A SUPPLEMENT TO THE FINAL IMPORT RISK ANALYSIS ON THE IMPORTATION OF FRESH TABLE GRAPES (*Vitis vinifera* L.) FROM THE STATE OF CALIFORNIA IN THE UNITED STATES OF AMERICA

JUNE 2000

Australian Quarantine and Inspection Service
GPO Box 858
Canberra ACT 2601
AUSTRALIA

CONTENTS

1 INTRODUCTION

On 13 January 2000, the Australian Quarantine and Inspection Service (AQIS) released the Final Import Risk Analysis (IRA) paper for the importation of fresh table grapes from the State of California in the United States of America (USA). The Final IRA presented the determination by the Executive Director of AQIS that imports of fresh table grapes from California USA would be permitted subject to the application of appropriate phytosanitary requirements. The release initiated a 30-day period for appeals against the IRA. As stated in *The AQIS Import Risk Analysis Handbook*, any stakeholder of the opinion that the process outlined in the Handbook had not been properly followed, including that the risk analysis failed to consider a significant body of relevant scientific or technical information, may appeal to the Director of Animal and Plant Quarantine. The appeal period closed on 14 February 2000. A total of 12 appeals were received.

Ten appeals were considered by the Import Risk Analysis Appeal Panel (IRAAP); two further appeals were received after the cut-off date for appeals and were not formally considered by the IRAAP. The members of the IRAAP were Mr Andrew Inglis as Chair (member, Quarantine and Exports Advisory Council (QEAC)), Dr Jim Cullen (member, QEAC), and Mr Geoff Gorrie and Professor Craig Pearson (AFFA). The IRAAP's recommendations were delivered on 30 March 2000. Attachment 1 provides a copy of the IRAAP's findings.

The IRAAP found no evidence that AQIS had ignored a significant body of relevant technical or scientific information in the Table Grape IRA, nor was there any evidence presented that the IRA was inconsistent with Government policy or lacked harmonisation with international standards. The IRAAP considered the issues highlighted by appellants regarding consultation and concluded, in general, the IRA was conducted as required by the Handbook for routine IRAs. As such, appeals under these categories were dismissed.

However, the IRAAP concluded that certain aspects of the IRA appeared not to have been conducted in a fully transparent manner. Consequently the IRAAP upheld appeals on the lack of transparency relating to the basis upon which decisions were made regarding two specific issues:

1. efficacy data for methyl bromide treatment; and
2. the risk of introduction of Pierce's disease.

Further, the IRAAP recommended the following course of action:

1. Within 90 days of the announcement of this decision, AQIS will (a) revise the IRA to improve its transparency and (b) advise stakeholders of this information.
2. The IRAAP, once satisfied that the two outstanding issues have been satisfactorily addressed, and clear explanations provided as to how the respective conclusions were arrived at, will consider that the appeal has been finalised and the Table Grape IRA concluded, at which time the import policy will become effective.

The list of appellants who raised these issues is presented in Attachment 2. Issues raised by appellants relating to efficacy data on methyl bromide are presented in Attachment 3 and Attachment 4 lists issues raised by appellants relating to the risk of introduction of Pierce's disease. A pest data sheet for Pierce's disease (causal agent, *Xylella fastidiosa*) is presented in Attachment 5.

This supplement to the Final IRA has been prepared to satisfy the recommendation of the IRAAP to revise the IRA to improve its transparency. All stakeholders have been provided with a copy of the supplement which is also available at the AQIS website: http://www.aqis.gov.au.

2 EFFICACY DATA FOR METHYL BROMIDE TREATMENT

2.1 Introduction

Methyl bromide (MB) is the most commonly used broad-spectrum quarantine treatment because of its high efficacy in killing insect and mite pests of a wide range of agricultural products, including fresh fruit and vegetables. It has been used by countries world wide, including Australia, Canada, New Zealand and USA, with reliable results (when applied correctly) over several decades since its discovery in 1932. In Australia, when a quarantine pest is detected on the surface of imported goods during on-arrival inspection, MB is generally used as a treatment. MB is also recommended for fruit fly disinfestation treatment against Queensland fruit fly in domestic trade.

AQIS has conducted a review and provided details of the MB treatment in the *AQIS Quarantine Treatments – Aspects and Procedures* prepared and made available to our overseas trading partners in June 2000 to ensure compliance with AQIS fumigation requirements. Many factors such as the handling, monitoring, temperature, concentration, fumigation time, loading and packaging of commodities can affect the efficacy of MB. On arrival inspection is routinely conducted to detect any live pests when fumigation is conducted offshore.

AQIS recommends internationally accepted dosages of MB fumigation for most fresh fruit and vegetables that are:

> $32g/m^3$ for 2 hours at 21ºC and above
> $40g/m^3$ for 2 hours at 16 - 20ºC
> $48g/m^3$ for 2 hours at 11 - 15ºC
>
> For each 5°C reduction below 21°C the fumigator must add $8g/m^3$ of MB to the fumigation dosage. For temperatures above 25°C, no dosage compensation is permitted by AQIS. In the case of table grapes, a minimum retention of MB concentration of 75% after 30 minutes of the commencement of the treatment, and 60% at the completion of the treatment, is required.

AQIS is closely monitoring compliance of pre-export MB fumigation, which is conducted overseas; non-compliant consignments are re-fumigated if live insects or mites are intercepted during on-arrival inspection, the reasons for failure of treatment are investigated with the exporting countries, and non-compliant overseas fumigators are deregistered.

As MB is listed as an ozone depleting substance by the Montreal Protocol (Anon., 1995) there is a phase out for general purpose uses early in this millennium (2005). AQIS also promotes best fumigation practice to reduce unnecessary usage and release of this chemical to the atmosphere.

2.2 History of the table grape import access request using MB

In 1990, AQIS received a request and supporting pest information from the Animal and Plant Health Inspection Service (APHIS), USA, seeking access for table grapes from California into Australia. In 1994, APHIS proposed a controlled atmosphere (CA) treatment with high carbon dioxide and low oxygen levels as a disinfestation treatment that could be applied in transit to control pests of quarantine concern. Since this was a novel treatment, AQIS requested that APHIS conduct research to evaluate its efficacy. APHIS submitted the results of the efficacy of the CA treatment in 1996 and provided additional data in 1997. However, AQIS consultants from the Commonwealth Scientific and Industrial Research Organisation (CSIRO) determined the data to be deficient and suggested that further research would be required. As this would take several years to conduct the necessary research, APHIS asked that AQIS consider access for Californian table grapes using MB fumigation treatment as an interim risk management option.

2.3 Use of MB for phytosanitary purposes

MB has been used extensively as a broad-spectrum disinfestation treatment for a wide range of products since Le Goupil (1932) in France first reported its insecticidal value (Bond, 1984). Since the 1930s, MB has been widely adopted for plant quarantine purposes because many plants, vegetables and some fruits were found to tolerate concentrations that were effective against the insects concerned.

MB's spectrum of activity and efficacy have been the object of considerable scientific research, and this has been confirmed in practice over a long period of time through post-treatment inspection of treated products. Fumigation of produce as a pre-export treatment (as a mandatory requirement or following the detection of live insects) or on-arrival (generally following the detection of live insects) is universal quarantine practice, at both international and domestic levels. A high percentage of cut flowers from Australia, particularly native species, are fumigated either immediately prior to export to secure phytosanitary certification, or on-arrival at their overseas destination following detection of live pests.

Over the years, many agricultural and horticultural commodities including cut flowers, fruit (cherry, coconut, pineapple, strawberry, watermelon), mushrooms, and vegetables (asparagus, okra, taro) imported from designated overseas countries have been compulsorily fumigated with MB on arrival in Australia. Cherries are regularly imported into Australia from the USA after fumigation with MB as a mandatory requirement for a range of pests.

Many countries require mandatory methyl bromide treatment for a wide range of fresh fruit and vegetables from exporting countries including Australia. For example, Canada – *Malus* spp. and *Vitis* spp.; Fiji – *Brassica* spp., *Capsicum* spp. and *Citrus* spp.; Indonesia -–*Actinidia* spp., *Ananas* spp., *Capsicum annuum*, *Carica* spp., *Citrus* spp.,

Cucumis sativus, Cucurbitaceae, *Cydonia* spp., *Fragaria* spp., *Lycopersicon* spp., *Malus* spp., *Musa* spp., *Persea americana*, *Prunus* spp., *Pyrus* spp. and *Vitis* spp.; New Caledonia – *Dioscorea* spp.; Papua New Guinea – all fresh fruit and vegetables; Solomon Islands – *Brassica* spp.; Tonga – *Allium sativum* and *Ananas* spp.; and Vietnam – *Actinidia* spp., *Ananas* spp., *Musa* spp., *Persea* spp., *Prunus* spp., *Pyrus* spp. and *Vitis* spp. (Anon., 2000b).

Australian states and territories allow a number of commodities to be fumigated with MB as a means of preventing spread of Queensland fruit fly through interstate trade (Standing Committee on Agriculture and Resources Management, 1996). In accordance with the Code of Practice for Management of Queensland fruit fly prepared by the Interstate Plant Health Regulation Working Group, MB is recommended for use on a wide range of crops which include abiu, apple, apricot, avocado, caimito, capsicum, carambola, cherimoya, chilli, citron, custard apple, date, feijoa, fig, grapefruit, guava, kiwifruit, lemon, lime, loquat, mandarin, mango, nashi, nectarine, orange, peach, pear, persimmon, plum, pomelo, quince, raspberry, sapodilla, strawberry, tamarillo, tangelo, tomato and wax jambu. Grapes are not included in this list as they are accepted domestically as not posing a risk of transferring Queensland fruit fly.

As MB is listed as an ozone depleting substance by the Montreal Protocol (Anon., 1995), use of this chemical for non-quarantine applications will be phased out by 2005. Use for quarantine and pre-shipment purposes is currently exempt from phase-out. However, MB will become less available and consequently is already becoming more expensive as its production is reduced. The Protocol also recommends parties minimise its application wherever possible and efforts are under way to identify alternative treatments to replace MB for these uses.

2.4 Efficacy of MB on pests related to table grape pests

It is clear from the literature that efficacy data for MB against the specific pests identified in the Californian table grape IRA are not available. However, there is substantial documentation of efficacy data against related species of pests within the same families. Furthermore, AQIS and quarantine agencies from other countries have been using this fumigant on similar pests and similar commodities for a long period and have built up a significant amount of historical data, which supports its efficacy. A large number of MB treatments have been developed by the United States Department of Agriculture (USDA) and these appear in the APHIS treatment manual (Anon., 1998).

Dr Peter Witherall, Station Leader at the APHIS – Plant Protection and Quarantine (PPQ) Station in Oxford, North Carolina, where fumigation policies and protocols were established, stated “In the USDA – APHIS Plant Protection and Quarantine Manual, we include a 2 hour MB treatment schedule, T101-I-2, for use on grapes for insects other than *Ceratitis capitata* (Mediterranean fruit fly) and *Lobesia botrana* (vine moth)”. Also, based on APHIS’s extensive experience in fumigating for quarantine control of surface pests on fruits and vegetables, this particular treatment schedule is considered to be very

efficacious for use against glassy-winged sharpshooter on grapes (Green, pers. comm., 2000).

It is also significant that the United States has employed a similar MB fumigation schedule as that proposed by AQIS, for importation of table grapes from Chile. Since 1994, APHIS have reported that no live pests have been found during inspection of fumigated grapes from Chile, which averaged over 25 million cartons per year (Obbink, pers. comm., 1998).

A fumigation schedule similar to T101-I-2 is used for the control of codling moth in cherries destined to Japan from the United States. Over the last five years, more than 3.75 million cartons of cherries were fumigated and shipped to Japan. Post-fumigation inspection of fumigated cherries revealed that all insects were dead (de Mange, pers. comm., 1998). AQIS currently accepts cherries from certain parts of California fumigated with MB, which has proved effective where it has been applied correctly. Research has shown MB to be effective against fruit flies and codling moth and this has enabled Australian access for cherries to Taiwan and apples to Japan.

Although there is no specific efficacy data of MB on the two species of mites, grape leaf bud mite (*Colomerus vitis*) and Pacific spider mite (*Tetranychus pacificus*), found on grapes, there have been numerous scientific reports supporting the effectiveness of the chemical against all stages of mites, including the resting egg stage. Work conducted in the United Kingdom reported that mites and their eggs were killed on heavily infested narcissus bulbs at MB treatments having a concentration and time (CT[1]) product ranging from 102-251 ghr/m^3 at temperatures between 19-24^{o}C (Gurney and Gandy, 1974). Also, investigations in Taiwan reported that acarid bulb mites (including eggs) on onion were effectively controlled by MB fumigation at a concentration of 42.5g/m^3 for 2 hours (Lee and Wen, 1980).

There is MB efficacy data on tortricid lepidopterans related to the two species, orange tortrix (*Argyrotaenia citrana* [Lepidoptera: Tortricidae]) and omnivorous leaf roller (*Platynota stultana* [Lepidoptera: Tortricidae]), associated with table grapes in California. In apples, work conducted on the codling moth (*Cydia pomonella* [Lepidoptera: Tortricidae]) indicated that a quarantine protocol of fumigation with 24g/m^3 MB for 2 hours followed by cold storage between 0.5°C and 4°C for 25 days, provided a high level of quarantine security against codling moth 1-day old eggs and diapausing 5th instars (Denterner *et al*., 1998). For plums, large scale tests conducted on codling moth with 48g/m^3 for 2 hours at 18.5^{o}C killed all 0-24 hour-old eggs exposed on the fruit in packing cartons (Leesch *et al*., 1999).

In similar trials, apples of several cultivars, either naturally or artificially infested with lightbrown apple moth (*Epiphyas postvittana* [Lepidoptera: Tortricidae]), were fumigated with MB at 0-48 g/m^3 for 2 or 2.5 hours at different temperatures. Chambers were loaded

[1] The CT factor is the product of concentration and time duration required in fumigating a given product to attain the desired level of mortality of insects.

to 40 or 80% of their capacities, supplemented or not supplemented by cold storage at 0°C for 21 days before or after fumigation. Treatment with MB at 12-24 g/m^3 (half the then specified USDA temperature-dependent rates) for 2.5 hours at ambient temperatures and 40% loading, without cold storage, also gave complete control (Terauds *et al*., 1978).

Based on efficacy data on related soft-bodied, small insects (Petty, 1987; Misumi *et al*., 1994), AQIS is satisfied that MB at the recommended rates would be effective against the two mealybug species on grapes, grape vine mealybug (*Pseudococcus maritimus*) and vine mealybug (*Planococcus ficus*); and species of sharpshooters including the glassy-winged sharpshooter (*Homalodisca coagulata*). All these insects belong to the order Hemiptera and the families Pseudococcidae, Coccidae and Cicadellidae, respectively.

For pineapple planting material infested with the pseudococcids *Dysmicoccus brevipes* and *Pseudococcus longispinus*, and the diaspid *Diaspis bromeliae*, fumigation with 32 or 40g/m^3 MB for 2-8 hours at approximately 23-35ºC and 20-25ºC respectively, effectively controlled these pests (Petty, 1987). For mandarins, the control of *Planococcus kraunhiae* and *Pseudococcus citriculus* were achieved with 48g/m^3 MB for 2 hours at 15ºC or above with 32% loading or below and this has been accepted as the fumigation standard for the export from Japan to the United States (Misumi *et al*., 1994).

2.5 Comment on the efficacy of MB against plant pathogens

Although several appellants raised the issue of efficacy of MB on plant pathogens, the final IRA identified no plant pathogens of quarantine concern that would be on the table grape importation pathway. Many of the plant pathogens associated with table grapes in California are also present in Australia or they are associated with parts of the grapevine other than the fruit clusters.

2.6 Other factors affecting efficacy of MB

Many factors such as the fumigation enclosure, concentration, temperature, fumigation time, loading ratio and packaging of commodities can affect the efficacy of MB. These are described below. Details of how MB treatment should be carried out can be found in the *AQIS Quarantine Treatments – Aspects and Procedures* (Anon., 2000c). In the final IRA, treatment procedures with MB are based on the guidelines detailed in this document.

