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The potential for the establishment of Pierce's Disease in Australian grapevines

FINAL REPORT
GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **DNR00/1**

Principal Investigator: **Peter Merriman**
Contributors: **Joanne Luck, Violeta Traicevski, Ross Mann & Jane Moran**

Research Organisation: Department of Natural Resources and Environment.

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The Place To Be

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Principal Investigator: **Peter Merriman**
Institute for Horticultural Development
Private Bag 15, Scoresby Business Centre, 3176
Tel: (03) 9210 9222 or Fax: (03) 9800 3521
Email: Peter.Merriman@nre.vic.gov.au

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Contributors

Dr Malik Malipatil	NRE, IHD Knoxfield
Ms Linda Semeraro	NRE, IHD Knoxfield
Ms Kyla Finlay	NRE IHD Knoxfield
Mr Mark Whattam	AQIS, Knoxfield
Dr Murray Fletcher	NSW Agriculture, Orange
Dr Brendan Rodoni	NRE, IHD Knoxfield
Ms Renae Sarec	NRE, IHD Knoxfield

Acknowledgements

Dr Peter Ridland	NRE, IHD Knoxfield
Dr Paul DeBarro	CSIRO Entomology Indooroopilly
Dr Chris Stansbury	AGWEST Bunbury
Dr Leann Beanland	NRE, SHC, Mildura
Dr Bob Emmett	NRE, SHC, Mildura
Dr Phil Phillips	UC Riverside
Dr Sandy Purcell	UC Berkley
Dr Bruce Kirkpatrick	UC Davis

EXECUTIVE SUMMARY

This report analyses the pathways for entry and establishment of: (a) the bacterium *Xylella fastidiosa,* causal agent of Pierce's disease (PD) in Australia and (b) the glassy winged sharpshooter (GWSS) *Homalodisca coagulata* which is the most devastating known vector of *X. fastidiosa.* It also presents an analysis of the potential impact of PD on wine grape production in Australia.

The report does not intend to represent an "official" import risk analysis under WTO guidelines which would require a more detailed analysis of pathways under specified protocols. Also the use of the terms "high, medium and low" in the report, to describe risk of entry and establishment may not exactly conform with the more precise use of these descriptors as specified under FAO guidelines.

Australian industry, quarantine agencies and diagnostic agencies are not well prepared to recognise and manage an incursion of PD. PD has not been reported in Australia and, in the event of an incursion, an outbreak may be initially undetected, by which time it may be too late for effective eradication and containment action.

Risk of Entry
For *X. fastidiosa,* pathways for entry are not considered to be totally secure. Current concerns are the risk of entry in symptomless host plants and in illegal imports of host plants by travellers or in mail consignments. AQIS has risk mitigation protocols for imported bud wood of grapevines which include fumigation, hot water treatment and three year PEQ indexing for pests and diseases. The risk of entry of *X. fastidiosa* in legally imported bud wood of grapevines is considered negligible. Many ornamental plants such as Hydrangea and Fuschia are symptomless carriers of *X. fastidiosa.* Current risk mitigation measures for these plants are less rigorous than for grapevines and involve a 3 month period of passive screening in PEQ. A concern is that infected but symptomless material could be inadvertently released for commercial production. The risk of entry by this pathway is considered more significant and categorised as moderate. The greatest risk of entry is through illegally imported infected budwood entering Australia, in the mail or with travellers, this risk is categorised as moderate.

For GWSS, the risks of entry of adult insects through PEQ, international mail or in traveller's luggage are considered negligible. At the time of writing the original draft report, available evidence indicated a significant risk of entry of eggs of GWSS in the rind of citrus fruit which was considered high. This assessment has now been updated to a rating of negligible following discussions with AFFA, who have provided the following additional information:

- advice from the University of California Riverside indicates that survival of eggs laid in rind of lemons was very low.
- CSIRO scientists who have visited areas affected by GWSS in California consider that egg masses, which appear as obvious brown scars, would be graded out during packing operations as would any adult insects.
- any egg masses or adult insects, which inadvertently are included in consignments, should be detected by enhanced risk mitigation measures recently introduced by AQIS. Inspectors at ports and airports have been provided with illustrated guides of GWSS in citrus. So far in excess of 10000 fruit have been inspected from California, since the introduction of GWSS, and no interceptions of eggs or adult insects have been recorded.

NB: the situation with GWSS in California is still evolving and there is little experimental evidence on which to base conclusions. Continual monitoring of the situation is essential and application of a conservative approach should be considered where appropriate.

Fruit of citrus or grapes is not reported to be a source of inoculum of *X. fastidiosa.*

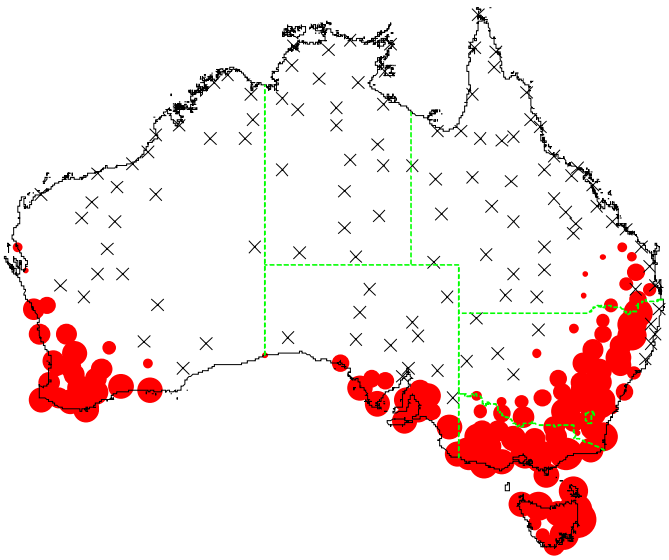
Risk of Establishment
If *X. fastidiosa* and GWSS entered Australia, the risks of establishment are considered high* because of the:

- Favourable climatic conditions
- Widespread distribution of host plants for both the bacterium and the GWSS
- Presence of native insects, that are known xylem feeders and the potential that these insects may be vectors of *X. fastidiosa*

** high risk assessment for establishment assumes a pathway for the introduced bacterium or insect to be distributed to host plants*

X. fastidiosa - survival is temperature limited and analysis in the US shows that the bacterium is rare where the average minimum temperature in the coldest month falls below 4 °C. Most Australian grape growing districts experience average minimum July temperatures of between 3-6 °C and it is expected that *X. fastidiosa* can therefore survive in most regions where grapes are produced in Australia.

GWSS - the predicted distribution of the GWSS is illustrated below. This map has been generated using climate data from regions in southern USA, where GWSS is known to occur, and comparing them with climate data from Australian grape and citrus production areas.



The predicted distribution of the GWSS (red spots) in Australia based on the CLIMEX best-fit model for the GWSS in California.

The impact of an Australian PD outbreak has been estimated on two scenarios:

1. In the absence of GWSS losses will increase linearly over time and will be manifest by slow spread of PD resulting in gradual death of vines
2. In the presence of GWSS losses are expected to increase exponentially over time resulting unacceptably high levels of death of vines as occurred in the Temecula region of Southern California

Chapter 1. Introduction

Brief Background to Problem

Xylella fastidosa and the glassy winged sharpshooter (GWSS) are two of the most important pest and disease organisms exotic to Australia. Pierce's disease (PD) is caused by the bacterium *X. fastidiosa* which is transmitted by the insect, *Homalodisca coagulata,* commonly known as the glassy-winged sharpshooter (GWSS), as well as other sharpshooter insects. The bacteria blocks the xylem vessels of plants and causes symptoms similar to water stress. The grapevine may die within 2 years of infection depending on the variety. Chardonnay and Pinot Noir are the most susceptible.

PD is a complex disease, not only because a range of sharpshooter insects spreads it, but also because both the bacterium and its insect vectors are known to occur in a wide range of host plant species. Furthermore, there are many different *X. fastidiosa* strains, which infect a variety of host plants.

X. fastidiosa is known to affect grapes, citrus, almond and other important horticultural plants. It is also reported in a range of ornamentals and amenity plants such as oleander, fuschia, hydrangea, honeysuckle, eucalyptus and elder trees as well as weeds and grasses such as blackberry, ivy and Bermuda grass. Neither the GWSS or *X. fastidiosa* have been detected in Australia.

Australian industry, government agencies and diagnostic scientists are not well prepared to manage an incursion of PD or the GWSS. An outbreak may go undetected for many weeks due to the lack of awareness of symptoms by growers and farm staff. By this time it may be too late for effective eradication and containment action.

The experience from California, where *X. fastidiosa* and sharpshooter insects (other than the GWSS) are endemic, shows that the disease can cause serious losses in riparian environments. However the introduction of the GWSS, which is a more voracious feeder, has raised the status of PD to a statewide emergency.

There is currently no effective control for the bacterium. Antibiotics generally control bacterial diseases, but there is an embargo on their use in plant industries in Australia. A second problem is that *X fastidiosa* is xylem restricted and therapeutic treatments need to be applied either as a soil treatment for root absorption or by injection into trunks. The only other effective management for perennial plants infected by bacterial plant pathogens is hygiene practices ie. removal of affected parts or of whole plants.

Australia has similar climatic zones and horticultural production to California and many of its key industries are aggregated around riverine systems that are favoured sites for some sharpshooter insects.

An invasion of either *X. fastidiosa* or the GWSS would trigger a number of quarantine actions, which would have immediate economic impact on plant industries. Economic losses would be incurred by the establishment of quarantine zones around infested sites, which would restrict movement of plants and plant products for local, interstate and international trade.

This report presents a background on PD, *X. fastidiosa* and the GWSS and analyses the potential risks, to the Australian grape and wine industry, of a PD outbreak. Awareness and response strategies are proposed to minimise the risk of a PD outbreak in Australia.

Industry Context

Much of the success of Australia's wine industry can be attributed to production efficiency achieved through extensive plantings of wine grapes and mechanisation. Many major plantings are grown inland where relatively warm dry conditions favour the fruit ripening process and reduce the risk of losses from foliar pathogens such as Botrytis and Mildews. This also means less expenditure on chemicals compared with that in cooler wetter regions. Regions proximal to the Murray and Murrimbidgeee Rivers, which produce over 50% of Australia's premium wine grapes, are examples of such a system where it is possible to efficiently produce large quantities of quality fruit which is a vital component in achieving the challenging export targets set by industry.

The Californian wine industry is very similar with many grapes produced along the Napa River. Pierce's disease has "cut a swathe of destruction" either side of the Napa River. The sharpshooter insects, in particular the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata,* feed on grapevine and, in the process, inoculates plants with the bacterium, *Xylella fastidiosa,* the causal agent of Pierce's disease (PD). This multiplies and blocks the xylem or water conducting tissues of vines and vines rapidly decline. Yield loss is severe and ultimately affected plants die.

The GWSS, is the most important vector of PD in California. The emergence of this highly polyphagous vector in California over the past three years has meant that vineyards are struggling to control the PD epidemic. Although the transmission efficiency of X. *fastidiosa* is low compared to the other known vectors (see Chapter 6), their ability to fly long distances, and in particular to feed on ligneous tissue has meant that PD is on the increase. There are no known successful control measures for the GWSS.

The similarity between California and the Murray/Murrumbidgee regions is striking. All the components, which favour PD and the GWSS (eg. Climate and production systems), are similar, including the presence of a related group of insects known locally as spittlebugs, which are also xylem feeders. Preliminary assessment indicates that the impact of PD would be equally devastating in Australia and would seriously compromise production efficiency, wine exports and national trade in canes and rooted cuttings from nurseries in affected areas.

Major output from the Project

The major output from this project is a more detailed knowledge of:

- the known vectors of PD, and related xylem feeding insects that already occur in Australian vineyards and adjacent habitats
- areas of Australia where climatic conditions are favourable for establishment of these vectors
- the potential risk for introduction of the GWSS and *X. fastidiosa* into Australia
- the economic threat of PD to the Australian wine-grape industry.

This report outlines the biology of PD and the GWSS and the likelihood of establishment of either the pathogen or the vector in Australia. This information has then been used to conduct a Pest Risk Assessment (PRA) that clearly demonstrates the risk to Australia.

Chapter 2.
Pest Risk Assessment (including economic impact)

The GWSS and *X. fastidiosa* have already cost Californian growers more than $30 million in the last 7 years. They pose a significant economic threat to Australia.

Three possible scenarios have been identified;

- **Scenario 1** The establishment of *X. fastidiosa*
- **Scenario 2** The establishment of the GWSS
- **Scenario 3** The establishment of both the GWSS and *X. fastidiosa*

Introduction

As the world trade environment has become more global the risk of movement of unwanted pests into new areas has become a frightening reality for many countries. The usual response to this is that countries erect trade barriers on phytosanitary grounds. The WTO recognises the legitimacy of phytosanitary measures (http://wto.org) but it can require member countries to justify regulations to avoid artificial phytosanitary barriers to trade.

In the past the assessment of the risk presented by pests was essentially based on the expert judgement of a group of experts. More recently this process has been formalised, particularly due to the requirements for transparency in international phytosanitary measures. This formal system is known as Pest Risk Assessment (PRA) and it is an elegant way of presenting and analysing the risk an exotic pest may pose to an area. There are many reasons for conducting a PRA and in this instance the PRA was initiated because of an emerging epidemic of PD (caused by *Xylella fastidiosa*) vectored by the newly established GWSS in California. The Californian grape industry is currently in crisis due to the combination of these two pests (see Chapter 1).

The PRA provides a simple scheme for deciding whether a risk exists for a particular area in this case the grape growing regions of Australia. The methodology is based on a binary decision tree, which asks a series of questions about the biology of the pest under assessment. The final stage involves an assessment of the economic and environmental impact of the pest under study. An in depth assessment of the environmental consequences of the introduction of these two pests was beyond the scope of this report and this part of the analysis was confined to economic impact. The end result of this process is an assessment of the risk of entry and consequent establishment of PD and the GWSS in Australia and the economic impact they may have for Australian grape growers and other industries.

In this report we have based the PRA on a scheme devised by the European Plant Protection Organisation (EPPO) (http://www.eppo.org/). The EPPO Pest Risk Assessment Scheme (standard PM 5/3(1), first approved in September 1997) provides detailed instructions, based on EPPO experience, for stages of Pest Risk Assessment covering: initiation, pest categorisation, probability of introduction, economic impact assessment. It provides a simple scheme for deciding whether a risk exists and was used in this report for assessment of the probability of establishment of *X fastidiosa* and GWSS.

PRAs normally comprise two sections, firstly a qualitative section which includes an economic assessment and secondly a quantitative section. Often the data is not available for a proper quantitative assessment and indeed this is the case for *X. fastidiosa* and GWSS in Australia. Consequently only the first section was completed. The answers to the questions in the decision tree were based on an extensive review of the published and current research overseas, and some preliminary local research. Details of this research and analysis are presented in the various chapters in this report.

A detailed PRA, on all known vectors of *X. fastidiosa* and all the known strains of X. fastidiosa*,* is beyond the scope of this report. Consequently two PRAs were conducted; one for the GWSS and one for the PD strain of *X. fastidiosa* and in addition the role of native xylem feeding insects was considered as "on shore" agents with the potential to spread *X fastidiosa.*

The PRA was complemented by an analysis of the potential entry pathways for *X. fastidiosa* and GWSS into Australia (see Chapter 7). It is evident that there is a significant risk for these pests entering Australia. These facts combined with the results of the PRA suggest that the Australian grape and wine industry has a serious threat to contend with.

The GWSS and PD pose a significant threat to Australia. The PRA analysis revealed three possible scenarios each with a different economic impact.

- Scenario 1 The establishment of *X. fastidiosa*
- Scenario 2 The establishment of the GWSS
- Scenario 3 The establishment of both the GWSS and *X. fastidiosa*

In this chapter each scenario is discussed in turn with some thought given to the possibility for eradication or containment of the pest. A more detailed analysis of the economic impact is given for the "GWSS and *X. fastidiosa*" combination as this represents a worst case scenario.

Scenario 1. The establishment of the PD strain of *Xylella fastidiosa* in the absence of a known vector.

The PRA clearly demonstrates that there is a risk of establishment of *X. fastidiosa* in Australia (Figure 2.1).

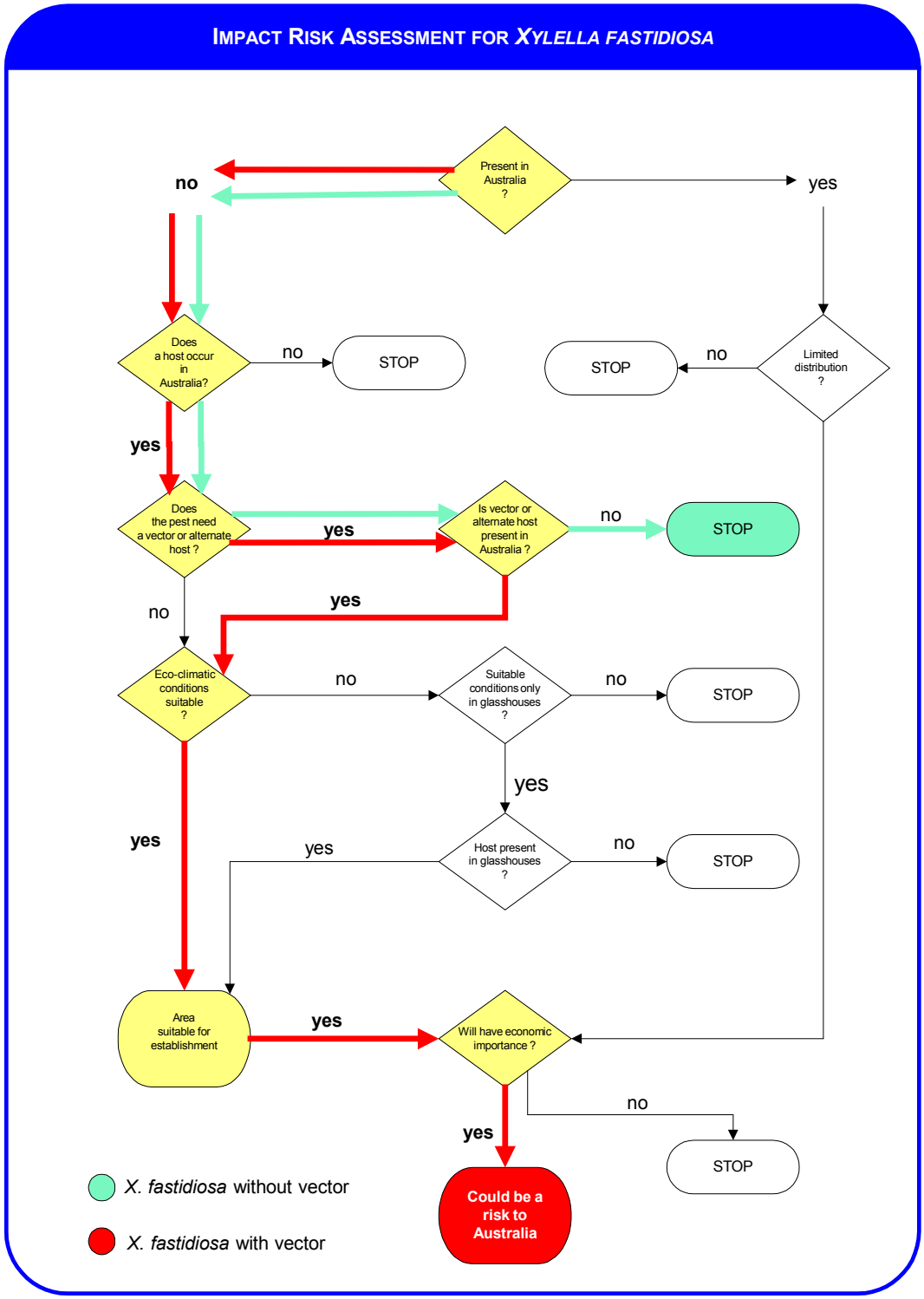


Figure 2.1. Pest Risk Assessment for the establishment of *Xylella fastidiosa* in Australia.

Working down the questions posed in the decision tree.

1. **Present in Australia? No.**
There are no records of any strains of *X. fastidiosa* in Australia and limited surveys have revealed no evidence for presence of the pathogen.

2. **Does a host occur in Australia? Yes.**
Suitable hosts are widespread in Australian grape growing regions (see Chapter 5 & Appendix II).

3. **Does the pest need a vector? Yes.**

4. **Is the vector present in Australia? Unknown**.
Overseas records show that a range of xylem feeding insects is known to transmit *X. fastidiosa*. Native Australian xylem feeding insects are not recorded as vectors of X. fastidiosa however, there is the potential that these insects could be vectors of *X. fastidiosa* should it become established in Australia (see Chapter 6).

5. **Eco-climatic conditions suitable? Yes.**

6. **Area suitable for establishment? Yes.**
Many areas of Australia have similar eco-climatic zones to *X. fastidiosa* epidemic areas in California (see Chapter 4).

7. Will have economic importance? Yes.
It is extremely difficult to predict the economic impact of *X. fastidiosa* in the absence of any known vectors. If Australian native insects were capable of vectoring the disease it is most likely that the epidemiology would be similar to that observed in Californian riparian environments prior to the introduction of the GWSS. In this situation the disease can be managed and losses can be kept to manageable levels. In California in the early 1900s losses due to *X. fastidiosa* in the absence of GWSS were estimated to be $10 million in total. Nonetheless it is worth remembering that more than 100 years ago, *X. fastidiosa* decimated an extensive grape industry, destroying and closing over 50 wineries (Gardener & Hewitt 1974).

Analysis of previous PD outbreaks (i.e. in the absence of the GWSS) shows the epidemic progression is linear, with levels of infection advancing relatively slowly from one, to four, to six percent of the vineyard over a three-year period (Larsen 2000 http:/www.malcolmmedia.com/gwsstext.html). If native insects are able to vector PD, a pattern of disease, and losses, similar to those caused by the Australian grapevine yellows phytoplasma might be expected.

In the absence of a highly effective vector like the GWSS, *X. fastidiosa* spread may be slow enough to contain and eradicate the disease, but success will depend on early detection.

Scenario 2. The Establishment of the Glassy winged Sharpshooter

The PRA clearly demonstrates a high risk for establishment of the glassy winged sharpshooter into Australia (Figure 2.2).

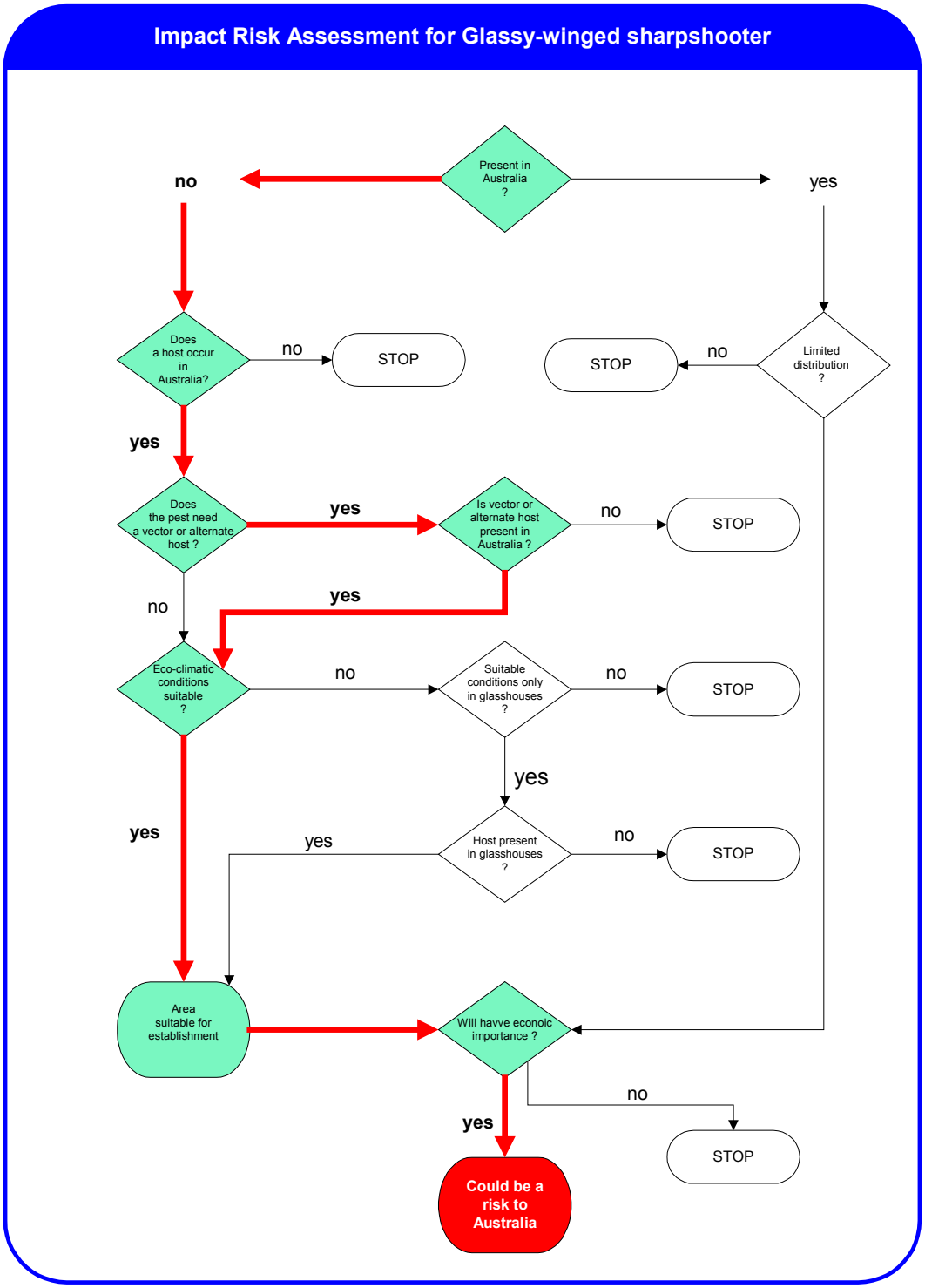


Figure 2.2. Pest Risk Assessment for the Glassy Winged Sharpshooter.

Working down the questions posed in the decision tree

1. **Present in Australia? No.** There are no records of the presence of the GWSS in Australia and preliminary surveys have shown no evidence of this (see chapter 4).

2. **Does a host occur in Australia? Yes.** Suitable hosts are widespread in Australian grape growing regions (see Chapter 5 & Appendix II)

3. **Eco-climatic conditions suitable? and Area suitable for establishment? Yes.** An analysis of climatic data (see Chapter 4) and the high number of alternative hosts (Appendix II) has revealed that many areas of Australia are suitable for the establishment of thriving populations of GWSS.

