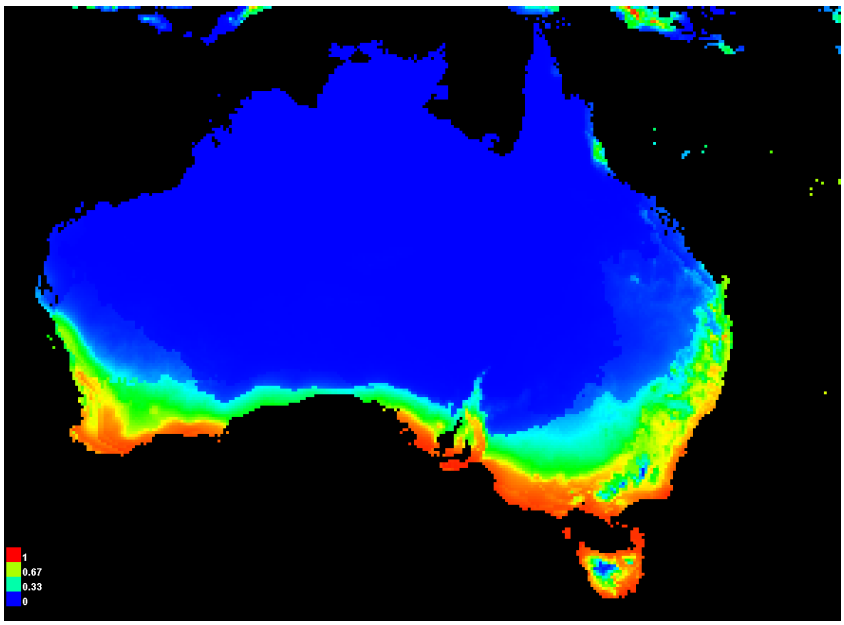
Preparedness for biological control of high-priority arthropod pests



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GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: UA 1201

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Research Organisation: **The University of Adelaide**

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PREPAREDNESS FOR BIOLOGICAL CONTROL OF FOUR HIGH-PRIORITY NON-NATIVE ARTHROPOD PEST SPECIES IN AUSTRALIAN VINEYARDS

1 ABSTRACT

This project takes a proactive approach in preparing the grapevine industry for the incursion of four high-priority insect pests, the glassy-winged sharpshooter, the vine mealybug, grape mealybug and the European grape vine moth. As a result, these pests have now been recognised as targets for biological control in Australia, the most important biological control agents have been selected and lists of potential non-target hosts needed for host specificity testing have been created. Furthermore, recommendations have been formulated for actions that could be undertaken either before or after the incursion of each of these pest species. The key outcome for the Grape and Wine Industry is better preparedness in the event of incursions by any of these pests.

2 EXECUTIVE SUMMARY

A key aspect of biosecurity is preparedness for an incursion of non-native pests. This project prepares the Australian grapevine industry for the incursion of four grapevine pests: The glassy-winged sharpshooter, the vine mealybug, the grape mealybug and the European grape vine moth. These pests have been recognised in the Industry Biosecurity Plan for the Viticulture Industry (version 2.0, 2009) as high priority pests that potentially threaten the productivity of the Australian wine industry. If one of these did arrive in Australia, then eradication would be the first priority response. But eradication is not always possible, in which case biological control is the second priority.

After an incursion, the spread of an invasive pest and the damage it causes can expand geometrically over time. Each year that passes without an effective solution translates into mounting losses in terms of either lost production, reduced quality and/or increased control costs. The industry can be spared substantial losses if a new pest can be rapidly brought under control. Preparation for an incursion of a high priority pest can substantially limit the time needed for an appropriate response and hence the damage incurred. This is the rationale behind this project.

Each of the pest species is covered in a chapter that consists of four sections.
The first section addresses the distribution of the pest species in its endemic range, the plant host range, the expected distribution in Australia and how this overlaps with the Australian wine growing regions, the presence of other plant hosts in Australia and the biology and pest status of the species.

In the second section, the potential biological control agents are selected. This selection is based on the efficacy and host specificity of the species that contribute to the biological control abroad. The taxonomic position, biology, ecology and endemic range of the selected biological control agents are also described.

In the third section, we discuss the possible native non-target hosts of the selected biological control agents and construct a list of non-target hosts that should be included in testing the host specificity of the new biological control agents. The final list of potential non-target hosts to be used in host specificity testing has been constructed in collaboration with experts on the native taxonomic groups to which the pest species belongs, taking into account taxonomic associations and ecological similarities between the native species and the pest. Further selection involved assessment of the native range of the species and accessibility and availability of the potential non-target host species for testing.

In the final section, we outline the procedure for host specificity testing.

As a result of this project, all four pest species have been approved as targets for biological control, which sets a precedent for Australia. Furthermore, for each of the four pest species, the response in case of an incursion has been formulated and the information required for the application for permits needed for host specificity testing has been collated. This information is summarised for each of the species below.

The **glassy-winged sharpshooter** (*Homalodisca vitripennis*), is a vector of the bacterium *Xylella fastidiosa* which causes Pierce's disease. Following a range extension from its endemic area in eastern USA to the major wine growing regions in California, Pierce's disease has caused very substantial losses to the grapevine and other industries there. If there is an incursion in Australia, then this species is likely to invade all major wine growing regions except Tasmania. The most efficient biological control agents overseas are two

Mymarid wasps, *Gonatocerus ashmeadi* and *G. walkerjonesi*, which are egg parasitoids of proconiine sharpshooters. Host specificity has not been convincingly established overseas. The non-target hosts that should be involved in host specificity testing are the native proconiine sharpshooters *Cofana spectra*, and one of the species *Ishidaella angustata*, *I. latomarginata* or *I. albomarginata.*

The **vine mealybug** (*Planococcus ficus*) is likely to be highly damaging to the Australian grapevine industry, as it causes sooty mould and transmission of grape leafroll and other viruses. Furthermore, this species is extremely difficult to control due to its hidden lifestyle under bark or in the root zone, and its close association with ants, from which it receives protection against natural enemies. An incursion would see all grape growing regions in Australia affected, apart from Tasmania. The main candidates for biological control include a number of parasitoid wasps that are already present in Australia and help to control the closely related citrus mealybug (*Pl. citrus*). However, it is recommended that a novel strain of the parasitoid wasp *Anagyrus pseudococci* from Spain is introduced in the case of an incursion. This strain has been proven to be a more efficient biological control agent in the USA than the strain that was already present locally. The parasitoid wasp is not known to be host specific. Therefore, host specificity testing may be needed before the introduction of this new strain. We recommend that the decision for host specificity testing, as well as the non-target host selection should be based on a survey of the current parasitisation of native Australian mealybugs by economically important parasitoids, including *A. pseudococci*. Based on availability, accessibility and expected overlap with the invasive pests, we suggest that the non-target list consists of *Pseudococcus eucalypticus, P. hypergaeus* and *Nipaecoccus ericiola.* Other recommended measures in case of an incursion are ant control and pheromonal attraction of male mealybugs and parasitoids.

As a result of this project, the priority status of the **grape mealybug** (*Pseudococcus maritimus*) in the new Biosecurity Plan for the Viticulture Industry has been downgraded. This decision was based on the species' limited invasiveness and the fact that it rarely causes economic damage to grapevine in its endemic range. As is the case with current mealybugs on Australian grapevine, the species is well controlled by a combination of generalist parasitoids and predators. However, in the case of an incursion, this species is likely to invade all major wine regions of Australia. Therefore, the invasion and the biological control achieved by generalist predators and parasitoids should be carefully monitored. Should pest status be reached, then we recommend importation of two encyrtid parasitoid wasps, *Acerophagus notativentris* and *A. angelicus* to test their host specificity prior to release. Based on availability, accessibility and expected overlap with the invasive pests, we suggest that the non-target list for host-specificity testing consists of *Pseudococcus eucalypticus, P. hypergaeus* and *Nipaecoccus ericiola.* The eventual outcome of the recommended survey of the introduced parasitoids of native mealybugs should be taken into account.

The **European grape vine moth** (*Lobesia botrana*) is the most damaging moth in its endemic range and has recently invaded the Americas. The parasitoid wasps that control this species vary greatly in their range and in efficacy depending on weather, climate, region and grape variety, making it an unlikely target for classical biological control. Furthermore, the most important parasitoid species in the endemic range has not been successfully reared. Hence we were unable to find a suitable parasitoid candidate for biological control. The current biological control measures overseas involve the judicial use of two strains of *Bacillus thuringiensis* in combination with mating disruption. The latter strategy works very well in several regions, has recently been tested in large-scale Australian vineyard trials as an eradication tactic for incursions of Biosecurity leafroller pests, and is greatly recommended

should the moth invade Australia. The possibility of non-target mating disruption of locally native *Lobesia* species seems unlikely, but could be explored.

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3 BACKGROUND

3.1 Introduction

A range of non-native species could threaten the productivity and the quality of wine grapes in Australia if any of them are introduced into this country, hence biosecurity preparedness is a crucial part of the grape and wine industry's biosecurity plan.

This project has used an innovative, pro-active approach to enhance preparedness of the grape and wine industry for the possible incursion of four high priority species - the glassy-winged sharpshooter, *Homalodisca vitripennis*, the European Grapevine moth, *Lobesia botrana*, the vine mealybug, *Planoccus ficus*, and the grape mealybug, *Pseudococcus maritimus*.

3.2 Current responses to incursions

The response to an invasive pest starts after its presence has first been discovered in Australia. The response consists of numerous sequential steps.

The first action is to investigate possibilities for eradication. If this is not deemed possible, this is followed by an assessment of the potential to contain the pest to a certain region. Often, neither eradication or containment are feasible, because most pest species are (a) highly invasive which is enhanced by traits such as high levels of reproduction and good migratory ability and (b) discovered long after the first incursion, which often means that the pest has become established in a large area and may have become adapted to local conditions.

When eradication or containment is not a viable option, the next step is to learn to 'live with it', i.e. move towards dealing with the on-going presence of the pest through the development of management strategies. The preferred approach in dealing with pests is to use an integrated pest management scheme, where biological control and minimal chemical treatment are used to keep the pest under an economic threshold. This implies the establishment of effective biological control agents.

The criteria that need to be met by biological control agents are that they are specific to the invasive pest and have a good numerical response to the pest population. It is highly unlikely that such host-specific and effective control agents are present in the invaded area, but they can often be found in the native region of the pest. After selecting the most promising candidate species, the process that will eventually lead to the importation and industrial use of the biological control agents consists of ten steps (Table 3.1):

This process of selection, specificity testing and assessment by the government generally takes years. In the mean time, the spread of the pest and the damage it causes can expand geometrically over time, i.e. each year that passes without an effective solution translates into mounting losses. It is easy to see that a speedy response to an invasive pest can spare the viticulture industries substantial losses. Furthermore, in the case of an incursion, the time pressure and the need to be seen to do something to prevent major losses could influence the care taken in selection process and hence pose a risk to biosecurity.

Table 3.1. The steps involved in the process that eventually leads to the importation and release of novel biological control agents (DAFF 2009).

1.	Approval of the target species as a candidate for biological control
2.	Offshore research on possible agents
3.	Construction of host-specificity test list to identify possible non-target hosts and development of test methodologies
4.	Obtaining permission to undertake specificity testing in contained use in Australia
5.	Obtaining a testing permit for the proposed biological control agents that are animals
6.	Specificity testing under quarantine containment in Australia
7.	Application to AQIS for the release of a biological control agent - if safe
8.	Risk analysis by Biosecurity Australia including input from stakeholders
9.	Release permits from AQIS and DEWHA
10.	Amending the live import list for biological control agents

The application to release a biological control agent (Table 3.1 pt 7) should contain the following elements (DAFF, 2009):

A. A summary of the proposed activity, including the proposed source of the agent, the number of individuals to be imported and the way in which the specimen(s) will be kept and transported within Australia and disposed of.
B. Information on the target species, including:
   - taxonomy
   - related Australian native and introduced species
   - native range
   - current distribution
   - pest status
   - documentation of approval for biological control
C. Information on the taxonomy of the biological control agent.
D. Information on the biology and ecology of the species, including but not restricted to:
   - The natural geographic range
   - Current distribution (i.e. has the species been used for biological control in any other countries?)
   - Related species
   - An estimate of the likely efficacy of the species
E. A description of the current status of the species in its native range.
F. A description of the current status of the species in Australia.
G. Information on where, when and how initial releases will be made.
H. A report on the results of host-specificity testing of the biological control agent, including the approved host specificity test list, an explanation of any variation from this list, testing methods, risk evaluation to non-target species and any evidence of laboratory artefacts.
I. An analysis of the overall potential impacts on the Australian environment of importing and releasing the species, including a statement on the likelihood that the species could become an environmental pest.

Applications for permits to import quarantine material and perform host specificity testing (points 4 and 5, Table 3.1) need to address points A – G and need to include details of the host specificity testing.

3.3 Improving the response time

It is clear that a large part of the applications for permits for host specificity testing involve the analysis of the literature to assess the likely efficacy of different potential biological control agents available overseas and the consultation of experts in Australia and overseas to select suitable biological control agents and native non-target hosts for host specificity testing. Most of this evaluation and consultation can be done in advance of an incursion. In this report, we prepare, as far as possible, the grape and wine industry for a possible incursion of four high priority species by:

- Preparation and submission of an application for recognition of these pests as targets for biological control;
- Development of the applications for:
  - A permit to import quarantine material to AQIS (Table 3.1 point 4,);
  - A testing permit for proposed biological control agents that are animals to the Department of the Environment, Water, Heritage and the Arts (DEWHA).

This preparation takes the process of the introduction of new biological control agents as far as far as it can be taken in the absence of the actual pest. It is clear that this preparation will both enhance the response time and improve the selection process.

4 PROJECT AIMS AND PERFORMANCE TARGETS

The overall objective of the project was to increase the readiness of the Australian grape and wine industry by preparing applications to allow importation of biological control agents to the Department of Sustainability, Environment, Water, Population and Communities, and to the Department of Agriculture, Fisheries and Forestry, covering the following invasive high priority pests that are not as yet in Australia:

- glassy-winged sharpshooter (*Homalodisca vitripennis*)
- vine mealybug (*Planococcus ficus*)
- grape mealybug (*Pseudococcus maritimus*)
- European grape vine moth (*Lobesia botrana*).

For each of the pests, applications should document:

- the risks that these pests pose, including the expected distribution in Australia;
- the most appropriate species of natural enemies to import for biological control of each pest species in priority order;
- the most appropriate source(s) of the agents;
- the range of Australian species that are potentially at risk from biological control agents;
- the procedures that should be carried out to test agent specificity and assure their ecological safety

The performance targets were the submission of applications for each of these species.

Three variations have been made to the performance targets.

Firstly, applications were prepared and submitted to recognise these high priority pest species as targets for biological control (Table 3.1 pt 1). This was not initially planned, but it is the first step required to achieve biological control.

Secondly, the "application to import biological control agents" has been interpreted as the "preparation of submissions to undertake host specificity testing in contained use" in Australia (Table 3.1, pt 4). The reason for this is that any application for importation of a novel biological control agent heavily relies on the outcome of host specificity testing. Actual host specificity testing was never a part of this project and can only be performed after an incursion of the pest species, as it requires a breeding program of the biological control agent in quarantine.

A third variation has been made because a suitable biological control agent for *L. botrana* could not be identified. This variation has resulted in alternative recommendations for the control of *L. botrana*, should it invade Australia.

5 METHODS

As detailed above, the project consisted of submission of the pest species to allow their approval as targets for biological control, and an analysis of the pest status, potential distribution in Australia, selection of biological control agents, selection of non-target hosts for host specificity testing.

5.1 Identification of potential high priority species as targets for biological control

Submissions have been written to request approval for recognition of each of the four potentially invasive grapevine pest species as a target for biological control (Appendix 1). These applications included the pest risk reviews already done for three of the four species by Plant Health Australia. The applications have been submitted to and approved by the Plant Health Committee of the Ministerial Council of the Department of Agriculture, Fisheries and Forestry (Decision 3rd May 2013; Ryan Genero, pers. comm.).

5.2 Pest status

Using the most recent literature, the pest status of the priority pest species was analysed in both the endemic region and in non-native regions that had become invaded by the pest. The reason to distinguish between these areas is that pest species are generally better controlled in their endemic region due to co-evolution with biological control agents.

5.3 Analysis of the potential geographic distribution in Australia

Estimating the area where a potentially invasive species might spread allows primary industries to make contingency plans if a non-native species is introduced. Modelling a species' climatic adaptation is one way to estimate the area that is under threat.

MaxEnt is a program that was developed to model the climatic adaptation of a species based on presence data and a range of geographically mapped climatic data (Phillips *et al.* 2006, Elith *et al.* 2011). We used MaxEnt to model the climatic adaptation of four non-native insects to determine the geographic range where the climate is suitable. These models were used to predict the likely regions in Australia where each species could establish breeding populations and spread after introduction. We also used the model predictions together with other information to suggest locations where biological control agents might be collected in future.

MaxEnt uses geographic locations where a species is known to occur as a key source of data on which the climatic adaptation models are based (Elith et al 2011). These data should be unbiased, or corrected for known biases, so obtaining a broad data set of localities that includes the known geographic range of a species is crucial. We used a range of sources to obtain locality data. Virtually all accessible primary publications that mentioned each species in the Web of Science and CAB Abstracts databases were used to initiate our locality database. These sources proved to provide an insufficient range of localities for all species, so we also conducted internet searches with *Google* and *Google Scholar.* Finally, experts and agricultural specialists were contacted to gain additional locality information.

High resolution climatic data were obtained from Worldclim with a 10 minute resolution based on the period 1950-2000 (Hijmans *et al.* 2005, Hijmans *et al.* 2013). We used 19 bioclimatic variables that were derived from these raw temperature and rainfall data (Table 5.1).

Our approach was to develop MaxEnt models for each species that were consistent with the published literature as well as the locality data that were used to fit the models. It is easy to over-fit the locality data to 19 bioclimatic variables. Inevitably, some judgement was used when choosing the final model for each species. The rationale for selecting the range of variables used in the models was (1) they explained at least 5% of the observed occurrence of geographic locations and (2) the form of the predictive curve was biologically reasonable, without abrupt discontinuities. The variables chosen and used in the final models are given in relation to each species below.

Note that it was assumed that location data that were used as inputs to the MaxEnt program were obtained by unbiased sampling. This assumption could not be assured, but there was no effective way to prepare bias grids for the species that were analysed as the extent of bias was largely unknown.

Table 5.1. Bioclimatic variables from BIOCLIM that were used in MaxEnt to model the geographic ranges of species (Hijmans *et al.* 2005).

Variable	Description
BIO1	Annual Mean Temperature
BIO2	Mean Diurnal Range (Mean of monthly (max temp - min temp))
BIO3	Isothermality (BIO2/BIO7) (* 100)
BIO4	Temperature Seasonality (standard deviation *100)
BIO5	Max Temperature of Warmest Month
BIO6	Min Temperature of Coldest Month
BIO7	Temperature Annual Range (BIO5-BIO6)
BIO8	Mean Temperature of Wettest Quarter
BIO9	Mean Temperature of Driest Quarter
BIO10	Mean Temperature of Warmest Quarter
BIO11	Mean Temperature of Coldest Quarter
BIO12	Annual Precipitation
BIO13	Precipitation of Wettest Month
BIO14	Precipitation of Driest Month
BIO15	Precipitation Seasonality (Coefficient of Variation)
BIO16	Precipitation of Wettest Quarter
BIO17	Precipitation of Driest Quarter
BIO18	Precipitation of Warmest Quarter
BIO19	Precipitation of Coldest Quarter

5.4 Considerations when selecting novel biological control agents

When selecting biological control agents for novel pests a wide range of aspects need to be considered. Among these, two features are of prominent importance. First, the control agents should be effective for the intended pest species in its novel environment and second, they should be specific, i.e. they should not attack native non-target species (Kuhlmann *et al.* 2006).

Among biological control agents, parasitoid wasps are the most likely candidates to combine these characteristics because they can provide excellent control (e.g. Godfray (1994), and because most parasitoid wasps attack a narrow range of hosts (Memmott *et al.* 2000).

However, there are differences in host specificity among parasitoid wasps, and these are often related to both the mode of parasitisation and the host stages attacked. The highest specificity is attached to endobionts of larvae and nymphs (Godfray 1994), for two reasons. First, endobionts have a more intimate physiological and biochemical connection with their host than parasitoids that don't have to deal with host immune defences (i.e. ectoparasites). Second, larval endobiont parasitoids attack growing host stages, and therefore their life-history and developmental strategy needs to be more finely tuned with that of the host than parasitoids that attack egg, pupal and adult stages. Based on this there is a preference for koinobiont larval endoparasitoids, followed by endoparasitoids of egg, pupa and adult stages (Strand 1986).

