SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE

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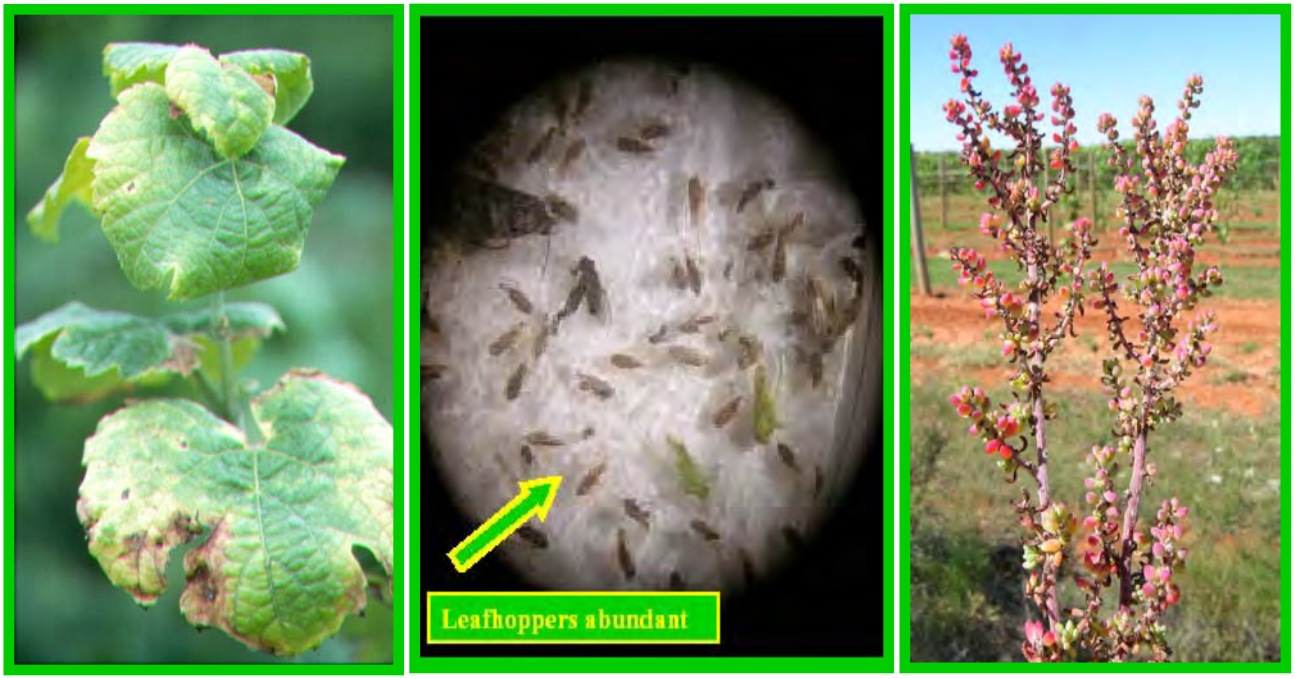
Grape and Wine Research and Development Corporation

# AUSTRALIAN GRAPEVINE YELLOWS – Source, Spread and Control




**FINAL REPORT to**
**GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION**

Project Number: **SAR 02/03**

Principal Investigator: **PA Magarey**

Research Organisation:
**South Australian Research and Development Institute**

Date: **May 2006**

# AUSTRALIAN GRAPEVINE YELLOWS

# Source, Spread and Control

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# TERMS OF THE REPORT

The following is a report about a series of investigations within Project Number SAR 02/03. For clarity and context, previous work that was continued and at times, expanded within SAR 02/03, is reported in separate chapters preceding those relating to the present work.

Examples include work undertaken in 2002/03 as part of GWRDC RITA Project 01/15-2, or in prior investigations initiated with assistance from the Riverland Vine Improvement Committee. The source of funding for each aspect of the work is detailed in the table below and is gratefully acknowledged.

The title of this publication refers to investigations into the source, spread and control of AGY. The body of work presented comprises descriptions of the investigations into determining the source of disease and the means by which it spreads – both are prerequisite to the development of a suitable control strategy. The present work makes no claim to have developed a control for AGY but rather to the laying a foundation toward the building of a suitable management strategy.

# SUMMARY OF PROJECT FUNDING

| Chapter | GWRDC | Riverland Vine Improvement Committee | RWIDC | Phylloxera Board | WGMB | SARDI |
|---|---|---|---|---|---|---|
| 1 | | | | | | ✓ |
| 2 | | ✓ | | | | ✓ |
| 3 | RT 01/15-2 | ✓ | | | | ✓ |
| 4 | SAR 02/03 | ✓ | | | | ✓ |
| 5 | | ✓ | | | | ✓ |
| 6 | | | | | | ✓ |
| 7 | | | | | | ✓ |
| 8 | SAR 00/2 | | | | | ✓ |
| 9 | RT 01/15-2 | | | | | ✓ |
| 10 | SAR 02/03 | | | | ✓ | ✓ |
| 11 | SAR 02/03 | | ✓ | ✓ | ✓ | ✓ |
| 12 | SAR 02/03 | | ✓ | ✓ | | ✓ |
| 13 | SAR 02/03 | | ✓ | ✓ | | ✓ |
| 14 | SAR 02/03 | | ✓ | ✓ | | ✓ |
| 15 | SAR 02/03 | | ✓ | ✓ | | ✓ |
| 16 | RT 01/15-2 SAR 02/03 | ✓ | ✓ | ✓ | | ✓ |
| 17 | SAR 02/03 | | ✓ | ✓ | ✓ | ✓ |
| 18 | SAR 02/03 | | ✓ | ✓ | | ✓ |
| 19 | SAR 02/03 | | ✓ | ✓ | | ✓ |
| 20 | SAR 02/03 | | ✓ | ✓ | | ✓ |
| 21 | SAR 02/03 | | ✓ | ✓ | | ✓ |

# CONTENTS

# ABSTRACT

In investigating the source and spread of AGY, none of more than 12,000 cuttings tested, transmitted disease. Surveys of >130 Chardonnay and Riesling vineyards and 50,000 Riverland, Riverina and Sunraysia vines showed levels of AGY ranged from 1-88% incidence with disease gradients found across vineyards near AGY-hot spots and wasteland vegetation where a native bluebush, ruby saltbush and climbing saltbush, tested PCR-positive for AGY. These are probably the primary hosts of disease. Intensive leafhopper trapping studies indicated that the green jassid and the common-brown leafhopper are prime candidate vectors. The prospects for further investigation are good.

# EXECUTIVE SUMMARY

A disease widespread with cause uncertain and control unknown, was investigated over many seasons up to 2005/06. Known as Australian grapevine yellows (AGY) this disease has been the subject of much confusion and debate. Reasons included a lack of awareness of the symptoms, of the source of disease and how it is spread. Additionally, the disease is epidemic and levels rise and fall from season to season. This made difficult the prediction of disease levels and the determination of its cause and severity.

The disease was similar to devastating yellows diseases in Europe and elsewhere and the threats these diseases posed enhanced local concerns about AGY, especially as they related to the risk of AGY being spread to new vineyards by dormant cuttings, at a time of rapid vineyard expansion in Australian viticulture. This was a recipe for anxiety and a call for action. Australian grapegrowers, particularly in the Riverland, Sunraysia and Riverina wanted some answers – especially concerning the source of disease, how it spreads and 'what can I do about it?'.

Experiments were conducted in a variety of trials from 1996/97 to 2005/06 on a diverse array of aspects concerning AGY. The symptoms of AGY were documented and a disorder, often confused with AGY and called Scaly Bark Stunt (SBS), was newly described (Chapter 2).

Extensive shadehouse- and field-experiments with cuttings taken from severely diseased vines showed that AGY was not spread by cuttings. Of some 12,000 cuttings tested, none showed AGY (Chapters 3 - 4). A small trial to test if AGY levels were reduced by pruning-off diseased canes made no difference (Chapter 5). The conclusion: factors other than propagation material provide the main source of disease. Interestingly, the latter experiment showed that AGY appears to survive in cordons and spurs and other vine tissue near where symptoms appear next season.

So, where did AGY come from?

To find out, two ways of surveying for AGY in vineyards were devised, tested and applied (Chapter 6 – 13). When a precise score of disease incidence is needed, a detailed arm survey which scores the AGY status of individual arms (cordons) on both sides of the vine, is best though it takes longer. A point survey that scores the disease status of only the facing sides of two rows at a time, is much quicker (averaging 20-30 vines/minute) but it will under-estimate levels of AGY by ~10% especially in taller canopies (Chapter 6).

In extensive surveys over a number of seasons, more than 140 vineyards and in excess of 58,000 vines in 2003/04 were examined and some typical features of AGY became apparent. Analyses of vineyard maps showed that AGY is an epidemic disease with peaks and troughs over the seasons (Chapter 7). The disease is widespread across the Riverland, Sunraysia and Riverina – very few vineyards of *cv*. Chardonnay or Riesling have no disease and yield losses vary from <1% to >30%. Trends in disease levels occur within regions at similar times. The first recorded peak in AGY in the Riverland was in 1978/79; the second in late 1999/00 – 2000/01. Disease levels usually remain high only for 1-2 seasons before declining and new peaks may not occur for a number of seasons. Also, within individual vines, symptoms come and go, the result of the combined effects of natural heat therapy and because diseased shoots die. There is potential to use the significant body of survey data, to gain further knowledge about the disease and how it spreads within the vine and the vineyard.

Additional surveys showed that that the source of AGY was not within vineyards and usually, not nearby (Chapter 8). The mystery of the source of AGY then began to be solved. Hot spots of

disease were located (Chapter 9) and then gradients of AGY across vineyards (Chapters 10 – 13), found in the Riverland were confirmed in the Riverina and Sunraysia. It became clear that the disease was originating either in wetland or riverine vegetation or in wasteland plants nearby. High levels of AGY were found to be associated only with native chenopods: a bluebush (yanga – *Maireana brevifolia*) and several saltbushes (ruby saltbush – *Enchylaena tomentosa*, and climbing saltbush – *Einadia nutans*) (Chapter 14). Then the breakthrough - these plants and an introduced species, false caper (*Euphorbia terracina*), tested positive in molecular (PCR) tests (Chapter15). This was the first record of AGY phytoplasma (AGYp) in native species in Australia.

Other progress is summarised:

- AGY is a disease of vineyards on the margins of viticulture *ie*. vineyards bounded by other vineyards have little risk of AGY and, to the contrary, vineyards on the interface between the viticultural region and wasteland vegetation, have a much higher risk of significant levels of AGY;
- the source of AGY is confined to zones within hot spots of dimension as small as ~100m. x ~50 m. and the infective distance of the presumed insect vector *ie* from source plant to the most distant new host, is ~ 600 - 1000 m.
- yanga bush is at least one of the main hosts that serve as an inoculum reservoir for AGYp in grapevines since AGYp was found in PCR-tests of 5 of 48 (10.4%) samples of yanga bush in the Riverland and in 6 of 81 (7%) from across all regions;
- AGY phytoplasma appear to be indigenous (native or naturalised) to the Australian and perhaps the Australasian region;
- the insect vector of AGY most likely feeds and/or breeds on one or more of these plant species; and that
- there is good prospect of locating the presumed leafhopper vector of AGY in or near one or more of the host plants identified above;

While studies of the source of AGY were continuing, so was the search for a means of spread. Insects were suspected as the vector of AGY and various trapping studies were made with help from Riverland Vine Improvement Committee and others (Chapters 16-19). An insect exclosure established over a commercial vineyard at Berri, SA, for three seasons showed that levels of AGY were significantly less than in an adjacent vineyard. This provided the first experimental evidence of a mobile vector for AGYp.

This project thus gave support to the hypothesis that an insect vector such as a leafhopper or a planthopper spread AGY from native plants to vineyards.

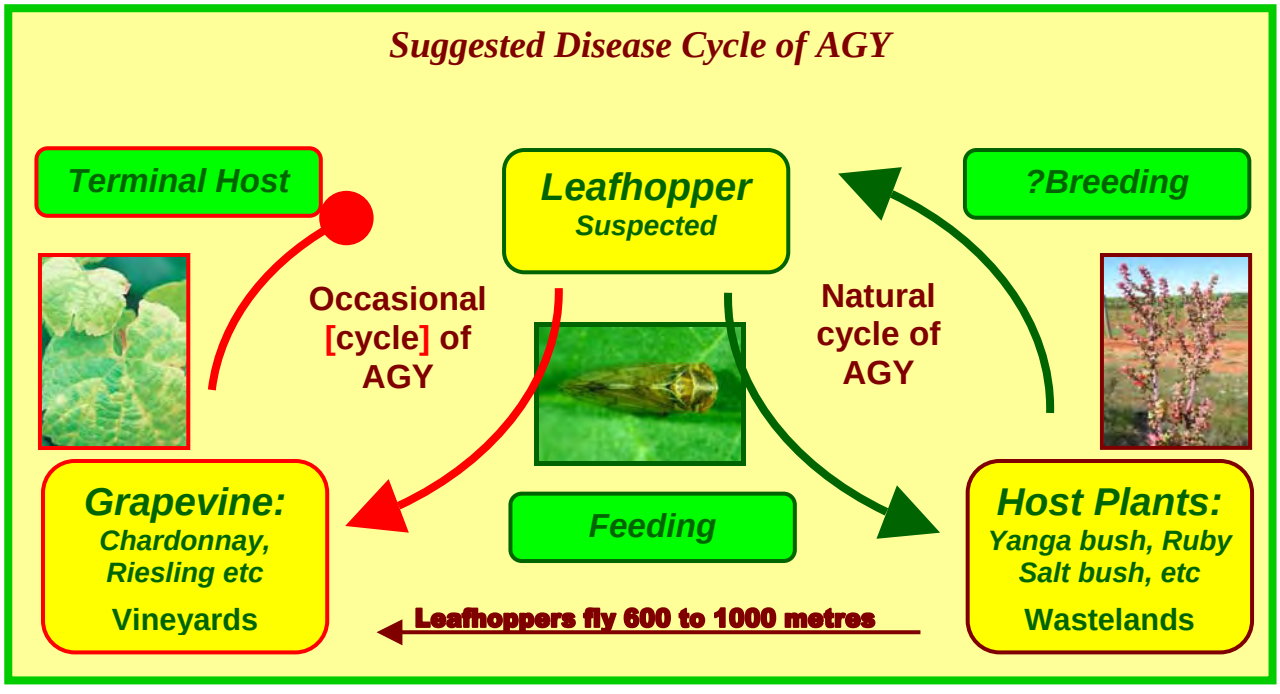
A series of trapping studies using sweep nets, light traps and sticky traps then showed that:

- yanga bush is the probable over-wintering host of *Orosius argentatus* (common brown leafhopper) in the Riverland and Riverina regions – previously not known for this almost ubiquitous leafhopper. This is of considerable interest especially given the finding of AGYp on that host;
- leafhoppers fly in high numbers on irregular occasions *viz.* warm nights with temperature $\geq 22\ ^{0}C$;
- they fly to light sources at near midnight and flight activity appears to be impeded by extreme temperatures *eg.* > 40 $^{0}$C during the day, or during windy and/or rainy weather conditions;
- it is probable that these characteristics also apply to the leafhopper vector of AGY and, if so, flight times of the vector and its inoculation of vines would be at irregular intervals each season dependent on the prevailing conditions especially temperature;

- Of the six most frequently found leafhoppers, *Austroasca sp.* and *Batracomorphus argentatus* were abundant and were most strongly correlated with high levels of AGY;
- *B. angustatus* is a prime candidate vector of AGY and should be investigated further since it transmits phytoplasma diseases elsewhere in Australia;
- it showed peak flights on only two occasions in the period of study and the dates of these were detailed with precision;
- Austroasca sp. are not considered vectors of AGY because they do not feed on phloem cells;
- *Orosius argentatus* was also abundant but trap counts showed it occurred at sites with varying incidence of AGY. Though it is not likely to be the prime vector of AGY, it should not be ignored.

The insect work showed that further investigations into the vector relations of AGY have good potential to identify the leafhopper species involved. Further detailed analyses of extensive vineyard mapping data will help identify aspects of the biology of the vector of AGY and provide an understanding of the disease in grapevines – knowledge that is essential for the development of a management strategy for AGY.

The present work has resulted in a clearer understanding of the source and means of spread of AGY and has described a potentially new disorder of grapes *viz.* scaly bark stunt (SBS). The knowledge gained on AGY has laid a foundation upon which to base continued investigation of the leafhopper(s) that spreads disease and the plant host(s) upon which it feeds. In summarising the present knowledge, a model to describe the disease cycle of AGY and how it spreads was detailed for the first time. The model summarises the aetiology and epidemiology of disease in the following diagram.




**Proposed life cycle of AGY on primary host plants and grapevines. Leafhopper insects are the vectors of AGY. They feed on plants such as the native yanga bush and occasionally on grapevines, transmitting AGY as they feed.**

Further progress in identifying the vector and the host plants and their biology is needed before a management strategy can be devised or a control developed for AGY. This work might be funded by GWRDC with assistance from local grape industry bodies in each of the major regions affected *viz.* Riverland, Riverina and Sunraysia, the Phylloxera and Grape Industry Board and also perhaps by Australian Research Council particularly since this project would suit a Ph. D. student or a post-doc programme.

It is likely to prove difficult to develop a control for AGY but for each of the yellows diseases for which an insect vector (agent of spread) has been determined, a successful control has been developed. For example, Flavescence dorée, the disease in France, is spread by a leafhopper that feeds only on grapevine. A single insecticide spray during dormancy is effective in controlling this otherwise serious disease. In Germany, Bois noir, a different form of yellows disease, is spread by other leafhoppers that feed on vegetation adjacent to vineyards. While more difficult to manage, effective weed control leads to control of that disease.

It is possible that the development of a control for AGY might require removal of the source of disease by removing native host plants, but this would not be welcome. Neither would the use of insecticide spraying be a desirable control measure. However, once the insect that spreads AGY has been identified and the conditions that favour its movement are known, there is potential to evaluate other forms of control such as light traps that might be used to attract leafhoppers on warm nights with temperatures above 22 $^{0}$ C, to reduce the incidence of disease in the vineyards nearby. More investigation of this is needed.

A number of presentations have been made to grower and industry groups in the Riverland and Riverina during the course of this project. In recent examples, a 20-page A5 coloured summary of the present work was presented to industry groups in the Riverland (Magarey *et al.* 2006a and b). While recognising that an understanding of AGY is not complete, it is appropriate that further details of AGY be shared in regional workshops in the Riverland, Riverina and Sunraysia so that Australian grapegrowers can learn more details of how identify the symptoms of AGY and to be informed of progress in understanding the source and spread of disease.

Several grape industry agencies assisted in the present project. These are acknowledged and cited in the fronts-piece in this report (see Terms of the Report).

**Investigations recommended re the source of AGY:**

To seek a better understanding of the epidemiology of AGY, which knowledge is critical to good prospects of finding the vector and a control for the disease:

- confirm the presence of AGYp through further PCR-tests for AGYp and other strains of '*Ca.* P. australiense' in the same three native and one introduced species at the same and different times of the season;
- sample from the native species in other hot spots and in areas with lesser AGY, to determine their likely role as primary hosts for AGYp;
- sample from other plant species in AGY hot spots, to obtain a better understanding of the array of plants that might be the primary hosts for AGY; and
- compare the isolates of phytoplasma from grapevine and native species by RFLP-analysis of tuf / rp to confirm if the strain detected is AGYp or another variant of '*Ca.* P. australiense'.

**Investigations recommended re the spread of AGY:**

- confirm through trapping and PCR testing the presence or absence of AGYp in the current high suspect vector insects;
- as a matter of urgency, the sticky traps that were placed in the Riverland and Riverina during 2005/06 be assessed to identify the types and frequencies of trapped insects, especially the leafhoppers and planthoppers with particular reference to *Batracomorphus angustatus* and *Orosius argentatus*;
- further investigate the biology and vector relations of leafhoppers and planthoppers that occur in association with hot spots for AGY in a fully resourced project of minimum duration three seasons commencing in season 2006/07 so as to allow continuity of data already collected;
- an extensive database of vineyard spatial and temporal disease assessment scores needs further analyses to elucidate details of the epidemiology of AGY disease and the biology of the leafhopper vector of AGY.
- confirm the attraction of leafhoppers to light sources at night to evaluate the possibility of using light-traps placed in the vicinity of primary host plants as an ecologically sound, low-risk management tool for the presumed leafhopper vector of AGY, to divert the insects from feeding in the vineyards and so reduce the incidence of AGY below an economic threshold.

**Future Strategy for Investigations into AGY**

1. It is proposed that more detailed PCR analyses of native plant species in and near the hotspots of AGY be undertaken to determine:
    1) which species are hosts of AGY; and
    2) is the pinking discolouration a quick diagnostic symptom of AGY?;
    3) is AGY related to Scaly Bark Stunt (SBS) and/or RSG symptoms on grapevine;
2. It is proposed to investigate the leafhopper and planthopper fauna of these native plants and undertake PCR analyses of the insects to determine which if any, spreads (vectors) the AGY phytoplasma with view to seeking a practical management strategy for AGY. See Figure 21.1 for a presentation of this plan;
3. It is suggested that a workshop be convened for the investigators of '*Ca.* P. australiense', the AGY pathogen, to bring synergy from the diverse expertise of scientists presently investigating AGY in an array of crops including pawpaw, strawberry, cucurbits, clovers and grapevine, and native plants of Australia and New Zealand including flax, the latter as probable primary hosts of the pathogen. Such a meeting should include key personnel as investigators of grapevine yellows overseas especially Dr Michael Maixner, from Bernkastel-Kues, Germany. The collective knowledge would greatly speed progress on investigating the disease on grapevine.

## Summary of Recommendations – Listed by chapter

### Chapter 2: Background

It was proposed that the present work would be undertaken:

- to expand investigations initiated during 2001/02 under GWRDC RITA Project RT01/15-2 and during projects sponsored in part by Riverland grape industry agencies;
- in attempt to answer some of the queries raised above with particular reference to those in the review by Randles (2000); and
- as a three year investigation to provide information about AGY as a disease, its source and spread and its epidemiology with regard to providing a platform of understanding of how a management strategy might then be developed.

### Chapter 3: Does AGY spread via Cuttings? 1

It is recommended that:

- the duration of bioassay be continued; and, to bring a more definitive conclusion to the work, specifically, that
- the existing propagation material be grown for at least another season to determine if symptoms a) show after a longer period of incubation in pots; or b) in field (vineyard) plantings of the material.

### Chapter 4: Does AGY spread via Cuttings? 2

It is recommended that:

- the premise that '*… propagation material from AGY affected vines provides a commercially significant source of disease*,' should be abandoned, and that;
- the source and spread of AGY be further investigated through studies of other factors such as a mobile vector.

### Chapter 5: Can Targeted Pruning Control AGY?

It is recommended that:

- The use of targeted-pruning of dormant canes to remove AGY from diseased vines is not recommended as a practice of value in reducing disease.
- Further studies of the epidemiology of AGY to determine how the disease moves to and within affected vines should consider the spur and proximal (near) sections of cordons and not the canes, as the likely site of overwintering inoculum.
- This work complements the findings of Chapters 3 and 4 and enhances their recommendation that, in pursuing epidemiology of disease and the primary sources of inoculum for AGY, the propagation material should be ignored.

### Chapter 6: Vineyard Surveys as a Tool to Investigate the Epidemiology of AGY

The type of survey for optimum efficiency depends on the planned use of the data. It is recommended that:

- the detailed arm (cordon) survey be used for epidemiological studies of the expression of AGY from season to season within and between vines; and that
- the faster point survey be used for all other studies including comparison of the relative level of AGY between vineyards, though noting that in vineyards with canopies taller than 1.8 – 2.0m, actual disease levels will be ~10% higher than shown by those surveys.

## Chapter 7: The Epidemic Nature of AGY

It is recommended that:

- weather data be analysed for associations of specific environmental conditions, in particular, in evaluating the influence of temperature on:
    a) the timing of disease expression on a seasonal basis; and
    b) the severity of disease expression on a regional basis and subsequent crop loss;
- the hypothesis that an insect vector spreads disease and its activity in the previous season influences levels of AGY in the next and subsequent seasons, be tested in further investigations.

## Chapter 8: Locating the source of AGY 1 – long distance transport is the norm

It is recommended that:

- investigations into the source of AGY be focussed on riverine and similar vegetation, the ecosystem(s) most likely to contain the primary host plant(s) and the source of inoculum for the disease.

## Chapter 9: Locating the source of AGY 2 – hot spots of disease occur

It is recommended that:

- the disease surveys be intensified, especially within the 'hot spot' localities of high disease in anticipation of finding higher levels of AGY on vineyard boundaries in vine blocks adjacent or near to the specific source of disease; and that
- more detailed assessment of levels of disease in additional localities in the Riverland and in other regions such as the Riverina be assessed to determine if the same pattern of occurrence of AGY existed in those districts.

## Chapter 10: Locating the source of AGY 3 – discovering disease gradients

It is recommended that:

- investigation be made to better define the possibility that disease gradients occur across vineyards that lie within the so-called 'hot spot' zones of high disease risk;
- an high priority be given to a detailed investigation of the plant species and leafhoppers and/or planthoppers present in the hot spots zones which include zones of permanent and/or semi-permanent shallow water adjacent to vineyards with high severity of AGY; and that
- the recent findings and clear descriptions of AGY and 'look-alike' diseases be presented to grapegrowers especially those in the high severity regions for AGY *viz.* the Riverland, Sunraysia and Riverina.

## Chapter 11: Locating the source of AGY 4 – defining disease gradients

It is recommended that:

- the hot spots of AGY be further investigated in pursuit of the source and means of spread of the disease; and that
- more detailed surveying be undertaken of vineyards within the zones of high risk of AGY to better define the disease gradients which, as a result, should provide more precise indicators as to the location of the primary host of AGY within those hots spots.

## Chapter 12: Locating the source of AGY 5 – refining disease gradients

The evidence from studies up to and including 2003/04 suggests that:

- the detailed vineyard surveys should be continued for a third season to confirm the specific disease gradients found in vineyards adjacent to riverine and/or wetland ecosystems and importantly, in attempt to reduce the number of candidate primary hosts;
- investigation of the suspect native vegetation at those localities should be focussed on common reed and selected Chenopod species such as bluebush and saltbush with view to finding the primary host of AGY;
- investigation of the suspect leafhopper vector of AGY should be focussed in the same location as the primary host plant (as above) – indeed, on that very host (once located); and that
- investigation be made of the location of the highly diseased *cv* Riesling vineyard near Griffith in the Riverina, in attempt to locate both the primary host and the leafhopper vector at that location.

## Chapter 13: Locating the source of AGY 6 – confirming disease gradients

It is recommended that the investigations continue to:

- seek the source of AGY using data from the detailed arm surveys and the point surveys to locate likely primary plant host(s); and
- focus on the 15 or so native plant species in the zones described above, one or more of which may be the primary source of AGY inoculum.

## Chapter 14: Reducing the Number of Host Plants to be Tested

In continuing the search for the primary host(s) for AGY, several points of investigation were recommended for immediate action *viz.*:

1. that the riverine/wetland species within hot spots of AGY be excluded from the list of plants to be sampled for AGYp, the phytoplasma associated with AGY in Australian viticulture;
2. that native chenopod and similar shrubs including yanga bush (short-leafed bluebush) (*Maireana brevifolia*) and ruby saltbush (*Enchylaena tomentosa*), in hot spots of AGY in the Riverland and at least one other region, be sampled for PCR-tests using specific primers for AGYp; and that
3. other plant species from within the hot spots be considered for PCR-testing as a second order of priority to determine the array of plants that might be the primary hosts for AGY and possible breeding-hosts for the insect vector of AGY.

## Chapter 15: The Role of Native Plant Species – Closing in on the Primary Hosts

Several points of investigation are recommended for immediate action *viz.*:

1. re-sample the same host species in the same localities at the same and different times of the season for PCR-tests for AGYp and other strains of '*Ca.* P. australiense', to confirm the presence of AGYp (and perhaps other strains of '*Ca.* P. australiense') in at least three native and one introduced species;
2. sample from the native species in other hot spots and in areas with lesser AGY, to determine their likely role as primary hosts for AGYp;
3. sample from other plant species in AGY hot spots, to obtain a better understanding of the array of plants that might be the primary hosts for AGY; and

4. at each point, compare the isolates of phytoplasma from grapevine and native species by RFLP-analysis of tuf / rp (as per Streten *et al.* 2005c) to confirm if the strain detected is AGYp or another variant of '*Ca*. P. australiense'; and thus,
5. seek a better understanding of the epidemiology of AGY disease which knowledge is critical to good prospects of finding the vector and a control for the disease.

**Chapter 16: The Role of an Insect Vector 1 - A Mobile Vector is Confirmed**

It is recommended that:

- investigations in pursuit of a leafhopper and/or planthopper vector of AGY be undertaken, preferably utilising modern PCR technology;
- an extensive database of vineyard spatial and temporal disease assessment scores be further analysed to elucidate details of the epidemiology of AGY disease and the biology of the presumed leafhopper or related vector of AGY.

**Chapter 17: The Role of an Insect Vector 2 - Sweep-netting for Leafhoppers**

It is recommended that:

- further insect trapping studies should be undertaken to investigate the role of *O. argentatus* and of cixiid and other leafhopper insects in the epidemiology of AGY;
- in particular, studies of the flights and occurrences of all leafhoppers and planthoppers in and near hotspots of AGY are warranted during the warmer months of the growing season.

**Chapter 18: The Role of an Insect Vector 3 - Surveys Using Light Traps**

It is recommended that:

- additional insect trapping studies are needed to determine the frequency of individual leafhopper and planthopper species in their accessing and feeding on grapevines and native species such as yanga bush and various salt bushes, to determine the identity of the insect vector of AGY.
- further investigation of the timing of and conditions for leafhopper flights is needed to resolve the factors that influence the movement of the vector of AGY, hence the timing and conditions which favour transmission of disease by the vector;
- studies similar to the present *viz.* using light traps, within both wasteland and vineyard settings are needed to resolve the flight patterns of the presumed vector of AGY;
- studies to confirm or deny the attraction of leafhoppers to light sources at night are suggested in pursuit of the possibility that light-traps placed in the vicinity of primary host plants might serve as an ecologically sound, low-risk management tool for the presumed leafhopper vector of AGY. The principle involved being that the majority of insects might be diverted toward the light source and sufficient numbers drawn away from feeding in the vineyards near by so as to reduce the incidence of AGY below an economic threshold. Alternatively, a device based on a UV insect 'zapper' that attracted and killed the insects might be better.

## Chapter 19: The Role of an Insect Vector 4 - Surveys Using Sticky Traps

The present study provided strong supportive evidence in favour of the hypothesis that a leafhopper or planthopper is vector of AGY which is transmitted from a native plant primary host growing in the vicinity of affected vineyards.

As a result, it is recommended that:

- as a matter of urgency, the sticky traps that were placed in the Riverland and Riverina during 2005/06 be assessed to identify the types and frequencies of trapped insects, especially the leafhoppers and planthoppers with particular reference to *Batracomorphus angustatus* and *Orosius argentatus*;
- further investigations into the biology and vector relations of leafhoppers and planthoppers that occur in association with hot spots for AGY, be initiated for season 2006/07 in a fully resourced project of minimum duration three seasons;
- the focus of these studies should be on deploying sticky traps, sweep netting and light traps in hot spot zones of AGY in association with PCR analyses of native plant host species, leafhopper insects and vines as appropriate;
- focus should also be on the occurrence, flight patterns and PCR status of the leafhopper species *Batracomorphus angustatus* and *Orosius argentatus*, among others;
- coupled with detailed analyses of the existing database of the occurrence and frequency of AGY within sectors (arms) of vines and other vineyard mapping data, the investigations will provide valuable understanding of the epidemiology of the disease and the behaviour of the insect vector(s);
- the studies should also include insect feeding studies in laboratory and field situations such as the placing of seedlings of *Maireana brevifolia* and other native species implicated in the AGY disease cycle (see Chapter 20), within netting-exclosures and in adjacent exposed sites, with insect traps nearby, to investigate which insects if any are associated with the development of infection by the AGYp.
- the success with the present project warrants the seeking of specialist advice on the development of these investigations in the dormant season 2006. It is recommended that advice is sought immediately from specialists with expertise in insect biology, insect taxonomy, molecular detection of phytoplasma, the taxonomy and ecology of native chenopods, and the biology and epidemiology of AGY as a disease, these experiments of optimum design are implemented as soon as possible in preparation for season 2006/07.

# Section 1: Project Details

## Chapter 1: Project Aims and Performance Targets:

### Objectives

To develop a practical control for AGY, by identifying:

1. the source of AGY – to determine the role of propagation material and/or insect vectors.
2. how AGY spreads to vineyards – to determine the source of an insect vector and if and when it migrates to vineyards and infects vines;
3. if and when AGY spreads within a vineyard – to determine if AGY spreads from diseased vines and/or weeds within the vineyard; and
4. how to prevent and/or manage AGY – to assess possible alternate hosts of disease, define vector-host relations and seek management options; and to evaluate possible remedial treatments for AGY through application of anti-senescence metabolites to affected vines.

### Outputs and Performance Targets as they appeared in the original application

**Outputs**

2. Resolution of the role of propagation material in the spread of AGY.
3. Knowledge of the role of insects in the spread of AGY.
4. A list of candidate alternate hosts of AGY.
5. Information about options for possible control strategies for AGY through changing vector/alternate host population dynamics.
6. Knowledge of the role of auxins in the expression of AGY symptoms.
7. AGY reference collection in culture.

**Performance targets**

1. In excess of 7,000 cuttings evaluated for expression of symptoms and presence of AGY by June 2004.
2. Levels of AGY assessed in an insect free environment and compared with levels in a commercial vineyard by June 2004.
3. Vegetation surveyed in Riverland and Sunraysia regions adjacent to ‘hot spots’ of AGY by June 2004 and a short list of species identified.
4. The host/vector relations initially studied for candidate insects by June 2005.
5. Field evaluation of at least ten vines for reduced symptom expression by June 2005.
6. At least 10 AGY infected plants of two species of alternate host in permanent glasshouse culture by June 2005.

Due to early success with the above objectives, it was considered best use of the project resources to retain focus on Outputs and Performance Targets 1 – 4. There remains benefit to pursue Items 5 when additional resources become available. It is expected that Item 6 will be the consequence of further progress in the projects objectives as the biology of Australian Grapevine Yellows is further understood.

### Exception Report to GWRDC

During the course of the project it became apparent that additional resources were necessary to fund molecular fingerprinting (PCR analyses) of AGY within a variety of potential host plants adjacent to vineyards with high levels of the disease. GWRDC agreed to provide a further $4,500 for these purposes. See Appendix 5.1 for details.

# Section 2: Introduction to Investigations

## Chapter 2: Background

### A Brief History of AGY

A new disease of Australian vineyards was observed first in the mid 1970's at CSIRO, Merbein, Victoria as a set of symptoms with cause unknown (Krake *et al.* 1999). It was reported for the first time in the Riverland, SA, in 1976, tentatively named (Magarey *et al.* 1985), then surveyed in other regions and found widespread across the country (Magarey *et al.* 1986b). It appeared on a number of varieties but the main ones affected were Riesling and Chardonnay (both then relatively new in Australian viticulture).

The disease, subsequently named Australian Grapevine Yellows (AGY) in 1981, showed some unusual characteristics. For instance, it was more severe in warmer inland regions such as the Riverland, Sunraysia (Victoria and NSW) and the Riverina (NSW). Moderate levels were seen in the Hunter Valley (NSW) while levels were lower in the cooler more southerly zones (Magarey *et al.* 1981). The disease also showed natural heat therapy, a phenomenon unique in terms of grapevine diseases. If daily maximum temperature exceeded $40^0$ C for 1-2 consecutive days, stunted and dying shoots with yellowed leaves and shrivelled bunches would begin to regrow within 10 – 14 days. This may account for why vines affected with AGY would often show symptoms for only one season before apparently recovering in the next.

These particular attributes of AGY made investigations difficult and added confusion in attempts by those unfamiliar with the disease to correctly identify symptoms. Indeed, much of that confusion remains today.

### Symptoms

Accurate diagnosis of AGY requires specific symptoms in four components of the grapevine: the leaves, the shoots, the bunches and the vine. Affected leaves turn yellow and curl downward (Figures 2.1-2.4). The yellowing (chlorosis) varies with seasons and perhaps other factors related to the type of pathogen associated (see Chapter 15). The chlorosis may be veinal (Figure 2.2), blotchy between the veins (interveinal) (Figure 2.2) or marginal (Figure 2.3). On red varieties, the chlorosis is red and is strikingly limited by the veins (Figures 2.27 – 2.29).

Affected shoots are stunted because shoot apices cease activity while the stems remain unlignified (they do not harden-off) and are rubbery though they snap easily when bent. Shoots die from the apex back, the more so in hot conditions (Figures 2.5 – 2.6). In cool seasons the lower internodes exhibit small (<1mm diameter) blotchy and oily-black raised pustules while the shoots take on a bluish hue.

Bunches shrivel and fall from flowering onwards as the leaves and shoots begin to show symptoms (Figure 2.5, 2.9, 2.10, 2.26). Young Riesling inflorescences usually collapse during flowering and rarely set fruit while in Chardonnay, fruit set frequently occurs but individual berries usually shrivel as they approach maturity (Figures 2.5, 2.9). Sometimes berries on affected bunches do not ripen properly and are bitter to taste.

An important feature is that AGY usually only affects several adjacent shoots on the vine leaving the remainder of the arm (cordon) unaffected (Figure 2.6). Rarely is the whole vine affected though when it is, crop loss is virtually complete. Diseased shoots die in the season of symptom expression or overwinter (Figures 2.20 – 2.21) often leaving affected spurs and/or section of the cordon barren or dead (Figures 2.23 and 2.23a).



**Figure 2.1a & b:** **Symptoms of Australian Grapevine Yellows (AGY) are distinctive once recognised correctly.**

**To Diagnose Australian Grapevine Yellows (AGY)**

**Look for four main features:**

- **Leaves - turn yellow, curl downward and fall early;**
- **Shoots - are stunted, fail to harden and die back;**
- **Bunches – shrivel from flowering onwards; and**
- **Vines – usually only a few shoots show symptoms.**

**The main varieties affected are cvs. Chardonnay & Riesling though cv. Sangiovese has shown moderate levels of disease.**




**Figure 2.1c - e:** **In cooler seasons, AGY-affected shoots show varying levels of black pustules on lower nodes of shoots. The pustules are more common on shoots affected with similar yellows diseases of grapes overseas (where the climate is cooler than Australia) – Photos: MW Maixner.**




**Figure 2.2 – 2.4: AGY-affected leaves showing the variation in yellowing (chlorosis) in white varieties such as Chardonnay and Riesling: sometimes it is veinal (2.2); blotchy between the veins (interveinal) (2.3); or marginal (2.4).**


**Figure 2.5: AGY-affected shoots first show symptoms at or near flowering (late October onwards). Leaves turn yellow and roll downwards while bunches begin to shrivel. Note the difference in bunch development on the unaffected shoots in the background.**

**Figure 2.6 – 2.8: Leaves on diseased shoots overlie each other like tiles on a roof (2.6). Shoot tips (apices) stop growing, stunting the shoot as leaves fall prematurely (2.7). Shoots remain green and do not harden-off (lignify) (2.8). In cool springs, small black pustules are seen at the base of shoots (on lower internodes).**




**Figures 2.9 – 2.10: From flowering onwards, especially in *cv.* Riesling, the young bunch (inflorescence) shrivels and falls (2.9). In *cv.* Chardonnay, though individual berries may set, most shrivel near harvest time so that affected shoots usually produce no fruit (2.10).**



**Figures 2.11: Typical view of AGY on *cv.* Chardonnay. Leaves tend to remain greener but curl downwards and roll more tightly than on *cv.* Riesling.**




**Figure 2.12 – 2.14: Often, only one or two shoots are affected per vine and then usually on the upper surface of the canopy (2.12 –2.13). In hot weather, yellowed leaves turn brown (necrotic) and fall early (2.14).**


**Figure 2.15: When the whole vine is affected, crop loss is effectively complete. This vine is severely diseased but may be nearly free of AGY next season.**

**Figure 2.16 – 2.18: The progression of AGY in shoots. Stems have a bluish-green hue, they fail to harden (2.16) and remain rubbery but snap easily when bent. Leaves turn brown (2.17) and fall leaving barren shoots (2.18).**






**Figure 2.19 – 2.21: AGY-affected shoots die back from the tip, often breaking at the nodes. AGY causes shoots to die either in the season of growth or over-winter. Sometimes the spur also dies.**


**Figures 2.22: Young vines affected with AGY often are systemically affected and show severe symptoms in the whole vine. Note the barren stunted shoots on this severely diseased arm.**

**Figure 2.23 & 2.23a: In the season following symptom expression, diseased shoots have died out. Where AGY-affected vines recover, previously affected sectors of the arm and spur remain barren.**



**Figure 2.24: Severe AGY in a young (two year old) *cv.* Riesling vine systemically affected. Usually symptoms do not appear until at least the third growing season. Note that all shoots are severely stunted and that crop loss is total on this vine.**


**Figure 2.25: Another vine with significant AGY infection on *cv.* Riesling. Note the disease on most shoots of that vine. Leaves of *cv.* Riesling with AGY show more chlorosis (yellowing) than occurs in Chardonnay.**


**Figure 2.26: AGY sometimes only affects part of the canopy. Here half of one vine is badly affected. It is not uncommon for AGY to appear in one sector of canopy that comprises adjacent diseased shoots from neighbouring vines. This is consistent with the pattern expected from an insect feeding at that one position in the canopy.**




**Figure 2.27 – 2.29:** **Red varieties turn deep red with a strikingly different pattern on leaves than in white varieties. In red fruited varieties, parts of bunches may collapse and die.**

**Figures 2.30 & 2.30a:** **Natural heat therapy is a phenomenon in which diseased shoots begin to regrow after 1-2 days of maximum temperature above 40°C. This may explain why diseased vines recover from AGY for one or more seasons.**







**Figures 2.31 & 2.31a:** **A block in the flow of the vascular system (the sap) has caused AGY-like symptoms. In this case, a broken shoot has yellowed leaves but … they are not curled downward, the shoot is pink in colour and has zig-zag growth – these are not symptoms of AGY. 2.31a shows the gnarled and broken shoot close -up.**


**Figures 2.32:** Scaly Bark Stunt (SBS) is often confused with AGY. Leaves, shoots, bunch stalks and berries are stunted. Different sized berries (hen-and-chicken effect) is common but yellowing of leaves is rare.


**Figures 2.33:** Usually all shoots on an SBS-affected vine are affected leaving a canopy that is 'stepped-down' in height compared to its neighbours. Often the leaves are a dark and sometimes a shiny green.


**Figures 2.34:** The bark on SBS-affected shoots has transverse cracks across the wood rather than the usual up and down (longitudinal) cracking. This effect causes the typical scaly bark appearance as seen here.

**'Scaly Bark Stunt' (SBS) – a Possible New Disorder Confused With AGY**

While surveying for AGY, a consistent set of symptoms of unknown origin was seen in many vineyards on from a few to many vines, especially *cv*. Chardonnay. The symptoms of this disorder have been confused with AGY showing AGY-like symptoms on ~10-15% of affected vines. In order to draw distinction from and reduce confusion with AGY, this disorder was given the name Scaly Bark Stunt (SBS) (Magarey 2003).

A preliminary description follows but refer also to Figures 2.32 – 2.34.

Compared with healthy vines, the following symptoms were noted:

**Leaves** are:

- small;
- dark green and sometimes rolled downward at the margins;
- later, especially when vines were under stress, yellow blotches may develop across the blade of some;
- interveinal and/or marginal burning (necrosis) sometimes follow.

**Shoots** are:

- short (stunted);
- usually hardened off (lignified) and with functional tips;
- affected uniformly *ie* usually most shoots on a vine are affected;
- some show zig-zag growth.

**Bunches** are**:**

- small because both bunch stalks and berries are small (stunted);
- sometimes also with hen and chicken effect (berries of different size);
- rarely, necrosis of berries also occurs.

**Bark on two-year old and older wood** shows:

- transverse cracking (fissures) in the thin layer of surface bark. This leads to,
- upward curling of the bark at these cracks to give a scaly appearance like the bark on silver birch trees.

**Vineyard**:

- affected vines occur singly or clumped - the clumping may reflect only the frequency of occurrence of the disorder rather than be indicative of any specific mode of transmission.

Scaly bark stunt may be a distinct disorder or it may be the separate expression of another disease such as AGY, or it may be a clonal variation *etc*. It has been seen on both *cvs* Chardonnay and Riesling and may occur on other varieties.

## Cause of AGY (Aetiology)

The nature of AGY as a disease was uncertain. At the time the present investigations began, there was much confusion and variation in opinion about the cause of AGY and its means of spread. There were only two possible causes: either a biotic agent or an abiotic factor, *ie.* a living, contagious parasite such as a plant pathogen, or an environmental or host factor.

In reviewing research associated with AGY for the Grape and Wine Research and Development Corporation (GWRDC), Randles (2000) reported that distinction between the two was needed because the impacts of an abiotic disorder and a biotic disease are very different. Disorders are limited in distribution whereas diseases are able to spread, multiply and cause severe epidemics. The control procedures for each are different too. Usually a disorder can be corrected by a simple direct measure such as in correcting a nutrient deficiency, whereas a disease requires control measures based on definition of the cause and source of disease, a knowledge of the disease cycle and how the disease spreads, and intervention based on interrupting the disease cycle. (Randles 2000) further indicated that '… it is essential to first determine whether (AGY) is an abiotic or a biotic disease as the latter presents more complex control options and an urgency arising from a need to avoid a possible epidemic'.

The review considered the most important gaps in knowledge of AGY at that time were:

- An unambiguous description of symptoms of AGY was not available to distinguish it from associated syndromes;
- Experimental transmission of AGY symptoms to grapevine or other plant species had not been achieved; and as a result
- No biological indicators were available for experimental and diagnostic studies; and
- AGY had not been expressed in plants grown in a controlled environment; and thus
- There was no model system in a controlled environment to test the effect of either heat or chemotherapy on AGY; and
- Growth parameters of affected plants in a controlled environment had not been measured;
- The rate of incursion of AGY (new disease) into pathogen 'free' vines was not known; and
- The patterns of spread of the AGY disease had not been analysed to provide spatial and temporal descriptors of dispersal.

In relation to the possible causal agent:

- The AGY phytoplasma had not been isolated into a standard phytoplasma host (*eg.* periwinkle - *Catharanthus sp*.).
- It was not known which potential agents beside phytoplasmas are present in grapevines and possibly associated with AGY.
- It was not known whether the AGY agent is endemic or introduced to Australia.

Koch's postulates, *ie.* the rules which need to be satisfied to identify a biotic agent as the pathogen causing disease (ill health), have been satisfied for all pathogen groups such as fungi, bacteria and nematodes but not for presumed pathogens such as phytoplasmas and some virus and viroids. This is because the latter agents are intracellular *ie.* they live inside the cells of plants and, to date, can not been grown in isolation and inoculated experimentally.

As a result, a range of experimental steps are needed to show these agents cause disease:

- an inability to relieve symptoms by the application of nutrients;
- an ability to transmit the disease by grafting; mechanical inoculation; or by an insect;
- the efficacy of antibiotics;
- the recognition of the agent by its shape *eg.* by electron microscopy; and
- detection by protein (antibody) methods or gene structure (nucleic acid) methods such as polymerase chain reaction (PCR) analyses.

Some of these tests concerning AGY were undertaken prior to the present work (in SAR 02/03). They included:

- symptoms In investigating AGY, the description of disease was taken as the set of symptoms first described for it (Magarey *et al.* 1985) and as presented in Figures 1-30 (above).
- nutrients Previous investigations into AGY analysed leaves and petioles and found no association with any nutrient deficiency or toxicity or any abiotic factor except an excess of nitrate nitrogen in diseased but not symptomless shoot tissue (Magarey *et al.* 1986b).
- transmission Attempts to transmit AGY by a variety of methods including green graft and chip budding and by direct propagation of cuttings from diseased vines were not successful (Magarey and Emmett, pers. comm.). The disease is yet to be cultured.
- antibiotics When antibiotics were pressure-injected into diseased vines during dormancy (Magarey *et al.* 1986b), tetracycline (Terramycin®) and not penicillin was effective in preventing symptoms in the following season (Figure 2.35). This inferred phytoplasma were associated with disease. That the treatment during the dormant period was effective indicated that the agent associated with disease was present in the season *before* symptoms appear and that the incubation period was therefore at least 8-9 months.

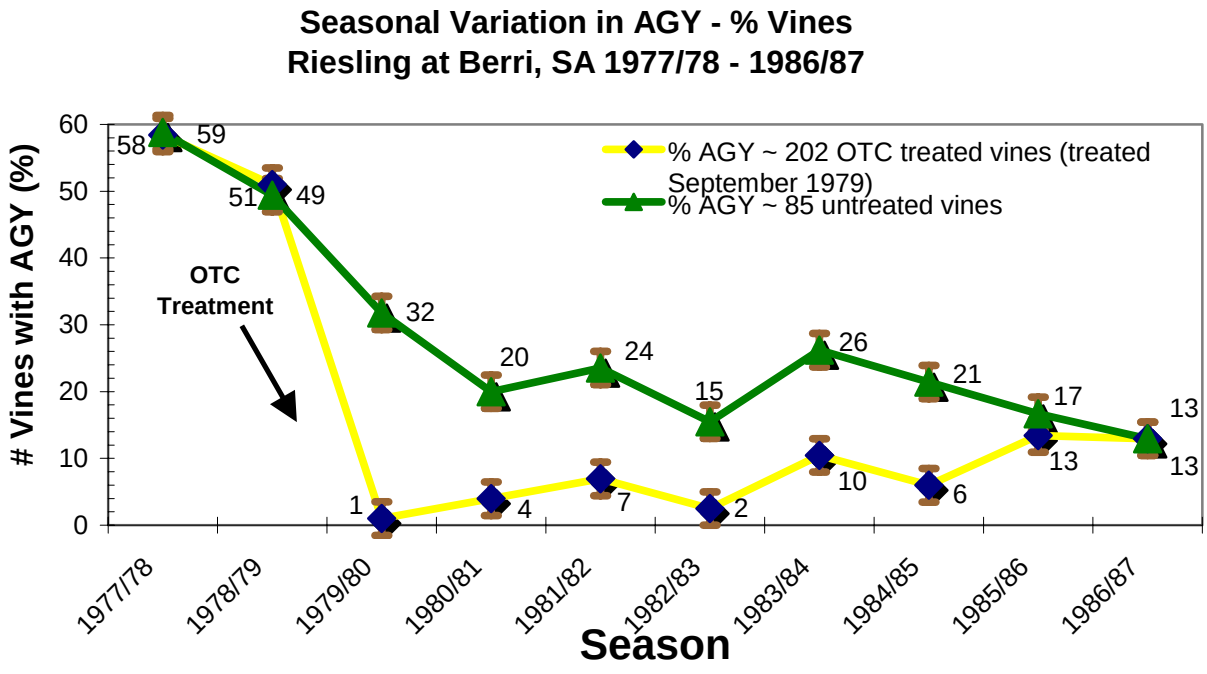



**Figure 2.35: The antibiotic tetracycline when applied to diseased vines during dormancy, significantly reduced the level of AGY in field experiments. This inferred that AGY was associated (?and caused by) phytoplasma organisms but more information was needed. (Data from Magarey *et al.* 1986b).**

- microscopy Studies with an high powered autofluorescence microscope revealed fluorescence in phloem cells at wavelengths similar to that of callose, in diseased but not symptomless shoot, petiole and leaf vein tissue (Figure 2.36). This further pointed toward phytoplasma as causal agents and the likely location of the pathogen (Magarey *et al.* 1986b).

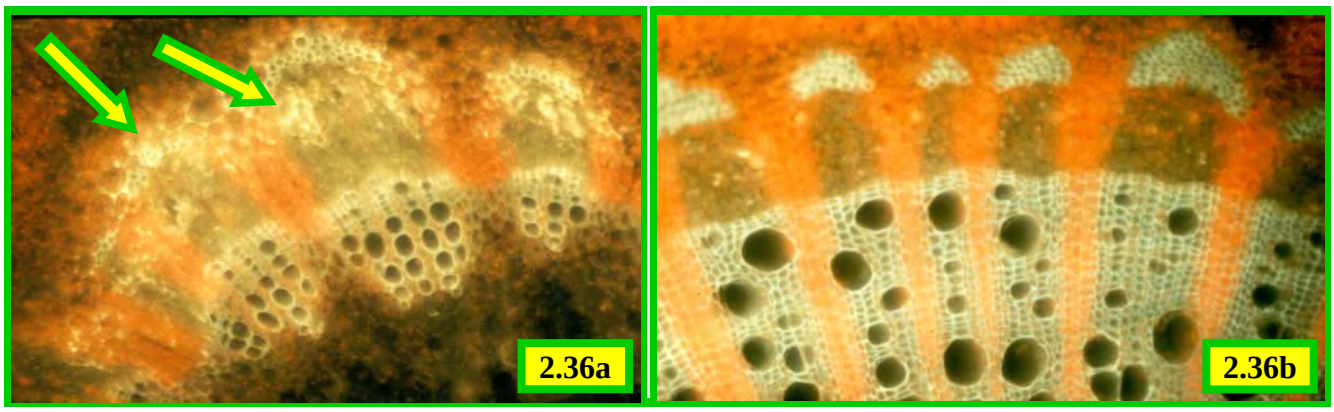



**Figure 2.36: Transverse section of *cv.* Riesling stems. Fig. 2.36a, at ~x100, with- and Fig 2.36b, at x~200, without-autofluroescence (yellowing) of the phloem sieve cells correlating with shoots with and without symptoms of AGY respectively. This autofluoesence occurs consistently with phytoplasma infected phloem and indicated where the phytoplasma (the presumed disease causing agents) were located.**

- electron microscopy Phytoplasma-like organisms were observed in the phloem of diseased but not symptom-less leaf veins tissue (Magarey *et al.* 1988);
- PCR Analyses Evidence to date had shown a frequent correlation of between 25% - 50% with the presence of symptoms and the detection of AGY phytoplasma by PCR analyses.

Given the above, there was good evidence that phytoplasma were associated with symptoms of AGY. However, Randles (2000) in acknowledging this association, indicated that in the absence of more definitive data, the phytoplasma had not been shown to the cause of disease, arguing the possibility that the symptoms might be caused by a complex of agents.

Randles (2000) further indicated that for all putative phytoplasma diseases the connection between a specific phytoplasma and a discrete disease like AGY is often not clear since these agents can occur in mixed infections with other phytoplasmas or with other pathogens. Different phytoplasmas can be associated with identical symptoms in the same host plants and some closely related phytoplasmas might cause different symptoms in different host plants. It ought not be assumed therefore that specific phytoplasmas cause specific diseases (Davis *et al.* 1998), though the evidence with regard to AGY (as above) pointed toward the strong association of AGY and phytoplasma as its causal agents.

The lack of data on the biotic nature of AGY was an important deficiency in knowledge of the disease (Randles 2000). That author considered it essential to determine the cause of disease and its epidemiology and suggested a 5-year plan to determine this and the mode of spread so as to develop control measures. He speculated that AGY might be a disease arising from outside the crop and its epidemiology could well be explained by occasional incursions of infective vectors.

In resolving the aetiology (cause of disease) for AGY, the (albeit tentative) evidence of a phytoplasma pathogen was strengthened by the similarity of symptoms with other yellows diseases overseas. Some of these were summarised by Daire *et al.* (1997), Maixner *et al.* (1995) and Magarey (1986) to show that:

- AGY is virtually identical in symptom expression with FD (Flavescence dorée) in France and elsewhere, and to (BN) Bois noir in Germany and elsewhere;
- These two diseases have been strongly associated with (two different) phytoplasma agents and are transmitted by at least two different leafhopper insect species;

**Industry Relevance**

In the late 1970's, the newly found AGY increased dramatically in severity causing significant economic loss to some vineyards (Magarey and Wachtel 1986b). Levels subsequently declined but in the late 1990's, there was a perception that AGY was again on the increase. This was particularly so in Riverland and Sunraysia vineyards. In a survey made during 2000/01, the average vineyard incidence of disease was ~11% (Magarey *et al.*, pers. comm.*)*. While no vineyard was free of the disease, some showed up to 50% of vines with AGY. These bore considerable crop loss. In addition, the epidemic (cyclic) nature of the disease meant that AGY was at some time, likely to further increase and reach more economically significant levels. Importantly, there was and still is no commercial control.

The controversy and concern which AGY had caused was eroding industry confidence in the use of *cv* Chardonnay as a variety important to Australian viticulture. Some investigators and industry leaders believed that AGY was sourced in propagation material. Fear of litigation from this had led two major suppliers to withdraw Chardonnay bud-wood from sale. This was concerning at a time when both the most susceptible varieties Chardonnay and Riesling were in strong demand for new plantings.

Others in the industry believed that AGY was spread from ground cover within affected vineyards and as a result (probably inappropriately) removed cover crops which are an asset to the vineyard environment.

Although the disease had been investigated off and on since 1976, the present investigators had the more recent opinion that AGY was associated with a complex interaction between the grapevine, a suspected leafhopper insect and an alternate host, probably a native plant that grows distant to most vineyards. The AGY National Technical Reference Group had rated as top priority the development of a management strategy for AGY and resolved that this should be the focus of the present investigation. They further directed that the present project should not seek financial justification by evaluating the economic loss from the disease in detail … they wanted some practical guidance toward a management strategy for AGY.

A brief estimate of the loss from AGY was made however, using assessments of disease incidence in more than 2050 *cv.* Chardonnay vineyards as surveyed by Hardy Wine Company in 2003/04 (Appendix 1). Various levels of disease severity from vineyard surveys (Chapter 6) were associated with measurement of yield decreases as a guide to estimate regional losses. Loss estimated from the Riverland, Riverina and Sunraysia vineyards in that season, totalled $28m.

Although the disease had caused some industry loss in recent years, the uncertainty about the biology of AGY and the lack of a control, had been more damaging. Confusion with diagnosing this disease in vineyards had also meant that other symptoms were implicated. These included restricted spring growth (RSG), late season leaf curl (LSLC) and an additional set of symptoms

now referred to as Scaly Bark Stunt (SBS). Of particular additional concern had been the then recent increase in levels of disease and the inferences that both grapevine propagation material and weed hosts spread disease both to and within vineyards.

Investigations by Beanland *et al.* (2002) in Sunraysia had focussed mostly on locating a 'within vineyard' source of disease but without success. They had painstakingly tested 34 weed-species as potential hosts of AGY (in 108 samples of mostly non-native species) and several thousands of leafhoppers collected from within and near vineyards but none tested positive for AGY. In attempts to transmit disease they found AGY in only four individuals of one leafhopper species *viz.* the common brown leafhopper, *Orosius argentatus*. Thus, basic questions of the source of AGY and how and when vines were infected remained unanswered.

Recent research by the present investigative team had suggested that propagation material was not associated with the spread of AGY and that the disease was probably transmitted to rather than from vineyards (see below). It was considered likely that an insect, probably a leafhopper, carried inoculum (infective matter that causes disease) to vineyards perhaps from a diseased native perennial. Evidence suggested that this perennial may grow almost exclusively in a riverine/wetland vegetation ecosystem perhaps some distance from most vineyards.

Clarification of the difficulty in diagnosing AGY, RSG, LSLC and SBS was also needed. It was envisaged that the proposed investigation would require the integration of expertise from the fields of entomology, botany, molecular biology and plant pathology and would benefit significantly from that synergism.

The foregoing suggested that it was necessary to further assess the role of propagation material and/or an insect vector (an insect that carries disease) in the spread of AGY. The attempts to control AGY would rely upon a good understanding of these matters.

**Potential for adoption by industry**

Some expected outcomes would be ready for immediate adoption *eg* determination of the role of grapevine cuttings in transmitting disease. Confirmation of the role of an insect and/or a native plant host in spreading disease would have significant bearing on the directions of future research toward a commercial control.

**Assessment of benefits to industry**

Prime benefits to industry include resolving levels of AGY in propagation material, understanding of the role of native vegetation and possibly an insect vector in disease spread.

At the time of commencement of the project, in vineyards of the Riverland, Sunraysia, Riverina and Hunter Valley districts, the loss caused by AGY was very variable. As will be shown in this report, this was related to the variable occurrence of the disease in different localities in each district. Beyond the Riverland, Sunraysia, Riverina and Hunter Valley districts, crop loss from AGY was and is effectively nil because levels of AGY were very low. Reasons for why this was so were considered to be of considerable value in determining the Australian grape industry's approach to resolving the enigma of AGY.

If levels in the Riverland and the other warm inland districts were to return to those seen in the late 1970's, crop loss would be much more severe than occurred at that time. Then only relatively few vineyards were significantly diseased but these were rendered un-economic by

AGY. The present scene is made more severe by the much-expanded area of susceptible varieties now planted and the resultant significantly increased proportion of the Australian industry devoted to *cvs*. Chardonnay and Riesling. The recent finding of AGY on varieties like Sangiovese has enhanced this potential loss.

In addition, Grapevine Yellows in Europe remained a highly destructive disease and was considered by AQIS as one of Australian Viticulture's ten most un-wanted diseases. Concern about AGY was largely founded on the risk that yellows diseases posed to the Australian industry.

**Proposal for Investigations in SAR 02/03**

It was proposed that the present work would be undertaken:

- to expand investigations initiated during 2001/02 under GWRDC RITA Project RT01/15-2 and during projects sponsored in part by Riverland grape industry agencies;

- in attempt to answer some of the queries raised above with particular reference to those in the review by Randles (2000);

- **as a three year investigation to provide information about AGY as a disease, its source and spread and its epidemiology with regard to providing a platform of understanding of how a management strategy might then be developed.**

***A widespread new disease with cause unknown,***
***and no known control…***

***… it varied in severity from season to season,***
***sometimes at high level causing significant crop loss.***

***It was similar to devastating yellows diseases overseas…***

***… a recipe for anxiety and a call for action:***

***What is the source of disease and how does it spread?***

## Section 3: Preliminary Investigations into the Source of AGY. Disease Distribution through Cuttings: Truth or a 'Myth-stake'?

*There are two aspects of this work. First, in investigating a control for AGY, it is necessary to find the source of disease. Though in the late 1990's this was unknown, there had been a long-standing premise held by some, that propagation material played a major role in spreading the disease. In response, with assistance from the Riverland Vine Improvement Committee, investigations were begun to find if that were so. Given progress in that study, GWRDC Project SAR 02/03 funded further work on several related matters to bring a successful conclusion to these investigations.*

## Chapter 3: Does AGY Spread via Cuttings? 1 Tests of propagation material for transmission of AGY - 2000/01 to 2001/02

### Introduction

A component of the Australian grape industry's anxiety about AGY was centred on the premise that the disease is transmitted in propagation material. In the 1990's, there was a high level of concern that the rapidly expanding industry was propagating AGY through cuttings taken from diseased vines. This thinking was supported by surveys undertaken in vineyards which reported symptoms thought to be AGY in the first season after planting (Constable *et al.* 2004). As a result, many nurseries had introduced the potentially damaging hot-water treatment as a standard protocol. Because of this, propagation quality was at times reduced by the hot-water treatment and, for fear of litigation, two major Australian nurseries had ceased selling *cv* Chardonnay cuttings. These problems were proving a significant cost and impedance to the industry.

In France, Flavescence Dorée (FD), a similar though more severe form of yellows disease of grapevines, had shown a transmission rate of ~0.1% *ie.* one cutting in 1,000 showed symptoms (Caudwell *et al.* 1997). Apart from this, little or no additional evidence had been provided to support the premise made by some Australian investigators.