4. **Will have economic importance? Yes.**
   Our current knowledge indicates that the GWSS causes no feeding damage on grapevine although it has caused minor damage on citrus rind (Blua *et al*. 1999). It is unlikely that the GWSS will become a pest (in its own right) on grapevine but it does pose a threat to other crops (see Chapter 5). An unknown risk is its potential to transmit other pathogens or organisms that have not yet been recognised as pathogens.

In the Americas, the silverleaf whitefly was recorded in the late 1800s and only recently (1986) it became an extreme economic pest because it was attacking crops it had not previously infested and was infecting them with viruses. In the US it has transmitted new plant-pathogenic viruses that had never previously affected cultivated crops, and induced plant physiological disorders. In Central America the emergence of whitefly transmitted viruses accounts for an estimated 20-100% of crop losses in tomato (Polston & Anderson 1997). The silverleaf whitefly, *Bemisia tabaci* Biotype B has recently moved to Australia from the US. It has moved around Australia on ornamentals and is now spread from Darwin to Tasmania (*pers. com.* DeBarro, 2001). The silverleaf whitefly is an extremely efficient vector of geminiviruses and the impact on Australian agriculture is still unknown.

The prospects for containment and eradication of the GWSS are poor. Blanket chemical sprays have not been effective in controlling the GWSS (see Appendix III). Imposing restrictions on the movement of plant material and plant produce may be the only real hope of limiting the spread of this insect (see Appendix III). This would be a costly exercise, with the expense of the regulation of movement of plant material. In addition the restrictions on trade have seriously impacted on the ornamentals industry (see Appendix III).

The bacterium now threatens California's $2.8 billion wine, table and raisin grape growing industry (http://www.fresnobee.com/voicescol18/story/0,2839,163970,00.html). In Australia, during the Fire Blight incident, apple and pear growers lost $2 m, due to interstate trade restrictions, even though trade was suspended for only 6-8 weeks (Kinsella pers com. 2001). Over $10m is estimated to have been lost by Tasmanian exporters of pears and apples during the Fire Blight incident. The lesson from California is that successful containment of the GWSS depends on a coordinated cross-industry approach. The difficulty is achieving consensus between industries, some of which are not severely impacted by GWSS, whose plants are transported between infected and non-infected sites. A cross industry approach will also be important for the Australian industries which are involved in intra and interstate transport of budwood and plant products which are hosts of GWSS and can inadvertently carry GWSS in consignments across Australia.

Scenario 3. Outbreak of GWSS in the presence of *Xylella fastidiosa*

The two previously described PRAs (Figure 2.1 and 2.2) clearly show that both the GWSS and *X. fastidiosa* have a high risk of establishment in Australia, which will have serious impacts on our grape and wine industry. This is confirmed by the predictive climatological analysis (Chapter 4) which demonstrates that the climate of most Australian grape-growing regions is favourable for the establishment of *X fastidiosa* and GWSS.

Introduction of the pathogen and vector into Australia could result in a repeat of the situation in Temecula, Southern California, where the spread of PD is exponential and high losses have been recorded within three years of infection. On a recent visit to Temecula by IHD scientists, one grower was reported as saying "…in 1997 I pulled 50 vines, in 1998 I pulled 500 vines, in 1999 I pulled 10 000 vines and stopped counting".

Overall, losses due to X. *fastidiosa* and direct control costs exceeded **$46 m** in three counties of California. This includes both direct expenditures and losses due to decreased production. There are over 8,300 acres of vineyard currently infected with PD on the North coast of California. About 17 percent of the area of growers in Napa County have some PD, compared to 8 percent in Sonoma County. Over the last five years, 775 acres in the North Coast have been replanted due to PD, of which 670 acres were in Napa County. The cost of replanting the 775 acres, including lost crop during replanting, was over $33 million or nearly $6.6 million/year for each of the five years. An additional 563 acres have been removed from production and not replanted due to PD, with 523 of those acres being in Napa County. The value of that land is estimated at $27.5 million (http://www.malcolmmedia.com/gwsstext.htm).

If the GWSS and X. *fastidiosa* become established in Australia, rural communities will be impacted. This would manifest as job losses, decline in local tourism, decline in local support businesses and decline in rural amenities.

Estimates of economic losses that may occur if PD and GWSS establish in Australia are difficult to estimate because the extent of the disease spread may differ due to the presence or absence of preferred breeding hosts for the GWSS and local climates. Sunraysia is very vulnerable due to it's striking similarity to the Temecula region both climatically (see Chapter 4) and in the mixture of crops grown, especially citrus (see Chapter 5). In regions like this the disease may spread at an exponential rate and crop losses may reach into the tens of millions within a couple of years. Cooler climate regions, where the average minimum July temperatures are below 4^{o}C, may experience only mild epidemics (see Chapter 4).

The chances of eradication of *X. fastidiosa* and GWSS are considered extremely low. Early detection is the key.

Containment may be successful in slowing the spread of PD. The isolation of some grape growing regions will assist containment action however corridors of alternative hosts (eg. citrus) which occur along the Murray Darling Basin are a significant problem (see chapter 5).

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Chapter 3.
History of Pierce's Disease in the USA and the biology of *Homalodisca coagulata* and *Xylella fastidiosa*

The Californian Epidemic

Pierce's Disease (PD) has recently re-emerged as a serious problem in southern Californian grape-growing regions. PD kills grapevines and there is no known cure. The disease was first recorded in Orange County, California in the 1880s by Newton B. Pierce (Pierce 1892) and resulted in the destruction of 16,187 Ha of vineyard in several regions of California (Goodwin and Purcell 1997). Until recently, PD outbreaks have been sporadic and generally confined to the Central Valley and Northern California. However, in the summer of 1999, wine grape growers in Riverside County experienced a sudden dieback of vines, in the premium grape-growing region of Temecula Valley. By mid-August more than 121 Ha of vines in the Temecula region were dead or dying with PD (Figures 3.1 and 3.2) (Anon, 2000).

Figure 3.1 Cleared PD infected vines in the premium grape-growing district of Temecula, Riverside County, California.

Figure 3.2. A 30-year-old vineyard in Temecula destroyed by PD.

The severity of the Temecula outbreak is due to the appearance of a new insect, the Glassy-Winged Sharpshooter (GWSS), *Homalodisca coagulata* (Figure 3.3) which transmits PD. UC extension officer, Phil Phillips first observed the GWSS in California in 1994, on Eucalyptus windbreaks growing adjacent to lemon orchards in Ventura County. This vector is a serious threat to Californian vineyards because it flies longer distances and is an indiscriminate and voracious feeder, able to feed on wood at the base of older grapevine stems. The presence of the GWSS in Southern California has exponentially increased PD, resulting in the current epidemic. There is now a growing concern that it may spread further north throughout the state's premium grape growing regions in the Napa and Sonoma Valleys, threatening more than 283,980 Ha of wine, table and dried grapes valued at $US 2.8 billion pa. (Anon, 2000).

Figure 3.3. The Glassy Winged Sharpshooter

The magnitude of the Temecula outbreak has resulted in the appointment of an emergency taskforce to develop strategies to contain and combat the GWSS and PD, with the Californian government providing $US6.9 million to fund PD research over the next 3 years. Pierce's Disease now replaces Phylloxera as the biggest problem facing Californian grape-growers today. Until recently, the disease has not been reported outside of the Americas (California, Florida, Texas, Mexico and Central America) however, in 1997 it was detected in Kosova, Yugoslavia (Berisha *et al*., 1997).

PD Symptoms

Vines develop symptoms of PD as a result of water stress caused by the blockage of xylem tissue with bacterial aggregates, gums and tyloses (Hopkins 1989). The major symptoms of PD include; marginal leaf necrosis, leaf abscission with petiole retention and irregular cane maturation. (Goodwin and Purcell, 1997). The first evidence of PD infection is the drying or scorching of grapevine leaf margins. The leaf usually dries over a period of days, leaving a series of concentric zones of discoloured and dead tissue. On red varieties such as Cabernet Sauvignon, a dark-reddish/purple band is formed between the necrotic margin and the green interior of the leaf (Figure 3.4a). The scorching develops inward from the margin and is continuous. On white fruit varieties such as Chenin Blanc, the necrosis is not continuous around the leaf margin and a chlorotic region is present between the green and necrotic area of the leaf (Figure 3.4b) (Goodwin and Purcell, 1997).

Figure 3.4a. PD symptoms on Cabernet Sauvignon leaf.

Figure 3.4b. PD symptoms on Chenin Blanc leaf.

Eventually the leaf blade sheds, leaving the petiole attached to the stem. Symptoms spread along the cane out towards the tips, which may dieback. The wood may fail to mature resulting in 'green islands' of tissue (Figure 3.4c). Bud break is delayed in the following season and chronically infected vines have restricted spring growth (Goodwin and Purcell, 1997). A PD affected grapevine normally dies 1-2 years after the first symptoms are observed.

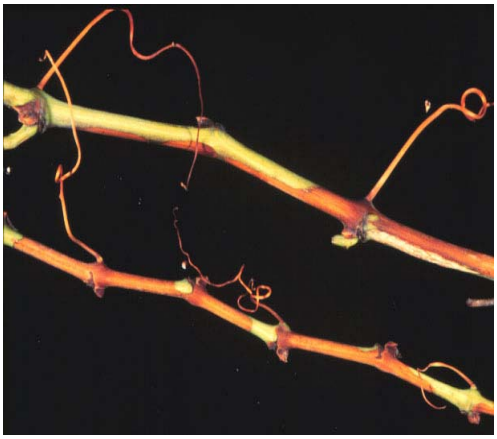

Figure 3.4c. PD infected grapevine canes with characteristic "green islands" symptom.

No European grapevine varieties are resistant to PD, however some are more tolerant eg. Cabernet Sauvignon, Cabernet Franc, Chenin Blanc, Merlot. The most susceptible varieties are French Colombard, Chardonnay, Pinot Noir and Barbera (Goodwin and Purcell, 1997).

The Bacterium- *Xylella fastidiosa*

The cause of PD remained a mystery until the bacterium was first isolated in the 1970s (Davis *et al.,* 1978). The difficulties experienced in isolating and culturing this xylem-limited organism led early workers to speculate that a virus caused the disease. The bacterium was eventually characterised and designated *Xylella fastidiosa* by Wells *et al*. (1987) and is the sole species belonging to this genus.

X. fastidiosa is a gram-negative bacterium confined to the xylem vessels of its host (Figure 3.5). Insects with piercing/sucking mouthparts feed on xylem sap and transmit the bacteria from diseased to healthy plants. Pierce's disease (PD) occurs when infected grapevines develop symptoms due to water stress caused by the bacteria blocking the water conducting system and restricting water movement through the vine. The vine generally dies in 1-3 years and there is no known cure.

There are many different strains of the bacterium, which occur in a wide range of hosts. Some of these strains have been associated with other diseases that cause significant losses in plants including peach, citrus, almond and elm. Importantly, there are many hosts in which *Xylella* resides in that do not express symptoms. These species can act as silent reservoirs (eg. blackberry or periwinkle). The bacterium may be acquired from these symptomless plants by xylem-feeding vectors, which transmit the bacterium to other crops such as grapevine. Important hosts for both *X. fastidiosa* and the GWSS are; Bermuda grass, Perennial Rye grass, Fescue grass, Mulberry, Citrus, Blackberry, Willow, Ivy and Stinging Nettle (Goodwin & Purcell 1997). Although the *X. fastidiosa* strain than infects citrus does not go to grapevine, citrus is considered one of the most important overwintering hosts for the GWSS.

The pathological relationships between *Xylella* strains have not been well characterised and need further study. Reciprocal transmission studies indicate that the PD strain is the same as the almond leaf scorch and the alfalfa dwarf strains (Davis *et al.*, 1981). The phony peach disease and plum leaf scald are also caused by the same strain (Wells *et al.*, 1981). PD strains and Periwinkle Wilt strains were both pathogenic to periwinkle but the PW strain did not cause symptoms when inoculated into grapevine (Davis *et al.*, 1983). The ragweed stunt strain infected plum and periwinkle but did not produce symptoms and did not infect grapevine, citrus and peach (Timmer *et al.*, 1983).

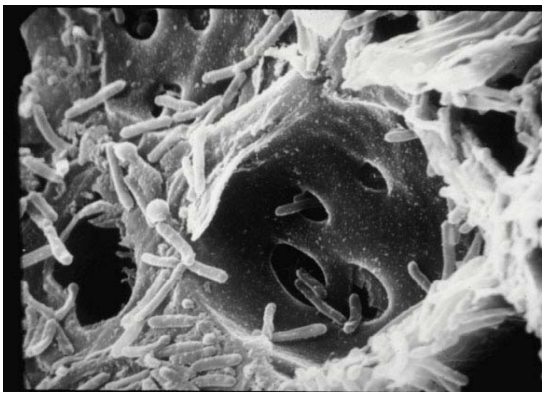

Figure 3.5 *Xylella fastidiosa* attached to xylem vessels (source http://www.nature.berkeley.edu/xylella)

The Vectors

All known vectors of the PD bacterium belong to the Cicadellidae family (Homoptera) in the subfamily Cicadellinae, many are commonly called sharpshooters. Spittlebugs or froghoppers (family Cercopidae) are also PD vectors. Cicadas (family Cicadidae) are xylem feeders but there have been no studies on their ability transmit PD.

The sharpshooter acquires the PD bacterium by feeding on the xylem sap of the infected host. The bacteria adhere to the sharpshooter's foregut and are subsequently transmitted to healthy plants. Until recently there were three main vectors of PD in California; the blue-green, the green and the red-headed sharpshooters. The recent appearance of the glassy-winged sharpshooter in California has dramatically changed the epidemiology of this disease. The GWSS is a very robust species (11.5-13.8mm in length) with an extensive host range, including ornamental and crop plants. It is also a very strong flier. The GWSS feeds on the larger basal stems of grapevines even during dormancy. Unlike the previously recorded vectors, which fed at the margins of the vineyard, the GWSS appears to fly much further into the vineyard.

Pierce's Disease has been present in California for over 100 years, vectored by the Blue-Green (BGSS), the Green (GSS) and the Red-Headed Sharpshooters (Figure 3.6). The BGSS naturally occurs in riparian vegetation and in residential or park landscapes where it feeds readily on a variety of ornamental shrubs or trees (eg. rose, fuschia and ivy) (http://www.cnr.berkeley.edu/xylella/bgss.html). These insects feed on the succulent new growth of grapevines. PD transmitted by these vectors can be managed by pruning out infected canes.

Figure 3.6 (a) Green sharpshooter (*Draeculacephala minevra*) (b) Blue-green sharpshooter (*Graphocephala atropunctata*) (c) Red-headed sharpshooter (*Carneocephala fulgida)* (d) Glassy-winged sharpshooter (*Homalodisca coagulata).* All images were sourced from (http://www.cnr.berkeley.edu).

Eighty to ninety percent of the BGSS breed in the riparian habitat. The other 10-20% migrate toward the vineyard and lay their eggs on vegetation near the edge of the vineyard (http://www.cnr.berkeley.edu/xylella/pd97.html). The green headed sharpshooter (GHSS) (Figure 3.6a) has been found on many herbaceous plants but prefers to feed and breed on grasses. The GHSS natural habitats are grasses and sedges along streams. It is commonly found in ditch banks, weedy hay fields and permanent irrigated pastures (http://www.cnr.berkeley.edu/xylella/rhss.html). The red-headed sharpshooter (RHSS) (Figure 3.6c) is another grass feeder with a broad host range but prefers habitats with sparser, less luxuriant plant growth (Purcell & Frazier, 1984).

The GWSS (Figure 3.6d) has two generations per year (Gill 1995), over-wintering as an adult in wooded areas, which provide protection from the low winter temperatures. Citrus orchards also provide excellent conditions for the GWSS over the winter months. As adults the GWSS can over-winter in grapevines and it is predicted that they are able to carry *X. fastidiosa* from the previous season and infect grapevines in spring, leading to chronic outbreaks of the disease. The GWSS is the only vector capable of feeding on the larger basal stems. Unlike the other sharpshooters the GWSS feeds on dormant trees and vines during the winter months (Purcell 1997). The GWSS spreads the disease through the vineyard much more rapidly that the other known vectors. (Figure 3.7).

Figure 3.7. Temecula vineyard (2000) destroyed by PD. The movement of disease through the vineyard has increased exponentially since the introduction of the GWSS.

PD in California was managed successfully until the GWSS was introduced, possibly on nursery material from Florida. The GWSS has catapulted the disease into a full-scale epidemic in Southern California, in the span of only three years.
Compared to the endemic Californian vectors:

- The GWSS flies much further
- It feeds on dormant tissue
- It is physically much larger and feeds more often
- It feeds on basal wood

GWSS oviposition occurs in winter to early spring and again in mid-to-late summer. Adults live for several months and lay small eggs side by side in groups of about 10, ranging from 1-27 (Turner & Pollard 1959). The eggs are laid within host plants inside the epidermis of the lower leaf surface and appear as greenish blisters (Turner & Pollard 1959). The upper leaf surface above the blisters may be marked over time by a yellowish elongated blotch. After hatching in seven to ten days, the old egg blister appears as a brown scar. The nymphs feed on leaf petioles or small stems while they progress through four moults before becoming winged adults in 10 to 12 weeks (Turner & Pollard 1959). The GWSS nymphs do not survive well on woody plants in their first and second instars.

In a recent visit to the Institute for Horticultural Development, Knoxfield, Rhonda Smith, an viticultural extension officer from Sonoma County, claimed that scientists and growers have only just realised the significant role citrus has played in the spread of GWSS. She said that "citrus was the key agent of spread for the GWSS because it was such a good host…….and that awareness was critical in keeping tabs on the GWSS". The GWSS was first detected (unofficially) by a home gardener, who inquired about her Magnolia which was covered in "white rain", Ms. Smith said. The "white rain" was later recognised as GWSS excrement.

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Chapter 4.
Predictive climatological analysis of Australian grape growing regions as favourable environments for the establishment of known Pierce's disease vectors.

- **Predictive temperature models show that most grape growing areas of Australia have suitable climates for the establishment of GWSS**
- **30 year meteorological data suggests that *X. fastidiosa* could establish in grape-growing regions.**

Introduction

Historically Pierce's disease has affected the Central Valley of Northern California since the 1880s. The blue-green sharpshooter (BGSS) was considered to be the most important vector until recently. The recent emergence of what is now termed a "super vector", the GWSS, has caused major devastation through premium vineyards in California.

The GWSS is a Native American insect, which originated in the East Coast regions of the USA. Until the mid 1990's the GWSS was not considered a threat to the West Coast as the eco-climatic conditions were not thought to be entirely suitable for it's establishment and spread. The GWSS is one of the major reasons that the East Coast does not have a thriving premium winegrape growing area (Purcell and Saunders, 1999). The recent devastation seen in California has alarmed the Australian wine grape industry, because climates for grape production in both regions are similar. In order to assess the potential threat to Australia the predictive model CLIMEX (Maywald & Sutherst 1989; Sutherst & Maywald 1985) was used to analyse whether climatic zones in Australia are suitable for the establishment of the GWSS.

CLIMEX is a computer program that can be used to predict the likely distribution of organisms (mainly agricultural pests) in new environments within countries or regions. It provides a method for predicting the establishment of introduced pests into particular climatic zones.

CLIMEX estimates the 'climatic preferences' of a species by linking the limits of the pest's distribution with long term average meteorological data for that region. This information can then be used to predict pest distribution for areas where it has not been introduced.

The GWSS was given priority due to its ability to colonise, reproduce and feed on a wide variety of host plants and the current situation in California. In this chapter we report on the use of CLIMEX to assess the potential of the establishment of the GWSS in Australia.

A comprehensive analysis of the likelihood of the establishment of all the known vectors of *X. fastidiosa* was beyond the scope of this report.

The prediction for the establishment of *X. fastidiosa* required a different approach. CLIMEX is unsuited to this type of organism since proliferation of systemic pathogens such as *X. fastidiosa* is largely independent of rainfall, humidity and day length. In this instance the analysis was done using historical meteorological data and the known distribution of *X. fastidiosa* in the USA.

Materials and methods

The GWSS

CLIMEX has two functions: **match climates** and **compare locations**. Both of these functions were used to analyse the likelihood of the establishment of the GWSS in Australia.

Match climates: CLIMEX allows the user to directly compare various climate parameters from a given location with any number of other locations. Data generated in this way can be put into maps to show potential distribution of a species. This function was used to directly compare the climates of the known locations of the GWSS in the USA to Australia. The analyses were based on temperature data alone as temperature is the most important climatic parameter for insect establishment, and there is no detailed information available on the biology of the GWSS.

Compare locations: Compare locations uses a combination of climate parameters (temperature and moisture) and stress indices (cold, hot, wet and dry) to predict the likelihood of the establishment of a pest in a given area.

The CLIMEX model assumes that climate is the major factor affecting the distribution of a species and it contains a number of climate templates eg Mediterranean or Temperate. Initially these standard templates were used in the analysis. The parameters of these templates were then modified to give a best fit for the known distribution of the GWSS in the USA, with special reference given to its distribution in California and the climatic zones in Australia where grapevines are grown. This modified template was then applied to Australia to give a final prediction of the likely extent of establishment of the GWSS.

Information on the known distribution of the GWSS was obtained from the USDA (http://plant.cdfa.ca.gov/gwss/gwmap.htm).

X. fastidiosa

The potential establishment was analysed by comparing the known distribution of *X. fastidiosa* correlated with minimum winter isotherms. (http://www/cnr/berkeley.edu). Australian minimum temperatures were obtained from the Bureau of Meteorology of the Commonwealth of Australia (http://www.bom.gov.au/climate/).

Results

The GWSS

Match climates: A number of matches were analysed and the results of two comparisons are given in Figure 4.1 a&b. Here the temperatures of Miami and Sacramento were matched with the temperatures in Australia. These two American locations were chosen because they represent two quite different climatic zones that are found in Australia and they are also locations where the GWSS is known to flourish. The maps show that GWSS, based on temperature data alone, would establish in nearly all but the very arid areas of Australia.

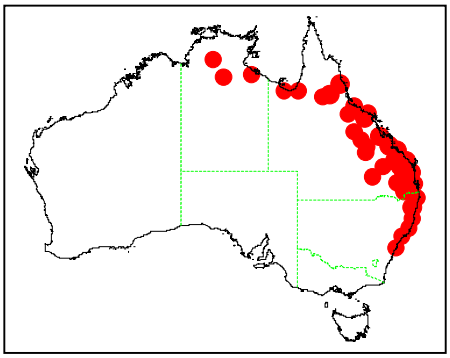

Figure 4.1a. Areas of Australia with temperatures similar to Miami (red spots).

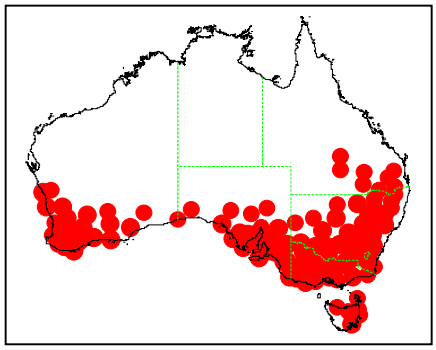

Figure 4.1b. Areas of Australia with temperatures similar to Sacramento (red spots).

Compare locations: The initial analysis for the USA using the CLIMEX climate templates is shown in Figure 4.2 a&b. This analysis revealed that neither template fitted the known distribution of the GWSS in the USA.

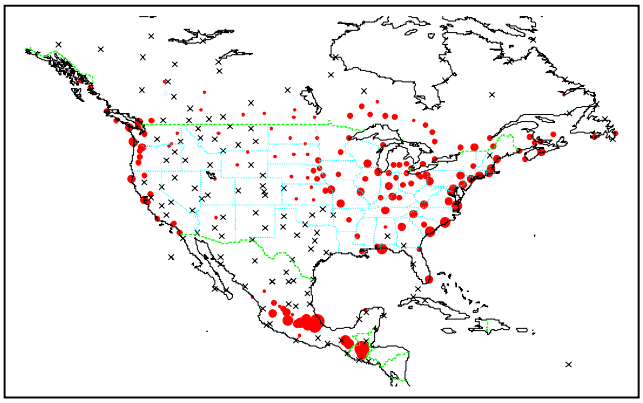

Figure 4.2a. Areas of the USA that fit the CLIMEX "Temperate" template (red spots).

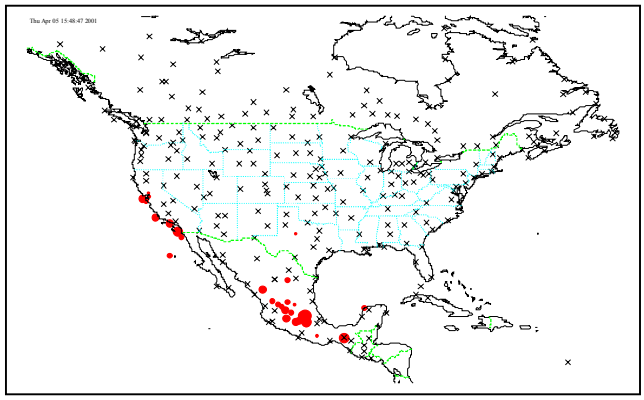

Figure 4.2b. Areas of the USA that fit the CLIMEX "Mediterranean" template (red spots).

The analysis of the Australian climate using the CLIMEX climate templates is shown in Figure 4.3 a&b.

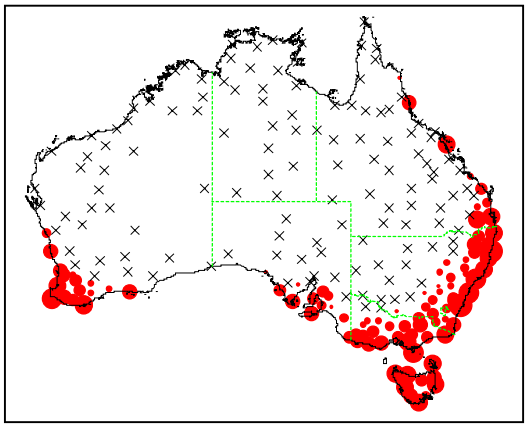

Figure 4.3a. Areas of Australia that fit the CLIMEX "Temperate" template (red spots).