Thus, in selection of biological control agents of our pests, we have given preference to larval endoparasitoids whenever possible. Furthermore, we did not select ectoparasites, fungi, viruses or bacteria as they are either less likely to be host specific, and/or more prone to host switching. Furthermore, we have taken into account proven efficacy and specificity elsewhere, in particular in regions that have a similar climate to Australia.

5.5 Selecting non-target hosts for host specificity testing: A general overview

The selected candidates for biological control need to be tested for host specificity, to assess whether it is safe for release. Host specificity testing is a regulatory requirement and a prerequisite to obtaining permission to release new biological control agents into Australia. During this process, the potential agent is thoroughly tested for its propensity to attack a number of potential non-target host species. However, because it is impractical to test a large range of species (Sands 1997, 1998) the tests need to be performed on the most likely non-target hosts (Kuhlmann *et al.* 2006). The selection of potential non target host species for testing requires careful consideration and consultation with the experts in the taxonomy and biology of both the potential non-target hosts and the proposed biological control agent. The final list requires approval from Biosecurity Australia.

There are several criteria for selecting the most likely non-target host species. Among the most important are shared host taxonomy and ecological similarity (Askew and Shaw 1986, Shaw 1988). However, spatial, temporal and morphological attributes, access and availability are also important factors.

Often, the procedure that has been followed to produce the list for specificity testing remains unclear (Kuhlmann *et al.* 2006), possibly because it involves consideration of such a wide range of factors. However, a transparent procedure is of importance and because of this, Kuhlmann *et al.* (2006) have developed a set of recommendations for selecting a non-target host list that involves the following steps:

Selecting non-target hosts on the basis of three categories

There are three categories of selection criteria that should all be considered in the selection of non-target hosts. These are:

Ecological similarities: Species that live in the same habitat or use the same host plant as the target species.

Phylogenetic and or taxonomic affinities: Species that are closely related to the target species.

Safeguard considerations: Beneficial and rare/endangered species that share one of the above characters with the target species.

Consultation with experts on the biology of the non-target species is needed to ensure the quality of the selection procedure.

Within these three categories, priority should be given to species that fit more than one criterion. This could produce a list more than fifty species, which is still impractical for host specificity testing. Therefore, this list is filtered, using two filters.

Filter 1: Remove species with attributes that do not overlap with the target species or with the biological control agent

Different climate requirements: Species that will not overlap in distribution with the biological control agent are ruled out

Phenological differences: Species that are unlikely to be affected by the biological control agent, because their life-cycles do not overlap, are given a lower priority.

Different host sizes: Species that, based on their size, are unlikely to be a target for the biological control agent are excluded.

This may reduce the list, but it may still not be feasible or necessary to test all of the species left. Therefore, a second filter is applied, based on feasibility.

Filter 2: Availability and accessibility

If a potential non-target species is not available or accessible in sufficiently large numbers to allow host specificity testing and it cannot be reared in sufficient numbers within a reasonable period of time, its inclusion into the list for host specificity testing is not warranted: The limited availability would preclude testing.

This procedure leads to the final test list. Depending on the outcome of the host specificity testing, the test list may need to be modified at a later stage.

In the following, we use the criteria suggested by Kuhlmann *et al.* (2006) to select lists for host specificity testing of the four arthropod vineyard pests.

6 THE GLASSY-WINGED SHARPSHOOTER

6.1 The target host, *Homalodisca vitripennis* (Glassy winged sharpshooter)

Taxonomy

The glassy winged sharpshooter (*Homalodisca vitripennis* (Germar) belongs to the order of Hemiptera, family Cicadellidae, subfamily Cicadellinae and tribe Proconiini.

Lifecycle and Biology

In southern California, the species has two generations per year (Blua *et al.* 1999). Oviposition occurs in late winter to early spring and again in mid-to-late summer. Adult glassy-winged sharpshooters (GWSS) live for about two months. Eggs are small and placed side by side in groups ranging from one to 27 with an average of ten inside the epidermis of the lower leaf surface (Turner and Pollard 1959). Eggs hatch in 1-2 weeks and the five nymphal stages feed on leaf petioles or small stems before becoming winged adults in 10 to 12 weeks (Turner and Pollard 1959).

Endemic distribution and plant host range

The glassy-winged sharpshooter is native to the south-eastern United States and north-eastern Mexico (Fig. 6.1). It has a very wide range of host plants; more than 175 species have been identified (Luck *et al.* 2001a, b, Hoddle *et al.* 2003).

Current distribution beyond its endemic range

Beyond its endemic range, the GWSS has invaded and established in southern California (Fig. 6.1), French Polynesia, Hawaii, Easter Island and the Cook Islands. Incursions overseas have most likely been caused by human transport (Petit *et al.* 2009).

Predicted distribution in Australia

Only locality records from its native range and a random sampling of locality records from its introduced range in California were used in modelling the geographic distribution of *H. vitripennis*. There are many more published records of the occurrence of *H. vitripennis* in California, so if all of them were used, the predicted geographic distribution of the species would have been biased in favour of Californian conditions. The MaxEnt modelling was replicated 10 times using a random seed. The model's predictions were extended to Australia to estimate the regions that are under threat from this species.

The final model of the distribution of *H. vitripennis* was based on four variables, BIO1 (48.5% contribution to the model predictions), BIO6 (23.6%), BIO18 (9.2%) and BIO19 (18.7%). The model predicts the know distribution well, and also predicts that *H. vitripennis* could extend its range northward in California as well as more widely in Mexico (Fig. 6.2). In Australia, all major wine grape growing regions, with the exception of Tasmania, could be threatened by an incursion of this pest (Fig. 6.3).

Host plants in Australia

Almost all of the 175 identified host plants of *H. vitripennis* used for feeding and breeding are present in Australia (Luck *et al.* 2001b). These include several crops such as grapes, citrus, summer and stone fruit, grains and many ornamentals. There is no reason to suspect that native Australian plants would not be a host to the GWSS, as the species has been observed to feed and breed on *Eucalyptus* (Luck *et al.* 2001a, b, Hoddle *et al.* 2003), and other Australian ornamentals grown in California (Rathé *et al.* 2012).

Pest status

The GWSS does considerable damage to woody plants by sucking sap (xylem) and spreading a film of faeces on crops. More importantly, GWSS is a vector of the xylem-dwelling plant pathogenic bacterium *Xylella fastidiosa*. This bacterium causes bacterial leaf scorch diseases on a wide range of crops and ornamentals. Australia harbours more than 90 known host species of this bacterium (Luck *et al.* 2001b, Luck *et al.* 2004). Among the diseases caused are Pierce's disease on grapes, leaf scorch diseases of almond, plum, peach, olive and

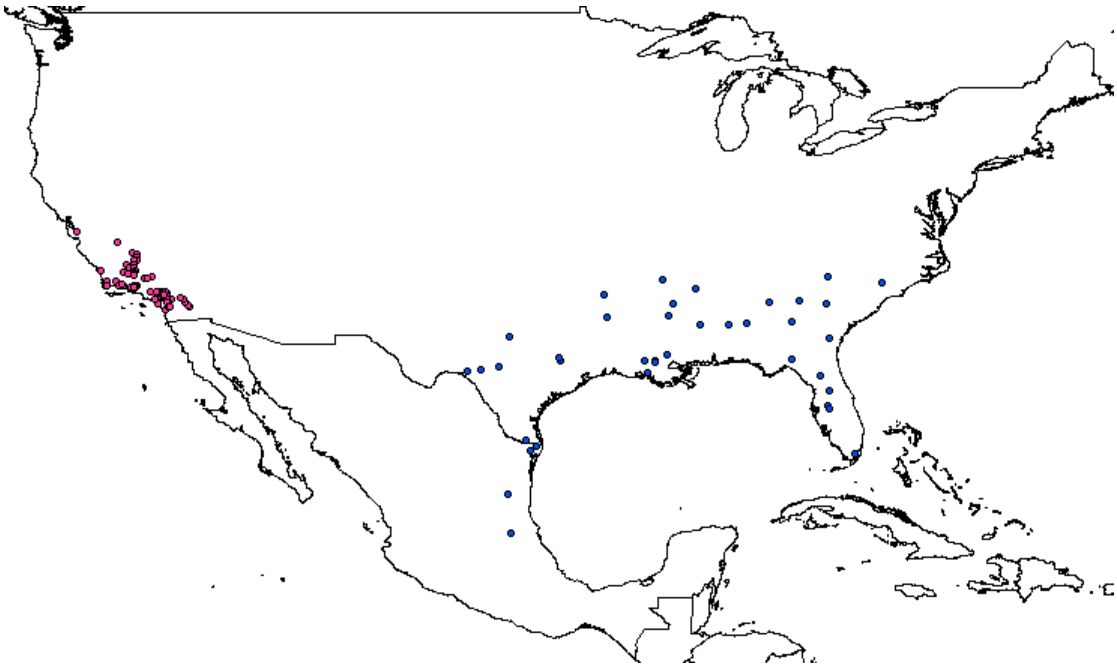


Figure 6.1. Locations in North America where *H. vitripennis* is known to occur (blue = native range; pink = introduced range).

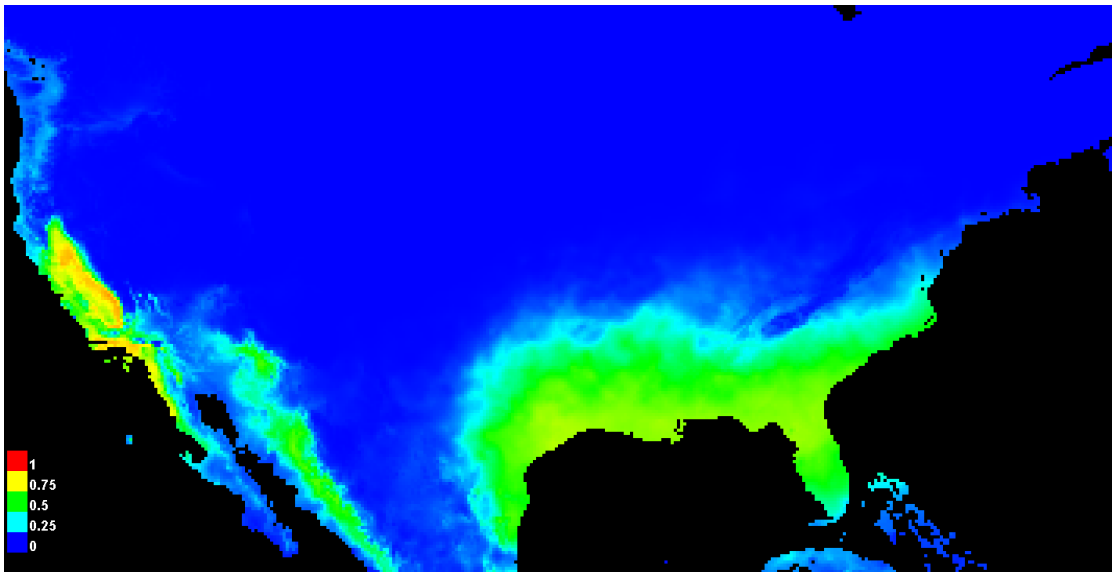


Figure 6.2. Potential geographic range of *H. vitripennis* in North America based on a maximum entropy model. (Blue = not predicted to occur; red = predicted to occur with near certainty).

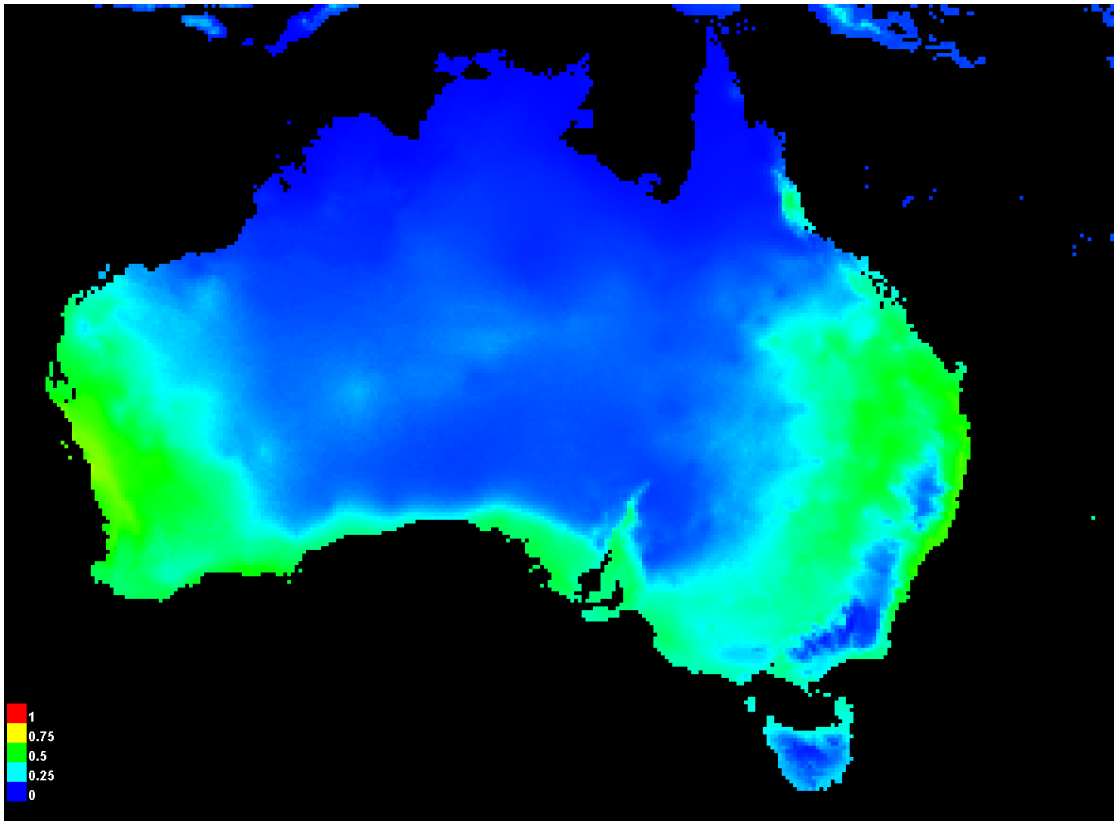


Figure 6.3. Potential geographic range of *H. vitripennis* in Australia based on a maximum entropy model. (Blue = not predicted to occur; red = predicted to occur with near certainty).

avocado, alfalfa dwarf and citrus variegated chlorosis. There is currently no cure for diseases caused by the bacterium.

The economic cost caused by the GWSS-*X. fastidiosa* combination is large. A study conducted by the University of California found that between 1994 and 2000, Pierce's disease caused nearly $30 million in losses and destroyed over 1,000 acres of grape vines and 30 km of ornamental oleander plantings in Northern California alone.

While the GWSS-*X. fastidiosa* combination has the potential to severely affect many agricultural crops and ornamental plants, research in California has also shown that the GWSS vectors *X. fastidiosa* into native vegetation that previously has had no prior association with the bacterium. This is particularly worrisome as it can lead to new disease epidemics not previously seen.

Biosecurity Australia has deemed the threat posed by GWSS to Australian horticulture and viticulture industries to be high. Five plant industries (citrus, nursery and garden, nuts, summer fruit and viticulture) have identified the species as a high priority risk. Pest specific risk reviews, fact sheets and contingency plans and diagnostic protocols have been developed as part of Industry Biosecurity Plans. These documents are available for download from
http://www.planthealthaustralia.com.au/pests/glassy-winged-sharpshooter/.

6.2 The proposed biological control agents *Gonatocerus ashmeadi* and *G. walkerjonesi*

Taxonomy

The parasitoid wasps *Gonatocerus ashmeadi* Girault, 1915 and *G. walkerjonesi* Triapitsyn, 2006 belong to the order of the Hymenoptera, family Mymaridae. Mymarid wasps are parasitoids of insect eggs.

Biology and ecology

The mymarid egg parasitoids *G. ashmeadi* and *G. walkerjonesi* are solitary endoparasitoids, i.e. one adult wasp is produced per host egg (Triapitsyn 2006). Various aspects of the biology and ecology of *G. ashmeadi* have been very well studied, and there is information about ovipositing behaviour (Velema *et al.* 2005), reproductive and developmental biology at various temperatures (Chen *et al.* 2006a, Pilkington and Hoddle 2006a), its functional response and superparasitism (Chen *et al.* 2006b), overwintering biology (Lopez *et al.* 2004), interspecific competitiveness (Irvin and Hoddle 2005, 2006) and population genetics (Vickerman *et al.* 2004, de León and Jones 2005).
As *G. walkerjonesi* was only described for the first time in 2006, the information about its biology is very limited. However, recent evidence suggests that the species may be better adapted to cooler climates than *G. ashmeadi* (Lytle and Morse 2012).

Endemic range

Triapitsyn (2006) suggested that the original host range of *G. ashmeadi* coincides with the original host range of GWSS. The species seems to be self-introduced into California before the introduction of *H. vitripennis* and established in low densities as an egg parasite of *H. liturata*, the local proconiine sharpshooter. Following establishment of *H. vitripennis* in southern and central California during the 1990s, *G. ashmeadi* simply switched back to its natural host, providing good control of the summer brood of the glassy-winged sharpshooter in interior southern California (Triapitsyn 2006, Lytle and Morse 2012).
It has been suggested that the origin of *G. walkerjonesi* is either native to southern California or originated from Las Flores, Masaya, Nicaragua, as specimens from both regions were found to be genetically identical (Triapitsyn 2006). The species was recovered from *H. liturata* prior to the introduction of GWSS into California (de León and Jones 2005, Lytle and Morse 2012).

Current distribution beyond its endemic range

Currently, *G. ashmeadi* occurs in Mexico (Nuevo León and Tamaulipas) and USA (California, Florida, Georgia, Louisiana, Mississippi, North Carolina, South Carolina, Texas and likely in Alabama and southern Arkansas; Triapitsyn 2006). The southern and south-eastern USA strains of *G. ashmeadi* were released in California against *H. vitripennis* (Morgan *et al.* 2002, Pilkington *et al.* 2005). The species was self-introduced into Oahu Island, Hawaii (USA). Furthermore*, G. ashmeadi* was intentionally and successfully introduced into Tahiti, French Polynesia, for biological control of *H. vitripennis,* from where it self-introduced onto all other islands of the archipelago and onto Easter Island (Petit *et al.* 2009).
The distribution of *G. walkerjonesi* has not expanded outside the endemic range given above (Triapitsyn 2006).

Expected efficacy as a biological control agent

In its original range, *G. ashmeadi* was found to be the most common natural enemy of *H. vitripennis* (Triapitsyn *et al.* 1998). Furthermore, whether introduced intentionally or self-introduced, the species very successfully controls the glassy winged sharpshooter in all areas where it has been introduced. Depending on the ambient temperature, the developmental time of *G. ashmeadi* is two to four times faster than *H. vitripennis* (Pilkington and Hoddle

2006b). Its efficacy seems to be particularly high in tropical areas (Petit *et al.* 2008, Grandgirard *et al.* 2009). For example, in Tahiti, the combination of temperature and humidity lead to overlapping generations of *H. vitripennis*, which implies a constant supply of eggs. This allowed parasitoids to build up in high numbers, lead to 95% control within a period of seven months after release (Petit *et al.* 2009), - a situation that may also occur in northern Australia (Rathe *et al.* 2012). Of the five egg parasitoids of *H vitripennis* that have been introduced to California (Pilkington *et al.* 2005), *G. ashmeadi* was found to be the most common natural enemy of *H. vitripennis* in California, Louisiana and Florida (Triapitsyn *et al.* 1998). Its efficacy is partly due to its rapid development relative to the host and in part to the fact that, at low parasitisation densities, the sex ratio upon emergence is very strongly female biased (Irvin & Hoddle 2005). The latter allows rapid establishment of the parasitoid population.

While the rate of parasitisation by *G. ashmeadi* is ~25% in California, this has contributed to a decrease in the spring generation by ~93% within five years (Hoddle 2010). However, the parasitisation rate in California is substantially less than that observed in Tahiti (Grandgirard *et al.* 2007, Petit *et al.* 2009) and, as explained above, it is likely that climatic effects, mainly temperature and humidity, are at the basis of these differences.