Fumigation enclosure

The fumigation enclosure must be well-sealed and capable of maintaining the minimum fumigant concentrations for the duration of the treatment. Where gas-tightness cannot be demonstrated the use of fumigation sheets is necessary. Sheets must have a permeability of less than 0.02 g mm day/m^2 (g/m^3) for MB and must be free from any defects, tears or holes (Anon., 2000c). Where fumigation chambers are used, these should be pressure

tested to ensure that they meet the minimum pressure test standard for effective fumigation. This is to ensure the effective use of the fumigant and to reduce the risk to personnel monitoring the fumigation.

Packaging material and stacking

Unless specified by AQIS, goods covered with or packaged in gas impervious materials (such as plastic wrapping or laminated plastic films, lacquered surfaces, aluminium foil, and tarred or waxed paper) must have the coverings or packaging opened, cut or removed prior to fumigation.

In California, grapes for export are typically packed into either Toyon Kraft Veneer (TKV) or extruded polystyrene (EPS) boxes (de Mange, pers. comm., 1999). Any box with processed wood ends and sides made of Kraft paper is called TKV. EPS boxes are composed of styrofoam. APHIS has performed tests on various packages used for grape imports in relation to gas penetration and evacuation. Thus far, APHIS has not refused the use of any of the commercial cartons/lugs used in the shipping of grapes. APHIS tested MB permeability of various types of boxes used for shipping Chilean grapes, including corrugated cardboard, wooden slats, corrugated hard plastic, extruded polystyrene (styrofoam). All were judged acceptable. Styrofoam was more sorptive than others but not to the extent that would prevent its use (de Mange, pers. comm., 1999).

Boxes of bagged grapes vary in weight (Nave, pers. comm., 2000); the most common weights are 7.2, 8.2, and 8.6 kg (or 16, 18 and 19 lb respectively). Single bags of grapes weigh between 0.68 and 0.9 kg (or 1½ and 2 lb) on average, and can be packed in a multitude of ways. Some boxes, for instance, are packed with single layers, while some are double layered. Plain-packed boxes (no bags) weigh either 9 kg (20 lb) (out of the Coachella Valley) or 9.5 kg (21 lb) (out of the San Joaquin Valley).

Loading ratio – free space

The loading ratio is the volume occupied by the commodity in a fumigation chamber divided by the total volume of the chamber. The loading ratio provides an indication of the free space that should be present in the fumigation chamber which in turn is directly related to both the fumigant dosage as well as the type of commodity being fumigated inside the chamber. For a known loading ratio, different dosages and commodities will influence the overall efficiency of fumigation. The maintenance of the correct loading ratio will also have a major impact in regulating the diffusivity of the fumigant into tiny crevices inside commodities where pests tend to breed.

Goods must be stacked with enough headspace (at least 50 mm) above and throughout the stack to allow for sufficient fumigant circulation around the whole consignment (Anon., 2000c). Sampling lines must be inserted during packing or stacking of goods to ensure that they are appropriately placed. For effective fumigation the fumigator must ensure that the goods are packed or stacked effectively. To treat large volumes

effectively, the fumigator must place the goods on pallets or skids. This will allow for sufficient penetration of the fumigant throughout the whole consignment.

Temperature

For fresh fruit and vegetables, the fumigator must use the fruit pulp temperature for dosage calculations (Anon., 2000c). The pulp temperature must be measured and included on the Fumigation Certificate. As a minimum, the fumigator must sample from at least one place in the bottom, in the centre and in the top of the consignment. Each temperature probe should be placed into the centre of a piece of fruit situated in the centre of the carton, where appropriate.

The minimum temperature (or fruit pulp temperature) for successful fumigation for AQIS quarantine purposes is 10°C unless specified otherwise (Anon., 2000c). For fumigation with a duration of 2 hours, at least 60% of the original fumigant must be retained at the end of the treatment unless otherwise specified by AQIS.

At temperatures below 10°C, MB has decreased effectiveness against pests as increased sorption of the gas by the body of the insect may counter balance the effects of decrease in respiration. Additionally, the resistance of insects may be weakened by the effects of exposure to low temperatures (Bond, 1984). Excessive fumigant uptake can also pose an increased safety risk, as the fumigant will be difficult to remove from the commodity. For example, with MB there is a moderate decrease in toxicity down to the boiling point (3.6ºC) and below this temperature, effectiveness declines sharply such that the amount of fumigant required to kill the insects increases dramatically (Bond, 1984).

The results of a fumigation may be influenced not only by the temperature prevailing during the treatment, but also by the temperature at which the insects are exposed, before the treatment (Bond, 1984). If the insects are in a cool environment, their metabolic rate will be low. If they are immediately fumigated at a higher temperature their physiological activity may still be influenced by their previous history, and the uptake of the fumigant may not be as great as if they had been exposed to the temperature of fumigation for a long time previous to treatment (Pradhan and Govindan, 1953). This phenomena can be of practical significance, particularly for certain species of insects that may go into a state known as diapause. For insects in this state, tolerance to some fumigants, e.g. MB and phosphine, may be several times greater than for non-diapausing insects (Bell, 1977a; Bell, 1977b).

Sorption

Sorption is the total uptake of gas resulting from the attraction and retention of the molecules by any solid material present in the system. Such action removes some of the molecules of the gas from the free space such that they are no longer able to diffuse freely throughout the system, or to penetrate further into the interstices of the material. Physical sorption varies inversely with the temperature and thus, is greater at lower temperatures (Bond, 1984). A volatile fumigant like MB having a boiling point of 3.6°C at atmospheric pressure would be less sorbed in a given situation. This fact has important

practical applications. It is one of the reasons why (a) dosages need to be progressively increased as the temperature of fumigation is lowered; and (b) AQIS does not permit MB fumigation at or below 10°C. As a general rule, those fumigants with higher boiling points tend to be more highly sorbed than the more volatile compounds (Bond, 1984).

CT product

CT is the product of concentration and time duration required in fumigating a given product to attain the desired level of mortality of insects. It is very important to maintain the desired CT product throughout fumigation to obtain the required level of insect mortality. The important variables influencing the CT product are: load size in the fumigation chamber, the leakage from the chamber during treatment and, the temperature at which fumigation occurs.

In order to regulate practical fumigation (in relation to the CT product), it is necessary to determine fumigant behaviour for the above variables. For this purpose, the fumigant level is monitored in the free space of the chamber during fumigation. Specific amounts of fumigant are added throughout the fumigation process to attain the cumulative CT product harmful to insect pests. This in turn will compensate for the fumigant losses, sorptivity of the fumigant and counter balance the time taken to attain specific concentrations inside the chamber.

Trials conducted by Agriculture Western Australia (AGWEST) showed that fumigation achieving a CT product of 52ghr/m^3 at 20°C were effective in killing western flower thrips (*Frankliniella occidentalis*). *F. occidentalis* was one of the quarantine arthropod pests identified in the final IRA document. MB at 54ghr/m^3 was also shown to be effective in killing more than 99% of western flower thrips (Anon., 2000a).

AQIS determined dosage rates for Californian table grapes as:

32g/m^3 for 2 hours at 21^{o}C and above
40g/m^3 for 2 hours at 16 - 20^{o}C
48g/m^3 for 2 hours at 11 - 15^{o}C

For each 5°C reduction below 21°C the fumigator must add 8g/m^3 of MB to the fumigation dosage. For temperatures above 25°C, no dosage compensation is allowed by AQIS.

2.7 Augmentation of the efficacy of MB treatment

AQIS is satisfied that MB fumigation used in conjunction with other mandatory phytosanitary import requirements, pre-shipment phytosanitary inspection and, on-arrival inspection will provide an appropriate level of protection against the introduction of the quarantine pests listed in the final IRA. In addition, sulphur dioxide treatment is required to reduce the risk associated with the introduction of black widow spiders.

MB has a long history of use against a wide range of pests, including mites. Similarly, sulphur dioxide disinfestation is known to be efficacious against mites. Sulphur dioxide has been reported to be efficacious against bulb mite on garlic and on onion at 50-100 g/m^3 (Bobrov, 1982). Work conducted in the USA found that MB and sulphur dioxide killed all forms of the northern fowl mites (adults, nymphs, larvae and eggs) and both chemicals were considered suitable for field use (Beerwinkle and Devaney, 1983).

Sulphur dioxide is also efficacious against the omnivorous leafroller, *Platynota stultana* (Yokoyama *et al*., 1999). Work conducted by Yokoyama *et al*. (1999) showed that low temperature storage combined with sulfur dioxide slow release pads caused 100% mortality of omnivorous leaf roller (2^{nd}-instar), which is the least susceptible stage to low temperature storage, after three weeks of exposure in table grapes. During the experiment, the temperature in the packed grape clusters decreased from ambient to 2^oC within two days after placement in storage.

Pre-export inspection is an important component and is considered herein as a verification assessment of the efficiency of the other risk mitigating measures. AQIS requires that APHIS sample and inspect all consignments after fumigation in accordance with official procedures for all visually detectable quarantine pests, including weeds and trash, as specified by AQIS in the final IRA document.

AQIS will complete an on-arrival inspection of a sample of table grapes consistent with the AQIS National Sampling Plan. On-arrival inspection, as with pre-export inspection, is an important component in the overall approach and is considered herein as a verification assessment of the efficiency of the other risk mitigating measures. This measure ensures that the entire risk control approach used has been effective in preventing and removing the quarantine pests of concern from the table grape importation pathway. This also serves as a *de facto* audit by AQIS of the efficacy of the entire process.

2.8 Conclusion

AQIS has given very careful consideration to the application for the importation of fresh table grapes from California in USA by conducting a detailed import risk analysis (IRA). AQIS has determined that subject to appropriate phytosanitary requirements, imports of Californian table grapes will not present a pest risk. Californian table grapes have been regularly exported to more than 20 countries, most of which do not require fumigation. AQIS considers that MB fumigation used in conjunction with other phytosanitary import requirements including a sulphur dioxide treatment, pre-shipment phytosanitary inspection and on-arrival inspection will provide security against the introduction of identified quarantine pests of table grapes.

2.9 References

Anonymous (1995). Montreal Protocol on Substances that Deplete the Ozone Layer. Report of the Methyl Bromide Technical Operations Committee, 1996 assessment. United Nations Environmental Program, Vienna, Austria.

Anonymous (1998). USDA Treatment Manual Interim Edition. USDA–APHIS, Frederick, Maryland.

Anonymous (2000a). Agriculture Western Australia (AGWEST) comments provided in the appeal against the Final IRA paper of AQIS on Californian table grapes.

Anonymous (2000b). AQIS Phyto. http://netdeployment.aqis.gov.au/phyto/asp/home.asp

Anonymous (2000c). AQIS Quarantine Treatments – Aspects and Procedures (Draft). AQIS: Canberra, Australia. http://www.aqis.gov.au/docs/appolicy/spsaus118ft.doc

Beerwinkle, K.R. and Devaney, J.A. (1983). Control of the northern fowl mite on inanimate objects by fumigation: field studies *Ornithonyssus sylviarum*. Poultry Science Champaign, Poultry Science Association 62(1): 43-46.

Bell, C.H. (1977a). The tolerance of the diapausing stages of four species of Lepidoptera to methyl bromide. Journal of Stored Products Research 13: 119-127.

Bell, C.H. (1977b). Toxicity of phosphine to the diapausing stages of *Ephestia elutella*, *Plodia interpunctella* and other Lepidoptera. Journal of Stored Products Research 13: 149-159.

Bond, E.J. (1984). Manual of fumigation for insect control. FAO Plant Production and Protection Paper No. 54, 432 pp.

Bobrov, L.G. (1982). Storage of potatoes and vegetables under the conditions of Kazakistan, 246 pp. (in Russian).

de Mange, B.P. (1998). Response to questions raised by AQIS concerning Californian table grapes. Letter to Dr Brian Stynes, AQIS from APHIS dated 3 December 1998.

de Mange, B.P. (1999). Response to your questions: grape pests. Letter to Dr T.K. Lim, AQIS from APHIS dated 3 July 1999.

Denterner, P.R., Alexander, S.M., Petry, R.J., O'Connor, G.M., Lester, P.J., Bennett, K.V. and Maindonald, J.H. (1998). Effect of combined methyl bromide fumigation and cold storage treatment on *Cydia pomonella* (Lepidoptera: Tortricidae) mortality on apples. Journal of Economic Entomology 91(2): 528-533.

Green, A. (2000). APHIS' letter to Sharan Singh (AQIS) on USA table grapes dated 10 May 2000.

Gurney, B. and Gandy, D.G. (1974). Methyl bromide for control of bulb scale mites, *Steneotarsonemus laticeps* (Halb.). Plant Pathology 23(1): 17-19.

Lee, H.S. and Wen, H.C. (1980). Field investigation of the acarid bulb mites on onion and their control. Journal of Agricultural Research of China 29(3): 211-218.

Leesch, J.G., Tebbets, J.S., Obenland, D.M., Vail, P.V. and Tebbets, J.C. (1999). Dose-mortality and large-scale studies for controlling codling moth (Lepidoptera: Tortricidae) eggs on "dAgen" plums by using methyl bromide. Journal of Economic Entomology 92(4): 988-993.

Misumi, T., Kawakami, F., Mizobuchi, M., Tao, M., Macida, M. and Inoue, T. (1994). Methyl bromide fumigation for quarantine control of Japanese mealybug and citrus mealybug of Satsuma mandarin. Research Bulletin of the Plant Protection Service, Japan, No. 30: 57-68.

Nave, K. (2000). Clarification of grape bag specifications. Communication from K. Nave, California Table Grape Commission, via John Baker, Produce Marketing Australia to AQIS dated 9 February 2000.

Obbink, B.J. (1998). Letter from California Table Grape Commission to Dr Marion Healy, AQIS dated 17 July 1998.

Petty, G.J. (1987). Control of mealy bugs and scale on pineapples by methyl bromide fumigation of planting material. Phytophylactica 19(3): 255-258.

Pradhan, S. and Govindan, M. (1953). Effect of temperature on the degree of susceptibility of insects to fumigation. Indian Journal of Entomology 15: 362-375.

Standing Committee on Agriculture and Resources Management (1996). Code of Practice for Management of Queensland Fruit Fly (Prepared by the Interstate Plant Health Regulation Working Group).

Terauds, A., Ireson, J.E., Rapley, P.E.L. and O'Loughlin, J.B. (1978). Post-harvest disinfestation of apples of the light-brown apple moth *Epiphyas postvittana*. Australian Journal of Experimental Agriculture and Animal Husbandry 18(91): 313-317.

Yokoyama, V.Y., Miller, G.T. and Crisosto, C.H. (1999). Low temperature storage combined with sulfur dioxide slow release pads for quarantine control of omnivorous leafroller *Platynota stultana* (Lepidoptera: Tortricidae). Journal of Economic Entomology 92 (1): 235-238.

_ EMBED
Word.Pict

3 RISK OF INTRODUCTION OF PIERCE'S DISEASE

3.1 Background to AQIS Consideration of Pierce's Disease Risk on Californian Table Grapes

AQIS has been concerned with the risk of introduction of Pierce's disease (PD) with table grape imports from California since the early 1990s. In May 1991, at a meeting with the APHIS, USDA Area Director Oceania Mr T.H. Russell, the then AQIS Senior Assistant Director (Plant Quarantine and Inspection Branch) Mr A. Catley, raised AQIS concerns over the occurrence of PD on table grape bunches from California (Catley, 1991). At this meeting, APHIS agreed to assess the likelihood of the transmissibility of PD via table grape bunches and indicated that they would seek AQIS approval of their research proposal in due course.

AQIS received the research proposal from APHIS in April 1992: "Determination of the concentration of viable *Xylella fastidiosa* and the efficiency of its vector transmission from grapevines with Pierce's disease after various intervals of transit storage conditions". AQIS accepted the research proposal as it was found to be technically sound (Singh, pers. comm., 1992).

