Engineering an invasion: classical biological control of the glassy-winged sharpshooter, *Homalodisca vitripennis*, by the egg parasitoid *Gonatocerus ashmeadi* in Tahiti and Moorea, French Polynesia

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Abstract The glassy-winged sharpshooter, *Homalodisca vitripennis* Germar (=*H. coagulata* Say) (Hemiptera: Cicadellidae), invaded Tahiti in 1999 and spread rapidly to the main island groups of French Polynesia becoming an important pest. It threatened agriculture, native biodiversity, and created serious social and recreational problems. Further, massive uncontrolled populations on Tahiti presented an elevated invasion threat to other South Pacific nations. In 2004, a classical biological control program against *H. vitripennis* was initiated in French Polynesia using the highly host-specific egg parasitoid *Gonatocerus ashmeadi* Girault (Hymenoptera: Mymaridae). After risk assessment studies indicated an acceptably low level of risk to non-target species, 13,786 parasitoids were released at 27 sites in Tahiti between May and October 2005. Here we present the results of *G. ashmeadi* and *H. vitripennis* population surveys during the first year of their interaction in French Polynesia (until mid-May 2006). The impact of *G. ashmeadi* on *H. vitripennis* was extremely rapid and high. Parasitism of *H. vitripennis* egg masses by *G. ashmeadi* has averaged 80–100% in Tahiti since the introduction of the parasitoid, and populations of *H. vitripennis* nymphs and adults have decreased by more than 90% since December 2005. Populations of *H. vitripennis* have been successfully maintained at this low level for more than 1 year. The same results were obtained in nearby Moorea where the parasitoid was probably spread by the unregulated transport of plants infested with parasitized *H. vitripennis* eggs. Population monitoring continues in order to determine if a stable equilibrium between the pest and the parasitoid has been reached.

Keywords Classical biological control · Impact assessment · Parasitoid introduction · Pest control · *Xylella fastidiosa*

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Introduction

The glassy-winged sharpshooter, *Homalodisca vitripennis* Germar (=*H. coagulata* [Say]) (Hemiptera: Cicadellidae), invaded Tahiti, French Polynesia in 1999 (Grandgirard et al. 2006). It reproduced and spread very rapidly in French Polynesia and is currently found throughout the Society Islands archipelago and has spread to Nuku Hiva in the

Marquesas archipelago (1,400 km northeast of Tahiti) and to Tubuai and Rurutu (600 km south of Tahiti) in the Australs archipelago (Grandgirard et al. 2006; Petit et al. 2007).

Homalodisca vitripennis is native to the southeastern USA and northeastern Mexico (Triapitsyn and Phillips 2000). Females lay their eggs within plant tissue, usually on the undersides of leaves of shrubs and trees. Adults and the five nymphal stages are xylophagous and extremely polyphagous, having been recorded feeding on more than 150 plant species in 34 plant families (Hoddle et al. 2003). Due to the low nutritional value of xylem, xylophages need to ingest large quantities of fluid. Consequently, *H. vitripennis* can consume up to 100 times its body weight per day (Brodbeck et al. 1993) and copious amounts of watery excreta are produced and continuously discharged as a result of prolific feeding.

In its home and introduced ranges (e.g., California USA), *H. vitripennis* is a major pest of agricultural, ornamental, and native plants because of its ability to vector the lethal xylem-dwelling plant bacterium, *Xylella fastidiosa* (Wells et al. 1987). In French Polynesia, this insect also caused numerous problems (Grandgirard et al. 2006). Firstly, it was a major public nuisance because: (1) massive densities of feeding adults and nymphs generated high quantities of excreta that literally rained from trees (hence its common French name 'mouche pisseuse', the pissing fly), and (2) numerous flying adults invaded houses and shops at night attracted to the lights. Secondly, thousands of adults and nymphs continuously feeding year round were suspected of retarding plant growth and causing declines in fruit production. Thirdly, *H. vitripennis* as a vector of *X. fastidiosa* represented a serious potential threat for Polynesian plants (agricultural, ornamental, native). While this bacteria has not been recorded in French Polynesia, many plants are asymptomatic reservoirs of the bacteria and could arrive in Tahiti (or could already be present) with imports from infected regions (notably the Americas). Finally, large uncontrolled *H. vitripennis* populations in French Polynesia represented an invasion threat to neighbors and trading partners. The immense population densities in French Polynesia made it impossible to contain this pest at ports and live adults, nymphs, and eggs on plants were often found in boats and planes leaving Tahiti. Consequently, *H. vitripennis* not only spread within French

Polynesia but also invaded Easter Island in 2005, likely as eggs transported on ornamental plants on flights from Tahiti (Sandra Ide, personal communication).

To minimize all of these problems, the feasibility of a classical biological control program against *H. vitripennis* using the exotic egg parasitoid *Gonatocerus ashmeadi* Girault (Hymenoptera: Mymaridae) was assessed. Classical biological control appeared the most appropriate solution for controlling this pest in French Polynesia because this technology has the potential to: (1) significantly and permanently reduce high pest population densities, (2) act without continuous human assistance over a wide geographic area, and (3) track *H. vitripennis* incursions into native habitats. The classical biological control program against *H. vitripennis* with *G. ashmeadi* was initiated in French Polynesia in 2004 in collaboration between the University of California (through its Gump South Pacific Research Station on Moorea and the Department of Entomology at Riverside) and the French Polynesian Department of Agriculture. Importantly, the program included non-target risk assessment prior to parasitoid release (Grandgirard et al. 2007a).

Gonatocerus ashmeadi is a solitary endoparasitoid attacking eggs of Proconiini sharpshooters (Cicadellidae: Cicadellinae: Proconiini) (Triapitsyn et al. 1998; Logarzo et al. 2003). It is native to southeastern USA and northeastern Mexico (Triapitsyn et al. 1998) where it is a common and effective parasitoid of *H. vitripennis* eggs. *G. ashmeadi* has several attributes that make it a promising candidate biological control agent against *H. vitripennis*: (1) egg to adult development is about four times faster than that of *H. vitripennis* (2 weeks vs. 2 months) (Pilkington and Hoddle 2006a; Setamou and Jones 2005); (2) its sex ratio is female-biased 1:3 (male:female) (Irvin and Hoddle 2005); (3) *G. ashmeadi* attacks *H. vitripennis* eggs on many different plant species in agricultural, urban, and wilderness areas (M. S. Hoddle, unpublished). In addition, it is the dominant parasitoid attacking invasive populations of *H. vitripennis* in southern California (Pilkington et al. 2005) and tropical climates appear very favorable for its development and reproduction (Pilkington and Hoddle 2006a, b). Rapid population suppression of *H. vitripennis* by *G. ashmeadi* was observed in Hawaii, where this self-introduced parasitoid was found in late 2004

(presumably arriving with host eggs). Initial parasitism estimates of *H. vitripennis* eggs in Hawaii were between 90 and 100%, and pest populations declined markedly and never reached the high levels observed in Tahiti (Bautista et al. 2005). Similar levels of control of *H. vitripennis* by *G. ashmeadi* were expected in French Polynesia because of climatic and habitat similarities.

