# **Disease Management in Macadamia**

Assoc. Prof. Andre Drenth The University of Queensland

Project Number: MC07003

#### MC07003

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# MC07003 - Disease Management in Macadamia



# **Final Report**

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The University of Queensland, Brisbane

September 2012







#### **Project Details**

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#### **Statement of purpose of the project:**

The project evaluated and optimised the management strategies of existing macadamia diseases; assessed and provided control measures for emerging diseases; and maintained vigilance for diseases/pathogens of potential biosecurity significance. Trials on chemical and biological products used in this project are research based. Therefore, the use of any of the products named in this report must be in accordance with the information provided by the Australian Pesticides and Veterinary Medicines Authority (APVMA) on products permitted for use on macadamia.

#### Project commencement date: 1 July 2007; Project completion date: 30 September 2012

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## **Media Summary**

This project MC07003 - disease management in macadamia has provided significant sciencebased outcomes for the Australian Macadamia Industry. Firstly, it provided effective management strategies for two major diseases - husk spot and Phytophthora stem canker/tree decline in macadamia. Secondly, it improved the understanding of the causal agent and the epidemiology of several emerging disease-systems such as husk rot and tree dieback. This knowledge underpins the development of control measures for these diseases. Thirdly, it highlighted new pathogens and diseases of macadamias in Australia and overseas.

This project generated additional data that enabled successful registration of a new product (Cabrio®) for husk spot control by APVMA. We have identified three new potential fungicides for future field assessment from laboratory screenings of several new fungicide products with softer chemistries and more environmentally suitable properties. These products may be suitable as a replacement of carbendazim. The project provided tools for screening and selection of husk spot resistance in the macadamia breeding program. The project provided results of laboratory and field trials of biological products for husk spot control. We developed a simple-to-use risk assessment tools to aid growers' decision for husk spot control and identified management practice that is vital to avert severe economic loss due to husk spot.

In addition to providing a clear understanding of the pathogen that causes stem canker and tree decline in macadamia orchards, MC07003 provided management strategy using phosphorus acid and metalaxyl to control *Phytophthora*-induced diseases. It developed a disease assessment tool and decision guide for application of phosphite to control *Phytophthora* control. It provided vital information that underpins the need to develop or adopt a nursery certification scheme for macadamia and facilitated two industry workshops to promote the benefit of a certification scheme to plant health.

The project showed that the fungi associated with macadamia dieback belong to the family Botryosphaeriaceae and its severity over time may shift its importance from minor to major disease of macadamias. The project showed that sudden death syndrome follows the onset of severe stress and the combinations of the stress factors together with the biological pressure from pathogens and pests favour the development of sudden death syndrome causing tree death.

Future R&D should evaluate the new products in field trials and provide the economic thresholds for biological control options for husk spot. A comprehensive integrated disease management strategy including the use of risk assessment and disease forecasting tools, cultivar resistance, biological and cultural control practices such as removal of sticktights from tree canopy for husk spot should be evaluated. Improved understanding of relationship of soil health-organic matter and *Phytophthora*-induced diseases is needed for sustainable management of the diseases. Good understanding of each disease system in macadamia is required.

## **Technical Summary**

The number of macadamia trees in commercial orchards has continued to increase in the east coast of Australia. Macadamias are affected by a range of pathogens that require a proactive response to manage and prevent development of disease causing an economic impact. Diseases such as husk spot caused by *Pseudocercospora macadamiae* that was first reported in Queensland in the early 1980s is now widespread in Australia causing over \$10 million loss in production annually if not adequately controlled each year (Newett, 1983; Jones, 2004). While other diseases such as *Phytophthora* tree decline progressively reduce tree production capacity over a large number of years.

This project has provided significant science-based outcomes for the macadamia industry. Firstly, it enabled effective management of two major diseases - husk spot and Phytophthora stem canker/tree decline in macadamia. It significantly advanced knowledge of the husk spot pathosystem; climatic factors that influence husk spot development (Miles *et al.*, 2010b), husk spot cycle (Akinsanmi and Drenth, 2011) and control options (Akinsanmi *et al.*, 2007; Akinsanmi *et al.*, 2008). Secondly, it improved our understanding of the causal agent(s) and epidemiology of several macadamia disease-systems such as husk rot and tree dieback, thus, underpins the development of control measures for these diseases. Thirdly, it highlighted other new and emerging pathogens and diseases of macadamias.

This project generated significant additional data to those previously obtained through our previous project MC03007 that enabled successful APVMA registration of a new product (Cabrio®) in September 2010 for husk spot control. Further *in vitro* screenings of prospective new products with softer chemistries and environmental suitability has identified three new potential fungicides that may be evaluated in field trials. The project provided evidence of the economic benefits of using optimum dose rates and adequate spray volumes and clearly demonstrated why spray coverage is vital to husk spot management. It showed that regardless of the mode of spray delivery mechanism, coverage is more critical than dose/unit area for husk spot control.

This project MC07003 provided risk assessment tools for husk spot. In order to derive estimates of disease risk, conidia dynamics should be considered in terms of relative rather than absolute conidial numbers during the season. The disease risk estimate is based on the prevalence of diseased husk in the tree canopy. Knowledge of host tree phenology, history of disease occurrence and the tendency to produce sticktights in some cultivars are critical for obtaining risk estimates of husk spot. For instance, in well-managed orchards with few trees with low sticktight level and routine fungicide spray applications, the risk of significant yield loss due to husk spot is low. Economic loss due to husk spot may be averted through routine monitoring of kernel quality which should dictate the start of harvest rather than using a preconceived harvest month.

This project provided the first comprehensive study on fruit stomatal abundance in macadamia genotypes that revealed its association with husk spot. This information will aid selection for disease resistance screening in the macadamia breeding and cultivar selection program MC09021 (Akinsanmi *et al.*, 2012).

Microscopic investigations revealed no direct mycoparasitism and no morphological alteration of *P. macadamiae* hyphae by the *Trichoderma* spp. tested. Although *P. macadamiae* mycelial growth was inhibited by putative volatiles produced by the *Trichoderma* spp. cultures, field trials showed no significant husk spot control with two commercial biological products used compared to the untreated control.

In addition to providing a clear understanding of the pathogen that causes stem canker and tree decline in macadamia orchards. MC07003 provided a chemical control strategy using phosphorus acid and metalaxyl, improved the understanding of factors that contribute to disease incidence and severity, and provided insights into the putative effects and interactions of soil health, tree nutrition and *Phytophthora*. In order to reduce pathogen transmissions from nursery to field, we facilitated two industry workshops towards the development or adoption of nursery certification scheme in macadamia. This project showed the first systematic evidence that *P. cinnamomi* can infect and colonise macadamia without any visual symptoms and that infections do not always result in visible lesions or cankers. This demonstrates the danger of assuming that asymptomatic macadamia trees are healthy and free of infection. Thus, asymptomatic trees may become severely affected under disease-conducive conditions.

These results indicate and support the importance of nursery accreditation in the macadamia industry. In addition, the results provide an impetus for the selection and development of disease resistant clonal rootstocks in macadamia. Inclusion of soil health management practices may provide an efficient and profitable management approach for the industry. Availability of nutrients from an organic matter source varies substantially among different kinds of organic matter mainly due to differences in C:N ratios. Different biological products and sources of organic matter including the readily available macadamia husks in macadamia orchard should be tested to determine the most effective amendment for quick recovery of diseased trees.

Other significant outcomes were produced in this project. It showed that the fungi obtained from macadamia dieback belong to the fungal family Botryosphaeriaceae. It showed that based on the severity of dieback observed in macadamia on the Atherton Tablelands this new development may over time shift the importance of this disease from minor to major in macadamias. The project showed that sudden death syndrome follows the onset of severe stress and the combinations of the stress factors together with the biological pressure from pathogens and pests favour the development of sudden death syndrome causing tree death. A proposed management strategy for sudden death syndrome is to improve or eliminate factors that cause severe tree stress including lack of adequate water and soilborne pathogens such as *Phytophthora*.

Future R&D should assess the effects of improved soil health management on reduction of disease incidence and severity; investigate different soil health treatments and management options for improving productivity in declining trees; assess macadamia species and varieties for selection of Phytophthora disease resistant rootstock; and develop an improved integrated management strategy for Phytophthora with the aim of reducing reliance on chemical applications. The frequency of application and effective concentration of phosphite in

macadamia roots for the control of Phytophthora diseases should be determined. An improved understanding of the economic impacts of Phytophthora diseases to individual growers is required to accelerate adoption of research outcomes.

Future research should focus on integrated management of husk spot through improved cultural control practices that include rapid degradation and removal of sticktights from the tree canopy using mechanical and/or biological approaches. Alternative chemicals to replace carbendazim and contribute to the existing chemical arsenals should be provided along with simple-to-use management decision making tools and forecasting system. Screening and selection of husk spot resistant varieties within the MC09021 and future breeding programs using phenotypic traits and molecular-assisted markers would provide long-term benefit to the industry.

Impact and assessment and control of other endemic diseases should be evaluated. The importance of any of the diseases may shift from minor to major if not adequately mobitored and controlled. For instance, under disease conducive conditions both husk rot and flower blight have the potential to cause significant yield loss. Although this project MC07003 has identified the causal agent of husk rot, information on the timing of infection and the conditions that predispose nut to infection still remained unresolved. A good understanding of the timing and the conditions required for infection is essential before any meaningful and effective disease management options are provided. Poor fruit set is often attributed to flower blight caused by *Botrytis cinerea*. This is an ongoing concern and a thorough understanding of the symptoms and the nature of flower blight caused by this pathogen and disease control measures at flowering should be provided.

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### **Chapter 1 General Introduction**

Australian macadamia production is rapidly increasing from the traditional major centres of production in the northern New South Wales from Nambucca Heads to South east Queensland in Bundaberg to Mackay and Western Australia with new orchards being established in Mackay and Emerald (Jones and Mayer, 2009). The rapid expansion of production area and aging of mature orchards provide ample opportunities for various pathogens from both within and outside Australia to limit productivity and quality. The increase in area and intensity of production require a proactive response to challenges from new and endemic native pathogens. A key challenge to adequate control of pests and diseases is the increasing restrictions and regulations of pesticide usage.

Although a number of macadamia diseases have been well characterized (Zentmyer, 1962b; Stephenson *et al.*, 2002; Akinsanmi *et al.*, 2009; Drenth *et al.*, 2009), the impact and ongoing management strategies of many of these diseases are still largely unknown and some diseases limit productivity including yield, quality and economic returns in one year to subsequent years to different degrees. Diseases such as husk spot, cause annual immediate reduction in yield and kernel quality, while other diseases, like *Phytophthora* stem canker and tree decline, progressively cause debilitating effects thereby reduce the trees production capacity over multiple years. Some plant pathogens may initially be found in limited areas within the country, but these may have the potential to cause significant problems for the whole industry. For instance, husk spot caused by fungal pathogen *Pseudocercospora macadamiae* was first reported from Maleny in Queensland in the early 1980s (Newett, 1983), it is now widespread to nearly all macadamia growing regions in Australia causing over \$10 million loss in production annually when the disease is not adequately controlled each year (Jones, 2004).

The nature, extent and impact of disease problems varies and changes over time. Therefore, instead of focusing on individual diseases at any point in time this project focused on overall plant health management of macadamias in Australia. The key strategy used in this project was to identify and quantify the disease problems which negatively impact productivity and quality. Then develop, test and implement cost effective disease management strategies for these diseases. The project provided a multifaceted approach to research in disease management as illustrated in Figure 1.1 and was designed to:

- 1. Improve the efficiency of husk spot control through improved timing, application and choice of chemical control, and to fully understand the disease cycle of the husk spot pathogen in order to deliver an integrated disease management strategy to the industry.
- 2. Assess management strategies for a range of other diseases such as husk rot, *Phytophthora* canker and gall canker and to investigate sudden death syndrome as an association of fungi and Scolytid beetles.
- 3. Increase the awareness among growers and processors of exotic diseases/pathogens of quarantine and biosecurity significance.



Fig. 1.1 Diagrammatic representation of the scope of MC07003

This document is broadly grouped into three categories according to the major aims of the project. Briefly, the first was evaluation of management strategies of existing macadamia diseases (Chapter 2). Second was assessment and control of emerging pathogens (Chapter 3) and third was maintaining vigilance on exotic diseases/pathogens of quarantine and biosecurity significance (Chapter 4).

Chapter 2 included major studies on control of husk spot (section 2.1), *Phytophthora*-related diseases (section 2.2) and husk rot (section 2.3). Studies on husk spot involved extensive field and laboratory trials, in particular, to provide data to support registration of a new fungicide–CABRIO by Nufarm Australia Pty Ltd. for use in macadamia and a replacement for carbendazim which came under review during the course of the project. Other husk spot control options including cultural, biological and disease resistant screening tools for macadamia breeding program were evaluated. In order to advance our knowledge on the husk spot disease system, epidemiological studies were performed to elucidate the husk spot disease cycle. Studies on *Phytophthora*-related diseases were to determine the incidence of *Phytophthora* diseases and the influence on tree phenology and yield, and to develop cost effective control options using phosphonates. Studies on husk rot disease system were designed to provide information on the identity of the causal agent and improve understanding of the disease system that will underpin the development of disease management strategy. Extensive industry engagement activities that promoted the research

findings and adoption o disease management strategies were held in tandem with the research trials.

Chapter 3 included information on assessment and control of emerging pathogens included emerging diseases such as kernel rot (section 3.1), gall-canker (section 3.2), Botryosphaeria dieback (section 3.3) and sudden-death syndrome (section 3.4). Management options were provided and their impacts on further development or spread of the diseases were assessed, followed by grower education and transfer of knowledge of research outcomes to reduce or prevent occurrence of the diseases in macadamia orchards.

Chapter 4 included information on potential pathogens of quarantine significance (section 4.1) and recent pathogens observed in macadamia (section 4.2). Routine diagnosis of diseased samples received from growers and industry consultants and those obtained from field visits constituted the research process in this section. The purpose of this section is aimed at safeguarding Australian production from invasive plant pathogens and any market access issues that may arise related to endemic pathogens.

Chapter 5 contained general discussion on disease management in macadamia (section 5.1) and future direction and R&D requirements (section 5.2) indentified through this project. This may serve as a guide to the industry in future disease management project.

Chapter 6 contained project outputs and outcomes as technology transfer which includes publications (section 6.1) and presentations (section 6.2) including workshops and growers' field days.

# Chapter 2 Evaluation of Management Strategies of Existing Diseases

### 2.1. Husk Spot

Husk spot caused by the fungus *Pseudocercospora macadamiae* (Beilharz *et al.*, 2003) is a serious disease of macadamias and it is considered the main disease that requires ongoing control on certain cultivars in Australia. The pathogen affects *Macadamia integrifolia*, *M. tetraphylla* and their hybrids in Australia, but it has not been reported from any other macadamia producing nations including the United States of America (Hawaii), Brazil, Guatemala, Costa Rica, China, Kenya, Malawi, South Africa, Thailand, and Zimbabwe. Therefore, information on husk spot is limited to studies in Australia.

Husk spot symptoms are initially expressed as chlorotic spots on mature-sized fruit, which over time turn into tan-brown lesions as shown in Fig. 2.1 (Beilharz *et al.*, 2003; Miles *et al.*, 2009). Husk spot results in premature nut drop giving rise to nuts with low kernel oil content that cause significant economic losses. Diseased fruit commonly abscise prematurely except when it becomes a sticktight (Akinsanmi *et al.*, 2007) from 14-20 weeks post anthesis, compared to natural abscission of mature fruit that commences at approximately 30 weeks post anthesis (Sakai and Nagao, 1985; Trueman and Turnbull, 1994; Akinsanmi *et al.*, 2007). It has been estimated that effective control of husk spot saves the industry in excess of \$10 million in lost production each year. HAL project MC03007 (Integrated management of husk spot disease in macadamias) has revealed a lot of background information about the pathogen and a number of large scale field trials have given rise to improvements in disease control.



#### Fig. 2.1. Husk spot symptoms

Information on the disease cycle of husk spot indicates that fungal spores are spread mostly by rain splash from lesions produced on husk (pericarp) on to developing fruit (Akinsanmi and Drenth, 2010). Conidia germinate at about  $26^{\circ}$ C in moist or high humidity conditions and can penetrate stomata of the macadamia fruit husk within 20 h (Miles *et al.*, 2009). The pathogen perpetuates in the tree canopy on diseased husks that failed to abscise known as 'sticktights' (Miles *et al.*, 2010a). Viable spores have been consistently obtained from diseased sticktights for over 30 months, and when sticktights were removed from the tree canopy it resulted in a significant reduction in disease incidence (Miles *et al.*, 2010a). Infection of fruit occurs most readily when fruit is at approximately 3 mm diameter (Akinsanmi *et al.*, 2007; Miles *et al.*, 2010b), thereafter, the pathogen incubates in the fruit for a period of 5-8 weeks, before symptoms are first observed on the pericarp, then sporulates from the resulting lesions 4-6 weeks later (Miles *et al.*, 2010b). How sticktights are formed is not known, but it is hypothesised to be related to disruption of the abscission layer at the base of the macadamia fruit pedicel as the result of various factors including hormone imbalances, photoperiod, temperature, moisture stress, and diseases (Taylor and Whitelaw, 2001; Hardner *et al.*, 2009). Occurrence of sticktights varies among macadamia cultivars. Irregular and continuous flowering outside the main flowering period in August - September (Sedgley, 1981; Trueman and Turnbull, 1994) causes overlapping of old and new fruits, which makes pest and disease management difficult, due to lack of a complete break between cropping seasons. This is heightened by the perpetual occurrence of sticktights in the tree canopy. However, physical removal of sticktights or infective host residues on a large-scale in commercial crops is generally a labour-intensive task and often not economical (Johnson and Stockwell, 1998; Marin *et al.*, 2003; Miles *et al.*, 2010a). A more cost-effective sustainable solution is utilising cultivars without sticktights that do not retain infective materials. Information on the relation of sticktights and husk spot severity in macadamia is scanty, and its usefulness as a selection tool in breeding programs is unknown. In this study, we hypothesize that the absence of sticktights in the tree canopy is a good phenotypic trait which may be able to be used as a selection tool for disease resistance.

HAL and macadamia industry funded studies on husk spot have provided the base understanding of components of the disease cycle including timing of infection (Miles et al., 2010b), source of inoculum (Miles et al., 2010a), period over which infection takes place (Miles et al., 2009) and mode of infection (Akinsanmi and Drenth, 2010); control strategies (Mayers, 1996; Stephenson et al., 2002; Akinsanmi et al., 2007; Akinsanmi et al., 2008) and developed methods which allow accurate assessment of disease incidence (how many fruits have lesions on the tree) and disease severity (amount of immature NIS dropped to the ground due to husk spot) (Akinsanmi et al., 2007). Accurate methods for disease assessment are essential tools for all field experimentation as they allow determination of the effectiveness of different disease management strategies (Akinsanmi and Drenth, 2011). Other significant advancement on our understanding of varietal differences (Akinsanmi et al., 2012), benefit-cost ratios of control options (Akinsanmi and Drenth, 2012) and alternative biological and cultural control options (Akinsanmi and Drenth, 2011) are outcomes included in this report. Despite the improvement in knowledge of husk spot pathosystem, husk spot still remains a major disease affecting macadamia in Australia, partly due to inefficiency of application of control options, disease management decisions and frequent failure to control the disease, particularly in 'wet years' when wet conditions in the orchard limit machinery access.

In order to implement sustainable integrated disease management strategies, the research activities on husk spot in this project were divided into six subsections: chemical control, cultural control, biological control, varietal resistance, epidemiological studies and economic impact of husk spot. Each sub-section focused on specific outcomes that contribute to integrated management of husk spot in macadamia.

The first subsection (2.1.1) on chemical control provided field data to support registration of a new fungicide–CABRIO (Nufarm Australia Pty Ltd.) that was identified as effective in our proceeding project (MC03007). Field trials were designed to determine if husk spot can be controlled with only copper or carbendazim or pyraclostrobin; to determine the optimal rate for efficacy of the pyraclostrobin fungicide and to test if pyraclostrobin efficacy in low spray volumes can be improved with addition of a silicon-based adjuvant. In order to select potential environmentally friendly fungicides as a replacement for carbendazim for field trials, the effects of different fungicide products on the *in-vitro* growth and performance of *P. macadamiae* were evaluated.

The second subsection (2.1.2) on cultural control consists of further studies on relation of conidia production and dispersal and sticktights in the tree canopy. This subsection also includes an assessment of management practices that may reduce the economic impact of husk spot.

The third subsection (2.1.3) on disease resistance consists of trials to evaluate the potential macadamia cultivars and germplasm in the breeding program for resistance to husk spot and to develop screening tools for husk spot resistance. These trials are meant to answer the questions: (i) how susceptible are macadamia varieties to infection and yield loss? (ii) In which varieties is the control of husk spot not economical? (iii) What is the relationship of lesion number to fruit abscission?

The fourth subsection (2.1.4) on biological control includes laboratory and field assessments of biological products. It also includes engagement with the industry stakeholders to evaluate and report the efficacy of alternative products used in husk spot management.

The fifth subsection (2.1.5) demonstrates the benefits of fungicide applications and factors affecting economic returns due to husk spot. The sixth subsection (2.1.6) provides information on the awareness campaign for husk spot and the international scientific community.

#### **2.1.1. Chemical Control**

#### 2.1.1.1. Registration and optimisation of new fungicide: CABRIO®

#### I. Introduction

Most macadamia cultivars are susceptible to husk spot and the disease is usually managed with fungicides. Husk spot control using two spray applications of a tank-mix of carbendazim or difenoconazole and copper, followed by an additional two applications of copper only at intervals of 4 weeks was first recommended (Mayers *et al.*, 2000) which was modified following MC03007 project (Akinsanmi *et al.*, 2007).

Out of the three registered products, carbendazim is the most widely used along with copper, but reliance on carbendazim poses serious doubts on its continual use. Especially, because carbendazim-based products may be withdrawn from use in Australia and the development of resistance to DMI fungicides by fungi similar to *P. macadamiae* (Karaoglanidis and Bardas, 2006; Karaoglanidis and Karadimos, 2006) bring forward the need for additional control options. Avoidance of continual use of the same product or chemicals of the same mode of action to control fungal pathogens is needed to provide more sustainable ways of managing fungicide resistance risk in macadamia production.

The previous project MC03007 identified pyraclostrobin (Cabrio) as an alternative fungicide for husk spot control (Akinsanmi *et al.*, 2008). Introduction of pyraclostrobin into the spray programs, alternating with other fungicides of different mode of action is required to minimize the risk of developing fungicide-resistant strains of the pathogen. The use of a more specific mode of action such as pyraclostrobin which has the ability to inhibit mitochondrial respiration by binding at the  $Q_o$  site of cytochrome b in fungi and other eukaryotes (Bartlett *et al.*, 2002), increases the risk of rapid occurrence and selection of resistant genotypes in the pathogen populations (Karaoglanidis and Karadimos, 2006).

Therefore, strategies such as limiting the number of applications, alternating with compounds from different cross-resistance groups and the use of mixtures with effective partner fungicides should be considered and such strategies need to account for the tolerance and resistance of different macadamia cultivars when applying fungicides and planning new orchards. In order to register Cabrio as a fungicide for husk spot control and make it available to growers, the effective rate, spray application practices and compatibility trials with other products used in the macadamia production system needed to be ascertained. Therefore, this project determined the optimum rate of Cabrio for husk spot control; and compared the effectiveness of Cabrio against husk spot if used in spray rotations with carbendazim and copper as a measure of fungicide resistance management.

#### II. Materials and Methods

#### Optimisation of pyraclostrobin rate

In order to determine the rate at which Cabrio would be most cost-effective for husk spot control, two field trials were established in Beerwah and Bangalow in the 2006-07 and 2007-08 seasons. Trees used were about 6 m high, 85-100  $\text{m}^3$  canopy volume, at 9 m x 4 m

spacing, with a history of annual occurrence of the husk spot disease and the presence of a high number of diseased pericarps providing a ready source of inoculum of *P. macadamiae* in the tree canopy (Miles *et al.*, 2010a). Treatments included Cabrio-only spray applications at three different rates; 0.2, 0.4 and 0.8 mL/L, and Cabrio (0.4 mL/L) mixed with the silicon-based adjuvant (DuWett), and an untreated control (Table 2.1). These treatments were compared with carbendazim-only, two copper-only products [cuprous oxide (Nordox<sup>®</sup> 500 copper, Swift & Co. Ltd. Australia) and Tri-base Blue<sup>®</sup> (tribasic copper sulphate, Nufarm Australia Ltd.)] and a tank-mix spray applications of carbendazim and cuprous oxide in the same field trials (Table 2.1). Each treatment was assigned to plots each consisting of three trees in a randomized block design with three replicates and husk spot incidence and severity was recorded as previously described (Akinsanmi *et al.*, 2007). All fungicides were applied at recommended rates using an airshear sprayer (Silvan Supaflo, 2000 L) which uses very low pressure (20 - 30 kPa) to distribute spray at low volume of high-speed air through 16 twin non-drip jet nozzles at approximately 3 L per tree. The spray regime was conducted at 4 weeks intervals (Table 2.1).

**Table 2.1.** Treatments and spray periods of fungicides to determine the optimal rate of Cabrio compared with sole spray application of carbendazim (Spin) or copper products or tank-mixture of Spin and copper.

	1	1 <sup>st</sup> application	2 <sup>nd</sup> application
	Rate $(100 L^{-1} of)$	(match-head fruit	
Treatments	solution)	size)	(+4 weeks)
Spin + Nordox(Cu)	50 mL + 400 g	Spin+Cu	Spin+Cu
Spin-only	50 mL	Spin	Spin
Nordox-only (Cu)	400 g	Cu	Cu
Tri-base Blue (TBB)	250 mL	TBB	TBB
Cabrio <sup>1</sup> /2	20 mL	Cabrio <sup>1</sup> /2	Cabrio <sup>1</sup> / <sub>2</sub>
Cabrio 1	40 mL	Cabrio 1	Cabrio 1
Cabrio 2	80 mL	Cabrio 2	Cabrio 2
Cabrio $\frac{1}{2} + A^A$	20 mL + 400 mL	Cabrio <sup>1</sup> ⁄2+A	Cabrio <sup>1</sup> /2+A
Untreated control	-	-	-

<sup>A</sup>Silicon-based adjuvant- DuWett

Spray applications with spreader and rotation trials for fungicide resistance management

In order to evaluate the efficacy of Cabrio in fungicide resistance management strategy with Spin + Cu and growers' spraying practices of inclusion of additives in tank-mix, two field trials were established at Beerwah and Bangalow in the 2008/09 season. Different treatments and an untreated control (Table 2.2) were assigned to plots as previously described (Akinsanmi *et al.*, 2007). Influence of two spray additives; DuWett and paraffinic oil (Biopest paraffinic oil, SACOA Pty Ltd, Nedlands, WA Australia) on efficacy of Cabrio and spray applications of Spin + Cu followed by Cabrio or Cabrio followed by Spin + Cu spray applications were compared (Table 2.2). The efficacy of a product, AERO (Nufarm Australia Ltd.) which contains dual active ingredients of pyraclostrobin and metriam, was also evaluated as a possible alternative for fungicide resistance management of pyraclostrobin (Table 2.2).

Treatment <sup>A</sup>	1 <sup>st</sup> spray application	2 <sup>nd</sup> spray application
Cabrio 1	Cabrio 1	Cabrio 1
Cabrio 1 +oil	Cabrio 1+ oil	Cabrio 1 + oil
Cabrio ½ + oil	Cabrio ½ + oil	Cabrio ½ + oil
Cabrio $\frac{1}{2}$ + DuWett (A)	Cabrio ½ + A	Cabrio ½ + A
Spin + Nordox(Cu)	Spin + Cu + oil	Spin + Cu + oil
Rotation 1	Spin + Cu +oil	Cabrio 1 + oil
Rotation 2	Cabrio 1 + oil	Spin + Cu + oil
Single spray	Cabrio 1 + oil	-
Aero <sup>B</sup>	Aero	Aero
Spin + Cu, Aero	Spin + Cu, Aero	Spin + Cu, Aero
Untreated control	-	-

**Table 2.2.** Fungicide treatments applied for managing fungicide resistance to husk spot

<sup>A</sup>Cabrio 1 at 40 mL, Spin at 50 mL, Nordox at 400 g and DuWett at 400 mL per 100 L <sup>B</sup>indicates AERO contains active ingredients – Pyraclostrobin and Metriam at 200 g/ 100 L

#### Data collection and statistical analysis

Husk spot disease incidence and disease severity data were collected following the same method as previously described (Akinsanmi *et al.*, 2007). Disease incidence is defined as the number of fruit that are visibly diseased with one or more lesions on the tree and disease severity is defined as the amount of abscised fruit that are visibly diseased relative to the total number of abscised fruit (Akinsanmi *et al.*, 2007). The harvest yield was determined by harvesting the fruit monthly starting from start of normal harvest period in mid March (Akinsanmi *et al.*, 2007). Disease incidence data were analysed as disease progress curve as previously described (Akinsanmi *et al.*, 2007; Akinsanmi *et al.*, 2008) with Kruskal-Wallis one-way analysis of variance performed to rank the treatments in order of efficacy against husk spot. In order to determine the effect of the treatments on premature nut drop, cumulative disease severity data was subjected to analysis of variance.

#### III. Results and Discussion

#### Optimisation of pyraclostrobin rate

Analyses of disease incidence and severity data from field trials using different fungicides and rates of Cabrio in the 2006-07 and the 2007-08 seasons showed that all Cabrio treatments were significantly similar or better than the tank-mix of carbendazim and copper and the untreated controls (Fig. 2.2). Significant differences were observed between the different rates of Cabrio. Overall, Cabrio 1+A was the most effective treatment for disease severity followed by Cabrio 2 and Cabrio 1 (Fig. 2.2) and the rankings of the treatment for disease incidence according to Kruskal-Wallis one-way analysis of variance showed that husk spot incidence was significantly reduced by Cabrio 1+A followed by Cabrio 2 and Cabrio 1 (Table 2.3). Efficacy of Cabrio only spray applications increased with increase in rates. The addition of DuWett to Cabrio improved its efficacy and showed that the efficacy of Cabrio 1+A was similar to Cabrio 2 (double rate) only. The results indicate that Cabrio significantly reduced premature abscission due to husk spot by more than 70%, over the untreated control and application Cabrio only at 40 mL/ 100 L (Cabrio 1) was similarly as effective as tank-mix of carbendazim and copper. Addition of a spreader or adjuvant to the fungicide spray remarkably improved the efficacy of Cabrio at 40 mL/100 L against husk spot. This showed that efficacy of Cabrio may be further improved with addition of an adjuvant. Although we could not determine the effect of Cabrio on lesion appearance, expansion and sporulation on individual fruit in the field or in a detached fruit assay, Cabrio acted directly against *P. macadamiae* in *in-vitro* assays (Akinsanmi, unpublished). Thus, the efficacy of Cabrio in field trials in reducing disease incidence and severity, implies that the product affects husk spot pathogenesis. Due to the mode of action and the nature of Cabrio as a strobilurin that inhibits mitochondrial respiration in fungi, spray application of Cabrio prior to infection or in the early stages of disease development may provide better effect and may capitalize on the preventive and curative activities of pyraclostrobin against spore germination (Bartlett *et al.*, 2002) than later in the season.

Disease incidence was significantly less in spray applications containing Spin + Cu compared to Spin only or Cu only spray applications (Fig. 2.2a). However, the effects of Spin + Cu on nut drop with husk spot was similar to Spin only, but was significantly different for Cu only (Fig. 2.2b). This confirms the recommendation of spray application of Spin + Cu is essential for adequate husk spot control (Mayers *et al.*, 1996; Mayers *et al.*, 2000). Spray applications using only the protectant fungicides (Cuprous oxide or Tri-base Blue) were not effective as other treatments containing Cabrio or Spin (Fig. 2.2).

Copper as an inorganic fungicide and mancozeb as a dithiocarbamate are protectant fungicides. Even though this group of fungicides can be redistributed on the plant surface with rainfall to protect exposed surface area, they can also be readily washed off by too much rainfall, therefore, leaving any new growth surface area unprotected until the next spray application. Protectant fungicides offer broad spectrum control for many different pathogens however, in order to be very effective, spray application may be required on a more frequent basis throughout the critical periods of husk infection. Spray applications containing these products may provide additional protection against other pathogens that may infect husk such as *Colletotrichum* sp. and *Phomopsis* sp. (Drenth *et al.*, 2009). Copper may be integrated into biological farming systems for husk spot control, but its application should be with great caution because continual application of copper over many years has been reported to result in the accumulation of copper in soils (Alva and Graham, 1991).



**Fig. 2.2** Means of the 2006-07 and the 2007-08 seasons (a) husk spot incidence measured as area under disease progress curve (AUPDC) and (b) weight of nut-in-shell (NIS) of nut drop with husk spot lesions at preharvest in trees treated with two spray applications of fungicides and the untreated and water treated controls. Spin + Cu is tank-mix of SpinFlo and copper, TBB indicates Tri-base Blue; Cu indicates Cuprous oxide (Nordox); A indicates DuWett and 'Spin + Cu, Aero' indicates second spray application with Aero. Cabrio  $\frac{1}{2}$ , 1 and 2 indicate rates at 20, 40 and 80 mL/100L, respectively. Lines on the bars indicate standard errors.

	Mean		
Treatment <sup>A</sup>	AUDPC	Rank	
Cabrio 1 + DuWett (A)	49.67	i	
Cabrio 2	80.09	ii	
Cabrio	96.74	iii	
Cabrio 1/2	99.56	iv	
Spin + Nordox (Cu)	103.65	V	
Spin + Cu, AeroB	105.61	vi	
Cabrio $\frac{1}{2} + A$	112.83	vii	
Spin only	132.65	viii	
Cabrio $\frac{1}{2}$ + Cu	135.72	ix	
Cu only	165.56	Х	
Tri-base Blue only	219.58	xi	
Untreated control	250.24	xii	

**Table 2.3** Rank of efficacy of fungicide applications on husk spot incidence as area under disease progress curve (AUDPC) according to Kruskal-Wallis one-way analysis of variance

Degrees of freedom = 12, Chi-square probability < 0.001, Value of H = 157.7, Adjusted for ties = 162.2

<sup>A</sup>Two spray applications at 4 weeks interval

<sup>B</sup> Spin+Cu was first application and Aero the second application.

#### Spray applications with spreader and rotation trials for fungicide resistance management

Generally, when Cabrio was used in rotation with Spin + Cu, husk spot severity was significantly reduced compared to the untreated control (Fig. 2.3). The efficacy of Cabrio 1 + oil followed by Spin + Cu + oil was similar to the reverse spray application and both rotation sprays were similar to Cabrio 1 only and Cabrio 1 + oil in both the 2007-08 (Fig. 2.3) and the 2008-09 seasons (Fig. 2.4). The results indicate that Spin + Cu and Cabrio 1 can be used interchangeably in rotation spray regime. Addition of copper to Cabrio did not improve the efficacy compared to Cabrio  $\frac{1}{2}$ -only treatment, but was better than copper only treatments. Husk spot incidence was significantly higher in the Cabrio  $\frac{1}{2}$  + Cu than Cabrio  $\frac{1}{2}$  treatment and Cabrio 1 (Fig. 2.2a). Although addition of copper to Cabrio 1 may have improved husk spot control, the costs-benefit ratio of Cabrio + Cu may outweigh Cabrio 1 spray application. However, alternating Cabrio-only with Spin + Cu or copper-only spray applications would be beneficial to reducing the risk of resistance to either of the fungicide.

Introduction of Cabrio into the disease control programs, alternating with the tank mixture of copper with Score or carbendazim products will minimize the risk of developing fungicide-resistance pathogen strains. The use of strategies such as limited number of applications, alternating with compounds from different cross-resistance groups and the use of mixtures with effective partner fungicides would greatly prolong the efficacy of the active ingredients for husk spot management. Alternating these products in spray program will reduce the risk for rapid selection of resistant genotypes within the pathogen populations which may more rapidly occur through the uninterrupted use of Cabrio every season (Karaoglanidis and Karadimos, 2006).

Although husk spot incidence was significantly lower in the Aero-only treatment than the untreated control, disease incidence was significantly higher in Aero than in the Cabrio 1-

only treatment. Pre-harvest nut drop with husk spot was significantly higher in the than Aeroonly treatment than Cabrio 1-only treatment (Fig. 2.3). When Aero was applied as the second spray application following Spin + Cu, husk spot incidence was significantly better controlled than the untreated control, but was similar with Spin + Cu only treatment and higher than the Cabrio 1-only treatments (Fig. 2.2a). The amount of pre-harvest nut drop with husk spot was significantly higher in the Cabrio 1-only, but similar with Cabrio 1+A treatment than Spin +Cu followed by Aero spray applications (Fig. 2.2b). However, this treatment was only applied at one location in Bangalow in the 2007-08 season, the consistency of this spray rotation regime for husk spot control could not be conclusively ascertained. Nevertheless, the results indicate a potential of pyraclostrobin-containing products as a replacement for pyraclostrobin-only product for husk spot control.



