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Biocontrol Science and Technology Publication details, including instructions for authors and subscription information: http://www.informaworld.com/smpp/title~content=t713409232 Pre-introductory risk assessment studies of Gonatocerus ashmeadi (Hymenoptera: Mymaridae) for use as a classical biological control agent against Homalodisca vitripennis (Hemiptera: Cicadellidae) in the Society Islands of French Polynesia Julie Grandgirard^a; Mark S. Hoddle^b; Jerome N. Petit^a; Diana M. Percy^a; George K. Roderick^c; Neil Davies^a ^a Richard B. Gump South Pacific Research Station, University of California, Berkeley, Moorea, French Polynesia ^b Department of Entomology, University of California, Riverside, CA, USA ^c Environmental Science, Policy and Management, University of California, Berkeley, CA, USA

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Pre-introductory risk assessment studies of Gonatocerus ashmeadi (Hymenoptera: Mymaridae) for use as a classical biological control agent against Homalodisca vitripennis (Hemiptera: Cicadellidae) in the Society Islands of French Polynesia

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Abstract

Homalodisca vitripennis (Germar) (=H. coagulata [Say]) (Hemiptera: Cicadellidae) invaded French Polynesia in 1999. A classical biological control program against H. vitripennis was initiated in 2004 aiming to introduce the exotic egg parasitoid Gonatocerus ashmeadi (Girault) (Hymenoptera: Mymaridae) to the Society Islands archipelago. Prior to any release, two risks were assessed: (a) continued uncontrolled H. vitripennis spread and proliferation in French Polynesia, and (b) non-target impacts by G. ashmeadi on indigenous French Polynesian cicadellids. The primary risk of H. vitripennis is its potential to vector the lethal plant bacterium, Xylella fastidiosa. While the presence of X. fastidiosa in French Polynesia has not yet been demonstrated, the presence of uncontrolled H. vitripennis greatly elevates the risk of a disease outbreak and thus represents a major threat for numerous plant species. Assessing the risk of G. ashmeadi introduction for native cicadellids first required an inventory of the Cicadellidae of the Society Islands, resulting in at least 14 cicadellid species (nine not previously recorded). The risk to these species of attack by G. ashmeadi was assessed using four criteria: (1) their phylogenetic relationships to known hosts of G. ashmeadi, and their similarity in (2) body size, (3) egg laying biology, and (4) ecology. All indigenous cicadellid species found were considered to be at low risk of attack because they differed greatly from all known hosts for G. ashmeadi: (1) none of the indigenous species are in the tribe Proconiini, (2) all were very small and, when possible to determine, (3) lay tiny single eggs, which (4) are deposited on the undersides of leaves of trees. These results persuaded the French Polynesian Government that the benefits of establishing G. ashmeadi for H. vitripennis control outweighed the serious potential risks associated with either delaying release or not releasing G. ashmeadi in French Polynesia. Releases of G. ashmeadi in Tahiti began in May 2005.

Keywords: Cicadellidae, ecological risk, invasion potential, invasive species, decision tree, non-target impacts, Xylella fastidiosa

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Introduction

The glassy-winged sharpshooter, Homalodisca vitripennis (Germar) (=H. coagulata [Say]) (Hemiptera: Cicadellidae), is indigenous to southeastern USA and northeastern Mexico (Triapitsyn & Phillips 2000). As an efficient vector of the xylemdwelling bacterium, Xylella fastidiosa, H. vitripennis is a major pest of agricultural, ornamental, and indigenous plants in its home and introduced ranges causing substantial economic loses in exotic crops such as European grapes (i.e. Pierce's disease) and citrus (i.e. citrus variegated chlorosis) (Wells et al. 1987; Redak et al. 2004). For host-plant records and feeding biology of this insect, see Hoddle et al. (2003) and Brodbeck et al. (1993).

