The host ranges of *Tetramesa romana* and *Rhizaspidiotus donacis*, potential control agents for giant reed, *Arundo donax.*

Introduction

Arundo donax, or giant reed, is a tall, woody grass that has been proposed as a target for biological control in New Zealand. It originates from Eurasia, but has become a serious environmental weed in many southern hemisphere countries and in North America. A global account of its distribution, impact and management can be found here: http://www.issg.org/database/species/ecology.asp?si=112 . Although still of limited distribution in New Zealand, it is consider to be a serious future threat to wetlands, riparian areas and other habitats. More information about its potential impact in New Zealand can be found in the application, or can be accessed here: http://www.landcareresearch.co.nz/science/plants-animals-fungi/plants/weeds/biocontrol/approvals/current-applications .

Two control agents have been selected for introduction to New Zealand

1. Arundo wasp, *Tetramesa romana* Walker (Hymenoptera: Eurytomidae)
2. Arundo scale, *Rhizaspidiotus donacis* (Leonardi) (Hemiptera: Diaspididae)

Arundo wasp lays its eggs into the shoots of giant reed, inducing a gall. Larvae feed within the shoot gall. Growth can be severely stunted and with sufficient attack stems can die. Like pest scales of pot plants and fruit trees, arundo scale extracts the products of photosynthesis from the phloem of giant reed rhizomes, reducing the productivity and growth rate of plants.

The New Zealand initiative can draw heavily on a biological control programme against giant reed that has been underway in USA and Mexico for over ten years. Both arundo wasp and arundo scale have been released in the USA and Mexico. Approval for release of these agents was granted only after extensive risk assessment (USDA 2009, 2010), including intensive research to determine that the agents were specific to giant reed. The results of these tests have since been published in peer-reviewed journals (Goolsby and Moran 2009, Goolsby et al. 2009). The arundo wasp was released first, and is causing reduction in giant reed vigour after only 5 years. Goolsby et al (2015) found that above ground biomass of *A. donax* decreased on average by 22% across 10 sites in Texas. This decline in biomass was negatively correlated to increased total numbers of *T. romana* exit holes in main and lateral shoots per site in 2014 compared to 2007. *Rhizaspidiotus donacis* has been shown to significantly reduce stem growth and rhizome growth in its native range (Cortes et al. 2011 a, b). It has been released in the USA and Mexico, and is now established there (Goolsby et al. 2011).

This report summarises the results of host range tests conducted before these agents were released in the USA (Goolsby and Moran 2009, Goolsby et al. 2009) and assesses whether this research is adequate to assess the potential host range of these insects in New Zealand. In the case of arundo scale, it also describes the results of a limited host range test conducted in a containment facility in New Zealand.

Host-range testing of Tetramesa romana

Female *T. romana* produce eggs parthenogenetically (no males required) and deposit them into shoot tips (Figure 1). Adults produce an average of 26 eggs in a lifetime. Oviposition results in gall tissue and shoot distension within 2 weeks (Moran & Goolsby 2009). Eggs hatch within 5 days of oviposition, and larvae feed on gall tissue. In the laboratory, at 27°C, the generation time of *T. romana* was 33 days (Goolsby and Moran 2009). Emerging adults leave characteristic emergence holes in damaged stems (Figure 1). Over 90% of exit holes made by emerging wasps were located within two nodes of the shoot tip. Field studies in Europe indicate *T. romana* has the potential to significantly impact *A. donax* by stunting growth and killing stems (in Goolsby and Moran 2009).

Figure 1. Left: *Tetramesa romana* probing the stem of a giant reed photo: http://CISR.UCR.EDU/giant_reed_arundo.html. Right: Emergence holes in a distended giant reed shoot photo: Quentin Paynter

Gall insects are endophytic herbivores that manipulate their host plants to produce tumor-like growths that provide the insects with food and shelter at the expense of the host plant. This intimate relationship requires a match between the genetics of the ability of the insect to induce a gall, and the susceptibility of the plant to gall formation (Craig et al. 1994). This unique combination of requirements means that most gall-

formers feed on a single host species, and on a particular part of the plant. This selects for high specificity in galling insects. Gall-forming has been recorded from a wide range of insect orders, but all are reported to highly host-specific or monophagous (Craig et al. 1994). This conclusion has been supported repeatedly by laboratory studies.