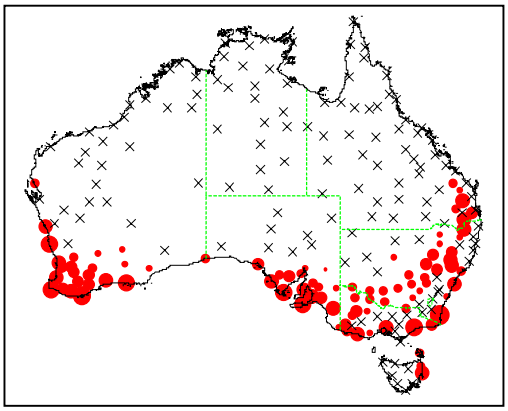

Figure 4.3a. Areas of Australia that fit the CLIMEX "Mediterranean" template (red spots).

This analysis showed that neither the Mediterranean nor the Temperate templates accurately described the known distribution of GWSS (Figures 4.2 a&b) in the USA. However because the Mediterranean template gave a reasonably accurate fit for grape growing regions in Australia (Fig 4.3 a & b) it was decided to manipulate this to achieve best fit for GWSS.

CLIMEX uses a series of parameters to model how the organism will react to climatic conditions such as temperature, moisture and day length. The parameters manipulated were the growth indices (temperature and moisture) and the stress indices (cold stress and heat stress) as these were considered to be most important for the insects' survival. These were adjusted until CLIMEX generated a distribution map that best reflected the known distribution of the GWSS in California (Figure 4.2). Only those parameters in the model that were modified from the standard template are shown (Table 4.1). This refined model was then applied to Australia to enable the generation of a map of potential GWSS establishment (Figure 4.4).

Table 4.1. Modified CLIMEX parameters used to fit the known distribution of the GWSS in California, shown with the standard parameters from the CLIMEX "Mediterranean" template.

Parameters		GWSS	Mediterranean template
Temperature			
	DV0	7	10
	DV1	12	16
	DV2	20	24
	DV3	29	28
	PDD	600	600
Moisture			
	SM0	0.2	0.1
	SM1	0.4	0.4
	SM2	0.7	0.7
	SM3	1.5	1.5
Cold stress			
	TTCS	0.07	0
	THCS	0	0.005
	DTCS	9	15
	DHCS	0.005	0.001
Heat stress			
	TTHS	35	30
	THHS	0.005	0.002
	DTHS	0	0
	DHHS	0	0

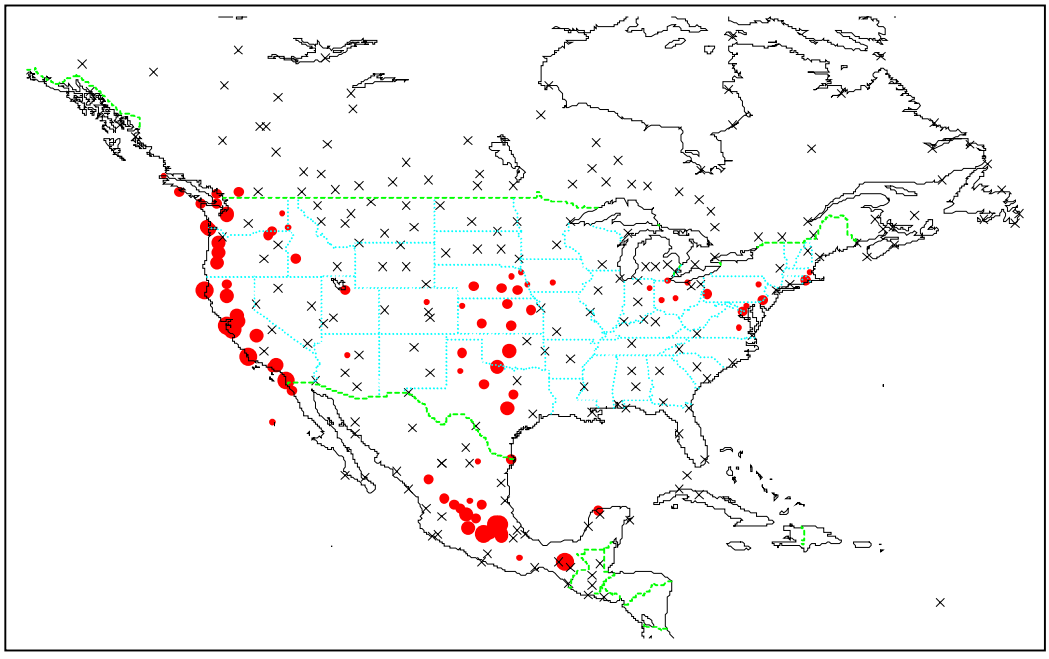

Figure 4.4. Predicted distribution of the GWSS (red spots) in California based on a modified Mediterranean CLIMEX climate model.

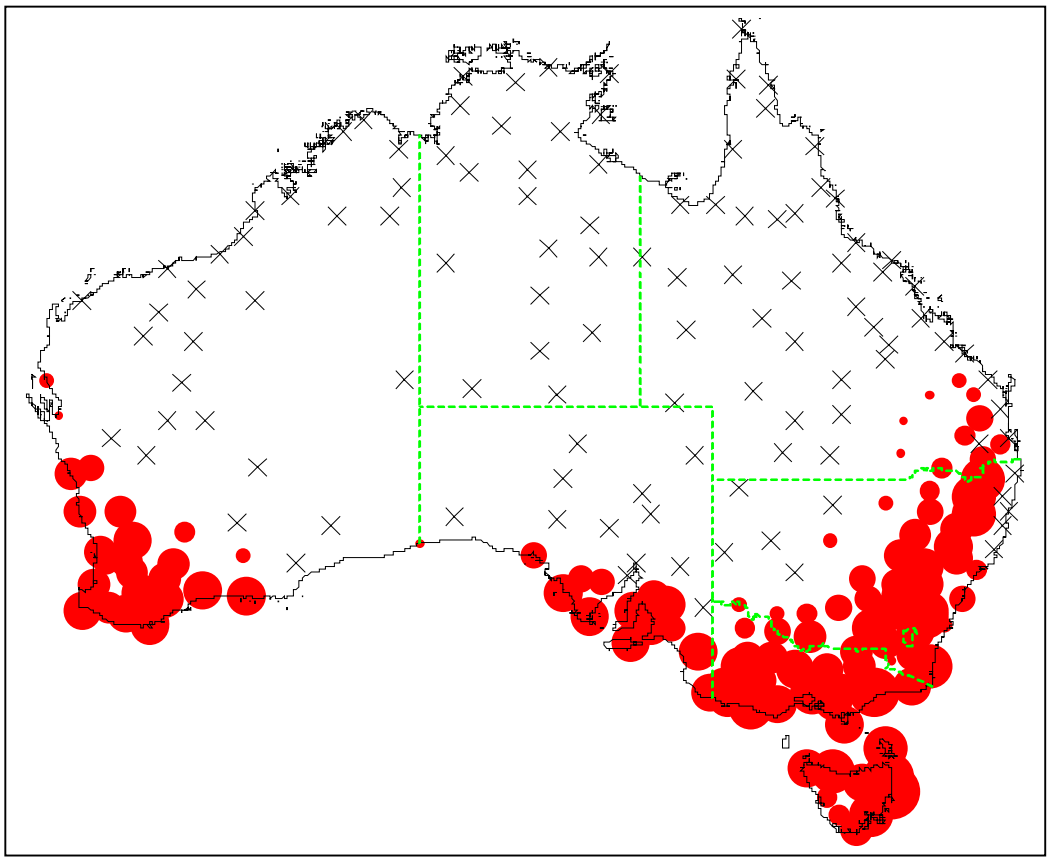

Figure 4.5. Predicted distribution of the GWSS in Australia (red spots) based on the CLIMEX best-fit model for the GWSS in California.

The CLIMEX model predicts that the majority of Australia's grape growing regions are highly suitable for the establishment of GWSS (Fig 4.5). The exception is the areas in the Northern Territory where this version of the model suggests that GWSS will not establish. However the "match climates" analysis suggests that the GWSS may well establish much further North into Australia's sub tropical regions (Fig. 4.1).

The predicted distribution of the GWSS in the USA based on a "Mediterranean" climate (Fig 4.5) still requires fine tuning because it did not encompass the areas on the East Coast of the USA that are known to be the origin of the GWSS.

Xylella fastidiosa

Current research has indicated that survival of the bacterium *Xylella fastidiosa* is temperature limited (Purcell, http://www.cnr.berkeley.edu). Figure 4.6 shows the distribution of *X. fastidiosa* in the USA correlated with the minimum winter temperature, shown as isotherms. Areas experiencing severe Pierce's Disease (PD) outbreaks are illustrated in red. The solid black lines are isotherms for average minimum January temperatures (from http://www.cnr.berkeley.edu). This demonstrates that the distribution of *X. fastidiosa* is limited if average minimum temperature in the coldest month fall below 4°C and the presence of the bacterium is rare if temperatures fall below 1.1°C. Figure 4.7 shows the average minimum July temperatures in Australia. It clearly indicates that most Australian grape-growing districts experience average minimum July temperatures between 3-6°C (purple and blue on the map). Based on this analysis *X. fastidiosa* could survive in most grape growing regions of Australia. Furthermore, severe PD outbreaks have been observed in Californian regions which experience light frosts (Purcell, 2001).

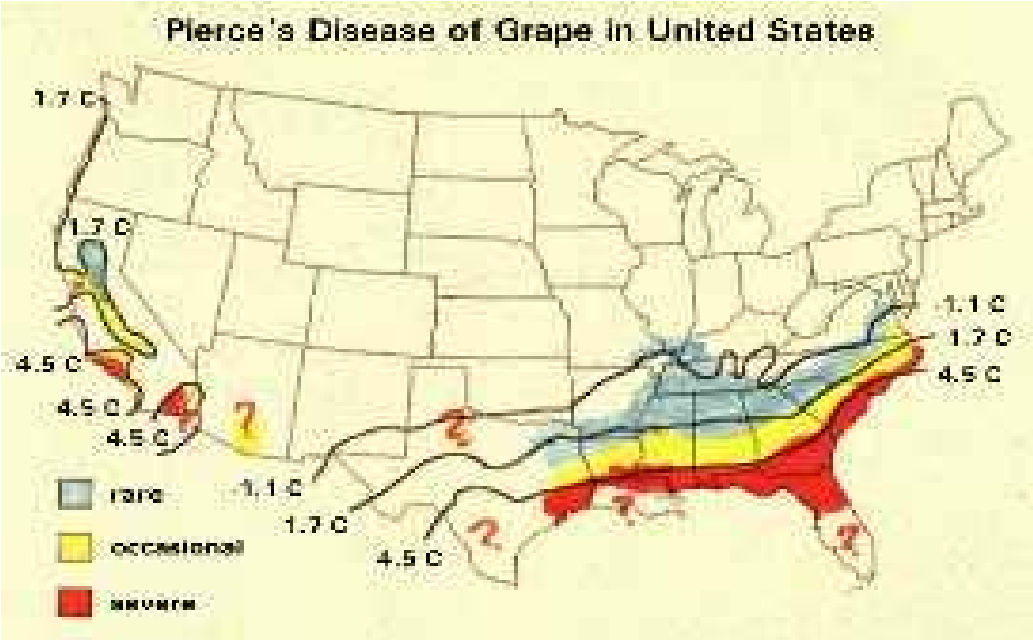

Figure 4.6. Correlation between average minimum winter temperature and occurrence of *X. fastidiosa* in North America.

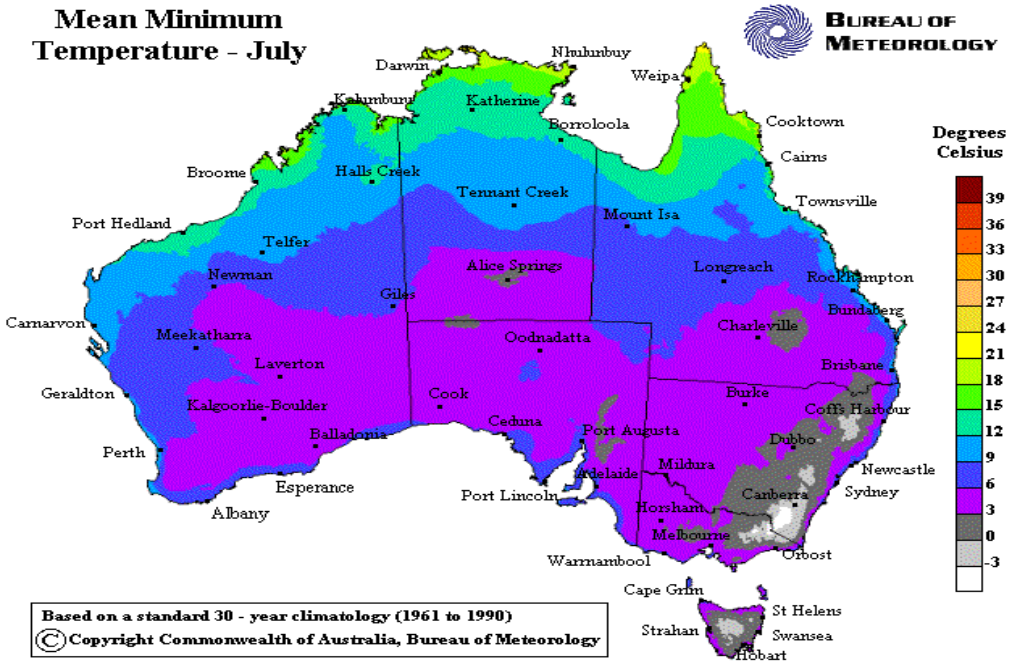

Figure 4.3. Mean July minimum temperature in Australia (30 year average). Areas in shades of grey represent temperatures below 3°C.

Discussion

This analysis provides compelling evidence for the establishment of both the GWSS and *X. fastidiosa* in the major grapevine growing regions of Australia.

The model that resulted from this analysis of the GWSS does not encompass all the known regions that the GWSS is known to inhabit in the USA. The model was based on a simple manipulation of climatic data, and basic cold and heat stress indices. This is due to the absence of detailed knowledge of the biology of the GWSS. If basic GWSS survival parameters like minimum and maximum survival temperatures, humidity tolerances, diapause and light requirements, were available a more realistic model could be produced. It must also be noted that CLIMEX does not take into consideration other factors such as: food supply, predators, local conditions, dispersal by wind, and disease. It is possible that the conditions provided by irrigation may extend the distribution of the GWSS to all grapevine growing regions of Australia including the arid center.

It takes more than a suitable climate for an insect to establish in a new region, the insect must also have access to feeding and breeding hosts. Australian grapevine growing regions provide the GWSS with ample feeding and breeding hosts and compelling evidence of this is provided in Chapter 5.

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Chapter 5.
Analysis of Alternative Hosts for *Xylella fastidiosa* and the Glassy-Winged Sharpshooter: Presence of Host Species in Australia

- ***X. fastidiosa* has 126 known host plants, 93 are present in Australia**
- **GWSS has 175 feeding and breeding hosts, all except one are present in Australia**
- **The GWSS's favoured overwintering host is citrus**

The availability of suitable hosts, and climate are fundamental for the establishment of both the GWSS and *X. fastidiosa*. Here we present results from a literature review to identifying the range of plant species, which may host *X. fastidiosa* and the GWSS and, whether they are present in Australia. This information is presented in Appendix I and Appendix II.

Part A. Alternative hosts of *Xylella fastidiosa* (for a comprehensive list see Appendix I)

X. fastidiosa is reported to have 126 host plants, 93 of them are present in Australia (see Appendix I). Many of the plants listed in Appendix I are either weeds which commonly occur in vineyards (eg. blackberry and ivy), amenity plants (eg. elm and oak) or horticultural commodity plants which are located close to Australian vineyards (eg. plum, peach and citrus).

Part B. Alternative Hosts of the Glassy-Winged Sharpshooter (for a comprehensive list see Appendix II)

The glassy-winged sharpshooter (GWSS) is reported to have 175 breeding and feeding hosts. All except one, are present in Australia (see Appendix II). The natural habitat of the GWSS is forest margins, however it feeds on commercial crops (eg. citrus, avocado and grape), ornamentals (eg. oak, sycamore, crepe myrtle) and natural vegetation (eg. eucalyptus Anon, 2000).

Some Australian vineyards are adjacent to citrus orchards, many have adjoining landscaped gardens which may contain ornamental hosts such as Agapanthus, Rose, Jasmine, crepe myrtle and/or trees such as Olive, Elm, Birch, Willow, all are suitable hosts for both GWSS and *X. fastidiosa*. Many Australian vineyards are located near native bush containing Eucalyptus, a favoured feeding and breeding host.

Blackberry and ivy (both GWSS and *X. fastidiosa* hosts) are ubiquitous across Australia as weeds in or around vineyards.

The distribution of commercial citrus plantations in Australia
The GWSS's favoured over-wintering host is citrus. Citrus has played a major role in the spread of the GWSS through California (Smith pers. com. 2001). Some Australian citrus orchards are located near grape-growing regions (Figure 5.1). The proximity of Australian citrus orchards to grape growing regions may increase the risk of the GWSS establishing (if introduced) into Australia.

Australia is the 4th largest citrus producing country in the Southern Hemisphere after Brazil, Argentina and South Africa. Citrus fruits are grown commercially in all states except Tasmania (Figure 5.1). Most of Australian citrus is grown in the major irrigated horticultural regions of New South Wales, along the Murray River in Southern New South Wales and northern Victoria (Sunraysia and Mid-Murray), the Riverland region of South Australia and The Central Burnett region of Queensland. Production in Western Australia accounts for the majority of the balance. New South Wales grows approximately 35% of Australia's citrus followed by South Australia 33%, Victoria 20%, Queensland 10%, Western Australia 2% and a small but growing industry in the Northern Territory (http://www.austcitrus.org.au/AboutIndustry.htm).

The Riverina region produces 90% of NSW citrus, and 35% of the total Australian production. The Riverina is also NSW largest winegrowing region, processing over 150,000 tonnes of grapes annually, approximately 60% of NSW total production. The Riverland is Australia's major horticultural producing region. Located in the central east of South Australia. The Riverland area grows 50% of the State's wine grapes, making it Australia's largest wine-producing region. Citrus is produced along the Murray in the northern zone in Victoria, centred around Mildura (Sunraysia).

The Murray River area, from Mildura to Swan Hill, currently produces 96% of the grapes grown in Victoria with 80% of Victorian wine production derived from this region (Australian Bureau of Agricultural and Resource Economics (ABARE). Production of Sunraysia grapes for dried, wine and fresh consumption is currently estimated at 350, 000 t (ABARE).

The proximity of Australian citrus orchards to grape-growing regions poses a significant risk for the establishment of PD based on the Californian's experience. A major problem emerging from the Californian PD epidemic is that citrus growers were initially unwilling to control the GWSS populations building up in their orchards, because the insect was not a real threat to their crop. The consequences are escalating populations of the GWSS currently moving through Kern and Tulare counties (Central Valley, California).

A map of Australia's grape and citrus growing areas was created from data from the viticulture and citrus centres across Australia (Figure 5.1). Government departments and private industries provided maps and/or approximate areas of the industries across Australia. The map was produced using the computer software package ArcView GIS 3.2. A Digital Chart of the World (DCW - 1:1,000,000) was used as a base map. Australia's grape and citrus growing areas and the potential establishment area of the glassy-winged sharpshooter were plotted onto the DCW. A map, illustrating the Australian grape and citrus growing regions, together with the predicted Australian GWSS distribution is included in Appendix V.

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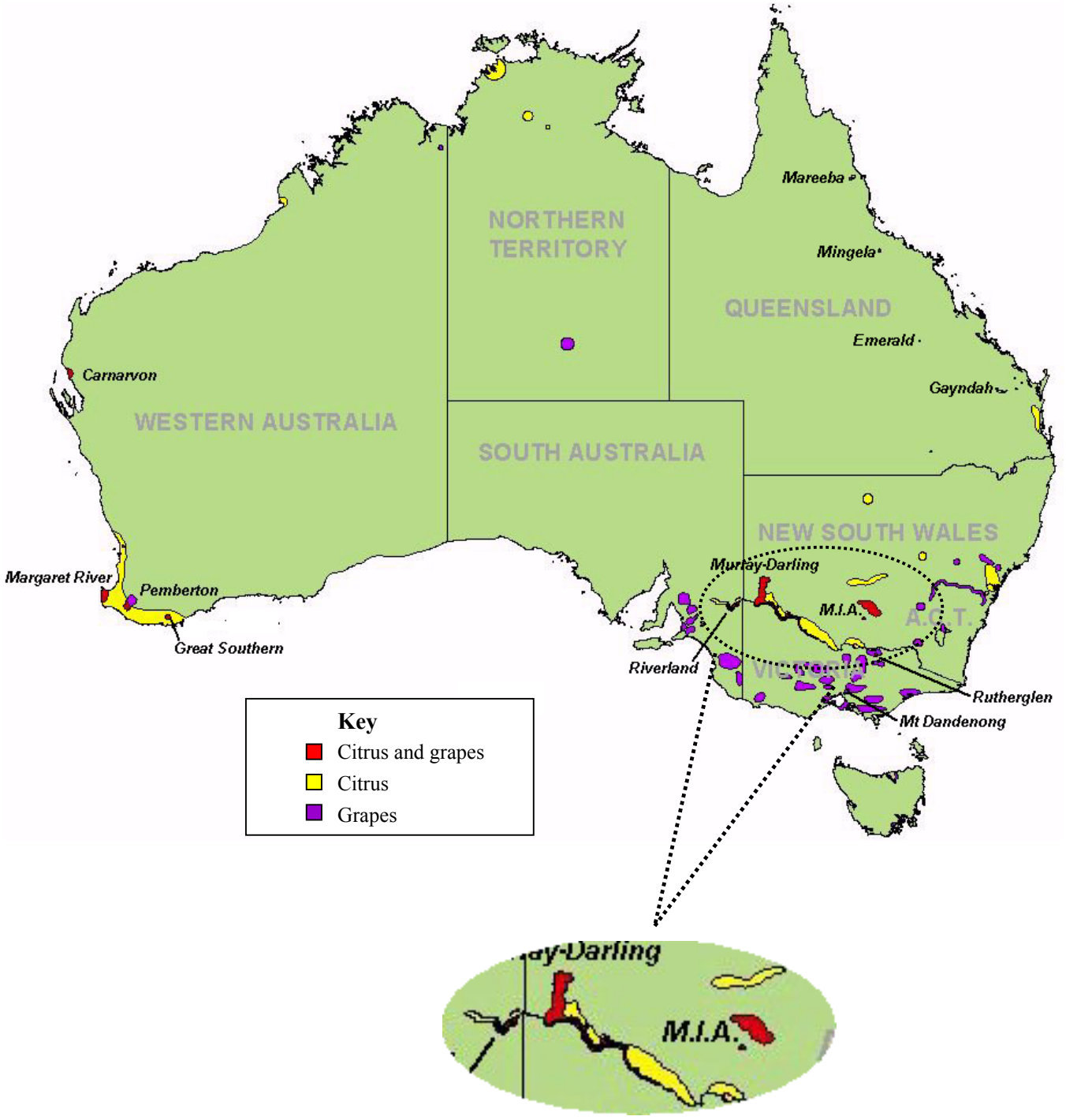

Regions along the Murray Darling river system, which include Riverland, the Riverina and Sunraysia districts, produce a major proportion of Australia's citrus and grapes

Figure 5.1. Map of citrus groves located near grape growing regions of Australia.

Chapter 6.
Analysis of the Occurrence and Distribution of "Sharpshooter" Insects in Australia and the potential for Australian xylem feeding insects to transmit Pierce's Disease (PD).

- **Australia has 14 species of xylem feeding insects which may potentially transmit *X. fastidiosa***

Introduction
The main vectors of PD are xylem-feeding insects belonging to the Cicadellinae subfamily (Homoptera) commonly known as sharpshooters. Spittlebugs and froghoppers (family Cercopoidea) are also vectors of the disease (Severin, 1950).

The principal vectors of PD in California are the blue-green (*Graphocephala atropunctata*, BGSS), the green (*Draeculacephala minerva*, GSS), the red-headed (*Carneocephala fulgida*, RHSS) and the glassy-winged (*Homalodisca coagulata*, GWSS) sharpshooter. The BGSS is a riparian inhabitant and the GSS and RHSS prefer grasslands. The GWSS is native to the East Coast of the USA and prefers forest margins however, in California it seems to be a voracious feeder and breeder on many plant species. The North American vectors have not been recorded in Australia although, up until now there had been no targeted surveys undertaken.

The Cicadellinae subfamily (sharpshooters) is not well represented in Australia (Naumann 1991). There are 14 species Australia wide, of these, three species have been recorded in Victoria (Ken Walker pers. com.), with the majority favouring the tropics. No Cicadellinae species have been reported as plant disease vectors.

The available data, documenting leafhopper (Superfamily Cicadelloidea) activity in Australian vineyards, is derived from two programs that monitored potential leafhopper vectors of Australian Grapevine Yellow disease, between 1982 and 1985, and 1996 and 1998, in North West Victoria. The survey's identified a number of leafhopper and planthopper species which are predominantly phloem and parenchyma feeders (Osmelak, 1989; Kelly *et al*., 1999). No known PD vectors or native Cicadellinae species were identified in those studies.

Two surveys were commissioned to evaluate Australian leafhoppers, sharpshooters and spittlebug fauna, associated with Victorian vineyards. Based on data on the Californian sharpshooters, the initial survey was undertaken in Victorian vineyards and associated habitats. The second, more focused survey, was conducted at several sites on the Mornington Peninsula based on Cicadellinae records from the Victorian Museum. The latter survey was conducted at regular intervals over summer (2000-2001).

Materials and Method

A) Literature review

An extensive literature review was conducted to determine Australia's known xylem feeding insects and their distribution. Consultation with insect taxonomists was also undertaken.

B) Leafhopper surveys

Sweep netting, suction trapping and light trapping were used to collect leafhoppers, planthoppers and spittlebugs in two surveys across Victoria.

Sweep netting involved sweeping a designated area for 20 sweeps using a butterfly net. The sample was placed in a "killing jar" containing ethyl acetate vapour for 5 minutes, to ensure all specimens were dead. A less vigorous method was used when sampling blackberries or the vineyard canopy to avoid tearing the nets or damaging the canopy. A fine mesh net was attached to the suction end of a converted leaf blower, where foliage was "vacuumed" over a 5-minute period.

Figure 5.1. Sweep netting grapevine canopy at St Leonard's, Rutherglen, Victoria.

Light trapping was undertaken at night when a permanent power source could be found in close proximity to a desired sampling area. A white sheet (1m X 1.5m) was suspended between two permanent features so that the bottom of the sheet was touching the ground. A mercury vapour lamp was suspended from a pole rested against the sheet on a 45° angle at a distance of 30cm from the sheet. Specimens were removed from the sheet with an aspirator. Specimens were then shaken from the aspirator vessel into the killing jar for 5 minutes.