Homalodisca vitripennis was first recorded in Tahiti (Society Islands of French Polynesia) in 1999 (Grandgirard et al. 2006). It reproduced rapidly and spread very quickly among the islands and archipelagos of French Polynesia. The species is currently found on all islands in the Society archipelago, on Nuku Hiva in the Marquesas, and on Tubuai and Rurutu in the Australs (Grandgirard et al. 2006; Petit et al. 2007). In Tahiti and Moorea, H. vitripennis populations have reached densities far exceeding those observed in its indigenous range or even in California where this pest invaded in late 1980s. Homalodisca vitripennis has caused several problems in French Polynesia. Its excessive watery excretion causes 'rain' under shade trees, hence the local name 'mouche pisseuse' (pissing fly), large numbers enter houses at night attracted to the lights, and it generates dried whitish excrement on plants/fruits, and retards plant and fruit growth (Grandgirard et al. 2006). While X. fastidiosa has not been recorded in French Polynesia, many plants are asymptomatic reservoirs of the bacterium and so it could already be present or might soon arrive from infected regions (notably the Americas) (Raju et al. 1983; Hopkins & Adlerz 1988). Recent laboratory and field experiments revealed that H. vitripennis might also have a negative impact on populations of generalist indigenous arthropod predators, in particular, species of indigenous spiders. Mortality of two spider species (the indigenous crab spider, Misumenops melloleitao, and the pan-Pacific orb-weaving spider, Cyrtophora moluccensis) was observed after they attacked H. vitripennis individuals (Suttle & Hoddle 2006). Field surveys revealed a decrease in M. melloleitao in areas where H. vitripennis has become common.

In addition to the real problems in French Polynesia and elevated risk of *X. fastidiosa* outbreaks, the immense propagule pressure emanating from Tahiti represents a major invasion threat to other South Pacific countries. Adult *H. vitripennis* have been found on airplanes originating from Tahiti arriving in Japan and in Australia. As predicted by invasion models (Hoddle 2004a), this insect has confirmed its high invasion potential in the South Pacific becoming established in Hawaii in 2004 (Hoover 2004), Easter Island (Chile) in 2005 (Sandra Ide, pers. comm. 2005), and the Cook Islands in 2007 (Disna Gunawardana personal comm. 2007). There was thus an urgent need to control *H. vitripennis* in French Polynesia and classical biological control appeared to be the most appropriate solution for permanently controlling this pest.

Based on the results of a long-term classical biological control program conducted by the University of California at Riverside, the California Department of Food and Agriculture, and USDA-ARS in southern California, the most promising natural enemy considered for release in French Polynesia was the solitary endoparasitoid *Gonatocerus ashmeadi* (Girault) (Hymenoptera: Mymaridae). This egg parasitoid is the dominant parasitoid attacking H. vitripennis in southern California (Pilkington et al. 2005). Gonatocerus ashmeadi is indigenous to southeastern USA and northeastern Mexico (Triapitsvn et al. 1998) where it is a common and very effective parasitoid associated with H. vitripennis. Gonatocerus ashmeadi is a specialist parasitoid that is only known to attack cicadellid eggs of species in the new world tribe Proconiini – this is true of both indigenous and colonized areas (Triapitsyn et al. 1998; Logarzo et al. 2003). In tropical areas where temperatures are elevated during the whole year, H. vitripennis correspondingly reproduces year-round as opposed to more temperate areas where fewer discrete generations per year of this pest are observed. California, for example, has approximately two H. vitripennis generations per year (Pilkington et al. 2005). Environmental conditions in French Polynesia were predicted to be favorable for G. ashmeadi allowing parasitoids to reproduce continuously throughout the year; effective control of *H. vitripennis* was thus expected (Pilkington & Hoddle 2006a,b). Rapid population suppression of H. vitripennis by G. ashmeadi was observed in Hawaii (suppression >90%) (Bautista et al. 2005) reinforcing anticipation of a positive outcome in French Polynesia given the climatic and habitat similarities.

In September 2004, the classical biological control program against H. vitripennis commenced when populations of G. ashmeadi from the University of California Riverside were established in the Service du Developpement Rural (SDR) quarantine facility at Papara (Tahiti). Before releasing this exotic parasitoid in the wild, preliminary studies were conducted of the potential impact of G. ashmeadi on indigenous species. These studies examined other species in the family Cicadellidae, which are the only species predicted to be at risk based on the known host specificity of the parasitoid. Such studies of risk assessment are very important because the introduction of a novel exotic species in a new environment might endanger the indigenous fauna, especially in small isolated islands such as those of French Polynesia (Perrings et al. 2002). The most egregious example of a 'biological control disaster' in French Polynesia was the unintentional extirpation of indigenous Partula snails on many islands by the predatory snail, Euglandina rosea, which was released for the biological control of the giant African land snail, Achatina fulica, in 1974 in Tahiti and in 1977 in Moorea (Murray et al. 1988). As a result of such 'accidents', biological control programs for arthropod pests are consequently now held accountable for greater levels of safety than has been required in the past (Simberloff & Stiling 1996; Van Driesche & Hoddle 1997; Hoddle 2004b; Stiling 2004). Determination of host specificity and assessment of expected levels of safety and impact are rapidly emerging as essential new components in the classical biological control of arthropod pests (Hopper 2001; Lynch et al. 2001). This article presents the steps in the risk assessment decision-making process for the release of the parasitoid G. ashmeadi against H. vitripennis in French Polynesia.

