk from gorse encroachment (P. Dale, pers. comm.). It is possible that the plant and the associated psyllid population have been lost in the subsequent three decades (P. Dale, pers. comm.). However, the host-plant species *O. solandri* is widespread and it is possible the *Anomalopsylla* species may be present on other *O. solandri* plants in the area (P. Dale pers. comm.).

The second Nationally Critical psyllid species, *Psylla* aff. *carmichaeliae* (Psyllidae), is also undescribed and is also known from only a single location, Woodside Creek, Marlborough, northern South Island. The psyllid population was examined most recently in 2008 (P. Dale, pers. comm.). The host-plant is *Carmichaelia torulosa*, and the conservation status of this plant species is "Threatened: Nationally Endangered" (Stringer et al. 2012). A third psyllid species, *Gyropsylla zealandica* (Psyllidae), has been placed in the "At Risk: Naturally Uncommon (Sparse)" category (Stringer et al. 2012). This is the largest psyllid in New Zealand but only a few specimens (15) have ever been collected (Dale, 1985). All records are from southern New Zealand, with locations ranging from 1220 m asl in the western South Island to near sea level on Stewart Island (Dale 1985). In addition, many New Zealand psyllid species are endemic to New Zealand and therefore have scientific value as local products of evolution.

1.5 Aims of the study

In New Zealand the importation and release of new organisms (including biological control agents) is regulated by the Hazardous Substances and New Organisms (HSNO) act (1998), administered by the Environmental Protection Authority (EPA). The purpose of the HSNO act is to "protect the environment, and the health and safety of people and communities, by preventing or managing adverse effects of.... new organisms". The EPA is required to consider the effects that a new organism is likely to have on native species (and on valued introduced species), and is directed to decline any application for the release of a new organism if significant displacement of a native species will occur as a result of the release. Given this regulatory framework, assessment of potential non-target impacts is an important part of any

application for release of a new organism in New Zealand. *Tamarixia triozae* has not been released as a classical BCA outside its native range before and little is known about its response to novel psyllid species. Given the wide distribution of *T. triozae* in its home range, it was assumed from the outset that *T. triozae* would be able to establish outdoors in at least some areas of New Zealand and hence the parasitoid could encounter non-target psyllid species outside the glasshouse or crop environment. The aims of the present study were to expose *T. triozae* to a range of psyllid species that occur in New Zealand and determine what, if any, non-target impacts *T. triozae* was likely to have if released into the New Zealand environment.

2 Methods

2.1 Development of species list for host range testing

Within weed biocontrol the centrifugal-phylogenetic approach to host range testing is well established and research has shown that the more closely related a non-target plant species is to the target weed species, the more likely it is to be attacked by exotic insects introduced to control the weed (Pemberton 2000). This approach is of less value, however, when assessing the risk of entomophagous insects introduced to control other insects, as insect phylogenies are often poorly understood and host utilisation by entomophagous insects is often not determined, or is poorly predicted, by taxonomic affinities (Hoddle 2004). Kuhlmann et al. (2006) analysed a number of host-range testing programmes for entomophagous insects, and concluded that although phylogeny was a valuable starting point for assessing host range, other criteria were as important. Kuhlmann et al. proposed that species lists for host testing of entomophagous insects should be assembled by considering species that fall into the following three categories: 1) ecologically similar species (i.e. species that live in the same the habitat as the target species, or in habitats immediately adjacent to the agricultural system used by the target species, species that share the same host-plants as the target species); 2) phylogenetically related species; 3) safeguard species (i.e. species that are beneficial, rare native species especially if related to the target species and/or species that are attacked by congeners of the entomophagous insect under consideration). The initial list that results may then need to be reduced using practical considerations (e.g. accessibility of species, ease of rearing) and further ecological filters (e.g. phenological asyncronization) (Kuhlmann et al. 2006).

Following this approach, an initial species list was established that contained all native members of Triozidae and Psyllidae, as *T. triozae* attacks a range of psyllid species within these families in its home range and the target pest (TPP) is a member of Triozidae. One exotic psyllid species, *Arytainilla spartiophila* (Psyllidae), was added to the list as this species has been introduced into New Zealand as a weed biological control agent. The resulting list of 68 species was then reduced by focusing on lowland native psyllid species (from both Psyllidae and Triozidae), as these species could occur in habitats adjacent to agricultural crops attacked by TPP (e.g. forest reserves, roadside margins, amenity plantings, abandoned agricultural land). The final list consisted of seven native species (Table 2) plus *A. spartiophila.* The seven native species represent the major taxonomic groups within the native psyllid fauna and all were easily collected within the Auckland region, an area where TPP is a widespread pest. Three species were of particular interest (*Trioza curta, Trioza panacis* and *Trioza vitreoradiata*) as they are parasitized in the wild by another *Tamarixia* species already present in New Zealand (R. Gardner-Gee, unpublished data).

The native psyllid species selected utilise native host plants from six families, two of which (Asteraceae and Fabaceae) contain species that are recorded as host plants of TPP in the USA (Wallis 1955). However, there are no confirmed reports of TPP breeding on plants within these families and it is likely that the records refer to adult feeding rather than breeding (Wallis 1955; Martin 2008). Within the USA, TPP breeds almost exclusively on plants in the family Solanaceae; the only other plants it is known to breed on in the wild are within the Convolvulaceae family (Wallis 1955). Laboratory studies indicate that TPP can also complete development on *Micromeria chamissonis* (Lamiaceae) (Wallis 1955; Martin 2008). Within New Zealand, TPP has been recorded breeding only on plants in the Solanaceae and

Convolvulaceae families (Martin 2008). No native psyllids utilise these two families in New Zealand (Dale 1985).

Table 2. Seven native psyllid species and their common hosts. Combinations marked with * were used in host specificity tests.

Psyllid family	Psyllid species	Host-plant family	Host-plant species	Host-plant details
Psyllidae	*Acizzia dodonaeae*	Sapindaceae	*Dodonaea viscosa**	Evergreen shrub growing to 1–3 m
	Ctenarytaina clavata	Myrtaceae	*Leptospermum scoparium**	Evergreen shrub and trees growing to 2–5 m tall
			Kunzea ericoides	
	Psylla apicalis	Fabaceae	*Sophora chathamica*	Genus members are small trees and shrubs
			Sophora fulvida	
			Sophora tetraptera	
			Sophora sp.*	
Triozidae	*Trioza curta*	Myrtaceae	*Syzygium maire*	Trees, up to 25 m
			*Metrosideros excelsa**	
			Metrosideros robusta	
			Metrosideros umbellata	
	Trioza "ohumata" (an undescribed species)	Asteraceae	*Brachyglottis kirkii**	A forest epiphyte or ground shrub to 3 m tall
	Trioza panacis	Araliaceae	*Pseudopanax crassifolius*	Shrubs and small trees, occurring in forest or scrub environments
			Psuedopanax discolor	
			Pseudopanax ferox	
			*Pseudopanax lessonii**	
	Trioza vitreoradiata	Pittosporaceae	*Pittosporum colensoi*	Genus members are trees and shrubs growing to 2–30 m tall
			*Pittosporum crassifolium**	
			Pittosporum eugenioides	
			Pittosporum tenuifolium	

2.2 Source of psyllids used in host range tests

Nymphs from multivoltine species (*Acizzia dodonaeae, Trioza curta, T. panacis, T. vitreoradiata*) were collected from the field on host-plant foliage and placed into holding containers for 1–3 weeks. Foliage stems were placed into water to maintain freshness. Adult psyllids that emerged during this time were transferred to large mesh cages (700 x 700 x 700 mm) containing clean potted specimens of their host plant and reared through multiple generations in unheated glasshouse units. Nymphs from these colonies were used as required for host-range testing. Univoltine species and some multivoltine species could not be effectively reared in this manner (*Arytainilla spartiophila, Ctenarytaina clavata, Psylla apicalis, Trioza* "ohumata"). For these species the nymphs used in testing were collected directly from the field. When direct use of field-collected nymphs was necessary, a sub-sample of each collection was placed into holding containers and monitored for the emergence of native parasitoids. This precaution was necessary due to the presence in New Zealand of at least one *Tamarixia* species, the eggs of which are indistinguishable from *Tamarixia triozae* eggs. *Tamarixia* adults did not emerge from any of the sub-samples from collections used for testing.

