Understanding the fundamental interactions between Woolly Apple aphid and pome fruit (Revised)

Dr Kevin Powell
Victorian Department of Primary Industries (VICDPI)

Project Number: AP06011

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Understanding the Fundamental Interactions between Woolly Apple Aphid and Pome Fruit

HAL FINAL REPORT APO6011
Dr K.B. Andrews & Dr K.S. Powell
December 2009

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Project Title: Understanding the Fundamental Interactions between Woolly Apple Aphid and Pome Fruit

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Media Summary

Victoria produces more than 25% of Australia's apple production. A major economic cost to apple growers is the use of insecticides to control pests. The woolly apple aphid (WAA) is a serious pest of apples in Australia and over the last ten years reports of its incidence have increased. It induces galls on foliage and underground parts of apple trees and also produces honeydew, which on the leaves and fruit forms a reservoir for sooty mould, reducing fruit quality and marketability and photosynthetic efficiency. Severe infestations on young trees cause stunting or even death. Woolly apple aphid is becoming a major problem for apple growers attempting to follow world best practice by adopting intensive production systems and reducing broad-spectrum pesticide usage. Over recent years, partly due to a reduction in use of pesticides, but also because of the use of susceptible rootstocks (including seedling stocks in 'conventional' plantings), WAA incidence has increased. Many growers are now routinely treating trees with chemicals to control woolly apple aphid. While this is effective on young trees, reliance on a single chemical group is unsustainable and will ultimately lead to WAA resistance to some pesticides.

The research team have utilised their expertise in insect physiology, electrophysiology and applied entomology to develop a better understanding of the feeding interactions between the pest and its host-plant that and further research development this will lead to improved management of woolly apple aphid.

Developments within this project have included:

(i) a Victorian grower survey highlighting woolly apple aphid distribution, infestation levels and management strategies

(ii) developing a woolly apple aphid artificial diet rearing system which will lead to an improved understanding of the insects nutritional requirements and how this impacts on its interactions with its host-plant. Further developments in artificial diet formulation will lead to improved woolly apple aphid management.

(iii) an electrophysiological method called the EPG (Electrical Penetration Graph) was used to examine in fine detail the feeding behaviour of two woolly apple aphid populations on resistant and susceptible rootstocks.

(iv) a comparison of the feeding behaviour and survival of two geographically distinct woolly apple aphid populations, using both the artificial diet system and the EPG system, indicate that future management of WAA will be improved by considering the genetic diversity and geographical range of woolly apple aphid populations.

Technical Summary

The woolly apple aphid (WAA; *Eriosoma lanigerum*) causes economic damage to apple production due to feeding activity on the roots and aerial parts of the apple tree. The aphids produce a white waxy filament which gives them their distinctive woolly appearance. Feeding in the leaf axils can destroy buds and heavy infestations can cause crop losses. In addition they excrete sticky "honeydew" which acts as a site for sooty mould development, reducing the quality and marketability of fruit. IPM strategies are used to control the woolly apple aphid, but choice and timing of applications are important particularly to avoid disruption of natural predators in the orchard. Woolly apple aphid is becoming an increasing problem in intensively produced orchards that have a high usage of insecticides and the potential exists for breakdown in rootstock resistance. The apple industry wants to increase the percentage of the crop grown under intensive production systems because these systems are considered world's best practice. Intensive production systems rely on dwarfing rootstocks to reduce tree size and increase precocity. Current dwarfing rootstocks are susceptible to WAA and the insect has increased in incidence over the last few years. Current options for control include insecticide application and 'resistant' rootstocks. Constraints to the use of chemical control measures include the insect's waxy excretions and its overwintering and feeding behaviour on roots, making chemical control more difficult. Some chemical options are also harmful to beneficial insects such as parasitic wasps, earwigs, lacewings and ladybirds. Whilst insecticide drenching is effective for young trees it is considered expensive by growers. The root-feeding activity of the insect also makes it difficult to detect until it appears in relatively large numbers above-ground. The cost of replanting onto so-called 'resistant' rootstocks is also an economic constraint to growers. The rootstocks only inhibit woolly apple aphid populations on the roots and do not impart resistance or tolerance to the scion. In order to make management options more effective against the woolly apple aphid several knowledge gaps, relating to the insects biology, need to be addressed.

This project has improved the current understanding of the fundamental biology of the woolly apple aphid, with the long-term aim of improving current management strategies. The project has utilised grower surveys, *in vitro* rearing systems and electrophysiological techniques. Major research findings when comparing two geographically isolated WAA were that these populations had fundamentally different nutritional requirements, as assessed by comparing survival on both simple sucrose-based and more complex liquid artificial diet formulations. This is the first time WAA has been reared *in vitro*. Feeding behaviour of the two WAA populations was also assessed *in planta*, on four apple rootstock types (Granny Smith, Northern Spy, MM106 and M793) using the electrical penetration graph (EPG) method and results indicated that rootstock resistance ratings are likely to differ depending on which 'population' is screened. This along with data from grower surveys suggests that that different WAA 'biotypes' are likely to exist within different apple growing regions of Australia. Further studies are recommended to determine the insects' geographical distribution and genetic diversity. In addition once the genetic diversity of woolly apple aphid is characterised, using molecular techniques already utilised overseas, artificial diet and EPG systems could be optimised and further utilised to develop an improved rootstock screening protocol. This fundamental background knowledge is required to develop a targeted and sustainable woolly apple management options for the Australian pome fruit industry.

Woolly Apple Aphid Survey

Introduction

Woolly apple aphid (WAA) *Eriosoma lanigerum* (Hemiptera: Aphididae) is known to affect apple growers across the state of Victoria. However, data on its distribution, impact and management strategies used in the different grower regions in Victoria is often anecdotal and or limited in detail. Obtaining such data from growers in the state provided baseline data from which further more detailed studies for specific regions could be conducted.

Material & Methods

In April 2008 a confidential survey (Appendix 1) was distributed to 300 apple growers in the Ardmona, Bacchus Marsh, Cobram, Gippsland, Harcourt, Kyabram, Metropolitan, Mornington Peninsular, North East, Shepparton, Stanley and the Yarra Valley districts. The aim of the grower survey was to gain an understanding of the level of impact WAA infestations have in Victorian apple orchards. Information was collected at 2 levels – the individual growers; and individual orchard blocks under the management of the grower.

The results of the survey were collated and analysed for summary statistics using GenStat© (VSN International). Due to the formatting of the survey, analysis was completed on both a grower and an orchard block level. Data compared on the individual grower level included: the presence/absence of WAA, the percentage of WAA infestation, chemical usage and management of WAA. A maximum of 3 individual orchard blocks were reported by each individual grower (responses were only requested for orchards containing WAA). Data compared on the individual orchard block level included: apple variety and rootstock, orchard age, tree vigour, canopy structure, location of WAA within the orchard and the severity of the WAA infestation.

Interactions between the variables reported in the grower survey, in regards to impacting on WAA infestation, were expressed using classification and regression tree analysis. The R statistical program© (The R Foundation of Statistical Computer) was used to perform this analysis.

Results

Survey respondents provided information on up to 3 orchard blocks impacted by WAA. A total of 56 (18.7% response rate) completed surveys were received, containing information on 131 orchards. Surveys were returned from 11 of the 12 districts targeted, however districts were combined into 7 broader district locations in order to increase sample number for statistical analysis. District locations appearing in statistical outputs are: Cobram, Gippsland, Harcourt, Mornington (Peninsular), Shepparton (incorporating Ardmona and Kyabram data), Stanley (incorporating North East data) and the Yarra Valley (incorporating Metropolitan data). No survey responses were received from Bacchus Marsh.

Woolly apple aphid - infestation levels and incidence

Of the 56 grower survey responses received, 54 reported the presence of WAA within their apple orchards (Table 1). The reported percentage of the apple orchard infested with WAA varied from 0-100%. Stanley and Gippsland reported the highest average percentage of WAA infestation, with 50% and 41% (respectively) of the apple orchard being affected by WAA. Harcourt (range 0.5-30) and Mornington (range 1-40) reported the lowest variation in percentage of WAA infestation, and also relatively low levels of WAA infestation of 11% and 17% (respectively). Shepparton had the highest number of grower respondents (21), and although experienced a high level of variation in percentage of WAA infestation (0.5-100), reported the lowest average percentage (2%).

Table 1. Grower survey results summarised by region.

region	no. of respondents	no. growers with WAA	average % of orchard with WAA	range % of orchard with WAA
Cobram	8	7	20	0-100
Gippsland	5	4	41	0-100
Harcourt	8	8	11	0.5-30
Mornington	4	4	17	1-40
Shepparton	21	21	2	0.5-100
Stanley	4	4	50	1-100
Yarra Valley	6	6	33	5-60

Woolly apple aphid - chemical control

Orchard growers reported a variety of chemicals that were used on the apple orchards to control WAA, or other orchard pests including codling moth and light brown apple moth. To allow for analysis of this data, chemicals were grouped by their active ingredient and chemical class (Table 2). A total of 9 chemical classes were identified, as well as the use of oil. Integrated Pest Management (IPM) was also commonly reported. A range of other chemicals only reported by a few growers were grouped for statistical analysis as "other" chemical treatment.

The use of chemicals varied across the regions (Table 3). Most growers used multiple chemical treatments, involving up to 6 chemical classes, in their orchard for the management of insect pests. The 3 chemical classes in most common usage across all regions were 1B, 4A and IPM. Chemical classes 2A, 3A, 11C and 16A were rarely used.

Table 2. Chemical active ingredients grouped by chemical class.

1A	1B	2A	3A	4A
Carbaryl Pirimicarb	Azinphos-methyl Chlorpyrifos Parathion-methyl	Endosulfan	Bifenthrin	Clothianidin Imidacloprid Thiacloprid

7B	11C	16A	22A	IPM
Fenoxycarb	Btk (*Bacillus thuringiensis*)	Tebufenozide	Indoxacarb	Isomate (IPM) Parasitic wasp Pheromone Organic

Table 3. Grower data for chemical insecticide class usage in apple orchard by region.

region	average no. chemical class	range no. chemical class	% of growers from region using each chemical class (n)				
			1A	1B	2A	3A	4A
Cobram	2.3	1-3	0	100 (8)	0	0	75 (6)
Gippsland	3.6	2-6	60 (3)	60 (3)	0	0	40 (2)
Harcourt	2.8	1-6	0	63 (5)	0	0	63 (5)
Mornington	2.8	1-6	0	75 (3)	0	0	50 (2)
Shepparton	2.1	1-4	5 (1)	86 (18)	5 (1)	10 (2)	52 (11)
Stanley	3.0	1-4	25 (1)	100 (4)	0	0	50 (2)
Yarra Valley	3.7	2-5	16 (1)	83 (5)	0	0	67 (4)

region	% of growers from region using each chemical class (n)						
	7B	11C	16A	22A	IPM	oil	other
Cobram	0	13 (1)	0	0	38 (3)	0	0
Gippsland	80 (4)	0	20 (1)	40 (2)	40 (2)	20 (1)	0
Harcourt	63 (5)	0	0	13 (1)	50 (4)	13 (1)	13 (1)
Mornington	0	0	25 (1)	25 (1)	50 (2)	25 (1)	25 (1)
Shepparton	14 (3)	5 (1)	0	10 (2)	10 (2)	19 (4)	0
Stanley	50 (2)	0	0	0	25 (1)	50 (2)	0
Yarra Valley	50 (3)	0	50 (3)	33 (2)	33 (2)	33 (2)	0

Table 4. Individual orchard block data for a number of variety plantings per region.

region	no. blocks	Fuji	Granny Smith	Pink Lady	Red Delicious	Sundowner	Mixture	Other
Cobram	15	0	10	2	1	0	0	2
Gippsland	11	5	2	1	0	0	0	3
Harcourt	19	6	3	3	2	1	0	4
Mornington	8	2	1	1	0	0	3	1
Shepparton	49	1	28	13	0	3	0	4
Stanley	12	2	1	1	2	0	2	4
Yarra Valley	17	4	3	3	0	3	2	2

Table 5. Individual orchard block data for a number of rootstock plantings per region.

region	no. blocks	M7	M26	MM106	MM111	Northern Spy	Seedling	Other
Cobram	15	1	0	2	0	4	4	4
Gippsland	11	2	1	0	1	3	0	4
Harcourt	19	1	1	0	5	2	8	2
Mornington	8	0	2	1	0	3	0	2
Shepparton	49	2	3	14	0	12	9	9
Stanley	12	1	0	5	1	2	0	3
Yarra Valley	17	6	2	0	1	1	2	5

Woolly apple aphid - varietal and rootstock selection

Details on the impact of WAA on orchard blocks were provided by the 56 growers surveyed for 131 individual blocks. Apple variety and rootstock plantings were reported for each of these individual orchard blocks (Table 4). Five main varieties were reported – Fuji, Granny Smith, Pink Lady, Red Delicious and Sundowner. Seven orchard blocks were reportedly planted with a "mix" of 2-3 varieties. Varieties only reported in low numbers (Cameo, Cider, Gala, Galaxy, Golden Delicious (GD), Jonathan, Lady William, Mutsu, Red Fuji Rosy Glow, Royal Gala, Smoothee GD) were grouped as "other" for statistical analysis. The apple varieties planted on 2 orchard blocks were of "unknown" origin; these were grouped for statistical analysis, but have been added to the "other" total in Table 4 for summary analysis.

Granny Smith was the most commonly planted apple variety, followed by Pink Lady and Fuji. By percentage of total orchard blocks reported for each region, Granny Smith was the predominant planting at both Cobram and Shepparton. Fuji was common at Gippsland, Harcourt, Mornington and in the Yarra Valley. Sundowner and Red Delicious were less common; however Red Delicious was the most predominant planting at Stanley (equal with Fuji).

There was no significant relationship between apple variety and rootstock selection. Six main rootstocks were reported – M7, M26, MM106, MM111, Northern Spy and Seedling (Table 5). Six orchard blocks were reportedly planted with a mix of 2 rootstocks (these were grouped for statistical analysis, but have been added to the "other" total in Table 5 for summary). Rootstocks reported in low numbers (M9, MM102, Mac-9 and VT Spy) were grouped as "other" for statistical analysis. The rootstocks planted on 14 orchard blocks were of "unknown" origin; these were grouped for statistical analysis, but have been added to the "other" total in Table 5 for summary analysis.

All rootstocks were regularly reported, however Northern Spy was the only rootstock present in all regions. By percentage of total orchard blocks reported for each region, Northern Spy was the most predominant planting in Cobram, Gippsland and on the Mornington Peninsular. Seedling and MM106 were the next most common rootstocks; MM106 was the predominant planting in Stanley and Shepparton, Seedling was common in Harcourt, Cobram (equal with Northern Spy) and Shepparton. M7 was the most common rootstock in the Yarra Valley. The age of the orchard block plantings ranged form <5 to +30 years, however most trees were between 10-20 years old. The majority of apple growers reported medium-strong vigour for the trees. The tree canopy was generally Central Leader or Vase; however Tatura Trellis was common in the Shepparton region (data not presented).

Woolly apple aphid - distribution and severity

The impact of WAA on individual orchard blocks was reported by 3 separate indicators:

(i) location of WAA on individual trees (base – trunk – canopy)

(ii) level of spread of the WAA throughout the orchard (even – patchy)

(iii) overall severity of the WAA infestation (low – medium – high)

WAA were reported on all aerial parts of the apple trees (base, trunk and canopy), either in isolation or in combination (Table 6). WAA migrate from the soil surface, or the base of the apple tree, to the trunk and canopy of the tree throughout the growing season, therefore it is expected to find the insect in all locations. The grower survey was conducting during April-May 2008 (autumn); the location of the WAA may be varied during the season but this information was not captured.

Table 6. Location of WAA on individual apple trees by region.

region	base	trunk	canopy	none
Cobram	2	2	4	1
Gippsland	1	0	1	1
Harcourt	8	0	0	0
Mornington	2	1	5	0
Shepparton	8	1	10	1
Stanley	0	0	7	0
Yarra Valley	0	0	7	0

region	base + canopy	base + trunk	trunk + canopy	all locations
Cobram	0	2	0	4
Gippsland	4	0	0	4
Harcourt	2	2	1	5
Mornington	0	0	0	0
Shepparton	6	6	2	15
Stanley	3	0	0	2
Yarra Valley	3	0	5	2

The level of spread of the WAA was indicated by the location of the pest insect being either evenly spread throughout the orchard, or only in patchy locations. Most growers reported patchy distribution of the insect (Table 7).