Testing for USA and Mexico

Field collection records in its native range indicate that *Tetramesa romana* only attacks *Arundo donax* and (to a lesser extent) the closely-related *Arundo plinii* (Goolsby & Moran 2009). Host range tests were conducted to confirm the host range revealed in native range studies.

No-choice tests and timed behavioural studies were used to determine the fundamental host range of two genotypes of the wasp collected from southern Europe. *Arundo donax* belongs to the family Gramineae. Based on the requirement of US regulators, thirty-five species of this family were selected for testing, including 29 native and economically valued species of grass or sedge. Six non-grass species commonly associated with giant reed in the USA were also tested (Goolsby and Moran 2009).

Probing of test plants (Figure 1) was most common on *Arundo* species. Females were observed to probe other gasses species with round stems (Table 1), but to a much lesser extent. Complete development was restricted to *A. donax* and *Arundo formosana* (Goolsby and Moran 2009, see table 1). This was true for both *T. romana* genotypes. *Arundo formosana* was clearly a marginal host as development on this plant took twice as long, compared to development on *A. donax*, and there was a significant difference between the number of offspring produced by *T. romana* females on *A. donax* and *A. formosana*. The mean number of offspring produced per female on *A. formosana* was a fraction (c. 6-14%) of that produced on *A. donax*. They also tested representatives from the core genera of the Arundinoideae tribe Arundineae (*Molinia, Hakonechloa, Phragmites*), none of which were hosts of *T. romana,* as well as more distantly-related plants that belong to other subfamilies within the PACMAD clade (*Aristida*, *Cynodon, Spartina*, *Uniola*, *Leptochloa*, *Danthonia*, *Cortaderia*, *Panicum, Sorghum, Zea, Saccharum*). Goolsby and Moran (2009) concluded that *T. romana* is specific to plants in the genus *Arundo.*

In summary, *T. romana* is clearly highly host-specific. Plant species from different genera in the same tribe (*Molinia, Hakonechloa, Phragmites*), were not hosts and relative performance on a plant species that is congeneric with the target weed (*A. formosana*) was so poor that a recent analysis of quantitative host-range testing data (Paynter et al. 2014) indicates that even *A. formosana* is unlikely to be a suitable field host for *T. romana*.

Tetramesa romana was released (USA 2009) and has established in the USA. Twelve of the tested grass species grew alongside giant reed in two sites where *T. romana* became established. Goolsby and Moran (2009) examined plants at these sites for the presence of galls or emergence holes. Attack was observed on *Arundo donax* but on no other grass species.

Testing for New Zealand

It was not considered necessary to undertake additional tests of species of environmental, cultural or economic importance in New Zealand. *Tetramesa romana* is not considered to pose a threat to native plants or valued exotic plants in New Zealand because:

- Gall-forming insects are universally highly specific to one, or a few host plants.
- *T. romana* has only been recorded from *Arundo* species in its native range.
- Extensive host-range testing for the USA confirms that *T. romana* is specific to the genus *Arundo* (see above).
- No damage to other grasses has been observed since *T. romana* became established in USA
- There are no native or otherwise valued *Arundo* species in New Zealand
- There are no native species in the sub-family Arundinoideae
- The exotic *Phragmites australis* is the only other species in the sub-family Arundinoideae that is established in New Zealand. It was extensively tested and is not at risk.

Host-range testing of Rhizaspiodiotus donacis

The development, survival, and reproductive output of the armoured scale *Rhizaspidiotus donacis* have been determined. Peak reproduction from field collections in the native range occurred between November and March, and the scale completed two generations per year (Moran and Goolsby 2010). Females collected in southern France and Spain produced an average of 85 live crawlers. Scale crawlers lived for less than two days in the absence of a host. On *A. donax* shoots held under a diurnally variable temperature regime (15–26°C), crawlers settled on leaf collars and axillary stem bases and completed the first instar within 14 days. Winged adult males emerged within six weeks and lived 1.7 days in vials. Immobile adult females were observed 75 days after crawler release. Survival from crawler to adult was 20–25%. The generation time for laboratory-reared females was 170 days and their average life span was 203 days.

Figure 2. *Rhizaspidiotus donacis* scales on leaf bases of Arundo donax photo: Goolsby et al 2009)

Testing for USA and Mexico

This scale insect has only been collected from *Arundo donax* in its native range, with one exceptional record of a collection from common reed (*Phragmites australis*), which is considered to be the result of misidentification of the host plant (Goolsby *et al.* 2009).