Materials and methods

Inventory of indigenous cicadellids in the Society Islands

Five islands within the Society Islands group were surveyed: the Windward Islands group: (1) Tahiti, and (2) Moorea between June 2004 and March 2005, and the Leeward Islands group: (3) Huahine, (4) Raiatea, and (5) Bora Bora, in March 2005. Different habitats and altitudes were sampled in the surveyed islands. Indigenous plant species were sampled by sweep netting at selected sites that had suitable

indigenous vegetation. Collected cicadellids were collected by aspiration, and placed in labeled vials with 95% ethanol. Some specimens were also collected using yellow pan water traps (yellow bowls 4 cm in height by 12 cm in diameter filled with water and soap ($\approx 10\%$) placed on the soil). When possible, cicadellids were identified to genus or species by comparison with collections of named specimens held at the British Museum of Natural History in London.

Assessment of ecological risks associated with releasing G. ashmeadi in the Society Islands archipelago in French Polynesia

Phylogenetic criteria. Gonatocerus ashmeadi has a relatively narrow host range that appears to be taxonomically restricted to the cicadellid tribe Proconiini. All records indicate that it parasitizes only the eggs of cicadellids in the tribe Proconiini in its home range (southeastern USA and northeastern Mexico) and introduced range in southern California (Triapitsyn et al. 1998; Logarzo et al. 2003). Therefore, cicadellids in the tribe Proconijni were considered at higher risk for attack than species in other tribes.

Size criteria. Gonatocerus ashmeadi is a large ($\sim 2 \text{ mm}$) solitary endoparasitoid that lays single eggs inside individual H. vitripennis eggs. Host eggs are typically laid in masses or clutches with two to 32 eggs (often around 10-12 eggs is a common clutch size) constituting an egg mass. Host eggs are laid side-by-side under the epidermis on the undersides of leaves, are readily visible (Irvin & Hoddle 2004), and usually covered with brochosomes, a chalky-white material (Velema et al. 2005). All known hosts of G. ashmeadi are proconiine cicadellids of moderately large size ($\sim 1.2-2$ cm in length) laying fairly large eggs ($\sim 2.5-3$ mm in length) in large obvious clutches. In southern California, small indigenous non-proconiine cicadellids (<7 mm) lay very small eggs that are almost impossible to detect visually. Eggs that are oviposited into plant material tend to be scattered individually on the undersides of leaves (occasionally petioles or very young stems are used as oviposition substrates). Eggs of this small size, which are laid in this manner, are not recognized by G. ashmeadi as being suitable host eggs (Hoddle & Boyd 2005). Even if they are recognized and attacked, the eggs are too small to support successful parasitoid development (Hoddle & Boyd 2005). Therefore, the size of non-target cicadellids in the Society Islands has been used as an additional source of information for assessing risk of attack by G. ashmeadi. Consequently, all collected indigenous cicadellids were measured from the anterior most tip of the head to the most distal tip of the abdomen, and the size range for each species was recorded.

Egg laying biology and ecology criteria. A posteriori non-target impact studies conducted on cicadellids indigenous to southern California have demonstrated that the risk of *G. ashmeadi* attacking non-target species depends greatly on the egg laying biology of these cicadellids and the plant part used for oviposition, and to a lesser extent, the type of host plant (Hoddle & Boyd 2005). *Gonatocerus ashmeadi* was tested against three species of indigenous cicadellids with different egg laying habits: the smoke-tree sharpshooter *Homalodisca liturata* Ball (big eggs laid in masses on leaves of trees in a manner similar to *H. vitripennis*), the Californian blue–green sharpshooters *Graphocephala atropunctata* Signoret (small eggs laid singly on young stems and petioles) and the green sharpshooter *Draeculacephala minerva* Ball (small eggs laid in clusters on grass similar to *H. vitripennis*). Laboratory experiments (choice and no-choice) and field data have demonstrated that *G. ashmeadi* attacked only the eggs of *H. liturata* (Hoddle & Boyd 2005). Eggs of *D. minerva* and *G. atropunctata* are not attacked by *G. ashmeadi* in the field or lab (Boyd, unpublished). *Gonatocerus ashmeadi* appears to parasitize cicadellids with similar sized eggs and egg laying habits to *H. vitripennis* (i.e. eggs in masses on the undersides of leaves of trees and bushes).