Based on the combined temperature and humidity requirements of *G. ashmeadi*, it has been suggested that implementing effective biological control of *H. vitripennis* in the southern dry irrigated regions may be slower than in the more humid tropical areas of Australia (D Morgan, cited by Rathe 2012). Therefore, in the southern dry, cooler areas of Australia a second parasitoid may be required. A recent survey shows that, in the cooler coastal parts of California egg masses of *H. vitripennis* are more commonly parasitised by *G. walkerjonesi* than by *G. ashmeadi* (Lytle and Morse 2012). This result indicates that *G. walkerjonesi* may be better able to deal with cooler climates than *G. ashmeadi*. This is important for the total control effort, as the pest will require control both in tropical areas and regions with a temperate Mediterranean climate (Fig 6.3). Therefore, and because other species were infrequently found on *H. vitripennis* egg masses (Lytle and Morse 2012), we have selected *G. walkerjonesi* as an additional potential biological control agent.

Other parasitoid wasps have been considered, as they have been introduced into California for the control of *H. vitripennis*. However, for some of these species (e.g. *G. triguttatus* Girault, 1916 and *G. fasciatus* Girault, 1911) establishment has so far remained very low (Irvin 2011), and this is likely due to the fact that these species were outcompeted by *G. ashmeadi* (Irvin and Hoddle 2005, Irvin 2011). This was also expected for *G. tuberculifemur* and *G. deleoni* (Irvin 2011, Irvin and Hoddle 2011), two so-called, 'new association species', i.e. species that did not have an earlier association with *H. vitripennis* in their native range. Furthermore, both *G. fasciatus* and the mymarid wasp *Anagyrus epos* Girault (Hymenoptera: Mymaridae) were not host specific. Of these, *G. fasciatus* is a gregarious species that lays more than one offspring per host egg, and has been shown to be able to parasitise egg masses of Cicadellini in the laboratory, even in choice tests (Boyd and Hoddle 2007). For *A. epos*, the host range was even wider, as laboratory experiments indicated that the species parasitises not only members of the Cicadellinae, but also significantly smaller species in a different subfamily (Krugner *et al.* 2008).

Previous research on the host specificity of G. ashmeadi

While the tribe Proconiini is absent in Australia, there are 13 known species of Cicadellinae belonging to the tribe Cicadellini (Fletcher 2009), so it is important that the host range of *G. ashmeadi* is critically examined. Three types of studies have been performed to assess host specificity of *G. ashmeadi*: literature studies, field studies and laboratory experiments. These are reviewed below.

Literature studies

Prior to the release of *G. ashmeadi* on Tahiti, Grandgirard (2007) performed a risk assessment using the literature. This study cites Triapitsyn *et al.* (1998) and Logarzo *et al.*(2003) in support of the contention that *G. ashmeadi* "…appears to have a narrow host range" and "…parasitizes only the eggs of cicadellids in the tribe Proconiini" (Grandgirard *et al.* 2007). Unfortunately, the original studies cited by Grandgirard (2007) do not demonstrate specificity of *G. ashmeadi* on proconiine species. Triapitsyn *et al.* (1998) investigated the efficacy of *G. ashmeadi* and some other *Gonatocerus* species for the parasitisation of egg masses of *H. vitripennis* and did not include any other species of Cicadellinae. Logarzo *et al.*(2003) examined two species of *Gonatocerus* from Peru (*G. triguttatus* Girault and an undetermined species of *Gonatocerus* near *ashmeadi* Girault) for their ability to parasitise egg masses of three species of proconiine sharpshooters: *Pseudometopia amblardii* (Signoret), *P. phalaesia (*Distant) and *Oncometopia* n. sp. Thus, the latter citation did not include *G. ashmeadi* and neither of the studies cited by Grandgirard *et al.* (2007) included any species outside the Proconiini.

Thus, the narrow host range of *G. ashmeadi* cannot be asserted from these studies. However, some laboratory and field experiments have been performed that may allow assessment of host specificity.

Field data

Soon after the release of *G. ashmeadi* on Tahiti, both *H. vitripennis* and *G. ashmeadi* unintentionally invaded all other islands of French Polynesia. This involved long distance dispersal overseas and was most likely aided by human transport of plant material (Petit *et al.* 2009). Although not structurally investigated, by 2008 non-target impacts by *G. ashmeadi* had not been observed on any of the islands in French Polynesia (Petit *et al.* 2009). It is worth while noting that the 13 species of Cicadellini present in French Polynesia are all considerably smaller than *H. vitripennis*, and may not be suitable hosts for that reason (Grandgirard *et al.* 2007).

Negative evidence indicates that *G. ashmeadi* specialises on Proconiini: In the southern USA, the only records of emergences of *G. ashmeadi* are from egg masses of species in the tribe Proconiini (Pilkington *et al.* 2005, Triapitsyn 2006). Native sharpshooter eggs in other tribes are laid singly, and seem to be embedded into the stem rather than deposited in groups just below the epidermal layer of leaves, as is characteristic of GWSS egg masses (Boyd cited in Pilkington *et al.* 2005). Therefore, while such data may indicate that native sharpshooters outside the Proconiini may be unlikely hosts for GWSS parasitoids in the field (Pilkington *et al.* 2005), it is also possible that this conclusion is partly based on the difficulty of detecting eggs of other Cicadellinae in the field.

Thus, field records so far support the notion that *G. ashmeadi* is a specialised parasitoid of Proconiini, but this support is not strong, as very few eggs of Cicadellini seem to have been collected.

Laboratory experiments

We have found only a single laboratory study that included *G. ashmeadi* and host species outside the Proconiini. Using both non-choice and choice experiments, Boyd and Hoddle (2007) tested specificity of *G. ashmeadi* using two species of Proconiini, one species of Cicadellini and one species of Draeculacephalini. The experiments were performed in petri dishes and in field cages. In this study, only the proconiine species were parasitised.

One might be surprised about this very small number of laboratory and field experiments that have been performed to test the specificity of *G. ashmeadi*. However, the near lack of such studies for the USA can be easily understood from the fact that *G. ashmeadi* was already self-introduced in California and Hawaii prior to the active releases of the wasp for biological control of *H. vitripennis*.

Host specificity of other Gonatocerus species

Experimental field studies were conducted in Argentina to determine whether free-living *Gonatocerus* parasitoids could locate and successfully parasitize eggs of 13 species among 4 subfamilies of Cicadellidae. More than 50% of the exposed egg masses of five out of six proconiine sharpshooters were attacked and one species in the Cicadellini was attacked at a very low rate (0.06%). The latter results were later confirmed and extended by further testing with eggs of 26 species of Cicadellidae from South America and Mexico (Logarzo *et al.* 2012, Table 1). In this study, egg masses of five out of seven Proconiini, and egg masses of two out of nine species of Cicadellini were parasitised in the field. For one of the species in the Cicadellini, field parasitisation rates reached 20%. Egg masses of species in other subfamilies were not parasitised. The study by Logarzo *et al.* (2012) also included no-choice laboratory experiments. In these experiments, the *Gonatocerus* species did not parasitise any eggs of the Cicadellini.

Table 6.1. Range of species tested, taxonomic affiliations and outcome for parasitisation with *G.* sp. near *tuberculifemur*. Data from Logarzo *et al.* (2012).

Subfamily	Tribe	# spp parasitised / tested in the laboratory	# spp parasitised / tested in the field	Was parasitisation by *Gonatocerus* spp observed?	Is the tribe present in Aus
Cicadellinae	Proconiini	6/6	5/7	yes	no
	Cicadellini	0/6	2/9	yes	yes
Deltocephalinae	Deltocephalini	0/1	0/1	no	yes
	Macrostelini	0/1	0/1	no	yes
	Euscelini	0/2	0/2	no	no
	Hecaliini		0/1	no	yes
Iassinae	Scarini	0/1	0/2	no	yes
Megophthalminae	Agallini		0/2	no	yes
Ledrinae	Xerophloeini		0/1	no	yes

Conclusion

In summary, circumstantial evidence indicates that *G. ashmeadi* is a specialist egg parasite of proconiine sharpshooters, but this is based on limited evidence from the field, and on host specificity testing that included a single species of Cicadellini. Experimental field results show that, in the field, not all *Gonatocerus* species are narrow specialists on proconiine cicadellids, but some also include species belonging to the Cicadellini. Therefore, we do not consider the available evidence sufficient to conclude that *G. ashmeadi* only parasitises proconiine cicadellids and, unlike Charles (2012) suggested for New Zealand, we conclude that host specificity testing should be performed before *G. ashmeadi* and *G. walkerjonesi* could be safely introduced into Australia.

6.3 Possible non-target hosts of *G. ashmeadi* in Australia

In the following, we use the method recommended by Kuhlmann *et al.* (2006) summarised above, to select the most likely non-target host of *G. ashmeadi*.

Taxonomic affinities, ecological similarities

Taxonomic affinities

The GWSS belongs to the tribe of Proconiini in the Cicadellinae. There are no representatives of the Proconiini in Australia and only 13 species in the Cicadellini (Fletcher 2009). Based on the above research (section 2.5 and Table 1) it is clear that *G. ashmeadi* is a specialised egg parasitoid of Cicadellinae and it seems likely that, within this subfamily, it specialises on Proconiini. For *G. walkerjonesi*, a specialisation on Proconiini needs to be ascertained.

Ecological similarities

Plant hosts: The GWSS lives on a wide range of plants and most of the 175 known host species are present in Australia. This includes both crops and non-crops, and both woody and herbaceous plants (Rathe 2012). Some of the native Australian Cicadellini (*Cofana* spp.) feed exclusively on grasses (Fletcher 2009, personal communication) and this can also be a plant host for GWSS. Therefore ecological similarities in plant hosts used by *H. vitripennis* and native invertebrate species are likely to be uninformative for the assessment of possible non-target hosts.

Evidence from laboratory and field surveys indicates that parasitism rates by *Gonatocerus* spp. vary among the host plants used by GWSS as an ovipositional host (Irvin and Hoddle 2004). Experiments show that *G. ashmeadi* is responsive to plant volatiles produced in response to ovipositing by GWSS, and that this level of attraction differs between plant species (Krugner *et al.* 2008). This indicates that *G. ashmeadi* may respond to volatiles produced in response to sharpshooter eggs in a range of plant species.

Parasitisation

Laboratory and field tests show that parasitisation rates by *G. ashmeadi* vary between plant hosts.

Because of the absence of any differentiation with respect to taxonomic affinities and the wide range of ecological connections of the host species, our initial test list contains all 13 Cicadellini present in Australia:

Filter 1: Spatial, temporal and morphological attributes

Spatial attributes: Climate

Based on the original distribution and incursion in the USA, an expected range of the glassy-winged sharpshooter has been modelled at an earlier stage using Climex modelling. (Merriman *et al.* 2001). Based on that modelling, the expected range included the eastern and south-eastern seaboards of Australia and the wine growing regions in WA. This would be convenient, as most of Australian native Cicadellini have a distribution that is limited to tropical or humid temperate climates. However, some species of *Ishidaella* are found as far south as Tasmania (Fletcher, 2009, personal communication). Furthermore, the expected distribution of GWSS in Australia needs to be put in perspective by the later incursions of the GWSS in French Polynesia and Hawaii, which clearly demonstrate an ability of GWSS to invade tropical areas.

Similar to earlier predictions of the distribution of GWSS, it has been suggested that humid, partially shaded and densely vegetated habitats are unlikely foraging areas for *G. ashmeadi* (Pilkington *et al.* 2005). However, this conclusion has become untenable after the

tremendous success of the introduction of *G. ashmeadi* in French Polynesia and Hawaii and the rapid control of GWSS after introduction on these islands. Thus, we suspect that the parasitoid can be very effective in tropical environments.

However, as neither the GWSS nor *G. ashmeadi* seem to thrive in unirrigated desert areas (Boyd and Hoddle 2007), and our climatic modelling indicates that the natural climate of California is only marginally suitable for GWSS, we conclude that the distribution of both the pest and the parasitoid in the drier regions of Australia will be restricted to the irrigated areas. Unfortunately, none of the Australian Cicadellini is typically a desert species (Fletcher, 2012, personal communication), so climatic considerations do not help to narrow the selection of potential non-host targets for host specificity testing.

Spatial attributes: Egg deposition
Depending on the climate, ovipositing by GWSS occurs in winter to early spring and again in mid-to-late summer, or in the tropical regions year round. Adults live for several months and lay eggs side by side in groups of about 10, ranging from 1-27 (Turner and Pollard 1959). The eggs are laid within host plants inside the epidermis of the lower leaf surface and appear as greenish blisters (Turner and Pollard 1959), and are protected by brochosomes, a glandular secretion that forms a protective layer over the eggs (Hix 2001). Any egg parasitoid would need to be adapted to deal with this protective layer, and may be adapted to searching for brochosomes (Velema *et al.* 2005).
Australian Cicadellini have a fairly solid ovipositor, which may suggest that the eggs are inserted into stems (M. Fletcher, personal communication). However, the ovipositing behaviour and eggs of most of the Australian Cicadellidae have not been studied, and it is unknown whether the native species use brochosomes to protect the egg masses.

Morphological attributes
All known hosts of *G. ashmeadi* are proconiine cicadellids of moderately large size (1.2 - 2 cm in length), that lay large eggs (2.5-3 mm in length) in large clutches (Boyd and Hoddle 2005). Egg size is likely to be an important factor in predicting parasitisation, because *G. ashmeadi* is a solitary parasitoid, and hence the egg needs to supply sufficient nutrients to allow the wasp larvae to complete its development. Circumstantial evidence supports the importance of egg size, as so far *G ashmeadi* has not been found to parasitise the egg masses of the considerably smaller native Cicadellini in French Polynesia (Petit *et al.* 2009).

In general appearance, GWSS is superficially similar to a number of Australian species, in particular in the tribe Thymbrini (Fletcher 2009). However, differences in biology between the Thymbrini precludes them becoming a non-target host (M. Fletcher, personal communication). Australian species of Cicadellini that are comparable in size to *H. vitripennis* are *Conoguinula coeruleopennis* and species of *Cofana*. The species of *Ishidaella* are significantly smaller than *H. vitripennis*. Identification of Ishidaella species relies on male genitalia, as they are relatively similar in appearance. This implies that inclusion of a single species of *Ishidaella* in the non-target host list should suffice (M. Fletcher, personal communication).

Filter 2: Access and availability

For the purpose of host specificity testing, a number of species show large similarities but some are rare and/or have a limited tropical distribution, such as *Conoguinula coeruleopennis*, while others are common. The Australian Cicadellini that are most common and widespread are *Cofana spectra*, *Ishidaella angustata* and *I. latomarginata* and *I. albomarginata* (Fletcher 2009). According to M. Fletcher, *C. spectra* could be fairly easily

obtained from Queensland, while a species of *Ishidaella* could be collected from Sydney gardens, and is likely to be one of the three listed above.

Conclusions

Based on above considerations, and in consultation with Prof. M. Fletcher, specialist on Australian Cicadellinae, the proposed non-target host test list includes:
Cofana spectra and one of the species *Ishidaella angustata*, *I. latomarginata* or *I. albomarginata*.

6.4 Host specificity testing

In the event of an incursion of the glassy-winged sharpshooter, the parasitoids *G. ashmeadi* and *G. walkerjonesi* will need to be subjected to host specificity testing. These species can be obtained through contacting Mark Hoddle at the University of California Riverside.
The non-target and target host species should be collected from the field and encouraged to lay eggs in captivity on the same plants they have been caught on and on grapevine.

Three types of tests should be performed in a Quarantine Approved Facility under controlled temperature and daylight conditions.

No choice experiments

No-choice testing provides the most conservative approach to determine host range as it places fecund female parasitoids in close proximity with the eggs of only a single species of host. These conditions therefore provide the greatest chance of drawing out non-target effects as the lack of host choice may lead to attempted parasitisation even if this were never to occur in nature.
These tests should be performed in enclosed arenas. Using a minimum of 20 replicates, egg masses of each of the two species of non-target hosts, should be presented to each of the parasitoids separately. Each set of replications should be accompanied by three control replicates using egg masses of the target host on grapevine to ensure that conditions are suitable for parasitisation.

Choice experiments

Choice experiments should be conducted in order to expose potential non-target effects in a semi-natural situation with egg masses of multiple potential hosts, each on one of their natural host plants including the target host. Such testing provides a more natural situation and is less conservative than no-choice tests in that wasps are less likely to parasitise an unusual host given a choice of hosts, one of which is the natural host (van Lenteren *et al.* 2006).
These tests should be performed enclosed arenas The parasitoids should be presented with a choice between eggs of one of the two species of non-target hosts on their native host plants and egg masses of *H. vitripennis*, either on grapevine or on the host plant of the native sharpshooter. There should be a minimum of 20 replicates.

Wind tunnel tests

Wind tunnel experiments should elucidate the attractivity of egg masses of *H. vitripennis and* the non-target hosts on grapevine. The methods are described in Kitt and Keller (Kitt and Keller 1998)

7 THE VINE MEALYBUG

7.1 The target host, *Planococcus ficus* (Vine mealybug)

Taxonomy

The vine mealybug (*Planococcus ficus* Ezzat & McConnell, 1956) belongs to the order of Hemiptera, suborder Sternorryncha, superfamily Coccoidea, family Pseudococcidae.

Lifecycle and Biology

The following description of the general lifecycle was taken from Daane *et al.* (2012). The vine mealybug overwinters under the bark of the trunk, on underground roots, or in ant nests. There is no diapause. On warm days, development may occur during the winter months, with completion of the first generation almost entirely under the bark. From spring to summer, the mealybugs can be found on all parts of the vine. Up to seven generations are completed and population density can increase rapidly, although high summer temperatures, in excess of 40°C, may slow the growth of the population. The rapid population increase in early summer is followed by an equally rapid decline after harvest, resulting from biological controls and abiotic mortality associated with high temperatures and vine senescence.

Endemic distribution and plant host range

The vine mealybug is Palearctic of origin, with its central distribution in the Mediterranean parts of Europe (Fig 7.1). The species is recorded on 24 host plants belonging to 17 plant families (Ben-Dov *et al.* 2013).

Current distribution beyond its endemic range

There are numerous uncertainties about the current distribution of *Pl. ficus* outside its endemic range, which are caused partly by difficulties to distinguish this species from *Pl. citri* (Gullan pers. com.), as the species are very closely related and even interbreed (Rotundo and Tremblay 1982).



Figure 7.1 Geographic distribution of *Pl. ficus* based on published records.

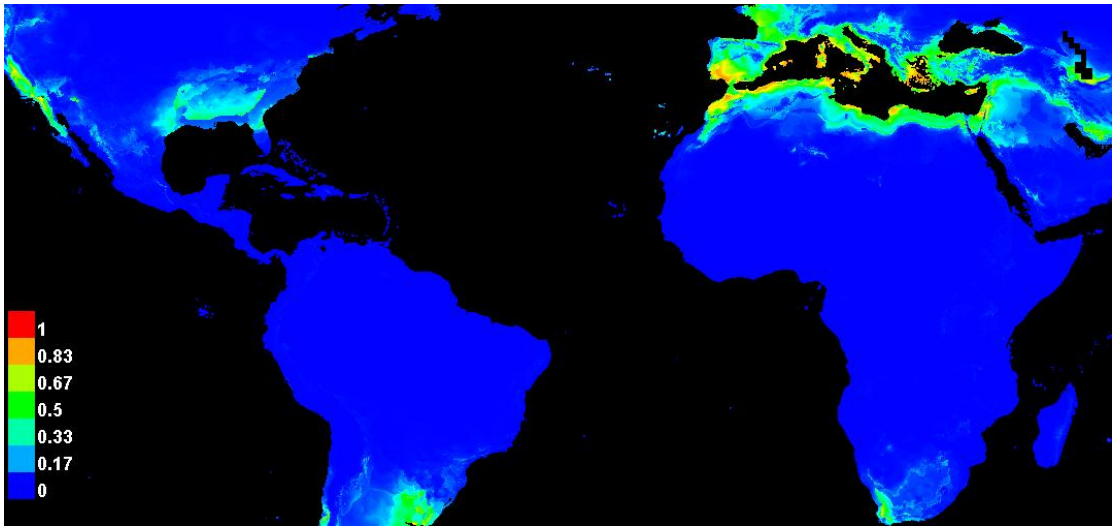


Figure 7.2. Predicted geographic range of *Pl. ficus* in Europe, Africa and the Americas based on a maximum entropy model. (Blue = not predicted to occur; red = predicted to occur with near certainty)

For example, while Ezzat and McConnell (1956) state that the species was present in many states of the USA in 1956, Gill (1994) contends that *Pl. ficus* was first identified in California in the Coachella Valley in the early 1990s, and that it has since spread into California's San Joaquin Valley and central coast regions, with new infestations reported each year (Daane et al 2012). Similarly, the species is listed as occurring in Chile (Ezzat and McConnell 1956), but a recent survey using molecular methods failed to identify the species from the country (Correa *et al.* 2012), and its presence in Chile had apparently been questioned before (Gonzalez 2011 cited in Correa et al 2012). According to the most recent overview by Daane et al (2012), the current distribution outside its endemic range covers California and Mexico, and Argentina (Mendoza), South Africa and some Atlantic Islands (Fig. 7.2). However, in some of these cases, presence has not as yet been confirmed using molecular data.