In February 1993, APHIS provided a copy of the research results entitled "Report of research. Infectivity of harvested grape cluster for Pierce's disease spread. Department of Entomological Sciences, University of California, Berkeley" by Dr Alexander Purcell, which was later published (Purcell and Saunders, 1995). Subsequently, AQIS circulated to all states, a Quarantine Circular Memorandum (Plants), QCMP 1993/12 in February 1993 seeking comments on the quarantine status of PD on table grapes imports from California (Dean, pers. comm., 1993). The QCMP included a copy of the research results of Dr Purcell. Responses were received from Queensland, Western Australia and South Australia. Queensland (Currey, pers. comm., 1993) and Western Australia (Brogan, pers. comm., 1993) supported AQIS decision to remove PD from quarantine concerns associated with the import of Californian table grapes. South Australia concurred that the risk of introducing PD was relatively low provided that no live and infected PD vectors were introduced with any such import (van Velsen, pers. comm., 1993). Subsequently, AQIS advised APHIS that PD was no longer a quarantine concern with imports of Californian table grapes to Australia.

3.2 Introduction to Pierce's Disease

PD has existed in California since it was first identified in the late 1880s. It is blamed for the eventual destruction of winegrape industries that flourished in Southern California until the late 19th century. PD has caused significant economic losses since it was first identified (Medley, 1998).

PD is caused by the bacterium, *Xylella fastidiosa*, and is spread by certain kinds of leafhoppers known as sharpshooters. PD is present from North America, through Central America and has been reported from some parts of northwestern South America. Recently, grapevines with symptoms of PD in Kosovo, Yugoslavia, were confirmed as harbouring grape virulent strains of *X. fastidiosa* (Berisha *et al*., 1996). It is present in some California vineyards every year, with the most dramatic losses occurring in the Napa Valley and in parts of the San Joaquin Valley. In Florida and other southeastern states, PD has precluded commercial production of European grape (*Vitis vinifera*) varieties, but some muscadine grapes and hybrids of American wild grape species with European grapes are tolerant or resistant to PD.

X. fastidiosa is limited to the xylem (the water conducting vessels of the plant) (Medley, 1998). *X. fastidiosa* is a small (0.25-0.50µm in diameter × 1.0-4.0µm in length), gram negative, nutritionally fastidious bacterium. For distribution, prevalence, host range, biology and epidemiology of the disease refer to the attached data sheet in Attachment 5.

In the Final IRA for Californian table grapes, AQIS did not stipulate any mandatory phytosanitary measures against the introduction of the causal organism of PD via the table grape importation pathway as table grape fruit clusters are not an epidemiological source of the inoculum for PD. This decision is based on the following points which takes into account the biology of the organism, the epidemiology of the disease and its interaction with vectors and potential vectors. Nonetheless, mandatory phytosanitary measures are required to mitigate the risk of introduction of the vectors of the disease.

3.3 Risk of Table Grape Fruit Clusters as Source of Inoculum

Table grape fruit clusters are not considered an epidemiological source of inoculum of *X. fastidiosa* for the following reasons:

- the spread (transmission and dispersal) of *X. fastidiosa* is through propagative material and vectors, not grape clusters;
- eggs of vectors (sharpshooters) are not laid on grape clusters;
- feeding preferences of vectors (sharpshooters) are not grape clusters;
- sharpshooter vectors are easily disturbed and unlikely to occur on harvested grape clusters as hitch-hikers;
- the concentration of *X. fastidiosa* in grape clusters is very low;
- grape clusters showing PD symptoms are not likely to be harvested;
- methyl bromide would be efficacious against the vectors;
- survival of the *X. fastidiosa* is low under normal in-transit cold storage regimes;
- likelihood of inoculum bearing fruit being fed upon by potential Australian insect vectors is extremely low; and

. the likelihood of establishment of PD through transmission by potential Australian insect vectors is low.

Each of the above reasons why PD is unlikely to be introduced and establish in Australia are discussed further in this paper.

Spread (transmission and dispersal) of X. fastidiosa is through propagative material and vectors, not grape clusters

Epidemiologically, *X. fastidiosa* is transmitted from vine to vine and dispersed via its vectors or by graft transmission. Over a greater range, propagative material is the pathway by which *X. fastidiosa* may spread (Smith *et al*., 1997). *X. fastidiosa* is not transmitted via contaminated pruning shears or by seed transmission (Smith *et al*., 1997; Varela, 2000).

Within the Americas, many genera of sharpshooters and spittlebugs serve as vectors of *X. fastidiosa* (Goheen and Hopkins, 1988). However, in California, the major vectors are the blue-green sharpshooter (BGSS, *Graphocephala atropunctata*), glassy-winged sharpshooter (GWSS, *Homalodisca coagulata*), green sharpshooter (GSS, *Draeculacephala minerva*), and the red-headed sharpshooter (RHSS, *Carneocephala fulgida*) (Gubler *et al*., 1999; Purcell, 1999b; Varela, 2000). Spittlebug vectors of Pierce's disease have been recorded in California (Delong and Severin, 1950), but none have been found on grapevines in California (Severin, 1950). Other sucking insects such as grape leafhoppers, are not vectors in California (Gubler *et al*., 1999).

The principal PD vectors in the Central Valley of California are the GSS and the RHSS (Purcell, 1999d; Purcell, 1999e). GSS and RHSS are also present in coastal areas but are more important as vectors of this disease in the Central Valley (Gubler *et al*., 1999). The BGSS is the most important vector in coastal areas (Purcell, 1999a).

A new PD vector, GWSS, has recently become established in southern California in the counties of Kern, San Bernardino, Santa Barbara, Ventura, Los Angeles, Orange, Riverside and San Diego (Anon., 2000b). This vector is a serious new threat to California vineyards because it moves faster and over greater distances into vineyards than the other species of sharpshooters (Gubler *et al*., 1999). Work conducted by Purcell and Saunders (1995) demonstrated the efficiency in transmitting PD by the GSS was 24% from vine to vine. Its role as a PD vector was based on the consistent occurrence of its breeding habitats near vineyards (Purcell, 1999d).

The most efficient vector of PD in California is the BGSS which possesses the ability to transmit the bacterium with approximately 88 to 90% efficiency from vine to vine (Purcell and Saunders, 1995; Varela, 2000). Purcell (2000) confirmed the efficiency of the BGSS and stated "We have done some preliminary trials of GWSS as a vector of Xf [*X. fastidiosa*] from vine to vine. It is clearly much less efficient than the blue-green sharpshooters used in our tests, perhaps only 20% as efficient".

Reports on the efficiency of the RHSS are not available, although it is likely that the efficiency would be similar to the GSS (Purcell, 1999e). Like the GSS, it was only rarely seen feeding on grapevines. Its role as a PD vector was based on the consistent occurrence of its breeding habitats near vineyards.

Purcell and Saunders (1995) conducted tests to determine the potential for California's most efficient vector (BGSS) of PD to acquire and transmit PD from grape clusters taken from PD-infected grapevines. Their studies also showed that the vectors, GSS and BGSS, could not transmit the bacteria to grapevines after six hours access feeding on fruit clusters from grapevines showing symptoms of PD. However, under similar trial conditions, 88% of GSS and 24% of BGSS transmitted the disease to grapevines after six hours access feeding on foliage of diseased vines. The results of their studies clearly demonstrated that no acquisition or transmission of the bacteria occurred from table grape fruit clusters. Furthermore, they noted that the highly artificial conditions in the studies were designed to maximise potential transmission and that in the field it would be unlikely that sharpshooters would feed at all on grape clusters as they do not provide the levels of nutrients and fluids required by sharpshooters.

APHIS, USDA (Green, pers. comm., 2000) has clarified that the following trading partners identify *X. fastidiosa* as a quarantine pest in propagative material, but not in fresh fruit. They are: Argentina, Brazil, Canada, Cook Islands, French Polynesia, Hungary, Indonesia, Madagascar, Malta, Mexico, New Caledonia, Paraguay, Switzerland, Tunisia, Uruguay, and the Inter-African group consisting of 30 African countries. For the European Union, table grape fruit are unrestricted but *Vitis* propagative material is prohibited. *X. fastidiosa* is identified as a prohibited pest but only for propagative material. For Chile, *Vitis* tissue cultures and all propagative material must be free from PD. Chile has very specific requirements for table grape fruit but neither the disease nor any vectors are listed to be of quarantine concern.

Hence, it is evident that harvested grape clusters are unlikely to serve as an epidemiological source of inoculum for vector spread of PD.

Eggs of vectors (sharpshooters) are not laid on grape clusters

Adults of BGSS overwinter mainly in riparian (riverbank) habitats, but also may be distributed at low density in areas with trees and shrubs. Eggs are laid singly in green tissues of leaves and stems beginning in April, depending on temperature (Guidicipietro, pers. comm., 2000; Varela, 2000). Most adults (80-90%) breed in riparian areas, hence the majority of the eggs are laid on riparian plants. Adults which have started to migrate will lay their eggs in vines at the edge of the vineyard. Their dispersal into the vineyard increases as natural vegetation dries up. Most overwintered adults die by the end of June (Varela, 2000).

GWSS overwinters as an adult and begins to lay egg masses in late February through May. The first generation matures as adults from late May through to late August. A

second generation of egg masses is laid starting in mid-June through to late September, which develop into overwintering adults (Anon., 2000a). Eggs of GWSS are laid side by side in clutches of 1-27, with the average mass consisting of 10 eggs (Guidicipietro, pers. comm., 2000). The eggs are laid under the lower epidermis of leaves, giving a greenish, water-blistered appearance (Guidicipietro, pers. comm., 2000). It is only on citrus fruits that egg masses may be deposited into the fruit rind when populations are high, and not on grape clusters.

GWSS probably first entered California as eggs on plants (Purcell, 1999c). GWSS egg masses were first detected on lemon trees in Ventura County in 1993 (Guidicipietro, pers. comm., 2000). Immature lemon fruit could be an oviposition host for GWSS. Old egg masses were found when the fruit were young. However, in work conducted by the University of California Cooperative Extension (Ventura County), 23,000 mature lemon fruit were examined and no viable egg masses were found. The California Department of Food and Agriculture (CDFA) concluded that mature citrus fruits, including lemons, do not pose an introduction potential for GWSS (Guidicipietro, pers. comm., 2000).

Both GSS and RHSS have three generations per year (Purcell, 1999b; Purcell, 1999d; Purcell, 1999f). They feed primarily on grasses. GSS breeds and feeds on water grass (*Echinochloa crusgalli*), Bermuda grass (*Cynodon dactylon*), perennial rye (*Lolium perennae*), and fescue grass (*Festuca* spp.). RHSS breeds and feeds primarily on Bermuda grass. They are found on grasses in wet spots, sump ponds, irrigation ditches, irrigated pastures or where the growth of grasses is lush and continuous all year. Adults stop reproducing in the autumn and begin to lay eggs in grasses as soon as temperatures are warm enough, usually in January or February.

From the above research it is apparent that eggs of all sharpshooters are laid on leaves or stems, and not on or among table grape fruit clusters. As consignments of table grapes from California to Australia are not permitted to have leaf material in the consignments, the risk of entry of larvae or eggs of the sharpshooters would be negligible.

Feeding preferences of vectors (sharpshooters) are not grape clusters

Purcell and Saunders (1995) noted in their studies referred to in the previous section, that the highly artificial conditions in the trial were designed to maximise transmission of *X. fastidiosa* from grapes. They further noted that in the field it would be unlikely that sharpshooters would feed at all on grape clusters, as they do not provide the levels of nutrients and fluids required. Their studies confirmed earlier studies (Purcell, 1975) that fruit clusters were not attractive feeding sites for the sharpshooter vectors of the disease as they were essentially xylem feeding suctorial insects.

BGSS has been reported as preferring green succulent growth, as the insects shift their feeding preference during the season to sites where succulent growth is available (Gubler *et al*., 1999). Like other xylem-feeding insects, BGSS prefers new growth on plants that are in a succulent condition. This was probably the major reason why they preferred riparian areas in California (Purcell, 1999a).

GWSS are reported to feed much lower on the cane than other sharpshooters in California. They will also feed on the larger (basal) stems of plants (Anon., 2000a), and even on dormant grapevines during winter (Purcell, 1999a).

RHSS and GSS are both reported to feed on succulent grasses, with the RHSS preferring slightly drier conditions as opposed to the GSS (Purcell, 1999d; Purcell, 1999f). It has been reported that RHSS and the GSS rarely feed on grapes (Purcell, 1999d; Purcell, 1999f).

To date no reports have suggested that the feeding preferences of sharpshooters include grape bunches. Purcell (2000) has stated "Grape bunches are a very low preference item for sharpshooter feeding, even more so when detached or even slightly dried in storage".

Sharpshooter vectors are easily disturbed and unlikely to occur on harvested grape clusters as hitch-hikers

Sharpshooters typically fly away when disturbed in the vicinity of fruit during picking. It is extremely unlikely that any sharpshooters would be associated with packed table grapes. In the event of any hitch-hikers on packed clusters, methyl bromide and sulfur dioxide treatment will eliminate them (See Section 1 for details on efficacy data on fumigation treatments). Consequently, the risks associated with vectors, such as the GWSS, feeding or hitch-hiking on consignments of table grapes would be negligible.

The concentration of X. fastidiosa in grape clusters is very low

Purcell and Saunders (1995) conducted trials to determine the efficiency of vectors in transmitting PD from grape clusters taken from PD-infected grapevines. As part of this work the authors investigated the incidence and fate of *X. fastidiosa* levels in the grape clusters exhibiting severe symptoms. Their research revealed that "isolations of *X. fastidiosa* from cluster stems and rachises were successful in only 5 of 24 samples [approximately 20%] 1 day after harvest. Concentrations of *X. fastidiosa* isolated from stems of diseased clusters were about 10 to 100 times lower than typical concentrations in grape petioles or leaf veins".

Grape clusters showing PD symptoms are not likely to be harvested

The likelihood of harvesting infected grapes showing PD symptoms for export or domestic markets is very low. The symptoms encountered, and defined as indicative of PD infection in the vine, include the shrivelling and raisining of, part of, or all of the fruit clusters above the site of infection (Gubler *et al*., 1999). Consequently, infected grape clusters on diseased vines would be shrivelled, dried, deformed, under-sized and would not be harvested because of low commercial quality.

Further to this, diseased vines would be unthrifty and would not produce any commercial yield of significance and would be removed as part of disease management practices

(Gubler *et al*., 1999). The symptoms of PD are produced by infections of the bacteria forming dense aggregates within the xylem vessels. These aggregates, along with gums and tyloses produced by the grapevine, restrict vascular flow of the xylem (Goheen and Hopkins, 1988).

Consequently, the likelihood of packing and exporting fruit with Pierce's disease would be very low.

Methyl bromide would be efficacious against the vectors

In the event of any sharpshooter hitch-hiking on packed bunches, methyl bromide treatment, as required by AQIS for Californian table grape imports, will eliminate these vectors (See the final IRA and Section 1 of this paper for details on efficacy data of methyl bromide fumigation treatments). Consequently, the risks associated with sharpshooter vectors, such as the GWSS, being introduced on treated table grape consignments from California would be very low.

Survival of X. fastidiosa is low under normal in-transit cold storage regimes

Harvested grapes are normally stored at 1-4^{o}C to maximise shelf-life. Wills *et al*. (1998) reported that grapes achieved a maximum storage life of four to six weeks if stored at this temperature range.

Research conducted by Purcell and Saunders (1995) in California indicated that levels of *X. fastidiosa* in infected grape clusters declined sharply after cold storage at 4°C. The bacterium was not recovered by dilution plating technique from infected grapes after 21 days of storage at this temperature.

Consequently, survival of *X. fastidiosa* in consignments of inoculum bearing table grapes, if any, would be very low at this cold storage regime.

Likelihood of inoculum bearing fruit being fed upon by potential Australian insect vectors is extremely low

The likelihood of xylem-feeding insects feeding on discarded grape bunches or their stems and stalks, is extremely low. Purcell (2000) stated "Discarded grape bunches or their stems and stalks would be no real threat for spittlebugs or sharpshooters picking up Xf [*X. fastidiosa*], as they have no proclivity to feed on such material and would only do so if there were no alternative available". Purcell goes further to state "the population levels of Xf [*X. fastidiosa*], in bunches from Pierce's diseased vines are very low to begin with, as assessed by the lack of transmission by an efficient vector and culturing. These [levels] drop even further in storage. Finally, xylem-feeders would not be likely to feed on even slightly desiccated bunches. I think grape bunches would not be a threat for introducing Xf [*X. fastidiosa*]."

Consequently, it is unlikely that Australian xylem-feeding insects would feed on discarded grape clusters as they have similar nutritional requirements to xylem-feeders in California.

