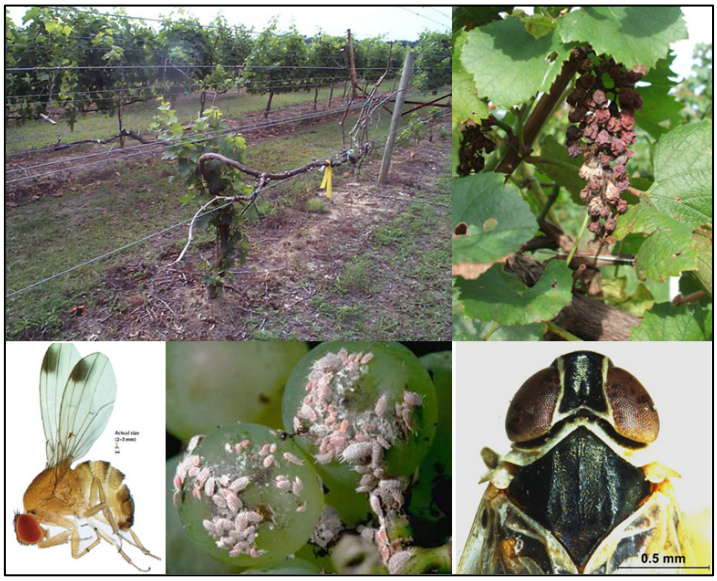
Australian Government

Australian Grape and Wine Authority

Department of Economic Development, Jobs, Transport & Resources

Developing and updating diagnostic protocols for key viticulture industry high priority exotic pest threats



FINAL REPORT to

AUSTRALIAN GRAPE AND WINE AUTHORITY

Project Number: DEP 1302

Principal Investigator: **Dr Mark Blacket**

Research Organisation: Department of Economic Development, Jobs Transport & Resources

Date: 24 July 2015

Contents

1. Abstract ……..…… 2
2. Executive Summary …… ……..…. 3
3. Background ……..…. 4
4. Project Aims and Performance targets ……..…. 4
5. Method ……. 5
6. Results/Discussion ……..……..….. 5
7. Outcome/Conclusion ……...….. 6
8. Recommendations ……. 7
9. Appendix 1. Communication …… 8
   - Appendices 1A – 1G: Draft diagnostic protocols for Cixiidae Planthopper, Vine mealybug, Grape mealybug, Spotted winged drosophila, *Xylella fastidiosa*, Flavesecence dorée phytoplasma and *Candidatus* phytoplasma solani (*Separate Documents x 7*) …….. 8
   - Appendix 1H: Training Workshop Manual (*Separate Document x 1*) ……... 9
   - Training Workshop ……... 9
10. Appendix 2. Intellectual Property ……..…… 15
11. Appendix 3. References …….. 15
12. Appendix 4. Staff …….. 15
13. Appendix 5. Other Data ……..…. 15
14. Appendix 6. Budget Reconciliation ……..……. 15

1. Abstract:

This project: 1) Developed / updated diagnostic protocols for seven high-priority exotic viticultural pests - Insects: Cixiidae planthopper, Vine mealybug, Grape mealybug, Spotted winged drosophila; Pathogens: *Xylella fastidiosa*, Flavesecence dorée phytoplasma and *Candidatus* phytoplasma solani. 2) Conducted a training workshop outlining the key diagnostic characters for each exotic pest.

These viticultural pests are associated with significant production losses overseas. The establishment of these pests in Australia would hinder produce export and grape movement. The best chance of control of these key pests is rapid identification, allowing a rapid response to contain and/or eradicate them if they were detected in Australia.

2. Executive summary:

The main objective of this project was to develop diagnostic identification resources (protocols) for seven high-priority exotic plant pests (insects & pathogens), for endorsement by the Subcommittee on Plant Health Diagnostics (SPHD) as National Diagnostic Protocols.