Risk assessment studies for non-target cicadellid species in French Polynesia commenced in May 2004 with the compilation of an inventory of French Polynesia's native cicadellid fauna. The risk of attack by *G. ashmeadi* was assessed by comparing native cicadellids with other known hosts for *G. ashmeadi* according to four criteria organized as a dichotomous decision tree: (1) taxonomy (parasitoid host range is limited to the tribe Proconiini), (2) morphology (body size), (3) egg laying behavior (single eggs or clusters), and (4) ecology (host-plants: grasses, shrubs, or trees). Based on the results, all native cicadellid species (at least 14 species) were considered to be at low risk of attack (Grandgirard et al. 2007a).

In April 2005, after careful consideration of the preliminary results from the non-target impacts surveys (particularly from the Society Islands), the French Polynesian Government decided that the risk of releasing *G. ashmeadi* was acceptably low compared to the continued spread of the pest and potential acquisition and vectoring of *X. fastidiosa* (Grandgirard et al. 2007a). The initial release of *G. ashmeadi* was on May 2005 on Tahiti, the major hub for domestic and international transport, and the island where *H. vitripennis* populations had reached the highest densities (Petit et al. 2007). Here, we present data on the population-level impacts on *H. vitripennis* in Tahiti and on the adjacent island of Moorea. Levels of parasitism of *H. vitripennis* eggs by *G. ashmeadi* were monitored and the abundance of *H. vitripennis* was compared before and after parasitoid releases at both release sites and paired non-release control sites. This Before After Control Impact Paired Series approach (Schmitt and Osenberg 1996) is a very powerful protocol to test ecological impacts caused by specific human-induced perturbations (such as natural enemy releases) rather than other factors influencing population sizes such as natural variation due to climate or topography.

Materials and methods

Parasitoid releases

Parasitoid rearing—*G. ashmeadi* was reared in the quarantine facility of the Service of Rural Development in Papara, Tahiti. The quarantine colony at Papara was established in September 2004 with 400 *G. ashmeadi* adults and parasitized *H. vitripennis* eggs that were imported from the *H. vitripennis* biological control program being run at the University of California, Riverside.

A nursery was established to provide plants for *H. vitripennis* oviposition and eggs were harvested by picking leaves and presenting them in vials of water for parasitoids to attack. To accomplish this, adult *H. vitripennis* were captured in the field and placed in screen mesh cages (70 cm × 70 cm × 80 cm) in a greenhouse with 16L:8D and natural temperature and humidity (approximately 26°C and 85% RH). Each *H. vitripennis* oviposition cage contained four host-plants, two green *Cordyline fruticosa* (syn. *C. terminalis*, *Taetsia fruticosa*) (Laxmanniaceae), for egg laying and two black eyed peas *Vigna senensis* (Leguminosae) for feeding. About 150 *H. vitripennis* adults were maintained in cages by daily introductions of field collected material to replace dead individuals. A green *Cordyline* variety was chosen over the red *Cordyline* variety, *Hibiscus rosasinensis* (Malvaceae), and *Morinda citrifolia* (Rubiaceae) because green *Cordyline* was relatively free of pests and diseases, promoted high oviposition rates by *H. vitripennis*, and was suitable for rearing the parasitoid because leaves bearing egg masses did not deteriorate quickly in quarantine after being removed from plants (J. Grandgirard, unpublished data). Plants in oviposition cages were replaced every 2 weeks.

Oviposited *H. vitripennis* egg masses in cages were collected 2 days after oviposition. The part of the *Cordyline* leaf containing the egg mass was excised as a 1 cm × 1 cm piece. Excised pieces of *Cordyline* leaf with egg masses were washed with a cotton wool ball dipped in a 10% bleach solution to kill potential micro-organisms and mites. Disinfested leaves were then rinsed thoroughly in water to remove any bleach residue. A microscope was used to count the number of *H. vitripennis* eggs in harvested egg masses. Weekly egg production

averaged 1,131 ± 167 (mean ± SE) with this rearing method for ten oviposition cages. To ensure maximum longevity, leaf pieces bearing *H. vitripennis* egg masses were inserted in water-saturated floral foam placed in a water-filled plastic box (21 cm × 9 cm × 2.5 cm). Each floral foam containing box held about 20–25 egg masses (i.e., approximately 250 eggs). Foam-containing boxes holding *H. vitripennis* eggs were used for parasitoid rearing.

Gonatocerus ashmeadi rearing was conducted in a controlled climatic quarantine facility with 16L:8D and 26°C, and approximately 72% RH. Parasitoid rearing cages (25 cm × 25 cm × 25 cm) were enclosed in larger cages (70 cm× 70 cm× 80 cm), to minimize the risk of parasitoid escape. Water-saturated foam containing boxes with excised *Cordyline* leaf pieces bearing *H. vitripennis* eggs were placed in rearing cages with new recently emerged (∼24–72 h of age) *G. ashmeadi*. A ratio of one parasitoid [sex ratio of 1:3 (male:female)] to ten *H. vitripennis* eggs was maintained in parasitoid rearing cages. Parasitoids were fed small droplets of honey on a yellow plastic stick (6.3 cm × 3.3 cm), the source of water for drinking was the foam pad bearing leaf pieces with

H. vitripennis egg masses which was watered every day. Adult *G. ashmeadi* were left in cages until death (approximately 7–10 days under these rearing conditions). New adult parasitoids emerged from parasitized *H. vitripennis* egg masses approximately 12 days after initial exposure to parasitoids. At the time of release from quarantine, approximately 20 generations of *G. ashmeadi* had been reared in quarantine with weekly parasitoid production averaging 611 ± 54 adults with a highly female-biased sex ratio (72 ± 1%).