Predicted distribution in Australia

The vine mealybug poses a significant threat to the Australian wine industry as it has demonstrated its ability to spread widely beyond its native range around the Mediterranean Sea (Fig 7.1).

The final geographic distribution model of the distribution of *Pl. ficus* was based on six variables, BIO8 (23.2% contribution to the model predictions), BIO6 (18.5%), BIO19 (18.1%), BIO1 (15.6%), BIO4 (14.9%) and BIO11 (9.7%). The model predicts the know distribution adequately, but locations in the interior of South Africa could arguably show greater likelihood of environmental suitability (Fig. 7.2). However, the present distribution and therefore the predicted distribution in Australia is likely to be affected by the area of land under irrigation, as mealybugs do not cope well with periods of extensive drought. Importantly, according to this model, all major wine grape growing regions In Australia could be threatened by this species, with the exception of Tasmania (Fig. 7.3).

Host plants in Australia

Most of the 24 host plant species of *Pl. ficus* that are listed on by Ben-Dov *et al.* (2013) are present in Australia. These include several crops such as grapes, citrus, apple, mango, figs, but also a number of ornamentals such as oleander and dahlia. Although the species has not been recorded on Australian native plants, the breadth of their host plants would suggest that some native species could function as host plants.

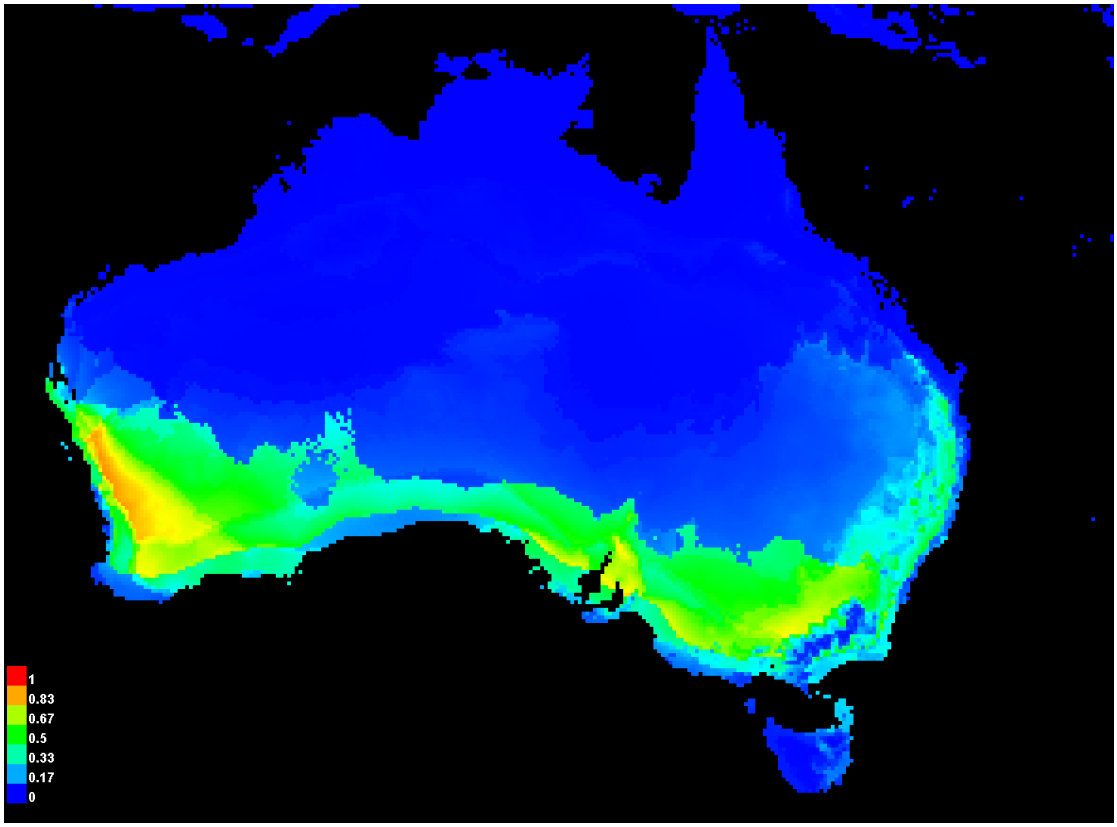


Figure 7.3. Potential geographic range of *Pl. ficus* in Australia based on a maximum entropy model. (Blue = not predicted to occur; red = predicted to occur with near certainty).

Pest status

Vine mealybugs are sap sucking insects that feed on leaves and, as it ripens, increasingly on fruit. Their presence can cause rejection of fruit. In addition, mealybugs may damage the vines directly and indirectly. Large populations may lower the vigour of the plant by feeding on phloem and may affect fruit quality by depositing honeydew on the fruit and leaves, on which sooty mould subsequently develops (Geiger & Daane, 2001). This can cause defoliation and fouled fruit, and is a problem of concern in the table grape industry (Bentley *et al*., 2008; Daane *et al.*, 2008a; Daane, *et al.* 2012). Furthermore, mealybugs are known to transmit several grape-vine leaf- roll associated viruses (Golino *et al.* 2002), which can be damaging to vines, the crop and wine quality (Daane *et al.* 2012). The control of grape leaf-roll disease is hampered by the fact that both mealybugs and viruses can survive on vine roots for many years after the vines have been removed (Bell *et al.* 2009).

Other options for control

Vine mealybugs are particularly difficult to control, as they spend a large part of their lifecycle hidden under the bark or in the root area of vines, which makes it difficult for parasitoids to find them (Daane *et al.* 2002). Furthermore, the species has a close association with ants, which defend them against parasitoids (Daane *et al.* 2002) and take them into their nests to survive periods of adverse conditions.

Chemical control of mealybugs is fraught with difficulty. First, mealybugs are covered by hydrophobic waxy secretions, which prevent the penetration of any water-based insecticide solutions (Franco *et al.* 2009). In addition, mealybugs are able to rapidly build up resistance to insecticides (Flaherty *et al.*, 1982; Walton & Pringle, 2004b; Franco, *et al.* 2009). Furthermore, due to the lack of selectivity of such pesticides, they reduce the levels of

natural biological control agents that suppress mealybugs and other arthropod pests (Daane *et al.*, 2006; Holm, 2008).

The viticulture Industry Biosecurity Plan 2009 has identified the vine mealybug as a high risk to the Australian Grape and Wine industry and has produced a Pest Risk Review, and a fact sheet, available for download from
http://www.planthealthaustralia.com.au/pests/vine-mealybug/.

7.2 The proposed biological control agents *Anagyrus pseudococci*, *Coccidoxenoides peregrinus*, *Leptomastix abnormis* and *Leptomastidia dactylopii*

Mealybugs have hundreds of natural enemies. Worldwide, the introduction of parasitoids, especially in the family Encyrtidae, has been the most successful strategy for controlling mealybug pests (Daane *et al.* 2012), with a number of spectacular successes (Moore 1988). In combination with the native Australian ladybird beetle *Cryptolaemus montrouzieri*, a range of parasitoids provide excellent control of the vine mealybug in northern America, provided insecticidal sprays are not used (Daane *et al.* 2012). Further improvement of biological control for mealybugs involves the control of honeydew seeking ants (Phillips and Sherk 1991). Combining information from Daane *et al.*(2001), references therein and Ben-Dov *et al.* (2013) and personal communication with Kent Daane, we constructed a list of parasitoid species involved in the biological control of *Pl. ficus* (Table 7.1).

The five main parasitoids of the vine mealybug are *Anagyrus pseudococci*, *Coccidoxenoides peregrinus, C. perminutus*, *Leptomastix abnormis* and *Leptomastidia dactylopii* (Table 7.1). These species have been introduced in the USA for the control of *Pl. ficus* and strains of all these species are already present in Australia, where they control *P. calceolariae*, *P. longispinus*, *Pl. citri* or *Nipaecoccus viridis* on citrus (Malipatil *et al.* 2000, Table 5.2). The strains of the five parasitoids that are currently present in Australia should be tested overseas for their efficacy to control *Pl. ficus*.

While strains of some of the species present in Australia will contribute to the control, it is likely that more effective strains are present in the native region of the vine mealybug. This was illustrated in California, where the introduction of a Spanish strain of *A. pseudococci* has lead to improved control of the control of *Pl. ficus* due to an improved numerical response in spring (Daane *et al.* 2008, pers. com).
Considering the serious consequences of this invasive pest for the grapevine industry, the first assessment should use molecular techniques to find the origin of the incursion, which could be South Africa, the Mediterranean, the USA or South America. Subsequently, the parasitoids that are most effective in controlling *Pl. ficus* in the region of origin should be included in host specificity testing. For now, we select the Spanish strain of *A. pseudococci* as optimal potential biological control agent. However, should the strain of *Pl. ficus* originate from another region in the Mediterranean, it is highly recommended to locally source the most effective strain of parasitoid wasp.

Taxonomy

The parasitoid wasp species *Anagyrus pseudococci* (Girault) belongs to the order of the Hymenoptera, family Encyrtidae.

Biology and ecology

The proposed biological control agent *A. pseudococci* a solitary parasitoid of immature and mature mealybugs and is the dominant natural enemy of the vine mealybug (Daane *et al.* 2008; Daane *et al.* 2012). The parasitoid has a development rate and temperature tolerances that closely match those of the vine mealybug (Gutierrez *et al.* 2008).

Endemic range

The species *Anagyrus pseudococci* occurs in several countries in the Afrotropical, Nearctic, Neotropical, Oriental and Palearctic regions (Noyes 2012). Strains of this species may have been transported on purpose or accidentally to many continents, including Australia.

Expected efficacy as a biological control agent

While the Spanish strain of *A. pseudococci* attains higher levels of control than other strains present in California (Daane pers. com.), it is unlikely that this species will provide sufficient control of *Pl. ficus* on its own. The species that currently parasitise *Pl. citri* in Australia will provide additional control. Further management of this pest will require other control measures in addition to the use of parasitoid wasps (Daane *et al.* 2012). These measures are outlined below, under the heading 'Other measures needed to control *Pl. ficus'*.

Table 7.1. The parasitoid wasps of the vine mealybug, based on references in Ben-Dov *et al.* (2013), Noyes (20012) and Noyes and Hyatt (1994). Also shown: presence of the parasitoid in Australia (based on Malipatil 2000). The species marked with a star have been introduced from overseas.

Known parasitioids of the vine mealybug	Present in	Present in Australia?
Aphelinidae:		
Coccophagus lyciminia (Walker)	Georgia	No
Marietta picta (Andre)	Georgia	No
Encyrtidae:		
Anagyrus agraensis	Iran, Tunisia	Yes
Anagyrus mirzai	Iran	No
Anagyrus pseudococci (Girault)	Israel, USA*, Iran, Georgia, Sicily, Argentine*	Yes
	Spain	No^
Coccidoxenoides peregrinus (Timberlake)	Israel, South Africa*, Tunisia, USA*	Yes
Coccidoxenoides perminutus (Timberlake)	South Africa	Yes
Homalotylus qualeyi Timberlake	Georgia	No
Homalotylus turkmenicus Myartseva	Iran	No
Leptomastix abnormis (Girault)	Abkhazia, Argentine*, Azerbaijan, Georgia, Israel, Italy, Tadzhikistan, Tunisia, Turkmenia, USA*	Yes
Leptomastidea dactylopii (Howard)	Iran, Georgia, South Africa, Tunisia	Yes*
Leptomastix flava Mercet	Iran, Israel	No
Neoplatycerus kemticus Trjapitzin & Trjapitzin	Egypt	No
Neoplatycerus palestinensis (Rivnay)	Israel	No
Pachyneuron concolor	Georgia	No
Pauridia peregrina Timberlake	Israel	No
Prochiloneurus aegyptiacus (Mercet)	n/a	No

^ This strain is more efficient than the strains currently present in the US (Daane pers. com.)

Host specificity

The species *A. pseudococci* is not a specialist species. Worldwide, this species is associated with 27 host species in 11 genera of mealybug (Noyes 2012). Of these 11 genera, 9 are represented in Australia, with 87 largely endemic species (Houston 2002) and nine species that are known to be widespread and economically problematic (Williams 1996). Given the broad host range of this parasitoid and the abundant presence of native species belonging to these subgenera, it is highly likely that the strain would be able to parasitise a range of native Australian mealybugs.

While this species may seem worryingly non-specific, it should be noted that *A. pseudococci* has been present in Australia for an unknown period of time. It is therefore possible that the Australian representatives of this species have become adapted to parasitise some native species. The introduction of a novel strain of the same species from overseas is therefore not expected to cause additional effects for potential non-target hosts. Furthermore, the association of parasitoids and mealybugs noted by Noyes (2012) has not been verified and is based on both primary and secondary literature. Therefore, it is likely that in many cases both the mealybug host and the parasitoid species have been misidentified (Noyes pers. com., Gullan pers. com.)

Thus, limited additional effects are expected from the importation of the Spanish strain of *A. pseudococci*. However, such effects will need to be tested prior to importation of this strain.

7.3 Possible non-target hosts of the Spanish strain of *A. pseudococci* in Australia

7.3.1 Category 1: Taxonomic affinities, ecological similarities

Taxonomic affinities

There are 3 species of *Planococcus* in Australia (Houston 2002), *Pl. citri*. *Pl. mali* and *Pl minor*. Of these species, only *Pl. minor* is not native. These species are all economically important pests, nationally and worldwide. The genus *Planococcus* may well be a monophyletic group, but so far, only the close taxonomic association between *Pl. ficus* and *Pl. citri* has been confirmed using molecular data (Downie and Gullan 2004).

Ecological similarities

Plant hosts:

Mealybugs predominantly live on perennial plants (Daane *et al.* 2012). As mentioned above *Pl. ficus* has been recorded on 24 host plants belonging to 17 plant families (Noyes 2012). Given the large range of perennial plant species that can be a host to the vine mealybug, it is likely that after incursion *Pl. ficus* would share plant host species with a number of native mealybug species, in particular with those with a wide host range.

Furthermore, *A. pseudococci* attacks hosts on a wide range of plant species. Noyes (2012) lists 20 plant species in 14 families, which includes plants from tropical, subtropical and Mediterranean origin. Thus, neither the plant hosts that harbour the vine mealybug nor those that harbour the mealybug species attacked by *A. pseudococci* help to narrow down the list of native non-target hosts.

Common native mealybug species with a wide host ranges include *Nipaecoccus ericiola, Ps. hypergaeus* and *Ps. calceolariae.*

Parasitisation:
As indicated above, the parasitoid *A. pseudococci* attacks all mealybug stages of *Pl. citri* and *Pl. ficus*, with a preference for the second, third and fourth nymphal stages. This implies that parasitisation is not limited to a certain size or instar. Hence information about the parasitisation behaviour does not help to limit the list of potential native non-target hosts.

Filter 1: Spatial temporal and morphological attributes

Spatial attributes: Climate
Based on the original distribution and incursion in the USA, an expected range of the *Pl. ficus* is throughout the wine growing regions in Australia (Fig 7.3). This range is shared with numerous native mealybugs including *Ps. hypergaeus*, *Ps. eucalyptus* and *N. ericiola*.

Spatial attributes: Egg deposition
The vine-mealybug overwinters under bark, in the root zone or in ant nests. In these positions, the mealybug cannot be attacked by parasitoid wasps and hence these attributes do not set it aside from any native mealybugs.

Morphological attributes
The sizes of Australian *Pseudococcus* species are not informative in the selection of non-target host species as the encyrtid wasps parasitise a range of instars and sizes of mealybug and will adjust the number of eggs and sex ratio to the size of the host.

Filter 2: Access and availability

For the purpose of host specificity testing a number of species show large similarities but some species rare, difficult to find and/or have a limited or mainly tropical distribution while others are common. In selecting species for host specificity testing, we have decided to focus on the native non-pest species that are most widespread and have the widest host range, as indicated in Williams (1985). This excludes a number of species. For example, *Pl. mali* is common on Tasmania, but has only been found once on the mainland (Williams 1985), while *Ps. calceolariae* and *Pl citri* are economically damaging mealybugs
Common species with a wide host range and a distribution in the grape growing regions of Australia are:

- *Ps. eucalypticus*: This species which occurs throughout Australia apart from NT; Its host plants are a range of Eucalyptus species.
- *Ps. hypergaeus*: This species has a very wide plant host range which includes both native and introduced species of plants. While this species has not been found in WA or NT it has been found in all other States in Australia and also occurs in New Zealand.
- *Nipaecoccus ericiola:* The species is common and widespread, but has not been found in Western Australia. It has a wide host range which includes both native and introduced species

Conclusion

Based on above considerations and in consultation with Prof. P. Gullan specialist on Australian mealybugs, Dr M. Malipatil specialist on parasitoids of Australian scale insects and Prof K. Daane specialist on biological control of mealybugs in the native range of *Pl. ficus*, the proposed test list for *A. pseudococci* includes *Ps eucalypticus*, *Ps. hypergaeus* and *Nipaecoccus ericiola*.

7.4 Host specificity testing

In the event of an incursion of the vine mealybug, the Spanish strain of the parasitoid *Anagyrus pseudococci* may need to be subjected to host specificity testing. This strain can be obtained from Koppert Biological Systems in Spain (www.koppert.es).

The non-target and target host species should be collected from the field and encouraged to reproduce in captivity on the same plants they have been caught on and on grapevine.

Three types of tests should be performed in a Quarantine Approved Facility under controlled temperature and daylight conditions.

No choice experiments

No-choice testing provides the most conservative approach to determine host range as it places fecund female parasitoids in close proximity to different developmental stages of a single species of host. These conditions provide the greatest chance of drawing out non-target effects as the lack of host choice may lead to attempted parasitisation even if this were never to occur in nature.
These tests should be performed in enclosed arenas. Using a minimum of 20 replicates for each developmental stage, the three non-target species of mealybugs should be presented to fecund females of *A. pseudococci*. Each set of replications should be accompanied by three control replicates using vine mealybugs on grapevine to ensure that conditions are suitable for parasitisation.

Choice experiments

Choice experiments should be conducted in order to expose potential non-target effects in a semi-natural situation with similar developmental stages of multiple potential hosts, each one on their natural host plants, including the target host. Such testing provides a more natural situation and is less conservative than no-choice tests in that wasps are less likely to parasitise an unusual host given a choice of hosts, one of which is the natural host (van Lenteren *et al.*).
These tests should be performed enclosed arenas. The parasitoids should be presented with a choice between the same developmental stage of a species of non-target hosts on their native host plants and of *Pl. ficus*, either on grapevine or on the host plant of the native mealybug. There should be a minimum of 20 replicates per developmental stage.

Wind tunnel tests

Wind tunnel experiments should be performed to elucidate the attractiveness of different stages of *Pl. ficus* and the non-target hosts on grapevine. The methods are described in Kitt and Keller (1998).

7.5 Further recommendations

We strongly recommend that a survey be performed of the current introduced parasitoid species that attack Australian native mealybugs that are not economically problematic, such as performed in New Zealand (Charles 2011). A similar survey in Australia should provide information about the effect of economically important parasitoids on native species.

Other measures needed to control Pl.ficus

Additional measures to control the population of *Pl.ficus* will include keeping ant numbers in check. The control of ants in the vineyard aids all invertebrate biological control agents, as ants protect mealybugs from predation and parasitisation in exchange for honeydew (Buckley and Gullan 1991, Phillips and Sherk 1991). Furthermore, the deployment of

predators such as the mealybug destroyer *Cryptolaemus montrouzieri*, lacewings and pirate bugs may also help to control vine mealybug densities. (Daane *et al.* 2012)
The use of pheromone mating traps for monitoring is also recommended. The use of mating traps is well established in several Australian horticultural industries, including the wine industry, for mating disruption of leafrollers and other mealybug species.

Interestingly, mating traps for *Pl. ficus* have been demonstrated to enhance the control of *Pl. citri* by attracting *A. pseudococci* (Franco *et al*. 2008, 2011). Therefore, the potential use of mating traps to enhance control by a range of parasitoids should also be investigated.