A range of high-priority plant pests were identified in the GWRDC Strategic RD &E Plan 2013-17, with no nationally endorsed protocols for most of these species. All of the pests are associated with significant loss in viticultural production overseas. The best chance of control of these key pests is rapid identification, allowing eradication efforts a reasonable chance of being successful. If any of these exotic pests did became established in Australia it would affect both local grape production and market access through reducing export opportunities. The establishment of these pests within specific wine growing regions would severely hamper movement of grape material within Australia and require quarantine measures to be applied.

This project re-examined, updated and collated available information on seven target pests from which draft diagnostic identification protocols were developed (or revised). New protocols were developed for: 1) Cixiidae planthopper, 2) Vine mealybug, 3) Grape mealybug, 4) Spotted winged drosophila and 5) Bois noir phytoplasma. Existing protocols were revised and further developed for: 6) *Xylella fastidiosa* and 7) Flavescence dorée phytoplasma (FDp).

Identification resources developed in this project included determining the diagnostic morphological characteristics of each exotic pest and comparing these with existing species in Australia. The protocols also established and documented which molecular identification protocols are suitable for use on each pest. The draft diagnostic protocols contain information on pest hosts, taxonomy, detection, and identification using both morphological and molecular characteristics, including comparisons with species in Australia with which the exotic pests could be confused in the field.

The one-day workshop conducted at the end of the project trained relevant diagnosticians, researchers and viticulture industry personnel from across Australia and New Zealand in the key identification features of each pest , including identification in the field.

The two main outputs of this project were: 1) Seven fully illustrated draft diagnostic identification protocols for the nominated priority exotic pests for use in Australia; and 2) A training workshop for relevant stakeholders in field and laboratory identification.

The project has significantly improved Australia's diagnostic capabilities that should reduce the establishment risk of these pests in Australia, by allowing for a rapid response to contain and/or eradicate them should an incursion occur.

3. Background:

This project provided an opportunity to develop, or update, national diagnostic protocols for seven exotic pests that have been identified as high priority biosecurity exotic pest threats to Australian viticulture in the most recent AGWA (formerly GWRDC) Strategic RD&E Plan.

The draft protocols developed have improved the Australian viticulture industry's preparedness in case of an exotic incursion, by allowing rapid and accurate diagnosis of specific plant pests. Each draft diagnostic protocol has been submitted to the AGWA and the Subcommittee on Plant Health Diagnostics (SPHD) for Plant Health Committee (PHC) endorsement. Once the protocols are endorsed by PHC they will be made accessible on their relevant websites. Protocol development for these pests, including updating the existing FDp and *X. fastidiosa* protocols, ensures that Australia is at the forefront of newly available diagnostic technologies based on international standards.

4. Project Aims and Performance targets:

The five objectives of this project were to:

1) Develop five, and update two, draft diagnostic protocols for key exotic viticulture pests to define the current morphological, biological and molecular detection methods that can be used for their accurate identification.

2) Clarify the key morphological and molecular characters that can be used to identify these pests in Australia, especially to develop resources that could be used for field identification.

3) Examine vineyard insect specimens preserved in collections from previous surveys, to establish which similar species are already present within Australia, and that these exotic species have not become established already.

4) Submit the protocols that incorporate internationally accepted diagnostic methods to SPHD for laboratory verification and endorsement by the PHC.

5) Produce training material and conduct a workshop to train diagnosticians and other key viticulture stakeholders in the identification of these exotic pests.

5. Method:

The current literature was reviewed and national / international experts consulted to ensure that the most up-to-date information was used in the development of the seven protocols.

For each insect taxa preserved specimens from reference collections were examined, to identify similar species with which they may be confused. This addressed knowledge gaps about the specific detection of these high priority insects and provides resources to target surveillance for these exotic pests in the field in Australia. The morphological characteristics for these exotic pests was examined and compared with similar species from Australia. This involved borrowing specimens from or travel to major institutions within Australia to examine preserved specimens, along with consultation with additional experts in identification of these groups of pests.