Parasitoid release method and release locations— *G. ashmeadi* was first released in Tahiti on May 2 2005 at Mahina (Fig. 1). The first release consisted of 541 parasitoids. Parasitoids were between 1 and 4 days old, of which 70% were female. All parasitoids had been provisioned with a honey meal before release. Parasitoids were liberated in plastic vials (height: 7 cm, diameter 2.5 cm) that were fixed to tree branches at the pre-selected release site. Vials were opened and parasitoids were left to disperse unassisted from vials. A total of 13,786 parasitoids were released between May and October 2005 at 27 sites located around most of Tahiti.
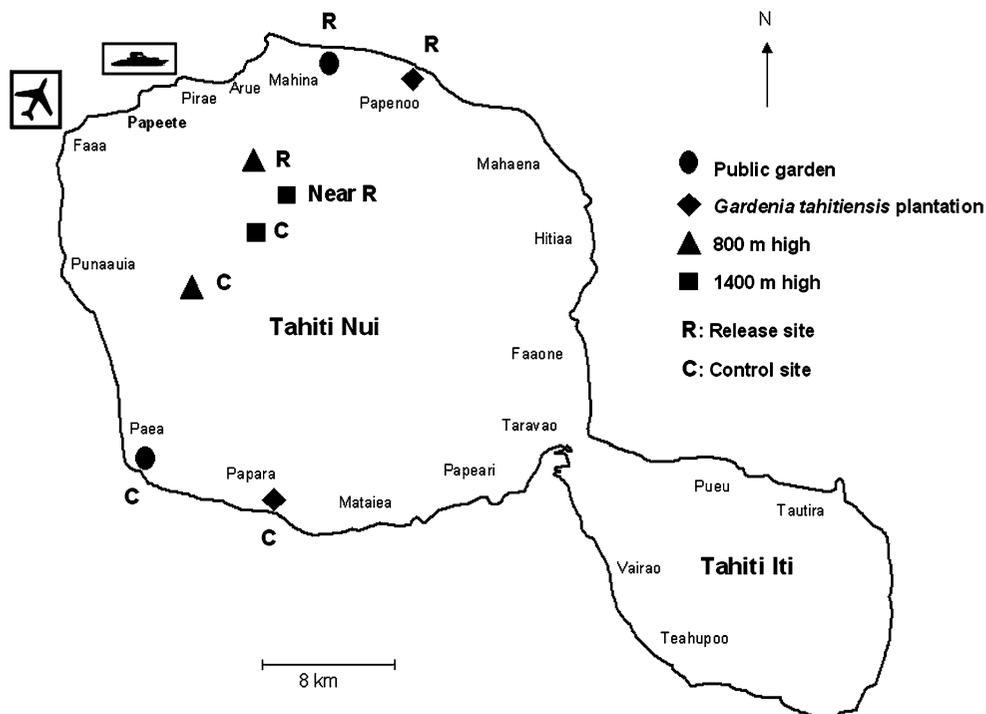
Fig. 1 Locations of the experimental paired-sites in Tahiti

Four paired-sites were regularly monitored in Tahiti between August 2004 and May 2006 (Fig. 1). Paired-sites (release site and non-release control site) were selected for similar climatic conditions, vegetation, pest abundance, and altitude. The Before After Paired Control Series design controls for temporal and spatial variation and the interaction between them. These data, together with the direct observation of parasitism rates, provide strong evidence that host population reductions were due to the parasitoid rather than other factors.

The study sites were located in different habitats to determine the influence of vegetation and topography on *H. vitripennis* densities and *G. ashmeadi* efficacy. Two paired-sites were at sea level. These urbanized areas were comprised almost exclusively of exotic ornamental vegetation. The first sea level paired-site was a public garden located at Mahina (release site) and Paea (control site). The second sea level paired-site was a *Gardenia tahitiensis* (Rubiaceae) plantation located at Papenoo (release site) and Papara (control site). All experimental sites were monitored every 2 weeks for *H. vitripennis* and *G. ashmeadi*. Additionally, two paired-sites were located at altitude in the mountains of Tahiti and were comprised of relatively undisturbed native vegetation. In these mountainous areas climatic conditions were more humid and cooler than at sea level. The first paired-site was located at Pirae at 800 m high (release site) and Punaauia (control site). The second paired-site was at 1,400 m and also located in Pirae (i.e., located along the same trail as the release site at 800 m, it was colonized before *G. ashmeadi* was released there, and will be referred to as the "near release site" in this paper) and Faaa (control site). Because of difficulty of access, these four high altitude sites were monitored approximately once a month. For selected study sites (i.e., Papenoo, Punaauia, and Faaa) temperature and relative humidity were monitored every hour using a Hobo® data recorder station (Hobo H8 Pro Series).

Parasitoids were first liberated at the release study sites at sea level (Mahina and Papenoo). Between 200 and 800 parasitoids were released each week in May and June 2006 to ensure parasitoid establishment. Parasitoids were then released at Pirae (800 m site) between July and October 2005. No parasitoids released at control sites and at the 1,400 m site. In September–October 2005, parasitoids were released at ports of entry and exit in order to reduce the risk of *H. vitripennis* transportation to uninfected islands or countries. During the same period, parasitoids were also released around the island of Tahiti except in the south western part of the island where the control sites at Paea and Papara were located (Fig. 1 and Table 1). The release location, number of parasitoids released, and the timing of releases are detailed in Table 1.

Determining the impact of *G. ashmeadi* on *H. vitripennis*

At each experimental site, *H. vitripennis* parasitism and abundance was monitored on ten individuals of a preferred plant species on which this pest feeds and oviposits. The same ten plants were sampled at each study site at each sampling interval. The plant species sampled were *G. tahitiensis* at the gardenia plantations (Papenoo and Papara), *Scaevola sp.* (Goodeniaceae) in the public gardens (Mahina and Paea), *Metrosideros collina* (Myrtaceae) at 800 m (Pirae and Punaauia), and *Vaccinium cereum* (Ericaceae) at 1,400 m (Pirae and Faaa).

Homalodisca vitripennis egg masses, nymphs, and adults were searched for and counted on each study plant for a fixed time period: 2 min 30 s for adults, 2 min 30 s for nymphs, and 2 min for egg masses. Egg masses were collected and returned to the

Table 1 The number of *Gonatocerus ashmeadi* released in different locations in Tahiti

Location	Period	Number of parasitoids
Experimental sites		
Mahina	May–June 2005	3,090
Papenoo	May–June 2005	3,484
Pirae	July–October 2005	1,652
Communication points		
Port	September–October 2005	1,271
Ferry	September–October 2005	1,317
Airport	September–October 2005	1,495
Global release		
Tahiti Iti	September 2005	900
East coast of Tahiti Nui	September 2005	225
West coast of Tahiti Nui	October 2005	352

laboratory. Harvested egg masses were placed individually in Petri dishes on a water-moistened filter paper, labeled, and maintained at 23°C until *H. vitripennis* nymphs or parasitoids emerged. The percentage of *H. vitripennis* egg masses parasitized was calculated (i.e., the number of parasitized egg masses/total number of parasitized and unparasitized eggs masses × 100). To obtain an accurate percentage parasitism estimate and to reduce bias due to small samples, data from several consecutive dates were used to calculate parasitism when less than five egg masses were collected at a particular date. The percentage of eggs parasitized by *G. ashmeadi* per egg mass (i.e., the number of parasitized eggs identified by a parasitoid emergence hole/total number of parasitized eggs and unparasitized eggs from which *H. vitripennis* nymphs emerged × 100) was determined by microscopic examination of emerged egg masses collected from the field.

