Final Report

Determining the extent and causes of abnormal vertical growth

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Delivery partner:

The University of Queensland

Project code:

MC15011

Project:

Determining the extent and causes of abnormal vertical growth MC15011

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Summary

Data obtained in this project show that abnormal vertical growth (AVG) costs the Australian macadamia industry over 2,000 tonnes loss in nut-in-shell production annually, which equates to an economic loss of about $10 million. This project has revealed at least 5-fold increase in the number of macadamia trees with AVG symptoms over the 2003 survey report. Approximately 0.2 million trees, covering over 900 hectares, have been affected in the Australian macadamia industry. There is a high-risk potential of AVG spreading in the affected orchards and developing in new production areas in Queensland and New South Wales.

In affected orchards, number of AVG trees constitutes 15% - 50% of the total trees. Although about 30% of the affected trees have been removed and replanted, AVG trees currently occupy over 700 hectares in Queensland and New South Wales. AVG causes severe seasonal fluctuation in flowering, resulting in yield losses ranging between 30% - 88%, depending on the variety. In addition to the significant reduction in yield, additional management of AVG trees in orchards costs each grower $2,200 - $2,500 per hectare annually. Estimates of return on investment showed that if the affected trees are not treated, it is more cost-effective to remove and replant. However, the risk of AVG re-occurring in the young trees erodes grower's confidence in future investment.

Differences in varietal susceptibility to AVG among the 75 varieties evaluated in this study have provided insights into the mode of genetic variation for AVG tolerance. Macadamia cultivars of Hawaiian origin (selections) were more susceptible than varieties developed/selected in Australia. No difference was found between seedlings and grafted trees or rootstocks. A confounding factor to the AVG distribution pattern is that AVG occurs mostly in soils that are prone to severe moisture stress.

Molecular study revealed that geminivirus DNA is integrated into the macadamia chromosomes. Detection of geminivirus in over 130 leaf samples with and without AVG symptoms was correlated with severity levels of AVG trees. Analysis of next generation sequencing data revealed no replication-competent of the geminivirus, which indicates that the geminivirus DNA is a mutated DNA, and therefore, there is no clear evidence of geminivirus infections associated with AVG. The similar levels of detection of bacterium (*Bacillus megaterium*) and fungi in the samples requires further studies to understand their roles in AVG disorder.

An outcome of the project activities is that the macadamia industry, Hort Innovation and research community are provided with the current impact of AVG. In addition, this project has looked critically at the cause of AVG, has put forward clear measurable research hypotheses and selection criteria (urgency, technical difficulties, required time-frame and industry importance) that allow for planning and investment into future research, short- and long-term management of AVG. Future direction for diagnostics, breeding and management of AVG should involve a full inter-related research project, focused on pathogenic interactions including detection, transmission studies and monitoring spread. The new project should have a strong linkage with the macadamia breeding program to develop genetic markers for AVG resistance.

Keywords

Bacillus; endogeneous geminivirus; endophytes; native tree; *Proteaceae*; tree nut;

Introduction

Abnormal vertical growth (AVG) is a disorder of macadamia trees that is characterized by excessive upright growth with few side branches and increased vegetative vigor resulting in reduced flowering, poor nut set and reduction in yield (O'Farrell, 2011). AVG appears to be more widespread and severe in the Bundaberg production area and on the Atherton Tablelands in Queensland (QLD). However, trees with similar symptoms have been observed in most production areas in QLD and New South Wales (NSW) (O'Farrell, 2011). Previous studies including the MC01030 (*Understanding 'abnormal vertical growth' of macadamia variety 344*) and the MC03012 (*Developing corrective treatments for maintaining macadamia nut production and normal growth - extended project*) projects associated AVG with agronomic characteristics including well-drained soils (O'Farrell, 2011). However, the cause of AVG was not established.

Information on varietal susceptibility is limited. Macadamia varieties 'HAES 344', 'HAES 741', and 'HAES 660' that are on deep red sands and red sandy clays (Farnsfield and Oakwood soil types) constitute most of the AVG trees in the Bundaberg area (Searle, 2016). Varietal susceptibility levels of 14 macadamia cultivars have been described (Searle, 2016). High susceptibility of 'HAES 344' was demonstrated when the variety was replanted in soils where AVG trees have been removed, under these conditions, AVG symptoms were observed in the young trees within five years(O'Farrell, 2011). This scenario suggests a pathogenic cause for AVG.

A preliminary study using next generation sequencing technique showed a promising evidence that AVG may be due to biotic agents (O'Farrell, 2011; Webb and Geering, 2015). The studies on spread and distribution pattern of AVG trees in the orchards (O'Farrell, 2011) and altered hormone balance (Fletcher and Mader, 2007) also suggest possible pathogenic involvement. At present, there is no cure for AVG and the cause is still unknown. The number of trees affected with AVG appears to be increasing from estimate of between 18,000 and 22,000 trees affected in 2004 to about 100,000 trees in 2017 (O'Farrell et al., 2016). This has reduced growers' confidence in replanting affected orchards and impaired further investment into areas at risk of AVG. In order to ascertain the status of AVG in the Australian macadamia industry, this 12-month scoping study was established to:

(i) Scope the impact of AVG disorder;
(ii) Review and update the agronomic observations on regional and varietal data with surveys of AVG disorder in Qld and NSW orchards;
(iii) Provide more information on the biotic cause of AVG; and
(iv) Develop a series of testable hypotheses with regards to the biotic nature of AVG.

Recommendations on the investment of resources for AVG research and management are provided to the stakeholders.

Methodology

1. How widespread is AVG?

a. Areas with AVG in the Australian macadamia industry

In order to provide information on the incidence of AVG in the Australian macadamia industry, a three-level hierarchical survey was carried out from March 2016 to October 2016. Industry-wide survey (Level 1) was a reconnaissance survey, used to determine the presence of AVG trees in the different macadamia production regions. The survey was through telephone and email contacts with macadamia consultants and growers in the five major macadamia producing regions in Australia. A subset of the growers and consultants in the reconnaissance survey was used in the semi-intensive survey (Level 2). The level 2 survey was performed to determine the extent of AVG in different farms based on the history, agronomic characteristics and size of farms affected. The information obtained from farm visits during the semi-intensive survey was used to select a subset of medium and large-scale farms for a more intensive survey (Level 3). In the intensive survey, using a structured face-to-face interview process, records of production data, direct and indirect economic costs, management practices, history of temporal spread, and varietal susceptibility were obtained from the growers.

b. Varietal susceptibility to AVG

A survey of severity of AVG on macadamia varieties and the effect of rootstock on AVG susceptible scion was carried out at five field sites in QLD. AVG severity was assessed using a modified rating scale developed by O'Farrell (2011). A weighted average of the severity ratings for each variety was used to classify the varieties into three groups.

2. Is AVG a significant economic importance to the industry?

Data obtained from the growers during the level 3 survey activities were used to estimate the impact of AVG to growers and the industry, including costs of AVG management, farm productivity and industry production. Economic impact was determined from the calculations of the revenue from sale of nut-in-shell (NIS), cash flow, Net Present Value (NPV) and Internal Rate of Return (IRR), based on three NIS price scenarios and broad assumptions as stated below:

- Different percentages of hectares with and without AVG.
- New orchard and replanting situations due to AVG.
- A constant mature yield of 3.4 tonnes NIS/ha for non-AVG trees, 1.5 tonnes NIS/ha for AVG trees.
- Annual production costs of $6,000 per ha for mature non-AVG trees and $8,500 for mature AVG trees.

3. What is the cause of AVG?

Over 250 samples including leaf, soil and wood tissues were obtained from both AVG and non-AVG trees from 11 macadamia production areas. Genomic DNA was extracted from 138 leaf samples and each sample was examined for the presence or absence of biotic agents including the geminivirus, bacteria and fungi using PCR amplification assays. New sets of more robust and efficient PCR primers than the previous sets developed by Webb and Geering (2015) were designed for the detection of geminivirus. Searches of the macadamia genome for integrated geminivirus sequences was performed. Multivariate procedure in GenStat statistical software was used to explore possible association of environmental factors: elevation (masl); historical annual rainfall (mm); mean minimum and maximum temperatures (°C); and relative humidity (%) with AVG incidence.

4. Research Workshop

A strategic workshop on AVG was held in Bundaberg on 16 February 2017. Twenty-one people were invited to the workshop, however, 18 participants including representatives of macadamia growers, researchers, industry consultants, Australian Macadamia Society and Hort Innovation attended the workshop. The workshop was the culmination of the MC15011 project activities. Outcomes of the workshop underpin the recommendations of future direction on AVG research and management in the industry.

Outputs

1. Analysis of the economic impact of AVG to the Australian macadamia industry.
2. Data on the current distribution of AVG.
3. Established that AVG is a growing problem.

4. Report of workshop on AVG in Macadamia.
5. A new set of more robust and efficient sets of PCR primers for detection of geminiviruses in macadamia.
6. Data on varietal susceptibility to AVG.
7. Data on association/interaction of biotic agents in AVG.

Outcomes

1. AVG disorder is a significant cost to individual growers and the industry. AVG causes economic loss of over 2,000 tonnes NIS loss in production annually, which equates to about $10 million loss to the Australian macadamia industry.
2. AVG increased the macadamia production costs of growers from average of $6,000 per ha to $8,500 per hectare.
3. Clear measurable research hypotheses and selection criteria were developed for future direction of investment on AVG disorder in macadamia.
4. A consensus of all stakeholders at the AVG workshop is a new full research project for 3-5 years should be established. The new research project should examine the cause of AVG, develop diagnostic tools for detection, transmission studies and monitoring spread.
5. Identified link with the macadamia breeding program for genetic solution for AVG.

Evaluation and Discussion

1. Macadamia production areas with AVG in Australia

This project has provided updated information on the spread of AVG in the Australian macadamia industry. The results revealed that AVG trees occupy over 900 hectares in the Australian macadamia industry. In affected farms, AVG trees constitute 15% - 50% of the total macadamia trees. Approximately 230,000 trees on 700 ha are currently affected with AVG in QLD and NSW. A survey in 2003 estimated 18,000 to 22,000 trees were affected with AVG (O'Farrell, 2011), thereafter, 100,000 trees were estimated with AVG (O'Farrell et al., 2016). This shows significant increase in the number of trees affected with AVG, thus, nearly 5% of the trees in the industry are currently AVG trees. About 30% of affected trees have been removed. Lack of confidence in the varietal susceptibility has impaired further replanting actions. AVG was observed, for the first time in the Sunshine Coast production region. This shows that AVG is now prevalent in all the major macadamia producing regions in Australia. Distribution of AVG trees in the farms is non-random in an aggregated pattern. This observation is similar to previous report on the spatial pattern of AVG (O'Farrell, 2011).

2. Effects of AVG on macadamia varieties and rootstocks

Seventy-five macadamia varieties including commercial cultivars, materials in the regional variety trials (RVT), seedlings and non-commercial varieties were classified into three groups (tolerant, moderately susceptible and highly susceptible). The criteria for classification included AVG severity rating (symptoms) and estimated yield reduction compared with non-affected trees. The classification of some varieties as tolerant based on symptom expression may be due to late onset of AVG (Searle, 2016). Generally, most of the varieties classified as highly susceptible such as 'HAES 344', 'HAES 741', 'HAES 246' and 'HAES 660' are Hawaiian selections. The Australian selections such as 'NG8', 'A16' and 'Own Venture' appear to be tolerant to AVG. A key difference between the Hawaiian and the Australian varieties is the proportion of *Macadamia integrifolia* genome in their genetic makeup (Peace et al., 2002; O'Farrell et al., 2016). The Hawaiian varieties tend to have higher *M. integrifolia* component than the Australian selections that contain high *M. tetraphylla* genome (Peace et al., 2002; O'Farrell et al., 2016). Using the highly susceptible cultivar 'HAES 344', no effect of rootstock or seedling was observed for AVG severity. Grafted trees with different 'tolerant' cultivars as rootstocks with AVG susceptible scions showed similar AVG severity compared with trees of susceptible rootstocks. However, trees of tolerant scions, grafted onto susceptible rootstocks in similar AVG conditions showed no AVG symptoms. This suggests that AVG transmission is not soil-borne via the rootstock.

3. Economic importance of AVG

Severe seasonal fluctuation in flowering occurs in AVG trees. Analysis of the data obtained from the growers revealed that AVG causes significant yield decline. Depending on the macadamia variety, 30% - 88% reduction in yield occurs in AVG trees. In comparison with non-AVG trees that produce average of 3.4 tonnes NIS per ha, NIS production in AVG affected trees range between 1 tonne per ha and 2.7 tonnes per ha. This shows that economic loss to the Australian macadamia industry is over 2,000 tonnes in NIS production annually, estimated at $5.00 /kg NIS, to be over $10 million loss in production annually due to AVG. In addition to the significant reduction in yield, growers with existing AVG trees incur additional production costs. These extra costs are incurred for AVG trees for removing broken branches and massive leaf litter from the orchard floor and for trialling management practices such as cincturing that costs $0.40 -$0.50 per tree. Therefore, an additional $2,000 - $2,500 per ha are incurred due to AVG, over the standard average annual cost of production of $6,000 per ha. At NIS price less than $5.00/kg, the cost of production on AVG ha is estimated at about $3,000 higher than the expected gross income. Analysis of return on investment showed significant improvement in replanted orchards.

4. Association of AVG disorder with environmental factors

AVG occurs at diverse elevations ranging from 14 masl at Baffle Creek in QLD to approximately 320 masl at Hogarth Range in NSW. A common abiotic factor in the AVG farms is soils with high risk of soil moisture stress. Macadamia trees in chocolate soils, basalt at Hogarth Range in NSW that are brown soils with a friable clay surface horizon overlying a tighter clay subsoil, are equally at risk as the trees in well-drained white sandy soils at Baffle creek and the deep red sands and red sandy clays soil types in the Bundaberg area (Searle, 2016). Therefore, there is a strong association of AVG with soil prone to severe soil moisture stress, mostly within the top 10 cm of the soil. This area is the most suitable and contains high proportion of fine roots in macadamia.