Goolsby *et al.* (2009) conducted no-choice tests using *R. donacis* collected in France and Spain against 47 plant species, including *Arundo formosana* and representatives from the core genera of the Arundinoideae tribe Arundineae (*Molinia, Hakonechloa, Phragmites*). They also tested more distantly-related plants that belong to the PACMAD clade (*Aristida*, *Bouteloua, Cynodon, Dichanthelium, Eragrostis, Muhlenbergia, Spartina*, *Sporobolus, Tridens, Uniola*, *Leptochloa*, *Danthonia*, *Cortaderia*, *Andropogon*, *Digitaria*, *Panicum, Pennisetum, Schizachyrium, Sorghum, Tripsacum, Zea, Saccharum*).

Two hundred crawlers were released on each test plant and on giant reed controls in no choice tests. Forty of the plant species tested supported no development of *R. donacis.* Normal development of *R. donacis* was observed on *A. donax* and *A. formosana* (about one third of the number reared on *A. donax*) indicating that it is a marginal host. Crawlers settled on *Spartina alterniflora* and *Leptochloa* sp. but failed to complete development. In follow-up laboratory studies using 1000 crawlers per plant, 10 live adult females were found on *Leptochloa virgata*, and one adult female on *Spartina alterniflora.* Very low numbers were reared on *Phragmites, Leptochloa* and *Spartina*. Indeed only a handful of males and no females were reared from *Phragmites*, while the numbers reared from *Leptochloa* and *Spartina* were *c.* 1% and 0.1% of the numbers reared from *A. donax*, indicating that these species are very poor hosts: Recent research that has investigated the predictive value of quantifying the relative performance of biocontrol agents on test and target plants during host-range testing indicates that *R. donacis* performance on *Spartina* and *Leptochloa*, relative to on *A. donax* was far below the threshold level that would predict host use in the field (Paynter et al. 2014). This was confirmed when field surveys were conducted at five locations in Spain and France at which *A. donax* (infested with *R. donacis*), co-occurred with two non-target species of concern and *R. donacis* was only found on *A. donax* (Goolsby et al. 2009. Six-month field

host exposures in Spain using potted *Leptochloa* plants entwined with heavily infested *A. donax* confirmed that *R. donacis* is specific to *Arundo* under field conditions (Goolsby et al. 2009).

Goolsby et al. (2009) concluded that *R. donacis* is specific to plants in the genus *Arundo* under field conditions. On the basis of this conclusion, approval for release of *R. donacis* into USA was granted (USDA 2010), and *R. donacis* was established in the USA and Mexico (Goolsby et al. 2011).

Testing for New Zealand

Testing completed in the USA indicated that the *R. donacis* is specific to *Arundo donax* in the field, but might produce occasional offspring on a few closely-related spp. There are no New Zealand native species in the same sub-family as *Arundo donax*, so all are only distantly related to giant reed. However, to confirm that New Zealand native species would not be at risk, three native species representing the sub-families most closely-related to the Arundinoideae (sub-families Chloridoideae and Micrairoideae) were tested. The species selected were the endemic *Isachne globosa* and *Zoysia minima*, and the non-endemic but indigenous *Spinifex sericeus*.

Tests were completed using the techniques described by (Goolsby et al., 2009) with minor changes. Infested stem samples were collected in Spain in January 2015. Stem samples were dissected and mother scales were removed with forces, placed in dried gel capsules, and refrigerated. When required, capsules were removed to a containment facility lab at *c*. 21 °C, and ambient photoperiod until crawlers emerged c. 6 – 8 weeks later. The capsule was examined under a microscope to ensure there were no contaminant species present. The capsule was attached to a test or control plant using an entomological pin, so that crawlers could disperse onto the plant. Tests began in late July, early August 2015. Scoring took place in November 2015. There were three replicates for each test plant.

A mean of 12.7 scales were observed on *Arundo donax* plants. There was no sign of scales on *Isachne globosa* or *Spinifex sericeus.* A single very small scale, probably a male, was observed on *Zoysia minima,* but was lost (Quentin Paynter, Landcare Research, pers. comm.).