Table 7. Level of spread of the WAA throughout the apple orchard by region.

region	even	patchy	none
Cobram	2	12	1
Gippsland	0	10	1
Harcourt	0	19	0
Mornington	1	6	0
Shepparton	4	44	1
Stanley	3	9	0
Yarra Valley	6	11	0

Growers were also requested to rate the overall severity of the WAA infestation. Most growers reported a low level of WAA infestation in the individual orchard blocks surveyed; high levels of infestation were only reported in Cobram, Stanley and Yarra Valley regions. The severity rating is very subjective, and may also differ between season, and it is not expected that all growers rated their levels of WAA infestation equally in comparison with other growers or regions.

Table 8. Overall severity of the WAA infestation by region.

region	low	medium	high	none
Cobram	6	6	2	1
Gippsland	6	4	0	1
Harcourt	9	10	0	0
Mornington	2	6	0	0
Shepparton	35	14	0	0
Stanley	6	3	3	0
Yarra Valley	5	9	3	0

In order to identify possible interactions between the variables reported in the grower survey, classification 'trees' were constructed for the 3 measures of WAA impact on the individual orchard blocks. The sample number from the grower survey was not sufficient to allow for prediction statistics; however the classification trees may still be reviewed as an indicator for what may be impacted on WAA populations.

Each split in the classification tree indicates the most likely variable impacting on the WAA population for each measure of interest. The variables splitting the tree are indicated on each 'branch'; the output at the base of each branch indicates the classification tree grouping. The first split is the most influential factor affecting the classification tree groupings, the second split the 2nd most influential, etc. The importance of the split order on the impact of the WAA population is highlighted by the decrease in font text size as the tree progresses.

Location of WAA on individual trees (base – trunk – canopy)

Region was the major factor for determining the location of WAA on individual apple trees (Figure 1). The canopy was the predominant location for WAA in the Mornington Peninsular, Stanley and Yarra Valley regions; a variety of locations for the WAA were reported in the other regions. The next most important variable for the location of WAA was variety; WAA were located on all locations (base, trunk and canopy) of Fuji and Granny Smith, however other varieties was more restricted in the location of WAA. Apple tree rootstock and canopy also influenced the location of the WAA.

Level of spread of the WAA throughout the orchard (even – patchy)

Region was also the major factor determining the level of spread of the WAA population throughout the apple orchard (Figure 2). Patchy distribution of WAA split the Cobram, Gippsland, Harcourt and Shepparton regions from the Mornington Peninsular, Stanley and Yarra Valley. Variety and rootstock separated these regions further into patchy and even WAA distribution.

Overall severity of the WAA infestation (low – medium – high)

The major determinant for the overall severity of WAA infestation was tree age; trees between 6-10 years old and >30 years old reported low levels of infestation, while trees <5 and 11-30 years of age reported a mix of infestation levels. The first tree split was again split by region (Figure 3), highlighting the importance of region on all 3 measures of WAA impact on the individual orchard blocks. Medium levels of WAA infestation were mainly reported in

the Mornington and Yarra Valley regions; the other regions required further splitting by rootstock, canopy and variety in order to group the outputs.

The grower data was used to generate a regression tree to provide an indication for the variables influencing the percentage of the orchard affected by WAA. This analysis aimed to indicate the impact of chemical usage in the orchard on the WAA population. Chemical usage was defined by the number of chemical classes reported, the presence/absence of each of the chemical classes, plus the presence/absence of Carbaryl or Sevin as part of a thinning program within the orchard.

The regression tree is interpreted similarly to the classification trees, except the number of growers grouped by each split and branch is displayed rather than the output influencing the split. The variables splitting the tree are indicated on each branch. Note that the total grower number used for the analysis (51) does not equal the total number of surveys received (56); some growers were censored from the analysis due to missing data points which were not acceptable for the R statistical package.

Region was once again the major factor influencing the occurrence of WAA within the orchard (Figure 4); chemical usage did not appear to influence the percentage of the orchard affected by WAA. Gippsland, Mornington, Stanley and the Yarra Valley clustered together, each recording high averages for the percentage of WAA reported (Table 1). Cobram, Harcourt and Shepparton also clustered; Shepparton was further split from the cluster however the sample number is low.

Throughout the classification and regression tree analysis, 2 main region groupings continued to occur. Cobram, Harcourt and Shepparton always clustered together; the cluster sometimes also included Gippsland. The Mornington, Stanley and the Yarra Valley also regularly clustered together. The variables influencing this clustering are extensive, however further investigation of these sites may reveal common methods for the management and control of WAA in apple orchards.

Figure 1. Classification tree indicating the variables impacting on the location of WAA on individual apple trees, as reported by surveyed growers.

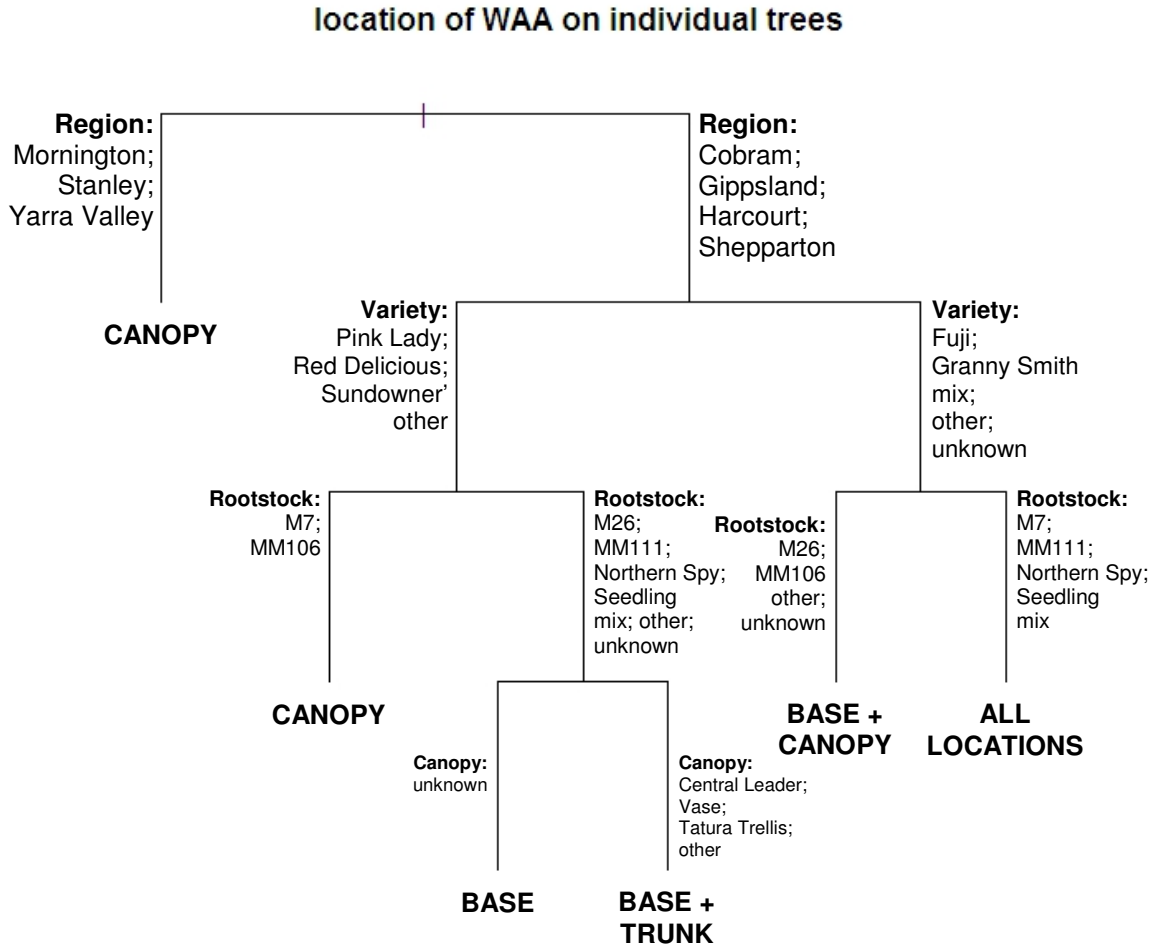


Figure 2. Classification tree indicating the variables impacting on the level of spread of WAA throughout the apple orchard, as reported by surveyed growers.

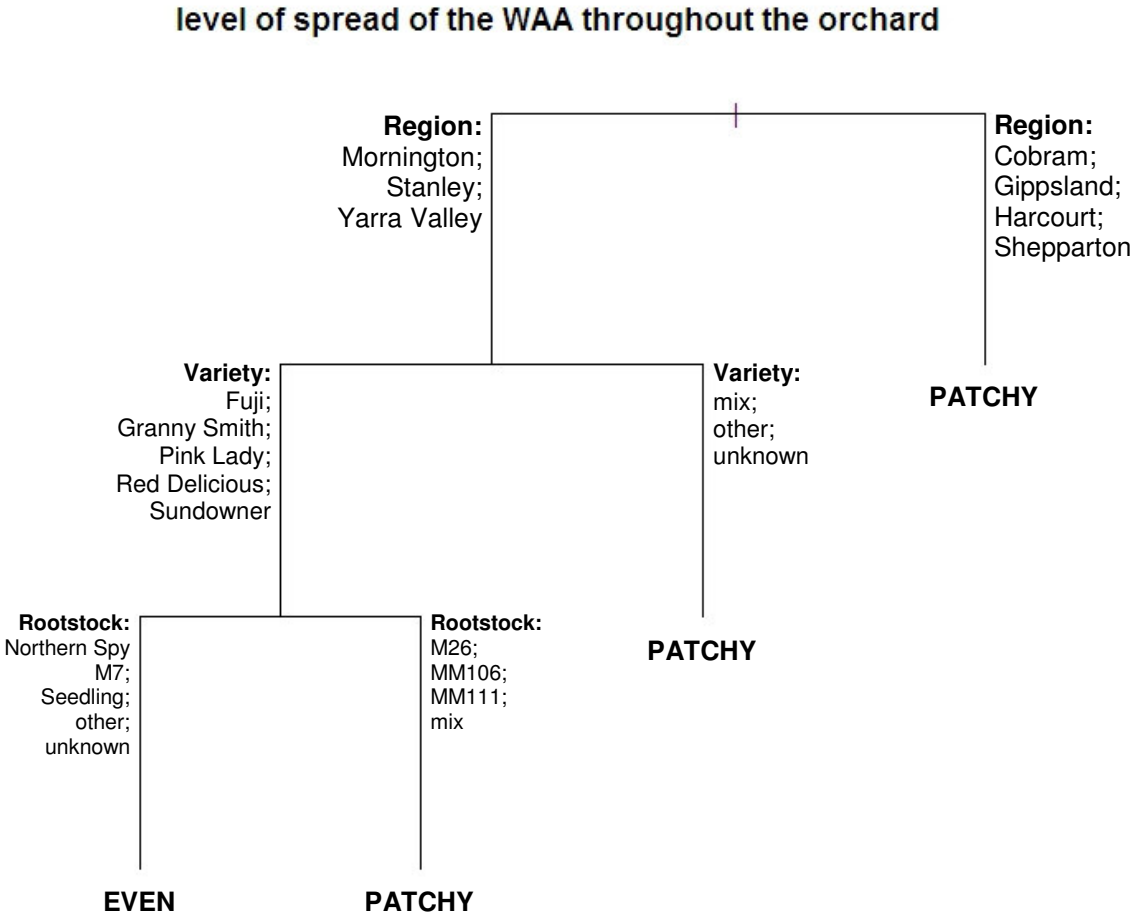


Figure 3. Classification tree indicating the variables impacting on the overall severity of WAA reported for each individual orchard block, as reported by surveyed growers.

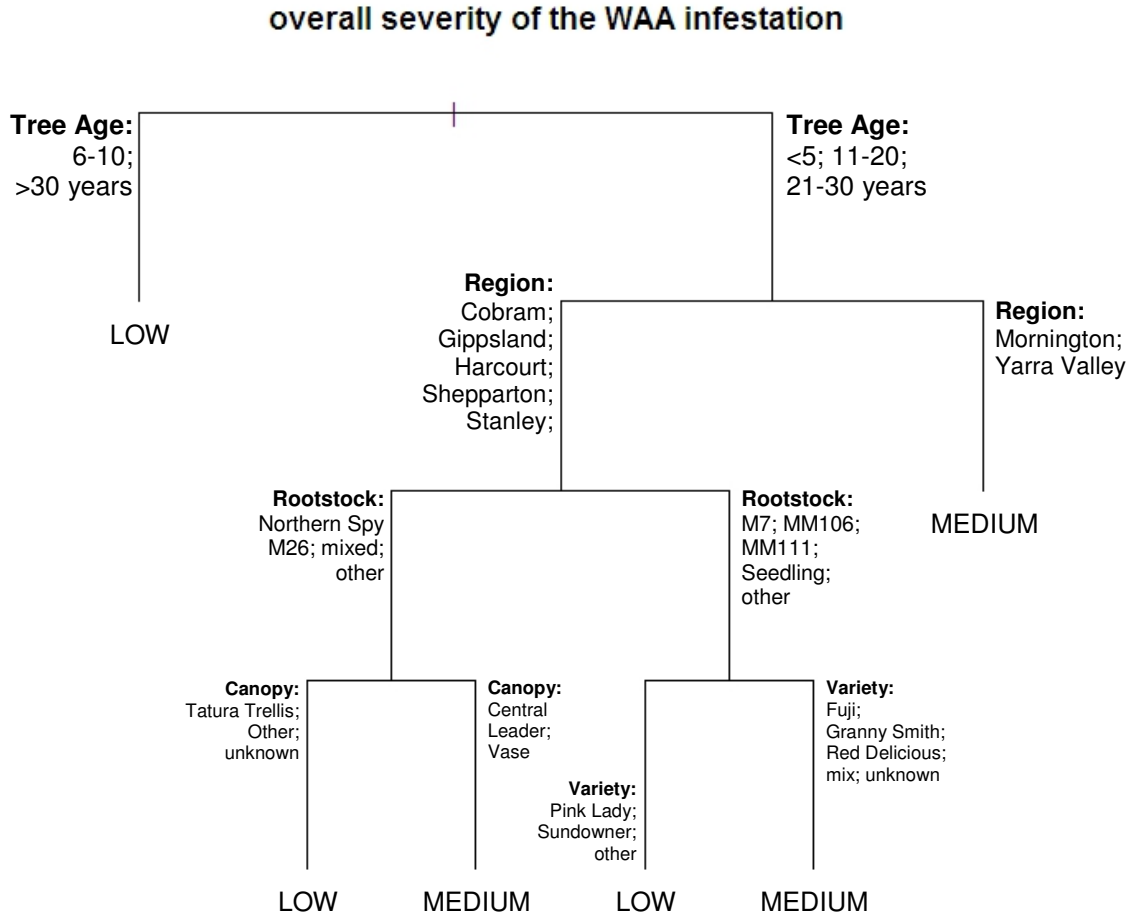
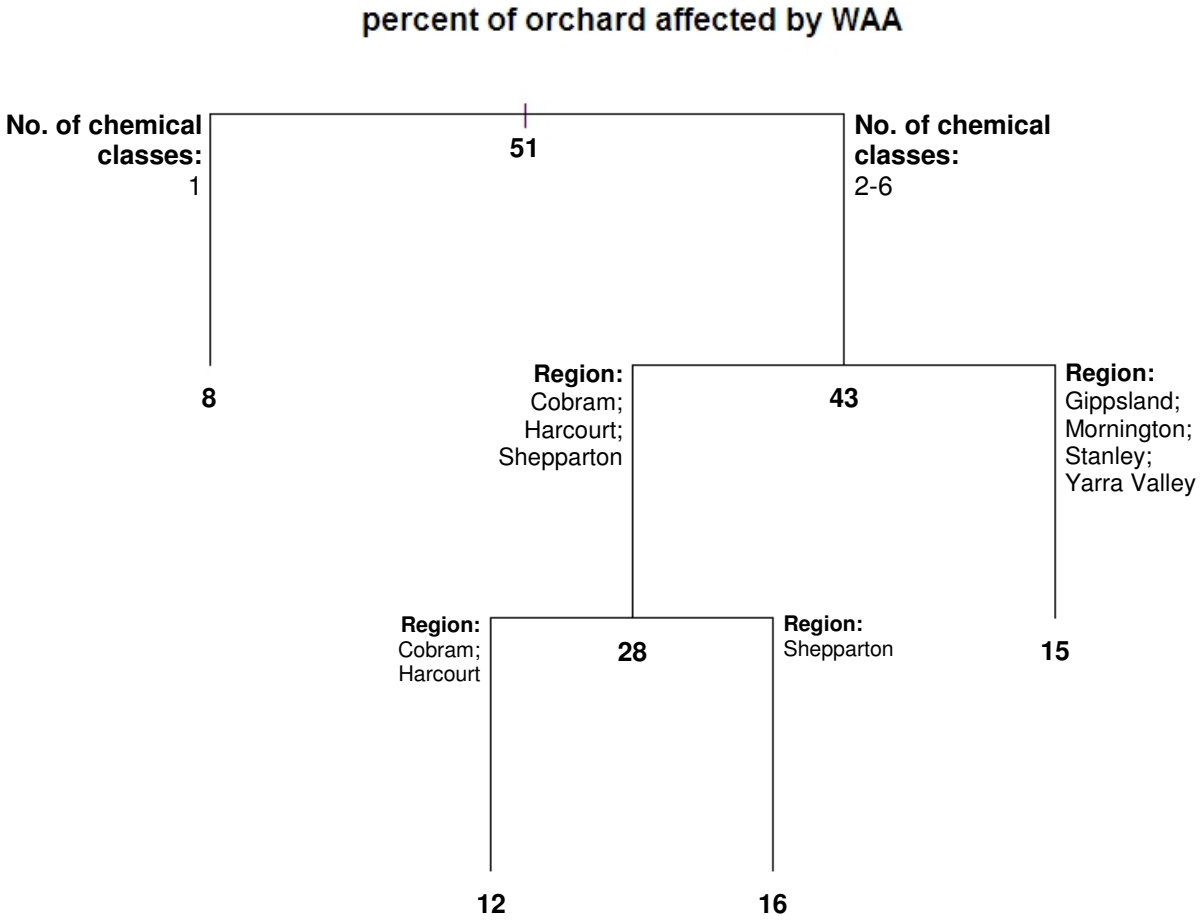


Figure 4. Regression tree indicating the variables impacting on the percentage of orchard affected by WAA, as reported by surveyed growers.