On Moorea (this island is immediately adjacent to Tahiti and separated by 17 km of uninterrupted sea), two *G. tahitiensis* plantations located in Pihaena and Hauru were monitored monthly between October 2004 and May 2006 (Fig. 2). These two sites were control sites (i.e., no parasitoids released), and were monitored to provide data on *H. vitripennis* abundance before parasitoid introduction onto Moorea and also to detect whether *G. ashmeadi* would arrive in Moorea from Tahiti prior to the planned release date (either through natural dispersal of adults or unintentional human mediated transport of parasitized eggs on plants).
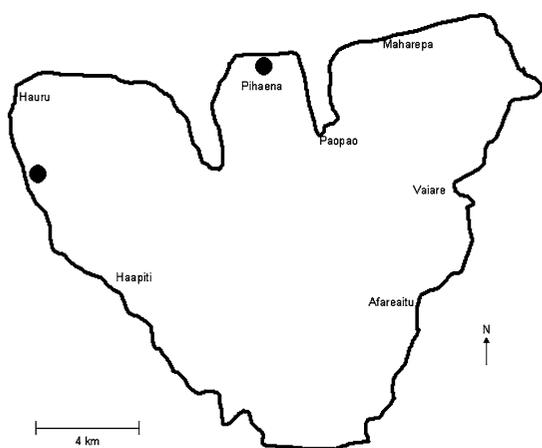
Fig. 2 Locations of the control sites in Moorea

To determine the impact of *G. ashmeadi* on *H. vitripennis* at the island scale, *H. rosasinensis* (Malvaceae) hedges located all around the islands of Tahiti and Moorea, were sampled for 1 min with a sweep net and the number of *H. vitripennis* nymphs recorded by site (see Petit et al. 2007). *H. rosasinensis* is an extremely common plant and a preferred host of *H. vitripennis*. *Hibiscus* monitoring sites were approximately 10 km apart, and the same sites were monitored by sweep net for *H. vitripennis* nymphs every 2 months.

Results

Before *G. ashmeadi* was introduced into Tahiti and Moorea, *H. vitripennis* adults, nymphs, and eggs were found year round at all study sites. The pest was present in very high numbers in urban habitats along the coast (i.e., *Gardenia* plantations and public gardens with ~50 nymphs/tree in Tahiti and 20 nymphs/tree in Moorea). *H. vitripennis* was less abundant at mountainous sites at high altitude (at 800 m there were ~10 nymphs/tree, and at 1,400 m, *H. vitripennis* densities were ~1 nymph/tree) (Figs. 3–6). Before *G. ashmeadi* was released, parasitism of *H. vitripennis* eggs by generalist parasitoids was <1% at all sites. Observed parasitism was due to undescribed species of *Centrodora* (Hymenoptera: Aphelinidae), and two mymarid species *Palaeoneura* sp. and *Anagrus* sp. (Grandgirard et al. 2007b). At Papenoo (*Gardenia* plantation), however, *Centrodora* sp. was found to be abundant and parasitizing on average 30% of the egg masses (Grandgirard et al. 2007b) (Fig. 3).

Impact of *G. ashmeadi* on *H. vitripennis* at sea level sites

Following the release of *G. ashmeadi* in May 2005, parasitism of *H. vitripennis* eggs increased rapidly at sea level in Tahiti. The percentage of egg masses parasitized was higher than 80% in the release sites (Mahina and Papenoo) just 1 month after the parasitoid release (Fig. 3). *G. ashmeadi* invaded the control sites rapidly as parasitized eggs were first found in August 2005 in Paea (public garden) and in September 2005 in Papara (*Gardenia* plantation). At

both the release and control sites, percentage of egg masses parasitized has remained very high during the first year since release (impact data are based on surveys from the first appearance of parasitoid at a site until May 2006). Average parasitism rates were 90% in public gardens (release site: 89.1 ± 2.6%, control site: 91.5 ± 2.5%) and 96% in *Gardenia* plantations (release site: 97.7 ± 1.3%, control site: 94.6 ± 2.5%). At each site, more than 95% of the eggs within a parasitized egg mass are parasitized. In Papenoo, parasitism of *H. vitripennis* egg masses by *G. ashmeadi* and *Centrodora* sp. was recorded. Among parasitized egg masses, *G. ashmeadi* parasitized a large majority of egg masses: 72.1 ± 3.3% against 18.1 ± 3.5% for *Centrodora* sp., and

9.7 ± 1.9% of the egg masses were parasitized by both parasitoid species (i.e., egg masses that contain eggs parasitized by *G. ashmeadi* and eggs parasitized by *Centrodora* sp.). Following colonization of the release and control sites by *G. ashmeadi*, numbers of *H. vitripennis* nymphs decreased markedly to very low densities, with less than one *H. vitripennis* nymph found per tree on average, corresponding to a 98–99% decrease in abundance from pre-release densities (public gardens: 98.5 and 99.1%, *Gardenia* plantations: 99.2 and 98.3%, for release and control sites, respectively) (Fig. 3). Densities of *H. vitripennis* nymphs have remained low since July 2005 to May 2006 at the release sites and since November 2005 at the control sites (i.e., impact was observed
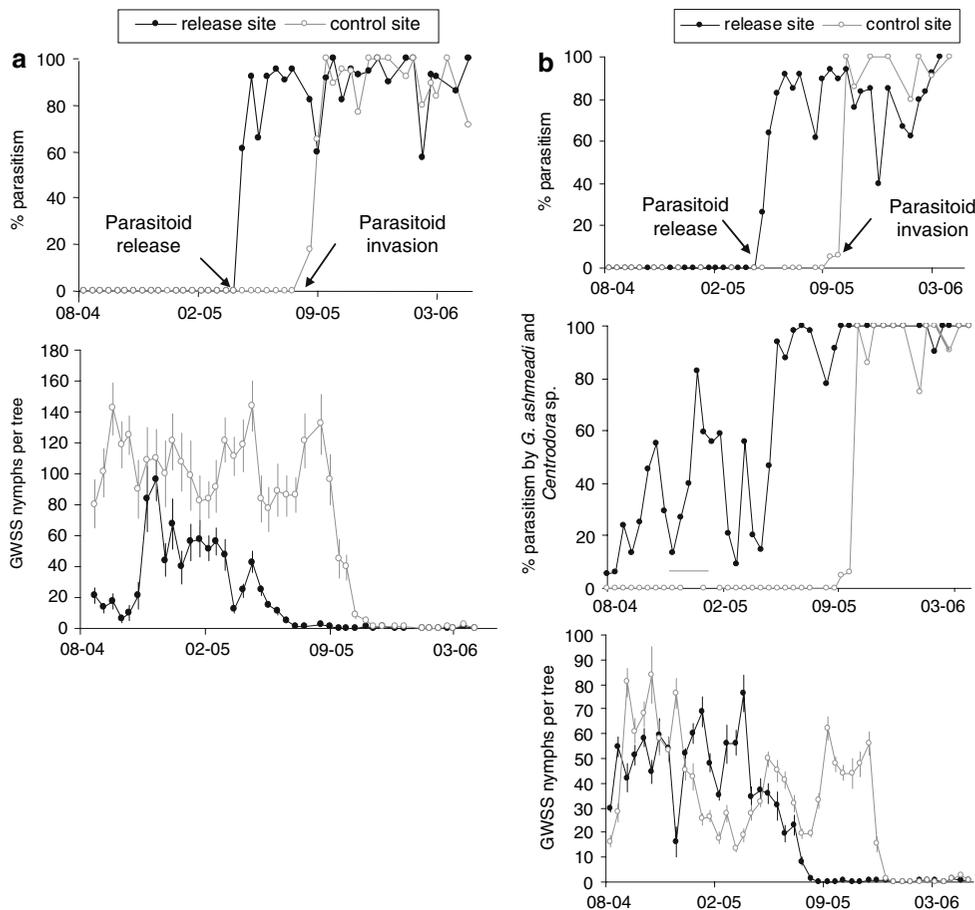
Fig. 3 Impact of *Gonatocerus ashmeadi* on *Homalodisca vitripennis* (*GWSS*) in release and control paired-sites located at sea level in Tahiti. (**a**) In public gardens. *Upper* percentage of GWSS egg masses parasitized by *G. ashmeadi*, *Lower* mean number (±SE) of GWSS nymphs found per tree for 2 min 30