This information on host exploitation by *G. ashmeadi* was used to develop a dichotomous risk-decision tree to assist in the identification of indigenous cicadellids in French Polynesia that would be expected to be at risk from unwanted attack by *G. ashmeadi* (Figure 1). This risk decision tree helped to ascertain the amount of micro-habitat sympatry with *H. vitripennis* and the subsequent likelihood of *G. ashmeadi* encountering non-target host eggs on various host plants. The risk decision tree was used to guide, and expedite decisions on which indigenous species should be surveyed and prioritized for potentially difficult, time consuming, and costly host specificity testing. The practical utility of this tree was extremely high when used at meetings and in documents to explain to government officials overseeing regulatory aspects of assessing the risk posed by natural enemies to indigenous species in French Polynesia.

The dichotomous risk-decision tree (Figure 1) for indigenous French Polynesian cicadellids comprises three main steps based on biological and ecological knowledge of potential host species and how foraging *G. ashmeadi* are most likely to respond to encounters with potential non-target hosts: (1) host-plant utilization by indigenous cicadellids (i.e. grasses vs. trees or shrubs); (2) part of plant used preferentially for oviposition (i.e. leaves vs. stems); and (3) oviposition habit (i.e. deposition of single eggs in host plants vs. egg masses). For example, the risk decision tree predicts that if

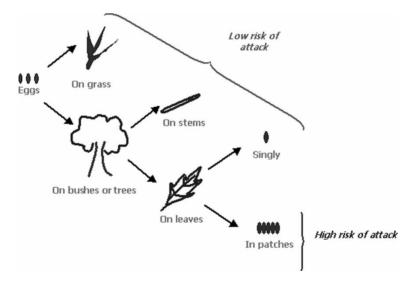


Figure 1. The dichotomous risk-decision tree used to determine the risk posed by *Gonatocerus ashmeadi* releases on cicadellids indigenous to French Polynesia. This tree enabled rapid assessment of the risk of attack by *G. ashmeadi* on eggs of indigenous cicadellids and was extremely useful for explanation of risk to regulatory officials from the French Polynesian Government who ultimately controlled the decision to release *G. ashmeadi* for classical biological control of *H. vitripennis*.

indigenous cicadellids lay single eggs on grasses, stems or leaves of bushes and trees the risk of non-target attack by *G. ashmeadi* is considered to be low. Conversely, for indigenous cicadellids that lay their eggs on leaves of trees and bushes in aggregated clutches, the risk of attack by *G. ashmeadi* is predicted to be high.

In an attempt to obtain accurate data on the oviposition ecology of indigenous French Polynesian cicadellids that would produce useful predictions from the riskdecision tree, stems and leaves from three species of indigenous trees, *Weinmannia parviflora*, *Metrosideros collina*, and *Vaccinium cereum*, which are known hosts of some species of indigenous cicadellids (Osborn 1934) were collected from different sites in Tahiti (i.e. Mount Marau, Fare Mato, Hamuta, and Te Maru Ata). Samples were held in the laboratory, indigenous cicadellid nymphs were reared out, and plant parts from which nymphs emerged were examined microscopically for emergence holes to ascertain if eggs were oviposited singly or in clutches or masses. Foliage samples were only collected from trees that were sampled by sweep netting and had either indigenous adult or nymphal cicadellids. This indicated that the plant being surveyed was a potential host for indigenous cicadellids, especially if nymphs were found.

Results

Inventory of indigenous cicadellids in the Society Islands

Previously reported species from the Society Islands include: Tharra tahitiensis (probably indigenous) (Nielson 1975) (= fassus tahitiensis = fassus insularis Osborn 1934), Tharra nitida (Nielson 1975), Exitianus plebeius (probably nonindigenous) (Ross 1968) (= Nephotettix plebius Osborn 1934), Balclutha virdinervis Matsumura (Knight 1987), and Sophonia orientalis Webb and Viraktamath (2004) (= S. rufofascia) (nonindigenous) (Polhemus 2001). We collected a total of 187 cicadellid specimens (Table I). Of these, 156 specimens were identified, constituting at least 14 different species: eight species were likely indigenous, three were nonindigenous, and three were of undetermined origin and could be either exotic or indigenous (Table II). Except for H. vitripennis, no Proconiini cicadellids were found, and all collected specimens were smaller than 10 mm. Unidentified specimens will likely be a mixture of endemic and