**Fig. 2.3** Mean weight of dry nut in shell (NIS) of preharvest fruit with husk spot lesion that dropped by mid-February in Bangalow from macadamia trees treated with fungicides in the 2007-08 season. Bars with the same letters are not significantly different according to Fisher's Protected LSD multiple comparison test.



**Fig. 2.4** Mean weight of dry nut in shell (NIS) of preharvest fruit with husk spot lesion that dropped by mid-February in Bangalow from macadamia trees treated with fungicides in the 2008-09 season. Bars with the same letters are not significantly different according to Fisher's Protected LSD multiple comparison test.

#### IV. Conclusions

Tank-mixture of carbendazim and copper produced better control than spray applications of either of the compounds alone. No significant improvement in husk spot control was observed by mixing copper with Cabrio compared to Cabrio applied alone. The efficacy of Cabrio is comparable to the tank-mix of carbendazim and copper, hence, Cabrio is a suitable alternative for husk spot control. A key advantage of using Cabrio is the reduction of copper sprays in the control program. The long-term reduction of copper in the crop protection system in macadamia will limit accumulation of copper in soils which is essential for improved soil health management. The ability of Cabrio to prevent infection and protect the developing nut at the critical periods of infection that cause premature nut drop makes it suitable for use as the first spray application at the match-head size nut stage.

Cabrio contains 250g/L of pyraclostrobin within the chemical Group 11. It has protectant and curative properties. Pyraclostrobin has been reported to effectively control a wide range of plant diseases in various crops including citrus, small fruits, peanuts, potatoes, sugarbeets, vegetables, stone fruits, tree nuts, turfgrass, and certain cereal crops (Bardinelli *et al.*, 2001). Ability of Cabrio to prevent spore germination and early growth of fungi makes application before infection an important management strategy. Pyraclostrobin has been reported to penetrate the leaf surface within minutes of spray application, and then diffuses within leaf tissue, stopping any mycelia growth inside the plant and protecting the opposite side of the leaf surface through translaminar movement (Bartlett *et al.*, 2002; Forster *et al.*, 2006). Due

to its lipophilic nature, pyraclostrobin remaining on the plant surface binds to and within the waxy layer of the cuticle. This ensures minimal loss of the active ingredient via evaporation and rain, irrigation or dew.

Cabrio is formulated as an emulsifiable concentrate and it is reported to have a low environmental risk profile. It is highly safe to foraging bees, safe to birdlife, has no soil residual and low mammalian toxicity. Cabrio is classified into a lower toxic level in S5 compared to carbendazim which is classed as a S7 toxic compound. APVMA (2003) report on Cabrio showed that under static conditions technical pyraclostrobin is rated as very highly toxic to aquatic organisms including fish, however, the strong binding properties of pyraclostrobin to soil particles is expected to limit the run-off of residues from treated areas and adsorbed to eroded soil particles. The risk from erosion is expected to be low and acceptable when pyraclostrobin is used in accordance with the proposed label and good farm management practices. The hazard to the aquatic environment is further mitigated by adsorption to sediment and dilution from non-treated areas in the catchment including the use of a spray buffer zones if water-bodies, watercourses or wetlands are downwind of the spray application area. Overall, any chronic hazard to aquatic organism is low with pyraclostrobin because it is rapidly adsorbed to sediment and rapidly degrades to less toxic metabolites under anaerobic conditions and in the field (APVMA, 2003).

Cabrio applied at 40 mL/100 L was effective compared to 20 mL/100 L rate. A double of application rate at 80 mL/100L improved husk spot control, however, disease incidence was similar to spray application at 40 mL/100 L. Even though the efficacy of Cabrio at 40 mL/100 L was enhanced by the addition of DuWett, the addition of paraffinic oil did not result in similar disease control compared with Cabrio-only application. It has been reported that addition of adjuvants in combination with pyraclostrobin did not often result in a significant improvement in control of cercospora leaf spot on sugarbeet compared to pyraclostrobin applied alone (Khan *et al.*, 2007). Therefore, we recommend that Cabrio should be registered and used at 40 mL/100 L for husk spot control in macadamia.

Resistance to strobilurin products including pyraclostrobin has been reported in various cropping systems (Vincelli, 2002; Avenot *et al.*, 2008; Wise *et al.*, 2009). Therefore, it is imperative to judiciously manage the routine use and application of Cabrio in macadamia to mitigate the high risk of *P. macadamiae* developing resistance to pyraclostrobin. Cabrio should be used in alternating spray application with other fungicides of different mode of action including copper. In order to manage fungicide resistance in husk spot, we recommend a maximum of two consecutive spray applications of Cabrio. This is very critical because of the residual source of inoculum (sticktights) with husk spot lesion that sometime persists in the tree canopy for several years. In which case, Cabrio should not be applied more than two consecutive times within or between seasons. An indicative of possible consecutive spray applications over two seasons for a two fungicide sprays per season program is shown below (Fig. 2.5). Protectant fungicides may be used between two Cabrio sprays or after two consecutive applications of Cabrio. Protectant fungicides used in this manner will help to slow (or reduce the chances for) the development of fungicide resistance on the farm.



Fig. 2.5 Possible Cabrio resistance management program with maximum of two consecutive spray applications of Cabrio within and between seasons.

V. Communications and Extension of Research Outcomes

• <u>Report to Nufarm Australia Ltd.</u>

A special report including data obtained from laboratory and field trials with pyraclostrobin was provided to Nufarm Australia Ltd. in the application for registration of Cabrio for husk spot control in macadamia to APVMA. In order to obtain the minimum residue limit (MRL) in macadamia an independent field trials were conducted by Nufarm. Cabrio was approved for husk spot control in macadamia on 3 September 2010 (Fig. 2.6).

CAUTION KEEP OUT OF REACH OF CHILDREN READ SAFETY DIRECTIONS BEFORE OPENING OR USING	
Nufarm	
Cabrio <sup>®</sup>	
Fungicide	
ACTIVE CONSTITUENT: 250 g/L PYRACLOSTROBIN SOLVENT: 705 g/L HYDROCARBONS, LIQUID GROUP 11 FUNGICIDE	
For the control of leaf speckle and leaf spot in bananas, downy and powdery mildew in g spot in macadamia and rust in almond as specified in the DIRECTIONS FOR USE table.	rapevines, <mark>husk</mark>
READ COMPLETE DIRECTIONS FOR USE BEFORE USING THIS PRODUCT.	
Cabrio is a registered trademark of BASF used under licence by Nufarm Australia Limited	
nufarm.com.au APVMA Approval I	No.: 58900/49315

Fig. 2.6 Image of Cabrio label for husk spot control in macadamia

• Growers' field days

Two industry field days were held March 10<sup>th</sup> & 13<sup>th</sup> in the 2008-09 season to demonstrate efficacy of Cabrio for husk spot control. The field days were held at two locations; Beerwah, QLD and Bangalow, NSW.

- Publications and Presentations
  - 1. Research on pyraclostrobin in macadamia. 18pp. Special Report to Nufarm Australia Ltd. August 2007.
  - 2. Pyraclostrobin is effective against husk spot. Southern African Macadamia Growers' Association Yearbook 17. 53-56, 2009.
  - 3. Macadamia diseases- Annual research updates: Presented at the Macadamia Pest Scout and Consultant meeting, Brisbane, 2007-2009.
  - 4. Husk spot management Australian macadamia industry MacGroup meetings, 2007, 2008, 2010, 2011.
  - 5. Best Practice group: Growers November meetings. Location: several in Queensland and New South Wales, 2009, 2010, 2011.

#### 2.1.1.2. Spray application practices and decisions for husk spot control

#### I. Introduction

Due to the wide variety of environmental conditions and different regions in which macadamias are grown, effective crop protection is a daunting task. Therefore, the decisions on whether to spray before any signs of infection (prophylactically) or not to spray are critical to achieving the desired effect. When spraying, growers are tasked with applying pesticide efficiently with maximum efficacy with the least environmental impact, but several factors including canopy volume and density, cultural practices, orchard layout and dynamics of pathogen affect pesticide efficiency and efficacy. In Australia, there is a large orchard to orchard variation in spray volumes applied to control pests and diseases. Traditionally, in agricultural crop production systems, high spray volumes (2000 L/ha) were used but through the adoption of modern spray technologies, current spray volumes (50-500 L/ha) have been greatly reduced. The reduction in spray volume has been driven largely by the use of finer spray qualities and the need to reduce spray hours and water usage efficiency. A key component of pesticide spraying is derived from an efficient spray application system that provide maximum efficacy.

In order to achieve adequate control of target diseases, spray coverage is very important except in fully systemic fungicides, where the amount of fungicide deposited per unit area is more important than the actual spray coverage (Cross *et al.*, 2001). The effects and importance of spray volume on spray deposits and the efficacy of the pesticide are well documented in various tree crops and these are often influenced by the tree canopy density (Travis *et al.*, 1987; Whitney *et al.*, 1989; Cunningham and Harden, 1998; Cross *et al.*, 2001). There are large variations in the geometric structure of macadamia orchards in Australia. Typical orchards contain multiple macadamia cultivars in the same row or block and with trees at different growth stages, tree sizes and canopy densities. Despite these within-orchard variations, it is a common practice on a given orchard to be sprayed in a similar way, using a fixed fungicide dose rate and spray volume throughout the season.

During spray application, minimal or no adjustments for spraying trees of different sizes, canopy and crop density are made. In most cases, this is due to lack of a clear understanding of how to make the necessary spray adjustments. The use of innovative pesticide spray delivery systems, such as low volume spray application, to reduce adverse environmental impacts may have inadvertently reduced effectiveness of fungicide spray applications for husk spot control. A number of systems for the adjustment of dose rate and spray volume according to orchard structure have been developed. The tree row volume (TRV) spray volume adjustment system in which the dose applied is varied by varying the spray volume at constant pesticide concentration in proportion to the TRV (Sutton and Unrath, 1984) and the simplified system, based on unit canopy row method, where dose rates are delivered per 100 m<sup>3</sup> of canopy (Furness *et al.*, 1998; Drew *et al.*, 2002). The use of the simplified system in westigated (Drew *et al.*, 2002).

There is no information on whether low spray volume is more effective than the dilute (high) spray volume with regards to the control of husk spot. Also, the effect of spray application efficiency on husk spot control is not known. The paucity of information concerning low volume spray application systems commonly used to control husk spot is a major concern.

Therefore, we sought to assess the effect of spray volumes and efficiency of spray applications on husk spot control, and determine the relative efficacy of dilute and concentrate rate spray applications on husk spot. This information will underpin the development of spray application parameters for adjusting or maintaining the same spray application volume throughout the season and orchard.

#### II. Materials and Methods

#### Experimental design

Field trials were established in the 2009-10 and the 2010-11 seasons to test the effect of fungicide spray application volumes on husk spot control. The trials were carried out on cultivar A16 under commercial cultivation in Alstonville, New South Wales, Australia. Trees used were 100 m<sup>3</sup> canopy volume with a history of annual occurrence of the husk spot disease and the presence of high number of diseased pericarps providing a ready source of inoculum of *P. macadamiae* in the tree canopy (Miles *et al.*, 2010a). Fungicides were applied using an airblast one-sided sprayer with DS Radak and 2000 L tank (Silvan Supaflo, Australia Ltd.) containing 150 g/100L cupric hydroxide (Kocide<sup>®</sup> Blue Xtra<sup>™</sup>, DuPont Australia Limited), 50 mL/100L carbendazim (SpinFlo<sup>®</sup>; Bayer CropScience, Australia) and 100 mL/100L synthetic latex spreader-sticker deposition aid (Bond<sup>®</sup>, Nufarm Australia). The sprayer was calibrated to give the required flow rates and volumes from the nozzles at 1800 -2000 kPa at the appropriate tractor ground speed. The appropriate rates per hectare were adjusted using the unit canopy row method (Furness et al., 1998; Drew et al., 2002) and spray volumes of 6.40 L (high), 5.12 L (moderate) and 3.12 L (low) were obtained of 100 m<sup>3</sup> tree canopy volume. In order to monitor the spray coverage efficiency, a fluorescent dye which glows under ultraviolet (UV) light was added into the sprayer and the relative spray coverage was determined on the fruit inspected at night using a UV light.

Fruit and leaf samples were obtained from the tree canopy and were also assessed under UV light in the laboratory. Each treatment was applied twice at 3-4 weeks interval per production season (Akinsanmi *et al.*, 2007) to a total of 15 data trees and different sets of trees were used for each treatment in the two production seasons. All trees received similar agronomic practices and insect populations were monitored and controlled by the application of insecticide sprays.

#### Effect of spray volume delivery on husk spot

In order to determine the effects of spray volumes on husk spot control, three levels of dilute spray volumes were used. The treatments consisted of dilute fungicide dose rate which was applied at low (3.12 L/tree), moderate (5.15 L/tree) and high (6.40 L/tree) spray volumes which correspond to 50%, 80% and 100% spray delivery efficiencies, respectively. Trees that received no fungicide spray served as the untreated control. Husk spot severity was monitored on each tree from December to March each year (Akinsanmi *et al.*, 2007; Akinsanmi *et al.*, 2008). In order to determine the efficacy and efficiency of the different spray volumes on husk spot control, the spatial distribution of diseased nut in the tree canopy was assessed in March each year. Each tree canopy was partitioned into three sections; the top, middle and bottom. Each section was approximately 25% of the tree canopy and the proportion of nut with husk spot lesion in each section was recorded.

#### Comparison of efficacy of dilute and concentrate spray volume on husk spot

High spray volume applied at 6.40 L/tree and concentrate (2X) spray volume applied at 3.12 L/tree was compared with an untreated control. Husk spot severity was monitored on each tree from December to March each year (Akinsanmi *et al.*, 2007; Akinsanmi *et al.*, 2008).

#### Data analysis

Area under disease progress curve (AUDPC) was calculated from disease severity data as described by Akinsanmi *et al.*(2007). The AUDPC data were log-transformed  $[log_{10}(x+1)]$  to stabilize variance. Significant differences between years, treatments and their interactions for disease severity and NIS harvested were analysed with the generalized linear model (GLM) procedure with normal distribution and identity as the link function in GenStat. Significant factors were separated and tested using Fisher's protected least significant difference (LSD) tests. Husk spot incidence data were square root-transformed before ANOVA.

#### III. Results and Discussion

Husk spot occurred in all treatments in both the 2009-10 and 2010-11 seasons, but the incidence was observed from the first week of January in the untreated controls while it was delayed in all the treated trees, giving rise to a higher disease incidence in the untreated control than in the treated trees. In the 2010-11 season, when conditions were very favourable for disease development, husk spot developed more rapidly which resulted in 100% disease incidence in the untreated control and average of 70% in treated trees in January compared to the 2009-10 season. Consequently, disease severity was significantly higher in the 2010-11 than in the 2009-10 seasons and therefore, the rates of nut drop among the treatments were significantly different in 2010 but were similar in 2011 (Fig. 2.7).

#### Distribution of husk spot in the tree canopy

The spatial distribution of nut with husk spot lesions in the tree canopy showed that spray volume influenced the position of diseased nut within the tree (Fig. 2.8). Overall, the percentage of diseased nut in the lower section of the canopy was significantly higher than nuts in the middle or upper section of the canopy height (Table 2.4) and the pairwise comparisons between the three heights of the canopy were significantly different (Table 2.5). This confirms the nature of husk spot disease and the spatial pattern of diseased nut is aggregated based on rain splash effect and proximity to inoculum source (Akinsanmi and Drenth, 2010). Hence, nut in the lower height are likely to be more readily infected due to gravitational effect on infective propagules than nut at the upper section of the tree. Results of the F tests based on the linearly independent pairwise comparisons among the estimated marginal means of each factor and their interaction showed that distribution of diseased nut in the tree canopy was significantly influenced by spray volume and canopy height (Table 2.4). Spray volume explained 32% while canopy height explained 22% of the variations in the spatial distribution of diseased nut in the tree canopy (Table 2.4). This indicates that both factors collectively contribute over 50% of husk spot incidence in the tree canopy. The pairwise comparison of the distribution of diseased nut among the spray volumes showed that high volume was significantly better than moderate and low volume, whereas moderate spray volume was not significantly different from low volume. Another significant contribution to husk spot incidence is abundance and distribution of sticktights in the tree canopy. The amount of sticktight in the upper canopy height was significantly less and in most cases no sticktights was observed at the upper canopy height than the middle and lower canopy heights. Sticktights were more abundant inside the tree canopy in the middle section than at the lower canopy height. The abundance of sticktights in the middle section of the tree canopy may explain the high husk spot incidence that was consistently observed in the middle section of the tree canopy (Fig. 2.8). When trees were sprayed, husk spot incidence was significantly less in the lower canopy height than the middle section of the canopy. This indicates that the proximity of the nut in the lower section of the canopy to sprayer/spray flow and spray deposit/coverage averts nut of increased infection from inoculum from the middle section.

**Table 2.4** Analysis of variance of husk spot incidence at different canopy heights within the tree canopy of A16 trees sprayed with high, moderate and low volume of tank mix of carbendazim and copper at dilute rate and an untreated control.

Source	df	Mean Square	F
Spray volume (SV)	3	68.04	29.76**
Canopy height (CH)	2	69.68	29.45**
$SV \times CH$	6	31.69	13.39**
Residual	48	2.366	

\*\* indicates *F*-test is significant at P = <0.001

**Table 2.5** Pairwise comparison of components of main factors of spatial distribution of diseased nut at different canopy heights (upper, middle and lower) within the tree canopy of A16 trees sprayed with various spray volumes (high, moderate and low) of tank mix of carbendazim and copper at dilute rate and an untreated control.

		Mean Difference	95% Confidence Interval for Difference	
Factor (I)	Factor (J)	(I - J)	Lower Bound	Upper Bound
Low	High	2.78*	1.66	3.91
Low	Moderate	0.91	-0.22	2.04
High	Moderate	1.87*	-3.00	-0.74
Untreated control	High	5.13*	4.00	6.26
Untreated control	Moderate	3.25*	2.12	4.38
Untreated control	Low	2.34*	1.21	3.47
Middle	Lower	1.65*	-2.63	-0.67
Lower	Upper	2.07*	1.09	3.05
Middle	Upper	3.73*	2.75	4.70

<sup>\*</sup>The mean difference is significant at LSD 0.05 level



**Fig. 2.7** Nut drop pattern of trees treated with different spray volumes at dilute rate of tankmix of carbendazim and copper and the associated total nut-in-shell (NIS) reject in the 2009-10 and the 2010-11 seasons. Enclosed graphs show progression of percent total reject and premium kernel of NIS produced over the nut drop period.



Fig. 2.8 Position of nut with husk spot lesions in the tree canopy of trees treated with different spray volumes at dilute rate of fungicide applications.

#### Comparison of efficacy of dilute and concentrate spray volume on husk spot

High volume dilute spray applications consistently reduced early nut drop due to husk spot compared to the low volume concentrate spray applications and the untreated control (Fig. 2.9). In both seasons, majority of nut drop occurred later in the season in the high volume spray applications compared to the low volume concentrate sprays. By early March about 23% and 67% of the total nut produced had dropped in the low volume concentrate spray in the 2009-10 and the 2010-11 seasons, respectively. Whereas, in the same period only 16% and 32% of the total nut produced had dropped in the high volume dilute spray. This period corresponds to 2.7% and 6.0% of total NIS reject in the 2009-10 and the 2010-11 seasons, respectively. The results show that high volume sprays provide better spray coverage, this prevents husk spot infection which resulted in lower disease severity than low volume spray.

High spray pressures and/or high water volumes would provide a more effective target spray of developing fruit within the tree canopy than low pressures and/or low water volumes during spray applications. Dense canopies are best penetrated by a combination of slower travel speeds and higher water volumes while finer sprays or higher spray pressure were shown to have little or no effect in penetration of such dense canopies (Wolf, 2006).



**Fig. 2.9** Nut drop pattern of trees treated with spray volumes at dilute and concentrate (2x) rates of tank-mix of carbendazim and copper and the associated total nut-in-shell (NIS) reject in the 2009-10 and the 2010-11 seasons.

#### IV. Conclusions

Irrespective of whether conditions are favorable or less conducive to husk spot development, the efficiency of spray applications is critical to effective husk spot control. In the 2010-11 season when conditions were favourable to husk spot development, application of high volume at optimum spray coverage efficiency provided the best husk spot control compare to low volume concentrate sprays and the untreated control.

Unlike pests, where coverage does not directly correspond with pest control and may result in more pests acquiring sub-lethal doses, coverage is essential for effective plant fungal disease control (Timothy *et al.*, 1999). Using sub-optimal spray volumes with registered rates as well as reduction in dose may result in poor disease control. Similar results have been reported in grapes, where the level of coverage achieved in the tree canopies was affected by the type of sprayer and operating parameters, as well as by the weather conditions, crop cultivar, growth stage, and trellising system of the vineyard (Wise *et al.*, 2010). A cautious approach is to use appropriate water volumes relative to the type of sprayer, nozzles and fungicide characteristics. Excessive spray volume may lead to undesirable effects and consequences. For instance, in citrus crops, high volume at 10 000 L/ha pesticide spraying with oscillating boom sprayers has been reported to result in low levels of pesticide retention on trees and high levels of off-target losses (Cunningham and Harden, 1999). The risk of spray drift is less with high volume spraying using a large droplet size and the larger nozzles reduce the possibilities of blockages. Studies have confirmed that sprayers which create large fraction of small droplets generally generate most drift (Combellack *et al.*, 1996).

Regardless of the mode or delivery mechanism of spray application, coverage of the developing nut is more critical than dose/unit area for husk spot control. Spray technologies that provide good coverage under a wide variety of conditions are essential to control husk spot and to reduce premature nut drop due to husk spot. Addition of an appropriate adjuvant to the fungicide application can significantly improve spray coverage and efficacy (McMullan, 1995).

#### V. Communications and Extension of Research Outcomes

- Publications and Presentations
- 1. Akinsanmi OA and Drenth A (2012) Economic returns from fungicide application to control husk spot of macadamia in Australia is influenced by spray efficiency, rates and costs of application. Crop Protection 41: 35-41.
- Femi Akinsanmi, John Pretorius, Kevin Quinlan, Paul O'Hare and Andre Drenth 2011. Husk spot control with fungicide spray applications: Is it profitable? <u>Australian</u> <u>Macadamia Society Ltd. News Bulletin</u> Vol. 39 (6): 62-66.

#### 2.1.1.3. Selection of fungicide products as putative replacement for carbendazim

#### I. Introduction

Fungicides continue to play an essential role in the effective control of plant diseases. At present, fungicide spray application is the most effective control option for husk spot disease in macadamia (Akinsanmi *et al.*, 2007). It has been projected that the impact of husk spot will be very high if the existing chemical control products used for husk spot control, in particular, carbendazim is deregistered, removed from sale, or the pathogen becomes resistant to the product. Therefore, research for alternative chemical options and a replacement for carbendazim are essential components of this project. There is potential for development of resistance in *P. macadamiae*. Given the enormous economic impact of husk spot disease to the industry, discovery of new and novel chemicals to manage this pathogen is an important priority in the current research program. The risk of emergence of resistance is naturally higher for fungicides that target single site than multi-site mode of action. Also, the possibility of resistance is believed to be higher for inhibitors targeting the primary metabolism of pathogens than inhibitors acting on secondary metabolism.

Fungicides with novel modes of action are being developed that include compounds that trigger defense mechanisms in the plant (Knight *et al.*, 1997). In a review of development of fungicides Knight *et al.* (1997) concluded that for the foreseeable future, new toxophores will be identified through a process of random screening, with natural products representing a rich source of fungicide leads. Prospective fungicides with low toxicity to humans and wildlife, low environmental impact, low residues in food, and compatibility with integrated pest management (IPM) programs are becoming increasingly important.

Identification of new fungicides for husk spot management which relies primarily on assessment of efficacy on trees in orchard conditions is time consuming, material and labour intensive, and requires relatively large quantities of test compounds. *In vivo* glasshouse screenings and field trials are expected to remain the dominant methods for characterizing new compounds. *In vitro* assays that first test the efficacy of the product on the growth or development of the fungal pathogen on fungicide-amended agar medium often supplement field trials. Combinations of these different methods are often needed for evaluating compounds derived from synthetic sources, for assessing natural products and compounds with only protective ability.

The main goal of this study was to identify a product of different chemical group from those which are already registered for husk spot control in macadamia which may serve as a replacement for carbendazim. The selection of prospective products based on efficacy in the *in vitro* assays before large-scale field testing is a judicious and an economical way of selection process of alternative products for the industry. This subsection contains results of laboratory trials on efficacy of fungicide products against the growth and development of *P. macadamiae*. The results provide a basis for the selection of potential fungicides for field trials. We have previously used this process to successfully select effective fungicides (Akinsanmi *et al.*, 2008).
# II. Materials and Methods

#### Fungicide products

Formulated fungicides of different mode of actions (Table 2.6) were evaluated *in vitro* for inhibition of mycelial growth of *P. macadamiae*. Stock suspensions were prepared in sterile distilled water and all subsequent dilutions were made in sterile distilled water. All concentrations are given as amount of active ingredient per volume in mg/L. Fungicide suspensions were incorporated into ½-strength potato dextrose agar (PDA, Difco) to produce a series of concentrations (0.01 mg/L, 0.05 mg/L, 0.10 mg/L, 0.15 mg/L, 0.25 mg/L and 0.50 mg/L) in 25 mL of the agar poured into 90-mm Petri plates. In the case of protectant fungicides, 1 mL of the fungicide solution of each concentration was spread on the surface of the agar after solidification of the medium, allowed to air-dry under aseptic conditions. Each Petri plate was inoculated with 4 plugs of mycelial disc (5-mm diameter) taken from the periphery of actively growing colonies on PDA.

Product name/code			Activity
	Active constituents	Chemical family	group code
Aero	pyraclostrobin & metiram	Methoxy carbamate & Dithiocarbamate	11 & M3
Amistar	azoxystrobin	Methoxy acrylate	11
Cabrio	pyraclostrobin	Methoxy carbamate	11
Cyren	thiophanate-methyl	Thiophanates	1
*DC-095	Unknown	Multi-site	Μ
*DC-096	Unknown	Carboxamides	7
*DC-097	Unknown	Unknown	-
*DC-099	Unknown	Carboxamides & Oximino acetates	7 & 11
Euparen Multi	tolyfluanid	Sulfamide	M6
Flint	trifloxystrobin	Oximino acetates	11
Folicur	tebuconazole	Triazole	3
Kocide Blue Xtra	copper hydroxide	Inorganic	M1
Nordox	cuprous oxide	Inorganic	M1
Norshield	copper fungicide	Inorganic	M1
Peratec	hydrogen peroxide & peracetic acid	Inorganic	М
Score (Foliar)	difenoconazole	Triazole	3
SpinFlo	carbendazim	Benzimidazole	1
Tri Base blue	tribasic copper sulphate	Inorganic	M1
Vision	pyrimethanil & fluquinconazole		9 & 3

 Table 2.6 Fungicide products tested in vitro for effect against P. macadamiae

\* indicates experimental products currently under development by the chemical company

#### Effect of fungicide on mycelial growth

Two isolates of *P. macadamiae* from our culture collection (Miles *et al.*, 2010b) were used in three replicate Petri dishes per isolate per concentration and were incubated at  $26^{\circ}$ C in darkness (Miles *et al.*, 2010b). Colony diameters were measured weekly for 4 weeks. Percentage growth inhibition was calculated on the basis of un-amended control plates. Experiments with each fungicide and each isolate were repeated at least once.

#### Data analysis

Effects of the fungicide on mycelial growth were compared with the untreated control from which the EC<sub>50</sub> levels (effective concentration causing 50% growth inhibition) were determined. Equipotent fungicide doses at the EC<sub>90</sub> levels were also determined from dose-response curves (Kataria *et al.*, 1991). Percent growth inhibition (*GI*) was calculated as  $GI = [(g_c - g_f)/g_c] \times 100$  where  $g_c$  and  $g_f$  are colony diameter on control and fungicide-amended agar plate, respectively.

### III. Results and Discussion

### Efficacy of fungicide products

Results showed significant (P < 0.001) differences exist in P. macadamiae fungal growth on PDA amended with the various fungicides at different concentrations (Table 2.7). There was significant fungicide and rate interaction (Table 2.7) which indicates that the efficacy of the products was influenced by rates of applications. This is exemplified in Fig. 2.10 where at 0.05 mg/L Cabrio significantly reduced colony growth within 18 days of incubation compared to SpinFlo at the same concentration, but SpinFlo was more effective at the same duration at 0.15 mg/L.

Table2.7	Analysis	of	effect	of	fungicide	at	different	rates	on	in	vitro	growth	of	Р.
macadamiae	2.													

Source of variation	d.f	Mean square	F pr.
Fungicide	13	4.15	< 0.001
Rate	5	2.31	< 0.001
Fungicide × rate	44	0.18	< 0.001
Residual	1065	0.01	

Inorganic fungicides whose mechanism of action is as protectant were excluded from the analysis of the *in vitro* efficacies data. The growth of mycelial plugs placed on culture media pre-coated with the inorganic product was similarly restricted. This confirmed the fungistatic nature of the compounds. When the mycelial plugs were removed and placed in control culture plates (untreated), growth of the fungal mycelium was observed. Therefore, the integration of these compounds with other products with fungicidal properties may provide a better disease control. This feature was confirmed with improved efficacy of SpinFlo when used in tank mixture with copper compared with SpinFlo alone (Akinsanmi *et al.*, 2008).



**Fig. 2.10** Progression of colony growth of *Pseudocercospora macadamiae* in culture media amended with fungicide at (a) 0.05 mg/L and (b) 0.15 mg/L.

Growth inhibition above  $EC_{50}$  occurred in Cabrio and Score at 0.01 mg/L (Table 2.8). Most of the fungicides attained  $EC_{50}$  at higher dose rates, 0.10 and 0.15 mg/L (Table 2.8). Overall, the growth inhibition was significantly higher and similar in SpinFlo, Score, DC-099 and Cabrio than other fungicides. Out of this group of four fungicides, only DC-099 is not currently registered for husk spot in macadamia. The second group of fungicides showing similar % inhibition of mycelial growth includes Folicur, Flint, Vision, Amistar and Aero. These results indicate that effectiveness against husk spot fungal growth is not specific to a specific chemical group. For instance, the in *vitro* efficacy of products that contained strobilurin was variable and the inhibitory effect of Cabrio was significantly better than Flint, Amistar and Aero.

	Rate (mg/L)						
Fungicide	0.01	0.05	0.10	0.15	0.25	0.50	
Aero	39	43	47	60	nd	nd	
Amistar	40	41	41	42	nd	nd	
Cabrio	55	59	60	64	nd	nd	
Cyren	nd	1	1	3	3	45	
DC095	nd	2	3	4	4	4	
DC096	nd	9	22	30	43	59	
DC097	nd	12	19	34	41	44	
DC099	nd	41	53	66	69	77	
Euparenmulti	34	39	38	39	nd	nd	
Flint	36	44	48	51	nd	nd	
Folicur	41	46	50	55	nd	nd	
Score	57	59	63	65	nd	nd	
SpinFlo	37	58	72	76	79	79	
Vision	36	35	46	52	nd	nd	

**Table 2.8** Growth inhibition (%) of *in vitro* colony growth of *P. macadamiae* on culture media amended with different rates of fungicide products compared with untreated control.

nd indicates not determined

Field conditions may alter the performance of some products compared to the *in vitro* assessments. Practical differences in the mode of action of these fungicides could have a major influence on their effectiveness in the field. For instance differences in protectant and systemic fungicides may influence field performance. It has been reported that among the strobilurin products there is a wide variation in their translaminar activity, redistribution in the vapour phase and mobility properties (Bartlett *et al.*, 2002). However, previous studies (Akinsanmi *et al.*, 2008) have shown that *in vitro* efficacy of fungicides against husk spot fungus often mirrors the efficacy of the products in field trials. As this dose rate increases, *in vitro* efficacy increased (Table 2.8). This indicates that using higher dose rates than the rates used in the *in vitro* assessments equipotent fungicide doses at the EC<sub>90</sub> levels may provide improved disease control in field trials.

It has been suggested that a high dose may be required to obtain effective control especially under high disease pressure, or where a sensitivity shift has eroded efficacy (van den Bosch *et al.*, 2011), but recent evidence suggests that, in most circumstances, a high fungicide dose will increase the speed at which fungicide resistance develops (van den Bosch *et al.*, 2011).

Therefore, appropriate dose rates for field trials must be pre-determined with the product suppliers before any large-scale field trials. A comparison cost of dose rate must also be considered.

### IV. Conclusions

In order to improve quality and yield, fungicides will continue to remain as essential tools for plant disease management. Thus, application of fungicide needs to be optimised for use in an IPM system. New chemicals with low mammalian toxicity and environmental impact as well as low residues in food and compatibility with IPM programs are very important features of new fungicides and may provide a strong competitive advantage over older products with less desirable environmental and toxicological aspects (Gullino *et al.*, 2000). A good balance between potency and safety is a major goal of new fungicides (Knight *et al.*, 1997). The availability of fungicides characterized by new modes of action can have a profound impact on disease control for certain crops, providing more choices for growers. However, the availability of new compounds cannot be seen as a replacement of old, traditional fungicides such as copper which continue to play a major role in disease control (Gullino *et al.*, 2000).

Most of the new compounds have different modes of action from those of the older fungicides and have increased specificity of their mechanisms of action greatly increases the risk of selecting fungicide resistant populations. This imposes significant limitations to their practical application as a sole product for disease management. Therefore, resistance management program must be carefully established. The new compounds with a lower toxicity and environmental impact from different chemical groups may serve as potential replacements of the existing chemicals for husk spot control for the macadamia industry.

Thiophanate-methyl was tested because it is in the same chemical family as carbendazim, but undergoes only very limited metabolic conversion compared to carbendazim, thus, may provide better control than carbendazim. Thiophanate-methyl is a systemic benzimidazole fungicide used in Australia as a broad-spectrum systemic fungicide for agricultural use. Although thiophanate-methyl breaks down in plants and the environment to form carbendazim which can lead to residues of carbendazim in treated commodities but in mammals it appears to undergo only very limited metabolic conversion to carbendazim. Because of concerns about potential foetal malformations and also evidence of testicular toxicity in laboratory animals following the use of benomyl, a compound structurally related to thiophanate-methyl and carbendazim, the APVMA (2010) commenced a review of the active ingredient. Although APVMA concluded that thiophanate-methyl did not induce birth defects in animal studies and it recommended that the restraint "DO NOT use this product in the home garden" should be added to all product labels to clarify that thiophanate-methyl products are for professional use only. Due to this concern and based on the results from the in vitro assessment, field trial with thiophanate-methyl in macadamia should not be conducted. The increased efficacy of DC096 and DC097 at 0.25 and 0.50 mg/L in the in vitro assays showed that these products could be explored in field trials in comparison with SpinFlo and copper mixture. The limited number of compounds with similar or better efficacy than carbendazim in this study indicates that new and novel chemistry, including those that activate natural plants resistance mechanisms and natural products should be explored as they become available for husk spot control.

# **2.1.2.** Cultural Control

#### 2.1.2.1. Husk spot fungal spores dispersal mechanism and disease occurence

#### I. Introduction

Previous studies have revealed that spores produced on the surface of the husk serve as a major source of inoculum (Miles *et al.*, 2010a). An understanding of the nature of pathogen dispersal mechanisms would provide useful information on disease epidemics. Putative contributions of different types of spores to the progress of disease can be partially inferred from spatial patterns of diseased fruit in the tree canopy (Sposito *et al.*, 2008). The level of aggregation of diseased fruit in the tree canopy is usually dependent on the distance to the inoculum source and the pathogen dispersal mechanism (Sposito *et al.*, 2008). For example, a high frequency of random patterns of diseased plants in a tree or orchard indicate involvement of airborne spores (Shaw and Royle, 1989; Sposito *et al.*, 2008), while a high level of disease aggregation often indicates short distance pathogen dispersal (McCartney and Fitt, 1998). Therefore, the degree of aggregation, the size, shape and orientation of aggregates will provide useful information that can be interpreted in relation to disease spread (Elmer *et al.*, 1998; Xu *et al.*, 2001).

Previous studies have shown that conidia germination and growth of P. macadamiae significantly increased in free water while temperatures above 34°C are lethal to the growth of P. macadamiae (Miles et al., 2010b) However, there is little information concerning the importance of these environmental factors on levels of husk spot observed in the field (Mayers, 1998). The role of asexual spores in long-distance spread and the possible involvement of ascosporic infection in husk spot epidemics have not been examined. In this study, we test the hypothesis that asexual spores of *P. macadamiae* are the only spore type involved in husk spot epidemics. Based on this null hypothesis, we expect diseased fruit to be present in highly aggregated patterns within tree canopies. Alternatively, if spores produced from sexual structures are involved then the diseased fruit will be randomly spaced within the tree canopy. This information may provide improved understanding of the epidemiology of husk spot in husk spot. In particular, the information will provide insight into how the pathogen has been able to spread from Maleny where it was first observed in 1981 to almost all macadamia producing areas and new plantations in south-east Australia. Based on the hypothesis that inoculum production potential in well-managed farms is low, this study tested this hypothesis through recording husk spot disease incidence and severity under two different management systems; fungicide-protected (managed) and unprotected (unmanaged) trees. In addition to the aggregation patterns, we also tested if rain and temperatures act as key environmental factors in husk spot epidemics through linking disease incidence and severity data over time to weather parameters. A better knowledge of the spatial pattern of husk spot and environmental factors influencing disease incidence and disease severity may aid the understanding of the mechanisms by which the disease spreads and under which environmental conditions disease presents itself over time.