Identification of psyllids in the field was based on host-plant association and general morphology. However, nymphs and/or adults were collected from all the colonies and collections used in the testing programme and stored in alcohol for further examination. All

specimens were examined by a psyllid taxonomist, Pam Dale, and all were found to be correctly identified.

2.3 General description and justification of host range tests

2.3.1 Oviposition tests

A range of choice and no-choice oviposition tests were carried out in small cages to gain information about the host range of *T. triozae* (Figure 1). Tests of this type are routinely used in host specificity testing, especially in containment situations where space limitations preclude large-scale tests (Van Driesche & Murray 2004). All tests were undertaken in containment conditions at 22ºC, with a 16:8 h photoperiod.

Preliminary no-choice tests were carried out to determine appropriate test conditions. Eight cages (vented "cookie jars") were established, each with three female *T. triozae* (3–10 days old, probably mated). Each cage contained either approximately 50 non-target psyllid nymphs (4^{th} and 5^{th} instars) on shoots of their host plant or 50 TPP nymphs (4^{th} and 5^{th} instar) on capsicum leaves. Foliage stems were placed in small vials of water and the vial necks were stopped with cotton wads to prevent the parasitoids drowning in the water. Psyllid nymphs used in these tests were sourced from laboratory colonies that were free of any parasitoids. Each cage was maintained for at least 7 days and every 2–3 days the old foliage was removed and fresh foliage (with psyllid nymphs) was added. The number of parasitoids was counted at each foliage change to ensure no mortality had occurred. Once removed from the cage, foliage was examined under binocular microscope and each psyllid nymph was inverted and examined for the presence of parasitoid eggs. In these eight cages, the initial 48-h results were the same as the 7-day results (i.e. if oviposition occurred at all, it was detected after 48 h). In all eight tests with TPP, *T. triozae* oviposited on TPP within 48 h. In the eight cages with non-target psyllids, oviposition was only detected in one of the cages, and in this case the oviposition occurred within 48 h. On the basis of these results the cage set-up described above was used in all subsequent screening tests and oviposition was checked after 48 h.

The screening no-choice tests were undertaken to determine if *T. triozae* would oviposit on non-target species in the absence of the target species (TPP). Eight psyllid species were tested (Table 2). For each psyllid species, a minimum of 15 cages with non-target psyllids were set up, along with 15 cages with TPP nymphs only (control cages). When possible, six cages were set up simultaneously (three with non-target psyllids, three with TPP) using the same cohort of *T. triozae*. If oviposition failed to occur in a control cage, the results from all cages using that cohort were discarded. To investigate *T. triozae* responses to non-target psyllids in more detail, one sequential no-choice test was also carried out using *T. curta* as the non-target species. Three female parasitoids were placed in a cage as per usual, and were then offered either TPP nymphs or *T. curta* nymphs over a 15-day period.

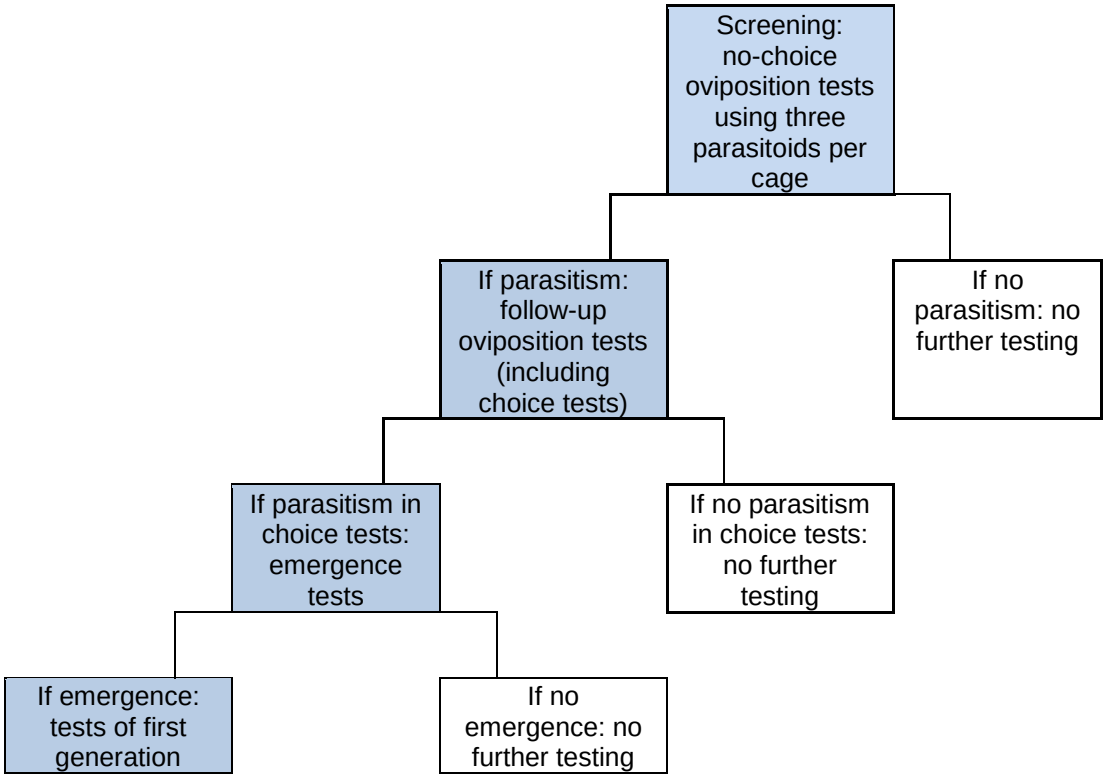

Figure 1. Flow chart indicating the decision framework used to guide host specificity tests with *Tamarixia triozae.*

2.3.2 Follow-up tests

If oviposition was detected in the screening tests, then follow-up tests were undertaken to determine parasitoid responses in choice situations and to gather further data on no-choice responses. Smaller cages were set up as per Table 3 and psyllid nymphs were examined for parasitoid eggs after 48 h. For these tests 20 cages were set up simultaneously (five of each treatment) using the same cohort of *T. triozae*. If oviposition failed to occur in the positive control cages, the results from all cages using that cohort were discarded.

The set up differed from the screening tests in that a single parasitoid was used per cage. The use of three parasitoids per cage in the initial screening tests can be regarded as a "worst case scenario" and these tests provide information about the likely behavior of a small group of parasitoids confined with a non-target host. A more realistic scenario, however, is that a solitary parasitoid encounters a non-target host, and testing with a single parasitoid per cage more closely simulates this scenario.