Protocols were developed identifying key morphological and molecular characters that could be used to recognize these exotic pests in Australia. The draft protocol documents include pest information, including:

a. General geographic distribution / biology information,
b. Taxonomic information,
c. Known host use,
d. Detection – Symptoms / Sampling / Methods,
e. Morphological identification – Field and Laboratory,
f. Morphological comparisons with other similar species in Australia,
g. Molecular identification,
h. Contact points for further information,
i. Up-to-date references,
j. Other relevant identification information & resources in Appendices.

These illustrated draft diagnostic protocols for both field and laboratory identification of the target exotic pests were used for a training workshop held for key stakeholders, at the end of this project.

6. Results/Discussion:

This project resulted in: 1) The production of draft diagnostic protocols for identification of seven key viticulture industry high priority exotic pest threats. These are attached as separate documents (see Appendices 1A – 1G), outlined further below. 2) A training workshop for key viticultural industry stakeholders, outlined further below.

Examinations of preserved specimens from Australian reference collections allowed recognition of these exotic pests from similar species and associated pests already in Australian vineyards. This activity has hightlighted the value of reference collections in diagnostics.

7. Outcome/Conclusion:

The project has achieved all of the objectives set out in the original project application, meeting all of the project milestones, as outlined in the table below. The development of the seven diagnostic protocols and the training workshop have benefited the viticulture industry through providing improved identification resources and awareness of these seven exotic pests and diseases, reducing the establishment risk in Australia.

Project Milestones:

Milestone	Title	Details	Output
a	Updated background information for seven viticulture industry high priority exotic plant pests	Update literature searches and collate background information on the seven targeted high priority exotic plant pests.	Development of the draft diagnostic protocols
b	Established laboratory procedures for diagnostic testing of the targeted plant pest species	Obtain reference material for each pest species from overseas, to be used as reference specimens and / or positive controls. Examine specimens of the exotic species and compare them with Australian native/endemic species with which they could be confused. Establish the procedures required for the diagnostic tests in the CHS laboratories at AgriBio.	Development of the draft diagnostic protocols
c	Completed (draft) protocols for the seven targeted pests submitted to SPHD as candidate National Diagnostic Protocols	Document laboratory protocols, including illustrations of diagnostic morphological characters and molecular test protocols if applicable. Write draft diagnostic protocols for Cixiidae planthopper, Vine mealybug, Grape mealybug, Spotted winged drosophila and Bois Noir phytoplasma. Revise and update the existing draft diagnostic protocols for Flavescence doreé phytoplasma and *Xylella fastidiosa*. Submit draft protocols to SPHD for review as candidate National Diagnostic Protocols.	Draft diagnostic protocols submitted to SPHD
d	Training workshop delivered at AgriBio	Prepare for and conduct a two-day identification training workshop for key stakeholders, involving up to 20 diagnosticians / surveillance personnel, representing each relevant Australian State and Territory.	Diagnostic Workshop conducted
e	AGWA Final Report	Prepare Final Report for submission to AGWA.	Final Report submitted

Recommendations:

1. This project has identified a need for further research and development of similar protocols for further related exotic species, for instance:

   a. There are at least 3 other species of Cixiidae (Planthoppers) and leafhoppers that are recorded as vectors of phytoplasmas in grapevines including at least one species which is considered to be equally as important as *H. obsoletus*. Further protocols are recommended; In particular a diagnostic protocol for the identification of the American grapevine leafhopper, *Scaphoideus titanus* Ball, which is the vector of FD phytoplasma. An epidemic of the grapevine yellows disease associated with FDp may not occur if this vector is not present. It is not known if this leafhopper is able to transmit phytoplasma species that are associated with grapevine yellows diseases in Australia.

   b. Recent research (Atallah *et al.* 2014) suggests that another exotic species of Drosophilidae - *D. subpulchrella* - closely related to SWD, is probably equally damaging to fruit;

2. The protocols for exotic insect pests developed in the current project were diagnostic "identification protocols" (following the SHPD protocol format, i.e. the DSWG sub-committee of SPHD). Further work would be useful to develop complimentary "surveillance protocols" for use in Australia for each of these groups of species. That is, documenting detailed methods for field (and produce) trapping / sampling for Drosophilidae, Cixiidae, and Pseudococcidae.