### II. Materials and Methods

#### Experimental sites and disease assessments

The development of husk spot epidemics in macadamia trees in managed (fungicideprotected), using routine annual fungicide applications of carbendazim and copper oxide and in unmanaged conditions, without any fungicides, were compared. In order to provide robust conclusions, disease incidence and severity data collected from 2004 to 2007 field trials in cv. A16 in Beerwah, Bundaberg and Bangalow as part of project MC03007 were used (Akinsanmi *et al.*, 2007; 2008).

### Husk spot epidemics models

The rate of increase of husk spot incidence  $(r_i)$ , and husk spot severity  $(r_s)$  in managed and unmanaged treatments were compared by analysis of variance. Combined data of field sites were used in the analyses because its effect was not significant. In order to improve the residual, a number of transformations contained in the generalised linear models of GenStat were fitted to both incidence and severity data and plots of the residuals were examined to determine homogeneity of variance and lack of fit. The models were compared using the coefficient of determination  $(R^2)$  of each model expressed by linear-by-linear model;  $y_i = \alpha + \beta/(1 + \delta x_i) + \varepsilon_i$  where  $\alpha$ ,  $\beta$  and  $\delta$  are linear parameters and  $\varepsilon$  is the error. The fitted values obtained from the model were used in the analyses to determine  $r_i$  and  $r_s$ .

### Influence of climatic factors on husk spot epidemics

The effect of climatic factors on disease incidence and severity was examined separately, using data of the daily minimum temperature (°C) recorded at 09:00 h and maximum temperature recorded at 18:00 h for the previous 24 h period, relative humidity (%) at 09:00 h and rainfall (mm) data which included number of rainy days ( $\geq 0.2$  mm) and total amount between assessment dates, average rainfall per month and accumulated precipitation from anthesis. The effect of each and combined climatic parameters on disease incidence and severity was analysed using the GLM for the all-subset regression link functions, which was used to produce a number of best models among all possible models. Terms with significant probabilities (P < 0.05) in the summary statistics were included in the final model.

### Diseased fruit distribution in the tree canopy

In order to examine if infection of fruit in the tree canopy was a random event or not, the relationship between the observed variance (v) and the expected binomial variance for a random distribution of binary data for husk spot incidence in each sampling quadrat was examined. The binary form of the Taylor's power law (Taylor, 1961; Hughes and Madden, 1992) was used. The relationship was examined for each treatment and year using the linear form [log(v) = log(a) + blog(m)] of the power law with the mean (m) and the estimate the values of *a* and *b* parameters. A random distribution of diseased fruit is inferred by a = b = 1, whereas, there is a constant level of aggregation for all disease incidence values when b = 1 and a > 1. When b > 1, the degree of aggregation a > 1 and b > 1 was tested by 'one-sided' *t*-test (Sposito *et al.*, 2008). Estimates of *a* and *b* were obtained with simple linear regression, and the significance of the relationship between log(v) and log(m) was determined by *F*-test, and the aptness of the model was evaluated by  $R^2$  and the pattern of the residuals plot.

### III. Results and Discussion

#### Progress of husk spot in the tree canopy of managed and unmanaged trees

There was a significant (P < 0.001) difference in disease incidence between the managed and unmanaged epidemics and among the years (P = 0.01), but not among the sampling quadrats, field sites and the interactions of the main effects. On average, at the onset of symptoms  $(I_0)$ , the number of fruit with husk spot symptoms was 38% higher in unmanaged than in the managed trees, while at the final assessment date  $(I_f)$ , the mean difference between unmanaged and managed trees was 57.4%. The mean difference  $(I_f - I_0)$  was 18% for managed and 37% for unmanaged trees. Husk spot disease incidence increased more rapidly in unmanaged than managed trees (Table 2.9). Except for managed epidemics in 2007 where  $r_i = 2.2$  and P = 0.07,  $r_i$  was consistently significantly < 1 in managed and > 1 in unmanaged epidemics (Table 2.9). The  $R^2$  values were generally higher in unmanaged than in managed trees, averaging 89% and 61%, respectively (Table 2.9). Overall, more rapid increases in husk spot incidence occurred in unmanaged trees compared to managed trees. This could be attributed to the initial number of diseased fruit, which is a reflection of the amount of primary inoculum present in the tree canopy. The high  $I_0$  in unmanaged epidemics resulted in higher  $r_i$  than managed epidemics. The mean percent difference in disease incidence between the  $I_f$  and  $I_0$  was higher in the unmanaged than managed epidemics, which indicates that infections were delayed in the managed epidemics due to the fungicide applied. Consequently, abscission of diseased fruit occurred earlier in the unmanaged epidemics than the managed epidemics.

**Table 2.9** Mean of the initial  $(I_0)$  and final  $(I_f)$  husk spot incidence, and parameter estimates from a linear regression of disease incidence on time (number of days between assessment dates) between fungicide-managed and unmanaged macadamia trees in different years

Treatment	Year	$I_0$ (%)	$I_{f}$ (%)	$r_i^{\rm A}$	$t(pr.)^{B}$	Intercept	$R^2(\%)^{\rm C}$	SE <sup>D</sup>
Managed	2004	4.9	12.1	0.3	0.02	4.8	35.5	2.4
Managed	2005	9.8	14.7	0.8	< 0.001	0.1	88.5	1.7
Managed	2006	1.6	9.0	0.3	0.05	-0.7	84.5	1.5
Managed	2007	1.3	54.7	2.2	0.07	1.2	36.0	17.5
Unmanaged	2004	28.4	52.4	1.4	< 0.001	27.0	77.7	4.8
Unmanaged	2005	68.0	76.0	5.1	< 0.001	0.4	98.2	4.4
Unmanaged	2006	31.0	94.0	3.1	0.05	2.6	84.8	15.6
Unmanaged	2007	43.2	97.6	3.4	< 0.00	42.7	96.5	4.3

<sup>A</sup> Rate of disease incidence increase per day.

<sup>B</sup> Probability associated with *t*-test.

<sup>C</sup> Coefficient of determination for goodness of fit.

<sup>D</sup> Standard error of observation.

#### Progress of nut drop with husk spot lesions

Generally, disease severity curves in managed and unmanaged epidemics followed similar wave-like pattern and differences in these patterns between years were not significant. The  $r_s$  values were relatively lower in managed than unmanaged epidemic for the respective years

(Table 2.10). The mean number of diseased fruit that abscised at  $I_0$  was 37% higher in unmanaged than in managed trees, and the difference between unmanaged and managed trees reduced to 5% at  $I_f$  (Table 2.10). On average,  $I_f - I_0$  was 44% for managed and 13% for unmanaged trees (Table 2.10). In unmanaged trees, higher numbers of abscised fruit were diseased at  $I_0$  than at  $I_f$  in the 2005 and 2006 seasons (Table 2.10). The  $R^2$  values were relatively higher in the unmanaged than managed trees with averages of 84% and 73%, respectively (Table 2.10). Unlike disease incidence,  $r_s$  did not appear to increase with  $I_0$ , indicating that other mechanisms not considered in this study contribute to fruit abscission in macadamia. Such mechanisms include limited availability of assimilates (Trueman and Turnbull, 1994), presence of growth regulators (Nagao and Sakai, 1985; Richardson and Dawson, 1993), and fruit removal force (Trueman *et al.*, 2000). This suggests that the fungicides applied in the managed situations possibly prevented infection rather than reducing abscission of diseased fruit.

**Table 2.10** Mean of the initial  $(I_0)$  and final  $(I_f)$  husk spot severity, and parameter estimates from a linear regression of disease severity on time (number of days between assessment dates) between fungicide-managed and unmanaged macadamia trees in different years

Treatment	Year	$I_0$ (%)	<i>I</i> <sub>f</sub> (%)	$r_s^A$	$t(pr.)^{B}$	Intercept	$R^{2}(\%)^{C}$	SE <sup>D</sup>
Managed	2004	47.4	80.0	3.4	< 0.001	19.2	68.3	14.8
Managed	2005	61.1	86.3	3.7	< 0.001	16.5	76.5	12.8
Managed	2006	0.0	100.0	2.2	0.083	24.8	76.2	14.2
Managed	2007	75.7	95.5	3.5	0.006	1.2	69.4	14.9
Unmanaged	2004	67.4	97.8	4.4	< 0.001	25.1	92.7	8.2
Unmanaged	2005	87.5	87.4	4.8	< 0.001	22.6	98.9	3.2
Unmanaged	2006	100.0	95.3	2.2	0.196	41.8	46.9	24.4
Unmanaged	2007	75.7	100.0	4.7	< 0.001	23.1	96.8	5.5

<sup>A</sup> Rate of disease incidence increase per day.

<sup>B</sup> Probability associated with *t*-test

<sup>C</sup>Coefficient of determination for goodness of fit.

<sup>D</sup> Standard error of observation.

#### Influence of climatic factors on husk spot

The generalised linear models of all possible subsets of the climatic factors; mean minimum and maximum temperatures, relative humidity, number of rainy days, accumulated precipitation from anthesis and amount of rainfall showed that number of rainy days was the most significant single climatic factor influencing husk spot incidence. Number of rainy days accounted for 15% of the total variance after the effect of the treatments (managed and unmanaged), which accounted for 46% of the variance. Other significant model subsets after number of rainy days ( $R^2 = 74\%$ ) containing one term were total amount of rainfall ( $R^2 =$ 70%) and accumulated precipitation from anthesis ( $R^2 = 67\%$ ). The  $R^2$  of the significant subset containing multiple terms with number of rainy days marginally improved the  $R^2$ values. Models containing a single factor/term showed that accumulated precipitation from anthesis ( $R^2 = 47\%$ ) followed by the amount of rainfall between sampling dates ( $R^2 = 45\%$ ) and number of rainy days ( $R^2 = 44\%$ ) produced significant effects on husk spot severity. The improvements of the  $R^2$  values of pairings of the terms were generally minimal, with the highest significant improvement with  $R^2 = 55\%$  occurred in the combination of amount of rainfall, accumulated precipitation and maximum mean temperature. Therefore, number of rainy days is the single most significant factor influencing husk spot incidence. This observation corroborates the predominance of rain-splash spore dispersal in husk spot epidemics. This indicates that frequent rain periods will heighten husk spot infection and consequently disease incidence. It appears the effect of temperatures on husk spot epidemics is minimal and is only important in conjunction with frequent rain days or prolonged wet conditions.

#### Diseased fruit distribution in the tree canopy

The relationship between the observed and expected variance was highly significant (P < 0.01) in all years. For both managed and unmanaged husk spot epidemics, there were significant relationships between the variance (v) and mean (m). In the managed epidemics, b was significantly (P < 0.05) > 1 in all the years, suggesting aggregation and the degree of aggregation increased with increasing disease incidence mean (Table 2.11). In addition, the relationship between the observed and the expected binomial variance in the managed epidemics; in 2005 and 2007 aggregation increased with increased with incidence mean (b > 1), in 2006 a near constant level of aggregation and in 2004 a constant random distribution (Table 2.11). These situations expressed in the relationship between the observed variance is almost constant forming a cluster of points around 1.2 (Fig. 2.11b). In the combined data of the managed and unmanaged epidemics the estimates of a were significantly > 1, indicating aggregation and the overall estimate of b was significantly > 1 (Table 2.11).

**Table 2.11** Parameters of the linear regression between the observed variance (v) and the random variance expressed as the mean (m) of the Taylor's power law [log(v) = log(a) + blog(m)] of husk spot incidence in managed and unmanaged trees in different years

Treatment	Year	$I_m \left(\%\right)^{\mathrm{A}}$	$a^{\mathrm{B}}$	$SE(a)^{C}$	$b^{\mathrm{B}}$	$SE(b)^{C}$	$R^{2}(\%)$
Managed	2004	7.9	0.27	0.34	1.69*	0.37	72
Managed	2005	8.0	0.71*	0.31	1.43*	0.32	70
Managed	2006	3.5	0.22	0.20	1.86*	0.32	81
Managed	2007	25.0	1.33*	0.62	1.10*	0.44	39
Unmanaged	2004	42.9	0.77	1.18	1.20	0.72	18
Unmanaged	2005	53.5	0.92	0.48	1.29*	0.28	72
Unmanaged	2006	45.7	2.67*	0.41	0.35	0.25	11
Unmanaged	2007	79.5	15.49*	3.10	6.65*	1.63	66
Managed mean		11.1	0.21*	0.09	1.87*	0.09	93
Unmanaged mean		55.4	3.31*	0.65	0.18	0.37	-
Overall mean		33.2	0.64*	0.11	1.38*	0.08	83

<sup>A</sup> Mean of disease incidence.

<sup>B</sup> Parameter estimate followed by \* is significant at P < 0.05 based on the *t*-test with  $H_o$ : a > 1 and b > 1.

<sup>C</sup> Standard error of the parameter.



**Fig. 2.11** Variance-mean relationships of husk spot epidemics. The disease incidence data from 2004 to 2007 were pooled for managed (a) and unmanaged (b) separately and used to estimate the general relationships between log (variance) and log (mean of incidence). The fitted lines correspond to linear relationship between respective variance and mean of zero intercept.

#### IV. Conclusions

Random distribution patterns of diseased plants were indicative of airborne ascospores while aggregated patterns of diseased trees were used to conclude that rain-splashed asexual spores were responsible for disease epidemics. The predicted values showed that degree of aggregation increased with an increase of the mean incidence. The observed year-to-year variations, especially in the unmanaged epidemics, were the result of differences in climatic factors and timing of specific disease management practices in the orchards, which influenced the initial level of infection  $(I_o)$ . Our results indicate that heterogeneity (i.e. disease aggregation) different from that expected for a random pattern occurred in both managed and unmanaged trees. This indicates minimal, if any, involvement of *P. macadamiae* sexual spores in husk spot epidemics in macadamia orchards in Australia.

Abscission of diseased fruit may have contributed to deviation of the spatial pattern from the random distribution (Waggoner and Rich, 1981), however, it is unlikely that it caused aggregation, because the difference between  $I_f$  and  $I_o$  was higher in unmanaged than managed epidemics for disease incidence. This indicates that at  $I_f$ , high numbers of diseased fruit remained in the tree. Therefore, aggregation could have arisen from the dispersal of the asexual spores from the erumpent stromata on the diseased husk to new fruit within close proximity. Generally, short distance spread of other fungi belonging to *Pseudocercospora* genus are attributed to rain-splashed asexual spores, and in fungi where the sexual state is known, long-distance spread is mostly caused by ascospores (McCartney and Fitt, 1998). In order to confirm if one or more genotypes of *P. macadamiae* is responsible for the husk spot epidemics in southeast Australia, comparison of different strains from several macadamia orchards would provide additional evidence of the type of spores involved.

### V. Communications and Extension of Research Outcomes

• Publications and presentations

Akinsanmi, O. A., and Drenth, A. (2010). Spatial pattern and the effects of climatic factors on husk spot disease in macadamia. Australasian Plant Pathology 39: 125-131.

### 2.1.2.2. Husk spot inoculum production and potential management using ethephon

#### I. Introduction

Spores produced on the macadamia husk surface are considered to be the major source of inoculum (Miles *et al.*, 2010a) from where spores are rain-splash dispersed onto developing fruit (Akinsanmi and Drenth, 2010). However, there is no information on whether *P. macadamiae* conidia are produced on any other parts of the macadamia including the leaf litter or other hosts. Although the contributions of sticktights with husk spot lesions (Fig. 2.12 a) to husk spot epidemic have been highlighted (Miles *et al.*, 2010a), the role of new infections on green husk to inoculum production during the cropping season has not been fully evaluated. In particular, whether out-of-season diseased fruit in an April-May flowering contribute more to husk spot epidemics than resident sticktights has not been determined. Husk spot symptoms are evident in August-September on fruit produced in the out-of-season April-May flowering (Mayers, 1996). This period coincides with the flowering and fruit set period of the main production season in August-July. At times, irregular and successive flowering may cause old and new fruits to overlap in the tree canopy (Fig. 2.12 b).

Ideally, flowering in Australian macadamia orchards is strongly seasonal, usually occurring during a brief period in August or September (Trueman *et al.*, 2000) and differences in flowering times are typically no greater than several weeks between cultivars and only a few days between trees within a cultivar (Moncur *et al.*, 1985; Stephenson and Trochoulias, 1994). Irregular flowering coupled with retention of diseased fruit from previous season in the tree canopy may enhance the perpetuation of *P. macadamiae* conidia throughout the year. A better understanding of the dynamics of *P. macadamiae* conidial production during the year would allow strategic estimates of disease risk to be derived for different periods of the growing season (Scherm *et al.*, 2008).

The effect of environmental conditions on *P. macadamiae* and husk spot incidence is significant. Moisture and temperature were found to be the key environmental factors affecting *in vitro* growth of *P. macadamiae* (Miles *et al.*, 2010b), while the number of rainy days is the single most important factor influencing disease incidence during the main production period (Akinsanmi and Drenth, 2010). It is unknown whether the time course of conidial production on husks is cultivar dependent (i.e. whether sporulation occurs earlier on early-blooming cultivars) or whether it depends solely on time of the year or environmental conditions.

An important question concerns the origin of sticktights. It is assumed that sticktights originate from failure of fruit abscission. However, it is quite possible that sticktights originate from stress on the plant during maturation giving rise to splitting of the husk at the final expansion phase. Several studies have considered the use of ethephon [(2-chloroethyl) phosphonic acid] or ethrel to promote fruit drop in macadamia (Nagao and Sakai, 1988; Nagao and Sakai, 1990; Richardson and Dawson, 1993; Trueman *et al.*, 2002). It is assumed that the use of ethrel to promote fruit drop will also reduce the number of sticktights. Ability to reduce or remove sticktights in the tree canopy may reduce the amount of husk spot inoculum in the tree canopy. Removal of sticktights through the use of ethrel, therefore, may also benefit husk spot disease control and thus improve the profitability for growers which have varieties with sticktights in their plantations.



**Fig. 2.12** Sticktights with visible husk spot lesion in the tree canopy (a) and overlapping of mature fruit and flowering production in macadamia (b).

Initial investigations as reported in the project MC03007 have revealed that the efficacy of ethrel is influenced by environmental conditions and ethrel does not have any effects on dried sticktights (Akinsanmi and Drenth, 2007). However, the amount of sticktights formed was reduced when water is not limiting (Akinsanmi and Drenth, 2007). Therefore, in this study we further tested the hypothesis that hot/dry weather in January/February gives rise to water stress and that this may lead to splitting of the husk tissue giving rise to more sticktights in the tree canopy.

Therefore, the objective of this research was to determine if *P. macadamiae* conidial production on old and new husks was seasonal in relation to the host phenology and environmental conditions. The relative presence of *P. macadamiae* conidia in other plant parts was evaluated and the effect of routine management practices with fungicide spray applications on dynamics of husk spot inoculum was determined. The effect of spray applications of ethrel to reduce the amount of sticktights formed in the season was examined in commercial orchard as a useful husk spot management tool. The information obtained from the studies would provide relevance of routine husk spot management on disease epidemics.

### II. Materials and Methods

#### Field site and sources of husk spot conidia in macadamia

In order to determine if other plant parts other than the husk produce and contribute to husk spot inoculum in the orchard, samples were obtained from macadamia twigs, leaves and husks. Dried and living (green) samples of each plant part were sampled. Samples were collected every month for 2 years (2007-2008) from cv. A16 and A38. The samples were obtained from bearing (10 years old) macadamia trees in Beerwah (26°85'S, 152°95'E), from trees that were sprayed annually with fungicides and those that were unsprayed with any fungicides. At least, 10 samples were obtained for each plant part per month. Leaf samples included those with or without any visible spots or lesions arbitrarily obtained from the tree

canopy and from litter under the tree. Green and old (sticktights) husks included those with visible husk spot lesions, while approximately five 200-mm dried and healthy (living) twigs were sampled from the tree canopy. The phenology stage of the tree at each sampling date was recorded.

Samples were placed in plastic bags and transported to the laboratory. The length of each twig, the surface area of each leaf, number of husk spot lesions on the surface of each fruit were recorded. The samples were misted with sterile distilled water, kept in sealable plastic bags and incubated in moist conditions at 25°C for 48 h. This process was adopted to allow determination of the number of conidia that could be produced at any given time during the season provided environmental conditions for sporulation were not limiting (Scherm *et al.*, 2008). Following incubation, the samples were washed in 50 mL of distilled water containing 0.05% Tween 80, filtered through two layers of cheesecloth, concentrated by centrifugation at 3,170 × g for 10 min. and conidial concentrations in four subsamples were determined with the aid of a haemocytometer. Conidia of *P. macadamiae* were distinguished and were counted from those of other species based on morphological characters, the conidia size and shape (Beilharz *et al.*, 2003).

#### Seasonal dynamics of husk spot inoculum in macadamia orchard

Samples of dried and green husks with husk spot lesion were obtained monthly from commercial orchard and processed to obtain *P. macadamaie* conidia as described prior. The amount of *P. macadamiae* conidia obtained at each sampling date was related to the prevailing weather conditions, tree phenology stage and management practice. Conidial concentrations of husk samples were expressed as numbers of conidia per number of lesions on the surface of the husk. While conidial concentrations were expressed as per unit area of each leaf and twig sample. Leaves were assumed to be elliptical in shape and the area was calculated as described by Mondal et al. (2007) and the surface areas of the twigs were calculated using the formula of area of a cylinder. Data were subjected to ANOVA and significant means were compared using Fishers' protected LSD.

### Husk spot management with ethrel

Field trials aimed at getting a better understanding of the factors involved in the formation of sticktights were established in a commercial orchard in Gympie in the 2006-07 season and repeated in the 2007-08 season. Treatments evaluated the effect of drought/water stress on the splitting of husk tissue and the formation of sticktights and the effect of spray application of ethrel on the retention of sticktights in the tree canopy. A row of 35 trees of cv. A16 was used and all trees were exposed to natural growing conditions, but in order to prevent water stress 15 trees were irrigated in dry conditions.

Data were obtained from 10 trees of both (irrigated and non-irrigated) treatments. However, half of the trees in each treatment were treated with ethrel, applied at 1.35 mL/L concentration using commercial spraying rig during harvest periods in April each year. The amount of sticktights at the start and end of each production season was recorded in each of the four plots. Plots were separated by at least a tree. In order to stablise variance, data were square-root transformed before analysis using the Generalized Linear Models in GenStat. Significant means were separated and compared using Fishers' protected LSD.

# III. Results and Discussion

#### Sources of husk spot inoculum in the tree canopy

Conidia of *P. macadamiae* were mostly recovered from both dried and green husks with husk spot lesion (Fig. 2.13). The conidia counts per unit area from the twig and leaf samples were near zero. Therefore, data from twigs and leaves were not included in the subsequent analyses. The cause of the low number of conidia recovered from twig and leaf are unknown. It is most likely that the conidia were deposited on the plant parts due to their close proximity to diseased husk which is the main source of inoculum in the tree canopy.



Fig. 2.13 Distribution of *Pseudocercospora macadamiae* conidial concentration on different macadamia plant parts in cvs. A16 (left) and A38 (right).

### Seasonal dynamics of husk spot inoculum production in the tree canopy

Husk spot inoculum was obtained from diseased husk throughout the season (Fig. 2.14). This indicates that developing fruit may be infected at any time of the growing seasons. This observation is congruent to field observations where fruits from out-of-season flowering show husk spot symptoms in August-September. The curves describing conidial production potential in relation to calendar date varied between years (Fig. 2.14). High conidia counts were recovered at the start of the main production season in spring (August-September) than other times on dried husks. Other peaks were observed between January and February where nuts were near maturity. This period coincides with timing of husk spot symptoms expression in the field (Akinsanmi *et al.*, 2007).

The pattern of inoculum count during the season appears not to be dependent on the weather conditions (Fig. 2.15). This could be as a result of the incubation protocol used to obtain the conidia in this study. This process determined the conidial production potential in which is the number of conidia that could be produced at any given time during the season provided environmental conditions for sporulation were not limiting (Scherm *et al.*, 2008). In contrast, if conidial numbers had been assessed directly on husks collected from the field without incubation, conidial counts would likely have been dominated by microclimatic rather than

seasonal effects (e.g. sporulation could have been suppressed by a sequence of consecutive dry days or conidia could have been removed by wash-off during a heavy rain) (Scherm *et al.*, 2008). Overall, the amount of conidia obtained was partly influenced by other factors including the management practices such as fungicide spray applications and harvesting.

The amount of conidia produced reduced by 67% to 86% after fungicide spray applications of carbendazim and copper mixture, in early October at the beginning of the main production seasons. Consequently, this resulted in a significant treatment effect on conidial concentrations (Table 2.12). Overall, the amount of conidia obtained from dried husk was always significantly higher, by up to 89% than green husk. Irrespective of the husk type, there was no significant interaction with the treatment (Table 2.12). This indicates that the conidial production potential was similar for the husk types irrespective of fungicide treatment. The notable effect of the fungicide application support the results of Miles et al. (2010a) where husk spot disease was observed to increase with the addition of diseased sticktights and reduced with the removal of sticktight in the tree canopy. Although there was significant (P > 0.165) (Table 2.12). This indicates the effect of the fungicide treatment was similar in both cultivars and is not influenced by husk type with P > 0.27 for the treatment × husk type × cultivar (Table 2.12).



**Fig. 2.14** Amount of *Pseudocercospora macadamiae* conidia obtained from dried and green husks with husk spot lesion throughout the seasons.



Fig. 2.15 Weather conditions during the assessments period of *Pseudocercospora* macadamiae conidial production.

Table 2.12 Analy	ysis of husk spot c	conidial concentrat	tions from dried and	green husk obtained
from fungicide-tr	reated and untreate	d trees of cultivar	s A16 and A38.	

							Le	sion
		Conic	dia/mL	Uni	t area		nu	mber
Source of variation	d.f.	v.r	F pr.	v.r	F pr.	<b>V.</b>	r	F pr.
Treatment	1	6.81	0.010	3.22	0.074	16.9	90	< 0.001
Husk type	1	5.53	0.020	10.02	0.002	6.	36	0.012
Cultivar	1	10.5	0.001	8.13	0.005	11.4	42	< 0.001
Treatment × husk type	1	0.15	0.697	0.39	0.534	0.4	17	0.493
Treatment × cultivar	1	0.09	0.762	0.61	0.435	1.9	94	0.165
Husk type × cultivar	1	6.62	0.011	8.91	0.003	4.0	)9	0.044
Treatment $\times$ husk type $\times$ cultivar	1	1.22	0.271	0.91	0.341	0.:	55	0.459
Residual	216							

v.r. indicates variance ratio; F pr. indicates probability Fisher's value.

#### Husk spot management with ethrel

Results of the field trials revealed variation in efficiency of ethrel to fruit drop. Spay application of ethrel significantly (P = 0.039) influenced the amount of sticktights formed or retained in the tree canopy and explained over 45% of the variance observed in the trial (Table 2.13). However, a significant number of sticktights still remain in the tree canopy which may carry-over inoculum between growing seasons.

It has also been reported that ethrel application causes undesirable effect such as severe leaf abscission (Kadman and Bental, 1983; Trochoulias, 1986; Richardson and Dawson, 1993; Trueman *et al.*, 2002). In addition, several factors have been reported to influence the efficacy of ethrel spray applications in macadamia (Stephenson and Gallagher, 1987a; Nagao and Sakai, 1988; Richardson and Dawson, 1993; McConchie, 2005). Year-to-year variation in rainfall amount and distribution and temperatures, locations, cultivars and timing of spray application are critical to effective accelerated fruit drop by ethrel. A combination of ethrel spray application and mechanical tree shaker has been reported to significantly accelerate fruit drop in macadamia compared to either of the methods alone (Trueman *et al.*, 2002). Reduced removal force of the green fruits has been attributed to sensitivity of macadamia fruit to ethrel (Trueman, 2003), but there is none or less effective uptake of ethrel by dried sticktights. Therefore, the combined use of tree shaker and ethrel may further accelerate the amount of both green (new) and dried (old) sticktights removed from the tree canopy.

**Table 2.13** Analysis of effect of irrigation to reduce water stress, ethrel spray applications in April on amount of sticktights retained at the end of the 2006 and 2008 production seasons in Gympie.

Source of variation	d.f.	v.r.	F pr.
Season	1	0.91	0.359
Irrigation	1	1.54	0.239
Ethrel spray	1	5.38	0.039
Season $\times$ irrigation	1	0.11	0.742
Season $\times$ ethrel spray	1	0.19	0.670
Irrigation $\times$ ethrel spray	1	1.23	0.289
Season $\times$ irrigation $\times$ ethrel spray	1	1.59	0.232
Residual	12		

v.r. indicates variance ratio; F pr. indicates probability Fisher's value.

### IV. Conclusions

Our results showed that knowledge of host tree phenology is critical to obtain estimates of disease risk based on the predictable seasonal pattern of conidial production potential from diseased husk within the tree canopy. Propensity of inoculum potential to increase throughout the season is due to the effect of climatic variation. The actual day-to-day infection risk and the seasonal risk estimates will be affected by short-term microclimatic variations and the host phenology. In order to derive these estimates of disease risk conidia dynamics should be considered in terms of relative rather than absolute conidial numbers during the season, based on the prevalence of the source of inoculum (diseased husks) in the tree canopy. This is because the actual conidial numbers may differ considerably at any given time. Absolute conidial numbers probably are of limited practical importance which is dependent on the overall inoculum potential in the orchard. In well-managed commercial orchards, with low levels of sticktights and routine use of fungicide spray applications that reduce new infections, it is expected that these orchards have low inoculum potential.

The ability of *P. macadamiae* to persist in sticktights in the tree canopy, from where inoculum is continually produced and the pathogen may survive on sticktights for several

years (Miles *et al.*, 2009), makes reduction or prevention of sticktights, in particular sticktights with husk spot lesion in the tree canopy, a critical management tool. Having a better understanding of the formation of sticktights may give rise to growing practices to prevent this from happening and the resulting information maybe be used in the breeding program to screen against varieties which have a tendency to form sticktights. However, due to several factors that influence efficacy of ethrel and the undesirable effects, further research is still needed to evaluate how sticktights can be removed from the tree canopy. Concerns that future yield and kernel quality may be adversely affected also need to be investiaged before extensive use of ethrel is recommended.

Removal of infective host residues in commercial crops is generally a labour-intensive task. In commercial situation, the efficiency of removal of sticktights from the tree canopy and the resultant reduction in disease incidence must justify the associated costs. We have observed that the removal of sticktights by a single person took up to 30 min per tree (Miles *et al.*, 2010a). Once the husks split and senescence, the abscission process without tree shaker will occur over a protracted period regardless of the application of ethephon. Planting of cultivars not prone to sticktights may be more beneficial and the most practical approach to managing sticktights in orchards and may provide additional benefits such as reduced incidence of other diseases and pests such as anthracnose caused by *Colletotrichum gloeosporiodes* and kernel grub.

# V. Communications and Extension of Research Outcomes

- Publications and presentations
- 1. Akinsanmi OA & AMS Crop protection IRG- June 2011 Husk spot management information sheet <u>Australian Macadamia Society Ltd. News Bulletin</u> Vol 39 (3).
- 2. Miles, A. K., Akinsanmi, O. A., Aitken, E. A. B., and Drenth, A. (2010). Source of *Pseudocercospora macadamiae* inoculum in macadamia trees and its use for characterising husk spot susceptibility in the field. Crop Protection 29: 1347-1353.

### 2.1.2.3. Cultural practices to reduce yield loss due to husk spot

### I. Introduction

How sticktights are formed is not well understood, but it is hypothesized to be related to disruption of the abscission layer at the base of the macadamia fruit pedicel (Hardner *et al.*, 2009). This could be the result of various factors including hormone imbalances, photoperiod, temperature, moisture stress, and diseases (Hardner *et al.* 2009; Nagao and Hirae 1992; Taylor and Whitelaw 2001). Previous studies in project MC03007 have revealed that optimal management of water relation is critical for reducing sticktights formation. Efforts have been made to determine if ethylene-generating compound such as ethephon (2-chloroethyl) phosphonic acid may reduce sticktights in the tree canopy. However, these have not been successful. A major challenge is that effectiveness of ethephon is influenced by several factors including timing of application, composition of spray mix, weather and cultivar interaction (Richardson and Dawson, 1993).

McConchie (2005) reported that *P. macadamiae* produces abscisic acid in culture and in infected macadamia husk tissue, however, the level required for fruit abscission was not determined. It has been estimated that it takes 3 days between induction of abscission and actual nut drop in macadamia (Nagao and Sakai, 1985). An increase in ethylene concentration was reported to precede the highest relative rate of abscission in macadamia (Sakai and Nagao, 1985), but ethylene may not be the only endogenous regulator influencing abscission in macadamia (Sakai and Nagao, 1985). Therefore, irrespective of the stage of growth fruit abscission may occur when all the contributing factors are optimal. For instance, when fruit development is impaired by poor tree nutrition, water stress and high temperatures (Trueman and Turnbull, 1994), premature fruit abscission could be accentuated. Conversely, if conditions for good nut development prevailed, fruit abscission before 'normal' harvest periods in March may not translate to actual yield loss.

Fruit abscission before the 'normal' harvest period does not always translate to eventual yield loss at the end of the seaon (Akinsanmi *et al.*, 2008). This could be due to a compensation effect on the remaining nuts for the amount of fruit that abscised at the early growth stage (Tobin *et al.*, 1993; Tobin *et al.*, 1997). However, if higher levels of disease-induced fruit abscission occurred very early in the season before full nut maturation, significant yield loss may occur, but, if nut maturity is reached before the early abscission, yield loss may be averted.

The common practice is to start harvesting based on a calendar based process from mid-March each year. Therefore, nut drop before this time regardless of its maturity is cleaned up or mulched as waste. Considering this, we hypothesize that harvesting process based on stage of nut maturation can significantly reduce the amount of yield and economic loss in macadamia compared to a calendar based at start of the harvest. Therefore, the objective of this study was to determine if routine monitoring of nut maturation with a view of starting harvest earlier than the usual practice may reduce impact of husk spot severity (proportion of abscised fruit with visible husk spot lesion) that otherwise would translate to yield loss.

# II. Materials and Methods

# Source of data and preharvest yield (loss)

In order to determine if the start of harvesting based on the calendar as commonly practiced in the industry is the ideal practice rather than start of the harvest based on actual kernel maturity, data obtained from a series of field trials including project MC03007 on cv. A16 at different locations between the 2004 and the 2011 seasons were analysed. All fruits that dropped from each tree between late January and early March were harvested every 2 weeks and recorded as preharvest yield. At each harvest, the proportion of fruit with husk spot lesions, NIS weights and maturity were recorded separately for the untreated and fungicide treated trees. Average of kernel recovery of 20% is used to calculate the value of preharvest fruit drop.

# Harvest yield

The harvest yield was determined by harvesting fruits monthly starting from mid-March (start of normal harvest period). All fruits were harvested from the ground for each treatment and de-husked separately using commercial de-huskers that exclude small non-commercial sized nuts (<15 mm diameter). NIS weights were recorded at each harvest and the harvest yield per treatment was determined as the total NIS weight harvested from mid-March until the end of fruit drop (July).

# Quality assessment

Kernel maturity was determined using a standard flotation method (Mason and Wills, 1983) About 100 dried nuts from each plot were cracked using a table-top cracking machine from which the percentage of immature kernels containing oil levels below 72% was calculated. Kernel quality parameters were assessed according to the AMS kernel assessment guidelines from the nuts dried in a fan-forced laboratory oven for 2 days each at 38°C, 45°C and 55°C, to about 1.5% moisture content. Kernel recovery (%) and quality assessment data were used to determine the value of the NIS harvested using \$2.50 / NIS kg at kernel recovery of 33% with maximum unsound kernel of 3.5%. The price is based on 10 years industry average.

# III. Results and Discussion

Macadamia cultivars require multiple harvests from the orchard floor after natural abscission of fruit. This is despite the fact that most fruits had already reached maximum oil content in March (Trueman *et al.*, 2000), well before peaks of natural abscission. This appears to influence the common timing of harvest starting in Mid-march. However, this study revealed that the nut drop pattern and the proportion of nut drop per harvest round varied with the management practice. Application of fungicide significantly delayed nut drop compared to the untreated controls (Fig. 2.16 - Fig. 2.19).

Although the number of harvest rounds varies depending on nut drop patterns, it has been reported in several cultivars from the major growing regions of Australia that there was no increase in kernel oil content after March (Trueman *et al.*, 2000). Kernel quality of preharvest fruit increases with time from January to March, but commercially acceptable quality was

attained before March. Hence, kernel quality of the nut that dropped before this period should be monitored. It has been suggested that large variations between cultivars in the timing of kernel maturation and subsequent drop are either differences in the activation of the biochemical pathways that prepare the fruit for abscission or the formation of the receptors that trigger the abscission process (McConchie, 2005). These differences may become significant with husk spot infection, where large amount of fruit may abscise at the preharvest period in cultivars that are sensitive to changes in biochemical processes for fruit abscission. *Pseudocercospora* infection may trigger cause biochemical changes for fruit abscission in macadamia (McConchie, 2005).