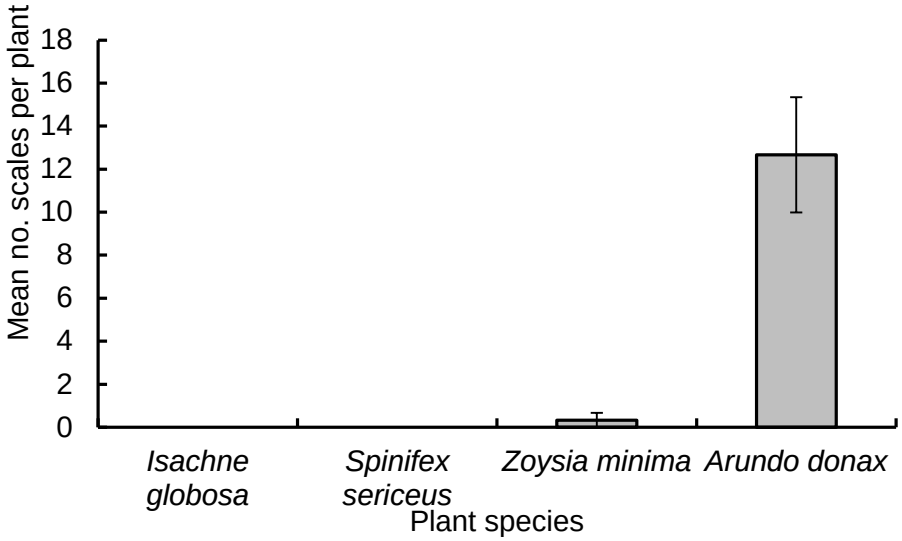

Figure 3. Results of host range tests using *R. donacis*which were conducted in containment in New Zealand.

These results add weight to the conclusion that can be drawn from field records and the extensive trials conducted for the USA (Goolsby et al. 2009); that *R. donacis* is specific to giant reed.

References

Craig, T. P., J. K. Itami, J. D. Horner and W. G. Abrahamson. 1994. Host shifts and speciation in gall-forming insects. Pages 194-207 *in* P. W. Price, W. Mattson, and Y. Baranchikov, editors. The ecology and evolution of gall-forming insects. USDA Forest Service, North Central Experiment Station. General Technical Report NC-174.

Goolsby JA, Moran PJ, Adamczyk JJ, Kirk AA, Jones WA, Marcos MA, and Cortés E. (2009). Host range of the European, rhizome-stem feeding scale *Rhizaspidiotus donacis* (Leonardi) (Hemiptera: Diaspididae), a candidate biological control agent for giant reed, *Arundo donax* (Poales: Poaceae) in North America. Biocontrol Science and Technology 19: 899–918.

Goolsby JA, Moran PJ (2009). Host range of *Tetramesa romana* Walker (Hymenoptera: Eurytomidae), a potential biological control of giant reed, *Arundo donax* L. in North America. Biological Control 49:160-168.

Goolsby JA, Moran PJ, Racelis AE, Summy KR, Martinez Jimenez M, Lacewell RD, Perez de Leon A, Kirk AA (2015). Impact of the biological control agent *Tetramesa romana* Hymenoptera: Eurytomidae) on *Arundo donax* (Poaceae: Arundinoideae) along the Rio Grande River in Texas. Biocontrol Science and Technology 26: 47-60. **DOI:** 10.1080/09583157.2015.1074980

Goolsby JA, Kirk AA, Moran PJ, Racelis AE, Adamczyk JJ, Cortes E, Garcia MAM, Jimenez MM, Summy, KR, Ciomperlik MA, Sands DPA (2011). Establishment of the armored

scale, *Rhizaspidiotus donacis*, a biological control agent of *Arundo donax.* Southwestern Entomologist 36: 373-374.

Goolsby JA, Cortes Mendoza E, Moran P, Adamczyk J, Marcos Garcia MA, Kirk A (2013). Evaluation of Spanish Arundo scale *Rhizaspidiotus donacis* (Hemiptera; Diaspididae) survival and fecundity on three new world genotypes of *Arundo donax* (Poaceae; Arundinoideae). Biocontrol Science and Technology 23: 499-506. DOI:10.1080/09583157.2013.772562

Moran PJ, Goolsby JA (2009). Biology of the galling wasp *Tetramesa romana*, a biological control agent of giant reed. Biological Control 49: 169-179. doi:10.1016/j.biocontrol.2009.01.017

Moran PJ, Goolsby JA (2010). Biology of the Armored Scale *Rhizaspidiotus donacis* (Hemiptera: Diaspididae), a Candidate Agent for Biological Control of Giant Reed. Annals of the Entomological Society of America 103: 252-263. doi: http://dx.doi.org/10.1603/AN09124

Paynter Q, Fowler SV, Gourlay, AH, Peterson PG, Smith LA, Winks CJ (2014). Relative performance on test and target plants in laboratory tests predicts the risk of non-target attack in the field for arthropod weed biocontrol agents. *Biological Control* 80: 133–142.