searching. (**b**) In *Gardenia tahitiensis* plantations. *Upper* percentage of GWSS egg masses parasitized by *G. ashmeadi*, *Middle* percentage of GWSS egg masses parasitized by *G. ashmeadi* and *Centrodora* sp., *Lower* mean number (±SE) of GWSS nymphs found per tree for 2 min 30 searching
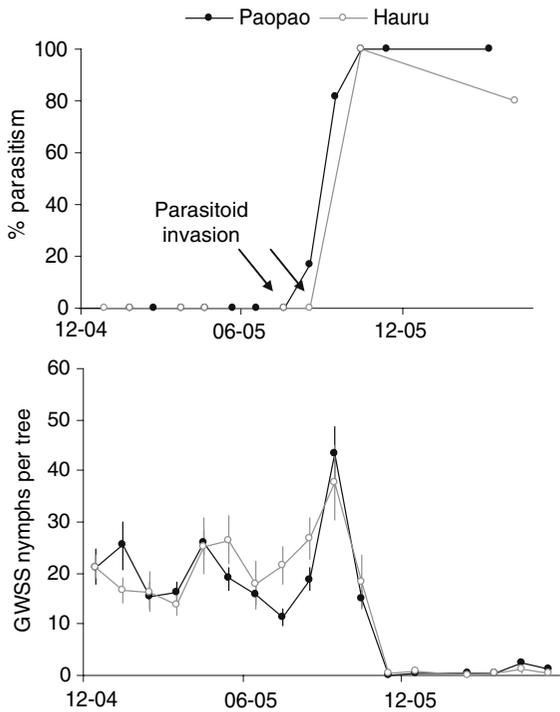
Fig. 4 Percentage of *Homalodisca vitripennis* found in sampled trees that were in the egg (*E*) stage, nymphal (*N*) stage, or adult (*A*) stage at the release site at Papenoo (*Gardenia tahitiensis* plantation). The density of *H. vitripennis* (mean number of adults, nymphs, and eggs/tree) is given for each date. *Gonatocerus ashmeadi* was released in May 2005
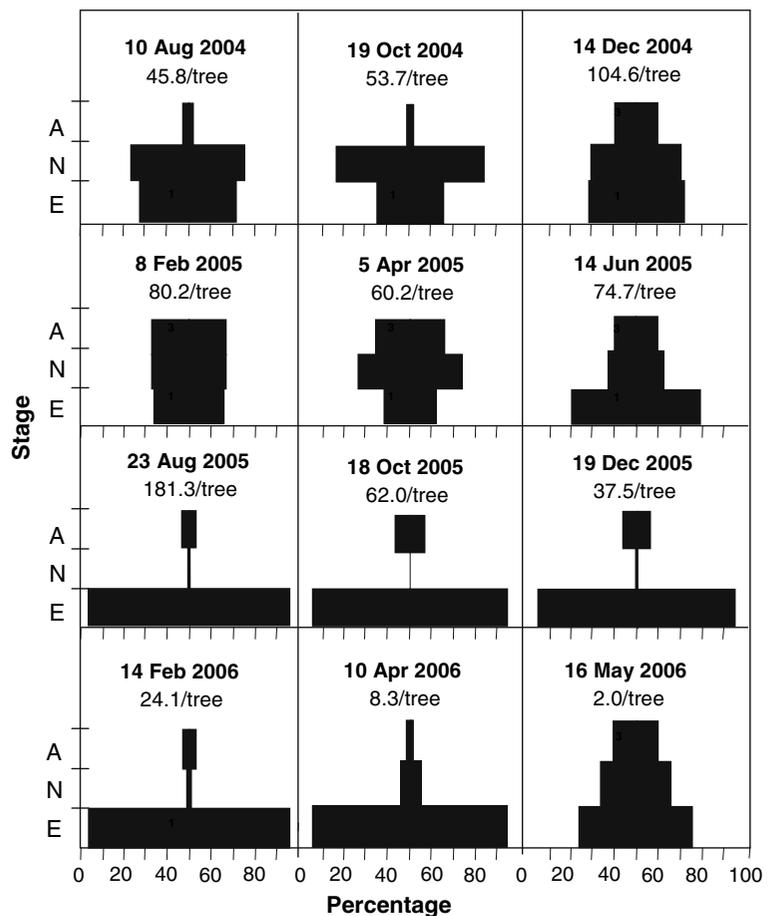
2–3 months after the sites were colonized by *G. ashmeadi*). A similar decrease was observed for adult *H. vitripennis* at the end of 2005-beginning of 2006. This decrease in adult abundance was followed by a decrease in egg mass abundance about 2 months later (February–April 2006). The observed decrease in *H. vitripennis* adult and egg mass densities is approximately 90% that of pre-parasitoid releases [adults: public gardens: 87.4% (release site) and 93.7% (control site), *Gardenia* plantations: 89.9% (release site) and 88.1% (control site); egg masses: public gardens: 93.7% (release site) and 70.9% (control site), *Gardenia* plantations: 90.6% (release site) and 92.2% (control site)].

The abundance of the different stages of *H. vitripennis* demonstrates that the age structures of *H. vitripennis* populations were affected by the introduction of the parasitoid *G. ashmeadi*. The variation of age structures is shown in Fig. 4 at the Papenoo (*Gardenia* plantation) release site. Data

were adjusted to 2 min sampling for each stage and only data obtained every 2 months are shown. Before the introduction of the parasitoid (August 2004–April 2005), nymphs were the most abundant stage (40–60%), followed by eggs (20–45%), and then adults (5–30%). The egg stage was not the most common life-stage found during sampling because eggs are more difficult to find than nymphs and adults, and *H. vitripennis* oviposits on many other host plant species (e.g. *Cordyline* sp.) and nymphs and adults can move from nearby plants. In June 2005, 1 month after the parasitoid release, the age structure of *H. vitripennis* changed: the egg stage became dominant ($\sim$60%), while nymphs represented $\sim$25%, and adult abundance remained relatively constant ($\sim$20%). Between August and December 2005, the abundance of adults was relatively similar to pre-release situation, these adults continued to lay eggs, and there was even an oviposition peak in August 2005. However, due to parasitism, fewer nymphs

Fig. 5 Impact of *Gonatocerus ashmeadi* on *Homalodisca vitripennis* (*GWSS*) in release (or near release) and control paired-sites located in the mountain in Tahiti. (**a**) 800 m altitude. (**b**) 1,400 m altitude. *Upper* percentage of GWSS egg masses parasitized by *G. ashmeadi*, *Lower* mean number (±SE) of GWSS nymphs found per tree for 2 min 30 searching

were successfully emerging and recruitment to this life-stage was significantly reduced. At this time, *H. vitripennis* eggs represented more than 80% of the

population, while nymphs comprised less than 5% of the population. In February–April 2006, the overall density of *H. vitripennis* was low and the age structure changed again. In this generation of adults, abundance was affected by the parasitoid (i.e., all *H. vitripennis* adults that had emerged before the introduction of the parasitoid were now dead). Because nymphal recruitment was reduced by parasitism, replacement of adults that were alive before parasitoid releases was not occurring to any significant extent. The observed decrease in adult densities occurred about 4 months after parasitoids were released all around Tahiti. In the most recent survey (May 16, 2006) adult *H. vitripennis* represented ∼5–10% of the population, nymphs are rare (<5%), and the population was composed primarily of eggs that will be parasitized. By May 2006, the overall density of *H. vitripennis* (∼2.0 *H. vitripennis*/tree) was extremely low and eggs represented about 50% of the population, nymphs 30% and adults 20%.