Using an average of 20% kernel recovery for premature fruit abscission, this study showed that the value of yield loss due to delay in start of harvest may be significant (Fig. 2.19) when decision to start harvesting is based on pre-conceived calendar period of mid March. Due to the nature of husk spot infection, preharvest fruit abscission may be significantly accelerated, resulting in 20-40% fruit drop before mid-March, depending on the season.



**Fig. 2.16** Cumulative percentage of total nut harvested from late January (15<sup>th</sup>-30<sup>th</sup>) to July each year from cv. A16 trees with (a) no fungicide spray applications (untreated), (b) spray applications with tank mix of SpinFlo+copper, and (c) spray applications with Cabrio only in the Northern Rivers macadamia production area (Alstonville and Bangalow). Vertical dotted line indicates most common start of harvesting in the industry.



**Fig. 2.17** Cumulative percentage of total nut harvested from late January (15<sup>th</sup>-30<sup>th</sup>) to July each year from cv. A16 trees with (a) no fungicide spray applications (untreated), (b) spray applications with tank mix of SpinFlo+copper, and (c) spray applications with Cabrio only in the Sunshine Coast macadamia production area (Beerwah). Vertical dotted line indicates most common start of harvesting in the industry.



**Fig. 2.18** Cumulative percentage of total nut harvested from late January (15<sup>th</sup>-30<sup>th</sup>) to July each year from cv. A16 trees with (a) no fungicide spray applications (untreated), (b) spray applications with tank mix of SpinFlo+copper, and (c) spray applications with Cabrio only in Bundaberg macadamia production. Vertical dotted line indicates most common start of harvesting in the industry.



**Fig. 2.19:** Estimate of value of preharvest yield loss (February – early March) in macadamia if calendar harvesting process starting from mid-March is followed. Assumptions: \$2.50 NIS/kg @ 33 % Sound Kernel Recovery (SKR); average preharvest SKR at 20%.

# IV. Conclusions

Fruit abscission in macadamia is potentially controlled by a complex biochemical processes involving abscisic acid, auxin and ethylene. Accelerated abscission of fruit following Pseudocercospora infection may be due to contribution of the fungus to the abscission process. Potentially, the effect of fungal infection may be negated with application of hormonal compounds such as auxin that inhibits abscission. It has been reported that the percentage of first grade nut in harvest increased from 0% at the end of December to about 95% in February within 50 days (Baigent, 1983). This indicates a rapid increase in the proportion of kernel that had attained 72% oil content in the harvests between January and February (Baigent, 1983). The pattern of fruit development is similar in commercial cultivars and late cultivars such as A16 that flower late and attained maximum kernel oil contents by March even though natural fruit abscission occurs from April to July (McConchie et al., 1996). Therefore, this study provides strong support that the decision to start harvest should not be based on pre-determined or preconceived harvest month, but should be based on the kernel quality of the abscised fruit. In which case, quality of the preharvest fruit drop should be routinely monitored, with the view to start harvest when commercially acceptable kernel recovery is attained.

# V. Communications and Extension of Research Outcomes

• Publications and presentations

Femi Akinsanmi, John Pretorius, Kevin Quinlan, Paul O'Hare and Andre Drenth 2011. Husk spot control with fungicide spray applications: Is it profitable? <u>Australian Macadamia Society</u> <u>Ltd. News Bulletin</u> Vol. 39 (6): 62-66.

# 2.1.3. Varietal Resistance

#### 2.1.3.1. Assessments of macadamia genotypes for resistance to husk spot

#### I. Introduction

P. macadamiae perpetuates in the tree canopy on diseased husks that fail to abscise known as 'sticktights' (Miles et al., 2010a). Occurrence of sticktights varies among macadamia cultivars. How sticktights are formed is not known, but it is hypothesised to be related to disruption of the abscission layer at the base of the macadamia fruit pedicel as the result of various factors such as hormone imbalances, photoperiod, temperature, moisture stress, and diseases (Taylor and Whitelaw, 2001; Hardner et al., 2009). Overlapping of old and new fruits makes pests and disease management difficult, due to lack of a complete break between cropping seasons. This is heightened by the perpetual occurrence of sticktights in the tree canopy which can carryover pests and pathogens. Physical removal of infective materials on a large-scale in commercial crops is generally a labour-intensive task and may not be economical (Johnson and Stockwell, 1998; Marin et al., 2003; Miles et al., 2010a). A more cost-effective sustainable solution is utilising cultivars without sticktights that do not retain infective materials. Information on the relation of sticktights and husk spot severity in macadamia is scanty, and its usefulness as a selection tool in breeding programs is unknown. In this study the hypothesis that the absence of sticktights in the tree canopy is a good phenotypic trait which may be able to be selected for to increase levels of disease resistance in the field was tested.

The development of appropriate methods for the evaluation of plant reactions to infection by pathogens is of paramount importance in breeding varieties with high levels of disease resistance (Infantino *et al.*, 2006). Successful screening for disease resistance is based on several factors including the knowledge of the plant and cropping system and pathogen biology, variability, genetic structure, the host-pathogen interaction and the availability of precise and accurate screening techniques (Infantino *et al.*, 2006). The use of resistant cultivars is widely recognized as the safest, most economical and most effective method for protecting crops from diseases (Johnson and Jellis, 1992). Pathogen entry into host tissue is a critical first step in the infection process. Recent studies of the infection process of *P. macadamiae* on macadamia fruit have shown that the conidia penetrate the pericarp via open stomata (Miles *et al.*, 2009). Observations of the penetration process found no evidence of appressoria formation or direct cuticular penetration, providing further evidence that the only entry point for the pathogen is through the fruit stomata (Miles *et al.*, 2009).

Stomata play an important role in gas exchange and water transpiration between the plant interior and the environment (Melotto *et al.*, 2006). Although commonly associated with the abaxial surface of leaves, stomata are also present on the epidermis of fruits, stems and flowers. Among other natural openings in the aerial part of the plant, stomata dominate in number and therefore represent one of the most important routes for entry of foliar pathogens (Melotto *et al.*, 2006; Zeng *et al.*, 2010). In certain tree crops such as sweet cherry, fruit stomatal abundance depends on genotype and is determined in response to environmental factors such as altitude, water availability,  $CO_2$ , and light (Peschel *et al.*, 2003).

In macadamia, there is no information on variability in fruit stomatal abundance and if the lack of stomata in the fruit surface serves as a natural resistance mechanism to husk spot. The use of resistant cultivars is widely recognized as the safest, most economical and most

effective method for protecting crops from diseases (Johnson and Jellis, 1992). The aim of the study was to test if severity of husk spot is correlated to fruit stomatal abundance. If there is a strong relationship between the fruit stomatal abundance and husk spot severity, this would serve as a useful trait to predict husk spot resistance/susceptibility of macadamia in breeding programs.

### II. Materials and Methods

#### Macadamia genotypes

Macadamia genotypes consisted of bearing trees (at least 10 years old) that are commercially grown (cultivars); seedling trees derived from cross-breeding (progeny); and wild macadamia germplasm. Data were obtained from the germplasm and progeny trials in Bundaberg and Alstonville. Where possible, three replicate trees were used, giving a total of 230 macadamia trees of 19 cultivars, 56 germplasm accessions and 40 progeny (Table 2.14).

### Prevalence of sticktights in tree canopy as a predictor of husk spot intensity

In order to test if the prevalence of sticktights in the tree canopy as a measure of potential inoculum pressure, is an effective phenotypic trait to predict levels of husk spot intensity (incidence, severity and lesion number), macadamia trees were assessed for prevalence of sticktights and husk spot intensity over three (2008-09, 2009-10 and 2010-11) production seasons. The prevalence of sticktights was assessed using a rating scale (Miles et al., 2010a) based on the number and distribution of sticktights in the tree canopy: 0 = clean and no sticktights,  $1 = \langle 5 \rangle$  pieces of sticktights, 2 = 5-10 sticktights,  $3 = \rangle 10$  sticktights and well distributed within canopy, 4 = few (<5) clusters of sticktights and 5 = several (>5) clusters of sticktights and well distributed within canopy. Prevalence data were collected twice a year at early (October-November) and mid (March-April). Disease incidence and severity were assessed in March and June. Lesion number was quantified as the average number of husk spot lesions per fruit on abscised diseased fruit per tree. In certain macadamia genotypes, disease incidence was recorded as zero at the assessment height upper level (2 m), but few abscised fruits were diseased, thus, provided disease severity and lesion number values. In order to account for this variation, disease incidence data were also treated as a discrete variable with values of either 0 or 1 as husk spot score, corresponding to no fruit from the tree has visual husk spot lesions (disease severity = 0%) or at least one abscised fruit from the tree has visual husk spot lesions (disease severity > 0%), respectively.

### Examination of stomatal abundance

In order to determine if fruit stomatal abundance can be used as a trait to predict resistance to husk spot, 10 fully expanded fruits of 21 genotypes were obtained from the tree canopy, transferred to the laboratory in moist polythene bags kept on ice. Samples were stored at  $-20^{\circ}$ C until stomatal abundance was quantified. Light microscopy was used to determine the stomatal abundance on sections of the epidermis taken from the middle region of the fruit using methods as previously described (Lux *et al.*, 2005). At least five thin sections were selected from each fruit and the number of stomata visible within the microscope view counted at five different points for each section. Stomatal abundance was recorded relative to the area of view under a 40x objective.

ID	Genotype category	Species group	Location	Selection <sup>A</sup>
Line 2-38	Breeding progeny	<i>M. integrifolia</i> /hybrid	Alstonville	Australia
Line 2-46	Breeding progeny	M. integrifolia/hybrid	Bundaberg	Australia
Line 3-15	Breeding progeny	<i>M. integrifolia</i> /hybrid	Bundaberg	Australia
Line 5-112	Breeding progeny	<i>M. integrifolia</i> /hybrid	Bundaberg	Australia
Line 6-16	Breeding progeny	M. integrifolia/hybrid	Bundaberg	Australia
Line 6-34	Breeding progeny	M. integrifolia/hybrid	Alstonville	Australia
Line 6-73	Breeding progeny	M. integrifolia/hybrid	Bundaberg	Australia
Line 6-79	Breeding progeny	<i>M. integrifolia</i> /hybrid	Bundaberg	Australia
Line 7-74	Breeding progeny	M. integrifolia/hybrid	Bundaberg	Australia
Line 7-83	Breeding progeny	<i>M. integrifolia</i> /hybrid	Bundaberg	Australia
Line 7-109	Breeding progeny	M. integrifolia/hybrid	Bundaberg	Australia
Line 8-87	Breeding progeny	<i>M. integrifolia</i> /hybrid	Bundaberg	Australia
Line 9-23	Breeding progeny	<i>M. integrifolia</i> /hybrid	Alstonville	Australia
Line 9-34	Breeding progeny	M. integrifolia/hybrid	Alstonville	Australia
Line 9-72	Breeding progeny	M. integrifolia/hybrid	Bundaberg	Australia
Line 10-71	Breeding progeny	M. integrifolia/hybrid	Bundaberg	Australia
Line 10-75	Breeding progeny	<i>M. integrifolia</i> /hybrid	Bundaberg	Australia
Line 10-84	Breeding progeny	M. integrifolia/hybrid	Bundaberg	Australia
Line 10-88	Breeding progeny	M. integrifolia/hybrid	Bundaberg	Australia
Line 10-93	Breeding progeny	M. integrifolia/hybrid	Bundaberg	Australia
Line 10-111	Breeding progeny	M. integrifolia/hybrid	Bundaberg	Australia
Line 11-20	Breeding progeny	M. integrifolia/hybrid	Bundaberg	Australia
Line 11-35	Breeding progeny	<i>M. integrifolia</i> /hybrid	Bundaberg	Australia
Line 11-79	Breeding progeny	<i>M. integrifolia</i> /hybrid	Bundaberg	Australia
Line 11-80	Breeding progeny	M. integrifolia/hybrid	Bundaberg	Australia
Line 12-48	Breeding progeny	M. integrifolia/hybrid	Alstonville	Australia
Line 14-25	Breeding progeny	M. integrifolia/hybrid	Bundaberg	Australia
Line 14-80	Breeding progeny	<i>M. integrifolia</i> /hybrid	Bundaberg	Australia
Line 14-90	Breeding progeny	M. integrifolia/hybrid	Bundaberg	Australia
Line 14-93	Breeding progeny	<i>M. integrifolia</i> /hybrid	Bundaberg	Australia
Line 16-20	Breeding progeny	M. integrifolia/hybrid	Alstonville	Australia
Line 16-33	Breeding progeny	<i>M. integrifolia</i> /hybrid	Alstonville	Australia
Line 16-41	Breeding progeny	M. integrifolia/hybrid	Bundaberg	Australia
Line 17-10	Breeding progeny	M. integrifolia/hybrid	Alstonville	Australia
Line 20-10	Breeding progeny	M. integrifolia/hybrid	Alstonville	Australia
Line 32-33	Breeding progeny	M. integrifolia/hybrid	Alstonville	Australia
Line 38-5	Breeding progeny	M. integrifolia/hybrid	Alstonville	Australia
Line 42-17	Breeding progeny	M. integrifolia/hybrid	Alstonville	Australia
Line 43-23	Breeding progeny	M. integrifolia/hybrid	Alstonville	Australia
Line 44-16	Breeding progeny	M. integrifolia/hybrid	Alstonville	Australia
H2	Cultivar	M. integrifolia	Alstonville	Australia
HY	Cultivar	Wild mixed/hybrid	Alstonville	Australia
NG18	Cultivar	M. integrifolia	Alstonville	Australia

Table 214	Source	of mac	odomio	anotypes
1 abic 2.14	Source	or mac	auanna	genotypes.

Cultivar Cultivar Cultivar Cultivar Cultivar Cultivar Cultivar Cultivar	Wild mixed/hybrid <i>M. integrifolia</i> Wild mixed/hybrid Wild mixed/hybrid <i>M. integrifolia</i> /hybrid <i>M. integrifolia</i> /hybrid	Alstonville Alstonville Alstonville Alstonville Both	Australia Australia Australia Australia
Cultivar Cultivar Cultivar Cultivar Cultivar Cultivar Cultivar	<i>M. integrifolia</i> Wild mixed/hybrid Wild mixed/hybrid <i>M. integrifolia</i> /hybrid <i>M. integrifolia</i> /hybrid	Alstonville Alstonville Alstonville Both	Australia Australia Australia
Cultivar Cultivar Cultivar Cultivar Cultivar Cultivar	Wild mixed/hybrid Wild mixed/hybrid <i>M. integrifolia</i> /hybrid <i>M. integrifolia</i> /hybrid	Alstonville Alstonville Both	Australia Australia
Cultivar Cultivar Cultivar Cultivar Cultivar	Wild mixed/hybrid <i>M. integrifolia</i> /hybrid <i>M. integrifolia</i> /hybrid	Alstonville Both	Australia
Cultivar Cultivar Cultivar Cultivar	<i>M. integrifolia</i> /hybrid <i>M. integrifolia</i> /hybrid	Both	
Cultivar Cultivar Cultivar	M. integrifolia/hybrid		HVP Australia
Cultivar Cultivar		Alstonville	HVP Australia
Cultivar	M. integrifolia + M. tetraphylla	Both	HVP Australia
	M. integrifolia/hybrid	Both	Australia
Cultivar	M. integrifolia/hybrid	Both	Hawaii
Cultivar	M. integrifolia	Both	Hawaii
Cultivar	M. integrifolia	Both	Hawaii
Cultivar	M. integrifolia	Both	Hawaii
Cultivar	M. integrifolia	Both	Hawaii
Cultivar	M. integrifolia	Alstonville	Hawaii
Cultivar	M. integrifolia	Both	Hawaii
Cultivar	M. integrifolia/hybrids	Alstonville	Australia
Germplasm	Wild mixed/hybrid	Alstonville	Australia
Germplasm	Wild mixed/hybrid	Alstonville	Australia
Germplasm	Wild <i>M. integrifolia</i>	Alstonville	Australia
Germplasm	<i>M. integrifolia</i> /hybrids	Alstonville	Australia
Germplasm	Wild mixed/hybrid	Alstonville	Australia
Germplasm	M. integrifolia. + M. ternifolia	Alstonville	Australia
Germplasm	M. integrifolia	Alstonville	Australia
Germplasm	M. integrifolia	Alstonville	Australia
Germplasm	Wild mixed/hybrid	Alstonville	Australia
Germplasm	Wild mixed/hybrid	Alstonville	Australia
Germplasm	Wild mixed/hybrid	Alstonville	Australia
Germplasm	Wild mixed/hybrid	Alstonville	Australia
Germplasm	M. tetraphylla	Alstonville	Australia
Germplasm	Wild mixed/hybrid	Alstonville	Australia
Germplasm	Wild mixed/hybrid	Alstonville	Australia
Germplasm	Wild mixed/hybrid	Alstonville	Australia
Germplasm	Wild mixed/hybrid	Alstonville	Australia
Germplasm	Wild <i>M. tetraphylla</i>	Alstonville	Australia
Germplasm	Wild <i>M. tetraphylla</i>	Alstonville	Australia
Germplasm	Wild M. ternifolia	Alstonville	Australia
Germplasm	Wild M. integrifolia	Alstonville	Australia
Germplasm	Wild M. integrifolia	Alstonville	Australia
Germplasm	Wild M integrifolia	Alstonville	Australia
Germplasm	Wild M integrifolia	Alstonville	Australia
Germplasm	Wild M tetranbulla	Alstonville	Australia
Germplasm	Wild mixed/hybrid	Alstonville	Australia
Germplasm	Wild M ternifolia	Alstonville	Australia
Germplaam	Wild mixed/hybrid	Alstonville	Australia
German		Alstonville	Australia
	Cultivar Cultivar Cultivar Cultivar Cultivar Cultivar Cultivar Cultivar Germplasm	CultivarM. integrifoliaCultivarM. integrifoliaCultivarM. integrifoliaCultivarM. integrifoliaCultivarM. integrifoliaCultivarM. integrifoliaCultivarM. integrifolia/hybridsGermplasmWild mixed/hybridGermplasmWild mixed/hybridGermplasmWild mixed/hybridGermplasmWild mixed/hybridGermplasmM. integrifolia/hybridsGermplasmM. integrifoliaGermplasmM. integrifoliaGermplasmM. integrifoliaGermplasmM. integrifoliaGermplasmM. integrifoliaGermplasmM. integrifoliaGermplasmWild mixed/hybridGermplasmWild mixed/hybridGermplasmWild mixed/hybridGermplasmWild mixed/hybridGermplasmWild mixed/hybridGermplasmWild mixed/hybridGermplasmWild mixed/hybridGermplasmWild mixed/hybridGermplasmWild mixed/hybridGermplasmWild M. tetraphyllaGermplasmWild M. tetraphyllaGermplasmWild M. integrifoliaGermplasmWild M. integrifoliaGerm	CultivarM. integrifoliaBothCultivarM. integrifoliaBothCultivarM. integrifoliaBothCultivarM. integrifoliaBothCultivarM. integrifoliaBothCultivarM. integrifoliaBothCultivarM. integrifoliaBothCultivarM. integrifolia/hybridsAlstonvilleGermplasmWild mixed/hybridAlstonvilleGermplasmWild mixed/hybridAlstonvilleGermplasmWild M. integrifoliaAlstonvilleGermplasmM. integrifolia/hybridsAlstonvilleGermplasmM. integrifolia/hybridsAlstonvilleGermplasmM. integrifolia. + M. ternifoliaAlstonvilleGermplasmM. integrifoliaAlstonvilleGermplasmM. integrifoliaAlstonvilleGermplasmM. integrifoliaAlstonvilleGermplasmWild mixed/hybridAlstonvilleGermplasmWild mixed/hybridAlstonvilleGermplasmWild mixed/hybridAlstonvilleGermplasmWild mixed/hybridAlstonvilleGermplasmWild mixed/hybridAlstonvilleGermplasmWild mixed/hybridAlstonvilleGermplasmWild M. tetraphyllaAlstonvilleGermplasmWild M. integrifoliaAlstonvilleGermplasmWild M. integrifoliaAlstonvilleGermplasmWild M. tetraphyllaAlstonvilleGermplasmWild M. tetraphyllaAlstonvilleGermplasm<

ID	Genotype category	Species group	Location	Selection <sup>A</sup>
76	Germplasm	Wild mixed/hybrid	Alstonville	Australia
77	Germplasm	Wild M. integrifolia	Alstonville	Australia
78	Germplasm	Wild mixed/hybrid	Alstonville	Australia
79	Germplasm	Wild M. tetraphylla	Alstonville	Australia
80	Germplasm	Wild M. tetraphylla	Alstonville	Australia
81	Germplasm	Wild M. tetraphylla	Alstonville	Australia
84	Germplasm	Wild M. tetraphylla	Alstonville	Australia
85	Germplasm	Wild M. tetraphylla	Alstonville	Australia
86	Germplasm	Wild M. tetraphylla	Alstonville	Australia
87	Germplasm	Wild mixed/hybrid	Alstonville	Australia
88	Germplasm	Wild M. ternifolia	Alstonville	Australia
89	Germplasm	Wild mixed/hybrid	Alstonville	Australia
90	Germplasm	Wild M. integrifolia	Alstonville	Australia
93	Germplasm	Wild M. tetraphylla	Alstonville	Australia
94	Germplasm	Wild M. tetraphylla	Alstonville	Australia
95	Germplasm	Wild M. tetraphylla	Alstonville	Australia
96	Germplasm	Wild M. tetraphylla	Alstonville	Australia
97	Germplasm	Wild mixed/hybrid	Alstonville	Australia
98	Germplasm	Wild mixed/hybrid	Alstonville	Australia
103	Germplasm	Wild M. integrifolia	Alstonville	Australia
106	Germplasm	Wild mixed/hybrid	Alstonville	Australia
107	Germplasm	Wild mixed/hybrid	Alstonville	Australia
109	Germplasm	Wild mixed/hybrid	Alstonville	Australia
110	Germplasm	Wild M. tetraphylla	Alstonville	Australia
111	Germplasm	Wild M. tetraphylla	Alstonville	Australia
112	Germplasm	Wild mixed/hybrid	Alstonville	Australia
160	Germplasm	Wild mixed/hybrid	Alstonville	Australia

<sup>A</sup>Hidden Valley Plantation, Australia

### Statistical analysis

All variates were tested for homogeneity of variance. Variances of disease incidence and lesion number data were stabilised using  $\log_{10}(x + 1)$  and  $(x + 1)^{0.5}$  transformation procedure in GenStat 11<sup>th</sup> edition, respectively. Preliminary analyses to confirm if location was a significant factor showed no significant effects, hence, data of both locations were combined in the subsequent analyses. In order to determine the relationship between prevalence of sticktights and husk spot intensity, polynomial regression analyses were performed separately on disease incidence, disease severity and lesion number data. In the regression analyses, prevalence of sticktights was used as the exploratory variates and terms were fitted with macadamia genotypes as the grouping factor on separate and estimate lines. The relationships were tested with linear, quadratic, cubic and quartic models in GenStat. The final models with P < 0.001 that explained the highest percent of variance were accepted. Subsequently, the overall relationship between prevalence of sticktights and macadamia genotypes was explored using a common line as the final model in the polynomial regression

analyses. Non-parametric tests using Kendall's rank correlation coefficients tau were performed to determine the extent of relationships among the variates. In order to confirm if significant differences exist in stomatal abundance among macadamia genotypes, a General Linear Model (GLM) analysis was performed on the stomata data and significant means were separated using Turkey's HSD algorithms tests (Tukey's Honestly Significant Difference Test). The associations between fruit stomatal abundance, disease intensity and prevalence of sticktights (as a measure of inoculum abundance) were explored using correspondence analysis. The quantitative variables (disease incidence, number of lesions, disease severity) were categorised into classes with defined boundaries that allows the classes to be easily linked with disease thresholds or attributes (Table 2.15). In order to represent the maximum possible error in the categories of prevalence of sticktights data, the five classes were reduced to three. Three contingency tables were built showing bivariate frequency distributions of the coded variables (Table 2.15). Chi-square tests using the maximum likelihood coefficient were applied to confirm the suggested patterns, and the null hypotheses stated as the independence of distribution of frequencies in each table, were rejected at P < 0.0001. A system of two axes that provided a framework that represents disease appearance and intensification was produced from the correspondence analysis. In order to examine if macadamia genotypes may be grouped according to their husk spot resistance and susceptibility classes, husk spot intensity, prevalence of sticktights and fruit stomatal abundance data were subjected to a multivariate analysis. The subroutine of discriminant analysis that used canonical variates analysis procedure in GenStat was used to produce a discriminant plot of the eigenvalues of the first two discriminant scores to group the macadamia genotypes.

				St	icktight	s <sup>A</sup>		
Variables	Levels	Category attributes	Values	ST1	ST2	ST3	d.f.	$\chi^{\rm B}$
Lesion num	nber (LN)	)						
	LN1	Intolerant	0 - 4	52	13	3		
	LN2	Average tolerant	5 - 10	8	10	5	4	39.25**
	LN3	High tolerance	>10	4	8	12		
Disease inc	cidence (I	OI)						
	DI1	Absence of disease	0	28	28	4		
	DI2	low incidence	1 -10	6	15	2	6	5/ 20**
	DI3	Average incidence	11 - < 50	0	6	8	0	54.59
	DI4	High incidence	$\geq$ 50	0	6	12		
Disease sev	verity (DS	5)						
	DS1	Low disease	< 5	39	20	0		
	DS2	Average disease	5 - 25	17	5	4	4	46.30**
	DS3	High disease	> 25	8	6	16		

**Table 2.15** Contingency tables of frequency distribution of disease variables with sticktight prevalence (ST) for correspondence analysis.

<sup>A</sup> ST1(rating 0 - 1): Low level of inoculum; ST2 (rating 2 - 3): Average level of inoculum; ST3 (rating 4 - 5): High level of inoculum.

<sup>B</sup> Chi-square test of using maximum likelihood method: \* indicates significance at P < 0.0001.

#### III. Results and Discussion

#### Predictors of husk spot intensity

A significant linear relationship (94.5%) was observed between the prevalence of sticktights and fruit stomatal abundance for each macadamia genotype (Table 2.16), suggesting a possible role of stomatal abundance in sticktight formation and that fruit stomatal abundance is a useful trait to predict genotype resistance/susceptibility to husk spot. However, the overall relationship with a single fitted line was best explained (18.5% variance, P = 0.005) by fitting a cubic curve with asymmetric maximum, falling to an asymptote:  $y = \alpha + (\beta + \beta)$  $\gamma x$  /(1 +  $\delta x$  +  $\varphi x^2$ ) +  $\varepsilon$ , where  $\alpha, \beta, \gamma, \delta$  and  $\varphi$  are parameters of the cubic curve and Kendall's rank correlation coefficient was 37% which indicates that both traits are somewhat linked. In some other crop systems, studies have highlighted complex stomatal-pathogen interactions (Elad, 1988; Kang and Buchenauer, 2000; Miles et al., 2009; Zeng et al., 2010). Significant variations were observed among the macadamia genotypes for disease intensity variables and prevalence of sticktights. A range of prevalence of sticktights ratings (0-5) exist in the macadamia genotypes populations. In order to determine if the range of sticktights prevalence represents normal distribution expected of a natural system, the actual prevalence rating data were compared to a theoretical model (expected distribution) using Q-Q probability distribution. The results showed that the observed prevalence of sticktights was near normal distribution (Kurtosis =  $-0.87 \pm 0.45$  standard error), and most genotypes had low to average sticktights ratings (0 - 2). Analyses to determine the influence of the sticktights on husk spot intensity showed that for each macadamia genotype, a significant polynomial relationships  $(R^2 > 83\%)$  exists between prevalence of sticktights and disease intensity variables (husk spot lesion number, disease incidence and severity, indicating that there was significant increment in husk spot in trees with a high sticktights ratings. However, the combined analysis of all the macadamia genotypes using a single regression line intercept showed the strength of the relationship to be about 16% for lesion number, 23% for disease incidence and 33% for disease severity. Significant relationships exist between fruit stomatal abundance and disease intensity, with about 66%, 92% and 75% of variances accounted for in the regression models with a common intercept for genotypes for lesion number, disease incidence and severity, respectively (Table 2.17). Fruit stomatal abundance varied significantly (P < 0.001) among macadamia genotypes (Fig. 2.20). Out of the 21 macadamia genotypes sampled, 660, A4 and 741 had the least number of stomata per unit area (Fig. 2.20).

	Separate lines, est	timate lines <sup>A</sup>	Common	line <sup>B</sup>	
	Variance	Variance	Best fitted		
Variables	explained (%)	model	explained (%)	model	
Lesion number	83.2	Quadratic	15.6	Linear	
Disease incidence	91.0	Quadratic	22.6	Quartic	
Disease severity	87.2	Cubic	32.6	Quartic	
Stomatal abundance	94.5	Linear	18.5	Quartic	

**Table 2.16** Relationship between prevalence of sticktights, fruit stomatal abundance and lesion number, disease incidence and severity as described by best fit models.

<sup>A</sup>Final regression model contained each macadamia genotype with fitted terms (x + bx)<sup>B</sup>Final regression model with a common intercept with fitted terms (a + x)

Table	2.17	7 Relatio	onshij	p between	frui	t stomata	and	disea	se	intensity	' va	riables (lesio	on numbe	r,
disease	in	cidence	and	severity)	as	described	by	best	fit	model	of	polynomial	regressio	n
analyse	es.													

	Separate lines, e	stimate lines <sup>A</sup>	Common 1	ine <sup>B</sup>
	Variance	Best fit	Variance	Best fit
Variables	explained (%)	model	explained (%)	model
Lesion number	97.4	Quartic	65.4	Cubic
Disease incidence	93.3	Linear	92.1	Quartic
Disease severity	97.1	Quartic	75.0	Cubic

<sup>A</sup>Final regression model contained each macadamia genotype (*G*) (Fitted terms:  $G + ST^*G$ ) <sup>B</sup>Final regression model with a common intercept (Fitted terms: Constant + ST)

#### Association of husk spot intensity variables with inoculum pressure

Examination of the contingency tables gave an indication of the relations among disease intensity variables (lesion number (LN), disease incidence (DI) and disease severity (DS)) and inoculum pressure (prevalence of sticktights (ST)). The first contingency table  $(LN \times ST)$ showed the reaction of macadamia fruit to husk spot infection. Of the 115 macadamia genotypes evaluated, 52 genotypes (45%) were intolerant to husk spot infection (LN1 × ST1), therefore, at low level of inoculum pressure (ST1) 45% of the genotypes abscised with few lesions (LN1). At high level of inoculum pressure (ST3) 12 genotypes (10%) were able to withstand high number of husk lesions (LN3) before abscission, than 5 genotypes (4%) at LN2 and 3 genotypes (3%) at LN1 (Table 2.15). Similar patterns as in LN × ST were observed for disease severity (ST  $\times$  DS) and disease incidence (DI  $\times$  ST). All the disease intensity variables were significantly (P < 0.0001) correlated. Correlation r = 0.55 was obtained for LN and DI, r = 0.75 for LN and DS, and r = 0.49 for DI and DS. Overall association among variables is shown in the correspondence analysis (Fig. 2.21). High inoculum pressure (ST3, on right hand-side) was closely associated with high disease intensity (DI3, DI4, DS3 and LN3) (Fig. 2.21). Conversely, low inoculum pressure (ST1) was associated with low disease intensity (LN1, DS1and DS2) (Fig. 2.21). The graph showed strong associations between the paths of increasing disease incidence, lesion number and prevalence of sticktights (Fig. 2.21). The three paths showed the same movement in the same direction (LN1-LN2-LN3; ST1-ST2-ST3; DI1-DI2-DI3-DI4), suggesting that increases in disease incidence and lesion number were primarily associated with inoculum pressure. In contrast to the path of lesion number that followed the path of increasing ST, the path of increasing disease severity (DS1-DS2-DS3) followed an upward movement (Fig. 2.21), suggesting that increase in abscised diseased fruit was independent of the number of lesions on diseased fruit. The closeness of DS1 and DS2 to ST1 compared to DS3 closeness to ST3 showed that any increments in abscission of diseased fruit was governed by other factors including inoculum pressure.




**Fig. 2.20** Relative stomatal abundance (number mm<sup>-2</sup>) in 21 selected macadamia genotypes. Bars with same letters are not significantly different according to Tukey's Honestly Significant Difference Test algorithms (P = 0.05, Mean Square Error term = 6.348, Harmonic mean sample size = 15.05)



**Fig. 2.21** A correspondence analysis showing the overall relationship between prevalence of sticktights ( $\diamond$ ) (ST: 1 = low inoculum potential, 2 = average inoculum potential and 3 = high inoculum potential) and husk spot intensity in macadamia based on disease incidence in tree canopy ( $\blacktriangle$ ) (DI: 1 = absence of disease, 2 = low incidence, 3 = average incidence and 4 = high incidence); lesion number on abscised fruit ( $\circ$ ) (LN: 1= low lesion number, 2 = medium lesion number and 3 = high lesion number); disease severity ( $\blacklozenge$ ) (DS: 1 = low disease severity, 2 = average disease severity and 3 = high disease severity). The arrowed lines show the path linking successive increments of the coded variables reflecting a logical increase.

#### Categorisation of macadamia genotypes into husk spot resistance-susceptibility groups

In the multivariate analysis of disease intensity parameters, prevalence of sticktights rating and relative stomatal abundance, the first two vector scores (scores 1 = 70.2% and scores 2 = 25.8%) explained about 96% of the relationships and the variations in the pathosystem (Table 2.18). The genotypes were grouped on the first two axes (Fig. 2.22). Both axes indicate that they are essential components for the development of husk spot infection and epidemic, partitioning the genotypes into their husk spot resistance-susceptibility levels (Fig. 2.22). The *x*-axis (score 1) classified the genotypes on the basis of their sensitivity to infection in terms of degree of infection, while the *y*-axis (score 2) classified the genotypes based on threshold for epidemic development in terms of infectious unit or transmission. Thus, y-axis represents the levels of risks to infection (retention of sticktights in the canopy). Fig. 2.22 shows that macadamia genotypes grouped in the top right corner (A16, Line 8-87) had high level of sticktights, hence, high levels of inoculum pressure and disease severity (for both lesion number and abscised diseased fruit). The genotypes near the centre of y-axis were able to withstand high number of husk spot lesions, despite moderate levels of sticktights. Overall, the risk of infection was low in 59%, moderate in 34% and high in 6% of the genotypes, while 88% were intolerant to infection, 6% were low or moderately tolerant to infection. When infected, disease severity was high in 6%, moderate in 25% and low in 6% of the population.

**Table 2.18** Scores of the latent vectors from canonical variate analysis of disease intensity variables, prevalence of sticktights and fruit stomatal abundance.

Variables	Scores 1	Scores 2	Scores 3	Scores 4	Scores 5
Disease incidence	-7.10	1.27	0.11	-0.06	-0.11
Stomatal abundance	0.03	-0.06	0.17	-1.55	-1.09
Prevalence of sticktights	1.79	9.06	-0.38	-0.11	-0.33
Lesion number	2.27	0.24	5.60	-2.92	4.78
Disease severity	5.81	-2.25	3.49	3.27	-3.82
% variation explained	70.22	25.78	2.94	0.63	0.43



**Fig. 2.22** Macadamia genotypes grouped on the basis of husk spot incidence, severity and lesion number, fruit stomatal abundance and prevalence of sticktights in the tree canopy. Score 1 (x-axis) represents sensitivity to infection. Score 2 (y-axis) represents the relative risk of infection. Labels of some genotypes are indicated.

# IV. Conclusions

This is the first comprehensive study to determine variability in fruit stomatal abundance in macadamia genotypes and to reveal its association with husk spot disease intensity variables. Essential components of husk spot infection and disease development which partitioned macadamia genotypes into their husk spot resistance-susceptibility levels were identified. This will aid selection for disease resistance screening in breeding and cultivar selection.

The presence of fruit stomata may also alter mechanical properties of the fruit surface inducing greater mechanical stress (Peschel *et al.*, 2003) and may be relevant to the process of husk splitting as the result of strain of exocarp of the fruit surface, possibly due to imbalance in water uptake and fruit turgor (Beyer and Knoche, 2002). Therefore, formation of sticktights may be related to the stress that is heightened by number of fruit stomata.

Pre-breeding selection of parents with desirable horticultural traits; low stomatal abundance and free of sticktights and their inclusion as selection tools for disease resistance screening may improve resistance in new macadamia cultivars, and contribute to cost-effective sustainable macadamia disease management.

It appears that the propensity of a diseased fruit to abscise is a genotypic effect and it is irrespective of the number of husk spot lesions. Therefore, macadamia genotypes with fruit that is able to withstand a high number of lesions before abscission may be considered as more tolerant to husk spot infection than fruit that abscised with one or two lesions.