5. Exploring biotic causes for AVG

Geminivirus was detected in about 56% of the leaf samples obtained from trees with AVG symptoms while positive detection occurred in 30% of the leaf samples from trees with no visible symptoms of AVG. Generally, geminivirus was not detected in *M. tetraphylla* samples. Fungi were detected in 76% of the AVG samples and 69% in the non-symptomatic samples. Detection rate of *Bacillus megaterium* in the AVG and non-symptomatic samples was similar. Searches of the macadamia genome revealed that the geminivirus sequences are integrated in the macadamia genome. However, results of the molecular assays suggest that the geminivirus DNA is a mutated DNA, thus, no conclusive replication-competent geminivirus was identified. This indicates no strong evidence of replicative form of geminivirus is involved in AVG infections. Bacterium was successfully isolated from the vascular system of leaf samples, however, further study is needed to characterize the bacterium, fungi, and other viral elements that were identified in the samples. The study should examine their roles in AVG.

6. Research Workshop

The conclusions of the stakeholders were unanimous that AVG is a significant issue in the Australia macadamia industry than previously thought. The participants agreed that a full research project on pathogenic interactions is needed for AVG. Clear recommendations were provided to the industry and Hort Innovation.

Recommendations

1. Further research is needed to clarify the roles of *Bacillus* species and fungal agents in AVG trees.
2. Future research is needed to examine the pathogenic interactions, diagnostics for detection and monitoring spread of AVG.
3. Future research is needed to confirm transmission and possible curative options for AVG.
4. Future research is needed to develop a varietal solution.

Scientific Refereed Publications

Journal article

None to report

Whole book

None to report

Chapter in a book or Paper in conference proceedings

None to report

Intellectual Property/Commercialisation

No commercial IP generated

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Appendices

1. MC15011 Project Details
2. Prevalence and significance of abnormal vertical growth of macadamia in Australia
3. Exploring the factors and biotic agents associated with abnormal vertical growth of macadamia.
4. Abnormal vertical growth financial planner profiles
5. Report of workshop on abnormal vertical growth in macadamia held on February 16, 2017 at Bundaberg Enterprise Centre, Bundaberg.

Appendix 1

Project Details

Project Code	MC15011
Project Title	Determining the extent and causes of abnormal vertical growth in macadamia
Project Type	R&D
Start Date	February 2016
End Date	February 2017
Service Provider	The University of Queensland
Industry	Australian Macadamia Industry
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Date of Report	28 February 2017

Horticulture Innovation Australia

AUSTRALIAN MACADAMIA SOCIETY

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Appendix 2
Prevalence and significance of abnormal vertical growth of macadamia in Australia

Summary

The number of trees affected with abnormal vertical growth (AVG) in the Australian macadamia industry has increased significantly. The proportion of affected trees in the industry has increased from 0.5% in 2003 to about 5% of the total number of mature macadamia trees currently in the industry. AVG causes the Australian macadamia industry an economic loss in excess of $10 million annually. Analysis of 75 varieties revealed significant differences exist among the varieties to AVG severity and there was no difference between seedling and grafted trees or rootstocks to AVG susceptibility. Varietal susceptibility of most of the Hawaiian varieties were classified as highly to moderately susceptible to AVG, whereas, most of the Australian varieties were tolerant to AVG.

1 Introduction

Symptoms of abnormal vertical growth (AVG) were first observed in 1970, on a farm in Baffle Creek in Queensland (QLD). The number of trees affected has spread to new areas and in the early 1990s, AVG was associated with major yield losses in several orchards in the Bundaberg production area. In 2003, the number of trees affected was estimated at between 18,000 and 22,000 trees (O'Farrell, 2004) and in 2007, the number of trees affected was estimated at 100,000 trees (O'Farrell et al., 2016). This constituted 0.5% and 1.7% of the total number of trees in commercial orchards in Australia in 2003 and 2007, respectively. AVG has been reported in commercial production areas of QLD and New South Wales (NSW), on a range of soils such as red ferrosols, yellow chromosol, kandosols and dermosols (O'Farrell et al., 2016). The soil types are characteristically well-drained with higher permeability and more rapidly draining at the surface and at 1 m depth (O'Farrell, 2011). Yield reduction of 25-75% has been reported in AVG trees compared with non-AVG trees of comparable age (O'Farrell, 2011).

Information on varietal susceptibility is limited. Most commercially grown Hawaiian selections are considered susceptible (Searle, 2016). All the cultivars are hybrids of *Macadamia integrifolia* and *M. tetraphylla*. Among the Hawaiian selections, cultivar 'HAES 344' and 'HAES 741' appear to be the most susceptible, followed by 'HAES 246' and 'HAES 508' (O'Farrell et al., 2016). Results of the field trials planted in 2008, to assess varietal susceptibility, and evaluate rootstock effects on AVG development in scion were inconclusive. No significant interactions was observed between rootstock and scion for any of the tree growth parameters (O'Farrell, 2011). Reciprocal grafting of AVG and non-AVG scions and rootstocks showed similar AVG symptoms. This suggests the mechanism of transmission is unlikely to be through rootstock-scion interaction. When new trees were replanted in soils where AVG trees had been removed, AVG incidence occurred in the young trees within five years. Therefore, AVG remains a serious constraint to macadamia production. In order to determine the extent and cause of AVG in the Australian macadamia industry, activities carried out in this study evaluated the occurrence, impact and risk of AVG in the Australian macadamia industry. The study updated information on varietal susceptibility to AVG.

2 Methodology

2.1 Survey of AVG in the Australian macadamia industry

In order to provide information on the occurrence of AVG in the Australian macadamia industry, a three-level hierarchical survey was performed (Table 1). An industry-wide survey (Level 1) of 32 macadamia consultants and growers in the five major macadamia producing regions including North, Central/Wide-bay, and Sunshine Coast in QLD and Northern Rivers and Mid-North Coast in NSW was performed. Participants were selected based on their long history of association with the Australian macadamia industry and regional roles with the growers. Information obtained on occurrence of AVG from the level 1 survey was used to select a subset of the participants for the regional survey (Level 2). Information on historical observations of AVG disorder, estimate of spread and impact in the regions were obtained from the regional survey. Eight growers were selected for the farm level survey (Level 3). The eight growers manage orchards with the highest number of AVG trees and long history of AVG, and have records of economic impact due to AVG in their orchards. Using a structured face-to-face interview process, data on production; direct and indirect economic costs; management practices; temporal spread, and varietal susceptibility were recorded.

Table 1: Detail and composition of survey levels of abnormal vertical growth in the Australian Macadamia Industry

SURVEY CATEGORY	LEVEL 1	LEVEL 2	LEVEL 3
Level	Industry-wide information	Regional survey	Farm level survey
Type	Prevalence/reconnaissance	Semi-intensive/detailed	Intensive/very detailed
Group	Growers and consultants	Growers and consultants	Growers
Statistics	32	18	8
Mode	Phone and email correspondence	Farm visit	Farm visit and face-to-face structured interview
Region	QLD (North, Central/Wide-Bay, and Sunshine Coast); NSW (Northern Rivers and Mid-North Coast)	Wide-Bay, Gympie/Bauple; Mountain Top, Canaiba; Hogarth Range	Bundaberg/Wide-Bay; Gympie/Bauple; Mountain Top/Jiggi

2.2 Survey of varietal susceptibility to AVG

A survey of tolerance or susceptibility of macadamia varieties to AVG was carried out at five field sites containing different varieties in 'AVG soils'. The sites were rootstock trial at Baffle Creek, AVG field transmission and Regional Variety Trial (RVT) 3 at Willa-Wirra, RVT2 at Hinkler Park Plantations; and variety/seedling trials at Winfield and West Red Hill. Effect of rootstock on AVG susceptible scion (Searle, 2016) was examined at Baffle Creek and Willa-Wirra. AVG severity level was recorded for each variety using a modified rating scale (Table 2). Due to variation in the number of trees assessed for each variety, the AVG severity rating scale was standardized based on weighted average (W_A). W_A was calculated as $[(N/(N-n))\ r)]/R$, where N is the total number of ratings of all varieties, n is number of ratings for the variety, r is the severity rating for the variety and R is the mean of overall ratings.

Table 2: Severity rating system used to describe phenotypic appearance of AVG.

SCORE	***DESCRIPTION***
0	No AVG - no visible vertical growth symptoms on any branch.
1	Suspicious AVG - vertical growth symptoms on 1 -3 inner branches, 'late onset'.
2	Mild AVG - distinct vertical growth appearance on most branches.
3	Severe AVG - vertical growth on lower branches and distinct crown symptoms.

Modified from O'Farrell (2011) classification system.

2.3 Economic impact of AVG

Estimates the economic impact of AVG were produced using the revenue from sale of nut-in-shell (NIS), cash flow, net present value (NPV) and internal rate of return (IRR) analyses. Economic impact of AVG was examined under orchard scenarios with different proportion of trees affected with AVG. The same assumptions based on 50-hectare irrigated orchard with average annual yield of 3.4 tonnes per hectare of NIS in unaffected orchard and 1.5 tonnes per hectare in AVG orchard were used in the calculations. Three cases of revenue at $3.00, $4.00 and $5.50 NIS per kg at 10% moisture content were used to develop the financial profiles of the 50-hectare orchard AVG scenarios using the *Macadamia Financial Planner Software.*

3 Results and Discussion

3.1 Macadamia production hectares with AVG

The three-level survey process used in this project has provided a more robust information on the prevalence of AVG in the Australian macadamia industry. The total production area with AVG trees in the Australian macadamia industry is over 900 hectares. At present, approximately 230,000 trees in QLD and NSW, covering over 700 hectares have AVG (Fig. 1). A survey in 2003 estimated the number of affected trees at between 18,000 to 22,000 trees (O'Farrell, 2011) and by 2007 the estimated number of trees had increased to 100,000 trees (O'Farrell et al., 2016). Therefore, the proportion of affected trees in the Australian macadamia industry has increased from 0.5% in 2003 and 1.7% in 2007 to nearly 5% of the total number of trees in commercial orchards.

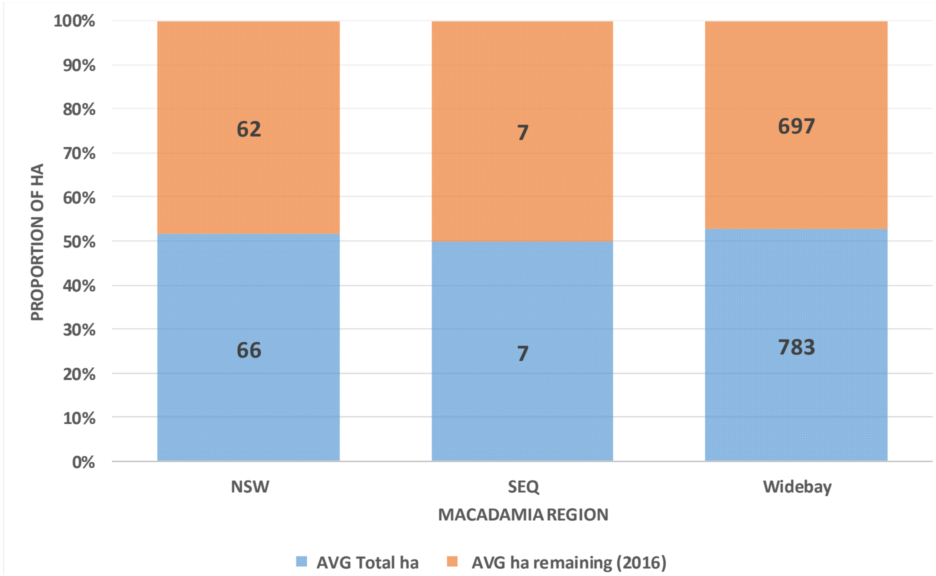

Fig. 1: Macadamia production hectares with AVG trees in New South Wales (NSW) and Southeast (SEQ) and Wide Bay districts in Queensland.

3.2 Macadamia producing areas with AVG

AVG was first observed on a farm in Baffle Creek in QLD and later in several orchards in the Bundaberg production area and in NSW (O'Farrell, 2004). In addition to orchards that have previously been reported with AVG, we observed for the first time, AVG trees in a new orchard in QLD and a farm in the Sunshine Coast production region. This shows that AVG is now prevalent in nearly all the major macadamia producing regions in Australia including Atherton Tablelands, Bundaberg-Wide Bay, Maryborough/Tiaro, Gympie/Bauple, and Sunshine Coast regions in QLD. In NSW, AVG trees exist in the Mountain Top/Jiggi, Canaiba, and Hogarth Range districts. Although over 30% of AVG trees have been removed in the affected farms, AVG trees currently constitute 15% - 50% of the total number of trees in the affected orchards. Plans to remove many affected trees are currently underway, but a major concern is the risk of temporal spread of infection and increase in the number of trees affected after replanting. Temporal spread of AVG in affected orchard is common and the distribution of AVG trees is in an aggregated pattern. This confirms previous report of the spatial pattern of AVG (O'Farrell, 2011).

3.3 Classification of varieties and effect of rootstock in AVG sites

Rootstock has no influence on AVG occurrence. In most cases, regardless of the rootstock (tolerant or susceptible), AVG symptoms occur if the scion is a susceptible variety such as 'HAES 344'. Similarly, no difference was found between seedling and grafted trees. Using the AVG severity rating scale, a range of macadamia varieties showed significant differences in AVG severity (Fig. 2). 'HAES 741' and 'HAES 344' cultivars were the most severely affected (highly susceptible) (Fig. 2). Based on the results of the severity ratings, growers' report (from survey) and observations in various AVG orchards, 75 macadamia varieties were classified into three AVG varietal susceptibility groups (Table 3). The results are comparable with previous reports of susceptibility of 14 varieties to AVG by Searle (2016). The varietal susceptibility groups of the 75 varieties are shown in figure 3.