Island Site		Maximum altitude (m)	Nb of specimens collected		
Tahiti	Fare Mato (Aorai)	1400	36		
	Hamuta	900	13		
	Jardin Vaipahi	0	1		
	Marau	1400	87 (59 Exitianus sp.)		
	Taharaa	925	1		
	Tahiti Iti	900	5		
	Te Maru Ata	800	20		
Moorea			5		
Raiatea	Tehuri	750	2		
	Mt Oropito	450	1		
	Trois cascades	50	3		
Huahine	Traversière	200	2		
	Mt Puhaerei	462	9		
Bora Bora	Mt Mata Pupu	236	2		

Table I. S	Sites surveyed	as part of the	inventory of	f cicadellids in t	the Society Islands.
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Ta	t Distribution] At: 20:51 27 December 2007 In Distribution of the competent of the competent of the competence of the co	collected between June 2004	and March 200	5 in the Society 1	Islands and identified at the t	ime of release decis	ion.
	ntent			Number of			
Status	Syb-family	Species	Size (mm)	specimens	Habitat	Island	Altitude (m)
Indigenous	Coe b diinae	Tharra tahitiensis	6-10	17	Weinmannia parviflora Metrosideros collina	Tahiti, Moorea	800-1430
	н С С С С С С С С С С С С С С С С С С С	Tharra sp. nov. 1	4-7	6	Various indigenous plants	Tahiti	800
	а Ш	Tharra sp. nov. 2	4-7	5	M. collina	Tahiti, Moorea	860
	_: کو	Tharra sp. nov. 3	4-7	7	W. parviflora	Tahiti	860
	ed F	Tharra sp. nov. 4	4 - 7	5	M. collina	Huahine	436
	Deltgcephalinae	Nesophyla? sp. nov. 1	4 - 6	17	W. parviflora	Tahiti	1000
	Iassisae	Batracomorphus sp. (close to B. pellucidus (Osborn))	6-8	6	M. collina	Tahiti	1430
	Selenocephalinae	Dryadomorpha cf. metrosi deri (Osborn) =Calotettix metrosideri	4-7	10	W. parviflora	Tahiti	800-1430
Nonindigenous	Deltocephalinae	Deltocephalus? sp.	4-6	5	grass-feeder, on Paspalum	Tahiti	
1 tonningenous	Dencorophiannae	Exitianus? sp.	1 0	59	grass-feeder	Tahiti	600 - 1400
	Nirvaninae	Sophonia rufofascia	4	3	polyphagous	Tahiti	700-1430
Nonindigenous/ Indigenous?	Deltocephalinae	cf. Balclutha sp.	4-6	9	W. parviflora	Tahiti, Raiatea, Bora Bora	1430
	Typhlocyvinae	Empoasca? sp.	3	6	grass-feeder?	Tahiti	860-1430
	Scarinae	Gyponana sp.	5-6	4	Polyphagous	Tahiti, Raiatea	0

nonindigenous species. None of the unidentified material belongs to the tribe Proconiini, and all unidentified specimens were small being about the same size as identified specimens. Specimen collections and species identifications are continuing for other islands in French Polynesia.

Almost all genera and species described previously from the Society Islands were recovered during surveys: *Tharra tahitiensis*, *Exitianus* sp. (=*E. plebeius*?), *Balclutha* sp (=*B. virdinervis*?) and *Sophonia orientalis* (Table II) (Osborn 1934; Ross 1968; Nielson 1975; Polhemus 2001). The only species that was not recovered during these surveys was *Tharra nitida* (Nielson 1975). This species is thought to have a highly restricted range, being found previously only on the Temehani Plateau in Raiatea. As part of the ongoing surveys of indigenous cicadellids this site in Raiatea, it is likely this species will be collected in the future.

Among the eight indigenous species identified, only two have been described before from the Society Islands (Osborn 1934): *Dryadomorpha metrosideri* and *Tharra tahitiensis* (Table II). Four new cicadellid species in the genus *Tharra* were collected during these surveys, as well as a species of *Batracomorphus*, which appears very similar to *B. pellucidus* (Osborn), and probably a new species of *Nesophyla* was found as well (Table II). Few specimens of any one species of indigenous cicadellids were collected at any one site, implying that indigenous species are uncommon in areas of indigenous vegetation in which surveys were conducted. The most abundant indigenous species found were *T. tahitiensis* and *Nesophyla* sp. (Table II). Survey results suggest that the most diverse cicadellid group in the Society Islands is the genus *Tharra* (subfamily Coelidiinae). Detailed taxonomic work on this genus with collected survey specimens is planned.