In the late 1970's, Dr RW Emmett, in Sunraysia, and one of us (PA Magarey) in the Riverland, had co-operated in attempts to transmit AGY. They had used ~700 cuttings taken from affected Chardonnay and Riesling vines and used these in various attempts to transmit AGY. For example, they attempted direct-propagation of cuttings, and the budding or grafting of affected material to a selection of indicator vines but none of the own-rooted or grafted vines showed typical symptoms of AGY when assessed the following season (Magarey and Emmett, pers. comm.).

In a further test during the dormant period 1997, the senior author in association with the Riverland Vine Improvement Committee selected 1,000 cuttings from a Chardonnay vineyard at Bookpurnong, SA. The cuttings were taken from vines that, in the previous season, showed symptoms of AGY, Restricted Spring Growth (RSG), Late Season Leaf Curl (LSLC) and/or Scaly Bark Stunt (SBS). They were then rooted and potted at Monash, SA, and assessed for AGY (and RSG, LSLC or SBS) late in season 1997/98.

No cuttings showed any symptoms of AGY.

However, the possibility persisted for a low level of affected cuttings (*ie.* fewer than 1/1000) to transmit the presumed phytoplasma pathogen of AGY. To do so, the titre (quantity) of the pathogen would need to be in appropriate balance with the vine it had invaded *ie.* it would need

to be low enough to allow an affected shoot to lignify adequately for survival but sufficiently high to be able to survive itself and induce symptoms at some time in its propagated life.

In the late-1990's, further transmission tests of AGY were conducted by the Victorian Primary Industries (Vic DPI) at Sunraysia Horticultural Centre (SHC), Irymple. This was part of a GWRDC-funded national program on AGY (Beanland *et al.* 2002) and was undertaken in conjunction with tests of the efficacy of hot-water treatments on propagation material. However, in these experiments, the replicate numbers were of the order of 400 cuttings/treatment, too low for a statistically valid test given the expected low rate of transmission of AGY.

Thus, a re-test of the premise was considered necessary. The low rate of transmission of FD in France meant that greater replication in our trials was necessary to detect possibly very low levels of AGY to resolve the disputed claims regarding the spread of the Australian disease.

The development of PCR (polymerase chain reaction) technology (Gibb *et al.* 1996; Liu *et al.* 1996) provided a help in these tests by allowing a specific test for the presence of the pathogen.

As a result, investigations recommenced in 2000/01 to test the hypothesis that AGY is transmitted to commercially significant levels in propagation material taken from diseased vines *ie.* to determine if grapevine cuttings spread the disease.

**Aim**

**To determine the role of dormant propagation material in the transmission of AGY in Australian vineyards.**

**Materials and Methods**

A bioassay for AGY in vineyard propagation material was needed. To establish this, the two most important varieties affected by AGY *viz.* Chardonnay and Riesling, were selected for sampling in vineyards of the Riverland and Sunraysia, two of the regions most severely affected by the disease. During the dormant seasons of 2000 and 2001, a total of ~ 12,500 cuttings were selected for bioassay from five of the most severely affected vineyards in those regions. Because in our previous experiments, no AGY had expressed in cuttings from any source of vines either diseased or symptomless, cuttings from symptomless vines were not collected for use as control plants; instead the replicate numbers of cuttings used in the bioassay were maximised to increase the likelihood of detecting AGY.

Two experiments were undertaken. To ensure that the selected material for each experiment contained the highest titre of the AGY pathogen, cuttings were selected only from arms of vines that had expressed symptoms of AGY for the previous two seasons as determined by visual surveys made by the senior author. These vines were sampled from across each vineyard but variation occurred in the detail of the sampling for each experiment.

**Experiment 3.1: Cuttings collected during dormancy 2000.** During 1999/00, the second season of surveying for disease in preparation for this trial, vines were tagged for presence of clear symptoms of AGY. During July and August 2000, a total of at least 4,000 cuttings were taken from these vines at three sites: two in Sunraysia (one each in NSW and Victoria) and one in the Riverland, SA (Table 3.1).

**Table 3.1 Experiment 3.1: The number of dormant cuttings collected in July and August 2000 for AGY transmission studies in an exclusion house at Berri, SA. 2000/01 to 2001/02.**

| Site # | Region | Location | Grower | Cultivar | Minimum # Cuttings |
|---|---|---|---|---|---|
| 1 | Sunraysia | Gol Gol North, NSW | A | Chardonnay | 1,500 |
| 2 | Sunraysia | Irymple, Vic | B | Chardonnay | 1,000 |
| 3 | Riverland | Berri, SA | C | Riesling | 1,500 |
| | | | | **Total** | **4,000** |

Sites 1 and 3 were commercial vineyards while Site 2 was at the Sunraysia Horticultural Centre.

**Experiment 3.2: Cuttings collected during dormancy 2001.** Some 8,500 cuttings were taken from four commercial vineyard sites in the Riverland: three of Chardonnay and one of Riesling (Table 3.2).

**Table 3.2 Experiment 3.2: The number of dormant cuttings collected in June and July 2001 for AGY transmission studies in an exclusion house at Berri, SA. 2001/02.**

| Site # | Region | Location | Grower | Cultivar | Minimum # Cuttings |
|---|---|---|---|---|---|
| 3 | Riverland | Berri, SA | C | Riesling | 6,000 |
| 4 | Riverland | Murtho, SA | D | Chardonnay | 1,000 |
| 5 | Riverland | Pyap, SA | E | Chardonnay | 1,000 |
| 6 | Riverland | Pyap, SA | F | Chardonnay | 500 |
| | | | | **Total** | **8,500** |

From Site 3, the same vineyard as in Experiment 3.1, additional cuttings were taken in a similar fashion from at least one arm on each of at least 55 vines in 4 rows to include canes that had shown clear symptoms of either AGY alone or with AGY and SBS when observed and tagged in March 2001. From Site 4, cuttings were harvested from at least 20 single-wire trellised, AGY-diseased vines each of which had shown some AGY in 1999/00 and clear symptoms on both arms during 2000/01. At both sites, at least 25 canes were cut from each targeted arm to include both symptom-bearing and symptomless shoots when observed during 2000/01. This required harvesting nearly all shoots on those arms. From Site 5, cuttings were taken from at least one arm of at least 12 vines that had expressed some AGY for the previous two seasons. From Site 6, several affected shoots were selected from each of ~ 30 vines selected at random to include those with severe expressions of AGY as observed in late season 2000/01. Most of these arms also expressed symptoms of SBS.

In both Experiment 3.1 and 3.2, the cuttings were treated according to standard commercial propagation practice excluding heat treatment. They were cut to ~30 cm length as necessary, then soaked in water, prior to storage at $4^0$ C and subsequent placing in a rooting medium in heat-beds, in Experiment 3.1, at Orlando Wyndham, Rowland Flat, SA, and in Experiment 3.2, at Loxton Research Centre (LRC), Loxton, SA. In both instances, the resultant rootlings were potted in 2L plastic bags at LRC, Loxton.

All potted rootlings were transferred to an insect exclusion-house established at Site 3 in Berri, SA, (see Chapter 16). The transfer was completed within 2 hours of potting to minimise the risk of infection from any presumed insect vector that might have fed on the small buds of the rooted cuttings. The rootlings were then maintained in the exclusion house for the duration of the respective experiments.


**Figure 3.1: Rootlings were grown from dormant cuttings harvested from AGY-affected *cvs.* Chardonnay and Riesling vines and grown in an insect exclusion house for up-to three seasons, Berri, SA.**

In the exclusion-house, the potted vines were assessed for the presence of symptoms typical of AGY, RSG, LSLC or SBS, initially at weekly intervals and later mostly monthly during the two growing seasons *viz.* during 2000/01 and 2001/02.

## Results

Of the 4,000 cuttings that were harvested from AGY-affected arms and vines in Experiment 3.1, only relatively few cuttings rooted sufficiently (average 4%) during the propagation process to survive the potting-up procedure (Table 3.3). The success rate for the 8,500 cuttings used in Experiment 3.2 was considerably higher (72%) but none of the survivors in either experiment expressed any symptom of AGY (or RSG, LSLC or SBS) during the two years of vine growth in these experiments.

**Table 3.3 The propagation of rootlings from cuttings selected from AGY-affected vines for tests of the transmission of disease in two studies in an insect exclusion house at Berri, SA, 2000/01 to 2001/02 [1].**

| Site # | Region | Cultivar | Minimum # Cuttings Collected | # Rootlings Propagated in 2000/01 (% propagated | # Rootlings Survived to August 2002 (% survived) [2] | # Rootlings with symptoms of AGY [3] |
|---|---|---|---|---|---|---|
| **Experiment 3.1. Cuttings collected during 2000** | | | | | | |
| 1 | Sunraysia | Chardonnay | 1,500 | 1,260 (84%) | 22 (2%) | Nil |
| 2 | Sunraysia | Chardonnay | 1,000 | 120 (12%) | 29 (3%) | Nil |
| 3 | Riverland | Riesling | 1,500 | 960 (64%) | 112 (8%) | Nil |
| | | **Total** | **4,000** | **2,340 (59%)** | **163 (4%)** | **0 (0%)** |
| **Experiment 3.2. Cuttings collected during 2001** | | | | | | |
| 3 | Riverland | Riesling | 6,000 | - | 4,997 (83%) | Nil |
| 4 | Riverland | Chardonnay | 1,000 | - | 754 (75%) | Nil |
| 5 | Riverland | Chardonnay | 1,000 | - | 312 (31%) | Nil |
| 6 | Riverland | Chardonnay | 500 | - | 77 (15%) | Nil |
| | | **Total** | **8,500** | | **6,140 (72%)** | **0 (0%)** |
| | | **Grand Total** | **12,500** | | **6,303 (50%)** | **0 (0%)** |

Note: [1] Experiment 3.1 commenced July and August 2000, and Experiment 3.2, June and July 2001.
[2] *ie.* through seasons 2000/01 to 2001/02.
[3] As assessed during the two seasons of Experiment 3.1 and for the one season of Experiment 3.2.

## Discussion

In both experiments, many cuttings died during the rooting process in the heat-beds. This was because many were AGY-affected (*ie.* symptom-bearing) and as a result, were pencil-thin (<9-10 mm diameter) and unlignified (they had not hardened-off). As a result, they lacked the internal reserves to root successfully. This is consistent with our observations of most AGY-diseased shoots on the vine, they die either in the season of symptom expression or over-winter.

Of the cuttings that rooted successfully, especially in Experiment 3.1, many died during subsequent propagation leaving few that survived through to 2001/02 (Table 3.3). Some of these losses were due to a failure in the potting procedure of Experiment 3.1 and it was not possible to distinguish which of the rootlings died as a consequence of AGY. The error in propagation method was corrected in the second experiment and as a result, the propagation rate in Experiment 3.2 was much higher (Table 3.3).

The exclusion house in which the potted rootlings were maintained was located at Site 3, the vineyard from which significant levels of AGY had been seen over a number of previous seasons (Magarey, *pers. comm.*) and from where the Riesling samples were taken for both experiments. This ensured that the bioassay was conducted in a locality where AGY was known to express clearly.

The insect-excluding nylon mesh within which the potted vines were maintained was designed to prevent access by insects of size larger than thrips (see Chapter 16). In this way, we attempted to exclude leafhoppers and other similar sized insects that had potential to be vector of AGY. As a result, any AGY symptoms that appeared could have been attributed to inoculum carried over in the cuttings and not the consequence of an insect spreading disease to the vines subsequent to propagation.

Overseas some yellows diseases are lethal to vines and while AGY is also lethal it is only lethal to shoots and occasionally, to arms. However, the death of AGY-affected shoots is extensive. In addition, diseased shoots are unlikely to be selected for propagation material and therefore, are considered most unlikely to be able to transmit the disease *via* the propagation process. Our experiments gave support to this conclusion.

However, it is conceivable that a grapevine shoot may be inoculated with the AGY-pathogen at titre insufficient to express symptoms. Such infected shoot material, though symptomless, could be expected to lignify and may therefore survive the season and be able to transmit the disease *via* the propagation process. For this reason, in these experiments, both diseased and symptomless shoots on the pre-selected diseased arms, were harvested to ensure maximum chance of selecting dormant canes able to transmit AGY.

However, in our experiments, none showed symptoms of AGY.

In Australian viticulture, we usually see no symptoms of AGY until the third season from planting. We suggest the reason for this is that insect vectors (and not disease from propagation material) introduce AGY to the newly planted cuttings in the field during in the first or second growing season at the earliest. As a result, the disease incubates in inoculated vines for at least one season, perhaps to build-up sufficient titre to cause symptoms, before showing only in the next (the third) season. Disease incidence is often then of the order of at least 10% (Magarey pers. comm.) *ie.* one in every ten vines might express some level of AGY. Also the distribution of that disease is usually in random clusters of AGY-affected vines or it shows as specific disease gradients across the vineyards (see Chapters 11 - 14). These patterns differ from that expected if planting material were the source of disease. For example, the disease would be expected to show as a completely random scatter or in some specific association with the vineyard source and/or clonal designation of the material. Neither appears to occur in Australian vineyards – for instance, we frequently observe uniform disease levels across a vineyard comprising adjacent plantings of propagation material from separate sources.

Thus the absence of AGY in any potted rootling in either experiment with rootlings grown from 1-2 seasons in the 'insect-free' environment of this trial suggested that AGY is not transmitted *via* the propagation material, or at least not at rates that could account for the level of disease seen in commercial vineyards.

Furthermore, in Section 4, we present other vineyard data as evidence to suggest that the main form of dispersion of AGY is a mobile vector and not propagation material. In fact, we propose that the source of AGY is external to the vineyard. These findings are contrary to the previous doubtful premise that AGY was spread by cuttings from diseased vines.

One reason for the differences we report and that published by previous authors (*eg* Constable *et al.* 2004), could be attributed to a difference in identifying symptoms of AGY. The latter authors took a broader range of symptoms as AGY than we adopted in this study (Chapter 2), for instance identifying AGY based on the occurrence of some of the key symptoms but not their co-incidence, whereas, we require the simultaneous presence of symptoms on all three vine organs *viz.* leaves, shoots and bunches.

Our experiments also suggest that grapevine is a terminal host of AGY *ie.* the disease moves into and not from vines.

## Conclusion

From these experiments which were completed prior to the commencement of project SAR 02/03, we concluded that:

- it was unreasonable to say that AGY was never spread by cuttings, despite the high number of vines we had tested; and
- since evaluation of the existing propagated material had been for at most two seasons, it was considered necessary to continue these studies to determine if cuttings take longer than this *ie.* till the third season, before expressing symptoms; and
- it was necessary to assess if vines may not show symptoms as potted rootlings but only in a field environment.

## Recommendation

It was recommended that:

- the duration of bioassay be continued; and, to bring a more definitive conclusion to the work, specifically, that
- the existing propagation material be grown for at least another season to determine if symptoms:
  - show after a longer period of incubation in pots; or
  - in field (vineyard) plantings of the material.

***To date***
***there seemed little risk***
***that AGY was spread in propagation material …***

***… but to be sure,***

***it was necessary***
***to extend and enlarge the bioassay.***

# Chapter 4: Does AGY Spread via Cuttings? 2
# Tests of propagation material for transmission of AGY - 2002/03 to 2004/05

## Introduction

A number of studies on the role of propagation material in the spread of AGY had been undertaken prior to the present project (SAR 02/03) (see Chapter 3). Two experiments in particular had indicated that levels of transmission of AGY had, at best, been very low but uncertainty as to the length of time that potted vines needed to express symptoms of AGY remained. That uncertainty meant that some sectors of Australian viticulture had remained unconvinced that the risk of transmission *via* cuttings was negligible and they remained assured of the pre-existing premise that propagation material was responsible for the spread of AGY. This in turn meant that for some, there were insufficient grounds for a commercially useful conclusion on this matter and that more evidence was needed.

A large number of potted rootlings taken from some of the most AGY-affected vineyards in the most severely affected regions of Australian viticulture remained as potted rootlings in an 'insect-free' environment and were available to the present project. As a result, opportunity existed to utilise the infrastructure deployed in the earlier experiments (Chapter 3) to continue the experimental work as described below.

In addition, some of the propagation material from the previous investigations had been planted out in a commercial vineyard and it was opportune to evaluate these for presence of AGY. This would provide additional data in pursuit of the hypothesis that AGY is not transmitted in commercially significant levels within propagation material taken from diseased vines *ie.* to determine if AGY is spread in grapevine cuttings.

## Aim

**To determine if dormant grapevine cuttings transmit AGY at commercial levels in Australian viticulture.**

## Materials and Methods

A number of rootlings survived the initial propagation process and the subsequent bioassay to 2001/02, as described in Chapter 3. Some of these rootlings were used in the continuing studies of this experiment (Table 4.1). Established from a collection of AGY-affected and symptomless cuttings from arms known to express symptoms of AGY for the previous two seasons, the rootlings were from highly diseased Riesling and Chardonnay vineyards in the Riverland (and Sunraysia). These were grown in the insect exclosure from 1 – 2 seasons but none had yet shown symptoms of AGY.

There were two aspects to the present work. First, continuing studies in the exclusion house, and second, three tests of rootlings grown in field-plantings.

**Experiment 4.1: Exclusion House Studies Continued – 2002/03.** In the dormant period of 2002, ~2,600 rootlings (Table 4.1) were re-potted on-site at Berri, with assistance from Orlando Wyndham, or off-site at LRC, and were then re-sealed within the exclusion house (see Chapter 3) for culture for season 2002/03. During that season, the rootlings were visually assessed for presence of AGY at regular monthly intervals with a final assessment in May 2003 at the end of

the second season inside the exclusion house. At that time PCR analysis was used to test any suspect AGY or 'look-alike' symptoms. To evaluate the efficacy of PCR analyses at that time of the season, a control series of samples was taken from affected shoots of five vines bearing symptoms typical of AGY. These vines were located immediately adjacent but external to the insect exclosure at Site 3. The leaf and shoot samples taken at both locations were kept refrigerated and sent cool at 10-15 $^{0}$ C, to the University of Adelaide PCR-laboratory at the Waite Institute, Adelaide, SA, for analysis.

**Table 4.1 Experiment 4.1: The number of potted rootlings used in tests for transmission of AGY in an insect-exclusion house at Berri, SA, 2002/03.**

| Site # | Region | Location | Grower | Cultivar | Season Cuttings Collected | Minimum # Cuttings |
|---|---|---|---|---|---|---|
| 3 | Riverland | Berri, SA | C | Riesling | 2000/01 | **135** |
| 3 | Riverland | Berri, SA | C | Riesling | 2001/02 | 2,450 |
| 4 | Riverland | Murtho, SA | D | Chardonnay | 2001/02 | 30 |
| 5 | Riverland | Pyap, SA | E | Chardonnay | 2001/02 | 26 |
| 6 | Riverland | Pyap, SA | F | Chardonnay | 2001/02 | 4 |
| | | | | **Total 2001/02** | | **2,510** |
| | | | | **Grand Total** | | **2,645** |

**Experiment 4.2: Field Plantings of Rootlings at Paringa, SA – 2000/01 to 2003/04.** In the dormant season 2001, rootlings of cv. Riesling had been propagated from cuttings selected from Site 3 (see Chapter 3) and maintained in the exclusion house for the growing season 2001/02 but they had shown no symptoms of AGY after the first season. Two field-plantings of this material were made.

First, in the dormant period (August) 2002, 1,482 of these Riesling rootlings were planted-out in a commercial vineyard at Paringa, SA. They were left to sprawl for their first season of growth (2002/03) then trained to the trellis wire in 2003/04. The vines were visually assessed for AGY in late both growing seasons *ie.* in February 2003 and again in 2004.

Second, in the dormant season 2003, 1,371 rootlings of *cv.* Riesling were planted in the same vineyard as above, except that the rootlings had been maintained in the exclusion house for a second season *viz.* 2002/03. They were trained to the trellis wire in the Paringa vineyard in their first field season (2003/04) and were visually assessed for symptoms of AGY in February 2004 by walking three replicates of two rows in each of two vineyard blocks planted to the test vines **(Figure 4.1).**

Two adjacent vineyard blocks at the same field site had been planted to cv. Chardonnay. These mature vines were surveyed at the same time, as above.


**Figure 4.1: Young Riesling vines were disease free when grown from dormant propagation material taken from vines affected with AGY for the previous two seasons. Paringa, SA.**

**Experiment 4.3: Field Planting of Rootlings at Pyap, SA – 2001/02 to 2004/05.** At the same time as in Experiment 4.2, *viz.* August 2002 after one season of growth inside the exclusion house, fifty-one of the cv. Chardonnay rootlings from Site 6 at Pyap, SA, were planted out in a single row in the same vineyard from which they were originally collected. They were planted ~400m from the mother-patch, adjacent to a second, mature planting of the same variety. The young vines were trained to the wire in the first season and visually assessed for AGY in February 2003, 2004 and 2005, as in Experiment 4.2. For comparison, the two adjacent mature blocks were similarly assessed.

**Results**

**Experiment 4.1: Exclusion House Studies Continued – 2002/03.** No symptoms of AGY were expressed in any of the 2,645 potted vines maintained in this study. However, there were some vines that, in May 2003, showed some symptoms that potentially could be confused with AGY. These comprised downward rolled leaves without the typical yellowing associated with AGY. PCR analysis of samples from these 14 potted vines tested negative for phytoplasma. In contrast, of the five leaf- and shoot-samples from field-grown vines adjacent to the exclusion-house, three (60%) tested positive for phytoplasma (data not shown).

**Experiment 4.2: Field Plantings of Rootlings at Paringa, SA – 2000/01 to 2003/04.** In February 2003, the first season of field observation, vineyard surveys of the rootlings planted in 2002, failed to find any symptoms of AGY. In the second in-field growing season, low levels of AGY were observed (Table 4.2).

**Experiment 4.3: Field Planting of Rootlings at Pyap, SA – 2001/02 to 2004/05.**
Vineyard surveys for AGY symptoms in the 51 field-grown Chardonnay vines from cuttings at Site 6, failed to find any disease in assessments from 2002/03 to 2004/05. This was despite the occurrence of AGY in the mother-planting near-by and in the immediately adjacent mature vines (Table 4.3).

**Discussion**

The evaluation of the incidence of AGY in the potted rootlings within the exclusion house provided further evidence that dormant propagation material does not transmit epidemiologically significant levels of AGY (Chapter 3). Compared to Experiments 3.1 and 3.2, the replicate numbers of this trial were necessarily decreased because the older rootlings required bigger pots which took up more space in the exclusion house. Never the less, we evaluated in detail in excess of 2,400 Riesling rootlings, yet none showed symptoms of AGY. This was in their second season of growth since being taken from mother-vines known to be severely diseased with AGY for two seasons. None of the potted vines maintained for the three seasons of our studies showed AGY symptoms.

Some rootlings within the exclusion house expressed downward rolling of leaves, a symptom similar in part and sometimes confused with AGY. However, we considered it typical of late season growth which occurred within the environment of a enclosed shadehouse. Lower portions of some shoots hardened-off while the distal (or upper) end of the shoots remained active and accumulated photosynthates. We have frequently observed that such growth (with excess carbohydrates) causes leaves to curl downwards. Contrary to symptoms of AGY, the leaves on these shoots had not turned yellow and neither had the shoots failed to harden (mature).

The negative response from the PCR tests of the rootlings we sampled, and the positive outcome in 60% of the PCR tests of the vines with symptoms typical of AGY, support our visual observations *ie.* that AGY did not occur in the rootlings we tested.

**Table 4.2 Experiment 4.2: Field tests of transmission of AGY *via* propagation of cuttings from diseased *cv.* Riesling vines at Paringa, SA. 2002/03 to 2003/04.**

| | # AGY Vines | # Vines | AGY Incidence | # AGY Vines | # Vines | AGY Incidence |
|---|---|---|---|---|---|---|
| | Year of Planting: 2002 | | | | | |
| Row # | Year 1 Assessed February 2003 | | | Year 2 Assessed February 2004 | | |
| **Western Block** | | | | | | |
| 644 - 645 | 0 | 282 | 0% | 3 | 282 | 1.1% |
| 646 - 647 | 0 | 282 | 0% | 1 | 282 | 0.4% |
| 648 - 649 | 0 | 282 | 0% | 1 | 282 | 0.4% |
| **Total** | **0** | **846** | **0%** | **5** | **846** | **0.6% a** [1] |
| **Eastern Block** | | | | | | |
| 648 - 649 | 0 | 318 | 0% | 2 | 318 | 0.6% |
| 650 - 651 | 0 | 318 | 0% | 4 | 318 | 1.3% |
| **Total** | **0** | **636** | **0%** | **6** | **636** | **0.9% a** |
| | Year of Planting: 2003 | | | | | |
| **Western Block** | | | | | | |
| 678 - 679 | - | - | - | 0 | 181 | 0% |
| 679 – 680 | - | - | - | 0 | 181 | 0% |
| 681 - 682 | - | - | - | 0 | 181 | 0% |
| **Total** | - | - | - | **0** | **546** | **0% a** |
| **Eastern Block** | | | | | | |
| 678 - 679 | - | - | - | 0 | 275 | 0% |
| 679 – 680 | - | - | - | 0 | 275 | 0% |
| 681 - 682 | - | - | - | 0 | 275 | 0% |
| **Total** | - | - | - | **0** | **825** | **0% a** |
| **Mature Chardonnay Block 1 (assessed after machine harvested)** [2] | | | | | | |
| 96 - 97 | - | - | | >5 | 202 | 2.5% |
| 109 - 110 | - | - | | >9 | 210 | 4.3% |
| **Total** | - | - | - | **>14** | **412** | **>3.4% b** |
| **Mature Chardonnay Block 2 (assessed after machine harvested)** [2] | | | | | | |
| 326 - 327 | - | - | | >7 | 124 | 5.6% |
| 332 - 333 | - | - | | >10 | 128 | 7.8% |
| **Total** | - | - | - | **>17** | **252** | **>6.7% c** |

Note: [1] Different letters in columns denote significant differences ($X^2_2 < 0.05$).
[2] Machine-harvested vines have lost foliage and actual scores for AGY will be at least 5-10% higher than these figures suggest.

**Table 4.3 Experiment 4.3: Field tests of transmission of AGY *via* propagation of cuttings from diseased *cv.* Chardonnay vines at Pyap, SA. 2002/03 to 2004/05.**

| Description | # AGY Vines | # Vines | AGY Incidence [1] | # AGY Vines | # Vines | AGY Incidence |
|---|---|---|---|---|---|---|
| | Year of Planting: 2003 | | | | | |
| | Year 1 Assessed February 2004 | | | Year 2 Assessed February 2005 | | |
| **Test Vines** | 0 | 51 | 0% **a** | 0 | 51 | 0% |
| **Adjacent Block**[2] | 13 | 116 | 11% **b** | - | - | - |
| **Mother-planting**[3] | 233 | 476 | 49% **c** | - | - | - |

Note: [1] Different letters in columns denote significant differences ($X^2_2 < 0.05$).
[2] The test vines were planted immediately adjacent to a mature block of *cv.* Chardonnay.
[3] The test vines were originally taken from diseased vines in the mother-patch located ~400m from where they were subsequently planted as rootlings.

In combination with our earlier studies (Chapter 3), the above evaluation of rooted cuttings for AGY in an 'insect-free' environment, has tested some 12,500 cuttings over a 1-3 year period of observation. This included some 7,500 Riesling and 5,000 Chardonnay cuttings collected in a process similar to that used in commercial viticulture – with one exception. We strongly biased our collection by harvesting cuttings from vines significantly affected by AGY for two consecutive seasons. This maximised the likelihood of finding positive transmission if it were to occur. In contrast, usual vine-improvement protocols stipulate that cuttings not be collected from mother vines that show symptoms. In addition, many protocols also recommend heat treatment of cuttings. While this has not been proven to have an influence on AGY-diseased cuttings (if such material exists!), it is a further procedure that should lessen the risk of transmitting AGY in propagation material.

In our studies, many diseased shoots lacked sufficient reserves and were unfit for propagation and, as a result, died during the rooting phase. However, that deficiency only served to strengthen the test under consideration *ie.* it affirmed that there is at most, a low risk of transmitting AGY by cuttings and highlighted the relative safety of normal nursery practice in propagating grapevine cuttings free of commercially significant levels of AGY.

A main reason we suggest as to why we failed to propagate AGY is that the disease kills shoots and most die before or during the propagation process. A possible second reason might be the observed occurrence of natural heat therapy in which the titre of the pathogen is likely to be reduced within diseased shoots. Field observations frequently show that severely diseased and stunted shoots that are dying back and dropping yellowed leaves, show a remarkable return to normal growth within 7-10 days of hot weather with maxima exceeding ~$40^0$C.

It is possible that these extremes of temperature substantially reduce and/or kill the AGY pathogen in affected shoots in a similar way that Caudwell *et al.* (1997) showed heat treatment at $50^0$C for 45min. controls flavescence dorée (FD) in dormant cuttings. Assuming it reasonable to transpose their (temperature x time) data for hardened cuttings and equate these with the minimum temperature x time required to reduce AGY within green shoots, then a natural occurrence of 37-$38^0$C for 10 hours, 39-$40^0$C for 4-5 hours, 42-$43^0$C for 2-2.5 hours or $45^0$C for 1 hour would be expected to disrupt the pathogen of AGY. Rare are the days when the temperature in the Riverland exceeds $38^0$C for 10 hrs or $40^0$C for 4-5 hours but it is not uncommon for the temperature to reach 42-$43^0$C for 2-2.5 hours or $45^0$C for 1 hour – the latter could be expected to substantially reduce the viability of the AGY pathogen in green shoots.

An alternative reason why AGY-affected shoots regrow after extreme heat is that under these conditions the membrane wall of the phytoplasma may be softened and the disease agents may become more pliable. This extra flexibility may enable them to pass through the sieve plates of the phloem whereas in cooler conditions, the AGY pathogen otherwise accumulates, blocking the phloem transport system (Magarey pers. comm.). Whatever the mechanism, the physiological processes of growth with AGY-affected shoot tissue swiftly return to normal growth after hot weather.

Given the above, it is conceivable that very high temperatures in the previous growing season would lessen the risk of transmitting AGY by cuttings in the season following, and to the contrary, there may be higher risk of transmitting AGY after cool seasons.

Despite high replicate numbers in our transmission tests and our lack of detecting AGY in cuttings, it is possible that low levels of AGY may still occur there. Repeat studies using even higher replicate numbers with more specific tests for the presence of the pathogen might find low levels of disease.

In assessing the rigour of our tests in which ~7,500 Riesling and ~5,000 Chardonnay cuttings were evaluated, suppose we failed to detect one or two diseased cuttings. As a result, AGY might have been transmitted at a level of one or two rootlings per 7,000 - that is, at a level of 0.01-0.04%. Also, it is conceivable that AGY might equally occur at an incidence less than our tests were able to define *viz.* less than one in 7,500 *ie.* < 0.01%.

However, such low levels of transmission would have negligible effect on commercial management of the disease. As our experiments demonstrated, field plantings of rootlings from diseased vines showed no disease in the first season of growth and at best, very low levels of disease in the second season, and it remains unclear whether that disease arose from within the cuttings or whether it came from the vines inoculated by a presumed mobile vector in the first season of planting. It noteworthy that levels of AGY in the Paringa vineyard were at least 100 x greater in mature vines than in our test rootlings. It thus appears that the level of AGY transmitted by propagation material (if any), is of no commercial consequence.

Our investigations elsewhere in this document (Chapter 16) would suggest that AGY usually must be re-introduced into vines each season. This is because many vines recover from the disease meaning that few express AGY from season to season. Also, we present evidence that AGY is native to Australasia and leads to disease in a number of introduced plant species when they are cultivated in that region (Chapter 15). Assuming that this holds true and given the observation that AGY is already widespread across Australian viticulture (Magarey, pers. comm.), there seems little ground for concern that that the disease is being spread *via* propagation material to new localities in Australia even at very low rates.

Our tests of the transmission of AGY in a large quantity of propagation material taken from diseased vines has shown that there was little or no risk of introducing AGY into a vineyard by cuttings and certainly not at rates comparable to those seen naturally occurring in affected vineyards.

**Conclusion**

- The premise that AGY is spread in commercially significant quantities through propagation material from diseased vines is not supported;
- Concern held by some sectors of the industry at the commencement of this project that AGY-affected cuttings are spreading the disease in non hot-water treated material, seems to be not justified; and
- Factors other than propagation material are the likely source of disease.

**Recommendation**

It is recommended that:

- the premise '… that propagation material from AGY affected vines provides a commercially significant source of disease,' should be abandoned, and that;
- the source and spread of AGY be further investigated through studies of other factors such as a mobile vector.

***It was a 'myth-stake' to think that dormant cuttings spread AGY and introduced the disease to new viticultural regions in Australia.***

***We needed to look elsewhere to find the source of AGY.***

## Section 4: Investigating the Epidemiology of Disease Is Pruning a Useful Management Strategy for AGY?

*Little is known of the epidemiology of AGY ie. about how AGY develops as a disease and under what conditions. This means that little is known about where the disease survives in the vine, how it spreads through the plant and across vineyards. We report here a brief investigation to better understand where the inoculum (the infective matter that causes disease) survives over-winter in the vine and to see if a simple pruning technique would be a useful management strategy.*

## Chapter 5: Can Targeted Pruning Control AGY? An investigation to find where AGY over-winters in the vine – 1998/99 to 2002/03

### Introduction

The pathogenic (disease-causing) agents associated with symptoms of AGY are believed to be phytoplasma, small bacteria-like organisms. The pathogens were first suspected after antibiotic injected into diseased vines led to complete recovery of these vines in the following season (Magarey and Wachtel, 1986b). The organisms were later seen in AGY-affected tissue in studies with an electron microscope (Magarey *et al.* 1988) and were associated with the sugar conducting cells (the phloem) of affected vines (Magarey *et al.* (1986b). In recent years, molecular tools such as PCR have been developed to detect AGY (Gibb *et al.* 1996; Liu *et al.* 1996). Constable *et al.* (2003a) showed that the phytoplasma are mobile in the vascular system (the plumbing) of affected vines, some are even transported to the roots. From the injection experiments with antibiotic and work we report elsewhere (see Chapter 16), it was apparent that AGY is introduced into the vine in the season or seasons before symptoms appear. They are inoculated to vines in year 1, then multiply over winter and early spring, to produce symptoms in late spring of the second season.

However, since the symptoms of AGY are usually localised to particular clumps of a few affected shoots on each diseased vine while the remainder is symptomless, it seemed reasonable to suppose that the bulk of the pathogenic agents is likely to survive in and/or near those soon-to-be diseased parts of the vine *ie.* the shoots.

This idea was supported by the outcome of a trial undertaken previously where vineyard levels of AGY were reduced by pruning previously diseased arms from dormant vines (Magarey and Wachtel, pers. comm.).

If this concept were so, it was considered possible that dormant shoots and/or the spurs and cordons were where AGY over-wintered and, as a result, a simple control strategy for disease might be developed through removing the dormant shoot growth. In other words, a less drastic pruning-strategy might prove a simple and effective control for AGY.

The following work, which began in 1997/98, evaluated this possibility in a trial at Berri, SA.

### Aim

**To determine if the disease agents for AGY over-winter in dormant canes and, if so, whether a strategy of targeted-pruning will control the disease.**

## Materials and Methods

In investigating if AGY were transmitted by propagation material, high numbers of *cv.* Riesling cuttings were taken from diseased vines at Berri, SA (Section 3). The method to identify the source vines for the experiments was outlined in Chapters 3 and 4. The basis was to score vines for AGY over two seasons and then selectively harvest the cuttings from those vines in the dormant period following the second season (June 2000).

This experiment presented opportunity to visually assess the pruned vines for a further three seasons, 2000/01 to 2002/03, scoring arms for AGY on 170 vines in each vineyard sector from which the cuttings were taken (the pruned section) and from the sector immediately adjacent in the same vineyard (the 'unpruned' control sector). The latter was pruned in the usual commercial manner (box-hedging) for that vineyard but diseased vines in the pruned sector were closely spur-pruned. All canes on the pruned vines were removed including both affected and symptomless material, leaving single buds on short (<1 cm) spurs on an otherwise bare cordon.

In the subsequent three seasons, all arms of all vines in the pruned sector and the unpruned control sector were monitored for AGY (see arm surveys, Chapter 6) and the percentage number of arms diseased in each sector were compared by Chi-square test of independence.

## Results

The incidence of AGY in both the pruned and unpruned control sector of the vineyard was the same ($P<0.05$) before the treatment was applied and remained in the first season after (Figure 5.1). The incidence of disease increased ($P<0.05$) in the pruned sector in the third season and then decreased.

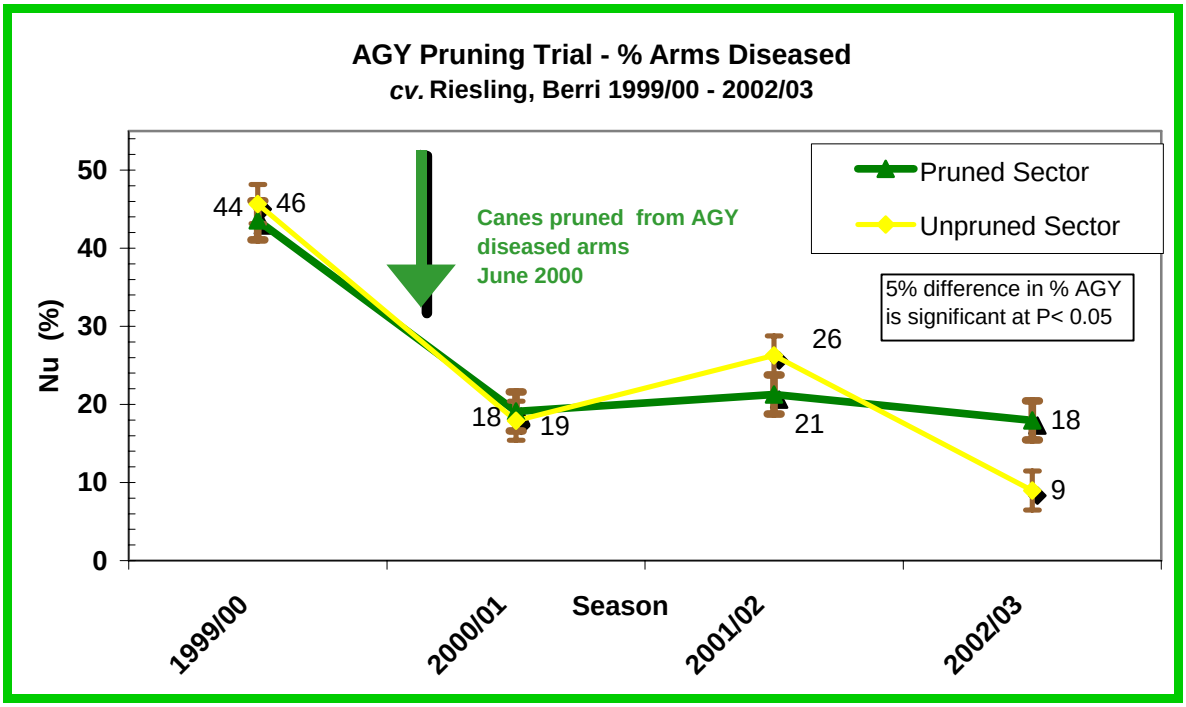



**Figure 5.1: Pruning diseased shoots from previously diseased vines failed to reduce the severity of AGY in the following seasons at Berri, Riverland, SA. 1999/00 - 2002/03.**

## Discussion

The reduction in the incidence of AGY in the season immediately following the pruning treatment was due to other factors than the pruning treatment. This was evidenced by the simultaneous rate of decrease in disease in both the pruned and the untreated control sections of the vineyard (Figure 5.1) and was consistent with the decrease in disease elsewhere in the region in that season.

Since there was no significant influence of the pruning treatment at that time, it seems unlikely that the subsequent difference was related to the pruning treatment applied in June 2000.

The lack of treatment response in the pruning trial suggested that the AGY phytoplasma are unlikely to reside in dormant cane material. Two additional observations support this view. One, diseased shoots usually do not survive the winter season and yet AGY may appear the next season regardless, and second, in our extensive testing of propagation material taken from the present vineyard (and others) (see Chapters 3 and 4), symptoms of AGY were not evident during the 1-3 seasons of observation that followed.

As a result, if our initial supposition is true *ie.* most of the AGY pathogen within the vine overwinters in close proximity to the shoots where symptoms express, then that inoculum must overwinter in the spurs and/or the sectors of the cordon nearest to the clusters of shoots that show symptoms.

This experiment only evaluated the removal of dormant cane material which appeared to not carry significant titre (quantity) of AGY inoculum. Given the previous more severe pruning trial in which diseased arms were culled and the vineyard level of AGY were reduced as a result the next season, it appears that the pathogen of AGY survives in cordons and spurs rather than the cane material used in propagation. This is a new finding in understanding the epidemiology of AGY.

## Conclusion

The inoculum for AGY symptoms:

- does not appear to overwinter (to significant levels) in dormant canes; but instead it
- appears to survive in cordons and spurs and other vine tissue at or near the site of symptom expression in the following spring-early summer.

## Recommendation

- The use of targeted-pruning of dormant canes to remove AGY from diseased vines is not recommended as a practice of value in reducing disease.
- Further studies of the epidemiology of AGY to determine how the disease moves to and within affected vines should consider the spur and proximal (near) sections of cordons and not the canes, as the likely site of overwintering inoculum.
- This work complements the findings of Chapters 3 and 4 and enhances their recommendation that, in pursuing epidemiology of disease and the primary sources of inoculum for AGY, the propagation material should be ignored.

***The concept of heavily pruning canes from AGY-affected vines to reduce disease next season seems to be ill-founded and unwarranted.***

# Section 5: Investigating the Spread of AGY Patterns of Disease over Time and Space 1

*Because the investigations in Sections 3 and 4 discounted the possibility of propagation material spreading commercially significant levels of AGY, other sources of disease needed to be pursued. We examined the patterns of occurrence of AGY within vineyards and the occurrence of affected vineyards within regions. We assess this information in terms of locating the main source of disease with plan to narrow-down the locations where we expect to find the primary source(s). To begin, though, we present a study of the survey methods used to assess the progress of disease epidemics over time, a critical process in our investigations.*

## Chapter 6: Vineyard Surveys as a Tool to investigate the Epidemiology of AGY Comparison of two methods of surveying for disease

### Introduction

A means of assessing the incidence and severity of AGY in the vineyard was needed to assist in our search for the source and spread of AGY. A time consuming, detailed survey method was being used to assess vineyard levels of disease. However, this method became cumbersome when the intensity of surveying needed to be increased to include a higher number of vineyards survey.

A comparison of survey methods was needed to ensure accuracy in scoring vines and vineyards for levels of AGY.

### Aim

**To evaluate two methods of surveying AGY as a measure of the incidence and severity of the disease in vineyards.**

### Materials and Methods

Two survey methods were used.

**Arm Surveys**. This survey provides the greater detail in scoring for disease. The survey-unit is a single arm (cordon). Both sides of each arm on each vine in the vineyard block was assessed while the assessor walked slowly, scanning one side of the row at a time. Arms were scored for presence or absence of AGY.

The number of vineyards assessed each season varied from 5-24. Surveys included from 5 to 30 rows/vineyard and from 50 to 300 vines/row. This ensured that the total number of vines in each block often exceeded 250-300 vines/vineyard, and that an array of sufficient length and breadth in each vineyard block that would reduce the influence of edge-effects and provide statistical rigour to analyse for differing patterns of disease over time and space (see below).

The data were entered in MS Excel® from which vineyard maps of disease were prepared and graphs of the varying incidence of AGY over time were plotted for each vineyard.

**Point Surveys.** This survey method was required because it was quicker than the arm surveys. The survey-unit comprised the foliage of one vertical-side of a single vine along the canopy row. Rows were selected in a uniform array across each vineyard. Visual assessment of both obverse

(facing) sides of the vine row was undertaken simultaneously while walking the centre of two rows of vines at slow pace. Each vine was scored for presence or absence of AGY. The reverse side of the vines were ignored.

A similar number of vineyard blocks and vines were surveyed as described above and the number of diseased vines in each plot was recorded in a regular grid-pattern across each vineyard. The number of plots usually ranged from 6-10 /vineyard but varied from 1 to 50 plots/vineyard.

**Statistical Rigour.** A preliminary study using statistical tests of independence (Chi-square, $\chi^2$) showed that at least 50 vines/plot was necessary to distinguish a 5% difference in disease incidence between plots at $P<0.05$. Thus where possible, the arm and the point surveys incorporated at least 50 vines/plot though this number was often far exceeded, scoring ~100 vines/plot. In the point surveys, an assessment of 50 vines/plot meant that one side of each vine of a minimum of 25 vines/row in each of two rows/plot were scored for AGY.

**Spatial Location.** In the second phase of surveys, especially from 2000/01, GPS technology was deployed to provide a geographical fix of each data position (plot) in the point surveys. ArcView® software provided the necessary tools to spatially orientate the data which were presented in MS Access®.

**Comparison of Surveys.** Since the point surveys were widely used in the second phase of investigations, some estimation of the relative accuracy of that survey method as compared to the arm surveys, was undertaken in three mature, commercial vineyards as follows: 1) *cv.* Riesling on a two-wire vertical trellis 1.5 m in height and 1.5m spacing along the row, at Berri, SA; 2) *cv.* Chardonnay with similar trellis and spacing, though 2m in vertical height, at Gol Gol, NSW; and 3). *cv.* Chardonnay with similar trellis and spacing, though 2.2m in vertical height, at Irymple, Vic. Tests of the differences between scores from the point survey compared with the arm survey of the same plots were undertaken using Chi-square ($\chi^2$) tests of the differences between disease scores from 48 - 50 vines/plot from 11, 7 and 6 plots/vineyard respectively.

**Results**

The scores for each plot are presented for both survey methods (Tables 6.1 - 6.3). In the *cv.* Riesling vineyard, for nine of eleven plots, the scores for incidence of AGY from the point survey were not significantly different from the scores from the arm survey while twice the point surveys scored less ($P<0.05$) (Table 6.1).

The vineyard score for the total block of 550 vines (11 plots) showed that the detailed arm survey score (mean AGY incidence of 6.4%) was not significantly different ($P<0.05$) from the score from the point surveys (mean incidence (4.0%). However, across all plots there was a consistent trend of lower disease scored in the point survey.

In the vineyard at Gol Gol, only one plot was scored the same ($P<0.05$). The other six scored significantly less ($P<0.05$) in the point surveys than in the arm surveys (Table 6.2). The overall mean scores also were significantly less ($P<0.05$) in the point surveys. In the vineyard at Irymple, disease scores were lower and only the aggregate score for all plots differed ($P<0.05$) though the point scores for all plots were numerically less (not significant at $P<0.05$).

**Table 6.1: Comparison of arm survey and point surveys - two methods used to assess vineyards for incidence of AGY in *cv.* Riesling, Berri, SA.**

| | Arm Survey | | | Point Survey | | |
|---|---|---|---|---|---|---|
| **Plot #** | **# AGY Vines** | **# Vines** | **AGY Incidence [1]** | **# AGY Vines** | **# Vines** | **AGY Incidence [1]** |
| 1 | 4 | 50 | 8% **a** | 1 | 50 | 2% **b** |
| 2 | 1 | 50 | 2% **a** | 1 | 50 | 2% **a** |
| 3 | 4 | 50 | 8% **a** | 4 | 50 | 8% **a** |
| 4 | 4 | 50 | 8% **a** | 3 | 50 | 6% **a** |
| 5 | 3 | 50 | 6% **a** | 4 | 50 | 8% **a** |
| 6 | 3 | 50 | 6% **a** | 2 | 50 | 4% **a** |
| 7 | 6 | 50 | 12% **a** | 2 | 50 | 4% **b** |
| 8 | 3 | 50 | 6% **a** | 1 | 50 | 2% **a** |
| 9 | 4 | 50 | 8% **a** | 2 | 50 | 4% **a** |
| 10 | 3 | 50 | 6% **a** | 2 | 50 | 4% **a** |
| 11 | 1 | 50 | 2% [2] | 0 | 50 | 0% [2] |
| **Total** | **35** | **550** | **6.4% a** | **22** | **550** | **4.0% a** |

Note:[1] Different letters in rows denote significant differences ($X^2$ $P < 0.05$).
[2] A zero score in plot 11, means that the $X^2$ test of independence is not valid.

**Table 6.2: Comparison of arm survey and point surveys - two methods used to assess vineyards for incidence of AGY in *cv.* Chardonnay, Gol Gol North, NSW.**

| | Arm Survey | | | Point Survey | | |
|---|---|---|---|---|---|---|
| **Plot #** | **# AGY Vines** | **# Vines** | **AGY Incidence [1]** | **# AGY Vines** | **# Vines** | **AGY Incidence [1]** |
| 1 | 9 | 50 | 18% **a** | 7 | 50 | 14% **a** |
| 2 | 9 | 50 | 18% **a** | 5 | 50 | 10% **b** |
| 3 | 21 | 50 | 42% **a** | 9 | 50 | 18% **b** |
| 4 | 26 | 50 | 52% **a** | 16 | 50 | 32% **b** |
| 5 | 28 | 50 | 56% **a** | 18 | 50 | 36% **b** |
| 6 | 11 | 50 | 22% **a** | 6 | 50 | 12% **b** |
| 7 | 9 | 50 | 18% **a** | 4 | 50 | 8% **b** |
| **Total** | **113** | **350** | **32.3% a** | **65** | **350** | **18.6% a** |

Note:[1] Different letters in rows denote significant differences ($X^2$ $P < 0.05$).

**Table 6.3: Comparison of arm survey and point surveys - two methods used to assess vineyards for incidence of AGY in *cv.* Chardonnay, Irymple, Vic.**

| | Arm Survey | | | Point Survey | | |
|---|---|---|---|---|---|---|
| **Plot #** | **# AGY Vines** | **# Vines** | **AGY Incidence [1]** | **# AGY Vines** | **# Vines** | **AGY Incidence [1]** |
| 1 | 11 | 48 | 23% **a** | 7 | 48 | 15% **a** |
| 2 | 11 | 48 | 23% **a** | 6 | 48 | 13% **a** |
| 3 | 1 | 48 | 2% [2] | 0 | 48 | 0% [2] |
| 4 | 2 | 48 | 4% [2] | 0 | 48 | 0% [2] |
| 5 | 6 | 48 | 13% **a** | 4 | 48 | 8% **a** |
| 6 | 7 | 48 | 15% **a** | 4 | 48 | 8% **a** |
| **Total** | **38** | **288** | **13.2% a** | **21** | **288** | **7.3% b** |

Note:[1] Different letters in rows denote significant differences ($X^2$ $P < 0.05$).
[2] A zero score in plots 3 and 4, means that the $X^2$ test is not valid.

**Discussion**

In the *cv.* Riesling vineyard, the more detailed and time consuming arm survey was not significantly better than the point survey in determining levels of AGY in a vineyard. However, consistently there were 2-3 less AGY vines recorded/50 vine plot in the point surveys. In the *cv.* Chardonnay vineyard at Gol Gol, the point surveys scored significantly less in all but one plot while at Irymple, the scores for each plot did not differ at $P<0.05$. In the latter, the significant difference between the aggregate scores, suggests that they may have been significantly less at higher levels of disease because there was a uniform trend of less disease found in the point surveys for each plot.

That the arm surveys give higher scores especially on vineyard aggregate scores is attributable to the greater precision of the detailed surveys. These score a survey-unit comprising both sides of a single arm as against the point survey which scores the full, facing canopy of a vine and then only on one side of the vine row. *ie.* it ignores the other (rear) side of the vine canopy row.

In a number of plots where AGY was found more frequently, the extra disease occurrences in the detailed survey were found whilst surveying the reverse side of the canopy. Our data suggest that the point survey scored an average of ~3% less disease than the arm survey in the Riesling vineyard and between ~10 % less in the taller Chardonnay vineyards. One reason for the difference is that the taller canopy reduced the assessor's vision of the reverse side of the canopy compared to the lower (Riesling) canopy, which permitted some vision of that reverse side.

The lighting at the time of the survey was uniform and there was no shadowing of the foliage on either side of the vine row. It is our experience that in very sunny conditions shadowing of the canopy makes the survey more difficult though not necessarily less accurate. As a result, surveying during the hours of 9am to 6pm are the best. However, even if shadowing were to influence the ability to detect disease, this could be expected to make little or no difference to the effectiveness of survey for a disease that is generally randomly scattered across vineyards. This is because the surveying a sample line across the vineyard includes an equal number of shaded as unshaded rows.

For an example of the output from the arm surveys, see Figure 8.1. The data on the disease status of each arm on each vine in that figure have been summarised to present the disease status of each vine. Figure 12.7 provides an example of the output from the point surveys. Each point represents the incidence of AGY in the associated 50 vine plot. The arm surveys score between 5-10 vines/minute depending on disease levels; the more disease takes longer to record. Whereas the point surveys can monitor between 20-30 vines/minute and being much quicker, are the more cost-effective for general vineyard surveys.

The outcome of this work provides some point of reference in comparing data from the point survey with those from the arm survey. The former is a simple method to assess for AGY with greater speed but it showed an expected loss of ability to score for disease where the level of scoring was focussed on disease incidence per vine. This loss seemed minor in lower trellises that permitted some vision of both sides but was significant in trellises taller than the assessor scoring for disease. The point surveys prove particularly useful where the relative incidence of AGY is important rather than the absolute value. For example, the point surveys have shown value and time efficiency in assessing relative incidence and severity of AGY across different parts of a vineyard. The more detailed arm survey remains the preferred method to record the occurrence of disease on each arm for specific studies of the epidemiology of disease at that level.

**Conclusion**

- The test of two survey methods (arm surveys *vs* point surveys) indicated that either method could be used when using single vines as the survey-unit;
- When the more detailed assessment of individual arms and a precise score of disease incidence is needed, the detailed arm survey which scores the AGY status of individual arms (cordons) on both sides of vine, is best though it takes longer, averaging 5-10 vines/minute;
- Point surveys that score the disease status of only the facing sides of two rows at a time, will under-estimate levels of AGY by ~10% in taller canopies that restrict the vision of the reverse side of the canopy but they are quicker averaging 20-30 vines/minute;

**Recommendation**

The type of survey for optimum efficiency depends on the planned use of the data. It is recommended that:

- the detailed arm (cordon) survey be used for epidemiological studies of the expression of AGY from season to season within and between vines; and that
- the faster point survey be used for all other studies including comparison of the relative level of AGY between vineyards, though noting that in vineyards with canopies taller than 1.8 – 2.0m, actual disease levels will be ~10% higher than shown by those surveys.

***Two survey methods were designed and tested:***

***Using …***
***1) the arm (cordon) as the unit of survey, is more accurate but is slower***

***2) the vine as the unit of survey, is faster and OK for some uses***
***but …***

***…it under-estimates AGY levels by 10%,***

***There are now tools to measure levels of AGY in vineyards.***

**Footnote: Measures of Incidence and Severity**

The term severity is used where multiple scores of disease incidence (the number of an individual present) are taken from a single plot to provide a measure of the severity (the amount or intensity) of disease in that plot. Whilst the term is also expressed as %AGY, it is a measure of disease severity in the same way that the accumulated score of the number of diseased individual berries provides a measure of disease severity on a bunch, for example in assessing powdery mildew. The number of vines with AGY in a vineyard is a measure of incidence; the proportion of multiple sites within the vineyard with AGY, is a measure of its severity.

# Chapter 7: The Epidemic Nature of AGY
## Studies of the temporal distribution of AGY from 1976/77 to 2004/05

### Introduction

Since AGY was first found in the Riverland in 1976, the disease has varied in severity from season to season across the various districts of Australian viticulture. From knowledge of similar yellows diseases overseas and from details about the epidemiology of AGY as it was being pieced together (as above), it was apparent that the likely source of AGY was either diseased grapevines or some alternative plant host *eg.* Bois noir in Germany was spread from weeds on the vineyard boundaries (Maixner 1993b). Of the yellows diseases of grapevine or other crops overseas, the usual means of spread had been an insect vector (an insect that carries and spreads disease agents). If this were so, it was likely that AGY was similarly spread and that some clear patterns of disease would be evident that would implicate the role of an insect. Thus, disease surveys were considered as tools to test this hypothesis.

In the late 1990's, the Australian industry was concerned about the increasing levels of disease in vineyards. In attempt to understand the disease and find its source, various surveys of disease were undertaken in different regions to provide critical data on the progress of disease in time and space. Analysis of these data, it was hoped, would assist navigate the direction of research in seeking to locate the main source(s) of disease. Also, it was considered important to determine if the same disease system were operative for AGY across Australian viticulture *ie.* were there the same patterns of spread and sources of disease. Trends in disease incidence and severity in different localities across Australia would provide evidence for that. We report here the monitoring of AGY over time, in various assessments of AGY as it varied in different seasons in different regions from 1976/77 to 2004/05.

### Aim

**To monitor the incidence and severity of AGY in vineyards from season to season to improve understanding of the epidemiology of AGY.**

### Materials and Methods

The main two varieties affected by AGY were selected for assessment, *viz.* Chardonnay and Riesling. Disease levels were monitored in three main regions of Australian viticulture including the Riverland, Sunraysia and the Riverina. As the pattern of disease spread became apparent through the surveys and the understanding of the epidemiology of AGY increased as result, the intensity of surveying was also increased. Two phases of survey were undertaken. In the first phase, detailed arm surveys were made while in the second phase, both detailed arm surveys and point surveys were undertaken (Chapter 6).

The vineyards were monitored by visual assessment of vines for AGY. Usually only one assessor was deployed to ensure consistency in scoring for disease and accuracy in comparing scores between seasons. Symptoms were assessed on the basis of the descriptions and illustrations in Chapter 2. These varied from some other investigator of AGY since we recognised as distinct from AGY the set of symptoms known as Scaly Bark Stunt (SBS). Accordingly, these were not scored as AGY.

Previous assessments by the senior author had shown that while symptoms of AGY appear with rapid onset from flowering (late October) onwards, they are usually fully expressed by mid-summer (mid-December) (data not presented). However, to ensure we recorded maximum disease incidence, the surveys were undertaken where possible, just prior to harvest each season (in the period from January to March).

In the first phase of surveying which followed the discovery of AGY, from 1976/77 to the mid-1980's, 11 vineyards were regularly assessed. In the second phase, from the late 1990's to present, many more vineyards were surveyed (Table 7.1). Of these, over 40 blocks were surveyed for at least consecutive seasons, some for seven or eight - these provided the basis for the data we report here.

Arm or point survey data for each vineyard were collated in MS Excel®, summarised to give a mean score of % AGY-infected vines or arms for that vineyard, and presented in a single graph of disease severity over the various seasons of assessment for that vineyard. As in earlier surveys, Chi-square tests of independence ($P<0.05$) were used to show differences in scores (Chapter 6).

**Table 7.1: The number and type of vineyards and their location, surveyed for incidence and severity of AGY, 1997-2005. [1]**

| Location | Chardonnay | | Riesling | |
|---|---|---|---|---|
| **Season** | **# Vineyards** | **# Vines** | **# Vineyards** | **# Vines** |
| **Riverland, SA** | | | | |
| 1997-1998 | | | 27 | 1,195 |
| 1998-1999 | | | 27 | 1,197 |
| 1999-2000 | | | 27 | 1,195 |
| 2000-2001 | 60 | 18,493 | 1 | 202 |
| 2001-2002 | 126 | 47,653 | 9 | 3,039 |
| 2002-2003 | 2 | 1,026 | | |
| 2003-2004 | 92 | 45,408 | 8 | 4,659 |
| 2004-2005 | 74 | 40,963 | 1 | 812 |
| **Total** | **354** | **153,543** | **100** | **12,299** |
| **Sunraysia (Vic and NSW)** | | | | |
| 2000-2001 | 2 | 483 | | |
| 2001-2002 | 2 | 990 | | |
| 2003-2004 | 9 | 6,198 | 1 | 300 |
| 2004-2005 | 7 | 7,116 | | |
| **Total** | **20** | **14,787** | **1** | **300** |
| **Riverina, NSW** | | | | |
| 2002-2003 | 8 | 3,460 | | |
| 2003-2004 | 23 | 6,090 | 11 | 3,750 |
| **Total** | **31** | **9,550** | **11** | **3,750** |

Note: [1] Figures are minimum estimates and include only those vineyards surveyed using the 'point survey' method of Chapter 6. Additional vineyards not listed were assessed in detail using the 'arm survey' - ~ 15 vineyards/season for the Riverland and three for Sunraysia.

## Results

Given the large amount of data collected, only a representative sample is presented below.

In assessing for AGY, nearly every vineyard surveyed (*ie.* of Chardonnay and Riesling), showed some level of AGY. The factor that varied was severity, not incidence of disease (see footnote in Chapter 6).

In the first phase of monitoring, from 1976/77 to the mid-1980's, the graphs show that AGY increased significantly ($P<0.05$) in the first three seasons of observation and then declined as rapidly to a low level (<5% incidence) within four or five seasons. As an example, in a *cv* Riesling vineyard in Renmark, Riverland, SA, AGY increased quickly from 1976/77 to peak at 86% of vines and 44% of arms diseased in 1978/79 (Figure 7.1). Levels declined to insignificant incidence within four seasons *ie.* less than 20% of vines affected. Thereafter they remained at low severity until increasing ($P< 0.05$) for a single season in 1985/86.

A *cv* Riesling vineyard at Loxton Research Centre, Riverland, SA, showed a similar progression of disease, peaking and declining in the same series of seasons except that AGY was only half as severe (maximum 42% vines affected in 1978/79) and took an additional year to reach very low levels in the vineyard (Figure 7.2). Both vineyards were greatly debilitated and rendered uneconomic during the three seasons of high disease (>20% vines with AGY).

A third Riverland vineyard of *cv.* Riesling, at Berri, SA, showed a similar progression of AGY though the increase ($P<0.05$) in disease had peaked (at ~59% vines affected) for season 1977/78 and again increased ($P<0.05$), albeit to a lesser severity (~24-26 % vines), in 1981/82 and in 1983/84 (data not shown).

In contrast, in the Victorian Sunraysia, a *cv* Chardonnay vineyard at Karadoc showed a peak in severity of AGY ($P<0.05$) in 1980/81, two seasons later than in the Riverland (Figure 7.3). Similarly, in vineyards in the NSW Riverina, at Griffith, the severity of AGY peaked in 1981/82. This trend was shown in both a *cv.* Riesling and a *cv.* Chardonnay vineyard block which otherwise showed similar increases and decreases in severity of disease (Figure 7.4).

In the second phase of monitoring, beginning in the late 1990's, the severity of disease in a *cv.* Riesling vineyard at Berri, Riverland, SA, increased significantly ($P<0.05$) from 1998/99 to peak in 1999/00 with 50% vines diseased (Figure 7.5). Levels declined ($P<0.05$) in 2000/01 but remained between 18 – 32% for the next five seasons until declining again in 2005/06. In a *cv.* Chardonnay vineyard near Berri, disease severity increased dramatically ($P<0.05$) from 1999/2000 to 2000/01 and declined over two seasons to 2002/03 before increasing again (Figure 7.6).

In a *cv.* Chardonnay vineyard in NSW Sunraysia, at Gol Gol North, high levels of AGY were observed throughout the 7 years of survey (Figure 7.7). There was a significant decline ($P<0.05$) from peak severity over two consecutive seasons in 1998/99 (50% vines diseased) to 2000/01 (22% vines) when the disease increased ($P<0.05$) to 45% vines in 2001/02. A subsequent decline led to stable levels of AGY since then at from 18 – 23% vines diseased. Figure 7.8 shows disease levels varying in Chardonnay at Irymple, Vic, in the period from 1999/00 to 2004/05 in a pattern almost identical to that at Karadoc, Vic, from 1979/80 to 1985/86 (Figure 7.3).