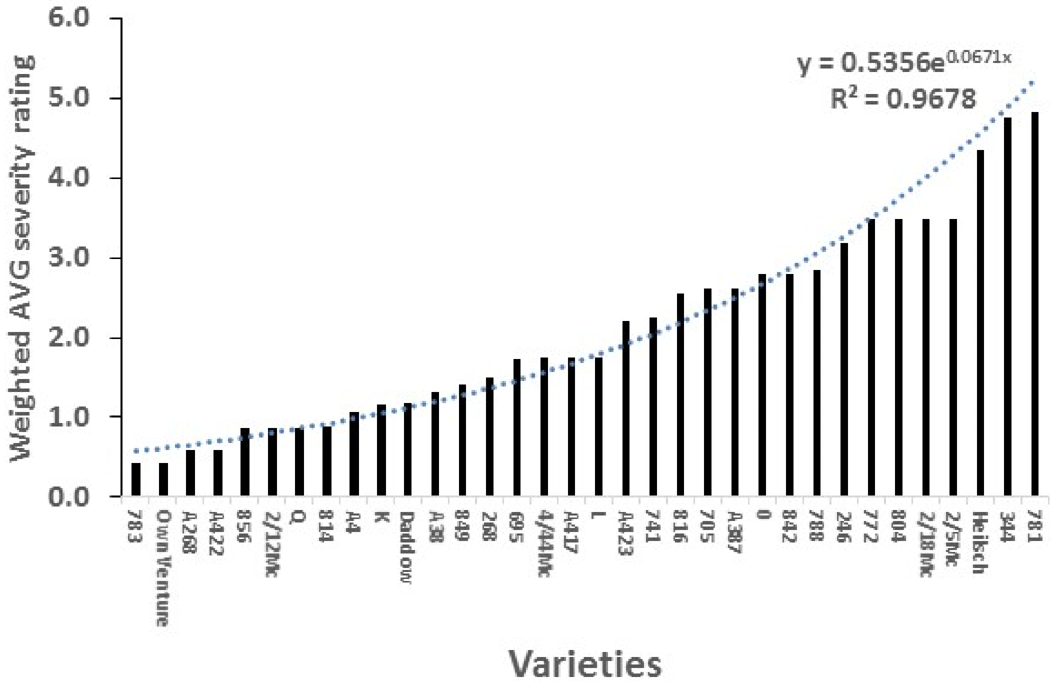

Fig. 2: Severity of macadamia varieties to abnormal vertical growth from weighted average of ratings in commercial orchards.

Table 3: Classification of macadamia varieties for susceptibility to AVG.

AVG VARIETAL CLASSIFICATION	ESTIMATED YIELD REDUCTION (%)	SEVERITY (SYMPTOMATIC RATING SCALE 0-3)	NUMBER OF VARIETIES*	EXAMPLES
TOLERANT	<30	<1.0	33	A16; Own Venture, NG8
MODERATELY SUSCEPTIBLE	30 - 50	1.0 -2.0	17	Beaumont; Daddow; A4;
HIGHLY SUSCEPTIBLE	>50	>2.0	19	HAES 344; HAES 741; HAES 816

**includes not commonly planted in commercial orchards and breeding lines.*

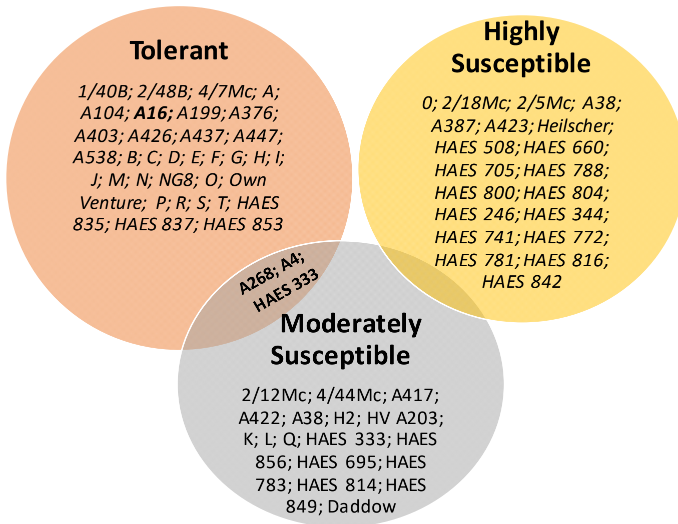

Fig. 3: Classification of macadamia varieties based on reactions (symptoms and yield reduction) to abnormal vertical growth.

3.4 Possible source of differences in varietal susceptibility to AVG

Generally, most of the susceptible varieties are the Hawaiian selections. In comparison with the varieties of Australian selection such as 'NG8', 'A16' and 'Own Venture' the Hawaiian varieties such as HAES 344', 'HAES 741', 'HAES 246' and 'HAES 660' are highly susceptible (Fig. 3). A key difference between these cultivars is the component of *Macadamia integrifolia* genome in their genetic makeup and pedigree (Peace et al., 2002; O'Farrell et al., 2016). For instance, cultivar 'A38' is an Australian selection and highly susceptible to AVG, its pedigree is 'HAES 344' (Hardner, 2016). The tolerant cultivar 'A16' is an Australian selection, along with 'A4'; 'Renown' and 'Norm Gerber series such as 'NG8', these varieties belong to the genetic 'genepool 6' with 45-85% *M. integrifolia* component in their genome (Peace et al., 2002). Therefore, it is most likely that varieties with high proportion of *M. tetraphylla* genome are more tolerant to AVG than varieties that contains high proportion of *M. integrifolia* genome. This offers a great opportunity for selection and breeding for AVG resistant germplasm.

3.5 Economic importance of AVG

Results from the survey clearly showed significant decline in yield in the affected trees. Similar rate of yield reduction was observed in most orchards with AVG trees (Fig. 4). Yield reduced from estimated farm average of 3.4 tonnes NIS per ha to about 1.0 - 2.7 tonnes NIS per ha. Using a 50-tonne NIS production orchard, at three different NIS price scenarios, the revenue per year obtained in AVG was significantly lower in AVG farm than non-AVG farm (Fig. 5). Loss in NIS production due to AVG, to the Australian macadamia industry, is estimated at over 2,000 tonnes NIS per annum. At $5.00 /kg NIS, this equates to over $10 million loss in production annually.

At least one additional full-time staff is required annually to manage 100 ha farm with AVG. The staff is required for extra farm management activities due to AVG trees such as clearing broken branches and massive leaf litter from the orchard floor; cincturing and other management practices that are specific to AVG trees. Therefore, cost of production in AVG farms increased by $2,000 - $2,500 per ha annually over the standard average macadamia production cost of $6,000 per ha. This indicates that when NIS price is lower than $5.00/kg NIS, the cost of production on AVG ha is estimated at about $3,000 higher than the expected gross income. Using $3.00 per kg NIS, in non-AVG mature orchards, IRR is 12.79% and NPV at 5% rate is about $2.5 million, whereas, in AVG affected mature orchard with 50% of the trees affected, IRR is 4.82% and NPV is -$63,430. These analyses revealed significantly reduction in income making macadamia production an unprofitable venture with AVG trees. If no curative treatment exists for AVG trees, the cost-benefit analysis revealed that it is better to remove and replant AVG trees. Although trunk cincturing increased flowering and nut yield of trees with mild to moderate branch symptoms of AVG (O'Farrell, 2011), this annual practice costs $0.40 -$0.50 per tree, and did not remediate vegetative symptoms. Thus, a more suitable option is required.

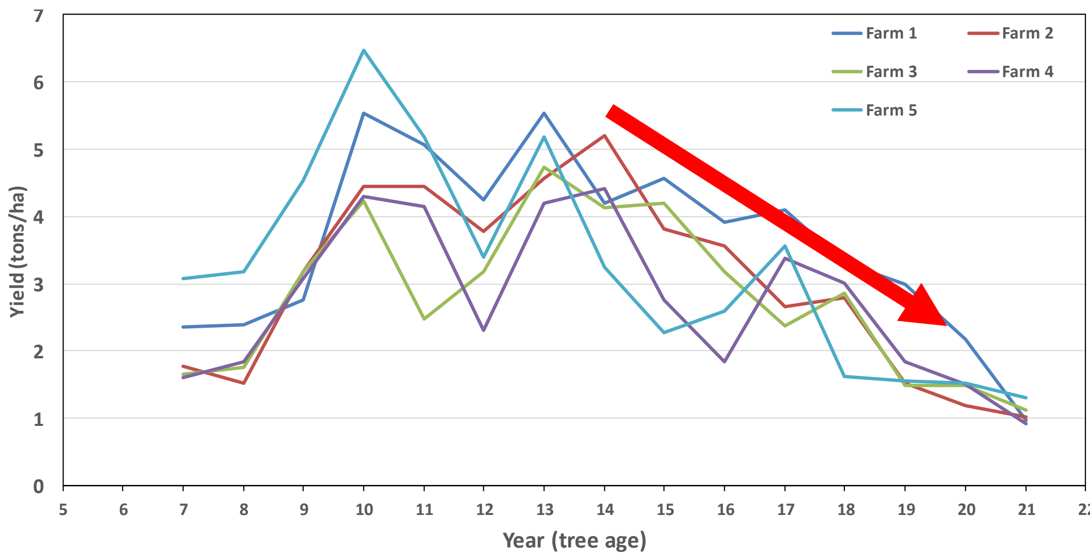

Fig. 4: Progression in yield reduction in macadamia orchards (farms) with abnormal vertical growth.

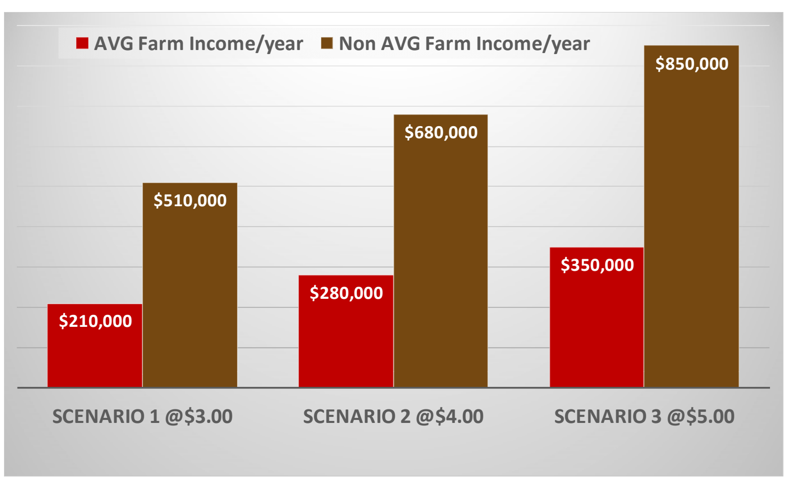

Fig. 5: Estimated revenue from AVG and non-AVG farms of expected 50-ton output at three price scenarios of NIS/kg.

4 Concluding Remarks

AVG incidence is increasing and a major constraint to production and new investment in macadamia industry. The rapid increase in the number of AVG trees from 0.5% to 1.5% and currently to over 5% of the total number of commercial macadamia trees in Australia, suggests high potential risk to the industry, if the spread is not controlled. AVG causes significant economic losses to both individual grower and the Australian macadamia industry. Up to 50% of the current non-AVG sites is at risk (O'Farrell et al., 2016), which creates a serious concern and potential detrimental impact to the industry. The varieties that displayed tolerance in conditions known to be conducive for AVG, offer the possibility of a breeding solution to AVG. These varieties could form the basis for further research to identify genetic markers associated with AVG resistance. However, the development of resistant varieties to AVG should be incorporated into the breeding program. Selection of AVG tolerant cultivars would reduce the risk of AVG occurring after replanting.

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Appendix 3
Exploring the factors and biotic agents associated with abnormal vertical growth of macadamia

Summary

Preliminary searches of the macadamia genome for integrated geminivirus sequences revealed some viral particles occur in the macadamia genome. Over 250 samples (leaf, soil and wood tissues) were collected from AVG symptomatic macadamia trees at three severity levels and asymptomatic trees. PCR assays with specific primers used to detect geminivirus, *Bacillus megaterium* and fungi in 138 leaf samples revealed putative interactions exist between the biotic agents in the samples. Results indicate that the presence of geminivirus correlates with increased severity to AVG. However, molecular assays including next generation sequencing used to confirm the identity of the pathogen revealed no evidence of geminivirus infections. No significant differences in the percentage of *B. megaterium* or fungal species was observed between AVG and non-symptomatic samples. *Bacillus* sp. was isolated from surface-sterilised vascular tissues of the leaf samples and a new set of more robust and efficient PCR primers were designed for the detection of geminivirus in this study.

1 Introduction

The cause of abnormal vertical growth (AVG) has not been resolved. Preliminary studies using universal phytoplasma PCR primers did not associate any phytoplasmas with AVG (O'Farrell, 2011). Similarly, soil nematode population did not show nematodes as the cause of AVG (O'Farrell, 2011). Anecdotal observations concluded insects are not likely a cause of AVG. More recently, molecular study using next generation sequencing detected a bacterium, *Bacillus megaterium,* and a bipartite geminivirus in three leaf samples from AVG trees (Webb and Geering, 2015). Although the study did not provide a clear contrast between non-AVG and AVG symptomatic samples, the discovery of biotic agents in the AVG samples raises the possibility of association of AVG with a biotic cause.

A combination or an interaction of the biotic agents is possible in the AVG syndrome. For instance, plant diseases caused by insect-transmitted viruses such as the geminiviruses transmitted by whiteflies (*Bemisia tabaci*) are the most economically important diseases of tropical and subtropical crops. The temporal increase in the number of affected trees along and across rows, coupled with the aggregated distribution pattern of AVG trees, could indicate an interaction between the biotic causal process and abiotic factors such as soil moisture stress. Clarity on biotic association with AVG will provide vital information and underpin a targeted research. Therefore, this study used molecular techniques to identify putative pathogens in AVG symptomatic samples and expand the range of macadamia samples tested for the presence of *B. megaterium* and geminivirus associated with AVG in macadamia. An expected outcome is that recommendations on how to diagnose and resolve the cause of AVG is provided.