Figure 5.2. Inter-row light trapping at St Leonard's vineyard, Rutherglen, Victoria.

Survey 1.
The first survey was conducted during February–April 2000, in and around commercial vineyards situated close to riparian environments, across 10 viticultural regions of Victoria. At each region, two vineyards were surveyed. Habitats selected for sampling included the vineyard canopy, inter-row grasses, cover crops, native vegetation, riparian vegetation and blackberries.

Survey 2.
In the second survey, samples were taken at 14 day intervals over three months. This study was designed to determine the presence of native Cicadellinae species in Victoria based on distribution information provided by the Victorian Museum (Ken Walker pers comm.). The same sampling protocols were used as in survey one. The second survey targeted undisturbed vegetation around Balnarring and the Mornington Peninsula, Victoria (November–February, 2000/2001).

Four sites were chosen, based on distribution records from the Victorian Museum. Cicadellinae species, *Ishidaella angustata*, had been recorded in Balnarring on the Mornington Peninsula. This species was targeted because its distribution was close to the viticultural areas on the Peninsula. Coolart Wetlands and Homestead, Balnarring; Hanns Creek Reserve, Balnarring; Baldry's Crossing, Greens Bush, Point Nepean National Park and Kings Falls, Arthur's Seat State Park were all sampled fortnightly. Vegetation sampled included native grasses, native trees and shrubs, weeds and ferns.

All sample collected in the field were returned to the laboratory and examined and identified using a stereomicroscope, and Cicadellinae keys.

Results

A) Literature review
An extensive literature search revealed a limited amount of data on the abundance and distribution of native xylem-feeding insects in Australia.

Most data presented in this report was derived from a survey for Australian Grapevine Yellows (AGY). Although the species found were predominantly phloem feeders, some are recorded as xylem feeders, which indicates a potential to transmit PD. The data obtained from the review of leafhopper and spittlebug fauna of Australia is presented in three parts: 1. leafhopper phloem feeders, 2. leafhopper xylem feeders and 3. data on spittlebugs present in Australia.

1. Phloem feeders in Australia
Results from the AGY survey revealed that *Orosius argentatus, Austroasca viridigrisea* and *Zygina zealandica* were consistently found in the highest numbers in the three vineyards sampled in Sunraysia. Other species recorded from the survey in moderate numbers were *Nesoclutha pallida, Austroasca torrida, Batracamorphus angustatus* and *Toya dryope* (Kelly *et al*., 1999). The leafhopper species identified in the AGY survey, which are part-time xylem feeders, are summarised in Table 6.1. Research has shown that phloem feeding insects such as leafhoppers, which feed on vines, do probe xylem as well as phloem during the process of identifying feeding sites. The risk is that they may acquire *X fastidiosa* and transmit this to other plants. The relative abundance of these species is also listed (Fletcher pers. com. 2001). Table 6.1 indicates that the xylem feeding leafhoppers are commonly found in Australia and may have the potential to transmit PD. No studies have been undertaken to determine bacterial transmission capabilities of these Australian insects although, AGY transmission (Beanland unpublished) and Tobacco Yellow Dwarf Virus (TYDV) transmission has been studied using Australian leafhoppers (Rodoni, pers. com. 2001).

Table 6.1. Xylem feeding leafhoppers identified in the AGY surveys and their xylem-feeding, (Osmelak *et. al.,*1989 and Kelly *et al.,*1999).

Species	% time xylem feeds	Abundance
O. argentatus	3%	Very common
Z. zealandica	5%	Common
B. angustatus	8%	Common
E. plebus	27%	Common

2. Xylem feeders in Australia

There are three types of full-time xylem feeding insects present in Australia. The Cicadellinae group (which sharpshooters belong to), spittlebugs and cicadas.

In Australia 14 species from 4 genera of the subfamily Cicadellidae are full-time xylem feeders. They are also in the same sub family as the sharpshooters (Cicadellinae). There is little data on the feeding and breeding hosts of these species and their life-cycles in general (Fletcher pers. com. 2001). Most published data focuses on the taxonomy of these species.

A review by Day & Fletcher (1994) of the Australian Cicadellinae species (Table 6.2) reveals that none of the Australian Cicadellinae species listed are pests on their observed hosts. No information is available on their ability to transmit xylem-limited bacteria, but in the absence of such data it is assumed that they all have the potential to transmit *X. fastidiosa*.

Table 6.2. Species of leafhoppers belonging to the Sub-family Cicadellinae and their distribution across Australia (Day and Fletcher, 1994). All species are xylem feeders and therefore, all have the potential to transmit PD.

Species	Hosts	Australasian Distribution
Conoguinula coeruleopennis	*Saccharum officinale* (sugarcane), grasses, coconut, teak (Verbenaceae) cotton (Malvaceae) Pipturus (Urtiaceae) (Day and Fletcher, 1994)	Qld, PNG, Indonesia
Cofana perkinsi	No data	Qld, PNG
Cofana spectra	Rice, sorghum, wheat, barley, sugarcane, grass (Young, 1979), rush, mulberry (Young 1979)	Qld, NT
Cofana unimaculata	Rice	Qld, PNG, India, Pacific Islands
Conogonia coerulescens	No data	Qld, India
Ishidaella albomarginata	No data	Qld, NSW, Tas
Ishidaella anemolua	No data	Qld
Ishidaella angustata	No data	NSW, Vic, Tas, WA
Ishidaella latomarginata	No data	Qld, NSW
Ishidaella naomiae	No data	Tas
Ishidaella pettimolua	No data	NSW
Ishidaella quadrata	No data	Tas
Ishidaella richmondensis	No data	Qld, NSW
Ishidaella tumida	No data	NSW

3. Cercopids (Spittlebugs) in Australia

Spittlebugs are xylem-sucking insects from the superfamily Cercopoidea (Homoptera). Most spittlebug nymphs live in a protective semi-liquid "spittle", derived from mixing a mucopolysaccharide with excreted xylem fluid, resulting in a froth which protect the nymphs from desiccation and some natural enemies (Thompson 1994). There are four known species that can transmit PD (Severin 1947, Purcell, A.H. (1980). In Australia there are 32 reported species (http://www.agric.nsw.gov.au/Hort/ascu/cecopid) (Table 6.3), none have been reported as pests on any agricultural crop, however some species from Indonseia have been shown to transmit the xylem-limited bacterium, *Pseudomonas syzigii* which causes Sumatra disease in cloves (Eden-Green *et al.* 1992). The ability to transmit a xylem-limited bacteria suggests these insects are capable of transmitting *X. fastidiosa*.

Table 6.3. List of Cercopid (Spittlebug) species and their distribution in Australia.

Family	Species	Distribution
Aphrophoridae	*Anyllis leiala*	NSW, Vic, Qld, ACT, Tas
	Bathylus albicinctus	NSW, Vic, Qld, ACT, Tas, NT
	Carystoterpa fasciata	NSW
	Carystoterpa pallida	NSW
	Clovia loxasema	NSW, QLD
	Clovia regalis	Qld
	Eoptyelus australis	NSW, Qld
	Eurycercopis nigrofasciata	Qld
	Neoaphrophora tiegsi	NSW, Vic
	Novaphrophora tasmaniae	Tas
	Philagra concolor	NSW, Qld
	Philagra fulvida	Qld
	Philagra parva	Qld, NSW, Qld, Vic, NT, SA, WA, Tas, ACT
	Philagra recurva	Qld
Cercopidae	*Aufidus trifasciatus*	Qld
	Aufidius lucidus	Qld
	Aufiterna kirkaldyi	Qld
	Eoscarta carnifex	Qld, WA
	Eoscarta vacuola	Qld
	Petyllis deprivata	NSW, Qld, Vic
	Tonnoiria tasmaniae	NSW, ACT, Qld, Tas, Vic
	Tonnoiria chinai	Qld
	Megastethodon urvillei	Qld
Machaerotidae		
	Chaetophyes admittens	NSW
	Chaetophyes compacta	NSW, Qld, Vic, Tas
	Chaetophyes vicina	NSW, Qld
	Hindoloides appendiculata	Qld
	Machaerota finitima	Qld
	Machaerota pugionata	Qld, NT
	Pectinariophyes reticulata	NSW, Qld, WA
	Pectinariophyes stalii	NSW, Qld, ACT, Vic, SA, Tas
	Polychaetophyes serpulidia	Qld, SA

Cicadas

Cicadas are flying, plant-feeding insects of the Order Homoptera, which also contains groups such as the leafhoppers, aphids, and scale insects. Cicadas are prevalent in Australia and have been found to feed on grapevines in Western Australia (amongst other crops) (Fletcher 2001).

Although they feed on xylem tissue, the risk of them transmitting PD is considered to be low, since they have not been recorded as vectors of plant disease.

B) Survey results

Leafhopper, spittlebug and planthopper species identified in the preliminary survey of Victorian vineyards are presented in Table 6.4. The results of the initial survey showed that there were no leafhoppers from the family Cicadellinae (the same family as the GWSS) present, although they have been previously recorded in Victoria (Table 6.2). Three species of spittlebugs were identified in this survey which may be capable of transmitting *X. fastidiosa*, based on their xylem-feeding behaviour. One of these Australian spittlebug species, *Pectinariophyes reticulata* is shown in Figure 6.1

Table 6.4. Leafhopper, Spittlebug and Planthopper species identified in survey of Victorian vineyards and associated habitats from 10 grape-growing regions; Pyrenees, Mildura, Mornington Peninsula, Swan Hill, Macedon Ranges, Yarra Valley, Nagambie, Gippsland, Ovens Valley and Rutherglen (Mann *et al*., 2001).

Family	**Sub-family**	**Species**
Leafhoppers		
Cicadellidae	Agallinae	*Austroagallia torrida*
	Deltocephalinae	*Arawa pulchra*
		Arawa sp.
		Diemoides sp.
		Exitianus plebius
		Horouta perparvus
		Recilia hospes
		Recilia vetus
		Orosius argentatus
		Orosius canberrensis
		Balclutha sp.
		Balclutha sp.
		Balclutha sp.
		Nesoclutha sp.
		Nesoclutha sp.
		Scaphoideus sp.
		Soractellus nigrominutus
		Stirellus fatigandus
	Eupelicinae	*Paradorydium sp.*
	Iassinae	*Batracomorphus angustatus*
	Idiocerinae	*Pascoepus illawarrus*
		Rosopaella sp.
	Macropsinae	*Macropsis sp.*
	Ledrinae	*Rubira sp.*
	Tartessinae	*Brunotartessus fulvus*
	Typhlocybinae	*Edwardsiana frogatti*
		Kahaono sp.
		Kahaono sp.
		Austroasca viridigrisea
		Zygina evansii
		Zygina zealandica
		Zygina nr. sidnica
	Xestocephalinae	*Xestocephalus sp.*
Spittlebugs		
Aphrophoridae		*Philagra parva*
		Anyllis leiala
Machaerotidae		*Pectinariophyes reticulata* (Figure 6.1)
Planthoppers		
Delphacidae		*Haplodelphax sp*
Dictyophoridae		*Hasta hastata*
Heteroptera		
Miridae		

Figure 6.1 *Pectinariophyes reticulata*, one of three Australian xylem-feeding spittlebugs identified in survey 1.

Since no Cicadellinae were collected in the first survey of Victorian vineyards (Mann *et al*., 2001), a more concentrated study was conducted on the Mornington Peninsula. The results are shown in Table 6.5.

Table 6.5 Leafhopper, Planthopper and Spittlebugs identified in a survey for potential Pierce's Disease vectors on the Mornington Peninsula.

Family	Subfamily	Species
Cicadelloidea (Leafhoppers)		
Cicadellidae	*Typhlocybinae*	*Zygina sp*
		Zygina sidnica
		Zygina evansii
		Kahaona sp (3 species)
	Deltocephalinae	*Nesoclutha sp*
		Balclutha sp
		Orosius argentatus
	Idiocerinae	*Rosopaella sp*
Eurymelidae		*Eurymeloides bicincta*
		Nanopoides maculosa
Membracidae		*3 unidentified species*
Fulgoridae (Planthoppers)		
Delphacidae		*Siphonta sp*
		1 unidentified species
Cercopoidea (Spittlebugs)		
Aphrophoridae		*Bathylus albicinctus*
		Neoaphrophora tiegsi
		Philagra pavra
		2 unidentified species

From over 500 insects collected from the Mornington Peninsula sites, 15 leafhopper, two planthopper and five spittlebug species were identified. No Cicadellinae were detected in the second detailed survey.

Discussion

The most comprehensive Australian leafhopper data is provided by the AGY surveys (see above). More data is needed on the native Cicadellinae and Cercopoidea species present in Australia since both have the potential to transmit PD.

Although no Cicadellinae were found in the surveys presented here, we cannot exclude the possibility that sharpshooters exist in Victoria. A Cicadellinae survey must be expanded to a national study over several seasons for a more accurate picture of the fauna present in Australia to ensure that any Cicadellinae present in Australia are identified.

Sandy Purcell (Entomologist, UC-Berkeley) has made a general statement that any xylem-feeding insect has the potential to transmit PD. Australia has many xylem-feeding insect species, including 14 species belonging to the same subfamily as the sharpshooters, Cicadellinae. Leafhoppers (Cicadellidae) are also present in Australia but are predominantly phloem-feeders with the exception of *Exitianus plebus*, which xylem feeds 27% of the time (Table 6.1). *Zygina zealandica* , which xylem feeds for 5% of the time, is another common Australian leafhopper, which is present in some Victorian vineyards in very high numbers (Mann *et al*., 2001; Constable & Glenn, 1999).

Transmission experiments with xylem feeding insects in Australia are not possible because current policy prevents the introduction of live cultures of *X. fastidiosa* to Australia. In the absence of such data it is assumed that the xylem feeders and some of the phloem feeding insects have the potential to transmit *X. fastidiosa*. The basis for this conclusion is that a range of sharpshooter insects is known to effectively transmit *X. fastidiosa* in North and South America. Sharpshooters acquire the bacteria whilst probing the xylem of infected plants. Cells of *X. fastidiosa* adhere to the lining of the mouthparts and then these are released when the insect probes another plant. It would appear therefore that the bacterium has no specific requirements, which limit its acquisition by certain sharpshooters and consequently Australian xylem feeding insects and possibly some phloem feeders may be vectors of *X. fastidiosa*.

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Chapter 7.
Pathways for entry of X*ylella fastidiosa* and the Glassy-winged sharpshooter into Australia.

- Five main pathways have been identified for the introduction of *X. fastidiosa* and the GWSS into Australia. The risk of entry is summarised as follows;

Pathway	Organism	Risk
Importation of grapevine canes	***X. fastidiosa***	**Negligible**
	GWSS	**Negligible**
Importation of ornamental hosts	***X. fastidiosa***	**Moderate**
	GWSS	**Negligible**
Importation of produce	***X. fastidiosa***	**Negligible**
	GWSS	**Negligible**
Passengers and luggage	***X. fastidiosa***	**Moderate**
	GWSS	**Negligible**
Overseas Mail	***X. fastidiosa***	**Moderate**
	GWSS	**Negligible**

The previous chapters have discussed the risks of *X. fastidiosa* and the GWSS establishing in Australia. This chapter analyses the possible entry pathways via either legal or illegal routes.

Australia maintains a managed risk quarantine policy for exotic organisms, which reduces the possibilities of entry and establishment to an acceptably low level.
Quarantine activities at the Australian border provide controls on the entry of people, animals, plants and goods that may introduce unwanted pests and diseases. Risks of all imported plant material are assessed prior to their arrival in Australia. Risk is determined by the type of plant material imported, its origin and the potential for it to carry exotic organisms that could escape and damage crops or the environment.

1. Legal pathways

Importation of nursery material
Grapevine material is classified as high risk for the purpose Post Entry Quarantine (PEQ) treatments. This category includes food plants, tree species for recreational and forestry

and ornamentals which are known to be hosts of high risk pests or pathogens. High risk materials are generally treated and grown at government facilities with extended periods of PEQ screening.

Moderate risk plants are the ornamental species that are not recognised as hosts of high risk pests or pathogens.

Moderate risk plants that are imported *in vitro* (tissue culture) are considered to be in the low risk category.

PEQ protocols for high, moderate and low risk plants are significantly different.

High risk grapevine material is:

1. Fumigated with methyl bromide.
2. Hot water treated at 50 C for a minimum of 20 minutes.
3. Held in a closed quarantine facility for one year and visually inspected for symptoms; followed by a second year of visual inspection in a quarantine screen house.

Details can be found under AQIS import conditions (ICON) at http:www.aqis.gov.au/icon/.

NB: *X. fastidiosa* can be eliminated from propagative material by hot water treatment of dormant cuttings. Immersion at 45 C for three hours or at 50 C for 20 minutes will destroy the bacterium (Varela *et al.,* 2001). The methyl bromide treatment is used particularly for treating insect infestations and will be effective against larval and adult life stages of insects including the GWSS. Its efficacy on eggs of insects such as GWSS is not clearly understood. However information from California indicates that grapevines are not a preferred host for GWSS and oviposition is considered unlikely.

The risk of entry of *X. fastidiosa* and GWSS on grapevine canes through PEQ is considered negligible

Medium risk plants are also treated with methyl bromide (MB) or dipped in a pesticide mixture (specified in ICON) for those species that are sensitive to MB. Hydrangea, lilac, fuchsia and honeysuckle are examples of medium risk plants that undergo a three-month PEQ holding period with at least two visual inspections for symptoms. The holding period may be at a government or accredited insect proof facility. A significant risk is that the plants listed above are reported to be symptomless hosts of *X. fastidiosa*. If material is imported from an area where *X. fastidiosa* is endemic there is a risk that infected plants may remain undetected and the bacterium inadvertently released.

The risk for entry of *X. fastidiosa* on ornamental host plants is considered moderate and the risk for the GWSS is considered negligible.

(b) Importation of produce

Consignments of horticultural produce destined for Australia are inspected and packed to an export standard and normally treated and inspected for exotic plant pests and diseases either off or on-shore. Consignments are inspected on arrival using internationally accepted protocols and additional fumigation treatment is required if live organisms are detected. Specified arthropod disinfestation treatments such as cold or fumigant chemicals are used to kill all life stages of nominated insects (especially fruit fly larvae and eggs).

There are many alternative hosts for *X. fastidiosa* and the GWSS however, citrus has emerged as having a pivotal role in the spread of the GWSS, through the Central Valley

of California. The role of citrus in the PD epidemic was strongly emphasised by Ted Batkin (Californian Citrus Research Board) at a recent symposium on PD (see Appendix IV).

Californian Citrus and the GWSS

The GWSS was probably introduced into California from Florida on citrus budwood. The presence of the GWSS on citrus fruit is now a serious problem for the Californian grape and wine industry. In the Central Valley of California, the GWSS has rapidly spread through the bulk movement of citrus fruit.

In Kern County (November 2000), valencias were picked in the morning, in the cool fall weather, which caused the normally active insect to become torpid, sluggish, and unable to fly. Large numbers of the GWSS "simply fell from the branches of citrus trees into picking buckets and packing bins in the groves". The infested bins were subsequently transported to packing sheds. Later in the day, as the temperature increased, the GWSSs became active and "clouds" of adults were reportedly swarming around the packing sheds. In Tulare County, truckloads of citrus have been so heavily infested with the GWSS that they were sent back to the growers (http://www.cfbf.com/archive/aa-1115a.htm).

Concerns about the GWSS on citrus fruit began in October, 2000, when packinghouse inspectors spotted the insect in about seven loads of early navels from the Arvin-Edison area of Kern County. Since then, sharpshooters have been found in more than a dozen of Tulare County's 55 packinghouses (http://www.cfbf.com/archive/aa-1115a.htm). Sharpshooters have now been found in shipments of valencias, navels and mandarins coming from Kern County.

Up until now, the citrus growers have not been concerned about the GWSS because it is not a significant pest on citrus fruit and the Citrus Variegated Chlorosis (CVC) strain of *X. fastidiosa* is not present in California. Additionally, it has not been cost-effective for growers to spray their crops (treatment costs $US300/acre and citrus is worth approximately $US50/acre). The government has now recognised the seriousness of the GWSS on citrus by announcing a subsidy for growers taking part in approved GWSS treatment programs. Restrictions on bulk movement of citrus have now been imposed on all unprocessed produce in California (November 2000) (http://www.cfbf.com/archive/aa-1115a.htm).

Californian Agricultural Commissioner, Lenard Craft, told growers and packers at a special citrus meeting in Exeter (Kern county) recently, **"for those shipping to Australia: They will not tolerate one sharpshooter, not even a dead one. Australia has a booming grape growing and winemaking industry……. Because the glassy-winged sharpshooter infects grapevines with deadly Pierce's disease, it's likely the Australians will carefully scrutinise citrus shipments coming from California"** (http://www.cfbf.com/archive/aa-1115a.htm)

Three potential risks have been identified associated with the importation of Californian citrus and other produce that may be a host for the GWSS (see appendix II):

1. Introduction of GWSS egg masses;
2. Introduction of GWSS adults;
3. GWSS adults carrying PD.

Export protocols in California require that citrus fruit are washed graded and packed to an export standard, then inspected and cold treated for shipment. These processes minimise the risk of adult GWSS being inadvertently trapped in export consignments.

However in the event that an insect is enclosed in an export pack, information indicates it can survive in a chilled shipment for up to 14 days (http://www.cfbf.com/archive/aa-1115a.htm).

Australian citrus importation
Information that GWSS lays eggs in the rind of citrus fruit is an obvious concern and was previously considered a significant pathway for entry of the insect to Australia. However, more recent information has altered this conclusion. Entomologists at the University of California, Riverside, have advised that survival of eggs laid in the rind of citrus is low. In addition CSIRO entomologists who visited California have established that egg masses in the rind form obvious brown scars. Their judgement is that such affected fruit would be easily graded out during packing operations, as would adults of GWSS, and any affected fruit that was not graded out would subsequently be detected during mandatory on-shore inspection of consignments. AQIS has provided inspectors illustrated guides on GWSS. So far an estimated 10,000 fruit have been examined in Australia since the onset of the GWSS epizootic in California and no egg masses or adult insects have been found.

The risk assessment of GWSS arriving in Australia in consignments of citrus fruit and other produce is now considered negligible although the situation should be reviewed regularly. There was little experimental data available at the time of writing on which to base risk management decisions and it can be expected that the GWSS situation in California will continue to evolve as the insect colonises new habitats. In view of the dynamic nature of the problem the situation should remain under constant review to identify any changes in the risk profile of GWSS which will need remedial action..

The risk of entry of GWSS eggs and adults in consignments of citrus fruit is considered negligible.

Fruit is not considered to be a pathway for *X. fastidiosa*

2. Illegal Pathways

(a) Passenger Luggage
Passengers travelling to Australia, carrying plants or plant products, from countries where PD and/or sharpshooters are present could be a significant pathway for *X. fastidiosa* or the insect vectors. Some passengers may smuggle material for commercial propagation; others may introduce a small amount of material for consumption or home planting. Data on the number of grapevine interceptions made by AQIS inspectors per year is not available.

AQIS do not have the resources to inspect every international passenger's luggage, although the increased budget foreshadowed for 2001/02 will increase levels of inspection.

Late last year (2000), several noteworthy interceptions were made which indicate that illegal introduction of host material is a significant problem. In September (2000) Merlot cuttings, originating from Yugoslavia, were intercepted at Tullamarine airport, Melbourne (Figure 7.1). [Pierce's Disease has been reported in Kosovo, Yugoslavia (Berisha *et al*, 1997)]. During the Sydney Olympics, (September, 2000) US-derived grapevine leaves and bunch rachises (unknown variety), were intercepted at Mascot Airport, Sydney (Figure 7.2). In the following month, Malbec cutting from Argentina were intercepted at Tullamarine airport, Melbourne (October 2000). All intercepted samples were forwarded

by AQIS to IHD plant pathologists who tested the material for *X. fastidiosa*. In each case the illegally imported material tested negative for the bacterium.

Figure 7.1.

Figure 7.2.

Figure 7.1. Merlot cuttings from Yugoslavia, intercepted at Tullamarine, in September 2000, tested negative for Pierce's Disease.

Figure 7.2. Rachises from an unknown table grape variety illegally imported from the US in September 2000, tested negative for Pierce's Disease.

Occasionally material is smuggled into Australia carrying an exotic disease. In September 2000, 5 kg of plums and plum budwood (Figure 7.3) were found concealed in an international passenger's luggage. The passenger had travelled on a flight from Serbia to Sydney carrying plums infected with the devastating exotic disease, Sharka, caused by the *Plum pox virus* (PPV). IHD virologist, Dr Brendan Rodoni examined the intercepted plums and confirmed the presence of PPV (Figure 7.3).

PPV is considered a major threat to Australia's $80 million stone fruit industry. In Europe, more than 100 million stonefruit trees are infected with Sharka. Last year the disease was detected for the first time in the USA, resulting in a major government campaign to contain its spread.

Figure 7.3 Plums infected with the exotic *Plum pox virus*, including a 20cm stick of budwood, intercepted at Sydney airport, September 2000.

If propagated, the infected plum budwood could have contributed to significant losses for the Australian stone-fruit industry.

The above examples clearly demonstrate the risks (to plant industry) presented by passengers, regularly violating quarantine laws. It also highlights the need for public awareness on PD and other exotic diseases, tighter quarantine controls and the requirement for a cohesive, national detection and diagnostic program for PD.
The risk for *X. fastidiosa* entering the country via this pathway is high, however the risk for the GWSS entering via this pathway is considered to be negligible.

(b) Entry through Overseas Mail
Incoming international packages containing plant material harbouring either *X. fastidiosa* or the GWSS (or both), could be a point of entry for PD and it's vector. Australia Post is responsible for the handling of all official mail items entering Australia. Traditionally, quarantine staff have played a secondary role in mail inspection, examining items referred by Australian Customs Service 'screeners' for quarantine clearance. This situation is gradually changing, with quarantine authorities taking a closer interest in mail surveillance.

The quarantine detector dog program involves both passive and active dogs — the passive dogs are used in passenger clearance, and the active (generally larger) dogs are used in mail exchanges and airport areas not accessible to the public. Each dog undergoes a minimum of five weeks of scent discrimination training and by the end of the course each dog will successfully respond to up to 15 different scents. These scents range from meat and meat products to fruit, vegetables, plant cuttings, eggs, as well as live birds and reptiles.

Data on the number of interceptions made at Australian international mail-exchanges is not available. Detector dogs are used as the first line of surveillance as well as X-Ray machines. If the dog detects any target material, the mail sack is opened and its contents are thoroughly checked. The risk of *X. fastidiosa* and/or the GWSS entry via international mail is dependent on the amount of surveillance used. Not all mail coming into Australia is screened and therefore it is possible for material containing disease to be introduced. Any gaps in screening mail or passengers can be perceived as a moderate risk for *X. fastidiosa* entry based on the above interceptions made during passenger clearance over a 2 month period. It is unlikely that the GWSS would survive in the mail system although entry through egg masses is possible.