Sophonia orientalis (=S. rufofascia) (Webb & Viraktamath 2004), the two-spotted leafhopper, is an introduced exotic cicadellid and was very common in all habitats surveyed. One collected species of cicadellid, *Gyponana* sp. belongs to the subfamily Scarinae and is a new record for the Society Islands. Scarinae are indigenous to the New World, and this species is therefore most likely to be an accidentally introduced nonindigenous species. Species in the genera *Balclutha* and *Exitianus* (both in subfamily Deltocephalinae) are known from the Society Islands (Ross 1968; Knight 1987), and species representation is widespread, with species being found throughout Micronesia and Polynesia, as well as other parts of the world. The indigenous or nonindigenous status of collected species in these two genera from French Polynesia will require sampling throughout the known range and population comparisons with genetic analyses.

Assessment of ecological risks associated with releasing G. ashmeadi in Society Island archipelago in French Polynesia

Phylogenetic criteria. None of the indigenous or potentially indigenous species of cicadellids known from the Society Islands are in the tribe Proconiini to which *H. vitripennis* belongs and which is the host clade for *G. ashmeadi*, and even in the Subfamily Cicadellinae (Table II).

Size criteria. All the cicadellids found in Society Islands were small (Table II). The largest indigenous species, T. tahitiensis, is between 6 and 10 mm in length ($\sim 2/3$ the

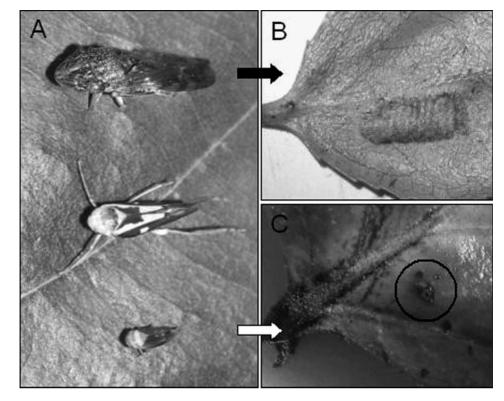


Figure 2. (A) Comparison of the size of the target cicadellid *Homalodisca vitripennis* (on the top) and two non-target cicadellids from the Society Islands: *Tharra tahitiensis* (in the middle, the biggest indigenous cicadellid found) and the undescribed species sp. nov. G. (on the bottom, which represents the common size of indigenous cicadellids found). (B) and (C): comparison of the eggs of the target cicadellid *Homalodisca vitripennis* (B, large eggs in a clutch or egg mass) and the undescribed species sp. nov. G (C, tiny single eggs) laid in *Vaccinium cereum*.

size of *H. vitripennis*) (Figure 2). All other *Tharra* species, including the undescribed species, range $\sim 4-8$ mm in length (Figure 2).

Egg laying biology and ecology criteria. Almost all the indigenous cicadellid species collected (nymphs and adults) were collected on indigenous trees or shrubs, in particular, *Weinmannia parviflora* and *Metrosideros collina*, and few have been collected from grasses (Table II). These collections suggest that most indigenous cicadellids preferentially feed and oviposit in trees and shrubs (see first step of the risk decision tree) which could potentially expose their eggs to foraging *G. ashmeadi* (Figure 1).

A total of 1055 leaves and stems of indigenous vegetation were collected and held for cicadellid emergence. Three *T. tahitiensis* nymphs were reared from small *W. parviflora* branches (stem+leaves), which suggests that this species reproduces on trees (first step of the risk decision tree) (Figure 1). Two nymphs were obtained from one branch and one nymph from another branch, which suggests that the eggs of this indigenous species are laid singly and not in clutches or masses (third step of the risk-decision tree). One nymph of *Nesophyla* sp. nov. 1 (4–6 mm) emerged from a leaf of *Vaccinium cereum* (second step of the risk-decision tree). Microscopic examination of leaves revealed emergence holes from which indigenous cicadellids emerged strongly suggesting that females lay a tiny single egg (<1 mm) on the underside of leaves (third step of the risk-decision tree) (Figure 2). The risk-decision tree indicates that these two indigenous species are at very low risk of attack by *G. ashmeadi* because of the habit of laying small single eggs. It is very likely that all species in the *Tharra* genus lay eggs similarly, and as a consequence all *Tharra* species found are probably at low risk of attack (i.e. six of the eight indigenous cicadellids identified thus far). No other nymphs emerged from the other 1055 plant samples indicating indigenous cicadellid eggs are most likely laid at low densities at the time surveys were conducted, as just 0.2% of collected indigenous plant material yielded cicadellids.

