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INVASION NOTE

Assessing invasion threats: novel insect-pathogen-natural enemy associations with native New Zealand plants in southern California

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Abstract The potential for novel pests to attack indigenous plants can be examined in non-native ranges of these plants. We used perennial native New Zealand plant species growing in botanic gardens and on public spaces in southern California to explore new associations between these plants, the bacterium *Xylella fastidiosa*, and its vector, the invasive insect pest, *Homalodisca vitripennis* (the glassy-winged sharpshooter), both of which are not yet present in New Zealand. Further, we examined the biocontrol potential of egg parasitoids against *H. vitripennis* on New Zealand plant hosts in southern California. We make the case for the inclusion of biocontrol as an early-response strategy against *H. vitripennis* should this pest invade New Zealand, and outline the steps required to make biocontrol part of a rapid-response management plan should an incursion and localized establishment occur.

Keywords Pre-emptive biocontrol · Glassy-winged sharpshooter · *Homalodisca vitripennis* · *Xylella fastidiosa* · Plant–insect–disease interactions · Sentinel plants

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Introduction

Risks of potential biological invasions can be assessed by exploring new associations in non-native ranges. Botanic gardens maintain plant collections from different geographic regions, providing natural laboratories where exotic species may develop new associations outside their native ranges. These novel interactions can inform biosecurity risk managers about the likely outcome of potential future invasions into geographic regions of interest (e.g. Messing et al. 2009). Moreover, new associations in exotic ranges can point towards biocontrol solutions against possible invaders. Here we refer to biocontrol as classical biological control, the introduction of specialist natural enemies from the pest's evolutionary area of

origin, commonly known as the home range. Since the mid-twentieth century many native and endemic New Zealand plant species have been grown in southern California's botanic gardens and arboreta, and in residential and municipal gardens, where since 1990 they became exposed to an invasive insect, the glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis* (Germar; Hemiptera: Cicadellidae).

GWSS vectors the xylem-dwelling phytopathogenic bacterium *Xylella fastidiosa* (Xf), which causes a variety of scorch-like diseases in plants of agricultural, ornamental, and biodiversity importance. The GWSS–Xf complex caused severe economic losses to a range of California's agricultural industries, urban landscape plantings, and possibly native plant species in wilderness areas (Pilkington et al. 2005). GWSS is highly polyphagous, developing on over 150 species in at least 35 plant families, expanding its plant host range as it encounters new species in invaded ranges (Hoddle et al. 2008).

GWSS is native to northeastern Mexico and southeastern USA, and has successfully invaded California as well as several Pacific islands (Petit et al. 2008). In California, GWSS is controlled by a complex of hymenopteran egg parasitoids, dominated by *Gonatocerus ashmeadi* (Girault) (Mymaridae) (Pilkington et al. 2005). Future incursion of GWSS into New Zealand is considered possible because of the high levels of trade and tourism from regions invaded by GWSS (Hoddle 2004). The long exposure of perennial New Zealand plants to the GWSS–Xf complex in southern California has allowed sufficient time for new associations to form. This exposure enabled us to examine the susceptibility of these plants to GWSS–Xf, as well as opportunities to assess the potential of natural enemies, especially egg parasitoids, for biocontrol of GWSS on New Zealand plant species.

Methods

In June 2012, New Zealand plant species were sampled in four southern Californian botanic gardens and arboreta as well as in amenity plantings in public spaces and on roadsides. The main sampling sites were Balboa Park and San Diego Botanic Gardens in the San Diego County, and Los Angeles Arboretum and Huntington Botanic Garden in the Los Angeles County. Two native New Zealand tree species, *Metrosideros excelsa* and *Myoporum laetum*, were able to be sampled outside of managed gardens because of their widespread urban plantings. Records of New Zealand plant species were obtained in advance from botanic gardens and arboreta, and attempts were made to find and sample every plant on these records. Plants were examined visually by two team members for a total person-time of 4–10 min per plant, depending on plant size. Presence/absence of GWSS eggs, nymphs, adults, or presence of shed exuviae was recorded as evidence of association between GWSS and the host plants. We were unable to synchronize the sampling period with high abundance of GWSS; yet disease presence in host plants builds up over extended periods, and we could assess host susceptibility to the pathogen even in the absence of direct evidence for vector-plant interaction. To determine if *X. fastidiosa* infections existed in the plants, samples of woody tissue were collected from all plants, regardless of whether an association with GWSS could be clearly established. We preferentially collected plant tissue that had visual symptoms typical of infection with *X. fastidiosa* (e.g. scorched foliage, chlorosis, wilt, dieback or stunted growth) to increase the chance of detecting *X. fastidiosa* in sampled plants. Presence of Xf-like symptoms does not always infer Xf-infection, because similar symptoms can be triggered by environmental stresses, nutrient deficiencies, or other phytopathogens. In addition, *X. fastidiosa* is not always evenly distributed throughout the plant host, and sampling tissue at random increases false-negative results (i.e. results in pathogen not being detected, despite being present in the plant) (Varela et al. 2001). Sampled woody tissue was preserved in two ways: directly into 95 % ethanol; and, dried in silica gel beads. Preserved material was moved into New Zealand under Ministry for Primary Industries permit number 2012046872, for molecular analyses. Extraction of DNA from the silica-dried samples was more productive than from ethanol-preserved tissue. DNA was extracted following Beard et al.'s (2013) protocol. Approximately 100 mg of dried plant tissue was used per DNA extraction. Samples were then screened for presence of *X. fastidiosa* with real-time PCR, using the protocol and primers designed by Harper et al. (2010).