The follow-up tests also examined parasitoid-induced mortality as well as oviposition, by counting the number of psyllid nymphs alive and dead after the 48-h test period. To be able to determine the extent to which mortality was due to the parasitoid, the follow up tests included "negative control" cages that contained psyllid nymphs (target and non-target) but not the parasitoid; data from these cages indicated the background level of mortality, which can be high in host-specificity testing (Berndt et al. 2009).

Table 3. Summary of follow-up test protocols. These tests were carried out if the initial screening no-choice tests detected parasitism of a non-target psyllid species.

	Negative control	Choice test	No-choice test	Positive control
Psyllids:	Non-target psyllid on naturally infested leaf with at least 20 late instars AND Target pest psyllid (TPP) on capsicum leaf (10 4th instars and 10 5th instars)	Non-target psyllid on naturally infested leaf with at least 20 late instars AND Target pest psyllid (TPP) on capsicum leaf (10 4th instars and 10 5th instars)	Non-target psyllid on naturally infested leaf with at least 20 late instars	Target pest psyllid (TPP) on capsicum leaf (10 4th instars and 10 5th instars)
Tamarixia triozae:	None	1 female	1 female	1 female
Cages:	Vented plastic pottles (100 mm diameter)			
Duration:	48 h			
Replicates:	15 per treatment			
Variables:	Egg numbers per psyllid nymph, numbers of live and dead nymphs			

2.3.3 Emergence tests

If the follow-up tests detected parasitism of a non-target psyllid species in a choice situation, emergence tests were then conducted to determine whether *T. triozae* was capable of completing its development on non-target species. The set-up for the emergence tests was similar to the follow-up tests (Table 4), but instead of assessment at 48 h, the parasitoids were removed at 48 h and the leaves were held within the cages for 3 weeks (usual emergence time for *T. triozae* is 14–16 days under quarantine conditions). At the end of this 3-week period cages were examined and the number of emerged *T. triozae* adults was recorded. The nymphs were not checked for parasitism or removed from the host leaf until the end of the 3-week period, as there is evidence that some psyllid species are intolerant of dislodgement. For example the lilly pilly psyllid (*Trioza eugeniae*) is gall forming, like *Trioza curta*. If lilly pilly psyllid nymphs are dislodged from their galls, they do not re-establish in their galls, or settle elsewhere, and eventually die of desiccation (Young 2003). For these tests 10 cages were set up simultaneously (five of each treatment) using the same cohort of *T. triozae*. If oviposition failed to occur in the positive control cages, the results from all cages using that cohort were discarded.

Table 4. Summary of emergence test protocols. These tests were carried out if the follow-up choice tests detected parasitism of a non-target psyllid species.

	No-choice	Positive control
Psyllid/s	Non-target psyllid on naturally infested leaf with at least 20 late instars	Target pest psyllid (TPP) on capsicum leaf (10 4th instars and 10 5th instars)
Tamarixia triozae	1 female (removed after 48 h)	1 female (removed after 48 h)
Cages:	Vented plastic pottles (100 mm diameter)	
Duration:	3 weeks	
Replicates:	10 per treatment	
Variable:	Numbers of adult parasitoids emerged	

2.3.4 First-generation performance tests

If emergence of parasitoid adults from a non-target host was detected, further tests were undertaken to assess the oviposition rates of parasitoids derived from non-target hosts compared with parasitoids derived from TPP (protocols given in Table 5). The ten replicates (cages) for each treatment were all set up at the same time. The parasitoids used for the 20 tests with *T. panacis*-derived *T. triozae* were from the same cohort. Similarly the parasitoids used for the 20 tests with TPP-derived *T. triozae* were from the same cohort. To assess emergence rates, a separate set of cages was set up following the same protocol, but parasitoids were removed after 48 h and the cages were incubated for 3 weeks. At the end of this 3-week period cages were examined and the number of emerged *T. triozae* adults was recorded.

Table 5. Summary of next-generation test protocols. These tests were carried out if emergence of *Tamarixia triozae* occurred from a non-target host.

	No-choice test 1	Positive control 1	No-choice test 2	Positive control 2
Psyllid/s:	Non-target psyllid on naturally infested leaf with at least 20 late instars	Target pest psyllid (TPP) on capsicum leaf (10 4th instars and 10 5th instars)	Non-target psyllid on naturally infested leaf with at least 20 late instars	Target pest psyllid (TPP) on capsicum leaf (10 4th instars and 10 5th instars)
Tamarixia triozae:	1 female from non-target host	1 female from non-target host	1 female from TPP	1 female from TPP
Cages:	Vented plastic pottles (100 mm diameter)			
Duration:	48 h			
Replicates:	10 of each treatment			
Variable:	Egg numbers per psyllid nymph			

2.4 Data analysis

Using the MASS (Venables & Ripley 2002) package with R (R Development Core Team 2012), negative binomial generalized linear models were used to model the numbers of parasitized psyllids under the various conditions. Emergence of *Tamaraxia triozae* in subsequent generations was analysed similarly. Mortality data were examined in a similar way with the number dead offset by the number of psyllids exposed to the test conditions. The model was used to predict the percentage mortality.

In some analyses, there was evidence of a significant effect of the cohort on the numbers observed. Calculating separate means for each cohort and averaging the result gave an almost identical result to the one obtained by simply removing cohort from the model. In those cases, the probability associated with the response under consideration was calculated with cohort as a blocking factor.

Analysis using a more straightforward Poisson model could not be used because of the degree of overdispersion which is typical of this kind of data. A negative binomial model estimates the overdispersion and gives more realistic estimates of relevant probabilities. The negative binomial model is one of the log family, a consequence of which is that the standard errors are on the log scale. Those standard errors have been added to and subtracted from the mean (also on the log scale) and the resulting three values back-transformed to give the values presented in the results Tables 6 and 7.

3 Results

3.1 Oviposition tests

Tamarixia triozae oviposited on TPP nymphs in all positive control cages, but did not oviposit on six of the eight non-target psyllid species tested (Figure 2). The parasitoid did lay eggs on two

native non-target species, *Trioza curta* and *Trioza panacis*. In the sequential no-choice test *T. triozae* consistently oviposited on *T. curta*, even after repeated exposure to its target host TPP (Figure 3).

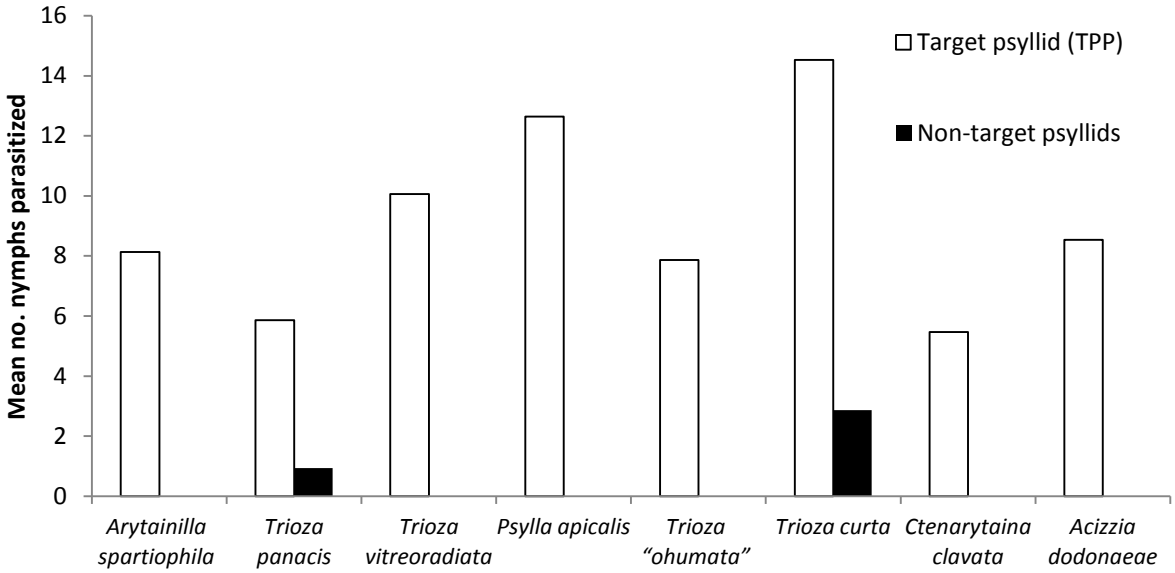

Figure 2. Mean number of nymphs with *Tamarixia triozae* eggs (parasitized nymphs) after 48 h in no-choice screening tests. In these tests *T. triozae* females (three per cage) were offered nymphs of either the non-target species (species given in x-axis) or the target species (TPP).