These results were presented to the Council of Ministers and the Conservation Committee in April 2005 for a decision to release *G. ashmeadi* in French Polynesia against *H. vitripennis* in May 2005. Before releasing *G. ashmeadi* in other archipelagos of French Polynesia (i.e. the Marquesas, Australs, and potentially the Tuamotus), a similar, detailed risk assessment study was recommended for each island group.

Discussion

The major environmental risk posed by a biocontrol program to combat *H. vitripennis* in French Polynesia is that the biocontrol agent, *G. ashmeadi*, might attack non-target cicadellids perhaps leading to the loss of rare and little known endemic species. We focused initial studies on non-target impact assessment for indigenous cicadellids in the Society Islands archipelago, as these would be the first islands to receive releases of *G. ashmeadi*. Only five cicadellid species (excluding *H. vitripennis*) had been recorded previously from the Society Islands. Our survey found at least 14 different species: eight were likely indigenous, three exotic, and three of undetermined origin (exotic or indigenous). The risk of non-target attacks by *G. ashmeadi* was assessed using four overarching and interrelated criteria: phylogenetic relationships between non-target cicadellids and known hosts for *G. ashmeadi*, and similarity in size, egg laying biology, and ecology.

The results revealed that the cicadellids found in Society Islands differed greatly from *H. vitripennis* and other known hosts of *G. ashmeadi*: none of the collected cicadellids is closely related to *H. vitripennis*. No representatives in the tribe Proconiini, or even in the subfamily Cicadellinae were found. All collected cicadellid species were very small compared to *H. vitripennis* ($\sim 2/3$ the size or smaller). When determined, the eggs laid on leaves of trees and shrubs were also small compared to *H. vitripennis*. The eggs of indigenous cicadellids (i.e. *Nesophyla* sp. nov. 1 and *T. tahitiensis*) are laid singly, and not in clutches that form egg masses typical for *H. vitripennis*.

Collectively, these data were applied to the dichotomous risk-decision tree that indicated that at least seven indigenous cicadellids species (*Tharra* species and *Nesophyla* sp. nov. 1) are at low risk of attack by *G. ashmeadi*. In addition, even if the target cicadellid *H. vitripennis* and the non-target cicadellids are found in the same habitat, the parasitoid might not recognize the indigenous cicadellid eggs as a potential host, because the chemical and physical cues it may use to locate host eggs might not be present on non-target species. Indeed, chemical cues such as volatiles or contact chemicals left by ovipositing cicadellid females may differ significantly between cicadellid subfamilies (e.g. brochosome usage). Moreover, the physical cues such as

the shape and the size of the indigenous and target species eggs are also different which may further reduce the likelihood of non-target attacks. Secondly, even if *G. ashmeadi* attacked eggs of non-target cicadellids, it might be unable to develop successfully in those eggs because of either the immune defense system of these non-Cicadellinae species, or the small size of the non-target species eggs are unlikely to provide appropriate resources for the development of *G. ashmeadi* (both in quantity and quality of resources). Therefore, the risk of *G. ashmeadi* attacking indigenous cicadellids inhabiting the Society Island group identified thus far can be considered to be very low.

While the risks are low they are not zero and therefore any decision to introduce a biocontrol agent (i.e. an exotic species) must also take into account the risk of NOT releasing the biocontrol agent. In other words, we also had to assess the ecological risks associated with uncontrolled *H. vitripennis* spread and proliferation in French Polynesia.

Potential acquisition and transmission of the lethal plant bacterium X. fastidiosa to the Polynesian flora. A major ecological risk associated with unregulated high density *H. vitripennis* populations in French Polynesia is the potential acquisition and vectoring of *X. fastidiosa* to agricultural, ornamental, and indigenous plants. In introduced areas, many plants which develop severe acute or chronic disease symptoms are 'ecologically naive' having had no evolutionary exposure to this bacterium. Typical disease symptoms exhibited by susceptible plant species are 'scorched' appearances, stunted growth, or production of undersized fruit (Hopkins & Purcell 2002).