Discussion

The grower survey conducted over the major apple-growing regions of Victoria provided sufficient data to conduct a preliminary statistical analysis of trends with WAA infested orchards. WAA was reported in seven apple-growing regions and was predominant in Gippsland, Stanley and the Yarra Valley, ranging from 33-50% of orchards infested. However, overall the % of each orchard with WAA present range from 0- 100%. This information alone is limited as in some regions few growers actually responded to the surveys.

There were three main variables which appeared to influence the impact of WAA:

Firstly region was one major factor that influenced the location of WAA on individual apple trees in orchard blocks. In the Mornington Peninsular, Stanley and Yarra Valley WAA appeared to predominate in the canopy whereas in other regions it could be found in multiple locations on the host plant. This observation suggests that WAA has preferential feeding sites in some geographic regions. Whether this is influenced by environmental conditions, as these are cooler higher rainfall regions, or differences in WAA genetic diversity is uncertain but it merits further investigation.

Secondly distribution within orchard blocks varied between region with the Mornington, Stanley and Yarra Valley regions being influenced by apple tree variety and rootstock selection, which were not factors in the distribution of WAA in other regions. Again this may be due to environmental variables or WAA genetic diversity. The overall severity of WAA infestation was heavily influenced by region (being the second split in the classification tree).

Thirdly, as WAA location, distribution and overall severity also appears to be related to rootstock this would indicate that WAA genetic diversity may be influencing interactions with rootstocks. With no information available on WAA genetic diversity in Australia this is difficult to confirm. If the genetic diversity of WAA could be mapped in Australia, the level of variation would assist in determining management options. For example, a low level of variation would improve the opportunity to manage WAA populations using host-plant resistance (i.e. selecting rootstocks with high resistance to the genotypes present); whereas if a higher level of genetic diversity occurred this would require a more integrated or region-specific approach to WAA management may be required. Molecular techniques have already been developed to characterise genetic diversity of WAA populations in the Western Cape province of South Africa (Timm *et al*., 2005) where a relatively low level of variation appears to occur. More recently eight microsatellite DNA markers have been developed for WAA in Chile (Lavandero *et al*., 2009) and this technique could be further developed to characterise WAA genetic diversity in Australia.

Recommendations

Although only 18% of growers responded to the survey the results do offer an insight into the relative distribution, damage and control measure in different geographical regions of Victoria. It may be worthwhile to conduct a targeted repeat survey of growers who did not respond, either by telephone or mail, to gain a more comprehensive data set for further analysis.

The information gained to-date could be used to target specific regions for follow-up field based studies on the insect populations in each region to determine, for example, the WAA population dynamics in different climatic conditions and with different management approaches should be compared. In addition targeted ground surveys should be conducted to collect WAA from different regions and characterise the genetic diversity across the apple growing regions of Australia using molecular techniques.

Artificial Diet Feeding Studies

Introduction

Artificial diets for insect rearing have been developed for many aphid species (Cohen, 2003). By developing an artificial diet studies on the nutritional requirements of sap-sucking insect pests can be conducted under controlled laboratory conditions. This allows us to understand the fundamental basis of host-plant interactions. It also allows us once we have developed an optimal diet to screen potential control agents such as novel antimetabolites (Powell *et al*., 2003), optimise application rates of known chemical insecticides and assess the impact of modifying the insects diet as a potential control option. It also offers the opportunity to compare nutritional requirements of different WAA populations. There is no published artificial diet for WAA and the nutritional requirements of WAA remain unknown. By developing an artificial diet and diet feeding system this will improve our understanding as to how nutritional quality of the host-plant may impact on the insects ability to establish and develop on susceptible and resistant apple cultivars.

In this study we compared the feeding behaviour of two WAA populations sourced from two areas in NSW, Australia on a range of artificial diet formulations and developed a novel diet bioassay system for WAA.

Material & Methods

Artificial diet experiments were performed on two geographically isolated WAA populations. The "Batlow" population was collected from commercial apple orchards in Batlow, NSW (Latitude: 35.3°S, Longitude: 148.22°E; 725m elevation; average max-min. 19.6-5). The Batlow population was collected on a number of occasions, from a range of host-plant apple trees. The "Albury" population was collected from a Crab Apple tree on a residential property in Albury, NSW (Latitude: 36.07°S, Longitude: 146.95°E; 165m elevation; average max-min. 22.1-8.7ºC). The Albury population was collected a number of times, but always from the same host-plant.

The two WAA populations were maintained in glasshouse conditions (23ºC, 12 hour day/night cycle) on ungrafted Granny Smith apple trees (Flemings Nurseries, Monbulk Victoria). The trees were enclosed within a purpose built insect-proof rearing cage to isolate the 2 populations (Figure 1). Insects were collected from the insect cages as required for experimentation. Insects were carefully handled with a fine paintbrush, placed inside the diet chambers, and sealed with a thin layer of Parafilm®. The design of the diet chamber was experimented in several trial studies to optimise orientation and environmental conditions and one diet chamber configuration, in which the insect feeding position is inverted and has a relatively high humidity within the chamber, was considered most suitable for optimising WAA survival (Figure 2). All artificial diet experiments were conducted under controlled laboratory conditions (23ºC, 12 hour day/night cycle).

Figure 1. WAA insect rearing cage for maintenance of stock cultures.

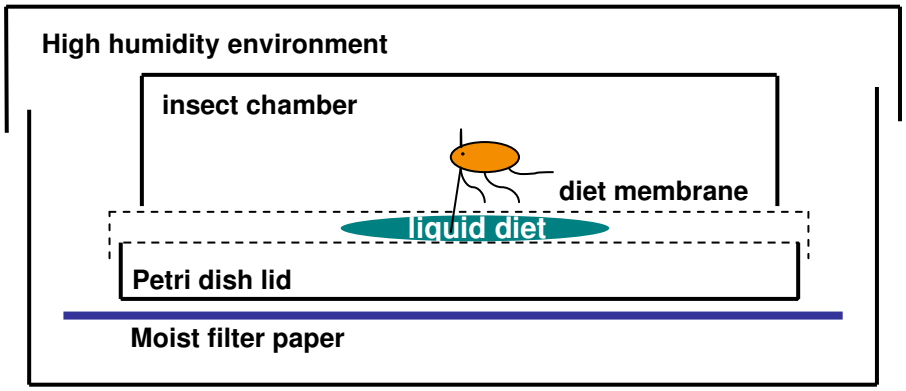


Figure 2. WAA inverted artificial diet chamber design. The liquid artificial diet was captured between 2 layers of Parafilm® plastic (dotted lines), through which the insect was able to penetrate mouthparts to feed. The diet chamber was inverted within a larger chamber so the diet surface was below the insects. The moist filter paper within the second chamber maintained humidity levels for the insects.

A range of artificial diet formulations were trialled in order to optimise WAA survival and development. Simple diets consisted of sucrose at a range of concentrations (5%, 10% and 20%). More complex diets (Table 1a and 1b), including DS-08 (developed during the course of the project), Catalayud (Catalayud *et al*., 1998), Febvay (Febvay *et al*., 1988), MED-1 (Mitsuhashi, 1974) and PT-07 (Trebicki *et al*., 2008) incorporated a range of amino acids and salts into the sucrose base diet formulation. None of the aforementioned diet formulations had previously been tested against WAA. The addition of an apple-based plant extract (made from ground roots and foliage) to either the Parafilm feeding surface or the diet was also trialled to test for natural attraction of the WAA to host-plant compounds. WAA survival on artificial diet formulations was generally compared a control of either water or no diet.

Table 1a. Chemical composition (mg/l) of three complex artificial diets trialled against two woolly apple aphid populations.

Ingredient	DS-08	Catalayud	Febvay	Ingredient	DS-08	Catalayud	Febvay
L-Alanine	1820	1787	1787	$MgCl_2*6H_2O$	1000		
ß-Alanine	80	62	62	$CuCL_2$	5		
L-Arginine hydrochloride	1556			$FeCL_3$	44.5	44.5	44.5
L-Arginine	3020	2449	2449	$MnCL_2$	6.5	6.5	6.5
L-Asparagine	2986	2986	2986	NaCL	25.4	25.4	25.4
L-Aspartic acid	883	883	883	$ZnCl_2$	8.3	8.3	8.3
L-Cysteine	296	296	296	Calcium Citrate	100	100	100
L-Cysteine hydrochloride	520			$CuSO_4*5H_2O$	8	4.7	4.7
L-Glutamic acid	1494	1494	1494	$MgSO_4*7H_2O$	2420	2420	2420
L-Glutamine	4480	4456	4456	KH_2PO_4	2500	2500	2500
L-Glycine	1666	1666	1666	$K_2HPO_4*3H_2O$	3000		
L-Histidine hydrochloride	1360	1360	1360	$CaCl_2*2H_2O$	320		
DL-Homoserine	4000						
γ-Amino butyric acid	200			Cholesteryl benzoate	25	25	25
L-Isoleucine	1648	1648	1648	**A**mino benzoic acid	100	100	100
L-Leucine	2360	2316	2316	Ascorbic acid	1000	1000	1000
L-Lysine hydrochloride	3511	3511	3511	Biotin	1	1	1
L-Methionine	724	724	724	Calcium Pantothenate	50	50	50
L-Ornitine hydrochloride	94	94	94	Choline Chloride	500	500	500
L-Phenylalanine	2945	2945	2945	Folic acid	12.4	10	10
L-Proline	1293	1293	1293	Iso Inositol	420	420	420
L-Serine	1243	1243	1243	Nicotinamide	51	100	100
l_Threonine	1272	1272	1272	Nicotinic acid	51		
L-Tryptophan	428	428	428	Pyridoxine hydrochloride	25	25	25
L-Tyrosine	386	386	386	Riboflavin	5	5	5
L-Valine	1909	1909	1909	Thiamine hydrochloride	25	25	25
Sucrose	200000	200000	289200	pH (Adjusted with KOH)	7.5	7.5	7.5

(a) Febvay *et al*. (1988). Influence of the amino acid balance on the improvement of an artificial diet for a biotype of *Acyrthosiphon pisum* (Homoptera: Aphididae). Canadian Journal of Zoology. 66: 2449-2453.

(b) Catalayud *et al*. (1998). Rearing the cassava mealybug *Phenacoccus manihoti* on a defined diet. Entomologia Experimentalis et Applicata. 86: 325-329

(c) Diet formulation DS-08 (unpublished) was developed during the project period.

Table 1b. Composition (mg/l) of two artificial diets, PT-07 and MED-1trialled against two woolly apple aphid populations.

Ingredient	PT-07	MED-1	Ingredient	PT-07	MED-1
L-alanine	1000	1000	MgCl26H2O	2000	2000
γ-amino butyric acid	200	200	KH2PO4	5000	5000
L-arginine hydrochloride	3000	4000	CaCl22H2O	32	31.15
L-asparagine	4000	3000	CuCl22H2O	3	2.68
L-aspartic acid	1000	1000	FeCl36H2O	23	22.28
L-cysteine	500	500	MnCl24H2O	8	7.93
L-cystine hydrochloride		50	ZnCl2	5	3.96
L-glutamic acid	1500	2000			
L-glutamine	6000	6000	Biotin	1	1
Glycine	400	200	Calcium pantothenate	50	50
L-histidine	1500	2000	Choline chloride	500	500
DL-homoserine		8000	Folic acid	10	10
L-isoleucine	1500	2000	Inositol	500	500
L-leucine	1500	2000	Nicotinic acid	100	100
L-lysine hydrochloride	1800	2000	Pyridoxine hydrochloride	25	25
L-methionine	1500	1000	Riboflavin	25	50
L-phenylalanine	1000	1000	Thiamine hydrochloride	25	25
L-proline	1000	1000	Ascorbic acid	1000	
L-serine	1000	1000	Sodium ascorbate		1000
L-threonine	1500	2000	Cholesteryl benzoate	25	
L-tryptophane	1000	1000			
L-tyrosine	200	200	Sucrose	50000	50000
L-valine	1500	2000			

*pH of both diets adjusted with KOH to 6.5

(d) PT-07: Trębicki P, *et al.,* 2008. Antimetabolic effects of *Galanthus nivalis* agglutinin and wheat germ agglutinin on nymphal stages of the common brown leafhopper using a novel artificial diet system. Entomologia Experimentalis et Applicata 131, 99-105.

(e) MED-1Mitsuhashi J. 1974. Methods for rearing leafhoppers and planthoppers on artificial diets. Review of Plant Protection Research 7, 57-67.

Artificial diet trials were monitored for the number of alive and dead insects daily. The data collated and formatted for survival data analysis to identify treatments having a significant impact on insect survival rate (Chisq $p < 0.05$). Survival analysis takes into account not only the time until death, but also the rate of death for insects under each treatment, and then plots the probability of insect survival for each treatment for each day of observation. The R statistical program© (The R Foundation of Statistical Computer) was used to perform the analysis.

Results

A total of 19 artificial diet experiments were performing on WAA insects (Table 2 a-c). Experiments varied in a range a factors, including insect life-stage (1st, 3rd instar, adult), WAA population source (Batlow or Albury), chamber humidity, chamber position (inverted or upright), chamber coating, and diet formulation. The main significant relationships related to diet formulation (diet no. 4, 5, 8-12, 14, 15) and WAA population (diet no. 12-15, 17-19). The maximum survival times for insects feeding on experimental diets displaying statistical significance for diet formulation and WAA population are included in Table 2 [where n = number of days]. The maximum survival times for other experiments and treatments are not shown.

WAA life-stage was a significant factor for survival in only one experiment, diet no. 6. In this experiment, 1st instars survived a maximum of 3 days, 3rd instars 4 days and adults 5 days. In this experiment no diet formulation was used and insects only had a 'water' control to feed on. Therefore it is likely that the increase survival time observed with increasing life-stage was due to food storage within the gut of the insect, rather than due to experimental conditions. The 1st instar life-stage was used for all artificial diet experimentation following this result. WAA insects responded with increased survival times to simple diet formulations containing 5%, 10% and 20% sucrose (diet no. 5, 8, 9, 10), however the complex diet formulation 'Febvay' provided the longest observed survival time for WAA (20 days, diet no. 10). 'Febvay' consistently extended the survival time of WAA, as observed in diets no. 4, 8, 9, 10 and 14. Overall the MED-1 diet, originally developed for planthoppers (Mitsuhashi, 1974) had no effect on WAA survival and was discontinued from further evaluation. Similarly diet PT-07 which was developed for leafhoppers (Trebicki et al., 2008) had only a marginal affect on WAA extending survival by 3 days (Table 2b).