Gonatocerus ashmeadi invaded Moorea around September 2005 (i.e., prior to planned releases) when parasitized eggs were first found at the control site in Pihaena. The parasitoid likely invaded Moorea as parasitized *H. vitripennis* eggs on ornamental plants transported from the parasitoid-infested areas of Tahiti to Moorea (there is no plant quarantine between Tahiti and Moorea and plant movement is unregulated). The results of surveys were very similar to those obtained in Tahiti. Following colonization of sites by *G. ashmeadi*, parasitism of *H. vitripennis* eggs

Fig. 6 Impact of *Gonatocerus ashmeadi* on *Homalodisca vitripennis* (*GWSS*) in two control sites (Pihaena and Hauru) in Moorea. *Upper* percentage of GWSS egg masses parasitized by *G. ashmeadi*, *Lower* mean number (±SE) of GWSS nymphs found per tree for 2 min 30 searching
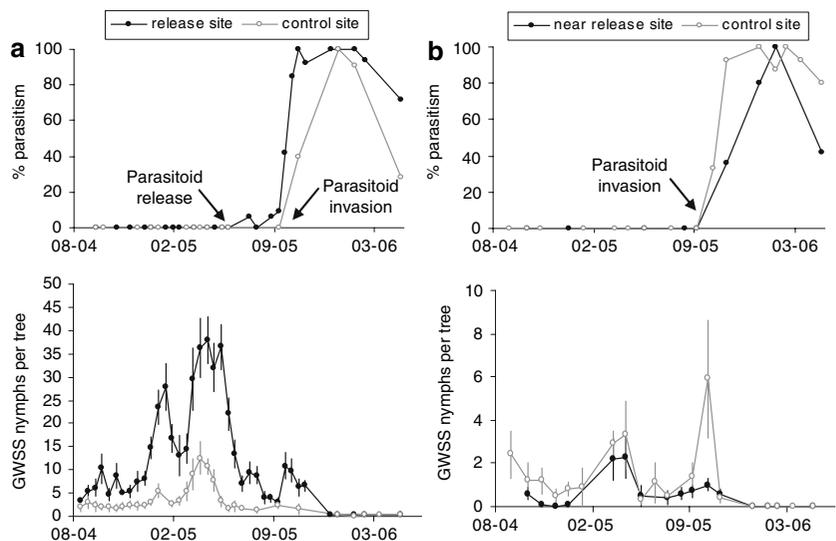
increased rapidly to more than 80% and numbers of *H. vitripennis* nymphs decreased by more than 95% from pre-release densities (Pihaena, 95.4%; Hauru, 97.2%) and have remained low since November 2005 (Fig. 5). A similar decrease was observed for adult *H. vitripennis* by March 2006 and for egg mass abundance by April 2006. The observed decrease in *H. vitripennis* adult and egg mass densities is around 90% that of pre-parasitoid releases (adults, 90.3% decrease in Pihaena, and 86.5% in Hauru; egg masses, 92.7% in Pihaena and 93.1% in Hauru).

Impact of *G. ashmeadi* on *H. vitripennis* at mountain sites

At 800 m, parasitoids were released on July 6 2006, and a total of 1,652 parasitoids were released between July and October 2006. However, parasitism was low (<20%) at this site even 2 months after releases began, suggesting that *G. ashmeadi* did not perform as well at high altitude as it had at sea level (Fig. 6). The release site was naturally colonized by parasitoids, presumably invading from sea level sites, at the end of September 2005. The non-release control site at medium elevation was colonized at the end of October 2005. Parasitoids then colonized high elevation sites located at 1,400 m in October (control site) and November 2005 (site located near the 800 m release site). Following colonization of the release and control sites by *G. ashmeadi*, parasitism increased very rapidly at all mountain sites and approached 100% and the numbers of *H. vitripennis* nymphs decreased by more than 92% in abundance from pre-release densities (800 m release site: 98.0%, control site: 92.7%; 1,400 m near release site: 100%, control site: 100%, no nymphs found) and have remained low since January—May 2006 (Fig. 6). The densities of adult *H. vitripennis* began to decrease in March 2006 and egg densities decreased in April–May 2006. The observed decrease in *H. vitripennis* adult and egg mass densities was around 85% that of pre-parasitoid releases at 800 m, but was only 30% for adults and 16% for eggs at 1,400 m. The percentage decrease was less important than that for other sites, since it is a proportion and the initial densities of *H. vitripennis* were small, but in absolute terms pest densities were reduced to the same levels as recorded in each of the study sites, either at sea level or in the mountains. In May 2006, parasitism

decreased by 28.6 and 80.0% according to the site studied. However, as the number of eggs laid by *H. vitripennis* at these high altitude sites was very low, this decrease did not result in a measurable increase in the number of nymphs that emerged compared to pre-release data. Indeed, the number of egg masses collected was low (12 egg masses for one site and less than eight for the other sites) suggesting that parasitism estimates might not be representative of overall parasitism of the target population. At all elevated sites, more than 95% of the eggs within an egg mass were parasitized.

Impact of *G. ashmeadi* on *H. vitripennis* at the island scale

In April 2005, before the parasitoid introduction, very high numbers of *H. vitripennis* nymphs were found all around the island of Tahiti with an average of 141.9 ± 11.2 nymphs per site (Fig. 7) (Petit et al. 2007). After the release of *G. ashmeadi*, an important decline in *H. vitripennis* abundance was observed. This decrease occurred firstly in the area of the initial release sites Mahina and Papenoo in August 2005 [northern part of the island (Figs. 1, 7)] with less than ten nymphs being found, while nymphs were still very abundant at all other sites. In October 2005, after the parasitoid had been widely released, *H. vitripennis* nymphs declined markedly at all sites, except in the south-west coastal area where no parasitoids were released (Fig. 7). By December 2005, *G. ashmeadi* had colonized the entire island of Tahiti (Petit et al. in preparation) and the number of *H. vitripennis* nymphs has been maintained (as of May 2006) at very low densities at all sites, with an average of less than one nymph per site (December 2005: 0.1 ± 0.1, February 2006: 0.5 ± 0.1, April 2006: 0.9 ± 0.3), a decrease of approximately 99% that of pre-parasitoid releases (Fig. 7). *H. vitripennis* was not abundant inland (mountains, valleys) as stated previously, here less than ten nymphs were found per inland site before the parasitoid release and this low level was maintained after the parasitoid introduction (Fig. 7).