USDA (2009). Field release of the Arundo wasp, *Tetramesa romana* (Hymenoptera: Eurytomidae) an insect for the biological control of *Arundo donax* (Poaceae) in the continental United States. Unpublished Environmental Assessment, USDA/APHIS/PPQ, 41p.

USDA (2010). Field release of the Arundo scale, *Rhizaspidiotus donacis* (Hemiptera: Diaspididae), an insect for the biological control of *Arundo donax* (Poaceae) in the continental United States. Unpublished Environmental Assessment, USDA/APHIS/PPQ, 32p + appendices.

Subfamily or family	Scientific name	Perpignan France					Granada Spain				
		Reps	Reps probed	Obs. T (h)	Number of probes	Reproduction	Reps	Reps probed	Obs. T (h)	Number of probes	Reproduction
Arundinoideae	*Arundo donax* Laredo	19	16	256	172a	Yes	13	11	97.7	124a	Yes
Arundinoideae	*Arundo donax* San Juan	27	22	200.2	152b/a	Yes					
Arundinoideae	*Arundo formosana*	10	4	112.3	19b/b	Yes	5	4	32.4	9b	Yes
Arundinoideae	*Phragmites australis A*	3	1	33	17a/a	No	2	2	13.1	6a	No
Arundinoideae	*Phragmites australis B*	6	3	55.6	11b/b	No					
Arundinoideae	*Phragmites australis C*	6	2	58.7	17b/a	No	3	1	20.9	1b	No
Arundinoideae	*Phragmites australis D*	10	1	133.1	6b/b	No	10	2	61.7	5b	No
Arundinoideae	*Phragmites australis E*	3	0	12.1	0	No					
Arundinoideae	*Phragmites australis F*	6	0	48.6	0	No	10	0	55.9	0	No
Arundinoideae	*Phragmites australis G*	4	1	34.8	1b/b	No					
Arundinoideae	*Phragmites australis H*						5	2	26.6	32a	No
Arundinoideae	*Molinia caerulea*	6	0	72.5	0	No					
Arundinoideae	*Hakonechloa macra*	4	1	23.2	5b/b	No					
Aristoideae	*Aristida purpurea*	6	0	75.8	0	No					
Centothecoideae	*Chasmanthium latifolium*	3	0	30.1	0	No	3	0	16.2	0	No
Chloridoideae	*Cynodon dactylon*	6	0	64.1	0	No					
Chloridoideae	*Spartina spartinae*	8	1	82.3	3b/b	No	3	0	9.8	0	No
Chloridoideae	*Spartina alterniflora*	4	1	13.5	2b/b	No	3	0	15.1	0	No
Chloridoideae	*Uniola paniculata*	10	4	120.3	31b/b	No	3	1	8.8	6b	No
Chloridoideae	*Leptochloa panacea*	6	1	62.1	6b/b	No					
Chloridoideae	*Leptochloa fusca*	6	0	73.7	0	No	1	0	3.5	0	No
Chloridoideae	*Leptochloa virgata*	2	0	22.6	0	No					
Danthonioideae	*Danthonia spicata*	3	0	25.6	0	No					
Danthonioideae	*Cortaderia selloana*	8	3	71.1	20b/b	No	3	2	17.3	4b	No
Panicoideae	*Panicum virgatum*	11	5	109.5	23b/b	No	6	2	31.1	4b	No
Panicoideae	*Panicum hirsutum*	5	0	33.5	0	No					
Panicoideae	*Panicum amarum*	2	0	18.8	0	No					
Panicoideae	*Sorghum bicolour*	9	0	85	0	No	4	1	23.6	2b	No
Panicoideae	*Zea mays*	12	3	143.6	9b/b	No					
Panicoideae	*Saccharum officinarum*	8	1	77.1	6b/b	No	3	2	19.3	7b	No