2 Methodology

2.1 Exploring Biotic Causes

2.1.1 Samples and DNA extractions

Over 250 samples including leaf, soil and wood tissues were obtained from AVG and non-AVG trees from 11 macadamia production regions (QLD - Atherton Tablelands, Baffle Creek, Bauple, Bundaberg, Tiaro, Winfield, and Wolvi; NSW - Alstonville, Hogarth Range, Jiggi, and Mountain Top). Genomic DNA was extracted from 138 leaf samples using the CTAB extraction protocol or the QIAGEN BioSprint 96 DNA Plant Kit following the manufacturers' protocols.

2.1.2 PCR assays for detection of pathogens

2.1.2.1 Detection and identification of geminivirus

In order to address the hypothesis that endogenous viruses are the cause of AVG, macadamia genome (Nock et al., 2014) was interrogated. This approach was used to confirm if geminivirus sequences are integrated in the macadamia genome. AVG and non-symptomatic samples were screened using PCR primers that amplified whole DNA component. PCR primers used to amplify target sequences from the total nucleic acid extract are detailed in Table 1. PCR for geminivirus was performed in 20 µl volume containing 50 ng of template and 10 mm of Mac_Gem F2 and Mac_Gem R2, using high fidelity Phusion Taq DNA polymerase. Next generation sequencing was carried out to analyse TempliPhi amplification products, with preferential amplification of circular over linear DNA, using a universal diagnostic assay for geminiviruses.

Table 1: PCR primer sequences used in this study for detection of target organisms.

Primer Name	Primer Sequence (5' to 3')	Target Organism	Source
Mac_Gem F2	GATTTAAAGAACCACAATGAT	Geminivirus	This study
Mac_Gem R2	CTTAATGCATCATTTACTGAAC	Geminivirus	This study
BmGYRB-F1	TTGAAGGTAATCGAGACAACAT	*Baccillus megaterium*	Webb and Geering (2015)
BmGYRB-R1	CCACAATAGGGTTCTCTAGCA	*Baccillus megaterium*	Webb and Geering (2015)
ProMatKF1	GTAATTTACGATCAATTCATTCA	*Macadamia*	This study
PromatKR1	AATGAGAAGATTGTTTACGGA	*Macadamia*	This study

2.1.2.2 Detection and identification of bacterium

In order to isolate the endogenous bacteria from the samples, freshly collected leaves and stems, from AVG symptomatic and non-symptomatic trees, were surface-sterilized in 2% sodium hypochlorite solution for 5 min. Thereafter, the tissues were washed in three changes of sterile water, followed by 60 s in 70% ethanol before they were rinsed thoroughly with sterile water and blot dried with sterile Whatman paper. The leaf and stem samples were plated onto nutrient agar Petri plates and incubated for 3 days at 30^{o}C. The colonies of the bacteria obtained were then subjected to gram reaction tests. PCR amplifications were done on both bacterial colonies and plant nucleic acid extracts using the primers BmGYRB-F1 and BmGYRB-R1 (Table 1). ProMatKF1 and PromatKR1 primers (Table 1) were used as endogenous primers.

2.1.2.3 Detection and identification of fungi

In order to detect any endogenous fungi in the leaf samples as a cause of AVG, PCR amplifications were performed using DNA barcoding primers of the elongation factor locus (Carbone and Kohn, 1999). These primers are also useful for *Fusarium* diagnosis.

2.2 Association of AVG disorder with environmental factors

Multivariate procedure in GenStat statistical software was used to explore possible association of environmental factors: elevation (masl); historical annual rainfall (mm); mean minimum and maximum temperatures (°C); and relative humidity (%) with AVG incidence.

3 Results and Discussion

3.1 Detection and identification of biotic agents in AVG

Searches of the macadamia genome revealed geminivirus DNA sequences is integrated in the macadamia genome. Using the Mac_Gem F2 and Mac_Gem R2 primers, geminivirus was detected in about 56% of the leaf samples from trees with AVG, while 30% positive detection occurred in samples from non-AVG (asymptomatic) trees (Fig. 1). No significant differences in the percentage of detection of *B. megaterium* or fungal species was observed between AVG and non-AVG plants (Fig. 1). Generally, geminivirus was not detected in the non-symptomatic *Macadcamia tetraphylla*, *M. jansenii* and *M. ternifolia* samples. Studies on varietal susceptibility showed low or no AVG symptoms in varieties with a higher component of *M. tetraphylla* in their genetic makeup, as described by Peace *et al.* (2002; 2003). Fungi was detected in 76% of the AVG samples and 69% in the non-symptomatic samples (Fig. 1). Analysis of the percentage detection at the three levels of AVG severity showed an increase in the percentage of geminivirus detected in the AVG samples, compared with the asymptomatic (none) samples. Lower rate of detection for *B. megaterium* was observed at the three levels of AVG severity compared with other biotic agents (Fig. 2). This suggests a possible interaction in the endophytic composition of the AVG samples compared with the non-AVG samples.

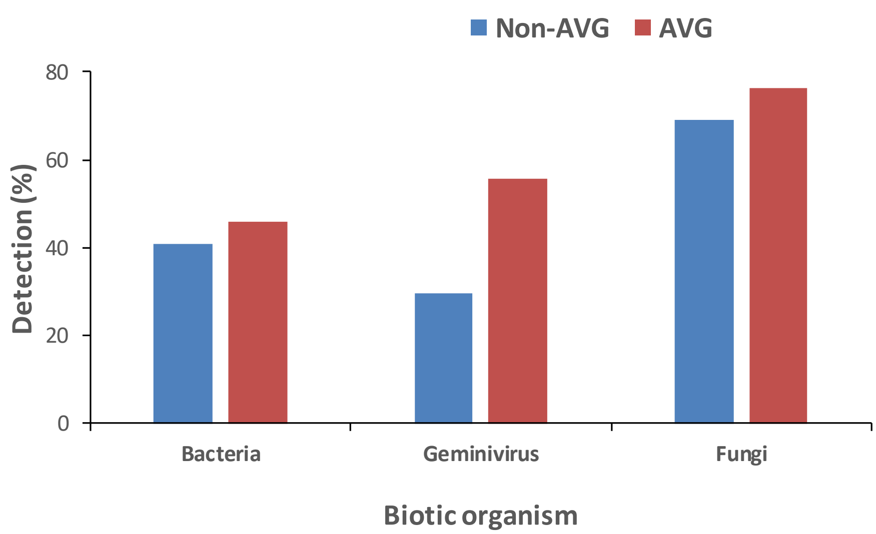

Fig. 1: Percentage of detection of geminivirus, Bacillus megaterium *and fungi from leaf samples obtained from AVG and non-AVG (asymptomatic) trees using PCR assays.*

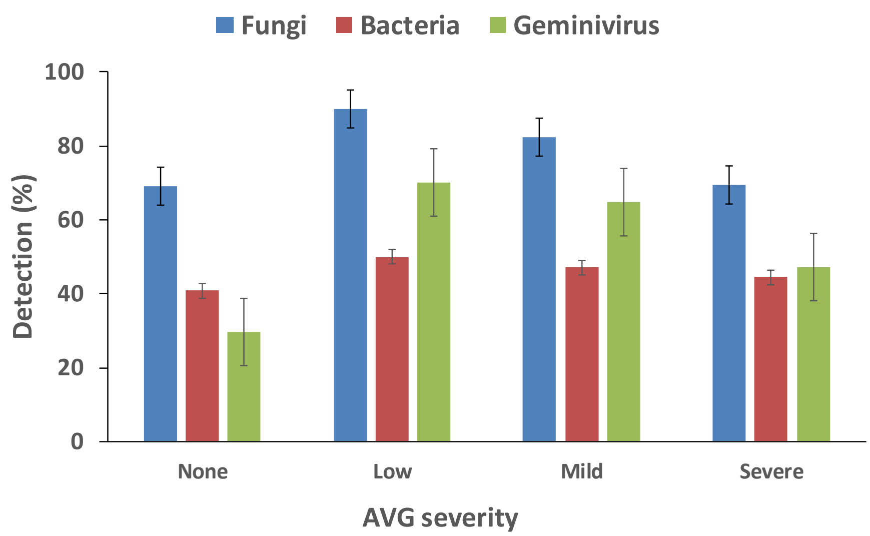

Fig. 2: Percentage of detection of geminivirus, Bacillus megaterium *and fungi from leaf samples obtained from AVG at three levels of severity and non-AVG (asymptomatic) trees using PCR assays.*

PCR products obtained using specific primers were directly sequenced and used to partially assemble about 2.8kb DNA-A component of the geminivirus (Fig. 3). Analysis of the DNA-A sequence revealed the presence of a replication origin that contains a nonanucleotide loop sequence (TAATATTAC), typical of geminivirus genomes. However, closer inspection of the coding sequence for the replication initiation protein (*Rep*), which is essential for viral replication, revealed the presence of numerous stop codons that would render the protein inactive. Subsequent analysis of the Sanger sequencing data revealed that eight premature stop codons were common to the *Rep* genes amplified from both AVG symptomatic and non-symptomatic leaf samples. Analysis of the traces did not reveal significant evidence of mixed bases at these codon sites. Molecular assays used to confirm if the geminivirus is activated and involved in the AVG infections did not detect conclusive replication-competent. This suggests that the geminivirus DNA is a mutated DNA and therefore, there is no strong evidence for replicative forms of the virus. In nature, phloem-feeding insects including various species of leafhoppers, a treehopper and whiteflies transmit geminiviruses. Geminiviruses are not transmitted through seeds, whereas many are graft-transmissible and some are mechanically (sap) transmissible. Plants infected with geminiviruses show a wide range of symptoms including stunting and distorted growth.

In addition to detecting plant viruses, DNA sequences obtained from the bacterial colonies were searched for the presence of cellular organisms that may play a role in the development of AVG. *B. megaterium* was the most abundant organism to be associated as an endophytic bacterium in macadamia tissues. Living cultures of the bacterium were isolated from the vascular system of selected leaf samples. *B. megaterium* is described as an endophytic organism known to influence plant growth and development by the production of phytohormones such as gibberellins, cytokinins and auxins (Ortíz-Castro et al., 2008). Based on the proportion of detection of endophytic organisms in the non-symptomatic samples compared with the AVG samples at different severity stages, it is plausible that the macadamia isolates of *B. megaterium* interacts synergistically with the other endophytic organisms to alter the physiological processes in the plant. Further studies are needed to characterize the fungi, bacterium and other viral elements observed in the next generation sequencing and determine their roles in AVG.

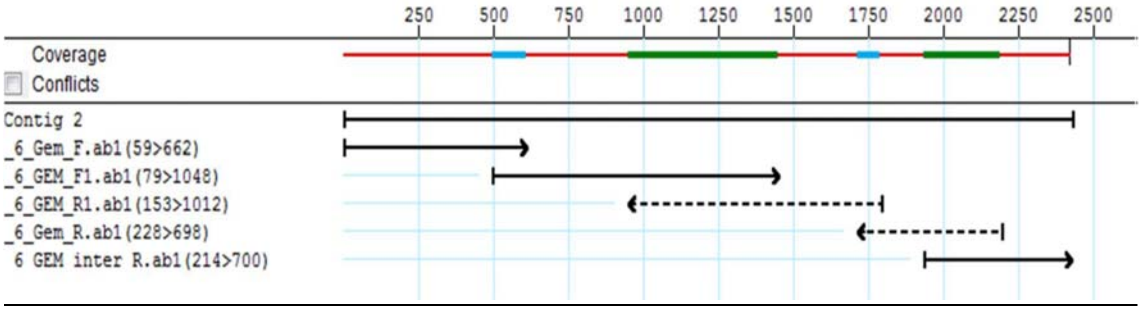

Fig. 3: Direction, position and size of the sequences based on PCR primers designed for geminivirus in abnormal vertical growth in macadamia.

3.2 Detection of biotic agents in macadamia varieties

Significant differences were observed in the percentage of PCR detection of the three biotic organisms in the macadamia varieties (Fig. 4). In the AVG susceptible varieties such as 'HAES 344', 'HAES 268' and 'HAES 741', disparity in the percentage of detection of the organisms was observed in the AVG samples compared with the non-symptomatic samples (Fig. 4). The reverse of the pattern was observed in the tolerant variety 'A4' (Fig. 4). Further studies are needed to examine the comparative composition of the endophytic organism and their roles in AVG.

3.3 Association of AVG disorder with environmental factors

AVG occurs at a range of elevations (14 masl - 320 masl). A common abiotic factor in the AVG farms is soils with high risk of soil moisture stress including the chocolate soils, basalt at Hogarth Range in NSW. These soils are brown soils with a friable clay surface horizon overlying a tighter clay subsoil. In contrast to the soils in the Bundaberg production region that are the deep red sands and red sandy clays soil types, these soils are not highly permeable and good drainage (Searle, 2016). Therefore, this indicates a strong association between soil moisture stress, not necessarily drainage, and AVG.