Experimental diet no. 10 compared Albury 1st instar WAA survival time on 5 diet formulations (Table 2b). Insects feeding on 'no diet' survived a maximum period of 5 days, in contrast to 16 days survival for insects feeding on 'Febvay' or '20% sucrose + plant extract painted onto the Parafilm'. Insects feeding on 'Febvay + plant extract' painted onto the Parafilm surface survived up to 20 days. The increase in probability of survival for insects feeding on these diet formulations is plotted in Figure 3. Although insects on the 'no diet' control rapidly died (4 days max. survival), insects feeding on the previously mentioned artificial diet formulations had a gradual rate of decline and survive for up to 5 times longer.

Experimental diet no. 14 compared the Batlow and Albury 1st instar WAA populations on diet formulations 'no diet' and 'Febvay'. Insect survival time reached a maximum of 7 days (Table 2c) which was low in comparison with diet no. 10. However, the Chisq p values were highly significant. This significance value was influenced by the delayed rate of death in the 'Febvay' x 'Albury WAA' treatment comparison. Figure 4 depicts the improved probability of survival for Albury WAA insects feeding on 'Febvay'.
The extended survival rate of the Albury population over the Batlow WAA population was replicated in experimental diets no. 12, 13, 14, 15, 18 and 19. 'Febvay' was the most common diet formulation used in these diet experiments; however the Albury WAA survived longer than Batlow WAA on a range of diet formulations. Experimental diet no. 18 used the laboratory developed diet formulation DS-08 (Table 1a). On this diet formulation, the Albury WAA populations survived twice as long as the Batlow WAA populations (Table 2c). The improved probability of survival for the Albury WAA population indicated the reduced rate of insect death in comparison to the Batlow WAA population (Figure 5).

Table 2a. Summary of experiments 1-7 examining WAA survival in an artificial feeding system [diet and population maximum survival time].

Experiment date **Diet no.**	14 / 15 NOV 2007 1 / 2	19 NOV 2007 3	22 NOV 2007 4	29 NOV 2007 5	03 DEC 2007 6	07 DEC 2007 7
Total insect no.	35/8	30	115	190	81	85
Treatment comparison **p value**	diet ns	life-stage x diet Ns	life-stage x diet < 0.05	diet < 0.01	life-stage x humidity ns	chamber x position < 0.001
WAA population **p value**	Batlow -	Batlow -	Batlow -	Batlow -	Batlow -	Batlow -
WAA life-stage **p value**	1st instar -	1st instar 3rd instar Ns	1st instar 3rd instar ns	1st instar -	1st instar 3rd instar adult < 0.05	1st instar -
Diet formulations **p value**	water Febvay ns	no diet Water 5% sucrose Ns	no diet [3] water [4] 5% sucrose [4] Febvay [5] < 0.01	Water [3] 5% sucrose [4] 10% sucrose [4] 20% sucrose [4] Febvay [4] < 0.01	water -	20% sucrose -
Other variables **p value**	- -	- -	- -	- -	4 humidity levels ns	3 chambers 4 positions < 0.001 / < 0.01

(f) Experiment date = date experiment initiated

(g) Diet no. = diet number in chronological order, or ease of reference to data

(h) Total insect no. = total number of insects used within the experiment, across all treatments

(i) Treatment comparison = factors examined within the experiment; the p value indicates significance (< 0.05 - < 0.001) across all treatment combinations (if only 1 treatment comparison, then this p value relates directly to the individual factor)

(j) WAA population = WAA population used for the experiment; p value indicates significance if population was a factor in the treatment comparison, - indicates no comparison

(k) WAA life-stage = WAA life-stage used for the experiment; p value indicates significance if life-stage was a factor in the treatment comparison, - indicates no comparison

(l) Diet formulations = diet formulations used for the experiment; p value indicates significance if diet was a factor in the treatment comparison, - indicates no comparison

(m) Other variables = other variables investigated in the experiment; p value indicates significance if variable was a factor in the treatment comparison, - indicates no comparison

Table 2b. Summary of experiments 8-13 examining WAA survival in an artificial feeding system [diet and population maximum survival time]. * = plant extract painted onto Parafilm; plant extract mixed with diet formulation unless * noted

Experiment date **Diet no.**	12 DEC 2007 8	18 DEC 2007 9	07 JAN 2008 10	08 FEB 2008 11	12 FEB 2008 12	15 FEB 2008 13
Total insect no.	140	448	241	251	497	203
Treatment comparison **p value**	diet x chamber coating < 0.001	Diet < 0.001	diet < 0.001	diet < 0.05	diet x population < 0.001	diet x population < 0.001
WAA population **p value**	Batlow -	Batlow -	Albury -	Batlow -	Batlow [7] Albury [10] < 0.001	Batlow [4] Albury [5] < 0.001
WAA life-stage **p value**	1st instar -	1st instar -	1st instar -	1st instar -	1st instar -	1st instar
Diet formulations	plant extract [5] water [5.5] water + plant extract [4] Febvay [11] Febvay + plant extract [6.5] 20% sucrose [6.5] 20% sucrose + plant extract [6]	5% sucrose [5] 5% sucrose + plant extract* [8] 10% sucrose [8] 10% sucrose + plant extract* [10] 20% sucrose [8] 20% sucrose + plant extract* [13] Febvay [8] Febvay + plant extract* [15] no diet [4]	no diet [4] Febvay [16] Febvay + plant extract* [20] 20% sucrose [9] 20% sucrose + plant extract* [16]	no diet [2] PT + 20% sucrose [3] MED-1 + 20% sucrose [3] DS-08 [3] Catalayud [3]	no diet [4] DS-08 [6] Catalayud [10] MED-1 [4] PT-07 [7]	Catalayud Febvay
p value	< 0.01	< 0.001	< 0.001	< 0.05	< 0.001	ns
Other variables **p value**	4 chamber coatings < 0.001	- -	- -	- -	- -	- -

Table 2c. Summary of experiments 14-19 examining WAA survival in an artificial feeding system [diet and population maximum survival time].

Experiment date **Diet no.**	26 FEB 2008 14	29 FEB 2008 15	04 MAR 2008 16	05 MAR 2003 17	05 MAR 2008 np 18	19 MAR 2008 19
Total insect no.	200	200	60	202	353	203
Treatment comparison **p value**	diet x population < 0.01	diet x population < 0.001	extract ns	population < 0.01	population < 0.001	diet x population < 0.05
WAA population **p value**	Batlow [5] Albury [7] < 0.001	Batlow [7] Albury [7] < 0.05	Albury -	Batlow [6] Albury [5] < 0.01	Batlow [5] Albury [10] < 0.001	Batlow [6] Albury [9] < 0.05
WAA life-stage **p value**	1st instar -	1st instar -	1st instar -	1st instar -	1st instar -	1st instar -
Diet formulations **p value**	no diet [5] Febvay [7] < 0.01	Febvay + plant extract [5] Febvay + plant extract* [7] < 0.01	Febvay -	Febvay -	DS-08 -	DS-08 DS-08 + plant extract* ns
Other variables **p value**	- -	- -	6 plant extract location in diet chamber ns	- -	- -	- -

(n) Experiment date = date experiment initiated

(o) Diet no. = diet number in chronological order, or ease of reference to data

(p) Total insect no. = total number of insects used within the experiment, across all treatments

(q) Treatment comparison = factors examined within the experiment; the p value indicates significance (< 0.05 - < 0.001) across all treatment combinations (if only 1 treatment comparison, then this p value relates directly to the individual factor);

(r) WAA population = WAA population used for the experiment; p value indicates significance if population was a factor in the treatment comparison, - indicates no comparison

(s) WAA life-stage = WAA life-stage used for the experiment; p value indicates significance if life-stage was a factor in the treatment comparison, - indicates no comparison

(t) Diet formulations = diet formulations used for the experiment; p value indicates significance if diet was a factor in the treatment comparison, - indicates no comparison

(u) Other variables = other variables investigated in the experiment; p value indicates significance if variable was a factor in the treatment comparison, - indicates no comparison

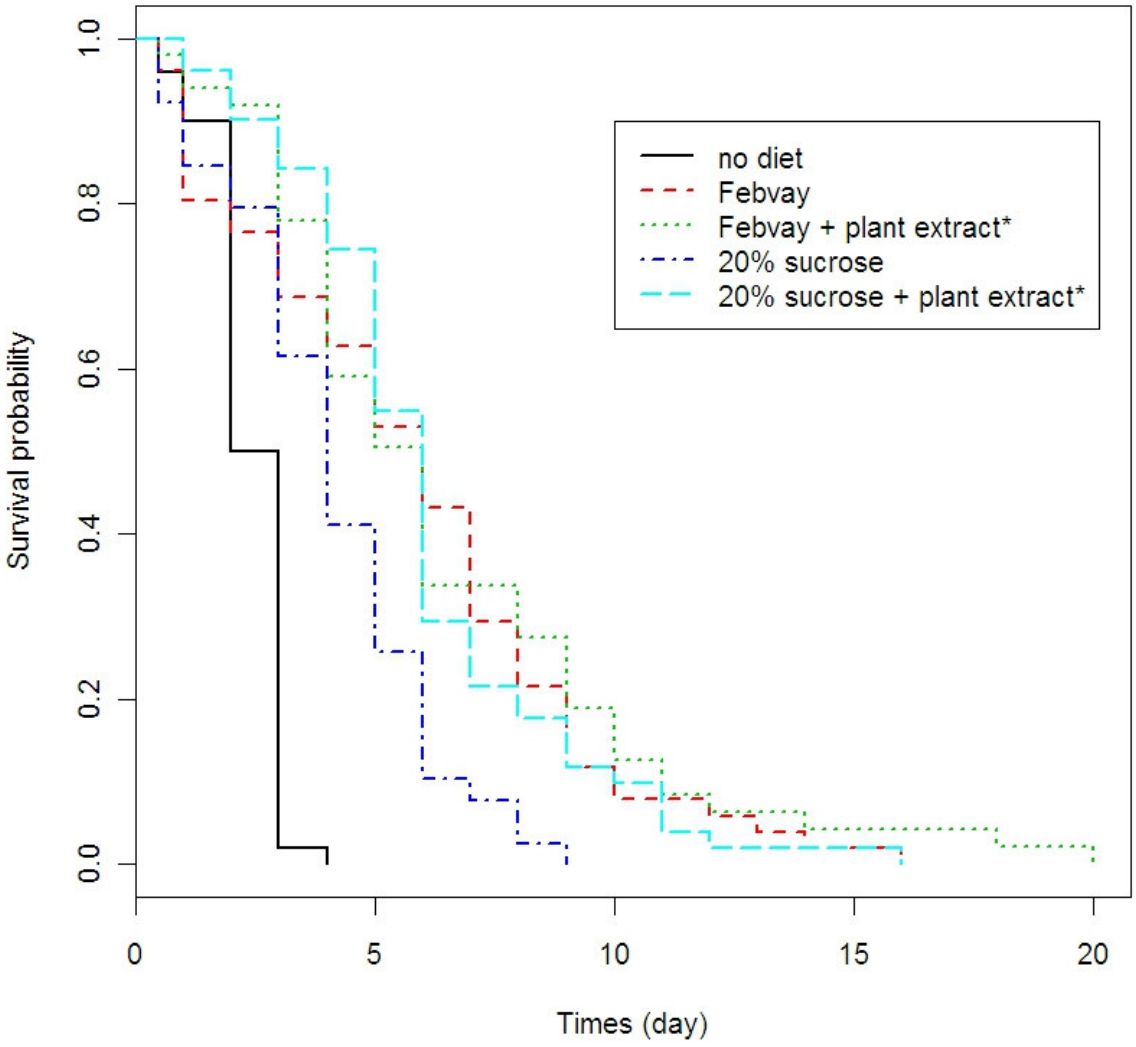


Figure 3. Plot of the probability of survival of woolly apple aphid on artificial diet formulations in experimental diet system no. 10.

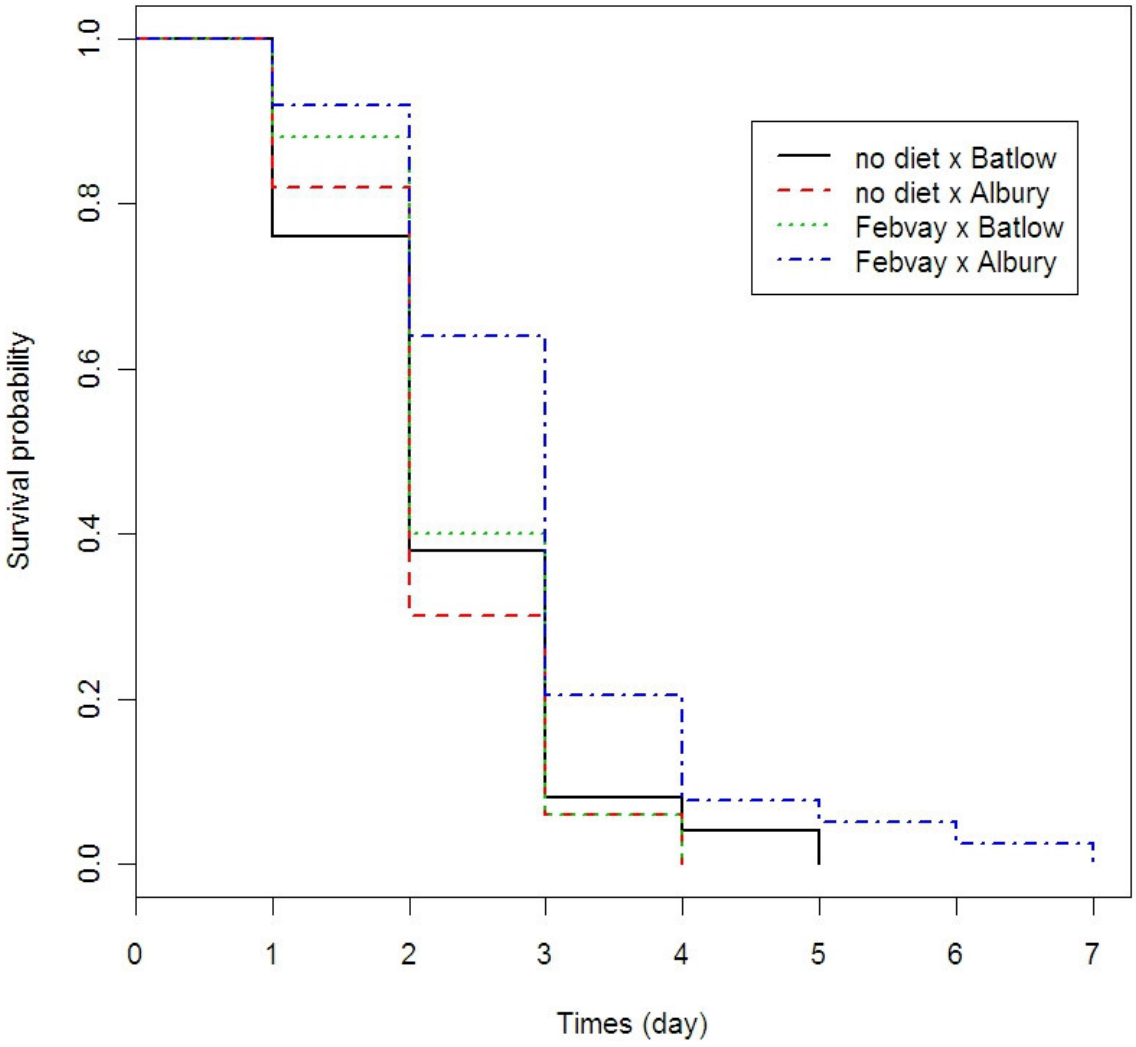


Figure 4. Plot of the probability of woolly apple aphid survival for treatment comparisons (diet x source population) in experimental diet system in diet no. 14.