In May 2001, the Australian Government included an increase in quarantine intervention in Mail Centres from approximately 2% to 100%. This will significantly increase the interception of material of quarantine concern such as budwood.

The entry for *X. fastidiosa* through the mail system is moderate and the entry for the GWSS is predicted to be negligible.

Conclusion

The risks of entry of GWSS and *X. fastidiosa* are summarised below;

Pathway	Organism	Risk
Importation of grapevine canes	***X. fastidiosa***	**Negligible**
	GWSS	**Negligible**
Importation of ornamental hosts	***X. fastidiosa***	**Moderate**
	GWSS	**Negligible**
Importation of produce	***X. fastidiosa***	**Negligible**
	GWSS	**Negligible**
Passengers and luggage	***X. fastidiosa***	**Moderate**
	GWSS	**Negligible**
Overseas Mail	***X. fastidiosa***	**Moderate**
	GWSS	**Negligible**

Californian authorities were first alerted about the GWSS when a home gardener rang to inquire about "white rain" covering her magnolia tree. The white rain was the GWSS excrement that is now recognised as the insect's hallmark. The insect's "calling card" and the ability by stakeholders to recognise other symptoms of Pierce's disease are essential requirements of an early warning system.

Proposed Action

A coordinated national awareness program is critical in educating growers and the general public on the dangers of breaching quarantine laws and its effect on the Australian grape and wine industry. A Pierce's Disease awareness campaign has recently been proposed which includes a national roadshow presented by a Californian PD and GWSS expert, in collaboration with Australian extension officers. Media releases, awareness brochures and shed posters will be distributed as part of the awareness campaign.

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Appendix I.

Alternative host list for *Xylella fastidiosa*. Where Y= present in Australia , N = not present in Australia and N(Genus) = Genus is present in Australia but not species.

Species name	Common name	Australia
Acacia longifolia Willd.	Sydney Golden Wattle[1]	Y
Acer sp.	Maple[2,3]	Y
Aesulus californica	Californian Buckeye[3]	N (Genus)
Ampelopsis arborea (L.) Koehne	Peppervine[4]	N (Genus)
**Amsinckia douglasiana*	Buckthorn Weed[1]	Y
**Artemisia vulgaris L. var. heterophylla Jepson*	Californian Mugwort[3]	Y
**Avena fatua L.*	Wild Oat[1]	Y
Baccharis halimifolia	Eastern baccharis[4]	Y
Baccharis pilularis	Coyote Brush[3]	Y
Baccharis salicifolia	Mule fat[3]	N (Genus)
**Bromus catharticus*	Rescue Grass[1]	Y
**Bromus rigidus Roth.*	Ripgut Grass[1]	N (Genus)
**Bromus sp.*	Russian Brome Grass[1]	N
**Callistephus chinensis Nees.*	China aster[1]	N
**Canna sp.*	Canna family[1]	Y
**Chenopodium ambrodioides L.*	Mexican tea[1]	Y
Citrus sp.	Citrus[10]	Y
Coffee arabicaL.cv. Mundo Novo	Coffee[11]	Y
Conium maculatum	Poison Hemlock[3]	Y
**Coprosma baueri Endl.*	Maddock family[1]	Y
**Cotoneaster rotundifolia Wall. Var. lanata Scheneid.*	Cotoneaster[1]	Y
**Cynodon dactylon*	Bermuda grass[1]	Y
Cyperus eragrostis	Nutgrass[3]	Y
Cyperus eragrostis	Tall umbrella plant[12]	Y
**Cyperus esculentus L.*	Yellow Nutgrass[1]	Y
*Cytisus scoparius Link	Scotch broom[1]	Y
**Daucus carota L.var. sativa D.C.*	Short White Carrot[1]	Y
*Digitaria sanguinalis (L.) Scop.	Common crabgrass[1]	Y
Duranta repens L.	Pigeon-berry[1]	Y
*Echinochloa crusgalli	Water grass[3]	N (Genus)
Echinochloa crus-galli (L.) Beauv.	Barnyard Grass[1]	Y
**Epilobium panniculatum Nutt.*	Panicled Willow Herb[1]	N (Genus)
**Eragrostis diffusa Buckl.*	Diffuse Love Grass[1]	N (Genus)
**Erodium cicutarium L'Her.*	Red-stem Filaree[1]	Y
Escallonia montevidensis DC.	Saxifrage family[1]	Y
*Syzygium australe	Brush cherry[1]	Y
**Festuca megalura*	Foxtail Fescue[1]	Y
Fragaria californica	Wild strawberry[5]	N (Genus)
**Franseria acanthicarpa (Hook.)*	Annual Bur-weed[1]	N
Fraxinus dipetala	Foothill Ash[1]	N (Genus)
Fraxinus latifolia	Oregon Ash[3]	N (Genus)
Fuschia magellanica Lam.	Fuschia[1]	Y
Genista monspessulanus	French Broom[3]	Y
**Godetia grandiflora Lindl.*	Godetia[1]	Y
**Hedera helix*	Ivy[1,3]	Y
**Heteromeles arbutifolia*	Tollon/Christmas Berry[1]	N (Genus)
**Holcus halepensis L.*	Johnson Grass[1]	Y
**Holcus sudanensis Bailey*	Sudan Grass[1]	N (Genus)
**Hordeum murinum L.*	Common foxtail[1]	Y
**Hordeum vulgare L.*	Barley[1]	Y
Hydrangea paniculata	Hydrangea[1]	Y
Juglans nigra	Black Walnut[5]	Y
**Lactuca serriola (L.) Hill*	Prickly Lettuce[1]	Y
**Lathyrus cicera*	Pea family[1]	N (Genus)
**Lathyrus clymenium*	Pea family[1]	N (Genus)

**Lathyrus sativa L.*	Grass Pea[1]	Y
**Lolium multiflorum Lam.*	Italian ryegrass[1]	Y
**Lolium temulentum L.*	Darnel[1]	Y
**Lonicera japonica Thunb.*	Japanese Honeysuckle[1]	Y
Marjorana hortensis Moench	Sweet marjoram[1]	Y
Medicago hispida Gaertn.	Bur clover[1]	Y
Madicago sativa	Alfalfa (Lucerne)[1]	Y
**Meliotus alba Desr.*	White Sweet Clover	Y
**Meliotus alba Desr.var.annua Coe*	Hubam Clover[1]	Y
**Meliotus indica All.*	Yellow Sweet Clover[1]	Y
**Meliotus officinalis Lam.*	Yellow Sweet Clover[1]	Y
Meliotus sp.	Sweet clover[1]	Y
Melissa officinalis L.	Garden Balm[1]	Y
**Mentha sp.*	Mint[1]	Y
Montia linearis	Miner's lettuce[5]	N (Genus)
Morus rubra L.	Mulberry[1,4]	Y
Nerium oleander	Oleander[1,5]	Y
**Oenanthe sarmetosa Presl.*	Water Parsley[1]	N (Genus)
**Oenothera hookeri T. &G.*	Evening Primrose[1]	N (Genus)
Parthencissus quinquefolia (L.) Planch	Virginia creeper[4]	Y
**Parthenocissus tricuspidata*	Boston ivy[1]	Y
**Paspalum dilatatum*	Dallis grass[1]	Y
**Pelargonium hortorum Bailey*	Fish Geranium[1]	N (Genus)
**Pennisetum clandestimum*	Kikuyu Grass[1]	Y
**Phalaris minor Retz*	Mediterranean Canary Grass[1]	Y
**Phalaris paradoxa L.*	Gnawed Canary Grass[1]	Y
**Phleum pretense L.*	Timothy[1]	Y
**Pittosporum crassifolium Cunn.*	Karo[1]	Y
Plantago lanceolata	Ribgrass[5]	Y
Platanus occidentalis L.	Sycamore[9]	Y
**Poa annua L.*	Annual bluegrass[1]	Y
**Fallopia convolvulus*	Black Bindweed[1]	Y
**Persicaria maculata*	Lady's thumb[1]	Y
Populus fremonti	Freemont cottonwood[3]	N (Genus)
Prunus dulcis	Almond[1,13]	Y
Prunus persica	Peach[8]	Y
Prunus sp.	Plum[4,3]	Y
Pyrus pyrifolia (N.L. Burm.)	Pear[7]	Y
Quercus s.	Oak[4,14]	Y
**Reseda odorata L.*	Common Mignonette[1]	Y
**Rheum rhaponticum L.*	Rhubarb[1]	Y
Rhus sp.	Sumac[4]	Y
Rosa californica	Californian Rose[3]	N (Genus)
Rosemary officinalis	Rosemary[1]	Y
**Rubus vitifolius*	Californian Blackberry[1]	N (Genus)
**Rumex cripsus L.*	Curly Dock[1]	Y
Salix sp.	Willow[1,3]	Y
**Sambucus caerulea Raf.*	Blue Elder[1]	N (Genus)
Sambucus canadensis L.	American elder[4]	N (Genus)
Sambucus mexicana	Blue Elderberry[3]	N (Genus)
**Setaria lutescens (Weigel) F.T. Hubb*	Yellow Bristle Grass[1]	N (Genus)
Solidago fistulosa Mill.	Goldenrod[4]	Y
**Sonchus asper (L.) Hill*	Prickly Sowthistle[1]	Y
**Symphoricarpos albus*	Snowberry[3]	Y
**Syringa vulgaris L.*	Lilac[1]	Y
Toxicodendron diversilobum	Poison Oak[3]	N
**Trifolium fragerum*	Strawberry Clover[1]	Y
**Trifolium hybridum L.*	Alsike Clover[1]	Y
**Trifolium incarnatum L.*	Crimson Clover[1]	Y
**Trifolium pratense L.*	Red Clover[1]	Y
**Trifolium repens L.*	White Clover[1]	Y
**Trifolium repens L. var. latum Mc Carthy*	Ladino clover[1]	Y
Ulnus sp.	Elm[6]	Y
Umbellularia californica	Bay Laurel[3]	N
Urtica dioica	Stinging Nettle[3]	Y
**Urtica gracilis*	Creek Nettle[1]	N (Genus)
Veronica sp.	Speedwell[1]	Y

**Vicia monanthus*	Vetch[1]	Y
**Vinca major*	Greater Periwinkle[3]	Y
Vitis californica Benth.	Wild grapevine[1]	N
**Xanthium canadense Mill.*	Cocklebur[1]	N(Genus)
**Zauschneria californicum*	Willow Herb[1]	Y

* experimental host: ie. one in which *X. fastidiosa* could be experimentally transmitted, using the blue-green sharpshooter. At the time these experiments were conducted, PD was predicted to be a virus, because the causal organism was difficult to isolate and culture.

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Appendix II.

Alternative hosts for the Glassy-Winged Sharpshooter, *Homalodisca coagulata*. (*Oviposition hosts) Y= present in Australia. Source: http://plant.cdfa.ca.gov/gwss/gwhllist.asp.

Species name	Common name	Australia
*Abelia sp.**	Abelia	Y
*Acacia sp.**	Acacia	Y
*Aeschynanthus sp.**	Basket plant	Y
*Agapanthus sp.**	Agapanthus	Y
*Agonis sp.**	Willow myrtle	Y
Albizia sp.	Silk Tree	Y
Aleurites sp.	Aleurites	Y
*Alnus sp.**	Alder	Y
*Althaea sp.**	Hollyhock	Y
*Amaranthus sp.**	Amaranth	Y
Ambrosia sp.	Ragweed	Y
*Ananas sp.**	Ananas	Y
*Annona sp.**	Annona (cherimoya)	Y
*Antirrhinum spp**	Snapdragon	Y
*Aptenia sp.**	Aptenia	Y
*Arbutus sp. **	Strawberry tree	Y
*Asclepias sp.**	Milkweed	Y
Asparagus sp.	Asparagus	Y
*Aspidistra sp.**	Iron Plant	Y
*Baccharis sp.**	Baccharis	Y
*Bauhinia sp.**	Bauhinia	Y
*Betula sp.**	Birch	Y
*Begonia sp.**	Begonia	Y
*Bougainvillea sp.**	Bougainvillea	Y
*Brachychiton sp.**	Bottle tree	Y
*Brunfelsia sp.**	Brunfelsia	Y
*Buxus sp.**	Boxwood	Y
*Camellia sp.**	Camellia	Y
*Campsis sp.**	Trumpet creeper	Y
*Canna sp.**	Canna	Y
*Capsicum sp.**	Pepper, chile	Y
*Cassia sp.**	Senna	Y
*Castanopsis sp.**	Chinquapin	Y
*Catalpa sp.**	Catawba	Y
*Ceratonia sp.**	Carob	Y
*Cercis sp.**	Redbud	Y
*Chenopodium sp.**	Lambsquarter	Y
*Chorisia sp.**	Floss-silk tree	Y
*Chrysanthemum sp.**	Chrysanthemum	Y
*Cinnamomum sp.**	Cinnamomum	Y
*Cissus sp.**	Grape Ivy	Y
*Cistus spp**	Rock rose	Y
*Citrus sp.**	Citrus	Y
*Clytostoma sp.**	Clytostoma	Y
*Coprosma sp.**	Coprosma	Y
*Cornus sp.**	Dogwood	Y
Cotoneaster sp.	Cotoneaster	Y
*Cupaniopsis sp.**	Cupaniopsis	Y
*Cycas sp.**	Cycad	Y
*Dietes sp.**	Dietes	Y
*Diospyros sp.**	Persimmon	Y
Elaeagnus sp.	Elaeagnus	Y
*Elaeocarpus sp.**	Elaeocarpus	Y
Erigeron sp.	Fleabane	Y
*Eriobotrya sp.**	Eriobotrya	Y
*Erythrina sp.**	Coral tree	Y
*Escallonia sp.**	Escallonia	Y
*Eucalyptus sp.**	Eucalyptus	Y
*Eugenia sp.**	Eugenia	Y

*Euonymus sp.**	Euonymus	Y
Eupatorium sp.	Boneset	Y
*Feijoa sp.**	Feijoa	Y
*Ficus sp.**	Fig	Y
*Fraxinus sp.**	Ash	Y
*Gardenia sp.**	Gardenia	Y
*Geijera sp.**	Geijera	Y
*Gelsemium sp. **	Yellow jessamine	Y
*Ginkgo sp.**	Gingko	Y
Gladiolus sp.	Gladiolus	Y
Gossypium sp.	Cotton	Y
*Grewia sp**	Grewia sp	Y
*Hardenbergia sp.**	Hardenbergia	Y
*Hedera sp.**	Ivy	Y
*Helianthus sp.**	Sunflower	Y
*Heteromeles sp. **	Tollon	Y
*Hibiscus sp.**	Hibiscus	Y
Howea spp.*	Sentry palm	Y
*Hymenosporum sp.**	Hymenosporum	Y
*Ilex sp.**	Holly	Y
*Jacaranda sp.**	Green ebony	Y
Jasminum sp.	Jasmine	Y
Juglans sp.	Walnut	Y
*Koelreuteria sp.**	Golden-rain tree	Y
Lactuca sp.	Lettuce	Y
*Lagerstroemia sp.**	Crepe myrtle	Y
*Lantana sp.**	Shrub Verbena	Y
*Laurus sp. **	Laurel	Y
*Ligustrum sp.**	Privet	Y
*Limonium sp.**	Statice	Y
*Liquidambar sp.**	Sweet gum	Y
*Liriodendron sp.**	Tulip tree	Y
*Macadamia sp.**	Macadamia	Y
*Magnolia sp.**	Magnolia	Y
Malus sp.	Apple	Y
Malva sp.	Mallow	Y
*Maytenus sp.**	Maytenus	Y
*Melaleuca sp.**	Honey myrtle	Y
Melia sp.	Chinaberry	Y
*Metrosideros sp.**	Bottlebrush	Y
*Michelia sp.**	Champak	Y
*Mirabilis sp.**	Umbrella wort	Y
Monarda sp.	Wild bergamot	Y
*Morus sp.**	Mulberry	Y
*Myoporum sp.**	Myoporum	Y
*Myrtus sp.**	Myrtle	Y
*Nandina sp.**	Nandina	Y
*Nephrolepis sp.**	Sword fern	Y
*Nerium sp.**	Oleander	Y
*Nicotiana sp.**	Tree tobacco	Y
Nyssa sp.	Tupelo	Y
Oenothera sp.	Evening primrose	Y
*Olea sp.**	Olive	Y
*Opuntia sp.**	Cactus	Y
*Osmanthus sp.**	Osmanthus	Y
*Pandorea sp.**	Pandorea	Y
*Persea sp.**	Avocado	Y
*Philadelphus sp.**	Mock orange	Y
*Philodendron sp.**	Philodendron	Y
*Phoenix spp**	Date palm	Y
*Phormium sp.**	Flax lily	Y
*Photinia sp.**	Photinia	Y
Phytolacca sp.	Pokeweed	Y
Pinus sp.	Pine	Y
*Pistacia sp.**	Pistachio	Y
*Pittosporum sp.**	Pittosporum	Y
*Platanus sp.**	Sycamore	Y

*Plumbago sp.**	Leadwort	Y
*Podocarpus sp.**	Podocarpus	Y
*Polygala sp.**	Milkwort	Y
*Populus sp.**	Cottonwood	Y
*Protea sp.**	Protea	Y
*Prunus sp.**	Prunus	Y
*Punica sp.**	Pomegranate	Y
*Pyracantha**	Pyracantha/Firethorn	Y
*Pyrus sp.**	Pear	Y
*Quercus sp.**	Oak	Y
*Raphiolepis sp.**	Raphiolepis	Y
*Rhamnus sp.**	Buckthorn	Y
*Rhododendron sp.**	Azalea	Y
*Rhus sp.**	Sumac	Y
*Robinia sp.**	Locust	Y
*Rosa sp.**	Rose	Y
Rubus sp.	Blackberry	Y
Rudbeckia sp.	Coneflower	Y
*Salix sp.**	Willow	Y
*Sambucus sp.**	Elderberry	Y
*Sapium spp**	Sapium	Y
Sassafras sp.	Sassafras	Y
*Schefflera sp.**	Umbrella tree	Y
*Schinus sp.**	Schinus	Y
*Simmondsia sp.**	Jojoba	Y
*Solanum sp.**	Solanum	Y
Solidago sp.	Goldenrod	Y
Sonchus sp.	Sonchus	Y
*Sorghum sp.**	Sorghum	Y
*Strelitzia sp.**	Bird of paradise	Y
*Syringa sp.**	Lilac	Y
Tecomaria sp.	Tecomaria	Y
Thuja sp.	Arborvitae	Y
*Trachelospermum spp**	Trachelospermum	Y
*Tristania sp.**	Tristania	Y
*Tulbaghia sp.**	Tulbaghia	Y
*Tupidanthus sp.**	Tupidanthus	Y
*Ulmus sp.**	Elm	Y
*Veronica sp.**	Speedwell	Y
*Viburnum sp.**	Viburnum	Y
Vigna sp.	Vigna	Y
*Viola sp.**	Violet	Y
*Wisteria sp.**	Wisteria	Y
Xanthium sp.	Cocklebur	Y
*Xylosma sp.**	Xylosma	Y
Yucca sp.	Yucca	Y
*Zantedeschia sp.**	Calla lily	Y
Zea sp.	Maize	Y

Appendix III.

Notes from "breakout sessions" held at the Pierce's Disease Symposium University of California, Davis December 10-12, 2000

Breakout Session #1 -- Epidemiology of Xylella Diseases, *Xylella* Host Reservoirs, Mechanism of Transmission to Plants, Insect/Plant Interactions

Session Chair – Edwin Civerolo
Facilitators – Susan Laughlin and Jim Brenner
Recorder – Joseph Morse

Participants: Rodrigo Almeida, Peter Andersen, Antonio Juliano Ayres, Kendra Baumgartner, Jim Brenner, James Campbell, Chung-Jan Chang, Edwin Civerolo, Brian Correiar, Heather Costa, Carlos Coviella, Michael Davis, Robert Denno, Greg Dwyer, Benjamin Filho, Juliana Garcia, William Gensler, Carmen Gispert, Tim Gottwald, Jennifer Hashim, Mark Hoddle, Donald Hopkins, Susan Laughlin, Carol Lauzon, Leland "Mac"Learned, Rui Leite, Jr., João R.S.Lopes, Andrew McElrone, Ana Marie Meneguim, Russell Mizell, David Morgan, Joseph Morse, Clifford Ohmart, Keiko Okano, Dan Opgenorth, Charles Pickett, Aurelio Posadas, Alexander Purcell, Allen Scoggan , Greg Simmons, Robert Steffens, Katherine Stevenson, Nick Toscano, Patrick Vail, Robert Wample, Magally Luque Williams, Gisela Wittenborn, Pedro Yamamoto

QUESTIONS OF SPEAKERS (THEIR ANSWERS AND THOSE FROM THE FLOOR FOLLOW):

Q: Regarding the epidemiology of *Xylella*; Florida has a very humid climate, long growing season; would humidity have a large impact on disease expression?

Unlikely that humidity would play a large role in disease expression; climate perhaps results in longer period of time when sharpshooter is able to feed, longer period of luxurious flush being available; quite long growing season; not a dormant period when grapes shut down

Q: Regarding the climatic graph shown by Purcell; is California less likely to see PD in the northern part of the state?

In the greenhouse, can see disease expression in as little as 6 weeks; a big factor is that the plant shuts down at night in California whereas it is quite warm at night in Florida; temperature may play a large role

In California northerly locations can see a vine recover when 4-5 canes were affected – perhaps from freeze therapy; something in the climate restrains disease spread and expression; winter climate may have a large impact on disease expression and movement

Should remember that we have a large range of climes in California; perhaps it looks bleak for PD in southern California; not yet clear how severe it may be in northern California – the winter climate may have a large impact; on the east coast have seen a correlation with warm winters – greater PD problems (a lot more phone calls regarding disease problems)

Is not due to freezing in the xylem – not getting that cold; but a relationship between climate and temperature

Q: Why do we not see PD in Europe; has never found a home in Europe despite sending a large amount of grape plant material there; no doubt was introduced, why never established?

Key may be that Europe may not have a good vector that overwinters (a guess)

Appears that much of the Mediterranean has a climatic match to where PD is severe in the US – so Europeans perhaps should be more concerned that they appear to be

Q: What is the invasion front of GWSS and PD in California?

GWSS trapping initiated this year – was not previously clear where it was present. Found GWSS in the counties of Tulare (3 locations), Fresno (7 locations), Sacramento (one location-Rancho Cordova), Butte (one location – Chico), and Contra Costa (one location –Brentwood)

Some concerns about how efficient the trap is; they may be blundering into it – perhaps the trap is really not very attractive

Movement of nursery plants appears to be a major route of movement; regulations initiated on July 17; this year, 52,000 inspections of plant material, less than 1% found to be infested with GWSS egg masses

We are looking for ways to improve survey and detection methods

Sacramento Co – figure that GWSS present perhaps 3-5 years; appears to not have moved great deal,

Butte Co – infestation centered around Hwy 99; perhaps oleander in highway median strip may have played a role in movement (initial find was an adult in a detection trap placed in a Crepe Mertle tree)

Contra Costa Co – homeowner called in; saw a GWSS poster in a nursery and called to say they saw it; was confirmed to be GWSS; 25 properties found to be infested; 200 + properties were treated; appeared to have come in on new landscape plant material.

Oregon – have reported numerous finds from nursery stock shipment; no reports yet of establishment

Experience from Georgia – in the past considered that elevations above 1200 ft didn't show PD; recently have isolated PD from plantings at 2000 ft

Surveys done in Temecula show a circadian pattern to movement of GWSS; at certain times of the day, appears to be attracted to the yellow trap

Needs to be some work on behavior of the insect; response to traps

Has been some work on different types of traps; yellow appears to be better than clear trap; Hix has been testing night-time traps; can see much more movement during warm days; will fly to parts of trees with lighter green flush; at certain times of the year is found on lemons more than other citrus varieties; during warm parts of the summer, appear to be everywhere – very active and numbers very high; behavior appears different

Weak citrus trees showing chlorosis have much larger GWSS populations; they are definitely attracted to yellow (e.g., yellow rain slickers).

Yellow is very attractive to leafhoppers in general – will draw them out of vegetation; for a more accurate and consistent census – perhaps other methods are needed, have used plants coated with stickem in Brazil to obtain a less biased sample

Q: What is the variability among species of sharpshooters regarding attraction to yellow and different colors?

There are two general categories of leafhoppers:

Nitrogen chasers – leave plants with low nitrogen levels to look for plants with higher levels; nitrogen level in plant is a good predictor of suitability

Nitrogen compensators – sedentary species; nitrogen less important; wait on plant until they get a xylem flush

This pattern has been noted in the past

Above could help us understand movement of GWSS, host plant choices

Q: Is there a good correlation between taxonomy and behavior of leafhoppers (per above)?

No – are examples of species closely related that show the 2 behaviors

Q: What is the best guess on when PD is transmitted in California?

April and May are still thought to be the key months; but with GWSS, it may be throughout the summer because of its ability to feed on larger size vines

With old vectors, could pull out vines 2-3 years after infection; showed "simple-interest" increase in number of infected vines

In Temecula, appears to be more of an exponential increase in diseased vines; is probably vine to vine movement of disease; as with citrus variegated chlorosis, get "compound-interest" increase in disease

In some varieties of grapes, there is tolerance to PD – complicates the pattern

So with PD / GWSS, may get more movement of bacterium later in summer after *Xylella* levels have built up in the host; big difference with GWSS is that it can infect older leaf tissue; *Xylella* is not pruned out

Q: Has anyone checked the hydraulic conductivity of the tissue when one sees Xylella in the plant?

Have done studies with inoculated plants – correlation of symptoms with movement of water; leaves with symptoms – can't move much water through; leaves back 3-5 nodes which will show expression in a week or two – are also not very conductive

Q: How does the bacteria move back down the vine?

There are certain times of the day when there may not be a great deal of pressure from the roots outward

Q: What is the fate of pulled vines?

They usually are left in a heap for a while; burned because of wood boring beetles.

Q: Do we know if GWSS feeds on pulled vines?

No – they'll move to healthy vines.

Q: What is the situation as far as inoculation and recovery of the PD *Xylella* strain in citrus?