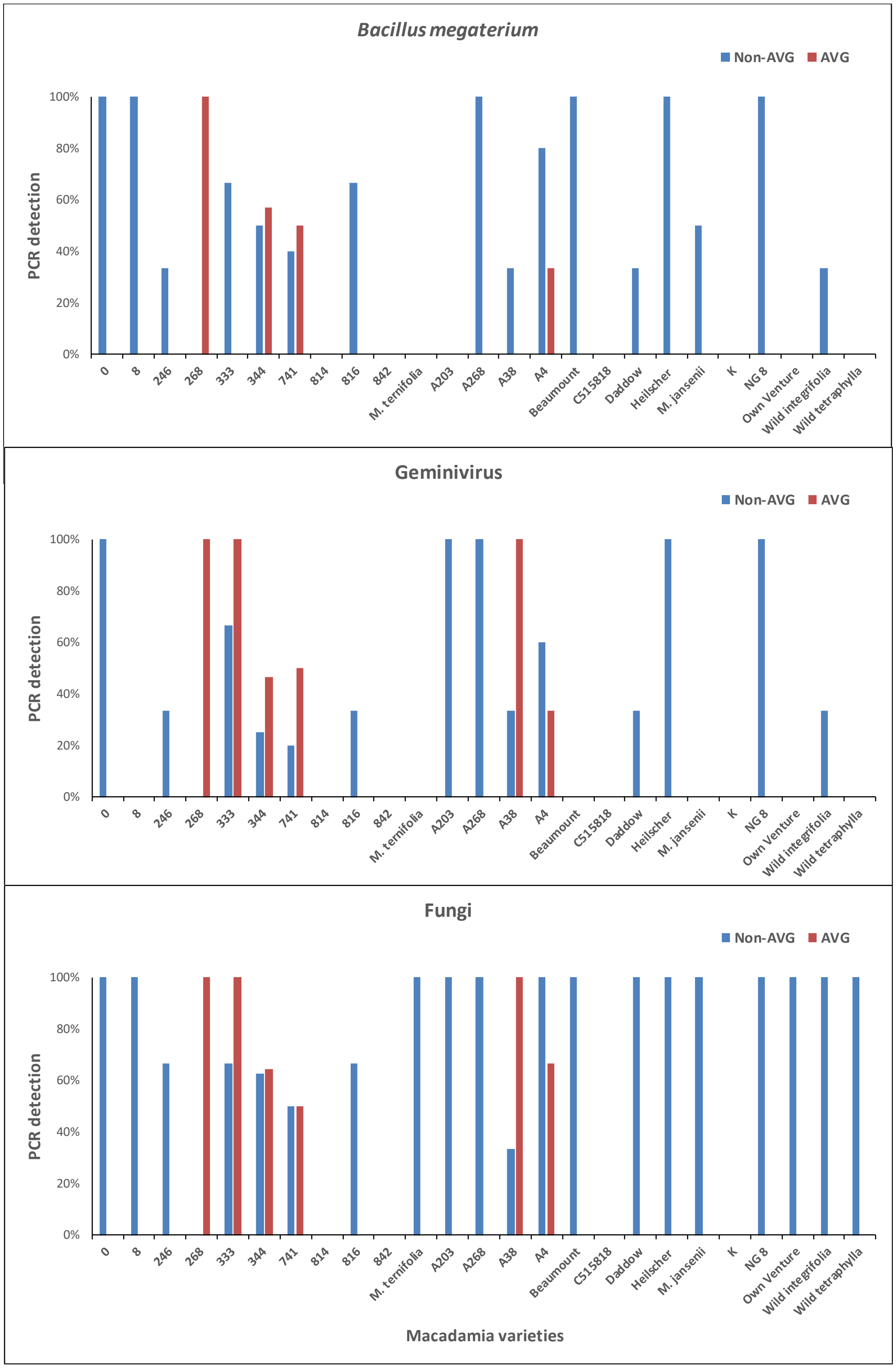

Fig. 4: Percentage of detection of three organisms in different macadamia varieties with and without AVG symptoms.

4 Concluding Remarks

The significance of the relationship between AVG and soil is not clear. The proposition that soil water stress might be involved in AVG is supported by the occurrence of AVG in diverse soil types with varying permeable and drainage characteristics. These soil attributes may influence soil health and could therefore alter the interactions between the external factors (soil water stress) and the endophytic biotic system.

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Appendix 4
Abnormal Vertical Growth (AVG) profiles

Developed by DAF Queensland and the University of Southern Queensland
Using the *Financial Planner for Macadamia* software.

Disclaimer

The information contained in Financial Planner for Macadamia software and this document is general in nature and has been prepared by the Department of Agriculture and Fisheries (DAF), the Queensland, University of Southern Queensland and other project stakeholders without taking into account the objectives, financial situation or needs of any particular investor, company or person.

All costs and returns projected by the software are based on the data entered into each profile. There may be differences between the assumptions included in program profiles and an investor's actual situation depending on their personal circumstances. The software also assumes that the rates of taxation and inflation associated with any given profile will apply over the whole life of the project. DAF Qld makes no representation about this assumption.

The costs and returns projected by the software and this document are indicative only. Any assumptions underlying the projections may have a significant impact on both costs and returns. Projections are by their very nature subject to the risks, contingencies and uncertainties associated with the industry. Accordingly, material differences may occur between projected and actual returns.

An investment in the macadamia industry is subject to the risks generally associated with horticultural operations. Before any investment is made in the macadamia industry, an investor should consider whether or not an investment in the industry is appropriate for their circumstances and if necessary seek professional advice.

Introduction

Profiles based on six different scenarios, each of which is modelled at three different average nut-in-shell prices were created in the *Financial Planner for Macadamia* software, to model a range of orchard scenarios. All of the scenarios are based on a 50-hectare irrigated macadamia orchard, which is close to the average size for Queensland macadamia farms and the assumptions listed in this document. The analysis term for each of the profile is 20 years.

Table 1: Details of criteria used for each profile (orchard scenario).

Profile	Criteria
1. New orchard establishment	• New 50 hectares, irrigated • Average yield
2. Established orchard no AVG	• Established 50 hectares, irrigated • Average yield
3. Established orchard 100% AVG	• Established 50 hectares, irrigated • 100% of trees AVG affected • Low yield
4. Established orchard 50% AVG	• Established 50 hectares, irrigated • 50% of trees unaffected, average yield • 50% of trees AVG affected, low yield
5. Full replant of 100% AVG affected orchard	• Established 50 hectares, irrigated • Full replant in year 1, average yield
6. Replant 50% AVG affected orchard	• Established 50 hectares, irrigated • Replant of 50% of orchard year 1 (affected trees), average yield • Remaining 50% of orchard unaffected, average yield

Three average prices were used for each of these scenarios, including:

1. $3.00 per kilogram nut-in-shell @ 10% moisture content
2. $4.00 per kilogram nut-in-shell @ 10% moisture content
3. $5.50 per kilogram nut-in-shell @ 10% moisture content

The information in this document includes tonnes of nut-in-shell (NIS) and kernel produced over the 20 years of the analysis, as well as net present value (NPV) and internal rate of return (IRR). In order to generate these investment data, capital growth is included. Initial farm values are based on setup costs for a new orchard. There is a 5% growth rate per annum applied to the farm value over the analysis term.

All profiles share the same assumptions and data in relation to property values and growth. This means that for the purposes of the following analyses, the prevalence of AVG has no impact on the capital value of the farm. In reality it is quite possible that the presence of AVG-affected trees on an orchard may negatively impact farm value, but without data to support this it has been excluded.

The following profiles also include undiscounted cumulative net cash flow over the 20-year analysis period. These cumulative cash flows exclude investment costs and asset sales but include any periodic capital expenditure. Profiles that include any form of tree replanting typically include the costs associated with both tree removal and replanting as part of periodic capital expenditure in the first year.

Profile assumptions

PARAMETERS	ASSUMPTIONS & DESCRIPTIONS
Analysis term	20 years starting in 2017
Inflation rate	0% (inflation has been removed as a factor in the analyses)
Depreciation method	Straight-line. Machinery is depreciated over 5 years, shed, and equipment over 20 years.
Taxation model	Custom rate @ 0% (taxation has been removed as a factor in the analyses)
Required rate of return	5%
Irrigation status	All the trees are irrigated
Orchard area	50 hectares (similar to average Queensland farm size)
Land area	Total land area is 53 hectares.
Land value	Initial value of land is based on $20,000/ha ($1,060,000 for the 53 hectares)
Tree age	All the trees in the orchard are the same age. The trees in the mature AVG and non AVG affected orchards are 20 years old.
Tree numbers	There are 15650 trees on the 50 hectares, planted at approximately 8m x 4m (313 trees/ha)
Tree value	Trees are valued at approximately $15/tree at planting ($234,000 for 50 hectares). Mature non AVG affected trees are valued at $100/tree. Mature AVG affected trees are valued at $50/tree.
Irrigation	Irrigation system cost is valued approximately $10/tree ($155,000 for the 50 hectares). Some savings are assumed when re-installing irrigation during tree replanting. Total costs are estimated at $100,000 for 50 hectares.
Land preparation	Land preparation costs to plant new trees are $1300/ha ($65,000 for the 50 hectares)
Tree removal	Tree removal and disposal for replanting costs approximately $20/tree ($312,000 for the 50 hectares)
Machinery capital costs	Machinery costs are $180,000 for the 50 hectares
Shed and equipment capital costs	Shed and equipment capital costs are $144,000 for the 50 hectares
Yield and quality	Expected mature yield is **3.4** tonnes/hectare for the non AVG affected trees and **1.5** tonnes/hectare for the AVG affected trees. The default density-based yield curve for 313 trees/hectare from the *Financial Planner* is used to model yield. Saleable kernel recovery is **33%.** Reject kernel recovery is **2%.**
Nut prices	$3.00, $4.00 and $5.50 per kilogram of nut-in-shell @10% are modelled. No price growth has been included in any of the profiles.
Costs	Annual costs for non-bearing trees is $2000/hectare. The percentage of mature costs applied in the first bearing year is 60%. Full costs are reached at 5 years after first bearing. Annual costs for non AVG affected mature trees is $6000/hectare. Annual costs for AVG affected mature trees is $8500/hectare (this includes the extra $2500/ha for managing AVG affected trees)
Capital purchases	There are no further capital purchases after the initial costs with lease costs included in annual costs.

Scenario 1: New orchard establishment

This scenario represents establishment of a new, 50-hectare irrigated orchard that is free of AVG. The three variants of this scenario model cash flows (Fig. 1) and rates of return using three different nut-in-shell prices ($3.00, $4.00 and $5.50). Table 2 below shows the projected total yield, initial and final values, rates of return and cumulative cash flow over a 20-year term.

Table 2: Projected total yield, initial and final values, rates of return and cumulative cash flow over a 20-year term in new orchard.

Establishment of a 50 hectare irrigated orchard unaffected by AVG				**Value**
Initial value of orchard (2017)				$1,838,000
Expected future value of orchard (2036)				$4,877,000
Total projected yield over 20 years (nut-in-shell)				1,929 tonnes
Total projected yield over 20 years (kernel)				630 tonnes
Profile	**Price/kg nut-in-shell**	**Net Present Value**	**Internal Rate of Return**	**Cumulative cash flow over 20 years#**
1	$3.00	$109,267	5.26%	$812,971
2	$4.00	$1,085,510	7.40%	$2,742,003
3	$5.50	$2,549,875	10.15%	$5,635,552

 # Cumulative cash flows are undiscounted and include periodic capital and exclude investment costs and asset sales

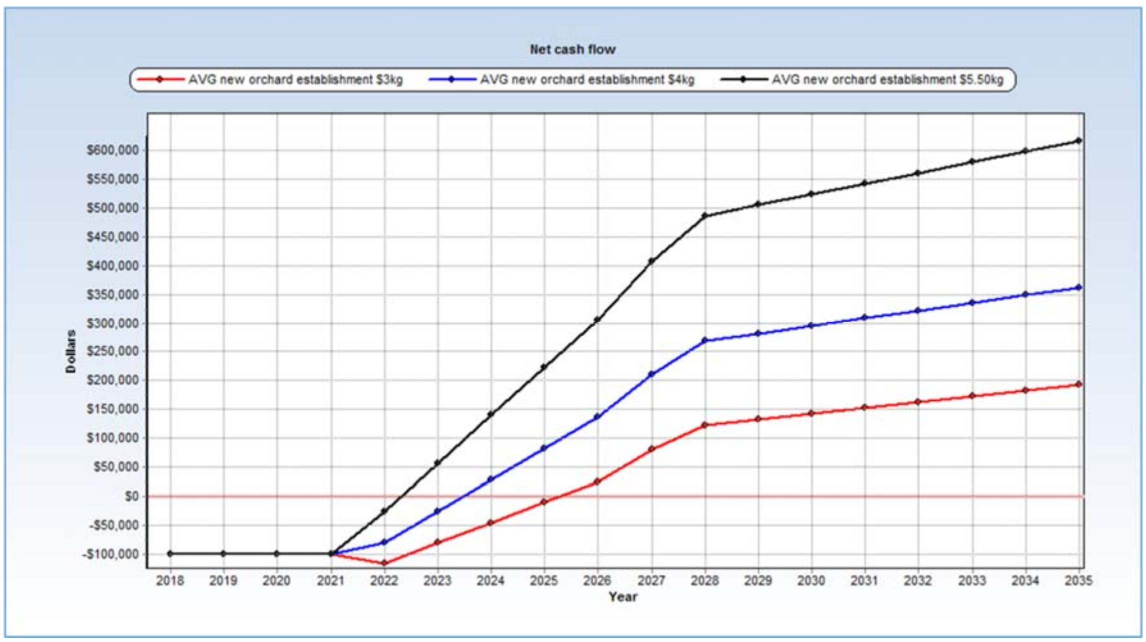

Fig. 1: Net cash flow in new orchard using three NIS price scenarios.

Scenario 2: Established orchard no AVG

This represents a 50-hectare irrigated orchard with tree 20 years old and no trees affected by AVG. The three variants of this scenario model cash flows and rates of return using three different nut-in-shell prices ($3.00, $4.00 and $5.50). Table 3 below shows the projected total yield, initial and final values, rates of return and cumulative cash flow over a 20-year term.

Table 3: Projected total yield, initial and final values, rates of return and cumulative cash flow over a 20-year term in established orchard with no AVG trees.