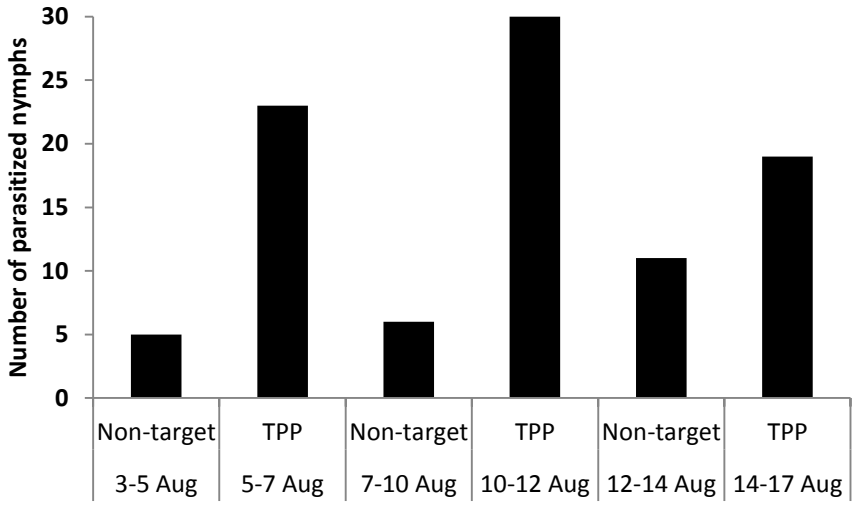

Figure 3. Number of nymphs with *Tamarixia triozae* eggs (parasitized nymphs) in a sequential no-choice test. In this test a single cage of *T. triozae* females (three per cage) were offered nymphs of either the non-target species (*Trioza curta*) or the target species (TPP) over a 14-day period.

3.2 Follow-up tests

Follow-up tests were conducted for two non-target psyllid species, *Trioza curta* and *Trioza panacis*. *Tamarixia triozae* parasitized both non-target species in choice and no-choice cages but generally laid fewer eggs on the non-target species than on the target TPP (Table 6). The

parasitoid had no significant effect on the mortality of *T. panacis* (Table 7). However, there is some evidence that the parasitoid did affect the mortality of *T. curta*, as there was a significant increase in mortality in the choice cages (21% of *T. curta* nymphs died) compared with the negative control cages without the parasitoid (12% of *T. curta* nymphs died) (Table 7). Under the same test conditions TPP mortality in cages with the parasitoid was 25–35%, whereas in negative control cages without the parasitoid TPP mortality was 4–7% (Table 7).

Table 6: Predicted number of nymphs parasitized by a single *Tamarixia triozae* female in 48 h (prediction -1 SE, prediction +1 SE). Differences between pairs of non-target versus target results are statistically significant if results within the pair have different letters following them.

Test type	Tests with *T. curta* as non-target		Tests with *T. panacis* as non-target	
	Non-target	Target (TPP)	Non-target	Target (TPP)
Choice	0.8 (0.6, 1.1) [a]	4.7 (4.0, 5.5) [b]	0.2 (0.1, 0.4) [a]	4.4 (3.5, 5.5) [b]
No-choice	3.0 (2.3, 3.9) [a]	5.6 (4.4, 7.1) [a]	0.2 (0.1, 0.4) [a]	6.1 (5.5, 6.7) [b]

Table 7: Predicted percentage of nymphs dead in 48 h (prediction -1 SE, prediction +1 SE). Choice and no-choice results with an * are significantly higher than the background level of mortality (indicated by the negative control results) for the species.

Test type	Tests with *T. curta* as non-target		Tests with *T. panacis* as non-target	
	Non-target	Target (TPP)	Non-target	Target (TPP)
Negative control	12.4 (10.4,14.8)	6.6 (5.3, 8.3)	7.7 (5.5, 10.8)	3.5 (2.6,4.9)
Choice	20.9 (17.8, 24.5)*	25.8 (23.1, 28.9)*	5.2 (3.7, 7.4)	32.1 (28.8, 36.0)*
No-choice	18.1 (15.4, 21.3)	34.5 (21.2, 38.1)*	12.8 (9.4, 17.5)	30.4 (27.2, 34.0)*

3.3 Emergence tests

Emergence tests were conducted for the same two species, *Trioza curta* and *Trioza panacis*. *Tamarixia triozae* did not complete development on *Trioza curta* nymphs: in the emergence tests no parasitoids emerged from *T. curta* after 3 weeks whereas *T. trioza* consistently emerged from TPP nymphs under the same conditions. To confirm this result, several additional investigations were undertaken. On one occasion, parasitized nymphs from the no-choice screening tests (26 *T. curta* and 237 TPP) were transferred to fresh leaf material and held for 21 days. No parasitized *T. curta* nymphs survived beyond 14 days and no *T. triozae* adults emerged from these nymphs, whereas *T. triozae* adults emerged from 44% of the parasitized TPP nymphs. In another investigation *T. triozae* adults were released into three large mesh cages each containing a host plant with 200+ late instar *T. curta* nymphs (10 female *T. triozae* and 4 male *T. triozae* per cage). Cages were examined regularly over a 4-week period but no additional adult parasitoids were seen within these cages.

Tamarixia triozae was able to complete development on *Trioza panacis* nymphs. No emergence was detected in the emergence tests (probably due to poor leaf condition), but further investigations were then undertaken, similar to those described above. *Tamarixia triozae* adults were released into three large mesh cages each containing a host plant with 200+ late instar *T. panacis* nymphs (10 female *T. triozae* and 4 male *T. triozae* per cage). Cages were examined regularly over a 4-week period. By the end of the 4-week period the cages contained between 40 and 160 adult *T. triozae,* clearly indicating that the female *T. triozae* originally released into the cages had laid eggs onto *T. panacis* and that a least a portion of those eggs had successfully hatched and completed development on *T. panacis*.

3.4 First-generation performance tests

The egg-laying performance of the first generation of *T. triozae* that had developed on *T. panacis* was compared with the performance of *T. triozae* that had emerged from TPP. Egg-laying was significantly affected by both the source of the parasitoids used in the test (P = 0.004), and the host offered to the parasitoids (P = 0.00001). There was no significant interaction between the terms. Parasitoids that had developed on the non-target psyllid laid

significantly more eggs on average, on both hosts, than parasitoids that had developed on TPP (Figure 4a). Emergence was also significantly affected by both the source of the parasitoids used in the test (P = 0.00009), and the host offered to the parasitoids (P = 0.01). There was no significant interaction between the terms. Parasitoids that had developed on the non-target psyllid produced significantly fewer adult parasitoids on average, on both hosts, than parasitoids that had developed on TPP (Figure 4b).