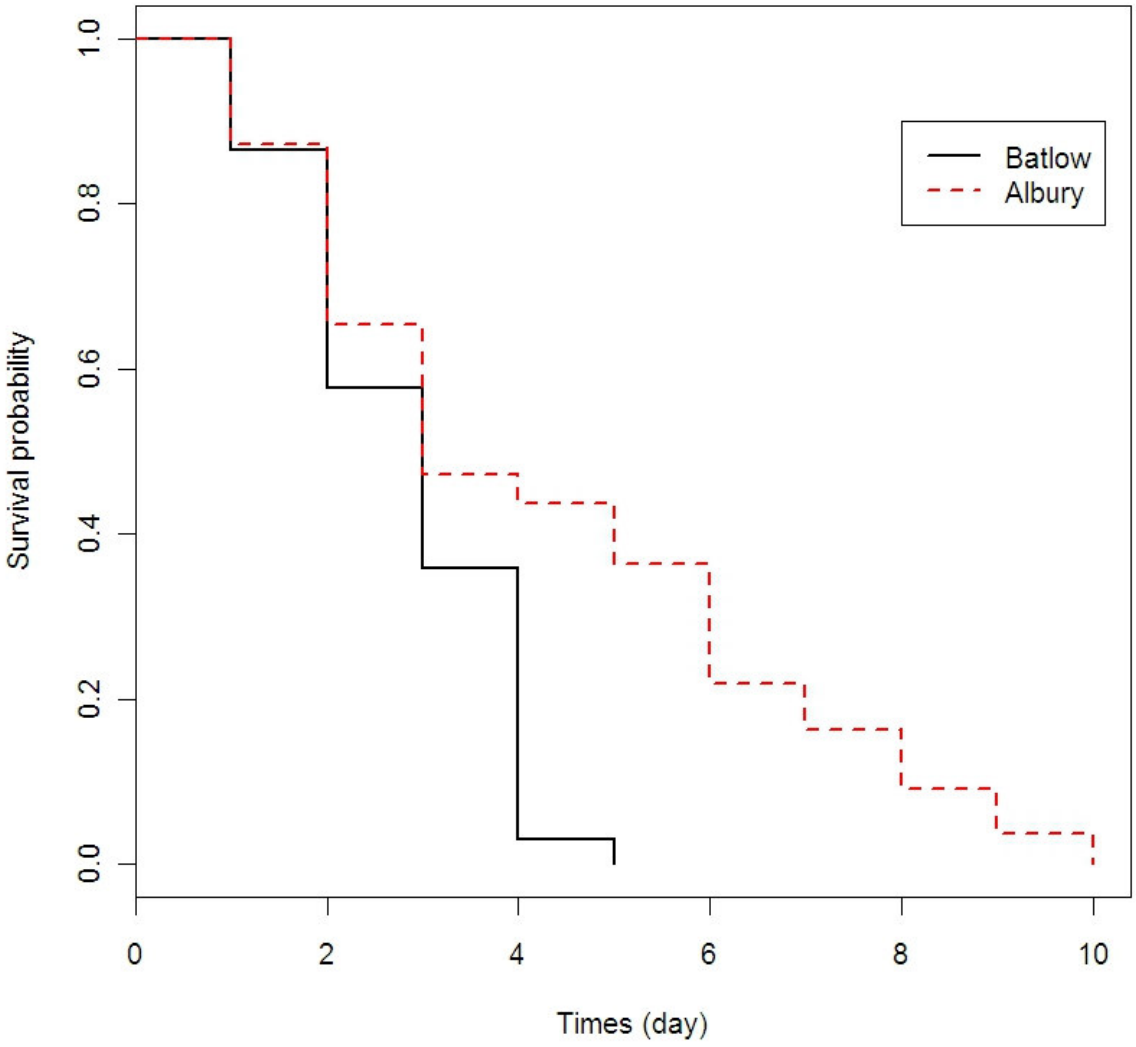


Figure 5. Plot of the probability of survival for two woolly apple aphid populations (Albury and Batlow) in experimental diet system diet no. 18.

Discussion

Extensive studies were conducted during the course of the project with several objectives in mind:

Firstly, to determine if WAA could be reared on an artificial liquid medium. By rearing WAA using an *in vitro* system this offers several opportunities to understand the basic nutritional requirements of the target pest. This knowledge of the insects nutritional requirements with further refinement, (i.e. diet optimisation, would allow an improved understanding of the potential impacts of either rootstock selection (as different rootstocks are likely to have different phloem sap chemical profiles) or orchard management (through changing water or nutrient management) on WAA.
This component of the project was relatively complex as there were no published data on either artificial diets or artificial rearing chambers for WAA. We therefore chose to screen four complex diets (used for other Hemiptera) and three simple sucrose-based diets in a series of 19 experiments. In addition we developed our own diet DS-08 during the course of the project and also incorporated an apple based plant extract within the diet system. Overall the best diet formulation used was the Febvay diet in combination with plant extract which allowed a maximal survival of 20 days. However, the Febvay diet has previously been developed for the pea aphid *Acyrthosiphon pisum* and it is most likely that with further development this diet formulation can be further optimised to improve WAA survival. It was interesting to note that an apple plant extract improved

A second objective of the project was to determine whether an *in vitro* system (combined with a suitable artificial diet) could be developed which allowed WAA to feed through a membrane and imbibe artificial diet. Although there are several published studies where a range of aphids have been reared in this way, prior to the completion of this project, there were no published studies for WAA. In initial trials we used a simple diet chamber with a double Parafilm membrane where the insect attempted to feed whilst in the upside-down position (as is the case with most published aphid rearing systems); WAA appeared to reject this feeding system and survival on diet was initial very poor. Visual observations using a low power microscope indicated that due to the length and thickness of the insects stylet it had difficulty in inserting its stylet with sufficient force to penetrate the membrane whilst in this position, However, once we modified the system by inverting the feeding chamber and enclosing it in a modified humidity chamber, we observed that WAA had no difficulty in penetrating the diet membrane and was indeed imbibing diet I(as evidenced by honeydew excretion.

A third objective was to determine if WAA population source affected survival times. When comparing two geographically distinct populations of WAA sourced from Batlow and Albury field sites it was apparent during the diet testing that each population had slightly different nutritional requirements. This finding suggests different WAA biotypes within Australia and could have impacts in determining optimal management strategies. Future studies should determine the geographical distribution and diversity of WAA biotypes and ultimately genotyping studies should also be conducted.

Recommendations

The development of an *in vitro* system for rearing WAA is the first reported study of its kind and with further refinement could even be used for *in vitro* screening of potential antimetabolites and novel insecticidal compounds. In particular the finding that the addition of apple plant extracts to the diet system improved survival may indicate that chemical components of the extract may either attract the insect to its host (in which case this may differ between rootstocks) or be essential components for WAA development. Characterisation of the chemical components of the extracts may provide useful insights into the interactions between the host plant and the insect and could also be important in determining if volatiles produced by the host plant also influence predator interactions with WAA.

Further studies, using genetically characterised WAA from different geographic regions of Australia, should be conducted to compare nutritional requirements of WAA genotypes. This information would aid in future management options in different apple growing regions of Australia.

Electrophysiological Feeding Studies

Introduction

The Electrical Penetration Graph (EPG) is an electrophysiological method which has been used for a range of sap-sucking insects including aphids, whiteflies, leafhoppers, phylloxera, planthoppers, thrips and mealybugs, to examine their feeding behaviour on host-plants. The EPG system is unique in that it allows real-time monitoring of insect feeding behaviour on crop plants and allows us to characterise and compare feeding behaviour on susceptible and resistant host plants. The direct current DC EPG system (Tjallinge, 1978) measures voltage in an electrical circuit produced where the insect forms one " insect" electrode and the plant forms a second " plant electrode (Figure 1.). When the insect begins to probe the plant surface its mouthpart or stylet on contact completes an electrical circuit and voltage fluctuations in the system are amplified, recorded and analysed using specialised computer software.

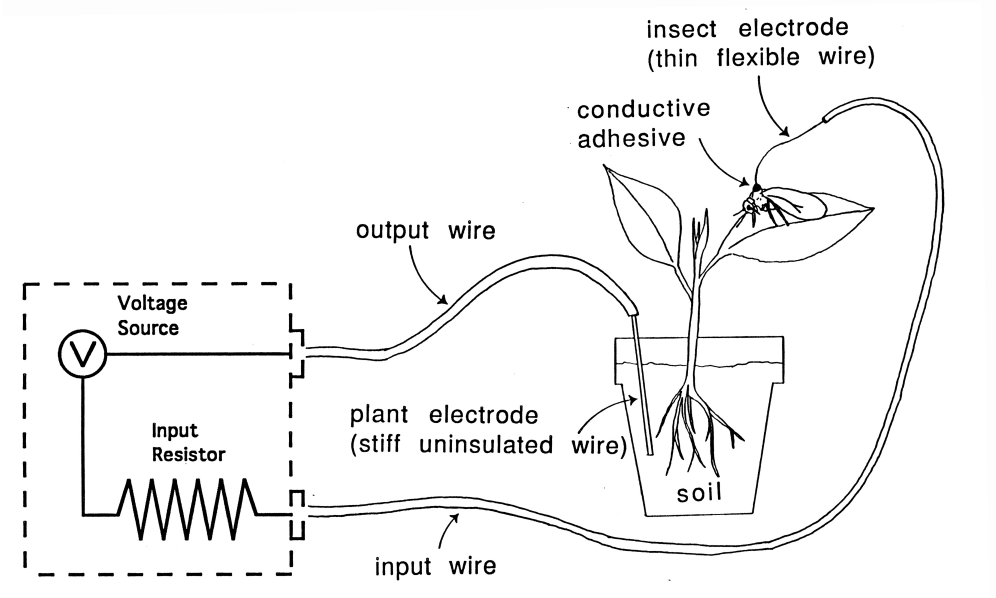


Figure 1. Diagram of the EPG components, where the amplifier (represented by the dashed box) is connected to the insect-plant system (from Walker, 2000). The main components of the system are labelled with arrows.

The EPG system has previously been used with woolly apple aphid populations in New Zealand (NZ) to characterise the electrical waveforms produced when WAA feeds on the susceptible apple (*Malus domestica*) cultivar ' Royal Gala' (Sandanayaka & Hale, 2003). It has also been used in NZ populations to compare WAA feeding behaviour and resistance characteristics of Northern Spy, Robusta and Aotea rootstocks. However, there is no

published EPG data comparing different WAA populations or using WAA populations sourced in Australia.
In this study we compared the feeding behaviour of two WAA populations sourced from two areas in NSW, Australia on susceptible (cv. Granny Smith) and resistant (Northern Spy, MM106 & M793) rootstocks to gain an improved understanding of rootstock resistance characteristics.

Material & Methods

EPG experiments were performed on two geographically isolated WAA populations. The "Batlow" population was collected from commercial apple orchards in Batlow, NSW (Latitude: 35.3°S, Longitude: 148.22°E; 725m elevation; average max-min. 19.6-5). The Batlow population was collected on a number of occasions, from a range of host-plant apple trees. The "Albury" population was collected from a Crab Apple tree on a residential property in Albury, NSW (Latitude: 36.07°S, Longitude: 146.95°E; 165m elevation; average max-min. 22.1-8.7ºC). The Albury population was collected a number of times, but always from the same host-plant.

The two WAA populations were maintained in glasshouse conditions (23ºC, 12 hour day/night cycle) on Granny Smith apple trees. The trees were enclosed within an insect cage to isolate the two populations. Insects were collected from the insect cages, as required, for experimentation and handled with a fine paintbrush. An insect electrode consisting of 2-3cm of 20µm diameter gold wire (Sigmund Cohn, Mount Vernon, New York) was connected to the back of the abdomen of late instar/adult WAA insects with conductive (water-based) silver paint. The insect electrode connected the WAA insects to the EPG monitor during recordings of feeding behaviour on apple trees (Figure 2).

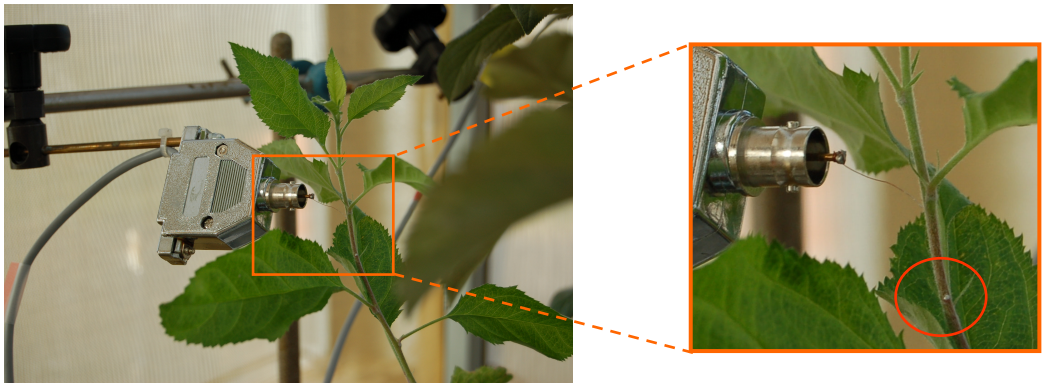


Figure 2. EPG insect electrode; the 20µm gold wire with the attached WAA insect is visible on the branch of the apple tree in the enlarged image.

WAA feeding behaviour was monitored using EPG on a range of apple rootstocks. Granny Smith was used as the control (susceptible) apple variety. Rootstock selection shared parentage and covered a range of WAA resistance levels:

1. **Northern Spy (seedling origin, intermediate resistance)**
2. **MM106 (Northern Spy x M1 cross, moderate resistance)**
3. **M793 (Northern Spy x M2, high resistance)**

This selection of apple variety and rootstocks was based on those commonly reported in the Victorian apple production in the grower survey. WAA were recording feeding on the branch of a whole, potted apple tree. A complete block consisting of one of each trial apple tree (Granny Smith, Northern Spy, MM106, M793) was completed for each experimental recording. The two WAA populations (Albury and Batlow) were run in separate experimental blocks. Susceptible control (Granny Smith) planting material was supplied by Flemings Nurseries, Monbulk Victoria and non-grafted rootstocks supplied by Rochford Rootstock Nurseries.
EPG recordings were performed using the Giga-8 series EPG amplifier (EPG systems, Wageningen, The Netherlands). Trials were conducted under controlled glasshouse conditions (23ºC, 12 hour day/night cycle) inside a faraday cage to reduce external noise interference (Figure 3). EPG recordings were generally for a continual period of 18 hours.

Figure 3. EPG set up for WAA feeding behaviour studies on apple trees. The apple trees are contained within a specially modified Faraday cage; branches were tied as required to prevent contact with the cage surface. The plant electrode was inserted into the moist soil of the potted tree; the insect electrode was positioned to allow WAA feeding on the branches of the tree. The EPG monitor is visible to the right of the Faraday cage.

EPG recordings were analysed using PROBE software (EPG systems, Wageningen, The Netherlands). Aphid EPG waveforms have been correlated to a number of feeding activities (Appendix 3), specifically relating to plant penetration, pathway, salivation and ingestion of a feed site. The analysis presented for WAA has been simplified to represent the major feeding activities: non-penetration (np), pathway (C) and salivation/ingestion - which include both phloem and xylem feeding (E). The time spent in each of these waveforms was collated as total time, mean time and percentage of time from entire recording. The frequency of the recordings was also determined. ANOVA was used to determine statistical significance ($p < 0.05$) between the rootstock and WAA population treatments.

Results

WAA EPG recordings were analysed based on 3 major waveforms: non-penetration (np), pathway (C) and salivation/ingestion (E). Non-penetration was defined as a 0V, flatline output. Pathway (C) was a composite waveform grouping of waveform A, B and C from the Tjallingii chart (Appendix 3). Pathway represents plant penetration by the insect, followed by searching through the plant tissue for a suitable feeding site. Waveforms are initially high in amplitude and erratic before settling into a search pattern. Potential drops (pd) are experienced when the insect penetrates a plant cell; "tasting" of the cell contents may be observed during a pd. Waveforms characterised as C for EPG analysis are presented in Figures 4-6.

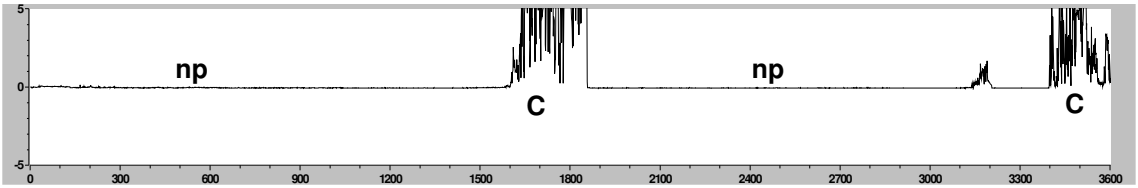


Figure 4. EPG recording displaying non-penetration (np) flatline interrupted by high voltage C waveforms. The recording returned to np following a short period of C.