Although in this study, we have only considered stomatal abundance, other morphological characters in addition to stomatal abundance, such as cuticular wax, surface layer thickness and thickness and compactness of palisade layer have been reported to also play important role in disease resistance mechanisms (Philip and Govindaiah, 1996). Since *P. macadamiae* enters the host through stomata a lot of these mechanisms are bypassed. Future research could investigate the suitability of mechanisms that regulate stomatal aperture as an immune response against invasion may be considered in future studies as additional resistance traits.

V. Communications and Extension of Research Outcomes

• Publications and presentations

Akinsanmi OA, Topp B and Drenth A (2012) Pericarps retained in the tree canopy and stomatal abundance are components of resistance to husk spot caused by Pseudocercospora macadamiae in macadamia. Euphytica 185:313-323

# **2.1.4. Biological Control**

# 2.1.4.1. Evaluation of potential biological agents against *Pseudocercospora macadamiae* for husk spot control

## I. Introduction

In recent years, public demands have stirred up greater awareness of environmental and health concerns and social responsibilities. The use of natural substances, organic and inorganic that have active antimicrobial properties may provide the desired environmental and human health impact and protect crops from pests and pathogens. However, there are several problems associated with the continuous use of inorganic fungicides such as copper including phytotoxicity to foliage, fruit set and development and the accumulation of heavy metal (copper) in the soil. Some of these inorganic compounds also have limited spectrums of activity.

The more general terms 'natural pesticides' and 'biopesticides' have been used to describe both microbial and plant-derived substances with fungicidal or fungistatic activity (Deliopoulos *et al.*, 2010). According to the Australian Pesticides and Veterinary Medicines Authority (APVMA) "A biological chemical product is an agricultural chemical product where the active constituent comprises or is derived from a living organism (plant, animal, micro-organism, etc.), with or without modification. This includes many products that are commonly referred to as 'botanicals', 'organics' or 'herbals' (where the active constituent comprises an extract derived from an organism rather than the whole organism, it may be accompanied by unidentified components)." Consequently, APVMA has classified biological products into four major groups; biological chemicals (e.g. pheromones, hormones, growth regulators, enzymes and vitamins), extracts (e.g. plant extracts, oils), microbial agents (e.g. bacteria, fungi, viruses, protozoa) and other living organisms (e.g. microscopic insects, plants and animals plus some organisms that have been genetically modified).

*Trichoderma* species have been known to be able to attack other fungi, through the complex mechanisms of mycoparasitism, which include direct growth of *Trichoderma* toward the target fungi, attachment and coiling of *Trichoderma* on target fungi, and the production of a range of antifungal extracellular enzymes (Harman, 2006). They are known to produce antibiotics that affect other microbes and act as biocontrol microbes (Weindling, 1934), increase plant growth and productivity (Lindsey and Baker, 1967) and induce disease suppression in soils (Chet and Baker, 1981). The potential of *Trichoderma* species as biocontrol agents of plant pathogens was first recognised in the early 1930s (Weindling, 1932, 1934) and subsequently they were applied successfully as biocontrol agents against several plant diseases in commercial agriculture (Howell, 2003). *Bacillus* spp. have been shown to control several diseases including early leaf spot of peanut (Kokalis-Burelle *et al.*, 1992), cercospora leaf spot of sugarbeet (Collins and Jacobsen, 2003), post-bloom fruit drop of citrus (Sonoda *et al.*, 1996), take-all and rhizoctonia root-rot of wheat (Ryder *et al.*, 1999).

The present study was established to elucidate the effect and the nature of interaction of *Trichoderma* spp. on mycelial growth of *P. macadamiae*. An understanding of the mechanisms of control is essential for timing of biocontrol application.

# II. Materials and Methods

#### Sources of isolates

The effect of *Trichoderma* spp. on the growth and development of *P. macadamiae* was examined with *Trichoderma* cultures of *T. koningii* + *T. harzianium*, *T. viride* + *T. harzianium* and 'Living soil' which contained a mixture of *T. koningii* + *T. harzianium* + *B. subtilis* + *Pseudomonas flourescense*. The biological agents were obtained from O'Grady Rural, Lismore, Australia. Each bioagent was tested against four isolates of *P. macadamiae*. All cultures were maintained on PDA and stored at 4 °C.

#### Dual culture tests

The effect of *T. koningii* + *T. harzianium* and *T. viride* + *T. harzianium* on the *in vitro* growth of four isolates of *P. macadamiae* were evaluated in the laboratory by dual culture technique as described by Denis and Webster (1971c). Petri dishes (90 mm) containing 25 ml of PDA were inoculated with 5 mm diameter mycelial plugs taken from an actively growing edge of 3 days old *Trichoderma* spp. and 10 day old *P. macadamiae* cultures. In the first experiment, the mycelial disks were placed at equal distance from each other on the surface of the medium on the same day. In the second experiment, due to the slow growth of *P. macadamiae* in culture, the mycelial plugs of the *Trichoderma* spp. were placed on the medium 7 days after *P. macadamiae*. Inoculated plates were incubated at 25°C in an incubator and the radial growth of *P. macadamiae* was measured 5 and 7 days after incubation. Controls without *Trichoderma* were maintained and each treatment replicated thrice and the experiments were repeated. Percent growth inhibition (*I*) of *P. macadamiae* radial growth was calculated as  $I = (C - T)/C \times 100$ , where C = colony diameter of pathogen in control, and T = colony diameter of pathogen in treatment.

#### Mycoparasitism and hyphal interactions

In order to demonstrate whether parasitism occurred (i.e. penetration of host hyphae by Trichoderma spp.), or whether growth inhibition of P. macadamiae was achieved at a distance from the hyphae of Trichoderma spp. (i.e. possible involvement of defined antibiotics and/or enzymes), microscopy studies were conducted. From the zone of interaction between the antagonist and P. macadamiae in dual culture plates, the mycelial mats were gently lifted with a needle and put in a drop of cotton blue stain on a microscopic slide and observed under light microscope for the presence of hyphal interactions. Direct mycelial interaction was examined between each pair of Trichoderma sp. and P. macadamiae on microscope slides. For each pair, approximately 1 mm agar blocks containing actively growing mycelium of opposing fungi were positioned 10 mm apart on a thin layer of water agar and PDA on sterile microscope slides. Slides were inoculated with the mycelial plugs of P. macadamiae and kept in a high humidity environment in sterile Petri dishes containing moist filter paper for 3 days before inoculating respective Trichoderma isolates. The plates were incubated in 12 h/12 h light /dark condition at 25°C. The zone of interaction between the antagonist and *P. macadamiae* was observed using light microscopy in a laminar flow from 2 days after dual inoculation.

#### Volatile inhibition tests

In order to determine if volatile substances are induced to inhibit fungal growth, the techniques described by Dennis and Webster (1971b) were used. Each *Trichoderma* treatment was centrally inoculated on 90 mm PDA plates with a 5 mm mycelial plug taken from 3 days old culture, 7 day-old plates of isolates of *P. macadamiae* each inoculated with 3 mycelial plugs were inverted on the *Trichoderma* plates by replacing the top of each Petri dish. Each pair of plates was sealed with parafilm tape and incubated at 25°C for 7 days. Control plates contained *P. macadamiae* without *Trichoderma*. The plates were replicated thrice and the experiment was repeated. Colony diameter of the *P. macadamiae* (inverted plate) was measured at 7 and 14 days after incubation. The percent inhibition of *P. macadamiae* radial growth was calculated.

#### Non-volatile inhibition tests

The effect of non-volatile inhibitors or metabolites produced by the biocontrol treatments on *P. macadamiae* was determined using the methods described by Dennis and Webster (1971a). Mycelial plugs of each treatment were added into 250 mL conical flasks containing autoclaved 100 ml potato dextrose broth. Inoculated flasks were incubated on an orbital shaker at 150 rpm for 10 days and 3 weeks in at 25 ( $\pm$ 2) °C. Thereafter, the cultures were aseptically filtered through two layers of Whatman no. 1 filter papers to remove the mycelial mat. The supernatant was further filtered through sterile minisart CE non-pyrogenic 0.2 µm filters. Each culture filtrate was added to molten PDA medium (40 °C) to obtain a final concentration of 10% (v/v) and poured 25 mL into the 90 mm diameter Petri plates. Each plate was inoculated with three mycelial plugs (5 mm) of *P. macadamiae* isolates and incubated at 25°C for 7 days. PDA plates without any amendment were used as control plates. The radial growths were measured at 10 and 15 days after incubation and the percent growth inhibition of was calculated.

# III. Results and Discussion

#### Dual culture tests

Results of pairing of four isolates of *P. macadamiae* with *T. viride*, *T. harzianum* and *T. koningii* were similar. Same day paired cultures showed that the biocontrol agents were fast growing and in most cases there were no lines of demarcation with *P. macadamiae* (Fig. 2.23). The *T. viride* + *T. harzianum* culture grew over *P. macadamiae* mycelial plugs, while *T. koningii* + *T. harzianum* culture did not affect the growth of *P. macadamiae* on the mycelial plug (Fig. 2.23). In the experiment where *P. macadamiae* was first allowed to grow for 7 days prior to pairing with mixed cultures of *Trichoderma* spp., a zone of inhibition was observed in the *T. viride* + *T. harzianum* with 9.3 % reduction of *P. macadamiae* colony diameter (Fig. 2.24). No zone of inhibition was observed in the *T. koningii* + *T. harzianum* culture, but caused 7.7 % reduction of *P. macadamiae* colony diameter (Fig. 2.24).



**Fig. 2.23** Growth of *Trichoderma* speices in dual culture with *P. macadamiae* 7 days after inoculation. Top plates: *T. viride* + *T. harzianum*; lower plates: *T. koningii* + *T. harzianum*.



**Fig. 2.24** Growth of *Trichoderma* species 7 days after inoculation in dual culture 14 days old *P. macadamiae.* Top plates: *T. viride* + *T. harzianum;* lower plates: *T. koningii* + *T. harzianum.* 

# Mycoparasitism and hyphal interactions

Microscopic observations to confirm whether there was any penetration of host *P. macadamiae* hyphae by *Trichoderma* spp. showed no interactions between the *Trichoderma* isolates and *P. macadamiae* (Fig. 2.25). The hyphae of the opposing cultures grew together, and there was no direct inhibition in either growth on microscope slides (Fig. 2.25). Similarly, *P. macadamiae* hyphae taken from the edge of the zone of interaction with the

mixed cultures of T. viride + T. harzianum showed no deleterious effect on the physical structures of P. macadamiae compared with untreated cultures.



Fig. 2.25 Micrograph of hyphae of *P. macadamiae* (Pm) and *Trichoderma* spp. (Tr). Left image: *T. viride*; right image: *T. harzianum* (right image).

#### Volatile inhibition tests

The results revealed that starting after 5 days of incubation, volatile compounds produced by *T. viride* + *T. harzianum* cultures caused significantly (p < 0.001) higher growth inhibition of *P. macadamiae* than *T. koningii* + *T. harzianum* cultures compared to the control treatments (Fig. 2.26). *P. macadamiae* mycelial growth was increasingly inhibited after incubation in both *Trichoderma* spp. cultures. At 14 days after incubation, mycelial growth of *P. macadamiae* was inhibited by 50% in *T. viride* +*T. harzianum* and by 25% in *T. koningii* + *T. harzianum* culture.

#### Non-volatile inhibition tests

Non-volatile inhibitors or metabolites produced by the biocontrol treatments showed no radial growth inhibition of *P. macadamiae*. The radial growth of *P. macadamiae* was similar in culture media amended with filtrates of the mixed cultures and the untreated control.



**Fig. 2.26** Effect of volatiles from *Trichoderma* species cultures on growth of *P. macadamiae*. TvTh indicates *T. viride* + *T. harzianum* while TkTh indicates *T. koningii* + *T. harzianum* and control indicates *P. macadamiae* only.

# IV. Conclusion

Although *Trichoderma* spp. have been reported to be parasitic (i.e. penetration of hyphae) on various plant pathogens, this microscopic investigation of the interaction zones of *P. macadamiae* and *Trichoderma* spp. failed to indicate any parasitic activities. No obvious direct mycoparasitism in the hyphae of *P. macadamiae* by the *Trichoderma* spp. cultures tested was observed and no morphological alteration of *P. macadamiae* hyphae was evident. *P. macadamiae* mycelial growth was inhibited by putative volatiles produced by the *Trichoderma* spp. cultures. The trial of fungal growth in dual cultures has clearly demonstrated that *Trichoderma* spp. may be inhibitory towards *P. macadamiae* and curtailed its growth in culture. The mixed *Trichoderma* spp. cultures induced a reaction by *P. macadamiae*, resulting in a clear inhibition zone.

The presence of an inhibition zone in dual culture without the hyphal contact suggests the possible discharge of diffusible growth inhibitory substances. The growth inhibition in the volatile trial suggests the clear inhibition zone observed in the dual culture was imposed by *Trichoderma* spp. These diffusible growth inhibitory substances may have imposed certain stress factors such as nutritional stress (Calistru *et al.*, 1997) on *P. macadamiae*. The imposition of a nutritional stress may be as a result of competition for limited resources used as a mechanism operating in dual culture interactions (Calistru *et al.*, 1997). Naturally occurring intervention between the microorganisms may contribute to preventing or limiting disease development. Although our initial results show that there is an interaction *in vitro* under certain conditions and in artificial culture media, any effect in the field needs to be independently verified using experimental field trials, as conducted in the next subsection (2.1.4.2).

V. Communications and Extension of Research Outcomes

• <u>Report to Australia Macadamia Industry</u>

Preliminary report on the findings of trials on biological control of husk spot was provided to the IDO for the macadamia industry in February 2009.

# 2.1.4.2. Field assessment of with biological products for husk spot control

# I. Introduction

Due to increasing number of organic orchards that are not able to use the conventional chemical options for husk spot control, there is a growing necessity to identify alternative, non-chemical approaches for husk spot control in macadamia. Previous research in MC96011 (Mayers *et al.*, 2000) showed that the efficacy of different biological control products tested applied singly or multiple times was poor and was comparable to the untreated controls. There have been few attempts to develop biological control products for diseases caused by *Cercospora* or *Pseudocercospora* species (Dubey and Singh, 2006; Galletti *et al.*, 2008) and to control fruit diseases in general (Sonoda *et al.*, 1996; Al-Dahmani *et al.*, 2003; Haggag and Saber, 2007; Hargreaves *et al.*, 2008). An alternative approach that has been tried has been to treat plants with compost teas (compost extracts). Anecdotal reports suggests that husk spot is suppressed when macadamia trees are sprayed with compost tea brewed with a mixture of several bioagents including *Trichoderma viride*, *T. harzianum*, *T. konigii* and *Bacillus subtilis*, whilst in other instances, the use of compost tea either had no effect on husk spot disease suppression or increased disease severity.

Compost tea that is brewed with a microbial food source such as molasses, kelp, rock dust, humic-fulvic acids with populations of beneficial microorganisms (Scheuerell and Mahaffee, 2002; Scheuerell, 2003) is emerging as a crop protection tool for organic agriculture for reducing incidence of foliar and/or soilborne diseases (McQuilken et al., 1994; Yohalem et al., 1994). These products also supply nutrients in a readily available form, which rapidly benefit plant growth through a direct contribution to plant nutrition. Compost tea has been reported to inhibit fungal conidial germination and growth when used as foliar sprays (Haggag and Saber, 2007). The premise for compost tea application is that the bioagents serve as fungal antagonists to P. macadamiae. In several other cropping systems, considerable attention has been paid to fungal antagonists mainly because of their potential for reducing the inoculum density of plant pathogens, inhibition of fungal pathogen growth and development, through the complex mechanisms of myco-parasitism and the production of a range of antifungal extracellular enzymes (Howell, 2003; Harman, 2006). Various Trichoderma spp. have been reported as fungal pathogen antagonists and they are known to produce antibiotics that affect other microbes and act as biocontrol microbes (Weindling, 1932, 1934), increase plant growth and productivity (Lindsey and Baker, 1967) and induce disease suppression in soils (Chet and Baker, 1981).

*Trichoderma* spp. have been applied successfully as biocontrol agents against several plant diseases in commercial agriculture (Howell, 2003). Although most applications have been applied to control root and soil pathogens, *T. harzianum* have been reported to be effective against fruit and foliar diseases, such as powdery mildews on pumpkins and *Botrytis cinerea* on strawberry and grapes (Harman, 2000). The inability of *T. harzianum* to extensively colonise newly formed leaf tissues requires the biocontrol agent to be applied frequently, and in some cases, every 10 days when disease pressure is high (Harman, 2000). Bacteria belonging to the genera *Bacillus* are considered to be safe micro-organisms and able to synthesize a vast range of beneficial substances for agronomical and industrial purposes and produce antifungal inhibition zones *in vitro* (Darbyshire and Greaves, 1973; Stein, 2005). They produce endospores that enable the organism to exist under different environmental conditions and survive for a long-term in storage (Collins and Jacobsen, 2003).

Although the biocontrol agents persists on flowers and fruits as a result of high percentage colonisation of immature fruits, the biocontrol agents cannot extensively grow on and colonise newly formed leaf tissues, hence, for these applications to be effective, the biocontrol agents must be applied frequently, in some cases every 10 days when disease pressure is high (Harman, 2000). The putative effect of *Trichoderma* spp. on growth of *P. macadamiae* in the *in vitro* studies (see 2.1.4.1), were assessed under field conditions over 2 years using commercially available biological products containing *Trichoderma* spp. and *Bacillus* sp. The aim of the field studies was to evaluate the efficacy of the biological products for husk spot control.

# II. Materials and Methods

#### Experimental design

Field trials were established in the 2009-10 and the 2010-11 seasons to test the effect of commercially available biological products on husk spot incidence and severity. Trees in the Centre for Tropical Horticulture research station in Alstonville were used. Trees were about 3-4 m high with annual history of husk spot in the A38 that contained high amount of sticktights and 849 with no sticktight and low history of husk spot. At least two trees were used per treatment which included tank-mix of carbendazim and copper, an untreated control and Superzyme<sup>TM</sup> and Seranade<sup>®</sup> max (Table 2.19). Prior to foliar application, the biologicals were activated in water for at least 6 h. Three rows of buffer trees separated carbendazim+copper treated trees and trees sprayed with biological and untreated control.

In the 2009-10 season only A38 trees were used with Superzyme, carbendazim+copper and an untreated control. Each tree was sprayed with 4 L spray volume at each application. Tankmix of carbendazim+copper was applied twice at 4 weeks interval starting at the match-head fruit stage, while Superzyme was applied 4 times at 2 weeks intervals. A plot was sprayed with Superzyme starting at 2 weeks pre-match-head and second plot were sprayed with Superzyme starting at match-head fruit stage. In the 2010-11 season the trial was repeated using the same experimental design in the A38 trees. Additional plots were included using 849 trees. In order to provide adequate source of inoculum in the 849 trees, 5 onion bags containing 50 g sticktights with visible husk spot lesions were randomly attached to branches inside the tree canopy. Carbendazim+copper tank mixture was sprayed twice at 4 weeks intervals, while Serenade and Superzyme were sprayed 3 and 4 times, respectively, at monthly intervals.

#### Data collection

Disease incidence and severity were assessed as described (Akinsanmi *et al.*, 2008). Number of husk spot lesions was recorded on 100 fruits arbitrarily obtained from harvested fruits. Data were analysed with ANOVA and compared with the untreated control.

Product	Main constituents	Rate (per L)	Source
Superzyme <sup>™</sup>	Trichoderma koningii, T. harzianum,	3.0 g	Zadco Pty Ltd.
	Pseudomonas putida and Bacillus subtilis		
Seranade <sup>®</sup> max	Bacillus subtilis	2.0 g	Nufarm Ltd.
SpinFlo +copper	Carbendazim + copper	0.5 mL + 2.5 g	Nufarm Ltd.

Table 2.19 Detail of products used in field trials for biological control of husk spot

# III. Results and Discussion

In the 2009-10 season similar high husk spot incidence was recorded in the A38 trees treated with the biological product (Superzyme) and the untreated control (Fig. 2.27). Husk spot incidence was significantly reduced with carbendazim+copper spray applications compared to both the untreated control and the biological sprays (Fig. 2.27). Husk spot incidence in the trees treated with the Superzyme was higher when spray application was started at pre-matchhead fruit stage than at the match-head stage. Similarly, the mean number of husk spot lesions on the abscised fruit was not significantly different between the two biological spray applications and the untreated control but it was significantly reduced in the carbendazim+copper applications (Fig. 2.28a). This indicates that the biological product did not prevent husk spot infection, compared to the chemical sprays. Disease severity was not significantly different between the untreated control and the pre-match head treatment with the biological, but disease severity was significantly higher in the pre-match head than the match-head biological treatments. In the 2010-11 season field trials, husk spot incidence was lower in the 849 plots than the A38 plots. Due to low husk spot incidence in 849 plots, husk spot incidence was similar in the 3 and 4 spray applications using the two biological products. The number of husk spot lesions on abscised diseased fruit was similar between carbendazim+copper, Serenade max and the untreated control in the 849 cultivar (Fig. 2.29).



**Fig. 2.27** Husk spot incidence progress in A38 trees sprayed with superzyme as biological product (Bio) starting at prematch-head (PreMH) and match-head (MH) fruit stage, carbendazim+copper and untreated control in the 2009/10 season.



**Fig. 2.28** Mean number of husk spot lesions in abscised diseased fruit in (a) the 2009/10 and (b) the 2010/11 seasons in cultivar A38 treated with carbendazim+copper, superzyme at prematch-head (pre-MH) and match-head (MH) fruit stage. Bars indicate standard error.



**Fig. 2.29** Mean number of husk spot lesions in abscised diseased fruit in the 2010/11 seasons in cultivar 849 treated with carbendazim+copper, superzyme and Seranade max. Bars indicate standard error.

#### IV. Conclusions

The mechanisms employed by biocontrol agents to effect biological control of plant diseases are many and complex, and their use varies with the kind of biocontrol agent, pathogen, and host plant involved in the interaction. Although the *in vitro* experiments showed the potential of *Trichoderma* spp. as biological control against husk spot, the results of our field trials did not produce any significant control of husk spot. In this study, we used 2, 3 or 4 spray applications of the biological products which appeared not to be effective, many more and more frequent spray applications may be required before any significant control may be achieved. Biocontrol mechanisms are influenced by the temperature, pH, and moisture of the plant and surface environment, and by other members of the microflora (Howell, 2003). The mechanism of control on macadamia fruit pericarp may need to be first established before adequate management of husk spot could be determined with biological products.

For practical implementation, a biocontrol agent should be effective under all circumstances where the target pathogen is capable of causing disease (Larkin and Fravel, 2002). Thus, biocontrol agents should be most active under the same conditions that the pathogen is most active. However, there are many aspects of the biocontrol interactions that may be affected by environmental factors that are favourable to the pathogen and subsequent disease development. Therefore, the efficacy of biocontrol rather than biocontrol agent populations or survival must be thoroughly evaluated under these different environmental conditions (Larkin and Fravel, 2002). Future research may use dual research pathways to identify effective biocontrol strategy. First, extensive screening and optimization of spray applications of current commercial biological products under field conditions. Secondly, intensive studies of

the microbial ecology of the husk spot pathosystem are required to identify any biological agents with antimicrobial properties against *P. macadamiae*.

Attempts to use other biological products such as compost tea have not provided any control for husk spot. Personal observations of use of compost tea as foliar applications in certain macadamia orchards appear to have increased husk spot severity. This may be due to the increased amount of food source from the compost materials that is made available for P. *macadamiae* growth and sporulation in the tree canopy.

V. Communications and Extension of Research Outcomes

• <u>Report to Australia Macadamia Industry</u>

Preliminary report on the findings of trials on biological control of husk spot was provided to the macadamia industry at the Annual pest consultants meetings in 2010 and 2011.

# 2.1.5. Benefits of fungicide applications and factors affecting economic returns

# I. Introduction

Decisions to apply fungicides are often based on expected financial returns. The goal of control strategies is aimed at reducing the risk of large crop losses. For spray applications to be profitable, it is expected that the control must adequately safeguard yield and minimise the risk of substantial yield losses. Application of fungicides incurs fixed and variable costs per hour, equipment and chemical costs and application costs which generally decrease by reducing spray volume and increasing ground speed (Whitney, 1968). The economic benefits and trade-offs concerning costs, effectiveness and economic benefits of different husk spot management strategies is unknown. Since fixed costs such as depreciation, interest on capital invested, taxes, insurance and housing vary widely among different macadamia orchard enterprises they were not assessed in this study. As a method for comparing disease control costs between gross income and the variable costs can be used (Quinlan, 2005). Therefore, in this study, we sought to investigate if the effectiveness and efficiency of fungicide spray applications for husk spot are significant contributors to the economic returns of macadamia production derived through improved yield and kernel quality.

# II. Materials and Methods

#### Experimental design and disease assessments

Field trials were as described in section 2.1.1.2. The treatments consisted of low, moderate and high spray volumes applied at dilute fungicide dose rate (DLV, DMV and DHV), low and moderate spray volumes applied at concentrate (2X) rate (CLV and CMV) and an untreated control (UC). In 2010, two additional treatments of dilute rate at high volume (DHV) applied three and four times were included (Table 2.19). Disease incidence and severity were as previously described (Akinsanmi *et al.*, 2007; Akinsanmi *et al.*, 2008). Yield and kernel quality assessments were recorded as previously described.

#### Costs and economic analysis

Variable costs associated with spraying, harvesting and processing operations were calculated using the duration, running cost of machinery and labour associated with these operations (Table 2.19). Machinery running costs were costs associated with fuel and oil only, while maintenance and housing costs as well as the labour for repairs were treated as fixed costs. The machinery costs for spraying was estimated at \$50 h<sup>-1</sup> and an approximate hourly award rate of \$30 h<sup>-1</sup> for labour was used. The machinery and labour costs for harvesting and processing (de-husking and sorting) were the same for all treatments and the untreated control, and were therefore excluded from the variable costs calculated. Based on 10 years industry average, NIS base price (*Bp*) of \$2.50 kg<sup>-1</sup> at kernel recovery of 33% with maximum unsound kernel of 3.5%, the actual NIS price (*Ap*) payable with price bonus/penalty for deviation from the standard kernel recovery of 33% and unsound kernel of 3.5% was calculated as;  $Ap = [Bp / 33\%] \times [KR - (UK - 3.5\%)] + f(y)$ , where *KR* is the actual kernel of harvest, *UK* is unsound kernel recovery of harvest, and *f*(*y*) is annual market

function of production determined by macadamia processors based on market demand and supply situation

#### Weather and data analysis

Daily weather data including rainfall (mm), highest and lowest maximum and minimum temperatures (°C), and sunshine hours recorded with a Campbell-Stokes recorder which only measures the duration of *"bright"* sunshine, which is less than the amount of *"visible"* sunshine, were obtained from the regional climate data station of the Australian Government Bureau of Meteorology nearest to Alstonville, New South Wales, 28.83°S, 153.43°E.

Gross margin income was calculated as difference between income and total variable costs for each treatment. The benefit of the spray applications was calculated as the change in gross margin income for each fungicide treatment compared to the untreated control income. In addition, benefit-cost ratios which represent the change in gross margin over change in total variable costs for each treatment as compared to the untreated control were determined.

# III. Results and Discussion

#### Husk spot assessment

Husk spot occurred in all treatments in both years but the incidence started in the first week of January in the untreated controls while it was delayed in the treated trees, giving rise to a higher disease incidence in the untreated control than in the treated trees. Disease development was significantly favoured by higher than normal rainfall in 2011 (Fig. 2.30), which resulted in 100% husk spot incidence in the untreated control by mid-January compared with an average of 70% in treated trees. During the months of December, January and February that correspond to the kernel oil accumulation phase, total rainfall for the 3 months was 379 mm, 258 mm and 98 mm in the 2011 season, whereas it was 106 mm, 56 mm and 189 mm in the 2010 season (Fig. 2.30).

The higher rainfall combined with reduced sunshine hours during the 3 months period to a mean of 5.5 h in the 2011 season compared to a mean of 7.2 h in 2010 provided better disease-favourable conditions in 2011 than in 2010, causing significant delays in kernel maturity in 2011. Results of the accumulated analysis of variance of the AUDPC showed significant (P < 0.001) differences between the treatments and years. Husk spot severity was significantly higher in 2011 than in 2010. Hence, the rates of fruit abscission among the treatments were significantly different in 2010 but were similar in 2011 (Fig. 2.31). High volume applications significantly reduced early fruit abscission (better disease control) compared to the lower volume applications and the untreated control (Fig. 2.31). Hence, the rates of fruit abscission were lowest in high volume spray applications compared to low volume spray applications.

Treatment	Dose category	Spray volume level	Coverage efficiency (%)	No. of applications	Spray volume (L/tree)	Tractor speed (km/h)	Spray duration (h/ha)	Chemical costs (\$/ha)	Labour costs (\$/ha)	Machinery costs (\$/ha)	Total costs (\$/ha)
UC	None	None	0	0	0	0	0	0	0	0	0
DLV-2	Dilute	Low	50	2	3.12	3.6	0.65	102.94	39.00	65.00	206.94
DMV-2	Dilute	Moderate	80	2	5.12	3.3	0.75	168.93	45.00	75.00	288.93
DHV-2	Dilute	High	100	2	6.40	2.8	0.89	211.16	53.40	89.00	353.56
DHV-3 <sup>A</sup>	Dilute	High	100	3	6.40	2.8	0.89	316.74	80.10	133.50	530.34
DHV-4 <sup>A</sup>	Dilute	High	100	4	6.40	2.8	0.89	422.32	106.80	178.00	707.12
CLV-2	Concentrate (2x)	Low	50	2	3.12	3.6	0.65	205.88	39.00	65.00	309.88
CMV-2	Concentrate (2x)	Moderate	80	2	5.12	3.3	0.75	337.46	45.00	75.00	457.46

**Table 2.20** Details and costs of spray applications with tank mixture of carbendazim and copper at different spray volumes, number of applications and fungicide dose rates and untreated control (UC) used in 2010 and 2011.

<sup>A</sup> Treatments were applied only in 2010.



**Fig. 2.30** Total monthly rainfall, highest and lowest maximum and minimum daily temperatures at Alstonville, New South Wales in Australia during the macadamia production seasons in 2010 and 2011.



**Fig. 2.31** Cumulative weights of abscised nut-in-shell (NIS) from macadamia trees sprayed at dilute (D) and concentrate (C) rates of carbendazim and copper fungicides using high (H), moderate (M) and low (L) volumes in the 2010 and the 2011 seasons. Values shown as suffix of the labels refer to number of spray applications. For instance, DHV-2 indicates dilute rate sprayed at high volume in two consecutive times. UC indicates the untreated control.

#### Kernel quality and gross margin

The effect of reduced sunshine hours and higher rainfall in the 3 months preceding harvest in 2011 resulted in reduced mean harvest yield to about 1500 NIS kg ha<sup>-1</sup> compared to about 3200 NIS kg ha<sup>-1</sup> in 2010. Consequently, harvest yield was significantly (P = 0.01) different between years and also among the treatments (Table 2.22). Harvest yield of each treatment compared to the 10-year industry average yield of 2500 NIS kg ha<sup>-1</sup> showed significant (P < 0.05) variations in yield (increase or decrease) occurred depending on the treatment. In 2010 all the treatments except DHV-4 and untreated control produced significant higher yield than the 10-year industry yield average (Table 2.21). However, in 2011 none of the treatments including the untreated control produced yield above the 10-year industry average yield. Overall, the mean change in harvest yield in both seasons in all the treated trees resulted in >10% yield increase above the untreated control (Table 2.22).

The lower profitability observed in 2011 than in 2010 can be attributed to the higher than normal wet conditions and reduced daily sunshine hours that affected fruit development, and in particular, delayed kernel maturity. This period coincided with the period of kernel oil accumulation phase which is critical for yield and kernel quality in macadamia (Jones, 1939; Stephenson and Gallagher, 1986, 1987b; Stephenson et al., 2003; Huett, 2004). Also, the conditions in the 2011 season were more favourable to husk spot development (Akinsanmi and Drenth, 2010; Miles et al., 2010b) and consequently the high rate of premature fruit abscission that occurred in the season. Due to higher NIS harvested at the start of harvest (harvest periods 1-3) in the untreated control than treated trees (Fig. 2.31), the value of NIS produced was consistently lowest in the untreated control (Table 2.21), which indicates poorer kernel quality occurred in the untreated control than in the treated trees. The quality and value of NIS produced with two spray applications (DHV-2) were similar to three (DHV-3) and four (DHV-4) spray applications (Table 2.21). Comparison of the dilute and concentrate rates when the conditions for disease development were more favourable in 2011 showed the values of NIS produced were significantly higher in the dilute rate treatments in DHV-2 than in the concentrate spray application treatments of CLV-2 and CMV-2 (Table 2.21).

The best gross margin incomes were obtained with dilute spray applications in both years. In the two spray applications, the gross margin was consistently highest in DHV-2 followed by DMV-2 and DLV-2, and was lowest in CMV-2 (Table 2.21). In 2010 the change in gross margin compared to the untreated control ranged from 8% - 35% in dilute spray applications and was 6% at CLV-2, but change was negative at CMV-2 (-4%) (Table 2.21). Whereas in 2011, change in gross margin compared to the untreated control increased by 5% - 17% in the dilute spray applications but was negative in CMV-2 (-12%) and zero in CLV-2 (Table 2.21). Overall, improved change in the gross margin over the untreated control except CMV-2 was achieved by spray applications (Table 2.22). The gross margins achieved with increased number of spray applications in 2010, was higher in DHV-3 than in DHV-4 or DHV-2 (Table 2.21). Results of the benefit-cost ratios showed that fungicide applications were beneficial in both years with dilute rate and volume applications (Table 2.21). The relative magnitudes of the benefit were higher in high dilute volume applications than low dilute volume sprays, and were generally less beneficial or more costly with concentrate spray volumes (Table 2.22).

**Table 2.21** Cumulative harvest yield of nut in shell (NIS), area under disease progress curve (AUDPC) and income parameters from macadamia trees sprayed against husk spot using different spray volumes, number of applications and dose rates and untreated control (UC) in 2010 and 2011. Values followed by the same letter, in the same year, are not significantly different at p = 0.05.

		Total	Mean		Gross			Benefit-
	Mean	harvest	NIS		margin	Change	in gross	cost
Treatment	AUDPC <sup>A</sup>	yield	value	Income	income	margin	income	ratio
		(kg/ha)	(\$/kg)	(\$/ha)	(\$)	$(\$)^{\mathrm{A}}$	(%)	
2010								
UC	398 a	2503 d	2.85 b	7,413	7,413	-		-
DLV-2	301 b	3006 c	2.90 ab	8,238	8,031	618	8	2.99
DMV-2	188 c	3574 b	2.90 ab	8,648	8,359	947	13	3.28
DHV-2	179 c	4151 a	2.99 a	9,475	9,121	1,708	23	4.83
DHV-3	169 c	3076 c	3.00 a	10,508	9,978	2,565	35	4.84
DHV-4	86 d	2496 d	2.99 a	10,477	9,770	2,357	32	3.33
CLV-2	225 с	3719 b	3.01 a	8,131	7,821	408	6	1.32
CMV-2	180 c	3084 c	2.96 ab	7,593	7,135	-278	-4	-0.61
2011								
UC	1670 a	1549 ab	2.12 c	3,177	3,177		0	
DLV-2	370 b	1722 a	2.35 bc	3,531	3,324	147	5	0.71
DMV-2	396 b	1393 b	2.47 b	3,706	3,418	241	8	0.83
DHV-2	368 b	1140 b	2.99 a	4,061	3,707	530	17	1.50
CLV-2	397 b	1691 a	2.32 bc	3,485	3,175	-2	0	-0.01
CMV-2	395 b	1408 b	2.17 c	3,254	2,797	-380	-12	-0.83

 $^{\text{A}}$  Calculated as the change in gross margin income as compared to the untreated control.

<sup>B</sup> Calculated as the change in gross margin income over change in total cost, as compared to the untreated control.

**Table 2.22** Change in harvest yield and gross margin of dilute and concentrate fungicide rates at different spray volumes compared to untreated control over two years with two fungicide spray applications.

Indicator	DLV-2	DMV-2	DHV-2	CLV-2	CMV-2
Change in harvest yield <sup>A</sup> (%)	17	23	31	33	11
Change in gross margin income <sup>B</sup> (\$)	382	594	1,119	203	-329
Change in gross margin income <sup><math>C</math></sup> (%)	6	10	20	3	-8
Benefit-cost ratio <sup>D</sup>	1.85	2.05	3.17	0.66	-0.72

<sup>A</sup> Calculated as the change in harvest yield as compared to the untreated control expressed in percentage.

<sup>b</sup> Calculated as the change in gross margin income as compared to the untreated control. <sup>C</sup>Calculated as the change in gross margin income as compared to the untreated control expressed as percentage.

<sup>D</sup>Calculated as the change in gross margin income over change in total cost, as compared to the untreated control.