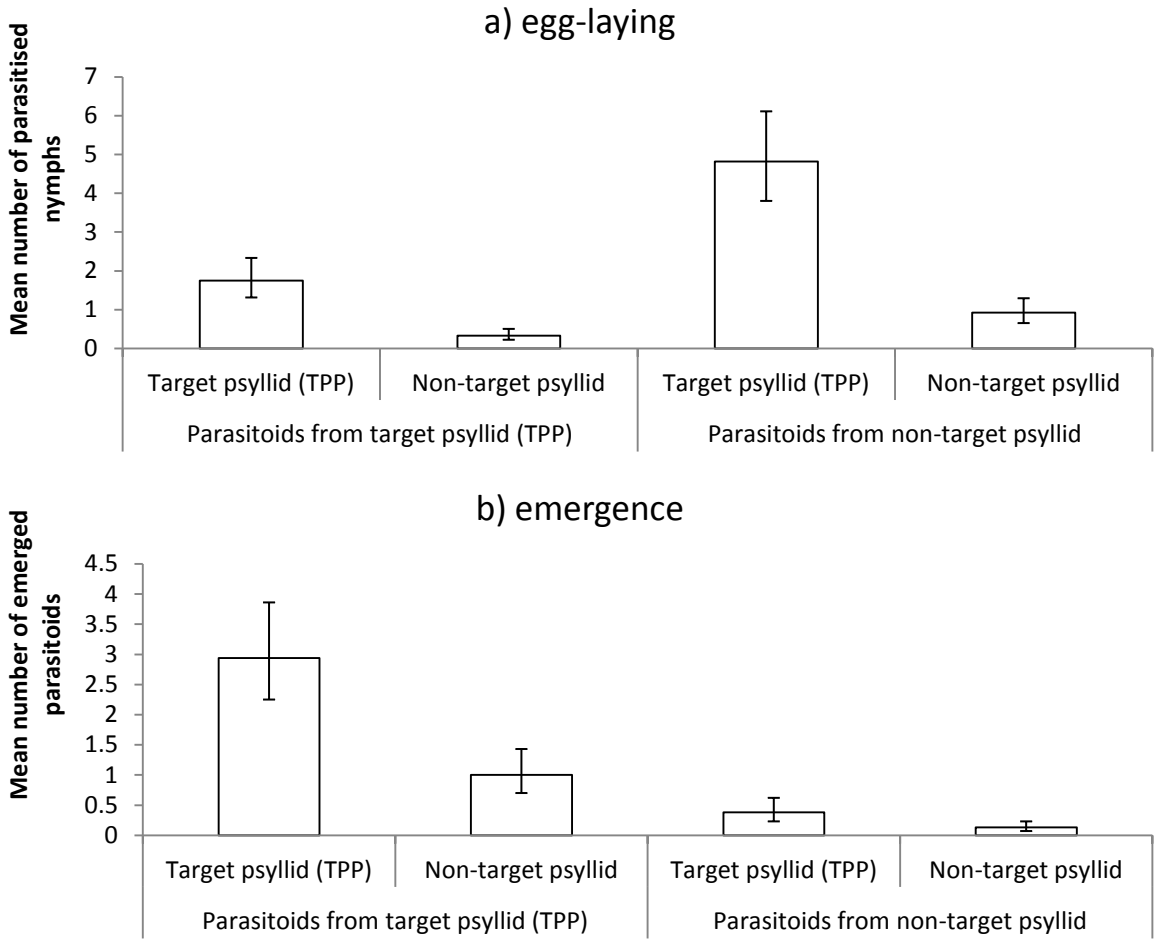

Figure 4. Performance of *Tamarixia triozae* adult females from different hosts, when offered the target psyllid (TPP) or a non-target psyllid (*Trioza panacis*), as measured by a) egglaying and b) emergence of adult parasitoids. The female parasitoids used in these tests had developed on either the target psyllid (TPP) or the non-target psyllid *T. panacis*.

4 Discussion

4.1 Interpretation of host range tests

The host range of a parasitoid species can be defined as the set of species that can support development of the parasitoid. A distinction is usually made between the physiological (or fundamental) host range and the ecological host range. The physiological host range consists of the species that can support parasitoid development under laboratory conditions, whereas the ecological host range consists of the species actually used by the parasitoid in the field for successful reproduction (Haye et al. 2005). The host range tests reported here provide the first information available on the physiological host range of the potential biological control agent, *Tamarixia triozae*. The parasitoid *T. triozae* did not lay eggs on six psyllid species it was offered in a series of small-scale no-choice tests. For each psyllid species, a total of 45 adult female *T.*

triozae were confined with nymphs of the species, in 15 separate cages. Under the same test conditions, *T. triozae* females laid eggs on its usual host, indicating that the parasitoids were in a physiological state that allowed them to readily attack an acceptable host (i.e. the parasitoids were competent). Given these results, these six psyllid species can be regarded as lying outside the host range of *T. triozae* and are unlikely to be parasitized by *T. triozae* in the wild. Five of the species are native (*Acizzia dodonaeae*, *Ctenarytaina clavata, Psylla apicalis, Trioza* "ohumata" and *Trioza vitreoradiata*) while one is an exotic beneficial (*Arytainilla spartiophila*).

Tamarixia triozae did oviposit on two native psyllid species (*Trioza curta* and *Trioza panacis*) in both choice and no-choice tests, but was unable to complete development on one of these species (*T. curta*). These results suggest that *T. curta* also lies outside the physiological host range of *T. triozae.* Successful development did occur on *T. panacis*, but the rate of egg-laying on *T. panacis* was low and the parasitoids that emerged from *T. panacis* had reduced ability to produce further offspring compared with parasitoids that emerged from their usual host (TPP). Hence, while *T. panacis* lies within the physiological range of *T. triozae,* it does not appear to be an optimal host for the parasitoid.

4.2 Extrapolation of test results to impacts in the field

Studies have shown that the physiological host range is often greater than the ecological host range of a species, a discrepancy that arises because laboratory tests cannot predict parasitoid host searching and other behaviours that occur in complex open environments (Froud & Stevens 2003; Haye et al. 2005). Furthermore, even if a non-target species does lie within the ecological host range of a parasitoid and parasitism occurs in the field as well as the laboratory, this may not significantly affect the non-target species. A major analysis of over 5000 insect introductions for classical biological control of insects found that only 87 (1.7%) of the introductions had any non-target effects recorded (Lynch et al. 2001). In the majority of these 87 cases, the data available indicated that the introduced agents utilised non-target hosts at a low level and did not generate sufficient mortality to cause population-level effects (Lynch et al. 2001). Quantitative evidence of severe impacts (e.g. > 40% long-term population suppression of either global or local populations) existed for at least ten introductions. However, when patterns of data recording and research effort are taken into account, the authors conclude that it is possible as many as 10% of all introductions have had serious population level effects (Lynch et al. 2001). Unfortunately, predicting detrimental non-target effects continues to be difficult (Parry 2009; Barratt et al. 2010). For example, even when levels of parasitism of a non-target host equals the parasitism rates on the target species, the effect on the two populations may be different (Barratt et al. 2010). Understanding the population level impacts of non-specific agents remains one of the major challenges in biocontrol research.