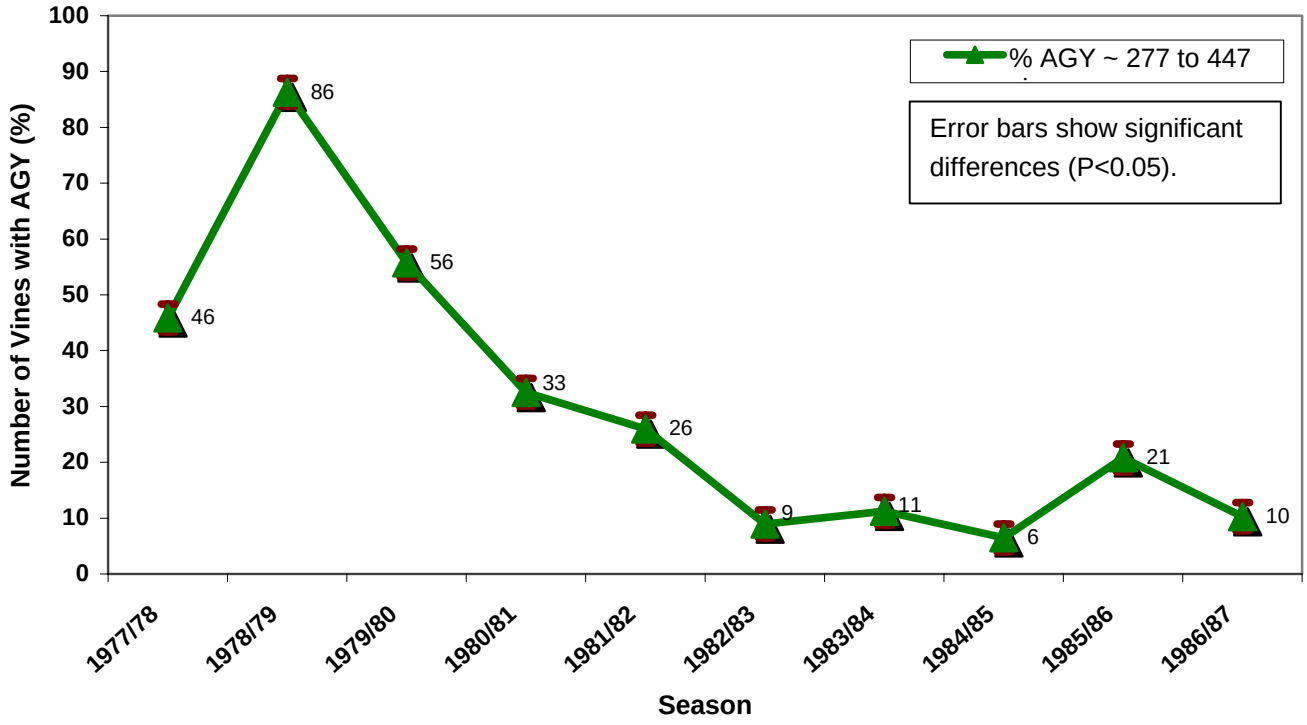



**Figure 7.1: The seasonal variation in % Riesling vines with AGY at Renmark SA. 1977/78 - 1986/87**

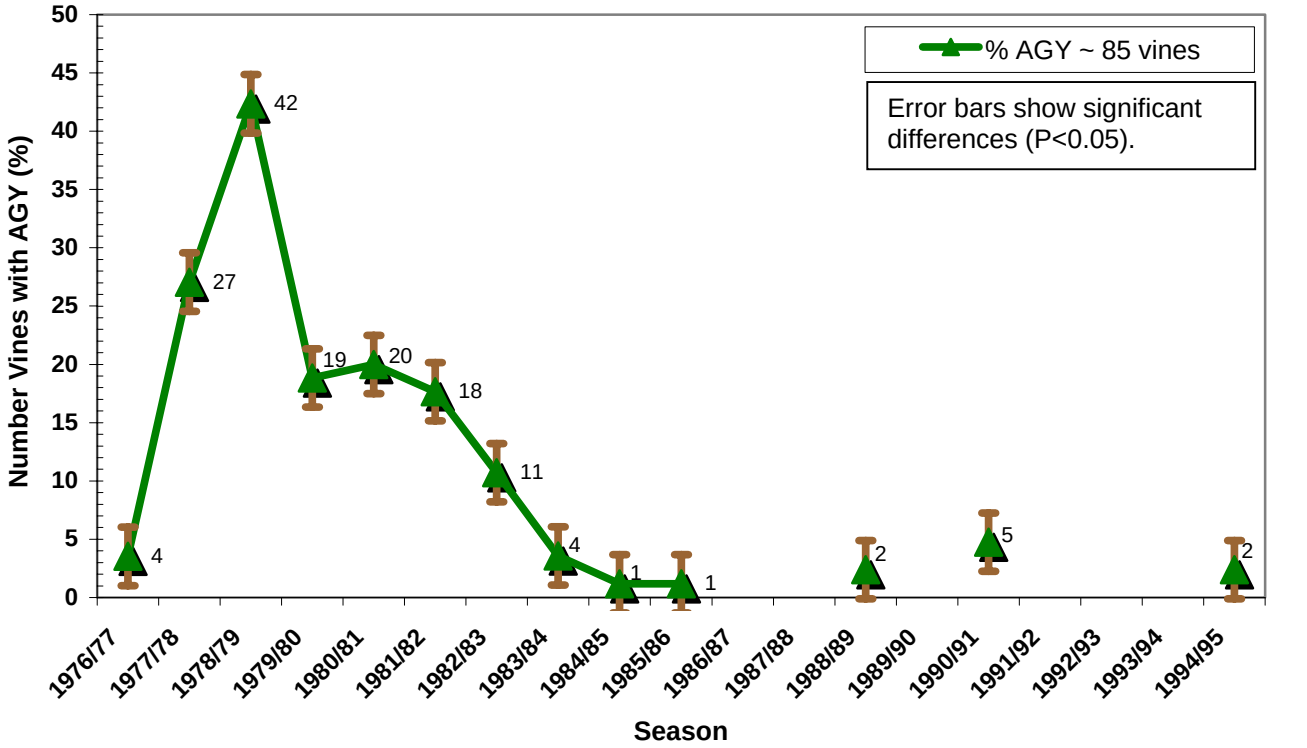



**Figure 7.2: The seasonal variation in % Riesling vines with AGY at LRC F Loxton, SA 1976/77 - 1994/95**

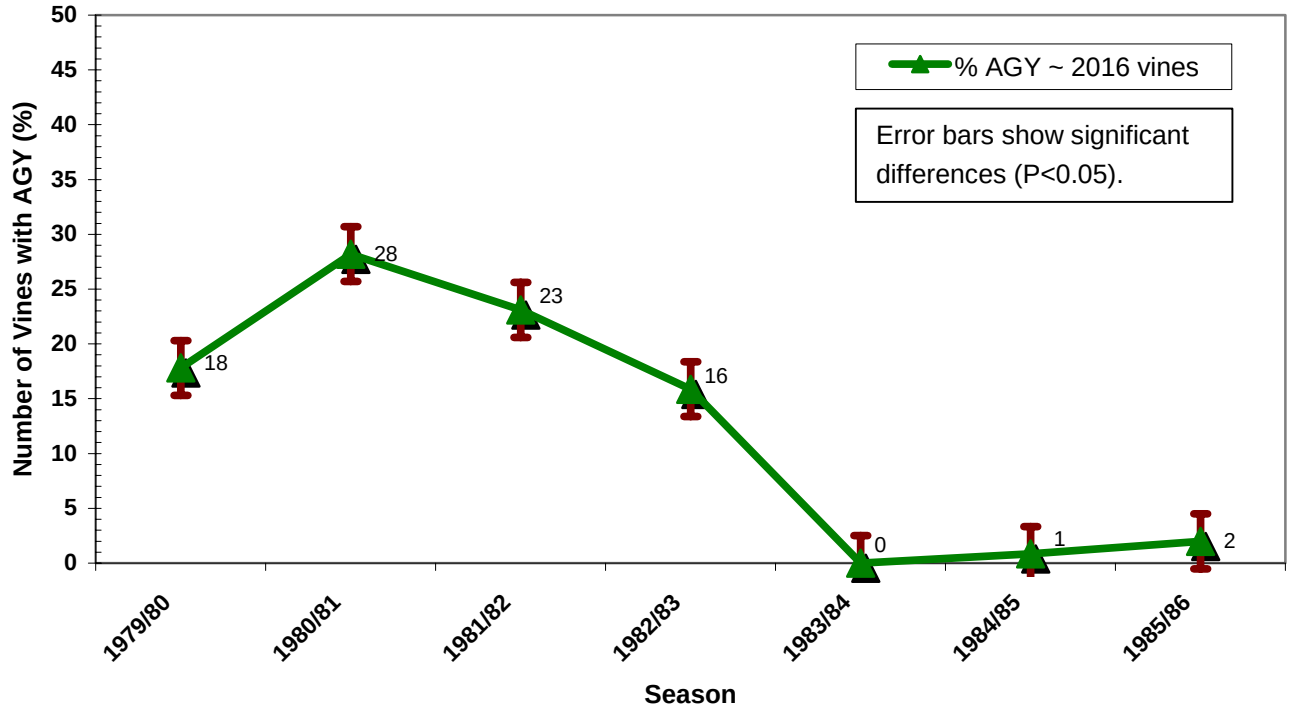



**Figure 7.3:** **The seasonal variation in % Chardonnay vines with AGY at Karadoc, Victoria 1979/80 - 1985/86**

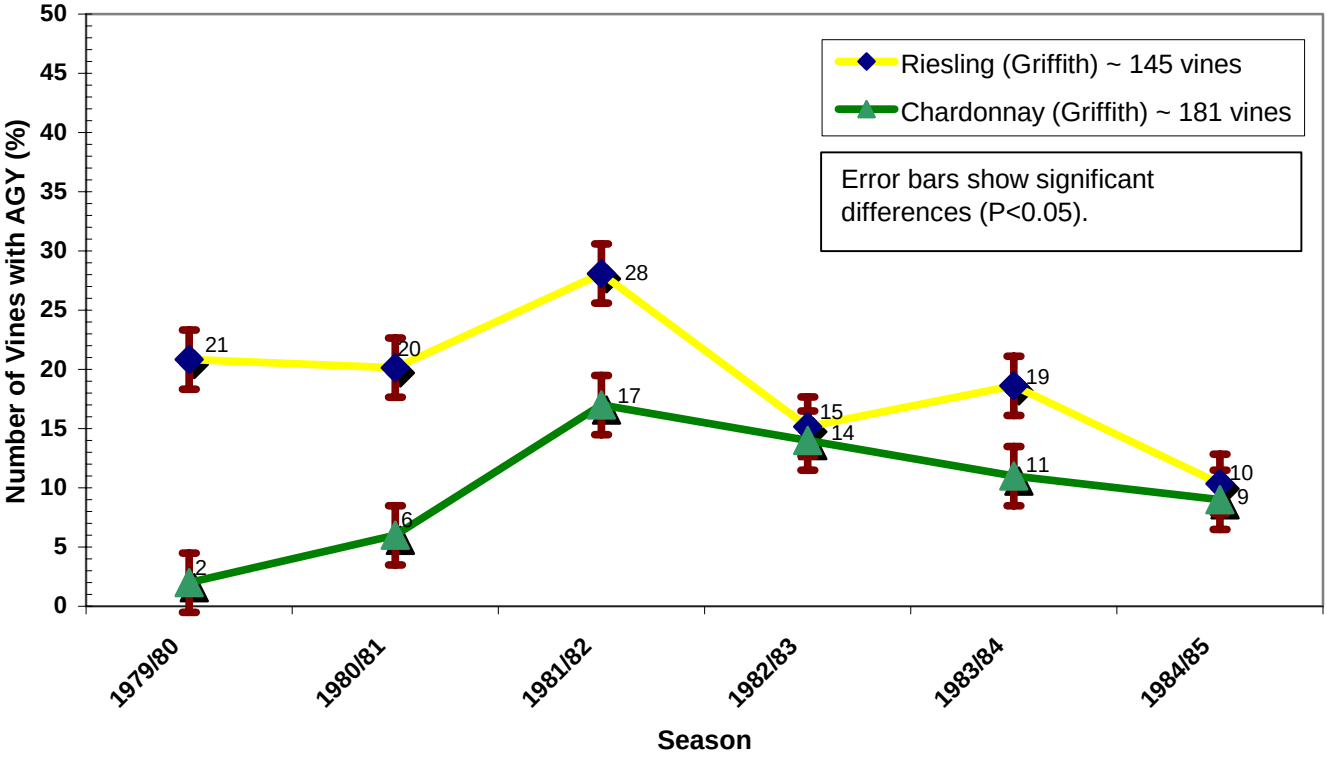



**Figure 7.4:** **The seasonal variation in % Riesling and Chardonnay vines with AGY at Griffith, NSW 1979/80 - 1984/85**

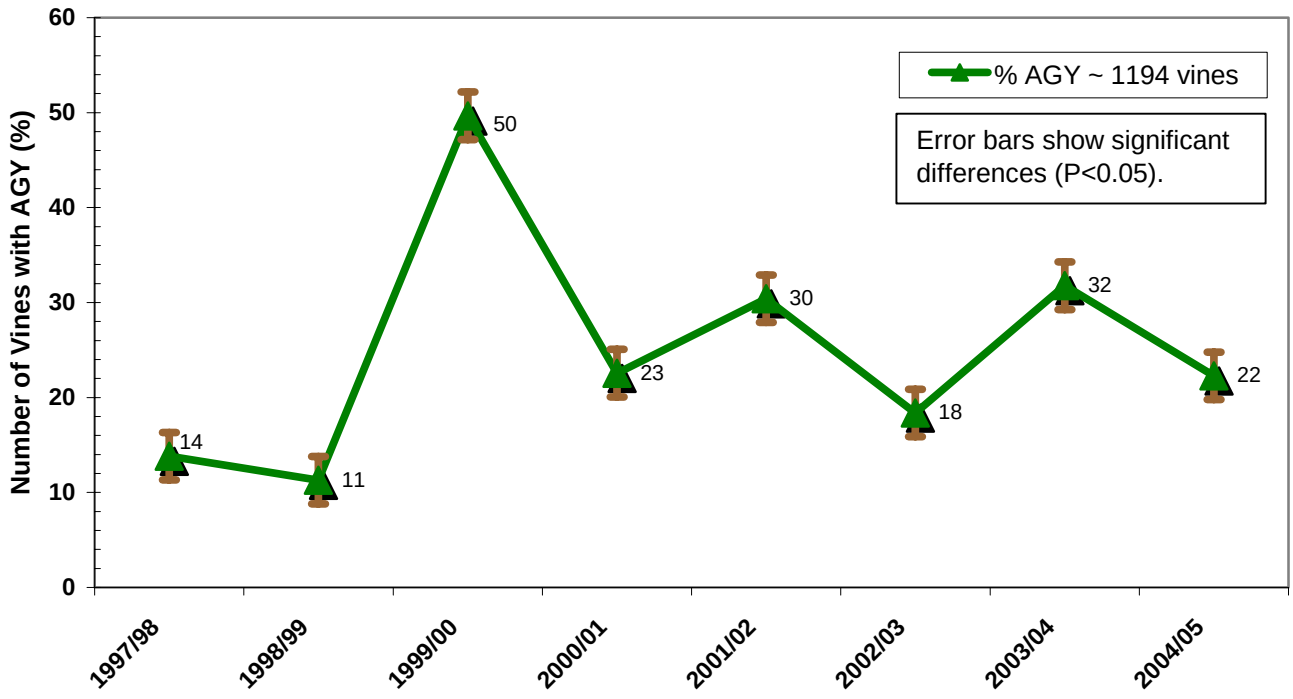



**Figure 7.5:** **The seasonal variation in % Riesling vines with AGY at Berri, SA. 1997/98 - 2004/05**

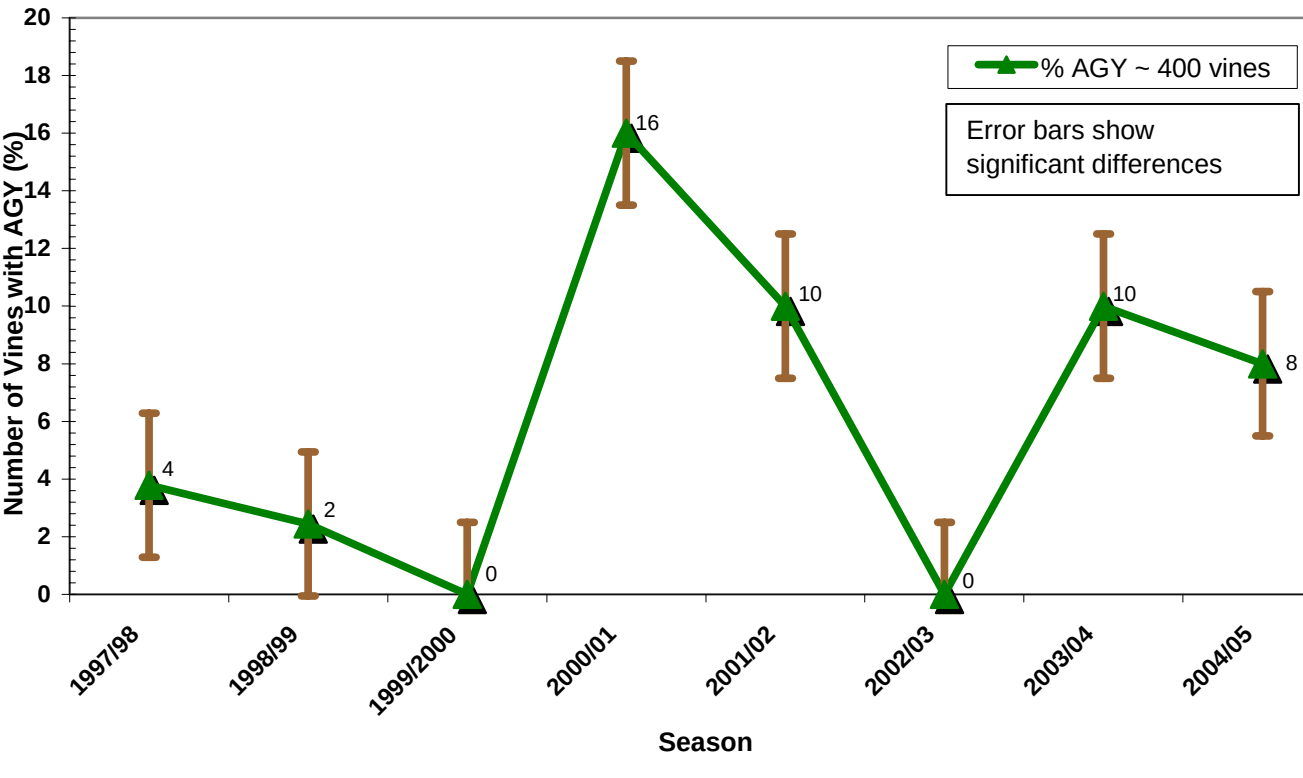



**Figure 7.6:** **The seasonal variation in % Chardonnay vines with AGY at Bookpurnong, SA. 1997/98 - 2004/05**

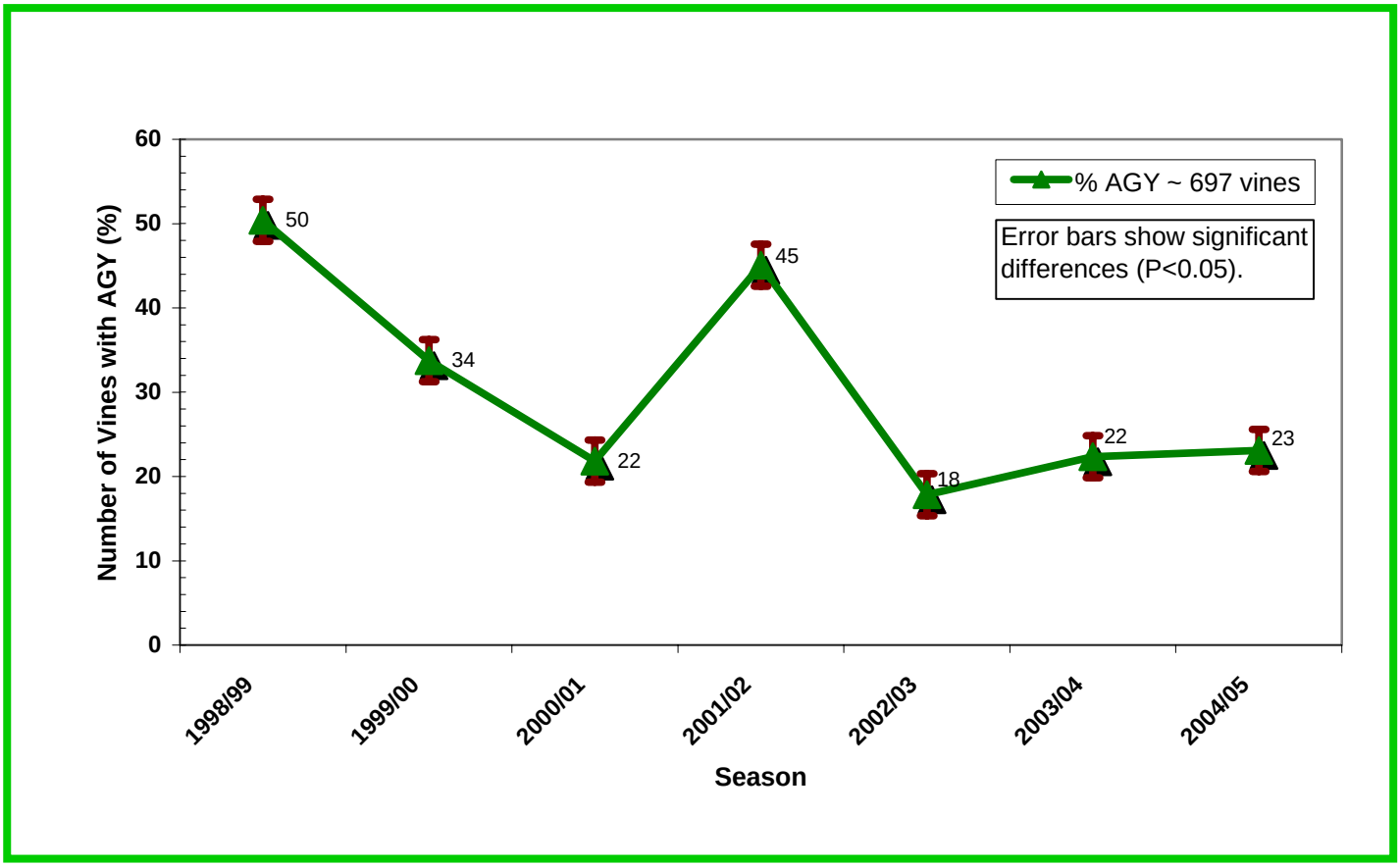



**Figure 7.7: The seasonal variation in % Chardonnay vines with AGY at Gol Gol, NSW, 1998/99 - 2004/05**

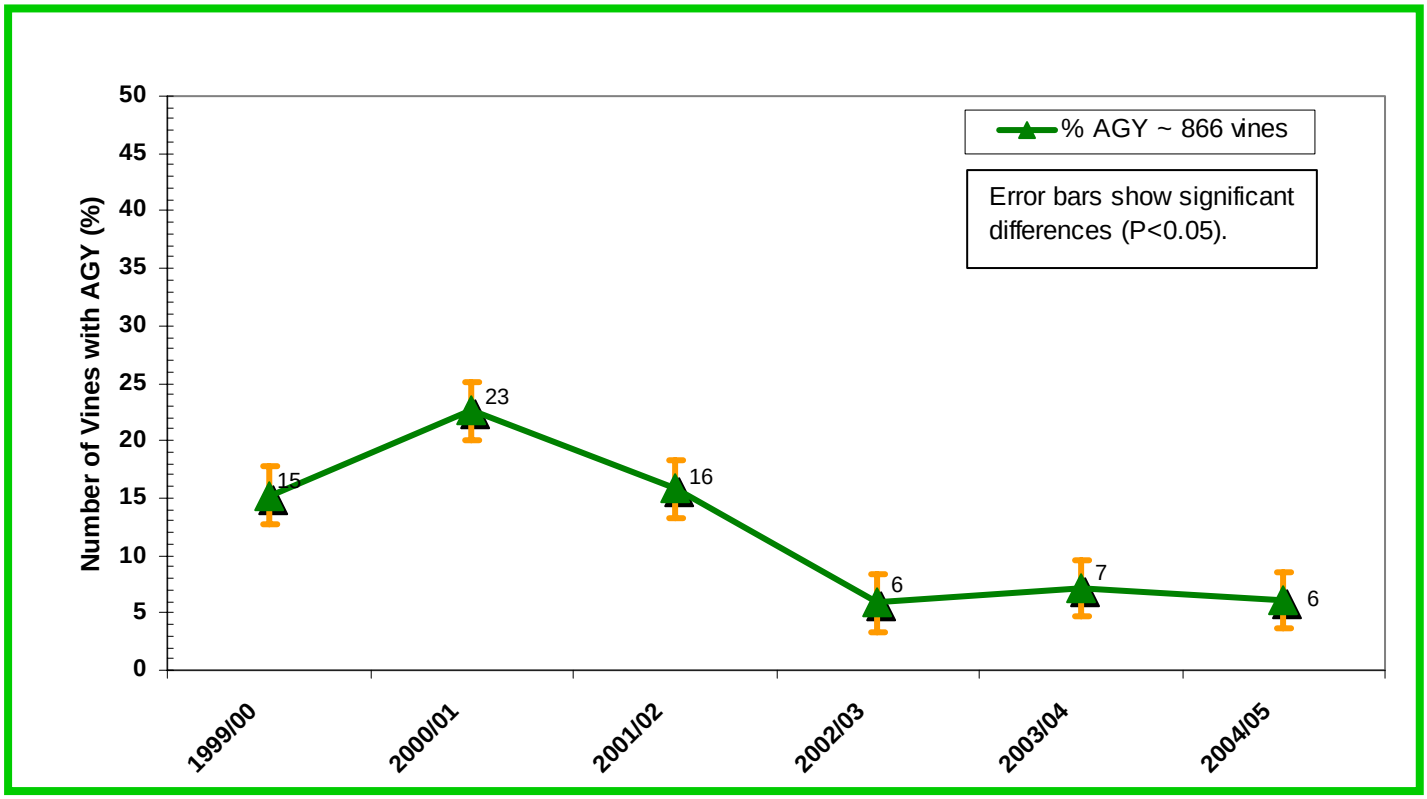



**Figure 7.8: The seasonal variation in % Chardonnay vines with AGY at SHC Irymple, Victoria, 1999/00 - 2004/05**

## Discussion

Extensive surveys of Chardonnay and Riesling vineyards in the Riverland, Sunraysia and Riviera regions showed that most vineyards were infected with AGY, but the severity of disease varied across the regions and seasons.

Despite these variations in symptom expression, there were strong similarities in the way AGY showed peaks and troughs in severity in different seasons. In the vineyards assessed in the first phase of monitoring from 1976/77 to the mid-1980's, the disease peaked significantly once in each region. In the Riverland, the peak occurred in 1978/79 (Figures 7.1 – 7.2) while in Sunraysia and the Riverina, it occurred two or three seasons later (Figures 7.3 – 7.4). On each occasion, the peak lasted only for one and sometimes two seasons before disease levels returned to low and insignificant levels in the following years. Despite this concerns about the disease continued.

A similar trend occurred in the second phase of monitoring from the late 1990's. This similarity is exemplified in the similar patterns from the two phases of survey as portrayed in vineyards from the first at Karadoc (Figure 7.3) and the second, at Irymple (Figure 7.8). Disease severity increased, at times dramatically, to levels similar to those observed in the first period. Some vineyards monitored showed more persistent levels of disease after the peaks and were regularly and significantly affected (Figure 7.5). Others showed the typical peak and subsequent decline to low levels (Figure 7.6).

The general pattern of disease showed typical epidemic characteristics affecting many vineyards at the same time with levels that varied from season to season. There are potentially many factors that influence the severity of disease expression each season. These include variation in primary inoculum loads (the titre of phytoplasma within the vine) and in the environmental conditions that favour spread and/or expression of disease.

A number of characteristics of AGY provide some clues to understanding the epidemiology of disease. Some that may have bearing in varying symptom expression are summarised below:

- Most diseased shoots die either within the season of symptom expression or in the subsequent dormant season (Chapter 2);
- Natural heat therapy is a phenomenon that causes affected shoots to regrow (Chapters 2 and 4); and
- Most affected vines recover from symptoms in the season following first expression (perhaps the result of the above two phenomenon).

These factors are consistent with and perhaps account for the relatively short duration of peak disease expression that was observed.

However, because

- vines are predisposed to disease (and presumably are inoculated with the pathogenic agents) in the season or seasons prior to symptom expression (Chapter 2); and
- the pathogenic agents may reside in the arms and spurs (the aerial parts) that bear the affected shoots each season;

it is conceivable that the titre of the pathogen in the season of symptom expression will be affected by the environmental conditions. For instance, there is probably an optimum temperature for multiplication of phytoplasma during the incubation period, that is the time between inoculation (when the disease agents are introduced into the vine) and disease expression in late spring. Thus for a given inoculum load, the temperature and other environmental

conditions may influence the severity of disease expressed the next season by influencing the rate of multiplication of the pathogen as it incubates in the vine during late dormancy and spring. This appears to be the case with diseases such as yellow speckle virus (Magarey *et al.* 1999) which will express more severely in seasons with warmer temperatures from late dormancy through early spring.

It is conceivable also that the severity of AGY in a given season may be influenced by the activity of an insect vector in the previous season – that is, how much inoculum is introduced to the vine and to a vineyard.

To test some of these possibilities and their influence on the cyclical occurrence of seasons with severe disease, it is of value to:

1. analyse weather data for associations between mean daily temperature in the period from late August to mid-November and the severity of AGY in that season. It could be that some factor such as temperature sum (degree-days), may account for the season to season variation in level of disease seen in vineyards. If successful, such associations could be used to forecast disease levels; and
2. analyse the same data for associations between the time of symptom expression (at flowering onwards) in Australia and in similar yellows diseases such as Bois Noir (Black wood disease) in Germany and other countries where that disease occurs relatively later in the growing season than does AGY.

Tests such as these could lead to significant progress in understanding how AGY occurs and in what conditions it spreads.

**Conclusion**

In studying the disease via extensive surveys over a number of seasons, some typical features of AGY became apparent:

- AGY is an epidemic disease with peaks and troughs that come and go as the conditions that favour the disease and its spread vary from season to season;
- AGY is widespread across the Riverland, Sunraysia and Riverina – very few vineyards of *cv.* Chardonnay or Riesling have no disease;
- losses from the disease vary as the severity of disease rises and falls;
- similar trends in disease increase and decline occur within regions at the same time;
- disease levels usually remain high only for 1-2 seasons before declining; new peaks may not occur for 5-7 seasons or more;
- the first recorded peak in levels of AGY in the Riverland was in 1978/79, the second in late 1999/00 – 2000/01;
- within individual vines, symptoms come and go, from the combined effects of natural heat therapy - a rare phenomenon for diseases but common with AGY – and because diseased shoots die in the season of symptom expression;
- there is potential to use the significant body of survey data on vineyard disease, to gain useful information about the development of symptoms and their continuity within the vine.

**Recommendation**

It is recommended that:

- weather data be analysed for associations of specific environmental conditions, in particular, in evaluating the influence of temperature on:
    a) the timing of disease expression on a seasonal basis; and
    b) the severity of disease expression on a regional basis and subsequent crop loss;
- the hypothesis that an insect vector spreads disease and its activity in the previous season influences levels of AGY in the next and subsequent seasons, be tested in further investigations.

***Extensive vineyard surveys showed some interesting aspects of AGY:***

***The disease is widespread across Australia and is epidemic …***

***… that is, it increases and decreases in severity from one season to the next;***

***More is to be gained by analysing all the survey data collected … to understand why the disease causes loss one year and not the next.***

***May be insects play a role in this …?***

# Section 6: Investigating the Source of AGY
# Patterns of Disease over Time and Space 2

*In the previous Chapters we investigated the pattern of disease in vineyards over a number of successive seasons. This revealed some useful epidemiological clues in how levels of AGY varied from year to year and when the vines were infected. As we continue the search for how AGY spread and from where it came, we now use intensive vineyard surveys over successive seasons to investigate the pattern of disease within and between vineyards. Can we find more clues about the location of the source of inoculum for AGY?*

## Chapter 8: Locating the Source of AGY 1 - long-distance transport is the norm
## Studies of the Spatial Distribution of AGY from 1976/77 to 1999/00

### Introduction

Given the observations of Chapter 7 and the preceding chapters, the possibility of an insect vector spreading disease became more significant. Investigations to determine how AGY spreads, now hinged on a simple question:

*'Does the disease come from within vineyards or is it introduced from outside?'*

The answer to this was needed to focus the present research in investigating a control strategy for AGY.

A partial answer had been provided in Section 3 through investigations of the role of propagation material – it seems that grapevine cuttings, at most, contributed inconsequential levels of inoculum to vineyard disease.

To resolve the matter further, preliminary surveys and resultant detailed maps of the spatial distribution *ie*. the occurrence of AGY across vineyards, had given some important clues. For instance, vineyard patterns of disease seemed to lack foci of infection but appropriate methods of statistical analysis were not available in Australia. So, in September 2000, two approaches to analysing AGY survey data were reviewed. This occurred during the principal investigator's travel to Germany and USA (see GWRDC Report SAR 00/2, Magarey (2000)). Methods of statistical-modelling data of the vineyard occurrence of a similar yellows disease in Germany were compared with GIS techniques from Iowa, USA.

The modelling in Bernkastel-Kues, Germany by one of us, Michael Maixner (Maixner 1993), showed greatest rigour for the necessary analyses while the GIS work showed value in portraying the spatial distribution of AGY within the vineyards. Both approaches offered potential for answering our question as we further investigated the patterns of spread for AGY.

### Aim

**To monitor the spatial distribution of AGY in vineyards to improve understanding of the epidemiology of AGY.**

## Materials and Methods

With the assistance of the statistical software package, PATCHY™, (Maixner 1993, 1993a), seven spatio-temporal statistical models were used to analyse comprehensive Australian data on the spatial incidence of AGY. These data were derived from detailed arm surveys (Chapter 6) to assess the AGY-status of each arm of each vine in 3-10 vineyards in three Australian regions for a period of 5 – 10 consecutive seasons over 15 years between 1976 and 2000 (Chapter 7). The vineyard surveys were conducted in the Riverland, (SA), Sunraysia, (NSW and Vic), and the Riverina, (NSW). We analysed 28 'site-by-season' vineyard combinations from these data.

## Results

Without exception, the statistical analyses showed a lack of regular arrays of AGY within the vineyards. Instead, frequently, there was a random scatter of small clumps of disease across the blocks assessed. Of the 28 combinations of 'site-by-season' tested, we present Figure 8.1 as a typical example of that pattern.

Two findings of the analyses using PATCHY™ were:

- AGY never occurred in foci of disease or in runs along rows or in clustered groups along edges of the vineyard; and
- In the test vineyards, AGY occurred in random clumps of diseased vines that varied in location from season to season *ie*. the disease occurred in different locations and in small clumps of different size across the vineyards.

## Discussion

Statistical analyses of the AGY survey data showed a random clustering of disease indicating that AGY does not spread from within the surveyed vineyards. If AGY were to spread either from diseased vines, diseased weeds or other vegetation in the vineyard, a regular focus or clumping of affected vines around these sources would be apparent from season to season. The contrary was observed – the random clumps were variable in their appearance and in their location between seasons.

Similarly, because there was no edge effect of aggregation of AGY-affected vines in association with vineyard boundaries, it is concluded that the disease does not originate from the immediately adjacent environment. Thus the source of AGY is unlikely to be located in any neighbouring vineyards, weeds or other vegetation in a zone at least ~300m around the surveyed vineyards. Typical highland Mallee vegetation which was abundant in close proximity to many of the test vineyards, was therefore excluded as a likely source of disease.

Given this evidence, it is likely that AGY originates from long distance transmission from vegetation *ie* from more than 300m distant from the vineyards we tested. If Mallee ecosystems are excluded, the only other major vegetation type in the regions we investigated was Murray Valley riverine and wetland vegetation. This appeared to be the ecosystem most likely to contain the plant(s) that are the primary inoculum sources for the disease and most worthy of further investigation.

## Australian Grapevine Yellows Survey

## Berri, SA - Vine Map

**Riesling - Survey 9 - 10 th March 2001;**

| Vine | Row 19 | 18 | 17 | 16 | 15 | 14 | 13 | 12 | 11 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | * | * | * |
| 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | * | * | * |
| 3 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | * | * | * |
| 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | * | * | * |
| 5 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | * | * | * |
| 6 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | * | * | * |
| 7 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | * | * | * |
| 8 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | * | * | * |
| 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | * | * | * |
| 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 |
| 11 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 |
| 12 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 13 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 |
| 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 |
| 15 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 |
| 16 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 17 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 |
| 18 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 19 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 20 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 21 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 22 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| 23 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| 24 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 25 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 26 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| 27 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| 28 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 29 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | * | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 30 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 31 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| 32 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 33 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | * | 0 | 1 | 0 | 1 | 0 | 1 | 0 |
| 34 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| 35 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | * | * | 1 |
| 36 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | * | 0 | 1 | * | * | 0 |
| 37 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | * | * | 0 |
| 38 | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | 0 |
| 39 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| 40 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 |
| 41 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 42 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |

| KEY | |
|---|---|
| **Symptoms** | |
| no AGY | 0 |
| AGY | 1 |
| missing | * |
| | |
| AGY Vines | 273 |
| Total Vines | 1180 |
| % AGY | 23.1 |
| | |
| **Orientation** | |
| 1 = N | |
| 2 = S | |
| North | |

Insect Exclusi

Pruning trial June-00

Pink tags=AG

| Pruning trial 2000/01 | |
|---|---|
| 1. Pruned vines in rows 6-8 | |
| AGY arms in 6-7 | #REF! |
| AGY arms in 8 | #REF! |
| Total arms | 68 |
| % AGY | #REF! |
| 2. Untreated vines (Rows 9-11) | |
| AGY vines | 52 |
| Total vines | 194 |
| % AGY | 26.8 |

| Exclusion House 2000/01 | |
|---|---|
| AGY vines | 8 |
| Total vines | 50 |
| % AGY | 16.0 |

**Figure 8.1: The typical spatial incidence of AGY in vineyards distant from disease hot spots is shown in this vineyard map from detailed arm- surveys for the disease at Berri, Riverland, SA. The disease occurs in random clumps of affected vines that vary in location from season to season. Cells marked 1 were vines with AGY; 0 were symptomless; and * were missing vines. Vines within the rectangle were enclosed in insect exclusion house (see Chapter 16).**

If this were so, then vineyards with highest levels of disease might be expected to occur in closest proximity to riverine and/or wetland localities.

This finding was contrary to popular theory held by some investigators that the disease was sourced by an insect vector that spread AGY from within the vineyard *ie.* from diseased vines, vineyard ground-cover or weeds, to healthy vines. It is interesting to note that the methods of transmission observed in yellows diseases overseas vary from our findings. Flavescence dorée (FD) in France is transmitted by a leafhopper insect which lives and feeds on vines (Schvester *et al.* 1963) and spreads disease from vine to vine in a somewhat radiating pattern. In contrast, Bois noir (BN) is also spread by a leafhopper but that insect lives on weed species within and adjacent to affected vineyards and produces significant edge-affects in disease patterns within the vineyard (Maixner 1994) Similarly, Pierce's Disease (Purcell *et al.* 1979) while not a yellows disease, is spread by a sharpshooter vector that lives in near-by riverine vegetation. Again, edge-effects are apparent in the neighbouring vineyards. The Australian disease appears to differ from each of these and the question remained, where is the source of AGY?

**Conclusion**

- The field surveys indicated that the source of AGY was not within the test vineyards but was most likely located somewhere beyond.
- Long distance transport of AGY via a possible insect vector from that source to the vineyard was occurring;
- The source of AGY inoculum was not located within a ~300m zone surrounding most diseased vineyards; and therefore
- The source is not primarily located in Murray Mallee ecosystems; but
- The primary source of AGY is likely to be found in riverine and/or wetland vegetation ecosystems.

**Recommendation**

- It is recommended that investigations into the source of AGY be focussed on riverine and similar vegetation, the ecosystem(s) most likely to contain the primary host plant(s) and the source of inoculum for the disease.

***More survey data:***

- ***… AGY usually occurs in scattered clumps, a sign that the disease comes from a long way off, perhaps via an insect;***

- ***… AGY doesn't come from within the vineyard or from usual Mallee vegetation, but more likely from river or wetland based plants***

***Let's set our sights on these areas to find the source of AGY…***

# Chapter 9: Locating the Source of AGY 2 - hot-spots of disease occur
## Studies of the spatial distribution of AGY in 2000/01 to 2001/02

### Introduction

In Chapter 8, it was shown that AGY occurred in random clumps of affected vines. This inferred that AGY was being spread some distance to those vineyards and that the source of disease did not lie either within those vineyards or within the adjacent vegetation in a zone ~300m around those vineyards. As a result, it was concluded that, because of its proximity to affected vineyards, highland (Mallee) vegetation and its associated ecosystem did not harbour the source of disease.

The only other major form of vegetation in the Riverland, Sunraysia and Riverina was riverine (Murray Valley or Murrumbidgee floodplain) and similar wetland vegetation. Consequently, these ecosystems were considered most likely to contain the primary host plant(s) and the source of inoculum. Although some vineyards with high levels of AGY had been found in these environments, a specific study of these localities was needed.

### Aim

**To monitor the spatial distribution of AGY in vineyards within riverine or similar ecosystems, in search for the primary sources of disease.**

### Materials and Methods

In January - March 2001, surveys of 91 sites were made using methods described above (Chapter 6), in vineyards of Chardonnay and Riesling in the Riverland and Sunraysia.

During 2001/02, GWRDC RITA Project RT01/15-2 was undertaken specifically to intensify the vineyard surveys in both riverine and Mallee vegetation systems in attempt to better define the spatial patterns associated with AGY and thereby locate the source of AGY. In January – February 2002, 240 vineyards each with at least 300 vines were surveyed in the Riverland. Some ~20 other sites were surveyed in Sunraysia (see Chapters 6 and 7 for details).

To test if disease levels were higher adjacent to the riverine floodplain and to define disease levels as vineyard distance from the river increased, disease severity was assessed across different localities in several districts, where possible, with transects covering Mallee or inland vegetation systems and riverine or wetland vegetation.

### Results

In 2000/01, surveys of the 91 vineyard sites showed higher severity of AGY in some localities and lower in others (data not shown). The higher severity seemed linked to specific localities or ‘hot spots’ of AGY and were associated with vegetation in riverine ecosystems and included vineyards growing along the river and/or near overflows from irrigation channels.

This finding led to a more detailed survey the next season (2001/02). In recent years, surveys of vineyards in the Riverland and Sunraysia had shown an increase in severity of AGY (data not shown).

Key findings of these surveys were:

- high levels of AGY were found in some vineyard localities and lower levels in others. Of a total of 269 Chardonnay vineyards assessed in several regions in 2001/02, 225 (84%) had disease severity with less than 20% vines affected. In contrast, 43 (16%) of the vineyards scored greater severity.
- when disease severity exceeded 20% vines/vineyard, some measure of crop loss occurred.
- the localities with high disease predominately occurred in zones close to riverine swamplands *ie.* within at most <1500m of these ecosystems. Figure 9.1 is a representative example of one of these broad transects.
- proximity to the riverine ecosystems alone did not seem to be significant for association with severe disease. The localities with high disease levels also occurred near permanent shallow water (lagoons and wetlands), irrigation overflows and the wastelands adjacent to those localities.
- low levels of AGY were found in other vineyards. These were almost universally further away *ie.* >1500 m from the above localities.
- in several vineyards within the hot spots of disease, a trend of increasing disease closer to the wetlands was evident. For example in one cv. Chardonnay vineyard at Loxton North (Baker's Lake), the severity of AGY increased (P<0.05) from 14%, 20% to 30% in successive sites within the vineyard in a line toward a drainage basin (Figure 9.1).

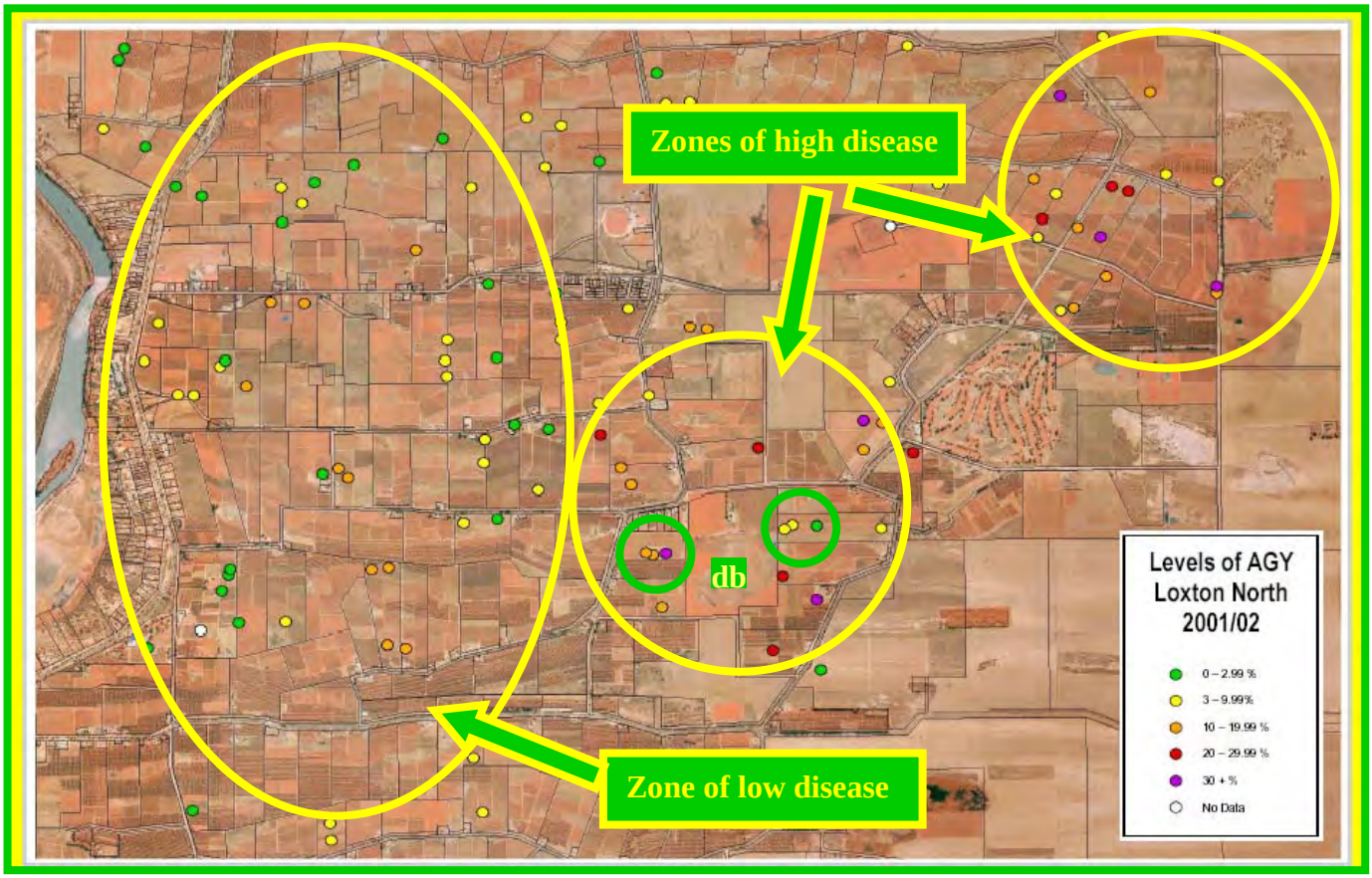



**Figure 9.1: Surveys in 2000/01 and 2001/02 identified for the first time localities with low or high incidence of AGY. In this example, at Loxton North, Riverland, SA, the localities with highest disease are furthest from the riverine ecosystems but are closest to drainage basins (irrigation overflows). The vineyards within the green circle showed increasing severity of AGY toward Baker's Lake, an irrigation overflow (drainage basin) at db.**

## Discussion

The surveys of 2000/01 and 2001/02 confirmed earlier evidence that in the majority of vineyards in all districts the severity of AGY is low and the disease was of no consequence. Of the vineyards assessed in 2001/02, 84% had disease severity scores below that considered threshold for crop loss *ie.* they showed disease severity less than 20% of affected vines. However, to the contrary, levels were higher than threshold and significant yield loss would have occurred in 16% of the surveyed vineyards.

The levels of AGY in the Riverina are less well defined but to date had paralleled those in the Riverland.

In recent years, surveys of vineyards in the Riverland and Sunraysia have shown an increase in severity in AGY. At the end of 2000/01, levels of AGY in most surveyed vineyards were just below those causing economic loss and industry concern was being expressed as to the source and spread of AGY.

The data from one district *eg.* Loxton North in the Riverland (Figure 9.1) and from other areas surveyed (data not shown), revealed that high levels of AGY occurred in definable zones not bigger than 1500 m x 1500m. It seemed reasonable to conclude that the source of AGY was located within these zones of high disease and was absent or in low numbers outside these zones.

These surveys provided the first substantial evidence of the location of the source of AGY in Australian vineyards. For the first time, the source of disease was associated with specific localities and vegetation ecosystems. These localities occurred in the Riverland and Sunraysia and were nearly always associated with permanent shallow water, such as occurred in lagoons or irrigation overflows or with adjacent wastelands (Figure 9.2).

The observed vineyard patterns of disease were consistent with the probability that an insect vector(s) of AGY does not live in or near the vineyards which showed only low levels of disease. Also, that the presumed vector probably lives and very likely, also sources AGY inoculum from non-Vitis plants *ie.* plants other than grapevines, more than ~300m from most vineyards. And quite the opposite, it would seem that the insect vector lives near vineyards with high levels of AGY within the hot spots identified in the above surveys.

**Figure 9.2: A typical scene in which high levels of AGY were seen consistently: A vineyard of *cv.* Chardonnay adjacent to a band of wasteland along an irrigation channel, in this case Gol Gol Creek at Gol Gol North, Sunraysia, NSW.**

## Conclusion

- AGY occurs in 'hot spots' – this was the first evidence that the source of AGY was nearby;
- The disease surveying process more closely defined (and better located) the zones from where AGY was naturally sourced and probably also, where the presumed insect vector associated with AGY naturally lives and feeds;
- It was possible that different patterns of AGY would be seen in the vineyards within the disease 'hot spots' *ie*. close to the source of disease; and
- Further studies were warranted, especially using disease surveys as a tool to better understand the epidemiology of AGY.

## Recommendations

It was recommended that:

- the disease surveys be intensified, especially within the 'hot spot' localities of high disease in anticipation of finding higher levels of AGY on vineyard boundaries in vine blocks adjacent or near to the specific source of disease; and that
- more detailed assessment of levels of disease in additional localities in the Riverland and in other regions such as the Riverina be assessed to determine if the same pattern of occurrence of AGY existed in those districts.

***… AGY sometimes occurs in hot spots …,***

***first sign that the disease is coming from nearby in some localities;***

***The source of AGY is now in sight!***

***And even more detailed surveys now are needed.***

# Chapter 10: Locating the Source of AGY 3 – discovering disease gradients Studies of the Spatial Distribution of AGY in 2002/03

## Introduction

The following work is a summary of investigations funded jointly by the present project GWRDC SAR 02/03 and the Riverina Wine Grape Marketing Board (WGMB). It comprises a portion of a report tabled to WGMB in March 2003 and is significant as part of the investigation to locate the source of AGY and to find how the disease spreads in Australian vineyards.

Surveys were planned to see if the same disease system (patho-system) occurs in the Riverina as in the Riverland and Sunraysia *ie.* to determine if the disease occurs in similar ways and is spread by the same or similar means from the same or similar host plants.

Monitoring local vineyards would enable levels of AGY to be assessed and assist locate the source of disease with view of determining the best course of investigation toward a commercial control of AGY in Australia.

## Aim

**To monitor the temporal and spatial distribution of AGY in vineyards adjacent to riverine or similar ecosystems in search for the primary sources of disease in the Riverina, NSW.**

Specific objectives included:

1. Information for growers - to present information about AGY to growers in the Riverina: Much had been said and thought about AGY; some of it confusing rather than clarifying grower understanding of the disease; and
2. Disease survey - to monitor levels of AGY in vineyards near Griffith, NSW, during 2002/03: The aim of this work was to assess the disease system present in that region.

## Materials and Methods

**Information for growers** In June 2002, the senior author presented the WGMB with background information on AGY and a proposal to investigate AGY in season 2002/03. A field day was planned to present symptoms and latest information on AGY. Subsequently, in January 2003, a brief survey of the levels of AGY was made in 12 vineyard sites near the Riverina districts of Bilbul and Hanwood.

**Disease survey** In January 2003, the incidence of AGY was assessed by point survey made by walking down 2 - 6 randomly selected rows per vineyard while scanning the foliage on both sides of the row and recording the presence or absence of AGY on each vine at 12 vineyard sites (Chapter 6). The presence of disease was scored if any AGY symptoms were seen on a vine. The percentage severity of AGY in each vineyard was then calculated. A global positioning device (GPS) was used to locate each sampling site and the data was used to determine precise locations of these vineyard observations.

The severity of AGY was assessed at each site and where levels of AGY were higher, brief survey was made of the near-by vegetation. Any special occurrences were noted.

Brief assessment of local insect populations was also made by one of us, Murray Fletcher, (Australia's leading leafhopper and planthopper specialist. This assessment was assisted by Leigh Pilkington, then a PhD student from the Faculty of Rural Management, The University of Sydney, at Orange, investigating Australian Lucerne Yellows, a disease similar to AGY).

The surveys included vegetation from within, near and distant from the vineyards and searched for differences in insect and plant species and compared these with a similar survey previously undertaken in the Riverland, SA. Observations were made for associations of vegetation types with high levels of AGY. These surveys are not reported here in detail though the outcomes of the assessments are recorded in Chapters 12 and 13.

Aerial reconnaissance was made of the surveyed vineyards and photos taken to enable the on-ground observations to be fitted into perspective for most of the localities we surveyed.

**Results**

**Information for growers** In a presentation to WGMB, details of progress in understanding the epidemiology AGY in Riverland and Sunraysian vineyards were given *ie.* of how the disease spreads over time and space and what factors may influence that spread. In reviewing a plan for further research in the Riverina the relevance of investigations in the Riverland was reviewed in relation to the expression of AGY in both regions. The possibility of capitalising on the benefits of sharing available resources was discussed and plans were made to further investigate the source and spread of AGY with view to developing a management strategy for AGY. That investigation was to link with the present project and has been reported more fully elsewhere (Magarey *et al*. 2003).

Given the confusion exists about AGY, it was decided that a field day on AGY be presented for Riverina growers and others in Australian viticulture. The objectives of this field day which could be presented in other regions where AGY was of consequence, would be to:

- show the symptoms of AGY;
- assist growers identify AGY and distinguish it from 'looks-like' symptoms;
- present latest data on the occurrence and severity of AGY in the Riverina (and elsewhere);
- present evidence on where AGY is sourced and how it spreads into vineyards; and
- outline progress in investigations toward the development of a strategy to manage AGY in Australia.

**Disease survey** Table 10.1 presents the data from the 2002/03 surveys. At sites # 3, 4 and 12, in excess of 10, 17 and 11% of vines respectively, were diseased. The higher of these levels approach the threshold estimated for economic loss *viz.* ~20% vines diseased.

The severity of AGY at the other sites surveyed was less and was of little commercial significance.

**Table 10.1: Levels of Australian Grapevine Yellows (AGY) in survey of *cv.* Chardonnay vineyards at Griffith, Riverina, NSW. 22 – 23rd January 2003.**

| Survey # | Grower # | Address | Location | Description | # Vines with AGY | # Vines Surveyed | % AGY | Approx. Distance to Free-standing Water | |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | Bilbul Rd | Beelbangera | Row 19 | 14 | 516 | **2.7** | **400 m** | Main Canal |
| 2 | 1 | Bilbul Rd | Beelbangera | Row 56 | 15 | 210 | **7.1** | **120 m** | Main Canal |
| 3 | 1 | Bilbul Rd | Beelbangera | Row 60 | 21 | 204 | **10.3** | **90 m** | Main Canal |
| 4 | 1 | Bilbul Rd | Beelbangera | Row 66 | 30 | 172 | **17.4** | **90 m** | Main Canal |
| 5 | 1 | Bilbul Rd | Beelbangera | Row 85 | 5 | 228 | **2.2** | **120 m** | Main Canal |
| 6 | 2 | Rosetto Rd | Beelbangera | | 6 | 312 | **1.9** | **500 m** | Main Canal |
| 7 | 3 | Kearey Rd | Bilbul | Sample Bk 1 | 6 | 340 | **1.8** | **50 m** | Small Canal |
| 8 | 3 | Old Willbriggie Rd | Hanwood | | 3 | 378 | **0.8** | **50 m** | Drainage Canal |
| 9 | 4 | Macedone Rd | Bilbul | | 10 | 570 | **1.8** | **150 m** | Drainage Canal |
| 10 | 5 | McCormack Rd | Yoogali | | 10 | 436 | **2.3** | **70 m** | Drainage Canal |
| 11 | 6 | Moseley Rd | Bilbul | | 13 | 526 | **2.5** | **30 m** | Drainage Canal |
| 12 | 7 | Kearey Rd | Bilbul | Adjacent to road | 41 | 358 | **11.5** | **50 m** | Small Canal |

## Discussion

**Past surveys**. In phase one surveys in the late 1970's, (see Chapter 7), the senior author with help from staff at NSW Agriculture, assessed levels of AGY in two vineyards, one Chardonnay and one Riesling, at the that Department's Viticultural Station over six consecutive seasons from 1979/80 (Figure 7.4). The incidence of AGY at that time illustrated the epidemic (up and down) nature of AGY. Levels in the same Chardonnay patch had increased from 2% in 1979/80 to 17% in 1981/82 then declined to 9% in 1984/85. Levels in the Riesling patch were higher but less variable than in the Chardonnay.

**Recent surveys** In the brief survey of 2002/03, no vineyard was free of AGY. The incidence scores for AGY varied from 0.8% to 17.4% vines affected (Table 10.1). This level was of the same order of severity as observed in the previous assessments some 20 years earlier.

Surveys in the Riverina and elsewhere in Australia provided evidence which suggested that the incidence of AGY will continue to vary (up and down) from season to season. However, it is noteworthy that in the earlier (phase one) surveys, levels of AGY increased x8-fold in only two seasons! An x11-fold increase (from 4% to 42%) was observed in three seasons in Riverland vineyards at that time (Figure 7.2).

If an increase of this proportion were to occur again, AGY would cause very significant crop loss in the Riverina.

**Disease patterns** A comparison of the incidence scores for AGY in various locations within the same vineyard in January 2003 at Beelbangera provided an estimator of disease severity at that location and led to some important observations about the possible locations of the source of the disease.

Importantly, there was progression of increasing disease severity from Sites 1 to 4 in the same vineyard from 2.7% to 17.4% in the space of about ~ 300m (Table 10.1). The aerial view of the same vineyard shows the perspective of that progression of disease in a gradient across the vineyard block (Figure 10.1). It was of interest to note the trend occurred from one end of the vineyard toward the main canal and that it seemed unrelated to the proximity of the drainage channel (on the left hand side of Figure 10.1).

The vegetation adjacent to areas of high levels of AGY included a range of native and introduced plant species.

The same pattern appeared to exist in other vineyards. Figure 10.2 shows an example.

**A Source of AGY?** The zone delimited in green in Figure 10.1 illustrates the possible location of the primary source of AGY near that vineyard. It represents an area adjacent to where (at least sometimes) still and/or shallow water occurs. That environment favours the growth of native vegetation including perennial shrubs, reeds and similar species which occurred there in higher frequency than further along the main canal. It was noteworthy that the perennials included the

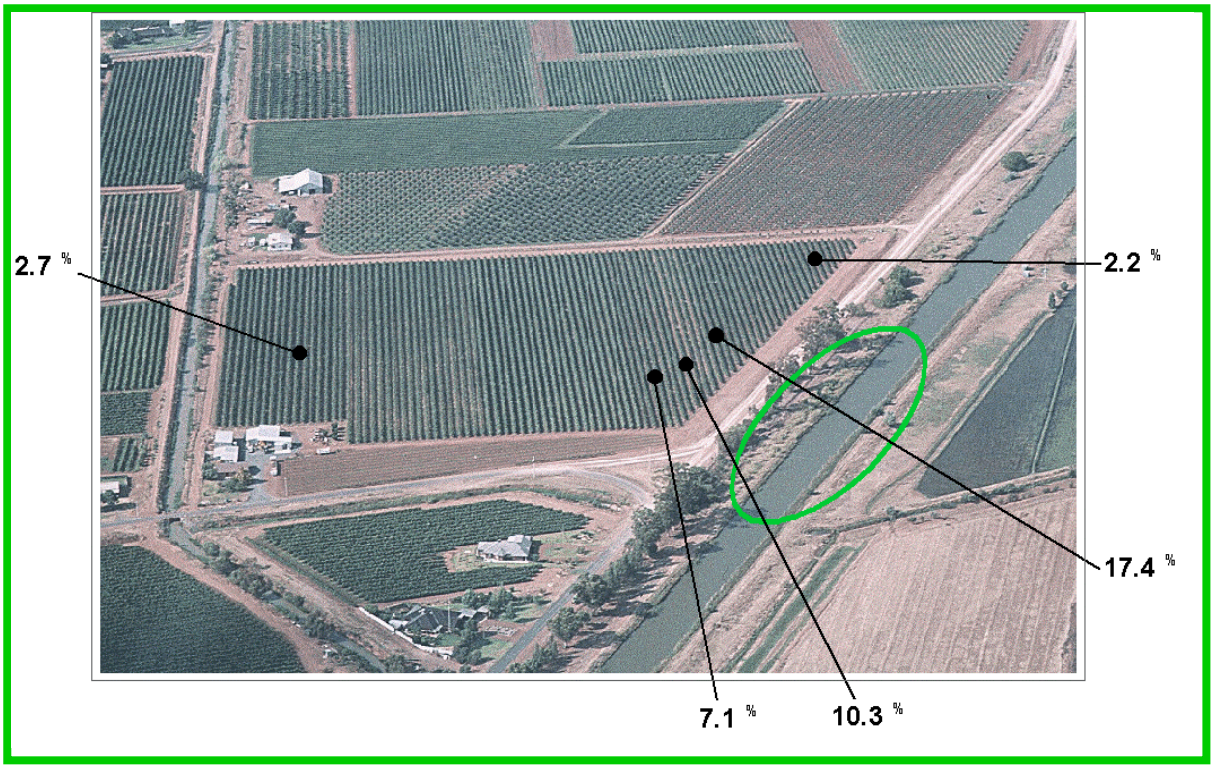



**Figure 10.1: Aerial view of vineyard Sites 1 – 5, Bilbul Rd, Beelbangera, near Griffith, NSW (see Table 10.1). Incidence of AGY is marked as surveyed during January 2003. Note the progressive increase in AGY toward the zone marked in green showing reeds and other vegetation near the main canal.**

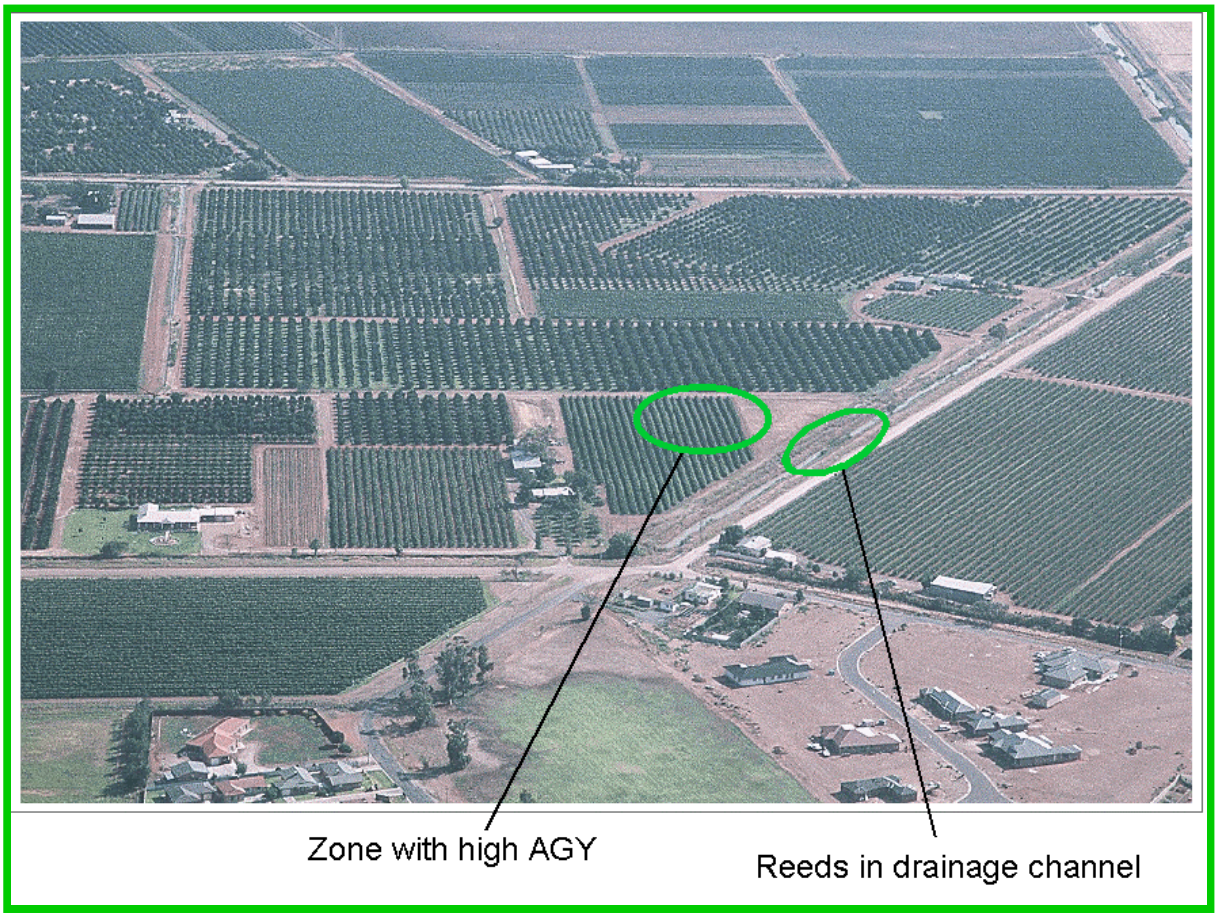



**Figure 10.2: Aerial view of vineyard Site 10 on McCormack Rd, Yoogali, near Griffith, NSW. Incidence of AGY as surveyed during January 2003, was highest in a vineyard clump marked in green on the photo above. Note the proximity to the drainage channel and a stand of reeds (circled in green) and other vegetation approximately 70m distant.**

Chenopod species such as *Maireana brevifolia* (yanga bush or short-leafed bluebush), *Enchylaena tomentosa* (ruby saltbush) and others. It is conceivable that these may favour higher levels of AGY, perhaps as source plants. Similarly, it is possible that this survey had (at long last) delimited the source area for AGY in that locality.