Additional to considerations of xylem-feeders preferences, natural decay of discarded bunches or their stems and stalks results in the discarded material being swiftly colonised by fungal and bacterial decay organisms (such as *Penicillium*, *Aspergillus* and *Botrytis* species). These species would also be saprophytically competitive towards any residual *X. fastidiosa* bacteria that might remain if any at all.

Likelihood of establishment of PD through transmission by potential Australian insect vectors is low

Purcell, in two articles (Purcell, 1989; Hill and Purcell, 1995) stated, "Most if not all xylem-feeding suctorial insects [order Hemiptera] are potentially Pierce's disease vectors". This statement, which has been widely quoted, is considered by AQIS to be an overstatement and simplification of the situation as many other features of the biology of any xylem-feeding insect will determine whether it is a vector or not of PD.

In the Americas, all known insect vectors of PD are confined to the order Hemiptera. More specifically, insects from the families Cicadellidae and Cercopidae are capable of transmitting the PD bacterium (Purcell *et al*., 1979; Varela, 2000). Insects from these families are commonly called sharpshooters or leafhoppers [Cicadellidae] and spittlebugs or froghoppers [Cercopidae].

In the family Cicadellidae, all vectors belong to the tribe Cicadellini, specifically the genera *Draeculacephala*, *Carneocephala*, *Graphocephala* (= *Hordnia*) and *Homalodisca*. These genera have been reported to be the most important vectors in the spread of PD in the United States of America (Purcell, 1989; Purcell *et al*., 1979). None of these genera are reported as occurring in Australia (Fletcher, pers. comm., 2000). Of the 14 species of Cicadellini in Australia (Day and Fletcher, 1994), none have been recorded on Vitaceae.

In the family Cicadellidae, the most likely candidates for transmission of PD in Australia are species in the genus *Ishidaella* as they are known to have broad host ranges. However, species in this genus have never been recorded on Vitaceae in Australia; hence it is believed that the risk of transmission of PD is negligible. Other Australian leafhoppers in the subfamilies Typhlocybinae, Agallinae, Iassinae and Deltocephalinae are unlikely to be vectors since Typhlocybinae are parenchyma feeders. The remaining subfamilies are phloem feeders and are less likely to transmit diseases of this type.

In the family Cercopidae, there are four spittlebug species that have been reported to be vectors of *X. fastidiosa* causing PD on grapevines and alfalfa dwarf on alfalfa in North America (Hewitt *et al*., 1949; Severin, 1950; Purcell *et al*., 1979). The four species are *Aphrophora angulata* (alfalfa), *Aphrophora permutata* (alfalfa), *Clastoptera brunnea* (alfalfa) and *Philaenus leucophthalmus* (grapevines and alfalfa), but none have been found on grapevines in California (Severin, 1950). Severin (1950) conducted tests on the

four species listed above for natural infectivity on healthy grapevines. Only *Philaenus leucophthalmus* showed some natural infectivity in transmitting the PD bacterium to grapevines; the remaining species all failed to cause infection on healthy grapevines. None of these species or genera are reported as occurring in Australia. Of the nine species of Cercopidae in Australia (Carver *et al*., 1991), none have been recorded on Vitaceae (Carver, pers. comm., 2000). One species, *Euryaulax carnifex*, is known to infest sugar cane (Carver *et al*., 1991). This implies that the importance of this family as a vector, when compared to the sharpshooter family, is relatively minor.

Cicadas (family Cicadidae) are also xylem feeders but so far have not been reported to be vectors.

All of the insects mentioned above can be considered as potential vectors of greater or lesser importance. However, in order for establishment of PD to occur in Australia, these potential vectors must acquire the bacterium from a host plant.

None of these potential vectors are known to be economically significant or feed on Vitaceae spp. (Carver, pers. comm., 2000). Hence, the feeding on discarded grape clusters by xylem-feeding or phloem-feeding insects would be exceptionally unusual. Additionally, in points covered previously, the likelihood of the bacterium being present in the discarded grape clusters has already been demonstrated to be negligible.

Consequently, the likelihood of these potential vectors acquiring the disease from discarded grape clusters would be extremely unlikely. Therefore, the potential for the transmission of PD in Australia by Australian vectors is remote.

3.4 Conclusion

In the Final IRA, AQIS did not recommend any specific phytosanitary measures to address the risks of PD being imported via the pathway of fresh table grapes from California. AQIS reached this decision based upon consideration of several important factors. The factors considered included the biology of the bacterium *X. fastidiosa*, the consideration that grape clusters showing PD symptoms are not likely to be harvested and survival of *X. fastidiosa* is low under normal in-transit cold storage regimes. Additional considerations included potential sharpshooter vectors of *X. fastidiosa*, the feeding preferences of vectors are not grape clusters, the eggs of vectors are not laid on grape clusters, and that methyl bromide would be efficacious against the vectors. AQIS also considered potential Australian vectors, the extremely low likelihood of inoculum bearing fruit being fed upon by these insects, and the low probability of establishment of PD through transmission by these insects.

AQIS is satisfied that scientific evidence shows that harvested grape clusters do not serve as an epidemiological source of inoculum of *X. fastidiosa*. This is further reinforced by the phytosanitary measures of other countries which serve to protect against the

introduction of PD on propagative material, but do not consider grape clusters. None of these countries have reported incidences of PD in their grapevines to date.

3.5 References

Anonymous (2000a). Background: questions and answers. http://www.cfbf.co/m GWPDBack.htm

Anonymous (2000b). Distribution of GWSS in California. http://plant.cdfa.ca.gov/gwss/gwmap.htm

Berisha, B., Chen, Y.D., Xu, B.Y. and Chen, T.A. (1996). Isolation of Pierce's disease bacteria from grapevines in Europe. Phytopathology 86: 119.

Brogan, N.M. (1993). E-mail communication with Sharan Singh on comments in respect to QCM 1993/12 Pierce's disease of grapes dated 15 March 1993.

Carver, M. (2000). Clarification of spittlebug species in Australia. Australian National Insect Collection (ANIC), CSIRO Entomology, Canberra ACT, 15 June 2000.

Catley, A. (1991). Californian grapes to Australia. Letter to Mr T.H. Russell, APHIS from AQIS dated 21 August 1991.

Carver, M., Gross, G.F. and Woodward, T.E. (1991). Hemiptera (bugs, leafhoppers, cicadas, aphids, scale insect etc.). pp. 429-509. In: Naumann, I.D. (chief editor). Insects of Australia (2nd edition). Volume 1. Melbourne University Press: Carlton, Australia 542 pp.

Currey, D.W. (1993). Response to Quarantine Circular Memorandum 1993/12. Letter to the Senior Assistant Director, AQIS from AQIS Queensland dated 5 March 1993.

Day, M.F. and Fletcher, J.J. (1994). An annotated catalogue of the Australian Cicadelloidea (Hemiptera: Auchenorrhyncha). Invertebrate Taxonomy 8: 1117-1288.

Dean, J. (1993). Risk of establishment in Australia of Pierce's disease through imported table grapes. Letter to Chief Quarantine Officers (Plants), AQIS from AQIS Plant Quarantine and Inspection Branch dated 23 February 1993.

Fletcher, M. (2000). Occurrence of sharpshooters in Australia. Orange Agricultural Institute, NSW Agriculture, Orange NSW.

Goheen, A.C. and Hopkins D.L. (1988). Pierce's Disease. pp. 44-45. In: Pearson, R.C. and Goheen, A.C. (eds). Compendium of Grape Diseases. The American Phytopathological Society (APS) Press: St Paul, Minnesota, USA 93 pp.

Goheen, A.C., Nyland, G. and Lowe, S.K. (1973). Association of rickettsia-like organism with Pierce's disease of grapevines and alfalfa dwarf and heat therapy of the disease in grapevines. Phytopathology 63: 341-345.

Green, A. (2000). APHIS letter to Sharan Singh (AQIS) on USA table grapes dated 10 May 2000.

Gubler, D., Stapleton, J., Leavitt, G., Purcell, A., Varela, L. and Smith, R.J. (1999). UC Pest Management Guidelines. http://www.ipm.ucdavis.edu/PMG/r302101211.html

Guidicipietro, M. (2000). APHIS response to AQIS questions raised at the bilateral technical meeting in Canberra on 17-18 May 2000.

Hewitt, W.B., Frazier, N.W. and Freitag, J.H. (1949). Pierce's disease investigations. Hilgardia 19(7): 207-264.

Hill, B.L. and Purcell, A.H. (1995). Acquisition and retention of *Xylella fastidiosa* by an efficient vector, *Graphocephala atropunctata*. Phytopathology 85: 209-212.

Medley, J.C. (1998). Pierce's Disease. http://aesrg.tamu.edu/Grapes/PierceDis.htm

Purcell, A.H. (1975). Role of the blue-green sharpshooter, *Hordnia circellata*, in the epidemiology of Pierce's disease of grapevines. Environmental Entomology 4: 745-752.

Purcell, A.H. (1980). Environmental therapy for Pierce's disease of grapevines. Plant Disease 64(4): 388-390.

Purcell, A.H. (1989). Homopteran transmission of xylem-inhabiting bacteria. In: Advances in disease vector research. Springer-Verlag: New York, USA 6: 243-246.

Purcell, A.H. (1999a). Blue-green sharpshooter.
http://www.cnr.berkeley.edu/xylella/bgss.html

Purcell, A.H. (1999b). General insect category.
http://www.cnr.berkeley.edu/xylella/geninsct.html

Purcell, A.H. (1999c). Glassy-winged sharpshooter.
http://www.cnr.berkeley.edu/xylella/oss.html

Purcell, A.H. (1999d). Green sharpshooter.
http://www.cnr.berkeley.edu/xylella/grnshrp.html

Purcell, A.H. (1999e). Red-headed sharpshooter.
http://www.cnr.berkeley.edu/xylella/rhss.html

Purcell, A.H. (2000). E-mail communications with D. Hunt-Sharman on harvested grape clusters as a quarantine risk to Australia dated 7 April 2000.

Purcell, A.H., Finlay, A.H. and McLean, D.L. (1979). Pierce's disease bacterium: Mechanism of transmission by leafhopper vectors. Science 206: 944-946.

Purcell, A.H. and Saunders, S. (1995). Harvested grape clusters as inoculum for Pierce's disease. Plant Disease 79: 190-192.

Severin, H.H.P. (1950). Spittle-insect vectors of Pierce's disease virus. II. Life history and virus transmission. Hilgardia 19(11): 357-382.

Singh, S. (1992). Pierce's disease proposal – APHIS. Internal minute/conversation record to Mr C. Hood, AQIS dated 22 April 1992.

Smith, I.M., McNamara, D.G., Scott, P.R., Holderness, M. and Burger, B. (eds). (1997). Quarantine Pests for Europe (2nd edition). CAB International: Wallingford, UK 1425 pp.

van Velsen, R.J. (1993). Risk of establishment in Australia of Pierce's disease through imported table grapes. Letter to Mr J. Dean, AQIS from APHIS dated 30 March 1993.

Varela, L.G. (2000). Pierce's disease in the North Coast. http://www.cnr.berkeley.edu/xylella/pd97.html

Wills, R., McGlasson, B., Graham, D. and Joyce, D. (1998). Postharvest: An introduction to the physiology and handling of fruit, vegetables and ornamentals. University of New South Wales Press, Sydney 262 pp.

_ EMBED Word.Pict

4 ATTACHMENT 1 – IMPORT RISK ANALYSIS APPEAL PANEL FINDINGS

IMPORTATION OF TABLE GRAPES FROM THE STATE OF CALIFORNIA IN THE UNITED STATES OF AMERICA

Dear appellant,

IRAAP Decision – Import Risk Analysis on the Importation of Table Grapes From California, USA

As previously advised on 10 March 2000, the Import Risk Analysis Appeal Panel (referred to as IRAAP or the Panel) convened on 22 and 29 March 2000 to consider appeals against the Import Risk Analysis on the Importation of Table Grapes from California, USA (the Table Grape IRA). This letter outlines the IRAAP's consideration of, and decision on, the appeals.

Background

On 13 January 2000 AQIS released the final IRA for the importation of table grapes from California, USA. The Table Grape IRA was conducted using the routine process described in the AQIS Import Risk Analysis Process Handbook (the Handbook). Stakeholders were advised that if, in their opinion, the process outlined in the Handbook had not been properly followed they could appeal in writing to the Director of Animal and Plant Quarantine by COB Monday 14 February 2000.

The IRAAP followed the guidelines established for the conduct of the appeal process. Those guidelines (at Attachment A) were developed with the assistance of legal advice from the Office of the Australian Government Solicitor.

Appellants were notified on 10 March 2000 that the Table Grape IRAAP would comprise Mr Andrew Inglis (Chair), Dr Jim Cullen, Mr Geoff Gorrie and Professor Craig Pearson. Appellants will note that the Chair of the Quarantine and Exports Advisory Council (QEAC), Professor Malcolm Nairn, excused himself from this IRAAP due to a potential conflict of interest. As a consequence, as the Deputy Chair of QEAC, I am Chair of this IRAAP. The Director of Animal and Plant Quarantine, who would normally sit on the IRAAP, excused himself from this IRAAP on the grounds that he had commented on the draft Table Grape IRA in his previous role. The Chief Plant Protection Officer and Deputy Chief Plant Protection Officer also excused themselves from this IRAAP on the grounds that they had been involved in the early stages of the IRA and may have a conflict of interest.

The Panel considered 10 appeals against the criteria outlined in section 4.5 of the Handbook. Section 4.5 states that: "any stakeholder of the opinion that the process

outlined in the Handbook has not been properly followed, including that the risk analysis failed to consider a significant body of relevant scientific or technical information, may appeal to the Director [of Animal and Plant Quarantine]".

Two further letters were received after the cutoff date for appeals. These were not considered formally by the IRAAP. However, the Panel noted that these correspondents raised issues also raised by appellants.

In reaching its recommendations, the IRAAP considered each appeal, the final Table Grape IRA and written briefing supplied by AQIS.

Recommendations

The IRAAP has considered the issues that formed the basis of appeals and has formulated its recommendations under the three broad headings of failure to consider a significant body of relevant scientific or technical information, consultation and transparency.

Failure to consider a significant body of relevant scientific or technical information

The IRAAP noted that several of the appellants had not commented on the draft IRA but had provided new scientific and technical material in their appeals using this as a basis for appeal on the grounds that AQIS "failed to consider a significant body of relevant scientific or technical information". The Panel was concerned that such an approach could be construed as delaying the IRA process and concluded that stakeholders should, wherever possible, take the opportunity to provide such material during the consultation period on a draft IRA rather than as part of an appeal.

Nevertheless, the Panel examined these appeals and concluded that there was no evidence that a significant body of relevant scientific or technical information had not been considered in the Table Grape IRA, nor was there any evidence presented that the Table Grape IRA was inconsistent with Government policy or lacked harmonisation with international standards. As such, appeals under these principles have been dismissed.

Consultation

The IRAAP considered the issues highlighted by appellants regarding consultation and concluded that although aspects of the consultative process demonstrated shortcomings, generally the IRA was conducted as required by the AQIS IRA Handbook on routine IRAs. As such, appeals under this category have been dismissed by the IRAAP.

The IRAAP notes that while the IRA was conducted using the routine process in accordance with the provisions of the Handbook, this process did not provide the opportunity for a level of industry and scientific consultation that would necessarily address all stakeholder concerns. In this regard, the IRAAP draws attention to two areas for improvement:

(a) the need for clear and comprehensive responses in the final IRA to issues raised following circulation of the draft IRA; and
(b) the desirability of a greater emphasis on public meetings of AQIS with industry as part of the consultation process.

The IRAAP also noted that there were unexplained delays in releasing the Pest Risk Analysis in response to a request from one stakeholder.

The IRAAP recommends that its comments regarding consultation be referred to the Quarantine and Export Advisory Council Policy Sub-Group on Managed Risk, for action.

Transparency

The IRAAP has concluded that certain aspects of the IRA appear not to have been conducted in a fully transparent manner. The Panel considers that in certain instances insufficient information is provided in the IRA, or is presented but not in a way which explains and supports how and why certain conclusions were reached. This is not to say that these conclusions are incorrect, but rather that the reader may not be able to reach the same conclusions on the basis of the information presented in particular sections of the IRA. The Panel believes that comments from several appellants demonstrate that further information is required in relation to the following issues:

1. efficacy data for Methyl Bromide treatment; and
2. the risk of introduction of Pierce's disease.