With *Tamarixia triozae*, the low level of parasitism observed on *T. panacis*, together with the evidence that *T. panacis* may not be an optimal host, suggests that *T. triozae* is not likely to cause high mortality in local populations if field parasitism did occur following release. In addition, both *T. panacis* and its hosts are widespread, occurring from Auckland in the north to Manapouri in the south, and from sea level to subalpine (Dale 1985). Given this distribution pattern, it is unlikely that *T. triozae* would locate and deplete all *T. panacis* populations, as several authors have noted that *T. triozae* has patchy distribution within its home range, occurring in abundance at one site but not at other nearby sites with abundant hosts present (Pletsch 1947; Johnson 1971). There is also some evidence that *T. triozae* has limited ability to locate hosts over distance (Johnson 1971). This may also allow some *T. panacis* populations to remain undetected by *T. triozae*.

Other *Tamarixia* species have been successfuly used in a number of biocontrol programmmes (Table 8). The *Tamarixia* species used appear to be monophagous within their home range (Zuparko et al. 2011). Despite this high level of host specificity, non-target effects have been reported for one species, *Tamarixia dryi,* introduced onto Reunion Island in 1974, to control the citrus psyllid *Trioza erytreae* (Aubert & Quilici 1983). On Reunion Island, *T. dryi* rapidly reduced its host numbers, eventually completely expirpating *T. erytreae*. Prior to the release of *T. dryi,* another psyllid*, Trioza litseae (eastopi),* was widespread and abundant, especially on its main host, a common Reunion Island shrub, *Litsea chinensis*. Parasitism of *T. litseae* by *T. dryi* was first detected in 1978, and heavy levels of parasitism were observed in 1980. By this stage the

target pest *T. erytreae* was considered to be locally extinct and by 1981–82 *T. litseae* had also dropped to extremely low abundance on its main host (Aubert & Quilici 1983). Although *T. litseae* was first described from Reunion Island, some authors do not appear to regard it as native there, and the successful control of *T. erytreae* on Reunion Island has been attributed to the ability of the parasitoid to maintain high numbers by using *T. litseae* as an alternative host (Aubert & Quilici 1983; Halbert & Manjunath 2004). Other authors have cited the host switching of *T. dryi* on Reunion Island as an example of unexpected severe non-target effects that can arise from classical biological control (Samways 2005; Parry 2009). Host-range testing was apparently not conducted prior to the release of *Tamarixia dryi* on Reunion Island. If tests had been done, then the non-target attack on *T. litseae* could have been predicted and accounted for in the decision making process.

Table 8. *Tamarixia* species that have been introduced into countries outside of their home range as biological control agents (based on Zuparko et al. 2011). *Tamarixia triozae* has been included for comparative purposes. *Two other hosts have been recorded for *Tamarixia radiata*, but are thought to be mistaken identifications (Wager-Page 2010).

Parasitoid	Introduced against	Number of parasitoid hosts recorded in home range	Areas where introduced	Non-target effects reported?	Provides some control in area of introduction?
Tamarixia dahlsteni	*Trioza eugeniae*	1	North America	No	Yes
Tamarixia dryi	*Trioza erytreae*	1	Reunion and Mauritius	Yes	Yes
Tamarixia leucaenae	*Heteropsylla cubana*	1	Asia and Africa	No	No
Tamarixia radiata	*Diaphorina citri*	1*	North America, South Americ,Asia, plus islands in the Pacific and Indian Oceans	No	Variable
Tamarixia schina	*Calophya schini*	1	North America	No	Yes
Tamarixia triozae	-	13	-	-	-

4.3 Extrapolation of results to psyllid species not tested

The native psyllids selected for testing were representative of the taxonomic diversity of the New Zealand native psyllid fauna; hence the results suggest that most native psyllid species are likely to lie outside the physiological host range of the parasitoid. However, the results also indicate that *T. triozae* may attempt to use novel species as hosts, and that *T. triozae* is capable of successfully developing in at least some of these novel species. Changes in parasitoid host use post-introduction are referred to as "parasitoid drift" and appear to be reasonably common (Follett et al. 2000). For example, 16% (51/313) of the exotic parasitoids introduced into North America for classical biological control have been recorded using novel native hosts (Hawkins & Marino 1997). Drift can arise through host switching, host range expansion or host shifting and may involve genetic change in the parasitoid population as it adapts to its new environment, or a parasitoid may be pre-adapted to use a non-target species (e.g. if the parasitoid responds to a chemical signal released by both the original and novel host) (Follett et al. 2000). *Tamarixia triozae* utilises a diverse range of psyllid hosts within its home range, suggesting some level of behavioural or developmental flexibility that may enable it to expand or change its host range in a new environment. If the laboratory results reported here are typical of *T. triozae*'s response to novel hosts, then 15–30% of native psyllid species in New Zealand (i.e. 10–20 species) could potentially be parasitized by *T. triozae* in the field. As noted above, not all parasitism will lead to successful development, or result in population level effects. Nonetheless, these figures do raise concerns, especially for rare psyllid species.

4.3.1 Rare psyllid species

Two threatened endemic psyllid species occur in lowland habitats that *T. triozae* could potentially invade. Both species are in the Psyllidae family, a family *T. triozae* is known to attack in its home range (Zuparko et al. 2011). Unfortunately, very little is known about the biology of either species and population numbers are thought to be too low to allow collection for host range testing (P. Dale, pers. comm.). It is possible that rare species may avoid attack if they are remote from agricultural areas where exotic parasitoids are likely to be concentrated, because their low numbers may not attract the exotic parasitoids. However, exotic parasitoids can invade natural habitats over time (Henneman & Memmott 2001; Barratt et al. 2007), hence such a refuge may be temporary rather than permanent. Louda et al. (2003) reviewed 10 projects that had quantitative data on non-target effects and concluded that non-target attack on rare species could accelerate their decline and increase the risk of extinction. Such impacts are considered

more likely if a natural enemy is able to maintain high population levels on another host (Louda et al. 2003).

5 Recommendations for future work

Concerns regarding non-target effects of biological control agents have increased markedly in the past three decades and there is wide agreement that agents with narrow host ranges are likely to be lower risk, more environmentally benign, and more cost effective to research than generalists. Despite this agreement, releases of agents with broad host ranges continue to occur, often because of extreme pest pressure that requires urgent action, or because more host-specific agents are not known for the target pest (Jenner & Kuhlmann 2009). Both these factors influenced the decision to import *Tamarixia triozae* into containment in New Zealand and begin host range testing. Very little published information on *T. triozae* was available at the outset of this project, and this report represents the first attempt to consider the safety of *T. triozae* as a biological control agent outside its region of origin. The host testing has proceeded with the working assumption that *T. triozae* was likely to establish in at least some areas of New Zealand. Test results indicate that some non-target parasitism is likely if *T. triozae* encounters novel psyllids in its new environment, so it is now important to gain a better understanding of the potential distribution of *T. triozae* in New Zealand. This is a cost-effective research option that could significantly improve our understanding of the risks *T. triozae* poses to the New Zealand psyllid fauna. In particular, climate modelling may be able to determine if the New Zealand alpine environment could create a significant refuge for native psyllid species.