To determine natural enemy activity, fresh GWSS egg masses collected in the field were kept in the laboratory at 26 °C (±1.9 °C) and 60 % RH until

emergence of either adult parasitoids or GWSS nymphs. Fresh egg masses could be distinguished from old egg masses by color: fresh egg masses are whitish-green, whereas old egg masses leave a brown mark on the leaf. While GWSS egg development takes around 8 days (Al-Wahaibi and Morse 2009), *G. ashmeadi* develops best if parasitism occurs in 3-days old GWSS eggs. Yet the parasitoid will also parasitise GWSS eggs that are younger and older than 3 days (Irvin and Hoddle 2005). We were unable to determine the accurate age of fresh egg masses at the time they were collected. Old egg masses, from which emergence had been completed, were scored as either parasitized or not, and the parasitoid species identified based on characteristic emergence patterns as either *G. ashmeadi* (single emergence hole per GWSS egg) or *Ufens* sp. (Trichogrammatidae) (multiple emergence holes per GWSS egg; Triapitsyn 2003).

Results

A total of 102 native New Zealand plants covering 39 species in 22 families were examined (Table 1 and supplementary Table S1). An association between GWSS and sampled plants species was evident in 25 % of samples: 26 out of a total 102 individual plants examined exhibited signs of sharpshooter activity (supplementary Table S1). This evidence of association should be weighed in the context that field sampling occurred when insect abundance in the surrounding environment was naturally low (M. S. Hoddle, pers. obs). *Xylella fastidiosa* was confirmed present in all locations sampled and in 51 % of samples (52 of 102; Table 1) and 72 % of plant species (28 of 39 species). Some plant species failed to yield adequate DNA for analyses; therefore, *X. fasitidiosa* infection may be underestimated.

Twenty-nine GWSS egg masses were collected from 11 individual plants, and 18 (62 %) were parasitized (Table 2). It was possible to determine the level of parasitism per egg mass in fresh egg masses (N = 8). The number of eggs in fresh masses ranged between 3 and 9 eggs per mass, and parasitism averaged 75 % (±12 %; mean ± SE) per mass. It is plausible that some fresh egg masses were collected too soon after they were deposited, and were not exposed to parasitism. Therefore it is possible that the parasitism rates we recorded underestimate true field parasitism. All parasitoids that emerged from fresh egg masses were identified as *G. ashmeadi*. GWSS nymph hatching success rate out of fresh egg masses was 16 % (±10 %; mean ± SE).

Discussion

Botanic gardens are curation sites for plants collected from around the world. Use of these collections has not traditionally been part of invasion risk-assessment by contributing geographic regions. Yet these live plant collections frequently form new associations with pests and diseases they never encountered in their native range. In essence, these plants are working as homeland sentinels that provide inexpensive pre-emptive screening for potential invasive pests and diseases (Messing et al. 2009; Better Border Biosecurity 2012). GWSS is considered at risk of invading New Zealand via trade and tourism in the South Pacific (Hoddle et al. 2008) and the climate is suitable for establishment of both GWSS and *X. fastidiosa* (Hoddle 2004). While the potential risk to the grape growing industry is well appreciated (Saunders et al. 2013), our results demonstrate that numerous indigenous New Zealand plant species are also susceptible to infection by *X. fastidiosa*. However, the consequences of infection (i.e. disease development and mortality rates) are not known for the plant species in which the pathogen was detected. Long-term monitoring and manipulative laboratory-based bioassay studies should be initiated to determine the fates of *X. fastidiosa*-infected trees that are native to New Zealand. Demonstrating susceptibility to GWSS and *X. fastidiosa* was achievable owing to the existence of specimens in botanic gardens and urban landscape plantings in southern California that put native New Zealand plants into direct exposure to this vector–pathogen complex. Detection of *X. fastidiosa* in three iconic New Zealand natives, pōhutukawa (*Metrosideros excelsa*), tītoki (*Alectryon excelsus*) and kauri (*Agathis australis*), is concerning. Our results and observations confirm that some New Zealand indigenous plant species can harbor this bacterium, but it remains unknown if these species will succumb to disease or act as long-lived pathogen reservoirs. Silent pathogen reservoirs could serve as disease sources threatening cropping systems, should vectoring insect populations feed on them and acquire the bacterium.