Under these circumstances, the IRAAP has upheld these appeals.

The IRAAP notes that the transparency of the process would have been enhanced if further information had been provided on the effectiveness of the United States Animal and Plant Health Inspection Service inspection and certification procedures.

Action

The IRAAP recommends the following course of action:

1. Within 90 days of the announcement of this decision, AQIS will (a) revise the IRA to improve its transparency and (b) advise stakeholders of this information.

2. The IRAAP, once satisfied that the two outstanding issues have been satisfactorily addressed, and clear explanations provided as to how the respective conclusions were arrived at, will consider that the appeal has been finalised and the Table Grape IRA concluded, at which time the import policy will become effective.

Any further comments that you wish to make on the process conducted by the IRAAP may be directed to me at the address on the first page of this letter.

Yours sincerely

Andrew Inglis
Chair of the IRAAP for the Importation of Table Grapes

30 March 2000

_ EMBED
Word.Pict

ATTACHMENT A

IMPORT RISK ANALYSIS APPEAL PANEL GUIDELINES

An Import Risk Analysis Appeal Panel is convened to consider appeals made by . stakeholders in accordance with the provisions of the IRA Handbook. The sections of the IRA Handbook that relate to convening an IRAAP are 4.5, 4.6 and 4.7.

IRAAP - Chair and Members

The IRAAP, as described in section 4.6 of the IRA Handbook, routinely comprises the Chair of the Quarantine and Exports Advisory Council as the Chair; the Director of Animal and Plant Quarantine, either the Chief Plant Protection Officer or the Chief Veterinary Officer as appropriate to the appeal and one other member of QEAC. The IRAAP membership will, in most cases, be balanced between scientific expertise and other fields of expertise such as economics, business management and communication.

If, when a person is approached to become the Chair or a Member of an IRAAP they believe that there may be a conflict of interest or perception of bias, that person shall declare those interests to the Chair or other Members as appropriate. In all cases, if the Chair or Member should decide to withdraw from the IRAAP, then a replacement is required.

Notification to appellants of the Chair and Members of the IRAAP will be undertaken by the IRAAP Secretariat.

IRAAP Meetings

The IRAAP must meet at least once during the course of the appeal process, in person. Follow up meetings may be in person if this is convenient or by way of teleconference.

Administrative arrangements

All papers associated with the appeal, including all correspondence with appellants, will be coordinated by the IRAAP Secretariat located in the Executive Secretariat of AFFA.

Conduct of Chair and Members

In considering an appeal, the Chair and Members of the IRAAP, wherever possible, should not make judgements on scientific issues that are within the scope of the actual appeal(s). The IRAAP's primary role is to review the process and will consider issues on a case by case basis.

The Chair of the IRAAP is the sole point of contact for appellants, or other parties. Any queries regarding the conduct of the IRAAP or deliberations or decisions made by the

IRAAP, must be directed to the Chair of the IRAAP, in writing, via the IRAAP Secretariat.

Dismissal of an appeal

Dismissal of an appeal requires majority support of the IRAAP. The Chairman will not exercise a casting vote.

Oral submissions to the IRAAP

The IRAAP will not consider oral submissions from any applicant unless the applicant can demonstrate to the IRAAP that they cannot present their case adequately by way of written submission.

45 Day period to hear appeals

The IRAAP will endeavour to consider appeals within the 45 day time period outlined in the IRA Handbook. Should circumstances arise where this may not be possible, eg volume of appeals received, then the Chairman of the IRAAP will write to appellants advising them of the delay and where possible, the date the IRAAP will conclude its deliberations and release its recommendations.

Consideration of appeals

The IRAAP will consider in detail the appeals provided, the final draft IRA and any written factual briefing from AQIS that may be requested by the IRAAP.

Announcement of decision

The IRA Handbook advises that if the appeal is upheld, the IRAAP refers its conclusion to the AQIS team or Risk Analysis Panel (in the case of non-routine IRAs) for rectification of the deficiency in the process. If the appeal is dismissed the policy is adopted.

The IRAAP Chair should formally notify the appellants, AQIS and the Minister and any other relevant interested parties of the IRAAP decision within 3 working days of the final decision being taken.

As mentioned previously, the Chair of the IRAAP is the sole point of contact for appellants, or other parties regarding the decision(s) of the IRAAP.

Action arising from a decision to uphold an appeal

Within a specified period of time of the announcement of the decision to uphold an appeal, AQIS is to advise the Chair of the IRAAP, in writing, of the action taken to remedy the issues raised.

5 ATTACHMENT 2 – APPELLANTS AND THEIR ADDRESSES

No.	Issue	Appellant Number	Total Number of Appeals
1	Efficacy data for methyl bromide treatment	1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 12	11
2	Risk of introduction of Pierce's disease	2, 3, 4, 5, 7, 8, 10	7

1 – Mareeba District Fruit & Vegetable Growers Association (Chairman: Mr Joe Moro)
PO Box 989
Mareeba QLD 4880
Appealed Issue No. 1

2 – South West Table Grape Growers Association Inc., Western Australia (President: Mr Roger Fahl)
RMB 3380
Harvey WA 6220
Appealed Issue No. 1, 2

3 – Australian Table Grape Association (Chairman: Mr Brian Woodford)
PO Box 746
Mildura VIC 3502
Appealed Issue No. 1, 2

4 – Dept. of Primary Industry and Fisheries (Director Horticulture: Dr N.R. Dasari)
Northern Territory Government
PO Box 990
Darwin NT 0801
Appealed Issue No. 1, 2

5 – Graham L. Hamdorf (Director/Partner)
E mail : hamdorf@vic.ozland.net.au
Appealed Issue No. 1, 2

6 – Far North Queensland Grape Industry Consultative Committee (Mrs Lucy Picco)
PO Box 33
Dimbulah QLD 4872
Appealed Issue No. 1

7 – Grape Growers Association of Western Australia (President: Mr Matt Katich)
PO Box 15
Midland WA 6936
Appealed Issue No. 1, 2

8 – Agriculture Western Australia (Chief Executive Officer: Mr G.A. Robertson)
3 Baron-Hay Court
South Perth WA 6151
Appealed Issue No. 1, 2

10 – National Vine Health Steering Committee (Chairman: Dr Bill Roberts)
PO Box 647
Magill SA 5072
Appealed Issue No. 1, 2

11 –Mr Peter Cottrell (Director)
Cottrell Farms Pty Ltd
Pilot Farm Road
Emerald QLD 4720
Appealed Issue No. 1

12 – New South Wales Agriculture (Director General: Mr K.P. Sheridan)
161 Kite Street
Locked Bag 21
Orange NSW 2800
Appealed Issue No. 1

_ EMBED
Word.Pict

6 ATTACHMENT 3 – ISSUES RAISED BY APPELLANTS ON THE EFFICACY DATA FOR METHYL BROMIDE TREATMENT

Appellant No. 1 & 6
Written data that methyl bromide used in combination with other phytosanitary measures will provide security against the introduction of this pest (Hemiptera) should be available.

There is no data stating these treatments will be effective on all pests and diseases occurring in California.

This disinfestation treatment data is not available [as such we demand] this treatment be disallowed. Efficacy data from other countries is not relevant to this application of importing fresh table grapes from California and is only relevant to those countries…

Appellant No. 2
The IRA states "no efficacy data against these specific pests"…the levels of treatment by methyl bromide are grossly inadequate to treat mites.

Appellant No. 3
The methyl bromide treatment will not affect pathogens, which are located in stems or stalks. This could include fungi such as *Monilinia fructicola* and powdery mildew, which are prolific producers of wind borne spores. This is a possible pathway for entry of these types of organisms and needs consideration.

Further analysis is also required of whether methyl bromide will be effective against all life stages of sharpshooters which are vectors of Pierces Disease, particularly the Glassy-Winged Sharpshooter which has only recently being described in California.

Treatment with methyl bromide will not in any way guarantee freedom from viral, bacterial or most fungal diseases.

Methyl bromide resistant… and gall insects may survive treatment and be undetected.

The Orange tortrix (*Argyrotaenia citrana*), the vine mealybug (*Planococcus ficus*) and the omnivorous leaf roller (*Platynota stultana*) are all noted as high-risk level quarantine pests (IRA table 1 page 11). AQIS proposes treatment with methyl bromide. However, AQIS admits "that efficacy data for methyl bromide against the specific pests identified in the IRA is not available" (IRA page 70). Despite this, AQIS proposes that the only protection against these high-risk level quarantine pests is a treatment for which efficacy data is not available and for which no scientific evidence is advanced in support.

AQIS also failed to properly investigate scientific information on the GWSS and control measures for this pest. Significantly, AQIS was not able to identify any credible

scientific evidence to support the view that the proposed control method (pre-shipment or post arrival fumigation with methyl bromide) was efficacious in eradicating GWSS eggs. The grape-leaf bud mite (*Colomerus vitis* – strain C) the grapevine mealybug (*Pseudococcus maritimus*) and the Pacific spider mite (*Tetranychus pacificus*) are all pests which are categorised as medium level risk quarantine pests. However, the same comments regarding the lack of scientific evidence to support the efficacy of methyl bromide fumigation apply equally to these pests.

The IRA identifies a variety of fungal, bacterial and viral pathogens but failed to address the risks associated with them in any satisfactory way or at all. While methyl bromide treatment was again proposed as a control measure AQIS failed to present any sufficient scientific evidence to support this as an effective treatment to ensure freedom from viral, bacterial or most fungal diseases.

The process under the SPS Agreement is described by the SPS Agreement as being scientifically-based. However, when AQIS admits that "efficacy data for methyl bromide against the specific pests identified in the IRA is not available" (IRA page 70) this is a clear indication of an absence of a scientific base for the conclusion by AQIS that methyl bromide is in all cases an effective phytosanitary measure to control the broad spectrum of pests in question.

The lack of scientific information regarding the efficacy of methyl bromide fumigation to control the quarantine pests listed above, or the efficacy of methyl bromide as a control agent generally, is a serious failure in the scientific process.

AQIS failed properly or at all to estimate the introduction potential of relevant insect pests and diseases in Australia and, in particular, failed to consider factors which might affect the likelihood of entry into Australia of relevant insects, other pests and disease including the lack of efficacy of the proposed treatment by methyl bromide of imported grapes.

Appellant No. 4
Methyl bromide (MB) fumigation, it is well known that this procedure has limitations in efficacy against many internal (as distinct from surface living) pests. This is why, until it was withdrawn, ethylene dibromide was used rather than MB in fumigating against fruit flies. These pests lay eggs beneath the fruit skin and the larvae feed within the fruit.

In Appendix 3, the 'Life history' section under GWSS notes that eggs are normally laid just under the lower leaf epidermis of host plants, but eggs are deposited in fruit if populations are high. This makes it clear that GWSS eggs must be considered as 'internal insects' and that consequently, unless and until acceptable experimental evidence is forthcoming, the efficacy of MB fumigation against them cannot be accepted. This is contrary to the finding (page 15) that methyl bromide disinfestation is determined to provide an appropriate level of protection against the introduction of medium- and high-risk quarantine pests.

Appellant No. 5
Even with the use of chemicals such as methyl bromide… it would be almost impossible to have 100% kill of all insects.

Appellant No. 7
The level of fumigation …inadequate to control certain pests and disease. Certainly it has been proven that the indicated rates by AQIS to date would not be an effective control and hence not acceptable.

Appellant No. 8
Both the Draft and Final IRA have assumed that fumigation will be efficacious against the target pests. AQIS admit that there is no efficacy data for the specified pests in the IRA. AQIS goes on to state in the Final IRA that "…AQIS and quarantine agencies from other countries have been using this fumigant on similar pests and similar commodities for a long enough period to build up a significant amount of historical data which supports its efficacy." None of this historical data is presented to stakeholders for consideration. In fact there is considerable evidence to show that many quarantine pests are not killed by the rates specified in the IRA.

AQIS Quarantine Circular Memorandum (Plants) (QCM(P)) 1990/32 in respect to proposed measures for strawberry fruit from the USA states "During October 1989 following the failure of the methyl bromide treatment used by Australian quarantine to kill all mites and insects on fresh Californian strawberries further imports were suspended pending a review of the quarantine risks and treatment options." The treatment that failed was 32g/m^3 for 2 hours at 21°C, the same as the rate proposed in the IRA.

The QCM(P) goes on to say that in respect to NZ strawberries the fumigation rate was increased from 32g/m^3 for 2 hours to 32g/m^3 for 3 hours with a CT product of 96ghr/m^3. Information provided by the US showed that western flower thrip are killed by fumigating with methyl bromide at the rate of 48g/m^3 (CT 96 ghr/m^3) for 2 hours at a temperature as low as 16°C. Red legged earth mite requires a CT of 144ghr/m^3 at 21°C, *Steneotarsonemus laticeps* (bulb scale mite) required a CT of 200ghr/m^3 at 18°C. Complete control of the stored product mite *Carpoglyplus lactis* was obtained when exposed to a CT of 144ghr/m^3. The European and Mediterranean Plant Protection Organisation [EPPO] has adopted a standard of 96ghr/m^3 at 20°C for control of *Rhizglyplus* spp. on various bulbs. Over wintering eggs of European red mite and eggs of other mite species have been shown to be highly tolerant to treatments with methyl bromide.

Trials conducted by AGWEST have shown that fumigation achieving a CT product of 52ghr/m^3 at 20°C were effective in killing western flower thrip. To translate this into the rates proposed, the fumigation will need to maintain 81% of initial gas dosage as a minimum throughout the treatment period. It should be borne in mind that a death rate of up to 80% in the untreated controls occurred that may indicate some interaction with pre or post harvest treatments that have assisted in the efficacy of the fumigation. AQIS

adopted this minimum gas concentration for all fumigation using 32g/m^3 for 2 hours at 21°C, (AQIS, 1993).

AQIS also state in ICON in respect to cut flowers that "The dosage required to kill soft-bodied insects on flowers is 32g/m^3 for 2 hours at 21°C, (T9030); the same as the scheduled dosage. For this reason, the fumigation must be conducted with the penetration and gas retention as near to perfect as possible, because a small amount of gas loss may render the treatment ineffective." (AQIS, 2000c).

Data from OEPP and EPPO Bulletin 23, (1993) shows that 54ghr/m^3 was effective in killing >99% of western flower thrip, although this was under an initial gas vacuum and the treatment ran for 4 hours.

If table grapes are fumigated at 32g/m^3 for 2 hours and assuming that the standard minimum gas concentrations are maintained, as specified in the AQIS fumigation handbook, a minimum CT product of 32ghr/m^3 at 21°C and above would be achieved.

From this it can be concluded that the AQIS statements in respect to fumigation are not consistent with the data. There is no evidence presented in the IRA to suggest that the proposed fumigation rates provide an appropriate level of protection against the target pests.

AQIS state in respect to issue 81, "The rates of methyl bromide for table grapes have been explicitly specified". No minimum gas concentration is specified, so this statement is incorrect. If the grapes are fumigated with a minimum gas concentration of 50% of initial dosage a different CT product will be achieved in comparison to grapes fumigated with a minimum gas concentration of 75% or 80%. Given that such variation could occur the rates have not been explicitly stated.

AQIS stated in respect to issue 81, "A loading ration not exceeding 80% is prescribed by APHIS for table grape imports from Chile, and is considered adequate by AQIS." The target pests and fumigation protocol used by the USA for Chilean grapes should be specified so stakeholders can form their own assessment.

AQIS state in respect to *Planococcus ficus* at Issue 42, "APHIS has confirmed that methyl bromide treatment will be efficacious against this insect." No data is presented to justify this statement. What CT product did APHIS achieve? Is this confirmation of the efficacy of the treatment based on anecdotal information or experimental data? Has it been published? How can stakeholders be satisfied that the rate proposed by AQIS is the same as that shown to be efficacious by APHIS?