Existing 50 hectare irrigated orchard unaffected by AVG				Value
Initial value of orchard (2017)				$1,838,000
Expected future value of orchard (2036)				$4,877,000
Total projected yield over 20 years (nut-in-shell)				3,411 tonnes
Total projected yield over 20 years (kernel)				1,113 tonnes
Profile	**Price/kg nut-in-shell**	**Net Present Value at 5% RRR**	**Internal Rate of Return**	**Cumulative cash flow over 20 years#**
1	$3.00	$2,450,642	12.79%	$3,932,780
2	$4.00	$4,576,008	20.78%	$7,343,680
3	$5.50	$7,764,057	34.06%	$12,460,020

 # Cumulative cash flows are undiscounted and include periodic capital and exclude investment costs and asset sales

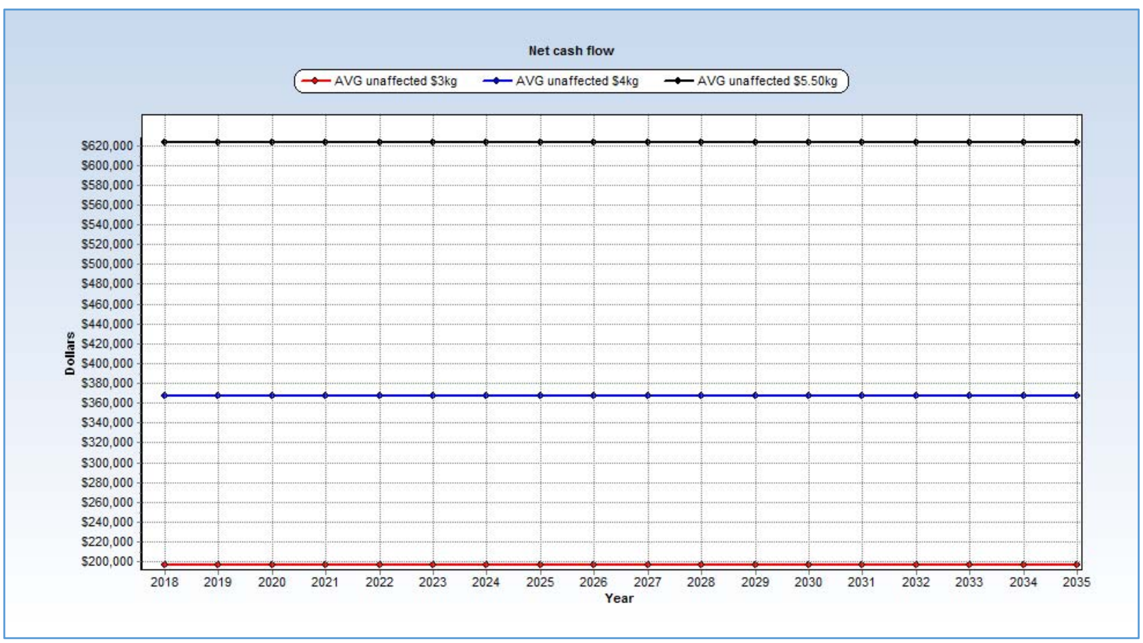

Fig. 2: Expected net cash flow in non-AVG affected orchard at three NIS price scenarios.

Scenario 3: Established orchard with 100% AVG affected trees

This represents a 50-hectare irrigated orchard with tree 20 years old and 100% of the trees affected by AVG. The three variants of this scenario model cash flows and rates of return using three different nut-in-shell prices ($3.00, $4.00 and $5.50). Table 4 below shows the projected total yield, initial and final values, rates of return and cumulative cash flow over a 20-year term.

Table 4: Projected total yield, initial and final values, rates of return and cumulative cash flow over a 20-year term in mature orchard with 100% AVG trees.

Existing 50 hectare irrigated orchard 100% affected by AVG				**Value**
Initial value of orchard (2017)				$1,838,000
Expected future value of orchard (2036)				$4,877,000
Total projected yield over 20 years (nut-in-shell)				1,505 tonnes
Total projected yield over 20 years (kernel)				491 tonnes
Profile	**Price/kg nut-in-shell**	**Net Present Value at 5% RRR**	**Internal Rate of Return**	**Cumulative cash flow over 20 years#**
1	$3.00	-$2,577,502	N/A	-$4,136,660
2	$4.00	-$1,639,841	0.7%	-$2,631,840
3	$5.50	-$233,349	4.34%	-$374,640

 # Cumulative cash flows are undiscounted and include periodic capital and exclude investment costs and asset sales

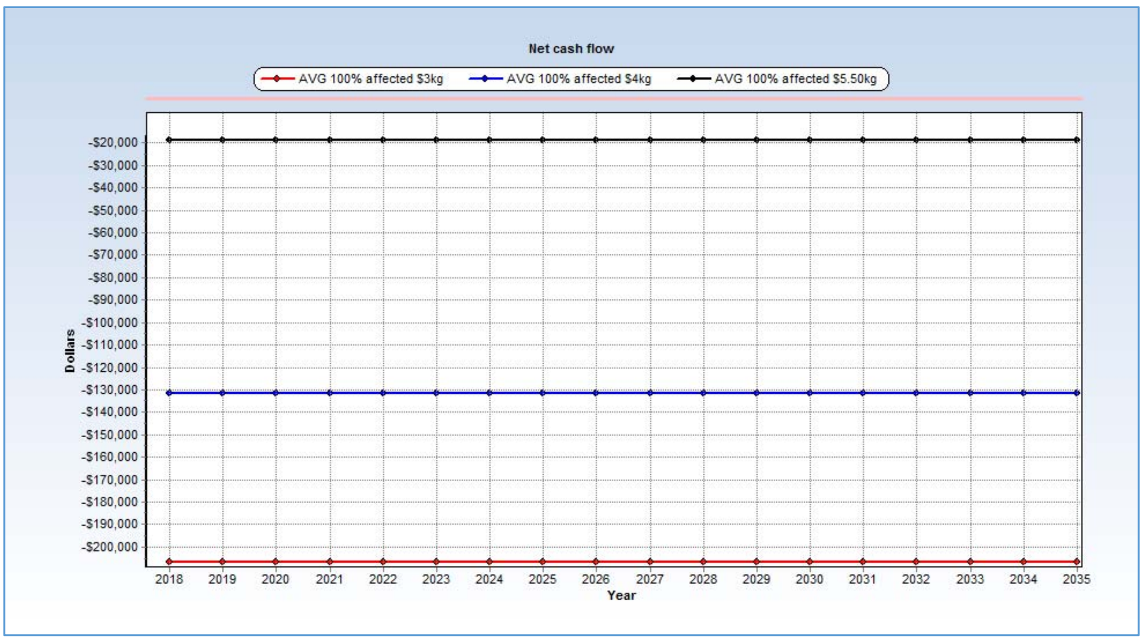

Fig. 3: Estimates of net cash flow (loss) in orchards with 100% AVG affected trees at three different NIS price scenarios.

Scenario 4: Established orchard with 50% AVG affected trees

This represents a 50-hectare irrigated orchard with tree 20 years old and 50% of the trees unaffected by AVG and 50% affected by AVG. There is no replanting. The three variants of this scenario model cash flows and rates of return using three different nut-in-shell prices ($3.00, $4.00 and $5.50). The table 5 below shows the projected total yield, initial and final values, rates of return and cumulative cash flow over a 20-year term.

Table 5: Projected total yield, initial and final values, rates of return and cumulative cash flow over a 20-year term in mature orchard with 50% trees affected with AVG.

Existing 50 hectare irrigated orchard 50% affected by AVG				**Value**
Initial value of orchard (2017)				$1,838,000
Expected future value of orchard (2036)				$4,877,000
Total projected yield over 20 years (nut-in-shell)				2,458 tonnes
Total projected yield over 20 years (kernel)				802 tonnes
Profile	**Price/kg nut-in-shell**	**Net Present Value at 5% RRR**	**Internal Rate of Return**	**Cumulative cash flow over 20 years#**
1	$3.00	-$63,430	4.82%	-$101,940
2	$4.00	$1,468,084	9.47%	$2,355,920
3	$5.50	$3,765,354	17.62%	$6,042,700

 # Cumulative cash flows are undiscounted and include periodic capital and exclude investment costs and asset sales

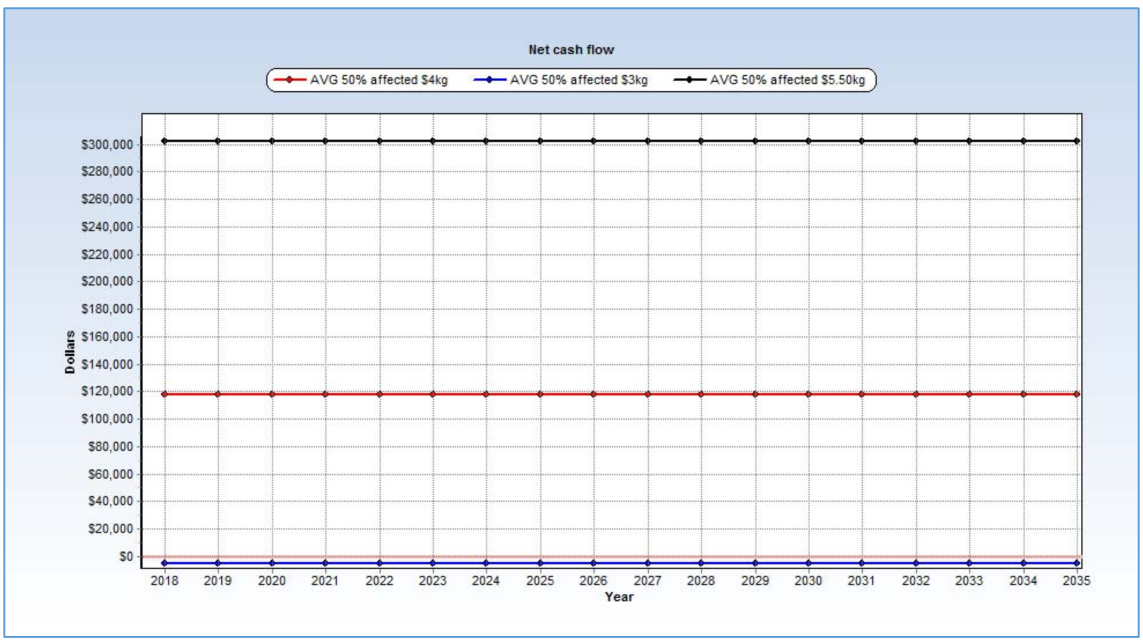

Fig. 4: Net cash flow in orchard with 50% AVG affected trees at three different NIS price scenarios.

Scenario 5: Full replant of 100% AVG affected orchard

This represents the replanting of a 50-hectare irrigated AVG affected orchard. New trees are unaffected by AVG and therefore produce average yield. Replanting occurs in year 1. The three variants of this scenario model cash flows and rates of return using three different nut-in-shell prices ($3.00, $4.00 and $5.50). The table 6 below shows the projected total yield, initial and final values, rates of return and cumulative cash flow over a 20-year term.

Table 6: Projected total yield, initial and final values, rates of return and cumulative cash flow over a 20-year term in mature orchard with 100% trees affected with AVG that is fully replanted.

Full replant of a 50 hectare irrigated orchard affected by AVG				**Value**
Initial value of orchard (2017)				$1,838,000
Expected future value of orchard (2036)				$4,877,000
Total projected yield over 20 years (nut-in-shell)				1,929 tonnes
Total projected yield over 20 years (kernel)				630 tonnes
Profile	**Price/kg nut-in-shell**	**Net Present Value at 5% RRR**	**Internal Rate of Return**	**Cumulative cash flow over 20 years#**
1	$3.00	-$568,828	3.82%	$100,971
2	$4.00	$407,415	5.79%	$2,030,003
3	$5.50	$1,871,779	8.33%	$4,923,552

 # Cumulative cash flows are undiscounted and include periodic capital and exclude investment costs and asset sales

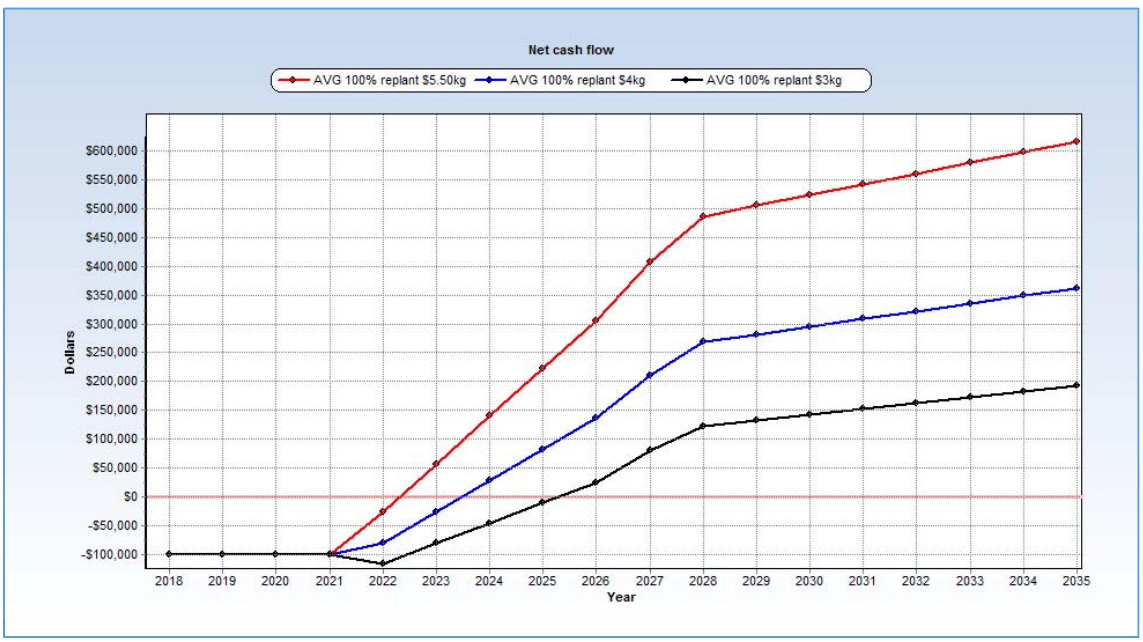

Fig. 5: Net cash flow in orchard with 100% AVG affected trees replanted at three different NIS price scenarios.

Scenario 6: Replant of affected trees on 50% AVG affected orchard

This represents the replanting of 50% of a 50 hectare irrigated AVG affected orchard (i.e. replanting of all AVG affected trees which amounts to half of all trees on the farm). New trees are unaffected by AVG and therefore produce average yield. Replanting occurs in year 1. The three variants of this scenario model cash flows and rates of return using three different nut-in-shell prices ($3.00, $4.00 and $5.50). The table 7 below shows the projected total yield, initial and final values, rates of return and cumulative cash flow over a 20-year term.

Table 7: Projected total yield, initial and final values, rates of return and cumulative cash flow over a 20-year term in mature orchard with 50% trees affected with AVG replanted.