3. During this project DEDJTR were one of twelve international laboratories participated in an international test performance study for two LAMP assays that have been developed for the detection of FD phytoplasma and *Ca.* P. solani under the umbrella of the EUPHRESCO project GRAFDEP2. The method was simple and easy to use and interpret and a score of 100% was obtained by DEDJTR scientists. If successful the kits will be made commercially available through Optigene (UK). The use of these kits could be incorporated into the National diagnostic protocols as a rapid laboratory based detection method for preliminary identification of FD phytoplasma and *Ca.* P. solani

Reference:

Atallah J, Teixeira L, Salazar R, Zaragoza G, & Kopp A. 2014. The making of a pest: the evolution of a fruit-penetrating ovipositor in *Drosophila suzukii* and related species. *Proceedings of the Royal Society B: Biological Sciences* **281,** 20132840.

8. Appendix 1: Communication:

Draft diagnostic protocols

The seven draft diagnostic protocols developed during this project, submitted to SPHD for endorsement by the PHC, are attached as separate documents:

Appendix 1A: Cixiidae planthopper – "Draft Diagnostic Protocol for *Hyalesthes obsoletus* Signoret", [New Draft Protocol, 44 pages]

Appendix 1B: Vine mealybug – "Draft Diagnostic Protocol for Vine Mealybug (*Planococcus ficus* (Signoret))" [New Draft Protocol, 34 pages]

Appendix 1C: Grape mealybug – "Draft Diagnostic Protocol for Grape Mealybug (*Pseudococcus maritimus* (Ehrhorn))" [New Draft Protocol, 31 pages]

Appendix 1D: Spotted winged drosophila – "Draft Diagnostic Protocol for Spotted Wing Drosophila (*Drosophila suzukii*)" [New Draft Protocol, 42 pages]

Appendix 1E: *Xylella fastidiosa* – "Draft Diagnostic Protocol for Pierce's Disease caused by *Xylella fastidiosa"* [Updated Draft Protocol, 37 pages]

Appendix 1F: Flavesecence dorée Phytoplasma – "Draft Diagnostic Protocol for *Flavescence dorée* Phytoplasma" [Updated Draft Protocol, 34 pages]

Appendix 1G: *Candidatus* phytoplasma solani – "Draft Diagnostic Protocol for *Candidatus* phytoplasma solani asscoiated with Bois Noir disease of grapevine" [New Draft Protocol, 38 pages]