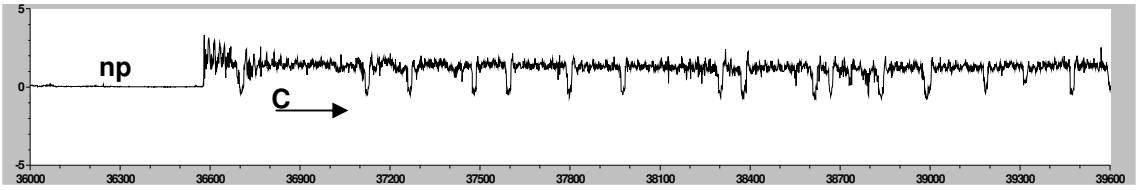


Figure 5. EPG recording displaying non-penetration followed by an extended period of C. Waveform C consisted of the initial high voltage plant penetration, followed by a period of searching, including potential drops (pd). A pd was defined by a rapid, but short, drop in voltage.

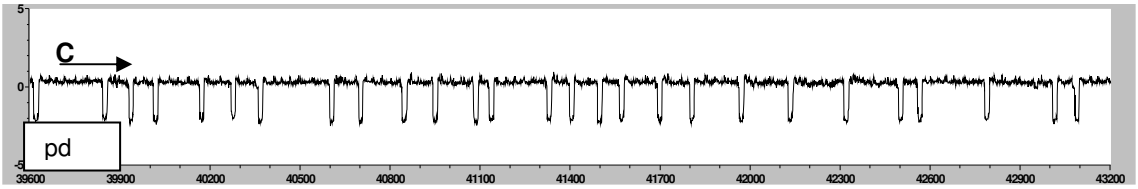


Figure 6. EPG recording displaying an extended period of C. A series of pd continued for an extended period of time indicating that the insect was searching and tasting a range of plant tissues, but not settling into a period of ingestion (E).

Waveform E was a composite of a range of feeding activities involved with salivation and ingestion (which includes both phloem and xylem ingestion). Waveform E is more regular in appearance than waveform C. Waveform E represents intracellular feeding activity and therefore occurs as a lower voltage level than waveform C; in effect, waveform E is an extended pd which may occur for several hours. Changes in waveform appearance characteristic of E are presented in Figures 7 and 8.

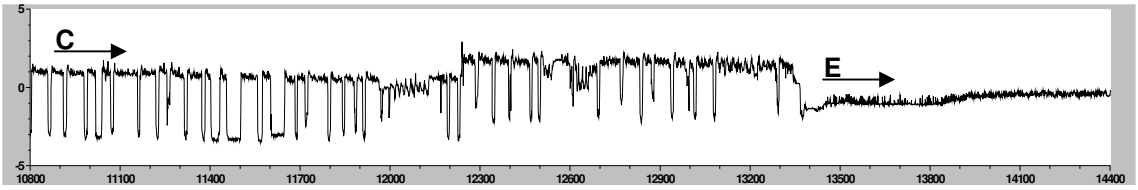


Figure 7. EPG recording displaying the transition for waveform C to E. The insect experienced an extended period of plant tissue searching and tasting prior to settling into an extended period of ingestion.

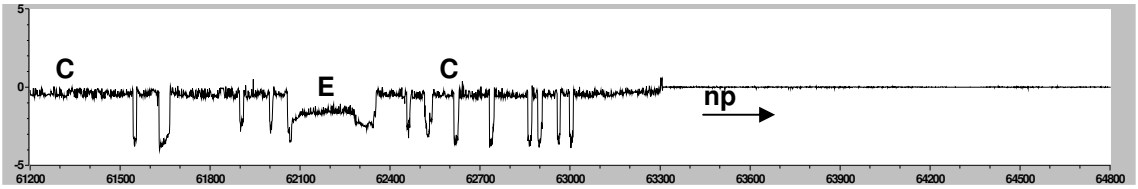


Figure 8. EPG recording displaying the transition between the 3 major waveforms. The insect initially displayed waveform C, followed by a brief period of E before returning to the C waveform. The insect then withdrew from the plant, displaying the EPG non-penetration pattern.

A total of 37 EPG recordings were attempted on all apple rootstocks (Granny Smith, Northern Spy, MM106 and M793); 19 of these recordings were with the Albury WAA population and 18 with the Batlow population. More than 50% of recordings failed due to the absence of waveforms C or E (Table 1), that is, only the non-penetration waveform was present and there was no indication in the EPG recording of the insect attempting to feed on the apple tree. The potential causes for the EPG failures may be due to individual insect health, environmental conditions during the recording or interference from the insects woolly covering. However the variables were comparable within the same block experiment and comparisons between differences in EPG recording success may be made based on rootstock and WAA population.

Significantly more Granny Smith (susceptible variety) trees experienced successful EPG recordings in comparison with the rootstock varieties (Table 1). The level of EPG recording success for the rootstocks reflected the expected WAA resistance levels, with M793 being more resistant than Northern Spy and MM106. However this relationship was not reflected when the 2 geographically isolated WAA populations, Albury and Batlow, were analysed in interaction with rootstock EPG recording success.

The Albury population displayed equivalent EPG recording success with Granny Smith and MM106, followed by Northern Spy and M793 showing less feeding activity (Table 1). The Batlow population displayed equivalent EPG recording success with Granny Smith, Northern Spy and M793, with less feeding activity on MM106. This mixed EPG recording success did not reflect the expected WAA resistance ratings for the rootstocks, and also indicated possible differences in feeding behaviour between the 2 WAA populations.

Table 1. EPG recording success for woolly apple aphid displayed as percentage of EPG recordings with C or E waveforms. Results presented as combined and separate WAA populations.

Rootstock	Combined [37]	Albury [19]	Batlow [18]
Granny Smith	46% a [17]	47% a [9]	44% a [8]
Northern Spy	35% b [13]	26% b,c [5]	44% a [7]
MM106	30% b,c [11]	42% a [8]	17% c,d [3]
M793	24% c [9]	11% d [2]	39% a,b [7]
P value	< 0.001	< 0.001	

(v) [n] = sample number; heading row = total EPG recordings attempted; data columns = number of successful EPG recordings

The EPG waveforms displayed on the successful EPG recordings for WAA were analysed for the presence in the np, C and E waveforms. The values for waveforms C and E were also combined in order to display the total time spent in probing (pathway) and feeding behaviour activity, in comparison with np (Table 2). Woolly apple aphid on Northern Spy and MM106 rootstocks displayed a higher percentage of time in the np waveform in comparison with Granny Smith and M793; however these relationships were not statistically significant. WAA spent a shorter than expected time in waveform E (ingestion); waveform C was the predominant feeding activity with an extensive number of repeated pd patterns observed. WAA feeding on the susceptible variety, Granny Smith, did not display statistically significant different feeding behaviour to WAA feeding on resistant rootstocks.

The EPG waveforms were also analysed based on WAA population (Table 3). Across of the apple rootstocks, the Albury WAA population spent a larger percentage of time in the np waveform, and less time in the C and E waveforms, in comparison with the Batlow WAA population. This difference in feeding behaviour between the Albury and Batlow WAA populations supported the apparent differences in dietary requirements displayed in the artificial diet studies. The geographically isolated WAA populations may therefore represent two WAA biotypes, where biological changes have been induced in the insect population due to different climatic conditions and host-plant food source.

The EPG waveform analysis by the interaction between the WAA population and apple tree rootstocks were not statistically significant (data not shown), however visual differences are present. Albury WAA spent 24-38% of the recording time in waveforms C and E across all rootstocks (9), while Batlow WAA displayed probing and feeding activity between 36-70% of the total recording time (Figure 10).

Table 2. Successful EPG recordings for woolly apple aphid expressed as total time, frequency, mean time and percentage of recording time spent in each waveform for each rootstock. Average values displayed ± standard deviation.

waveform	rootstock	total time (sec)	frequency	mean time (sec)	% recording time
Np	Granny Smith	36675 ± 25274	2.8 ± 1.6	14344 ± 14528	58 ± 39
	Northern Spy	40868 ± 19805	2.8 ± 2.0	19196 ± 13213	68 ± 33
	MM106	40020 ± 21555	3.5 ± 1.9	14453 ± 10035	70 ± 32
	M793	25723 ± 25161	2.0 ± 0.7	14731 ± 15981	40 ± 39
	ANOVA p	ns	ns	ns	ns
C	Granny Smith	16668 ± 18568	7.9 ± 8.9	4633 ± 7647	26 ± 29
	Northern Spy	13192 ± 13141	5.9 ± 6.6	2962 ± 3056	21 ± 20
	MM106	8157 ± 6940	7.6 ± 6.9	1742 ± 2408	15 ± 12
	M793	21261 ± 21219	5.8 ± 5.4	4672 ± 5761	33 ± 33
	ANOVA p	ns	ns	ns	ns
E	Granny Smith	10186 ± 11077	5.8 ± 8.4	2307 ± 3674	16 ± 17
	Northern Spy	7416 ± 11312	3.9 ± 5.9	1038 ± 1279	12 ± 17
	MM106	9423 ± 15750	5.1 ± 6.9	1077 ± 988	15 ± 24
	M793	17816 ± 19168	4.3 ± 5.7	8602 ± 16419	27 ± 30
	ANOVA p	ns	ns	ns	ns
C + E	Granny Smith	26855 ± 25350	13.7 ± 17.2	3797 ± 5120	42 ± 39
	Northern Spy	20608 ± 21561	9.9 ± 12.4	2724 ± 2619	32 ± 33
	MM106	17580 ± 21408	12.7 ± 13.6	1780 ± 2288	30 ± 32
	M793	39077 ± 25161	10.1 ± 11.1	6752 ± 7327	60 ± 39
	ANOVA p	ns	ns	ns	ns

Table 3. EPG successful recordings expressed as total time, frequency, mean time and percentage of recording time spent in each waveform for each woolly apple aphid population across all rootstocks. Average values displayed ± standard deviation.

waveform	rootstock	total time (sec)	frequency	mean time (sec)	% recording time
np	Albury	40691 ± 20484	3.0 ± 1.4	15303 ± 9177	70 ± 32
	Batlow	32689 ± 24995	2.7 ± 1.9	16065 ± 16485	50 ± 39
	ANOVA p	Ns	ns	Ns	< 0.05
C	Albury	8938 ± 10553	7.3 ± 7.4	1798 ± 2805	15 ± 17
	Batlow	20055 ± 18463	6.7 ± 7.1	5205 ± 6684	31 ± 28
	ANOVA p	< 0.01	ns	< 0.01	< 0.01
E	Albury	9172 ± 12197	5.0 ± 6.9	1730 ± 3076	14 ± 19
	Batlow	12056 ± 15507	4.8 ± 7.0	3863 ± 10015	19 ± 23
	ANOVA p	Ns	ns	Ns	ns
C + E	Albury	18110 ± 20990	12.2 ± 14.2	2036 ± 2807	30 ± 32
	Batlow	32111 ± 24995	11.5 ± 14.0	5055 ± 5745	50 ± 39
	ANOVA p	< 0.01	ns	< 0.01	< 0.05

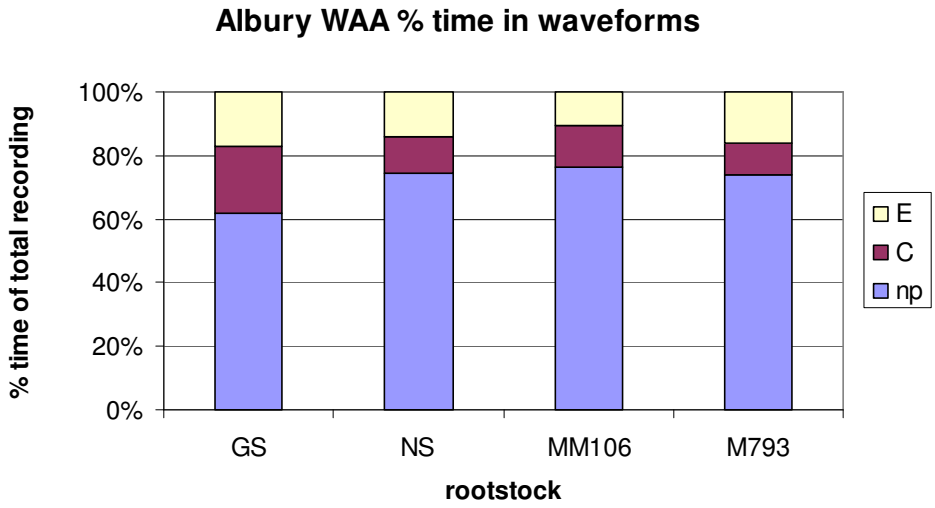


Figure 9. EPG successful recordings expressed as percentage of the Albury woolly apple aphid population spent in each waveform for each apple tree rootstock.

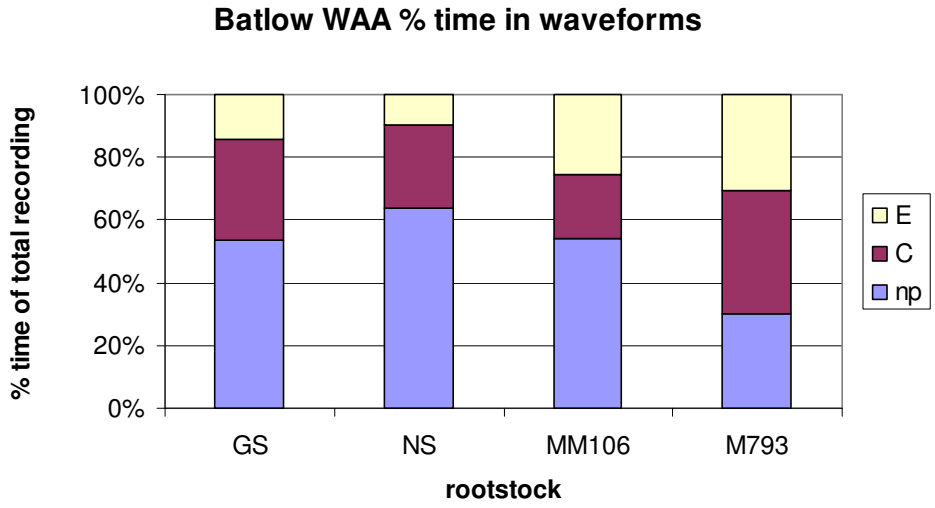


Figure 10. EPG successful recordings expressed as percentage of time the Batlow woolly apple aphid population spent in each waveform for each apple tree rootstock.

Discussion

To-date very few studies have been conducted using the Electrophysiological Penetration Graph (EPG) technique for WAA and those published studies were conducted on New Zealand populations of WAA, which may have different genetic characteristics to WAA populations in Australia. Two geographically isolated WAA populations were screened against 4 apple rootstock types in our study and this is the first published report of its type to specifically use Australian WAA populations. Three major waveform types were described and further waveform analysis is being currently being prepared to submit to a peer reviewed journal.

When comparing pooled data of WAA populations the level of EPG recording success reflected the expected WAA resistance ratings with susceptible Granny Smith having more EPG successful recordings than the highly resistant M793 rootstock. However when comparing the two WAA populations 'Albury' and 'Batlow' the recording success differed and relative resistance ratings changed depending on population used. This result highlights firstly that the two populations used in our study are most likely to be two separate ' biotypes' of WAA. Further evidence for this hypothesis is supported by data reported in Chapter 2 where each population differed in their survival on artificial diet formulations and hence their nutritional requirements. It also highlights that resistance ratings are general and not 'biotype' specific. The finding that WAA biotypes may occur in Australia can have important implications for management of the pest in terms of rootstock recommendation or other management options.

Interestingly, susceptible Granny Smith did not experience significantly more ingestion activity (waveform E) than the resistant rootstocks (Northern Spy, MM106 and M793). Unexpectedly the WAA resistant M793 experienced more recording time in waveform E than Granny Smith, however this result is not significantly different and may be skewed by the difference in sample number (Granny Smith 17 successful EPG recordings, M793 9 successful recordings). This result does however show that WAA are feeding on resistant rootstocks, allowing for variation in rootstock success under field conditions. A more detailed analysis of the EPG recordings will assist in defining the differences between susceptible Granny Smith and resistant rootstocks.

Recommendations

As we only selected two geographically isolated WAA populations in this study a more extensive survey of WAA populations is recommended, in the major production areas of both Victoria and New South Wales, combined with a genetic study to fully characterise the genetic diversity of WAA in different geographical regions. Once characterised the EPG system, combined with the artificial diet system, offers much potential for relatively rapid screening of individual predominant WAA genotypes against specific apple rootstocks.