French Polynesia has imported numerous plant species indigenous to the Americas over a period of many years. It is thus possible that X. fastidiosa is present in French Polynesia in hosts that do not readily express disease. With the arrival of a vector such as H. vitripennis, it is possible that the X. fastidiosa could be acquired and vectored to susceptible host plant species. Host plant surveys for X. fastidiosa have been conducted in Tahiti since September 2004. Three different tests were conducted to detect X. fastidiosa: (1) ELISA tests on Gardenia tahitensis, Cordyline sp., Morinda citrifolia. (2) PCR tests of xylem fluid of periwinkle (Catharanthus rosea), which are highly sensitive to infection with X. fastidiosa, and (3) deployment of sentinel plants and examination of other hosts suspected of expressing disease caused by X. fastidiosa followed by extraction of xylem fluids and culturing of bacteria on selective media for identification. Another detection approach used successfully in southern California (Bextine et al. 2005) is to collect *H. vitripennis* and assay excised heads for bacteria using real time PCR. To date, X. fastidiosa has not been detected in French Polynesia, however, the results of these experiments were not known before the parasitoid release. As plant movement between the Americas and French Polynesia continues, there is a risk that X. fastidiosa will establish and H. vitripennis may then acquire and vector this bacterium to agricultural, ornamental, and indigenous host plants.

Homalodisca vitripennis has been observed feeding on numerous indigenous plant species including Metrosideros collina, Weinmannia parviflora, Glochidion sp. and Dodonea sp. at altitudes ranging 600 and 1400 m in Tahiti. If X. fastidiosa is present in French Polynesia these plants may be susceptible to lethal infection resulting in widespread chronic epidemics could occur. Metrosideros excelsa (Pohutakawa) is indigenous to New Zealand and is a common ornamental in coastal areas of southern

California. Pohutakawa is readily fed on by the indigenous blue-green sharpshooter and occasionally by *H. vitripennis* both of which vector *X. fastidiosa*. Xylem extraction studies have revealed *X. fastidiosa* in 60% of sampled Pohutakawas in some areas of southern California (Boyd, unpublished). The implications of infection are unknown but being monitored, and could be indicative of possible future outcomes for *M. collina* in French Polynesia. This *Xylella-Homalodisca* threat will continue to expand as *H. vitripennis* densities and its range within French Polynesia and the South Pacific increase.

Other risks to Polynesian flora. Even in the absence of X. fastidiosa, H. vitripennis at the densities observed in French Polynesia is likely to have a negative impact on the exotic and indigenous flora. The huge quantities of xylem fluid ingested daily by H. vitripennis nymphs and adults is suspected to weaken plants by inducing water-stress that leads to leaf yellowing, defoliation, and growth retardation (Grandgirard et al. 2006). Dried excreta on fruit appear powdery white and reduce the aesthetic appearance of fresh produce. The leaves of many plants (e.g. Cordyline sp.) are made unsightly by the presence of hundreds of large brown 'scars' which are necrotic tissues resulting from oviposition and subsequent eclosion of H. vitripennis nymphs.

Decision to introduce G. ashmeadi in French Polynesia

The data presented in this paper were those available to the authorities (i.e. the government of French Polynesia) when they made their decision to release G. ashmeadi from quarantine. At the time of the release decision, the risk posed by G. ashmeadi to non-target cicadellids was shown to be low for several specific species and most likely to be low for other indigenous species. Some level of uncertainty persisted, however, because the inventory of indigenous species was incomplete, some collected specimens were still undescribed, and details of the biology/ecology of some species unconfirmed. Yet the uncertainty of non-target attacks by G. ashmeadi and the magnitude of those attacks should they occur could not have been reduced further without very substantial additional cost (i.e. a lot more research). In addition, the failure to reduce H. vitripennis population densities (through the introduction of G. ashmeadi) increased substantially the likelihood that H. vitripennis would invade other islands/countries, and that it might acquire and transmit the lethal plant bacterium X. fastidiosa to the Polynesian flora. Balancing these risks, the French Polynesian Government considered that the benefits of controlling H. vitripennis with G. ashmeadi outweighed possible negative consequences and decided to release G. ashmeadi from quarantine: establishing populations in the field was initiated in May 2005.

In order to provide accurate data on the risks of introducing an exotic arthropod natural enemy, it is essential to develop accurate methods of risk assessment. In the present case, important knowledge on the host range of the parasitoid, *G. ashmeadi*, and the behavior, ecology and taxonomy of indigenous cicadellids from southern California facilitated the development of a dichotomous risk-decision tree. This tree was very helpful in prioritizing research areas and explaining to government authorities and regulators the risk exotic natural enemies posed to the French Polynesian ecosystem. A similar tool and approach can be applied to other island groups or countries where *G. ashmeadi* introduction against *H. vitripennis* is being considered (e.g. Easter Island, the Cook Islands).

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