AQIS state in respect to *Colomerus vitis* at Issue 51, "Because of its extremely small size and difficulty of detection by inspection AQIS considers that methyl bromide fumigation is required to reduce the risk of the leaf curl strain of *C. vitis* entering Australia on Table grapes from California." No evidence is presented in the IRA to suggest that the methyl bromide rates proposed will be efficacious against this mite. What level of security is

_ EMBED
Word.Pict

afforded given that the treatment's efficacy against the pest is unknown and the pest may not be adequately detected in fruit by visual inspection?

If any mandatory pre-export disinfestation treatment is carried out, details of the minimum gas concentration or CT product must be provided, in addition to dosage, treatment duration, and grape pulp temperature, loading ratio and date.

It is well documented that invertebrates take some time to die following fumigation. Dr D. Hardie (pers. comm.) reports that recent trial work on western flower thrips indicated that there was a delay in mortality and it was not uncommon to find them still alive up to 48 hours following fumigation. It has also been an AQIS procedure to wait for a specified time to ensure that all pests have died prior to re-inspecting consignments following fumigation. This aspect has not been fully documented in the IRA.

Bean thrips lay eggs in the fruit… Do the eggs pose a risk… and is fumigation efficacious against the eggs?

AQIS state in respect to *Tetranychus pacificus* at Issue 53, "AQIS considers that because of its small size, *T. pacificus* might not necessarily be detected in fruit on visual inspection and that mandatory methyl bromide fumigation is required to provide security against its introduction." What level of security is afforded given that the treatment's efficacy against the pest is unknown and the pest may not be adequately detected in fruit by visual inspection?

Appellant No. 10

In the absence of supportive MB efficacy data on all life cycle stages of quarantine pests, Californian table grape exporters should be required to treat all product with MB prior to shipment – ie mandatory Option A (plus mandatory SO_2 + CO_2 Treatment). NOTE it has not been stated categorically, in the IRA, that shipment at $<1.5^0$C and SO_2 + CO_2 treatments are mandatory.

AQIS has not provided evidence of the effectiveness of MB on all life cycle stages of the quarantine pests. There is considerable doubt that eggs laid in berries, by several of the listed pests, would not be killed.

AQIS has failed to provide references in which the effectiveness of methyl bromide (MB) fumigation on product in plastic sleeves has been demonstrated at the listed dosages. It is known that this packaging restricts fumigant movement (both infiltration and aeration) and the stated rates of fumigation may not reflect this.

No specific evidence has been provided to support the statement (p. 10) that MB will protect against the introduction of the orange tortrix, omnivorous leafroller and the vine mealy bug. This is a significant omission, since these pests are high risk and may have life cycle stages present in bunches.

Since most viruses enter the rachis, peduncles and fruit, it would seem appropriate that they appear on the pathway, until more evidence exists about the effectiveness of MB on all vectors.

Appellant No. 11

AQIS's response to Issue 86 was "Efficacy data for methyl bromide against the specific pests identified in the IRA is not available". Table grapes berries [*sic*] and bunches can pack in tightly against each other impeding airflow, this could be compounded by the use of bunch bags. Surely the fumigation process of bunch bagged grapes should be scientifically proven to give adequate results in a documented form before being implemented.

Appellant No. 12

I believe fumigation is a critical component of the proposal and should be supported through efficacy trials conducted on some of the more resistant target pests like the orange tortrix and omnivorous leaf roller, which are difficult to detect by inspection. As I have already stated fumigation with methyl bromide at 32 g/m^3 for 2 hours at 21^oC is marginal for insect control, particularly where research on lepidopterous species indicate it can be in excess of three time that rate. I do not believe it is sufficient, as stated by AQIS, to base ones assessment of the treatment on historic data. These observations will include instances where the treatment has failed even to control susceptible soft-bodied insect species.

7 ATTACHMENT 4 – ISSUES RAISED BY APPELLANTS ON THE RISK OF INTRODUCTION OF PIERCE'S DISEASE

Appellant No. 2
All decisions were based on the likelihood of presence on the pathway. The IRA appears dismissal of the bunch stalks. A bunch consists of more than the berries, and as such provides a range of sites for the presence of pests and disease. Pierce's disease for example, can be in the bunch stalks as shown by Purcell and Saunders (1995). Their work showed the disease could survive in bunch stalks for up to 21 days at 4°C. The IRA quoted this work but claims it is not on the pathway. Surely AQIS has used its own interpretation on this issue. We believe that interpretation may not be valid. The possibility of air freighting grapes has not been assessed, nor denied, in this IRA. Such an action would be well within the stated 21 days. As this disease can render whole areas unsuitable for vine production, and the press statement from Mr Hickey included the importance of Pierce's disease, then we believe the risk to be too high. Similarly, viruses are claimed to not be on the pathway. This is true of berries, but the woody parts of the bunch stalks are capable of carrying such viruses. Again, indications that *decisions may have been made to fit the outcome.*

The IRA does not discuss potential hitch-hiker pests that may be of concern to other horticultural producers.

Appellant No. 3
Failed to obtain from the areas where relevant… the glassy winged sharpshooter… the newly established disease in USA, Pierce's disease currently occur in California reliable biological information (life cycle, host range, epidemiology, survival etc.) as this information was necessary in order to estimate the establishment potential of such insects, pests and diseases in Australia.

Many of these quarantine pests are strong flyers with large host ranges (eg Glassy winged sharpshooter). AQIS should have considered the placing of restrictions on opening containers when ambient temperatures exceed certain limits.

The glassy-winged sharpshooter is the vector for several bacterial plant pathogens, particularly *Xylella fastidiosa*. This bacterium is the causal agent for Pierce's disease of grapes, phoney peach disease, variegated chlorosis of citrus, alfalfa dwarf, almond leaf scorch and oleander leaf scorch. Since the 1990's GWSS has expanded through various Californian counties to become a very serious pest. It is widely distributed in the Table Grape Growing regions of California.

Pierce's Disease has been found to occur in bunch stems and stalks and as such is a possibility via the pathway. Australia has insects related to the sharpshooters and these may have the ability to act as a vector. Dr Sandy Purcell is considered to be the world's leading expert on Pierce's disease. His statement all xylem feeding suctorial insects are potential vectors of Pierce's disease should not be taken lightly. This disease has for all

practical purposes already destroyed the wine grape-growing region of Temecula in southern California. Despite the severe threat that GWSS (and Pierce's disease) pose for Australia's grape growing industry, AQIS has relied on anecdotal evidence rather than scientific process.

AQIS was aware that GWSS was a serious insect pest for grapes and included it as a quarantine pest (IRA page 9). AQIS should have concluded that it was a major quarantine issue for this IRA because of the importance of Pierce's disease bacterium *Xylella fastidiosa*. Instead, and despite all evidence to the contrary, GWSS was classified as merely having a medium risk level.

AQIS also failed to properly investigate scientific information on the GWSS and control measures for this pest. Significantly, AQIS was not able to identify any credible scientific evidence to support the view that the proposed control method (pre-shipment or post arrival fumigation with methyl bromide) was efficacious in eradicating GWSS eggs.

The Appellants submit that: the quantity of imported fresh table grapes entering Australia under the proposed options A and B; the strong possibility of the fruit being infested with GWSS eggs, and other pests and diseases; and the free movement of imported grapes within Australia would ensure the establishment and spread of GWSS and other pests and diseases in the production areas once entry has occurred.

AQIS has not presented any research to support its conclusions in respect of the proposed control measures for GWSS. No entomological evidence is produced. AQIS has not even fully researched the GWSS life cycles. For an insect pest as devastating to our Australian Industry, this is a completely unacceptable scientific approach by AQIS.

This IRA is in no way consistent with Australia's conservative approach to pest and disease risk management, especially as all the risks associated with GWSS and the other high-risk insects were not investigated through a logical, scientific process.

The likelihood of establishment of GWSS or other pests in Australia through the number and frequency of consignments of imported table grapes into Australia is very high.

Appellant No. 4

On page 19, the phytosanitary requirements listed for GWSS are listed as "methyl bromide fumigation, inspection". In Appendix 3 it is stated that individual may secrete themselves within the fruit bunches, which clearly suggests that inspection would not be a reliable method of ascertaining their presence.

In Appendix 3, the 'Life history' section under GWSS notes that eggs are normally laid just under the lower leaf epidermis of host plants, but eggs are deposited in fruit if populations are high. This makes it clear that GWSS eggs must be considered as "internal insects" and that consequently, unless and until acceptable experimental evidence is forthcoming, the efficacy of MB fumigation against them cannot be accepted. This is contrary to the finding (page 15) that methyl bromide disinfestation is determined

to provide an appropriate level of protection against the introduction of medium and high-risk quarantine pests.

GWSS is, as acknowledged in Appendix 3, a pest of high economic importance and the IRA fails in the measures proposed to deal with it.

Appellant No. 5
Economic factors aside, the problem relating to the introduction of pests and diseases is a real one. The last thing the Australian wine industries need is the introduction of imported pests and diseases such as Pierce's disease…GWSS…

Appellant No. 7
Risks of entry, establishment and spread of pests and diseases, and their potential impacts are not fully evaluated.

Firstly a bunch of grapes are more than just berries. AQIS must also include all diseases that affect the peduncles, laterals, rachises and subrachises and assess the risk that they pose. Issue 76 is wrong as it ignores… *Xylella fastidiosa*.

Appellant No. 8
The IRA does not consider *Xylella fastidiosa* (Pierce's disease) to be present on the pathway. However the vectors of the pathogen occur on the fruit, (AQIS, 2000) and the bacterium is reported to be present on fruit clusters by Purcell and Saunders, (1995). Therefore it may be reasonable to assume that the pathogen is on the pathway.

In California (USA), all tested members of the subfamily Cicadellinae (including *Carneocephala fulgida*, *Draeculacephala minerva* and *Graphocephala atropunctata*) were vectors of the grapevine strain, (AQIS, 1999). All these are xylem feeding suctorial insects which acquire the bacterium rapidly on feeding (less than 2h) on the infected grapevines or an alternative host, (AQIS, 1999). No information is presented as to their presence on the pathway, albeit as a hitchhiker and the risk these vectors pose.

AQIS state in respect to Pierce's disease at issue 16, "The disease organism that causes Pierce's disease itself has a low risk of entering Australia on grapes since Purcell and Saunders (1995) showed that it does not survive more than 24 hours on grapes when subjected to storage at 1°C". AGWEST has been unable to find where Purcell and Saunders, (1995), state or infer that the disease organism that causes Pierce's disease does not survive more than 24 hours on grapes when subjected to storage at 1°C. Purcell and Saunders, (1995), demonstrated that the disease exists for up to 21 days in storage at 4°C. It should be noted that this is only one report and there is no indication that the experiments have been repeated with similar results. Given the seriousness of Pierce's disease this work should be seen as an indicator rather than definitive proof.

An AQIS press release from 14 February 2000, stated that 'Keeping out Phylloxera and Pierce's disease - the principal high-risk pest and disease of quarantine concern to Australian growers - were some of the main considerations in the risk analysis, according

to AQIS Executive Director Paul Hickey". Both Phylloxera and Pierce's disease did not appear to be the main considerations in the assessment as both were considered by AQIS not to be on the pathway. The risk of the disease being transmitted by infected vectors present in consignments, in particular *Homalodisca coagulata*, was not considered.

The PRA states that the Estimated Risk of *Xylella fastidiosa* is low, as the table grapes are trash free and the disease vectors are not present in California, yet the PRA also states that in California all tested members of the sub family Cicadellinae were vectors of the grapevine strain of *Xylella fastidiosa*. Which statement is correct?

Another area of concern is the apparent inconsistency between the close scrutiny of New Zealand apples for Fireblight, yet Pierce's disease was hardly considered in this IRA. Both diseases are serious and would have a major impact on Australian horticultural industries. It is easy to understand AQIS' close scrutiny of the risks NZ apples pose to the Australian pome fruit industry. What is difficult to understand is the low level of investigation made to Pierce's disease, despite the pest being on the pathway. Pierce's disease is considered the most formidable obstacle to growing *Vitis vinifera* in North America and the main reason for the failure to establish vineyards in Florida, an area ideal for the grape industry, (AQIS, 1999).

AQIS state in respect to the potential for Australian insect species to transmit Pierce's disease at issue 41, "However, species in this genus have also never been recorded on Vitaceae in Australia; hence it is believed that the risk of transmission of Pierce's disease is negligible". No evidence is presented to back this statement. Pierce's disease can infect a large number of hosts other than Vitaceae which may allow the establishment of the disease in Australia.

The family Cercopidae are also vectors of Pierce's disease (AQIS, 1999 and University of California, Berkeley, 1999). The family Cercopidae is represented in Australia, (Naumann, 1993).

Quarantine leaflet 34 states that "Although many leafhoppers of the subfamily Cicadellinae occur in Australia they may not be suitable vectors; however, the risk remains and vectors have been known to adapt to the introduction of a new pathogen.

These aspects have not been adequately considered in the IRA.

AQIS state in respect to issue 76, "AQIS is of the opinion that there is no necessity for testing table grape fruit for fungal spores because the IRA has identified that no diseases of quarantine concern occur on the fruit."

Xylella fastidiosa - Purcell and Saunders (1995), demonstrated that grape clusters contained *Xylella fastidiosa*.

The IRA has not considered the risk of potential disease hitchhikers that are present in California but not in Australia. It is important to identify these, assess the risk and, if necessary, put in place appropriate measures.

Carneocephala fulgida is present in California (Bentley, et al, 1998 and AQIS, 1999). Bentley, et al, 1998 states that "The green sharpshooter and the red-headed sharpshooter also are present in coastal areas, but they serve as the primary vectors in the Central Valley." AQIS, 1999 states that "In California (USA), all tested members of the subfamily Cicadellinae (including *Carneocephala fulgida*, *Draeculacephala minerva* and *Graphocephala atropunctata*) were vectors of the grapevine strain." AQIS, (1999) goes on to state "The green sharpshooter (*Draeculacephala minerva*) and the redheaded sharpshooter (*Carneocephala fulgida*) are the most important vectors in the Central Valley and other areas of north and (South) America. [*sic*]".

Interestingly in the same data sheet AQIS, (1999) state that the **"**Estimated risk**:** Low, as the table grapes are trash free and the disease vectors are not present in California. These oversights were pointed out to AQIS in AGWEST's response to the draft however they were not addressed in the final IRA. This pest is a vector of *Xylella fastidiosa* and therefore it is critical that it is assessed accurately and a data sheet provided.

Appellant No. 10
Pierce's disease. The bacterium, vectors and alternative hosts continue to be researched in CA. New vectors were identified in 1999. The reference used to support the AQIS decision (that PD was not on the pathway), in fact suggests otherwise (Purcell and Saunders 1995). It demonstrated that bunch to bunch transmission did not occur, but that sharpshooters fed on diseased vines readily transmitted the bacterium into bunches. Australia must be protected from both the bacterium (ie in bunches) and the known vectors.

AQIS has no evidence to support the statement that we have few, if any, potential PD vectors in Australia (issue 41).

8 ATTACHMENT 5 – PEST DATA SHEET ON PIERCE'S DISEASE (CAUSAL AGENT, *XYLELLA FASTIDIOSA*)

***Xylella fastidiosa* Wells, J., Raju, B., Hung, J., Weisburg, W.G., Mandelco-Paul, L. and Brenner, D.J., 1987 [Bacteria: Xanthomonadales: Xanthomonadaceae]**

Synonyms and changes in combination: Pierce's disease (PD) is a disease of grapevines which has been reported to cause significant economic losses since it was first identified in southern California in the 1880's (Medley, 1998). The causal agent of PD is *Xylella fastidiosa*, a bacterium which is limited to the xylem (i.e. water conducting vessels of the plant) (Medley, 1998). *X. fastidiosa* was first identified in 1973, prior to this the disease was though to be a result of a virus (Hewitt *et al*., 1949).

Different strains of *X. fastidiosa* cultured from different hosts by the same techniques cause various diseases in other plant hosts. Two such diseases are phony disease of peach and plum leaf scald, which were previously listed and described separately by EPPO (Anon., 1986). Another strain causes citrus variegated chlorosis in South America. The grapevine-virulent strains do not infect peach, whereas the strains virulent in peach do not cause disease in grapevine (Hopkins, 1988). Relationships between strains are still in the process of being categorised. Taxonomic efforts to classify strains using molecular methods (Chen *et al*., 1995) have raised questions as to the reciprocity of strains of *X. fastidiosa*. Host-range and cross-inoculation studies are still needed to determine host-specialised forms (pathovars) within *X. fastidiosa* (Purcell and Hopkins, 1996).