**Proximity to shallow water:** In investigating this fact more fully, it was interesting to note the estimates of the distance from the survey sites to the nearest still/shallow water (see the right hand column of Table 10.1). It was significant that high levels of AGY *viz.* a severity >10%, only occurred in vineyards within 100m of some form of waterway. While high disease scores did not occur every time a vineyard was within that 100m zone, in this limited survey, every time (in three of three occasions) where AGY was in 'above average' severity, that site was close to shallow water.

As a further example in a different locality, at Yoogali (Table 10.1, Site 10), an unusual, cluster of AGY-affected vines was also of interest. Though the mean vineyard severity score was low (2.3%), a small group of diseased vines were clustered in one corner of the site (Figure 10.2). The vineyard owner had indicated that this clumping had occurred in at least several previous seasons. There was no obvious sign of any difference within the vineyard that may have accounted for the observed levels of AGY at that location but that portion of the vineyard lay closest (~70m) to the section of the drainage channel where reeds and other shrubs and non-grass species were growing.

As a result of these observations from a limited survey and only for one season, there is evidence to hypothesise that proximity to environments which include shallow water has a bearing on the severity of AGY. In fact, such areas may contain the primary source of disease and may contain the primary host plants that carry AGY.

There are many other factors which could account for the observed occurrence of higher levels of AGY near these environments and the above suggestions needed to be interpreted with caution. This is especially so since the presence of channels with permanent or semi-permanent still and/or shallow water was not always associated with high levels of AGY in nearby vineyards.

Despite this, the association of higher AGY severity with areas with or without of shallow water could be explained by the presence or absence of the particular plant host in sufficient number or the lack of that plant or plants being infected with AGY. We would hypothesise that the primary source of AGY inoculum in those localities is a plant host of variable occurrence but that it is in someway associated with the shallow water/wetland/lagoon ecosystems.

The above observations pointed (though not conclusively) to the following:

- if permanent still or shallow water is within 100m of a Chardonnay or Riesling vineyard, then levels of AGY may be high (*ie.* >10-15%), but
- high levels of AGY only occur near localities with permanent or semi-permanent still or shallow water, and to the contrary,
- vineyards more than 400-800m from permanent still or shallow water are likely to have low levels of AGY.

Thus, it appeared that the vegetation system that develops in or near channels, canals and drainage basins favours the growth of the alternate hosts of AGY. A vineyard that is placed more than 800m from an ecosystem that grows near shallow water, may have some AGY but is not at risk from high levels of the disease.

Many other factors may account for these observations but the above concepts (hypotheses) need to be tested by further observation and investigation.

**Summary of surveys in the Riverina in 2002/03** The survey undertaken during January 2003 gave only a brief snapshot of the vineyard system where AGY was found in that region. None-the less, it confirmed similar findings from the Riverland and Sunraysia and raised some interesting points that warranted further investigation.

We found that:

- the levels of AGY were relatively minor in most locations; but
- potentially significant levels (*ie.* >10% incidence) occurred in 25% of the locations (3 of 12) surveyed (*ie*. significant from an epidemiological if not economical viewpoint);
- the incidence and pattern of AGY in the Riverina was similar to that observed in the Riverland and Sunraysia;
- more extensive monitoring was required to obtain better assessment of the severity of AGY in the Riverina; but although
- the levels of AGY increased within zones ~100m of some habitats with permanent or semi-permanent water. This pattern was consistent with recent observations in SA; and
- the existence of disease gradients (a trend of increasing levels of AGY across a vineyard) was potentially very significant in the search for the source of AGY;

**Conclusion**

- It is likely that the same disease system for AGY occurs in the Riverina as in Riverland and Sunraysia. As a result, the findings from South Australia and Victoria can probably be applied to the Riverina and *vice versa*;
- The Riverina investigations added evidence in support of the hypothesis that AGY is associated with specific ecosystems that are associated with riverine wetlands, lagoons and the like, and/or with associated wastelands and uncultivated areas.
- Despite the caution, several observations from the Riverina were consistent with observations in the Riverland – that there is a positive association between high levels of AGY and the proximity to permanent still/shallow water. This warranted further investigation at both locations.

**Recommendation**

It is recommended that:

- investigation be made to better define the possibility that disease gradients occur across vineyards that lie within the so-called ‘hot spot’ zones of high disease risk;
- an high priority be given to a detailed investigation of the plant species and leafhoppers and/or planthoppers present in the hot spots zones which include zones of permanent and/or semi-permanent shallow water adjacent to vineyards with high severity of AGY; and that
- the recent findings and clear descriptions of AGY and ‘look-alike’ diseases be presented to grapegrowers especially those in the high severity regions for AGY *viz.* the Riverland, Sunraysia and Riverina.

***AGY is associated with specific ecosystems***
***these are***
***… riverine wetlands,***

***and/or***

***… wastelands and uncultivated areas.***

***The disease system in the Riverina***
***matches the system in***
***the Riverland and Sunraysia.***

# Chapter 11: Locating the source of AGY 4 – defining disease gradients Studies of the spatial distribution of AGY in 2002/03

## Introduction

Given the findings of the previous chapters, an understanding about the possible nature and source of AGY was becoming clearer.

Because AGY was first detected only in 1976 when *cvs*. Riesling and Chardonnay had started to become popular as varieties in Australia, some investigators had been operating on the theory that AGY was introduced into Australia through infected cuttings. As shown above (Chapters 3 and 4) this now appeared unlikely. Also as mentioned earlier (Chapter 5), the advent of PCR technology had allowed identification of the phytoplasma disease agents associated with yellows diseases. Studies of the phytoplasma associated with AGY had led to recognition that the presumed pathogen was also associated with disease on other hosts eg. New Zealand flax (*Phormium tenax*) (Padovan *et al*., 1995), and that the pathogen AGY was a distinct and previously un-named phytoplasma now known as '*Candidatus* Phytoplasma australiense'.

Other key findings from the literature were:

- although a number of similar yellows diseases occurred in grapes overseas and were also associated with phytoplasma disease agents, none of these was associated with '*Ca.* P. australiense';
- the closest relative to the presumed Australian pathogen in any host including grapevine was the phytoplasma associated with Bois Noir in Germany and elsewhere, but this was not '*Ca.* P. australiense'; and
- in contrast, in Australia, phytoplasma virtually identical with AGY had been found associated with pawpaw dieback in Northern Territory, with strawberry lethal yellows in Queensland and the yellows disease of flax in New Zealand. In each of these crops, the agent was indistinguishable from '*Ca.* P. australiense'.

Since '*Ca.* P. australiense' has not been detected overseas, it is suggested that:

- AGY is native to the Australasian region; and if so,
- AGY occurs naturally in native plants and as a result, a native plant (or plants) is (are) the likely primary host of AGY. And if so, that
- grapevine is not a natural host for the AGY pathogen.

Given the above, some understanding about the possible nature and location of the primary host plant can be deduced.

Since phytoplasma are not seed-borne, it is not likely that the primary native host of AGY is an annual plant. This is because an annual plant would not be able to transfer the pathogen from one generation to the next and each new generation of the host plant would need to be inoculated with the pathogen for '*Ca.* P. australiense' to survive - this was considered an unlikely event in a natural system.

Thus, it seemed more likely that the primary host of AGY would be a perennial native species and therefore perhaps a woody plant.

Also, if the host species were only short in stature, the native plant(s) in question might naturally invade some vineyards and be accepted by vineyard managers … and thus be a source of disease there. But, since our studies of patterns of AGY in vineyards had suggested that the disease was not sourced from within vineyards, it was reasonable to conclude that the native host of AGY was not there. This might be because it was taller than plants normally accepted by growers as ground cover or as inter-row plants. As a result, one reasonable assumption is that the presumed primary native host of AGY is taller than usual ground cover species accepted within vineyards and therefore likely to be taller than > 0.5 m when mature.

Another possibility is that the plant favours growth in untilled environments and as a result, is uncommon within vineyards.

**Hypotheses redefined** As a result of the above, some more specific hypotheses as developed earlier and formulated here, are suggested in relation to the source and spread of AGY:

1. the AGY phytoplasma are indigenous (native or naturalised) to the Australasian region and so inhabit native plants; and as a result,
2. a native plant (or plants) is/are the likely primary host of AGY;
3. the primary host of AGY is a plant woody and perennial in nature and taller than 0.5m in height when mature; and that
4. grapevine is not a natural host for the AGY pathogen; and
5. grapevine is a terminal host of AGY and does not transmit epidemiologically significant quantities of the pathogen in propagation material;
6. a mobile biotic agent(s) spreads (is vector of) disease with movement largely into and not from vineyards;
7. the vector(s) is one or more species of leafhopper or planthopper insect;
8. AGY is sourced from plant(s) that are located more than 300m from most vineyards surveyed to date; and
9. the source of AGY lie(s) within clearly defined localities (disease hot spots) of dimension not more than 1500m x 1500m.

A more focussed set of aims for the survey-based aspects of the project were thus devised:

1. to complete the studies on defining the 'hot spot zones' of high disease; in attempt
2. to identify the primary host(s) of AGY expected within the hot spots;
3. to assess the role of the presumed leafhopper/planthopper vector(s) of AGY in native vegetation associated with riverine and/or wetland ecosystems or adjacent wastelands;
4. to further the knowledge of the source of AGY inoculum; and,
5. to find how AGY is spread, …and, in so doing,
6. to assist in the development of an effective control.

The outcomes of Chapter 9 and the above reasoning, led to new hypotheses which warranted testing.

**Aim**

**To investigate the possibility that the source (primary host) of AGY grows in swamplands or associated wastelands close to vineyards in zones of high disease.**

## Materials and Methods

In season 2002/03, vineyard monitoring using the detailed arm survey and the point survey (Chapter 6) was continued but with specific design to monitor along single transect lines within those vineyards. Some 28 Riesling and 30 Chardonnay blocks were assessed in the Riverland, 12 blocks of Chardonnay in the Riverina and 5-6 blocks of Chardonnay were assessed in Sunraysia.

Where possible, the presence or absence of AGY was scored for each vine in at least 50 vines/block in at least 3-5 blocks/vineyard. This level of replication means that a 5% difference in the AGY severity score could be distinguished using $\chi^2$ statistic in tests of independence ($P<0.05$) as in Chapter 6. Data points were plotted using MS Excel™.

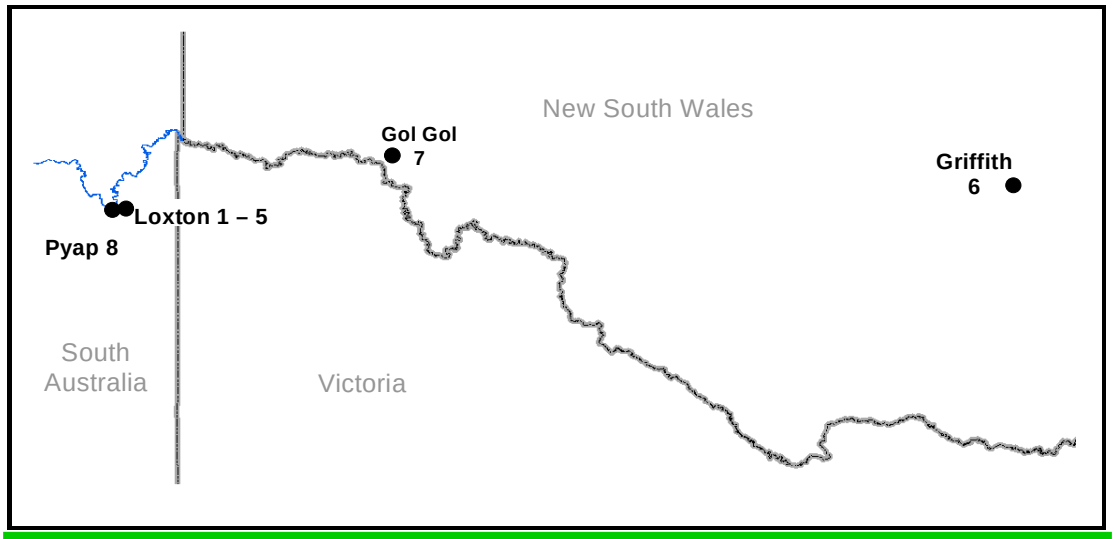



**Figure 11.1: Map showing the location of the sites of some of the AGY transect - surveys in the Riverland, Riverina and Sunraysia during 2002/03.**

The Riverina data from the surveys outlined in Chapter 10 were graphed and are re-presented here for comparison.

## Results

The survey transect line through the vineyards was labelled **A** ➔ **B**, where **A** was the distal end of the transect *ie.* furthest from riverine and/or wetland ecosystems and **B** at the proximal end *ie.* closest to that vegetation system.

A large body of data was collected in this phase of the investigation. A representative sample of the data from the transect surveys, including at least one from each region, is presented graphically in Figures 11.2 – 11.11 to illustrate the trends that were consistently observed.

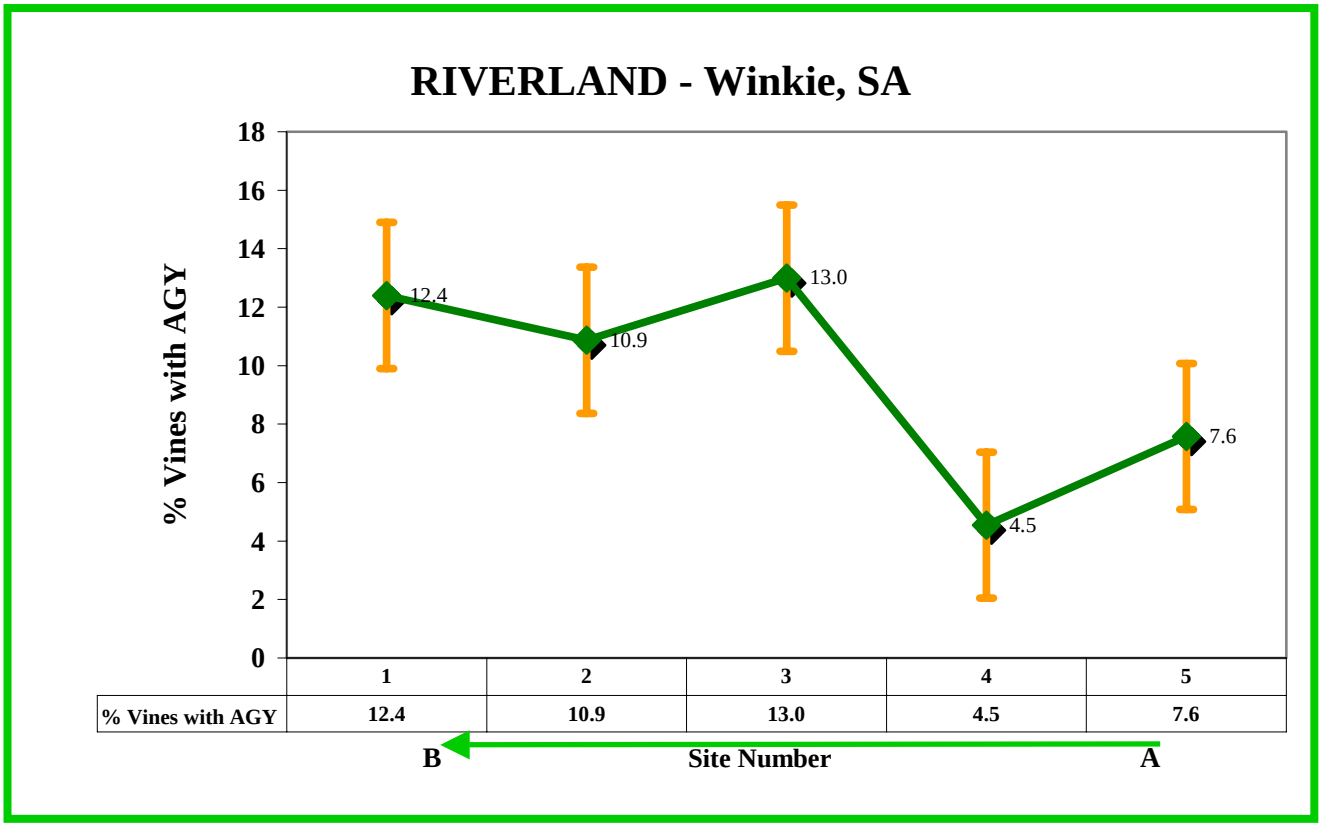



**Figure 11.2**: **The gradient in severity of AGY along a vineyard transect at Winkie, Riverland, SA, 2002/03. A is furthest away and B is closest to the Puddletown drainage basin and adjacent wasteland.**

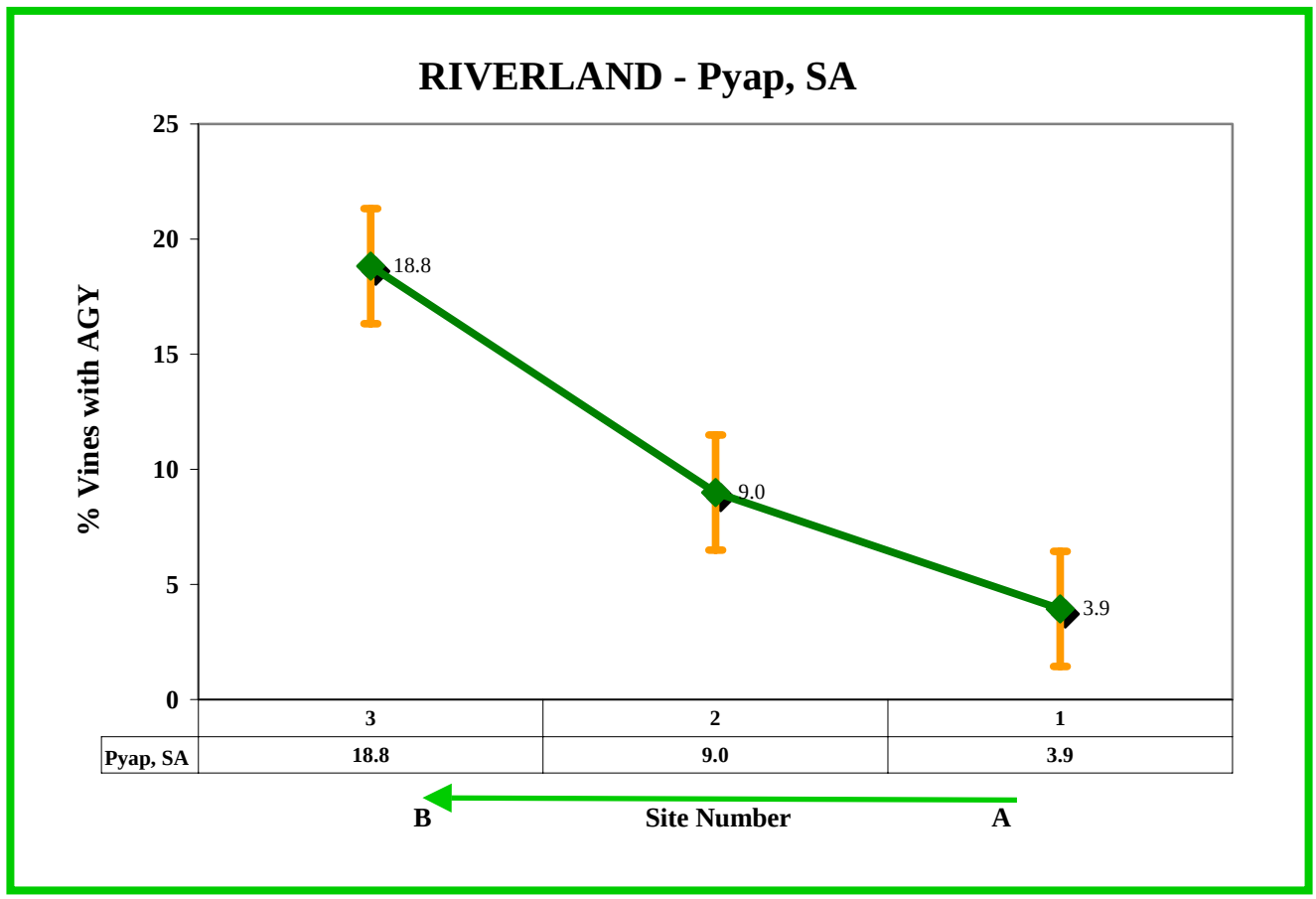



**Figure 11.3**: **The gradient in severity of AGY along a vineyard transect at Pyap, Riverland, SA, 2002/03. A is furthest away and B is closest to the River Murray and adjacent wasteland.**

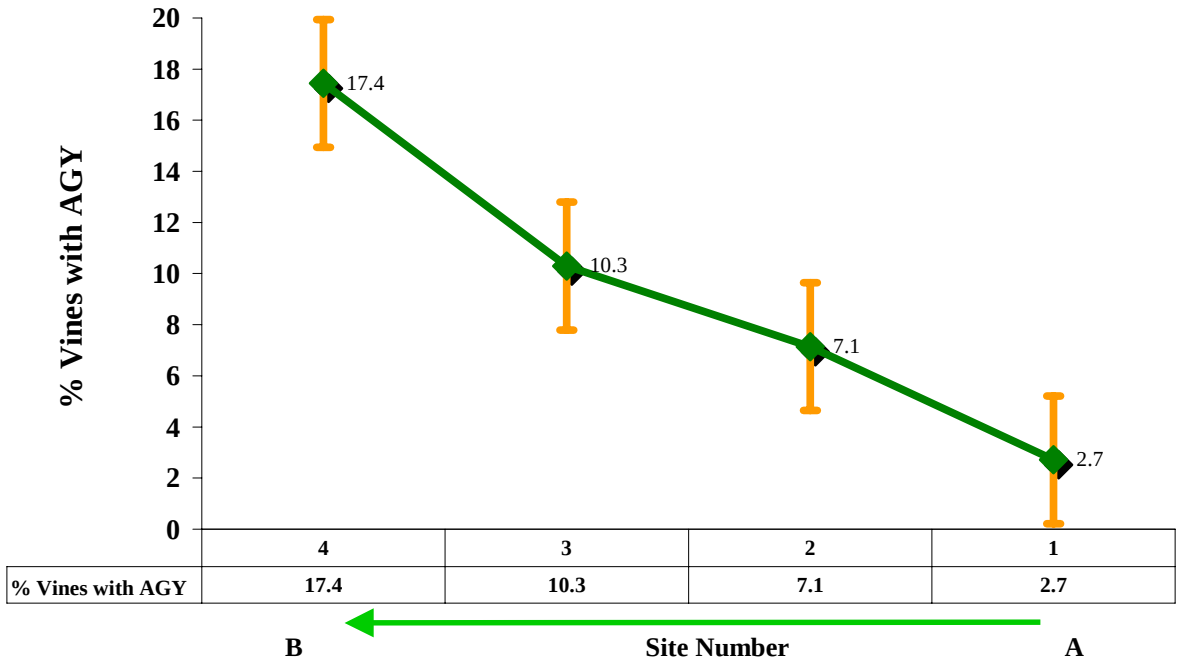



**Figure 11.4:** **The gradient in severity of AGY along a vineyard transect at Griffith, Riverina, NSW, 2002/03. A is furthest away and B is closest to a major canal and adjacent wasteland.**

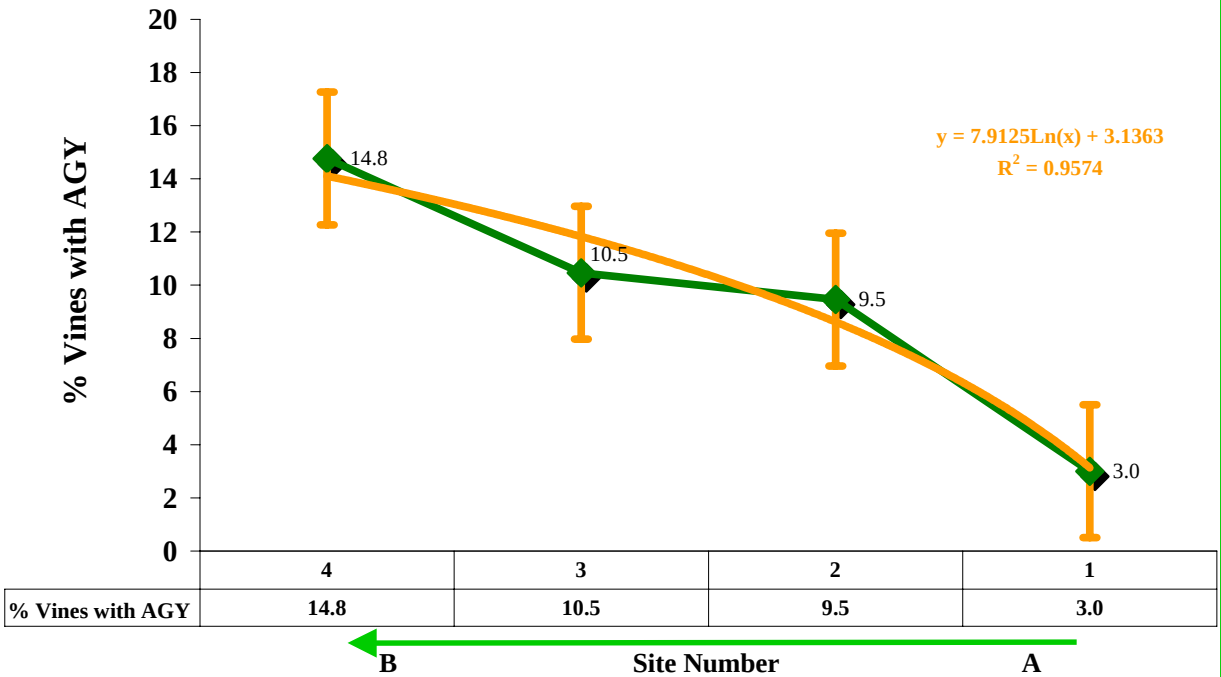



**Figure 11.5:** **The gradient in severity of AGY along a vineyard transect at Gol Gol, Sunraysia, NSW, 2002/03. A is furthest away and B is closest to Gol Gol Creek and adjacent wasteland.**

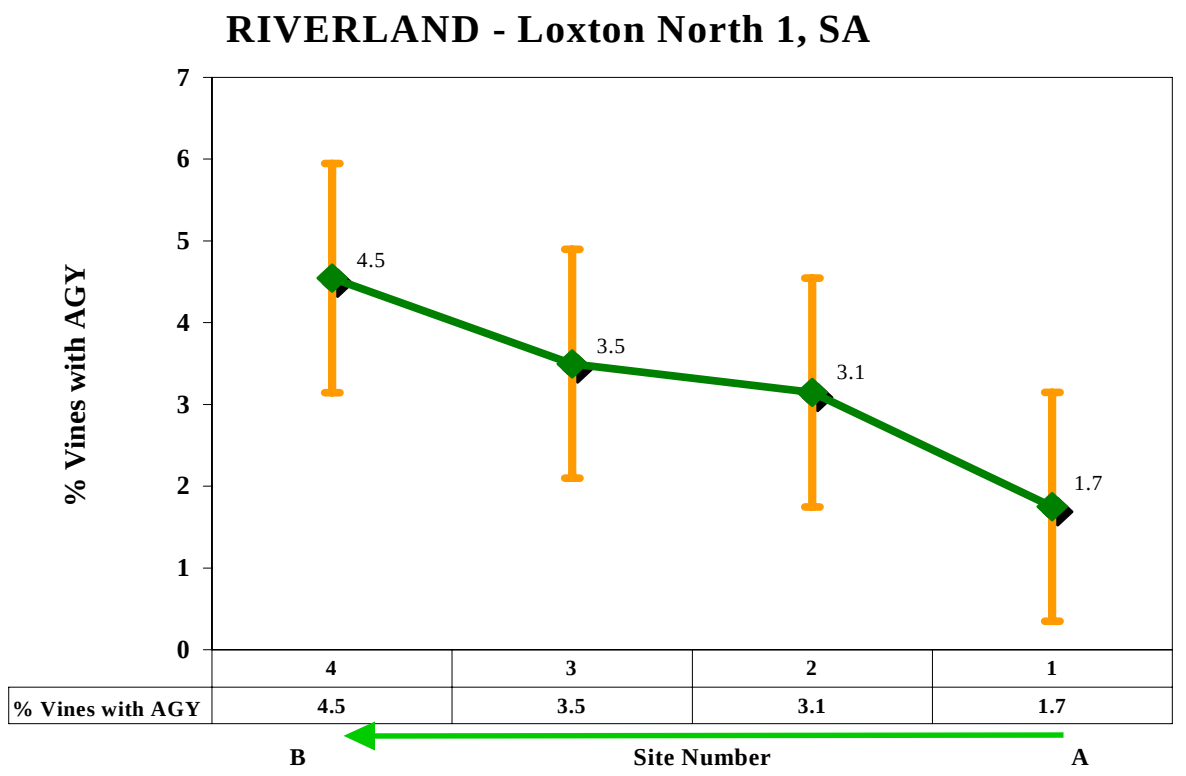



**Figure 11.6:** **The gradient in severity of AGY along a vineyard transect at Site 1, Loxton North, Riverland, SA, 2002/03. A is furthest away and B is closest to Baker's Lake and adjacent wasteland.**

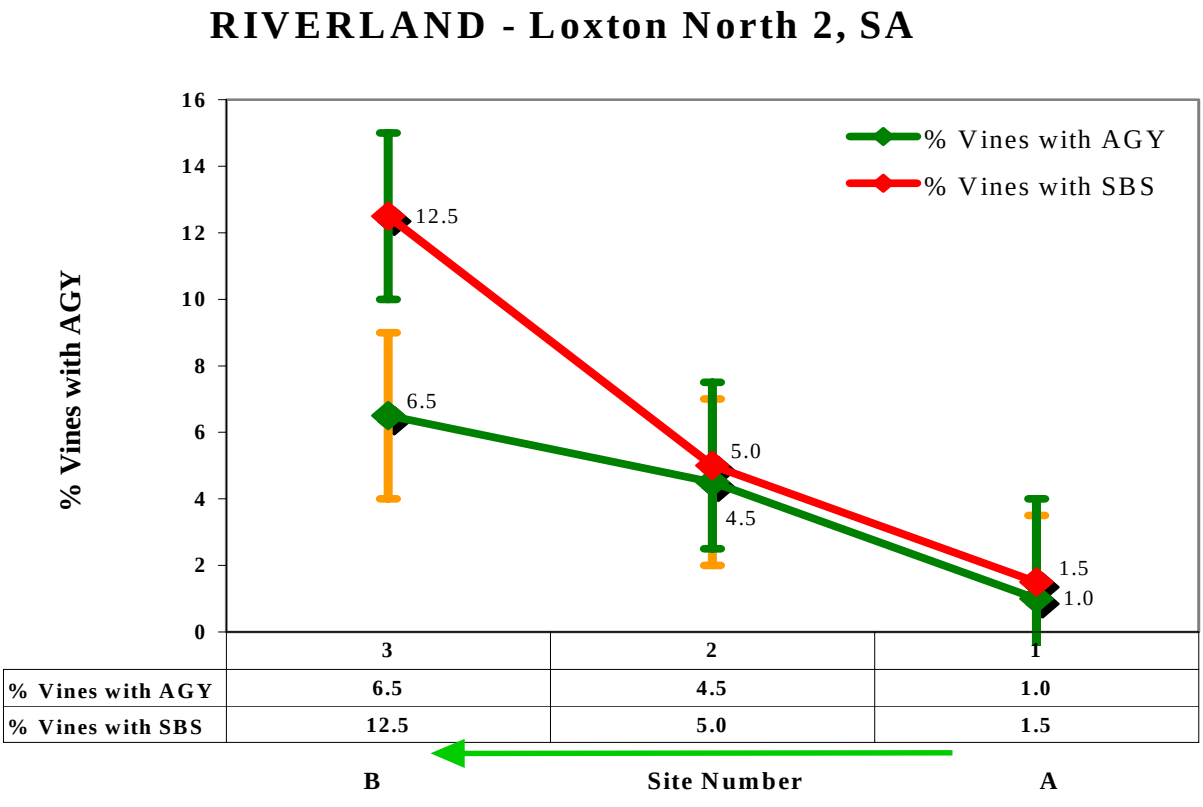



**Figure 11.7:** **The gradient in severity of AGY along a vineyard transect at Site 2, Loxton North, Riverland, SA, 2002/03. A is furthest away and B is closest to Baker's Lake and adjacent wasteland.**

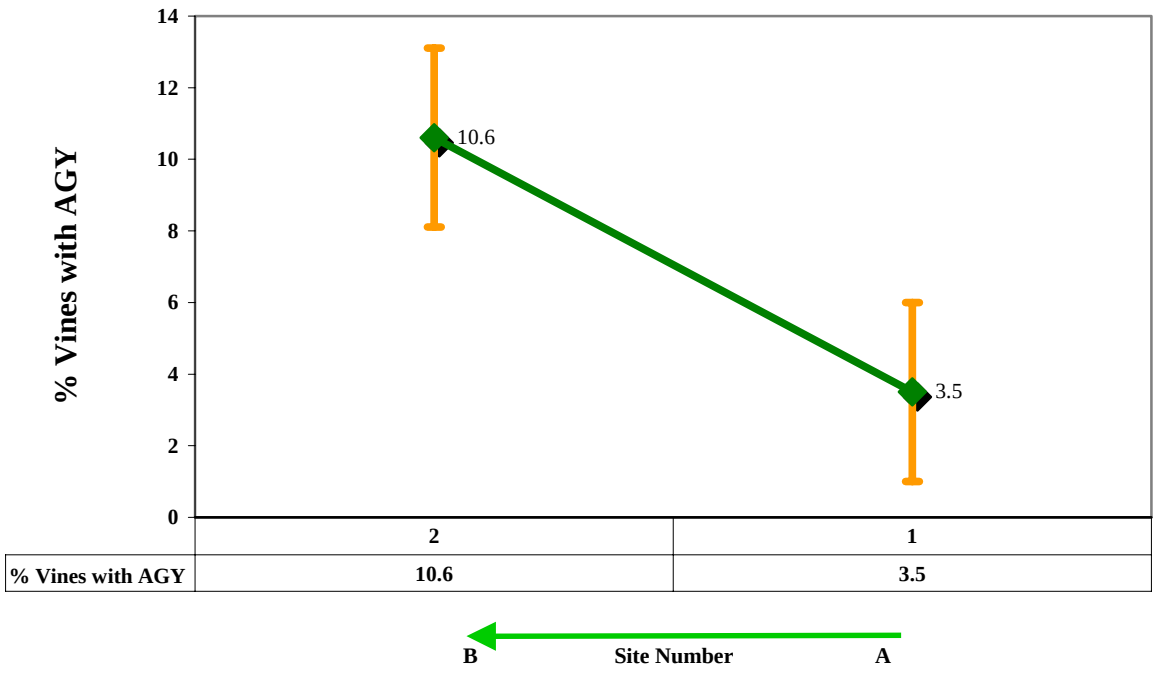



**Figure 11.8:** The gradient in severity of AGY along a vineyard transect at Site 3, Loxton North, Riverland, SA, 2002/03. A is furthest away and B is closest to Baler's Lake and adjacent wasteland.

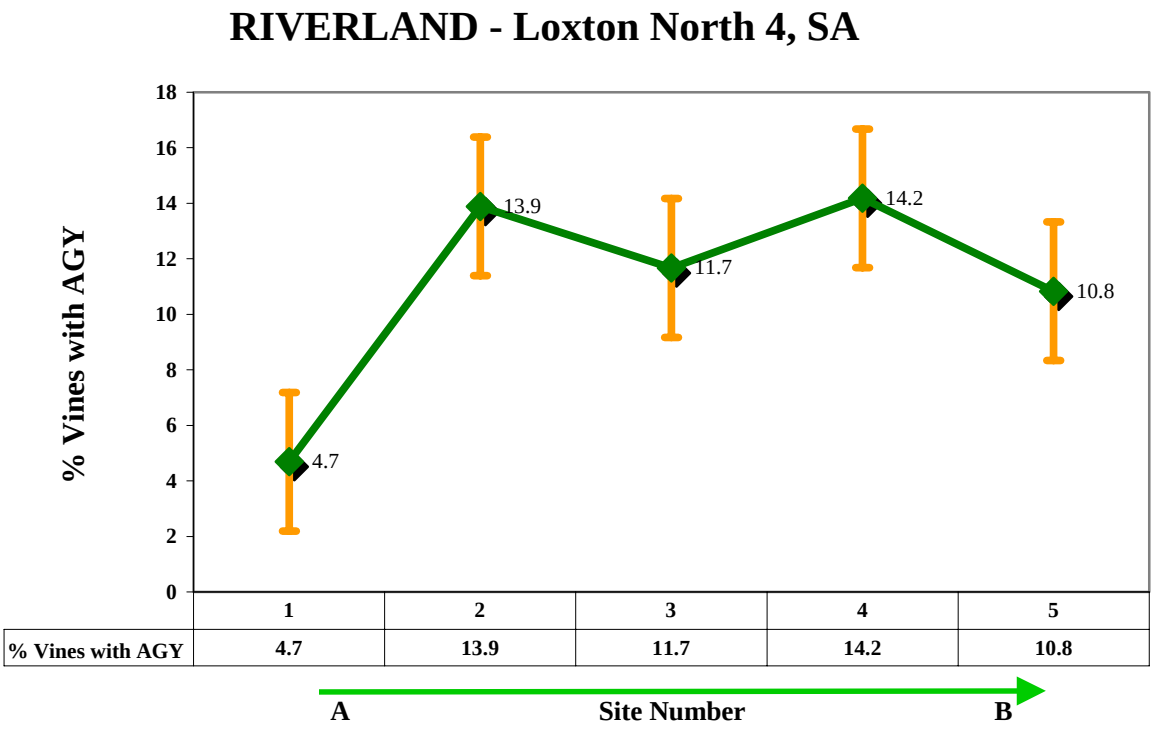
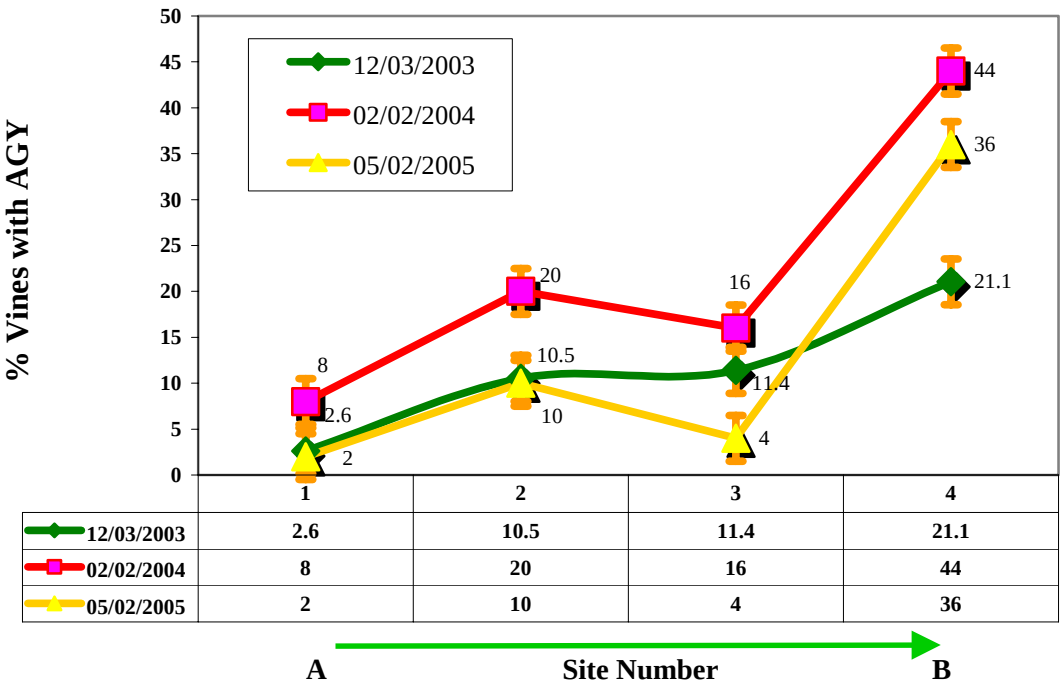



**Figure 11.9:** The gradient in severity of AGY along a vineyard transect at Site 4, Loxton North, Riverland, SA 2002/03. A is furthest away and B is closest to Baker's Lake and adjacent wasteland.




| | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 12/03/2003 | 2.6 | 10.5 | 11.4 | 21.1 |
| 02/02/2004 | 8 | 20 | 16 | 44 |
| 05/02/2005 | 2 | 10 | 4 | 36 |

**Figure 11.10: The gradient in severity of AGY along a vineyard transect at Site 5, Loxton North, Riverland, SA for three consecutive seasons 2002/03 – 2004/05. A is furthest away and B is closest to the drainage basin known as Baker's Lake, and adjacent wasteland.**

## Discussion

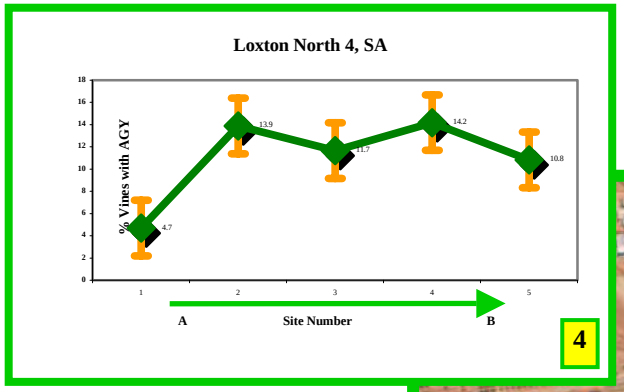
In all survey sites except in the vineyard at Loxton North 1 (Figure 11.6), there was a significant disease gradient ($P<0.05$) from A to B. The Loxton North 1 vineyard showed the same trend as occurred at the other sites but disease severity was (only just) too low to distinguish a difference better than $P< 0.06$.

While disease levels varied from vineyard to vineyard and between regions, the slope of the gradient across an individual vineyard was similar in each transect in the various hot spots for AGY irrespective of the region, the vineyard or the severity of AGY in that vineyard.

In each case, the disease increased toward the location of some form of riverine vegetation ecosystem – either from the River Murray flood plain (Figure 11.3) or from irrigation canals (Figure 11.4 and 11.5) or drainage basins (Figures 11.2 and 11.6 – 11.10).

The transect surveys showed consistent and well-defined gradients of AGY across vineyards located within the previously designated hot spots of disease. Higher levels of AGY occurred on the edges of the vineyard closest to permanent still/shallow water and associated wastelands such as exist in irrigation overflows and similar ecosystems. These areas may be considered zones of 'high inoculum load'.

To assist interpretation of the data, the transect lines at Loxton North, Riverland, SA, have been orientated above an aerial photo. of the site (Figure 11.11). The trend lines point to the centre of that location and would suggest that the inoculum for AGY flows out from the figurative 'hill of disease' at that site – which was at that time, a substantial drainage basin known locally as Baker's Lake (Figure 11.12).



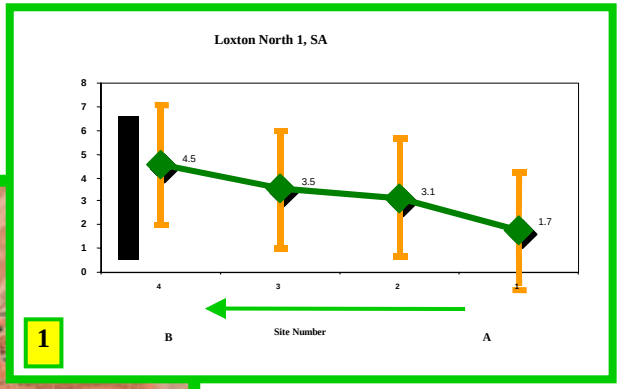
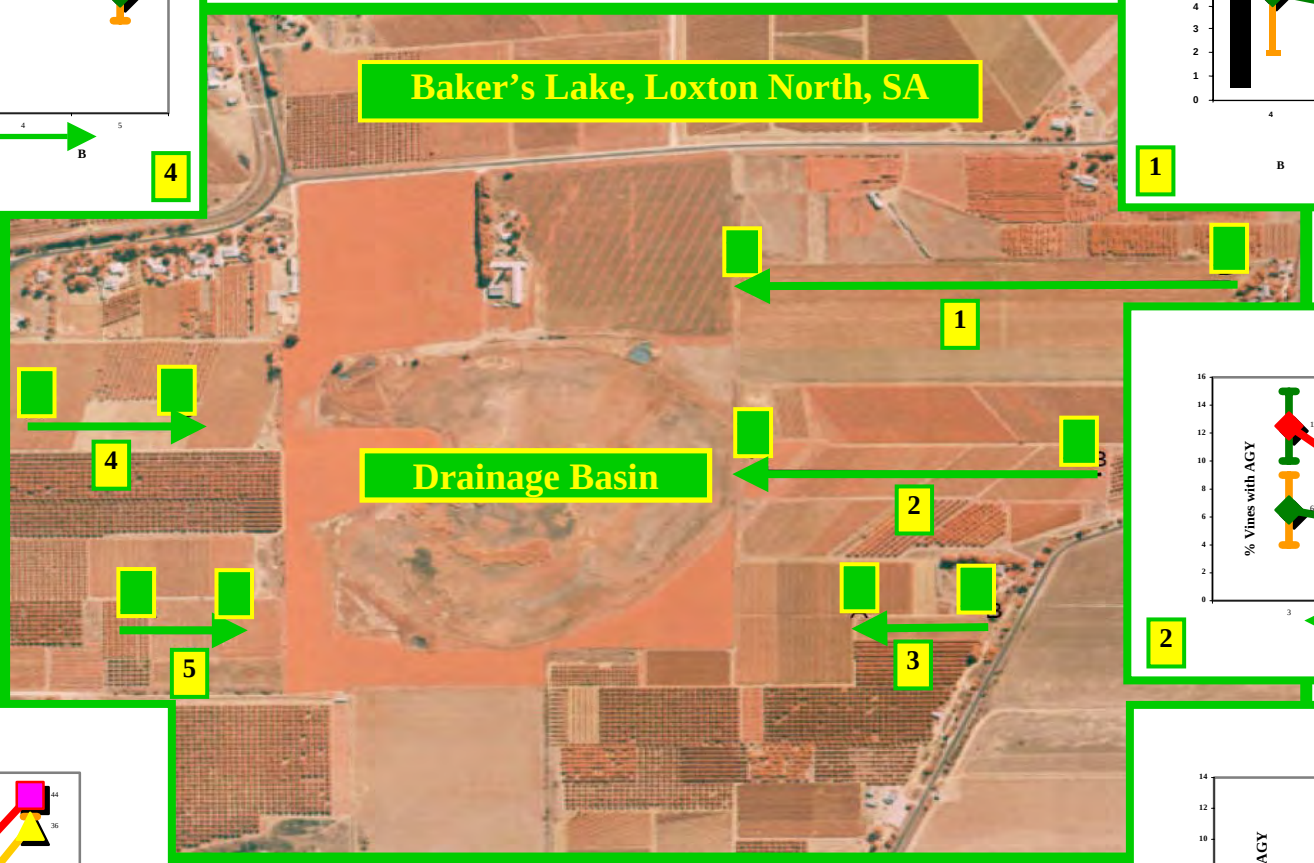
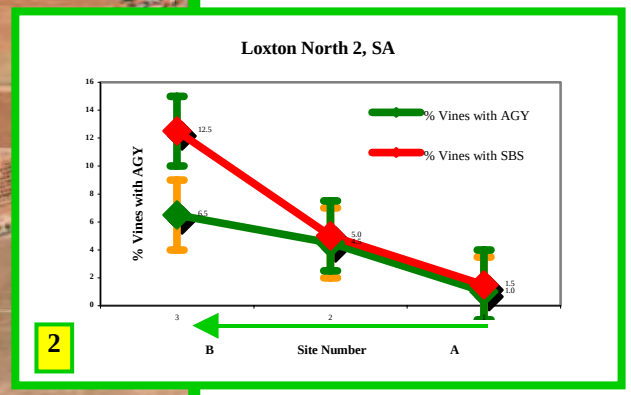
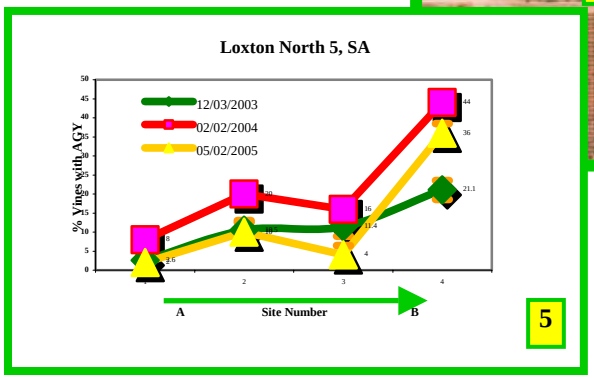
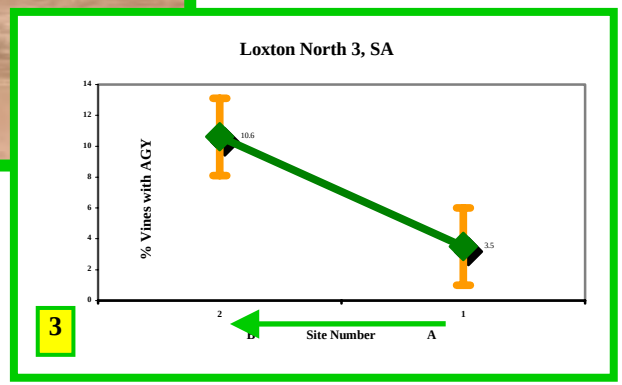




**Figure 11.11: The gradients in severity of AGY along vineyard transcends around a drainage basin (Baker's Lake) at Loxton North, Riverland, SA. All point toward the drainage basin and/or the adjacent wastelands.**



The consistent trend of disease increase suggests that the source of AGY lies in the hill of disease and implicates the vegetation within or in close proximity to these and any other vineyard which exhibits a significant gradient in disease severity. It would suggest that the so-called riverine vegetation environment might contain or comprise the source of AGY. As a result, it seems that the primary plant host of AGY lies within the boundary of the zone we have described above.

**Figure 11.12: A view of Baker's Lake, Loxton North, SA, in 2002, the last year the drainage basin contained water. It was cleared of reeds later that year and has been dry since.**

This finding may appear to contradict the conclusions drawn in Chapter 8 where the random scatter of diseased clumps across vineyards suggested long-distance transport of AGY occurred frequently. However, the above disease gradients were not present unless the vineyard was located in a hot spot for AGY and unless the vineyard was close to some form of riverine ecosystem or un-tilled wasteland surrounding that zone. It was rare for a disease gradient to be present in vineyards adjacent to pure Mallee vegetation. It seemed that the vineyard sites that had been initially surveyed and subsequently analysed for the studies of disease patterns had, fortuitously, never included vineyards sufficiently close to riverine and associated ecosystems. Thus the gradients were not present in the vineyards described in Chapter 8.

As presented above, several hypotheses were developed from evidence collected in 2001/02. One of these was that the plant which we presume is the primary host of AGY, lives within the disease hots spots. The transect surveys of Figures 11.2 – 11.11 present data which support that premise.

In the vineyards surveyed above, infection is most likely to have resulted from short distance transport of inoculum. Often there was a significant decrease in level of disease *eg.* from 17% to 3%, over a distance of only a few hundred metres across a vineyard. The slopes of the gradients infer that the proximal edge of the vineyards (side B) was very close to the source of AGY. It would also suggest that the presumed insect vector did not usually travel or was not usually infective over distances of more than several hundred metres. This would also account for the random clumping of disease in vineyards not in close proximity to the source of AGY.

The gradient of AGY across a vineyard often decreased to a very low level of disease at the distal end of the transect (side A). That level (often ~3% severity) may reflect a background infectivity of AGY from long-distance transmission of disease. This level of severity is similar to that which is evident in vineyards surrounded by other vineyards (see Chapter 12) rather than by riverine vegetation as above.

The strong association between high levels of AGY and the proximity of specific ecosystems such as marshlands and permanent still/shallow water is a significant finding and has focussed investigations seeking the source of disease in Australian vineyards. One benefit will be a reduced input of time and finances required for further investigations to resolve where AGY comes from and how it spreads in vineyards.

A second benefit is to screen native vegetation types as likely the primary hosts that provide the source of inoculum of AGY.

The transect surveys infer that the source of AGY is among the vegetation in and/or near that seen in Figure 11.12. This includes a number of grass species and similar annuals which we excluded in the discussion above. It however includes a number of native species such as common reed (*Phragmites australis*). While technically a grass, this is a perennial. The native plants within the hot spot zones also included about 15–20 plants from Chenopodiacae and similar families *viz.* shrubs such as bluebush (*Maireana spp.*) and saltbush like Ruby saltbush (*Enchylaena tomentosa*). These species warrant further investigation.

While there are many positive correlations of riverine/wetland ecosystem and high severity of AGY that support the hypotheses presented above, there are also some anomalies. For instance, frequently there were both irrigation and drainage channels adjacent to the vineyards in the Riverina but AGY was not at high level near some of these. This suggests that one or more specific plant species or population of plant species was either not sufficiently infected with AGY or was absent in that ecosystem which as a result, was not infective for AGY. Alternatively, the suspect plant host species might need to be preconditioned in some way before it is inoculated and/or before it becomes a source of inoculum for the disease to spread to the neighbouring vineyards.

The transect surveys described above were unidirectional meaning that they followed a single transect line in each vineyard. While these surveys appear to have been highly successful in defining a gradient in disease across the vineyard, a bidirectional survey using a simultaneous East – West and North – South transect line would appear to provide a more accurate (two dimensional) guide to the gradient in AGY severity and could be expected to lead to a refinement of our understanding of the location of the source and means of spread of AGY.

## Conclusion

A number of conclusions arise from this work:

- vineyards in close proximity (300 – 500m) to hot spots of AGY showed a graded pattern of increasing AGY along transect lines oriented toward those hot spots;
- there is a strong association between high levels of AGY and the proximity of specific riverine ecosystems such as swamps, lagoons, drainage basins, creeks, marshlands and permanent still or shallow water;
- permanent shallow water and/or associated wastelands is positively correlated with the disease gradients in the vineyards nearby; and
- it appears that the location of the primary source of AGY inoculum and thus the main plant host(s) for AGY, are located within the zones identified *ie.* within the so-called 'hills of disease' within the hot spots;
- there are a number of native species which are implicated as potential host plants of AGY. These include common reed and bluebush and saltbush species.
- it appears that these hot spots have a maximum dimension of ~600m x ~1,000m encompassing the shallow water and/or the wastelands near by;
- the presumed insect (leafhopper vector) that carries AGY would appear to frequent or arise from the hot spots of disease;
- the presumed vector of AGY appears to usually fly or be infective over only relatively short distances (300 – 800m) from the source of AGY inoculum;
- the hot spot ecosystems show significant potential for further investigations into the source and mechanism of spread of AGY; and
- a bi-directional transect survey (with E-W and N-S lines of survey) would increase our ability to use the vineyard surveys to interpret the disease gradients and as a result, the direction of inoculum flow into the vineyard, thus assisting pinpoint the location of the source of disease.

## Recommendation

It is recommended that:

- the hot spots of AGY be further investigated in pursuit of the source and means of spread of the disease; and that
- more detailed surveying be undertaken of vineyards within the zones of high risk of AGY to better define the disease gradients which, as a result, should provide more precise indicators as to the location of the primary host of AGY within those hots spots.

***Rethinking the hypotheses and getting closer!***

- ***AGY increases the closer the vineyard is to specific sites;***

- ***Swamps, lagoons and wastelands,***
***Reeds, saltbush and bluebush - all are in question;***

- ***One of these plants is probably the primary host of AGY …;***

***… But, which one(s)?***

# Chapter 12: Locating the Source of AGY 5 – refining disease gradients Studies of the spatial distribution of AGY in 2003/04

## Introduction

The surveys in 2002/03 provided evidence in support of the hypothesis redefined in Chapter 11 *ie.* that the primary host of AGY was probably a native perennial located near some vineyards and not others. Consistent disease gradients were found across a number of vineyards in different regions affirming that the source of AGY probably lies close to the edge of vineyards within those hot spots. This work infers that more intensive surveying of disease gradients should be a useful tool to pinpoint the location of the primary host plant(s) for AGY.

Some 15 – 20 native plants were reported as possible primary hosts for AGY including a number of native reed species and shrubs. Many other species (~200 in total) might be involved in the life cycle of disease. The zones which contained these potential sources of AGY had been narrowed within the hot spots to areas in the vicinity of 600m x 1000m.

The above progress was founded principally on detailed transect survey data for only one season. Transect and other surveys during season 2003/04 were therefore again undertaken 1). to determine if the pattern of expression of AGY seen in vineyards during 2002/03 was a usual expression of disease epidemiology; and, if practical, 2). to more precisely determine the location of the suspected source of disease. It was expected that this would assist in better identifying the plants suspected as the source of AGY and it may lead to isolating the supposed leafhopper vector of the disease.

## Aim

**To re-test the possibility that the source (*ie*. the primary host) of AGY grows in riverine swamplands or associated wastelands close to vineyards in zones of high disease.**

## Materials and Methods

In season 2003/04, the vineyard surveys of Chapters 6 to 11 were continued but on a more intensive scale. Point surveys assessed the severity of AGY within 8-30 plots/vineyard in a total of 156 *cv.* Riesling or Chardonnay vineyards: 107 were in the Riverland, 36 in the Riverina and 13 in Sunraysia.

Within most vineyards, for each plot comprising an average of 50 vines/plot, the severity of AGY was scored (as in Chapter 10) and GPS readings were taken. Point surveys were made along two to six transects/vineyard averaging 4-5 plots/transect. The data were entered in MS Access ™ database and plotted and analysed either using MS Excel™ with fitted regression curves, or GIS kreiging software from the Environmental Systems Research Institutes (ESRI) ArcMap 9.1 Spatial Analyst extension (Krivoruchko *et al.* 2004). Kreiging uses the semi-variogram, a function of the distance and direction separating two locations, to quantify the spatial autocorrelation in the data. The semi-variogram is then used to define the weights that determine the contribution of each data point *ie* the scores of the severity of AGY along transects in the vineyard, to predict new values at locations not sampled. It thus produces a predicted surface of values from single data points – their separate scores and the orientation each contributes statistically to the predicted surface. Significant differences in severity scores were presented using different colours for different scores at $P<0.05$.

**Results**

AGY generally occurred at higher severity than in the previous season (see Figures 7.5 – 7.8). The worst affected was a Riesling vineyard in the Riverina in which ~85% of vines showed AGY. This was a young (two year old) vineyard and many vines were severely and systemically affected. Crop loss at that site was estimated by the grower to be ~30% and there was a significant lack of suitable shoot material for the next season's fruitfulness (Figure 2.24).

A representative sample of the data from the point surveys in each region is portrayed in Figures 12.1 – 12.16. Typical examples of the two-dimensional transect surveys are presented in Figures 12.1 and 12.2. Significant ($P<0.05$) gradients in the severity of AGY occurred in vineyards within the hot spots when surveyed in some directions but not others. Kreiging and statistical analyses using the GIS software also showed significant ($P<0.05$) trends within vineyards near riverine/wetland ecosystems but generally not elsewhere. For examples, see Figures 12.7, 12.9, 12.11, 12.15 and 12.16.

The exceptions occurred in relatively small areas of vineyards that were adjacent to some forms of Mallee vegetation (Figure 12.10).

A typical gradient in the severity of AGY is presented for Gol Gol North, NSW, as a representative example from Sunraysia (Figure 12.1). This site will be used as a case study to illustrate trends seen consistently at other sites across the regions.

The survey for the Gol Gol vineyard comprised eight transects along a south-north grid and five across an east-west grid. The south-north transects showed a significant ($P<0.05$) progression of increased severity in AGY toward the north end (Figure 12.1). The fitted logarithmic regression line ($R^2 = 0.7045$) showed disease increased from the southern end (**A**) (9% severity) to the northern end (**B**) (23% severity). In contrast, the east-west transects in the same vineyard showed a trend of disease increase that was barely significant (at $P<0.05$) from the eastern edge (15% severity) to the western edge of the vineyard (20%) (Figure 12.3). The fitted logarithmic regression gave an $R^2 = 0.5126$.