In summary

A range of suggestions and recommendations have been formulated above. For convenience, these are summarised below in two categories: those activities that could be performed at any time and those that should be performed upon an incursion.

To be done at any time:

- Investigate overseas whether the five Australian parasitoids of *Pl. citri* (*Anagyrus pseudococci*, *Coccidoxenoides peregrinus, C. perminutus*, *Leptomastix abnormis* and *Leptomastidia dactylopii*) could provide efficient control options for *Pl. ficus.*
- Investigate the extent of parasitisation of Australian native mealybugs by parasitoids that contribute to the control *Pl. citri.*
- Construct a library of DNA signatures of *Pl. ficus* from different regions in the world. This will allow rapid assessment of the origin of the incursion and hence improve selection of biological control agents.
- Educate growers regarding the effects of insecticides on biological control of mealybugs and the close mutualism between mealybugs and ants.

Upon an incursion of *Pl. ficus*:

- Investigate whether the five Australian parasitoids of *Pl. citri* (*Anagyrus pseudococci*, *Coccidoxenoides peregrinus, C. perminutus*, *Leptomastix abnormis* and *Leptomastidia dactylopii*) could provide efficient control options for the invasive strain of *Pl. ficus.* Quarantine would not be needed for these experiments.
- Use molecular tools to identify the origin of the incursion.
- Identify the species that is considered the most effective biological control agent in the region of origin.
- If the most effective biological control agent in the region of origin of the incursion turns out to be a strain of a species already present in Australia and this has been verified using molecular techniques, proceed directly to apply for permission for the release. It is highly unlikely that the new strain would be more damaging to native mealybugs than a strain of the same species that has been in Australia for more than 50 years.
- If the most effective biological control agent in the region of origin of the incursion is different from any of the parasitoid species already present in Australia, perform host specificity tests using some widespread native species that occur in and around vineyards and have a wide range of host plants. These species are *Nipaecoccus ericiola* and *Pseudococcus hypergaeus* and *Ps. eucalypti*. The selection process of these non-target hosts overlaps with the non-target hosts for the grapevine moth and is further discussed in Chapter 8, section 3.

8 THE GRAPE MEALYBUG

8.1 The target host, *Pseudococcus maritimus* (Grape mealybug)

Taxonomy

The grape mealybug (*Pseudococcus maritimus* Ehrhorn) belongs to the order of Hemiptera, suborder Sternorryncha, superfamily Coccoidea, family Pseudococcidae.

Biology

The biology of this species is very similar to that of *Pl. ficus*, with a number of differences. Unlike *Pl.ficus*, the species has a period of winter dormancy and has only two generations in California's interior valleys (Geiger and Daane 2001). Therefore, the population builds up less rapidly than that of *Pl. ficus*. In addition, while tended by ants, the species is not known to survive adverse periods inside ant nests (Daane *et al.* 2012).

Endemic distribution and plant host range

The grape mealybug is widely distributed in the United States and the southern part of Canada (Fig 8.1). It is not a major pest there and it was especially difficult to obtain authoritative reports of locations where it has been found, except in California and Washington, where it is a minor pest of wine grapes. We are indebted to Debra Creel, who painstakingly recorded locality information from all labelled specimens that are deposited in the U. S. National Museum. Without her help, this modelling exercise would not have been possible. Note that one specimen in the U.S. National Museum is from Leeton, NSW. However, Gimpel and Miller (1996) stated that only three specimens of *Ps. maritimus* have been authenticated outside the USA. They reviewed all specimens in the U.S. National Museum, so the record from Leeton is likely to be a misidentification.
The species is recorded on 81 host plants belonging to 37 plant families (Ben-Dov *et al.* 2013). It was first reported on vines in California in the 1950s (Ben-Dov 1994, Ben-Dov *et al.* 2013).

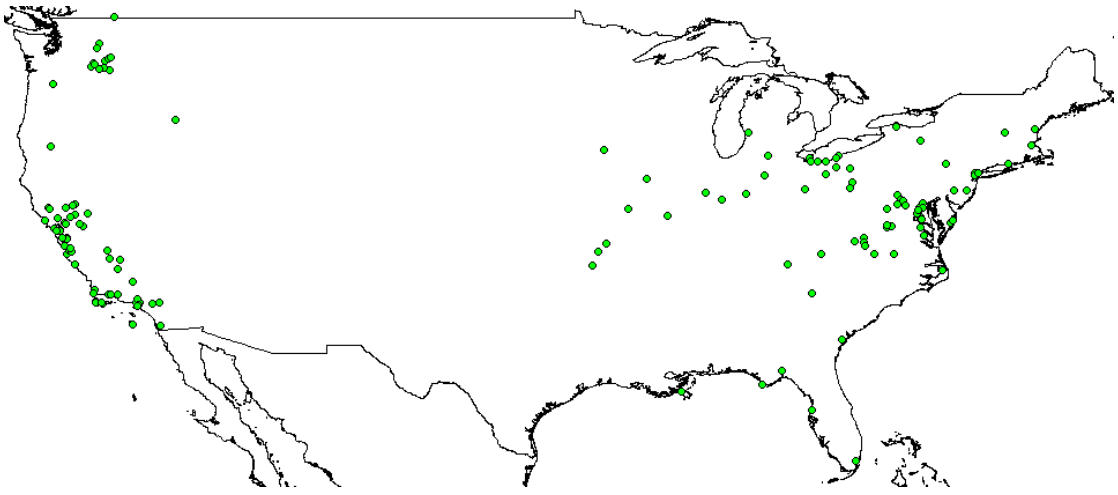


Figure 8.1. Geographic distribution of *Ps. maritimus* based on published records and museum specimens.

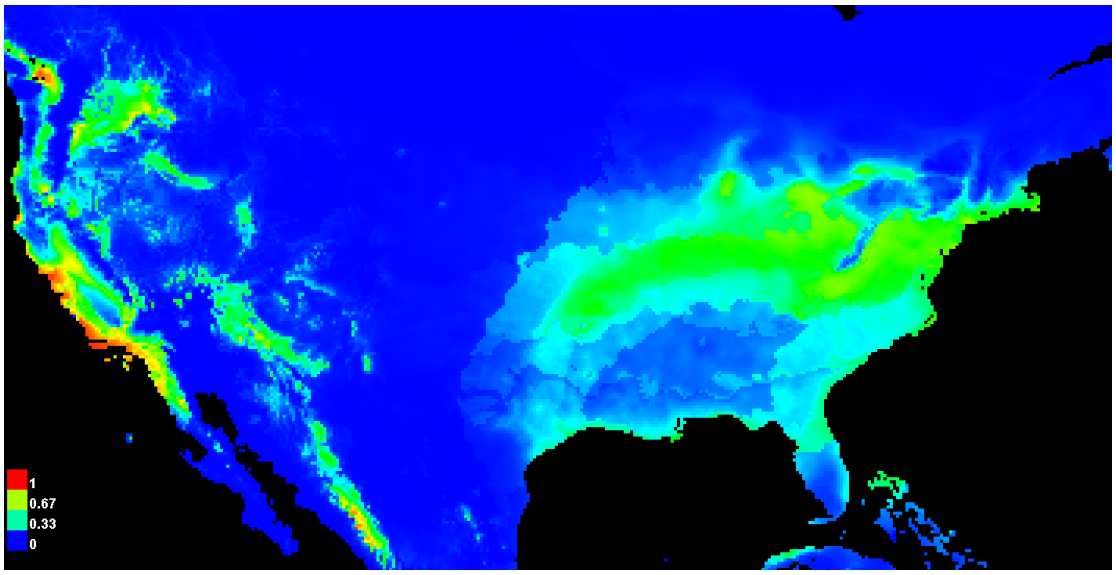


Figure 8.2. Predicted geographic range of *Ps. maritimus* in North America based on a maximum entropy model. (Blue = not predicted to occur; red = predicted to occur with near certainty)

Current distribution beyond its endemic range

The grape mealybug is now known to occur in all northern American vineyard regions. Outside northern America, the grape mealybug has become established in Bermuda, Guatemala, French Guyana, Colombia, Argentina, Brazil, Chile, Poland, Armenia Azerbaijan, Indonesia and Puerto Rico (Ben-Dov *et al.* 2013).

Expected distribution in Australia

Maximum entropy modelling is likely to have incorporated biased predictions due to incomplete geographical records from the south-eastern USA. Thus the final model should be considered to be tentative (Fig. 8.3). Three variables were incorporated into the final model, BIO1 (37.9% contribution to model predictions), BIO19 (36.1%), and BIO6 (26%). The model predicts the locations where *Ps. maritimus* is known to occur based on the data used to construct the model. Of the pest species covered in this report, the grape mealybug is predicted to have the greatest invasion potential in southern Australia (Fig. 8.3). However, the data indicate that grape mealybugs do not cope well with periods of extended drought (Daane *et al.* 2012). For large parts of Australia, this would imply that they can only be present in irrigated areas, which includes nearly all vineyards.

Host plants in Australia

Most of the 81 host plant species of *Ps. maritimus* that are listed by Ben-Dov *et al.* (2013) are present in Australia. These include several crops such as grapes, pear, apricot and many ornamentals (Bartlett 1978, Flaherty *et al.* 1982b). There is no reason to suspect that native Australian plants would not be a host to the grape mealybug, as the species has been observed to feed on *Grevillea*, and several Fabaceae (Ben-Dov 1994).

Pest status

Grape mealybugs are sap sucking insects that feed on leaves and fruit, and excrete excess sap as honeydew. Honeydew is a medium for growth of black sooty mould on grape bunches and leaves, causing defoliation and fouled fruit. Sooty mould is a problem of

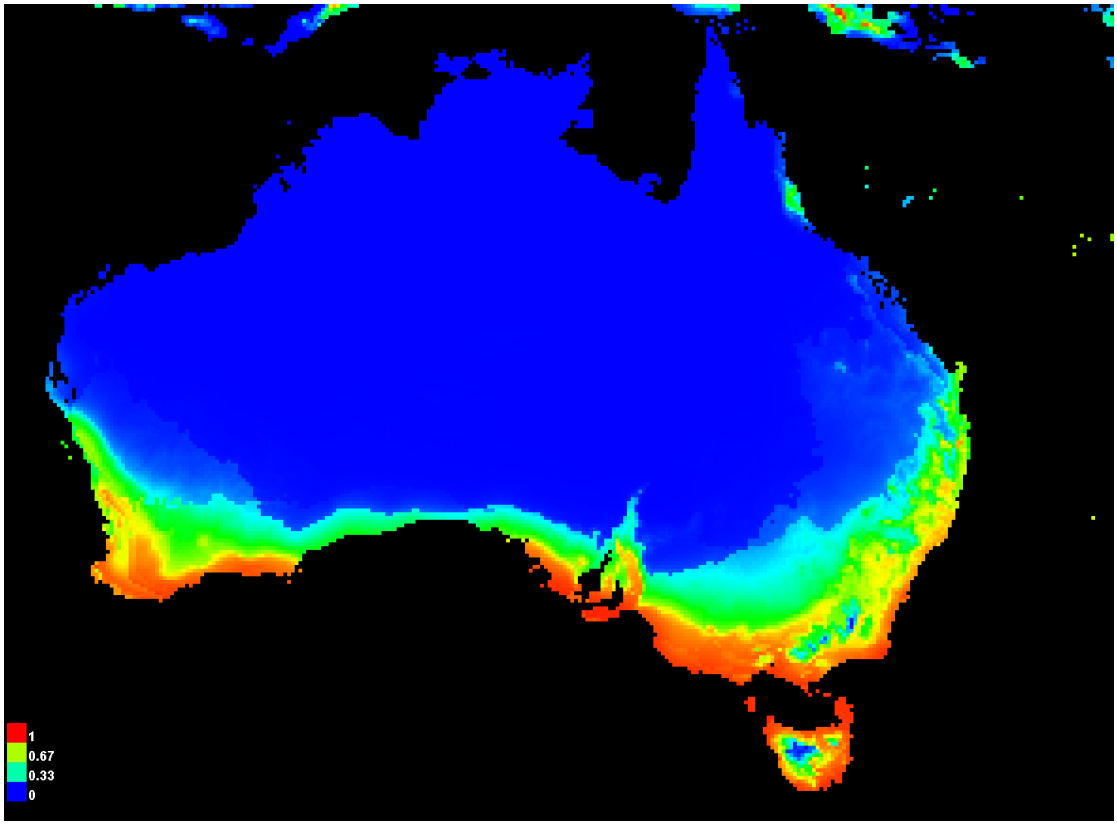


Figure 8.3. Potential geographic range of *Ps.maritimus* in Australia based on a maximum entropy model. (Blue = not predicted to occur; red = predicted to occur with near certainty).

concern in the table grape industry. Furthermore, mealybugs are known to transmit several grape-vine leaf-roll associated viruses (Golino *et al.* 2002), which can be damaging to vines, the crop and wine quality (Daane *et al.* 2012). However, while *Ps. maritimus* is present throughout large parts of the USA, its presence rarely reaches pest status. The occasional outbreak of *Ps. maritimus* generally results from pesticide usage removing the beneficial insects (Daane *et al.* 2012).

While the viticulture Industry Biosecurity Plan 2009 has identified the species as a medium - high risk to the Australian Grape and Wine industry, the status of high priority pest has recently been downgraded. A Pest Risk Review is available for download from www.planthealthaustralia.com.au/pidd and can be found in Appendix 1.

8.2 Management strategies and possible control agents: *Acerophagus notativentris* and *A. angelicus*

Mealybugs have hundreds of natural enemies. Worldwide, the introduction of parasitoids, especially in the family Encyrtidae, has been the most successful strategy for controlling mealybug pests (Daane *et al.* 2012), with a number of spectacular successes (Moore 1988). In combination with lacewings and with the native Australian ladybird beetle *Cryptolaemus montrouzieri*, parasitoids provide excellent control of the grape mealybug in North America provided insecticidal sprays are not used (Daane *et al.* 2012). Further improvement of biological control for mealybugs involves the control of honeydew seeking ants (Phillips and Sherk 1991). Combining information from Daane *et al.* (1998), references therein, (Ben-Dov *et al.* 2013)) and the Chalcidoidea database (Noyes 2012), we constructed a list of parasitoid species involved in the biological control of *Ps. maritimus* (Table 8.1).

Of the seven parasitoids that are currently involved in the control of Australian mealybugs on citrus, five species may contribute to the control of the grape mealybug upon an incursion in Australia (Table 8.1). The potential control provided by wasps already present in Australia could be investigated in quarantine in the USA.

Table 8.1. Known parasitoid wasps of the grape mealybug, based on combined data from Geiger and Daane (2001) and Ben-Dov *et al.* (2013) and references therein, including presence and hosts in Australia, based on Malipatil *et al.* (2000) and in the USA. Species: citrophilous mealybug (*Pseudococcus calceolaria)*, longtailed mealybug (*Ps. longospinosus*), and citrus mealybug (*Planococcus citri*). A question mark behind the name of the parasitoid indicates that parasitisation of the grape mealybug is not certain or uncommon.

	Present in Australia?	Mealybug hosts in Australia
Aphelinidae:		
Coccophagus gurneyi (**?**)	**yes**	citrus
Encyrtidae:		
Acerophagus notativentris	no	
Acerophagus angelicus	no	
Aenasius paulistus	no	
Anagyrus pseudococci	**yes**	long-tailed
Anagyrus yuccae	no	
Coccidoxenoides peregrinus	**yes**	citrus
Leptomastix abnormis	**yes**	citrus
Leptomastix dactylopii (**?**)	**yes**	citrus
Tetracnemoidea brevicornis	**yes**	long-tailed, citrophilous
Tetracnemoidea peregrina	**yes**	long-tailed, citrophilous
Pseudleptomastix squammulata	no	
Zarhopalus corvinus	no	
Signiphoridae:		
Chartocerus subaeneus	no	

The egg parasitoid *Ophelosia keatsi* Girault has been observed to parasitise *Ps. viburni* (Malipatil *et al.* 2000). Because this mealybug species is relatively closely related to *Ps. maritimus* (Downie and Gullan 2004 Hardy et al 2008) and the egg parasitoid is already present in Australia, its potential to parasitise *Ps. maritimus* should be investigated.

The parasitoid species that are present in Australia may not be the most effective biological control agents for *Ps. maritimus*. In North America, the encyrtid wasps *Acerophagus notativentris* and *A. angelicus* are the dominant parasitoids of this species (Flaherty *et al.* 1982a, Grimes and Cone 1985, Grasswitz and Burts 1995, Geiger and Daane 2001, Daane *et al.* 2008). Therefore, these two are the most suitable new biological control agents to consider. These two species of Encyrtid wasps could be considered as potential biological control agents if, despite good education of growers with respect to the effects of the use of

insecticides on natural enemies and the importance of ant control, the grape mealybug turns out to cause economic damage in Australia.

Taxonomy

The parasitoid wasps *Acerophagus notativentris* (Girault) and *A. angelicus* (Howard) belong to the order of the Hymenoptera, family Encyrtidae.

Biology and ecology

Both proposed species of *Acerophagus* are gregarious endoparasitoids, which means that they place more than one egg in a host. The *Acerophagus* species place more eggs in larger mealybug hosts. For *A. notativentris*, this can be up to ca. 25 eggs in a 4 mm host, for *A. angelicus*, it is around 5 eggs in a large host (Daane *et al.* 2008). These species have been successfully cultured at the University of California, Berkeley (Daane et al 2002).

Endemic range

Both species of *Acerohagus* can be found in a large number of states in the USA, including Oregon, Washington (Grimes and Cone 1985; Grasswitz and Burts 1995) and California (Geiger and Daane 2001). There is no evidence that they have been purposefully or accidentally exported to other continents.

Expected efficacy as a biological control agent

In its original range, *A. notativentris* and *A. angelicus* each provided approximately 45% parasitism of grape mealybug crawlers in spring (Daane *et al.* 2008). This level of control is relatively low for an efficient biological control agent. The combination of these two species is deemed to be more effective for mealybug control when combined with predators, specifically with the mealybug destroyer *C. montrouzieri* (Daane *et al.* 2012), which is a common Australian native species and is grown commercially in Australia. Importantly, control of ants in the vineyard aids the biological control agents, as ants protect mealybugs from predation and parasitisation in exchange for honeydew (Buckley and Gullan 1991, Phillips and Sherk 1991).

Host specificity

The genus *Acerophagus* are specialist koinobiont gregarious parasitoids of mealybugs (Daane 1999). While *A. notativentris* has been shown to readily parasitise a large range of mealybug species, Flanders (1947) claims that it only develops successfully on the grape mealybug. Experiments by Daane (1999), who screened seven mealybug species (*Ps.calceolariae, Ps. comstocki, Ps. longispinus, Ps. maritimus, Ps. viburni, Planococcus citri* and *Ferrisia virgata*), indicate that only *Ps maritimus* and *Ps longispinu*s were suitable insectary hosts for *A. angelicus*. This is an interesting result, as *Ps maritimus* and *Ps. longispinus* are more distantly related (Downie and Gullan 2004), while *Ps. viburni,* which is more closely related to *Ps maritimus*, was not parasitised. While this result may suggest a wider host range than initially expected, contamination between mealybug cultures within an insectary is a common occurrence (Gullan pers. com.). Therefore, it is also possible that the results of the laboratory experiment indicate parasitisation of a single species only.

If Daane's experiment (1999) is taken to suggest host specificity, this would seem in contrast with the information obtained from the universal Chalcidoidea database (Noyes 2012). According to the database, the range of mealybug hosts that are associated with *A. notativentris*, include *Dysmicoccus ryani*, *Eurycoccus jessica*, *F. virgata*, *Ps. affinis*, *Ps. comstocki*, *Ps. maritimus* and *Ps. obscurus*. This represents seven species from four possible genera which are widely distributed over the phylogeny (Downie and Gullan 2004). The range for *A. angelicus* seems to be even wider, and includes 15 species from six genera, *D.*

brevipes, D. ryani, *F. virgata*, *Phenacoccus gossypii*, *Ph. madeirensis*, *Ph. solani*, *Planococcoides njalensis*, *Pl. citri*, *Pl. ficus*, *Ps. adonidum*, *Ps. calceolariae*, *Ps. gahani*, *Ps. longispinus*, *Ps. maritimus* and *Spilococcus implicatus*.
In contrast to the specificity suggested by Daane, this may seem worryingly non-specific. However, it should be noted that (a) the association of parasitoids and mealybugs mentioned in Noyes (2012) has not been verified and is based on both primary and secondary literature. Therefore, it is likely that in many cases both the mealybug host and the parasitoid species have been misidentified (Noyes pers. com., Gullan pers. com.) and (b) the ability of a parasitoid to attack a host species does not necessarily imply that the parasitoid can successfully develop in the host, as was demonstrated by Daane (1999).