Full replant of a 50 hectare irrigated orchard affected by AVG				**Value**
Initial value of orchard (2017)				$1,838,000
Expected future value of orchard (2036)				$4,877,000
Total projected yield over 20 years (nut-in-shell)				2,670 tonnes
Total projected yield over 20 years (kernel)				872 tonnes
Profile	**Price/kg nut-in-shell**	**Net Present Value at 5% RRR**	**Internal Rate of Return**	**Cumulative cash flow over 20 years#**
1	$3.00	$869,527	7.16%	$1,916,549
2	$4.00	$2,420,332	11.01%	$4,586,520
3	$5.50	$4,746,538	16.75%	$8,591,463

 # Cumulative cash flows are undiscounted and include periodic capital and exclude investment costs and asset sales

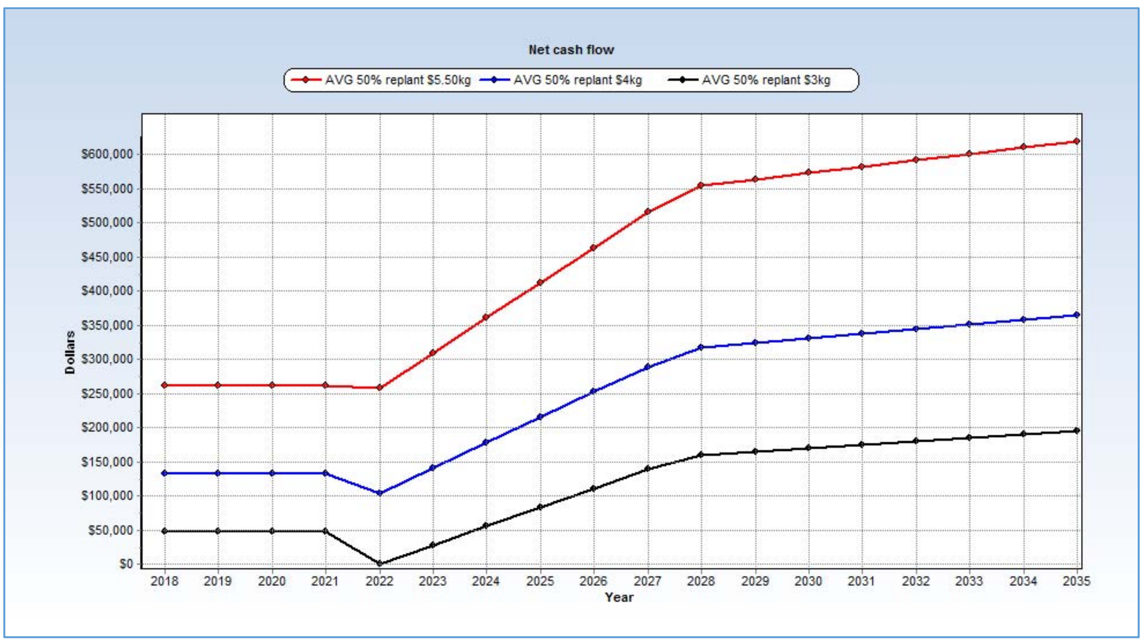

Fig. 6: Net cash flow in orchard with 50% AVG affected trees replanted at three different NIS price scenarios.

Acknowledgements

We gratefully acknowledge the contributions of Mr Shane Mulo and Mr Paul O'Hare of the Department of Agriculture and Forestry, Queensland and Associate Prof Geoff Slaughter (University of Southern Queensland) for developing these profiles using the *Financial Planner for Macadamia* software.

Appendix 5 – Report of Workshop on Abnormal Vertical Growth in Macadamia

Dr Femi Akinsanmi
The University of Queensland
Project Number: MC15011

Paul O'Hare (Workshop Facilitator)
Department of Agriculture & Forestry, Queensland

Bundaberg Enterprise Centre,
Bundaberg QLD 4670
16th February 2017

Executive Summary

Representatives of the various stakeholders including macadamia growers, researchers, industry consultants, Australian Macadamia Society and Hort Innovation participated in a strategic workshop on Abnormal Vertical Growth (AVG) disorder. The workshop was designed to scope the size of the AVG problem, review the research findings on AVG and provide recommendations for a strategy moving forward. AVG is now a significant disorder in macadamia that causes over 80% reduction in yield in affected cultivars. Additional costs of managing AVG trees each year is about $2,500 per ha. Since AVG symptoms were first observed in 1970, at a farm in Baffle Creek, Queensland and was reported to be responsible for major yield losses in macadamia in Bundaberg in the early 1990s, over 900 ha have been associated with AVG. Over 600 ha in Queensland and New South Wales have existing AVG trees. After much deliberations, based on clear measurable research hypotheses and selection criteria (urgency, technical difficulties, required time frame and industry importance), it was agreed that a new full research project for 3-5 years should be established. The new research project should further enumerate the cause of AVG, develop diagnostic tools for detection, transmission studies and monitoring spread. The project should have strong linkage with the breeding and cultivar improvement projects, develop genetic markers for selection of resistant varieties, explore differences among varieties, breeding lines and progeny to AVG and demonstrate alternative management options.

Table of Contents

AVG workshop report

A. Background

Abnormal vertical growth (AVG) is a disorder that affects macadamia trees. The effect on trees is characterised by abnormally upright branching pattern associated with poor flowering resulting in significant reduction in yield. Since AVG was first reported in commercial orchards in the mid-1990s, it has been found in macadamia growing regions in Queensland and New South Wales. Previous research (*MC03012 - Developing corrective treatments for maintaining macadamia nut production and normal growth - extended project*) was unable to provide conclusive evidence on the cause of AVG, but has provided numerous vital information on the prevalence, symptoms, susceptibility of certain macadamia cultivars and showed that the incidence of AVG is higher on soils that are highly permeable and free draining.

MC15011 project - '*Determining the extent and causes of abnormal vertical growth*' was established to determine the current impact of AVG and examine the potential cause of the disorder including the role of biotic agents in its development and spread. A key component of the MC15011 project is a research-grower workshop. This report serves as the minutes of meeting of the workshop held as detailed above. The objectives of the AVG workshop were to:

(i) Scope the size of the AVG problem;
(ii) Review the research findings on AVG; and
(iii) Develop recommendations for a strategy moving forward.

B. Workshop participants

Table 1 shows the list of all the invited participants, details of their involvement in AVG and representatives of the various stakeholders including:

1. Growers - The three growers that produce 30% of the total production in Australia with significant hectares of AVG trees;
2. Researchers - Scientists and participants of research projects on AVG;
3. Industry consultants - Representing those with long history of association with AVG management and research;
4. Industry representative - Productivity manager of the Australian Macadamia Society;
5. Macadamia breeder - Project leader of the macadamia breeding and improvement project; and
6. Representatives of Hort Innovation.

C. Program of event & presentations

The agenda is shown in Table 2. Below is the summary of each presentation and the ensuing discussions.

1. Welcome and preambles – Dr Femi Akinsanmi

- Introduced of the participants and recognised their expertise and contributions to the previous and current (MC15011) research projects.
- Stated that the workshop is the culmination of the MC15011 project's 12-months scoping study.
- Highlighted the desire of the industry to have conclusive answers to the following questions:
    - How serious is AVG & what is the risk to the industry?
    - Is the area with AVG increasing & how many hectares have AVG?
    - Which variety is tolerant & why?
    - What is the cause of AVG?
    - What is the likelihood of effective & sustainable AVG management?

2. Introduction (including purpose and structure of workshop) - Paul O'Hare

- Emphasised the three main objectives.
- Highlighted the structure and expectations of the workshop.

3. Impact on production – Dr Femi Akinsanmi

Presented summary of data collected from growers in QLD and NSW on impact of AVG:

- AVG is a significant risk to the Australian macadamia industry
- AVG causes severe seasonal fluctuation in yield (due to inconsistency in flowering).
- AVG trees yield ranges between **1 – 2.7 t/ha** (mostly approx. 1 t/ha) compared with 3.4 t/ha in non-AVG trees from similar orchards. Fig.1 shows similar progressive decline in yield in AVG trees from five farms.
- **30% - 88%** yield losses occur in the existing trees due to AVG.
- 100% loss in production for 3-5 years when trees are removed & replanted.
- Based on three nut-in-shell price scenarios, significant income losses occur in AVG trees, sometime gross income/ha was less than production cost/ha. Figure 2 is an example of income from a 50 ha farm with AVG trees compared with similar farm size without AVG.
- Additional management costs of **$2200 - $2500 per ha each year** are required to manage AVG trees.

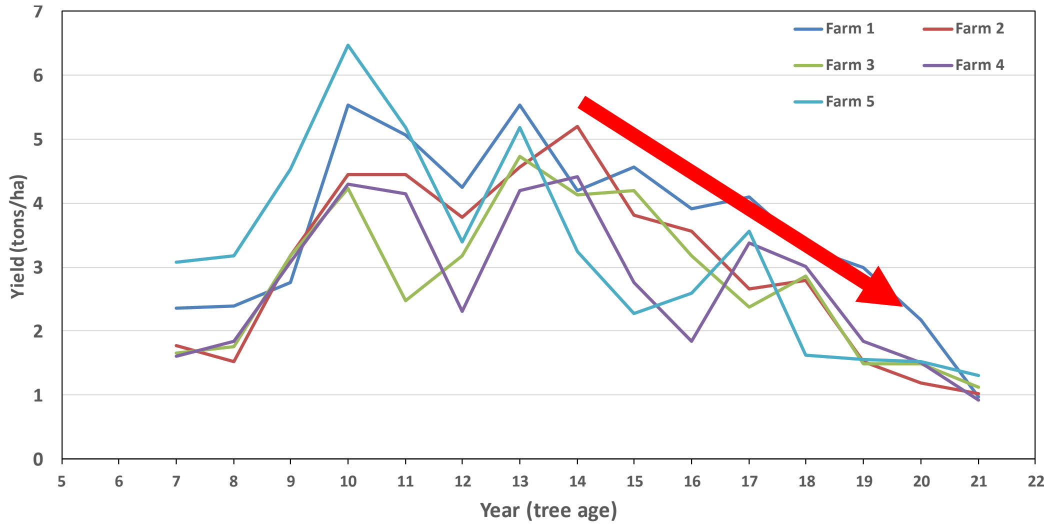

Fig. 1: Trends in production in trees with AVG disorder in five selected farms.

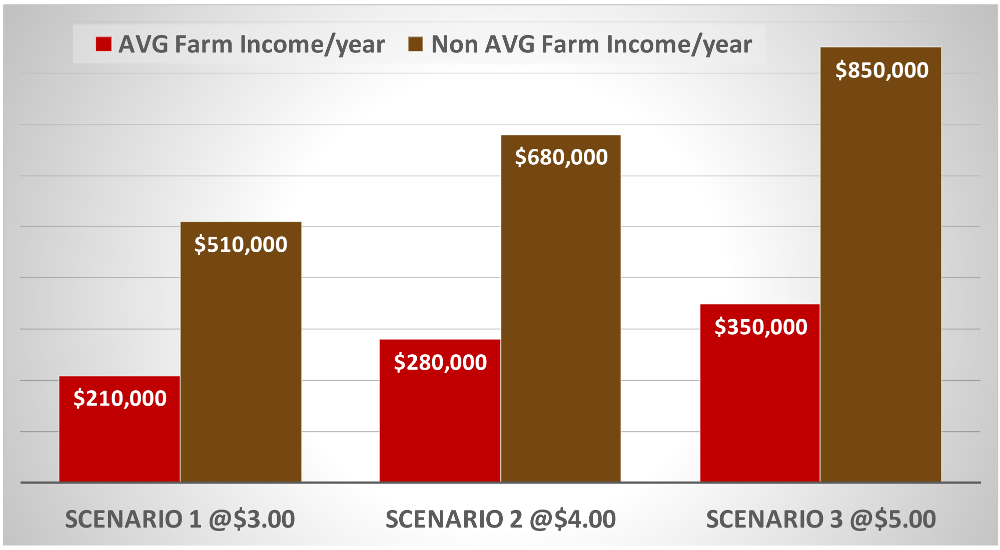

Fig. 2: Differences in income from a 50 ha farm with and without AVG trees using three different nut-in-shell (NIS) at $3.00; $4.00 and $5.00 dry NIS/kg scenarios.

4. *Overview of AVG historical concepts - Dr Chris Searle*

Outlined historical observations on AVG:

- AVG disorder was first noted in 1970s at CSRs Baffle Creek orchard, but became more prominent in mid to late 90s as Bundaberg orchards began to mature.
- A possible related disorder is vining on Atherton Tablelands.

- Anecdotal evidence suggested AVG is worse in upright varieties (344/741) in better-drained areas of orchard whereas vining is worse in the same varieties but only in wet areas.
- Momentum for AVG project started late 90s and a project was first commissioned in 2002 – led by Pat O'Farrell (DAF Q).
- Two facilitated workshops have been held on AVG, but information to date only showed strong association between varieties and soil and differences between cultivars.
- AVG can lead to 80% yield loss.
- Worse in apically dominant cultivars on freely draining soils.
- Worse in hotter/drier areas - west of Tolga/west of Lismore.
- Spread in orchard appears disease like, and worse in a replant situation.
- Cincturing, at the right time, can partially restore yield.
- Anecdotal reports of AVG in South Africa and Brazil. Vining is a problem in Hawaii.

5. Previous research on association of AVG with abiotic factors (MC03012) - Pat O'Farrell

Summarised the information obtained from the previous research trials on role of soil water in AVG disorder:

- Water stress symptoms described by Stephenson et al (1987, 2003) in glasshouse and lysimeter experiments have not been recognized as similar to those in AVG affected trees.
- AVG and non-AVG occur in the same irrigation block suggesting that, if water is involved, it is interacting with some other factor.
- Permeability relationship – low PAWC in soils of highly permeable?
- Climate association – elevated Etc in warmer, dryer climates?
- Temporal increase – intensification of effect as trees become larger (increased water requirement) and harvesting practices impact soil condition?
- Reduced fine root presence – unfavourable soil condition (hard-setting) in surface?