A more extensive analysis of EPG waveforms is warranted in order to observe differences in feeding behaviour across the selected rootstocks and to separate the E waveform into phloem and xylem components which may be useful in distinguishing resistance characteristics at the cellular level.

Technology Transfer

Introduction

During the course of the project a variety of techniques and approaches were used to disseminate knowledge associated with project outputs and these differed depending on the target audiences. Each of the transfer methods is highlighted along with the predominant target audience.

Methods and Results

International Conferences (target audience – predominantly scientists)

The following is an abstract from an oral presentation given at the XXIII International Congress of Entomology, Durban, South Africa 6-12 July 2008.

Development of an artificial diet rearing system for woolly apple aphid (*Eriosoma lanigerum* H.)

*Dario Stefanelli, Kevin S. Powell**
Department of Primary Industries, BioSciences Research Division, Rutherglen, Victoria, Australia

Introduction: Woolly apple aphid (WAA), *Eriosoma lanigerum* (Hausmann), is one of the economically important pests of apple, *Malus domestica* (Borkh.). Artificial diets are an important tool to study insect development, feeding behaviour, symbiont activity and to screen for novel antimetabolites which may aid in their control. Prior to this study, artificial diets were available for a range of aphids but not WAA. The aim of this study was to develop an *in vitro* rearing system using artificial diets for WAA.

Methods: Two WAA stock populations were reared under greenhouse conditions on susceptible apple seedlings. Two feeding chamber designs ("conventional" and "inverted"), three sucrose concentrations (5%, 10% and 20%), and one diet formulation (Febvay *et al*, 1988) were tested. In all experiments a no diet control was used. Fifty first instar nymphs were utilised for each treatment and control. The geographical provenience of the aphid populations were tested separately as factors.

Results: The "inverted" chamber design was best suited for WAA feeding and utilised for all subsequent testing. Five and 10% sucrose concentration were statistically different from the control but suboptimal in terms of insect development and survival. Twenty percent sucrose and the Febvay diet more than doubled the survival time, up to 19 days, and evidence of ecdysis was observed. There was a significant difference in the survival curve and duration between the two aphid populations, which is indicative of genetically distinct characteristics.

Conclusions: An *in vitro* feeding system was developed for WAA which could be used to screen antimetabolites.

National Conferences (target audience – predominantly scientists)

The following is an abstract from a submitted oral presentation for the Australian Entomological Society's 39th Annual General Meeting & Scientific Conference, September 2008, Orange, New South Wales.

Interactions between Woolly Apple Aphid and pome fruit: Stage 1 - development of an artificial feeding system

Stefanelli, DA (1), KS. Powell (2) & KB. Kingston (2)*
(1) Department of Primary Industries, Knoxfield Centre, Knoxfield VIC 3180; (2) Department of Primary Industries, Rutherglen Centre, Rutherglen VIC 3685

The Woolly Apple Aphid (WAA), *Eriosoma lanigerum* (Hausmann), has recently re-emerged as a serious pest for the apple industry, partly due to the introduction of susceptible dwarfing rootstocks into the production system. Current research is examining interactions between the WAA and pome fruit by reviewing the biology of the pest insect, specifically the dietary requirements and feeding behaviour. The development of an artificial feeding system allows for determination of the nutritional intake required by WAA for survival and reproduction, and establishes a model system for testing antimetabolites which may be applied as novel control agents against the pest insect. While artificial feeding systems exist for a range of aphid species, the chamber design and diet composition required further development for application to this study. Glasshouse reared, first instar WAA insects were utilised for the artificial feeding system experiments. Two chamber designs were trialled (conventional vs. inverted) using a range of diet formulations that varied in sucrose concentration, amino acid and vitamin composition. The modified, inverted chamber design was determined most suitable to WAA artificial feeding system experiments. A simply diet formulation containing 20% sucrose, and a complex diet containing sucrose plus a range of amino acids and vitamins, resulted in statistically improved insect survival times compared to controls containing no diet solution. The maximum WAA survival time in the artificial feeding system was 20 days. Possible future applications of the artificial feeding system, plus other techniques being utilised within the project, will be discussed.

The following is an abstract from an oral presentation given at the 40th Annual General Meeting & Scientific Conference, September 2009, Darwin, NT.

Woolly Apple Aphid dietary requirements and feeding behaviour studies suggest the presence of differential biotypes in Australian apple orchards

Andrews, KB (1), DA. Stefanelli (2) & KS. Powell (1)*
(1) Department of Primary Industries - Biosciences Research Division, Rutherglen Centre, Rutherglen VIC 3685 (2); Department of Primary Industries, Knoxfield Centre, Knoxfield VIC 3180

The Woolly Apple Aphid (WAA), *Eriosoma lanigerum* (Hausmann), is a reoccurring pest for the apple industry. Current research is examining interactions between the WAA and pome fruit by investigating the biology of the pest insect. An artificial feeding system was developed to determine the nutritional requirements of WAA, while the electrophysiological technique EPG has been employed to investigate the insects feeding behaviour. Two geographically isolated populations of WAA were established in a glasshouse environment on potted apple trees; first instar insects were utilised for artificial feeding system experiments, late instar and adult insects for the EPG trials. Artificial feeding trials examined a range of diet formulations varying in sucrose concentration, amino acid and vitamin composition. A simple diet formulation containing 20% sucrose, and a complex diet containing sucrose plus a range of amino acids and vitamins, resulted in statistically improved insect survival times compared to controls containing no diet solution. The maximum WAA survival time in the artificial feeding system was 20 days. The two geographically isolated WAA populations displayed differential survival rates on artificial diet formulations indicating the presence of environmental biotypes. The presence of biotypes was supported through EPG trials where WAA insects displayed selective feeding behaviour on a range of apple rootstocks. The potential consequences of WAA biotypes for the future management of the pest insect will be discussed.

Industry journals (target audience – predominantly growers)

Copy of article published in "Top Grower", Volume 2, No. 1, March 2007 (Fruit Growers Victoria Ltd).

DPI Notes

Searching for woolly apple aphids

Kim Kingston, Research Scientist, DPI-Rutherglen

Scientists at the Department of Primary Industries (DPI) are seeking the assistance from apple orchardists in the region to locate populations of the woolly apple aphid.

The woolly apple aphid causes economic damage to the apple industry due to feeding activity on the roots and aerial parts of the apple tree. The high level production of the "honeydew" waste products from the woolly apple aphid result in the development of a sooty mould on the fruit, reducing the quality and marketability of the product. IPM strategies may be employed to control the woolly apple aphid, however the insect is becoming an increasing problem in intensively produced orchards that have a high usage of insecticides.

A new project, funded by HAL, has been developed by DPI to improve the current understanding of the biology of the woolly apple aphid, with the long-term aim of improving current management strategies. Scientists and extension staff based at DPI-Rutherglen and DPI-Tatura have been engaged on the three-year project to:

- Determine the essential dietary requirements of the pest insect through the development of an artificial feeding system. The artificial feeding system will allow for the testing of alternative control strategies for the woolly apple aphid;
- Compare the feeding behaviour of the woolly apple aphid on susceptible and resistant apple varieties. Feeding behaviour studies may provide insight into the impact of orchard management on the pest insect;
- Identify important plant volatiles that may increase the level of attraction of natural predators and parasitoids. Potentially, the application of the plant volatiles to the orchard production system may increase the rate of re-colonisation by beneficial insects and therefore reduce the impact of the woolly apple aphid on apple production.

The project is only in the initial stages, and a population of the woolly apple aphid is urgently required.

Collections of the insect are required from non-insecticide treated field populations in order to establish a laboratory population. If you currently have woolly apple aphids in your orchard that could be collected, please contact Kim Kingston at DPI-Rutherglen, phone 02 60304607.

Left. The woolly apple aphid may also establish on the interior branches of the apple tree. Although not visible on this photograph, the production of the "honeydew" waste product from the insect on the surface of the fruit may lead to the development of a sooty mould.

The characteristic white, woolly excretion from the woolly apple aphid makes the insect easy to identify on the trunk of this apple tree (Right).

The following is a Copy of an article published in “Premium Pickings”, Volume 18, No. 2, June 2009 (DSE, DPI).
http://www.dpi.vic.gov.au/DPI/nrenfa.nsf/LinkView/18A4011423807FC5CA256F19000B861DE99188EA90D34C39CA257157002515B9

When Woolly Apple Aphid gets ‘wired’

Henry Schneider and Kim Andrews - DPI Tatura and Rutherglen

Department of Primary Industries (DPI) researchers are collecting electrical data to better understand the feeding behaviours of the Woolly Apple Aphid (WAA), in the hope of understanding how to manage the pest insect and prevent costly crop losses to apple growers. The project is funded by Horticulture Australia and based at DPI Rutherglen Centre.

The WAA is a major pest in apple orchards and controlling the pest is often difficult and costly. An infestation can be detrimental to the fruit quality of the current season, and may also impact the following season’s flowering capacity.

This project aims to interpret WAA feeding behaviour on a range of apple trees in an effort to better understand what makes apple trees attractive to WAA (Image 1).

Individual WAA will be ‘wired’ by attaching a very fine gold wire, about the diameter of a human hair, on to the back of the insect. A metal probe is inserted into the soil of a potted apple tree (Image 2). When the aphid penetrates the plant surface - like a mosquito puncturing human skin - an electrical circuit is completed and the aphids feeding behaviour is electronically recorded.

Image 1. Woolly Apple Aphid infestation on Granny Smith

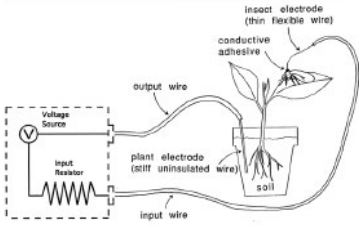


Image 2. EPG electrical circuit, when the insect penetrates the plant surface the feeding behaviour is recorded. (Image supplied by Greg Walker, University of California-Riverside)

The ‘wired’ technique is known as Electrical Penetration Graph (EPG). EPG has previously been used to examine the feeding behaviour of a wide range of sap-sucking insects.

The unique signals recorded by EPG allow for the interpretation of WAA feeding behaviour, providing knowledge of the apple trees’ resistance mechanism against insect damage.

Dr Kim Andrews is undertaking this research to determine why WAA may find certain apple sap tastier that others and a list of possible feeding responses for WAA have been identified (Table 1).

“Studying insect feeding behaviour, on a range of apple trees, under controlled conditions, will allow us to predict WAA infestations under different management conditions in the field,” Dr Andrews said.

Dr Andrews will initially monitor WAA feeding behaviours on Granny Smith apple plants, and then compare the EPG signals with a range of rootstocks, including Northern Spy and MM106.

Future research projects could utilise EPG to investigate WAA feeding behaviour under a range of management regimes to interpret the impact of fertiliser and water application on the pest insect.

For further information on the Woolly Apple Aphid project please contact Dr Kim Andrews at DPI Rutherglen on (02) 6030 4607.

Electrical signal	Feeding behaviour	Resistance rating (possible mechanism)
None	No feeding	Strong resistance (repellent)
Cell penetration patterns only	Plant surface penetration but no feeding	High resistance (structural)
Cell penetrations and short periods of feeding patterns	Plant surface penetration followed by multiple “tasting” events	Resistant (nutritional)
Cell penetration and extended periods of continuous feeding patterns	Plant surface penetration followed by prolonged feeding events	Susceptible (no structural or nutritional blockers to WAA feeding behaviour)

Table 1. Potential interpretations of WAA feeding behaviour from EPG electrical signals.

Image 3. Dr Kim Andrews preparing apple trees to be wired with WAA at DPI Rutherglen Centre

Copy of article published in Apple and Pear Industry Report 2006-2007.
http://www.horticulture.com.au/reports/industry_annual_reports.asp#a_1

Pests & Diseases

AP06011
UNDERSTANDING THE FUNDAMENTAL INTERACTIONS BETWEEN WOOLLY APPLE APHID AND POME FRUIT

Kevin Powell - VIC Department of Primary Industries

Woolly apple aphids cause economic damage to industry due to their feeding activity on the roots and aerial parts of the tree. In addition, the high level of production of "honeydew" waste products from the woolly apple aphids results in the development of a sooty mould on the fruit, reducing the quality and marketability of the product. IPM strategies may be employed to control the woolly apple aphid, however, in intensive orchards where susceptible dwarfing rootstocks are used as well as a high usage of insecticides the insect is becoming a major problem. Further, while the use of resistant rootstocks is possible in less intensive orchards there is potential for breakdown in rootstock resistance.

This project will improve the current understanding of the biology of woolly apple aphid, with the long-term aim of improving current management strategies. The aims of the project are to:

- determine the essential dietary requirements of the pest through the development of an artificial feeding system. The artificial feeding system will allow for testing of alternative control strategies for woolly apple aphid
- compare the feeding behaviour of woolly apple aphid on susceptible and resistant apple varieties. Feeding behaviour studies may provide insight into the impact of orchard management on the pest insect
- identify important plant volatiles that may increase the level of attraction of natural predators and parasitoids. Potentially, the application of the plant volatiles to the orchard production system may increase the rate of re-colonisation by beneficial insects and therefore reduce the impact of woolly apple aphid on apple production.

In the first six months of the project the team has developed a system for rearing woolly apple aphids under glasshouse and laboratory conditions. Two aphid populations from different regions have been established which will be used in the first stages of the project to develop an artificial diet feeding system.

Woolly apple aphid in commercial apple orchard

Parasitized woolly apple aphid laboratory culture

Copy of article published in Apple and Pear Industry Report 2007-2008.
http://www.horticulture.com.au/reports/industry_annual_reports.asp#a_1

Promote and Facilitate Intensification of Orchard Production

UNDERSTANDING INTERACTIONS BETWEEN WOOLLY APPLE APHID AND POME FRUIT

A better understanding of the biology of the woolly apple aphid is being developed with the aim of identifying better management strategies.

The woolly apple aphid causes economic damage to the industry due to feeding activity on the roots and aerial parts of the apple tree.

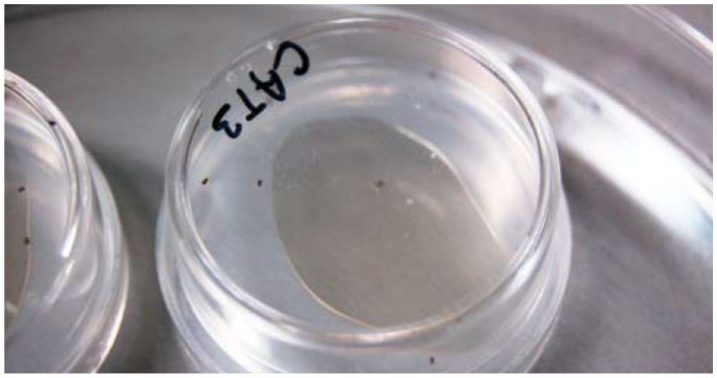
Feeding in the leaf axils can destroy buds and heavy infestations can cause crop losses. In addition, they excrete sticky 'honeydew' which acts as a site for sooty mould development, which reduces the quality and marketability of fruit.

IPM strategies are used to control woolly apple aphid but the choice and timing of applications are important, particularly to avoid disruption of natural predators in the orchard.

Woolly apple aphid is becoming an increasing problem in intensive orchards that have a high usage of insecticides and the potential exists for breakdown in rootstock resistance.

This project will:

- Determine the essential dietary requirements of the pest through the development of an artificial feeding system.



Feeding chamber for rearing woolly apple aphids on a liquid artificial diet

- Compare the feeding behaviour of the woolly apple aphid on susceptible and resistant apple varieties. Feeding behaviour studies may provide insight into the impact of orchard management on the pest insect.
- Identify plant volatiles that may increase the level of attraction of natural predators and parasitism.

The project team has developed systems for rearing woolly apple aphids under glasshouse and laboratory conditions.

Two aphid populations from geographically distinct regions have been fed on artificial diet formulations. This bioassay feeding system could be further developed as a rapid tool for testing new protectants.

Early indications are that the two populations have different dietary requirements for optimal development and hence different biological behaviours. This has implications for screening rootstocks for aphid resistance.

A survey of Victorian growers to assess extent of woolly apple aphid damage, distribution and management revealed that in some orchards, up to 100 per cent of trees were affected and predominant control was insecticide application.

In the next year, the feeding behaviour of woolly apple aphid will be tested using an electrophysiological approach to help understand the mechanisms of susceptibility and resistance.