Pooideae	*Triticum aestivum*	3	0	13.1	0	No					
Pooideae	*Distichlis spicata*	3	0	20.5	0	No					
Pooideae	*Sporobolus wrightii*	6	1	31.4	1b/b	No					
Bambusoideae	*Oryza sativa*	3	0	18.8	0	No	2	0	9.8	0	No
Bambusoideae	*Arundinaria gigantea*	6	2	65.3	10b/b	No					
Cyperaceae	*Schoenoplectus maritimus*	5	0	74.3	0	No					
Juncaceae	*Juncus acutus*	10	3	128.5	7b/b	No					
Typhaceae	*Typha domingensis*	4	0	34.1	0	No					
Arecaceae	*Sabal mexicana*	3	0	22.4	0	No					
Juglandaceae	*Carya illinoinensis*	3	0	35.3	0	No					
Salicaceae	*Salix exigua*	3	0	33.1	0	No					
Asteraceae	*Baccharis neglecta*	4	0	43.6	0	No					
Oleaceae	*Fraxinus berlandieriana*	4	0	30.3	0	No					

Table 1. Feeding and reproductive success of two genotypes of *Tetramesa romana* on a range of grasses (after Goolsby and Moran 2009).

			Live and dead females	Total all stages
sub/family	**Test species/control**	**Replicates**	**mean ± SE**	**mean ± SE**
Arundinoideae	*Arundo donax* (pooled)	29	**23.07 ± 4.24**	**33.28 ± 5.63**
	A. formosana	3	5.00 ± 2.89	11.00 ± 5.63
	Phragmites australis A	5	0	0.60 ± 0.6
	Phragmites australis B	4	0	0
	Phragmites australis C	3	0	0
	Phragmites australis D	6	0	0
	Molinia caerulea	3	0	0
	Hakonechloa macra	3	0	0
Aristidoideae	*Aristida purpurea* var. *longiseta*	3	0	0
Centothecoideae	*Chasmanthium latifolium*	2	0	0.5 ± 0.5
Chloridoideae	*Bouteloua hirsuta*	3	0	0
	Cynodon dactylon	3	0	0.33 ± 0.33
	Dichanthelium acuminatum	3	0	0
	Eragrostis intermedia	3	0	0
	Eragrostis spectablilis	3	0	0
	Leptochloa fusca ssp. *Uninervia*	3	1.00 ± 1.00	1.33 ± 1.33
	Leptochloa panicea ssp brachiata	3	0	1.33 ± 1.33
	Leptochloa virgata	2	3.50 ± 3.50	2.00 ± 2.00
	Muhlenbergia capillaris	3	0	0
	Spartina alterniflora	3	6.67 ± 6.17	4.33 ± 3.84
	Spartina spartinae	3	0	0
	Sporobolus wrightii	3	0	0
	Tridens albescens	3	0	0

	Uniola paniculata	3	0	0
Danthonioideae	*Cortaderia selloana*	5	0	0
	Danthonia spicata	1	0	0
Panicoideae	*Andropogon glomeratus*	3	0	0
	Digitaria cognata	3	0	0
	Panicum amarum	3	0	0
	Panicum hirsutum	3	0	0
	Panicum virgatum	3	0	0
	Pennisetum ciliare	3	0	0
	Saccharum officinarum	3	0	0
	Schizachyrium scoparium	3	0	0
	Sorghum bicolor	3	0	0
	Tripsacum dactyloides	3	0	0
	Zea mays	3	0	0
Pooideae	*Elymus virginicus*	1	0	0
	Triticum aestivum	3	0	0
Bambusoideae	*Arundinaria gigantea*	3	0	0
Ehrhatoideae	*Oryza sativa*	3	0	0
Cyperaceae	*Cyperus articulatus*	3	0	0
	Schoenoplectus maritimus	3	0	0
Eriocaulaceae	*Eriocaulon decangulare*	3	0	0
Typhaceae	*Typha domingensis*	3	0	0
Arecaceae	*Sabal mexicana*	3	0	0
Juglandaceae	*Carya illinoinensis*	3	0	0
Salicaceae	*Salix exigua*	3	0	0
Asteraceae	*Baccharis neglecta*	3	0	0
Oleaceae	*Fraxinus berlandieriana*	3	0	0

Table 2. Results of no-choice host range tests for *Rhizaspidiotus donacis,* values are mean numbers of scale insects per plant (Goolsby et al. 2009).