5.1.1 Alpine refuges

Parasitism of the native fauna can only occur if *T. triozae* actually encounters an acceptable native psyllid host. Spatial refuges for psyllid species have been mentioned above, but climate refuges may also be important. *Tamarixia triozae* is recorded mainly from warm dry / arid areas in its home range, and New Zealand's alpine and sub-alpine areas may be climatically unsuitable for the parasitoid (D. Logan, unpublished data). This is an area that deserves further research. Although only five native psyllid species are exclusively alpine (i.e. only occur in alpine herbfields), another 25 species are found from lowland to alpine situations and another 17 species occur from lowland to sub-alpine situations (Dale 1985). Hence almost 70% of the native psyllid fauna occur in alpine to subalpine situations. One other species occurs only in the subantarctic islands (Dale 1985). Climate may protect these species from potential *T. triozae* parasitism in all or part of their ranges.

6 Summary

The aim of this study was to evaluate the potential for deleterious effects by *T. triozae* on native or valued psyllid species in New Zealand. *Tamarixia triozae* was known to parasitize a number of psyllid species in its home range, and as no information was available about the response of the parasitoid to novel hosts, host range testing was considered necessary prior to any application for release in New Zealand. Testing was carried out using seven common native psyllid species and one beneficial (an exotic psyllid species introduced for weed biocontrol). *Tamarixia triozae* did not oviposit on the beneficial psyllid species or on five of the seven native psyllid species it was exposed in no-choice tests. *Tamarixia triozae* did oviposit on two native psyllid species in both choice and no-choice tests, but was only able to complete development on one of these species (*Trioza panacis*). The rate of egglaying on *T. panacis* was low and the parasitoids that emerged from *T. panacis* had reduced ability to produce further offspring compared with parasitoids that emerged from their usual host (TPP). The native psyllids selected for testing were representative of the taxonomic diversity of the New Zealand native psyllid fauna; hence the results suggest that most native psyllid species are likely to lie outside the physiological host range of the parasitoid. Nonetheless, a significant portion of the native psyllid fauna could be attacked by *T. triozae* in the field, if it is assumed that *T. triozae* will overlap with most native psyllids in space and time. Climate modelling will be an important focus for future work.

7 Acknowledgements

This report draws extensively on both published and unpublished work undertaken by Peter Workman during his time with Plant & Food Research. John Charles, Nicholas Martin, Graham Walker and Peter Cameron have provided useful discussion and comment on earlier drafts of this report. Aleise Puketapu, Ngaire Larsen and Joanne Poulton provided invaluable help with field work, insect rearing and host testing. Thank-you also to Patrick Connolly who undertook the statistical analysis of the host range tests. Overseas researchers have been generous with their assistance, especially Esteban Rodriguez-Leyva and John Trumble.

8 References

Aubert B, Quilici S 1983. Biological control of the African and Asian citrus psyllids (Homoptera: Psylloidea),through Eulophid and Encyrtid parasites (Hymenoptera: Chalcidoidea) in Reunion Island. In: Garnsey SM, Timmer LW, Dodds JA eds. Ninth Conference of the International Organization of Citrus Virologist, 9−13 November 1983, Argentina. University of California, Riverside, USA. Pp. 100-108.

Barratt BIP, Ferguson CM, Bixley AS, Crook KE, Barton DM, Johnstone PD 2007. Field parasitism of nontarget weevil species (Coleoptera : Curculionidae) by the introduced biological control agent *Microctonus aethiopoides* Loan (Hymenoptera : Braconidae) over an altitude gradient. Environmental Entomology 36(4): 826-839.

Barratt BIP, Howarth FG, Withers TM, Kean JM, Ridley GS 2010. Progress in risk assessment for classical biological control. Biological Control 52(3): 245-254.

Berndt LA, Sharpe A, Withers TM, Kimberley M, Gresham B 2009. Risks to non-target species from potential biological control agent *Cotesia urabae* against *Uraba lugens* in New Zealand. Release of *Cotesia urabae* for biological control of the pest gum leaf skeletoniser. New Zealand, Unpublished application to Environmental Risk Management Authority. Pp. 1-24, Appendix 2.

Boucek Z 1988. *Tamarixia leucaenae* sp. n. (Hymenoptera: Eulophidae) parasitic on the leucaena psyllid *Heteropsylla cubana* Crawford (Hemiptera) in Trinidad. Bulletin of Entomological Research 78: 545-547.

Bravo ME, Lopez LP 2007. Principales plagas del chile de agua en los Valles Centrales de Oaxaca. Agroproduce 7: 12-15.

Burckhardt D 1994. Psylloid pests of temperate and subtropical crop and ornamental plants (Hemiptera, Psylloidea): A review. Trends in Agricultural Sciences, Entomology 2: 173-186.

Butler CD, Trumble JT 2012. The potato psyllid, *Bactericera cockerelli* (Sulc) (Hemiptera: Triozidae): Life history, relationship to plant diseases, and management strategies Terrestrial Arthropod Reviews 5(2): 87-111.

Dale PJ 1985. A review of Psylloidea (Insects: Hemiptera) of the New Zealand subregion. Unpublished thesis, University of Auckland, Auckland.

Elmhirst J 2005. Crop profile for greenhouse tomato in Canada. Ottawa, Pesticide Risk Reduction Program, Pest Management Centre, Agriculture and Agri-Food Canada. Pp. 46.

Follett PA, Duan J, Messing RH, Jones VP 2000. Parasitoid drift after biological control introductions: Re-examining Pandora's box. American Entomologist 46(2): 82-94.

Froud KJ, Stevens PS 2003. Importation biological control of *Heliothrips haemorrhoidalis* by *Thripobius semiluteus* in New Zealand - a case study of non-target host and environmental risk assessment. In: Van Driesche RG ed. 1st International Symposium on Biological Control of Arthropods, 14-18 January 2002, Honolulu, Hawaii. Pp. 366-369.

Halbert SE, Manjunath KL 2004. Asian citrus psyllids (Sternorrhyncha: Psyllidae) and greening disease of citrus: A literature review and assessment of risk in Florida. Florida Entomologist 87(3): 330-353.

Hawkins BA, Marino PC 1997. The colonization of native phytophagous insects in North America by exotic parasitoids. Oecologia 112(4): 566-571.

Haye T, Goulet H, Mason PG, Kuhlmann U 2005. Does fundamental host range match ecological host range? A retrospective case study of a *Lygus* plant bug parasitoid. Biological Control 35(1): 55-67.

Hayes L 2005. Biological control agents for weeds in New Zealand: A field guide. Lincoln, New Zealand, Landcare Research.

Henneman ML, Memmott J 2001. Infiltration of a Hawaiian community by introduced biological control agents. Science 293(5533): 1314-1316.

Hoddle MS 2004. Analysis of fauna in the receiving area for the purpose of identifying native species that exotic natural enemies may potentially attack. In: Van Driesche RG, Murray T, Reardon R eds. Assessing host ranges for parasitoids and predators for classical biological control: A guide to best practice. Morgantown, West Virginia, Forest Health Technology Enterprise Team. Pp. 24-39.

Jenner W, Kuhlmann U 2009. Ecological theory vs. practice: Have non-target concerns led to increased use of monophagous agents? In: Mason PG, Gillespie DR, Vincent C eds. Third International Symposium on Biological Control of Arthropods, 8-13 February 2009, Christchurch, New Zealand. FHTET, USDA Forest Service. Pp. 45-55.

Jensen DD 1957. Parasites of the Psyllidae. Hilgardia 27: 71-99.

Johnson TE 1971. The effectiveness of *Tetrastichus triozae* Burks (Hymenoptera: Eulophidae) as a biological control agent of *Paratrioza cockerelli* (Sulc.) (Hymenoptera: Psyllidae) in north central Colorado. Unpublished MSc Thesis thesis, Colorado State University.