6. Report on survey of prevalence and variety– MC15011 - Dr Femi Akinsanmi

- AVG occurrence in some farms: 15% - 50% of total trees.
- At least 340 ha have history of AVG. *(It was agreed that the figure presented is too conservative. Growers agreed to update with the actual ha with AVG). Post workshop* **updated figure is over 900 ha.**
- Less than 30% of production area with AVG has been replanted since 2002.
- Current area with existing AVG trees is **over 620 ha (updated).**
- Distribution pattern of AVG on all farms is non-random (aggregated).
- Soils prone to severe moisture stress have high risk for AVG, not necessarily well-drained red soils, but all soils including heavy clayey soils, prone to severe moisture stress in the top 15 cm of soil around the fine root zone.
- Elevation, rainfall and ambient temperatures have no significant direct effect on AVG occurrence.
- Significant differences exist among varieties (new data for 52 more varieties).
- Rootstock has no effect on AVG disorder.
- It appears that non-Hawaiian cultivars are more tolerant, i.e. cultivars of Hawaiian origin (selection) and imported to Australia have higher AVG incidence (very susceptible) than varieties of Australian origin (developed/selected in Australia).
- Tolerant cultivars are considered to have about 30% reduction in yield compared with same cultivars with no AVG.

7. Report on Biotic factors as putative causative agents of AVG - Dr Andrew Geering

- Geminiviruses are a major group of pathogenic viruses in the tropics; circular, ssDNA genome.
- The presence of geminivirus DNA integrated into macadamia chromosomes correlates with increased susceptibility to AVG as shown in figure 3.
- Results of Geminivirus DNA sequenced suggest mutated DNA, not replication-competent and therefore, no strong evidence for replicative forms of virus. At this stage, there is no clear evidence of geminivirus infections in AVG.
- There is no significant differences in the presence of *Bacillus megaterium* or *Fusarium* spp. between AVG symptomatic and non-AVG symptomatic leaf samples.
- Future direction could include

- Plant breeding solutions.
- Test virulence of bacteria - virulence associated with mobile genetic elements.
- Broaden scope of investigation to include Phytoplasmas.
- Apply selective biocides such as antibiotics to test for amelioration of symptoms.
- Test for transmission of symptoms by grafting to macadamia seedling.
- Test for persistence of symptom in cutting-grown plants in pasteurized soils.

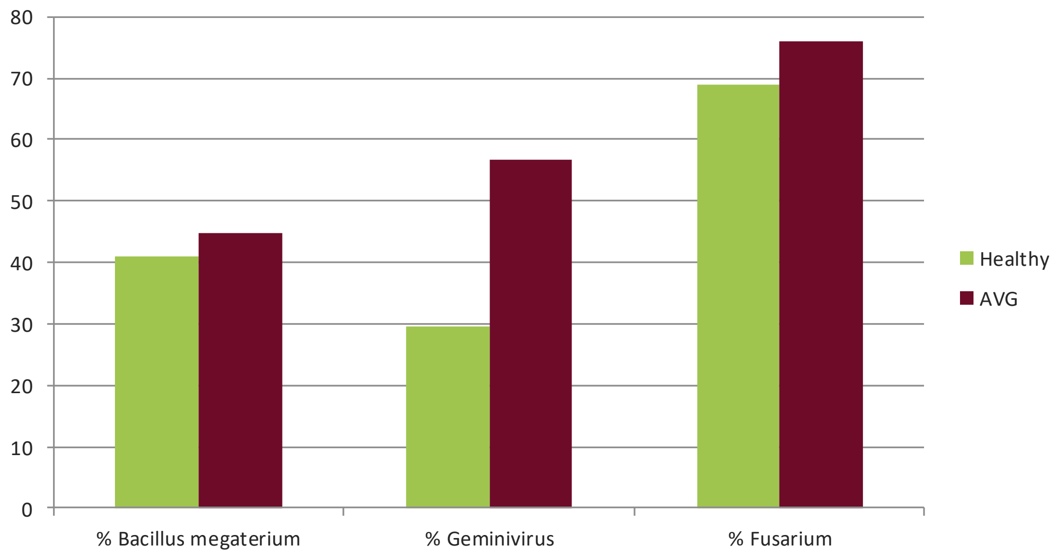

Fig. 3. Correlation between putative pathogens and AVG for all cultivars sampled including non-symptomatic ('healthy') samples.

8. *Risk of AVG occurrence & influence on growers' confidence for expansion – Dr Chris Searle*

- Plantings have expanded rapidly since 1990s in Bundaberg district. AVG occurs extensively throughout district.
- AVG is present at Maryborough/Tiaro. In the past AVG was only found west of highway at Gympie, it has now been found in the east region and at a site at Glasshouse Mountains.
- AVG is a problem for growers on Jiggi plateau in NNSW.
- Both remaining orchards on Atherton Tablelands have ongoing AVG problems.
- New areas with soil types similar to areas with known AVG are serious risk sites for macadamia production.
- Growers reported that AVG is a major consideration in the acquisition of new land and farms.
- Extrapolation of future areas for macadamia could be drawn from the University of New England ST15002 project ('*Multi-scale monitoring tools for managing Australian Tree Crops: Industry meets innovation*') based on avocado producing areas in Australia.

9. *Proposed future research plan & AVG management - Dr Femi Akinsanmi*

An integrated approach for future research project was proposed. This includes three inter-related research themes: sustainable management, pathogenic interactions and varietal assessment.

a. Sustainable management: Moisture stress improvement trials, to focus on on-farm activities for both existing AVG trees and new planting systems, designed to maintain good soil moisture conditions around the root zone. A second component of the management research is curative trials that is based on strategic and well-informed applications of 'pesticides' for AVG control.

b. Pathogenic interactions: Future research activities should finalise the biotic cause to a more conclusive end. This pathogenic interaction section could constitute a PhD research study. Research activities should include:
    a) Assays for rapid transmission studies to confirm the spread of AVG;
    b) Use of biocide to confirm pathogenic cause;
    c) Complete the identification process of any biotic agents and their roles in AVG; and

d) Examine the microbial composition and diversity in macadamia.

c. Varietal assessment: Future research should examine the presence of the biotic agents in selected varieties, breeding lines and germplasm. The project should explore the basis and the propensity of the Hawaiian cultivars to develop severe AVG, based on their origin and history of domestication. The project should have clear link to the breeding and regional varietal projects.

D. General discussion points during presentations

a. **Yield loss:** Generally, AVG produces less than 3kg/tree compared with 12kg/tree in non-AVG trees
b. **AVG ha:** The actual production area with AVG is far higher than reported – due to confidential nature of the issue.
c. **Significance of AVG**: The scale of the problem is under-estimated.
d. **Internal Rate of Return (IRR):** The calculation should be extended from the 20 years to 30 years of investment. It would show that the IRR of AVG is very low compared with replanted farms and farms with no-AVG.
e. **Soil and water:** Issues with soil and water relations were discussed. It was concluded that water is a factor and most likely a trigger, but not the cause of AVG. Issue of increased water (irrigation) and mulch as a management tool was discussed. Growers pointed out that it could not be the sole management tool because previous attempts have been unsuccessful to improve yield. A question was asked if soil temperature was measured as part of the soil water trials.
f. **Mulching:** A grower trial that used mulch was discussed. It was agreed that the trial was not properly set-up, monitored and was prematurely terminated by the grower.
g. **Cincturing:** Trunk cincturing as a management tool was discussed. Although the practice improved yield, only in trees with low severe AVG, annual practice is required to improve yield. This practice was found to be too difficult, expensive ($0.50/tree), detrimental to the tree and poses a work health and safety risk.
h. **Plant hormone:** Previous attempts at using plant growth regulator was discussed. Spray applications of uniconazole restricted vegetative growth. Further investigations should determine the plant hormones or processes that cause increased flowering after cincturing, to identify if the process can be induced through other mechanisms and as an alternative to cincturing.
i. **Varieties:** The range of tolerant and susceptible varieties was discussed. A clear definition/description of tolerant is that yield loss is minimal (less than 30%) compared to the susceptible varieties with over 50% yield losses. The importance of breeding for AVG tolerance, including using tolerant parents in crosses was discussed. Future research project should examine the breeding progenies/materials for the presence/absence of the biotic agents.

E. Agreement and recommendations on strategy moving forward

After much deliberations, based on clear measurable research hypotheses and selection criteria (urgency, technical difficulties, propensity to achieve results and priority), it was agreed that

1. The findings from MC15011 project are promising, therefore, the project should be expanded into a new full research project.
2. A new research project of 3-5 years should be established to further enumerate the cause, explore differences among varieties to AVG and demonstrate alternative management options.
3. Activities proposed under the pathogenic interactions research theme should constitute the new research project. These should include the following:
    1) Temporal spread and detection studies;
    2) Transmission studies and identification of associated biotic agents;
    3) Comparative examination of internal (endophytic) microbial composition and diversity in AVG and non-AVG trees and its association with origin (genepool) of cultivar selection.
    4) Screening of varieties and germplasm in breeding and RVT projects.
    5) Examination of what influence flowering response after cincturing.
    6) Application of biocides to confirm pathogenic interaction and as management tool.
    7) Develop molecular diagnostic tools including gene expression to examine infections, response and identify genetic markers for AVG resistance.

F. Other research ideas that should be considered and link to other projects

It was agreed that these research ideas should be a separate sub-program from the recommended new project (pathogenic interactions). The additional ideas include:

1. Testing of future macadamia progenies in RVT project on AVG sites.
2. Genome sequencing of AVG and non-AVG susceptible varieties and genetic inheritance of tolerance.
3. Modelling of AVG risk in future macadamia areas based on the mapping of avocado growing areas from the ST15002 project.

PS: The research ideas 1 & 2 in this section F are more suitable under MIVIC.

Table 1: Details of stakeholders and participants at the AVG workshop

a. Participants in attendance

	Name	Organisation	Contact	Rationale
1	Paul O'Hare	DAF QLD	Paul.O'Hare@daf.qld.gov.au	Workshop Facilitator
2	Dr Femi Akinsanmi	Uni of Qld	uqoakins@uq.edu.au	MC15011 Project Leader
3	Dr Andrew Geering	Uni of Qld	a.geering@uq.edu.au	MC15000 Project participant & senior scientist on initial DAF Qld report of biotic cause
4	A/Prof. Bruce Topp	Uni of Qld	bruce.topp@daf.qld.gov.au	Macadamia Breeder & MC15011 project member
5	Dr Chris Searle	Suncoast Gold macadamia	Chris.Searle@suncoastgold.com.au	AVG expert and industry consultant
6	Lindsay Bryen		melmac@nor.com.au	Macadamia grower and consultant with AVG leadership
7	Pat O'Farrell	DAF QLD	Patrick.Ofarrell@daf.qld.gov.au	AVG expert & leader of previous AVG projects
8	Scott Alcott	Macadamia Farm Management	scottallcott@bigpond.com	Macadamia grower – with orchards with significant AVG
9	Clayton Mattiazzi	Hinkler Park	ClaytonM@hinklerpark.com.au	Macadamia grower – with orchards with significant AVG
10	Matt Burns	Hancocks Farm Company Pty Limited	mburns@hfc.com.au	Macadamia grower – with orchards with significant AVG
11	Robbie Commens	Australian Macadamia Society	Robbie.Commens@macadamias.org	AMS - Macadamia Industry Productivity Manager
12	Corrine Jasper*	Hort Innovation	Corrine.Jasper@horticulture.com.au	Hort Innovation Industry relations
13	Kathryn Young *	Hort Innovation	Kathryn.Young@horticulture.com.au	Hort Innovation R&D – MC15011
14	Andrew Pearce	Macadamia Direct	welcomecreek@bigpond.com	Macadamia consultant & grower with history of AVG
15	Ian Vimpany	Vimpany & McSkimming Consulting	Vimpany1@internode.on.net	Macadamia consultant & grower with history of AVG in NSW
16	Dr Prati Pandit	Uni of Qld	p.pratibalasailesh@uq.edu.au	Molecular biologist on MC15011 project
17	Scott Hill	Vimpany & McSkimming Consulting		Macadamia consultant
18	John	Hancocks Farm Company Pty Limited		Macadamia grower

* by teleconference phone link

b. Invited participants (apologies for absence)

	Name	Organisation	Contact	Rationale
1	Dr Anthony Kachenko	Hort Innovation	Anthony.Kachenko@horticulture.com.au	Hort Innovation R&D team leader
2	Dr Andre Drenth	Uni of Qld	a.drenth@uq.edu.au	MC15000 Project team member
3	Dr Ben Callaghan	Hort Innovation	Ben.Callaghan@horticulture.com.au	Hort Innovation R&D plant health program manager
4	Matt Webb	DAF QLD	Matthew.Webb@daf.qld.gov.au	First author on the initial DAF Qld report of biotic cause

Table 2: AVG workshop agenda

Time	**Item**	**Person**
8:00 am	Tea and coffee for 8:30 am start	
8:30 to 8:35 am	Welcome	Femi Akinsanmi
8:35 to 8:50 am	Introduction (including purpose and structure of workshop)	Paul O'Hare
8:50 to 9:00 am	Impact on production (feedback from growers)	Femi Akinsanmi
9:00 to 9:15 am	Overview of AVG historical concepts	Dr Chris Searle
9:15 to 9:30 am	Previous research on association of AVG with abiotic factors (MC03012)	Pat O'Farrell
9:30 to 9:45 am	Report on survey of prevalence, variety assessment & impact – MC15011	Dr Femi Akinsanmi
9:45 to 10:00 am	Report on Biotic factors as putative causative agents of AVG –MC15011	Dr Andrew Geering
10:00 am	**Morning tea**	
10:30 to 10:45 am	Risk of AVG occurrence & influence on growers confidence for expansion	Chris/Pat
10:45 to 11:00 am	Proposed future research plan & AVG management	Dr Femi Akinsanmi
11:00 to 11:45 am	Discussion on future research	led by Paul O'Hare
11:45 to 12:30 pm	Discussion on AVG management	led by Paul O'Hare
12:30 to 1:00 pm	Agreement on strategy moving forward	led by Paul O'Hare
1:00 pm	Meeting close	Corrine Jasper
1:05 pm	**Lunch**	