Thus, the validity and reasons for the host specific development *A. notativentris* should be further explored. If the species is host specific, this could be the result of an ability of *A. notativentris* to deactivate the specific immune reaction of *Ps. maritimus* and closely related species. Such a finding would imply that the parasitoid could be specific for a small subset of closely related mealybug species.
However, it is also possible that evolution of defensive reactions of host species to the parasitoid is at the basis of host specificity in the native range. In that case, it should be expected that the Australian native species may be susceptible to the proposed *Acerophagus* species, because they did not co-evolve with the parasitoids.

Thus, if, on the basis of monitoring the incursion, it is decided that a biological control agent is required for the control of *Ps. maritimus*, host specificity testing will need to elucidate whether the proposed *Acerophagus* species will attack and successfully reproduce on Australian native mealybugs.

No host specificity testing has been performed for any of these parasitoid species outside its native range.

8.3 Possible non-target hosts of *Acerophagus notativentris* and *A. angelicus* in Australia

Category 1: Taxonomic affinities, ecological similarities

Taxonomic affinities
There are 27 native and 5 introduced species of *Pseudococcus* in Australia (Houston 2002). Of the native species, two are economically important pests, nationally and worldwide. However, selection of host species should not necessarily be based on the current understanding of the taxonomy as the genus *Pseudococcus* is unlikely to be a monophyletic group (Downie and Gullan 2004).
The species that is most morphologically similar and closely related to *Ps. maritimus* is the introduced species *P. viburni* (Miller *et al.* 1984), which is reported as the most important underground mealybug pest in Australia (Williams 1985), and is a pest of several other crops including greenhouse tomatoes (Schoen and Martin 1999) and apples (Ward 1966). Although they are in the same genus, the native *Pseudococcus* species are unlikely to be closely related to the *Ps. maritimus-viburni* group Species in the genus *Dysmicoccus* (27 species in Australia, which includes the pineapple mealybug *D. breviceps* and the sugarcane mealybug *D. boninsis* and *Erium globosum* seem to be more closely related to the Ps. maritimus- viburnum complex than the native Pseudococcus species (Downie and Gullan 2004).
However, if the reported association of *A. notativentris* and *A. angelicus* with *F. virgata* is correct (Noyes 2012), this would imply that taxonomic affinities should not be the most

important predictors for the identification of potential non-target species, as *P. maritimus* and *F. virgata* are only distantly related.
Thus, taxonomic considerations do not considerably narrow down the test list of potential non-target host species.

Ecological similarities
Mealybugs predominantly live on perennial plants (Daane *et al.* 2012). As mentioned above *Ps. maritimus* has been recorded on 81 host plants belonging to 37 plant families (Ben-Dov *et al.* 2013) including wide spread Australian genera such as *Acacia* (Fabaceae) and *Grevillea* (Proteaceae). Given the large range of perennial plant species that can be a host to the grape mealybug it is likely that after incursion *Ps. maritimus* would share plant host species with a number of native mealybug species, including mealybug species on native plants such as *E. globosum*, which specialises on *Acacia* species

Native species with a wide host range are *Nipaecoccus ericiola, Pseudococcus hypergaeus* and *P. calceolariae.* In addition*, P. eucalypticus* seems to be a widespread species in the wine growing regions and is found on a wide range of *Eucalyptus* species. Because *P. calceolariae* is an important pest on citrus and causes export problems to the US, we do not include this species in the non-target host list.
Based on a relatively wide range of host plants compared to other native mealybugs, *P. eucalypticus*, *P. hypergaeus* and *N. ericiola* could be candidates for host specificity testing.

Filter 1: Spatial, temporal and morphological attributes

Spatial attributes: Climate
In the USA, the distribution of *A. angelicus* follows that of the grape mealybug (Flaherty *et al.* 1982a, Grimes and Cone 1985, Grasswitz and Burts 1995, Geiger and Daane 2001, Daane *et al.* 2008).

Spatial attributes
Mealybugs are sap-feeding insects that survive on the phloem of perennial plants. Female *Ps. maritimus* feed mainly on grape-vine leaves but move to the trunk for oviposition (Flaherty et al 1982) where on average 57 eggs are produced in an ovisac (Grimes and Cone 1985). This trait does not necessarily set the species apart from native mealybugs.
Variation in the size of the circulus, an adhesive pad on the venter of the mealybugs that provides a grasp on the leaf surface, indicates that there may be variation in the amount of time spent by native mealybugs on the leaf area (Gullan pers. com.). *Ps. maritimus* has a large circulus and may spend a large part of its time on leaves. However, because *Ps. maritimus* is not exclusively found on leaves, there is *a priori* no reason to suppose that leaf dwelling habits set this species apart from any native *Pseudococcus* species, when it comes to parasitisation.

Morphological attributes
The sizes of Australian *Pseudococcus* species are not informative in the selection of non-target host species as the encyrtid wasps parasitise a range of instars and sizes of mealybugs and will adjust the number of eggs and the sex ratio to the size of the host (Gullan pers. com.).

Filter 2: Access and availability

For the purpose of host specificity testing a number of species show large similarities but some species are difficult to find and/or have a limited or mainly tropical distribution while others are common. The most widespread native species of *Pseudococcus* in Australia, *P. calceolariae*, is an economically important pest both nationally and world-wide. In selecting

species for host specificity testing, we have decided to focus on the native non-pest species that are most widespread and have the widest host range, as indicated in Williams (1985). These species are:

- *P. eucalypticus*: This species which occurs throughout Australia apart from NT; its host plants are a range of Eucalyptus species.
- *P. hypergaeus*: This species has a very wide plant host range which includes both native and introduced species of plants. While this species has not been found in WA or NT it has been found in all other States in Australia and also occurs in New Zealand.
- *Nipaecoccus ericiola:* The species is common and widespread, but has not been found in Western Australia. It has a wide host range which includes both native and introduced species

Conclusions

Based on above considerations and in consultation with Prof. P. Gullan specialist on Australian mealybugs, Dr M. Malipatil specialist on parasitoids of Australian scale insects and Prof J. Noyes specialist in Chalcidoid wasps the proposed test non-target host list for *A. pseudococci* includes: *Pseudococcus eucalypticus P. hypergaeus and Nipaecoccus ericiola.*

8.4 Host specificity testing

Should it be necessary to import novel biological control agents for the grape mealybug, then it is recommended that the parasitoid wasps *Anagyrus notativentris* and *A. angelicus* are subject to host specificity testing. These wasps are not currently reared commercially, but they can be obtained from the laboratory of Prof. Kent Daane (http://www.ipm.ucdavis.edu/).

The host specificity tests are similar to those described for the vine mealybug in Chapter 7 section 5.

9 THE EUROPEAN GRAPEVINE MOTH

9.1 The target host, *Lobesia botrana*

Taxonomy

The European grapevine moth (*Lobesia botrana* Denis & Schiffermüller) belongs to the order of Lepidoptera, suborder Dytrisia, superfamily Tortricoidea, family Tortricidae, tribe Olethreutini.

Biology

The following is a summary from Ioratti et al (2012) and references therein. Adults of *L. botrana* are crepuscular. During twilight, virgin females release a pheromone on the canopy at several stations, which attracts the males (Roelofs *et al.* 1973). The average adult life-span is 2-3 weeks, during which females produce 50 – 80 eggs. Eggs are deposited singly or in twos on or close to buds or flowers. They hatch at 75 degree-days above a threshold of 10 °C. There are five instars and larval development is completed in approximately one month. The larvae feed on flowers and buds, pea size or ripened berries. The pupal stage is protected by rolled leaves or inflorescences tied with silk and lasts approximately 14 days in non-diapausing individuals. Diapausing pupae are usually found under bark, in the soil or leaf-litter. They emerge as adults in the following spring.

The number of generations produced per year strongly depends on the climate, temperatures, and humidity. There is a single generation per annum in northern Europe, three-four in southern France, Spain, Greece, Italy and California, and five in Central Asia. In Egypt, Israel and Crete some populations do not undergo diapause but spend the winter in the larval stage. Detailed data related to the temperature dependent development at different localities can be found in Venette *et al.* (2003)

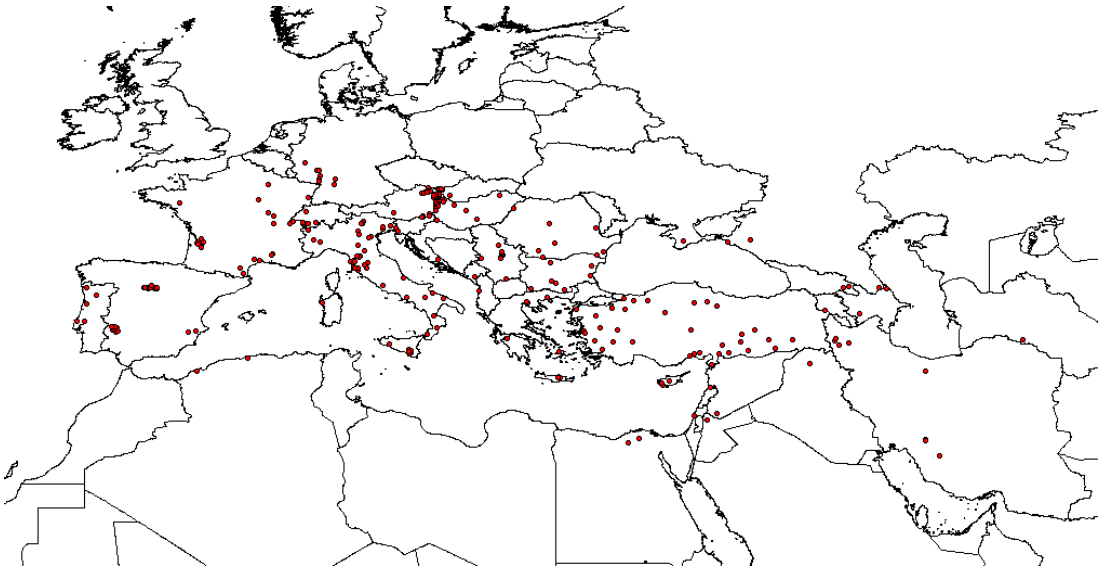


Figure 9.1. Locations where *L. botrana* is known to occur that were used to develop the MaxEnt model.

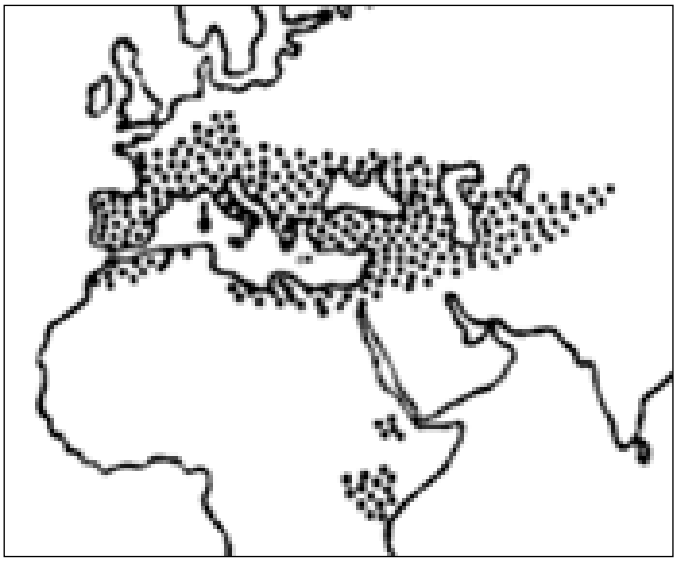


Figure 9.2. Geographic distribution of *L. botrana* (Carter 1984, p. 165).

Endemic distribution and plant host range

Lobesia botrana was originally restricted to Austria, but extended its range in the late 1800s and early 1900s to include southern Europe (Marchal 1912), Northern Africa, the Middle East and Central Asia (Gilligan *et al.* 2011 Fig. 9.1 and 9.2).

Larvae of *L. botrana* have been recorded from 40 species of plants on 20 families, including apple, almond, jujube, kiwi, nectarine, olive, pear, pomegranate, persimmon, plum, raspberry, *Ribes* species and rosemary (Gilligan *et al.* 2011). It has been hypothesized that a preference for grapevine has only evolved recently (Marchal 1912), as the species prefers to oviposit on Daphne (Thymelaecae) and that a higher larval survival on vine and lower predation risk in vineyards, might be among the fitness related factors explaining evolutionary host shift to, and worldwide adaptive success on, grape vines (Torres-Vila and Rodriguez-Molina 2013).

Current distribution beyond its endemic range

In the first decade of this century, *L. botrana* has invaded Chile (2008), Argentina (2008) and North America (2009, Gutierrez *et al.* 2012), probably as a result of inadvertent human

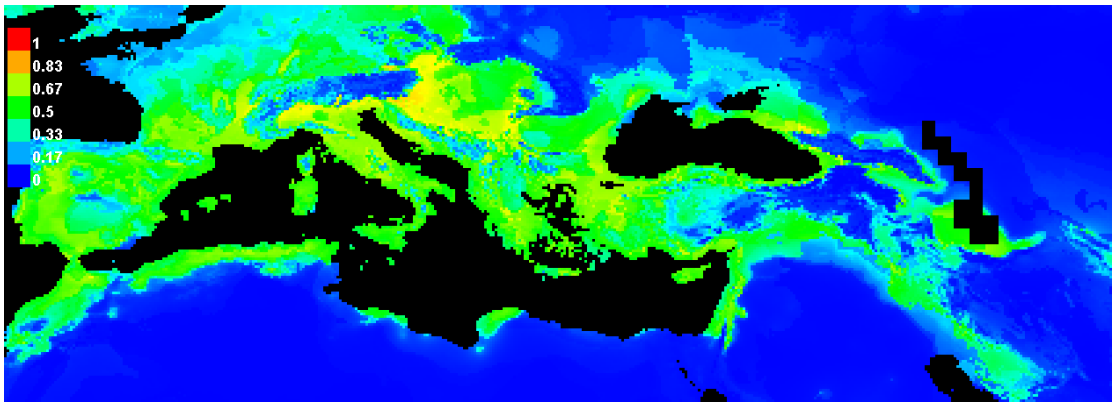


Figure 9.3. Predicted geographic range of *L. botrana* in Europe, Asia and Northern Africa based on a maximum entropy model. (Blue = not predicted to occur; red = predicted to occur with near certainty).

transport (Gilligan et al 2011, Ioratti et al 2012). Its history of invasions indicates that it poses a threat to the Australian wine industry.

Predicted distribution in Australia

An extensive range of locations where *L. botrana* is reported to occur throughout Europe, Northern Africa, the Middle East and Central Asia was used to develop a climatic model of its distribution.

The final model of the distribution of *L. botrana* was based on six variables, BIO13 (47.6% contribution to the model predictions), BIO6 (16.3%), BIO7 (13.3%), BIO5 (8.5%), BIO16 (7.3%) and BIO9 (7.0%). The model predicts the know distribution well (Fig. 9.3). All major wine grape growing regions In Australia could be threatened by this species (Fig. 9.4).

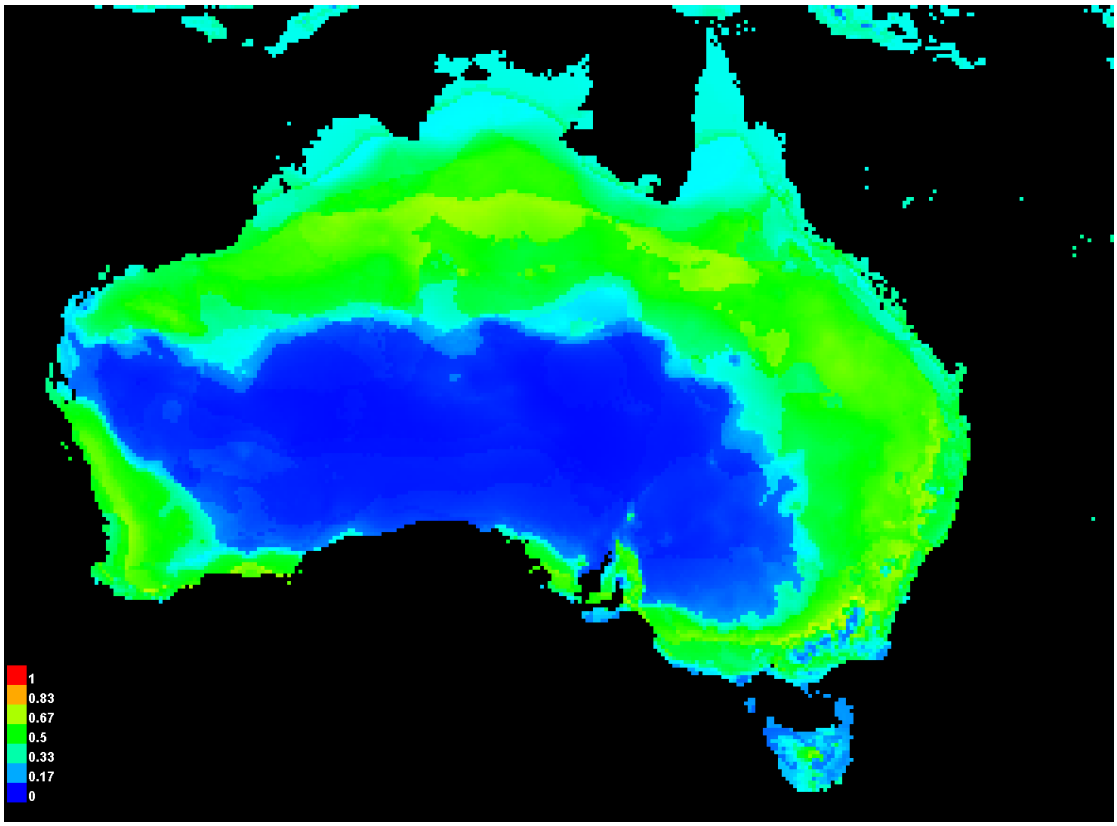


Figure 9.4. Potential geographic range of *L. botrana* in Australia based on a maximum entropy model. (Blue = not predicted to occur; red = predicted to occur with near certainty).

Host plants in Australia

Nearly all of the 40 species of host plants mentioned by Gilligan *et al.* (2011) are present in Australia. Many of these are crops and are particularly important in the horticultural areas in Australia. These areas coincide with the predicted distribution of *L. botrana* (Fig 9.4).

Pest status

The European grape vine moth is the major pest of grapes in the Mediterranean basin (Thiéry and Moreau 2005, Ioriatti *et al.* 2011). The flower-consuming generation does not generally cause as much damage as the later generations that consume berries. While the consumption of pea-sized berries causes reduced yield, the most damage is done by the

consumption of ripened berries. The presence of larvae, webbing and rotten berries leads to downgrading of table grapes. More importantly, damage caused to ripened berries leads to infections of *Botrytis cinerea*, which causes bunch rot and decreases wine quality.

Based on invasiveness and negative effects, the new viticulture Industry Biosecurity Plan, which is soon to be released, has identified *L. botrana* as a high risk to the Australian Grape and Wine industry. However, no Pest Risk Review or fact sheet has as yet been produced.

9.2 The proposed biological control agents

Integrated pest management control strategies for *L. botrana* include a range of practices. Selective insecticide control, supported by monitoring using pheromone traps and egg scouting, is combined with biological control using *Bacillus thuringiensis* var. *kurstaki* and var. *azawai*, predators and mating disruption by sex pheromones (Ioriatti *et al.* 2011, Ioratti *et al.* 2012).

Parasitoid wasps

Parasitoid species associated with *L. botrana* belong to various families including Ichneumonidae, Chalcididae, Pteromalidae, Eulophidae and Trichogrammatidae. The presence and efficacy of species varies between regions and even between grapevine cultivars within a region, between years (Moreau *et al.* 2010) between strains (Pizzol *et al.* 2010) and between generations (Ioratti *et al.* 2012). Often the efficacy is high during the first two generations and it declines in the overwintering generation. The most frequently observed and most efficient species in European vineyards is the larval parasitoid *Campoplex capitator* (Ichneumonidae), with parasitisation rates of up to 53% (Xuereb and Thiéry 2006). However, difficulties with mass-rearing of this parasitoid have so far prevented releases in Europe (Ioratti *et al.* 2012). Combined, the variability in efficacy, the lack of specificity and the inability to breed efficient parasitoids has so far hampered biological control of the moth by parasitoids. As a consequence, the release of parasitoids for the control of *L. botrana* is not a current practise in Europe (Moreau *et al.* 2010, Ioriatti *et al.* 2011, Ioratti *et al.* 2012) and has, as far as we could determine, not been attempted in the United States (Gutierrez *et al.* 2012, Ioratti *et al.* 2012).