Higher levels of AGY occurred in the parts of vineyards nearest native vegetation. This was observed at hot spot sites in Sunraysia and in the Riverland at Winkie (Puddletown), Eckert's Creek and Loxton North (Baker's Lake). The GIS analyses (kreiging) showed that the likely flow of inoculum is sourced in the localities adjacent to the vineyard sectors with highest levels of disease. The disease scores for AGY in a number of vineyards near a swamp (natural drainage basin) near Winkie, SA, known locally as Puddletown. The vineyards closest to the swamp showed highest levels of disease, except for a single site within one vineyard at the upper right-hand corner of Figure 12.6 - That part of the vineyard was bounded by irrigated lucerne and native scrublands.

In other surveys, vineyard sites near some native vegetation have higher severity at one end of the vineyard (Figure 12.11) while vineyards distant from native vegetation and surrounded only by other vineyards show low levels of AGY (Figure 12.13).

This pattern was repeated in other locations and in different regions surveyed *viz.* the Riverland, Riverina and Sunraysia. Figures 12.8 – 12.16 show further examples of this consistent trend.

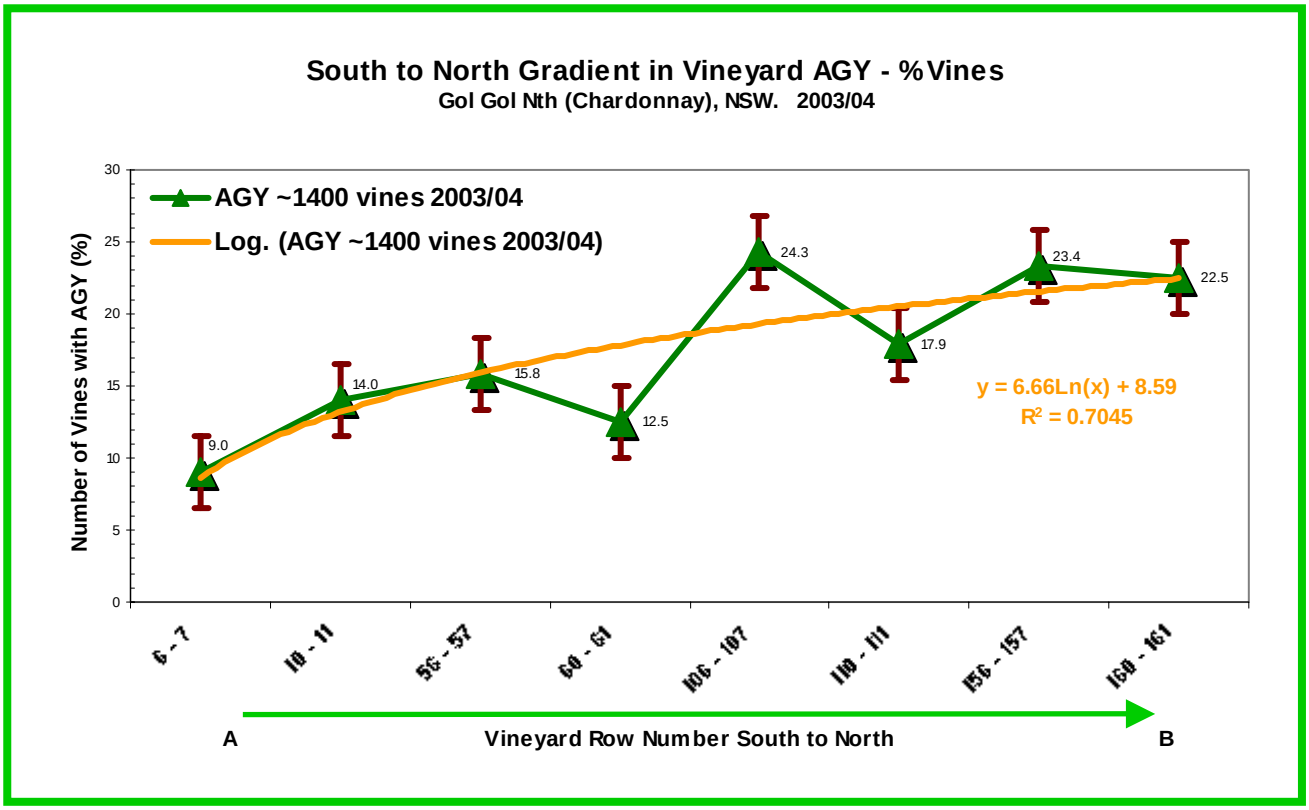



**Figure 12.1: The gradient in severity of AGY along a south-north vineyard transect at Gol Gol Nth, NSW, 2003/04. A is adjacent to vineyards; B is closest to Gol Gol Creek and wastelands (see Figure 12.4).**

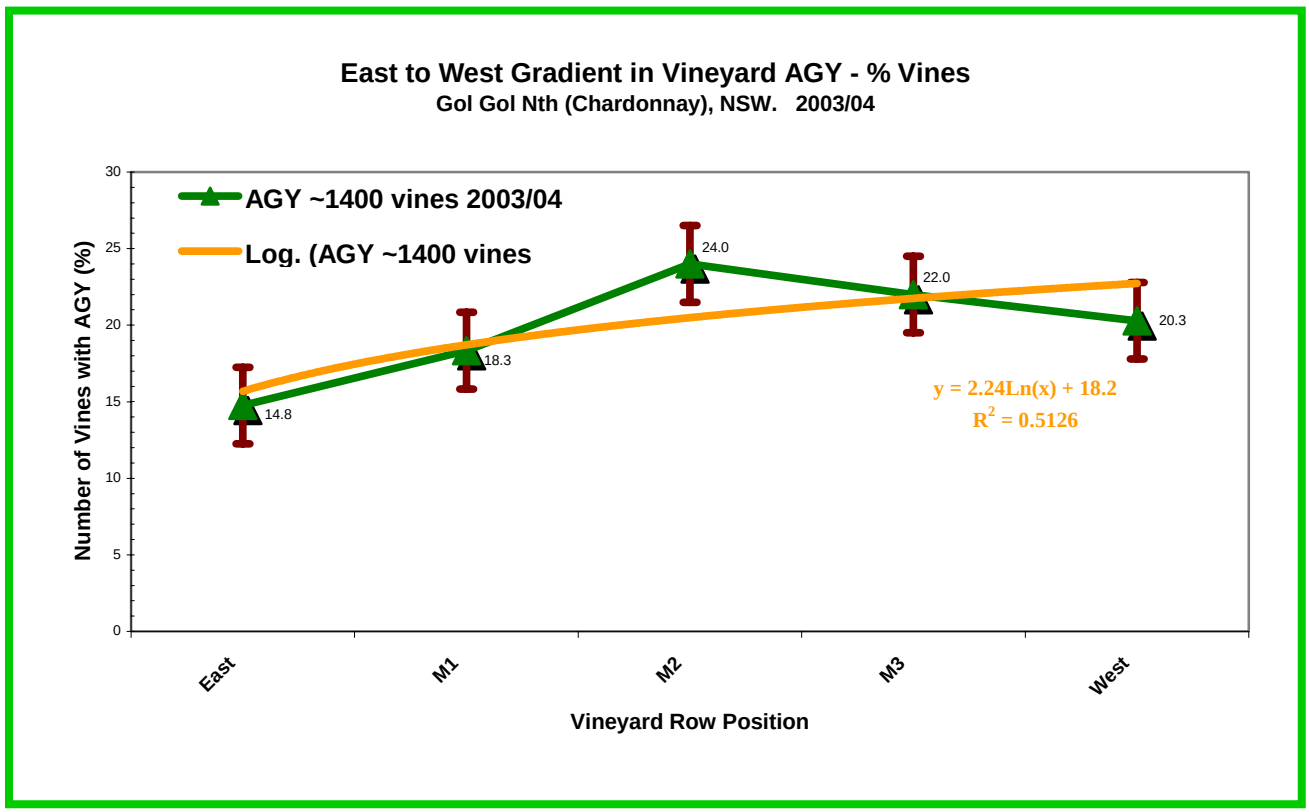



**Figure 12.2: The gradient in severity of AGY along an East-West vineyard transect at Gol Gol Nth, NSW, 2003/04. Other vineyards are adjacent at the eastern and western ends.**

## Discussion

The high level of AGY in one Riesling vineyard in the Riverina (with 85% vines uniformly affected, often systemically) was of considerable concern because that vineyard was only two years old. As a result, it was probable that the vineyard was subject to very high inoculum loads in its first season of growth. The environment surrounding that vineyard provides a significant site at which to investigate the source of inoculum and to locate the vector of AGY, since both are likely to be in very high number there.

Key findings of the surveys in each region generally affirmed the observations made in 2002/03 with regard to the location and occurrence of hot spots of AGY. This was also true for the location and nature of disease gradients across vineyards. The analyses of the more intensive survey data collected in 2003/04 than in 2002/03, showed finer variations in AGY gradients for each vineyard and these showed some points of interest (see below).

**Hot spots of AGY**:

- as an example, Figure 12.3 shows zones of high and low severity of AGY across different localities in the Riverina, NSW. This was typical of the zones of disease seen in previous seasons, for example, in the Riverland (Figure 9.1).
- the consistent response observed over two seasons suggests that localities across regions can be rated for risk of AGY according to characteristics of the zone in which the Riesling and Chardonnay vineyard is located;
- each locality can be classified as follows:
    - **high severity**: when vineyards are adjacent to or within ~500m of riverine/wetland ecosystems;
    - **medium severity**: when vineyards are at greater than 500 – 1,000m distance from riverine/wetland ecosystems and/or adjacent to some atypical Mallee vegetation (not yet well-defined); and
    - **low severity**: when vineyards are surrounded by other vineyards and/or in vineyards surrounded by typical highland Mallee vegetation.

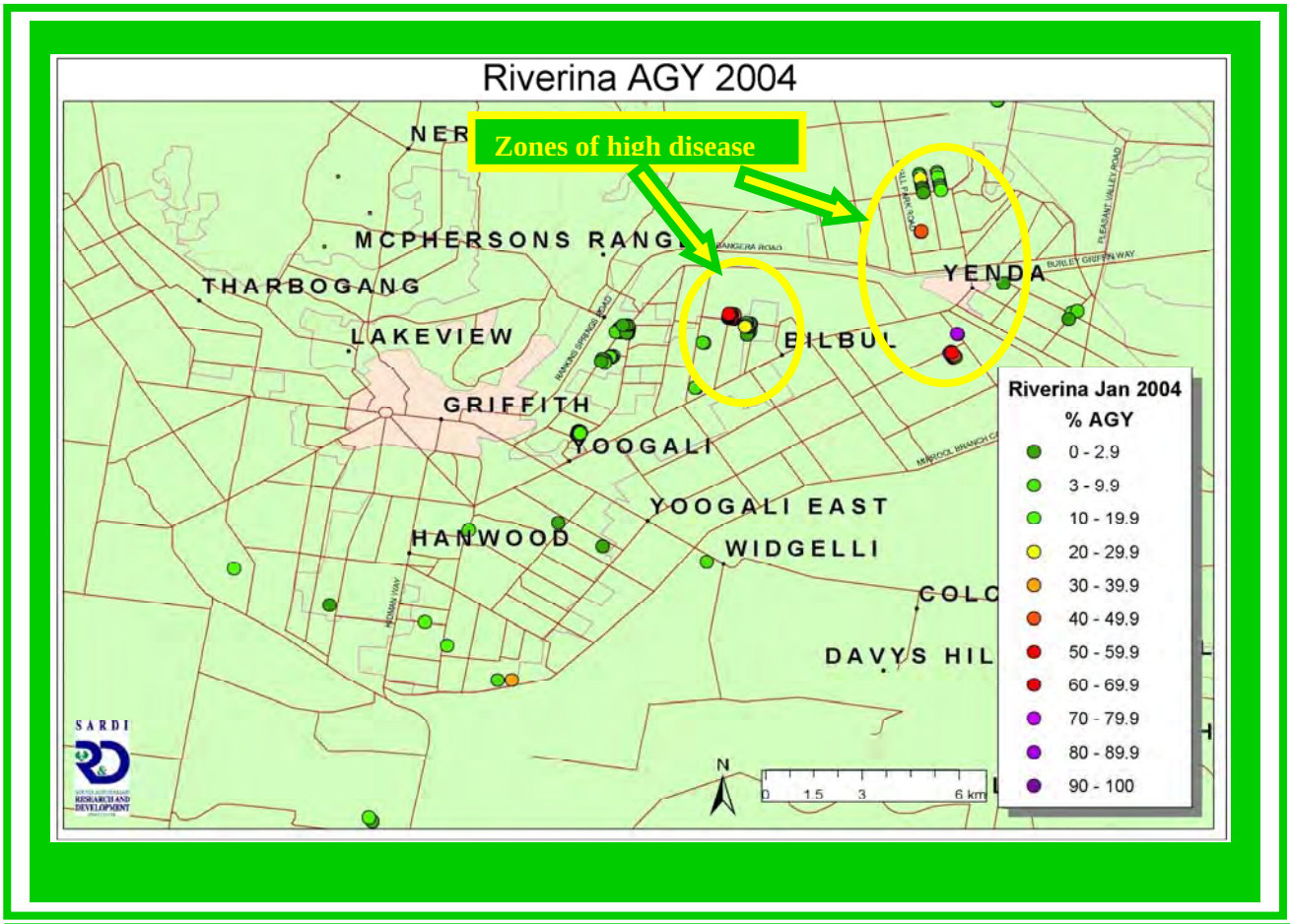



**Figure 12.3: Hot spots of AGY occur in various regions, typically near wetland/riverine ecosystems and adjacent wastelands like these at Yenda and Bilbul, near Griffith, Riverina, NSW. 2003/04.**

**Disease gradients** These were observed across the regions in 2003/04 in the same pattern as observed in 2002/03.

Since the survey of the Gol Gol vineyard in 2002/03 showed a disease gradient toward the northern end (Figure 11.5) and the more intensive two directional transect survey in 2003/04 (Figures 12.1 & 12.2) showed only a uni-directional gradient toward that end, we are confident this slope points to where the source of AGY is located for that vineyard.

Additional examples of survey data with similar disease gradients are shown for Riverland vineyards in Figures 12.6 – 12.16 with trends ($P<0.05$) in the severity of AGY occurring across vineyards located within disease hot spots in Winkie, Eckert's Creek and Loxton North. Patterns of disease there were typical of the Gol Gol case study and provided evidence that the source of AGY lies close to those vineyards also.

The southern end of the Gol Gol vineyard was surrounded by other vineyards while the northern end was ~500m from Gol Gol Creek (Figure 12.4 a) which has a permanent supply of water for irrigation. Along the creek banks there was a wasteland of native vegetation comprising various species which consisted predominantly of various saltbush and bluebush on the edges of the creek and common reed at the water's edge (Figure 12.4 a -c).


**Figure12.4a: A steady increase in severity of AGY occurred along the south-north (A → B) vineyard transect at Gol Gol North, NSW, in 2003/04 (see Figures 11.5 and 12.2). At the north end is Gol Gol Creek. The vineyard at C is regularly and severely affected by AGY (see Figure 7.7).**

**Figure 12.4b: A view of Gol Gol Creek shows the predominant lower story species are reeds and various chenopod shrubs including yanga (bluebush) and saltbush.**


**Figure 12.4c:** Yanga (bluebush) (A) and cumbungi (bullrush) (B) occurred frequently in the riverine and/or wetland vegetation that predominates in the AGY hot spots identified in the surveys of 2002/03 and 2003/04.

To compare data from the Gol Gol vineyard for 2002/03, the scores from the eight transects in 2003/04 were combined to present an average equivalent to the four transects of 2002/03. The re-plot in Figure 12.5 can then be compared with Figure 11.5. In 2002/03, the severity of AGY at the northern end of transect was 5-times more ($P<0.05$) than at the southern end. In 2003/04, the average for the vineyard (19%) was nearly double that of the first season (10%) but although the difference in severity of AGY between the northern and southern ends was again significant ($P<0.05$), in the second season, levels at the north end were only 2.5-times those at the south.

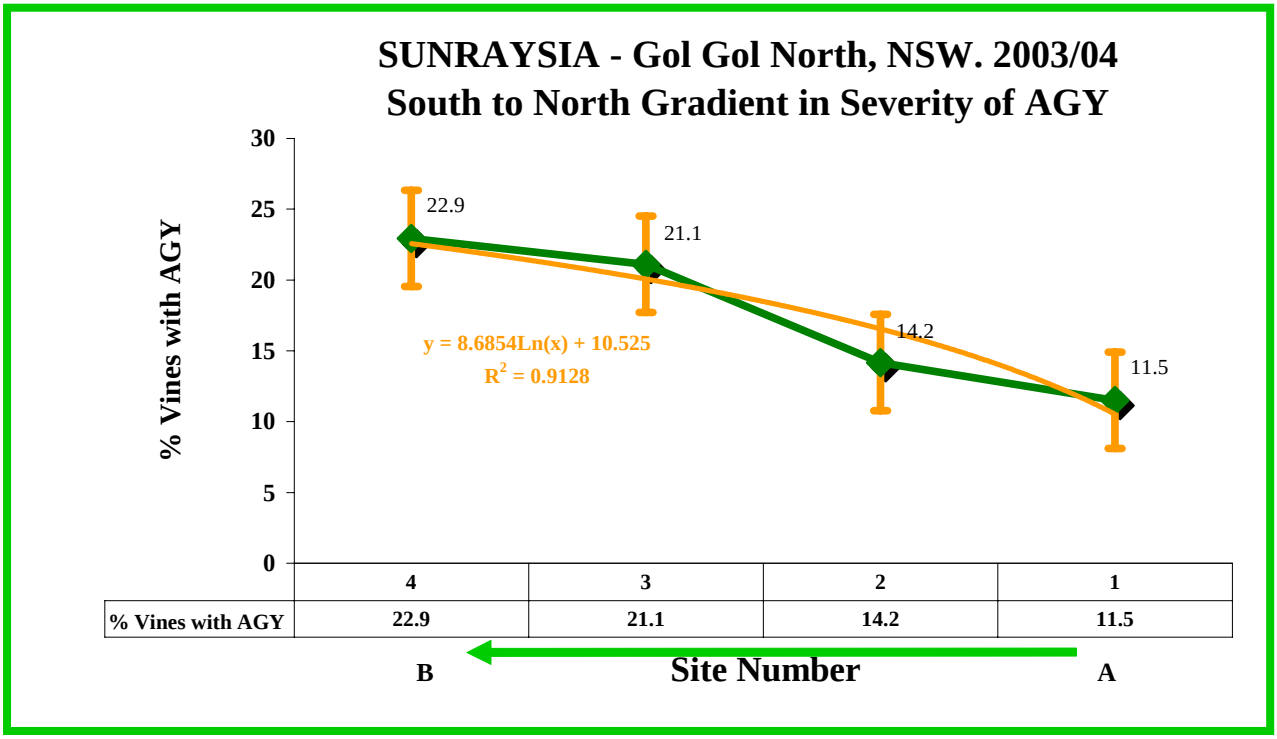



**Figure 12.5:** The gradient in severity of AGY along a south-north vineyard transect at Gol Gol Nth, NSW, 2003/04 re-plotted as a 'four-transect average' from 12.2 for comparison with **Figure 11.5.**

Closer analysis of data from this vineyard showed that the southern end expressed a three-fold increase in severity of AGY in 2003/04 compared to 2002/03 while closest to the creek *viz.* the northern end, showed a two-fold increase. If this finding holds true for other seasons and other vineyards, it would infer that in the second season, even though the inoculum flow across the vineyard in general increased, the distance of spread from its presumed point of origin at the northern end increased proportionately more.

Evidence with other leafhopper-vectored diseases overseas, suggests that disease severity is directly related to the frequency of feeding attempts by infective insects (Maixner 2006). The pattern of disease observed in 2003/04 thus suggests that the disease agent was more mobile in the second season increasing disease in sectors of the vineyard more distant from the northern end *ie* more distant from its source. Consequentially, the difference between the two seasons might be due to factors such as the increased flight distance of the (presumed) leafhopper vector rather than an increased frequency of flight numbers.

This possibility should be further tested using data from the other transect surveys undertaken in the present project. An understanding of the flight patterns and flight distances of the presumed leafhopper vector should provide sound basis for determining the factors that favour movement of inoculum into the vineyard, hence the factors that lead to the severity of disease expressed from season to season (see Chapters 7 and 8). Environmental conditions such as prevailing temperature are likely to play a significant role in insect activity and infectivity. Because disease gradients across vineyards occur in relation to their orientation to riverine vegetation irrespective of which geographic orientation, wind direction is unlikely to be a factor.

Given that AGY does not spread from vine to vine (Chapter 8) and that the vineyards adjoining the Gol Gol site at the southern end separated that part of the site from possible inoculum sources in adjacent native vegetation, the lower level of AGY at that end was to be expected. This held true also for the western boundary of the site which was surrounded by other vineyards while the eastern boundary was adjacent to a grassland paddock (12.4a).

The survey data from 2003/04 provide further evidence that the source of AGY is located in the vegetation displayed in Figures 12.4, 12.10 and 12.12. These show the ecosystem adjacent to higher levels of AGY in vineyards at Gol Gol and at Winkie and Eckert's Creek in the Riverland, SA, respectively. The main species of native plant present at these sites were common reed and the chenopod shrubs *eg* saltbush and bluebush but others present included the bulrush and several species of sedge. It is likely that one or more of these species or of others nearby are the primary host plant(s) for AGY.

This finding further supports the hypothesis (Chapter 11) that AGY is native to Australasia, that the pathogen is resident in native plants and that these hosts occur in some form more prevalent or grow more favourably at locations external to vineyards but within a ~900m radius of permanent still/shallow water.

As a result of this work, the zones which harbour the source of primary inoculum are likely to comprise areas as small as ~100m x 50m and the number of native plant species as candidate primary hosts of AGY has been reduced from 100's to a relatively few (15-20) species. Their location has been delimited to specific zones which are typified by untilled land with or without the presence of permanent or near permanent shallow water.

On rare occasions, dry-land Mallee vegetation also appears to provide a source of AGY. For example, see Figures 12.11 & 12.12.

To test the veracity of and to refine these findings further, resurveys of hot spots of disease for a third season and a search for AGY-infected native plant hosts is required. This should reduce the number of plants suspected as primary host of AGY to a more manageable level and ultimately lead to the finding of the supposed leafhopper vector(s) of AGY.

**Conclusion**

The investigations of hot spots of AGY and of disease gradients within vineyards during 2003/04 showed that:

- the source of AGY is confined to hot spot zones as small as ~100m x ~50m;
- there is a strong positive association between high levels of AGY and proximity (<500m) to native vegetation comprising riverine and/or wetland ecosystems;
- there is lesser correlation between levels of AGY and some forms of dry-land Mallee vegetation;
- vineyards within 500 – 1,000m of riverine and/or wetland vegetation had moderate risk of AGY; whereas
- the presence of vineyards around another means that vineyard is likely to have only low levels of AGY;
- AGY is a disease of vineyards on the margins of viticulture. In other words, vineyards that are bounded by other vineyards have little risk of AGY and, to the contrary, vineyards on the interface between the viticultural region and riverine and/or wetland vegetation, have a much higher risk of significant levels of AGY;
- The number of native plant species that are considered possible primary hosts of AGY was now limited to at most 15-20 species. The main plants in question were the chenopod shrubs yanga (short-leafed bluebush, *Maireana brevifolia*) and ruby saltbush (*Enchylaena tomentosa*) among others, the grass species, common reed (*Phragmites australis*) and several species of sedge.

**Recommendation**

The evidence from studies up to and including 2003/04 suggests that:

- the detailed vineyard surveys should be continued for a third season to confirm the specific disease gradients found in vineyards adjacent to riverine and/or wetland ecosystems and importantly, in attempt to reduce the number of candidate primary hosts;
- investigation of the suspect native vegetation at those localities should be focussed on common reed and selected Chenopod species such as bluebush and saltbush with view to finding the primary host of AGY;
- investigation of the suspect leafhopper vector of AGY should be focussed in the same location as the primary host plant (as above) – indeed, on that very host (once located); and that
- investigation be made of the location of the highly diseased *cv* Riesling vineyard near Griffith in the Riverina, in attempt to locate both the primary host and the leafhopper vector at that location.

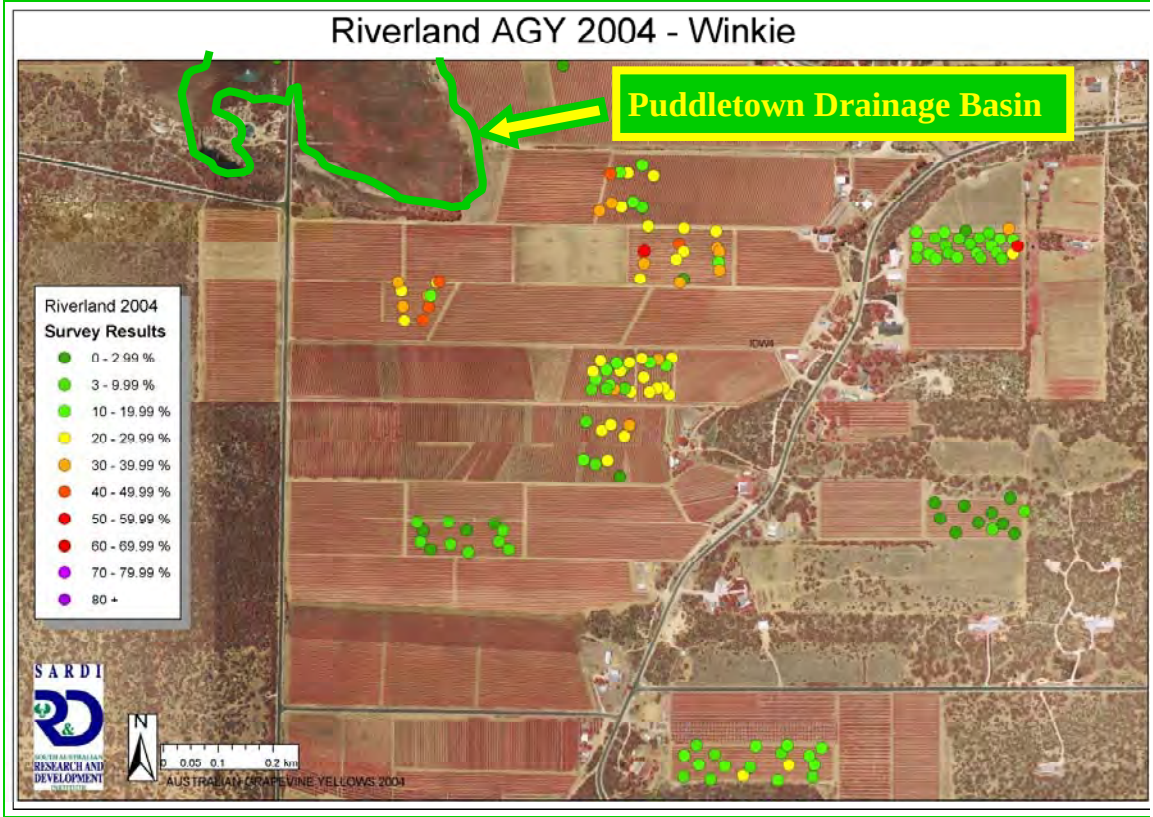



**Figure 12.6:** **Point-survey scores for severity of AGY in vineyards at Puddletown, near Winkie, SA. 2003/04.**

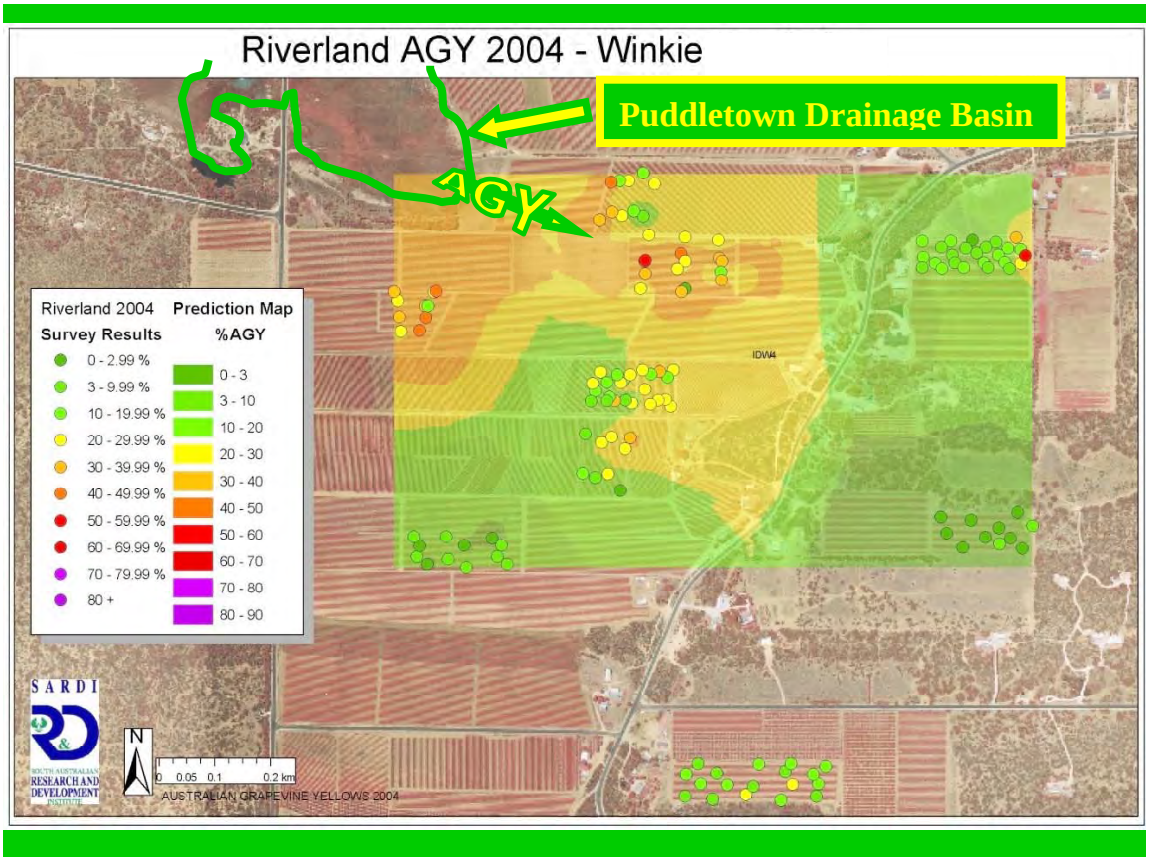



**Figure 12.7:** **Kreiging indicated that zones of high AGY lay near Puddletown basin at Winkie, SA, 2003/04, and suggested that the flow of AGY inoculum was from there into the vineyards.**

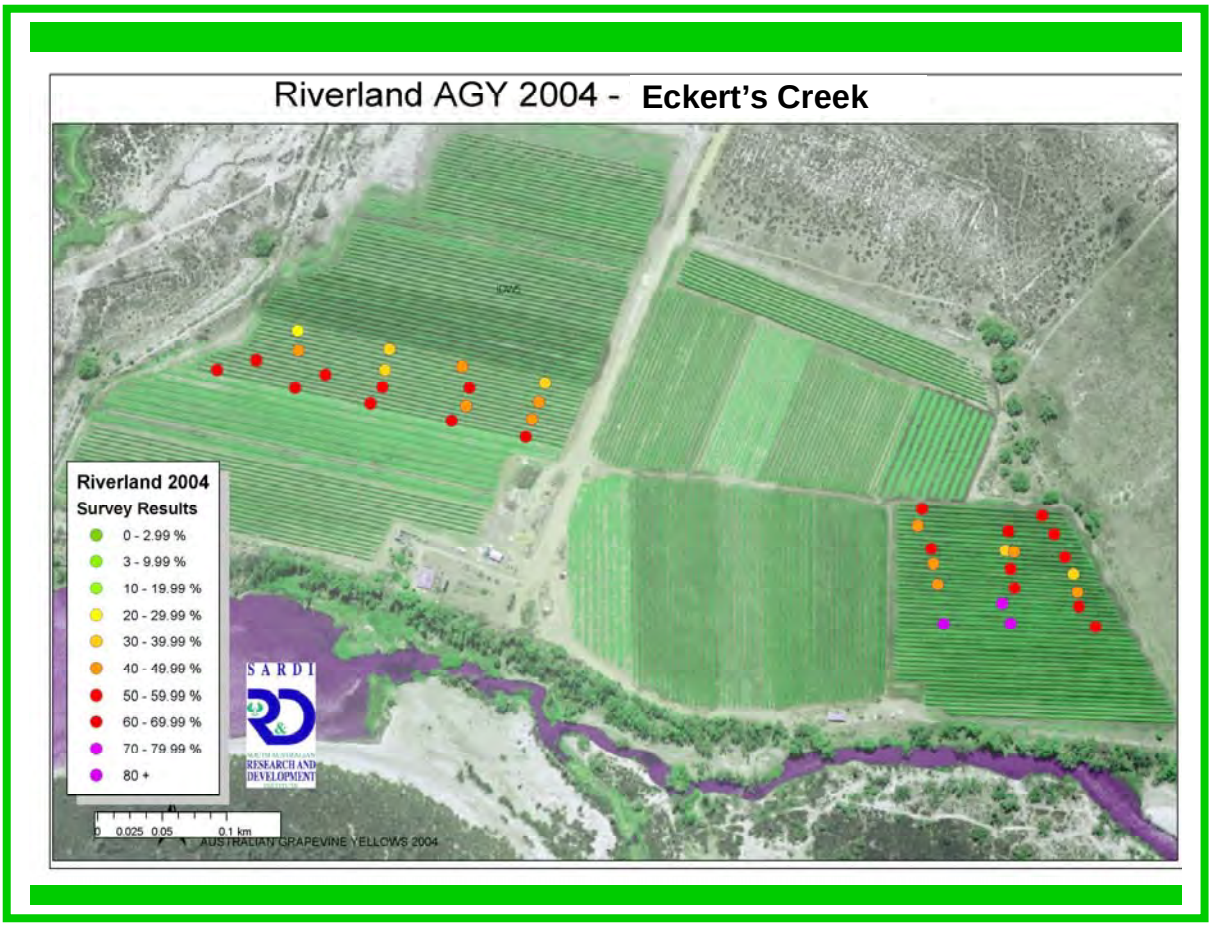



**Figure 12.8:** **Point-survey scores for severity of AGY in vineyards near Eckert's Creek, SA. 2003/04.**

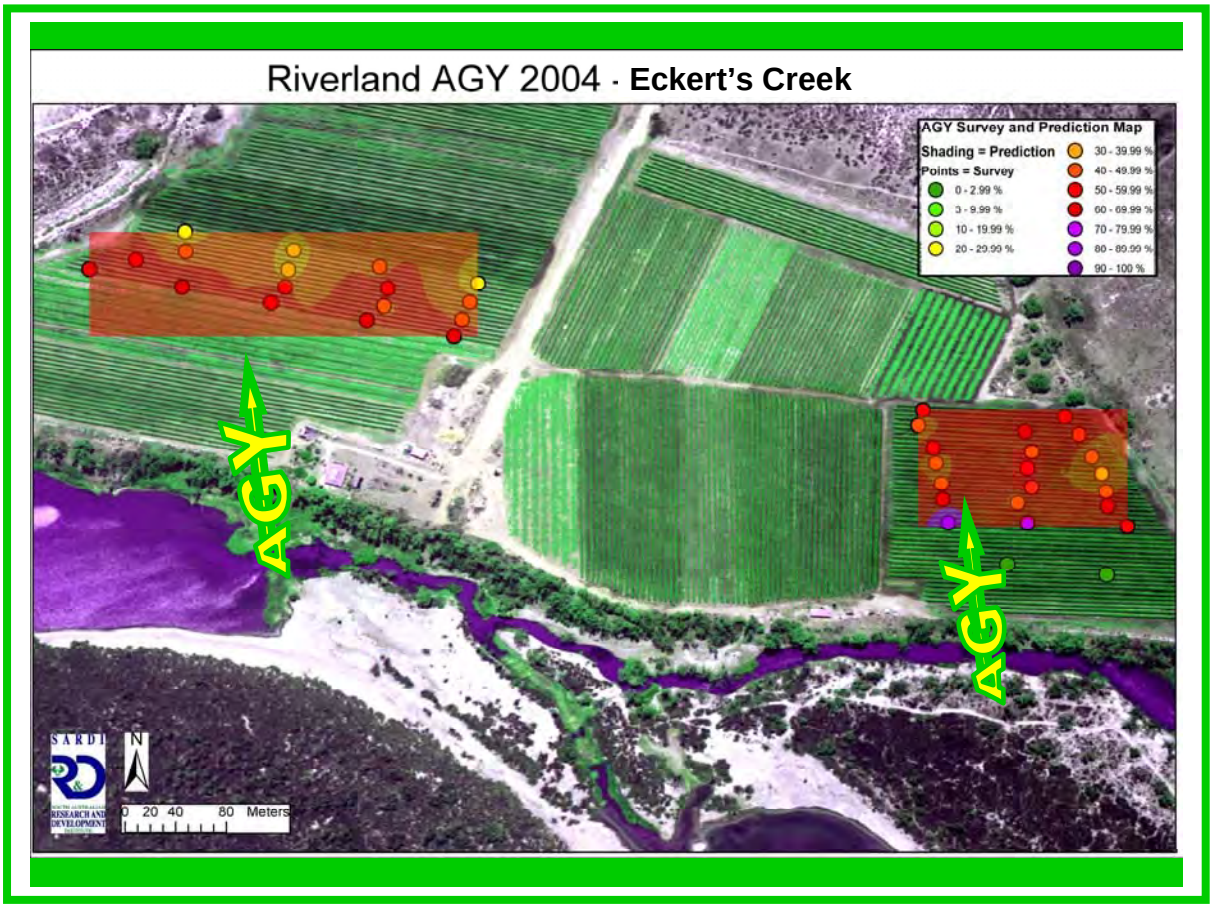



**Figure 12.9:** **Kreiging indicated that zones of high AGY lay near Eckert's Creek, SA, in 2003/04 and suggested that the flow of AGY inoculum is from there into the adjacent vineyards.**

**Figure 12.10: The riverine/wetland ecosystem typically associated with high AGY in adjacent vineyards, here at Eckert's Creek, Riverland, SA. 2003/04.**


**Figure 12.11: Kreiging indicated zones of high AGY lay near some forms of Mallee vegetation (like at A - see Figure 12.12) near Winkie, Riverland, SA, 2003/04, but not in others (B).**

**Figure 12.12: The vegetation, possibly including the primary host of AGY, adjacent to a small hot spot of AGY near Winkie, Riverland, SA – see Figure 12.11.**

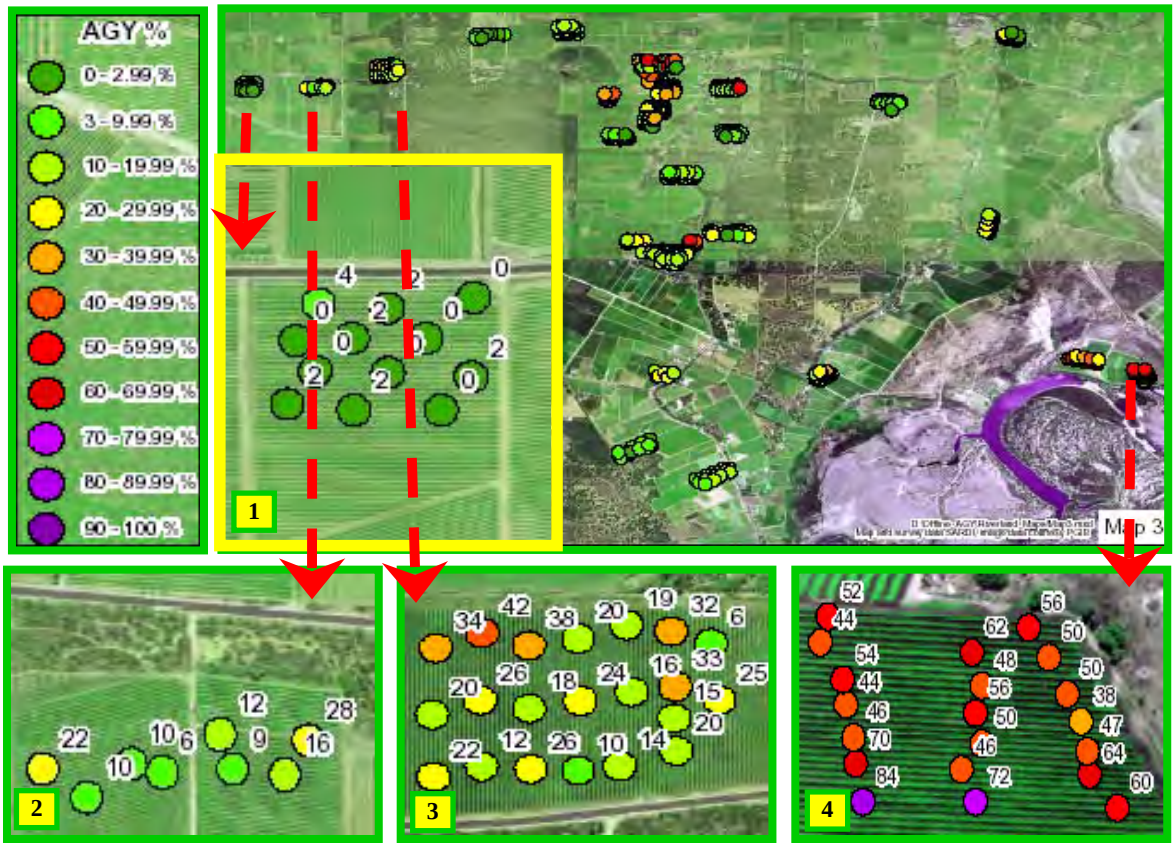



**Figure 12.13: Analyses showed AGY levels are: low in vineyards surrounded by other vineyards 1; higher near some types of native vegetation 2 & 3; and highest near riverine and/or wetland ecosystems 4. Data are for vineyards near Glossop and Berri, SA, in 2003/04.**

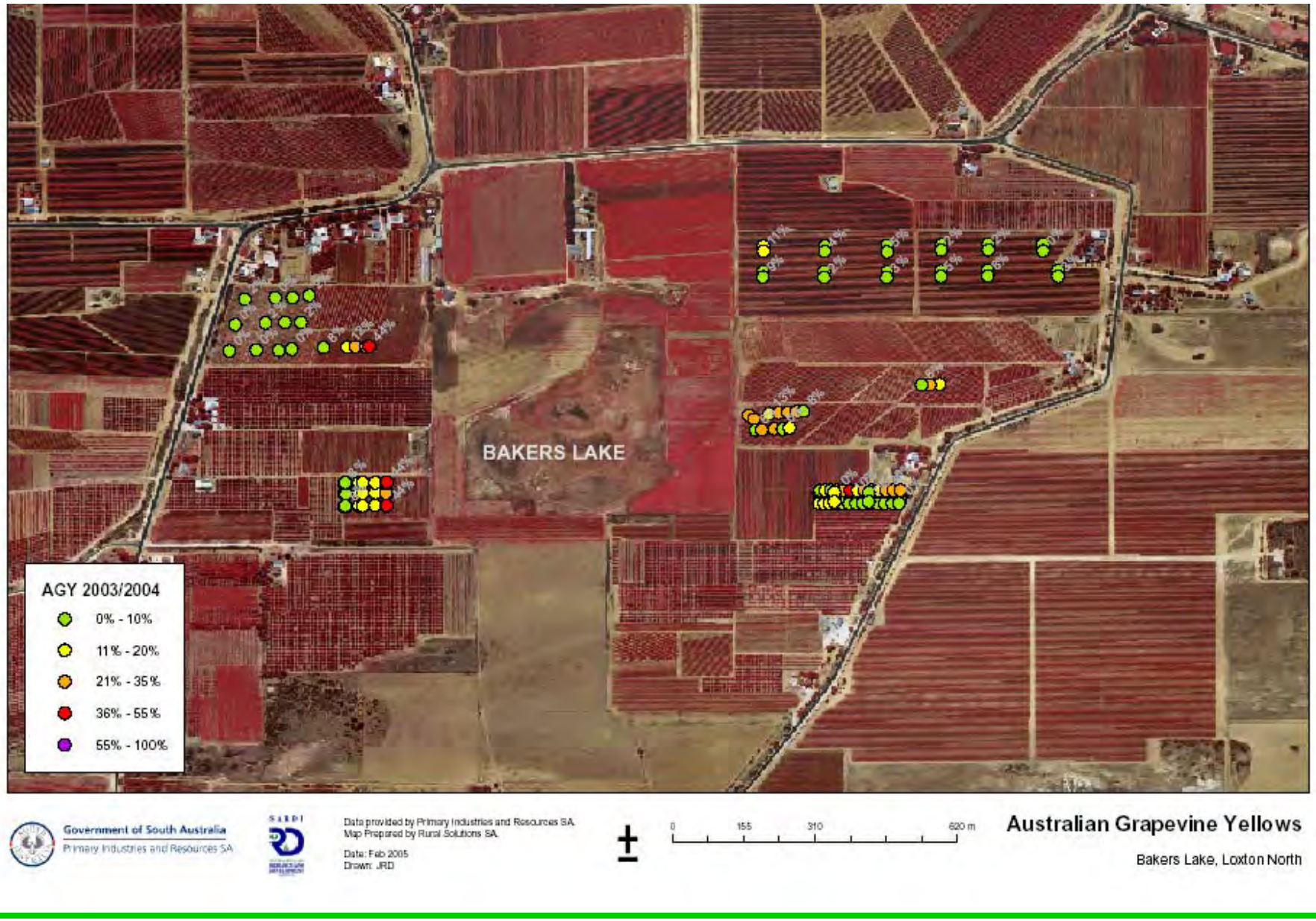



**Figure 12.14:** **Point survey data for vineyards adjacent to Baker's Lake, Loxton North, SA. 2003/04.**


**Figure 12.15:** **GIS analyses of point survey data shows a zone of high AGY to the left of this figure *ie.* within or near Baker's Lake, Loxton North, SA. 2003/04**


**Figure 12.16:** GIS analyses of point survey data shows a zone of high AGY to the right of this figure *ie.* within or near Baker's Lake, Loxton North, SA. 2003/04.

***Narrowing the hot spots …***

***… now as small as 100m x 50m.***

***AGY is a disease of the margins (edges) of viticulture ...***

***… it comes from swamps and/or wastelands,***

***… and doesn't come from other vineyards***

***Surveying for a third season is needed to check the findings to date.***

***High disease in some young vineyards in Riverina is of concern – why is this?***

# Chapter 13: Locating the Source of AGY 6 – confirming disease gradients
# Studies of the spatial distribution of AGY in 2004/05 and 2005/06

## Introduction

The surveys of vineyards within hot spots of AGY during 2003/04 re-affirmed the strong disease gradients seen near vegetation associated with wetlands and wastelands in 2002/03. The more intensive surveying of that season enabled greater precision in defining the locations of the source of AGY within the hot spot zones. Areas as small as ~100m x ~50m. comprising native vegetation adjacent to permanent shallow water were identified in the Riverland and Sunraysia. Similar trends had been evident in the Riverina in 2002/03.

As a result of this, the number of native plants as possible primary hosts of AGY was reduced to some 15-20 species within each zone. The main plants in question were a number of perennial grass species, *eg* the common reed (*Phragmites australis*), the bulrush (*Typha orientale* - cumbungi), several species of Juncaceae (the rushes), of Cyperaceae (the sedges) and Chenopodiaceae and related shrubs. The latter included yanga (short-leafed bluebush, *Maireana brevifolia*) and ruby saltbush (*Enchylaena tomentosa*).

In seeking the source and spread of AGY, further investigation of these plants was needed but the cost of PCR analyses prevented molecular assessment of that number of suspect species within the various hot spot zones across the regions. Further rationalisation of the number of suspect plants was needed.

The intensive surveying of 2003/04 had been undertaken for only one season but good progress had been made in reducing the number of species in question. Assessment of these outcomes was needed for an additional season to validate the conclusions from Chapter 12 and greater precision in targeting plant species as possible hosts of AGY was needed before taking multiple samples for the molecular tests.

## Aim

**To confirm the occurrence of specific disease gradients across vineyards within hot spots of AGY to reduce the number of native plants as candidate primary hosts of AGY in riverine vegetation and/or in associated wastelands.**

## Materials and Methods

During seasons 2004/05 and 2005/06, the incidence and distribution of AGY within and between vineyards in the Riverland and Sunraysia was assessed using point and arm surveys as described in Chapter 12, except that vineyard numbers were reduced while the intensity of sampling was increased. In 2004/05, more than 100 Riesling and Chardonnay vineyards were re-assessed in the Riverland and ~10 in the Sunraysia. In the second season only several were surveyed in each region.

To assist the spatial location of the survey data points, in most vineyards, GPS readings were linked to each disease score for each plot of 50 vines across usually more than five transects in a regular grid across each vineyard. As before, data were entered within MS Excel™ or MS Access™ and analysed using statistical tests of independence based on the Chi-square ($X^2$) statistic or using the GIS kreiging software ESRI ArcMap 9.1™ spatial analyst extension (Chapter 12). Statistically separate disease scores were plotted using different colours to portray differences at $P<0.05$.

## Results

The vineyards surveyed in 2004/05 showed that levels of AGY across the regions were similar to those in 2003-04. For perspective of the relative changes from each region in the levels of disease assessed in 2004/05, refer to Figures 7.5 – 7.8. These data affirmed that the incidence of AGY varies over time and space across the regions surveyed – that is, that the levels AGY vary from locality to locality, from vine to vine within the vineyard, and from season to season.

Representative samples of the transect surveys are presented below. Data from the 2003/04 surveys of the gradient of AGY in the vineyard at Gol Gol, Sunraysia, NSW, were presented in as a case study in Chapter 12 (Figures 12.2 and 12.3). Similar data from 2004/05 and 2005/06 are presented in Figures 13.1 – 13.4 for comparison. The pattern of a graded increase in AGY across the vineyard was statistically significant ($P<0.05$) in both 2004/05 and 2005/06 but only for the south to north and not the east to west transect.

Three additional case studies for 2004/05 and 2005/06 from the Riverland, SA, are presented to summarise the outcomes found across the two regions. Data and associated photos of these sites are from vineyards in hot spots at Puddletown, near Winkie, (Figures 13.5 - 13.9), Eckert's Creek, near Berri (Figures 13.10 - 13.13) and from Baker's Lake, Loxton North (Figures 13.14 - 13.17). The outcomes at these sites were consistent with data from the initial case study at Gol Gol, NSW, showing disease gradients ($P<0.05$) across vineyards within the AGY hot spots. There was a trend of increasing disease toward the riverine and/or wasteland vegetation at each location, irrespective of the geographic orientation of that locality in relation to each vineyard.

## Discussion

The disease gradients seen in all seasons of survey were consistent in character. They were present in each vineyard assessed within each hot spot in each region and were uniform in pattern across the vineyards. They were independent of geographical orientation (compass point) but were uni-directional, in each case being upwardly graded in the direction toward riverine and/or wasteland vegetation. The data for 2004/05 and 2005/06 confirmed earlier observations of this and pointed to the probability that the source of AGY lies within the hot spot zones of riverine and associated vegetation aligned to the edges of the vineyards identified above.

The rate of spatial decrease in AGY across the vineyard at Gol Gol occurred with similar slope in the four seasons in which intensive surveying was undertaken. For instance, in 2004/05, the level of AGY was highest (24% of vines affected) closest to Gol Gol Creek at the northern end of the vineyard 255 m. from the creek, and lowest (8% affected vines affected) furthest south from the source areas, 650 m. from the creek (Figures 12.4a and 13.1). This was virtually the same disease level as occurred at that site in the season before (23% to 9%) (Figure 12:1) when disease levels in the vineyard were effectively the same (20% vines affected in 2004/05 and 19% in 2003/04). However, in 2005/06 and 2002/03 when disease levels were less (*viz.* 14% and 10%% vines affected respectively) AGY was at 18% and 10% respectively at the northern end, lower than in the earlier seasons of survey, and was absent from the southern end in 2005/06 while 3% vines (base levels seen in many vineyard across the regions surveyed) were affected in 2002/03.

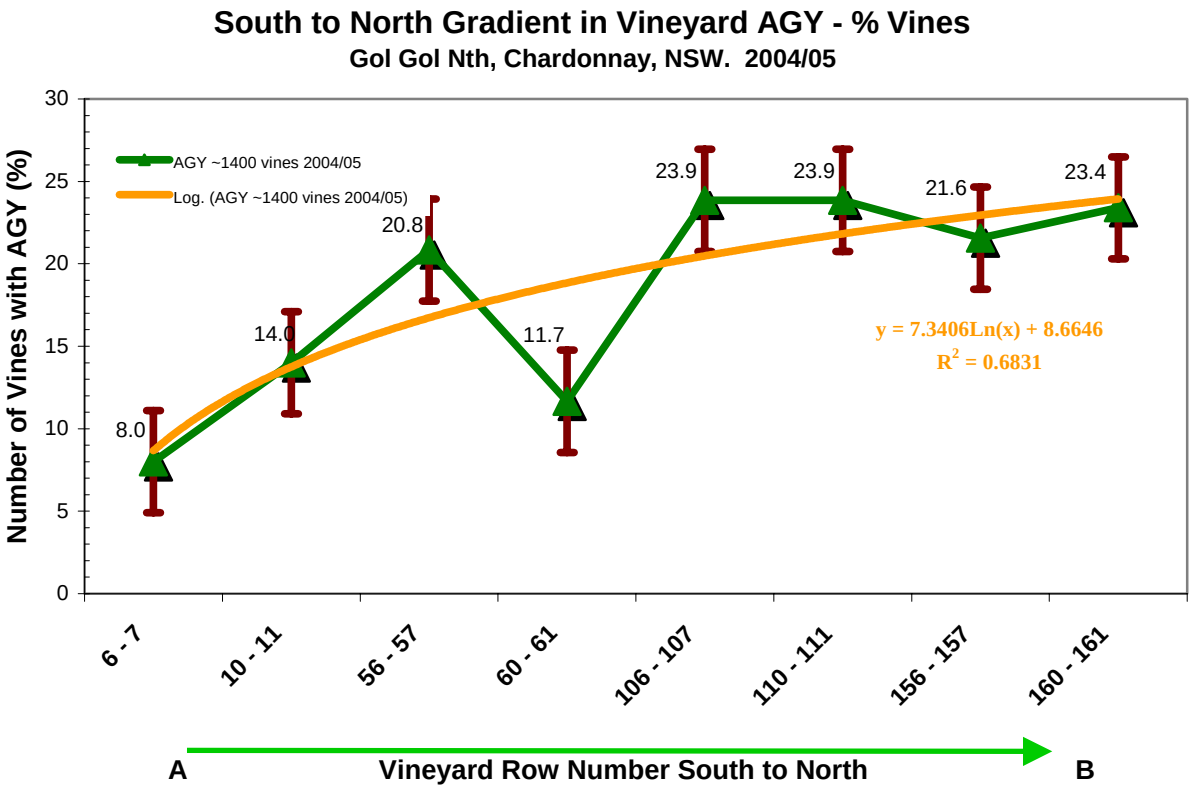



**Figure 13.1**: **The gradient in severity of AGY along a south-north vineyard transect at Gol Gol Nth, NSW, 2004/05. A is adjacent to vineyards; B is closest to Gol Gol Creek and wastelands. (see Figure 12.2).**

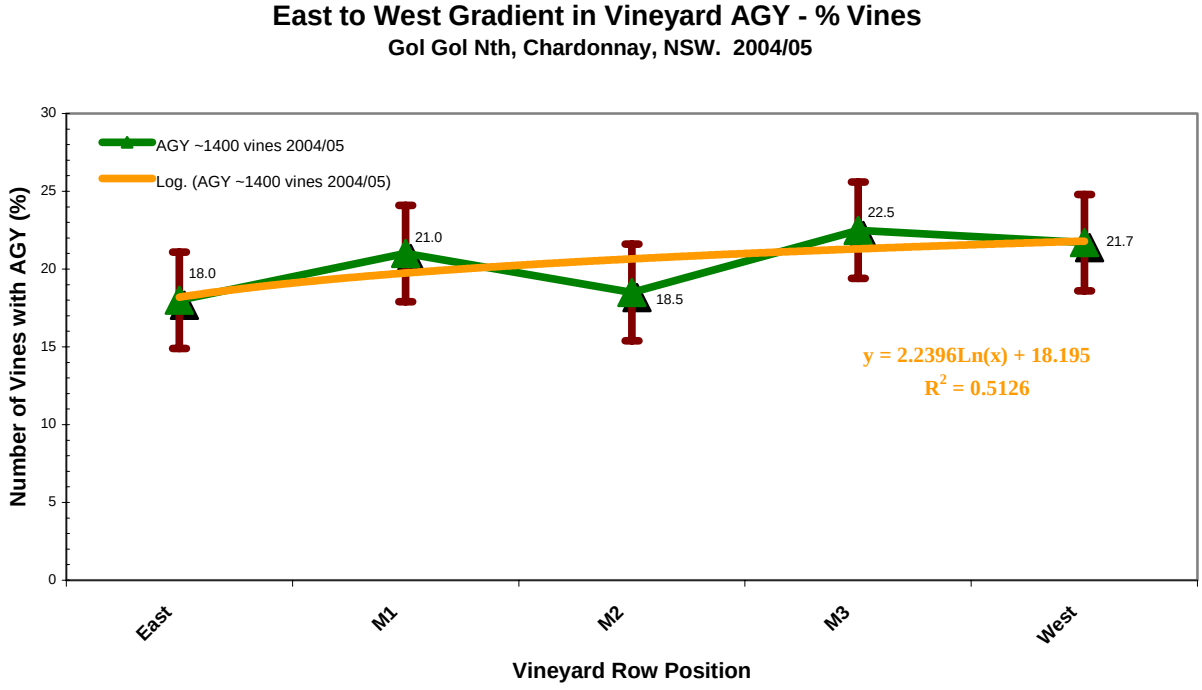



**Figure 13.2**: **The gradient in severity of AGY along an East-West vineyard transect at Gol Gol Nth, NSW, 2004/05. Other vineyards are adjacent at the eastern and western ends (see Figures 12.3 – 12.4).**

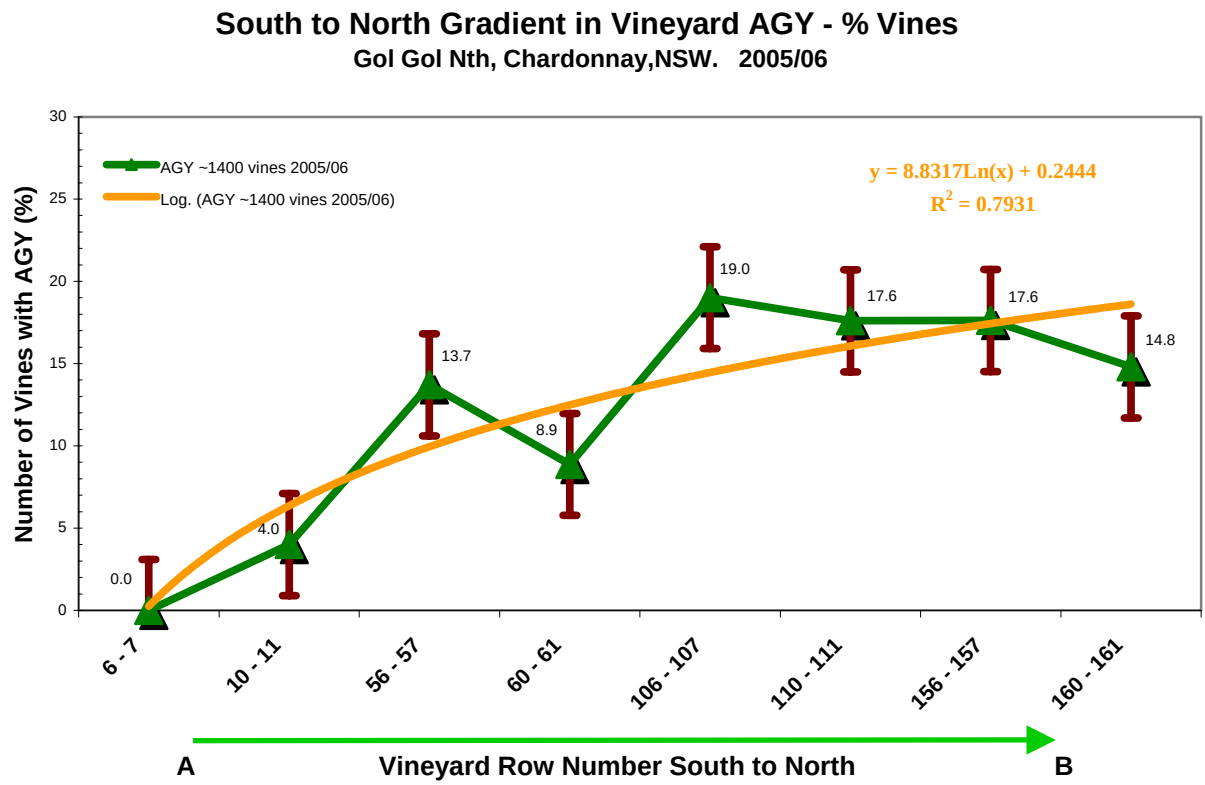



**Figure 13.3**: **The gradient in severity of AGY along a south-north vineyard transect at Gol Gol Nth, NSW, 2005/06. A is adjacent to vineyards; B is closest to Gol Gol Creek and wastelands (see Figure 12.2).**

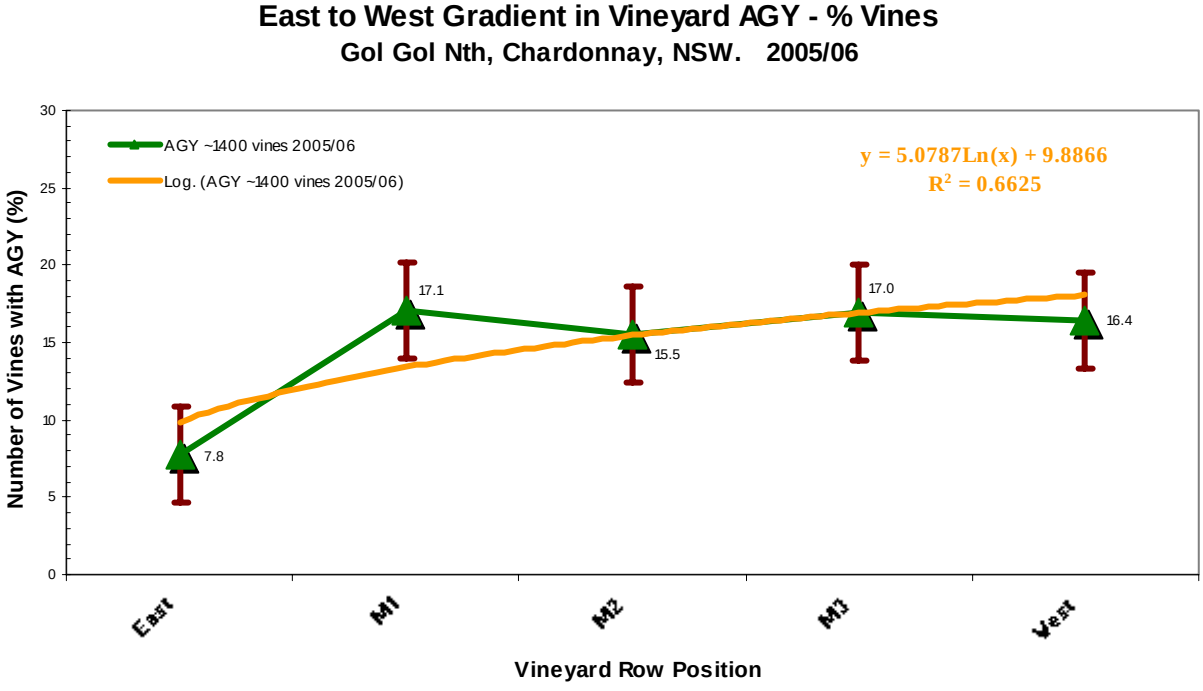



**Figure 13.4**: **The gradient in severity of AGY along an East-West vineyard transect at Gol Gol Nth, NSW, 2005/06. Other vineyards are adjacent at the eastern and western ends (see Figures 12.3 – 12.4).**