Common name(s): Common names of PD are Anaheim disease; California disease; California vine disease and Pierce's disease of grapevines.

Different strains of the bacterium also cause the following diseases on the following crops: Alfalfa dwarf; almond leaf scorch; citrus blight; citrus variegated chlorosis (CVC); decline ("declinio"); dwarf lucerne; leaf scald; leaf scorch; phony disease of peach; plum leaf scald; stunt; variegated chlorosis (Anon., 1999; Medley, 1998).

Host(s): The principal host of *X. fastidiosa* is grapevine (*Vitis vinifera*) and also the American species *V. labrusca* and *V. riparia*. Other American species used as rootstocks (*V. aestivalis*, *V. berlandieri*, *V. candicans* and *V. rupestris*), and hybrids derived from them are resistant, as is *V rotundifolia* (Goheen and Hopkins, 1988). Almonds (*Prunus dulcis*) and lucerne (*Medicago sativa*) are minor cultivated hosts of the grapevine-infecting bacterium, and numerous wild plants and weeds can carry it without symptoms (e.g. wild grasses, sedges, lilies, various bushes and trees) (Raju *et al*., 1983; Hopkins and Adlerz, 1988).

Peaches (*Prunus persica*) are another major host (phony disease), attacked by a distinct form of *X. fastidiosa* also found in *P. salicina* (causing leaf scald). All cultivars, forms and hybrids of peach are attacked, whether on their own roots or other rootstocks. Plums (*Prunus domestica*), almonds (*P. dulcis*), apricots (*P. armeniaca*) and the wild *P. angustifolia* were reported susceptible to phony disease before the association with *X.*

_ EMBED Word.Pict

fastidiosa was established. This reported range partly overlaps that of the grapevine-infecting strain and further studies are now needed to clarify the host ranges of the different strains (Smith *et al*., 1997). Various perennial weeds of orchards such as *Sorghum halepense*, may act as reservoirs for the peach-infecting strain (Yonce and Chang, 1987).

X. fastidiosa in the wide sense also attacks *Acer rubrum* (red maple, scarlet maple); *Morus rubra* (red mulberry); *Platanus occidentalis* (American plane, buttonwood) (wilt and leaf scorch); *Quercus rubra* (northern red oak, red oak); *Ulmus americana* (American elm, white elm); and *Vinca minor* (dwarf periwinkle; lesser periwinkle) (stunt) (Smith *et al*., 1997). Strains from *Ulmus* and from *P. occidentalis* are not reciprocally infectious (Sherald, 1993). The bacteria involved are not known to be transmissible to grapevine. Until their relationships and pest significance have been clarified, they can all be regarded as potentially dangerous for the EPPO region (Smith *et al*., 1997).

X fastidiosa causes a newly recognised disease, called citrus variegated chlorosis, in Brazil (Lee *et al*., 1991; Beretta *et al*., 1992). The disease affects mostly oranges (*Citrus sinensis*); it has been observed especially on cultivars Pera, Hamlin, Natal and Valencia. It occurs on trees propagated on all commonly used rootstocks in Brazil including lemandarin (*C. limonia*), Cleopatra mandarin (*C. reshni*) and *C. volkameriana* (Smith *et al*., 1997). The disease has not been observed on Tahitian limes (*C. latifolia*) or mandarins (*C. reticulata*), even when the trees were planted in severely affected orange groves. Some weed species are also hosts and act as reservoirs of infection (Smith *et al*., 1997).

Plant part(s) affected: Systemic.

Distribution: The distribution of *X. fastidiosa* on grapes are: Argentina; Brazil; Central America; Costa Rica; El Salvador; Guatemala; Honduras; India; Mexico; Nicaragua; Panama; Paraguay; Peru; South America; United States (Alabama, California, Florida, Georgia, Mississippi, Missouri, North Carolina, South Carolina, Texas); Venezuela.

The geographical distribution of PD on grapevines appears to be related to the ability of the bacteria to survive winter temperatures (Varela, 2000). PD seems to be restricted to portions of North America with mild winters (Anon., 2000a). PD is present across the southern states from Florida west through southern Texas to California. In the eastern states it extends up to Virginia. In the west it has not been found north of California or equivalent latitudes. In general, the disease is less prevalent and less severe in areas where winter temperatures are colder i.e further north, more inland from ocean influences and at higher altitudes (Anon., 2000a).

Winter climate is a key factor in delimiting the areas where *X. fastidiosa* can persist from one season to the next (Anon., 1999). Wet winters promote survival of high vector populations and favour disease spread in regions with dry summers. In temperate climates with regular freezing winter temperatures, infections of *X. fastidiosa* established in grapevines during the early growing season are most likely to persist until the following year (Purcell, 1981).

Biology

Life history: The biological information on *X. fastidiosa* refers to the strain on grapes. *X. fastidiosa* is a gram negative, xylem-limited bacterium, measuring 0.25-0.50µm in diameter and 1.0-4.0µm in length. The bacterium has a convoluted cell wall of several layers and extracellular fibrous strands.

Infections of the bacteria form dense aggregates within the xylem vessels. These aggregates, along with gums and tyloses produced by the grapevine restrict vascular flow of the xylem (Goheen and Hopkins, 1988). Goheen and Hopkins (1988) also suggested that a phytotoxin produced by *X. fastidiosa* may also play a role in the development of the disease. Symptoms appear when a significant amount of xylem is blocked (Varela, 2000).

Symptoms of PD first appear as water stress in midsummer as a result of blockages in the xylem caused by the bacterium (Medley, 1998). The occurrence of the following four symptoms in mid- to late summer indicates the presence of PD: (1) leaves become slightly yellow or red along margins in white and red varieties, respectively, and eventually leaf margins dry or die in concentric zones; (2) fruit clusters shrivel or raisin; (3) dried leaves fall leaving the petiole (leaf stem) attached to the cane; and (4) wood on new canes matures irregularly, producing patches of green, surrounded by mature brown bark or wood (Medley, 1998; Gubler *et al*., 1999; Anon., 2000a). When new growth occurs on infected canes it can be delayed and is usually stunted. Leaves on stunted shoots can have a yellow mottling between the major veins. Depending on the grape variety, death of the entire vine usually occurs in one to five years (Medley, 1998).

Usually only one or two canes will show PD symptoms late in the first season of infection (Gubler *et al*., 1999). Symptoms gradually spread along the cane from the point of infection out towards the apex and more slowly towards the base. By mid-season some or all fruit clusters on the infected cane may wilt and dry (Gubler *et al*., 1999). Tips of canes may die back, and roots may also die back. Vines deteriorate rapidly after appearance of symptoms. Shoot growth of infected plants becomes progressively weaker as symptoms become more pronounced.

In the following year, some canes or spurs may fail to bud out. New leaves become chlorotic (yellow) between leaf veins and scorching appears on older leaves. From late April through summer infected vines may grow at a normal rate, but the total new growth is less than that of healthy vines (Gubler *et al*., 1999). In late summer leaf burning symptoms reappear.

Leaf symptoms vary among grape varieties (Gubler *et al*., 1999). Grape varieties such as Pinot Noir and Cabernet Sauvignon have highly regular zones of progressive marginal discolouration and drying on blades. In the varieties Thompson seedless, Sylvaner, and Chenin Blanc, the discolouration and scorching may occur in sectors of the leaf rather than along the margins. Climatic differences between regions can affect the timing and severity of symptoms, but not the type of symptoms (Gubler *et al*., 1999). Hot climates accelerate symptom development, as moisture stress is more severe even with adequate soil moisture.

Some vines infected during the season appear to recover from PD the first winter following infection (Varela, 2000). Recovery from PD depends on the grape variety. In Cabernet, recovery is high while in Barbera, Chardonnay and Pinot Noir it is low. In more tolerant cultivars, the bacterium spreads more slowly within the plant than in more susceptible cultivars (Varela, 2000). Once the vine has been infected for over a year (i.e. bacteria survive the first winter) recovery is much less likely (Varela, 2000). Young vines are more susceptible than mature vines, possibly because the bacteria can move more quickly through younger vines than through older vines. Rootstock species and hybrids vary greatly in susceptibility. Testing of rootstock plants show that *V. riparia* is rather susceptible; *V. rupestris* (St George) and 420A are very tolerant. Rootstock does not confer resistance to susceptible *V. vinifera* varieties grafted on to it. Climate, variety and age determine how long a vine with PD can survive (Varela, 2000). One-year old Pinot Noir or Chardonnay can die the year they become infected, whereas chronically infected 10-year-old Chenin Blanc or Ruby Cabernet can live for more than five years. Long before that, however, these chronically infested vines will cease to bear a crop (Varela, 2000).

X. fastidiosa is transmitted and dispersed via its vectors or by graft transmission. Propagative material is the pathway by which *X. fastidiosa* may spread (Smith *et al*., 1997). *X. fastidiosa* is not transmitted via contaminated pruning shears or by seed transmission (Smith *et al*., 1997; Varela, 2000).

Within the Americas many genera of sharpshooters and spittlebugs serve as vectors of the bacterium (Goheen and Hopkins, 1988). However, in California, the major vectors are the blue-green sharpshooter (*Graphocephala atropunctata*), glassy-winged sharpshooter (*Homalodisca coagulata*), green sharpshooter (*Draeculacephala minerva*), and the red-headed sharpshooter (*Carneocephala fulgida*) (Gubler *et al*., 1999; Purcell, 1999b; Varela, 2000). Spittlebug vectors of Pierce's disease have been recorded in California (Delong and Severin, 1950), but none have been found on grapevines in California (Severin, 1950). Other sucking insects such as grape leafhoppers, are not vectors in California (Gubler *et al*., 1999). Refer to the "Vector relationships" heading for more information on the insect vectors of *X. fastidiosa*.

The laboratory observations from Purcell and Saunders (1995) work on harvested grape clusters as inoculum for PD showed that the number of viable *X. fastidiosa* decreased with time spent in cold storage at 4°C. Research conducted by Purcell and Saunders (1995) in California indicated that *X. fastidiosa* in infected grape clusters declined sharply after cold storage at 4°C. The bacterium was not recovered from infected grapes after 21 days of storage at this temperature. This data supports the observations made by Varela (2000). Further, experimental cold therapy of diseased grapevines suggests that freezing temperatures can eliminate the bacterium directly from plants (Purcell, 1980).

Positive identification of *X. fastidiosa* can be obtained by three methods: culturing the bacterium on selective media, serological test such as ELISA (enzyme linked immunosorbent assay) or PCR (polymerase chain reaction) (Varela, 2000).

For cultural diagnosis a specialised media has been developed for isolating and growing the PD bacterium. Petioles are used to isolate the bacteria. An advantage of this technique is that it does not give false positives and it is a comparatively inexpensive diagnostic test. The disadvantages are that it is time consuming, colonies may require one to three weeks to develop, microbial contaminants cloud or obscure results and the bacteria can only be isolated from petioles during the summer and early fall (Varela, 2000).

ELISA is based on using antiserum to detect the presence of the bacteria. This technique is fast and relatively inexpensive. It is useful to confirm the presence of *X. fastidiosa* in symptomatic plants after June. The disadvantage is that it does not provide as sensitive detection of the bacterium as PCR.

PCR enzymatically amplifies specific parts of the bacterium's DNA. This is the most sensitive technique to detect small numbers of bacteria in plants. It is specific for *X. fastidiosa* but has the disadvantages that it is expensive, cannot determine if the bacteria are dead or alive or how many bacteria are present in the sample (Varela, 2000).

Using ELISA, commercial laboratories can confirm if vines harbour *X. fastidiosa*. Samples taken from August through October of symptomatic leaves which are still attached to green portions of canes (live tissue), generally give the most reliable test results (Varela, 2000).

Vector relationships: All sucking insects that feed on xylem sap may be potential vectors of *X. fastidiosa*. However, all known vectors of *X. fastidiosa* are limited to the Homoptera suborder (Purcell, 1999c). The main vector groups are leafhoppers (family Cicadellidae) in the subfamily Cicadellinae. Many of these are commonly called sharpshooters. Spittlebugs or froghoppers (family Cicadidae) are also vectors (Purcell, 1999c). Cicadas (family Cicadidae) are also xylem feeders but there are no published reports of their being tested as vectors. In California, the only recorded vectors are the blue-green sharpshooter (BGSS, *Graphocephala atropunctata*), green sharpshooter (GSS, *Draeculacephala minerva*), red-headed sharpshooter (RHSS, *Carneocephala fulgida*), and the glassy-winged sharpshooter (*Homalodisca coagulata*) (Goheen *et al*., 1973; Purcell 1999a; Varela, 2000).

Vectors acquire the bacterium by feeding on infested plants. The bacteria appear to adhere to the foregut (between the needle-like mouthparts and the stomach) and are then transmitted during feeding by infective vectors into other plants. Vectors remain infective after picking up the bacterium until they moult their external skeleton. After moulting, insects must feed again on an infected plant before they can acquire and transmit the bacterium (Purcell, 1999c).

BGSS is the most important vector in the North Coast (Varela, 2000) and in coastal areas (Gubler *et al*., 1999; Purcell, 1999a). The most efficient vector of PD in California is the BGSS which possesses the ability to transmit the bacterium with approximately 88 to 90% efficiency from vine to vine (Purcell and Saunders, 1995; Varela, 2000). Purcell (2000) confirmed the efficiency of the BGSS and stated "We have done some preliminary trials of GWSS as a vector of Xf [*X. fastidiosa*]

from vine to vine. It is clearly much less efficient than the blue-green sharpshooters used in our tests, perhaps only 20% as efficient".

Purcell and Saunders (1995) conducted tests to determine the potential for California's most efficient vector (BGSS) of PD to acquire and transmit PD from grape clusters taken from PD-infected grapevines. Their studies also showed that the vectors, GSS and BGSS, could not transmit the bacteria to grapevines after six hours access feeding on fruit clusters from grapevines showing symptoms of PD. However, under similar trial conditions, 88% of GSS and 24% of BGSS transmitted the disease to grapevines after six hours access feeding on foliage of diseased vines. The results of their studies clearly demonstrated that no acquisition or transmission of the bacteria occurred from table grape fruit clusters. Furthermore, they noted that the highly artificial conditions in the studies were designed to maximise potential transmission and that in the field it would be unlikely that sharpshooters would feed at all on grape bunches as they do not provide the levels of nutrients and fluids required by sharpshooters.

The principal PD vectors in the Central Valley of California are GSS and RHSS (Purcell, 1999e; Purcell, 1999g). GSS and RHSS are present in the North Coast and are vectors under some circumstances (Varela, 2000). Both species are also present in coastal areas but are more important as vectors of this disease in the Central Valley (Gubler *et al*., 1999).

Work conducted by Purcell and Saunders (1995) demonstrated the efficiency in transmitting *X. fastidiosa* by the GSS was 24% from vine to vine. Its role as a PD vector was based on the consistent occurrence of its breeding habitats near vineyards (Purcell, 1999e).

Reports on the efficiency of the RHSS are not available, although it is likely that the efficiency would be similar to the GSS (Purcell, 1999g). Like the GSS, it was only rarely seen feeding on grapevines. Its role as a PD vector was based on the consistent occurrence of its breeding habitats near vineyards.

A new PD vector, GWSS, has recently become established in southern California in the counties of Kern, San Bernardino, Santa Barbara, Ventura, Los Angeles, Orange, Riverside and San Diego (Anon., 2000c). This vector is a serious new threat to California vineyards because it moves faster and greater distances into vineyards than the other species of sharpshooters (Gubler *et al*., 1999). GWSS occurs in unusually high numbers in citrus and avocado groves and on some woody ornamentals (Gubler *et al*., 1999).

Vector biology and life cycle:

Blue-green sharpshooter (BGSS, *Graphocephala atropunctata*)
BGSS is considered to be the most important *X. fastidiosa* vector for coastal California, from San Diego in the south through Mendocino counties to the north (Purcell, 1999a). It is reported as occurring from Central America through British Columbia and is often abundant in coastal Oregon and Washington. In California, it can be found in the foothills of the Sierra Nevada mountains along streams or less abundantly in riparian (stream bank) vegetation along rivers in the Sacramento and

San Joaquin Valleys (Purcell, 1999a). Along the foothills and mountains of the Pacific coast, BGSS occurs mostly along streams or springs, the margins of forest openings and in a variety of ornamental landscapes.