Kuhlmann U, Schaffner U, Mason PG 2006. Selection of non-target species for host specificity testing. In: Bigler F, Babendreier D, Kuhlmann U eds. Environmental impact of invertebrates for biological control of arthropods: Methods and risk assessment. Wallingford, UK, CABI Publishing. Pp. 15-37.

La Salle J 1994. North American genera of Tetrastichinae (Hymenoptera: Eulophidae). Journal of Natural History 28(1): 109 -236.

Liefting LW, Weir BS, Pennycook SR, Clover GRG 2009. '*Candidatus* Liberibacter solanacearum', associated with plants in the family Solanaceae. Internation Journal of Systematic and Evolutionary Microbiology 59: 2274-2276.

Liu T-X, Zhang Y-M, Peng L-N, Rojas P, Trumble JT 2012. Risk assessment of selected insecticides on *Tamarixia triozae* (Hymenoptera: Eulophidae), a parasitoid of *Bactericera cockerelli* (Hemiptera: Trizoidae). Journal of Economic Entomology 105(2): 490-496.

Lomeli-Flores JR, Bueno R 2002. Nuevo registro de *Tamarixia triozae* (Burks) parasitoide del psílido del tomate *Paratrioza cockerelli* (Sulc) (Homoptera: Psyllidae) en México Folia Entomológica Mexicana 3: 375-376.

Louda SM, Pemberton RW, Johnson MT, Follett PA 2003. Nontarget effects - The Achilles' heel of biological control? Retrospective analyses to reduce risk associated with biocontrol introductions. Annual Review of Entomology 48: 365-396.

Luna Cruz A 2010. Toxicidad de cuatro insecticidas sobre *Tamarixia triozae* (Burks) (Hymenoptera: Eulophidae) y su hospedero *Bactericera cockerelli* (Sulc) (Hemíptera: Psyllidae). Unpublished thesis, Institucion de Ensenanza e Investigacion en Ciencia Agricolas, Montecillo, Texcoco, México.

Lynch LD, Hokkanen HMT, Babendreier D, Bigler F, Burgio G, Gao ZH, Kuske S, Loomans A, Menzler-Hokkanen I, Thomas MB, Tommasini G, Waage JK, van Lenteren JC, Zeng QQ 2001. Insect biological control and non-target effects: A European perspective. In: Wajnberg E, K. SJ, C. QP eds. Evaluating Indirect Ecological Effects of Biological Control. Wallingford, UK, CABI Publishing. Pp. 99-125.

Martin NA 2008. Host plants of the potato/tomato psyllid: A cautionary tale. Weta 35: 12-16.

McGuinness CA 2001. Conservation requirements of New Zealand's nationally threatened invertebrates. Wellington, Biodiversity Recovery Unit, Department of Conservation.

Ouvrard D 2012. Psyl'list - The World Psylloidea Database.

Parry D 2009. Beyond Pandora's box: Quantitatively evaluating non-target effects of parasitoids in classical biological control. Biological Invasions 11(1): 47-58.

Pedley RIF, Workman P 2007. Survey of *Trioza vitreoradiata* predators and potential for biological control of *Bactericera cockerelli* New Zealand Institute for Crop & Food Research Limited. Pp. 1-12.

Pemberton RW 2000. Predictable risk to native plants in weed biological control. Oecologia 125(4): 489-494.

Pletsch DJ 1947. The potato psyllid *Paratrioza cockerelli* (Sulc), its biology and control. Montana Agricultural Experiment Station Bulletin 446: 1-95.

R Development Core Team 2012. R: A language and environment for statistical computing. Vienna, Austria, R Foundation for Statistical Computing.

Rojas PR 2010. Biología de *Tamarixia triozae* (Hymenoptera: Eulophidae) parasitoide de *Bactericera cockerelli* (Hemiptera: Psyllidae). Unpublished thesis, Colegio de Postgraduados, Montecillo, Texcoco.

Rojas PR, Rodríguez-Leyva E, Lomeli-Flores JL, Liu TX 2009. Ciclo de vida de *Tamarixia triozae* (Hymenoptera: Eulophidae) parasitoide de *Bactericera cockerelli* (Hemiptera: Triozidae). XXXII Congreso Nacional de Control Biológico, Zacatecas, México. Pp. 153-156.

Romney VE 1939. Breeding areas of the tomato psyllid, *Paratrioza cockerelli* (Sulc). Journal of Economic Entomology 32: 150-151.

Samways MJ 2005. Insect Diversity Conservation. Cambridge, Cambridge University Press.

Stringer IAN, Hitchmough RA, Larivière M-C, Eyles AC, Teulon DAJ, Dale PJ, Henderson RC 2012. The conservation status of New Zealand Hemiptera. New Zealand Entomologist 35(2): 110-115.

Teulon DAJ, Workman P, Thomas KL, Nielson MC 2009. *Bactericera cockerelli:* Incursion, dispersal and current distribution on vegetable crops in New Zealand. New Zealand Plant Protection 62: 136-144.

Tuthill LD 1952. On the Psyllidae of New Zealand (Homoptera). Pacific Science 6: 83-125.

Van Driesche RG, Murray T 2004. Overview of testing schemes and designs to estimate host ranges. In: Van Driesche RG, Murray T, Reardon R eds. Assessing host ranges for parasitoids and predators for classical biological control: A guide to best practice. Morgantown, West Virginia, Forest Health Technology Enterprise Team. Pp. 68-89.

Vega CJL 2010. Determinación de alimentación de preferencia de *Tamarixia triozae* (Burks) (Hymenoptera: Eulophidae) sobre estadios de *Bactericera cockerelli* (Sulc.) (Hemiptera: Psyllidae). Unpublished thesis, Universidad Autónoma Agraria Antonio Narro, Saltillo, Coahuila, México.

Venables WN, Ripley BD 2002. Modern Applied Statistics with S. Fourth Edition ed. New York, Springer.

Wager-Page S 2010. Proposed Release of a Parasitoid (*Tamarixia radiata* Waterston) for the Biological Control of Asian Citrus Psyllid (*Diaphorina citri* Kuwayama) in the Continental United States: Environmental Assessment, June 2010. Riverdale, APHIS, United States Department of Agriculture.

Wallis RL 1955. Ecological studies on the potato psyllid as a pest of potatoes. Technical Bulletin U.S. Department of Agriculture 1107: 1-25.

Workman P 2008. Greenhouse assessment of potential biological control agents for pests of capsicums and tomatoes New Zealand Institute for Crop & Food Research Limited. Pp. 1-32.

Workman P, Davidson M 2007. Potential biological control agents for greenhouse pests in New Zealand New Zealand Institute for Crop & Food Research Limited. Pp. 1-45.

Workman P, Nielson MC, Teulon D 2006. Potato/tomato psyllid in New Zealand: Immediate options for biological control New Zealand Institute for Crop & Food Research Limited. Pp. 1-16.

Young GR 2003. Life history, biology, host plants and natural enemies of the lilly pilly psyllid, *Trioza eugeniae* Froggatt (Hemiptera: Triozidae). Australian Entomologist 30(1): 31-38.

Zuparko RL, De Queiroz DL, La Salle J 2011. Two new species of *Tamarixia* (Hymenoptera: Eulophidae) from Chile and Australia, established as biological control agents of invasive psyllids (Hemiptera: Calophyidae, Triozidae) in California. Zootaxa(2921): 13-27.