Project: AP06011

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6 Woolly apple colonies on pot grown apple plants

Parasitised Woolly Apple Aphid

Copy of article published in Apple and Pear Industry Report 2008-2009.
http://www.horticulture.com.au/reports/industry_annual_reports.asp#a_1

Diet could hold the key to woolly apple aphid control

A better understanding of the feeding behaviour of the pest woolly apple aphid (WAA) – especially in modern high density growing systems – will enable the development of sustainable management options.

WAA is a consistent pest for the Victorian apple industry. Successful insecticide treatment is complicated by the woolly protective coating produced by the aphid. Natural predators (earwigs and wasps) are successful under field conditions, although these beneficials are detrimentally affected by the application of insecticides for other pest insects in the orchard.

The planting of resistant rootstocks could impact on WAA population outbreaks; however, changes in management practices – towards (susceptible) dwarfing rootstocks – have limited this control option.

New approaches for the control and management of WAA are therefore required to limit the damage caused by this pest insect in future growing systems.

This project, scheduled for completion by the end of the year, is investigating the feeding physiology of WAA.

Artificial diet studies and examination of the feeding behaviour of WAA through the use of the electrical penetration graph (EPG) aim to understand the feeding requirements of WAA for survival and reproductive development. A successful artificial diet system provides a model for the rapid testing of novel control agents against the pest insect, as well as providing a chamber for close analysis of nutritional requirements.

EPG records the feeding response of WAA to a range of apple rootstocks, allowing for comparisons in feeding behaviour and determination of resistance mechanisms.

An understanding of WAA's feeding behaviour is important in the development of long-term and sustainable management options.

Analysis of a survey of Victorian apple growers, completed as part of the extension component of this project, indicated large variations in WAA infestation rates across and within apple growing regions.

Variable responses for rootstock resistance were also recorded.

A presentation of research data on artificial diets for WAA was made at the International Congress in Entomology held in Durban, South Africa, and research discussions took place with WAA researchers at Stellenbosch University.

This year EPG will be utilised to examine the feeding behaviour of WAA on a range of apple rootstocks.

Project AP06011

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Radio rural program (target audience – predominantly growers and general public)

Copy of webpage promoting the ABC Radio Goulburn Murray Rural Report, 04 June 2009.

http://www.abc.net.au/rural/vic/content/2009/06/s2588984.htm

ABC Rural

WOOLLY APPLE APHID

By Warwick Long

Thursday, 04/06/2009

The Woolly Apple Aphid is a small pest milimetres in size that can cause downgrades in commercial fruit and disrupt fruit picking.

A new research project is being designed to look into the feeding and living habits of the aphid in a effort to better understand the creature and how to control it.

Kim Andrews a research scientist form the Department of primary Industries in Rutherglen says that some high tech equipment is being used to monitor the incsect.

A strand of wire about the width of a human hair is attached to the back of the insect and to the plant and when the animal feeds a circuit is completed and researchers can understand where and why the bug is feeding.

In this report: Kim Andrews, Research Scientist, Department of Primary Industries, Rutherglen

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- Woolly Apple Aphid
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In addition at the commencement of the project in consultation with Fruitcheque Staff at DPI Cobram and Tatura an Adoption Strategy was developed (Appendix 2).

International Scientific Exchange (target audience – predominantly scientists)

During the course of the project research discussions were held and linkages developed. Renowned EPG expert Professor Freddy Tjallingii (Wageningen University) was invited to DPI Rutherglen during the course of the project through DPI funded Visiting Scientists program
For discussions on a range of EPG topics. Dr Powell also visited Dr Ken Pringle, woolly apple aphid researcher, at the University of Stellenbosch in South Africa, for discussions on WAA research.

Discussion

Industry and scientists where kept well informed of project objectives and results during the course of the project. The technology developed during the course is available for further development in future related projects.

Acknowledgements

The survey component of this project could not have been possible without the assistance of apple growers in Victoria who are gratefully acknowledged for their support. We would also like to thank Matt McMahon (Batlow Apples, NSW) and Wolfgang Schwarz (DPI - Rutherglen) for access to woolly apple aphid populations. Mirko Milinkovic supplied Granny Smith seedling trees (DPI Knoxfield) for maintenance of insect populations. David Williams (DPI-Tatura) was an invaluable intellectual resource because of his breadth of knowledge on Australian horticultural industries,

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Appendix 1

Woolly Apple Aphid Questionnaire

A research project into Woolly Apple Aphids on apples is being investigated by entomologists at DPI Rutherglen in collaboration with Horticulture Australia Limited. The research is looking at developing improved control measures particularly using information on how aphid populations are distributed and interact with orchard management operations.

To help establish background information for the project, we are requesting that growers take the time to do this quick survey about their experience with woolly apple aphid on their orchards.

This should take about 15 minutes to complete with a reply-paid envelope provided.

Question 1.

(a) Have you seen Woolly Apple Aphid in your orchard? (please circle) Y / N?

(b) What % of your apple orchard is affected?.....…….....%

(c) Please provide further information on up to 3 blocks affected by Woolly Apple Aphid.

BLOCK 1 Variety……Rootstock (if known)……

Planting date or age in years:……

Tree vigour rating: □ Low □ Medium □ Strong

Canopy type □ Central leader □ Tatura Trellis □ Vase □Other ……..… (Please specify)

Woolly aphid severity (tick all which are relevant):

Individual trees: Woolly apple aphid found on: □ Tree base □Trunk □ Canopy

Orchard block: Woolly apple aphid found: □Evenly spread □Only in patches

Overall severity of Woolly apple aphid infestation: □ Low □Medium □ High

BLOCK 2 Variety……Rootstock (if known)……

Planting date or age in years:……

Tree vigour rating: □ Low □ Medium □ Strong

Canopy type: □Central leader □Tatura Trellis □Vase □Other …… (Please specify)

Woolly aphid severity (tick all which are relevant):

Individual trees: Woolly apple aphid found on: □ Tree base □Trunk □ Canopy

Orchard block: Woolly apple aphid found: □Evenly spread □Only in patches

Overall severity of Woolly apple aphid infestation: □ Low □Medium □ High

BLOCK 3 Variety……Rootstock (if known)……

Planting date or age in years:……

Tree vigour rating: □Low □Medium □Strong

Canopy type □Central leader □Tatura Trellis □Vase □Other …… (Please specify)

Woolly aphid severity (tick all which are relevant):

Individual trees: Woolly apple aphid found on: □ Tree base □Trunk □ Canopy

Orchard block: Woolly apple aphid found: □Evenly spread □Only in patches

Overall severity of Woolly apple aphid infestation: □ Low □Medium □ High

Question 2: Please provide information on your orchard management:

(1) Which chemicals have you applied against woolly apple aphid in your orchard over the last 2 years?

……
(2) Which chemicals do you use to control codling moth, LBAM and OFM in your orchard?

……

(3) Do you use Carbaryl or Sevin as part of your thinning program? Y / N

(4) Do you use any other method to control Woolly Apple Aphid? If so please comment.

……

Question 3.

In which of the following apple growing regions are you located (please circle):

North East Bacchus Marsh Shepparton/Ardmona Cobram Stanley

Yarra Valley Kyabram Harcourt Mornington Peninsular Gippsland

THIS SURVEY IS CONFIDENTIAL AND DATA WILL ONLY BE AVAILABLE TO THE PROJECT TEAM. IF YOU PREFER, YOU CAN SEND THIS BACK WITHOUT YOUR ADDRESS.

We would find it valuable to collect samples from different regions to see whether there is a difference in Woolly Aphid populations. If you are willing for samples to be taken from your orchard please provide your contact details.

□ I agree to a sample being made available for the research project.

NAME ……....

ADDRESS ……

PHONE …… EMAIL:…….

Thank you for your participation. Please use the reply-paid envelope and return by FRIDAY 25th APRIL

For more information about this survey contact: Cathy Mansfield at DPI Tatura on 03 5833 5225.

Appendix 2

Woolly Apple Aphid Project Adoption Strategy

1. Trigger

The Woolly Apple Aphid (WAA) project began after a particularly bad year for WAA in apple crops around the state of Victoria. Growers were looking for solutions and a series of workshops were held. The growers meetings identified that shifts in management of other pest such as pheromone use for codling moth may have allowed the usually suppressed aphid population to develop. Another issue these meetings raised was that problems were worse on dwarfing rootstocks which are being used in most new plantings of apples. Choice of chemicals may have been suppressing natural enemies.

2. Issue

The issue is that little is known about why WAA prefer particular apple tree varieties and rootstocks. Knowledge gained in this project could help growers make decisions on rootstocks when planting new blocks. This work also could create strategies to reduce impact of WAA on currently planted orchards.

The project aims to identify weakness and opportunity for management of WAA by understanding their physiology better. This will determine suitable methods to test WAA in a range of different feeding substrates. To do this laboratory cultures must be maintained and suitable methods of testing feeding behaviour in relation to a range of variable must be developed.

This project will produce outcomes which will feed into a following research project.

3. Engagement

Networks are required with apple growers who have apple blocks which have been affected by WAA to collect insect for culturing and testing, these same networks are needed to collect more detailed information about the behaviour and management of the insects.

HAL, Apple and Pear Australia to update them on the project to ensure ongoing support
Apple growers to understand why the project exists and what it aims to achieve.
Networks need to be developed with key researchers in the field including:

Dr Ken Pringle, Stellenbosch University, South Africa has done both WAA management and monitoring.
Dr Freddy Tjallingii, Wageningen University, The Netherlands. Has conducted considerable work on aphid feeding behaviour

4. Current Situation

What is the actual situation? Are things good enough?

Growers had trouble particular trouble with WAA in the summer of 2006.

Survey of impacts of woolly aphid was undertaken in Victoria in (April 2008) which investigated the characteristic of WAA damage in orchards. This has given the project a clear idea of the current situation.

Research

No-one has developed and artificial diet until this project , because you need to understand basic nutritional requirement before you can understand resistance /susceptibility mechanisms.

EPG studies

Past research includes Tjallingii's aphid EPG work and preliminary work in New Zealand. We need to do this with Australian a WAA population because they could behave differently i.e. have different nutritional requirements.

5. Outcomes (Project Vision)

Outcomes by the end of the project life?

This project aims to study the feeding behaviour, gather information about the woolly aphid physiology so that non-chemical and chemical control methods can be developed to manage this pest.

It will test if it is possible to manipulated apple crops to be more resistant to woolly apple aphid.

Addition test selected woolly aphid populations for host plant resistant .In the future this project could provide resistant rootstock recommendations.

The major outcome is a better targeted IPM program which takes into account the characteristics of the woolly apple aphid population.

6. Understanding the end users

The end users of this research are two-fold:

In the short term it is the second research team that will use the knowledge gained in this project to test a range of control options.

In the long term the end users will be apple growers so that understanding their requirements will be very important to determine whether the control methods will suit their needs and requirements. It is important to understand what they look for in their current control measures e.g. quick to undertake? Will it fit in with their IPM program? Further work will be needed in this area.

7. What change needs to happen?

A product/output for practice change in the orchard will not be produced within the life of this project. The extension work will focus on ensuring the product of the research is suited to grower's requirements. It will examine the decision making growers currently use to assess their WAA control methods.

8. Partners (who can help us deliver the outcomes)

Apple and Pear Australia, Fruit Growers Victoria and Australian Fresh Fruit Company - Can help us reach apple growers, build awareness of the project and feed back project information to our target audience. These groups can also be used to test the suitability of our products.

Consultants and Chemical resellers will need to understand both the project and how the products of the project fit in with Orchard pest and diseased management strategies.

Other state government extension staff to ensure that a national awareness of the projects aims and findings.

Nurseries to ensure they understand the outcomes latest rootstock research.

9. Resources and Capability

Half-a-day per week to complete the extension component of this work. Five days a week to complete research components.

10. Risks

Assumption is that we can develop artificial diet feeding system

11. Activities

Research

Development of artificial diet
Characterization of Woolly Apple Aphid feeding behaviours on rootstock material

Extension

Survey to understand current situation

Questioning of apple growers to understand their decision making and requirements for WAA control methods to ensure product of research is suitable

Awareness raising of the project through a range of methods-media etc

Testing assumptions of project team that project outputs will lead to achieving change.

12. Theory of action

Bennett's Hierarchy has been produced.

13. Monitor and Evaluate.

Review strategy with team to determine what success looks like for this project. (Ongoing)

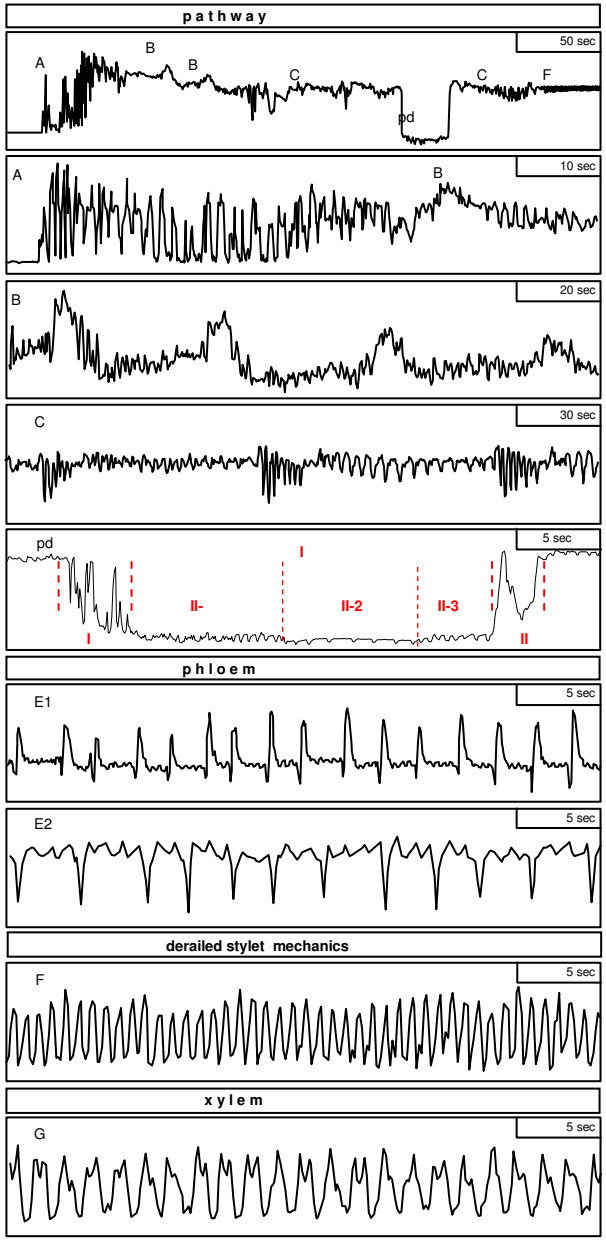
14. Communication Plan

Will focus on industry funding bodies and a scientific audience for peer-review.

15. Exit Strategy

This projects outputs are to be used by the next project team.

Appendix 3 EPG Waveforms and Correlations



EPG wave form[1]	relative[2] amplitude	rep. rate	volt. level	el. origin	plant tissue	aphid activity	remarks
	|===== characteristics ===|				|================ correlations ==========|		
A	100	5-10	e	R	epidermis	cuticle penetration	first wave-form, electrical stylet contacts
B	75	0.2-0.3	e	R	epidermis / mesophyll	sheath salivation	Waveforms overlap. Therefore, ABC mostly lumped as 'stylet pathway' activity (C) in EPG analysis.
C	30	mixed	e	R	all tissues	many activities during pathway	
pd	.		i	emf	all living cells	stylet puncture	3 phases, II with 3 sub-phases:
II-1						salivation | ?	non-pers. virus inoculation
II-2						salivation | ?	non-pers. virus inoculation
II-3						ingestion	non-pers. virus acquisition
E1e	.	2-7	e	emf	mesophyll (?)	unknown	same activity as E1?
E1	.	2-7	i	emf	sieve elements	salivation	persistent virus inoculation
E2							
w	.	4-9	i	emf	sieve elements	(watery?)	salivation persistent virus
p	5	0.5-4	i	R	sieve elements	(passive)	ingestion acquisition
F	5	11-19	e	R/emf	all tissues	derailed stylet mechanics	'penetration difficulties'
G							
w	.	4-9	e	emf	xylem	active ingestion	'drinking'
p	0-60	4-9	e	R	,,	unknown	only occasionally shown

[1] p: peaks, w: waves. [2] useless to provide for emf signals.