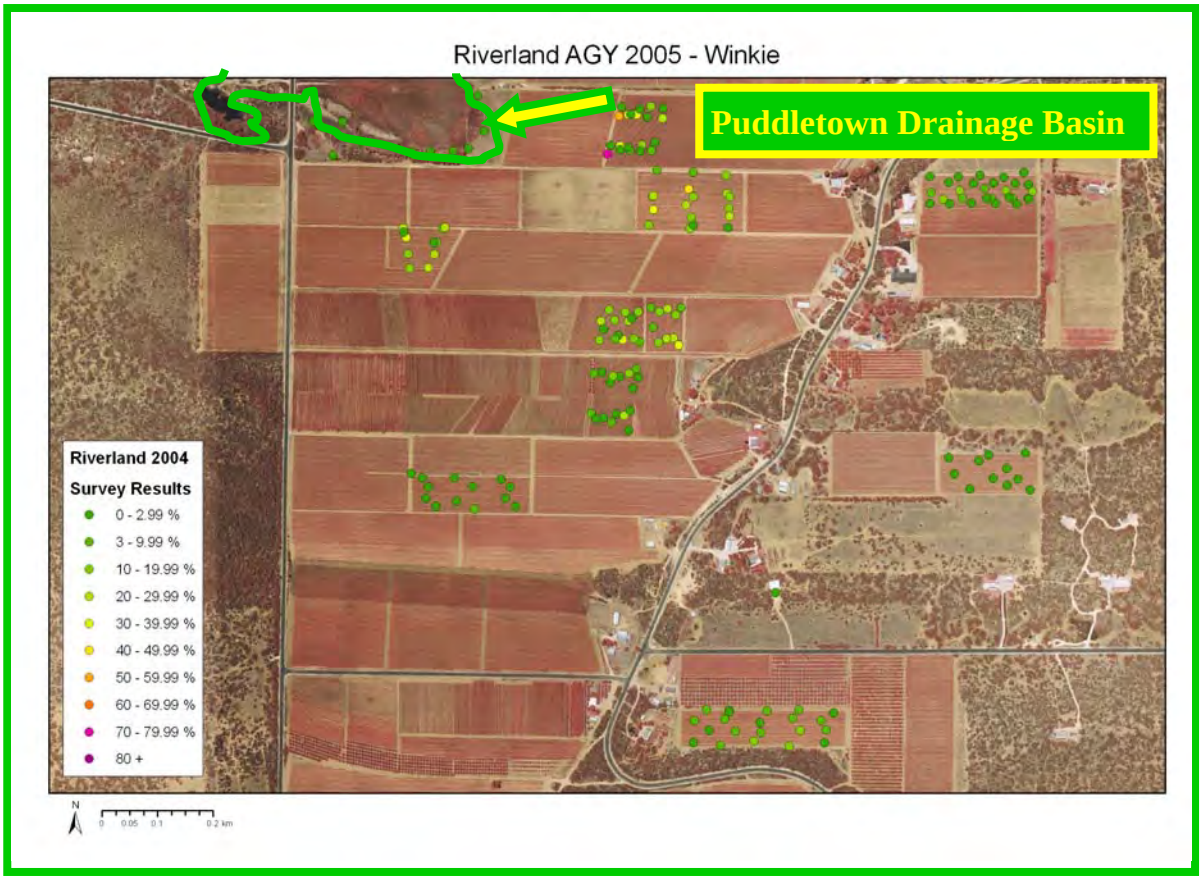



**Figure 13.5: Point-survey scores for severity of AGY in vineyards at Puddletown, near Winkie, SA. 2004/05.**

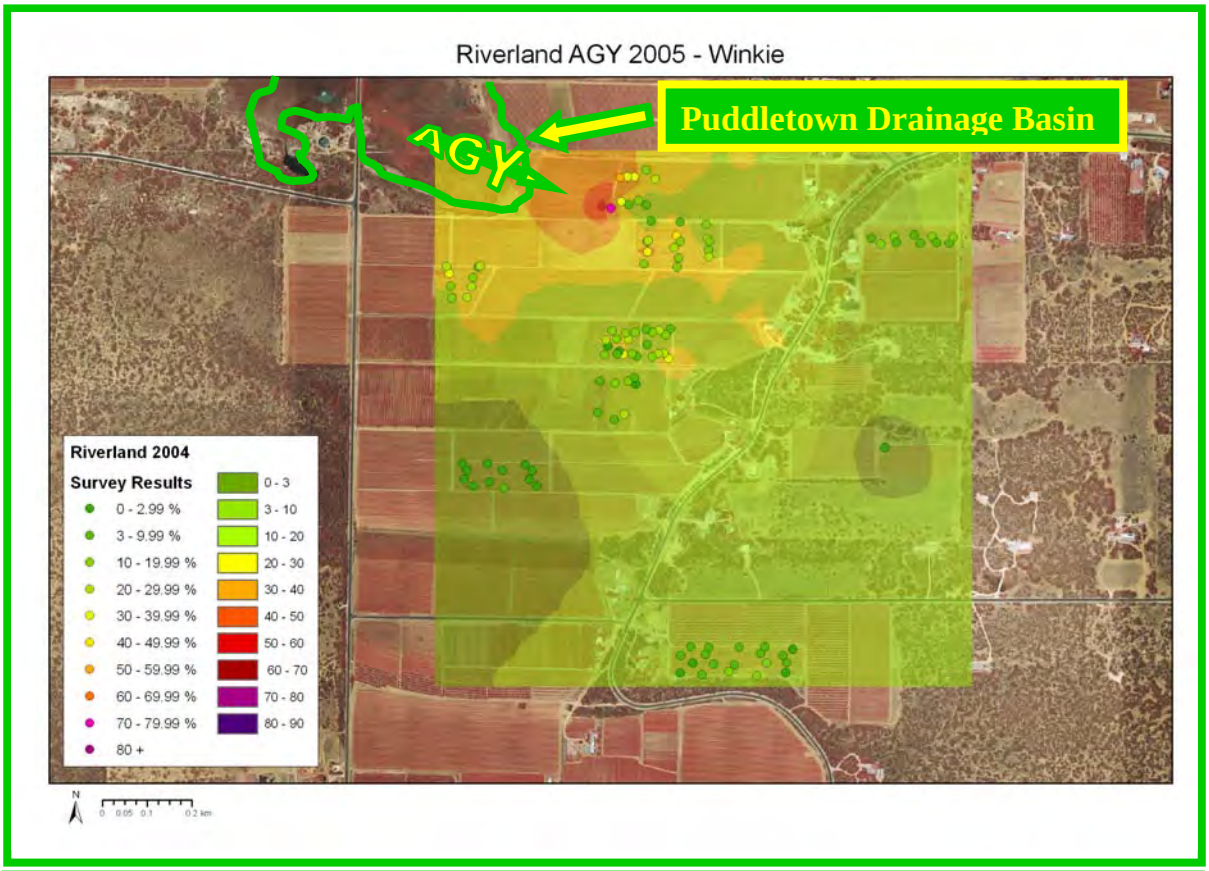



**Figure 13.6: GIS kreiging analyses of scores for severity of AGY in vineyards at Puddletown, near Winkie, SA. 2004/05, confirmed the patterns seen the previous season.**

**Figure 13.7: View of Puddletown Swamp (drainage basin) near Winkie, SA, showing scattered yanga bush (foreground), rushes (mid) and common reed (background).**



**Figures 13.8 & 13.9: A dense stand of yanga bush adjacent to highly diseased vineyards at Puddletown Swamp. Note the pink discolouration of many of these plants.**


**Figure 13.10:** **Point-survey scores for levels of AGY in vineyards near Eckert's Creek, SA. 2004/05.**

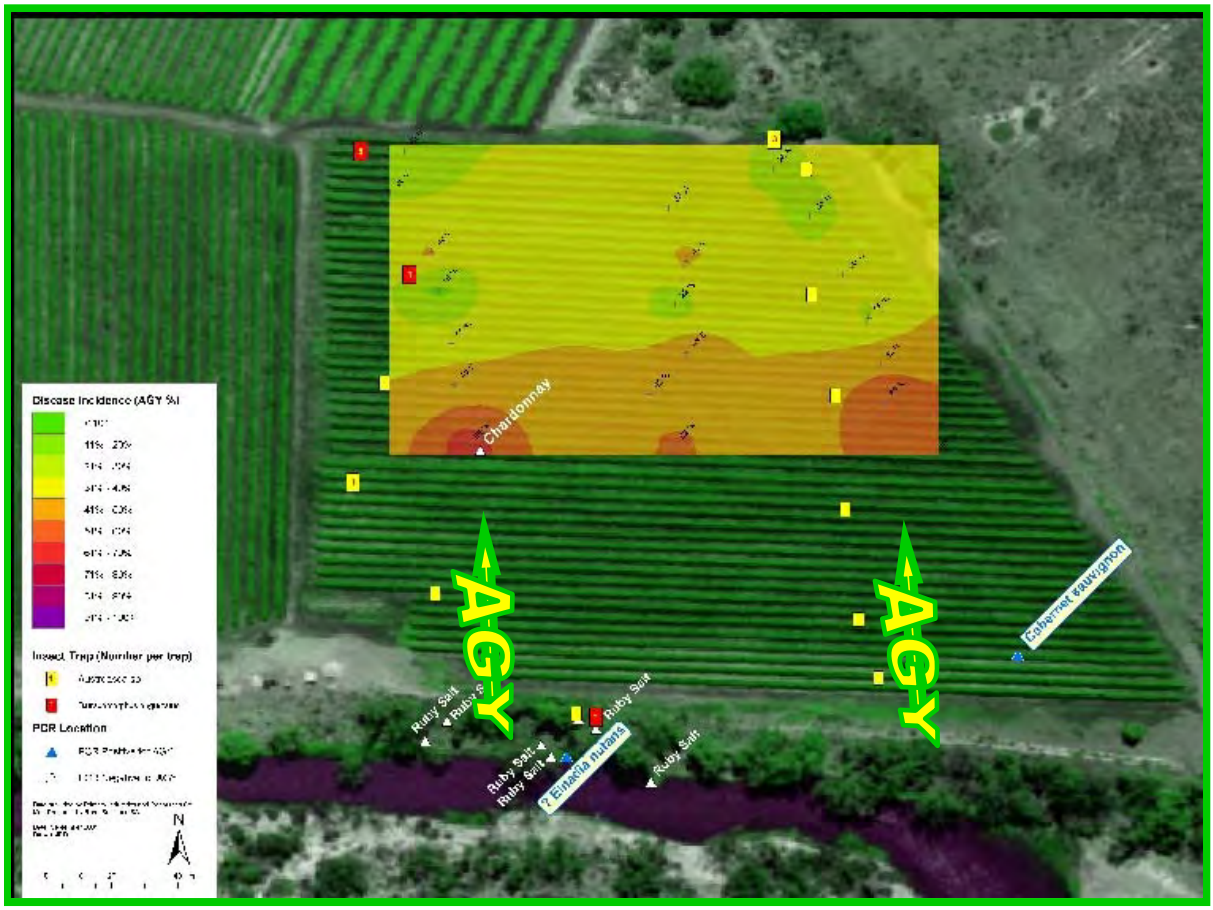



**Figure 13.11:** **Kreiging indicated that zones of high AGY lay near Eckert's Creek, SA, in 2004/05 and suggested that the flow of AGY inoculum is from there into the adjacent vineyards.**

**Figure 13.12: The ecosystem along Eckert's Creek, SA, appears to be a source of AGY. The possible link of permanent shallow water and high levels of AGY has not been resolved.**

**Figure 13.13: Typical vegetation along Eckert's Creek, SA, containing yanga bush, ruby saltbush and other Chenopod species. This ecosystem is typical of roadside vegetation implicated as a source of AGY inoculum at other sites.**


**Figure 13.14: GIS kreiging analyses of point survey data shows a zone of high AGY to the left of this figure *ie.* within or near Baker's Lake, Loxton North, SA. 2004/05.**

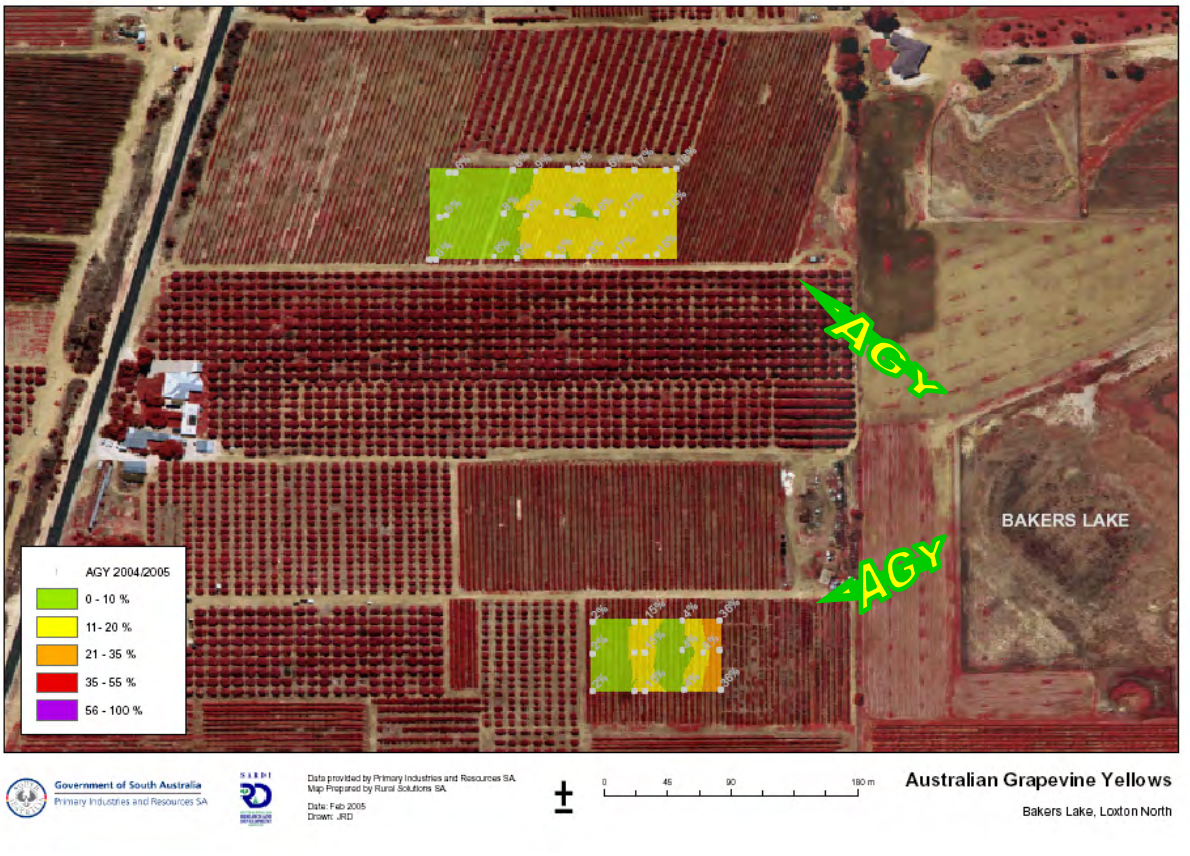



**Figure 13.15: GIS kreiging analyses of point survey data shows a zone of high AGY to the right of this figure *ie.* within or near Baker's Lake, Loxton North, SA. 2004/05.**

**Figure 13.16: A view of Baker's Lake, Loxton North, SA, in 2002, looking in the same direction as Figure 14.1. Note the abundant bird life as an indicator of the permanency of the water at that time.**

**Figure 13.17: A dense stand of yanga bush on the west side of Baker's Lake, Loxton North, SA, in 2002. This is a view of a similar aspect as presented in Figure 14.2.**

One explanation for this difference is that the movement of AGY inoculum was less extensive from the supposed source(s) of disease in the adjacent vegetation north of the vineyard in those seasons (refer to Figure 12.4a). If the hypothesis of a leafhopper insect vector for AGY is assumed, then because the movement of inoculum did not reach to the southern end of the vineyard 650 m. from the creek in 2005/06, it barely reached that distance in 2002/03 and in the other two seasons, it reached that distance in greater titre, the movement (flight distances) of the vector must be similar.

Using similar assumptions on the movement of a supposed insect vector of AGY as above, at the Puddletown site, the disease gradients inferred a flight distance of between 400 – 1300 m. depending on the assumed location of the inoculum source. At the Eckert's Creek site, the upper limit of movement was not defined because the level of disease in the surveyed vineyards was so high, but flight distance was in excess of 300 m., while at Baker's Lake, the flight distance might be as little as 300 – 400 m. with upper limit being not greater than 800 – 1200 m..

Thus at least for the seasons of the study, it appears that the outer limit of infective spread of the disease was identified *ie.* the distance from the source of disease to the most distant affected vines. If leafhoppers do vector AGY, the distance from source to the distal edge of the vineyard may be the maximum distance over which that insect travels, at least on a regular basis. That distance appears to be ≤ 800 - 1200 m. in the vineyards of our case studies.

Since disease levels were confined to the zones described above, it appears that AGY is not spread in appreciable quantities beyond the distances presented. The data also indicate that the source of AGY is probably limited to relatively small geographical areas of dimension as low as 100 m. x 50 m. at Eckert's Creek to as large as ~ 400 m. x ~ 500 m. at Gol Gol, Puddletown and Baker's Lake.

In each season of study at the Gol Gol site, the disease gradients in these zones were sharply defined. The test vineyard had been surrounded for a number of seasons by other vineyards on two sides, a citrus orchard on part of one of those sides and by a bare, grassed paddock on the other. Figure 12.4a shows that the fourth side, the side with the highest disease, was bound by a vineyard and a small planting of citrus that adjoined riverine vegetation and an associated wasteland with a variety of native and some introduced plant species. The predominant species were of the Chenopodiaceae family (saltbush and bluebush) including a predominance of yanga bush and similar woody native perennial shrubs (Figure 12.4b). Common reed was prominent (Figure 12.4c) and though these had been cleared in 2002 or 2003, they had since regrown on the edges of the creek banks.

At the Puddletown site, levels of AGY increased toward vegetation comprising a variety of plant species associated with wetlands. These included a predominance of common reed, sedges and rushes in some parts of the adjoining vegetation (Figure 13.7) and of yanga bush interspersed. On one side if the swamp, next to the highest levels of disease in the adjacent vineyards, there was a predominance of yanga bush (Figures 13.8 – 13.9), although common reed was also present in lower numbers.

At the Eckert's Creek site, the vineyards were bounded on three sides by vineyard and on the fourth by grassed River Murray floodplain vegetation (Figures 13.10 and 13.11). On the high disease side adjacent to the creek (on the lower edge of these figures), the predominant native species apart from grasses, were the Chenopods, saltbush and bluebush, and some bulrush. The uniformity of gradients in the two vineyards for the three seasons of study suggests that the

inoculum is sourced in the riverine vegetation along the ~800 m. of creek bank at the edge of Eckert's Creek (Figures 13.12 and 13.13).

At Baker's Lake, the disease gradient was sharply focussed with high levels of AGY at the western edge of the vineyards in Figure 13.14 (left side of figure). The upper two vineyards showed this most clearly while the lower (more southerly) vineyard showed a more diverse pattern of AGY disease. On the western-side of the wetlands at Baker's Lake, Figure 13.15 shows clear zones of high disease in the eastern side of the vineyards *ie* on the side adjacent to the swamplands despite the vineyards otherwise being surrounded by vineyards and/or citrus orchard. The source of disease appears to be coming from the lake ecosystem (Figures 13.16 – 13.17) associated with the vegetation on that side of lake. The predominant native plants present there were common reed, yanga bush and other chenopod shrubs.

Concurrent studies further investigated the occurrence of native plants within these more precisely defined zones – see Chapter 14 for a description of these.

**Conclusions**

A number of findings resulted from this work:

- the disease gradients seen in previous seasons of study were affirmed in vineyards adjacent to riverine and/or wetland vegetation;
- the source of AGY is confined to zones within hot spots of dimension as small as ~100m. x ~50 m.;
- the infective distance of the presumed insect vector *ie* from source plant to the most distant new host is 400 - 1200 m.; and
- the number of native plant species considered candidate primary hosts of AGY was further reduced, to ~ 15 species.

**Recommendations**

It is recommended that the investigations continue to:

- seek the source of AGY using data from the detailed arm surveys and the point surveys to locate likely primary plant host(s); and
- focus on the 15 or so native plant species in the zones described above, one or more of which may be the primary source of AGY inoculum.

***Disease gradients:***

- ***occur consistently in vineyards in hot spots;***
- ***occur over distances of 400 -1200m;***

***and***

***the primary hosts of AGY are among < 15 native plant species***

# Section 7. Further Investigations into the Source of AGY
## Searching for a Primary Host of AGY

*With data for season 2004/05 (Chapter 13), the surveys of AGY across the regions (Chapters 10 – 13) had now included three, season-replicates. This gave confidence in the observation that levels of AGY increase toward riverine/wetland and wasteland vegetation adjacent to vineyards inside hot spots of disease and that the source of AGY lay within specific localities in those ecosystems. It was now time to have a close look at these areas to determine which plants were the best to sample for PCR-tests in the search for the hosts of AGY. First we surveyed then we sampled the native vegetation.*

## Chapter 14: Reducing the Number of Host Plants to be Tested
## Vegetation Surveys of Possible Host Plants

### Introduction

Consistent, well-defined disease gradients across these vineyards pointed to the primary hosts of AGY being numbered among 20 or fewer native plant species located within zones as small as 100m x 50 m (Chapter 13).

In detailed studies of several vineyard sites over several seasons, the vector of AGY apparently was infective over distances averaging 600m (range from 400 – 1200m) from the supposed source of inoculum, that is, from the supposed location of the primary host plant(s). As a result, the sites where the presumed pathogen of AGY (AGYp) was believed to be sourced were better defined but not sufficiently to reduce the list of potential host plants to less than 20 native species.

Overseas, yellows diseases of grapevine are associated with similar but different phytoplasmas than AGYp which has been consistently associated with AGY (Padovan *et al*., 1995; Habili *et al*. pers. comm.). The lack of AGY outside Australasia and other field evidence (Chapter 11) implied that AGY is native to the region and thus, the primary host plants would be native species.

The cost of PCR-tests restricted the number of native plant species that could be sampled in the search for AGYp. This paper reports a brief review of the native vegetation in hot spots of AGY in an attempt to reduce the number of suspect primary host plants for which finances were available for PCR-testing. This was a forerunner to intensive molecular testing of samples (Chapter 15), in the search to identify the primary host(s) of AGYp.

### Aim

**To minimise the number of candidate native plants to be sampled for PCR-testing in search for the primary host of AGY.**

### Materials and Methods

In the three seasons from 2002/03, brief visual assessment of the vegetation in and near hot spots of AGY was made in at least six sites including three in NSW (two in Sunraysia and one in the Riverina) and three in the Riverland of SA. More detailed survey of the vegetation was made at several of these sites identifying the species and their abundance (data not shown). Riverine and associated un-tilled wasteland closest to the vineyards that had expressed distinct disease gradients within the hot spots, was selected for assessment. The vegetation in these localities was scored for presence or absence either of easily identified native species or of main sub-groups of

species most commonly found. Populations of plants at each site were subjectively rated as follows: Score 0 = plants absent; Score 1 = some individuals present but rare or low in number; Score 2 = moderate numbers present either in scattered clumps or in thin spread across the site; and Score 3 = high numbers present either in dense stands or evenly spread across the site.

The scores for each plant or plant group at each site were then summed to give an aggregate score across all sites. The plants with highest incidence scores were then selected for sampling for PCR analysis unless disqualified by the presence of zero scores at some sites (Table 14.1).

**Results**

Table 14.1 lists the seven native plants which scored highest for incidence in surveys of vegetation in riverine and adjacent wasteland environs closest to highest levels of AGY in nearby vineyards. Two genera of reed - the common reed and bulrush (cumbungi) - and some chenopod shrubs including yanga and various saltbushes, ranked the top five in the present survey.

**Table 14.1: Incidence of various native and non-native plant species within hot spots of AGY adjacent to high levels of disease in nearby vineyards. 2002/03 – 2004/05 [1].**

| Hot spot Site Season | Yanga | Common Reed | English Bulrush | Ruby Saltbush | Other Chenopods | Sedges | Lignum |
|---|---|---|---|---|---|---|---|
| **Gol Gol, NSW 1** | | | | | | | |
| 2002/03 | 3 | 1 | 1 | 1 | 1 | 0 | 1 |
| 2003/04 | 3 | 1 | 1 | 1 | 1 | 0 | 1 |
| 2004/05 | 3 | 2 | 1 | 1 | 1 | 0 | 1 |
| **Gol Gol, NSW 2** | | | | | | | |
| 2002/03 | 2 | 1 | 1 | 1 | 1 | 1 | 1 |
| 2003/04 | 3 | 1 | 1 | 1 | 1 | 1 | 1 |
| 2004/05 | 3 | 2 | 1 | 1 | 1 | 1 | 1 |
| **Griffith, NSW** | | | | | | | |
| 2002/03 | 2 | 2 | 2 | 1 | 1 | 0 | 0 |
| 2003/04 | 2 | 2 | 2 | 1 | 1 | 0 | 0 |
| **Puddletown, SA** | | | | | | | |
| 2002/03 | 1 | 2 | 2 | 1 | 1 | 3 | 0 |
| 2003/04 | 1 | 2 | 2 | 1 | 1 | 3 | 0 |
| 2004/05 | 2 | 2 | 2 | 1 | 1 | 3 | 0 |
| **Eckert's Creek, SA** | | | | | | | |
| 2002/03 | 1 | 1 | 1 | 2 | 1 | 0 | 1 |
| 2003/04 | 1 | 1 | 1 | 2 | 1 | 0 | 1 |
| 2004/05 | 1 | 1 | 1 | 2 | 1 | 0 | 1 |
| **Baker's Lake, SA** | | | | | | | |
| 2002/03 | 1 | 3 | 3 | 1 | 1 | 1 | 0 |
| 2003/04 | 2 | 0 | 0 | 1 | 1 | 1 | 0 |
| 2004/05 | 3 | 0 | 0 | 1 | 1 | 1 | 0 |
| **TOTAL** | **34** | **24** | **22** | **20** | **17** | **4** | **9** |
| **Mean Score/Site** | **2.0** | **1.4** | **1.3** | **1.2** | **1.0** | **0.9** | **0.5** |
| **% Grand Total** | **24%** | **17%** | **16%** | **14%** | **12%** | **11%** | **6%** |

**Note[1]: Incidence scored as follows: 0 = plants are absent or in very low number; 1 = low in number; 2 = moderate in number either in scattered clumps or thinly populated; and 3 = high in number either in dense stands or evenly spread.**

**Discussion**

As discussed in Chapters 12 and 13, it was concluded that the most likely candidate primary host plants included ~ 20 native species.

When determining which of these were to be sampled for PCR tests, both the incidence (Table 14.1) and the continuity of occurrence of each species were considered. For instance, while common reed and bulrush ranked second and third in abundance across all sites, there was a significant reduction to zero in their occurrence at one site, thus calling in question the role these plants played as potential primary hosts of AGY.

The site was at Baker's Lake, Loxton North. The lake there existed as a result of the open-channel irrigation system then operative in that district. The riverine/wetland ecosystem which had developed at the site was reliant upon both irrigation drainage- and channel overflow- water. However, in winter 2002, this supply ceased when the channel delivery was upgraded. Pipes replaced the open channels and, since end-of-run overflows no longer occurred, the lake dried and the site was bulldozed to assist rehabilitation of the area.

As a result, the water-based (riverine/wetland) ecosystem (Figures 11.12 and 13.16) was obliterated leaving the adjacent untilled wasteland as the predominant ecosystem in that locality (Figures 13.17, 14.1 and 14.2). If the riverine/wetland vegetation were significant in the disease cycle of AGY, it would be expected that rehabilitation of the site would lead to a reduction in levels of disease in the nearby vineyards. However, given that AGY only expresses symptoms in the season following infection (Chapter 16), that reduction in vineyard disease would not be expected in the first season, *ie.* in 2002/03, though it would be subsequently *ie.* in 2003/04 and 2004/05.

The level of AGY in transect across one of the vineyards adjacent to the former Baker's Lake, is shown in Figure 11.10 for seasons 2002/03 to 2004/05. Disease levels were not reduced in that period but to the contrary, in both seasons after 2002/03 levels of AGY were increased ($P<0.05$) before the owner removed the vineyard in 2005.

This result typified those in each *cv.* Chardonnay vineyard which abutted the former lake. A review of the levels of AGY showed that the disease remained high in the ends of the vineyard closest to the wetland (compare disease scores in Figure 11.11 with those in Figures 12.14 - 12.16 and Figures 13.14 - 13.15).

A closer review of the disease gradients in each vineyard over the three seasons permitted some estimation of the relative proximity of the supposed source of AGY inoculum. By comparing the disease scores for 2002/ 03 in Figures 11.6 - 11.10, for 2003/04, in Figures 12.15 - 12.16 and for 2004/05, in Figures 13.14 - 13.15, it was apparent that the level of AGY was higher in the eastern edges of the vineyards that were to the west of the wetland than in the western edges of the vineyards that were to the east. Thus, the level of AGY 'lake side' of the western vineyards was higher than 'lake side' of the eastern vineyards.

For example, in season 2003/04, levels of AGY on the 'lake side' of the western vineyards averaged ~44% vines, while across the 'lake', levels on the western edges averaged significantly less ($P<0.05$) *viz.* between 11 – 28% disease. This inferred that the source of AGY was not mid-way between the eastern and western vineyards but rather was closer in proximity to those in the west. By assessing the slope of the decline of disease across the vineyards at each site (Figure 12.14), it appeared that the source of AGY lay within ~200 – 300m of the western vineyards and

up to ~500 – 700m from the north eastern-most vineyard. The vegetation that lay within the zone thus described comprised an almost pure population of yanga bush in wasteland adjacent to the former lake (Figures 13.7 and 14.2) and a stand of irrigated lucerne (Figure 14.1).

These observations inferred that while the source of AGY lay within the broad boundaries of the riverine/wetland ecosystem *ie.* within the confines of the Baker's Lake locality, the primary source plant was neither a riverine nor a wetland species. Instead, the source of AGY was more likely a native plant or plants within the wasteland ecosystem. Thus the water-based native plants that were bulldozed *viz.* common reed (Figure 14.3), bulrush (Figure 13.16) and some of the sedges (Figure 13.7), were excluded as the likely primary host plants of AGY, despite their high relative abundance at other sites. The stand of lucerne was also excluded as a primary host since it was not a native species (see earlier). As a result, the chenopod shrubs at Baker's Lake *viz.* yanga and saltbush, were the plants most suspect as the primary hosts of AGYp.

In a similar process of measurement and deduction, the relative location of native vegetation in the vicinity of high levels of AGY in vineyards at Puddletown Swamp (Figure 12.7) suggested that a stand of yanga bush there was the most likely primary source of AGY inoculum (Figures 13.7 – 13.9).

These conclusions were consistent with observations at other sites with high levels of AGY. For example, at Gol Gol, NSW, common reed and bulrush were present in abundance (Table 14.1) along the edges of the creek (Figure 12.4b and 12.4c). However, in 2002 (or 2003), these riverine species were substantially removed when the creek was dredged. The reeds regrew next season but in each of the following seasons they were cutback. Significantly, in the adjacent vineyards, the levels of AGY did not decline after the dredging or in the following seasons (Figure 7.7). In addition, common reed and bulrush are found in some localities in moderate to high numbers while only low levels of AGY occur in the vineyards near-by. This evidence infers a lack of involvement of riverine species as inoculum sources for AGY.

Transposing this inference to the site at Eckert's Creek (Figure 13.11), led to the assumption that a zone of more mixed vegetation including native chenopods (Figure 13.13) was more likely to contain the AGY host plant than the adjacent riverine vegetation (Figure 13.12) which, as a result, was discounted for sampling for PCR-tests.

Similarly, at other sites, plants with less frequency of occurrence and with zero scores at some locations *viz.* sedges and lignum (Table 14.1), were also discounted as candidate primary host species.

In consequence, the chenopod vegetation, *viz.* yanga (short-leaf bluebush, *Maireana brevifolia* (R.Br.) Paul G. Wilson, Chenopodiaceae), and related chenopods, mostly hardy shrubs, rather than the riverine/wetland species were considered to have the highest probability of being the source of AGYp. This led to a significant reduction in the number of plant species (from ~20 to 3-5) considered as probable primary hosts of AGYp to be targeted for examination *via* PCR.

**Figure 14.1: A view to the east of Baker's Lake, Loxton North, SA, in 2005/06. The vineyards adjacent on the distant side of the former drainage basin have consistently shown higher levels of AGY on their closest boundaries despite the lake being drained and bulldozed in 2002.**

**Figure 14.2: A dense and almost pure stand of yanga bush on the western edge of Baker's Lake, Loxton North, SA, in 2005/06. The vineyards adjacent on the right had high levels of AGY on their closest boundaries. Note the mounds of dirt on the left, remnants of the reeds and cumbungi that had been bulldozed.**

## Conclusions

Evidence from vegetation surveys for suspect host species suggested:

- that Riverine/wetland vegetation including the common reed (*Phragmites australis*), the bulrush (*Typha orientale* - cumbungi) and several species of Juncaceae (the rushes) and of Cyperaceae (the sedges) were unlikely to be the primary host(s) of AGY;
- that one or more native chenopod shrubs, including yanga bush (short-leafed bluebush) (*Maireana brevifolia*) and ruby saltbush (*Enchylaena tomentosa*), were the most likely candidate primary hosts of AGY in Australian viticulture; and
- that there is good prospect that these and similar species will test PCR-positive for AGYp.

## Recommendations

In continuing the search for the primary host(s) for AGY, several points of investigation were recommended for immediate action *viz.*:

1. that the riverine/wetland species within hot spots of AGY be excluded from the list of plants to be sampled for AGYp, the phytoplasma associated with AGY in Australian viticulture;
2. that native chenopod and similar shrubs including yanga bush (short-leafed bluebush) (*Maireana brevifolia*) and ruby saltbush (*Enchylaena tomentosa*), in hot spots of AGY in the Riverland and at least one other region, be sampled for PCR-tests using specific primers for AGYp; and that
3. other plant species from within the hot spots be considered for PCR-testing as a second order of priority to determine the array of plants that might be the primary hosts for AGY and possible breeding-hosts for the insect vector of AGY.

**Figure 14.3: Common reed (*Phragmites australis*) and several other species were abundant in and near shallow water in hot spot zones of AGY, in a number of districts in Australian viticulture. Despite this, vegetation surveys linked with disease gradients across vineyards, discounted these native plants as probable primary hosts of AGY.**

***The primary host plant for AGY:***

- ***is not wetland or riverine vegetation;***

***but***

- ***is probably one or more native chenopods,***

***and it is time they were PCR- tested!***

# Chapter 15: The Role of Native Plant Species – Closing in on the Primary Hosts PCR Analyses of Suspect Host Plants

## Introduction

Surveys of vineyard disease and of adjacent vegetation in hot spots of AGY (Chapters 10 – 14), had shown that: 1) the source of AGY usually lay within specific localities; 2) the most likely candidate primary host(s) of disease occurred in wasteland close to the edge of the vineyards inside hot spots of disease, rather than in riverine/wetland vegetation; and that 3) several native chenopod shrub species including *Maireana brevifolia* (yanga bush) and *Enchylaena tomentosa* (ruby saltbush), were considered the most likely primary host or hosts of the AGY phytoplasma.

Phytoplasma (mycoplasma that occur in plants) have been investigated by RFLP analyses (restriction fragment length polymorphism) and sequence analyses of their DNA, specifically of the 16S rRNA gene. As a result, they were differentiated into 15 major groups (*viz.* 16 Sr I-XV) (Firrao *et al.* 2005; Lee *et al.* 1993, 1998, 2000; Schneider *et al.* 1993; Seemüller *et al.* 1998).

Phytoplasma from a number of these groups have been associated with similar yellows-disease symptoms of grapevines from a diversity of locations across Europe, Israel, Eastern USA and Australia (Magarey 1986). The symptoms we described in Chapter 2 for AGY appear to be almost identical with each disease and though varietal and climatic/environmental differences seem to occur, a common sensitive cultivar is Chardonnay on which symptoms are generally very consistent across the regions where grapevine yellows has been recorded.

Phytoplasma belonging to the various groups have been associated with the diseases cited in Table 15.1.

The 16 Sr XII group (the stolburs) is divided into two subgroups, Serbian stolbur of pepper (16 Sr XII-A) and Australian grapevine yellows (16 Sr XII-B) (Lee *et al.* 1993, 1998; Schneider *et al.* 1999).

The stolbur group (16 Sr XII-A) includes the phytoplasma associated with the yellows disease Bois Noir (BN), known by that name in France and as Vergilbungskrankheit (VK) in Germany. It is the most widespread grapevine yellows and occurs all over Europe (Daire *et al.* 1997; Langer *et al.* 2004) and elsewhere *eg* in France, Germany, Italy, Spain, Switzerland, Austria, Slovenia, Serbia and Croatia, and in Israel (Daire *et al.* 1997) and Lebanon (Choueri *et al*. 2003). More recently, it was reported from Hungary (Palermo *et al.* 2004). It is found on a wide range of wild and cultivated herbaceous plants (Marcone *et al.* 1997; Schneider *et al.* 1997), the chief of which are the bindweeds (*Convolvulus arvensis* L. and *Calystegia sepium* L.) and stinging nettle (*Urtica dioica* L.) (Langer *et al.* 2004).

The AGY group (16 Sr XII-B) has, to date, been found in Australasia (Streten *et al.*2005b) and more recently in Bolivia (Jones *et al.* 2005) and Israel (Gera *et al.* 2005). It is known as '*Candidatus* Phytoplasma australiense' ('*Ca.* P. australiense') (Davis *et al.* 1997a) and according to (Streten *et al.*2005b), comprises four strains. One is AGYp (16 Sr XII-B, *tuf*-Australia I; *rp*-A). This is widespread across Australia and is found in a variety of hosts including the introduced species, grapevine, strawberry, pumpkin and cottonbush (Streten *et al.* 2005b). Two other strains are found in New Zealand; one exclusively.

## Table 15.1 Characteristics of the 16 Sr Phytoplasma Associated with Yellows Diseases of Grapevine? [1]

| Ribosomal Group [1] | Group Type Name | Group Common Name | Grapevine Yellows Disease | Location/Country |
|---|---|---|---|---|
| 16 Sr I<br>16 Sr I-A | | Aster Yellows | AGY (BVGY)<br>GY<br>Nth American GY | Buckland Valley, Victoria, Australia,<br>Italy,<br>New York and Virginia, USA |
| 16 Sr II | | Faba Bean Phyllody | ?AGY (TBB) | Australia |
| 16 Sr III | | Western-X Disease | Nth American GY | NY and Virginia, USA |
| 16 Sr IV | | | - | |
| 16 Sr V[2] | | Elm Yellows | FD – Flavescence dorée<br><br>PGY – Palatinate GY | France, Italy, Serbia, Slovenia, Spain, Switzerland etc<br>Palatinate, Germany |
| 16 Sr VI | | | - | |
| 16 Sr VII | | | - | |
| 16 Sr VIII | | | - | |
| 16 Sr IX | | | - | |
| 16 Sr X | | | - | |
| 16 Sr XI | | | - | |
| **16 Sr XII-A[2]**<br>**VK Type I**<br>**VK Type II**<br>**16 Sr XII-B** | **'*Ca.* P. solani'**<br><br><br>**'*Ca.* P. australiense'** | **Stolbur**<br><br><br>**(four strains)** | **BN – Bois noir, Legno nero**<br>**VK - Vergilbungskrankheit**<br><br>**AGY** | **Chile, Croatia, Italy, Hungary, Germany, Serbia, Spain**<br>**Australia, ??Chile**<br>**Australasia, Bolivia, Israel** |
| 16 Sr XIII | | | - | |
| 16 Sr XIV | | | - | |
| 16 Sr XV | | | - | |

Note:

1. This table is not complete but lists the x15 groups designated within the 16 Sr Group (Lee *et al.* 1998; Firrao *et al.* 2004).
2. *Ca.* P. solani and Ca. P. vitis are not yet officially described Candidatus species within the 16 Sr V group. Some investigators have designated subgroups in the 16 Sr V Group as 16 Sr V-C, and 16 Sr V-D. These and the types in Group XII-A are not yet as well defined as the groups or "official" subgroups like XII-A and -B, and their real geographic distribution is not completely known. These have been cited to give an indication of the possible variation among and within these tentatively classified groups of phytoplasma, the taxonomy of which is still in its infancy!

In Australia, the group '*Ca.* P. australiense' is associated with a number of diseases of important crop hosts. These include AGY (Padovan *et al.* 1995, 1996), lethal yellows of strawberry (SLY1 and SLY2) (Padovan *et al.* 1998; 2000b), green petal of strawberry (SGP) (Padovan *et al.* 2000b), dieback of papaya (papaw) (PDB) (Gibb *et al.* 1996; Liu *et al.* 1996) and more recently, yellow leaf curl of pumpkin (PYLC) (Streten *et al.* 2005a). '*Ca.* P. australiense' was also associated with a single garden specimen showing witches' broom on mung bean (MBWB) (Schneider *et al.* 1999) and with witches' broom of Paulownia (Bayliss *et al.* 2005).

Only a relatively few (~80) non-crop species have been assessed during surveys for phytoplasma in Australia (Davis *et al.* 1997b, 2003; Schneider *et al.* 1999; Streten *et al.* 2005c). '*Ca.* P. australiense' was found in only a few (<12) of these. Examples include the introduced species *Gomphocarpus physocarpus* (cottonbush) with two symptoms – a reduced yellow leaf (CBRYL) and a witches' broom (CBWB), *Melilotus sp.* tentatively identified as hexham scent (Streten *et al.* (2005c); Don Hutton, pers. comm.), *Medicago polymorpha* (burr medic), a *Trifolium sp.* (clover) (Streten *et al.* 2005c) and in a single report, periwinkle (*Catharanthus [formerly Vinca] roseus*) (Davis *et al.* 2003) with phyllody. Recently, Saqib *et al.* (2006) in SW Western Australia, found '*Ca.* P. australiense' in *Trifolium pratense* (red clover), several other pasture legumes and *Cucumis myriocarpus* (paddy melon) while Habili *et al.* (pers. comm.) in Adelaide, South Australia, detected the pathogen in liquidambar with yellows and retarded growth.

In New Zealand, '*Ca.* P. australiense' is associated with diseases of native plants *viz. Phormium* (flax) yellow leaf (PYL) (Liefting *et al.* 1998; White *et al.*, 1998), *Cordyline australis* (cabbage tree) sudden decline (CSD) (Andersen *et al.* 2001) and *Coprosma* lethal decline (CLD) (Andersen *et al.* 2001), and an introduced plant, strawberry lethal yellows (SLY) (Andersen *et al.* 1998).

Streten *et al.* (2005c) found AGYp in Queensland on the Australian natives, *Exocarpus cuppressiformis* (native cherry) and *Jacksonia scoparia* (dogwood). Thus in New Zealand, '*Ca.* P. australiense' has been associated with several native and one introduced species while in Australia, these phytoplasma have been associated with an array of introduced hosts but only two native species.

The four strains of '*Ca.* P. australiense' phytoplasma as per Streten *et al.* (2005b) are:

1. AGYp strain (16 Sr XII-B, *tuf*-Australia I; *rp*-A) - comprising AGY, PDB, PYLC, SLY1, SGP and CBWB, from across Australia;
2. New Zealand I strain (16 Sr XII-B, tuf-New Zealand I; rp-B) – comprising SLY2 and CBRYL, both from south-eastern Qld;
3. New Zealand II strain (16 Sr XII-B, tuf-New Zealand II) – comprising PYL, CLD and SLY, from NZ; and the
4. Mung bean strain (16 Sr XII-B, rp-C ) – comprising MBWB, from north-western Western Australia.

In vineyard surveys in the Buckland Valley, Victoria, during 1995/96, an occurrence of grapevine yellows was found with symptoms typical of AGY but with unusually high levels. Of five vineyards surveyed in the Ovens Valley district in December 1995, three in Merriang South showed vineyard incidence <1% of vines affected whereas in comparison, two vineyards in the near-by Buckland Valley had incidences of 2% and 7 % respectively (Magarey unpublished data). Given the cool viticultural region in which they were located, this inferred either that a different and more severe strain of AGY was present or a vector was present either with greater infectivity or in greater relative abundance than elsewhere. As a result, Gibb *et al.* (1999) undertook PCR-tests and found a 'variant' of AGYp which they termed Buckland Valley grapevine yellows (BVGYp). Later, Constable *et al.* (2002) showed that though BVGYp was more closely related to the Aster Yellows (16 Sr I) group than to '*Ca.* P. australiense' (16 Sr XII), and it probably represents a new group of phytoplasma.

The detection of '*Ca.* P. australiense' and more particularly, the AGYp strain, in plants in the vicinity of vineyards would implicate those species as possible primary host reservoirs of the AGYp. This chapter reports the search for the hosts of '*Ca.* P. australiense' and specifically for AGYp. Success in this would assist in locating the vector(s) of AGY in Australian viticulture.

Vineyard surveys over several previous seasons had shown that vegetation associated with high levels of AGY in adjacent vineyards had potential to host AGYp. At various times, some 20 - 30 PCR-tests had been undertaken for '*Ca.* P. australiense' and/or AGYp in samples selected at random from native and other plants in hot spots for AGY (data not presented). The samples had included a grass *viz.* common reed (Figure 14.1), several chenopod shrubs, and *Eucalyptus* trees. The latter included river box, *E. largiflorens*, a tree which grows in river floodplain environs (Figure 12.4a), areas positively correlated with high AGY. None of the samples tested positive for phytoplasma and the high cost of PCR-tests limited more extensive sampling.

Though AGY is widespread in the Riverland, Sunraysia and Riverina and occurs in nearly every Chardonnay vineyard surveyed, there is an apparent lack of disease in host plants well-known for expressing phytoplasma diseases elsewhere. For example, there has been only a single report of '*Ca.* P. australiense' in periwinkle in Australia (Davis *et al.* 2003) and yet that host is a good indicator of yellows disease and is used as a repository for various yellows pathogens overseas (Langer *et al.* 2004; Marcone *et al.* 1999; Vibio *et al.* 1994). Although periwinkle is not uncommonly planted in domestic and civic gardens across the Riverland, symptoms of phytoplasma (*eg.* phyllody – causing leaf-like petals) has been seen in only a single garden plant at Loxton and that specimen tested PCR-positive but for a phytoplasma similar to TBB (tomato big bud) and not AGYp or '*Ca.* P. australiense' (data not shown). At no time has AGYp been found in these periwinkle elsewhere in southern Australia.

This observation is consistent with the view that AGY is transferred to grapevines from specific areas and that the vector of disease is not usually abundant or at least is not regularly infective to transmit disease over long-distances, though in some seasons this may occur. The host-vector relations of AGYp may also be so specific that it does not include many alternate hosts.

In order to resolve this and in attempt to identify the likely host plants of AGY, PCR-tests were undertaken on a range of native chenopod and other similar plants identified at local sites within hot spots of disease (Chapter 14).

**Aim**

**To identify the primary host plant or plants for AGYp within the hot spot zones of disease.**

**Materials and Methods**

**Sampling for PCR analyses** Detailed surveys for AGY in vineyards of Riverland, Sunraysia and the Riverina (Chapter 13) were used to designate prime sites for AGY. Vegetation surveys (Chapter 14) were used to designate 3-5 chenopod and related woody native plant species from which samples were collected in the Riverland at Puddletown Swamp, near Winkie, from Eckert's Creek, near Berri, from Baker's Lake, Loxton North, and from other locations where AGY occurred at lesser levels, *eg.* from near stands of yanga at Winkie and Loxton. Samples were taken from less precisely designated sites in the Riverina (see Chapter 10). A number of the sites in both regions are illustrated (Figures 10.1, 12.6, 12.8, 12.10, 12.14, 12.7 –12.9, 12.12, 12.13, 12.16 and 12.17).

In March 2005, native plant species with the highest population aggregate scores were sampled from in or near wastelands within AGY hotspots in the Riverland, SA. Several specimens were also taken of non-native species at some of these locations (Table 15.2). Additional samples were collected in May 2005 from similar locations in the Riverina near Griffith, NSW, (Table 15.3).

In selecting the plant samples, preference was given to specimens that showed some evidence of disease, especially sectorial discolouration and death of shoots, similar in general nature to those expressed in AGY (see Chapter 2) or systemic diseases.

**PCR analysis** The samples selected included leaf and stem material from candidate plants. The specimens were placed in plastic bags and kept at 4-5 $^{0}$C prior to dispatch 12 hrs later in an insulated sealed container to Waite Diagnostics, University of Adelaide, Glen Osmond, SA, for analysis. For nucleic acid extraction and PCR analysis, RNeasy Mini columns (Qiagen, Germany) were used to extract total nucleic acids from young stems and leaf veins (MacKenzie *et al.*, 1997). Nested polymerase chain reaction was used to detect AGYp.

First step PCR was carried out using the primers fP1 (Deng & Hiruki, 1991) and rP7 (Schneider *et al.*, 1995). The PCR products from this step were diluted 1:15 in water and subjected to a second PCR using the AGYp specific primers, AUSGYF1 and AUSGYR2, (Davis *et al.* 1997a). These primers were derived from the 16 S ribosomal RNA and gave an AGYp-specific PCR band of 644 bp (Figure 15.1).

**Results**

The PCR-analyses of several native and non-native species in the Riverland are presented in Table 15.2. A total of eight of 88 plants tested positive for AGYp. These included several species of native, woody, perennial Chenopodiaceae of which yanga predominated – AGYp was found in 5 of 48 samples of the latter most of which showed some level of chlorosis (pinking/reddening) of leaves and/or stems with or without sectorial dieback of branches.

Other chenopods which tested positive for AGYp were ruby saltbush (1 of 28 samples) and climbing saltbush (1 of 5) both of which also showed some level of pinking/reddening of leaves and/or stems. No other phytoplasma was observed including TBB (Tomato Big Bud).

The PCR-analyses of native and non-native species in the Riverina are presented in Table 15.3. Only one sample in 56 tested positive for AGYp - that was from a yanga bush that had green leaves on pink stems. Two samples tested positive for TBB. One was ruby saltbush with green leaves on pink stems; the other was tentatively identified as climbing saltbush, having red leaves on reddened stems.

**Table 15.2 Incidence of AGYp in various plant species collected from the Riverland, SA, detected by nested-PCR then using specific primers for AGYp. April 2005.**

| Host | Botanical Name | # Samples | # +ve for AGYp | % with AGYp |
|---|---|---|---|---|
| Yanga bush | *Maireana brevifolia* | 48 | 5 | 10.4 |
| Ruby saltbush | *Enchylaena tomentosa* | 28 | 1 | 3.6 |
| Climbing saltbush | *Einadia nutans* | 5 | 1 | 20.0 |
| False caper | *Euphorbia terracina* [1] | 1 | 1 | 100 |
| Narrow-leaf hopbush | *Dodonaea viscosa* | 1 | 0 | 0.0 |
| Lignum | *Muehlenbeckia florulenta* | 1 | 0 | 0.0 |
| Sudax [1] | | 1 | 0 | 0.0 |
| 'Spiky leafed' plant | *Unidentified* | 1 | 0 | 0.0 |
| **Total** | | **88** | **8** | **9.1** |

Note: [1] A non-native (introduced) plant.

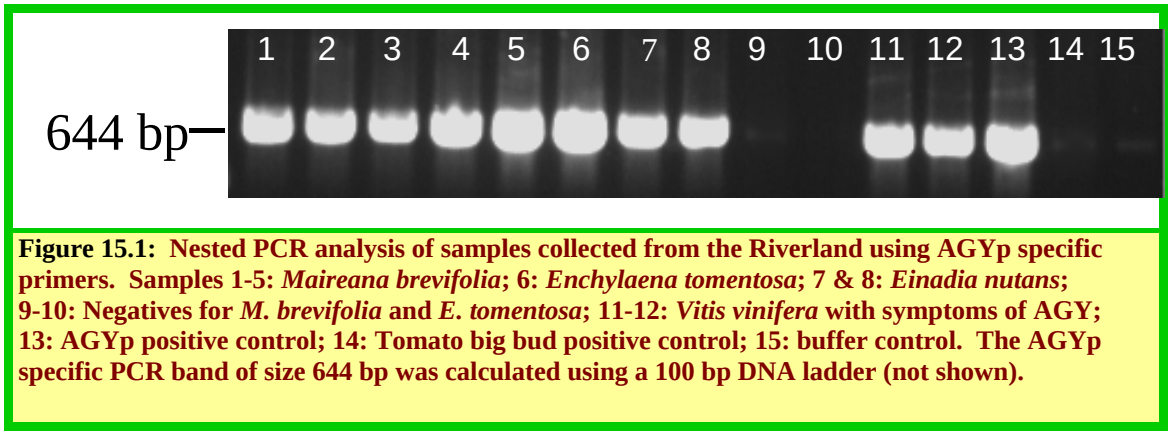



**Figure 15.1: Nested PCR analysis of samples collected from the Riverland using AGYp specific primers. Samples 1-5: *Maireana brevifolia*; 6: *Enchylaena tomentosa*; 7 & 8: *Einadia nutans*; 9-10: Negatives for *M. brevifolia* and *E. tomentosa*; 11-12: *Vitis vinifera* with symptoms of AGY; 13: AGYp positive control; 14: Tomato big bud positive control; 15: buffer control. The AGYp specific PCR band of size 644 bp was calculated using a 100 bp DNA ladder (not shown).**

**Table 15.3 Incidence of AGY and TBB in various plant species collected from the Riverina, NSW, as detected by nested-PCR then using specific primers for AGYp. May 2005.**

| Host | Botanical Name | Sample numbers | # +ve for AGYp | # +ve for TBBp |
|---|---|---|---|---|
| Yanga bush | *Maireana brevifolia* | 33 | 1 | 0 |
| Ruby saltbush | *Enchylaena tomentosa* | 6 | 0 | 1 |
| Climbing saltbush | *Einadia nutans* | 6 | 0 | 1 |
| Wireweed | *Polygonum aviculare* | 1 | 0 | 0 |
| Convolvulus | *Convolvulus spp.* | 2 | 0 | 0 |
| Prickly pear | *Opuntia stricta* [1] | 1 | 0 | 0 |
| Miscellaneous unknown species | - | 7 | 0 | 0 |
| **Total** | | **56** | **1** | **2** |

Note: [1] A non-native (introduced) plant.

## Discussion

PCR-tests were made on the few species of native, perennial chenopod shrubs that remained in contention as primary hosts of AGY after assessing the vegetation in disease hot spots - Table 14.1. These species best fitted the evidence that had been accumulated in seeking to locate the hosts of AGY (see Chapters 2 to 14). The positive PCR-responses from plants in hot spots of AGY across the Riverland and from the single plant in specimens of chenopod and non-riverine species sampled in the Riverina, were therefore of significance.

This is the first record of AGYp in Australian native plants and the first of a strain of '*Ca.* P. australiense' in natives in southern Australia. It raises the prospect that other native plants within hot spots for AGY will also be found with AGYp and that other phytoplasma may be found in *M. brevifolia* and related shrub species.

Walsh *et al.* (2006) stated (seemingly incorrectly) that '*Ca.* P. australiense' has so far, been detected only in introduced plant species when Streten *et al.* (2005c), in investigating alternate hosts of SLY in southern Queensland, had detected '*Ca.* P. australiense' in two native species *viz. Exocarpus cuppressiformis* (native cherry) and *Jacksonia scoparia* (dogwood). The former is a tree and the latter is most commonly a small tree to 4m (Cunningham *et al.* 1992).

Streten *et al.* (2005c) also found TBB (tomato big bud) phytoplasma in several species including *Chenopodium carinatum* (creeping goosefoot) and the native species, *Callitris baileyi* (Bailey's Cypress pine).

The finding of '*Ca.* P. australiense' in a possible *Melilotus species* near Toowoomba (Streten *et al.* 2005c), is of considerable interest. *Melilotus alba* (bokhara clover), a legume, has been recorded from Griffith and Wanganella, NSW, (Cunningham *et al.* 1992) and is a common weed in cotton growing regions ( see Cotton Industry web-site). We observed what appeared to be this species in abundance along irrigation channels at Griffith close to unusually high levels of AGY in a nearby *cv.* Riesling vineyard (Chapter 10). Levels were extreme *viz.* > 70% vines affected and this seemed unaccounted for by the presence of low numbers of chenopod shrubs at that site. In addition, since *M. indica* (hexham scent) is common to riverine vegetation, both species should be further investigated as possible hosts of '*Ca.* P. australiense' and potential feeding sites of the vector(s) of AGY. However, we consider it unlikely that their role as a primary host is significant since these plants are annual forbs and not perennials but they may be associated as secondary hosts of AGYp.

As discussed in Chapter 12, our data from several Riverland vineyards associated a related legume *Medicago sativa* (lucerne), with high levels of AGY. In one vineyard, at Puddletown (see top right in Figure 12.6), AGY and more particularly, SBS (see Chapter 2), were at high levels adjacent to a stand of irrigated lucerne. In a second vineyard, near Glossop, levels of AGY were highest in the corner adjacent to lucerne (Figure 12.13 block 3) and, at a third site in the same district, levels of AGY were high when a stand of lucerne was present while yanga was lacking (data not shown). Additionally, the zone of high disease at Baker's Lake had both a dense stand of yanga and a recently re-invigorated stand of lucerne present (Figure 14.1). Lucerne yellows phytoplasma had been found previously at the Baker's Lake site (Murray Fletcher and Leigh Pilkington, pers. comm.). As a result, further PCR-tests are needed to determine if *M. sativa* is also a host of '*Ca.* P. australiense' and/or AGYp. It could play a significant role as a local source of AGY.

The plant host range for any given phytoplasma will be an expression of the nature of the vector (or vectors) that transmits it and its feeding behaviour (Lee *et al.* 2000, as quoted by Streten *et al.* 2005c). This is likely to also hold true for AGYp. On the basis of surveys for hosts of phytoplasma in south eastern Queensland, Streten *et al.* (2005c) suggested that either the vector of '*Ca.* P. australiense' has a narrow host range or it has a limited number of species susceptible to it. In Australia, '*Ca.* P. australiense' has been found on many hosts in a broad spread of geographical areas. This is true also for the AGYp strain, suggesting that is has long been endemic in the region and perhaps also that either the vector is widespread and feeds on may hosts (*ie.* is polyphagous) or that a number of species transmit the pathogen. Evidence from the vector associations with other yellows and phytoplasma diseases suggests that it is likely that one species will be the main vector though a number of other leafhoppers may occasionally transmit AGYp. Similarly, a number of phytoplasma may at varying and usually low levels also cause AGY symptoms in grapevine and each of these pathogens are likely to be vectored by a different leafhopper or planthopper.

Interestingly, the vector of '*Ca.* P. australiense' has not yet been identified in any crop/plant system in Australia whereas in New Zealand, the planthopper, *Oliarus atkinsoni* (a Cixiid planthopper), transmits the New Zealand strain (Boyce *et al.* 1953; Liefting *et al.* 1997). Since *O. atkinsoni* is a monophagous species *ie.* it feeds only on *Phormium sp.*, and is essentially limited to New Zealand (Liefting *et al.* 1998; Andersen *et al.* 2001), Streten *et al.* (2005c) concluded that it is unlikely to transmit '*Ca.* P. australiense' in Australia. However, similar cixiids may do so, especially given that these planthoppers are sole vectors of the stolbur group overseas (Langer *et al.* 2004).

The presence of native plants PCR-positive for AGYp in hotspots of AGY in the geographically divergent regions of the Riverland and the Riverina indicated the probability that AGYp is transmitted from these hot spots to infect grapevines. This provides further evidence that a leafhopper or a planthopper or other mobile vector transmits the disease.

Evidence in Chapter 3 suggests that grapevine is probably a terminal host of AGY. This is similar to Bois Noir in Germany and France where the planthopper *Hyalesthes obsoletus* spreads the stolbur phytoplasma to grapevine which seems to be a dead end host (Boudon-Padieu 1999 quoted by Mori *et al.* 2002). If this is true for AGY, the phytoplasma AGYp did not spread across southern Australia in grapevine and there must be another reservoir of inoculum across the regions in which the disease has been found.

Of the ten phytoplasma diseases found in Australia and associated with the four strains of '*Ca.* P. australiense', six are listed associated with the AGYp strain (Streten *et al.* 2005c). These seem to be more geographically widespread than the other '*Ca.* P. australiense' strains. For instance, AGYp has been found in south-eastern Queensland causing two diseases on strawberry, in south-eastern Queensland, central Northern Territory and in north-western Western Australian in a disease of pumpkin, in south-eastern Queensland and Western Australia in papaw dieback, and in nearly every viticultural region across the southern half of the Australian continent from Western Australia to Hunter Valley, New South Wales, and to Stanthorpe, Queensland, associated with AGY (data from surveys by the senior author are not shown). In addition, symptomatic *G. physocarpus* were collected at different locations in south-eastern Queensland. This widespread distribution of AGYp suggests that its (supposed) insect vector(s) is/are equally well-distributed across the continent.

This distribution and our finding of AGYp in three Australian native species in Southern Australia coupled with our earlier evidence (Chapter 11), suggests that the AGY phytoplasma may be indigenous (native or naturalised) and that an Australian native plant (or plants) is (are) the primary host (or hosts) of these pathogens. [The occurrence in New Zealand of the

*Phormium* yellows strain of '*Ca.* P. australiense' on many NZ native hosts and the occurrence of '*Ca.* P. australiense' in two native plants in Queensland (Streten *et al.* 2005c), suggests that the group may be native to Australasia though some members have been found outside this region (Gera *et al.* 2005; Jones *et al.* 2005).

It seems probable that yanga bush, ruby saltbush and/or climbing saltbush are the primary host(s) of AGYp. Yanga, and ruby saltbush in particular, are native perennials that rapidly re-colonise recently disturbed soil. They grow well in wasteland environments but do not tolerate tillage while all three species grow to a height of 1m or more when mature (Cunningham *et al.* 1992).

These results support the specific items in the hypotheses that were presented in Chapter 11; in particular:

*Item 1* *the AGY phytoplasma are indigenous (native or naturalised) to the Australasian region and so inhabit native plants; and as a result,*

*Item 2.* *a native plant (or plants) is/are the likely primary host of AGYp;*

*Item 3.* *the primary host of AGY … is a perennial … taller than 0.5 m. …;* and

*Item 9.* *… the source of AGY lie(s) within clearly defined localities (disease hot spots) of dimension not more than 1500 m. x 1500 m.*

Evidence from investigations in subsequent seasons (Chapters 12 and 13) indicated that the source areas for AGY were likely to be localities as small as 100m x 50m and it was in these areas that the native shrubs were found with AGYp.