Have isolated PD strains of *Xylella* from citrus in Florida – it can survive in citrus and one can recover it 6-8 weeks later. In Florida, it appears that the levels of *Xylella* is so low in citrus, it is unlikely that it is a major host of *Xylella*; may be different in California because of the very large numbers of GWSS found on citrus (citrus doesn't appear to be a major host plant in Florida for some reason)

Purcell has tried putting PD strain into citrus on 3 occasions – can't recover it

Above may be a difference between different PD strains – can't generalize with different PD strains; also differences in age of wood (does better in older wood)

Q: What is the situation regarding movement of the bacterium back down the vine?

Hartung calculated that the rate of movement just by bacterial multiplication is similar to what is observed – perhaps this is why pruning works

Oak leaf scorch – followed recovery on a monthly basis; over 2 years, get a cycle of recovery similar to air temperature (even though root temperature may not go too low)

Citrus to coffee transmission ok; coffee to citrus transmission occurs but with more difficulty; at present are calling these two similar strains – may change in the future

BRAINSTORM LIST OF CRITICAL RESEARCHABLE QUESTIONS

Process Used:

1. Participants suggested critical researchable questions and the recorder tried to capture these as a short "sound byte"

2. As a group, we went back over the list of questions, clarified/modified the sound byte, numbered them, and selected the most important ones for movement to a short list (underlined means they were moved to the short list)

3. A subcommittee was appointed to review all items on the short list, revise it, and assign each of the topics on the long list as applying to key topics on the short list

SHORT LIST -- OVERALL RESEARCHABLE QUESTIONS (AS REVISED BY THE SUBCOMMITTEE):

1. What can be done to prevent hosts from becoming diseased due to *Xylella* (infection can be very different from disease expression)?
    a. Prevent infection (overlaps with #3)
    b. Prevent disease expression in infected plants

2. Research what makes plants attractive to GWSS; what are the dynamics of movement of GWSS among various host plants; how does this relate to pathogen transmission (where does it acquire the pathogen)?

3. Can epidemiology and empirical methods be used to understand pathogen transmission and help with control strategies?

4. Need to better understand the fundamental mechanisms involved in GWSS feeding biology (overlaps with #5) and transmission of the pathogen (#4); determine how this can help with control strategies; determine how this affect infection and disease

5. Better understand basic bacterial physiology in the plant and in the insect. How does it *Xylella* grow? How does it move? How does it acquire nutrients (e.g., carbon)?

6. Improve detection methods and develop standards for differentiating *Xylella* strains. Do this in both plants and insects.

7. Develop control mechanisms for *Xylella* (use viruses, genomics, by engineering cross protecting strains, host plant resistance, other methods)

8. Develop ways of rearing GWSS in the lab (overlaps with other sessions) for continuous supply of all life stages necessary for many projects

9. What sort of disease mapping data should we gather, who is going to manage this, and how might it affect management?

LONG LIST OF RESEARCHABLE QUESTIONS (AFTER REVISION; UNDERLINED ITEMS WERE CONSIDERED KEY ISSUES; THE NUMBER AFTER EACH REFERS TO WHICH ITEM(S) ON THE SHORT LIST IT RELATES TO):

1. Needs to be some work done on the behavior of GWSS; response to traps; try to develop more effective traps; tie this research into what makes various plants attractive to GWSS -- 2

2. Can epidemiology and disease transmission studies (also modeling / empirical information about disease transmission) help us better time control strategies of various sorts? Try to catch the disease before it reaches the exponential growth phase; Better understand what happens to inoculations during the winter -- 3

3. Can we understand the importance of other host plants of *Xylella* (or of leafhoppers) -- 3, 6

4. How long does GWSS need to feed on infected tissue in order to be able to transmit; need to better understand basic biology of the Proconiini Tribe – time to transmit, mechanism of transmission and acquisition; how easily recognize these in the field, better diagnostic tools -- 4

5. How might we disrupt feeding and transmission -- 4

4+5 = better understand the mechanism of feeding biology and *Xylella* transmission, and how to disrupt them

6. What are the mechanisms that affect bacterial spread in the vascular system, what are the mechanisms that underly this – is it pulled through the vascular cells; does the insect affect movement; what is the key to spread within the vascular system (how does it get from cell to cell); relationship of infection to disease -- 5

7. Are there differences between different host plants – might need some examination microscopically -- 5, 7

8. How is the bacterium able to grow when it is so carbon limited? Do they start to self provision; How do they access the bioavailable carbon? -- 5

9. Basic need to study the microorganism – its position in the plant, in the insect; its basic physiology -- 5

10. How does the bacteria spread; what limits its spread within the vascular system? Does GWSS affect this? -- 5

11. Is GWSS partially a phloem feeder? Does this have any consequences regarding it potentially being a phytoplasm vector? (work has already been done – statement that it is an obligate xylophage) -- 4

12. Once GWSS acquires the bacterium, what is the process that affects transmission to other plants? -- 4

13. Can we relate GWSS populations to the likelihood of disease transmission? -- 2, 3

14. What percentage of GWSS carry different strains of *Xylella*; do individual GWSS carry multiple strains? -- 3,6

Need rapid diagnostic tests to tell *Xylella* strains apart both in the plant and in the sharpshooter -- 6

15. What sort of disease mapping data should we gather, who is going to manage this, and how might it affect management? -- 9

16. Does the bacterium use the cell wall of the plant for carbon acquisition? -- 5

17. Presence or abundance of viruses (phages) that affect *Xylella*; are they involved in genetic modification of the bacterium? -- 7

18. Possibility of engineering a cross protecting strain of *Xylella* -- 7

19. Is the *Xylella* in the xylem of the plant multiplying in functional or dysfunctional vessels; are GWSS able to pick up more from one or the other? -- 4, 5

20. What are the factors that underly metapopulation dynamics of GWSS? -- 2

21. What are the factors that are responsible for various levels of tolerance in different host plants (e.g., different grape varieties) – study host plant resistance -- 7

22. Population size of the bacterium – how does it affect the likelihood of spread? -- 3

23. Are there any systemic pesticides that might affect both GWSS and *Xylella*? -- 7

24. Timing of fertilization, pruning, and pesticide applications – to affect GWSS and pathogen transmission -- 2,4

25. Understand the impact of water stress, temperature, and fertilization on colonization and infection of CVC or PD – tie these into chemistry and physiology of *Xylella* / the plant -- 3

26. Need to look at the situation on a broader plant community scale – is there a complex of plants in some areas which favor disease spread -- 2

27. Need for better integration of approaches to dealing with reducing PD spread -- 7

28. How does the insect distribute its time moving and feeding on various host plants – how does this affect transmission of *Xylella?* -- 4

29. Better understand how infection results in disease -- 1

30. Real need to develop a good method for rearing GWSS in the lab – would help provide more reliable and consistent (age, not infected vs infected) insects for various studies; will probably need a wide range of host plants; will probably need to vary plants over the year; need to break GWSS diapause -- 8

31. Need to define environmental conditions which result in GWSS producing eggs throughout the year -- 8

32. Chemicals used to kill the bacterium – may not be accepted by the public due to the concern over bacterial resistance development -- 7

33. Need to screen GWSS pesticides we have for impact on the bacterium -- 7

34. Mating system of GWSS – are males or females more involved in disease transmission? -- 2, 4

35. Need to understand acoustical behavior of GWSS (as used during mating) -- 2

36. Need to understand mating behavior; use of sound in mate attraction; possible use in monitoring (males often very mobile; females may mate only once) – potential for novel control strategies (do males feed much less); need to understand this system better -- 2

37. More information needed on host attraction; olfactory or visual cues key? -- 2

Breakout Session 2 - Molecular Genetic and Genomic Approaches to Understanding and Controlling Pierce's Disease of Grapes

Session Chair - Douglas Cook
Facilitators - Linda Manton and Jim Brenner
Recorder - Michael Reid

Participants: Louis Aung, Barbara Baker, Kendra Baumgartner, Dovraina Bock, George Bruening, James Campbell, Chung-Jan Chang, Edwin Civerolo, Doug Cook, Donald Cooksey, Korsi Dumenyo, Helene Feil, William Feil, Ken Frankel, William Gensler, David Gilchrist, Deborah Golino, Noel Keen, Bruce Kirkpatrick, Joao Paulo Kitajima, Alan Krivanek, Jan Leach, Jiang Lee, Wenbin Li, Jim Lincoln, Steven Lindow, Joyce Loper, Carole Meredith, Martin Mochizuki, Patricia Monteiro, Heather Patterson, Gregory Pogue, Paul Predici, David Ramming, Ana Claudia Rasera da Silva, Paul Richardson, Adib Rowhani, Frederick Ryan, Peter Salm, Stephen Smith, Stephanie Stilwagen, Kristin Summerfelt, Alan Tenscher, ChrisTown, Diane Ullman, Marie-Anne Van Sluys, M. Andrew Walker, Barney Ward

I. Questions Of Speakers, And Answers

Genetic Engineering Approaches (Dave Gilchrist)

Is there any danger in using 35S as the promoter?
Yes, can use controllable genes if that becomes critical (35 S negative). Genomic libraries used in the first place - had hoped to identify promoters. Rely on multiple insertions - 18/500,000 worked. Options are open and time frame is short so you can change systems rapidly.

Roots that die from the explants, do you detect bacteria?
Haven't tried that yet.

How long does the process take?
Weeks. Initiation of the root through to death.

What strain are you using?
A broad host range ATCC15-834.

Does it carry the second tDNA?
Comment. Uually *Agrobacterium* rhizogenes has two tDNAs one of which contains the auxin synthesis genes. It would be interesting to know because it might influence results on resistance, maybe...

Breeding (Andy Walker)

Any evidence for vector resistance?
Haven't looked for vector resistance.

Not much evidence for insect resistance in Florida?
There are some feeding preferences among different cultivars of *vinifera* and muscadine - end result is that you get infection - don't need that many insects.

How great are the chromosomal differences among the species and varieties?
Breeding is free within *Vitis*, not so easy with *Muscadinia*, different chromosome numbers. Very distinctive. *Rupestris* x *Rotundifolia* is fertile 38 chromosomes, F1 strange segregation, F2 segregates normally.

In the injection infection... can it be done mechanically?
Have done studies trying to perfect the technique. Syringe and graft inoculations both work, but difficult to do it quickly. Pin-prick has higher efficiency.

Does it have to be 'intravenous'?
Best inoculations occur under rapid growth conditions, drop is almost instantaneously absorbed into the stem.

Resistance largely quantitative - could it be something to do with structural features of the xylem?
Certainly I think that a number of genes will be involved. Movement in the xylem is important. Definition of resistance is important and there are many.

Resistance in *Muscadinia* is no movement, no expression. What is the nature of the resistance in Mortensen's cultivars?
Range, some do move but don't express. So movement does occur in some *rotundifolia*. Downward movement is the more important component.

With the majority of the bunch grape types that he developed - is there downward movement?
Yes in some, and we need to look at all them more closely? Less movement, and no response to the bacterium.

Are there any resistant rootstocks?
We haven't found any - the Olmo rootstocks are *Vits* x *rotundifolia*, but are not resistant.

Discoloration of the cane is a phloem characteristic and I'm surprised that you use that?
Well we use presence of the bacteria as well. The cane symptoms mimic what we see on the leaves. I'd like to hear why that occurs.

Have you observed tyloses in the resistant cultivars infected by *Xylella*?
Haven't looked, but we want to - could be a simple process.

Genomics (Brazilian Group)

What's your expected time frame?
Depends on the project - 1 year to four years, we have 21 projects. Bottleneck is the establishment of a protocol for genetic manipulation of *Xylella*. Proteomics and microarray probably will be on time in 1 to 2 years. Microarray will be ready for use next year.

Could you say a few words as to how the project was planned - stunningly ambitious - who set the objectives?
It is not a project, it's a program - there was a call for projects and there was a subject list seeking applications for the program. We had 50 project proposals for the first call - approved 10. For the second call, 30 proposals, approved 11. A committee chooses the projects. People started to work independently, and we then decided we needed to network to help improve the problem solving. It's a functional genomic program, with 21 projects.

Is it possible for you to share the contents of XDM (the medium) with those that are interested?
You'll have to contact the laboratory - she will provide the e-mail, not yet published.

Brazil went through a *Xylella* episode earlier than California. What's your feeling - are you winning the battle? What strategies are best?
I'm not sure - we still have a lot of Xylella, and then we have citrus canker which is a bigger problem. Depends on the geographic area.

What are the *Xanthomonas* strains that you're sequencing?
When will the sequence of the PD *Xylella* (grapevine strain) be finished?
By January next year first draft, together with the analysis of the strains done by JGIL.

Composition of the xylem sap... can you tell us more on what you're doing?
There is a huge interest in knowing the composition - very small amounts of fluid, very hard to extract, plant physiologists working on that.

Guttation streams will be rich in organic acids and sugars - are they sorbitol or something else?
What we see is that *Xylella* is not capable of using polysaccharides - can use glucose, glycerol. Lots of amino acid receptors in the membrane - could be alternative sources. Xylem is acidic, therefore composition is relevant (common). Found a lot of ion receptors in the membranes.

Are cytokinins involved?
Have looked for relevant genes in the genome - haven't seen any.
Comment - inorganic salts, amino acids, Krebs cycle intermediates - good for PD strain, not for others.

Genomics (Doug Cook)

Why not a full-on genomics project?
A deep sequencing project would get you the majority of the genes. In house or subcontracting for ESTs. If you sequence the genome of grapes - $50 million to 125 million. Tools required much more difficult than an EST profile. Better to do the EST's because you go directly to functional genomics. You do need both approaches, but the longer you delay on a full-blown sequencing project, the more you lose in time and money. All the agronomic traits are buried in there. Could involve a consortium, French, Australians.

If you had the money, which genome would you sequence?
I don't know - Cabernet Sauvignon.

Is there a short list of varieties?
Cabernet sauvignon would be good, and *rotundifolia*? Unlikely to get more than one. NSF not interested in another plant genome. DOE doesn't have the resources in its 'plant' budget. Needs political will. May be easier for grapes because of the political welling point.

Do you know about the repetitive sequences in the genome?
I don't know for grapes. Citrus is not too far away from grapes, but they're nothing like the other taxa.

Genomics (Paul Predki)

Finished and unfinished genomes. If you fail to find a sequence vs. citrus to what extent can you be sure that it's really missing?
It's context dependent. When you don't see a gene in the draft genome, you look for neighbors. If they're missing it's more likely that a whole region is deleted.

The collaborators will do the finishing?
We don't know exactly how it will play out. We'll take it to a high draft level (where we're at now), and then we'll ask the communities to decide whether it's worth finishing them. Looking for ways to finish them.

What you've done is admirable - is the community going to do it?
The 64 Kb region that's missing - could those be the genes involved in pathogenicity. Can provide a list later. Haven't spent time on the biology - now is the time to ask 'what genes'.

40% of the orf's in *Xylella* have no assignable function - is that general to other bacteria?
Yes.

With the gross differences you see - it's dangerous to assume those are the reasons for the host range differences and other phenotypic differences. Comparative biology is critical. What are your plans?
We don't have specific plans - we're geared up to become a machine for hgp. Now moving to postgenomic. Developing functional genomic technologies. Sorting out what we're going to do. Huge amount of information about a few genotypes. Need to know that the things that we think are important features are truly important.

Have you noticed differences in the number of virus-related genomes?
They share a lot of homology - haven't looked in all that much detail.

Frame shifting. How much information do you lose from frame shifting?
Took the subset of genes for which we have a match. BLAST will cut out a few nucleotides here and there. Looked at the subset of our genes for which we considered them finished quality and when we do the comparisons, there were just under 10% of the genes frame-shifted with respect to each other. Therefore either non-functional or not genes in one organism or the other and many of them are the 'hypothetical' genes.

Genomics (Jan Leach)

To what extent does *X. oryzae* cause a xylem problem?
Silica holds the rice up, but purportedly it is analogous.

Is there any evidence that there are diferences in secondary walls?
Resistance is not created equal in rice. Phenotypes are different – no one's looked at xylem structure. Some Japanese work has looked at hydathode structure relationships. Secondary wall structure is changed in response to infection in the resistant lines.

Differential display showed some genes expressed only in the resistant strain. Have you seen any genes only expressed in the susceptible strain?
We're doing that subtraction now - that's a good question.

Besides peroxidase what have you seen?
Yes, many PR genes, pectinase, PLD one or two in the secondary walls.

Is the peroxidase accumulating more in the wall of the resistant rice?
Definitely. Purely hypothetically, we suppose that it could be the thickness of the wall, last step in lignin deposition?

Gap between entrance into the pore and into the xylem - how does it occur?
The epithin has large spaces... the tracheid finishes at that space - they move through the spaces as the guttation droplet is pulled back in, in the flooded tissue of the epithin.

The pore is a modified structure of the stomates isn't it?
It looks like one, but it's smaller, and it's open, and the hydathode is the whole structure. The outer cells are dead.

Is the movement external to the membrane, or do the bacteria penetrate the cell?
We've never seen it, in all the microscopy we've done.

How much damage does *X. oryzae* cause to rice?
In seedling infection in India - devastating. In the adult phase, up to 30% loss. Can be controlled by R genes in some parts of the world.

Presence or absence of motility genes? Comment that there are no motility genes in the genome of *Xylella fastidiosa*.
I don't know about that. You can see spread in the plants, quite effectively.

Genes coding for the type III harp system - are they clustered?
Yes, Frank White has analyzed. 20 - 30 Kb. Systems are very conserved.
Comment - no avr genes or harp-type systems (in Xf?).

II. Brainstorm on High Priority Research Questions and Approaches

Overall Issues

- Should figure out the basic biology - is there really an interaction with host cells?
  Need to delimit the essential step in *Xylella* infection

  - Are there quorum-sensing activities in *Xylella*?
  - Host range of different *Xylella* strains
- What about natural reservoir plants?
  - Is the bacterium detrimental to the insect or just 'hitching a ride'?
  - Are biofilms involved?
- Need a good model system for *Xylella*/Plant interactions
  - Tobacco, Tomato, *Medicago*, *Arabidopsis*, *Catharanthus*
  - Define optimal conditions for rapid expression in grape?
  - Characterize other hosts with amenable properties
  - Should be highly tractable Tobacco, Tomato, *Medicago*, Rice
- Need a surrogate for *Xylella* that give some of the symptoms
  - Mimic some aspect of the disease that's not *Xylella*
  - Chemical or microbial

<u>The *Xylella* Genome</u>

- Compare different microbial pathovars
  - Sequence based?
  - Microarray-based,
  - Informatics based
- Look at plasmid differences between the strains
- Use heterologous systems to test bacterial gene function and test for responses by the plant

<u>Grape genomics</u>

- Parallel measurements of gene expression in plant, microorganism, and insect
  - microarrays
  - CDNA AFLP
- Study ecology and population biology related to the insect and the bacterium using genomics

<u>Proteomics</u>

- Characterizing proteins of the insect/plant/bacterium and their interactions
- Unusual or unique proteins expressed in *Xylella*

<u>Breeding approaches</u>

- Integration of quantitative genetics with molecular biology
- QTL analysis of the plant response

<u>Resistance genes</u>

- What could be considered a virulence gene? How do you identify?
  - No homology
- Search for resistance genes in heterologous systems

<u>Biotechnology approaches</u>

- Evaluate candidate defense response genes, antagonistic proteins
- Search for xylem-targeting mechanisms
- Disrupt pathogen or insect life-cycle
  - Characterize toxins that might be expressed in *Xylella* to control the vector
  - Disruption of pathogen behavior by disrupting quorum sensing
  - EPS genes, fimbriae, toxins
  - Biochemistry/metabolomics
- Sap composition - organic, inorganic, and other components
  - Seasonal differences
- Macromolecules and other metabolites of any kind produced by *Xylella*
- Look at the components of the host response
- Do different strains grow differently, do they use different metabolic pathways?
- Need basic microbial physiology, mutants, pressure response genes, temperature, oxygen levels, susceptibility signalling, nutrient uptake, membrane transport mechanics
  - What are the bioenergetics of such a dilute system?
- What are the differences between resistant and sensitive species
  - Composition of sap, structure of xylem, etc.

Gum, tyloses, and other plant responses

III. Necessary Enabling Technologies (For Genomics Research on This System)

- Need a good model system for *Xylella*/Plant interactions
- Need a surrogate for *Xylella* that give some of the symptoms

Xylella Genomics

- Need comparative tools
- Need efficient transformation systems
- Find heterologous systems to test bacterial gene function and test for responses by the plant

Grape genomics

- Need efficient transformation systems
- Transcriptional profiling
- EST libraries
- Whole genome sequencing
- Parallel informatics
- Build a physical map of grape
- Deep BAC libraries
- Organize a unigene set that can be shared

Proteomics

- Characterizing the protein components of the insect/plant/bacterium and their interactions

Breeding approaches

- Integration of quantitative genetics with molecular biology
- QTLs
- Need to organize and characterize the wide range of available germplasm
- Microsatellites (could be in genomics too)
- Protoplast fusion

Biochemistry/metabolomics

- Metabolic profile of the host environment
- Metabolic profile of the bacterium

Informatics

- Need a central repository and central website to access information
- Community driven – need a steering committee of biologists to coordinate

Breakout Session #3 -- Ecology of Sharpshooters: Prospects and Limitations of Biological Control and Chemical Control of Sharpshooters

Session Chair – Robert F. Luck
Facilitators – Susan Laughlin and Jim Brenner
Recorder – Joseph Morse

Participants: David Akey, Ted Batkin, Kendra Baumgartner, Jim Brenner, Harold Browning, Kent Daane, Robert Denno, Greg Dwyer, Curtis Engle,J uliana Garcia, Raymond Hix, Mark Hoddle, Susan Laughlin, Leland "Mac"Learned, Roger A. Leopold, Robert Luck, Philip Manson, Steve Matthiasson, Russell Mizell, Joseph Morse, Len Nunney, Clifford Ohmart, Keiko Okano, Dan Opgenorth, Charles Pickett, Aurelio Posadas, Rick Redak, Jim Rudig, Frank Sances, Allen Scoggan, Greg Simmons, Michael Sipiora, Daniel Smith, Robert Steffens, Richard Stouthammer, Nick Toscano, Helmut Van Emden, Lucia Varela, Gisela Wittenborn, Randall Wittie

I. QUESTIONS OF SPEAKERS (THEIR ANSWERS AND THOSE FROM THE FLOOR FOLLOW):

Q: Isn't a key issue the relationship between GWSS and disease incidence – our hope is to keep disease out of the vineyard; ties in with epidemiology studies

Perhaps classify we should vineyards as having L, M, and H levels

Q: What is the number of vectors required to maintain economic disease levels?

Is so low that that line of research may not be productive (on east coast, with intensive pesticide use, were not able to reduce phony peach disease levels)

Are now trying to figure out what is a productive short term strategy; looking at transmissionin the vineyard may be too late – need to keep well to the left on the exponential growth curve

Brazilian sticky card sharpshooter levels – can be quite low (0.5/ card/ several weeks) and still have very high CVC levels

Probably yellow sticky card levels not useful in accurately reflecting population levels or disease incidence

In Temecula, research is looking at treatments in citrus and trying to determine what the long term impact is

Perhaps thresholds will change depending on what material (pesticide) is used in vineyards

Besides controlling the vector in citrus, there may be many other sources of GWSS – riparian plants or urban plants

Q: In southern California, GWSS numbers appear to be attenuating somewhat – is this adaptation by natural enemies or GWSS? Are these changes biologically significant with respect to disease incidence?

Perhaps not; probably is significant reduction in terms of GWSS numbers

Parasitoid levels appear to be increasing some in last several years

Q: Imidacloprid and other neonicotinoids – do they result in a reduction in feeding below the level needed to transmit bacterium?

Statement made that there is a reduction in bacterium transmission by chemical control

Levels in California are quite higher than Florida and they can't grow grapes

N = # of sharpshooters is part of the epidemiology equation; but should also try to work on the other factors in the equation

Q: Should look at the acquisition side of the equation; Is it guaranteed that GWSS will pick up *Xylella* from infected tissues?

Literature abounds with models about controlling vectors; practical experience in the field is that it is much more difficult to reduce transmission than model predict

Q: Most of Hoddle's presentation dealt with egg parasitoids; are there many nymphal predators? Anything known about pathogens?

Have observed some spider and Reduviidae activity; history of fungal pathogens in California poor; have seen a solitary vespid wasp that seems to specialize on GWSS (detect by putting out pots of peet moss)

Q: Why is GWSS as rare as it is in Florida? – in some areas very difficult to find; what is keeping it low?

Q: Is interspecific competition a factor in GWSS' home range?

Doesn't appear to be

In Florida don't see much oviposition of GWSS on citrus

Q: Levels of egg parasitism in GWSS 2nd generation in California (85% or higher) – are we getting effective biological control?

Are still getting large GWSS numbers going through the winter

Problem is low parasitism in the first generation

In some species, need >97% mortality to reduce the population

Problem is perhaps that stages other than GWSS eggs are not seeing much predation or parasitism

High population levels of GWSS seeing in California versus low levels in native range – is not an uncommon pattern within invasive species; perhaps a lack of natural predators and parasitoids

Interesting that first generation that has low parasitism is also the time of year from Purcell's data (but with blue-green sharpshooter) when pathogen transmission is most critical

Q: So what level do we need to reduce GWSS populations to?

We don't know

Q: Are there any birds preying on GWSS – are GWSS distasteful?

Q: Why does GWSS have such a predator avoidance response – implies it has evolved to avoid predators

Don't appear to be any nymphal parasites of GWSS

In some collections can get 100% egg parasitism; early in the year during the 1st generation, get quite low levels of parasitism – this is where the problem is

May be some problem in parasitoids tracking GWSS in time and space; unless we better understand movement of GWSS over time and hosts, may be difficult to figure out how effective the parasitoids are
Vespid – may be a very effective local predator but are often very nest site limited

Q: Pattern of egg masses availability over the year is bimodal; what do parasitoids switch to in early summer before the 2nd generation comes on – maybe the eggs are present on other hosts?

Q: Are eggs aggregated spatially?

Widely distributed on citrus but mostly on new flush – this is only one host – may need to look at many other host plants

With dissections – research is looking at maturity of eggs in GWSS females; did find a fresh egg mass last week – perhaps a few eggs present during much of the year

Key point – are a lot of other host plants we are not looking at; we appear to missing part of the puzzle

By focusing so much on citrus – may be getting a misleading picture (a lot of what we are seeing may be leafhoppers moving through the citrus)

Appears to be a drop in GWSS levels in Temecula; 1999 – 15/trap/wk in grapes; this year down to 1.5 – is in areas not near treated groves

Q: Due mostly to parasitism? Insecticide applications?

Have hot spots – placement of traps appears critical

Q: Once GWSS becomes established, is there much that can be done?

Tulare county -- In eradication mode, was probably too late in terms of size of population
Kern County is not in eradication mode; it was too large at detection.