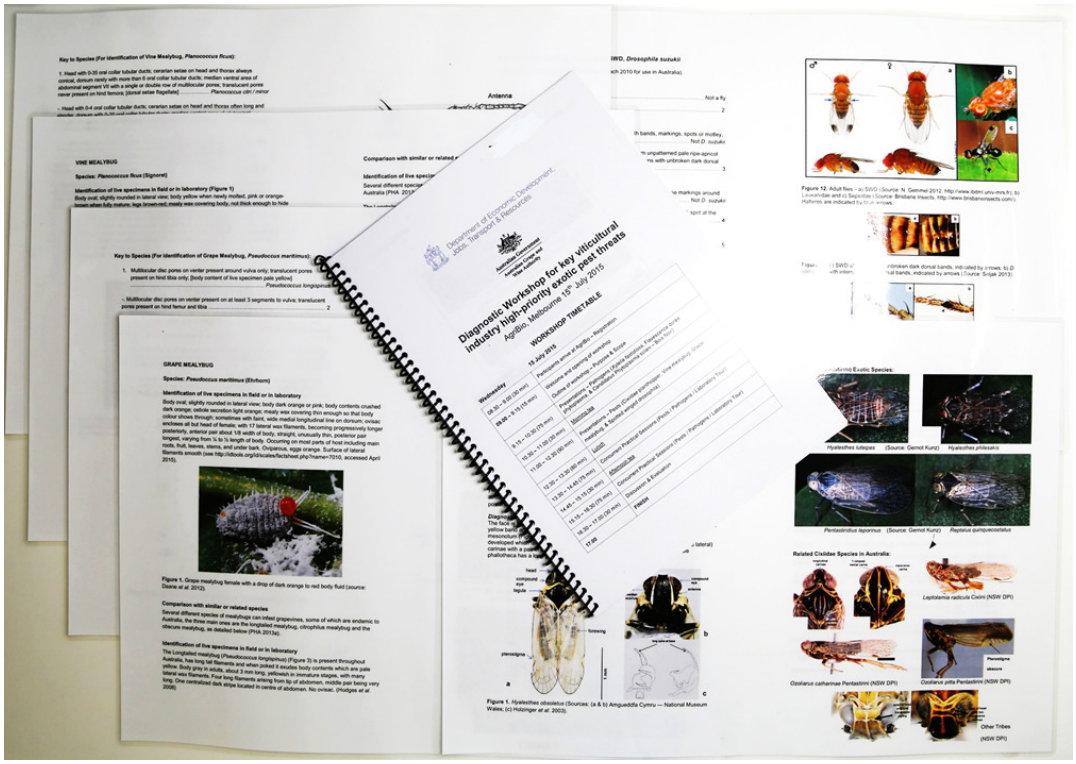
The seven diagnostic protocols (Appendices 1A – G) that were developed / updated

Training Workshop

A one-day diagnostic training workshop was held at AgriBio (Bundoora, Victoria) on the 15th July 2015. Each of the project personnel participated as presenters.

The workshop program (shown below) involved morning presentations outlining each of the draft diagnostic protocols (covering both morphological and molecular identification), followed by practical sessions in the afternoon that re-enforced the key diagnostic morphological characters for each pest / disease. Printed training material was provided to workshop participants (i.e. a training manual); this consisted of copies of all of the mornings presentations. The manual is attached to this report as a separate document: ***Appendix 1H*** [87 pages]. Identification in both the field and the laboratory were covered in the workshop.

Twenty-one participants attended the workshop including: industry representatives, researchers, & diagnosticians. These participants were invited from across Australia and New Zealand, the attendees are listed in the table below. The participants provided very positive feedback regarding the workshop, a summary of the workshop survey scores and comments are presented below.



Workshop Training Manual (Appendix 1H),
& other training material used during the workshop at AgriBio

Diagnostic Workshop Participants (AgriBio, 15th July 2015):

	Name	Agency
1	Greg Baker	SARDI, GPO Box 397 Adelaide, 5001, SA
2	Adam Broadley	Department of Agriculture, VIC
3	Brenda Coutts	Department of Agriculture and Food, Western Australia, 3 Baron-Hay Court, South Perth, 6151, WA
4	Kerry Degaris	The Australian Wine Research Institute, Waite Precinct, PO Box 197, Glen Osmond, 5064, SA
5	Robin Eichner	Department of Agriculture, VIC
6	Brian Englefield	Wine Grape Growers Australia, VIC
7	John Hawtin	Dried fruits Australia, 31 Deakin Avenue, Mildura, 3500, VIC
8	Elise Heyes	R&D Program Manager, Australian Grape and Wine Authority, SA
9	Greg King	Biosecurity Vic, DEDJTR, Knoxfield Centre, 621 Burwood Hwy, Knoxfield, 3180, VIC
10	Stewart Learmonth	Department of Agriculture and Food, Western Australia, WA
11	Dongmei Li	MPI, New Zealand
12	David Madge	Biosciences Research, DEDJTR, Mildura Centre, Cnr 11th St & Koorlong Ave, Irymple 3498, VIC
13	Andrew Manners	Biosecurity Queensland Ecosciences Precinct, 41 Boggo Road Dutton Park, 4102, QLD
14	Daniel Mansell	Biosecurity Vic, DEDJTR, Attwood Centre, 475 - 485 Mickleham Rd, Attwood 3049, VIC
15	Sue Pederick	SARDI, GPO Box 397, Adelaide, 5001, SA
16	Ben Rose	Viticulturist and Valuer, 1944 Healesville Kinglake Road, Toolangi, 3777, VIC
17	Ainsley Seago	NSW Department of Primary Industries, 1447 Forest Rd, Locked Bag 6006 Orange, 2800, NSW
18	Lucy Tran-Nguyen	Department of Resources, GPO Box 3000, Darwin, 0801, NT
19	Mark Walpole	Aquila Audax Enterprises, VIC
20	Melanie Weckert	NSW Department of Primary Industries, National Wine & Grape Industry Centre, Locked Bag 588, Wagga Wagga, 2678, NSW
21	Maryam Yazdani	School of Agriculture, Food and Wine, Adelaide University, SA