Woody plants, including grapevines, are favoured for feeding and reproduction. The list of plants on which it regularly feeds is enormous, but it favours some plant species over others, especially for laying eggs (Purcell, 1999a). The most common plants on which it is found in California include grape, blackberry, elderberry, mugwort, stinging nettle, and snowberry (Purcell, 1999a). In ornamental landscapes in residential areas or parks, it favours roses, fuschia, ivy and a variety of ornamental shrubs or trees. Unlike other important *X. fastidiosa* vectors in California, BGSS commonly occurs on commercial grapevines near riparian (riverbank) vegetation (Purcell, 1999a). The principal breeding habitat for BGSS is riparian vegetation, although ornamental landscape plants may also harbour breeding populations. As the season progresses, these insects shift their feeding preference, always preferring to feed on plants with succulent growth (Purcell, 1999a).

Adults are long-lived and there is usually only a single generation per year. A few adults may lay eggs a few weeks after they mature, resulting in a partial second generation, but most females require a period of cool temperatures to mature reproductively and do not lay eggs until the following spring (Purcell, 1999a). A high percentage of adults survive the winter, but not much is known of their behaviour during winter (Purcell, 1999a). Adults overwinter mainly in riparian habitats, but also may be distributed at low density in areas with trees and shrubs. Eggs are laid singly under the epidermis of leaves (along veins or petioles) and stems in April, depending on temperature (Guidicipietro, pers. comm., 2000; Varela, 2000). Most adults (80-90%) breed in riparian areas, hence the majority of the eggs are laid within riparian plants. Adults that have started to migrate will lay their eggs in vines at the edge of the vineyard. Their dispersal into the vineyard increases as natural vegetation dries up. Most overwintered adults die out by the end of June.

The flightless immatures (nymphs) emerge from late April or early May through July and remain on the same plant where they had emerged from as eggs, thus the majority of the nymphs are found on riparian plants. Nymphs become adults between late June and the end of August. As adults begin to emerge in late June they move deeper into the vineyard. At the beginning of September, when grape foliage is less succulent, sharpshooters begin to move back into nearby natural habitats (Varela, 2000).

Like other vectors; adult BGSS retain infectivity with *X. fastidiosa* for an indefinite period (Purcell, 1999a). Thus adults that acquire the bacterium during the autumn can introduce *X. fastidiosa* into plants during the following spring (Purcell, 1999a). The spatial pattern of PD in north coast California vineyards reflects the spring dispersal pattern of BGSS adults (Purcell, 1999a).

Green sharpshooter (GSS, *Draeculacephala minerva*)

_ EMBED
Word.Pict

GSS is considered to be one of two important species of insect vectors for *X. fastidiosa* and alfalfa dwarf diseases in the Central Valley of California. It also occurs in coastal areas in grasses and sedges along streams. Although it has been found on many species of herbaceous plants, it strongly prefers to feed and reproduce on grasses. It is most common on water grass (*Echinochloa cruz-galli*), fescues, perennial rye grass and Bermuda grass (Purcell, 1999e). Its most common habitats are ditch banks, weedy hay fields and permanent irrigated pastures, anywhere that its preferred grasses continue to grow throughout the year. For this reason it is common in orchard or vineyard cover crops only when there are suitable host plants in the cover crop at all times of the year. It is only rarely seen feeding on grape (Purcell, 1999e). Its role as a *X. fastidiosa* vector is based on the consistent occurrence of its breeding habitats near vineyards.

There are usually three generations per year in California. Beginning in February and March, females insert eggs into the leaves of winter annual or perennial grasses (Purcell, 1999e). Nymphs emerge from late February through March. Second generation eggs are laid beginning in April or early May. Nymphs from the second generation reach maturity during the latter part of June through July, during which time third generation eggs are deposited. Adults stop reproducing in the autumn and begin to lay eggs in grasses as soon as temperatures are warm enough, usually in January or February (Purcell, 1999e). Adult colouration can vary from bright green through dull brown during winter, while during late spring and summer, the upper surface of adult green sharpshooters is a bright grass green. In some areas, most adult green sharpshooters are brown during autumn and winter (Purcell, 1999e).

Red-headed sharpshooter (RHSS, *Carneocephala fulgida*)

RHSS along with GSS, is considered to be one of the important species of insect vectors for *X. fastidiosa* and alfalfa dwarf diseases in California's Central Valley. It occurs from Mexico and western Arizona north to northern California. It is similar in appearance to GSS but is smaller and has reddish colouration on the pointed crown of its head. This sharpshooter also occurs in coastal areas in grasses and sedges along streams. Although it has been found on numerous species of grasses and sedges, by far its most common host plant for feeding and laying eggs is Bermuda grass (*Cynodon dactylon*) (Purcell, 1999f). Its most common habitats are ditch banks, orchard cover crops; weedy hay fields and permanent irrigated pastures, anywhere that its preferred grasses continue to grow throughout the year (Purcell, 1999g). RHSS prefers more open or sparser plant growth compared to GSS, and may be found in small patches of Bermuda grass along roadsides, ditches, or the margins of alfalfa fields where the grass growth is not succulent or dense enough to support high populations of GSS (Purcell, 1999g). Like GSS, it is only rarely seen feeding on grape (Purcell, 1999g). Its role as a *X. fastidiosa* vector is based on the consistent occurrence of its breeding habitats near vineyards.

There are usually four generations per year in central California. Eggs of each generation are laid in mid-March, mid-May, early July, and mid-August. Approximately 25 days are required for development from egg to adult. Adults are

active during winter, but they are much less abundant and more widely scattered than green sharpshooter adults during this time (Purcell, 1999g).

Glassy-winged sharpshooter (GWSS, *Homalodisca coagulata*)
GWSS is distributed in southern United States and northern Mexico except in very arid areas. Its range has recently extended into southern and central California (Purcell, 1999d). Currently GWSS have been recorded as occurring in the counties of Kern, San Bernardino, Santa Barbara, Ventura, Los Angeles, Orange, Riverside and San Diego (Anon., 2000c). These insects thrive on a variety of common plants and have spread rapidly from Ventura to the Mexican border; they have also been found in the San Joaquin Valley (Anon., 2000b).

The host list for GWSS includes 73 plant species (Anon., 2000b). It feeds on a wide variety of trees, woody ornamentals and annuals. Crepe myrtle and sumac are especially preferred (Anon., 2000b).

Under high population pressure, some immature green lemon fruit have been used for egg laying (Guidicipietro, pers. comm., 2000). GWSS egg masses were first detected on lemon trees in Ventura County in 1993. Immature lemon fruit is a oviposition host of GWSS (Guidicipietro, pers. comm., 2000). However, in work done by the University of California Cooperative Extension (Ventura County), 23,000 mature lemon fruit were examined and no viable egg masses were found. Old egg masses, laid when the fruit was young, were found. The California Department of Agriculture concluded that mature citrus fruit, including lemons, do not pose an introduction potential for GWSS (Guidicipietro, pers. comm., 2000).

Adults are about ½ inch (13-14 mm) long, dark brown in colour with small yellow dots on head and thorax. Wings are membranous and translucent with reddish veins. The insect overwinters as an adult and begins to lay egg masses in late February through May. The first generation matures as adults from late May through to late August. A second generation of egg masses is laid starting in mid-June through to late September, which develop into overwintering adults (Anon., 2000b). Eggs of GWSS are laid side by side in clutches of 1-27, with the average mass consisting of 10 eggs (Guidicipietro, pers. comm., 2000). The eggs are laid under the lower epidermis of leaves, giving a greenish, water-blistered appearance (Guidicipietro, pers. comm., 2000).

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

Unlike the current sharpshooter vectors associated with *X. fastidiosa* in California (BGSS, GSS and RHSS), the GWSS is much larger, appears to fly much further

and certainly in greater numbers into commercial agricultural plantings than native Californian sharpshooters (Phillips, 1999). The ease with which GWSS moves into the middle of agricultural plantings extends the threat of *X. fastidiosa* infections from a primarily vineyard boarder problem to potentially a vineyard-wide problem, even on the largest plantings. Populations of GWSS have already moved into Temecula vineyards in northern San Diego County and have been associated there with new confirmed findings of PD in vineyards where PD was previously undocumented (unlike vineyards a few miles to the south which are closer to riparian areas and have had a long history of PD vectored by BGSS). GWSS is not confined to riparian areas because of its wide host plant range. It can easily develop large populations on dooryard ash , eucalyptus, macadamia, or stone fruit trees (Phillips, 1999). The large numbers this insect generates in crop or non-crop plantings increases the likelihood of bacterial transmission from even the smallest source.

Economic importance: The infestation in Temecula (southern California) has been severe with more than 30% of the vineyards in the area experiencing outbreaks of PD due to GWSS feeding and inoculation (Anon., 2000b). Estimated damage as a consequence of PD have been valued at US$33 million over the past five years in the Riverside County of California (Anon., 2000b). Goheen and Hopkins (1988) described PD as the principal limiting factor in production of *Vitis* in the gulf coastal plains of the USA.

Without an effective control, the GWSS could spread throughout the San Joaquin Valley, threatening nearly 800,000 acres of wine, table and raisin grapes, more than 70% of the state's vineyards, with PD (Anon., 2000b). It is estimated that the sharpshooter and PD threaten more than $14 billion in agricultural crops in California including commercial agricultural crops of almonds, alfalfa, apricots, cherries, citrus, peaches and ornamentals. In addition to commercial agricultural and nursery crops, the California Department of Transportation (CalTrans) estimates it could suffer at least $52 million in losses if oleander along 2,100 miles of freeway median is lost due to oleander leaf scorch caused by *X. fastidiosa* (Anon., 2000b).

References:

Anonymous (1986). Data sheets on quarantine organisms No. 137, peach phony bacterium. Bulletin OEPP/EPPO Bulletin 16: 25-28.

Anonymous (1999). Crop Protection Compendium Global Module – 1999 Edition. CAB INTERNATIONAL: Wallingford, UK.

Anonymous (2000a). An Introduction to Pierce's Disease. http://www.cnr.berkeley.edu/xylella/page2.html

Anonymous (2000b). Background: questions and answers. http://www.cfbf.com/GWPDBack.htm

Anonymous (2000c). Distribution of GWSS in California. http://plant.cdfa.ca.gov/gwss/gwmap.htm

Beretta, M.J.G., Lee, R.F., Derrick, K.S., Davis, C.L. and Barthe, G.A. (1994). Culture and serology of a *Xylella fastidiosa* associated with citrus variegated chlorosis in Brazil. pp. 830-831. In: Proceedings of the International Society of Citriculture. Volume 2 cultural practices, diseases and their control: 7th International Citrus Congress, Acireale, Italy, 8–13 March 1992, International Society of Citriculture, Catania, Italy.

Chen, J., Lamikanra, O., Chang, C.J. and Hopkins, D.L. (1995). Randomly amplified polymorphic DNA analysis of *Xylella fastidiosa* Pierce's disease and oak leaf Scorch pathotypes. Applied and Environmental Microbiology 61: 1688-1690.

Delong, D.M. and Severin, H.H.P. (1950). Spittle-insect vectors of Pierce's disease virus. I. Characters, distribution, and food plants. Hilgardia 19(11): 339-356.

Goheen, A.C. and Hopkins D.L. (1988). Pierce's Disease. pp. 44-45. In: Pearson, R.C. and Goheen, A.C. (eds). Compendium of Grape Diseases. The American Phytopathological Society (APS) Press: St Paul, Minnesota, USA 93 pp.

Goheen, A.C., Nyland, G. and Lowe, S.K. (1973). Association of rickettsia-like organism with Pierce's disease of grapevines and alfalfa dwarf and heat therapy of the disease in grapevines. Phytopathology 63: 341-345.

Gubler, D., Stapleton, J., Leavitt, G., Purcell, A., Varela, L. and Smith, R.J. (1999). UC Pest Management Guidelines. http://www.ipm.ucdavis.edu/PMG/r302101211.html

Guidicipietro, M. (2000). APHIS response to AQIS questions raised at the bilateral technical meeting in Canberra on 17-18 May 2000.

Hewitt, W.B., Frazier, N.W. and Freitag, J.H. (1949). Pierce's disease investigations. Hilgardia 19(7): 207-264.

Hopkins, D.L. (1988). *Xylella fastidiosa*: A xylem-limited bacterial pathogen of plants. Annual Review of Phytopathology 27: 271-290.

Hopkins, D.L. and Adlerz, W.C. (1988). Natural hosts of *Xylella fastidiosa* in Florida. Plant Disease 72(5): 429-431.

Lee, R.F., Derrick, K.S., Beretta, M.J.G., Chagas, C.M. and Rosetti, V. (1991). Citrus variegated chlorosis: A new destructive disease of citrus in Brazil. Citrus Industry, October 1991, pp. 12-14.

Medley, J.C. (1998). Pierce's Disease. http://aesrg.tamu.edu/Grapes/PierceDis.htm

Phillips, P.A. (1999). The Glassy-winged Sharpshooter - A serious new "PD" vector for California Vineyards. http://ucceventura.xlrn.ucsb.edu/IPM/IPMHome.htm

_ EMBED
Word.Pict

Purcell, A.H. (1975). Role of the blue-green sharpshooter, *Hordnia circellata*, in the epidemiology of Pierce's disease of grapevines. Environmental Entomology 4: 745-752.

Purcell, A.H. (1980). Environmental therapy for Pierce's disease of grapevines. Plant Disease 64(4): 388-390.

Purcell, A.H. (1981). Vector preference and inoculation efficiency as components of resistance to Pierce's disease in European grape cultivars. Phytopathology 71(4): 429-435.

Purcell, A.H. (1999a). Blue-green Sharpshooter. http://www.cnr.berkeley.edu/xylella/bgss.html

Purcell, A.H. (1999b). Central Valley Guidelines for Pierce's Disease. http://www.cnr.berkeley.edu/xylella/central-valley-guidelines.html

Purcell, A.H. (1999c). General insect Category. http://www.cnr.berkeley.edu/xylella/geninsct.html

Purcell, A.H. (1999d). Glassy-Winged Sharpshooter. http://www.cnr.berkeley.edu/xylella/oss.html

Purcell, A.H. (1999e). Green Sharpshooter. http://www.cnr.berkeley.edu/xylella/grnshrp.html

Purcell, A.H. (1999f). Prepared Remarks for the Hearing of the California Assembly Agricultural Committee, October 12, 1999. http://www.cnr.berkeley.edu/xylella/ap.htm

Purcell, A.H. (1999g). Red-headed Sharpshooter. http://www.cnr.berkeley.edu/xylella/rhss.html

Purcell, A.H. (2000). E-mail communications with D. Hunt-Sharman on harvested grape clusters as a quarantine risk to Australia dated 7 April 2000.

Purcell, A.H. and Hopkins, D.L. (1996). Fastidious xylem-limited bacterial plant pathogens. Annual Review of Phytopathology 34: 131-151.

Purcell, A.H. and Saunders, S. (1995). Harvested grape clusters as inoculum for Pierce's Disease. Plant Disease 79: 190-192.

Severin, H.H.P. (1950). Spittle-insect vectors of Pierce's disease virus. II. Life history and virus transmission. Hilgardia 19(11): 357-382.

Sherald, J.L. (1993). Pathogenicity of *Xylella fastidiosa* in American elm and failure of reciprocal transmission between strains from elm and sycamore. Plant Disease 77:200-203.

Smith, I.M., McNamara, D.G., Scott, P.R., Holderness, M. and Burger, B. (eds). (1997). Quarantine Pests for Europe (2nd edition). CAB International: Wallingford, UK 1425 pp.

Varela, L.G. (2000). Pierce's Disease in the North Coast. http://www.cnr.berkeley.edu/xylella/pd97.html

Yonce, C.E. and Chang, C.J. (1987). Detection of xylem-limited bacteria from sharpshooter leafhoppers and their feeding hosts in peach environs monitored by culture isolations and ELISA techniques. Environmental Entomology 16: 68-71.

_ EMBED
Word.Pict