The same results were obtained in Moorea (Fig. 8). Before the parasitoid was discovered in Moorea, very high numbers of *H. vitripennis* nymphs were found all around the island with approximately 100 nymphs per site (April 2005: 98.3 ± 15.3, June 2006: 87.3 ± 9.8, August 2006: 103.3 ± 16.5). The parasitoid was

discovered in Moorea in September 2005, but might have already been present as early as August 2005 in the north-west of Moorea, where a decrease in the abundance of *H. vitripennis* nymphs at two sites was observed. Since October 2005, after the parasitoid was found in Moorea, a major decline in *H. vitripennis* abundance was observed. This decrease occurred firstly in the area of the north-west part of Moorea, which was likely the first area colonized by the parasitoid because human settlement is concentrated in this area. By December 2005, *G. ashmeadi* colonized the entire island of Moorea (Petit et al. in preparation) and the number of *H. vitripennis* nymphs has been maintained at very low densities at all sites, with an average of less than one nymph per site (December 2005: 0.4 ± 0.2, February 2006: 0.7 ± 0.2, April 2006: 0.5 ± 0.2), a decrease of approximately 99% that of pre-parasitoid releases.

Discussion

Gonatocerus ashmeadi was released in Tahiti between May and October 2005 to control high density *H. vitripennis* populations. No releases were made on the adjacent island of Moorea, but the parasitoid was

found there in September 2005. *G. ashmeadi* became established on both islands, including high elevation sites, in just 2–3 months post-release. Dispersal of the parasitoid from Tahiti to Moorea was likely facilitated greatly by humans moving plants within and between islands (Petit et al. in preparation).

Our study using paired parasitoid release and non-release control sites demonstrated that *G. ashmeadi* had a rapid and major impact on pest densities and the population age-structure of *H. vitripennis*. By December 2005, a dramatic decrease in *H. vitripennis* abundance was observed at sea level all around the islands of Tahiti and Moorea (>95% or higher at most sites). The simultaneous study of paired control and release sites before and after parasitoid releases demonstrated that the observed decrease in pest densities resulted from the action of *G. ashmeadi* rather than natural temporal fluctuations due to climatic or geographical variations between sites. Before *G. ashmeadi* introduction, pest abundance fluctuated seasonally at both sites. Once *G. ashmeadi* was released, parasitism increased rapidly and was followed by a rapid and substantial decrease in *H. vitripennis* abundance (>95%) at the release site while parasitism remained undetectable and pest abundance remained high at the non-release control site. Once the

Fig. 7 Number of *H. vitripennis* nymphs collected during 1 min with a sweep net in *Hibiscus rosasinensis* edges in Tahiti. The parasitoid was released in May 2005 in study sites (Mahina and Papenoo, North) and all around the island (except South–West) in September–October 2005
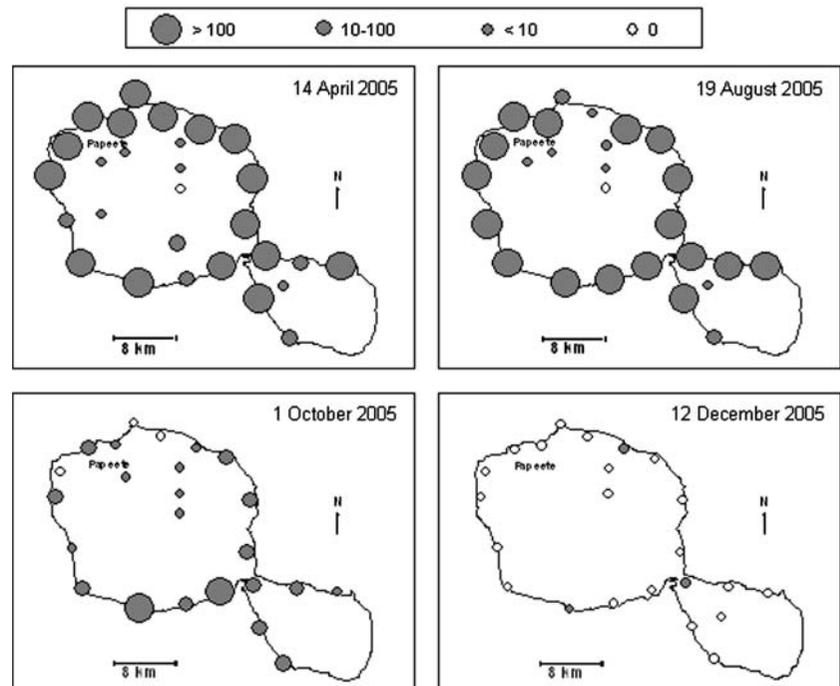
Fig. 8 Number of *Homalodisca vitripennis* nymphs collected during 1 min with a sweep net in *Hibiscus rosasinensis* edges in Moorea. The parasitoid was discovered in September 2005
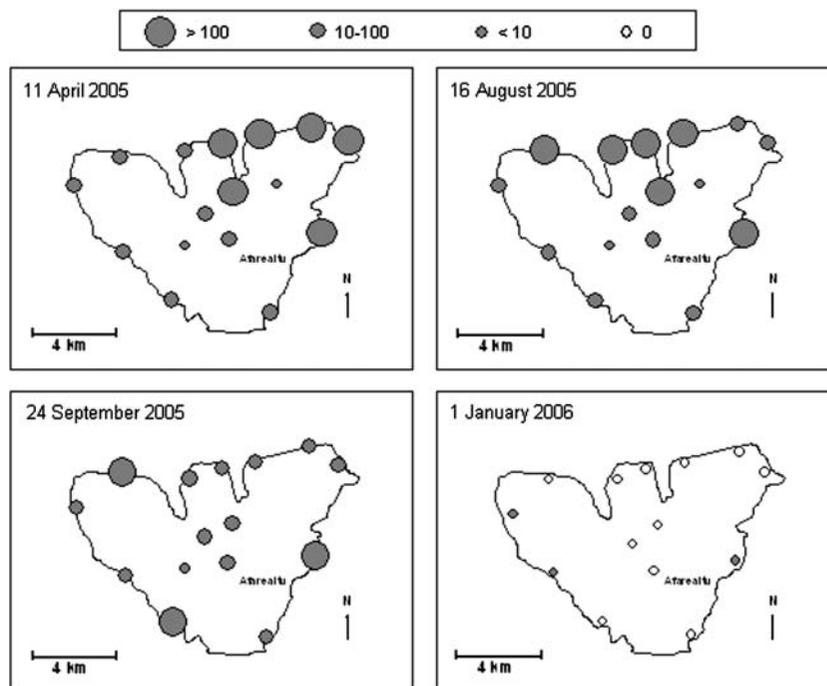
parasitoid invaded the non-release control site, the impact was similar to that observed at the release site.