Mating Disruption

Mating disruption is generally very selective with minimal off-target effects, and can be highly efficacious when deployed either alone or in combination with other compatible insect-control technologies (e.g. selective insecticides) to either eradicate or manage population incursions of Biosecurity pests. The use of mating disruption for the control of *L. botrana* is well established (Ioriatti *et al.* 2011, Gutierrez *et al.* 2012, Ioratti *et al.* 2012). Mating disruption mostly makes use of handheld dispensers that are distributed over the vineyard at a density of 500/ha. While this control is highly effective and has low environmental impact, it is a rather costly method (±$ 160 Aus/ ha). Mating disruption is currently deployed in 140,000 ha of European vineyards. Its further uptake in Europe is hampered by socio-economic factors and lack of interest in innovation in much of the European wine industry (Ioriatti *et al.* 2011).

Ioratti *et al.* (2011) explains how this technique was successfully introduced into the province of Trentino, Italy and this example is illustrative of what could happen in Australia:
Before the introduction of mating disruption, 60% of the growers controlled the vine moth with two insecticide treatments per year (mainly chlorpyrifos methyl), 31% with a single treatment and 9% did not treat. Now, twelve years after the first introduction of mating disruption in 1998, 90% of growers use mating disruption and insecticides are only

sporadically used in small isolated vineyards during years that are favourable for pest development. Crucial in the success of the adoption of the mating disruption technique in Trentino was a close collaboration between research scientists, extension entomologists and growers associations, as well as an initial support for growers to purchase the dispensers.

Recent advances in the formulation of mating disruption products have resulted in a new range of proprietary products which are well suited to rapid, mechanized, large-scale application from ground or air. One of the newer products - SPLAT^{TM} LBAM (ISCA Technologies, Riverside, California, USA) - was tested by SARDI in a series of large-scale vineyard trials undertaken as part of a CRC Plant Biosecurity project using LBAM as a research model for *L. botrana*. These trials demonstrated that the mechanised application of SPLAT^{TM} can treat extensive areas of host crop and surrounds to suppress and potentially eradicate the target pest at lesser cost, treatment time and dependence on local landscape features compared to the standard twist-tie technology (Woods *et al.* 2012).

In California, chlorpyrifos is not registered for seasonal use in vineyards and avoidance of disruption of the natural control of native pseudococcids pests was major concern of the control program for *L. botrana*. This was achieved by using selective insecticides at crucial times during the first and second generation of larval emergence, in combination with mating disruption (Ioratti *et al.* 2012).

In Australia, close collaborations between research scientists, extension entomologists and the grapevine growers' organisations already exist. Furthermore, the Australian grape and wine industry is innovative and highly educated. Most growers are aware of the presence of beneficial insects and concur with the general public in a preference for the use soft insecticidal control. Therefore, we believe that mating disruption would receive great support from the industry in the case of an incursion of *L. botrana* and that a program to introduce this technique would have a large chance of success.

Host specificity

The pheromonal bouquets used in mating disruption are highly species specific (Mafraneto and Carde 1994, Ioriatti *et al.* 2011). In addition, mating disruption only works on the local scale of the vineyard, as male *L. botrana* only fly up to 100 m with an average of 50 m from where they eclose (Roehrig and Carles 1981, cited in Ioratti *et al.* 2012).

9.3 Possible non-target hosts of mating disruption pheromones of *Lobesia botrana*

Because mating pheromones are highly species specific, work on a relatively short range and are deployed in the context of the vineyard, it is highly unlikely that they will attract native species and disrupt their mating patterns.

Furthermore, it is unlikely that any of the >16 species of *Lobesia* occur in the main vine growing ranges, as they are documented to occur in the coastal regions of northernmost Western Australia, the Northern Territory, Queensland and New South Wales (Horak 2006). The host plants of native *Lobesia* species include flowers of *Melaleuca quinquenervis* (Myrtaceae) of *Litchi sinensis* (Sapindaceae), berries of *Lantana* sp. (Verbenaceae) and seed pods of *Buckinghamia celsissima* (Proteaceae; Horak 2006 and references therein). The native *Lobesia* species have not been recorded on grape vine. Conversely, none of known families known to host native *Lobesia* species have been recorded as hosts families for *Lobesia botrana*. However, given the wide range of host plants of the latter species, this is not necessarily informative.

Casting a wider net, PhD student Feng Yi (Adelaide University, unpublished) has surveyed South Australian vineyards for the occurrence of tortricid larvae in 2011 and 2012. The only tortricid species he found so far on grapevine is the light brown apple moth (*Epiphyas postvittana*).

However, assessment of possible mating disruption of native moths in Australian vineyards could be performed at any time, by purchasing and deploying mating disruption dispensers (e.g. Isomate®-EVGM in the USA, Isonet L®,Lplus®, LE® in Europe) in traps in vineyards at the appropriate density and investigating the attraction of native moth species. In addition, wind-tunnel experiments could be performed to assess the attractiveness of the pheromones to native species.
The native species of *Lobesia* that should be involved in these trials is *L. physophora*, as it the most common species and has a wide diet choice, indicated by the fact that it feeds on both flowers and fruit of Lantana and Litchi (Horak 2006). Furthermore, *L. extrusana* should be included, as it seems to have a more southerly distribution that the other species, which occur mainly in the northern parts of Australia.

Conclusion

No suitable parasitoid species is as yet available for the control of *L. botrana* in grapevine. Overseas development of methods for the mass-rearing of *Campoplex capitator* should be monitored. In case of an incursion of *L. botrana*, we do not recommend to move such attempts to Australian shores, as this is best done in the home range of the parasitoid. Control measures for *L. botrana* involve mating disruption in spring and summer, using *Bacillus thuringiensis* at crucial periods in the lifecycle in combination with measures to enhance the density of predators in the vineyard (Thomson and Hoffman 2009, Penfold and Collins 2011).

10 OVERALL OUTCOMES AND RECOMMENDATIONS

10.1 Outcome

This project set out to improve the preparedness for the Grape and Wine industry for the incursion of four high priority pest species. The outcome of this is that these species have now been approved as targets for biological control, their expected range in Australia has been modelled, the potential biological control agents have been evaluated, the most efficient agents have been selected, and for each pest species information has been collated that allows easy preparation of applications for hosts specificity testing in the case of an incursion (Chapters 6-9).

10.2 The next steps in case of an incursion

It is important to note that the information provided about the biological control agents is the best available at the time that this report was produced. This implies that, in the case of a future incursion of any of the four pest species that are the subject of this report, the information provided should be checked against the recent developments in the biological control of these species overseas.

After necessary amendments, the details about the host specificity testing should be elaborated. The address of an AQIS-approved quarantine premise with appropriate containment level to hold the organism(s) must be supplied. Where appropriate, the host material or the media used for transportation of the agent should be specified. Specific details that need to be added are, where and when the tests will take place, what containment level is applied, where exactly is the biological material sourced from and how is it contained during transport. The chapter should then be collated with an application form for a permit to import quarantine material (http://www.daffa.gov.au/aqis/import/application/forms/biological-materials) and submitted to AQIS to apply for an import permit.

Furthermore, a testing permit for proposed biological control agents that are animals is required from the Department of the Environment, Water, Heritage and the Arts (DEWHA). The updated information in the chapters covers Part 1 of the standard Terms of Reference. This text should be accompanied by a completed form that can be downloaded from http://www.environment.gov.au/biodiversity/trade-use/permits/forms/invertebrates.html and submitted as a hard copy to DEWHA. An email or fax may be submitted to initiate the process, but permits will not be completed until the hard copy has been received.
A $150 fee is required for non-government organisations.

Voucher specimen(s) at the most recognisable stage must be lodged at a recognised institution and documents proving the product source are required where applicable.

10.3 Further recommendations

Dispersed in this report are numerous recommendations for actions that can be performed either prior to or after the incursion of each of these problematic vineyard pests. Here, we briefly summarise these recommendations.

Sharpshooters

A preliminary investigation of the egg-laying behaviour of native proconiine sharpshooters should provide some insight into the feasibility of host specificity of the proposed mymarid egg parasitoids. This study should focus on the proposed native non-targets hosts *Cofana spectra*, and *Ishidaella angustata*, *I. latomarginata* or *I. albomarginata*.

Mealybugs

An experimental evaluation is needed of the potential of Australian mealybug parasitoids *Anagyrus pseudococci*, *Coccidoxenoides peregrinus, C. perminutus*, *Leptomastix abnormis* and *Leptomastidia dactylopii* to control the vine mealybug and of the Australian strain of *Anagyrus pseudococci* for the control of the grape mealybug. This should be performed in quarantine overseas.

A library should be constructed of DNA signatures of *Pl. ficus* from different regions in the world. This will allow rapid assessment of the origin of the incursion and hence improve selection of biological control agents

It should be investigated whether the parasitoids of economically important mealybug species in Australia attack native mealybugs. In New Zealand, none of the introduced parasitoids have been found on native mealybug species, even though some of these introduced species had been present for 80 years (Charles and Allan 2002). In Australia, the effect of introduced parasitoid species on non-target native mealybug hosts has not been investigated (Gullan pers. comm.). Such an undertaking would help with the selection of novel parasitoids. Currently, the lack of information on the non-target effects combined with the lack of distinction between initial parasitisation and successful development of the parasitoids makes it very difficult to predict the potential effect of newly introduced parasitoids for the control of *Pl. ficus* and *Ps. maritimus*.

The validity and reasons for the host specific development of *A. notativentris* should be further explored. This can currently only be done overseas.

The awareness of growers of the mutualisms between ants and mealybugs should be increased. Control of mealybugs is easier achieved in conjunction with the control of ants (Gullan 1997). This is particularly important for the control of *Pl. ficus* (Daane *et al.* 2002, 2012).

Upon an incursion of any of the species in this report, it is recommended that measures are undertaken to enhance generalist arthropod predators in the vineyard (Daane *et al.* 2012). Examples of such measures can be found in Thomson (2009) and Penfold and Collins (2011). Furthermore, for the mealybug species, the use of parasitoid wasps in conjunction with releases of the mealybug destroyer *Cryptolaemus montrouzieri* is highly recommended.

The European grapevine moth

For the European grapevine moth, no convincing biological control agents have been found and hence the recommendations focus on other control measures. Assessment of possible mating disruption of native moths in Australian vineyards could be performed by purchasing and deploying mating disruption dispensers (e.g. Isomate®-EVGM in the USA, Isonet L®,Lplus®, LE® in Europe) and testing their attractivity to a range of native male tortricids in a wind tunnel and in vineyards.

APPENDIX 1: THE APPLICATIONS FOR RECOGNITION AS A TARGET OF BIOLOGICAL CONTROL

All four pests have been recognised as targets for biological control. The applications that form the basis of this recognition have been inserted below.

To:
The Standing Committee/Ministerial Council Department of Agriculture, Fisheries and Forestry

Re:
Request for approval of the Glassy Winged Sharpshooter (*Homalodisca vitripennis*, Hemiptera, Insecta) as a candidate for biological control

Date: 12/12/12

Summary

- The Glassy Winged Sharpshooter (GWSS) has been identified by Plant Health Australia as a high risk to five primary industries (viticulture, citrus, summer fruit, nuts, and nursery and garden industries).
- GWSS is a leafhopper that develops on, and causes severe damage to, a very wide range of crops and ornamentals.
- The GWSS is a vector for a bacterium that causes leaf scorch diseases on woody plants, including grape, almond, citrus, stone fruit, olive, and avocado and oleander.
- In California, the economic impact of the GWSS-bacterium combination has been immense.
- The bacterium also forms new associations with native vegetation, causing novel epidemic diseases.
- In the case of an incursion, a rapid response will mitigate the economic and environmental impact.
- Approval of the GWSS as a target for biological control will enhance possibilities for a rapid response, sparing a wealth of horticultural industries substantial losses.

We request approval of the glassy winged sharpshooter *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae), as a candidate for biological control.

Background

The Glassy Winged Sharpshooter (GWSS) is native to the southeastern United States and northeastern Mexico. It has invaded and established in southern California, French Polynesia, Hawaii, Easter Island the Cook Islands. Incursions have most likely been caused by human transport.

Economic and environmental impact

The GWSS does considerable damage to woody plants by sucking sap (xylem) and spreading a film of feces on crops. More importantly, GWSS is a vector of the xylem-dwelling plant pathogenic bacterium *Xylella fastidiosa*. This bacterium causes bacterial leaf scorch diseases on a wide range of crops and ornamentals, and Australia harbours more than 90 known host species of this bacterium. Among the

diseases caused are Pierce's disease on grapes, leaf scorch diseases of almond, plum, peach, olive and avocado, alfalfa dwarf, and citrus variegated chlorosis.
There is currently no cure for diseases caused by the bacterium.

The economic cost caused by the GWSS-*X. fastidiosa* combination is large. A study conducted by the University of California found that between 1994 and 2000, Pierce's disease caused nearly $30 million in losses and destroyed over 1,000 acres of grape vines and 30 kilometres of ornamental oleander plantings in Northern California alone.

While the GWSS-*X. fastidiosa* combination has the potential to severely affect many agricultural crops, urban ornamental and landscape plants, research in California has also shown that the GWSS vectors *X. fastidiosa* into native vegetation that previously has had no prior association with the bacterium. This is particularly worrisome as it can lead to new disease epidemics not previously seen.

Biosecurity Australia has deemed the threat posed by GWSS to Australian horticulture and viticulture industries to be high. Five plant industries (citrus, nursery and garden, nuts, summer fruit and viticulture) have identified the species as a high risk, and have developed pest specific risk reviews, fact sheets and containment plans as a part of their Industry Biosecurity Plans. A copy of the Pest Risk Review for the viticulture industry is attached for further information about this threat. The other documents are available for download from www.planthealthaustralia.com.au/pidd.

GWSS: A target for biological control

In Australian vineyards, only limited use of insecticides is allowed after flowering. Spraying often disrupts biological control of other vineyard pests. Thus, in the case of an incursion, the viticulture industry needs to rely on biological control of this pest. Parasitoid wasps are the most commonly used biological control agent against GWSS. In California a number of species of parasitic wasp have been released. The release of one of these species, *Gonatocerus ashmeadi,* has lead to a reduction of GWSS by 90 – 95% in the first year after release on Hawaii, Tahiti and 9 islands in French Polynesia.

Now is the time to recognise GWSS as a target for biological control

When an incursion occurs, the spread of an invasive pest and the damage it causes can expand geometrically over time. Each year that passes without an effective solution translates into mounting losses. Rapid control of GWSS, as a result of good preparation for the possible incursion, can spare a wealth of Australian horticulture and viticulture industries substantial losses.

Approval of the GWSS as a candidate for biological control is part of this preparation. It will importantly lower response time, because it enables submission of a list of non-target host species that should be tested before applications for importation of selected biological control agents for the GWSS can be submitted.

Therefore, we apply for the glassy winged sharpshooter, *Homalodisca vitripennis*, to be approved as a candidate for biological control.

Yours sincerely,

Application for recognition of the glassy-winged sharpshooter as a target for biological control

Michael Keller (Assoc. Prof. Applied Entomology, The University of Adelaide)
Greg Baker (Head Entomology, South Australian Research and Development Institute)
Katja Hogendoorn (Research Associate, The University of Adelaide)

Attachment: Pest Risk Review for the Glassy Winged Sharp Shooter
(Author: Plant Health Australia)

Plant Health AUSTRALIA

Pest Risk Review

ADFA ATGA WGGA Wine Grape Growers Australia wfa Winemakers' Federation of Australia

Glassy-winged sharpshooter

COMMON NAME	Glassy-winged sharpshooter (GWSS)
SCIENTIFIC NAME	*Homalodisca coagulata* (Say 1832)
SYNONYMS	*Homalodisca triquetra; Phera vitripennis*

May 2004

The information contained in this PRR was largely extracted from the Draft Review of Post Entry Quarantine Protocols for the Importation of Grapevine (Vitis) into Australia.

The scientific and technical content of this document is current to the date published and all efforts were made to obtain relevant and published information on the pest. New information will be included as it becomes available, or when the document is reviewed. The material contained in this publication is produced for general information only. It is not intended as professional advice on any particular matter. No person should act or fail to act on the basis of any material contained in this publication without first obtaining specific, independent professional advice. Plant Health Australia and all persons acting for Plant Health Australia in preparing this publication, expressly disclaim all and any liability to any persons in respect of anything done by any such person in reliance, whether in whole or in part, on this publication. The views expressed in this publication are not necessarily those of Plant Health Australia.

Part of plant/commodity affected

Stems, leaves, shoots, fruits.

Biology

Symptoms

The pest causes spoilage of fruit from the residue of its excrement and direct feeding damage.

Identification

Adults are about ½ inch (13-14 mm) long, dark brown in colour with small yellow dots on their head and thorax. Wings are membranous and translucent with reddish veins.

Want more info?

If you would like more information, or to download a copy of the Industry Biosecurity Plan, visit **www.planthealthaustralia.com.au**, email **admin@phau.com.au** or phone (02) 6260 4322.

Life history

The insect overwinters as an adult and begins to lay egg masses in late February through May. The first generation matures as adults from late May through to late August. A second generation of egg masses is laid starting in mid-June through to late September, which develop into overwintering adults (Anon., 2000b). Eggs of GWSS are laid side by side in clutches of 1-27, with the average mass consisting of 10 eggs (Guidicipietro, 2000). The eggs are laid under the lower epidermis of leaves, giving a greenish, water-blistered appearance (Guidicipietro, 2000). Eggs are deposited in fruit if populations are high. Nymphs hatch in about two weeks and proceed to feed into leaf petioles or small stems. A second peak in adult activity occurs in the summer during the months of July and August. Peak oviposition from these first generation adults occurs in August.

GWSS feeds and reproduces on a wide variety of trees, woody ornamentals and annuals (Purcell, 1999d). The GWSS uses its needle-like mouth to actively suck the xylem sap of plants. It continues feeding only where it finds suitable concentrations of key nutrients. As the nutrient content of various plants decreases, the insect will move in search of new hosts (Anon., 2000b). GWSS feeds on the larger (basal) stems of plants, including grapevines (Anon., 2000b), and has been reported to feed on dormant grapevines during winter (Purcell, 1999d). GWSS inhabits citrus and avocado groves and some woody ornamentals in unusually high numbers. At immediate risk are vineyards near citrus orchards (Purcell, 1999d).

H. coagulata has become a serious pest in California's table grape growing areas as it is a vector of *Xylella fastidiosa*, the organism for Pierce's disease (PD), phoney peach disease, almond leaf scorch, variegated chlorosis of citrus and oleander leaf scorch.

Research on possible biological control agents is in progress in California. A mymarid (Hymenopteran) has been identified as an egg parasite but so far no adequate control agents have been detected.

Dispersal

H. coagulata is endemic to eastern USA and was first noted in California in 1989. Since that time it has spread rapidly through the grape growing regions of the state. *H. coagulata* is larger, flies further and occurs in much higher numbers in commercial agricultural plantings than other Californian sharpshooters.

Since *H. coagulata* is a large species, it moves rapidly away when disturbed and would not hide within the fruit clusters.

Host range and distribution

Host range

The host list for GWSS includes 73 plant species (Anon., 2000b). It feeds on a wide variety of trees, woody ornamentals and annuals. In Ventura County, California it has been observed breeding on

native plants. It also attacks numerous ornamental hosts and is a serious pest of fruit and ornamentals. Crepe myrtle and sumac are especially preferred (Anon., 2000b).

Betula sp. (birch); *Buxus* sp. (box wood); *Citrus* spp.; *Eucalyptus* sp.; *Euonymus japonicus; Fagus* spp. (oak); *Fraxinus* sp. (ash); *Hibiscus spp.; Kalmia* spp. (laurel); *Lagerstroemia indica* (crape myrtle); *Macdamia integrifolia (*macadamia nut); *Nicotiana* spp. (tree tobacco); *Persea americana* (*avocado); Philodendron* spp.; *Plantanus* spp. (sycamore); *Prunus armeniaca* (apricot); *Prunus persica* (peach); *Prunus* spp.; *Quercus* sp.; *Rhus* spp. (sumac); *Ulmus parvifolia, Ulmus pumila* (Chinese elm).

Distribution

Mexico, USA.

Pest risk analysis

The following risk analysis for angular leaf scorch is based on the methodology in Biosecurity Australia's guidelines on Import Risk Analysis for Plants and Plant Products (2001).