Although AGYp was associated with native chenopods bearing pink discolouration of leaves and/or stems, the significance of this association with symptoms is not known. It is possible that AGYp will be found in symptomless plants, especially if AGYp is native to Australia.

Despite our evidence above, the possibility that the alternate hypothesis *viz.* that AGYp has been introduced to Australasia, should not be discounted because the organism has to date, been found predominantly in introduced plant species.

In attempt to resolve which hypothesis is correct, more sampling and PCR-testing of native vegetation is needed:

- to confirm the present observations, ie. re-sample the same species of hosts in the same localities for PCR-tests to confirm the findings cited in Tables 15.2 and 15.3, noting that there may be a seasonal difference in the recovery of AGYp from these hosts as apparently occurs in the rate of detection of AGYp from grapevine (Gibb *et al*. 1999) – detectable from November to March with a peak in January and February;
- to determine the likely role of those species as primary hosts for AGYp by sampling from these species in other hot spots and in areas with lesser AGY. For example, one or more of these species may not be infected with AGYp in other areas of high disease and may therefore be a passive carrier of the AGY inoculum (ie. not be involved in the life cycle of AGY) and/or to the contrary, one or more of these plants may prove to be the only species with AGYp within all hot spot zones;
- to sample from other plant species to obtain a better understanding of the array of other species that may be primary host plants for AGY;
- to seek a better understanding of the epidemiology of AGY disease. This information is crucial and should lead to good prospects of finding a control for the disease.

The detection of AGYp in additional species in the vicinity of vineyards would implicate these species as hosts of '*Ca.* P. australiense' and of AGYp in particular. Monitoring these hosts should then provide insight into the identity of the vectors of AGY in Australia since it is likely that the vector of AGYp will feed on one or more of these plants, at least for sufficient time to acquire the pathogen and perhaps also for sufficient time to breed *ie.* complete its own life cycle on that host.

**Conclusions**

Surveys of native vegetation and PCR-tests of suspect host species suggests that:

- three native chenopod shrubs and one introduced plant which tested positive for AGYp viz. yanga bush (short-leafed bluebush) (*Maireana brevifolia*), ruby saltbush (*Enchylaen*a t*omentosa*), climbing saltbush (*Einadia nutans*), and false caper (*Euphorbia terracina*), are the primary host or hosts of AGY in southern Australia;
- this is the first record of AGYp in native species in Australia;
- this is the second report of '*Ca*. P. australiense' in native species in Australia and the first in southern Australia;
- yanga bush is at least one of the main hosts that serve as an inoculum reservoir for AGYp in grapevines – since AGYp was found in 5 of 48 (10.4%) samples of yanga bush in the Riverland and in 6 of 81 (7%) from across all regions;
- AGY phytoplasma are indigenous (native or naturalised) to the Australian and perhaps the Australasian region;
- the insect vector of AGY most likely feeds and/or breeds on one or more of these plant species; and that
- there is good prospect of locating the presumed leafhopper vector of AGY in or near one or more of the host plants identified above.

**Recommendations**

Several points of investigation are recommended for immediate action *viz.*:

- re-sample the same host species in the same localities at the same and different times of the season for PCR-tests for AGYp and other strains of '*Ca*. P. australiense', to confirm the presence of AGYp (and perhaps other strains of '*Ca*. P. australiense') in at least three native and one introduced species;
- sample from the native species in other hot spots and in areas with lesser AGY, to determine their likely role as primary hosts for AGYp;
- sample from other plant species in AGY hot spots, to obtain a better understanding of the array of plants that might be the primary hosts for AGY; and
- at each point, compare the isolates of phytoplasma from grapevine and native species by RFLP-analysis of *tuf / rp* (as per Streten et al. 2005c) to confirm if the strain detected is AGYp or another variant of '*Ca*. P. australiense'; and thus,
- seek a better understanding of the epidemiology of AGY disease which knowledge is critical to good prospects of finding the vector and a control for the disease.


**Figure 15.2: Typical cluster of yanga bush (*Maireana brevifolia*) in a wasteland adjacent to a vineyard with AGY at Loxton, SA. One of these specimens tested positive in PCR-tests for AGY phytoplasma (AGYp) in April 2005.**


**Figure 15.3: Close up view of yanga bush (short-leafed blue bush) which tested positive for AGYp in a wasteland at Loxton, SA, April 2005. Note the pink discolouration in sectors of the plant – it is not known if this is a symptom of AGYp in these plants or if it is a natural or abiotic phenomenon such as a sign of high salinity.**


**Figure 15.4: A stand of yanga bush adjacent to high levels of AGY in vineyards at Puddletown Swamp, near Winkie, SA. The pink discolouration is common at many sites where AGY is at high level nearby but the relevance of this is not known. Some of these plants tested PCR-positive for AGY phytoplasma (AGYp).**


**Figure 15.5: Ruby salt bush (*Enchylaena tomentosa*) with reddened leaf tips typical of the plants which tested positive for AGYp in the Riverland, SA.**


**Figure 15.6: A site of sampling for PCR-tests at Loveday, Riverland, SA, where AGYp was found in an introduced species, false caper (*Euphorbia terracina*). Look in the shrubbery for the yellow sticky insect-trap.**


**Figure 15.7: Typical wasteland setting which Australian native chenopods and similar shrubs colonise easily after the land has been disturbed. It is ecosystems such as this that are positively correlated with high levels of AGY in the vineyards near-by and where specimens PCR positive for AGYp were collected.**

***PCR-positive at last!***

***The primary host of AGY is likely to include the native plants:***

- ***Yanga bush (Maireana brevifolia),***
- ***Two saltbushes:***
  - ***Ruby saltbush (Enchylaena tomentosa)***
  ***and***
  - ***Climbing saltbush (Einadia nutans)***

***among others.***

***This is the first time AGY has been found in native plants.***

***It is time to do a lot more testing of native plants for AGY***

## Section 8. Does an Insect Spread AGY? Searching for a Vector

*Three native plant species were found with AGYp. It seemed likely that these would contribute to at least a portion of the primary reservoir of AGY - that is, of the inoculum from which vineyards are infected. If this were so, the vector of AGY will feed on these plants which would give us the best chance of locating the insects. Evidence inferred that the vector was a leafhopper or a planthopper, but it was important first to establish if it was in fact, a mobile agent such as an insect, and if so, which one(s)? Studies on this and more, are presented here.*

### Chapter 16: The Role of an Insect Vector 1 - A Mobile Vector is Confirmed Studies Using an Insect Exclusion House – 2000/01 to 2005/06

**Introduction**

Leafhopper and/or planthopper insects are implicated as vectors of phytoplasma diseases wherever a vector is known (Tsai 1979; Langer *et al.* 2004). Interestingly for the yellows diseases of grapevine, though many different phytoplasma have been associated, only three natural vectors are known:

1) the leafhopper *Scaphoideus titanus* transmits flavescence dorée (16 Sr V) in France, Italy and elsewhere (Schvester *et al.* 1963, 1969; Vidano 1964; Mori *et al.* 2002);
2) the leafhopper *Oncopsis alni* (Schrank) transmits Palatinate grapevine yellows (16 Sr V) in Germany (Maixner *et al.* 2000); and
3) the planthopper *Hyalesthes obsoletus* Signoret, is vector of Bois Noir (BN) (16 Sr XII) in Germany, France and Italy (Maixner 1994; Sforza *et al.* 1998; Alma *et al.* 2002).

The BN phytoplasma belongs to the stolbur group (Table 15.1) of which there are two sub-groups:

1) '*Candidatus* Phytoplasma solani' (16 Sr XII-A), the phytoplasma taxonomically most closely related to AGYp, the phytoplasma associated with AGY (Schneider et al. 1999); and
2) '*Ca.* P. australiense' (16 Sr XII-B) comprising four strains one of which is AGYp – (see Chapter 15).

The members of the 16 Sr XII group are associated with disease in several crops including grapevine. Significantly, all known vectors of the group are cixiid planthoppers (Family Cixiidae). Of the stolbur (16 Sr XII) group in Australasia, the only known vector is a cixiid, *Oliarus atkinsoni*, which transmits *Phormium* (flax) yellows (16 Sr XII-B) in New Zealand (Liefting *et al.* 1997).

Of the other phytoplasma diseases within Australasia, the leafhoppers *Orosius argentatus*, the common brown leafhopper, and *Batracomorphus angustatus*, the large green jassid, transmit tomato big bud (16 Sr VI-A, TBB) (Hill 1943, Grylls 1979) which has been found in grapevine (Gibb *et al.* 1999). In addition, both insects have been associated with transmitting potato purple top wilt while the latter insect also has been implicated in transmission of pawpaw yellow crinkle and at least one other phytoplasma not yet found in grapevine including lucerne witches' broom (Grylls 1979, Weintraub *et al.* 2006). Thus, considering the vectors of yellows (phytoplasma) diseases of grapevines and other crops both within and outside Australia, the most likely transmitting agent of AGY is an insect vector, and the most likely insect is a leafhopper if not a planthopper.

Earlier studies to identify the vector of AGY had failed to positively link with any insect though the role of the common brown leafhopper, *Orosius argentatus*, was investigated (Osmalek *et al.* 1989). Beanland *et al.* (2002) reported two specimens of *O. argentatus* had tested PCR-positive for AGY. This was the first time an insect tested positive for AGY, but the authors expressed doubt that this species was the major vector of the disease.

Evidence presented in earlier chapters (especially Chapter 15) showed that wasteland vegetation was associated with high levels of AGY in vineyards. Given our findings of AGYp in native chenopod vegetation in or near the hot spots of disease, the hypothesis that the vector of AGY will feed and/or breed on plants such as these and fly from there to infect vineyards nearby, seemed strengthened. To resolve this matter, further tests were needed.

Concurrent with investigations into the source of AGY, we evaluated the above hypothesis in an attempt to determine how the disease was spread *ie.* to investigate the role of a mobile vector such as an insect in transmitting the disease. The field experiment reported here was undertaken at Berri, SA, from 1999/2000 to 2005/06. Early progress in this has been presented in previous reports (Magarey *et al.* 2003, 2005).

**Aim**

**To establish the role of a mobile vector in the spread of Australian Grapevine Yellows within vineyards of the Riverland.**

**Materials and Methods**

An insect exclusion-house was established to surround 50 mature vines comprising two equal rows each of 25 vines in a commercially operated vineyard of *cv.* Riesling trained on a double-wire, vertical trellis at Berri, SA. The vineyard had shown high levels of AGY in the seasons up to and including 1999/00. In May 2000 (during dormancy), the vines were shrouded and sealed within fine-meshed white shade cloth (Figures 16.1 – 16.3a) to prevent or at least significantly restrict the free movement of any insects bigger than thrips, including leafhoppers in particular.

Inside the exclusion house, the vines were placed under a stringent insecticide regime specifically targeting leafhoppers. This involved sprays in both late dormancy and throughout each season that the exclusion house was maintained, *viz.* up to and including 2002/03. The work was undertaken with the assistance of the Riverland Vine Improvement Committee.

**Figure 16.2: Left: Inside the sealed, insect exclusion house at Berri, SA, in August 2000; Right: in October 2000. During the first season (2000/01), levels of AGY were identical inside and out. There-after, levels declined significantly inside and remained low for the next three seasons.**

**Figure 16.3: Left: Site of the insect exclusion house at Berri, SA, October 2000; and Right: after it was removed and vineyard practices returned to normal in 2003/04 (right).**

The exclusion house was removed during dormancy 2003 (Figure 16.3) and normal vineyard practices were recommenced on the two rows of vines previously enclosed.

During 2000/01 and the five subsequent seasons, levels of AGY were surveyed on 100 arms [cordons] of the 50 enclosed vines and on 248 arms on a total of 124 vines outside. The latter comprised 25 vines/row in each of the five rows immediately adjacent to the exclusion house (with one vine missing). The survey recorded the incidence of any AGY on each arm assessed at least once during each season with a final assessment in January-February just prior to harvest (see Chapter 6 for detail). Disease scores were expressed as a percentage of arms that showed any AGY for each block of vines (*ie.* inside *vs.* outside) and these scores were plotted for comparison (Figure 16.4).

To assess the level of insect intrusion into the exclusion house, yellow sticky traps were maintained at four sites along the two rows for the duration of the experiment.

## Results

In the first growing season after the vines were enclosed *viz.* 2000/01, the incidence of AGY was identical inside and outside the exclusion house *viz.* 12% (Figure 16.4). In the second season, the incidence inside the exclusion house decreased significantly ($P<0.05$) to 4%, one fifth the incidence on vines outside *viz.* 21%. Thereafter, levels inside remained significantly lower (≤5% arms affected).

On the vines outside the exclosure, the incidence of AGY remained at higher levels and over a three year period, varied significantly ($P<0.05$) from 16% - 23% until 2004/05, the first season after the vines were re-exposed. In that season the incidence outside dropped significantly such that the difference in the levels was only just significantly higher (at $P<0.05$) than inside the exclusion house *ie.* 11% outside *vs.* 5% inside. This trend was repeated in season 2005/06 when the incidence outside declined further to be identical with the incidence on the previously shrouded vines.

No leafhopper was observed on the yellow sticky traps within the exclusion house though smaller insects such as thrips, were present. A number of flies, mosquitoes and other non-Hemiptera insects were present and in different seasons, light brown apple moth (LBAM) and mealy bug each appeared in plague proportions. The LBAM pupated within the exclusion house and the parasitoids and predators of mealy bug were either killed by the spray regime or excluded by the fine meshed cloth of the exclusion house. Both events bore dramatic testimony to the efficacy of biological control for each pest!

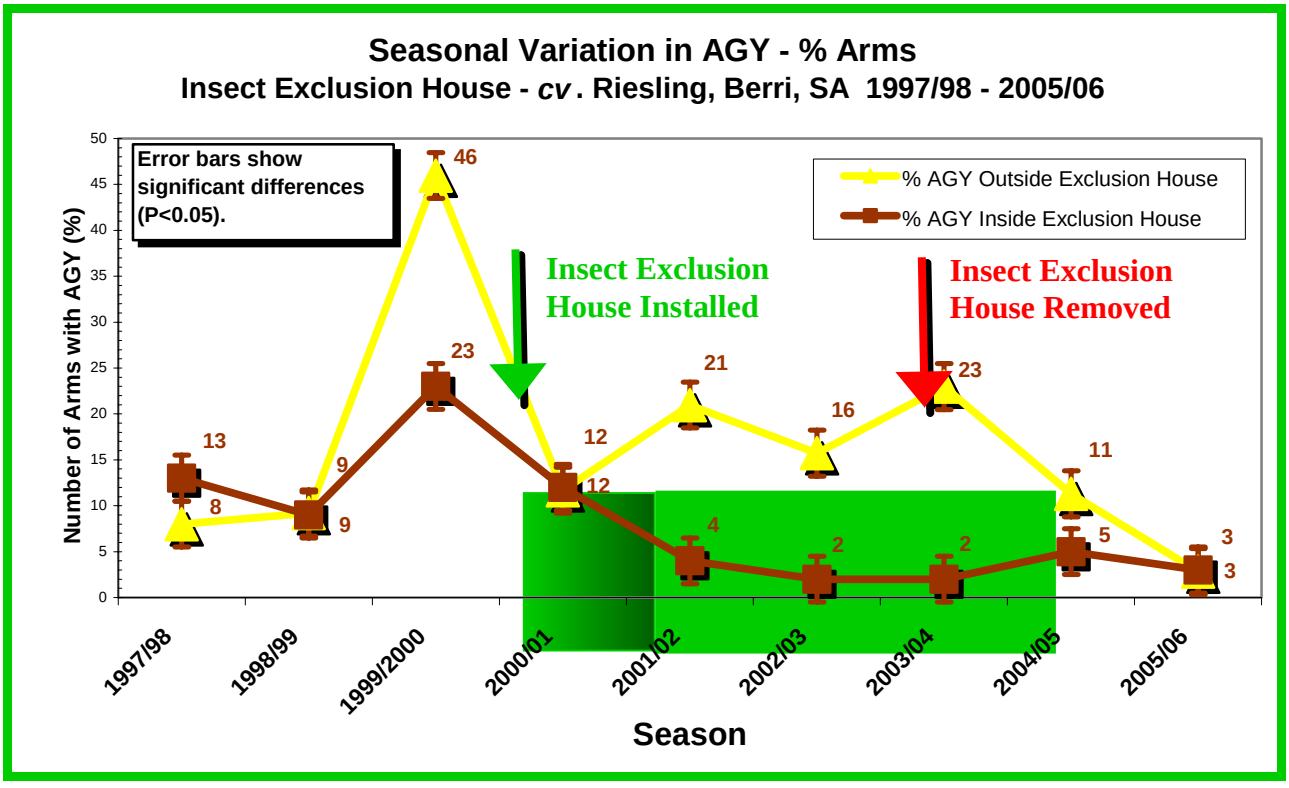



**Figure 16.4 The decline in incidence of AGY on arms (cordons) inside an insect exclusion house established in a cv. Riesling vineyard, Berri, SA, 1997/98 – 2005/06. The shaded area shows the time of influence of the exclusion house:**
**Light green = No effect evident because vines were inoculated the season before symptoms show.**
**Dark green = Effect continues for one season after the house is removed.**

**Discussion**

The shrouding of a segment of the vineyard with insect-excluding cloth led to confirmation of some aspects of the epidemiology of AGY and provided interesting clues on others.

For instance, the identical incidence of AGY inside and outside the exclusion house in 2000/01 *ie.* in the first season after the vines were enclosed, indicated several truths:

- First, neither the shade cloth *per se*, nor the resultant minor change in the microclimate within the exclosure, *ie.* slightly raised temperature and higher humidity (data not shown), nor any other factor, inhibited expression of disease. This gave credence to our test of the hypothesis that the exclusion house would serve as a physical barrier to exclude a potential mobile vector for the disease.
- Second, a predisposing factor for AGY disease, such as the presumed phytoplasma pathogen of AGY (AGYp), was present within the vines before the exclusion house was erected *ie*. the pathogen must have been introduced at least in season 1999/2000 and it then over-wintered in the vines. This concept was discussed in Chapter 5 where it was concluded that the disease causing agent probably survived in established woody vine tissue such as diseased cordons rather than in annual growth such as canes.
- Third, as a rider to the above, the expression of symptoms did not require insect activity in early season 2000/01 *ie.* in the spring and early summer immediately prior to symptom expression, but this must have occurred sometime in the previous season, or earlier.
- Fourth, consequently, the incubation period of AGYp within the grapevine is at least 7-8 months, probably more, prior to symptoms appearing reasonably suddenly from flowering onwards from late October through early November. That is, incubation must have commenced late last season at the earliest (*ie*. in March or April) - see discussion later in this chapter. We have no evidence that precludes an incubation period which might even exceed 12 months; and
- Lastly, Gibb *et al.* (1999) found that AGYp were detected most frequently in vines in the Sunraysia, in February. One possible explanation for their finding is that there was an higher titre of AGYp in the vines at that time. If so, it is reasonable to suppose that the titre of phytoplasma similarly might be high at that time in other plants such as the native chenopods which tested PCR-positive for AGYp – see Chapter 15. If this were so, it is reasonable then also to suppose that, in the native AGY patho-system, this would be the optimum time for a vector to acquire the pathogen. Thus it is possible that an insect that acquired the pathogen in mid-late summer, might become infective within several weeks and transmit the disease in February – March in the season prior to symptom expression in the vines. If this holds true, inoculation may have occurred in at least by February – March 2000.

In studies of the physiology of grapevines at Wagga, NSW, Hackett (pers. comm. 2006) removed all leaves from various plots of *cv.* Chardonnay in January. He found that 9.1% of 1152 untreated control vines showed symptoms of AGY in the following season, whereas in the leaf removal treatments, none (0%) of 36 vines showed symptoms the next season. This indicated that leaf removal had a significant ($P<0.05$) effect on the expression of AGY and suggest several options of interest with regard to the timing of the feeding of the supposed insect vector of AGY:
Option 1). the vector fed on the vines and inoculation occurred prior to January - the phytoplasma had insufficient time to be transferred beyond the foliage and were removed with the foliage;
Option 2). the vector fed after January and the lack of foliage on treated vines prevented inoculation; and less likely,

Option 3). the lack of reserves or some other physiological factor within bare vines prevented symptom expression.

While this experiment was inconclusive in resolving these options, it showed the potential of further related experiments in which leaf removal (or the shrouding of vines) at different times could be used to determine when inoculation by the vector of AGY occurs.

In our experiments, in the second season (2001/02), the incidence of AGY declined significantly to ≤4% and thereafter remained low. This suggested that the physical presence of the exclusion house was instrumental in reducing the level of disease and indicated the role of a mobile vector bigger than thrips in the expression of AGY within vineyards. Given the strong association of leafhoppers and/or planthoppers as vectors of other yellows diseases and of phytoplasma diseases in general (see above), this work gave strong supportive evidence of a leafhopper vector for AGY.

The outcomes of experiments in Queensland with PDB (pawpaw dieback, associated with AGYp – see Chapter 15), corroborates this finding. Elder *et al.* (2002) demonstrated total control of PDB using insect exclusion houses constructed with two fine-mesh cloths similar to that used in the present experiments while Walsh *et al.* 2006 used similar exclosures of three grades of mesh and demonstrated the complete success of exclosures with the two finer mesh cloths and good control with the coarsest mesh cloth. These experiments gave further credence to the idea that both AGY and PDB, considered the same strain of 16 Sr XII-B phytoplasma as AGYp (*viz.* 16 Sr XII-B, *tuf*-Australia I; *rp*-A - Streten *et al*. 2005b), are transmitted by a leafhopper or a planthopper.

Data in Figure 16.4 show the progression in number of diseased arms inside and outside the exclusion house. Field observations revealed that the AGY-affected arms occurred on vines that were scattered across the vineyard (see Figure 8.1). This is likely the pattern of disease that directly reflects the infection sites, hence the feeding and inoculation sites of the vector. If so, the survey methodology that recorded the fine detail in scoring individual arms of each vine for presence or absence of disease, also provides a good descriptor of the likely feeding activity of the vector in the vineyard. The reduced number of diseased arms on vines within the exclusion house would therefore reflect a reduction in insect vector feeding on the shrouded vines.

Analyses of the data from the above experiment gave opportunity to gain insight into aspects of the epidemiology of AGY. Two levels were investigated.

1. The Vine as a Survey Unit: Analyses of the spatial and temporal occurrence of diseased vines during the time in which they were enclosed in the exclusion house was reviewed in relation to disease expression on individual vines (Table 16.1). These showed that 24% of the 50 enclosed vines expressed symptoms in at least one season. None was diseased for all seasons of observation.

Of the eight vines that expressed AGY in the first season within the exclusion house, three (37%) were diseased in more than one season *ie*. they exhibited some level of 'recurring' disease, while six others (16%) of the remaining 38 vines first showed symptoms only in the second or third season of influence of the exclusion house. However, because there was negligible likelihood of insects re-inoculating vines during the time the vines were shrouded (no leafhoppers were found on sticky traps inside the house, whereas they were recorded on vines outside the house), it was likely that these expressions of disease were the result of varying titre of AGYp within these vines.

**Table 16.1 The occurrence of AGY on arms of vines shrouded by insect excluding shade-cloth then exposed in a commercial vineyard at Berri, SA, 2000/01 – 2005/06.**

| | Vine # | Arm[1] | 2000/01[2] | 2001/02[3] | 2002/03 | 2003/04 | 2004/05 | 2005/06 |
|---|---|---|---|---|---|---|---|---|
| Row 2 | 11 | N | AGY | | | | | |
| | | S | AGY | | | | | |
| | 13 | N | AGY | | | | | |
| | | S | AGY | | | | | |
| | 17 | S | AGY | | | | | |
| | 19 | S | | AGY | | | | |
| | 22 | N | | AGY | | AGY | AGY | |
| | | S | | | | | AGY | |
| | 26 | N | AGY | | AGY | | AGY | |
| | | S | AGY | | | | AGY | |
| | 28 | S | | AGY | | | | |
| | 33 | S | AGY | AGY | | | | |
| Total # | Arms | | 8 | 4 | 1 | 1 | 4 | Nil |
| | Vines | | 5 | 4 | 1 | 1 | 2 | Nil |
| Row 3 | 11 | S | AGY | | | | | |
| | 16 | S | | | AGY | | | |
| | 22 | N | | | | AGY | | |
| | | S | AGY | | | | | |
| | 23 | S | | | | | AGY | AGY |
| | 25 | S | | | | | | AGY |
| | 31 | N | AGY | | | | | |
| | | S | AGY | | | | | |
| | 33 | N | | | | | | AGY |
| Total # | Arms | | 4 | Nil | 1 | 1 | 1 | 3 |
| | Vines | | 3 | Nil | 1 | 1 | 1 | 3 |
| Total # | Arms | | 12 | 4 | 2 | 2 s | 5 | 3 |
| | Vines | | 8 | 4 | 2 | 2 | 3 | 3 |

[1] N = North, and S = South arm of vines.

[2] **Light green = No effect of the exclusion house because vines were inoculated one season before symptoms showed.**

**AGY** = Arms expressed AGY symptoms in the **first season** inside the exclusion house.

[3] **Dark green = The period of influence of the exclusion house continued for one season after the house was removed.**

**AGY** = Arms expressed AGY symptoms **only after the first season** inside the exclusion house.

That is, in any season that the levels of the AGY pathogen dropped below a threshold level, symptoms were no longer expressed. These vines remained infected but symptomless (latent infection) until the titre then increased above the presumed threshold and symptoms 'reappeared' for one or more subsequent seasons.

Five of eight vines diseased in the first season within the exclusion house (*ie.* 63% of total) showed full remission of symptoms, *ie.* not latent infection. This was consistent with observations of the season-to-season level of remission of symptoms on vines elsewhere in our field surveys. The reductions in symptom expression, *viz.* remission or latency, are likely the result of the combined effect of natural heat therapy and host hypersensitivity (Chapters 2 and 4, and Figure 2.30 and 2.30a).

Greater precision in understanding the influence of these factors on the titre of the AGY pathogen could be gained through analysis of the large body of vineyard data available from the surveys previously undertaken (Chapters 6-13). Analyses of the temporal and spatial distribution of both diseased vines and diseased arms would facilitate a better understanding of the frequency of disease remission, latency and recurrence *etc*, which in turn would lead to greater knowledge of the epidemiology of disease. For instance, comparison of the levels of recurrence of AGY within a vine compared to the relative levels of new inoculations would give valuable knowledge of when vectors were active in vineyards.

Given the pattern of repeated symptom expression of AGY on the vines within the exclusion house and that the exclusion house was highly effective in preventing new infections, it seems plausible that all symptoms on these vines resulted from infection prior to season 2001/02.

This supports the view that:

- a mobile vector of AGY such as a leafhopper is vector of the disease; that
- the number of diseased vines in a vineyard at any one time, comprises a portion of vines with recurring (latent) disease and a portion newly inoculated; and that
- the relative levels of recurring disease *vs* newly expressing disease varies from season to season; and that
- the level of insect activity is similarly variable and likely accounts for the greatest increases in incidence of AGY in vineyards as seen for example, in the peaks in disease incidence in Figures 7.1 – 7.8.

The pattern of expression of symptoms on vines within the exclusion house was consistent with earlier experiments in which tetracycline antibiotic (Terramycin®, oxytetracycline-hydrochloride) was pressure-injected into diseased vines (Magarey *et al.*, 1986b). In that work, assessment of the incidence of AGY in vines was also made at two levels. For instance, Figure 2.35 illustrates the reduction in the number of diseased vines and Figure 16.5 shows the reduction in the number of diseased arms in the same trial (Magarey *et al.* unpublished data). The latter level of assessment allows direct comparison with the present investigation for the period when the number of diseased arms declined within the exclusion house. There is a strong resemblance between the rate and extent of decline in disease in Figure 16.4 (effect from excluding insects) and Figure 16.5 (effect from removing the pathogen).

From these two graphs, it seems reasonable to suggest that the minor variations in incidence in AGY on a season to season basis could be related to variations in pre-existing titre of AGYp within vines (latent infection) and that only the major increases are related to insect vector activity transmitting disease and adding new inoculum. If this holds true, the leafhoppers might well fly and introduce significant levels of new disease only in occasional seasons. These events would be evidenced by higher levels of disease in vineyards in the season following.

For example, in Figure 16.4, the peak in incidence of AGY in season 1999/2000 might be attributed largely to new infections from insect vector activity in the previous season whereas the subsequent variations might be attributed largely to within vine fluctuations in titre of AGYp and in Figure 16.5, the peak in 1983/84 suggests that the insects flew in higher numbers or were otherwise highly infective, in the previous season. Further analyses of these data would prove valuable in this regard and would lead to possible resolution of the environmental factors associated with insect vector activity.

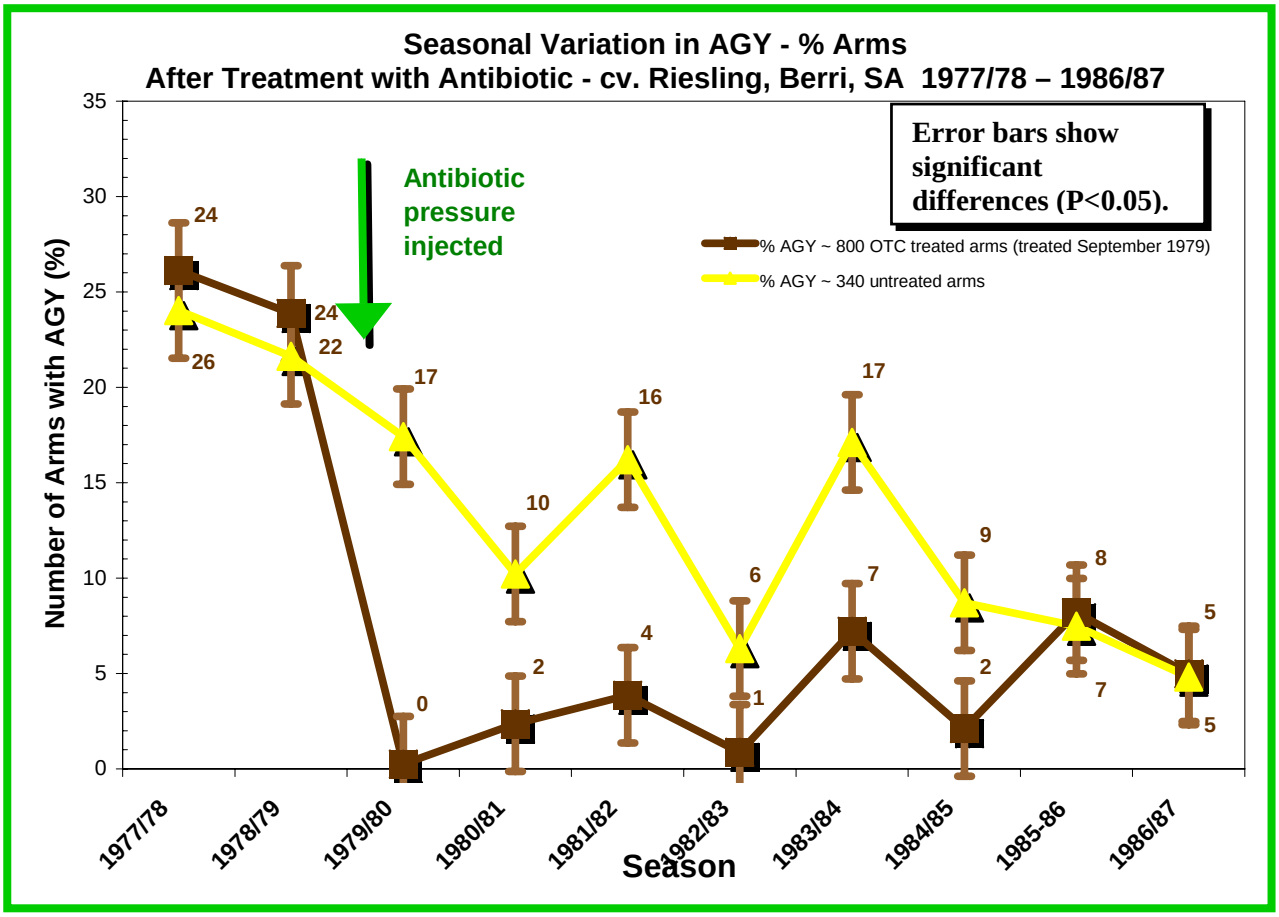



**Figure 16.5: The effect of antibiotic treatment of AGY-affected vines on the incidence of disease in a *cv.* Riesling vineyard at Berri, SA 1977/78 to 1986/87. The response lasted six seasons though the residues of antibiotic remained detectable for only ~30 days.**

2. The Arm as a Survey Unit: In attempt to identify better the 'within-vine' fluctuation in titre of AGYp, the survey data from vines within the exclusion house were re-analysed in relation to disease expression on individual arms (Table 16.1).

Symptom remission: Of 8 vines (12 arms) that expressed AGY in the first season within the exclusion house, half (four vines) were diseased on both arms - of these, three recovered fully the next season while the fourth expressed symptoms consistently, albeit in alternate seasons. The other half (four vines) were diseased on only one arm – of these arms, three recovered fully the next season and the fourth recovered after the second season inside the exclusion house. Thus, of the 8 vines diseased in the first season, seven (88% of vines) and 11 of 12 arms (92% of arms) recovered *ie.* showed remission of symptoms in the absence of insects.

Symptom recurrence: Of three arms that expressed some level of recurring symptoms, one was diseased only in the first and second season. Of the other two, both were diseased only in the fourth season having then been 're-exposed' to insect feeding for one season.

New symptoms: Nine additional arms expressed AGY only after the first season. Of these, five (56%) expressed symptoms in the period 2001/02 and 2003/04 *viz.* the period in which the exclusion house is likely to have had an effect in preventing insects from feeding on the vines. The other four (44%) expressed symptoms first only after they were re-exposed to insect feeding.

Although the number of occurrences was low and a statistically rigorous conclusion is not able to be drawn from the above data, it seems more likely that the 'newly diseased' arms within the exclusion house were inoculated previously (ie. prior to the exclusion house being established) and as expressed above, only showed AGY as the titre of the pathogen within the vine oscillated above a threshold for symptom expression viz. they might have been the consequence of a resurgence latent infection as the titre in those arms increased following a temporary remission of symptoms. This was true at least for 56% of those arms above.

To further investigate this possibility, data for two time periods from the two plots *viz.* inside and outside the exclusion house, were presented against the number of seasons in which symptoms were expressed on each arm (Tables 16.2 and 16.3).

**Table 16.2. Comparison of the number of AGY-affected arms on *cv.* Riesling vines inside or outside an insect exclusion-house in a vineyard at Berri, SA. 2000/01 to 2003/04 [1]**

| # Seasons with AGY | # Arms Outside | % Total | # Arms Inside | % Total |
|---|---|---|---|---|
| **0** | **113** | **46 a** | **83** | **83 b** |
| **1** | **102** | **41 a** | **14** | **14 b** |
| **2** | **24** | **10 a** | **3** | **3 b** |
| **3** | **9** | **4** | **0** | **0** |
| **4** | **0** | **0** | **0** | **0** |
| | **248** | **100** | **100** | **100** |

Note: [1] Because symptoms appear in the season after vines are inoculated, seasons 2000/01 to 2003/04 constitute the period when the influence of the insect exclusion house was operative in preventing new infection.
[2] Different letters in rows denote significant differences ($X^2_2 < 0.05$).

**Table 16.3. Comparison of the number of AGY-affected arms on *cv.* Riesling vines inside or outside an insect exclusion-house in a vineyard at Berri, SA. 2000/01 to 2005/06**

| # Seasons with AGY | # Arms Outside | % Total | # Arms Inside | % Total |
|---|---|---|---|---|
| **0** | 96 | **39 a** | 79 | **79 b** |
| **1** | 108 | **44 a** | 16 | **16 b** |
| **2** | 30 | **12 a** | 3 | **3 b** |
| **3** | 12 | **5 a** | 2 | **2 a** |
| **4** | 2 | **1** | 0 | **0** |
| **5** | 0 | **0** | 0 | **0** |
| **6** | 0 | **0** | 0 | **0** |
| | **248** | **100** | 100 | **100** |

Note: [1] Seasons 2000/01 to 2003/04 constitute the period when the influence of the exclusion house was operative, and seasons 2004/05 to 2005/06 constitute the period in which the vines were re-exposed to insect feeding.
[2] Different letters in rows denote significant differences ($X^2_2 < 0.05$).

Table 16.2 showed that for each category for which analyses were possible in the three seasons following 2000/01, thus including the three seasons in which the exclusion house was operative in reducing the access of leafhopper vectors to the shrouded vines, the number of diseased arms was significantly higher ($P<0.05$) on vines outside the house than inside. This comprised more arms diseased in each category *viz.* arms diseased for one season only, for any two seasons or for any three seasons. Table 16.3 showed that in the period comprising both the above duration and the following two seasons in which the vines were re-exposed to insect activity and thus to new infection, there was little change in the relative proportion of arms diseased – again there were significantly fewer ($P<0.05$) diseased arms recorded in the plot that inside exclusion house.

But if the insects were actively transmitting AGY when the vines were re-exposed, it would be expected that there would be a similar increase in the number of newly diseased arms in both plots. To assess this, a more detailed comparison of data for the two vineyard plots was made from 2003/04 (the last season influenced by the exclusion house) to 2004/05 and 2005/06 (the first and second seasons influenced by the re-exposure to insect activity) (Table 16.4).

In the first season in which the vines were re-exposed (*viz.* 2004/05), there were 15 arms outside the exclusion house that newly expressed disease. This was 13.2% of 113 arms previously symptomless for the duration of our trial. This was a significantly higher ($P<0.05$) percentage in comparison with the new disease seen on only two (2.4%) of 83 arms that were within the exclusion house plot. In 2005/06, there were equally low numbers of new infections for both plots *viz.* 2.5% arms on vines in the exclusion house plot and 2.0% on vines on the outside plot. This evidence suggests that there was little insect vector feeding activity in the two seasons of re-exposure of the vines and raised the possibility that the high levels of new disease in the exposed plot was the result of insect vector feeding in the second season prior to symptom expression *ie.* in 2002/03. Thus, AGYp might have an incubation period within vines as long as 19 - 20 months or more.

In the first season in which the vines were re-exposed (*viz.* 2004/05), 13 (9.6%) of 135 arms in the outside plot and (17.6%) of arms in the inside plot expressed symptoms on arms previously diseased. In the second season, there was less disease but similar relative levels on previously diseased vines *viz.* 3.3% of arms in the outside plot and 5.3% arms within the inside plot. These levels are not significantly different from the levels of new symptoms as outlined previously and this finding concurs with that above, *ie.* that the level of new symptoms and probably also of feeding by the insect vector, was low in both seasons.

In summary, during the two seasons for which the vines were re-exposed, most 'new' expressions of AGY appeared on previously diseased arms. It was thus likely that most symptoms appeared as a result of latent infection as the titre of the AGY pathogen fluctuated above and below a threshold for symptoms on previously diseased arms. This suggests that, at least in seasons 2004/05 and 2005/06 *viz.* the seasons in which the previously shrouded vines were re-exposed to insect activity, the vector was not active in the vineyard and few new inoculations occurred and that. Observations in other vineyards suggest that this also occurred in these seasons across the Riverland region.

The numbers of vine replicates used in the antibiotic experiment (Figure 16.5) was higher than deployed in the insect exclosure experiment. Thus the data set from that experiment offers potential for detailed analysis of the relative level of new expression of AGY on arms *vs* the levels of repeat symptom expression where carry-over of inoculum has been substantially reduced by the effect of antibiotic. Along with analyses of the mapping data from the annual disease surveys undertaken over many seasons in commercial vineyards as presented in earlier chapters (Magarey, unpublished data sets – see Chapters 6-13), this should provide a better understanding of the epidemiology of AGY and of the biology of the insect vector in terms of the seasons in which leafhopper activity might be identified and the frequency of flights. This would assist definition of factors such as temperature degree-day scores associated with vector feeding and disease expression in terms of remission of symptoms and longevity of inoculum in vines.

**Table 16.4. Comparison of the number of seasons in which AGY expressed symptoms on arms of 124 *cv.* Riesling vines outside *vs* 50 vines inside an insect exclusion-house in a vineyard at Berri, SA, from 2000/01 to 2005/06 [1].**

| | 2003/04 | | | | | | | | | 2004/05 | | | | | | | | | 2005/06 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Arm # | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 | Row # | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 | Row # | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |
| 1 | 3 | 3 | 0 | 1 | 0 | 0 | 0 | 0 | | 0 | 3 | 3 | 1 | 1 | 0 | 0 | 0 | 0 | | 0 | 3 | 3 | 1 | 1 | 0 | 0 | 0 | 0 |
| 2 | 0 | 1 | 0 | 3 | 0 | 0 | 0 | 0 | | 0 | 0 | 1 | 1 | 3 | 0 | 0 | 0 | 0 | | 0 | 0 | 1 | 1 | 3 | 0 | 0 | 0 | 0 |
| 3 | 0 | 1 | 1 | 3 | 0 | 0 | 1 | 0 | | 0 | 0 | 1 | 1 | 4 | 0 | 0 | 1 | 0 | | 0 | 0 | 1 | 1 | 4 | 0 | 0 | 1 | 0 |
| 4 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | | 0 | 1 | 1 | 0 | 2 | 0 | 1 | 1 | 0 | | 0 | 1 | 1 | 0 | 2 | 0 | 1 | 1 | 0 |
| 5 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | | 0 | 1 | 1 | 1 | 2 | 0 | 0 | 0 | 0 | | 0 | 1 | 1 | 1 | 2 | 0 | 0 | 0 | 0 |
| 6 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| 7 | 1 | 2 | 0 | 0 | 0 | 0 | 1 | 0 | | 0 | 1 | 2 | 0 | 1 | 0 | 0 | 1 | 0 | | 0 | 1 | 2 | 0 | 2 | 0 | 0 | 1 | 0 |
| 8 | 1 | 2 | 0 | 1 | 0 | 0 | 1 | 0 | | 0 | 1 | 2 | 0 | 1 | 1 | 0 | 1 | 0 | | 0 | 1 | 2 | 0 | 1 | 2 | 0 | 1 | 0 |
| 9 | 1 | 1 | 1 | 1 | 2 | 0 | 0 | 0 | | 0 | 2 | 1 | 1 | 1 | 2 | 0 | 0 | 0 | | 0 | 2 | 1 | 1 | 1 | 2 | 0 | 0 | 0 |
| 10 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
| 11 | 1 | 3 | 0 | 0 | 1 | 0 | 0 | 0 | | 0 | 2 | 3 | 0 | 0 | 1 | 0 | 0 | 0 | | 0 | 2 | 3 | 0 | 0 | 1 | 0 | 0 | 0 |
| 12 | 1 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | | 0 | 1 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | | 0 | 1 | 2 | 0 | 0 | 1 | 0 | 0 | 0 |
| 13 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 |
| 14 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 |
| 15 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 16 | 2 | 1 | 3 | 0 | 1 | 0 | 1 | 0 | | 0 | 3 | 1 | 3 | 0 | 1 | 0 | 1 | 0 | | 0 | 3 | 1 | 3 | 0 | 1 | 0 | 1 | 0 |
| 17 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| 18 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 0 |
| 19 | 3 | 0 | 2 | 1 | 0 | 0 | 0 | 0 | | 0 | 3 | 0 | 2 | 1 | 0 | 0 | 0 | 0 | | 0 | 3 | 0 | 2 | 1 | 0 | 0 | 0 | 0 |
| 20 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 |
| 21 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 22 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 23 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| 24 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| 25 | 0 | 2 | 1 | 1 | 0 | 1 | 2 | 0 | | 0 | 0 | 2 | 1 | 1 | 0 | 1 | 3 | 0 | | 0 | 0 | 2 | 1 | 1 | 0 | 1 | 3 | 0 |
| 26 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| 27 | 0 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 0 | 0 |
| 28 | 0 | 2 | 3 | 0 | 1 | 0 | 0 | 0 | | 0 | 1 | 2 | 3 | 0 | 1 | 1 | 0 | 0 | | 0 | 1 | 2 | 3 | 0 | 1 | 2 | 0 | 0 |
| 29 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 |
| 30 | 1 | 1 | 1 | 0 | 2 | 0 | 0 | 0 | | 0 | 1 | 1 | 1 | 1 | 3 | 0 | 0 | 0 | | 0 | 1 | 1 | 1 | 1 | 3 | 0 | 0 | 0 |
| 31 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 |
| 32 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | | 0 | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 0 | | 0 | 0 | 0 | 2 | 0 | 1 | 1 | 0 | 0 |
| 33 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 |
| 34 | 0 | 1 | 0 | 0 | 0 | 0 | 2 | 0 | | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 3 | 0 | | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 3 | 0 |
| 35 | 0 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 0 | 0 |
| 36 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 37 | 1 | 1 | 1 | 0 | 2 | 0 | 0 | 0 | | 0 | 1 | 1 | 1 | 0 | 2 | 0 | 0 | 0 | | 0 | 1 | 1 | 1 | 0 | 2 | 0 | 0 | 0 |
| 38 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| 39 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | | 0 | 2 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | | 0 | 2 | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| 40 | 1 | 2 | 1 | 0 | 1 | 0 | 0 | 0 | | 0 | 1 | 3 | 1 | 0 | 1 | 0 | 0 | 0 | | 0 | 1 | 3 | 1 | 0 | 1 | 0 | 0 | 0 |
| 41 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 |
| 42 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 43 | 2 | 1 | 0 | 2 | 1 | 1 | 0 | 0 | | 0 | 2 | 1 | 1 | 2 | 1 | 1 | 0 | 0 | | 0 | 2 | 1 | 1 | 2 | 1 | 1 | 0 | 0 |
| 44 | 2 | 2 | 0 | 0 | 0 | 1 | 0 | 0 | | 0 | 2 | 2 | 0 | 0 | 0 | 1 | 0 | 0 | | 0 | 2 | 2 | 0 | 0 | 0 | 1 | 0 | 0 |
| 45 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| 46 | 1 | 1 | 2 | 1 | 1 | 0 | 0 | 0 | | 0 | 1 | 1 | 2 | 1 | 1 | 0 | 0 | 0 | | 0 | 1 | 1 | 3 | 1 | 1 | 0 | 0 | 0 |
| 47 | * | 1 | 2 | 1 | 1 | 0 | 0 | 0 | | 0 | * | 1 | 2 | 2 | 1 | 0 | 0 | 0 | | 0 | * | 1 | 2 | 2 | 1 | 1 | 0 | 0 |
| 48 | * | 1 | 1 | 0 | 1 | 0 | 2 | 0 | | 0 | * | 1 | 1 | 0 | 1 | 0 | 2 | 0 | | 0 | * | 2 | 1 | 0 | 1 | 0 | 2 | 0 |
| 49 | 1 | 2 | 2 | 2 | 3 | 0 | 0 | 0 | | 0 | 1 | 2 | 3 | 2 | 3 | 0 | 0 | 0 | | 0 | 1 | 2 | 4 | 2 | 3 | 0 | 0 | 0 |
| 50 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**Note:** 0, 1, 2, 3, and 4, = # arms expressing AGY in any zero, one, two, three or four seasons of the study from 2000/01 to 2005/06 where each vine comprised two arms *viz.* arm # 1, 2, 3, 4, 5, & *etc* down the columns.

Table 16.4 illustrates some of these trends showing that most vines are diseased for a few seasons and few vines are diseased for most.

This knowledge is likely to greatly assist our understanding of the activity and dispersal of the leafhopper vector for AGY and as a result, for possible control measures and/or management options – see Chapter 19.

**Conclusions:**

- Levels of AGY within an insect exclosure were significantly less ($P<0.05$) than in an adjacent vineyard plot, providing the first experimental evidence of a mobile vector for AGYp in viticulture;
- This work affirmed the project hypothesis that AGY is vectored by an insect such as a leafhopper or a planthopper; and
- Further detailed analyses of extensive vineyard mapping data will be useful in identifying aspects of the biology of the vector of AGY and in gaining an understanding of the disease in grapevines – knowledge that is essential for the development of a management strategy for AGY.

**Recommendations**

It is recommended that:

- investigations in pursuit of a leafhopper and/or planthopper vector of AGY be undertaken, preferably utilising modern PCR technology;
- an extensive database of vineyard spatial and temporal disease assessment scores be further analysed to elucidate details of the epidemiology of AGY disease and the biology of the presumed leafhopper or related vector of AGY.

***The insect exclusion house worked.***

***It showed that AGY is spread by a mobile vector***

***very likely a leafhopper or a planthopper***

***Detailed analyses of vineyard mapping data will show much about AGY and how it spreads and survives in the vineyard***

***It is time to do these analyses now***

# Chapter 17: The Role of an Insect Vector 2 - Sweep-netting for Leafhoppers Studies of the Common Brown Leafhopper – 2002/03

## Introduction

The findings to date indicated the probability that native plants such as yanga bush and saltbush were the primary hosts of AGY (Chapter 15) and more recently, that the vector of AGY was an insect, a leafhopper or a planthopper (Chapter 16). The common brown leafhopper, *Orosius argentatus*, (Evans) (Hemiptera: Cicadellidae), is widespread in the regions where AGY occurs and had been investigated as a potential vector of the disease in several previous studies (Osmalek *et al.* 1989; Beanland *et al.* 2002). The lack of strong evidence to implicate this leafhopper left unanswered the question: 'Was *O. argentatus* the missing link in AGY epidemiology?'

The vectors of all other phytoplasma diseases in which the epidemiology and transmission biology have been determined are phloem-feeding Hemiptera, either leafhoppers (Cicadomorpha: Cicadellidae) or planthoppers (Fulgoromorpha: Fulgoroidea). As presented in Chapter 15, Flavescence dorée in France is transmitted by the leafhopper *Scaphoideus titanus* Ball (Cicadellidae: Deltocephalinae: Scaphoideini) (Alma 2002) while Bois Noir (BN, also known in Germany as Vergilbungskrankheit, VK), is transmitted by the planthopper *Hyalesthes obsoletus*, Signoret (Cixiidae) (Maixner 1994). *Phormium* (flax) yellows in New Zealand is caused by the same phytoplasma as AGY (though a different strain) and is transmitted by another cixiid, *Oliarus atkinsoni* (Ushiyama *et al.* 1969).

The pattern of varying severity of AGY within vineyards and of the incidence between vineyards showed a positive correlation with wasteland vegetation associated with the irrigation overflows, swamps and the like. Our previous surveys suggested that the vector does not live in vineyards but spreads from an alternative host in the above vegetation ecosystems. For instance, the random distribution of AGY within some vineyards (Chapter 8) indicated that the primary host is not present and rather is distant from those vineyards while the strong disease gradients in others near wastelands and wetlands (Chapter 12) indicated that the primary host plants were close-by.

An hypothesis was developed suggesting that:

- the wasteland areas harbour the primary host;
- the primary host(s) are native plants such as yanga bush (a bluebush) and saltbush and that these are not normally not present in vineyards; and that
- these hosts harbour a native leafhopper or planthopper which is the natural vector of AGY; and that
- among other leafhoppers and planthoppers, a prime candidate vector of AGY was the common brown leafhopper, *Orosius argentatus*.

To test this hypothesis, a brief sweep-net survey of native vegetation in the Riverland, SA, and Riverina, NSW, was undertaken in attempt to identify an insect vector for AGY.

## Aim

**To survey the insect fauna of native vegetation in and near swamplands and wastelands in search for a leafhopper or similar vector of Australian Grapevine Yellows.**

## Materials and Methods

A brief foray was made to determine the frequency and association of leafhopper species within hot spots of AGY by sweep netting a more or less random selection of native and various other possible host plant species. The netting was undertaken in vineyards, swamplands, irrigation overflow areas, adjacent wastelands and other areas near high intensity AGY vineyards in South Australia and NSW. A total of 28 samples was taken in this preliminary survey undertaken in cold temperatures in May and June 2002 respectively.

At the same time that the sweep netting studies were undertaken, samples of a random collection species were collected from the native plants (principally chenopods), in brief survey of their AGY status via PCR analyses.




**Figure 17.1 –17.3: Sweep netting for leafhoppers in and near hots spots of AGY, near Loxton, Riverland, SA.**

## Results

The most common insect located in the sweep nets was the common brown leafhopper, *Orosius argentatus* (Evans) (Cicadellidae: Deltocephalinae: Opsiini) which was present in 14 of 15 samples swept from yanga bush, *Maireana brevifolia* (R.Br.) (Chenopodiaceae). *O. argentatus* was absent from 13 samples from 10 other plant species/communities.

*O. argentatus* was also present in large numbers in a single sweep net sample from yanga bush growing near an AGY-affected vineyard near Griffith, NSW in June 2002.

The brief survey of native plants and PCR analyses for AGY were reported elsewhere (Chapter 15). The tests were negative for AGY.

**Figure 17.2: The common brown leafhopper (*Orosius argentatus)* was the most common leafhopper found in sweep netting studies in hotspots of AGY during winter. It appears that yanga bush may be its natural overwintering host. [photo: NSW DPI]**

## Discussion

Our previous studies showed that leafhoppers are the suspected vectors of AGY and that yanga bush and saltbush species are the suspected primary hosts and sources of disease. Mapping of disease incidence over many seasons in a number of different viticultural regions in Australia revealed a random scatter of AGY symptoms within some vineyards and distinct disease gradients in others. This suggested an insect vector was present near the latter and that it acquired AGY from native vegetation and flies into vineyards. Sweep net sampling was carried out in vineyard groundcover and nearby vegetation, focussing primarily on the overflow areas, to determine the leafhoppers present.

A total of 27 samples was taken in the Berri, New Residence, Loxton and Pyap areas of South Australia between 29$^{th}$ – 31$^{st}$ May 2002 when grape foliage was senescent. *Orosius argentatus* (Evans) (Cicadellidae: Deltocephalinae) was the most common leafhopper found and was in all 14 of the samples swept from yanga bush (*Maireana brevifolia* (R. Br) PG Wilson) (Chenopodiaceae). This leafhopper was also present in large numbers in a single sweep net sample from yanga bush growing close to an AGY affected vineyard near Griffith, NSW, on 18 June 2002. *Orosius argentatus* was absent from a number of samples from a number of other plant species/communities within or near the South Australian vineyards and from two samples from similar vegetation in Griffith. This close positive association between *O. argentatus* and yanga bush at both Loxton and Griffith suggests that this native species is a natural host for that leafhopper. The absence of *O. argentatus* from any other plant species implies that, in winter, this otherwise polyphagous leafhopper (feeds on many hosts) has returned to its natural host for overwintering. The positive association between *O. argentatus* and native Chenopodiaceae has more recently been reported by Getachew *et al.* (2005).

Other authors have reported *O. argentatus* as vector of tomato big bud and papaya yellow crinkle phytoplasmas (Hill 1943; Grylls 1979; Weintraub *et al.* 2006) and this species is the only leafhopper to date to have shown a positive PCR result for AGY phytoplasma (Beanland *et al.* 2002). However, despite these occurrences, given the abundance of *O. argentatus* in the regions in which we have observed AGY, it would seem unlikely that this species is a principal vector of AGY. If the leafhopper had any reasonable level of vector efficiency, its huge numbers would overcome any possible lack in transmission efficiency and the incidence of AGY would be expected to be much greater. Not-withstanding, the possibility of this species being the vector of AGY should not be discounted.

Thus, the vector of AGYp (and of '*Ca.* P. australiense') had remained unidentified in any crop/plant system in Australia. In contrast, in New Zealand, the planthopper, *Oliarus atkinsoni* (a cixiid planthopper), is known to transmit the New Zealand strain of '*Ca.* P. australiense' (Boyce *et al.* 1953; Liefting *et al*. 1997). Since *O. atkinsoni* is a monophagous species *ie.* it feeds only on *Phormium sp*., and is essentially limited to New Zealand (Liefting *et al*. 1998; Andersen *et al.* 2001), Streten *et al.* (2005c) concluded that it is unlikely to transmit '*Ca.* P. australiense' in Australia. However, similar cixiids may do so, especially given that these planthoppers are sole vectors of the stolbur group overseas (Langer *et al.* 2004).

Further investigations in search of these insects and studies of the flights and occurrences of all leafhoppers in and near hotspots of AGY are warranted. Of particular benefit would be investigations during the warmer months of the growing season.

**Conclusion**

The findings of this survey need to be considered in the perspective that the sampling was undertaken in winter when the activity of adult leafhoppers is limited. However, several points are made in conclusion:

- Yanga bush (narrow-leafed blue bush - *Maireana brevifolia*) is the probable over-wintering host of *O. argentatus* (common brown leafhopper) in the Riverland and Riverina regions – previously this was not known for this almost ubiquitous leafhopper;
- This is of considerable interest given the finding that yanga bush was the most frequent repository of AGYp in our search for the primary host of AGY; and
- It is possible that *O. argentatus* is involved in transmitting AGY; so
- Further sweep netting studies in the warmer months of November to February are warranted.

**Recommendations**

- Further insect trapping studies should be undertaken to investigate the role of *O. argentatus* and of cixiid and other leafhopper insects in the epidemiology of AGY;
- In particular, studies of the flights and occurrences of all leafhoppers and planthoppers in and near hotspots of AGY are warranted during the warmer months of the growing season.

***Yanga bush is the overwintering host***
***of the common brown leafhopper (Orosius argentatus)***

***… this is a new finding for that insect!***

***More insect trapping studies are needed during the growing season***

# Chapter 18: The Role of an Insect Vector 3 - Surveys Using Light Traps Studies of the Flights of Leafhoppers – 2004/05

## Introduction

In investigating the role of an insect vector in the epidemiology of AGY, two techniques had been used: insect exclusion - using fine- meshed netting to exclude insects from a vineyard site (Chapter 16), and sweep netting – using fine meshed netting to trap insects from non-vineyard vegetation in and near wastelands in disease hot spots (Chapter 17). Conclusions drawn from these studies were that an insect, probably a leafhopper or a planthopper was the vector of disease and that the common brown leafhopper (*O. argentatus*), though the only leafhopper positively associated with a presumed primary host of AGY *viz.* yanga bush (*Maireana brevifolia*), and the only leafhopper to-date found positive for AGY (on only a few individuals among several thousand tested by Beanland *et al.* (2002)), it was considered not likely that leafhopper was more than an occasional vector of AGY.

Thus, it remained for other techniques to be used in attempts to identify the vector of AGY. Sweep netting during the warmer months was suggested (Chapter 17) and this approach, though deployed in brief, had not been successful so far (Beanland *et al.* 2002; Magarey *et al.* – unpublished data). One probable reason for this was that, to be successful, sweep net surveys need to be done at the time when the leafhoppers are active. Most studies have been carried out during the day and were able to give a leafhopper "score" only at a fixed and brief point in time when it seemed likely that the vector of AGY was a casual or accidental feeder of the grapevine. A hit and miss approach to finding an occasional visitor to the vineyard was not likely to succeed.

Previous studies on leafhopper vectors of grapevine and other phytoplasma diseases overseas seem not to have investigated the diurnal flight times of the insect in great detail. Among exceptions to this was Bressan *et al.* (2006). In that study, transparent sticky traps (10.5 cm x 15 cm) were placed in the vineyard to assess the flight of *Hyalesthes obsoletus*, the cixiid planthopper vector of BN in Germany and elsewhere. Those traps were monitored every 2 hours during the daytime – including evenings and early mornings.

A lack of resources prevented our undertaking such a significant trapping study to investigate the vector of AGY in detail. However, in order to further study the role of an insect vector in the spread of disease, a small pilot experiment was undertaken using light traps at night to capture an assessment of insect activity at times and over longer time frames than sweep netting could accommodate.

## Aim

**To make a preliminary assessment of the flight activity of leafhoppers in attempt to understand more of the movement and activity of these insects as probable vectors of AGY**

## Materials and Methods

A small pilot trial, a light trap was established at a single site in suburban Loxton, Riverland, South Australia. The nearest commercial viticulture was ~2 km and agricultural paddocks ~600m. distant. A domestic light fixture (100 Watt incandescent lamp) provided the source of light (Figure 18.1) and was deployed at irregular time intervals. Initial observations indicated that the flight of leafhoppers was associated with warm nights, for instance, on nights when ambient temperature at 10 pm exceeded $20^0$ C. As a result, the light trap was activated only when insects of any sort were active at the light source.

A sticky trap used in studies of lucerne yellows by one of us (Leigh Pilkington), was constructed from a standard 8.5cm diameter clear plastic Petri dish in which the internal surface of the base plate had been coated with 1.5 - 2 mm of Tanglefoot ® non-drying glue (Australian Entomological Supplies Pty Ltd, PO Box 250, Bangalow, NSW, 2479 Australia). These provided excellent low-cost sticky traps for the present experiment.

For uniformity of operation, the light trap was usually deployed between 10 pm and 2 am and the sticky trap was placed in position only after the light source had been activated for at least 45 minutes. The trap was left *in situ* (Figure 18.1) usually for between 30-60 minutes.

The number of leafhoppers trapped per plate was counted under a low-powered microscope without differentiation of the types and species of leafhoppers since the capacity to identify these insects was not available to the project.


**Figure 18.1: A domestic light fixture provided a light source and a Petri Dish the sticky trap for pilot studies on the flight of leafhoppers in the Riverland, South Australia, 2004/05.**

## Results

The predominant insects caught in the light traps were leafhoppers though a wide range of insects was observed. Initial observations indicated that the flight of leafhoppers was positively associated with warm conditions at night. One evidence of this was the observation that, at least in the Riverland, SA, the insects most commonly trapped in light fittings, such as in the diffuser around a domestic light bulb, were leafhoppers, and then often in large numbers at irregular intervals. These were observed in greater abundance on warm, humid nights (Table 18.1; see Figure 18.3c) and not at all in many nights when the temperature was below $18^0$ C (data with zero scores are not presented). By example, note the very low numbers of leafhoppers trapped in the evening of 30th November 2004 and again on 20th March 2005 when the ambient temperature was low (< $22^0$ C) by the end of the period of exposure. The plate in Figure 18.3b illustrates this low number.

In general, moderate to high numbers of leafhoppers were trapped when the ambient temperature exceeded $21^0$ - $22^0$C (Figure 18.3c) unless the daytime maximum temperature had been extreme *ie.* in the low 40 $^0$C's or the conditions had been windy – both situations seemed to curtail the flight of leafhoppers. An example of the latter occurred on the evening of 18th December 2004 when leafhopper numbers were low despite the ambient temperature being 26 $^0$C – the maximum that day had been very hot ($42^0$C).

**Table 18.1 Numbers of leafhoppers on Petri dish sticky traps at a light source, Loxton, Riverland, South Australia. 2004/05.**

| Date[1] | Trap Up[2] | Trap Down[3] | Temperature[4] | Conditions | # Leafhoppers[5] |
|---|---|---|---|---|---|
| 30/11/2004 | 12:10 am | 12:50 am | 26C | calm, o/cast, balmy | 310 |
| 05/12/2004 | 11:40 pm | 12:15 am | 26C | bit humid | 73 |
| 4/10/2004 | 11:15 pm | 12:30 pm | 22C | calm | 89 |
| 2/11/2004 | 11:00 pm | 01:00 am | 25C-23.5C | calm | 83 |
| 10/01/2005 | 11:00 pm | 11:50 pm | 27C | calm | 41 |
| 17/12/2004 | 12:40 am | 1:10 am | 21C-20C | cool, calm | 35 |
| 17/12/2004 | 10:40 pm | 11:35 pm | 26C-25C | calm | 34 |
| 08/04/2005 | 09:30 pm | 10:30 pm | 26C-25C | calm, max 33C | 28 |
| 09/04/2005 | 11:30 pm | 12:30 am | 26.5C-25.5C | calm, max 36C | 28 |
| 04/12/2004 | 011:10 pm | 12:10 am | 22C-21C | calm, max 32C | 23 |
| 18/11/2004 | 01:00 am | 02:00 am | 26C-27C | RH very low | 19 |
| 03/12/2004 | 11:20 pm | 12:00 am. | 22-21C | | 15 |
| 18/12/2004 | 11:20 pm | 11:55 pm | 26C | calm, max 42C | 14 |
| 01/04/2005 | 06:30 pm | 09:30 pm | 24C | calm at sunset, max 33C | 13 |
| 30/11/2004 | 11:30 pm | 12:30 am | 23C-20C | light rain | 4 |
| 20/03/2005 | 10:00 pm | 12:30 am | 21C-18C | | 3 |

Note: [1] **Date** is the day on which the trap was placed in position, so that on 30/11/2004 at 12:10 am, a trap was placed in position until 12:50 am that night; and late that next night though still the same date, a second trap was placed in position from 11:30 pm to 12:30 am on 1/12/04.