Once a site was colonized by *G. ashmeadi* (either by release or natural colonization), the parasitism of *H. vitripennis* egg masses increased rapidly to a very high level (80–100%). As *G. ashmeadi* attacks the eggs, its impact was first seen on the abundance of *H. vitripennis* nymphs, which occurred about 2 months after a site was colonized. Adult *H. vitripennis* declined markedly about 4–6 months after parasitoid arrival at a site. After the adult decline, eggs became less abundant about 6–7 months after parasitoid arrival. By May 2006 (1 year after parasitoid releases began), all life stages of *H. vitripennis* were affected and pest population densities declined dramatically.

Our results also demonstrated that the presence of a resident parasitoid, *Centrodora* sp., that attacked moderate numbers (∼30%) of *H. vitripennis* eggs in certain locations (Papenoo) before the introduction of *G. ashmeadi* did not interfere with the success of this biological control program using a specialist parasitoid species. *Gonatocerus ashmeadi* appeared to outcompete *Centrodora* sp. in Papenoo, with parasitism levels by *Centrodora* declining in favor of *G. ashmeadi*. Such a result was expected since *G. ashmeadi* develops at least two times faster than

Centrodora sp. in *H. vitripennis* eggs, and as *H. vitripennis* eggs became less abundant *Centrodora* sp. likely focused on exploiting hosts used before the invasion of *H. vitripennis* into French Polynesia (Grandgirard et al. 2007b).

The sites monitored in this study were located in different habitats: coastal urban habitats at sea level vs. interior natural habitats at high altitude. Therefore, it was possible to determine the influence of vegetation and topography on *H. vitripennis* densities and *G. ashmeadi* efficacy. Before the parasitoid was introduced, *H. vitripennis* was found to be more abundant at sea level than in the mountains. These results are consistent with those found in studying the spatial distribution of *H. vitripennis* on different islands of French Polynesia which showed that *H. vitripennis* is more abundant in urban habitats than in undeveloped valleys and mountains (Petit et al. 2007). Urban habitats are more favorable for *H. vitripennis* reproduction than natural habitats because people grow numerous exotic plants which are well watered, fertilized, and cut regularly providing excellent growth characteristics for oviposition and feeding by *H. vitripennis*. In natural habitats the diversity of host plants is reduced and plants are not artificially maintained. It is possible that climate might slow *H. vitripennis* reproduction at high altitude

in comparison to sea level sites. Weather station data from high elevation sites show that the mean daily temperature during this study was $27.0 \pm 0.1^{\circ}$C at sea level, $20.5 \pm 0.1^{\circ}$C at 800 m, and $15.5 \pm 0.1^{\circ}$C at 1,400 m high. Such temperature differences might slow the efficacy of the parasitoid at high altitude, as *G. ashmeadi* development and reproduction is low at 20°C (Pilkington and Hoddle 2006a).

In contrast to sea level sites, the establishment of *G. ashmeadi* at high altitude was more difficult. Even though parasitoids were released regularly (every week or every 2 weeks) for 4 months for a total of 1,652 parasitoids in Pirae at 800 m, the released parasitoids had no clear impact on *H. vitripennis* abundance. Parasitism became very high and was followed by an important decline in *H. vitripennis* abundance only after a natural invasion of the site by thousands of *G. ashmeadi* colonizing progressively up the mountains from sea level. This finding implied that very high numbers of parasitoids were necessary for successful establishment at high altitude sites and that the number of parasitoids released for establishment was insufficient. The failure of parasitoid establishment at relatively low population density suggests that an Allee effect limited the establishment of *G. ashmeadi* in the mountainous high altitude sites. An Allee effect seems responsible for many parasitoid establishment failures and arises when the number of parasitoids released at the same time and place is low (less than 100 and often even less than 1,000), leading to difficulties in finding a mate and other problems (Hopper and Roush 1993). In the present case, several possible factors might have led to an Allee effect: (1) The low initial density of *H. vitripennis* may have resulted in parasitoids being unable to find host eggs to parasitize and exceptionally high numbers of parasitoids were needed to overcome the problem of host scarcity and subsequent mate finding following successful parasitization. (2) The native plants at high altitude are less diverse than at sea level, and if the relatively few native plants bearing *H. vitripennis* egg masses had trichomes that could impede egg laying parasitoid populations could not build to substantial levels on more suitable plants (i.e., glabrous leaves) because they were not available. (3) Climatic conditions might have been unfavorable for the successful establishment of the parasitoid. Indeed, the parasitoid was released during the cool-dry season (May–

October, July and August being the coolest months in the year), and relatively low temperatures (daily average temperature recorded in August 2005 at 800 m high: 18.9°C) might have impeded parasitoid establishment, and spread significantly. This idea is supported by the fact that establishment of *G. ashmeadi* at all mountain sites occurred at the end of the cool-dry season when temperatures were higher (daily average temperature recorded in October 2005 at 800 m high: 20.3°C). (4) Finally, the sampling method may have underestimated parasitism, since only one plant species was sampled and it was possible that *G. ashmeadi* preferred other neighboring plant species that were not sampled in this study. Consequently, when many parasitoids become present they may exploit hosts on less preferred plants due to high competition for resources.

In summary, our results have demonstrated that *G. ashmeadi* has provided excellent control of *H. vitripennis* in Tahiti and Moorea just 1 year after its introduction into Tahiti. The biological control agent rapidly and markedly reduced the target pest population and as a consequence many problems associated with this pest have diminished too. The excessive feeding on plants, watery excrement (referred to as rain), and nocturnal home invasions by hundreds of *H. vitripennis* attracted to lights are no longer problems. Farmers have declared their fruit production has improved in comparison to previous years, possibly reflecting diminished water-stress caused by fewer feeding *H. vitripennis*. The risk of spread of *H. vitripennis* to other islands and countries has been greatly reduced because pest populations have been greatly suppressed. If the plant bacteria *X. fastidiosa* arrives accidentally in Tahiti (or is already present), the risk of pathogen transmission and impact on native, ornamental and agricultural crops has been greatly reduced because vector densities have been reduced by >90% on average by *G. ashmeadi*. Following the release of *G. ashmeadi*, no more islands of French Polynesia have been infested by *H. vitripennis* suggesting that a significant reduction in propagule pressure via biological control has occurred. Programmatic surveys in Tahiti and Moorea will continue until at least May 2007 to determine how the parasitoid reacts to the recent decrease in the egg mass abundance and to determine if a stable equilibrium between the pest and the parasitoid is reached. Based on our findings so

far, we predict that populations of *H. vitripennis* will likely be maintained at near current levels by *G. ashmeadi*, representing a dramatic improvement compared to the pre-release situation. Seasonal fluctuations might be observed, especially in altitude, with higher densities of the pest expected during the cool season due to lower parasitism. Major storm events could also induce a reduction in parasitism and promote pest outbreaks but they are likely to be brought quickly back under control by the responding increase in parasitoid populations. Large-scale pesticide applications for the eradication or control of unwanted terrestrial arthropod invaders may also potentially disrupt biological control of *H. vitripennis* by *G. ashmeadi*.

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