Initially tried to eradicate GWSS over 25 or so square miles; some people didn't want them on their property; couldn't use aerial treatments; basically used lightweight insecticide on limited scale

Present plan is to set up a barrier program; treat more aggressively once gets into agricultural areas

Eventually will need to know the relationship between GWSS levels and disease incidence

Get phone calls – why spraying sharpshooters when don't know what economic level is

Program in Temecula based on pesticide efficacy data had – used best guess on effective materials with least impact on the environment; Only sprayed several hundred acres with chlorpyrifos; Don't really know what numbers GWSS must be reduced to

CDFA would have liked to eradicate GWSS in Tulare Co. if possible but the ultimate objective was to limit its spread; wasn't initially clear how large a population we were dealing with; Did the best job they could with treatments available

Q: Eradication difficult where homeowners may not agree to participate; citrus growers are invested in IPM – are there selective or biorational pesticides available for use even if they have lower efficacy?

Beauveria – if used in oil formulation, is effective even in dry climates; Advantage is that with most xylem feeds they are on the terminals, more exposed

Possible solution to lab rearing problem – provide a host buffet (perhaps deal with by growing plants hydroponically; perhaps with sodium lamps for long day lengths)

Q: Does this tie in with what is going on in the field?

Field studies in Florida have shown that GWSS adults move tremendously

II. BRAINSTORM LIST OF CRITICAL RESEARCHABLE QUESTIONS

Process Used:

4. Participants suggested critical researchable questions and the recorder tried to capture these as a short "sound byte"

5. As a group, we went back over the list of questions, and placed various questions under sub-category headings

6. We did not prioritize the list

II.A. GENERAL RESEARCH QUESTIONS:

1. What is the probability that GWSS will pick up *Xylella* from infected plants? How does this vary among different plants and with different *Xylella* strains?

2. Can the pathogen be displaced by other *Xylella* strains within the sharpshooter?

3. Use of cross protection – can we inoculate with an innocuous strain?

4. Need an efficient way of detecting which type of *Xylella* we are looking at

5. Need some economic / political studies on separating impacts on citrus from impacts on grapes. Can we perhaps avoid problems by not growing grapes near citrus?

6. A mapping of the extent of *Xylella* throughout California in grapes is needed – in commercial vineyards and almonds (and other crops and flora). Would help with epidemiology studies and show the public how important this problem is.

7. Is PD being spread by infected plant material or propagation?

8. Need to determine what the target level of GWSS suppression is to: a. reduce disease incidence, b. to free up restrictions on nursery shipments

9. Need to know more about the genetics of GWSS – is it just one population or a mix of several?

10. Extend Purcell approach – add impact of spatial structure to the disease triangle

II.B. GWSS GENERAL ECOLOGY QUESITONS:

1. Study dispersal ecology of GWSS – look at movement between various host plants, attraction to various plants, use pesticide or other applications. What are the consequences to GWSS if it is forced to feed on suboptimal plants – impact on fitness, mating success, etc. Use a broad scale experimental approach to manipulate regional GWSS levels. Look a food web structure impacts – fertilization of plants may uncouple the system and provide escape from natural enemies.

2. What is the basic ecology of disease spread – how it is affected by GWSS density, time budget for GWSS as affected by temperature and other factors, variability in host plant susceptibility, spatial patchiness of the insect – its distribution

3. Shouldn't ignore the fact that there is co-evolution between *Xylella* and GWSS

II.C. BIOLOGICAL CONTROL:

1. Until we can mass rear GWSS, we are limited in what we can do

2. Augmentation of parasitoids and predators should be studied

3. Why do trichogrammatids not prefer some host plants (e.g., lemons)?

4. Is impact on native sharpshooters a problem in bringing in new natural enemies?

5. Need studies on entomopathogens; strains of pathogens developed specifically for GWSS (need to clearly identify agent being used; needs to be standardized as to potency, formulation, etc.)

6. Impact of pesticides on GWSS parasitoids; importance of plant nectaries

7. Prospect for nymphal and adult parasitoids, predators, and pathogens

II.D. CHEMICAL CONTROL:

1. Use of fungi in oil as a biopesticide – can be quite effective

2. Also look at other biorationals

3. Look broadly at impact of pesticides used for GWSS control on non-target organisms and humans. Impacts of pesticides on animals that are dependent on GWSS – spiders, egg parasitoids, etc.

4. Look at difference between knockdown and repellency – are we causing increased dispersion with some pesticides; what other chemicals might be repellant?

5. What does imidacloprid do as far as stopping feeding by GWSS; also look at effect on *Xylella* transmission; potential for pesticide hormolygosis

6. Use theoretical approach regarding the 4 parameters in disease transmission – study with field experiments

II.E. CULTURAL CONTROLS:

1. Evaluate various host plants for use in monitoring, as possible trap crops

2. Perhaps manipulate host plant status to make a super trap crop; perhaps develop a suicide host

3. Evaluate the combined push / pull strategy – use both attractive and non-attractive trap crops

4. Look at cover crops being used in grapes or other commodities and impact on GWSS populations; are some of these good reservoirs for *Xylella* or good hosts for GWSS?

5. Impact of vineyard management practices on the manifestation of disease: monoculture, irrigation, shutting off irrigation, non-contour planting on eroded soils, impact of chemical fertilizers on compromising the immune system of the grapevine

II.F. GWSS PHYSIOLOGY

1. Studies on feeding behavior; (e.g., acquisition does not equal infectivity)

2. Studies on mating and reproductive behavior; impact of interactions between host plants and *Xylella*

3. Is there a time when GWSS ovaries are nonfunctional?

4. Study endosymbionts in GWSS

5. Is there a cost to GWSS in carrying *Xylella*?

6. Is there any resistance of GWSS individuals with respect to carrying *Xylella*?

II.G. INSECT REARING

1. Need to be able to mass-rear GWSS for many purposes – physiological studies, biocontrol, pesticide, other studies

II.H. NEED FOR COLLABORATIVE STUDIES:

1. Need to assemble all published work on PD/GWSS, etc. – perhaps develop a web site database

2. Statewide Coordinator has been appointed by the Governor (Robert Wynn) – will provide coordination, clearinghouse – one idea to put together is an annual research meeting

3. Progress in mass rearing may require a large collaborative effort; look at lab adaptation of mass reared insects

4. Need to make sure we don't leave important participants out of future meetings; list of all participants, addresses, email addresses, area of expertise, what projects working on in relation to *Xylella* diseases / sharpshooters, etc., 5-10 most relevant publications (based on what has been developed for whitefly research)

5. Need to broaden the funding base for research on PD/GWSS

6. Need to add high powered mathematicians for future modeling efforts

Breakout Session 4 - Xylem Physiology and relationship to Pierce's Disease (WaterStress): Mechanisms of Pathogenesis in Xylem Diseases

Session Chair - Michael Reid
Facilitators - Linda Manton and Jim Brenner
Recorder - Michael Reid

Participants: Peter Andersen, Louis Aung, Kerry Britton, Robert Denno, William Gensler, Andrew Groover, Raymond Hix, John Labavitch, Carol Lauzon,Jiang Lee, Wenbin Li,Andrew McElrone, Carole Meredith, Russell Mizell, Edward Norberg, Rick Redak, Thomas Rost, Ken Shackel, Melvin Tyree, Robert Wample, Ed Weber

I. Questions Of Speakers, And Answers

Xylem Structure And Function

When you look at the ways it moves - how does it go up and down?
Very difficult question. It seems to me that as soon as a bacteria hits a pit membrane, some go up and down instantly - a sequence of those would result in movement through the plant in short order.

Freeze stems in liquid nitrogen and see the frequency of seeing *Xylella* with ice or with air - certainly cavitation could get them a long way very quickly. University of Ottawa has a cryoscanning EM system - all done frozen –100°C. Petiole freezing would be best because it's a small tissue.

Problem with the system is that the new vector feeds lower down. Not at the leaf - stem cordon and trunk. Grape vines often refill their vessels - water goes down in the winter and back up in the spring.

Observation: Harder to get bacteria from the petioles than from the stems. Controversy about that.

If the bacteria is embolized downward, how will it pass the pit membrane?
Would have to dissolve the membrane to pass.

Refilling the cavitated vessels - does the membrane become damaged and then repaired?
In Vermont the vessels become air filled - sublimation - but the pit membranes are ok. Root pressure pushes the water column up. Bubbles are dissolved. We presume that you can't repair the pit membranes.

Water Stress And PD

What's in the salivary exudate - lipoproteins, other proteins? How does that relate to the sheath material? Priming of the site - could it institute plant responses?
Chemical mediation of inoculation and transmission and host plant responses.

Xylem Chemistry

When you did the amino acid profile - did you look at PD vs. non-PD xylem chemistry?
Problem is getting at the right stage - you have to impose dramatic pressures. *Xylella fastidiosa* is highest in the small vessels of the leaf, desiccation at the leaf and petiole. Challenge is to get it at the genesis of infection - certain vessels are infected and others are not. If we could sample individual vessels, using a time-course that would be useful.

Comment - You obviously have a biofilm in there - chemotactic responses. In a biofilm there will be attachment and detachment signals. Physical movement is one area, and chemical mediation of movement. Become planktonic and then stick somewhere else.

Low carbohydrate concentration - is that not important?
No, it is very important from the insect perspective - will use it as an energy source like anything else.

Can you grow *Xylella fastidiosa* without a carbohydrate source?

Dogma is that *Xylella fastidiosa* will perish with high carbohydrate concentration, but one speaker said their medium had sugar as their major energy source - controversy. Standard media for many different pathovars all have very low sugar. In planta the bacterium needs as an energy source - glucose? Organic acids? But the genome sequence of CVC doesn't have the enzymes for gluconeogenesis which means that the glucose will be important for the bacterium in planta.

Have you compared sensitive and susceptible plants?
The insect clearly responds to xylem fluid chemistry, but it's hard to know about the bacterium. Inducible and constitutive functions in the xylem may be key.

Clarification - you can kill the bacterium with sugars?
Can't remember off hand.

Wouldn't it be interesting if you could kill the bacterium by persuading the grape to send a pulse of sugar from starch hydrolysis through the xylem?
Comment - *Xylella* grows quickly in young shoots, and not in older wood.

Why?
Lots of possibilities - chemical, anatomical, hard to speculate.
Preferred feeding site...aphids probe and pull out, probe and pull out to find the phloem.

Does GWSS select the site ahead, or does it also probe?
Insects probe inversely proportional to the quality of the host. Has to sample the substrate in order to do that. When you look at the salivary sheath, unlike aphids, where you see 'avoidance', glassywing and the rest of the group goes straight through.

Is there a depth they don't go beyond?
Mouthparts are 2mm or longer - feed on 3-5 year old oak stems. Distance to reach the functional xylem at least 2 mm.

Young tissue - primary xylem or secondary xylem?
Bacteria can go up 3 cm per day in the young shoots. Can't get any symptoms on plants without young shoots.

Has anyone looked for other bacteria inhabiting the xylem in any of your studies?
Bruce Kirkpatrick and Sandy Purcell are considering other endophytes, and Don Hopkins at UFL interested in cross protection possibilities. The endophyte situation could be significant.
Clarification - looking for survivors in Napa vineyards for endophytes that are antagonistic.

Is the *Xylella fastidiosa* in GWSS the same strain as *Xylella fastidiosa* in the blue-green sharpshooter?
I guess that there are many strains - simultaneously in one insect? - don't have the primers to differentiate the different strains. If a single insect harbored multiple strains - might their be competition between the strains? Genome has chitinase, there has to be attachment of the bacterium to the foregut in fluid moving at 1 m/sec. Proteins and chitin interaction; foregut is lined with chitin.

Fluid dynamics - look at microorganisms growing on rocks near waterfalls, etc. Could be some similarities.
Good point - we have to start thinking outside the box.

Comments about insertion of the mouthparts and cavitation events. When it probes does it get into the xylem and then get out again?
I'd be careful about assuming that there is cavitation when the insects feed - PVC/gasket/condom imagery for sealing the vessel tight. Payoff for the insect evolutionarily is to avoid the defensive compounds that are extraxyllary.

Occlusion And Bacterial Products

Elaborate with the cut flower systems - to what extent is water stress dependent on bacterial cells themselves?
Used to use india ink; inject a small amount of it... small enough to go through the xylem and then plug up the pits.

Is much known about the localization of infection - why do some elements have *Xylella* and adjacent ones not?
Something might be produced by the bacteria that protect them (like a boil) Staph produce fibrin to protect themselves. Bacteria will produce catalase. Resistance in plants is a plant response walling off the bacterium. With Pierce's disease it's a matter of high bacterial concentration. In muscadine grapes you have more gums. That's an outcome.

Couple of questions - when Mark Matthews spoke - do we have a good sense of the timing of development of the disease?
Whole vascular system is exposed in cut flowers - a massive insult or introduction of microbes.
Impression with *Xylella* is that you get discrete inoculations with the sharpshooter then something happens over a more extensive period of time.

Do you have a sense of how the events seen in flowers and attributions of blockage might relate to the longer term development? How hard do they have to suck, and what is the tension in the xylem?
You're not measuring the tension in the xylem but the water potential of the whole leaf. Only way to get the potential in the stem, you need to cover it and let it equilibrate. Lower tension values then are found.
For a *Prunus* species under high VPD. Exposed transpiring leaf - 15-18 bar. Adjacent non-transpiring leaf - 9 bar. Mid-day values in Florida of 10 bar. No irrigation for a month - 15-18. What are you measuring in the pressure chamber? That's the key thing. A factor of 2 is important.

Xylem Development

Do you think that the role of calcium could be mediated by bacterial growth?
Yes, one thing that I was curious about was what the developing xylem looks like in a diseased plant. Changing Ca levels and direct interactions with the cell walls - you can imagine that tracheary element differentiation could be perturbed leading to some of the disease symptoms.

Could e.g. serine proteases and calcium from *Xylella fastidiosa* be causing premature apoptosis in the xylem cells?
Yes you can imagine that.

Are there calmodulin-like proteins from the bacteria or the plant that may lead to the timing of cell death?
Not sure... at lower levels calcium influx is involved in cell wall synthesis.

Sugars could be playing a role also?
I was interested in the talk - changes in secondary wall structure seemed to be involved in resistance. Drought can change the ultimate structure of the xylem. I wonder if altering some cultural practice might have a beneficial effect with regards to increasing resistance to PD.
That's a possibility - the citrus group have had some success in delaying the development of symptoms with cultural practices in Brazil. Heavy rain followed by drought. Analogous with PD - symptoms are worst in September.

Could it be that drought application could result in resistant secondary walls?
Susceptibility of *Vitis vinifera* is so great that it's unlikely to make it.

Why does wild grape in the riparian zones, Vc, never die from PD? Could active oxygen be part of a defense mechanism?

Free radicals - strong levels of H202 in PD infected vines... maybe the plant can sometimes win. H202 could be a function of the diseased state of the plant. The levels in PD infested vines are enormous.
Potential involvement of free radicals and peroxide also indicates the complexity of the system. Secondary wall synthesis requires free radicals. Can imagine that the presence of an invader at the same time you're differentiating wall might complicate the problem.
Could explain the situation in Brazil. Comparison of wild and vineyard grapes - so much pruning in the vineyard - that the wild riparian grapes could be outgrowing the disease.
Comment on water stress talk... Webber saw classic PD symptoms due to physical damage of the stems (cut with a picking knife) where the shoot didn't wither - instead of typical water stress, basal leaf senescence, rapidity with which the stress develops may be important.

Is that systemic rather than sectoral stress?
Maybe there are normally root symptoms in a slow desiccation. Or could it be that some xylem vessels are damaged and not other. All water stress doesn't necessarily look alike.

II. Brainstorm on High Priority Research Questions and Approaches

Xylem Structure And Development

- Comparative anatomical analysis between resistant and susceptible grapes and other plants - structure, shape of pits, etc.
- Effect of *Xylella* on cambial activity
- What is the role of the live xylem cells?

Xylem Chemistry

- Xylem chemistry comparison between resistant and susceptible grapes and other plants
- Clarify the importance of carbohydrate to *Xylella fastidiosa* viability and multiplication
- Monitor changes in xylem chemistry over time with respect to bacterial population
- Response of the natural xylem fluid to water stress - does this affect insect preferences?
- Alter xylem chemistry to alter growth of bacteria and GWSS performance
- Soil drenches are being tested
- What are the effects of foliar fertilization on xylem chemistry?
- What is the role of the living cells on xylem chemistry?
- What is the impact of *Xylella fastidiosa* infection on carbohydrate storage in xylem parenchyma
- Are there plants where GWSS feeds but *Xylella* doesn't grow - e.g. Monocots?
- Why does *Xylella fastidiosa* move in some plants but not cause symptoms?
- Can we differentiate the effects of water stress and spread of the bacteria (high humidity vs. low humidity)
- Can changes in xylem chemistry affect pit membrane porosity?

Xylem Occlusion

- What occludes the xylem? - relative contributions of the bacteria and of bacterial and plant products
- Examine the timing - what is the progression (including cell by cell) throughout the plant?
- What is the effect of dead *Xylella fastidiosa*?

Bacteria/Biofilms

- Look at normal microbiota of xylem and adjacent tissues
- How do the bacteria attach in the plant and the insect?
- What's the effect of xylem temperature and other abiotic factors on bacterial growth and spread?
- Are there extracellular proteins that *Xylella fastidiosa* may produce that affect osmotic balance (like Cholera toxin)?
- Explore *Xylella fastidiosa* trophic metabolic pathways
- Examine factors involved in induction of bacterial EPS formation?

Biology And Biophysics Of Bacterial Transport

- Understanding xylem structure at leaf and branch junctions - could they cavitate naturally at that point?
- Are *Xylella*-infected vessels full (of water) or empty?
- Is there lateral movement of *Xylella*?
- Does the bacteria do anything to xylem structure or chemistry that affects vector performance or *Xylella fastidiosa* spread in the plants?
- What's the role of insect salivary enzymes in infection?
- What is the rate of spread of *Xylella fastidiosa* up and down in stressed and unstressed vines?
- How do the bacteria dissolve through the pit membrane?
- Find ways to monitor movement and fate of *Xylella fastidiosa*
- Tag *Xylella* with GFP
- Internal biomarker
- Confocal microscopy with vital stains.

Collaborative Opportunities

- Rapid screening system - branch of grape in *Xylella fastidiosa* filtrate
- Classical biochemical approach - live cells, dead cells, bacterial products
- They grow slowly - 12 days in culture to build up population
- *Xylella fastidiosa* /GWSS website for information and collaboration

Breakout Session #5 -- Prospects for Chemical and Biological Control of *Xylella fastidiosa*

Session Chair – Donald Cooksey
Facilitators – Susan Laughlin and Jim Brenner
Recorder – Joseph Morse

Participants: John Andrews, Kerry Britton, Jim Brenner, Thomas Burr, Chung-Jan Chang, Donald Cooksey, Korsi Dumenyo, Donald Hopkins, Alan Jones, Bruce Kirkpatrick, Umesh C. Kodira, Conrad Krass, Susan Laughlin, Carol Lauzon, Jiang Lee, Wenbin Li, Steven Lindow, Kaishu Ling,Joyce Loper, Jo Luck, Robert Luck, Philip Manson, Carole Meredith, Thomas Miller, Patricia Monteiro, Joseph Morse, Len Nunney, Dan Opgenorth, Robert Page, Gregory Pogue, Martin Romantschuk, Scott Soby, Helmut Van Emden, Robert Wample, Barney Ward, Ed Weber, Magally Luque Williams, Gisela Wittenborn

II. BRAINSTORM LIST OF CRITICAL RESEARCHABLE QUESTIONS (because of time constraints during this last session, not time to ask speakers questions)

Process Used:

7. Do to limited time available for this particular breakout session, we were not able to address questions to the various speakers and went directly to brainstorming a list of researchable questions

8. Participants suggested critical researchable questions and the recorder tried to capture these as a short "sound byte"

9. Session Chair and Facilitator went back over the items and placed them under the appropriate category

Understanding the basic biology of *Xylella*

1. Some basic studies need to be done with *Xylella*; how it interacts with hosts and non-hosts; where the pathogen is located in the host; Purcell's 4 examples of types of hosts – understand why different plants are acting differently (what is the mechanism?); Examine cases where *Xylella* is non-systemic

2. Look at what environmental factors affect bacterial movement in the vine

3. Differences in behavior of solitary *Xylella* cells versus aggregated cells – differential gene expression

4. Assuming see differences (3) Examine density dependent gene expression inhibitors

5. Are there preferential sites for cell to cell movement of *Xylella*?; Are the competitors inhibiting this movement? Do with GFP to visualize.

6. Are there preferential sites for *Xylella* interaction with the plant – attachment and aggregation? Or is this random? (also should be examined in the insect mouthparts)

7. In GWSS, examine whether there are several strains of *Xylella* in the same bug, are they competing?

8. When see different titers of *Xylella* – is this different numbers of cells within a limited number of xylem vessels or differences in the number of vessels colonized?
With pathogenicity – appear to be getting movement from vessel to vessel.
In non-pathogenic *Xylella* – multiplies in vessels it is put in (by the vector) but then stops

9. Better understand the role of plant response in relation to disease (*Vitis vinifera* – most of the plug is *Xylella*; muscidine grapes – combination of bacterial cells and gums/tyloses)

10. Better understand the microbial ecology of the xylem in grapes

11. Examine the mechanism involved in cold therapy (what are the critical temperatures; probably are not freezing)

12. Examine host range of different *Xylella* strains; differences between strains

13. Is there horizontal plasmid transfer between different *Xylella* strains?

14. Determine whether *Xylella* is actually forming a biofilm in the xylem – if so, what is biofilms role is in causing disease?

Chemical control of *Xylella*

15. If we find a good bioprotectant agent (Kirtpatrick's work) – how often would it need to be applied; could this be done during the dormant season? (may be hard to do)

16. Use *Xylella* to express toxins to kill GWSS

Biological control of *Xylella*

17. Develop mutants of *Xylella* -- gum minus and pillus minus – look at the importance of attachment (also important within GWSS mouthparts – look at it there also); also make GFP or other reporter constructs to examine where the gene is expressed

18. Look at phages or other existing endosymbionts (non-pathogenic strains of *Xylella*) that might be used as soon as possible

19. Methods of introduction -- introduce perhaps at the nursery level; Can we use GWSS or other organisms to transfer these control agents?

20. Determine how the phage kills the bacteria; introduce that agent into the plant

21. Look at other endophytic bacteria in grapes; look at e.g. *Agrobacterium* as a possible biocontrol agent (after manipulation)

22. Is virulence separable from colonization? Many biocontrol strategies would require a biocontrol agent that could colonize the same sites as *Xylella* with similar or better fitness as *Xylella*.

23. Risk assessment or strategies to engender public acceptance of genetically modified organisms

24. Examine relationship between *Xylella* and GWSS – block acquisition by GWSS, multiplication, or transmission

Host resistance

25. Are there rootstocks that do not harbor *Xylella*? (perhaps design a rootstock that one buds much higher on the plant); Genetically engineer a rootstock or scion to produce bactericidal products

26. Determine population genetic fine structure of *Xylella* within and among plants in relationship to disease

27. Look at how resistant grapes resist *Xylella* – examine mechanism of resistance to *Xylella*

Collaboration

28. Maintain good cultures of various strains of *Xylella* – need for microbial database of who has what strains – archive infected plant and GWSS material

Appendix IV.

GWRDC Travel Report
PD Conference attended in UC-Davis, Dec 2000

Project Title	**Analysis of the potential for the establishment of Pierce's Disease in Australian grapevines**
Project No	
Organisation	Institute for Horticultural Development
Location	Knoxfield, Victoria
Supervisor	Peter Merriman
Staff	Joanne Luck

Objectives
• To attend Pierce's Disease Conference • To validate Australian PD diagnostic protocol

Pierce's Disease Symposium
University of California, Davis USA
December 2000

Summary

An international Pierce's Disease symposium was held in December 2000 at the University of California, Davis. The Secretary of the Californian Department of Food and Agriculture (CDFA), William Lyons stated that there is a PD issue on his desk every day of the week. This is now the most significant problem currently facing Californian Agriculture which is highlighted by the $US 47 million already spent on Pierce's disease at the state and federal level. Spraying has reduced glassy-winged sharpshooter (GWSS) numbers, however it has not made an impact of the spread of the disease. The complicating factor in controlling the disease is both the insect and the bacteria have over 100 alternative plant hosts. The conference was held over three days including speakers from California, Florida and Brazil. Briefly, there is still a great deal to learn about the basic biology of the insect vector and the bacterium, before an effective control can be offered. Expectations are pinned on the genetic engineering of PD resistance in *V. vinifera* but unfortunately this may be 10 years away.

Tuesday 12 Dec 2000
Conference session topics

1. General Introduction
2. Epidemiology of *Xylella* diseases, *Xylella* host reservoirs, mechanisms of transmission to plants, Insect/Plant interactions
3. Molecular genetic approaches to Understanding and Controlling Pierce's disease of Grapes.
4. Ecology of sharpshooters, biological and chemical control
5. Xylem physiology and relationship to Pierce's Disease
6. Prospects for chemical and biological control of *Xylella fastidosa*

For "Breakout" discussion group notes for each session see Appendix 1

1. General Introduction

William Lyons, Secretary, CDFA.
Overview of CDFA's program on PD

- USDA has spent $200 million last year alone on 5 invasive exotic diseases which cost the US $100 billion/year
    1. Dutch Elm
    2. Asian Longhorn beetle
    3. Gypsy Moth
    4. Plum Pox (Pennsylvania)
    5. Citrus Canker (Florida)
- The GWSS is native to Texas and Florida and Invasive to California, therefore by breaching state borders it has been as damaging as an exotic pest.
- PD is the number one priority on the National Invasive Species Council's agenda with the GWSS moving towards the Napa Valley threatening a $9 billion industry

Citrus Research Board-Ted Batkin
Economic impact of PD to Agricultural Crops in California

- Predicts losses associated with PD due to quarantine barriers, crop loss, jobs and an impact on tourism.
- Almond Leaf Scorch (Golden Death) is now being identified more frequently due to grower awareness. Losses have been experienced in Los Angeles and Costa counties-there is 570, 000 acres of almonds in California. Growers are starting to be concerned about GWSS. Can inoculate PD strain of *Xylella fastidiosa* into Almond and get ALS.
- Phony Peach Disease (also caused by *X. fastidiosa*) -Californian growers are not recognising the potential impact of the GWSS. 80-95% of fruit is grown in San Joachin Valley. It is a serious problem in Georgia where many trees have been lost.