[2] **Trap Up** is time the trap was in place adjacent to the light source in Figure 18.1.

[3] **Trap Down** is time the trap was removed from the light source.

[4] **Temperature** is ambient temperature at the time the trap was operative.

[5] **# Leafhoppers** is the number counted/Petri dish trap and includes all leafhoppers present without discrimination between types, species *etc.*

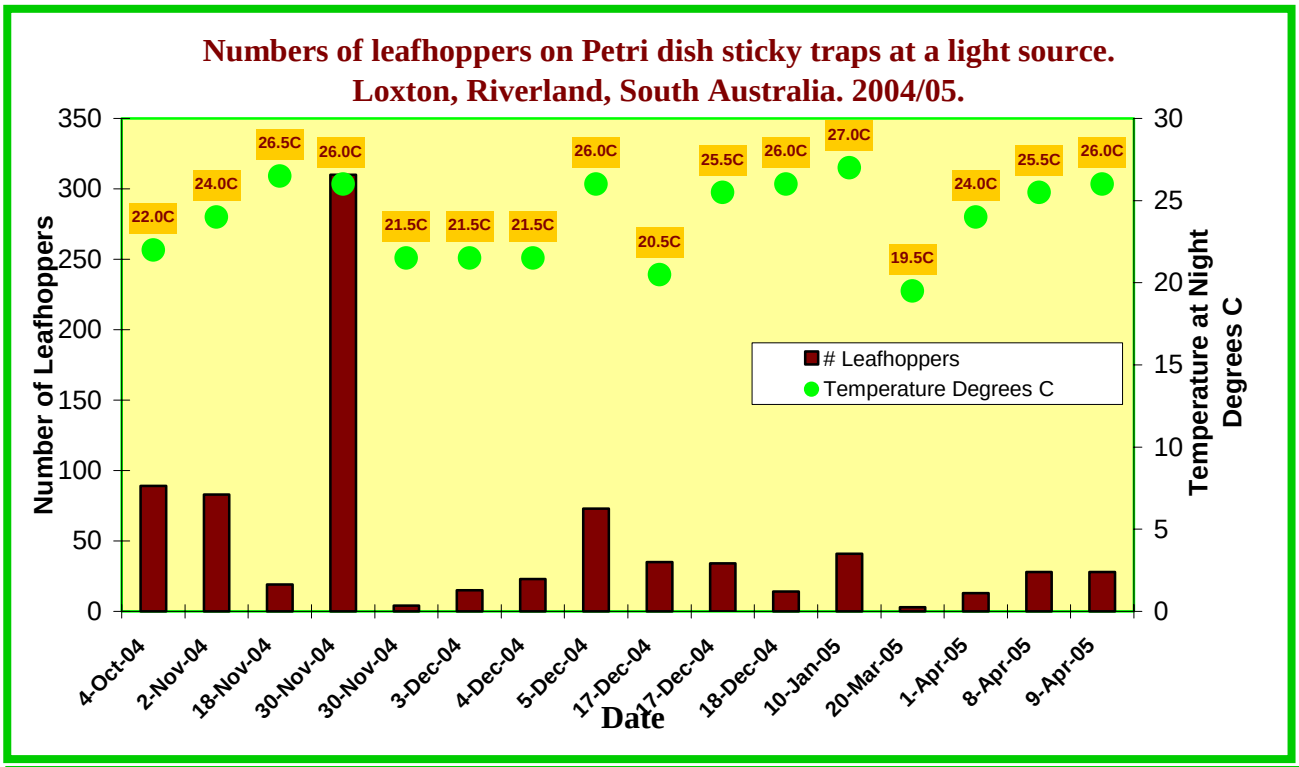



**Figure 18.2: Graph showing the chronological relationship between flight activity of leafhoppers and night temperature. See Table 18.1 for details of times traps were up and prevailing conditions especially daily maxima where they were >40 $^0$ C *eg* On 18$^{th}$ December 2004, RH was very low.**

Large numbers of leafhoppers were trapped just after midnight on 30th November 2004 during a calm and balmy (humid) night (see Figure 18.3c). These conditions were consistent with previous observations when large numbers of leafhoppers were evident on warm, thundery nights following warm to hot days without much wind.

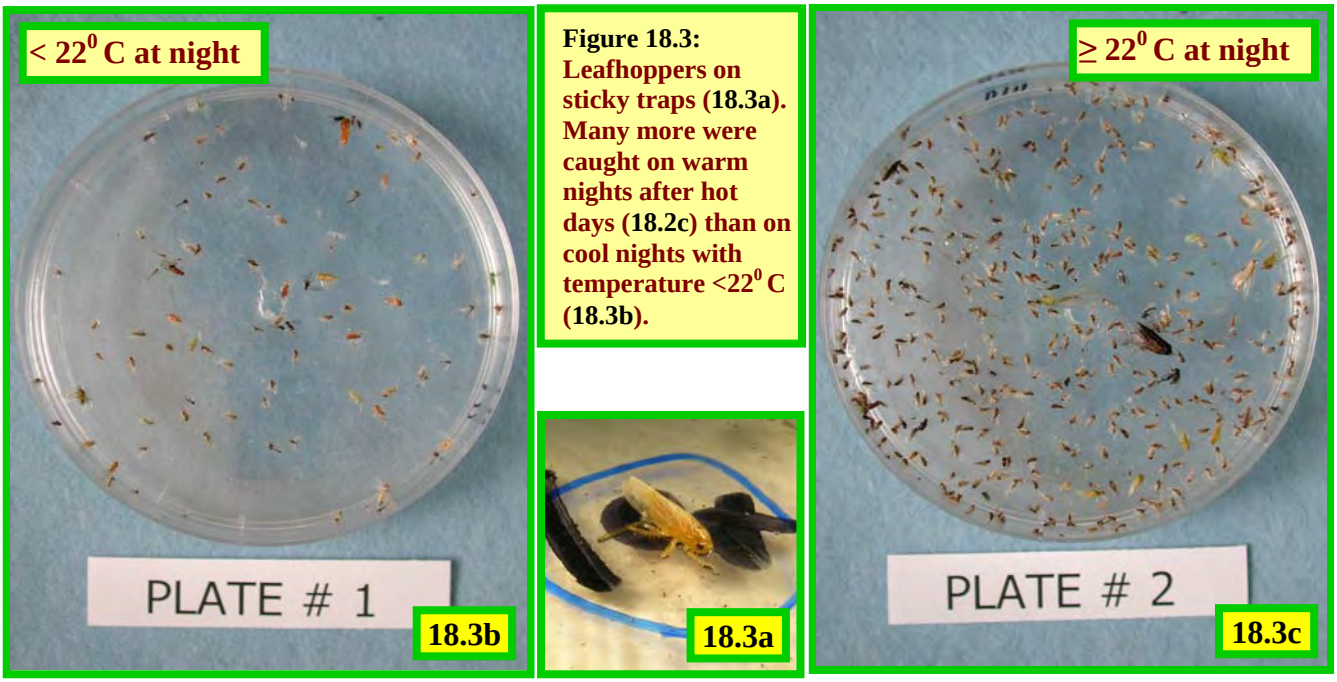



**Figure 18.3: Leafhoppers on sticky traps (18.3a). Many more were caught on warm nights after hot days (18.2c) than on cool nights with temperature <22⁰ C (18.3b).**

## Discussion

This pilot investigation into the flight patterns of insects showed a number of interesting aspects of relevance to our search for a vector for AGY. These may be summarised as below.

In relation to leafhoppers:

- They were the predominant insect caught in the traps on warm nights;
- They fly at night (around midnight);
- They are attracted to light;
- They fly at irregular times viz. they fly in huge numbers (mass migration?) on some occasions and in few numbers at other times;
- There is evidence for a tentative rule as a guide to the conditions that favour the flight of leafhoppers: high numbers are probable when night time minimum temperature is ≥ 22 $^0$C;
- Conversely, few leafhoppers fly on nights with temperature < 22 $^0$C;
- Extreme high temperatures during the day (>40 $^0$C) and windy conditions impede the flight of the insects that night.

These findings agree in part with a study of leafhopper activity including that of *O. argentatus* (Osmelak *et al.* 1989). These authors showed that the leafhopper flew on irregular occasions and sometimes in very large numbers. Our findings are of interest in that Bessan *et al.* (2006) reported that *H. obsoletus* planthopper vector of BN in Germany showed peak flight activity in late evening before sunset whereas Lessio *et al.* (2006) showed the peak flight activity of *Scaphoideus titanus*, the leafhopper vector of FD in France occurred some time over night. Our data suggest peak flight activity of leafhoppers occurred during darkness in the vicinity of midnight.

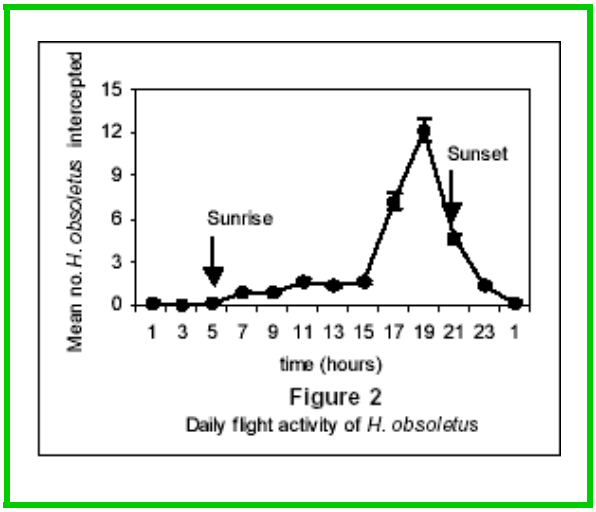



**Figure 18.4: Flight activity of *Hyalesthes obsoletus* planthopper, the vector of Bois noir in German vineyards. This showed peak flight activity in late evening before sunset.**

**Data from Bressan et al. (2006).**

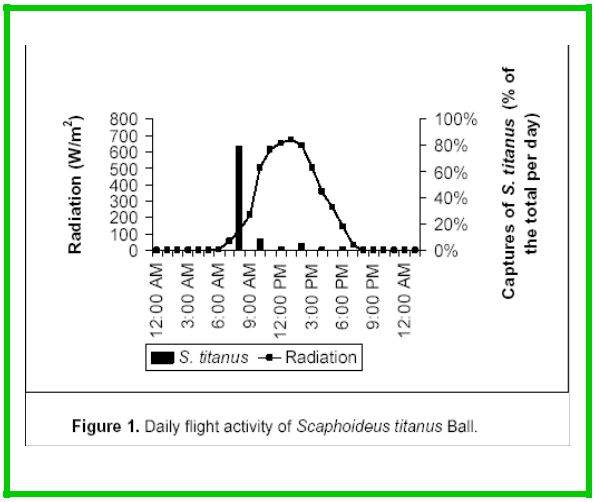



**Figure 18.5: Daily flight activity of *Scaphoideus titanus* leafhopper, the vector of Flavescence dorée in French vineyards. This showed a peak flight activity between 9:00 pm and 8:00 am.**

**Data from Lessio et al. (2006).**

Our preliminary studies do not provide data to compare flight numbers (if any) during day-light hours but observations made during the evening of high vector activity showed little presence of leafhoppers at the traps until an hour or two after sunset, into the evening and toward mid-night. Also, evidence from later studies (Chapter 19) suggest the probability that most leafhopper activity did occur during the night time.

From the above preliminary evidence it is possible that the general characters of the leafhoppers we observed might also apply to the vector of AGY *ie.* regarding the conditions under which it might prefer to fly and feed. There are many factors that likely interact on each insect species and its tendency for flight and its preferences for transmitting disease as a consequence. However, our evidence suggests in the broadest terms, it is likely that the vector of AGY will fly at night and at irregular intervals each season depending on prevailing temperature and wind speed among other such factors.

If the vector proves to have as strong an affinity for night flights and toward light sources, this raises the possibility that light-traps might be placed in the vicinity of primary host plants to provide an ecologically useful management tool for the vector of AGY. The majority of insects might be attracted to the light in sufficient numbers so as to reduce the number that fed in the vineyards and to reduce the rate of inoculation of AGY below that which caused economic loss. Further studies on this are warranted.

## Conclusions

Preliminary studies with light traps showed that leafhoppers:

- fly in high numbers on irregular occasions having warm nights with temperature ≥22 $^0$C;
- fly to light sources at night near midnight (Note: our experiment did not determine if daytime activity also occurred but observations suggested this was not likely);
- flight activity appears to be impeded by extreme temperatures *eg.* > 40 $^0$C during the day, or during windy and/or rainy weather conditions;
- it is possible that these characteristics also apply to the leafhopper vector of AGY and, if so, flight times of the vector and its inoculation of vines would be at irregular intervals each season dependent on the prevailing conditions especially temperature.

## Recommendations

Given the above pilot trial, a number of recommendations follow:

- additional insect trapping studies are needed to determine the frequency of individual leafhopper and planthopper species in their accessing and feeding on grapevines and native species such as yanga bush and various salt bushes, to determine the identity of the insect vector of AGY.
- further investigation of the timing of and conditions for leafhopper flights is needed to resolve the factors that influence the movement of the vector of AGY, hence the timing and conditions which favour transmission of disease by the vector;
- studies similar to the present *viz.* using light traps, within both wasteland and vineyard settings are needed to resolve the flight patterns of the presumed vector of AGY;
- studies to confirm or deny the attraction of leafhoppers to light sources at night are suggested in pursuit of the possibility that light-traps placed in the vicinity of primary host plants might serve as an ecologically sound, low-risk management tool for the presumed leafhopper vector of AGY. The principle involved being that the majority of insects might be diverted toward the light source and sufficient numbers drawn away from feeding in the vineyards near by so as to reduce the incidence of AGY below an economic threshold. Alternatively, a device based on a UV insect 'zapper' that attracted and killed the insects might be better.

***Leafhoppers fly in big numbers at night***

***… but only when temperatures ≥ 22$^0$ C***
***and when day time conditions were favourable***

***… these factors probably apply to the vector of AGY***

***and could account for inoculation of vines***
***at irregular intervals.***

## Chapter 19: The Role of an Insect Vector 4 - Surveys Using Sticky Traps Studies of the Occurrence of Leafhoppers - 2002/03 to 2004/05

### Introduction

In investigating the occurrence and frequency of leafhoppers in and near hot spots of AGY, it was apparent that more detailed studies were needed than described in the previous chapters. It was considered necessary to deploy sticky traps to determine the leafhopper population at these localities over a longer period of time than was able to be achieved through sweep netting (Chapter 17) or via the light traps (Chapter 18).

The following trapping studies were undertaken to monitor the population of insects and in particular, of leafhoppers, in the zones where AGY was at high incidence and to compare these with similar assessments in localities with low disease incidence. Data from this study would facilitate identification of the range of insects one or more of which might be vectors of AGY.
The studies we report began in 2003/04 but were undertaken principally in season 2004/05 and have continued in 2005/06.

### Aim

**To investigate the insect- and particularly, the leafhopper-fauna of vineyard and non-vineyard localities in and near hot spots of AGY, to assist in identifying the vector of AGY.**

### Materials and Methods

A grid of sticky traps was established in the 12 hot spot locations for AGY in the Riverland, SA, and ten in the Riverina, NSW, (with some also in the Sunraysia, NSW), for seasons 2003/04 to 2005/06. These traps were placed either within the canopy of vineyards or on stakes at height of 0.5 - 1.5m in vegetation within or near the boundary of vineyards. Locations were selected for the occurrence of specific gradients in the incidence of AGY, as in Chapters 11-13. The grid work of traps usually 5-20 traps/site, was placed along those gradients such that the population of insects could be monitored at sites with progressively varying levels of incidence of AGY.

**Figure 19.1: Yellow sticky traps were placed in a grid along decreasing incidence of AGY, in this case, in vegetation on the boundary of a vineyard adjacent to plants that tested PCR-positive for AGYp.**

**Figure 19.2: Sticky trap here nearly hidden amongst the vegetation of yanga bush (*Maireana brevifolia*) near a vineyard with high level of AGY, at Winkie, Riverland, SA. 2004/05.**

The traps were constructed from Petri dishes in which the base plate was painted with a non-drying glue, as described in Chapter 18. In this case, the lids were painted yellow and the base plates were inverted and fitted inside the lids, so as to expose the glued surface to the exterior. A hole (~5mm diameter) drilled in the lid and the base p1ate, enabled the yellow sticky traps to be affixed by wire to a post or to the vine trellis wire *etc*. (Figures 19.1 and 19.2)

Operation of the traps, Traps were replaced every fortnight (2 weeks) from the end of dormancy until leaf-fall so as to allow insect movement in and near vineyards to be monitored for the duration of the growing season. Thereafter, the traps were exchanged at 4-6 week intervals.

Identification of the insects was undertaken by one of us (Murray Fletcher) at the end of season 2004/05 for the Riverland grid only. Detailed assessment of individual species was undertaken using microscopy to identify most species (Figure 19.3) but some remained identified only to genus. The traps from 2005/06 in the Riverland and the other locations remain to be assessed when finances for this work can be obtained.

**Figure 19.3: Low powered microscopy was used to help identify the leafhoppers trapped in the survey of vineyard and adjacent vegetation.**

## Results

Total Number of Leafhoppers To date, initial screening of the traps has revealed at least 20 different leafhopper species are present in the vineyard sites surveyed (Table 19.1). Of these the most frequently present are cited in the ranked list of Table 19.1. These include a number of potential leafhopper vectors of phytoplasma which were common in the vicinity of severely diseased vineyards *ie.* in the hot spots of AGY we surveyed.

The most frequently occurring was the common brown leafhopper, *O. argentatus.* The next in frequency were *Austroasca sp., Batracomorphus angustatus* and *Orosius canberrensis.* These species are of interest, especially since *B. angustatus* is known as a vector of several phytoplasma diseases including TBB (see discussion in Chapter 16).

The low numbers of cixiid leafhoppers trapped was of note. The hypothesis that an insect of this type is a vector of AGY, given their association as vector of flax yellows in NZ and with BN in Germany and elsewhere in Europe (Chapter 16), is not supported by the low numbers trapped in this survey, although this does not require the hypothesis to be rejected at this stage.

The data on which leafhoppers were trapped at each site, in what frequency and timing but these have not yet been analysed. Potentially there is much understanding of the biology and the timing of flights that can be elucidated by these analyses which are warranted. However, some simple correlative analyses have been undertaken as a first step in this process.

**Table 19.1 Combined total number of leafhoppers trapped across all traps in the Riverland during 2004/05.**

| Leafhopper ID | # Trapped[1] | Ranking[2] |
|---|---|---|
| *Orosius argentatus* | **860** | **1** |
| *Austroasca sp* | **177** | **2** |
| *Batracomorphus angustatus* | **137** | **3** |
| *Orosius canberrensis* | **68** | **4** |
| *Dikraneurini* | **66** | **5** |
| *Empoascini* | **57** | **6** |
| *Limotettix sp* | 24 | **7** |
| *Austroagallia torrida* | 15 | **8** |
| *Xestocephalus sp* | 15 | **8** |
| *Cixiidae* | 5 | **10** |
| *Arawa sp* | 3 | **11** |
| *Delphacidae* | 3 | **11** |
| *Eurymelinae (Ipoini)* | 2 | **13** |
| *Acanthucalis macalpini* | 1 | **14** |
| *Achilidae* | 1 | **14** |
| *Austroagallia sp* | 1 | **14** |
| *Eupelicinae (Paradorydium brighami)* | 1 | **14** |
| *Issidae* | 1 | **14** |
| *Issidae nymph* | 1 | **14** |
| *Opsiini* | 1 | **14** |
| **Summary Total # Trapped** | 1,439 | |

[1] # Trapped is the total number of leafhoppers in each class from a grid of sticky traps in 12 vineyards sites with 5-20 traps/site.
[2] Ranking is Total Number of insects trapped ranked in ascending order.

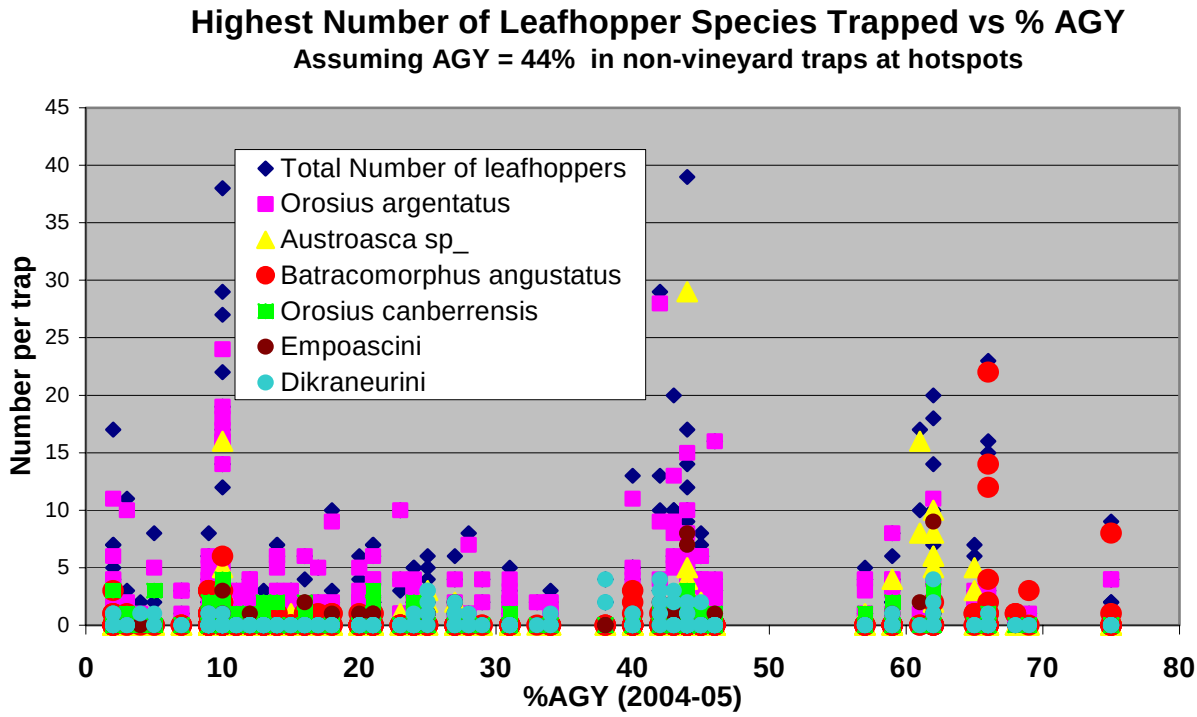



**Figure 19.4:** The number of leafhoppers of each type at each trap (Y axis) is shown in relation to the incidence of AGY in vineyard at or nearest to that site (X axis). Many species of leafhopper were common but not many where levels of AGY were also high.

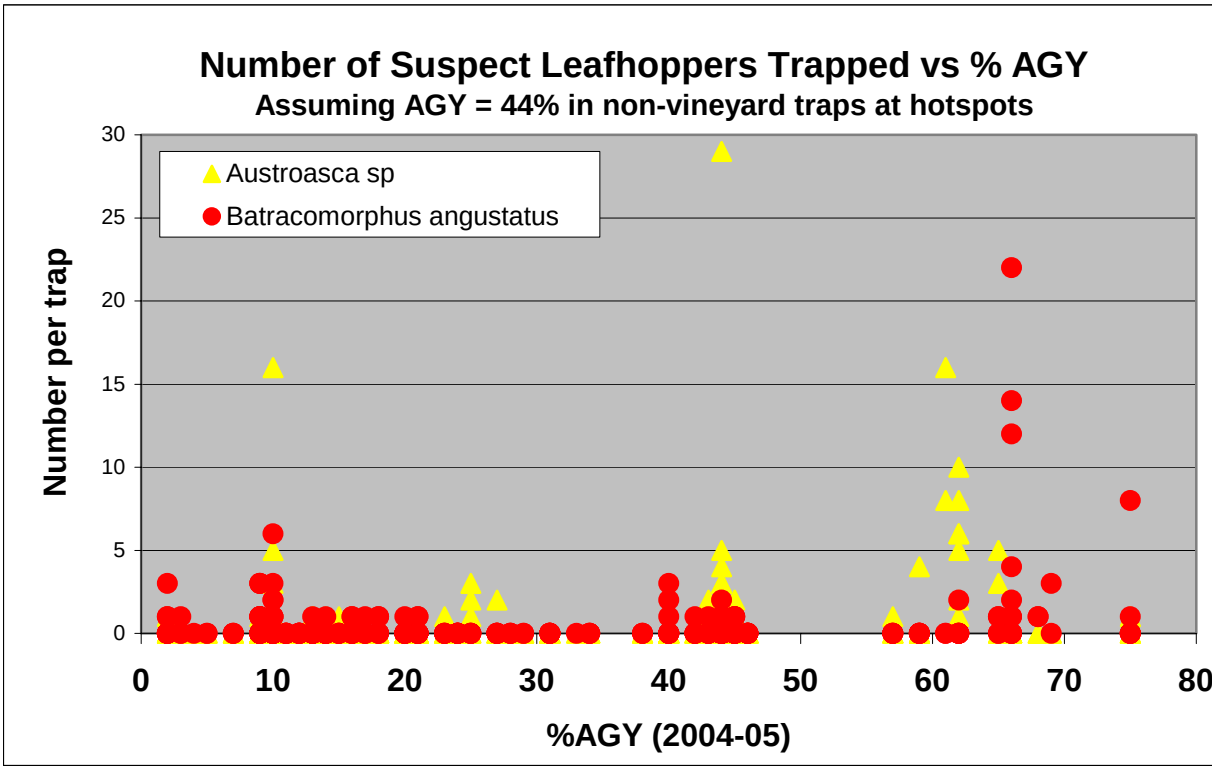



**Figure 19.5:** Two species of leafhopper were common where levels of AGY were high. Only one of these remains as prime candidate vector of AGY – the large green jassid, *Batracomorphus angustatus.*

Association with AGY Some simple analysis of the trapping has been undertaken to show the association of the occurrence of leafhoppers in each trap with the incidence of AGY at or near that trap. This process of correlation by association provided the data expressed in Figures 19.4 and 19.5.

**Discussion**

Figure 19.4 presents the array of the number of leafhoppers per sticky trap as they occurred in association with traps located at sites with varying levels of AGY. A cut off was made to include only the six most frequently observed leafhopper species trapped because the others were significantly fewer in number (Table 19.1). To facilitate the plotting of data, where a trap was in a non-vineyard site, *eg.* located in native vegetation near a hot spot for AGY, an average score for AGY was arbitrarily assigned to that trap by adopting the incidence of disease in the adjacent vineyard. The mean score assigned was 44 % AGY.

To distinguish which leafhopper(s) might vector AGY, the plots on Figure 19.3 where levels of AGY are low and insect numbers are high, or where AGY incidence is high and insect numbers are low, were discarded. For instance, any insect might easily have been detected across the spectrum of AGY incidence at these sites and bear no significance to the epidemiology of disease. Of interest are only those species which occur at high number where the incidence of AGY is also high. Again an arbitrary score needed to be assigned to what constituted a high incidence of AGY and that chosen was the presumed threshold for economic loss estimated at ~20% incidence (Chapter 2).

The data in Table 19.1 and Figure 19.4 (without statistical analysis) showed the high abundance of *O. argentatus* (Figure 19.6) but its frequent occurrence across all traps at all levels of AGY implied a low likelihood that this species was the primary vector of the disease. This supported earlier deductive reasoning (see Chapters 17 and 18). To the contrary, the insect might be present ubiquitously but acquire the pathogen only at specific times *eg.* in mid-season. Thus, it remains possible that *O. argentatus* is vector of AGY and this species should not ignored.

**Figure 19.6: The common brown leafhopper, *Orosius argentatus,* is abundant in vineyard localities where AGY is in high incidence and may spread AGY at times but is unlikely to be the main vector of AGY.**

A ‘sieve’ placed across the data in Figure 19.4 to select leafhoppers with high numbers at sites above the AGY threshold as discussed, presented the data shown in Figure 19.5. Recognising the constraints of the survey system we used and the lack of more rigorous statistical analysis to-date, the data showed only two candidate species remaining in contention as primary vector of AGY: *Austroasca sp* and *Batracomorphus angustatus* (Figure 19.5).

These species ranked number two and three respectively in total numbers present in the sticky traps and both occur at high number where AGY is also at high incidence. *Austroasca sp* also occurred in low numbers across the array of traps (Figure 19.5), but they are known to feed only on parenchyma tissue and have not been recorded as a vector of yellows or related diseases.

The green jassid, *B. angustatus*, also occurred across the spectrum of AGY incidence and was at high level (>5 specimens/trap) at one location where AGY was at low level (Figure 19.5) but this species has been reported to transmit the tomato big bud phytoplasma (16 Sr VI-A) (Grylls 1979) which has been found associated with yellows symptoms in grapevine (Gibb *et al.* 1999) (Chapter 17). In addition, it is reported as transmitting potato purple top wilt and has been implicated (not proven) with pawpaw yellow crinkle and at least one other phytoplasma not yet found in grapevine including lucerne witches' broom (Grylls 1979, Weintraub *et al.* 2006).

Thus, two leafhoppers showed potential as candidate vectors of AGY, by virtue of their positive correlation with high levels of AGY near the traps on which they were captured. However, the *Austroasca species* are unlikely vectors since they feed principally on parenchyma tissue and to date, phytoplasma have not been found in other than the sugar conducting phloem cells. This supports our interest in *Batracomorphus angustatus* as a prime candidate vector of AGY.


**Figure 19.7: The green jassid, *Batracomorphus angustatus*, is abundant in vineyard localities where AGY is in high incidence and is a prime candidate vector of the disease.**

In further analysis of the data, the trap scores for the above two leafhopper types were plotted against the date on which these species were recorded in the traps (Figure 19.8).

In 2004/05, there were only two peak flights of *B. angustatus*, one in early October and the other in late November. Since the traps were exchanged on a fortnightly basis, it was not possible to define more precisely when the flight occurred. However, our study using the light trap (Chapter 18) pinpointed more detail. Two nights when leafhoppers flew in great abundance were respectively, the 4-5$^{th}$ October 2004 and 30$^{th}$ November 2004 (Table 18.1). These single-night events coincided with the periods in Figure 19.6 when the peak flights occurred.

It is reasonable then to suppose that *B. angustatus* flies at night with temperatures ≥22 $^{0}$C. The absence of the insect flights on other occasions when the temperature was within this zone *eg* on 2$^{nd}$ November and 5$^{th}$ December 2004 (Table 18.1), may be the result of the developmental cycle of the insect not coinciding with conditions that favoured flights of the adults.

Only two major flights of *B. angustatus* were recorded and only one of these occurred in the period from mid-season when transmission of AGY might be expected. Given further knowledge of the vector of AGY (once identified), it is reasonable to suppose some ability to predict the level of inoculation each season based on the number and intensity of flights. As a result, it is speculated that some ability to predict the levels of AGY within a region next season might be possible.

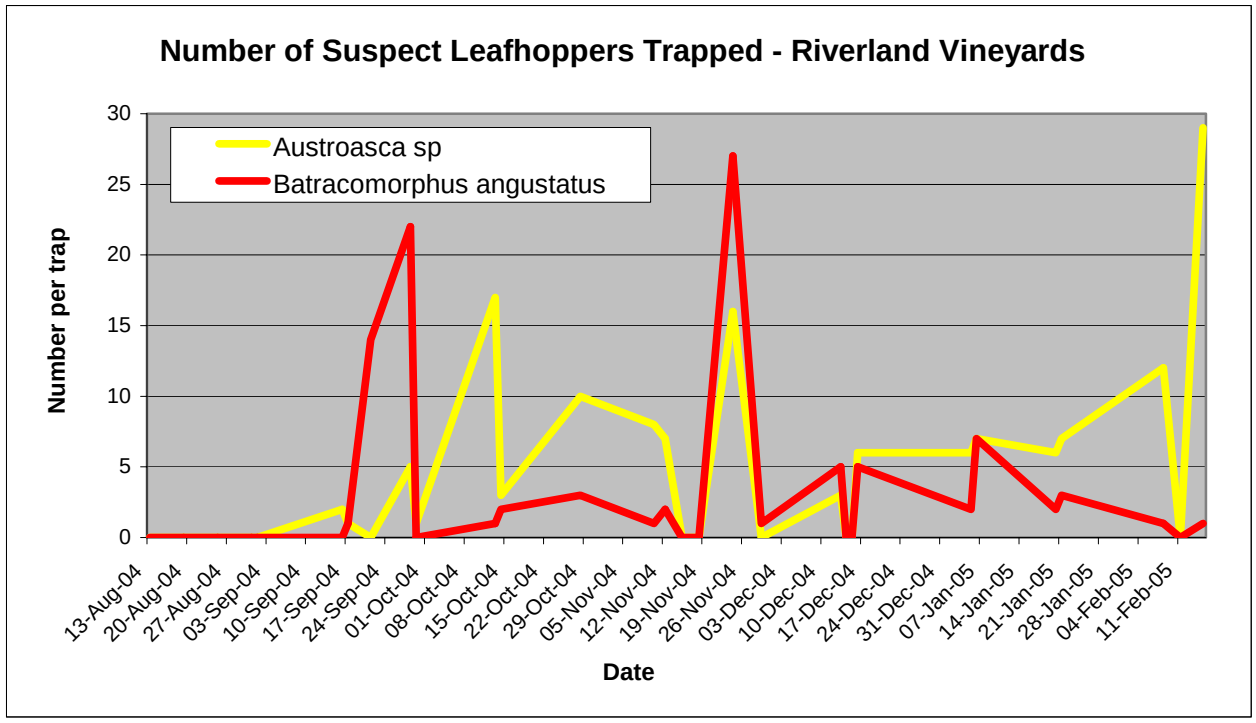



**Figure 19.8: A possible vector of AGY, the large green jassid, *Batracomorphus angustatus*, had significant flights on only on two occasions: in spring and mid-summer. The dates of these flights coincided with the night of peak flights shown in Table 18.1.**

During 2005/06, a similar survey of the insect fauna in vineyard localities with hot spots of AGY was undertaken but to date the resources are not available to categorise and identify the trapped leafhoppers. The potential for this study is considerable given the outcomes from the data presented above. However, the traps will need to be examined while reasonably fresh because, over time, the trapped specimens deteriorate. As a result, the male insects will need to be taken off the sticky trap and examined in ethanol for genitalia structures. The females may become unidentifiable. This should therefore be undertaken soon (*viz.* May – July 2006).

Given the potential for success indicated in this present investigation, there is hope to achieve the project objective of identifying an insect vector of AGY through analyses of the above mentioned traps and in further investigation in season 2006/07. PCR analysis of trapped *B. angustatus* will provide valuable corroborative evidence of the association of this species with transmission of AGY if results are positive.

## Conclusions

- Of the six most frequently found leafhoppers, *Austroasca sp*. and *Batracomorphus argentatus* were abundant and were most strongly correlated with high levels of AGY;
- *B. angustatus* is a prime candidate vector of AGY and should be investigated further;
- *B. angustatus* showed peak flights on only two occasions in the period of study and the dates of these were detailed with precision;
- *Austroasca sp.* are not considered vectors of AGY because they do not feed on phloem cells;
- *Orosius argentatus* was also abundant but trap counts showed it occurred in all sites with varying incidence of AGY. Though it is not likely to be the prime vector of AGY, it should not be ignored;
- Further investigations into the vector relations of AGY have good potential for success in identifying the leafhopper species involved.

## Recommendations

The success of the present study supported the hypothesis that a leafhopper or planthopper is associated with AGY as vector of the disease.
As a result, it is recommended that:

- as a matter of urgency, the sticky traps that were placed in the Riverland and Riverina during 2005/06 be assessed to identify the types and frequencies of trapped insects, especially the leafhoppers and planthoppers with particular reference to *B. angustatus* and *O. argentatus*;
- further investigations into the biology and vector relations of leafhoppers and planthoppers that occur in association with hot spots for AGY, be initiated for season 2006/07 in a fully resourced project of minimum duration three seasons;
- the focus of these studies should be on deploying sticky traps, sweep netting and light traps in hot spot zones of AGY in association with PCR analyses of native plant host species, leafhopper insects and vines as appropriate;
- focus should also be on the occurrence, flight patterns and PCR status of the leafhopper species *B. angustatus* and *O. argentatus*, among others;
- coupled with detailed analyses of the existing database of the occurrence and frequency of AGY within sectors (arms) of vines and other vineyard mapping data, the investigations will provide valuable understanding of the epidemiology of the disease and the behaviour of the insect vector(s);
- the studies should also include insect feeding studies in laboratory and field situations such as the placing of seedlings of *Maireana brevifolia* and other native species implicated in the AGY disease cycle (see Chapter 20), within netting-exclosures and in adjacent exposed sites, with insect traps nearby, to investigate which insects if any are associated with the development of infection by the AGYp.
- the success with the present project warrants the seeking of specialist advice on the development of these investigations in the dormant season 2006. It is recommended that advice is sought immediately from specialists with expertise in insect biology, insect taxonomy, molecular detection of phytoplasma, the taxonomy and ecology of native chenopods, and the biology and epidemiology of AGY as a disease, these experiments of optimum design are implemented as soon as possible in preparation for season 2006/07.

***Many leafhoppers were trapped***
***but only two show good potential as vector of AGY:***

- ***the green jassid (Batracomorphus angustatus)***

***and***

- ***the common brown leafhopper (Orosius argentatus)***

***The green jassid flew in abundance only twice in season 2004/05***

***Further studies of these insects should prove valuable.***

## Section 9. Suggested Model of AGY Disease Cycles

*Given the understanding of AGY as a disease, how it is spread and from where, the following diagrams are presented as preliminary models of how the disease spreads, how it is expressed and of insect feeding process during inoculation of vines.*

### Chapter 20: A Model to Describe AGY – Life cycle and insect vector feeding Some proposals

The following graphical representations of the factors involved in the disease are presented.

**Proposed Model of the Disease Cycle for AGY**

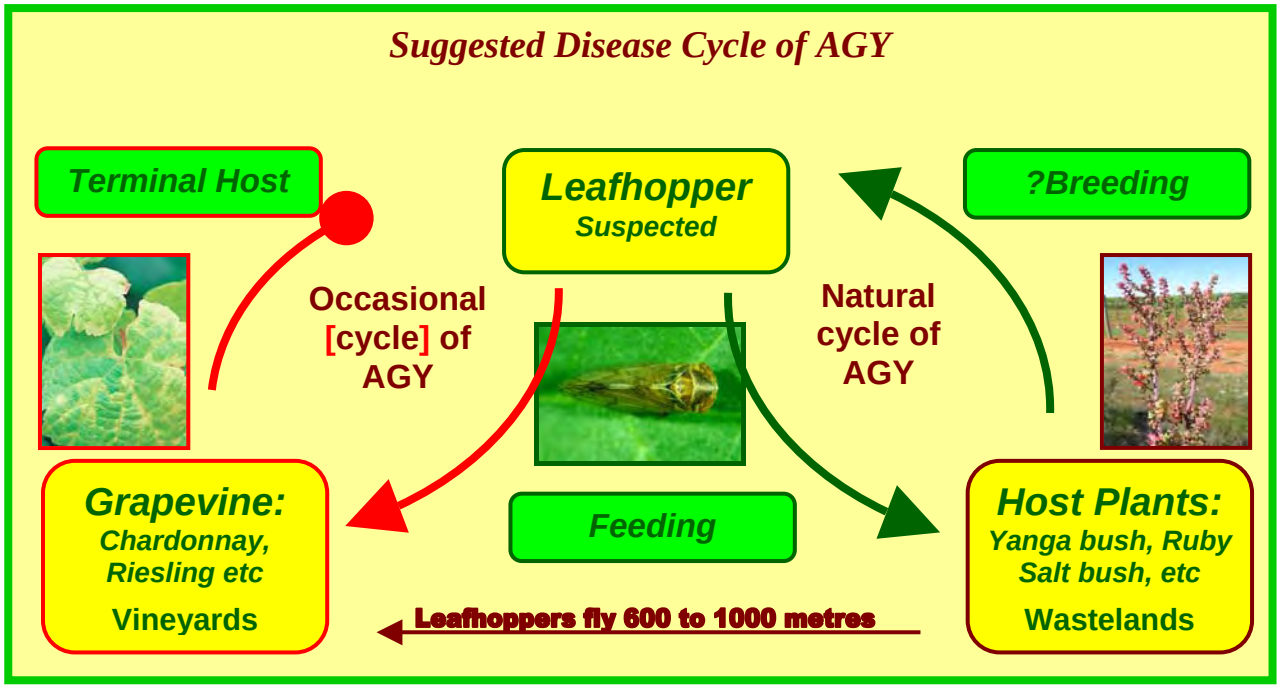



**Figure 20.1: Proposed life cycle of AGY on primary host plants and grapevines. Leafhopper insects are the vectors of AGY. They feed on plants such as the native yanga bush and occasionally on grapevines, transmitting AGY as they feed.**

The varying levels of AGY in vineyards on a season by season basis is accounted by the dynamic between the level of recurring disease (A), the rate of new infections (B), the level of remission (C) and the level of new expression of symptoms on vines which remained latently infected but showed no AGY last season (D).

Hence:

**Disease Incidence (% AGY) = A + B – C + D**

where A = % recurring disease;
B = % new infection;
C = % remission; and
D = % new infection on a vine previously in remission.

The level of recurring disease (A), *ie.* symptoms showing season by season, is probably increased by factors including the initial titre of inoculum in the vine (IT) *ie* last season and the rate of multiplication (ROM) of AGYp in the vascular fluid of vines since dormancy this season, and it is decreased by factors including the level of natural heat therapy (NHT) and host hypersensitivity (shoot death, SD).

Thus, in crude mathematical expression:

% Recurring Disease (A) = % Disease Incidence last season * ((IT * ROM) - NHT – SD)

where

- it is important to note that the underlying mathematical relationship between these factors is not known; but
- IT is dependent on many factors relating to the vector, the host, cultivar susceptibility, and the environment including the initial titre of inoculum introduced into the vine and the rate of multiplication (ROM) of the phytoplasma within the vine between time of inoculation and symptom expression;
- ROM is likely influenced by the prevailing temperature especially from late dormancy to flowering, and by other factors that influence AGYp within vines as they incubates during the lengthy (≥7-8 month) period between inoculation (perhaps in early to mid-summer last season) and symptom expression this season (in late spring and early summer).
- NHT is dependent on the number of days ≥ $40^0$C in the growing season (the period from September to March);
- SD is dependent on factors such as IT, vine vigour and prevailing temperatures – shoots dieback more quickly in hot weather.

If the equation for % recurring disease calculates a high score, next season AGY will reappear at the same rate in previously diseased sites (*viz.* the previously diseased arm) (A); if a low score, AGY will be in remission and a proportion of the previously diseased arms will be symptomless (C).

The rate of new infection (B) is determined by many aspects of the insect vector as it interacts with its host plant or plants (perhaps the yanga bush and ruby salt bush), the susceptibility of the grapevine cultivar, the environment (prevailing temperatures and RH) and the distance to susceptible vineyards (≤ 600 – 1000 m.).

The initial titre (IT) of inoculum in a vine will be dependent on an array of factors, the principal of which is the distance of the host plant from the vineyard since this has the major influence in determining the frequency with which the leafhoppers will browse on the vines. Other factors include firstly, the prevailing temperature and its influence on the frequency of and distance travelled during flights of the leafhoppers (flights appear to require prevailing temperatures ≥23-24$^0$ C), and secondly, the efficiency of vector transmission (TE).

Many factors will influence transmission efficiency (IE) including the number of insects present/vine, the proportion of insects infected with AGY and the inoculation efficiency of infected insects (% successful inoculations/feeding attempt).

**Model of the Interactions Associated with Symptom Expression in AGY**

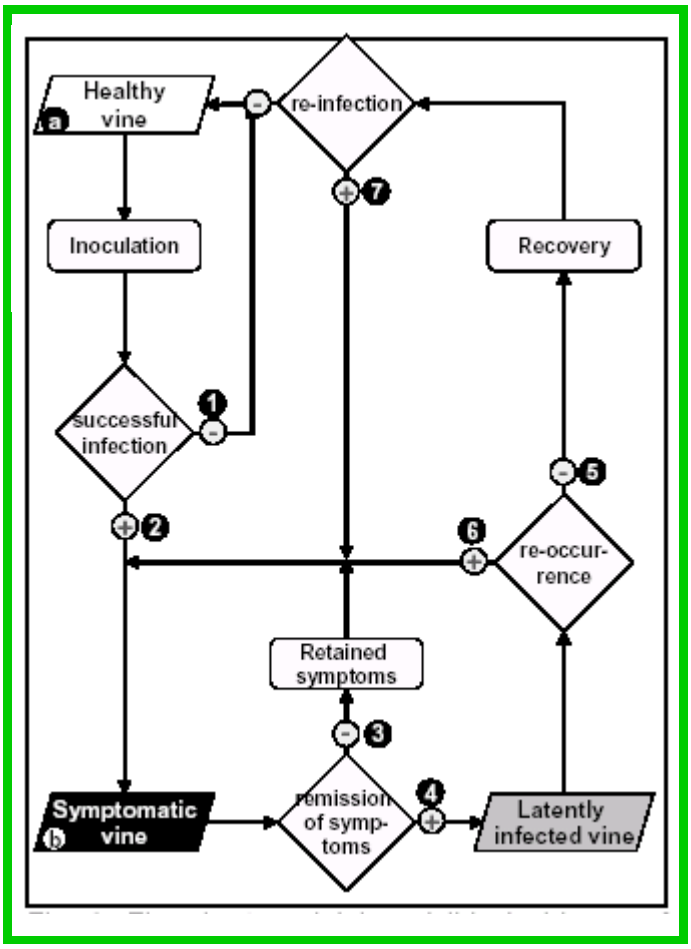



**Figure 20.2: Flow chart showing the expression of symptoms of AGY as the result of the interaction between numbers of factors.**

**Diagram courtesy of Maixner (2006) where it was applied to the dynamic equilibrium for expression of Bois noir in European vineyards.**

## Model of the Cycle of Infection and Infectivity of the Leafhopper Vector of AGY

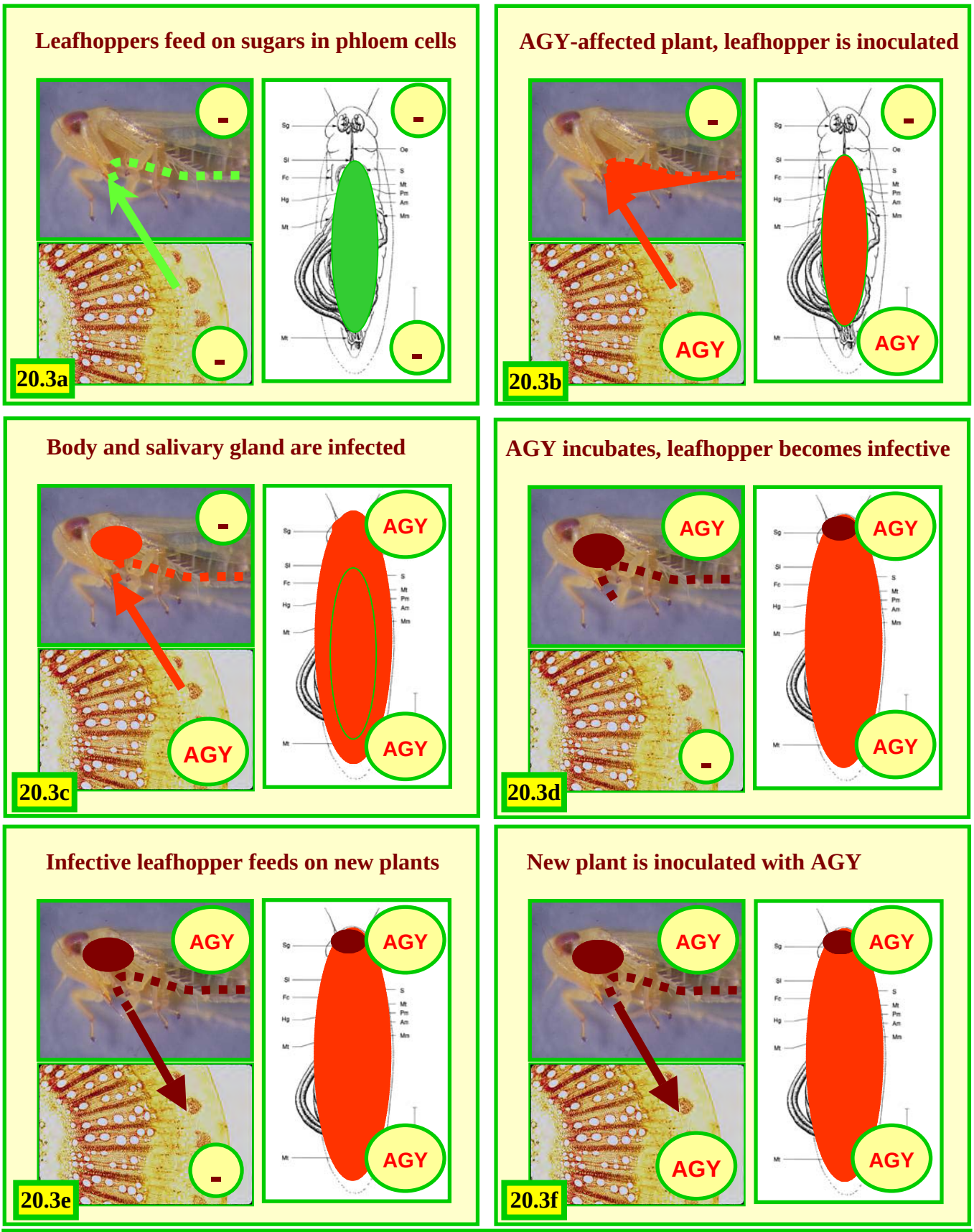



**Figure 20.3: The cycle of infection of leafhoppers as the larvae feed on AGY-infected plants and the leafhopper is inoculated, becomes infected and soon becomes infective. The adult leafhopper later emerges and flies to new plants and, as it probes looking for sugars in the phloem cells, it injects infected saliva and inoculates the new plant as it feeds.**

**Diagram modelled on original from MW Maixner who adapted concepts from Tsai & Perrier (1996).**

## A Possible Mechanism of Infection and Symptom Expression for AGY

For AGY at least, the vine is infected the season prior to symptom expression when the phytoplasma are introduced to the foliage by the leafhopper vector(s). The pathogen then soon (prior to senescence) travels down the phloem from the leaves in a benign movement to the spur and sometimes to localised (adjacent) portions of cordon tissue. Here the pathogen resides and incubates for 7 - 9 months - perhaps inoculated in December to February and showing symptoms from flowering onwards.

Under some rare but favourable conditions, AGY may induce disease in the season of its inoculation.

With AGY on relatively rare occasions, and with North American Grapevine Yellows (NAGY) usually the pathogen moves past the cordons into the trunk and/or roots where it would also incubate. This 'extra' movement may result from sheer titre with which the pathogen is inoculated eg if the vector feeds on the vine for longer, or is otherwise more infective.

Especially with NAGY, this extra (systemic) movement may also be influenced by a lack of immediate pathogen-induced host response to infection that would otherwise kill or reduce the pathogenicity of the pathogen and maybe also by a higher titre and/or smaller size/greater variation in the morphology of the NAGY pathogen allowing greater (less restricted) movement through phloem sieve plates.

With AGY and NAGY, in late dormancy as temperatures increase, sap flow recommences and sugars are 'mobilised', favouring two pathogen processes:

1) the surviving pathogen titre begins to replicate in vascular fluids at a temperature dependant rate - thus, higher (toward optimum) late-winter early-spring temperatures lead to increased titre and increased consequences (as described below);
2) the multiplied pathogen (increased titre) proliferates (moves along) the now distally mobilised phloem (sugar) pathways to the developing buds and young shoot growth.

This leads to two or more pathogen-induced host responses:

1) a disrupted hormonal development of the host, resulting in:
    a) a disrupted (reduced and/or prevented) acropetal development of the phloem (and ?? other) tissue within infected shoot, petiole and leaf tissue leading to a reduced/prevented function of secondary phloem tissue within shoots and as a result, unlignified shoots which progressively die (don't know why shoots and/or cordons are killed - is it directly from lack of 'nutrition??' via phloem or is it from direct pathogenicity?);
    b) a reduced development/function or death of apical buds and as a result, reduced/ceased apical growth and stunted shoots;
    c) a reduced development of flowering processes or later disruption/blocking of the phloem transport system (see below) killing the inflorescences or preventing normal development of bunches and berries which shrivel over time.
2) an host-defence response to the presence of the pathogen, resulting in:
    a) the deposition of '?callose'-like deposits in (phloem) cells containing the pathogen - these deposits 'coat' and kill the pathogen or reduce their further direct pathogenicity;
    b) the pathogen propagules, being 'coated' and now physically bigger and/or more prone to aggregate, accumulate at and partly or completely block phloem sieve plates, reducing phloem transport;

c) the leaves (food factories) continue to produce photosynthates which accumulate because of the reduced flow down the phloem highway from factories (leaves) through petioles and shoots to spurs, trunks and roots;
d) negative feedback mechanisms then lead to reduced chlorophyll content in leaves, reducing photosynthesis causing leaves to turn yellow in patches, sometimes along veins;
e) the accumulated photosynthates cause:
   • leaves to curl downward then senesce and fall early; and may be also
   • nitrate toxicity (necrosis) of inflorescences.

If the above can be disproved or approved:

1) we will have made progress in understanding the disease, how it is caused and perhaps how it may be corrected;
2) perhaps we can determine when the normal development of tissues of shoots, buds, petioles, leaves and bunches is disrupted and how;
3) this could lead to a better understanding of whether the application of plant hormones such as auxins, might correct symptoms and lead to the possible development of a control;
4) maybe we can determine if there is a 'coating' and killing of the pathogen and if so, gain a better understanding of whether the phytoplasma are likely to be transmitted by taking cuttings from diseased vines *ie.* is the disease transmitted by propagation material and do nurserymen and commercial growers need to have concern about this or do our studies provide supportive evidence that this is not an issue?
5) maybe we can better understand the basis of pathogenicity and hence reasons why NAGY is often systemic and AGY localised in their grapevine hosts; and
6) maybe we can gain an understanding if native hosts (non-Vitis) are likely to show symptoms and so help in the search for the as yet, undetected alternate host of AGY.....!

## Chapter 21: A Proposal for Investigation
### A suggested experiment

The followng is a diagramatic representation of the process recommended (see text in erlier sections of this report) to assist in deriving a better understanding of AGY, its source and spread.

Live insects (leafhoppers) are to be collected and fed on a sucrose medium to collect the contents of their salivary glands. This would be PCR-tested for AGYp to determine if the phytoplasma is present in the insect – to see if the insect is an active vector of AGY. The leafhoppers would then be introduced on to caged host plants *eg.* yangabush seedlings and periwinkle, and these would be PCR-tested to determine if the phytoplasma were transmitted to these plants. Caged leafhoppers could then be fed on PCR-positive plants to see if those insects were able to acquire the phytoplasma from the test plants.

A bioassay system such as portrayed below was presented by Michael Maixner and has been used in studies on Bois noir in Gremany. The process is swift and effective in determining the vector of disease and allows rapid resolution of the vector – host feeding relationships.

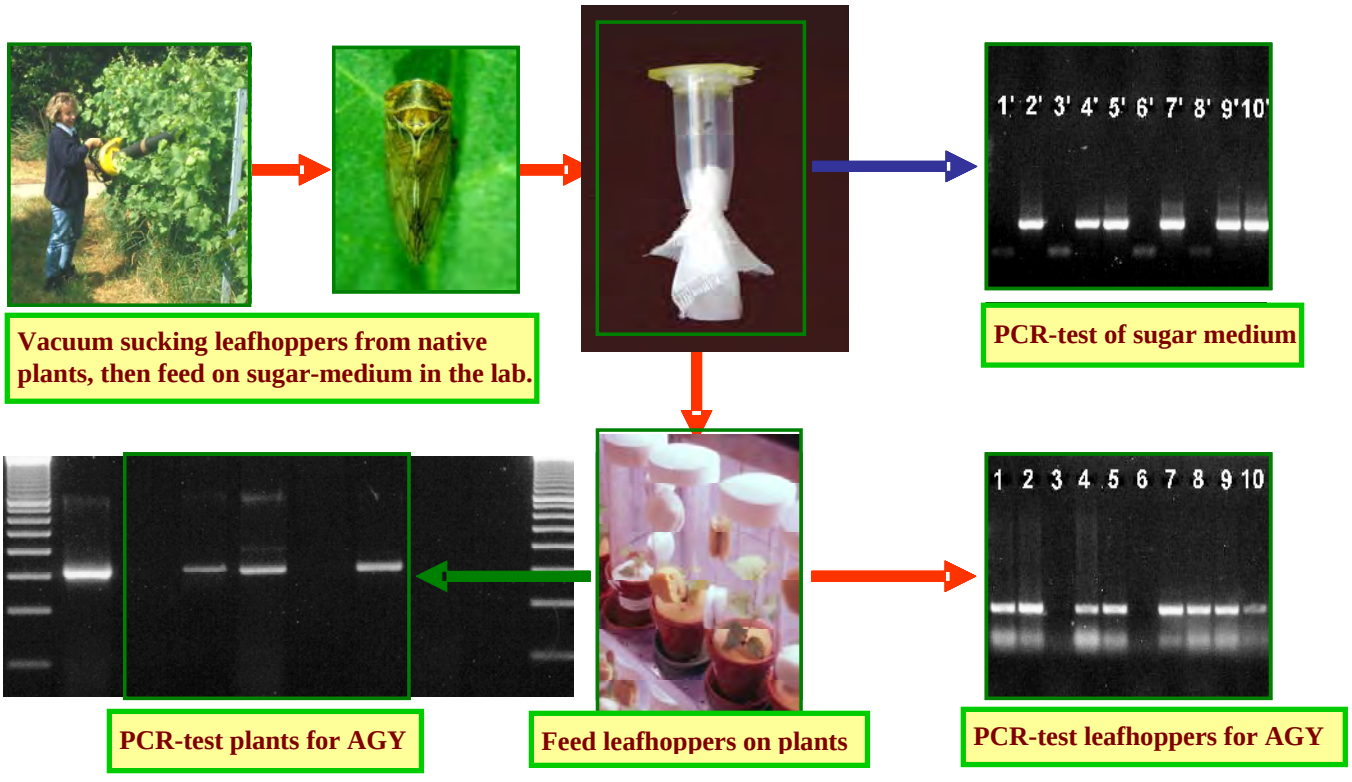



**Figure 21.1: Representation of the process avalible to rapidly test whether an insect such as a leafhopper is a likely vector of disease and to determine the vect-host feeding relationships. Diagram courtesy of MW Maixner.**

# Appendix 1. Crop Loss Estimates A brief review

*GWRDC Project SAR 02/03 operated under guidance from the Grapevine Yellows National Technical Reference Group which was under the auspices of the National Vine Health Steering Committee. Given the imbalance between the aims of the project and the resources available to achieve those aims, it was decided that the project give priority to the most important aspects of the disease – to understand the epidemiology and to find a management strategy - and not give input to an economic evaluation of the losses caused to industry. Given the interest in AGY and the debate about the losses it caused, the following study was undertaken separate from SAR 02/03. It is reported here for completeness.*

## Introduction

The levels of AGY (incidence and severity) are of considerable concern in a number of vineyards particularly in the Riverina, Riverland and Sunraysia. It is estimated that crop loss becomes economic when the incidence of AGY exceeds ~20% vines per vineyard. Levels of AGY in worst affected vineyards in Griffith, NSW, and Riverland were above 80% indicating severe crop loss was occurring in those vineyards. The owner of one of these vineyards estimated a loss of 30%.

The current project was directed not to investigate the economic consequences of AGY but discussion from a number of sectors led to the need for a brief review of the yield losses caused by AGY to be undertaken.

The following paper presents that review but it excludes for reasons of confidentiality some of the base data.

## Aim

**To estimate the losses caused by Australian grapevine yellows in the Riverland, Riverina and Sunraysia.**

## Materials and Methods

- Intensive mapping of the vineyard incidence of AGY was undertaken in the course of project SAR 02/03 – see Chapters 7-13;
- Each season a Riverland winery, Hardy Wine Company, Berri, SA, undertakes regular crop surveys of all their growers for a number of diseases including AGY. Before the surveys begin, their staff are trained in the recognition of AGY by the senior author. Vineyard levels of AGY were rated and categorised as high, medium or low.

**Yield Relationships - Estimating Crop Loss (2004 prices)**

**Assumptions for the Riverland:**

**Disease assessments:**

- AGY disease incidence was assessed by BRL surveyors rating vineyards at: 1 = nil AGY; 2 = low levels; 3 = medium levels; and 4 = high levels of AGY.

**Yield assessments:**

- Yield loss (actual data) was measured for three (season x block) reps for one property. These figures were used to derive estimators of crop loss where unaffected yield averaged 24 t/ha and the average yield loss was calculated at 14.8%.
- These crop loss estimates were used as multipliers on BRL scores for area (ha) in each disease category. For instance, BRL assessors attributed disease level 4 to 194 ha; we attributed an average of 60% crop loss to this area; level 3 to 204 ha at 20% loss; level 2 to 335 ha at 10% loss and level 1 to 557 ha at nil loss.

**Prices and Production [2003/04]:**

- **Chardonnay $897/tonne, 75,688 tonnes;**
- **Riesling $454/tonne, 3,994 tonnes; and**
- **Sangiovese $200/tonne, 820 tonnes,**

where Cost of AGY ($) = $ Value of Production (for 3 cvs) x Mean Crop Loss (14.7%);
Mean Crop Loss (%) = [$ Value Possible (if no AGY) - $ Value Actual Production (given BRL assessments of area to each AGY level and our assessment of crop loss for each level)]/ $ Value Actual Production.

**Results**

**Table 1. Estimated Crop Loss from AGY in Three Australian Viticultural Regions**

| Region | Farm Gate Loss ($) [2003/04] | Industry Loss ($) [2003/04] |
|---|---|---|
| **Riverland** | $10.4 m | $41.4 m |
| **Riverina** | $5.3 m | $21.0 m |
| **Sunraysia** | $12.9 m | $51.6 m |
| **TOTAL** | **$28.5 m** | **$114.1 m** |

***AGY is an economic problem for Australian viticulture***
***but varies from season to season in the losses it causes.***

***There is no known control for AGY***

***so that***

***… it is expedient to capitalise on progress to date***
***and complete investigations to find the source and spread of disease***

***before***

***the disease increases again and causes greater loss.***

# Appendix 2. Literature on AGY

*Two lists of publications are presented. The first is a list of references cited in the text above and the second is a list of papers on Australian Grapevine Yellows produced from the Loxton Research Centre.*

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## Section 2.2: Papers on AGY produced from Loxton Research Centre

### A. RESEARCH PUBLICATIONS

### Refereed Papers


- Magarey, P.A. and Wachtel, M.F. (1985). A review of the present status of Australian Grapevine Yellows. Agricultural Record 12(17):12-18.

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# INDEX