Entry potential

Entry potential: Medium

As individuals are likely to disperse on disturbance when fruit is picked but may secrete themselves within the fruit bunches.

Establishment potential

Establishment potential: Medium

As the pest is not parthenogenetic and requires a fertilised female or both sexes to be present.

Spread potential following establishment

Spread potential following establishment: High

As it is highly mobile and has demonstrated a rapid spread rate in California.

Economic impact

Economic impact: High

As is a vector of Pierce's disease.

Conclusions

Overall risk: High

References

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Application for recognition of the grape mealybug as a target for biological control

To:

The Standing Committee/Ministerial Council Department of Agriculture, Fisheries and Forestry

Re:

Request for approval of the grape mealybug (*Pseudococcus maritimus*, Hemiptera, Insecta) as a candidate for biological control

Date:

12/12/12

Summary

- The grape mealybug has been identified by Plant Health Australia as a high risk pest to Australian horticulture and viticulture industries
- The damage caused by grape mealybug includes weakening of the vine through sap sucking, and spread of honeydew on leaves and fruit which provides a medium for fungi and bacteria
- Furthermore, the grape mealybug has been identified as a vector of grape leafroll viruses
- Approval of the grape mealybug as a target for biological control will enhance possibilities for a rapid response

We request approval of the grape mealybug, *Pseudococcus maritimus (*Erhorn) (Hemiptera: Pseudococcinae), as a candidate for biological control.

Background

The grape mealybug is native to North America, where it is the primary mealybug pest in vineyards. It is invasive and has become established in Argentina, Azerbaijan, Brazil, Chile, Egypt, Georgia, Gibraltar, Guatemala, Iran, Mexico, New Zealand, Peru, South Africa, and Sri Lanka.

Impact on grapevine

Grape mealybugs are sap sucking insects that feed on leaves and fruit, and excrete excess sap as honeydew. Honeydew is a medium for growth of black sooty mould on grape bunches and leaves, causing defoliation and fouled fruit. Sooty mould is a problem of concern in the table grape industry.
Furthermore, this species is a known vector of Grape leafroll viruses.

The viticulture Industry Biosecurity Plan 2009 has identified this species as a medium - high risk to the Australian Grape and Wine industry, and has produced a Pest Risk Review, which is attached for further information about this threat.

Grape mealybug: A target for biological control

Application for recognition of the grape mealybug as a target for biological control

In Australian vineyards, only limited use of insecticides is allowed after flowering, and spraying often disrupts biological control, in particular of mealybugs. Thus, in the case of an incursion, the viticulture industry needs to rely on biological control of this pest. In its native range, the grape mealybug is effectively controlled by a range of parasitoid wasps. Importation of host specific parasitoid wasps is therefore a promising option for biological control.

After incursion of an invasive pest, a rapid response is of the essence for containment and control. This requires preparation. Approval of the grape mealybug as a candidate for biological control is part of this preparation, as it allows application to approve of a list of non-target host species that should be tested before application for importation of additional selected biological control agents.

Therefore, we apply for the grape mealybug, *Pseudococcus maritimus,* to be approved as a candidate for biological control.

Yours sincerely,

Michael Keller (Assoc. Prof. Applied Entomology, The University of Adelaide)
Greg Baker (Head Entomology, South Australian Research and Development Institute)
Katja Hogendoorn (Research Associate, The University of Adelaide)

Attachment: Pest Risk Review for the Grape Mealy bug (Author: Plant Health Australia)

Plant Health AUSTRALIA

Pest Risk Review

ADFA ATGA WGGA Wine Grape Growers Australia wfa Winemakers' Federation of Australia

Grape mealybug

COMMON NAME	Grape mealybug
SCIENTIFIC NAME	*Pseudococcus maritimus* (Erhorn 1900)
SYNONYMS	*Dactylopius maritimus* (Ehrhorn, 1900); *Planococcus maritimus* (Erhorn, 1900); *Pseudococcus bakeri* Essiz, 1910; *Pseudococcus omniverae* Hollinger, 1917

May 2004

The information contained in this PRR was largely extracted from the Draft Review of Post Entry Quarantine Protocols for the Importation of Grapevine (Vitis) into Australia.

The scientific and technical content of this document is current to the date published and all efforts were made to obtain relevant and published information on the pest. New information will be included as it becomes available, or when the document is reviewed. The material contained in this publication is produced for general information only. It is not intended as professional advice on any particular matter. No person should act or fail to act on the basis of any material contained in this publication without first obtaining specific, independent professional advice. Plant Health Australia and all persons acting for Plant Health Australia in preparing this publication, expressly disclaim all and any liability to any persons in respect of anything done by any such person in reliance, whether in whole or in part, on this publication. The views expressed in this publication are not necessarily those of Plant Health Australia.

Part of plant/commodity affected

Leaves and fruit.

Biology

Symptoms

The problem from the grape mealybug arises from the honeydew excreted. This honeydew accumulates on fruit and foliage, supporting the growth of dark sooty mold fungus. Sooty mold presents a cosmetic problem, mainly of concern in table grapes. In wine grapes this is not an issue. Where grape mealybug is expected to be a problem, it is more severe on late-ripening varieties. Early-maturing varieties are harvested before the second (summer) generation has had much of an impact. Injury is usually not severe, and natural enemies generally provide control.

Want more info?

If you would like more information, or to download a copy of the Industry Biosecurity Plan, visit **www.planthealthaustralia.com.au**, email **admin@phau.com.au** or phone (02) 6260 4322.

Identification

The grape mealybug is white to pink in colour, having a flattened ovoid shape. Filaments protrude around the perimeter, with the two longest at the rear. The mealybug's back is obviously segmented and usually covered with white, waxy secretions.

Life history

Small, white waxy insects; adult males only are winged. The wingless females lay up to 600 eggs in an ovisac. The early instar stage males and females appear similar. The male passes through three nymphal instars, then forms a flimsy cottony cocoon about 3 mm long where pupation occurs.

The juveniles and adult females form clusters on leaves and at the base of berries. Grape mealybugs overwinter under old wood as eggs or newly hatched crawlers. Eggs are usually laid under old wood, however in spring eggs are at times laid within the grape clusters. Honey dew causes down-grading of bunches of grape berries. It is most severe on varieties that produce clusters close to the base of the shoot.

Dispersal

Grape mealybugs are mobile but do not disperse quickly.

Host range and distribution

Host range

Alternanthera pungens (khaki weed), *Camellia sinensis* (tea), *Citrus* spp. (orange, lime), *Cydonia oblonga* (quince), *Gladiolus* spp. (gladiolus), *Juglans regia* (walnut), *Lens culinaris* (lentils), *Malus domestica* (apple), *Manihot esculenta* (cassava), *Medicago sativa* (alfalfa), *Parthenium* spp. (parthenium weed), *Prunus armeniaca* (apricot), *Prunus persica* (peach), *Pyrus communis* (pear), *Solanum tuberosum* (potato), *Solidago sempervirens* (golden rod), *Vitis vinifera* (grape vine).

Distribution

Argentina, Azerbaijan, Brazil, Chile, Egypt, Georgia, Gibraltar, Guatemala, Iran, Mexico, New Zealand, Peru, South Africa, Sri Lanka, United States of America (California, Hawaii).

Pest risk analysis

The following risk analysis for angular leaf scorch is based on the methodology in Biosecurity Australia's guidelines on Import Risk Analysis for Plants and Plant Products (2001).

Entry potential

Entry potential: Medium-High

Grape mealybugs may thrive in fruit clusters and are mobile.

Establishment potential

Establishment potential: Medium-High

The grape mealybug has a broad range of hosts.

Spread potential following establishment

Spread potential following establishment: Low-Medium

Mealybugs tend not to disperse quickly.

Economic impact

Economic impact: Medium

They do not reduce crop yield, but contaminate fruit and foliage. Susceptibility to grape vine mealybug damage varies by variety.

Conclusions

Overall risk: Medium-High

Moderate to high in fruit, broad range of hosts, and some grape mealybug stages are difficult to detect due to their small size. Grape mealybug is important in California as a pest of grapes, particularly in areas of the Kern and Tulare Counties. It is sometimes found in the Salinas and Santa Clara Valleys, but it is not considered important there. In 1978 and 1979 grape mealybug outbreaks were also reported in Napa Valley vineyards.

References

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To:

The Standing Committee/Ministerial Council Department of Agriculture, Fisheries and Forestry

Re:

Request for approval of the vine mealybug (*Planococcus ficus*, Hemiptera, Insecta) as a candidate for biological control

Date: 12/12/12

Summary

- The vine mealybug has been identified by Plant Health Australia as a high risk to Australia's viticulture industry
- Among mealybugs, the vine mealybug is by far the most damaging to grapevine
- The damage caused includes weakening of the vine through sap sucking, spread of honeydew on leaves and fruit, which provides a medium for fungi and bacteria to grow, and transmission of grapevine leafroll associated viruses
- In the case of an incursion, a rapid response will mitigate the economic impact.
- Approval of the vine mealybug as a target for biological control will enhance possibilities for a rapid response, in particular because one of the main control agents, a parasitoid wasp, is already reared in Australia for the control of a related species.

We request approval of the vine mealybug, *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcinae), as a candidate for biological control.

Background

The vine mealybug is native to the Mediterranean and therefore well adapted to most of Australia's wine growing regions. It is an invasive species that has become established in Afghanistan, Argentina, Azerbaijan, Brazil, Canary Islands, India, Iran, Iraq, Mexico, Pakistan, Saudi Arabia, South Africa, Tucuman, and the USA. Vine mealybugs are sap sucking insects that feed on many different parts of the vine: trunk, canes, leaves, clusters and sometimes the roots.

Impact on grapevine

Wherever they occur, vine mealybugs are the most damaging of all mealybug species in vineyards. They contaminate grape bunches with egg sacs, larvae, and adults, and they excrete excess sap as

honeydew, which is a medium for growth of black sooty mould on grape bunches and leaves, and causes defoliation and fouled fruit. However, the primary concern is that this mealybug transmits at least five viruses that are associated with grape leafroll disease, which can destroy whole vines.
The viticulture Industry Biosecurity Plan 2009 has identified the vine mealybug as a high risk to the Australian Grape and Wine industry, and has produced a Pest Risk Review, and a fact sheet. A copy of the Pest Risk Review is attached for further information about this threat.

Vine mealybug: A target for biological control

In Australian vineyards, only limited use of insecticides is allowed after flowering, and the use of these often disrupts extant biological control, in particular of mealybugs. Thus, in the case of an incursion, the viticulture industry needs to rely on biological control of this pest. Most successful biological control programs of mealybugs in the vineyard rely primarily on parasitic wasps of the family Encyrtidae that are mealybug specialists. One of the main encyrtid wasps used in control of the vine mealybug, *Anagyrus pseudococci*, has already been introduced into Australia for the control of the citrus mealybug. Mating disruption, ant control and importation of new species of parasitic wasps are additional promising options. However, even to release *A. pseudococci* for the control of vine mealybug, the species needs to be approved as a target for biological control.

Now is the time to recognise vine mealybug as a target for biological control

After incursion of an invasive pest, a rapid response is of the essence for containment and control. More often than not, there is a substantial lag time between a first incursion and the detection of the pest, during which the pest can establish relatively uncontrolled. This scenario is highly likely for the vine mealybug, as it is very similar in morphology to the citrus mealybug already present in Australia. Therefore, especially in the case of the vine mealybug, detection of an incursion should trigger rapid steps to control and contain further spread.

Approval of the vine mealybug as a candidate for biological control will substantially lower response time, because it allows targeted release of an effective parasitic wasp species that is already reared and used in Australia for the control of citrus mealybug. Furthermore, it allows seeking approval of a list of non-target host species that should be tested before applications for importation of additional selected biological control agents can be submitted.

Therefore, we apply for the vine mealybug, *Planococcus ficus,* to be approved as a candidate for biological control.

Yours sincerely

Michael Keller (Assoc. Prof. Applied Entomology, The University of Adelaide)
Greg Baker (Head Entomology, South Australian Research and Development Institute)
Katja Hogendoorn (Research Associate, The University of Adelaide)

Attachment: Pest Risk Review for the Vine Mealy bug (Author: Plant Health Australia)

Plant Health AUSTRALIA

Pest Risk Review

ADFA ATGA WGGA Wine Grape Growers Australia wfa Winemakers' Federation of Australia

Vine mealybug

COMMON NAME	Vine mealybug
SCIENTIFIC NAME	*Planococcus ficus*
SYNONYMS	n/a

May 2004

The information contained in this PRR was largely extracted from the Draft Review of Post Entry Quarantine Protocols for the Importation of Grapevine (Vitis) into Australia.

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Background

The vine mealybug has been considered an economic pest of grape since the late twentieth century and is found throughout much of the Mediterranean region. It is primarily located in Italy, Spain, France, Israel and Egypt, as well as in South Africa, Azerbaijan, India, Pakistan and Argentina. It was first identified in California in 1994 in the Coachella Valley.

Part of plant/commodity affected

Whole plant including fruit.

Want more info?

If you would like more information, or to download a copy of the Industry Biosecurity Plan, visit **www.planthealthaustralia.com.au**, email **admin@phau.com.au** or phone (02) 6260 4322.

Biology

Symptoms

Vine mealybugs derive their nutrients from the phloem tissue and are capable of feeding on many different parts of the vine: trunk, canes, leaves, clusters and sometimes the roots. They negatively impact crop quality and yield by contaminating clusters with egg sacs, larvae, adults, and honeydew. Often the honeydew leads to mold growth, defoliation and fouled fruit. The vine mealybug is particularly troublesome because it excretes significantly more honeydew than other mealybug species. This leads to large amounts of mold on clusters and leaves; thus, making it exceedingly difficult to clean the fruit at harvest.

Identification

Vine mealybugs are soft, oval-bodied pests that are pink in colour with a white waxy covering that extends into filaments along the margins of their bodies.

Planococcus ficus is morphologically very similar to *Planococcus citri* and can only be reliably distinguished serologically.

Life history

Adults over-winter in the adult stage under the bark in the lateral canes. Females secrete a profusion of fine wax threads during egg laying. Each female lays up to 500 eggs which take a week to hatch in summer temperatures, longer in winter. The youngest instars (crawlers) are the dispersive stage. It feeds by inserting mouthparts into plant tissues. Crawlers emerge from under the bark during spring and early summer and move to the shoots where they infest young buds. They then move into the leaves from mid-summer to infest the fruit bunch. After the crop is picked they remain on the leaves but move to under the bark as winter approaches. The female emerges after the 3rd instar. The 2nd instar male spins a cocoon and emerges as a winged adult. The life-cycle last 3-4 weeks in summer but is longer in winter.

There are generally three generations a year which, in the Northern Hemisphere, start at the end of May, the second half of July and the end of August/beginning of September respectively. The species is more likely to be found on the leaves during the growing season than the grapevine mealybug (*Pseudococcus maritimus*) and is more sensitive to low temperatures. This species is protected by ants which feed on the honeydew. *P. ficus* causes damage by producing honeydew which drops onto the fruit bunches and serves as a substrate for sooty mould. In addition the mealybug itself infests fruit bunches to feed.

Hybrids between *Planococcus ficus* and *Planococcus citri* and have been demonstrated experimentally. Only *P. citri* is recorded in Australia. Both species are implicated as vectors of grapevine leafroll – associated virus 3 and corky bark. *P. ficus* also transmits the souring yeast *Hanseniaspora vinae*.

A serious infestation of this species on grapes in California was first noted in 1994 with infestation in the main vine growing areas in 1998 (Warner, 1999).

Dispersal

P. ficus can be dispersed by the wind or spread in soil on farm machinery.

Host range and distribution

Host range

Allotropa mecrida; Anagyrus pseudococci; Chartocerus subaeneus; Chrysopa carnea; Clausena josefifi; Coccidoxenoides peregrinus; Dichrostachys glomerata; Ficus benjamini; Ficus carica (fig)*; Leptomastidea abnormis; Leptomastix dactylopii; Leptomastix flavus; Leptomatidea abnormis; Leucopis alticeps; Malus pumila (apple); Mangifera indica* (mango)*; Pachyneuron concolor; Pauridia peregrina; Persea americana* (avocado)*; Phoenix dactylifera* (dates)*; Prochiloneurus bolivari; Prochiloneurus pulchellus; Prosopis farcata; Punica granatum* (pomegranate)*; Salix* spp.*; Styrax officinalis; Tephrosia purpurea; Vitis vinifera* (grapes)*; Zizyphus spina-christi*.

Distribution

Afghanistan; Argentina; Azerbaijan; Brazil; Canary Islands; Crete; Cyprus; Egypt; France; India; Iran; Iraq; Israel; Italy; Lebanon; Libya; Pakistan; Portugal; Sardinia; Saudi Arabia; Sicily; Spain; South Africa; Tucuman; Tunisia; USA.

Pest risk analysis

The following risk analysis for angular leaf scorch is based on the methodology in Biosecurity Australia's guidelines on Import Risk Analysis for Plants and Plant Products (2001).

Entry potential

Entry potential: High

As it occurs in the fruit bunches.

Establishment potential

Establishment potential: High

Hosts are abundant and common in southern Australia.

Spread potential following establishment

Spread potential following establishment: Medium

It can be dispersed in the wind and spread in soil on farm machinery.

Economic impact

Economic impact: High

It causes spoilage of fruit.

Conclusions

Overall risk: High

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To:

The Standing Committee/Ministerial Council Department of Agriculture, Fisheries and Forestry

Re:

Request for approval of the Grape Berry Moth (*Lobesia botrana*, Lepidoptera: Tortricidae) as a candidate for biological control

Date: 12/12/12

Summary

- The grape berry moth has been identified by Plant Health Australia as a risk to Australian horticulture and viticulture industries
- The grape berry moth is an invasive species, that has spread to many countries worldwide, despite good quarantine measures
- The damage caused by grape berry moth includes feeding on all stages of fruit, spreading fungal disease like *Botrytis*, and attracting secondary insect pests to grapes
- Approval of the Grape Berry Moth as a target for biological control will enhance the possibility for a rapid response in case of an incursion.

We request approval of the Grape Berry Moth, *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae), as a candidate for biological control.

Background

The grape berry moth is native to Southern Italy. It is an invasive species that is now found throughout Europe, North and West Africa, and the Middle East. It has invaded eastern Russia, Chile and more recently Japan and the USA. The importance of this species as grape vine pests has recently been reviewed by Ioratti *et al.* (2012). The grape berry moth prefers grapes as a host, but larvae are also reported on blackberry, currants, olive, summer fruit, persimmon, kiwi, pomegranate, and a wide range of non-crop host plants. It is transported by humans, as eggs or larvae on fruit stems and flowers, or as adults.

Impact on grapevine

In Europe, the Grape Berry Moth is a major pest of grapevines that is responsible for great economic losses and frequent insecticide applications. The larvae of the grape berry moth feed on all stages of fruit, hollowing out the ripe berries and contaminating the produce with webbing and excrement. The feeding damage exposes the ripe berries to infection by fungi, in particular *Botrytis*, which leads to the rejection of grapes for wine making. It also attracts secondary pests, such as ants and fruit flies.

Grape Berry Moth: A target for biological control

In Australian vineyards, only limited use of insecticides is allowed after flowering. Moreover, frequent spraying disrupts the extant biological control in the vineyard. Therefore, in the case of an incursion, the viticulture industry needs to rely to a large extent on biological control of this pest. In its native range, several species of parasitic wasps contribute substantially to the control of grape berry moth. Two of these (*Campoplex capitator* and *Dicaelotus inflexus,* Hymenoptera: Ichneumonidae) stand out, because they locally control the pest and highly specific. Furthermore, mating disruption using pheromone traps is successful at low population densities, which would be achievable if biological control agents contribute to suppression.

Now is the time to recognise Grape Berry Moth as a target for biocontrol

After incursion of an invasive pest, a rapid response is of the essence for containment and control. To achieve this, one needs to be prepared for the incursion. Approval of the grape berry moth as a candidate for biological control is part of this preparation, as it allows application to approve a list of non-target host species that should be tested before applications for importation of selected biological control agents can be submitted.

Therefore, we apply for the grape berry moth, *Lobesia botrana*, to be approved as a candidate for biological control.

Yours sincerely,

Michael Keller (Assoc. Prof. Applied Entomology, The University of Adelaide)
Greg Baker (Head Entomology, South Australian Research and Development Institute)
Katja Hogendoorn (Research Associate, The University of Adelaide)

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APPENDIX 2: INTELLECTUAL PROPERTY

Due to the nature of the project no valuable intellectual property was produced.

APPENDIX 3: REFERENCES

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