- Oleander -The combination of Oleander Leaf Scorch and GWSS was first noted in Ventura County. The oleander trees were planted by Caltrans as a freeway barrier. When the disease was associated with GWSS Caltrans sponsored research on the disease.
- Impact on nursery industry-most concerned are the ornamental growers, due to the high cost of inspection and treatment plus delays in shipping.
- Californian citrus-growers are not concerned about GWSS infestations on citrus because it is not a significant pest on citrus in the absence of the Citrus Variegated Chlorosis strain of bacterium, *Xylella fastidiosa* (currently destroying citrus in Brazil).
- The treatment for GWSS is $US 300/acre- the net value of citrus is $50/acre therefore treatment is not a considered option. (The Californian government are now subsidising growers involved in a spray treatment program.)
- There is currently no treatment for GWSS on citrus or stonefruit exported from California
- Southern Kern County table grape pickers work in the low temperatures of the morning. The GWSS are much slower at low temps and drop into their picking bins, which are then trucked to packing sheds in North Kern county. As the day warmed up, clouds of GWSS were reported around the packing sheds. Quarantine regulations were imposed which halted the movement of fruit. These regulations were later modified in order to get fruit moving again.
- Other states are now concerned about movement of material that may contain the GWSS. No further information.
- Tablegrape exporters are confident their treatment is working
- Stonefruit and Citrus don't have GWSS treatment for export

2. Epidemiology of *Xylella* Diseases, *Xylella* host reservoirs, Mechanism of transmission to plants, Insect-Plant interactions

Alexander (Sandy) Purcell, **UC-Berkeley**

Overview and Epidemiology of PD and the Californian Situation

- Almond Leaf Scorch is just a curiosity at the moment but he felt that the situation could soon change for the worse, threatening the Californian almond industry.
- Can inoculate PD strain into almonds and get ALS.
- GWSS no latent period
- Xf persists in GWSS until it moults
- Not many bacteria needed for transmission (~200)
- Transmission efficiency varies with each host.
- GWSS not as effective as BGSS 20% bacteria transmitted/insect/grapevine compared to 95%/ insect/grapevine
- PD does not move rapidly in grapevine
- PD is winter climate limited
- Optimum temperature for growth is 28-29°C at 32°C it drops off rapidly (in-vitro studies).
- Environmental influences
- Climate, surrounding habitat, vectors, viticultural habitat
- Edge effect with BGSS is stable over 100 years, GWSS has altered the whole epidemiology of PD
- Temecula went from having no disease to complete devastation within 3 years.

Don Hopkins, University of Florida, Leesburg

Ecology of Xylella diseases in Florida and Eastern US

- Cannot grow *V.vinifera* at all in the south-eastern states because PD and GWSS are indigenous. PD has been detected as far north as Virginia. Leaf Scorch disease of shade trees occurs further north in Pennsylvania and New York. The Xf in larger trees may be more protected from the cold.
- After OLS was found in California, DH detected it in Florida.
- Tissue Specific but not host specific
- Xylem of leaf petiole in infected grapevine is completely plugged. Location of bacteria can be related to type of plant. Can get a sectoring of the disease. Xf in Leaf Scorch tends to be in leaf petiole and vein. In turkey oak LS, Xf is found in the petiole only. In a Xf die-back/decline disease (without leaf scorch) the bacteria is more likely to be found at the base of the branch. In Dwarfing disease (Phony Peach) the bacteria are found in the older wood or roots.

- Avirulent or weakly virulent strains were inoculated into young plants and then challenged with virulent strains with some success.
- PD strain has been isolated out of citrus (experimentally) but it is not a good host (multiplicative).

Armando Bergamin-Filho, **University of Sao Paulo FAPESP**
Epidemiology of Citrus Variegated Chlorosis (CVC) and Coffee Leaf Scorch in Diseases in Brazil

- CVC first reported in 1987 in Sao Paulo
- By 1994 it was in several states and today it is found in all citrus growing regions of Brazil.
- Symptoms;chlorosis, stunting, witches broom, small hard fruit.
- There are now 68 million disease citrus trees in South America. The infection rate is six times higher in summer than winter.
- They monitor for sharpshooters using yellow sticky traps and trap plants which are painted with a sticky solution.
- There is a significant role of water stress and temperature on infection.
- Coffee Leaf scorch was first recorded in 1995
- Leaf Scorch is not a common symptom. The main symptom is shortened internode length, leaf chlorosis, leaf fall, reduced yield and size of bean, shoot dieback, death may take several years. The citrus strain can be transmitted to coffee.
- In December 2000 CLS was found in Costa Rica where coffee is the main crop. In Costa Rica citrus is used as an over-story for the coffee plantation.
- ABF believed that the xylem becomes non-functional as soon as a hole was made by the vector piercing the xylem.

Tim Gottwald, **USDA/ARS**
Application of epidemiological analyses of *Xylella*-caused diseases to development of effective disease management strategies

- Brazil has 1.6 million tress and 30% are infected
- Paraguay and Argentina also affected
- Vector belongs to the Proconlini tribe
- Disease more prevalent in the south, where there is more irrigation (vector), in younger trees.
- 12 vector species involved in transmission of CVC
- The original infection most likely came from infected nursery material
- Disease management strategy

i. Early detection
ii. Avoidance-disease-free nurseries
iii. Control of vector population
iv. Systematic and repeated surveys
v. Pruning and roguing to reduce inoculation source
vi. Cu++ protection
vii. Tolerance
viii. Resistance-long-term strategy (10-20years)

- Nurseries priority is healthy rootstocks
- Symptoms are evaluated with PCR and ELISA

Wednesday 13 Dec 2000
Session 2 Molecular Genetic and Genomic Approaches to understanding and Controlling Pierce's Disease of Grape

Andrew Walker UC-Davis
Overview of PD:Genetic sources and application to resistance

- Resistance naturally occurs in Muscadine species
- Use IC-PCR and ELISA to test PD presence
- St George rootstock allows movement of Xf but doesn't express symptoms
- There is no movement of Xf and no symptoms of PD when injected into Muscadine rotundifolia

- Vitus champinii-resistance is not uniform
- V.smalliana-orlando seedless and Suwanee evaluated

- All lack vinifera quality re-fruit characters and wine quality
- In 1968 Mortensen found that V simpsonii, V smalliana and V. shullenok Contained 3 independently inherited genes and they were susceptible to PD if they were homozygous for the recessive genes
- Resistance to Xf is probably quantitative.
- First progeny were analysed this summer where AW saw a segregation of Xf resistance in table and raisin grape varieties.
- The historic reliance on cultivar names will change. AW hopes that R gene tranformant will be accepted as a clone. There will be 10 years to complete wine evaluation from study.
- Phil Phillips believes there is not time. It took 7 years to spread from 2 GWSS foci now there are multiple foci throughout California. The rate of spread will be much quicker. He believes there is probably only 3 years to find a solution.

Ana Claudia Rasera da Silva, Bloco da Quimica Fina e Biotechnologia, Brasil

Xylella Genomics in Brazil

- CVC strain of Xf sequenced and 3838 genes have been identified.
- Sugar is the major source of energy and it has a very efficient metabolism. They identified genes encoding a toxin, antibiotics, ion sequestration, EPS adhesion genes (similar to xanthan gum). Xf is not able to synthesise cobalbumin. With the DNA sequence data, Eliana Lemos has developed a new minimal media which Xf will grow on at faster rate that conventional media. Have contacted her and she has forwarded this protocol.
- The Proteome analysis is now underway
- Differential expression of genes is dependent on host, response to oxidative stress and quorum sensing-all being investigated using Microarray.
- They are cloning and disrupting genes involved in adhesion and invasion.
- Fastidian gum
- Less viscous than xanthan gum, chemical characterisation, low yield due to slow growth.
- Path test on citrus=6 months
- Path test on tobacco=1 month
- Also looking at Cataranthus roseus as host path test=1 month
- Further work on citrus and glasshouse temperature control has allowed symptom expression in 1 month now.

Douglas Cook, UC-Davis

Application of Plant Genomics to PD:EST profiling and data mining

- Transcriptional profiling-correlation between conditions and gene function
- Arabidopsis has 1/4 size genome compared to grapevine
- 67% of genes are ESTs
- V vinifera has 500 Mbp genome
- 105 Expressed Sequence Tag ESTs characterised
- <300 genomic sequences in database
- Australian EST project has shown that 50% of ESTs are unique to *V.vinifera*.
- And 27% have no matches to plant ESTs therefore cannot use *A.thaliana* as model

Strategy

i. cDNA libraries
ii. ESTs
iii. Data mining, database
iv. Functional genomics-microarray

AW wants to look at genes expressed in

i. Susceptible infected
ii. Susceptible uninfected
iii. Resistant infected
iv. Resistant uninfected

- Are susceptible genes suppressed in a resistant reaction? Or is there an R gene(s) expressed in resistant reactions?

Paul Predki Dept of Energy/Joint Genome Institute
Bacterial Genomics:From whole genome sequence to PD

- Sequencing facility can sequence bacterial genome in 1 day. PD strain had already gone to Brazil to be sequenced before this facility was up and running.
- Have already sequenced the oleander strain (Ann1 strain) and the almond strain (Dixon strain) which may be identical to the PD strain. The whole genome was shotgun cloned into pUC vector and sequenced. Robots are used to isolate 50, 000 plasmid preps/day. They use dye-terminator cycle sequencing and the DNA sequencer operates 24 hours/day.
- There is an unedited assembly of small insert libraries which takes 24 hours, a hand edited assembly of small insert which can take 20-30 days and the editing of the larger insert libraries can take much longer.
- Oleander had 86% conservation of genes with the CVC strain

Jan E. Leach, Kansas State University
Molecular and Cellular Biology of Xylem-Limited Bacteria from *Xanthomonas* to *Xylella*

- Bacterial blight, *Xanthomonas oryzae* pv. *oryzae* lives inside xylem in rice moving through the epithem and exiting plant via the hydrothore.
- There is no wounding of the xylem in transmission -which is via winds and driving rain. Resistance is conferred by a single dominant R gene which provides effective control of the disease.
- Can infiltrate leaf using syringe and bacteria will find its way into the xylem
- All members of the avirulence gene family are using to Xanthomonas.
- Avr genes are important to aggressiveness
- Pit walls are composed of primary wall parenchyma
- Pit aperture measurement-the secondary wall increased in thickness and the pit narrows in a resistant reaction.

David Gilchrist, University of California, Davis
Genetic Engineering: Functional screening for resistance and gene transfer in vegetative tissue of grape

- Transformation using *Agrobacterium rhizogenes* using hairy root culture.
- MRNA isolation from infected and healthy grapes
- No current work presented just proposed research on gene delivery to grapes

Session 3 Ecology of Sharpshooters: Prospects and limitations of Biological Control and Chemical Control of Sharpshooters

Robert Luck, UC-Riverside
Overview of Ecological Research Needed to Address GWSS

- GWSS life cycle, 5 instars
- 2 generations/year
- 2 peak trap catches and 2 peak egg laying periods
- 131 hosts recorded
- 95 are trees, shrubs and vines
- 93 are oviposition hosts
- Must discriminate between sampling and feeding.
- Reduction in vector population has not led to a reduction of disease.
- Density of GWSS correlates with plant concentration of essential amino acids.
- Amino acids differ in different hosts, spatially, diurnally and seasonally.
- N and C are present in 1% of xylem fluid
- Is search for host non-random and cue driven?
- Is *Xylella*'s association with the GWSS fortuitous-some papers say yes but it is not clear?
- Does Xf manipulate the nutritional composition and pressure of the xylem to enhance its spread or does Xf manipulate the GWSS to enhance transmission?

Russ Mizell, University of Florida

Sharpshooter Movement Between Host Plants in Florida

- GWSS overwinters on wild plum in Florida and then spreads Xf into peach orchards causing phony peach disease.
- Claims that the yellow sticky traps are not blunder traps but definitely attract.
- The salivary sheath is left in the xylem after probing.
- Plant and excreta chemistry are monitored -slide of test-tube full of GWSS excreta (clear, like water) attached to branch of crepe myrtle with gauze over a single GWSS.
- GWSS abundance is proportional to excreta rate.
- GWSS doesn't really like peach.
- There are rootstock and scion effects on GWSS
- 150 different cultivars of peach in the field and GWSS numbers
- Females feed more than males
- GWSS moves for food and oviposition
- Moves in the evening from 6.30pm til dark
- During mating the female elevates and points posterior towards outwards on leaf.
- In mark and re-capture studies more males are caught (2.5X). RM believes that the vector is cued by plant chemistry.
- He placed dead and live insects on branches which did not attract more insects to the host
- It is not a scent cue that drives the GWSS to feed on its host.
- RM removed the xylem from the plant and it did not have any effect on probing
- Nymphs require a subset of conditions which is not the same as the adult. Non-hosts can be based on many factors;climate, daytime, xylem chemistry
- Suicide hosts-adult lays eggs on host but nymphs do not feed on this host.
- Could set up non-host barrier with suicide crops.

Rick Redak, UC Riverside

Expectations and Limitations of Chemical Control for GWSS

- Nursery quarantine (CFDA) strategies

1. Survey
2. Establish GWSS-free area for shipment
3. Ship plants free of GWSS

- Nursery owners are not happy with the current quarantine restrictions, costly in treatment and delays in shipping
- Technically possible to control GWSS and obtain pest free status
- Insecticide is only effective approach
- Monitoring
- Registration and labelling
- Comfort levels
- Contradictory demands by other states
- Environmental effects of heavy pesticide use for effective quarantine
- Timing of pesticide application is critical
- Reduction of non-grape habitat can significantly reduce GWSS in vineyard
- Imidicloprid can kill GWSS in a matter of hours however GWSS may only need one hour to re-inoculate a plant with X.fastidiosa.

Mark Hoddle, UC-Riverside.

Prospects and Limitations of Chemical Control for GWSS

- Biological control with parasitoid wasps
- Inability to mass rear wasps is limiting factor
- Studied wasps from Florida (GWSS natural environment) Louisiana, Mexico
- Wasps effective on 2nd generation but mortality is not as high in the first generation therefore GWSS adults are still in high enough numbers to go into over-wintering.

Sandy Purcell, UC-Berkeley
Modelling infection pressure with GWSS: What are the Economic Thresholds?

- Chronic PD-no removal by pruning
- GWSS feeds on woody stem
- 50% transmission in <3 hours
- 15% loss to PD/year in Ventura county
- no compensation for removal of crop
- vegetation management
- sticky trap catches in Kern county have been reduced from 800/card to 12/card by vegetation management
- In Temecula diseased vines were not removed

Session 4: Xylem Physiology and Relationship to Pierce's Disease (Water Stress): Mechanisms of Pathogenesis in Xylem Diseases

Melvin Tyree, US Forest Service, Vermont
Xylem Structure and Function

- Xylem is dead tissue
- Pathology of xylem-Dutch elm disease
- Air cannot enter xylem vessel or else it becomes non-functional
- Cryo-scanning could demonstrate whether Xylella is growing in a water-filled media or if it is an air-filled chamber

Andrew Groover, Forest Genetics, Davis
Xylem Development

- Xylem is composed of multiple cell types
- There is a programmed cell death of xylem tissue where the entire cell contents autolyse leaving a secondary cell wall
- Differentiating tracheid elements may produce cells that X.fastidiosa likes
- Calcium influx drives cell death
- Cell corpse must withstand negative pressure, provide mechanical support and resist cavitation
- A secreted serine protease, trypsin, co-ordinates secondary wall synthesis and cell death
- Xylem cell death occurs 96 hours after cell differentiates

Mark Mathews, UC-Davis,
Water Stress and Xylem Function in Grapes

- Water stressed grapevines do not have the same symptoms as PD infected vines
- Water stress is expressed by general chlorosis and senescence. Leaves are abscised at stem unlike PD vines which retain the leaf petiole on leaf shed.
- The theory of a toxin being involved in PD needs to be re-evaluated
- Water deficit reduced diameter and number of xylem vessels in grapevine
- Is plugging of xylem plant or bacterial in origin?
- Can bacteria seed a new embolism upon entry into adjacent vessel?
- Water stress is not the only factor in PD but it is critical to the syndrome.

Peter Andersen
Xylem Chemistry and Feeding preferences of the GWSS

- GWSS can feed 6-8 hours without movement
- Probing of the xylem required high energy levels to suck against the negative pressure of the xylem.
- GWSS is carbon-limited which is very unusual for an insect
- Can consume 100-100,000 times it's body weight in one day
- The xylem fluid is composed of 99% water, it has an unbalanced organic composition and non-essential amino acids dominate.

- The xylem fluid in the GWSS contains organic acids, amino acids, it is low in carbohydrates
- 54% of the amino acids in the grapevine xylem are glutamine
- GWSS extracts 100% of the organic acids from the xylem, essentially a filter-feeder
- Crepe myrtle is the preferred host for the GWSS adult and soybean is the preferred host for the nymph.
- The nymphs have different nutritional requirements than the adult
- Glutamine is the primary determinant for feeding
- Glutamine concentration is highest in the crepe myrtle in the middle of the day which is when the GWSS feeds the most
- Florida has only 1% of the GWSS numbers that California now has (even though it originated from Florida). BUT Florida still cannot grow *Vitis vinifera* because of PD. This is ominous for the future of grape-growing in California.

Michael Reid, UC-Davis, DANR/Davis

Bacteria and Bacterial products in Xylem Occlusion

- ***Bacteria and occlusion of xylem in cut-flowers, reason for short vase-life***
- Bacteria accumulate in pit membranes
- Bactericides increase vase-life-application to PD?

Session 5: Prospects for Chemical and Biological Control of *Xylella fastidiosa*

Bruce Kirkpatrick, UC-Davis

Prospects and Challenges of Controlling *Xylella fastidiosa* with Bactericides

- Chemotherapy for PD
- Found that muscadine resistant to PD had higher levels of Zinc, copper, manganese and iron. Were these inhibitory to Xf? He did plate inhibition and field trials.
- Foliar sprays-no phytotoxicity observed but didn't achieve inhibition of PD
- dripline delivered and injected into trunk of vine.
- A young infected vine cannot be turned around with chemotherapy

Donald Cooksey, UC-Davis

Prospects for Biological Control for *Xylella fastidiosa*

- Non-pathogenic strains of Xf may competitively displace pathogen
- Bacteriophages may control pathogen
- Avirulence genes in bacterium?
- Virulence genes –xanthan gum biosynthesis. Xanthan may block xylem
- Could have control through degradation of the xanthan gums-xanthan degrading enzymes isolated from gram positive soil bacteria

Thomas Miller, UC-Riverside

Paratransgenesis to Prevent PD and other Plant Disease

- Paratransgenesis-Genetically altered endosymbionic bacteria in insects-"Influential passengers"
- Whitefly carried Wolbachia endosymbiont
- Investigating possible inhibitory behaviour (on *Xylella fastidiosa*) of other bacteria carried by GWSS

Steve Lindow, UC-Berkeley

Constraints to Biological Control

- PD plumbing problem
- Cellulase important virulence factor
- No type III secretion system
- Sporadic plugging events in all or most elements
- Biofilm colonisation
- Biocontrol through

1. competition
2. Antibiosis
3. Hyperparasitism
4. Induced host resistance
5. Alteration of pathogen behaviour

6. Signal antagonists

- There was a public forum held after the conference where health and environmental risks were raised over the residential spraying for GWSS. Representatives from organic growers associations and environmental groups

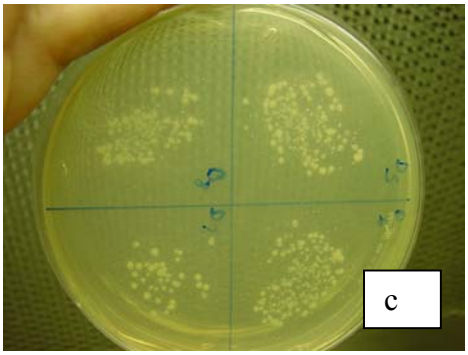
Australian PD Diagnostic Validation
Dr Bruce Kirkpatrick's lab (UC-Davis) was visited after the conference to establish whether our Australian developed diagnostic protocols could detect *Xylella* in diseased grapevines.

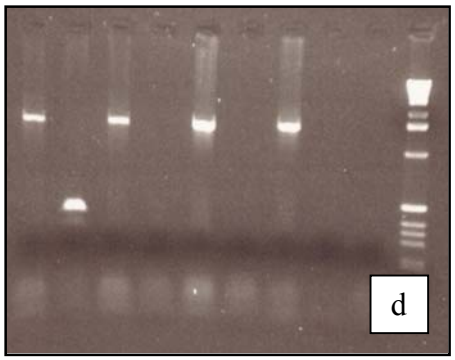
Infected merlot was sampled from Dr Kirkpatrick's glasshouses. This merlot was symptomatic and had already tested positive for *Xylella* using PCR testing methods designed by Florida . Leaf samples were collected (Figure 1b) and DNA was extracted for PCR. A culture of *Xylella fastidiosa* was used to inoculate agar medium (Figure 1a) which was made at our Plant Health laboratory in Knoxfield, Victoria. After 7 days, minute *Xylella* colonies were observed on the plates (Figure1c). confirming that our Australian prepared media would support the growth of *Xylella fastidiosa* if present in grapevine material. *X.fastidiosa* specific products were successfully amplified using PCR protocols adapted from US and Italian methods (Figure 1d). This test validated two critical diagnostic techniques we have developed for use in the event of a PD out-break. It was not possible to "work up" the tests in Australia in the absence of the target organism therefore this validation has given us confidence in detecting this exotic organism in Australian grapevines.

Visit to Napa Valley
Hosted by Dominic Pecchenino and David Michul, Beckstoffer Vineyards

One day was spent in the Napa Valley with Phillip Manson from Winemakers New Zealand. We visited six vineyards owned by Beckstoffer Vineyards, some of which demonstrated the PD and Phylloxera problems in the region. The Napa growers have dealt with PD for over 100 years and it appears they have learnt to live with it. The Blue-Green Sharpshooter is the most common vector in the Napa, with "misses" sporadically occurring close to the edge of the blocks, near riparian habitats. The growers were worried about the GWSS being introduced to the area from Southern California. They believed that this insect would probably be introduced on ornamental nursery stock and it was just a matter of time before it was introduced into the Napa Valley. Early this year, the GWSS was detected in Sonoma County which is directly adjacent to Napa County.

Figure 1
(a) Dr. Jo Luck inoculating *X. fastidiosa* to Australian PD agar medium
(b) PD infected Californian Merlot leaves sampled for DNA testing
(c) *Xylella fastidiosa* colonies on PD agar medium
(d) Agarose gel containing DNA specifically amplified from *Xylella fastidiosa* (lane 2)





Appendix V.

Map of potential areas for GWSS establishment in Australia

POTENTIAL ESTABLISHMENT AREAS OF THE GLASSY-WINGED SHARPSHOOTER IN ASSOCIATION WITH AUSTRALIA'S VITICULTURE AND CITRUS INDUSTRY

Citrus Areas
Viticulture Areas
Citrus and Viticulture Areas
Potential Establishment Areas

0 100 500
KILOMETRES

NORTHERN TERRITORY
QUEENSLAND
WESTERN AUSTRALIA
SOUTH AUSTRALIA
NEW SOUTH WALES
VICTORIA
TASMANIA

Darwin
Katherine
Mataranka
Kununurra
Broome
Mareeba
Mingela
Alice Springs
Emerald
Rockhampton
Bundaberg
Wallaville
Childers
Gayndah
Howard
Biggenden
South East Queensland
St George
BRISBANE
Stanthorpe
Bourke
Canarvon
Swan Valley
PERTH
Margaret River
South West Western Australia
Pemberton
Great Southern
Narromine
Mudgee
Upper Hunter Valley
Lower Hunter Valley
Gosford
Windsor
SYDNEY
Lachlan River
Murray-Darling Irrigation
Murray - Darling Region
Clare Valley
Riverland
Barossa Valley
Eden Valley
Adelaide Hills
ADELAIDE
McLaren Vale
Mypalonga
Murray-Darling NW Victoria
Murray Region
Murrumbidgee Irrigation Area
Cowra
Central East NSW
Young
Canberra
Langhorne Creek
Padthaway
Tocumwal
Tumbarumba
Rutherglen
Goulburn Valley
Bendigo
Pyrenees
Ovens Valley
Coonawarra
Grampians
Kiewa Valley
Central High Country
Macedon Ranges
Ballirat
Sunbury
MELBOURNE
Yarra Valley
West Gippsland
East Gippsland
Geelong
Far South West
Mornington Peninsula
South Gippsland
Launceston
Hobart

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Map Information

Pierce's Disease (PD) is a lethal, vector borne bacterial disease of grapevines that has emerged as a serious threat to California's US $2.8 billion dollar viticulture industry.
The main vectors of PD are xylem feeding insects belonging to the Cicadellinae subfamily (Homoptera) commonly known as sharpshooters. Recent devastation of vineyards in Southern California is attributed to the introduction of a new pest, the glassy-winged sharpshooter (Homalodisca coagulata) (GWSS). The bacterium that causes PD (Xylella fastidiosa) and the GWSS have not been recorded in Australia.

This study predicts the potential distribution of the GWSS in Australia based on US data. The distribution is overlayed with Australia's viticulture and citrus production areas.
Citrus is the preferred breeding and feeding host of the GWSS and has significantly attributed to the spread of PD in Southern California. The Californian's are now reviewing the risk of growing grapes near citrus orchards (Pierce's Disease Symposium, December 2000)

Reference: Thematic Map Data Source
Data Source:
** CLIMEX, CSIRO Entomology, Indooroopilly, QLD*
** Queensland Fruit and Vegetable Growers, Brisbane Market, QLD*
** NSW Agriculture, Orange, NSW*
** MIA Horticultural Council (Riverina), Griffith, NSW*
** Department of Natural Resource and Environment, Sunraysia Horticultural Centre, Mildura, VIC*
** SunLIS, Mildura, VIC*
** Victorian Mineries Tourism Council, Melbourne, VIC*
** Hema Maps, Wine Map of Australia, Eight Mile Plains, QLD*
** South Australian Research and Development Institute, Loxton, SA*
** Citrus Growers SA, Berri, SA*
** Department of Primary Industries and Fisheries, Alice Springs and Katherine, NT*
** Agriculture Western Australia, Midland, WA*

Base Map Information
Base Map of Australia hase been produced using the DNRE Corporate Geospatial Data Library 1:1 000 000 Digital Chart of the World (DCW)

Map Projection - Universal Transverse Mercator (AGD 1966 Datum)

Map Data and Cartography - Spatial Vision Ref SV000302.

For Further Information, please contact

Violeta Traicevski, Institute for Horticultural Development, Department of Natural Resource and Environment 621 Burwood Hwy, Knoxfield, Victoria, 3180; Email: violeta.traicevski@nre.vic.gov.au

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