Table 1 Plant species indigenous to New Zealand and their susceptibility to *Xylella fastidiosa*. Plants were sampled in southern California, where they are grown as ornamentals, and where *X. fastidiosa* and its highly effective vector, *Homalodisca vitripennis*, co-exist. *Vitis vinifera* (grape vine), is not native to New Zealand; it represents a highly susceptible host

Species	Plant family	New Zealand native/endemic	Number of samples taken	Number of samples that tested positive
Species that tested positive for presence of *X. fastidiosa*				
Agathis australis	Araucariaceae	Endemic	2	1
Alectryon excelsus	Sapindaceae	Endemic	5	2
Brachyglottis sp.	Asteraceae	Native	1	1
Coprosma repens	Rubiaceae	Endemic	8	4
Coprosma robusta	Rubiaceae	Endemic	1	1
Cordyline australis	Asparagaceae	Native	4	1
Cordyline sp.	Asparagaceae		2	1
Corokia cotoneaster	Argophyllaceae	Endemic	2	2
Corokia macrocarpa	Argophyllaceae		1	1
Corynocarpus laevigatus	Corynocarpaceae	Endemic	2	2
Haloragis erecta	Haloragaceae	Endemic	1	1
Hebe[a] spp.	Plantaginaceae	Endemic	6	6
Melicope ternata	Rutaceae		1	1
Melicytus ramloros (*ramiflorus*?)[b]	Violaceae	Endemic subspecies	1	1
Meryta sinclairii	Araliaceae	Endemic	4	3
Metrosideros excelsa	Myrtaceae	Endemic	12	6
Metrosideros kermadecensis	Myrtaceae	Endemic	1	1
Myoporum laetum	Scrophulariaceae	Endemic	8	2
Phormium colensoi (syn. *cookianum*)	Xanthorrhoeaceae	Endemic	1	1
Phormium tenax	Xanthorrhoeaceae	Native	2	1
Pittosporumcrassifolium	Pittosporaceae	Endemic	4	2
Pittosporum eugenioides	Pittosporaceae	Endemic	1	1
Pittosporum tenuifolium	Pittosporaceae	Endemic	12	7
Pittosporum umbellatum	Pittosporaceae	Endemic	4	2
Vitex lucens	Lamiaceae	Endemic	2	1
Vitis vinifera	Vitaceae	Non-native	3	3
Species that tested negative for presence of *X. fastidiosa*[c]				
Leptospermum scoparium	Myrtaceae	Endemic	1	0
Lophomyrtus sp.	Myrtaceae	Endemic Genus	1	0
Pisonia brunoniana	Nyctaginaceae	Native	1	0
Pittosporum sp.	Pittosporaceae		2	0
Podocarpus spicatus	Prumnopityaceae	Endemic	1	0
Reclassified as *Prumnopitys taxifolia*				
Podocarpus totara	Podocarpaceae	Endemic	3	0
Pseudopanax lessonii	Araliaceae	Endemic	1	0
Pseudopanax sp.	Araliaceae		1	0
Solanum aviculare	Solanaceae	Native	1	0
Sophora microphylla	Fabaceae	Endemic	1	0
Sophora tetraptera	Fabaceae	Endemic	1	0

[a] The genus *Hebe* was recently placed under *Veronica* (Heenan 2012)

[b] Specimen label reads *Melicytus ramloros*, which does not exist. The specimen is likely *M. ramiflorus*

[c] Negative signal for *X. fastidiosa* in this snapshot survey does not guarantee these plant species are immune to the pathogen

Table 2 Parasitism rates on *Homalodisca vitripennis* egg masses collected in southern California from plant hosts native to New Zealand

Plant host	Number of egg masses collected	Number of egg masses parasitized by *G. ashmeadi*	Number of egg masses parasitized by *Ufens* sp.	Percent egg masses parasitized
Alectryon excelsus	1	0	0	0
Coprosma repens	5	NA[a]	2	Minimum 40
Hebe[b] sp.	1	1	0	100
Metrosideros excelsa	10	4	0	40
Myoporum laetum	6	6	0	100
Pittosporum spp.	2	1	1	100
Pseudopanax lessonii	4	3	0	75
Total	29	15	3	62