WORKSHOP TIMETABLE

Wednesday	**15 July 2015**
08.30 – 9.00 (30 min)	Participants arrive at AgriBio – Registration
09.00 – 9.15 (15 min)	Welcome and opening of workshop Outline of workshop – Purpose & Scope
9.15 – 10.30 (75 min)	Presentations – Pathogens (*Xylella fastidiosa*, Flavescence dorée phytoplasma, & *Candidatus* Phytoplasma solani – Bois Noir)
10.30 – 11.00 (30 min)	Morning tea
11.00 – 12.30 (90 min)	Presentations – Pests (Cixiidae planthopper, Vine mealybug, Grape mealybug, & Spotted winged drosophila)
12.30 – 13.30 (60 min)	Lunch
13.30 – 14.45 (75 min)	Concurrent Practical Sessions (Pests / Pathogens / Laboratory Tour)
14.45 – 15.15 (30 min)	Afternoon tea
15.15 – 16.30 (75 min)	Concurrent Practical Sessions (Pests / Pathogens / Laboratory Tour)
16.30 – 17.00 (30 min)	Discussion & Evaluation
17.00	**FINISH**



Diagnostic training workshop held at AgriBio, July 2015.

Workshop participant survey - Summary of Feedback

Workshop:
Diagnostic Workshop for key viticultural industry high-priority exotic pest threats
15th July 2015 (AgriBio, Melbourne)

Number of surveys: Sheets filled in (scores / comments) by 19 (of 21) participants (90%).

Q 1. Did this activity meet your expectations?

Not at all | 1 | 2 | 3 | 4 | 5 | Exceeded expectations

Results: Average Score 4.4 (88%, Exceeded expectations)
Comments:

- Good content, very informative;
- It was nice to see both disciplines. all talks and demonstrations were very interesting;
- Very good;
- Knowledgeable presenters with talks and practical re-enforcement very good
- Would have been good to have more hands on identification of specimens
- A lot of DNA discussion was way over my head but I still found it very interesting

---

Q 2. What is your overall rating of this activity?

Poor | 1 | 2 | 3 | 4 | 5 | Excellent

Results: Average Score 4.6 (92%, Very Good – Excellent)
Comments: (None provided by participants)

Q 3. How do you rate the balance between input sessions, activities and discussions?

Poor | 1 | 2 | 3 | 4 | 5 | Excellent

Results: Average Score 4.5 (90%, Very Good – Excellent)
Comments:

- Enough time given for other questions
- Good balance between theory and practical
- Ideally more diagnostic activity
- More identification activity would have been good

Q 4. How relevant was the course content to your workplace?