# **IV.** Conclusions

This study shows that fungicide application to control husk spot in macadamia is generally profitable compared to no fungicide applications. However, the magnitude of the benefits is dependent on the efficacy, rates and costs of fungicide applications, weather conditions, cultural practices, such as routine monitoring of abscised fruit, and the market price of macadamia. Our results revealed important conclusions for the management of husk spot.

First, it indicates that fungicide control, even as prophylactic applications, provides financial benefits over unsprayed management options. Second, high volume dilute spray applications consistently provide greater benefit than other treatments under conditions that are favourable or sub-optimal for disease development. Third, the use of higher fungicide dose rates does not provide any additional benefits. Fourth, using at least the recommended two spray applications provides economic benefits compared to no fungicide application. Due to the incessant and high amount of rainfall in 2011, the third (DHV-3) and fourth (DHV-4) sprays could not be applied, hence, this study did not provide data in support of additional spray applications in high disease pressure conditions to the recommended two spray applications. However, in 2010, two spray applications (DHV-2) produced similar or higher benefit-cost ratios, and thus were more cost-effective than 3 or 4 spray applications. Therefore, it is crucial for a grower to assess the weather conditions (forecasts) between October and December each year to determine if it is conducive for husk spot infection before deciding to increase the number of spray applications.

V. Communications and Extension of Research Outcomes

- Publications and Presentations
- 1. Akinsanmi OA and Drenth A (2012) Economic returns from fungicide application to control husk spot of macadamia in Australia is influenced by spray efficiency, rates and costs of application. Crop Protection 41: 35-41.
- Femi Akinsanmi, John Pretorius, Kevin Quinlan, Paul O'Hare and Andre Drenth 2011. Husk spot control with fungicide spray applications: Is it profitable? <u>Australian</u> <u>Macadamia Society Ltd. News Bulletin</u> Vol. 39 (6): 62-66.

9 PS	JUL Pathogen	of the month – July 2011
IS & LASCOR	a	d. f
z, maye	D C Fig. 1. Chlorotic spot (a); sporulating tan-brown less fruits (c); electron and light micrographs of fungal s conidioma (f). Photo credits: Andrew Miles (a, b,d, Disease: Husk spot	sion (b); husk spot symptoms on diseased macadamia tructures penetrating through stomates (d, e); and f); Olufemi Akinsanmi (c); and P.W Sutherland (e).
	ost production annually. Husk spot has not been re ncluding the United States of America (Hawaii), Bra Africa, Thailand, and Zimbabwe.	Host Range: Species of macadamia, including
acauaiii	pathogen was first documented by B. L. Oxenham in 1951 on specimens collected from Nambour, Queensland. The pathogen was initially identified as <i>Cercospora</i> sp., then <i>Cercoseptoria</i> sp., and finally as <i>Pseudocercospora</i> macadamiae in 2003.	Macadamia integrifolia, M. tetraphylla, M. ternifolia, and their hybrids. Impact: Its greatest impact is extensive premature abscission of fruit. Direct yield losses of up to 40% have been reported. Current commercial
	Disease symptoms: Husk soot symptoms	the second second states are second states to second states to second states and states are set.
pora II	develop very slowly and first appear on the green husk (pericarp) as chlorotic spots that later turn tan to dark brown (Fig. 1). A characteristic feature of the spot is that it is harder than the surrounding tissue.	macadamia varieties are susceptible and the impact increases with tree age. Husk spot also impacts upon the saleable edible kernel, it increases operational costs at the processing stage and a harvested crop with high levels of immature kernel is downgraded, resulting in lower financial returns.
ucercospora n	develop very slowly and first appear on the green husk (pericarp) as chlorotic spots that later turn tan to dark brown (Fig. 1). A characteristic feature of the spot is that it is harder than the surrounding tissue. <i>P. macadamiae</i> conidia are produced on the dark brown spots and appear as greyish velvet mat (Fig. 1b). Conidia are easily dispersed from the diseased husk by rain splash onto developing fruits.	macadamia varieties are susceptible and the impact increases with tree age. Husk spot also impacts upon the saleable edible kernel, it increases operational costs at the processing stage and a harvested crop with high levels of immature kernel is downgraded, resulting in lower financial returns. <b>Control:</b> An integrated management approach consisting of a combination of control measures including routine spray applications of fungicides, cultural practices to reduce the source of inoculum and use of more tolerant varieties have reduced the impact of husk spot.

# 2.1.6. Media and Scientific Community Awareness Campaign for Husk Spot

http://www.appsnet.org/Publications/potm/Jul11\_POTM.pdf

# 2.2. Phytophthora blight, root rot and trunk canker

# 2.2.1. Phytophthora in macadamia

The soil borne, water loving Oomycetes belonging to the genus *Phytophthora* are significant plant pathogens worldwide. It is estimated that Phytophthora diseases cost the Australian plant industries approximately \$200 million a year (Irwin et. al., 1995). More than 100 phytopathogenic Phytophthora species have been described so far causing diseases in several plant species and tree crops (Bazan De Segura, 1970; Zentmyer et al., 1978; Zentmyer, 1980; Combrink and Labuschagne, 1996; Erwin and Ribeiro, 1996; Drenth and Guest, 2004; Kroon et al., 2011). In macadamia few Phytophthora species have been reported to cause various diseases in several countries. In Hawaii, P. capsici and P. cinnamomi are associated with quick decline syndrome, stem canker and bleeding from the trunk (Zentmyer, 1962b; Ko and Kunimoto, 1994), P. capsici was found to infect macadamia raceme, tree bark and wood tissue and caused girdling of mature trees leading to their death (Ko and Kunimoto, 1995), and P. tropicalis is associated with sap bleeding and quick decline (Keith et al., 2010). In Costa Rica, P. palmivora was reported to attack macadamia (Desegura, 1970). In Kenya, P. cinnamomi caused root rots and trunk cankers (Mbaka et al., 2009). In South Africa and Australia, P. cinnamomi is associated with stem canker, tree decline and dieback (Pegg, 1973; Serfontein, 2008).

Phytophthora blight caused by *P. capsici* results in the death of whole racemes after prolonged periods of wet weather ca. 1 to 2 weeks heavy rain (Hunter *et al.* 1971). Phytophthora blight is not common in macadamia in Australia but potentially may cause losses after prolonged periods of wet weather.

Trunk canker caused by P. cinnamomi is the most widespread disease in all macadamia producing countries. It is characterised by furrowed deep cankers on the trunk and irregular areas of dead bark extending from the soil line to several feet high in mature trees (Pegg 1973). In some cases, cankers may extend into branches, and trees appear chlorotic and stunted. Infections are first observed as discolouration of the bark at the base of the tree, often with gum exudation. When this bark is scraped away, the outer wood is discoloured with a shade of brown that generally extends to ground level. Symptoms of macadamia trees infected with *Phytophthora* are diverse and varied. In Australia, according to Pegg (1973) symptoms of macadamia tree infected by P. cinnamomi include a gradual decline of the tree with usually pale or yellow green leaves instead of dark green. Under conditions of moisture stress, leaves of infected trees often wilted and tend to abscise giving the diseased trees a sparse appearance. New leaf flushes and shoot growth are usually absent or sparse. Branches also dieback as the disease persists and fruit set is usually poor (Pegg, 1973). In Kenya, P. cinnamomi has been reported to cause root rot and trunk canker (Mbaka et al., 2009). In South Africa and Australia, P. cinnamomi has been associated with stem canker, tree decline and dieback (Pegg, 1973; Serfontein, 2008), but unlike with avocado, P. cinnamomi is generally not considered to cause a major root rot disease problem in macadamia (Serfontein, 2008). At this point in time it is unclear what damage Phytophthora does to the root system. The use of herbicides to control weeds under the trees and mechanical harvesting do not promote root health under the tree and in effect may exacerbate any root health problems in macadamia.

Generally, in order to reduce diseases caused by *Phytophthora* sp. in tree hosts, precautions should be taken to prevent wounding of trees and improve drainage around the tree trunk.

Further spread of the disease from nursery stocks should be avoided by using clean soil or potting mix and good hygiene practices in the nursery. Many orchardists apply phosphonates on a regular basis to control Phytophthora trunk canker. Application is either applied on orchard wide basis or in specifically targeted to unthrifty looking trees. Although Phosphonate is a highly effective product in reducing the impact of Phytophthora in other tree crops such as avocado, significant improvements through changes to timing and method of application have been made. Timing is important as phosphonate is easily transported in the Phloem, thus depending on the time of year it either accumulates in the root system or may accumulate in the fruit. Application methods have changed from effective and cheap, but time consuming trunk injections to foliar sprays which are less effective. The latest research in other crops is based on application of phosphonate to the trunk with silicon based products such as Pentrabark which facilitate efficient and rapid uptake. Trunk application has several advantages and may turn out as a cheap and effective way to control trunk canker. Some experimental work is needed to establish the impact of Phytophthora on yield before symptoms are apparent on the trunk and application of phosphonate at different rates and intervals to determine the economic impact of disease and the effectiveness of control.

Although there is little quantitative information on the economic or yield loss due to Phytophthora diseases in macadamia, about 60% of macadamia yield loss is estimated to be due to Phytophthora diseases in Kenya (Mbaka et al., 2009; Mbaka et al., 2010), and in Hawaii, 53% of trees inoculated with P. cinnamomi died within seven months after planting (Keith et al., 2010). In Australia, hundreds of macadamia trees were reported to have been killed or rendered unproductive as a result of planting nursery trees with P. cinnamomi trunk cankers (Pegg, 1981). Linde et al. (1999b) established non-host specialisation in the P. cinnamomi pathogen population and Mbaka et al. (2010) only observed an independent and continuous phenotypic variation among Kenyan P. cinnamomi isolates from macadamia but no pathogenic variation. Population genetic studies have shown that the P. cinnamomi population in both Australia and South Africa show very little diversity within each country as well as between countries (Linde et al., 1999a). This uniformity in the pathogen population makes field data more easily applicable among different sites and locations within Australia. It is expected that isolates of P. cinnamomi from different sources including location and hosts of the same mating types have similar growth patterns, morphology and physiological behaviour, and have considerable ability to produce a range of pathogenic phenotypes (Zentmyer, 1980; Zentmyer and Guillemet, 1981; Dudzinski et al., 1993).

*P. cinnamomi* is an important oomycete soilborne pathogen with a global distribution which infects over 3000 susceptible plant species of which about two third are located in the Australasian agricultural, horticultural and forest ecosystems (Zentmyer, 1980; Perez-Martinez, 2008). Among the over 100 phytopathogenic *Phytophthora* species described so far causing diseases in several plant species, *P. cinnamomi* is among the most widespread and destructive *Phytophthora* species that threatens native biodiversity (Zentmyer, 1983; Drenth and Guest, 2004; Brasier, 2009). There are several publications on the interaction of *Phytophthora* spp. and tree crops (Bazan De Segura, 1970; Zentmyer *et al.*, 1978; Zentmyer, 1980; Combrink and Labuschagne, 1996; Erwin and Ribeiro, 1996; Drenth and Guest, 2004). In particular, *P. cinnamomi* causing root rot in avocado (*Persea americana*) has been the focus of several studies (Zentmyer, 1961; Zentmyer *et al.*, 1978; Darvas *et al.*, 1984; Zentmyer, 1980; López-Hèrrèra and Pèrèz-Jimènèz, 1995; Perez-Martinez, 2008). There is limited information concerning the effect of *Phytophthora* on macadamia despite few *Phytophthora* spp. having been reported to cause various diseases in several macadamia producing countries.

In Australia, appearance of symptoms of macadamia tree decline often follows extreme environmental conditions such as prolonged water-logging associated with cyclonic weather or drought. Anecdotal observations indicate that certain underlying factors such as soil fertility, poor irrigation, poor drainage, tree age, and rootstock-scion interaction predispose macadamia to tree decline often associated with *P. cinnamomi* (Akinsanmi and Drenth, *unpublished*). Macadamia trees are able to grow without any aboveground symptoms in *P. cinnamomi* infested soil and sometimes are able to curtail or outgrow the development of *Phytophthora* stem canker (Pegg, 1981; Akinsanmi and Drenth, *unpublished*). However, it is not known if this 'infection abatement' characteristic is a resistance mechanism or a consequence of certain physiological processes of the tree. Infection abatement may be influenced by environmental conditions such as optimal nutrition and water relations or may be related to seasonal carbohydrate fluctuation and root growth and development (Stephenson *et al.*, 1989; Firth *et al.*, 2003).

Studies on the distribution of the macadamia root system, its morphology, periodicity, turnover and longevity showed that the root system of macadamia has unique features for its adaptations to seasonal dry and flooding conditions and to low available soil phosphorous in the Australian ecosystem (Firth et al., 2003). In contrast to other Proteaceae species, the proteiod roots in macadamia occur in large quantities in the entire fibrous root system at a deeper level in the soil, root hairs occur in abundance on the proteiod rootlets and the proteiod roots retain their functions in relatively dry conditions for more than a year compared to non-proteoid fibrous roots that may be functional for less than 2 years in relatively dry conditions, before decay after the onset of wet soil conditions (Lamont, 1973; Firth et al., 2003). A significant feature of the macadamia root system is the predominance of fine fibrous roots (<1 mm in diameter) which are hardened and non-fleshy compared with the roots of many tropical tree crops such as avocado, thus, are less likely to suffer tissue collapse in dry surface soil and have the capacity to regenerate new lateral growth from desiccated roots following drought (Firth et al., 2003). In grafted macadamia trees, the root system is relatively shallow and spreading, with a short taproot and most of the fibrous root system is near the soil surface, compared to ungrafted trees with a longer taproot system (Firth et al., 2003). In Australia, new root growth occurred predominantly in autumn, but some new fibrous roots are produced in early winter and spring, the peak of root flushes and root development appear to be active after the completion of leaf expansion phases in early spring (September/October) and in early autumn (March/April) (Stephenson et al., 1986; Firth et al., 2003). Early investigations indicated macadamia roots are resistant to P. cinnamomi (Zentmyer, 1960), but more recent reports showed that P. cinnamomi can be isolated from necrotic rootlets, cause root rot and necrosis and is able to invade macadamia rootlets without apparently affecting tree health (Pegg, 1981; Serfontein, 2008; Mbaka et al., 2009).

# 2.2.1.1. Incidence and pathogenicity of Phytophthora cinnamomi

# I. Introduction

There is little quantitative information on the prevailing *Phytophthora* species affecting macadamia and the prevalence of *Phytophthora*-related diseases in macadamia orchards is not known. Determining whether this is the only *Phytophthora* species causing disease in macadamia is essential if the plant, pathogen and environmental interactions are to be understood. Whilst control measures for most *Phytophthora* diseases are similar (Akinsanmi and Drenth, 2004), it is important to investigate whether other species of *Phytophthora* are, or have the potential to be more virulent or aggressive towards macadamia. If needed, more rigorous disease control, including maintaining optimum tree health, should be applied, and continued research is imperative for the long term growth and sustainability of the industry. Thus, a survey of macadamia orchards was undertaken between 2007 and 2010 to determine the incidence of *Phytophthora*- related diseases, the identity of the prevailing *Phytophthora* species and the associated symptoms.

# II. Materials and Methods

# Survey of distribution of Phytophthora-related diseases in macadamia orchards

Surveys of 30 macadamia orchards in the Bundaberg, Gympie, Sunshine Coast, Mackay and Northern Rivers growing regions were undertaken during farm visits to macadamia commercial orchards between 2007 and 2010. Survey method and sampling procedure used were based on examination of trees in adjacent rows from a randomly chosen, central point in each block. Trees were examined for visual symptoms including chlorosis, new shoot growth (suckers), unthrifty trees with decline, dieback, and sparse canopy. The percentage of trees and cultivars affected was recorded.

Soil samples were taken from symptomatic and asymptomatic (dark green full canopy) trees and analysed in the laboratory. Root samples were examined for root rot and representative samples were collected arbitrarily from symptomatic and asymptomatic trees in each field. Samples were stored in sealed plastic bags and kept refrigerated at 4°C. Sample size was determined based on an estimated disease incidence from interview with the growers/orchard manager. Samples from nursery beds and/or potting mix were included in the survey on the same basis to determine the incidence of *Phytophthora*, therefore, the potential for introducing infected materials to new orchards.

# Identity of Phytophthora species in macadamia orchards

A total of 241 soil samples from 30 orchards were examined for the presence of *Phytophthora*. Replicate soil samples taken from around the root zone of symptomatic and asymptomatic trees were bulked to form a composite sample from which subsamples were baited for *Phytophthora* using germinated New Zealand blue lupins roots (*Lupinus angustifolius*) according to the method described by Pratt and Heather (1972). This procedure was used to confirm the presence of active *Phytophthora* in the soil samples. Isolates of *Phytophthora* were obtained from diseased lupins, purified and the species identified using a PCR-based technique (Drenth *et al.*, 2006). Infested soil samples collected from pineapple

and avocado with obvious *Phytophthora* root rot caused by *P. cinnamomi* were used as positive controls. In addition, DNA samples of *P. palmivora* (UQ3689), *P. heveae* (UQ6234) and *P. cinnamomi* were included as controls. In addition to the lupin baiting method, direct total genomic DNA was extracted from the soil samples using the MoBio Laboratories Inc, PowerSoil® DNA Isolation Kit following the manufacturer's procedure.

# Pathogenicity tests of the prevailing Phytophthora species in macadamia orchards

Wound inoculation methods were used to test the pathogenicity of *Phytophthora* species isolates obtained from macadamia orchards. Grafted trees in potting bags were inoculated in the glasshouse using mycelial plugs (10 mm) taken from the actively growing margin of colony growth on Campbell's vegetable juice (V8) agar and control agar plugs were cut from plates of sterile V8 agar. The plugs were inserted below the bark of the stem, slightly peeled back with a sterile scapel. The plugs were held in place with parafilm (Fig. 2.32) until end of the trial. In order to confirm if macadamia trees can withstand *Phytophthora* infection, inoculated trees grown in potting bags were subdivided into two groups. The first group were fertilised on a regular basis with Osmocote® while the second group was not fertilised.



**Fig. 2.32** Stem wound inoculation with mycelial plugs of *Phytophthora* sp. Isolate placed below the slightly peeled bark of the grafted trees held in place with parafilm.

# III. Results and Discussion

# Identity of Phytophthora species in macadamia orchards

Twelve of the 241 (~5%) soil samples were positive for *Phytophthora*. Samples obtained from symptomatic and asymptomatic trees were positive for *Phytophthora*. Positive symptomatic samples showed tree decline and canker symptoms. About 92% (11 out of 12)

positive samples were identified as *P. cinnamomi* and a soil sample obtained from symptomatic tree with canker symptoms was identified as containing *P. nicotianae*. This study confirmed *P. cinnamomi* as the dominant *Phytophthora* species associated with macadamia in Australia. Its presence in symptomatic and asymptomatic trees suggests that in some cases macadamia may be tolerant to infection without showing any symptoms. The presence of *P. nicotianae* in a soil sample near a tree with a canker lesion is not evidence that this species is responsible for the disease.

# Pathogenicity tests of prevailing Phytophthora species in macadamia orchards

In this study, samples that tested positive for *Phytophthora* using the lupin baiting method were the same in direct soil DNA extraction methods. There was no correlation between symptomatic tree, location or *Phytophthora* presence. Our results showed that canker was induced in stem inoculated with selected *P. cinnamomi* isolates through a wound inoculation technique. Previous reports from several countries have confirmed *Phytophthora* canker occurs on trunks and lower branches of macadamia trees (Zentmyer, 1960; Hine, 1961; Zentmyer and Storey, 1961; Brodrick, 1973; Pegg, 1973; Mbaka *et al.*, 2009) and these reports often associate infection that occurred through wounds.

In order to confirm if macadamia trees can withstand *P. cinnamomi* infection, inoculated trees grown in potting bags that received regular fertilizer treatment did not show any obvious canker symptoms. The inoculated site showed a slight discoloration compared to the control inoculation (Fig. 2.33). It has been reported that in certain cases, without any treatments and no apparent reason, Phytophthora canker development on macadamia trees ceased after a period of time and *P. cinnamomi* was unable to be isolated from such cankers (Pegg, 1981). It is uncertain if macadamias are able to curtail or outgrow the development of *Phytophthora* canker, or if the physiological processes of the trees can restrict continuing invasion of the tree trunk by *P. cinnamomi*.



**Fig. 2.33** Effect of *Phytophthora cinnamomi* on macadamia stem wound-inoculated using mycelial plugs in (a) non-fertilised, (b) Osmocote-fertilised trees and (c) control trees inoculated with sterile mycelial plugs.

# **IV.** Conclusions

This study presents the first evidence that *P. cinnamomi* can infect and colonise macadamia without any visual symptoms. Infections did not always result in lesions or canker. This demonstrates the danger of assuming that asymptomatic macadamia trees are healthy and free of infection which may become severely impacted when disease-conducive conditions prevail.

V. Communications and Extension of Research Outcomes

• <u>Publications and Presentations</u>

Akinsanmi OA and Drenth A (2012) Disease management workshops as July 2012 AMS Macgroup meetings in QLD and NSW.

# 2.2.1.2. Effect of Phytophthora cinnamomi on macadamia

# I. Introduction

Phytophthora cinnamomi is an important oomycete soilborne pathogen with a global distribution which infects over 3000 susceptible plant species of which about two third are located in the Australasian agricultural, horticultural and forest ecosystems (Zentmyer, 1980; Perez-Martinez, 2008). Among the over 100 phytopathogenic Phytophthora species described so far causing diseases in several plant species, P. cinnamomi is among the most widespread and destructive Phytophthora species that threatens native biodiversity (Zentmyer, 1983; Drenth and Guest, 2004; Brasier, 2009). There are several publications on the interaction of *Phytophthora* spp. and tree crops (Bazan De Segura, 1970; Zentmyer et al., 1978; Zentmyer, 1980; Combrink and Labuschagne, 1996; Erwin and Ribeiro, 1996; Drenth and Guest, 2004). In particular, P. cinnamomi causing root rot in avocado (Persea americana) has been the focus of several studies (Zentmyer, 1961; Zentmyer et al., 1978; Darvas et al., 1984; Zentmyer, 1984; Botha et al., 1990; López-Hèrrèra and Pèrèz-Jimènèz, 1995; Perez-Martinez, 2008). There is limited information concerning the effect of Phytophthora on macadamia (Macadamia integrifolia, M. tetraphylla and their hybrids) despite few Phytophthora spp. having been reported to cause various diseases in several macadamia producing countries.

Unlike avocado, where P. cinnamomi infects and destroys the feeder roots which leads to death of the tree (Zentmyer, 1984; López-Hèrrèra and Pèrèz-Jimènèz, 1995; Pegg et al., 2002; Perez-Martinez, 2008), there is more confusion as to whether P. cinnamomi causes root necrosis and root rot disease in macadamia (Ko and Kunimoto, 1976; Serfontein, 2008). Mbaka et al.(2009) reported that root rot and trunk canker are major constraints to macadamia production in Kenya, where the above-ground symptoms observed in the macadamia trees were associated with the direct result of rot of the fine feeder roots as described for avocado. In Australia, P. cinnamomi has been found to cause stem cankers above the soil line which girdle macadamia trees (Pegg, 1973), however, it is not known whether root rot or necrosis and/or stem canker is the main cause of tree decline or death in macadamia. It is believed that zoospores of the pathogen are attracted to the root elongation zones in macadamia similar to avocado, where chemical signals from the root tip apex serve as the attractants (Zentmyer, 1980; Perez-Martinez, 2008) and electrical signals generated in the rhizosphere also mediate in the infection process (van West et al., 2002). In avocado, P. cinnamomi only invades the small feeder roots, but under special conditions, it may infect and colonise secondary roots (Perez-Martinez, 2008).

As a member of the Proteaceae, macadamia produces proteoid roots which are temporary, and continually replaced by extension of the main root axes (Lamont, 2003) and the root system has unique features for its adaptations to seasonal dry and flooding conditions and to low available soil phosphorous in the Australian ecosystem, its centre of origin (Firth *et al.*, 2003). A significant feature of the macadamia root system is the predominance of fine fibrous roots (<1 mm in diameter) which are hardened and non-fleshy compared with the roots of many tropical tree crops such as avocado, thus, are less likely to suffer tissue collapse in dry surface soil and have the capacity to regenerate new lateral growth from desiccated roots following drought (Firth *et al.*, 2003). Although there is no information on the morphology and effect of waterlogging on macadamia roots, many species that produce proteoid roots grow under permanently or seasonally waterlogged conditions (Lamont, 2003) and proteoid roots are more readily formed in waterlogged conditions than in moist or dry conditions

(Lamont, 1976). In species that evolve or are well-adapted to flooding, the risk of asphyxiation is minimised by internal long-distance apoplastic gas transport pathways (Jackson and Armstrong, 1999). In macadamia trees grafted onto seedling rootstock, the root system is relatively shallow and spreading horizontally, with a short taproot and most of the fibrous root system is near the soil surface, compared to seedling trees with a longer taproot system (Firth et al., 2003). New root growth occurs predominantly in autumn, but some new fibrous roots are produced in early winter and spring, the peak of root flushes and root development appear to be active after the completion of leaf expansion phases in early spring (September/October) and in early autumn (March/April) (Stephenson et al., 1986; Firth et al., 2003). In Australia, rootstocks are commonly used in avocado and macadamia production systems to enable selected scions to be vegetatively propagated, shorten time in the nursery and to reduce the variation that occurs between seedlings. In contrast to the avocado industry where the necessity to overcome various soil-related problems including Phytophthora root rot have led to the development of clonal rootstocks and selection of Phytophthora root rot resistant rootstock selections (Ben-Ya'acov and Michelson, 1995; Huett, 2004; Smith et al., 2011), the majority of the Australian macadamia orchards have been established using seedling rootstocks derived from open pollinated seed collected from the M. integrifolia cultivar H2 (Trochoulias, 1992; Huett, 2004; Hardner et al., 2009).

Avocado and macadamia orchards are in similar environments, often in close proximity in soils infested with P. cinnamomi. Since P. cinnamomi is the most destructive and important pathogen of avocado in Australia (Pegg et al., 2002), avocado trees are managed for Phytophthora using an intensive management program (Pegg et al., 1985), whereas macadamia trees receive no or limited control measures. It is common when macadamia trees are in close proximity to avocado trees for *Phytophthora* diseases to appear and be very severe on avocado but often absent or limited in the adjacent macadamia trees. It is not known if this is because macadamia roots are able to adapt to or tolerate Phytophthora infections compared to avocado roots or if there is host specificity among the Australian P. cinnamomi isolates in macadamia and avocado rootstocks. In this study, we sought to test if macadamias are more resilient to P. cinnamomi infection compared to avocado. Specifically, we aimed to determine (i) if P. cinnamomi causes root necrosis and root rot in macadamia; (ii) the effect of soils with different levels of P. cinnamomi on germination, root development and growth of macadamia seedlings; and (iii) the effect P. cinnamomi infection on the growth of chemically-treated and untreated young grafted macadamia trees. The outcomes of this study may have significant implications on Phytophthora disease management strategies and disease resistance rootstock selection programs in macadamia.

# II. Materials and Methods

# Plant materials

*M. integrifolia* trees of cultivars 816 and 842 grafted onto H2 seedling rootstock were used in field trials. Macadamia cv. 816 was selected because it was frequently observed as more severely affected/diseased by *Phytophthora* compared to other macadamia cultivars in Australia, while 842 was selected because it showed no or limited symptoms in various orchards (Akinsanmi, *unpublished*). The trees were 2-year-old at planting. In the glasshouse trials, fresh nuts (seeds) of four commonly grown macadamia cultivars (A4, 246, 816 and 842) and common rootstock (H2) were obtained from open pollinated trees from orchards

containing blocks of each cultivar. The nuts were surface sterilised in 70% ethanol and rinsed in three changes of sterile distilled water before planting directly or pre-germinated. In order to obtain 'pre-germinated' nut, the sterilized nuts were kept in moist conditions at room temperatures (22-25°C) until germinated (cracking of shell and emergence of hypocotyls). In this study, nut that were planted directly without pre-germination are considered 'non-germinated'.

# Source of P. cinnamomi cultures

In order to obtain 'soil culture', soil samples were collected from around the roots of macadamia and avocado trees showing symptoms of *Phytophthora* infection in the field as described by Smith *et al.* (2011). Briefly, replicate soil samples taken from around the root zone of diseased trees were bulked to form a composite sample from which subsamples were baited for *Phytophthora* using germinated New Zealand blue lupin roots as described by Pratt and Heather (1972), from which *Phytophthora* isolates were obtained, purified and the species identified using PCR-based technique (Drenth *et al.*, 2006). Soil samples that contained active *P. cinnamomi* were used as 'soil culture' (+*Pc*) in laboratory trials. A site in commercial orchards at Duranbah, northern New South Wales in Australia from where avocado trees had been removed because of *Phytophthora* root rot caused by *P. cinnamomi* was confirmed using the lupin baiting method. The field site is a krasnozem soil with high water retention characteristics, rich in organic matter and top soil high cation exchangeable capacity >34 mg equivalent per 100g soil.

#### In vitro assessment of direct effect of P. cinnamomi on macadamia roots

In order to determine if P. cinnamomi causes root necrosis or root rot in macadamia, seedlings of five macadamia cultivars (H2, A4, 246, 816 and 842) produced from pregerminated nuts planted in pasteurised sandy soil in the glasshouse until roots were about 50 mm long were used. Ten seedlings of each cultivar were baited for *Phytophthora* in the +Pcsoil culture. The seedlings were separately immersed in soil suspension through holes in lids of 100 mL clear plastic cups containing distilled water with ca. 200 g of +Pc soil culture and 10 seedlings of each cultivar were immersed in soil suspension in the plastic cups containing distilled water with ca. 200 g of autoclaved soil (-Pc). Each cup contained two seedlings, and cups containing New Zealand blue lupin seedlings immersed in +Pc soil and -Pc soil cultures served as controls. Cups were kept under 12 h fluorescent lights at 25°C, routinely agitated to aerate the suspension and roots were observed for rot every week for 3 weeks. The trial was repeated and at the end of each trial, the macadamia roots were removed, washed under running tap water and assessed for rot or necrotic lesions. In order to test for the presence of P. cinnamomi in the roots, the roots were surface sterilised in 10% bleach (sodium hypochlorite) solution, rinsed in sterile distilled water before plating about 10 mm sections taken from the root tips in V-8 agar plates amended with antibiotics (50  $\mu$ g mL<sup>-1</sup> each of Penicillin and Polymixin and 100  $\mu$ g mL<sup>-1</sup> of Pimaricin).

# In-situ assessment of effect of P. cinnamomi on macadamia seedling development

In order to evaluate the influence of *P. cinnamomi* on the development of macadamia seedlings, two nut types (pre-germinated and fresh surface-sterilized non-germinated nuts) of each of H2, 246 and A4 were planted at approximately 30 mm depth in 100 mL pots with perforated bottom in four different substrates: +Pc soil, pasteurised potting mix, 1:1 and 1:2
ratios of mixture of +Pc soil culture and pasteurised potting mix. Each nut type consisted of a total of 20 nuts per cultivar, and each pot contained one nut. At planting, each pot was saturated with sterile distilled water and kept at  $25^{\circ}C \pm 3^{\circ}C$  in the glasshouse. The pots were watered weekly with sterile distilled water and the percentage of seedlings that emerged was determined. Different parameters including seedling height measured from soil line to tip of topmost leaf, leaf area measured as cumulative perimeter of all leaves on each seedling, and stem diameter measured at the soil line were recorded monthly for 5 months. Seedling vigour was determined using seedling height data and was calculated as  $(h_f/h_i)^{[1/(tn-1)-1]}$ , where  $h_f$  and  $h_i$  are the final and the initial seedling heights, respectively, and  $t_n$  is the number of days between  $h_f$  and  $h_i$ . At the end of the trial, the seedlings were removed from the pots, washed under slow running tap water, blot dried and the weight of the whole seedling and the root weight were recorded. Roots were observed for root rot and necrosis. Presence or absence of P. cinnamomi in the substrate was confirmed using the baiting method with germinated New Zealand blue lupins seedlings as described above. In order to stabilise variances of the parameters measured, seedling vigour data were transformed with  $(x + 1)^{0.5}$  while the other parameters (seedling height, stem diameter and leaf area) were transformed with  $\log_{10}(x+1)$ before performing generalized linear model procedure with normal distribution in GenStat 11<sup>th</sup> edition. Data were analysed as a nested structure with nut type nested within cultivar and means tested using least significant (P < 0.05) difference tests.

#### Field trials on the performance of macadamia trees in Phytophthora-infested soil

The performance of cvs. 816 and 842 on H2 seedling rootstock in soils containing high levels of P. cinnamomi was assessed under field conditions. The performance of the macadamia cultivar were compared with commercial avocado Phytophthora root rot resistant clonal rootstock 'Dusa' and susceptible seedling rootstock 'Reed' with Hass as scion (Smith et al., 2011). Trees were planted at a spacing of 3 m x 3 m and irrigated with a single sprinkler per planting space as required. A total of six macadamia trees of each cultivar were planted among three trees each of the avocado rootstocks. At planting 60 g of a commercial general compound fertiliser (CK77, CK Life Sciences International (Holdings) Inc. and Rustica Plus, Campbells Fertiliser Australasia) was applied to the soil around each tree. Three macadamia trees of each cultivar were planted in holes pre-treated with 60 g of metalaxyl-M (Ridomil® Gold 25 G, Syngenta) and treated with potassium phosphonate applied as a bark application of 20% v/v potassium phosphonate in 2% v/v bark penetrant Pulse® (Nufarm Australia Ltd) using a paint brush to about 1 m above ground level at planting, while three macadamia trees of each cultivar were planted 'untreated' with any fungicides. In order to give the avocado trees the opportunity to begin to grow vigorously to produce a more favourable root: shoot ratio before infection, all the six avocado trees were treated with 0.1% v/v potassium phosphonate solution a day before planting in holes that were pre-treated with 60 g of metalaxyl-M and thereafter, potassium phosphonate was applied monthly to the avocado trees either as a foliar spray or as a bark application for 9 months as described by Smith et al. (2011). Tree health of both macadamia and avocado trees was assessed using a *Phytophthora* diseased tree health rating scale of 0-10, where 0 =vigorous and healthy, to 10 = dead tree (Darvas et al., 1984; Gabor et al., 1990), yearly for 4 years. The height of each macadamia tree was measured from the soil line to the canopy apex and the tree canopy volume was classified as dense, light or sparse. The effect of treatment on macadamia trees was determined as percent growth reduction using the mean height of the treated trees  $(h_t)$  and untreated trees  $(h_n)$  of each cultivar and calculated as  $[100((h_t - h_n)/h_t)]$ . Data were analysed with General Analysis of Variance procedure in GenStat, with cultivars nested within crop and date of assessment as covariate. The roots of the trees were observed for any presence of root necrosis and root rot. Samples of proteoid and secondary roots were obtained from the macadamia trees and tested for the presence of *Phytophthora* as described above and soil samples were confirmed as containing *P. cinnamomi* at the start and end of the trial using the procedure described above.

#### III. Results and discussion

#### Direct effect of P. cinnamomi on macadamia roots

Roots of lupins in +Pc soil were severely infected and rotted within 7 days, whereas, lupin roots in -Pc soil and all roots of macadamia cultivars in both +Pc and -Pc soils appeared healthy with no visible necrotic lesion or rot and the growth of the seedlings was similar. After 3 weeks, *Phytophthora* was not recovered from any sections of macadamia roots tested.

#### Indirect effect of P. cinnamomi on macadamia seedling development

The percentages of seedlings that emerged from the substrates were significantly different (Fig. 2.34). Overall, 51% of pre-germinated and 35% of non-germinated nut emerged, while only 31% of nut planted in +Pc soil emerged compared to 54% in 1:1 mixtures, 42% in 1:2 mixture and 53% in potting mix. The average seedling emergence of all substrates was 72% in H2, 40% in A4 and 23% in 246. Comparison of the pre-germinated and non-germinated nut for each cultivar showed that 81% of seedling emergence occurred in the non-germinated H2 and 63% in the pre-germinated H2 compared to higher seedling emergence in the pregeminated than the non-germinated in A4 and 246 (Fig. 2.34). Cultivar as a main factor in the analysis accounted for 55-72% of the variations in the above ground parameters of stem diameter, seedling height and vigour and leaf area (Table 2.23) whereas substrate alone accounted for <10% of the variations in the above ground parameters, but >30% in the below ground parameters (root weight and seedling weight). There was no significant (P = 0.08) substrate × cultivar interaction for all the parameters except marginally for stem diameter, where a reduction in stem diameter occurred in +Pc soil in H2. Although there was no significant interaction between substrate and nut types per cultivar for seedling vigour, the effect of substrate was dependent on the nut type planted (Fig. 2.35). Seedling vigour of pregerminated nut was consistently and significantly reduced when planted in +Pc soil, compared to other substrates (Fig. 2.35). However, the effect of substrates on seedling vigour of non-germinated nut was dependent on the cultivar. In H2 the effect of potting mix on seedling vigour of non-germinated nut was similar in +Pc soil and 1:2 mixture, whereas, in 246 and A4 effect of +Pc soil was more severe than potting mix (Fig. 2.35). Overall, seedling vigour was better in the pre-germinated nut than the non-germinated nut (Fig. 2.35). The mean root of both H2 nut types obtained from potting mix was significantly higher than those of +Pc soil and their mixtures (Table 2.24).