[a] Three egg masses were too old to determine parasitism status

[b] The genus *Hebe* was recently placed under *Veronica* (Heenan 2012)

Exploring new associations in botanic gardens and urban landscapes in exotic ranges need not be limited to identifying future invasion risks. The risk-assessment exploration conducted here investigated the activity of natural enemies attacking GWSS eggs on New Zealand plants. Effectiveness of biocontrol systems involving a pest that feeds on multiple plant host species can vary: some plants can affect natural enemies of their herbivores positively; others may have negative effects (Price et al. 1980). Therefore it was important to assess the effectiveness of natural enemy activity against the target pest on New Zealand plants.

Biocontrol may be effective for slowing invasion speed and lowering propagule pressure, yet it is rarely used to manage invasive pests until they are widespread, often after all other management options have failed (Fowler et al. 2000). Use of biocontrol agents as an early-response tool targeting GWSS would include mass releases of egg parasitoids upon first detection of this pest, in anticipation of natural enemies destabilizing pest establishment, spread, and colonization rates. Allee effects often affect pest populations at this incipient phase of establishment. Natural enemies may therefore make small pest populations more vulnerable to extinction by stochastic events (Drake and Lodge 2006; Petit et al. 2008). Early biocontrol efforts with highly host specific natural enemies could therefore provide self-diffusing control at the invasion front, should eradication efforts with pesticides fail.

However, by the time GWSS is detected in a new range its populations may be too widespread for eradication to be feasible. Because there is no pheromone or other sensitive and species-specific monitoring tool, current monitoring efforts in New Zealand rely on yellow sticky traps, which are unlikely to be effective at detecting low density incursions into a country that is free of the pest. In addition, chemical control in urban and wilderness areas as part of an eradication effort is unlikely to be publicly acceptable. Biocontrol should therefore be prioritized as an early response to a GWSS incursion in New Zealand.

We suggest that potential GWSS invasion into New Zealand is a suitable model system to proactively develop the concept and best-practice standards for using biocontrol as suppression tactic during the early phases of an invasion event when established pest populations are small and highly localized. Obvious steps include feasibility studies on the suitability of the early and rapid deployment of potential biocontrol agents identified as being suitable prior to incursion. These studies could include assessment of efficacy of biocontrol agents in exotic ranges, and should also include host-range tests to assess risk to non-target species—an important step of the pre-importation regulatory process. Completing these required steps ahead of pest arrival can save considerable time (often years) and would provide biocontrol as one component of an early-response toolbox for rapidly responding to an incursion event.

Biocontrol against GWSS has been spectacularly successful throughout the invaded range (summarized in Charles and Logan 2013). In southern California, where five species of egg parasitoids attack GWSS eggs, we found evidence of two species parasitizing the pest on New Zealand plant hosts: *G. ashmeadi* and *Ufens* sp. *Gonatocerus ashmeadi*, which provided excellent control in the entire invaded range, is likely

to be safe to introduce to New Zealand, but may not be climatically suited to cover the entire range vulnerable to invasion by GWSS (Charles 2012; Charles and Logan 2013). The next logical step in a pre-emptive plan would be to assess the feasibility of additional egg parasitoid species for potential use in New Zealand, similarly to the assessment done for *G. ashmeadi*. Another component in the work plan pre-empting GWSS invasion would be to search for reservoirs of *X. fastidiosa* in ornamental plants that were imported into New Zealand from areas where *X. fastidiosa* exists. These asymptomatic plants would act as pathogen reservoirs from which uninfected GWSS could acquire and spread *X. fastidiosa*.

Other countries have anticipated invasion by GWSS (e.g. Rathé et al. 2012) and the pre-emptive biocontrol response outlined here need not be limited to New Zealand or to GWSS. Other high-risk pests (e.g. brown marmorated stinkbug, *Halyomorpha halys* [Hemiptera: Pentatomidae], also widely established in southern California and intercepted regularly in New Zealand; C. Duthie, pers. comm.) could be considered for pre-invasion assessment. The concept of preventative biocontrol for managing invasive pests at an early stage of the invasion process is compelling economically and environmentally. Regulatory barriers are likely to be manageable, and the main limitation will come from failure to shift scarce funding from managing existing pest problems to proactively working on pests with future invasion potential. We argue that such a paradigm shift needs serious consideration in the light of ever-expanding biological invasions.

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