Not Relevant	1	2	3	4	5	Relevant

Results: Average Score 4.4 (88%, Relevant)
Comments:

- Good time to see specimens
- Especially for emergency situations
- We only get a few grape samples
- Greater awareness of exotic pests of grapevines
- Useful for in-field recognition of pests and diseases

Q. 5. My key learnings were…
Comments:

- Characteristics of exotic pests of vines
- Differences between PCR techniques
- Different range of plant pests which affect grapevines
- Details of different identifiers for exotic pest species
- The amount of work in the field
- Range of vectors
- Understanding characteristics of each pest and disease
- Some familarity with grapevine EPPs discussed
- Identification of pests. Getting to know the experts in identification
- Improved diagnostic protocols
- Importance of diagnostic networks to be able to access specialists to assist with suspected biosecurity interception identifications
- Complexity of some identification requirements - capabilities of diagnostic services available
- This is an excellent facility
- Morphological features, update information on diagnostics of exotic pests
- Key identification characteristics, keep these out of Australia

---

Q. 6. What learnings can you transfer to your work?
Comments:

- Updated information regarding SWD & diagnostics
- Timing of turn around on assessment of exotic pests
- Molecular
- Identification in the field
- Send more samples
- Send / submit samples
- Identification & actions
- Handbook
- Protocols will be available for public use. How to obtain photos for field use to aid detection
- Disease diagnostics especially for Phytoplasmas
- Biology, basic diagnostics of these pest threats. Key diagnostic experts
- General awareness of exotic pests and disease identification
- DNA barcoding of mealybugs
- Make growers in my industry aware of these exotic pests

Q. 7. How would you rate the processes and activities used by the facilitators?

Not effective	1	2	3	4	5	Very effective

Results: Average Score 4.5 (90%, Very effective)
Comments:

- Good to see "real" samples of insects
- Would have preferred more specimen identification, less tour

---

Q. 8. How would you rate the skills and knowledge of the facilitators?

Poor	1	2	3	4	5	Excellent

Results: Average Score 4.9 (98%, Excellent)
Comments:

- Clearly very knowledgeable and were able communicate this to a group of varying audience

---

Q. 9. Were there any aspects of this workshop that could be improved in the future?
Comments:

- No
- In the time frame, it was good. Another day would be good for DNA analysis for those who are interested but no opportunity for exposure
- Within the limitations of a one-day workshop I don't think you could substantially improve on the workshop. With more time you could go into more detail on the diagnostic protocols and identification of insects
- How to use identification knowledge to encourage farmers to be aware - they will get it first
- Would have preferred more specimen identification, less tour
- No
- No it was all informative

Q. 10. Any other comments?
Comments:

- Excellent
- Great workshop. Thanks!
- Thanks for inviting me!
- Excellent venue!
- The cross section of the audience was good - funders, researchers, taxonomists, consultants, growers
- From limited specific knowledge to an improved knowledge now I know who to contact if suspect problems
- Thank you it was all interesting

9. Appendix 2: Intellectual Property:

The IP from the project will be placed in the public domain. The IP from this project includes improved knowledge of seven high-priority exotic insects pests: Cixiidae planthopper, Vine mealybug, Grape mealybug, Spotted winged drosophila; & diseases: *Xylella fastidiosa*, Flavesecence dorée phytoplasma and *Candidatus* phytoplasma solani. The diagnostic protocols have been submitted to SPHD for endorsement and publication on their website. There is a route to market as the developed protocols will be made available to industry through diagnostic laboratories including Crop Health Services (DEDJTR) and other national diagnostic services.

10. Appendix 3: References

The seven protocols developed, or updated, during this project (Appendices 1A – 1G) each include a full list of relevant references specific to that particular pest or disease. Therefore, they are not listed again in this section.

11. Appendix 4: Staff

Dr Mallik Malipatil (Project Supervisor), 10% for 12 months.
Dr Mark Blacket (Chief Investigator), 20% for 12 months.
Dr Linda Semeraro, 10% for 12 months.
Dr Fiona Constable, 10% for 12 months.

12. Appendix 5:

Data acquired during this project has been incorporated into the Draft Protocols (Appendices 1A – 1G). Note, most of the information included in these protocols was obtained from published sources (e.g. journal articles), or is available publically online (e.g. previous diagnostic protocols, images etc).

13. Appendix 6: Budget reconciliation

The End of Project Financial Statement has been submitted online, via AGWA's Clarity Investment Management System.