Fig. 2.34 Percentage of seedling emergence of pre-germinated (open bars) and nongerminated (shaded bars) macadamia nut of three cultivars 246, A4 and H2 planted in potting mix and soil containing *Phytophthora cinnamomi* (*Pc*). Bars in each graph with the same letter are not significantly different at P<0.05.



**Fig. 2.35** Seedling vigour of pre-germinated (open bars) and non-germinated (shaded bars) macadamia nuts of cultivars 246, A4 and H2 planted in potting mix and soil containing *Phytophthora cinnamomi* (*Pc*). Bars in each graph with the same letter are not significantly different at P < 0.05.

**Table 2.23** *F*-probability values of the accumulated analysis of variance of parameters measured on two nut types (pre-germinated and non-geminated) of three macadamia cultivars planted in four different substrates containing different levels of *Phytophthora cinnamomi*.

					Seedling		
		Root	Stem	Leaf			
Source of variation	df	weight	diameter	area	Height	Vigour	Weight
Treatment (substrate)	3	0.092	0.409	0.086	0.233	0.842	0.308
Cultivar	2	< 0.001	< 0.001	< 0.001	< 0.001	0.005	0.004
Treatment × cultivar	6	0.043	0.080	0.468	0.130	0.413	0.254
Cultivar/nut type	3	0.162	0.149	0.386	0.158	0.241	0.488
Treatment × cultivar/nut type	9	0.136	0.361	0.908	0.900	0.783	0.726

**Table 2.24** Means of key seedling growth parameters from pre- and non-germinated nut types of macadamia H2 cultivar planted in four different substrates containing different levels of *Phytophthora cinnamomi*. Values followed by the same letter in each column are not significantly different at P = 0.05.

Treatment		Seedling	Seedling height	Leaf area
(substrate)	Root weight (g)	weight (g)	(mm)	$(mm^2)$
+Pc soil	2.07 b	7.19 ab	81.07 c	3902 c
1:1 ratio mixture <sup>a</sup>	1.34 bc	6.14 bc	113.87 a	9465 a
1:2 ratio mixture <sup>a</sup>	1.24 c	5.53 c	88.12 bc	4827 bc
Potting mix	2.97 a	8.30 a	102.39 ab	6904 b
2251 0 2				

<sup>a</sup> Mixture of +Pc soil and potting mix.

There were significant variations in the volumes of roots obtained from different substrates (Fig. 2.36). Root density of pre-germinated nuts was severely reduced in +Pc soil, followed by 1:1 mixture and sparsely reduced in 1:2 mixture when compared with potting mix (Fig. 2.36). It appears the root density was not severely affected when non-germinated nut was planted in +Pc soil compared to the pre-germinated nut (Fig. 2.36a). This study on *in-situ* germination of macadamia nuts and the subsequent emergence of seedlings from a Phytophthora-infested substrate clearly showed that H2 is more tolerant than A4, while 246 is the least tolerant to Phytophthora infection. The inconsistent seedling emergence of nongerminated 246 and A4 nuts indicates that these cultivars should be pre-germinated before planting. In the 1970s, M. tetraphylla was favoured for seedling rootstock due to perceived superior nursery performance including more even and consistent germination, growth and stronger root system (Trochoulias, 1992; Hardner et al., 2009), but more recently, due to scion incompatibility, M. integrifolia rootstock is now favoured (Nagao and Hirae, 1992). Hence, since the early 1990s, the majority of Australian macadamia orchards have been established with *M. integrifolia* H2 seedling rootstock (Trochoulias, 1992; Huett, 2004; Hardner et al., 2009). However, the non-uniformity of seedling rootstocks coupled with the effect of rootstock-scion interaction on susceptibility to Phytophthora put the Australian macadamia industry at a great risk of Phytophthora and other soil-related problems. This necessitates the need for concerted research efforts toward the development of clonal and selection of *Phytophthora*-resistant rootstocks.



**Fig. 2.36** Appearance of roots of macadamia H2 cultivar obtained from pre-germinated (left picture) and non-germinated (right picture) in each a - d, after planting in substrates containing *Phytophthora cinnamomi* (*Pc*) (a), 1:1 mixture (b), 1:2 mixture of +*Pc* soil and pasteurised potting mix (c) and potting mix only (d). Bars represent 10 mm.

#### Field trials on the performance of macadamia trees in Phytophthora-infested soil

Lupin baiting of the soil obtained from the field trial confirmed the soil contained *P*. *cinnamomi* at the start and end of the trial. Differences in tree health of treated and untreated macadamia trees were evident from 18 months after establishment compared to 6 months after establishment in both avocado rootstocks. Tree health ratings of treated macadamia cultivars were significantly (P = 0.05) different from the untreated trees. At 18 month after planting the mean tree health rating in both treated macadamia cultivars was zero, while in the untreated 816 was 1.25 and in 842 was 0.60, but at 4 years after planting the mean tree health ratings of the treated macadamia cultivars were significantly (P < 0.001) different from both treated avocado rootstocks. The initial tree health rating at 18 month after planting in both treated avocado rootstocks was >5 when treated macadamia cultivars still appeared vigorous and healthy, while at the final assessment (4 years after planting), the mean tree health ratings in the treated avocado trees was 5.45 in Dusa and 8.40

in Reed rootstocks. The macadamia tree canopy was sparse in untreated trees and untreated 816 (Fig. 2.37a) trees were more severely affected with extensive tree dieback compared to 842 (Fig. 2.37b). The effect of *Phytophthora* on tree canopy of untreated trees was significant compared to treated trees (Fig. 2.37). Trunk canker occurred only in the untreated macadamia trees. Tree growth reduction of 842 was significantly (P = 0.02) different from 816, at 18 months after planting, mean tree growth of 816 reduced by 32.5% while 842 reduced by 9% when compared to the treated trees. But at 4 years after planting tree growth of the untreated 816 had reduced by 60% while tree growth of 842 had reduced by 40%.

Although the macadamia cultivars used in this study were grafted onto H2 seedling rootstocks, the scion significantly influenced the effect of *Phytophthora* on the tree. Thus, when trees of a less tolerant scion are planted in Phytophthora-infested soil without any chemical control applications at planting, tree growth may be drastically reduced just like we observed in the untreated 816 trees in this study. These results could explain the general observations in several macadamia orchards in Australia, where certain cultivars appear to be more severely affected by *Phytophthora* than others. Similar observations have been reported in California, where canker was observed to occur on *M. integrifolia* seedlings as well as in the *M. integrifolia* scion and the *M. tetraphylla* rootstock (Zentmyer and Storey, 1961). The variations in the scion-rootstock interaction, as observed in this study, could have contributed to the effect of *Phytophthora* on the *M. tetraphylla* rootstock that is regarded as less susceptible to P. cinnamomi (Zentmyer and Storey, 1961). Although variability in nutrient utilization among macadamia cultivars has been reported (Stephenson and Cull, 1986), there is no information on the interaction between nutrition and the physiological processes in macadamia. The effect of this interaction may be expressed in the scion x rootstock relationship to *Phytophthora*.



**Fig. 2.37** Appearance of 3-years-old grafted macadamia cultivars 816 (a, c) and 842 (b, d) on H2 seedling rootstock after planting in *Phytophthora cinnamomi* 'killing field'. The untreated macadamia trees are represented in (a) and (b), while trees treated with potassium phosphonate and metalaxyl-M at planting are represented in (c) and (d). Bars represent 0.5 m.

#### IV. Conclusions

This study confirmed that macadamia trees may be infected with *P. cinnamomi* without any visual effect on tree health. Our results are congruent to previous observations from a survey of macadamia plantations in south-east Queensland (Pegg, 1981) and in California (Zentmyer and Storey, 1961), where macadamia seedlings planted in soil infested with P. cinnamomi on which severely diseased avocado trees had been removed showed good growth (Zentmyer and Storey, 1961). Results of the field trials showed that macadamia trees are more tolerant to infection than avocado trees, thus, can withstand prolonged association with P. cinnamomi without showing any visual effect on tree health (growth and canopy) or trunk canker. Once macadamia roots are established the effect of P. cinnamomi on the tree performance is limited and the tree is able to tolerate *Phytophthora* infections if good soil nutrition is maintained. Generally, *Phytophthora* significantly affected germination, seedling emergence, seedling vigour and root weight of macadamia, when nuts were planted in infested soil, but the extent of the effect is dependent on cultivar. In our field trials, trunk canker occurred only in the untreated macadamia trees. However, it is uncertain if infections were initiated on the trunk directly or through wounds. Previous reports from several countries have confirmed *Phytophthora* canker occur on the trunks and lower branches of macadamia trees and these reports often associate infection occurred through wounds (Zentmyer, 1960; Hine, 1961; Zentmyer and Storey, 1961; Brodrick, 1973; Pegg, 1973; Mbaka et al., 2009). In certain cases, without any treatments and no apparent reason, Phytophthora canker development on macadamia trees was observed to cease after a period of time and P. cinnamomi could no longer be isolated from diseased (cankers) area (Pegg, 1981). It is uncertain if macadamias are able to curtail or outgrow the development of Phytophthora canker, or if the physiological processes of the trees can restrict continuing invasion of the tree trunk by P. cinnamomi. Since, macadamia trees planted in P. cinnamomi infested soil grew well without any visual symptoms of infection, it is not known if this 'infection abatement' characteristic contributes to the apparent low rating of visual tree health assessment of treated trees observed in this study.

Results of the *in vitro* assessment of the direct effect of *P. cinnamomi* on the roots of five macadamia cultivars showed that macadamia roots are tolerant to *Phytophthora* attack, which is similar to previous observations (Pegg, 1973). It appears *P. cinnamomi* only invades small feeder roots, but under special conditions may infect other parts of the roots (Perez-Martinez, 2008). It has been reported that the formation of proteoid or cluster roots is genetically controlled, but their initiation, growth and exudation of nutrient-solubilising compounds is influenced by a range of physical, chemical and perhaps even biological factors (Lamont, 2003; Shane and Lambers, 2005). Although it is not known when proteoid roots are formed in macadamia, the ability to initiate root clusters in proteaceae species typically takes several months (Lamont, 1973; Lamont, 2003). Hence, this could be the reason why no symptoms (necrotic or rot) were observed on the roots of the macadamia seedlings accessed in this study. This is in contrast to other reports where root rot and root necrotic lesion were reported in macadamia (Serfontein, 2008; Mbaka et al., 2009). Other reports showed that P. cinnamomi was isolated from necrotic rootlets of macadamia (Ko and Kunimoto, 1976; Pegg, 1981), it is possible that the rootlets referred to in the studies are synonymous to proteoid roots. In conclusion, successful establishment of macadamia trees in the field depends largely on producing healthy nursery stock in substrate that is free of *Phytophthora*. Application of control measures to reduce *Phytophthora* infective propagules from the root zone at planting will allow the roots of the newly planted trees to grow and become established before being exposed to incessant Phytophthora attack.

V. Communications and Extension of Research Outcomes

• Publications and Presentations

Akinsanmi OA and Drenth A (2012) Disease management workshops as July 2012 AMS Macgroup meetings in QLD and NSW.

#### 2.2.1.3. Application of Phosphonate in macadamia

#### I. Introduction

*Phytophthora* tree decline is common in macadamia orchards in Australia and the actual productivity loss has not been quantified. A major concern is how to improve productivity and growth of trees with decline symptoms and trunk canker. At present, management strategies developed for controlling *Phytophthora* diseases in other tree crop systems (Darvas *et al.*, 1984; Pegg *et al.*, 1985; Ali *et al.*, 2000; Hardy *et al.*, 2001; Browne and Viveros, 2005; Vawdrey and Westerhuis, 2007), in particular, for controlling *Phytophthora* root rot in avocado have been adopted for controlling *Phytophthora* diseases in macadamia. However, it is not know if these control strategies are effective for *Phytophthora* control in macadamia.

The use of metalaxyl and phosphite to control *Phytophthora* diseases in various tree cropping systems has generally been successful, but their efficacy varies depending on the cropping system (Guest and Bompeix, 1990; Singh *et al.*, 2003). Metalaxyl is an acylalanine fungicide with an asymmetrical carbon atom that gives rise to a pair of optical isomers or enantiomers resulting in the biological activity against oomycetes (Singh *et al.*, 2003). Metalaxyl inhibits hyphal growth through inhibiting rRNA synthesis in oomycetes, is translocated upward in the xylem and provide systemic activity against oomycetes in plants (Browne and Viveros, 2005). The removal of S<sup>+</sup> optical isomer that causes phytotoxicity in plant from metalaxyl has enabled its continued use as a fungicide.

Phosphonates or phosphites marketed as inorganic and organic salts of phosphonic acid have a complex mode of action acting directly on the pathogen and indirectly in stimulating host defence responses to ultimately inhibit pathogen growth (Guest and Bompeix, 1990; McDonald *et al.*, 2001; Browne and Viveros, 2005). The mobility of phosphite through the phloem causes it to accumulate in the prevailing sink tissues at the time of application which makes the mode and timing of application critical. Effectiveness of phosphite in suppressing *Phytophthora* depends on its concentration in the plant tissues (Jackson *et al.*, 2000). The concentration of phosphite in plant tissues is known to be directly related to its application rate (Jackson *et al.*, 2000). Phosphite is considered to have low phytotoxicity (Guest and Grant, 1991; Singh *et al.*, 2003). However, severe phytotoxicity reactions have been reported in a wide range of plant species after phosphite application (Ali *et al.*, 2000; Pilbeam *et al.*, 2004). In addition, evidence for the negative effects of phosphite on growth and development of plants because it intensifies the deleterious effects of phosphorous deficiency in plant has been reported (McDonald *et al.*, 2001; Varadarajan *et al.*, 2002).

Nevertheless, both metalaxyl and phosphite are commonly used to control *Phytophthora* diseases, after a balance between optimal concentrations for disease control, phosphate metabolism and minimal phytotoxicity reactions have been achieved. Therefore, it is critical in macadamia production system to provide the strategic timing and rates of chemical applications for the control of *Phytophthora* that is well aligned to the tree phenological events. Deployment of effective and sustainable disease control strategies including chemical control depends on a good understanding of the host-pathogen interaction, the contributing factors, the agronomic requirement of the crop and the characteristics of the pathogen, in this case, Phytophthora as a soilborne pathogen whose alibility to survive, reproduce and cause disease is also influenced by soil characteristics (Lazarovits et al., 2001). In this study, we sought to determine the rates of foliar and trunk applications of phosphite in macadamia to

control *Phytophthora* tree decline. Therefore, the specific aims of this study were to: (i) determine the maximum and optimal rate of phosphite as foliar sprays, trunk injections and bark spray application in macadamia; (ii) examine the effectiveness of the application methods of phosphite for the rejuvenation of macadamia trees in decline; and (iii) determine the efficacy of bark spray application of phosphite and soil drench application of metalaxyl on recovery of declining macadamia trees. The information from this study would provide the basis for part of the management of *Phytophthora* diseases in macadamia.

#### II. Materials and Methods

#### Foliar application of phosphite in macadamia

In order to determine the maximum and optimal rate of phosphite for foliar application for the control of *Phytophthora* tree decline in macadamia, a series of dose rates of (Agri-Fos®) 600, Agrichem Loganholme, Australia), equivalent to 1.5, 2.4, 3.0, 3.6, 4.8, 6.0 and 7.2 g/L phosphite were applied to 3-year old potted grafted trees in the glasshouse and 5-year-old trees of cultivar 849 in a macadamia orchard. In the glasshouse trial, each treatment at pH 5.9 was sprayed on the foliage of 2-3 trees using a hand-held sprayer till run-off and trees that were sprayed with water served as control. The trial was repeated on different sets of cultivar 849 trees. In the field trial, a total of 65 trees were used, of which 63 trees were partitioned to contain 7 treatments; 2.4, 3.0, 3.6, 4.8, 5.0, 6.0 and 7.2 g/L phosphite and two trees served as untreated control and the treatments were applied once in mid autumn (April) by spraying the tree canopy to run off with a knapsack sprayer. All trees were assessed weekly for phytotoxicity to the leaves until 21 days after foliar application. Severity of phytotoxicity reactions on leaves was rated as 0 = no phytotoxicity; 1 = 1% - <10% leaves with minor chlorotic reaction; 2 = 10% - <30% of leaves show low necrotic and severe chlorotic reactions;  $3 = 30\% - \langle 50\% \rangle$  of leaves with mild severe necrotic reaction; 4 = 50% - 60% of leaves are severely necrotic; and 5 = >70% of leaves show very severely necrotic reaction. In order to determine if the phosphite concentration is cumulative, at least three additional trees in the field trial received 2 or 3 foliar applications using 2.4 g/L phosphite rate at monthly intervals, resulting in total of 4.8 g/L and 7.2 g/L phosphite, respectively. Phosphite concentration in the plant was assessed at 7 and 21 days after each application. Root samples for phosphite analysis were collected from the feeder roots and fruit samples (nut containing kernel) were obtained from the tree canopy in the field trial. Samples were sent to a commercial analytical laboratory that uses a PLA005 analytical method with a phosphorous acid detection limit of 5 mg/kg of tissue on an air dry basis (SGS Australia Pty Ltd.).

#### Trunk application of phosphite in macadamia

A field trial using macadamia trees that had mild tree decline symptoms with approximately 80 m<sup>3</sup> canopy volumes was established to determine the maximum and optimal rate of phosphite applied as trunk injection on macadamia. Different concentrations of phosphite were injected into 19 year-old trees at 0.5 m above ground level, using a 20 mL spring-loaded syringe (Chemjet<sup>®</sup>, Bongaree, Qld, Australia), inserted into 25 mm deep holes drilled with a hand drill in the trunk. A series of 5 mL and 7 mL of undiluted potassium phosphonate (Agri-Fos® 600) were injected as a single unit, three units each of 5 mL and 7 mL and 4 units of 5 mL applications. These correspond to a total of 3, 4.2, 9, 12 and 12.6 g/L phosphite. Three trees were injected per treatment and untreated trees served as control. The effect of treatment

on foliage was determined using the phytotoxicity rating of the tree canopy as described prior. In order to observe if timing of phosphite application is critical for effective control, the macadamia trees were injected in autumn to coincide with nut harvest and root flushing periods (Stephenson *et al.*, 1986). In a second trial, phosphite was applied in autumn as a bark application of 20% v/v phosphite (200 g/L phosphonic acid) in 2% v/v bark penetrant Pulse® (Nufarm Australia Ltd) to the trunk of 15 trees until slight run-off using knapsack spray unit to about 1 m above ground level as previously described (Smith *et al.*, 2011). The applications were repeated at the same period in the following season. Concentrations of phosphite in the root and fruit were determined as described above, and the samples were obtained from both the side and the opposite side of point of injection at 7 and 21 days after application.

#### Efficacy of phosphite applications on macadamia tree decline

The efficacy of phosphite application method on rejuvenation of 15-year-old macadamia trees of cultivar 849 with obvious dieback and decline symptoms, with or without stem canker associated with P. cinnamomi (Akinsanmi and Drenth, unpublished) was determined using a bark application of 20% v/v phosphite in 2% v/v bark penetrant Pulse® and foliar spray application of 2.4 g/L phosphite. Foliar application was applied based on dilute spray volume on 100 m<sup>3</sup> canopy volume macadamia trees(Drew et al., 2002) using an air blast onesided sprayer with DS Radak and 2000 L tank (Silvan Supaflo, Australia Ltd.), while bark application was applied using a knapsack spray unit as describe prior. Phosphite was applied in late autumn (May) and repeated in the mid spring (October) seasons to a row of 40 trees per treatment, while a row of 40 trees that received no phosphite served as untreated control. At the start of the trial, and in May of each year for 3 years, each tree was assessed for disease severity based on a rating scale of 0-5, where 0 = dark green leaves, healthy tree; 1 =mild pale to yellow leaves of the tree canopy; 2 = severe pale to yellow leaves obvious signs of tree stress (shoot regrowth from rootstock); 3 = stem canker, gummosis and sparse canopy with pale to yellow leaves; 4 = stem canker, very sparse tree canopy and obvious dieback; and 5 = extensive branch dieback or dead tree with stem canker present (Fig. 2.38).

#### Comparison of phosphite trunk spray and metalaxyl soil drench on macadamia tree decline

The effect of soil drench application of metalaxyl and bark application of phosphite on rejuvenation of 8-year-old macadamia trees cultivar 849 with decline symptoms was examined and compared with untreated control trees. Each treatment was applied biannually at late autumn (May) and mid spring (October) to a row of 15 trees for 3 consecutive years. Phosphite was applied as a bark application of 20% v/v phosphite in 2% v/v bark penetrant Pulse® to the trunk of trees as described above while metalaxyl-M (Ridomil Gold 25G, Syngenta Crop Protection Pty Ltd, Australia) was applied at 60 g granules per meter of canopy diameter around the tree and irrigated immediately after to incorporate the granules into the soil. Data on the disease severity ratings were recorded as described prior.



**Fig. 2.38** Pictorial representation of *Phytophthora* tree decline severity ratings in macadamia. Rating scale of 0-5, where (a) 0 = dark green leaves and healthy tree; (b) 1 = mild pale to yellow leaves of the tree canopy; (c) 2 = severe pale to yellow leaves obvious signs of tree stress (shoot regrowth from rootstock); (d) 3 = stem canker, gummosis and sparse canopy with pale to yellow leaves; (e) 4 = stem canker, very sparse tree canopy and obvious dieback; and (f) 5 = extensive branch dieback or dead tree with stem canker present.

#### Data analysis

Data measuring the effects of different phosphite rates applied as foliar sprays, trunk sprays and trunk injections on tree canopy were analysed and significant means were separated and compared using the least significant different test at 5% significant level. Disease severity ratings data were  $log_{10}(x + 1)$  transformed to stabilise variance, thereafter, the effect of the treatments on disease severity of each year was analysed separately using the generalized linear model procedure which excludes intercept in the model in SPSS [IBM SPSS Statistic version 19], then, data were combined and analysed with year as a covariate. Treatment means were compared using the Bonferroni test with the mean difference significant at the 0.05 level. Progressive improvement across the 3 years for each data by each treatment was evaluated using repeated measures procedure in SPSS. Pairwise comparison between the years was performed and significant differences were evaluated using the Bonferroni test.

#### III. Results and Discussion

#### Foliar application of phosphite in macadamia

In the glasshouse trial, at 7 days after spray application phytotoxicity was only evident at the highest rate of 7.2 g/L phosphite with browning of the tips of the leaves, and at 14 days after application leaf burns was evident in four other treatments (Fig. 2.39 a). Similar phytotoxicity ratings were recorded at the 14 and 21 days after spray application. Therefore, treatments were significantly (P < 0.05) differentiated in their phytotoxicity ratings at 14 days after applications (Fig. 2.39a). The mean phytotoxicity ratings of trees treated with 2.4 g/L phosphite or less were not significantly different from the water sprayed control, but were significantly different from applications at higher rates (Fig. 2.39 a). Significant differences were observed among the treatments in the field trial (Fig. 2.39 b). Although phytotoxicity ratings of trees sprayed with 2.4 g/L phosphite were similar to those sprayed with 3.0 g/L phosphite, it was significantly lower than ratings of other treatments with phytotoxicity ratings of > 2 (Fig. 2.39 b). Increasing phytotoxicity ratings of the tree canopy occurred with increasing number of foliar application (Fig. 2.40), consequently, the amount of phosphite in the plant (root and fruit) increased with number of applications (Fig. 2.40). Concentration of phosphite in the plant tissues sprayed to a total of 7.2 g/L rate (three foliar applications) was significantly different from the 2.4 g/L (one foliar application) and 4.8 g/L (two foliar applications) and the untreated control (Fig. 2.40).



**Fig. 2.39** Mean ratings of phytotoxicity on leaves of macadamia trees at 14 days after foliar applications of a series of phosphite on (a) in a glasshouse trial and (b) in a commercial orchard. Bars in each graph with the same letter are not significantly different with Least Significant Different test at P < 0.05.



Number of application

**Fig. 2.40** Concentrations of phosphonic acid in the roots and nuts (kernel) of macadamia trees that received one, two and three monthly foliar spray applications of 2.4 g/L phosphite starting at the root flushing period in mid autumn. Samples were obtained at 21 days after each application. Lines on bars indicate standard error.

#### Trunk application of phosphite in macadamia

At 7 days after trunk injection, there was no visual effect on the tree canopy in any of the treatments except in the trees injected to a total of 12 g/L and 12.6 g/L phosphite where very severe necrotic and leaf burns occurred within 7 days in all the trees. However, the amount of phosphite in the root and nut samples was below the detection level, <5 mg/kg. At 21 days after trunk injections, the mean phytotoxicity ratings ranged from 0.2 in the single unit applications of 5 mL (3 g/L) and 7 mL (4.2 g/L) to 4.3 in the multiple unit applications of total of 12 g/L and 12.6 g/L phosphites. Generally, phytotoxicity ratings in the single unit applications was <1, showing no or little leaf burns at the margins of the leaves. Mean phytotoxicity rating in the 12 g/L phosphite application was 2.3, where <30% of leaves in the tree canopy showed slight necrotic reactions. Varied amounts of phosphite were detected in the root and fruit samples (Fig. 2.41). The amount detected in the root of multiple unit applications ranged from 24 mg/kg to 263 mg/kg, while in the single unit application the mean amount detected was 7 mg/kg. Phosphite detected in the fruit samples of multiple unit applications was 35 mg/kg only in the 12.6 g/L phosphite application (Fig. 2.41). Phosphite was detected in samples obtained from both sides of the injection points which showed lateral mobility of phosphite occurred in macadamia trees. In the 20% v/v bark applications trials, no

phytotoxic reactions occurred in the tree canopy and the amount of phosphite detected in the root and fruit samples was <5 mg/kg.



Fig. 2.41 Mean concentrations of phosphonic acid in the roots and nuts of macadamia collected at 21 days after trunk injection with different rates of phosphite. Lines on bars indicate standard error.

#### Efficacy of phosphite applications on macadamia tree decline

There was no significant difference in the mean disease severity ratings between the trunk and foliar phosphite applications. However, disease severity ratings in both treatments significantly (P = 0.038) reduced from the initial disease severity rating of ~1.0 to <0.5 in the third year, but disease severity ratings in the untreated control trees marginally increased from mean of 1.0 to >1.3 over the 3 years period. Based on disease severity ratings, improvement from macadamia tree decline and stem canker was not significantly (P = 0.836) different between the years in both foliar and trunk phosphite applications.

#### Comparison of phosphite trunk spray and metalaxyl soil drench on macadamia tree decline

The biannual application of both treatments reduced disease severity ratings of the affected trees from 12 month after applications. Unlike in the untreated control where disease progressed over time, both phosphite and metalaxyl significantly controlled macadamia tree decline (Fig. 2.42). There were no significant differences between years (P = 0.316) and in the treatment by year interaction (P = 0.360). Although the rate of disease control in both phosphite and metalaxyl treated trees were expressed as logarithm  $y = a - b \ln(x)$  functions with  $R^2 > 95\%$ , the rate of improvement of diseased trees with phosphite was better than the metalaxyl treated trees (Fig. 2.42). The logarithm function showed incremental expression of macadamia tree decline in the untreated controls (Fig. 2.42).



**Fig. 2.42** Progression of disease severity on 15 macadamia trees affected by macadamia tree decline following bark application of phosphite (20% v/v) phosphite and 2% v/v bark penetrant) or soil drench of metalaxyl-M (60 g per meter of canopy diameter) applied at root flushing periods in autumn and spring each year for four years and an untreated control. Rating scale of 0-5, where 0 = dark green leaves and healthy tree and 5 = extensive branch dieback or dead tree with stem canker present.

#### IV. Conclusions

Prior to this study, information on the effectiveness of phosphite as trunk injections, trunk or foliar sprays and soil drenches with metalaxyl on diseases caused by *Phytophthora* species in macadamia production system has been anecdotal or limited (Nagao et al., 1992). Through a series of field trials, we found that trunk and foliar sprays of phosphite and soil drenches with metalaxyl are effective against macadamia tree decline and stem canker caused by P. *cinnamomi* in commercial orchards. This is similar to reports in various tree cropping systems where metalaxyl and phosphite were successfully used to control diseases caused by Phytophthora (Darvas et al., 1984; Pegg et al., 1985; Ali et al., 2000; Hardy et al., 2001; Browne and Viveros, 2005; Vawdrey and Westerhuis, 2007). However, the rate of improvement or rejuvenation of macadamia tree decline was better in phosphite than the metalaxyl treated trees. Unlike phosphite, metalaxyl has low adsorption and high mobility, thus, can be rapidly leached from sandy soils which are low in organic matter (Sharom and Edgington, 1982) and extensive biodegradation of metalaxyl may have occurred which could have led to a significant premature loss of fungicidal efficacy (Bailey and Coffey, 1985). Therefore, it is imperative for growers to consider the soil type in their orchards before application of metalaxyl as soil drench for macadamia tree decline and stem canker.

Our findings showed that macadamia is sensitive to phosphite trunk injections at rates higher than 4.2 g/L with severe visible phytotoxicity while foliar application of phosphite above 2.4 g/L showed significant phytotoxicity. Therefore, these rates represent the maximum rate of phosphite that should be applied to macadamia. The application of control treatment in autumn and spring were effective against macadamia tree decline and stem canker. This result is congruent to control of *Phytophthora* pod rot and stem canker of cocoa, where under plantation conditions, annual or 6-monthly applications of phosphite was consistently reported to provide long-term control (Guest et al., 1994). These periods are concurrent to periods of predominant new root growth in macadamia (Firth et al., 2003). The root flushing periods in macadamia result in considerable increase in the capacity of the fibrous roots to regenerate new lateral growth from desiccated rots following drought or from decay after wet soil conditions, thus, the fibrous roots contribute to the increase in nutrient absorption capacity of the large surface area of proteoid roots which may enhance the tolerance and/or recovery of macadamia to extreme weather conditions (Firth et al., 2003) and the effect of root or soilborne diseases. However, further studies are still required to determine if annual phosphite application is as effective as biannual phosphite applications in macadamia.

Phosphite applied as trunk injection resulted in relatively higher detection of phosphrous acid in the plant tissues than other methods of application. This is similar to the report that injections of phosphite at flush maturity were more effective for the accumulation of phosphonate in feeder roots than when trees were actively flushing (Pegg *et al.*, 1990). Although macadamia trees were injected in autumn during root flushing period, phosphite caused severe phytotoxic leaf burns. This could be because major assimilates, carbohydrates and nitrogen compounds, are continually cycled within macadamia tree (Huett, 2004). Hence, trunk injection application methods may be limited to severely diseased and non-productive macadamia trees. Pilbeam *et al.* (2000) reported that trunk injection of phosphite is expensive and impractical as a routine treatment for extensive areas but can be used to treat severely defoliated trees caused by *P. cinnamomi* infections. Therefore, in Australia, where macadamia production is highly mechanised, foliar applications of phosphite may be the most practical method, as these could be applied along with other foliar applications.

The highest rate of phosphite foliar spray of 7.2 g/L tested in this study was severely phytotoxic to macadamia, causing severe burning of the leaves in both glasshouse and field trials, whereas, 2.4 g/L phosphite showed no or little phytotoxic leaf burn at the tips of leaves which were not significantly different from both the water sprayed and untreated controls. This indicates that 2.4 g/L is the maximum rate of foliar applications of phosphite application in macadamia. Similar marginal and tip necrosis has been reported previously in a variety of plants in response to the application of phosphite (Pilbeam *et al.*, 2000; Barrett *et al.*, 2003). Previously, the lowest concentration of phosphite at which leaf burn has been reported is 0.4% phosphite following foliar application in mandarins and azaleas (de Boer and Greenhalgh, 1990; Pilbeam *et al.*, 2000), but in this study, foliar application of 0.25% phosphite led to necrosis of the leaf in macadamia. This may be due to sensitivity of macadamia to phosphorus in the plant.

As a Proteaceae, macadamia is well-adapted to soils with low available phosphorous and have efficient phosphorous uptake mechanism that they experience phosphorus toxicity at lower rhizosphere phosphorous than non Proteaceae crop plants (Hawkins *et al.*, 2008). Differences in phosphite uptake and retention ability among plant species, phosphite concentration in plant tissues, the rates of phosphite applied and phytotoxicity vary among plant species (Ali *et al.*, 2000; Pilbeam *et al.*, 2000; Barrett *et al.*, 2004). Since phosphite is

known to negate the acclimation of plants to phosphate deficiency by disrupting the induction of enzymes and obstruct the signal transduction pathway by which plants perceive and respond to phosphate deprivation at the molecular level (McDonald *et al.*, 2001; Varadarajan *et al.*, 2002), this could make Proteaceae more sensitive to phosphite than other plant species. Application rate of phosphite is a significant factor that influences phosphite concentration in plant tissues which may have stimulated a direct response of macadamia tree to counteract *P. cinnamomi* infection (Jackson *et al.*, 2000; McDonald *et al.*, 2001), thus, resulting in an increased proliferation of new root growth required for the tree development and productivity. The phosphite concentration in the plant increases with repeat applications of phosphite, therefore, macadamia trees showed increasing phytotoxicity and phosphite concentration with increasing application rate and number of applications. Timing of phosphite application is also crucial as source-sink relationship at the time of application also affects phosphite concentrations in plant tissues (Whiley *et al.*, 1995).

In conclusion, this study provides the first actual data and information on effective application rates of phosphite in macadamia. It indicates that phosphite and metalaxyl have the potential to manage *P. cinnamomi* in macadamia. The low phosphite rate above which phytotoxicity occurs in macadamia may be because macadamia is well adapted to low soil phosphorous having evolved in phosphorous deficient soils in Australia. It is generally accepted that phosphite does not eradicate the pathogen (Pilbeam *et al.*, 2000) and the issues of metalaxyl degradation (Sharom and Edgington, 1982) and high risk of *P. cinnamomi* resistance to metalaxyl showed that it is essential to adopt integrated disease management strategies including cultural control methods to control macadamia tree decline and stem canker. Plant hygiene practices are essential to minimise the spread of *P. cinnamomi* from nursery plants to field establishment. More research still needs to be done to determine how to maximise the control of *P. cinnamomi* while minimising any harmful effects of phosphite on plant and in the ecosystem.

#### V. Communications and Extension of Research Outcomes

• Publications and Presentations

Akinsanmi OA and Drenth A (2012) Disease management workshops as July 2012 AMS Macgroup meetings in QLD and NSW.

### 2.2.2. Decision guide for Phytophthora management with phosphite

A decision guide for application of control measures for Phytophthora diseases using Phosphite was developed for macadamia growers. Specific management strategy that is based on the level of disease severity (Fig. 2.38) should be applied. The management strategy has been classified into four categories: healthy, maintenance, curative and restorative strategies (Table 2.25). In addition a guide to the timing and volume of application was developed (Table 2.25). Application for a minor use permit with a revised rate of phosphite as trunk and foliar spray applications obtained in this project for the macadamia industry, is currently with the APVMA

**Table 2.25** Decision guide for application of phosphite to control Phytophthora diseases in macadamia

Application strategy	Severity level	Description	Application method	
		Scale		
Healthy	None	0	<b>None</b> (Maintain good management practices)	
Maintenance	Low -Medium	1 - 2	Foliar	
Curative	High	3 - 4	Trunk or Foliar	
Restoration	Very High	5	Trunk	

Table 2.26 Guide to timing and dose rate of phosphite application to macadamia

Application of chemical control	Foliar spray	Trunk spray
Timing	After leaf flush i.e. at root flush (hardened and non-fleshy new leaf)	Root flush
Number/year	<ul><li>1 - 4 (1-2 = low-medium severity;</li><li>2-4 times for high severity)</li></ul>	1-2
Dose rate	Max. 0.25% Phosphite	20% Phosphite + 2% bark penetrant
Spray volume	Dilute	Wet slightly until run-off (around the trunk to about 1 m above soil level)

## 2.3. Husk Rot

Husk rot (Fig. 2.43), or sometimes referred to as 'anthracnose', is likely to be present in most mature macadamia trees, without causing any yield losses (Fitzell 1994). The symptoms include diffuse black spots that are up to 1 cm in diameter that rapidly spread and eventually end in a dark coloured greasy decay of the husk (Fitzell, 1994). In some years premature shedding of nuts can occur. Economic effects are not known, however the first report of husk rot threatening yields in Australia was made in 1991 (Loebel, 1991), in the USA in 1957 (Brooks and Olmo, 1957) and confirmed present in Hawaii as early as 1962 (Zentmyer, 1962a). Fitzell (1994) quotes premature drop of up to 20% and an epidemic in Northern Rivers NSW was threatening to cost the local industry an estimated \$6 M (Quinlan 2004).In Australia, anecdotal report indicates that cv. 344 is frequently more infected than other cultivars.

Husk rot disease can be caused by a number of pathogens such as *Phomopsis*, *Colletotrichum, Lasiodiplodia* and *Stilbella* which are all common fungi. Despite this, the disease occurrence is sporadic but for unknown reasons it is frequently reported from the Gympie region in Queensland. The sporadic nature of husk rot outbreaks in the region is most likely due to an association with weather events and/or insect activity. In order to develop some control measures for this disease it is important to determine what causes the onset of this disease so the risk factors can be determined and treatment methods developed. A link between insect damage and the occurrence of other damage to the husk such as hailstorm damage needs to be determined. Due to the sporadic nature it is to be expected that targeted timing of fungicide application may contribute to preventing economic damage.



Fig. 2.43 Husk rot symptoms with visible pin-hole injury

## 2.3.1. Identification of the causal agent of macadamia husk rot

#### I. Introduction

In humid conditions, fruiting bodies will appear in the lesions and infection can cause up to 20% on immature fruit to drop. Some organisms that have been considered capable of causing husk rot are *Colletotrichum* sp., *Phomopsis* sp., *Lasiodiplodia* sp. and *Stilbella* sp. (Fitzell, 1994). However, there is no information on any systematic studies to determine if any specific organism is responsible for husk rot. This study used morphological and molecular methods to identify the organisms associated with husk rot in macadamia and performed pathogenicity test to confirm the causal agent. Identifying the causal agent of diseases is important in an effort to develop effective management strategies for this disease.

## II. Materials and Methods

#### Isolation of organisms associated with the symptoms

Samples of macadamia fruits displaying symptoms of husk rot were collected from macadamia orchards in the Gympie region. Pure cultures were isolated from the diseased samples and grown on PDA plates and DNA of each isolate was extracted using the Promega DNA extraction kit. Representative isolates with the same morphological and cultural characteristics were selected and sequenced.

#### Identification of organisms using DNA sequencing

PCR primers specific to the internal transcribed spacer region (ITS) were used on each isolate. PCR amplifications were performed using a Peltier-Effect Cycling (PTC<sup>™</sup>-100 thermal programming controller, MJ Research Inc., Waterson Mass. USA) under the following conditions: initial denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 30-90 s, annealing at 55-60 °C for 30-90 s and extension at 72 °C for 60s, with a final extension at 72 °C for 10 min. The total 30 µl PCR reaction volume contained 25 ng of template DNA, 1x PCR buffer (Fisher Biotech, Australia), 1.5 mM MgCl<sub>2</sub>, 0.5-2.0 U of Taq DNA polymerase (Fisher Biotech, Australia), 0.14-0.60 mM of each primer and 0.10-0.21 mM dNTPs. PCR products were separated on 1.0% agarose gels (DNA grade agarose, Progen industries Ltd., Queensland, Australia), stained with ethidium bromide and visualized under UV light. Fragments were excised from the agarose, purified using a Roche High-Pure PCR Product Purification Kit (Mannheim, Germany) according to the manufacturer's protocols, and sequenced in the Big Dye Terminator<sup>™</sup> Version 3.1 at the Australian Genome Research Facility (AGRF) Ltd, University of Queensland, Queensland, Australia. Sequence data were analysed using Vector NTI Advance 10.3.0 (Invitrogen Corporation, Austria) and similarity searches were performed for similar nucleotide sequences from the GenBank database.

#### Pathogenicity tests

Macadamia fruits of cv. 344 with no visible disease symptoms or injury were obtained from the tree canopy of trees with no husk rot. The fruits were subdivided into two lots: non-sterilised and surface sterilized by soaking in 10% NaOCl solution for 5 mins, then rinsed in

three changes of sterile distilled water, and air dried in the laminar flow. An hierarchical process was used to confirm pathogenicity (Fig. 2.44). Fifteen fruits of each fruit lot were injured using a sterile needle creating 5-10 pin-holes per fruit and 20 fruits were injury-free. Then 5 fruits each were incubated under room conditions  $(25^{\circ}C\pm 2^{\circ}C)$  for 3 days in three conditions; (1) in moistened sterile nylon bags with two husk rot-diseased fruits, (2) moistened with  $10^{6}$  spore concentrations of pure culture of either *Colleotrichum* sp., or *Phomopsis* sp., and (3) moistened with sterile distilled water. After incubation, each fruit was inspected for husk rot symptoms and Koch's postulates were tested for diseased fruits. Non-sterilised and sterilised fruit with and without pin-holes that were inoculated with sterile water served as control.



Fig. 2.44 Hierarchical process for determining the causal agent of husk rot.

#### III. Results and Discussions

#### Identity of isolates

Overall, 23 fungal isolates in the genera *Phomopsis*, *Colletotrichum* and *Nigrospora* were obtained from husk rot symptomatic husks. The majority (83%) of the isolates belonged to genus *Phomopsis*, while 13% of the isolates were *Colletotrichum gloerosporoides* and 4% were *Nigrospora sphaeria*. Results of the BLAST searches of *Phomopsis* sp. were similar to *Diaporthe* species, the telomorphic stage of *Phomopsis* sp. and *Diaporthe* phaseolorum was the most common species with high DNA similarity values with the *Phomopsis* isolates.

#### Pathogenicity of causal agent

Results of the pathogenicity tests showed that fruit incubated with diseased fruit and inoculated with pure culture of *Phomopsis* sp. produced husk rot only in fruit pin-hole injury treatments (SID, SIP, NID and NIP). The control and injury-free treatments did not produce husk rot.

#### IV. Conclusions

Results of this study confirmed that *Phomopsis* sp. is the causal agent of husk rot of macadamia. Although other fungi were isolated from diseased materials, these fungi were associated with the disease but not the primary causal agent. Only fruit with injury was infected in the pathogenicity study, demonstrating that infection by *Phomopsis* sp. may actually be secondary and may only occur once the fruit pericarp is predisposed to injury. However, it is unknown if insect or other factors are responsible for inducing the injury in the field conditions. More research is needed to develop an effective management strategy. Due to the sporadic nature of this disease and the highly variable levels of disease incidence, field trials to assess the effectiveness of control methods are seriously hampered.

#### 2.3.2. Survey of husk rot severity and economic impact

#### I. Introduction

Although husk rot has been reported to be threatening yields in Australia (Loebel, 1991), the economic impact of husk rot has not been evaluated. This study was established to determine the relative incidence of husk rot, and if injury is a factor in its occurrence. Understanding of the factors associated with husk rot incidence will aid in managing the disease.

#### II. Materials and Methods

#### Prevalence of husk rot

Macadamia orchards in the Glasshouse Mountains and Gympie regions were surveyed for husk rot in the 2009-10 seasons. The proportion of fruit that abscised with husk rot was recorded in each orchard. Samples of abscised fruit were also obtained from various orchards and examined microscopically for the presence of injury, injury with husk rot or husk rot only. In order to determine the annual prevalence and severity of husk rot, the 8-years historical data between the 2000-01 and the 2007/08 seasons collected by Dr Henry Drew in the Gympie district were analysed. Husk rot severity was scored weekly from mid-December to February each year. Severity score ratings of 1-5: 1 = very low; 2 = low; 3 = medium; 4 = high and 5 = very high were used.

#### Association of injury with husk rot

The association of injury with husk rot incidence was evaluated in field trials. Field trials were established using 15 trees each in two rows of cv. 344 in an orchard in Gympie. Treatments included injured and non-injured fruits. Pin-hole injuries were made on developing fruits in the tree canopy of cv.344 in December in the 2007-08 seasons. The injured fruits and fruits on selected racemes without artificial injury were tagged and monitored for husk rot.

#### III. Results and Discussions

#### Prevalence of husk rot

Husk rot severity was significantly different among years (Fig. 2.45), and the incidence varied annually and at times it occurred in different orchards. Since the 2000-01 season, husk rot occurred on at least 3 orchards in the Glasshouse Mountains and Gympie regions and has been recorded on 26 orchards in the same year in the 2001-02 and the 2006-07 seasons. In addition, within the same orchard and tree row, significant differences occurred among the trees.



**Fig. 2.45** Mean husk rot severity score in macadamia orchards assessed in the Glasshouse Mountains and Gympie regions between the 2000/01 and the 2007/08 seasons. Severity score ratings of 1-5: 1 = very low; 2 = low; 3 = medium; 4 = high and 5 = very high. Bars indicate standard errors.

#### Association of injury with husk rot

About 89% of fruit sampled with husk rot had injury and none of the asymptomatic fruit samples had visible injury (Fig. 2.46). Types of injury observed included husk splitting or microscopic pin-hole size. No husk rot was observed on fruit with artificial injury in the field trials. Incidence of husk rot was erratic with no definite pattern in the orchards. This suggests that other factors in addition to the microorganism may be involved in husk rot pathosystem.



Fig. 2.46 Mean proportions of husk rot symptomatic and asymptomatic (healthy) fruit sampled for association with pericarp injury.

## IV. Conclusions

This study confirmed that the incidence of husk rot varied between seasons, blocks and rows. It appears other factors are associated with infection by *Phomopsis* sp.

# Chapter 3 Assessment and Control of Emerging Pathogens and Diseases

There are frequent reports and observations of the occurrence of new diseases, pathogens and disorders in the Australian Macadamia Industry. Some of these have economic significance. For example, we have observed 'black kernel' in sound NIS and have isolated microbes from infected kernels and the recent occurrence of sudden tree death in NSW associated with the bark beetle *Cryphalus niger* and a Ceratocystis fungus. With a growing industry and increasing mature orchards, it is important to monitor new developments and provide prompt control measures.

# 3.1. Kernel rot

#### I. Introduction

The germination behaviour of macadamia seeds (nuts) of different varieties varies greatly. In some cultivars, germination is very erratic ranging from four weeks to eight months after sowing. Several factors including thickness of shell, time of storage of the seeds and conditions of storage between harvesting and planting, have been attributed to this erratic germination behaviour (Hamilton, 1957; Hardner *et al.*, 2009). In Australia, after natural abscission from the tree, macadamia nuts are harvested from the ground, thereafter the pericarp (husk) is mechanically removed and the resulting nuts are dried for storage or further processing. It has been noted that when the shell is cracked before planting, the majority of the embryos rot rather than germinates (Hamilton, 1957). It is not known whether this is due to injury to the embryo in cracking or the involvement of other factors. It has been reported that mechanically cracking of the shells actually lowers the percent of germinating seed, therefore, it is not recommended (Hamilton, 1957).

In order to determine the factors that contribute to erratic germination, non-germination and post-emergence death of macadamia seedlings, we compared H2 fruit that was harvested from the tree canopy with fruit obtained from the orchard floor in early March. Thus, we sought to address the following questions: 1) is the soil the source of kernel rot? 2) are kernels from the orchard floor contaminated with micro-organism? 3) can the germination of macadamia kernel be improved by obtaining nuts directly from the tree canopy?

#### II. Materials and methods

#### Is soil the source of kernel rot?

One hundred nuts each from the tree canopy and from orchard floor were subdivided into 2 lots of 50 nuts. In order to remove any surface contaminants, the first lot was surface sterilised in 25% NaOCl and 70% ethanol solution prior to planting in autoclaved soil, whereas, the second lot was planted without surface sterilisation in autoclaved soil. The percentage of nut that germinated clean and unclean (with micro-organisms), the percentage of cracked shell [rotten kernel or non-germinated], uncracked shell [rotten kernel or intact kernel] were recorded after 3 months.

#### Are kernels from the orchard floor contaminated with micro-organisms?

A total of 100 nuts obtained from both the tree canopy and 100 nuts obtained from the orchard floor were manually dehusked, surface sterilised in NaOC1 and ethanol solution, cracked under aseptic conditions and small pieces of kernel were aseptically removed and placed on Potato Dextrose Agar plates. The plates were incubated at room temperature for 10 days. The number of kernel pieces with micro-organisms and total number of colonies (bacterial and fungal) were recorded. In order to determine if the germination of macadamia kernel can be improved by obtaining the nuts directly from orchard floor, 100 nuts sourced from the orchard floor were treated with Chipco<sup>®</sup>Rovral<sup>®</sup> liquid fungicide and the percentage that germinated clean was recorded.

#### III. Results and discussion

Nut deterioration occurred more in cracked shell than uncracked shell. Fungicide coating of nuts before planting did not reduce kernel rot in uncracked shell. The results showed that irrespective of fungicide treatment or no treatment >90% of the nuts harvested off the tree canopy germinated clean without any micro-organisms. In contrast, only about 30% of nuts from orchard floor germinated of which 13-22% germinated clean (Fig. 3.1). Results showed that nut germination cannot be improved with fungicide treatment compared to surface sterilisation with bleach and ethanol.

No micro-organisms were observed in kernel obtained directly from the tree, but a total of 10 colonies (24%), consisting of 5 fungal and 5 bacterial colonies, were obtained from kernels of nuts harvested from the orchard floor.

Even though only H2 was used in this study, the overall significance is that micro-organisms are a significant contributor to erratic germination behaviour of macadamia nuts especially when nuts are obtained from orchard floor rather than from the tree canopy. The potential for a uniform and high percentage of nut germination is maximized when nuts are obtained directly from the tree canopy. Subsequent erratic germination may be attributed to genetic differences, time and conditions of storage between harvesting and planting. In a commercial situation, where large amount of nuts are required it is advisable that they be harvested the same day as they dropped from the tree. This would potentially reduce/limit the deteriorating conditions and kernel rot incidence and will improve production efficiency for nursery operators. Although it appears that surface sterilisation of nuts harvested from the surface of the shell, it is important to know that no significant improvement can be made when nuts are already affected by deterioration and kernel rot (Fig. 3.2).

#### **IV.** Conclusions

A range of factors may cause macadamia seedling blight. These factors are often extrinsic to the germinating nut. Direct exposure to sunlight, overhead irrigation, pests and pathogens are factors that can cause seedling blight. In order to compensate for the damage to the primary hypocotyl, a generally weaker secondary shoot may be produced from the cotyledon (Fig. 3.2), but often, the blighted geminating nuts are destroyed. Geminated nuts forming secondary hypocotyls should be avoided as rootstock because the resulting seedlings may not be vigorous as those obtained from clean nut. Ideally, nuts for nursery use should be obtained from the tree canopy. However, in mechanized commercial situation where nuts are harvested from the ground, clean-up a day prior to harvest in dry condition may reduce kernel infection. A factsheet (see below) has been provided to aid better understanding of the importance of growing healthy plants from nursery to orchard.



Fig. 3.1 Comparison of germination of macadamia nuts harvested from the orchard floor that were surface sterilised and non-treated. Bars represent standard error.



Fig. 3.2 Poorly germinated macadamia nuts. Note: absence of hypocotyl development.



Fig. 3.3 Germinated macadamia nuts with blighted hypocotyls. Note the secondary shoot in the second nut from right.

## V. Communications and Extension of Research Outcomes

- <u>Publications and Presentations</u>
- 1. Akinsanmi OA and Drenth A 2011 Macadamia fact sheet: Plant health- growing healthy trees from nursery to orchard. <u>Australian Macadamia Society Ltd. News</u> <u>Bulletin</u> Vol. 39 (1): 52-53.

# Macadamia Fact Sheet

Plant health – growing healthy trees from nursery to orchard

Olufemi Akinsanmi (Femi) and Andre Drenth, University of Queensland, Tree Pathology Centre, Brisbane

#### Introduction

This fact sheet provides succinct information on diseases and conditions that affect orchard establishment and productivity of macadamia trees from the nursery. Nursery practices contribute to both short- and longterm conditions of trees planted in orchards. Trees to be planted must be healthy i.e. free of diseases, with good root network and very resilient. The following information should be considered.

#### Seed

Poor seed health reduces germination, seedling establishment and gives rise to poor tree establishment. Seed should be harvested from the tree or collected the same day it is abscised from the tree and dehusked immediately. This maintains a high percent seed germination, reduces contamination by micro-organisms causing kernel rot or poor germination.



Rotten seed (left) and poorly geminated macadamia seed (right).

#### Seedling

A range of factors may cause poor performance of macadamia seedlings after normal germination. These factors are mostly extrinsic to the germinating nut and include; sunburn, excessive watering, pesticides, poor handling and pathogens. These may cause trees to suddenly dieback in the nursery or many years later in the orchard. Certain symptoms like seedling blight (brownish blotches on the foliage and withering of the entire plant without rotting) may become apparent early in the nursery. In order to compensate for the damage to the primary hypocotyl or shoot, secondary shoots may be produced but these are generally weaker than the primary shoot. Poor handling during potting may result in abnormal stem or root configurations like 'J-roots', 'swirl-roots' and 'J-stems'.



Germinated macadamia nuts with blighted hypocotyls. Note the secondary shoot in the second nut from right.



Normal (far left), abnormal root and stem configurations (middle) and tree that suddenly died due to poor root system (right).

#### Grafted tree

Grafted trees are prone to a number of debilitating conditions including graft incompatibility, dieback syndrome, and foliage diseases. Generally, good hygiene, nutrition and watering practices may reduce the incidence of these conditions. Use sterilised or clean materials for grafting, avoid over watering, especially if using overhead irrigation, monitor root development and use adequate fertiliser regime.

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Phomopsis die-back disease (left) and Alternaria blight (right) close-up of leaf infection on grafted trees.

#### **Field establishment**

Macadamia trees that are planted in the field should be free of pests and pathogens. For instance, trees infected with Phytophthora may not survive under field conditions, and those that survive are more prone to tree decline in later years. Poor root system also contributes to poor tree establishment in the orchard. It is advisable to check for healthy root system prior to planting. Thereafter, good soil health management practices should be followed.



From top left: planted trees with poor and good root development; plant die-back; poor root network in potting mix; and damaged tree due to Phytophthora infection.

### Further information

Further information is available from Email: uqoakins@uq.edu.au

#### Farming sometimes requires change More of the same won't help

Soil Health - Tree Health - Sustainable Income Canopy management - Tree removal - Erosion control

Light penetration - Plant diversity – Ground cover

> Promote active microbial biomass Crop Protection Management You need a management change normac459@optusnet.com.au Phill McCarthy 0428861097

## Harvesting & Dehusking

Experienced northern rivers operator available for harvesting with two harvesters:

- MacMaster Harvester with multiple heads and dehuskers. Suit 5 – 12 meter rows
- Carrero Harvester small jobs welcome.
- Dehusking line available, with registered weigh station.

Family concern - satisfaction guaranteed

Contact Paul Scott at Southridge Farms

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### 3.2. Gall canker

During the previous project MC03007 the causal agent of gall canker was identified as *Nectria pseudotrichia*, a fungus that is commonly found in tropical and subtropical environments. Cross inoculation studies revealed that the fungus has a wide host range and can colonise other hosts including avocado, mango and certain eucalypt species. The disease was observed in young and bearing-age trees in several orchards in NSW and QLD but the economic importance of the disease is still unknown.

#### Fungicide testing

In anticipation of possible need for fungicide intervention, a range of fungicides were evaluated for their efficacy against the fungal pathogen using an *in vitro* assay. Ten different fungicides at different concentrations (0.05, 0.10 and 25 mg/L) were tested against the fungus. Results showed significant differences among fungicides and concentrations (Fig. 3.4). At least, four potential fungicides (Folicur, Score, SpinFlo and Vision) were identified for possible fungicides for further testing under field conditions.



**Fig. 3.4.** Mean diameter of mycelial growth of *Nectria pseudotrichia* isolates at 7 days on culture media amended with different fungicides. Lines on bars indicate standard error.

#### Disease monitoring and survey

A management approach where the galls were excised from the affected tree trunks was trialled. Results indicate that redevelopment of canker and gall occurred on the edges of the previous canker. However, when the galls were excised and the tree sprayed with SpinFlo, further redevelopment of the galls and canker in the affected part ceased. In order to determine the impact gall-canker on macadamia production, diseased trees were monitored

for tree death and/or decline. Results indicate that gall-canker appears to be 'tolerated' by affected macadamia trees and has no effect on yield and/or tree performance. Development of diseased non-bearing trees was not affected. In Bundaberg, gall-canker symptoms were observed on 2-years old newly planted grafted trees. Visual appearance of tree canopy of symptomatic and asymptomatic trees was similar. In all the three major macadamia nurseries surveyed, no gall-canker symptom was observed. Annual reports of industry pest scouts and consultants, at the AMS annual pest consultant meetings, on any noticeable effects of gall-canker on production revealed no detrimental impact on tree growth and production.

It is proposed that in order to control the spread of the disease, tree with visible signs and symptoms of infection should not serve as a source of budwood. Currently, control by surgery (excise gall) and fungicide application of affected trees appear to be unnecessary. Further monitoring and awareness campaigns for any noticeable detrimental effect by growers and consultants should continue.

## 3.3. Tree dieback in Atherton Tablelands

#### I. Introduction

The Australian macadamia production is expanding to northern Queensland areas of Mackay, Atherton Tablelands and Emerald. However, these areas are prone to severe tropical rainfall and cyclones. In 2005, following cyclone Larry's devastating impact in northern Queensland about 25% of the macadamia orchards in the Atherton Tablelands were completely destroyed while severely damaged trees in the surviving orchards were replanted or severely pruned to rejuvenate the trees and since then diseases have become the main concern to the growers in the district (O'Farrell *et al.*, 2006; Weinert *et al.*, 2007).

In May 2010, several 3 years old trees of cultivar A268 that were replanted following the cyclone episode were reported to show unusual disease symptoms. The symptoms were initially thought to be due to nutritional disorders, but all attempts to rectify the symptoms through addition of fertilizer failed. Severity and the number of affected trees continued to increase in an affected orchard at Tolga. Interestingly, most of the affected trees were replanted on the same sites where macadamia trees had been removed (Fig. 3.5). According to the growers, the symptoms were first noticed on few trees immediately following the wet season in March 2010. Therefore, in order to determine the prevalence of the symptoms, a survey of macadamia orchards in the district was performed in June, 2010. Identity of microorganisms associated with the symptoms was determined from samples obtained from the affected trees



**Fig. 3.5** A block of 3-years old macadamia trees with dieback symptoms adjacent to mature trees (foreground) without dieback symptoms. Arrows indicates dieback symptoms in affected trees.

#### II. Observations and discussion

#### Survey of diseases in the district

Five orchards were surveyed for the presence of the symptoms in the Atherton Tablelands region. Out of the five orchards surveyed, dieback symptoms were observed in three orchards and it was very severe in one orchard at Tolga in a block of 3 years old cv. A268. It was reported that cyclone Larry-damaged trees were uprooted and converted to wood chips in the orchard before replanting with A268. In the second orchard, very mild dieback symptoms were observed on few (<10) scattered branches of mature bearing trees. In the third orchard, dieback symptoms were observed on a branch of one mature (>10 year old) tree. Other diseases observed include *Phytophthora* tree decline which was the most prevalent disease observed in all orchards and husk spot which was observed on a few trees.

Characteristic dieback symptoms were observed in all affected trees with distinct gummosis (Fig. 3.6) below the point of dieback. Above the point of gummosis, dieback occurred with dried or dead and brown coloration beneath the bark of the branches or stem (Fig. 3.7) and brown or dried leaves remained attached to the dead branches (Fig. 3.8). Leaves (green) of the affected trees showed purplish blotches and reddish coloration of the leaf veins (Fig. 3.9). In addition, the cross-sections through the affected branch showed dark discoloration of the wood (Fig. 3.7).



**Fig. 3.6** Point of gummosis on macadamia trees cv. A268 from which branch or leaves above turned brown or dead (A). (B) shows brown discoloration beneath the point of gummosis on the main trunk and (C) shows point of gummosis at tree branch region.



**Fig. 3.7** Discoloration of the stem and branches of macadamia trees cv. A268 with dieback symptoms. A. cross-section of affected stem above point of gummosis; B. lateral discoloration above the point of gummosis (arrow); and C. advancing discoloration and browning of stem above point of gummosis. Arrows indicate point of gummosis.



Fig. 3.8 Macadamia trees cv. A268 with dieback symptoms showing dried leaves that remained attached to branches.



Fig. 3.9 Leaf of macadamia trees cv. A268 showing purplish blotches and reddish vein on trees with dieback symptoms.

#### Symptoms

It is uncertain what caused the injury at the point of gummosis that possibly served as the entry point for the pathogens. In similar cases of sudden tree death syndrome in the Northern Rivers region, involvement of insects, especially Scolytid beetle, was considered as the main cause of the injury to the branches and trunk. In the last two years, very few (3-5) incidences have been reported in the Northern Rivers region. Although the beetle can potentially attack healthy trees, they gravitate more readily to trees that are already stressed by Phytophthora or drought. Generally, stem discolouration was <2mm in depth and did not cover a large area. The purple and reddish coloration of the leaves appear similar to boron or phosphate deficiencies. Leaves in nutritionally deficient trees abscise readily, but the leaves in the diseased trees remained attached to the branches. In addition, symptoms of nutritional disorders appear throughout the tree canopy, but only the affected branches showed symptoms in tree dieback caused by pathogens. In order to confirm if a nutritional factor was responsible for the leaf symptoms, plant nutrient analyses were performed separately on both asymptomatic and symptomatic trees. The results showed that all the major and trace elements except manganese were within the recommended levels for macadamia trees (Table 3.1).

#### Isolation and identification of pathogenic organism

Samples were obtained from symptomatic and asymptomatic plants. Samples were observed for visible signs and microscopic structures of insects, fungi and bacteria. The oozing technique was used to test for the presence of bacteria in the diseased materials. Direct microbiological isolation techniques for fungal pathogens were made at the point of gummosis and between the advancing margins of diseased and healthy tissue in diseased branches. Results of the tests for bacteria were negative and no fungus was obtained from asymptomatic tissues, whereas, fungi were consistently isolated from symptomatic samples.

Morphological characteristics on PDA and DNA sequencing of the ITS region of the fungi allowed the identity of the fungal isolates to be confirmed. Most of the isolates were identified as belonging to the *Botryosphaeriaceae* species complex. The morphological characters of the isolates were spreading dark green or black colonies and the conidia were dark brown, striate, ellipsoidal, uniseptate and produced in ascostromatic pycnidia. Isolations from small lesions below the point of gummosis on branches produced 2 fungal genera out of which *Pestalotiopsis* sp. constituted 60 % and *Epicoccum* sp. constituted 40% of the total isolations. While all isolations made from above the point of gummosis between healthy and diseased tissues produced fungi identified as *Pestalotiopsis* sp. and *Botryodiplodia*-type colonies including *Dothiorella/Botryodiplodia* sp. Fungal isolates obtained from isolations made from different orchards with similar dieback symptoms were identified as *Lasiodiplodia* sp. and BLAST searches of the ITS sequences showed high similarity with *L. theobromae*. All the fungi expect *Epicoccum* sp., are part of the Botryosphaeriaceae complex.

Nutriont	Unit	Diaback treas	Haalthy trees	Recommended level
Nutitent	Unit	DIEDACK LIEES	meaning mees	Kecommended level
Nitrogen (Kjeldahl)	%	1.6	1.6	1.4-1.5
Nitrate Nitrogen	mg/kg	50	50	
Phosphorus	%	0.08	0.09	0.08-0.10
Potassium	%	0.9	0.95	0.4-0.7
Sulfur	%	0.17	0.16	0.16-0.25
Calcium	%	0.57	0.57	0.5-0.9
Magnesium	%	0.1	0.1	0.07-0.10
Sodium	%	0.01	0.01	< 0.02
Chloride	%	0.05	0.05	< 0.05
Manganese	mg/kg	3800	3600	100-1000
Iron	mg/kg	41	58	40-200
Copper	mg/kg	4.6	4.1	4.5-10
Zinc	mg/kg	8.3	7.3	6-15
Boron	mg/kg	22	22	40-75
N/P Ratio		20	18	
N/K Ratio		1.8	1.7	
N/S Ratio		9.4	10	

**Table 3.1** Status of nutritional analysis of leaves of cultivar A268 from asymptomatic (healthy) and dieback symptoms and expected recommended range for macadamia.

#### Pathogenicity assays

Pathogenicity tests using wound inoculation techniques were performed with representative isolates of Botryosphaeriaceae species complex. Mycelial plugs from 6 isolates on PDA were inoculated separately on to wounded stems of 6-months-old macadamia trees in a greenhouse. Stem inoculated with sterile PDA plug served as control. After 4 weeks of regular daily watering followed by 3 weeks water stress (no watering of pots), 60% of the inoculated test plants withered and became irreversibly desiccated, while 30% recovered as watering resumed but declined 2-3 weeks later, reproducing the symptoms observed in the field (Fig. 3.8). In contrast, the control plants recovered from water stress and remained alive. *L. theobromae* was re-isolated from all infected plants. Results indicate that isolates identified as *Dothiorella/Botryodiplodia* sp. induced dieback in potted trees in the glasshouse 3 months after inoculation.

#### **III.** Conclusions

Currently, more than 2000 names are linked to this family Botryosphaeriaceae including teleomorph and anamorph states of which *Diplodia*, *Botryosphaeria*, *Fusicoccum*, *Dothiorella*, *Lasiodiplodia* and *Sphaeropsis* (Slippers and Wingfield, 2007). Although some fungi in the family can infect through natural openings of healthy plants and survive in the hosts as endophytes, most of the species of the Botryosphaeriaceae cause disease symptoms such as dieback and cankers on numerous woody and non-woody hosts, especially in combination with stress-inducing environmental conditions (De Wet *et al.*, 2008), such as water stress (Ma *et al.*, 2001) and extreme temperatures (Michailides and Morgan, 1992).

The taxonomy of genera of the Botryosphaeriaceae with *Diplodia*-like anamorphs (*Diplodia*, *Lasiodiplodia* and *Dothiorella*) is commonly confused because they have similar typically dark ovoid conidia with thick walls that are difficult to distinguish from each other (De Wet *et al.*, 2008). These genera are well-known pathogens, with a cosmopolitan distribution and are able to habit as saprobic, parasitic and endophytic (Denman *et al.*, 2000).

Outbreaks of Botryosphaeriaceae-related diseases can cause significant yield loss (Ma *et al.*, 2001), spread rapidly and become very destructive when disease-favourable environmental conditions prevail in extensive area. Botryosphaeriaceae-related diseases pose a serious threat to several crops including grapevine decline and cankers (Taylor *et al.*, 2005), stem blight of blueberry (Polashock and Kramer, 2006), apple ring spot (Tang *et al.*, 2012), panicle and shoot blight of pistachio (Michailides and Morgan, 1992) and mango dieback (Slippers *et al.*, 2005). Botryosphaeria disease is now considered the single greatest threat to the California pistachio industry (Ma *et al.*, 2001).

In macadamias *Botryosphaeria ribis* (*Dothiorella ribis*) has been reported to induce branch canker and dieback (Herbert and Grech, 1985; Grech *et al.*, 1994). In dieback symptoms, the leaves on affected branches look pale and lack a healthy sheen that eventually dry out but stayed attached to the branch. In canker symptoms the bark of affected branch become dark with slight water-soaked appearance and the wood has a brown-purple discoloration in narrow lines along the stem. However, the disease is regarded as minor in macadamia.

IV. Communications and Extension of Research Outcomes

• <u>Technical report</u>

Akinsanmi OA. Status of macadamia diseases and sudden tree dieback in Atherton Tableland. Australian Macadamia Industry December 2011.

## 3.4. Sudden death syndrome

### I. Introduction

The incidence of sudden death in macadamia trees as the results of association between scolytid beetle, mainly *Cryphalus* sp., and fungal pathogen(s) has been erratic. Fungi within the Botryosphaeriaceae group were obtained from infected trees.

In the last 5 years, the incidence has been reported in five orchards in the Northern New South Wales production region. Distinct signs of the beetles exit points are evident on the branches and trunk of affected trees (Fig. 3.10). The presence of these signs on the plant parts distinguishes sudden death syndrome from tree decline and dieback in macadamia.



Fig. 3.10 Signs of points of exit of beetles (white arrows) on macadamia branch on trees with sudden death syndrome. Black arrows indicate gummosis.

### II. Observations for developing management strategy

### Transmission studies to determine if beetles are a vector of the fungal pathogen

Samples of *Cryphalus* sp. were obtained from trees with sudden death syndrome. The beetle samples were divided into two lots. Beetles in the first lot were surface sterilized in 10% NaOCl solutions, then rinsed in three changes of sterile distilled water before plating in PDA, whereas beetles in the second lot were plated directly (unsterilized) on PDA plates. The plates were incubated under room conditions. The plates were observed for growth of microorganisms and at 14 weeks after inoculation, no fungal growth was observed on the plates demonstrating that *Cryphalus* sp. may not be a vector of the fungal pathogen. Information obtained from molecular studies also failed to reveal presence of any fungi in the beetles.

#### Field observations

Field observations and discussions with growers of affected orchards showed that sudden death syndrome follows the onset of stress due to factors other than the observed pest organisms. It appears that a combination of stress factors together with the biological pressure from pathogens and pests favours the development of sudden death syndrome. The symptoms can develop rapidly and irreversibly causing tree death.

Observation of the distribution of affected trees showed that there is no defined disease distribution pattern in the affected orchards. Although the insect beetles can potentially attack healthy trees, they gravitate more readily to macadamia trees that are already stressed possibly due to *Phytophthora* infection and drought.

#### Management strategy

The proposed management strategy is to improve or eliminate the factors that cause severe tree stress including lack of adequate water and soilborne pathogens such as *Phytophthora*. The use of irrigation to supplement rainfall and improvement of soil health to reduce the impact of soilborne pathogens on tree health are critical to the management of sudden death syndrome. Field observations suggest prevention is the most effective strategy. Once the sudden death syndrome is initiated, the affected tree will eventually die.

#### **III.** Conclusions

It is critical to understand the ecological role, the pathogenicity, diversity and host-pathogenenvironment interaction that are mediated by farming practices to reduce the threat posed by the syndrome.

## 3.5. Plant health in macadamia nurseries

### I. Introduction

Nursery plants can be an important carrier of many pests and pathogens, including exotic organisms that threaten not only ornamentals but also agricultural crops and forests. There are numerous historical examples of major pathogens and pests that were introduced to farms, areas, or countries via the nursery trade that have caused widespread and catastrophic epidemics (Parke and Grünwald, 2012). Generally, methods to prevent the movement of pests and pathogens via the domestic nursery trade are based on certification, endpoint inspections and quarantine.

The use of pesticides in nursery operations may result in asymptomatic conditions of infected materials before planting in the field. Pesticides may temporarily suppress disease severity and diseased roots in infested pots or potting media may be asymptomatic. In Australia nursery certification schemes exist in some crops such as avocado, where the Avocado Nursery Voluntary Accreditation Scheme (ANVAS) is operated under strict hygiene conditions where the plants in the nursery are inspected and tested twice each year by government inspectors. The scheme was developed for participating nurseries to offer trees for sale that are certified free of Phytophthora root rot and avocado sunblotch viroid and that are true-to-type with respect to the variety offered for sale.

At present, there is no accreditation scheme for macadamia nursery practitioners and therefore, growers are left to decide and accept trees from the nursery of their choice without any quality guidelines. Sometimes within a few years after planting in the orchard, significant numbers of young trees develop large multiple Phytophthora cankers and died. The following case studies clearly underpin the importance for the macadamia industry to have a nursery accreditation scheme. Based on poor establishment of trees from certain nurseries, a workshop was organised with key stakeholders to substantiate the need and facilitate the development of a nursery accreditation scheme for the Australian macadamia industry.

#### II. Nursery-field tree pathway conditioned trees to disease

#### Case study 1

Certain macadamia growers experienced repeated failures of newly planted trees that originated from container-grown rootstocks. This practice caused poor root development (Fig. 3.11) which was not obvious under nursery conditions because of regular fertilizer regime, frequent watering and regular pesticide usage.

#### Case study 2

Extensive tree death (Fig. 3.12) was observed in two newly established macadamia orchards in Bundaberg. The trees were planted in 2006 and 2008 and all affected trees showed typical *Phytophthora* stem canker symptoms. About 50% of soil samples taken from around the root zone of symptomatic plants and 75% of root samples obtained from affected trees tested positive for *P. cinnamomi*. Samples taken from healthy plants and between the rows were negative for *Phytophthora*. Trees with mild dieback symptoms that were treated with

Phosphorus acid in 2006 recovered and showed similar tree canopy appearance as healthy trees.

#### Case study 3

In order to confirm if *Phytophthora* sp. observed in the field samples that caused tree death and stem canker (Fig. 3.13) also occur in macadamia nurseries, a survey of certain macadamia nurseries was conducted. Samples were obtained from potting mixes in the nurseries were tested for *Phytophthora*. *P. cinnamomi* was detected from the potting mix samples obtained from dieback-affected plants. *Phytophthora* sp. was also detected by direct isolation from roots of affected plants. Water samples taken from the source of irrigation in each nursery were negative for *Phytophthora*.



Fig. 3.11 Macadamia tree with poor root development and stem canker symptoms



Fig. 3.12 Young macadamia tree death due to *Phytophthora*-induced disease. Arrow indicates stem canker.



Fig. 3.13 Young macadamia trees with Phytophthora stem canker symptoms.

#### III. Workshop - Plant health in macadamia nursery

Two series of workshops on the importance of maintaining good plant health in both nursery and field conditions were held during this project MC07003. The first workshop was targeted at key stakeholders including industry representatives, nursery operators/managers, and crop protection scientists. The second workshop was targeted at a wider audience including growers and crop consultants.

The first workshop was held on 2<sup>nd</sup> June 2009 to educate the nursery managers on the importance of managing diseases and pests in the nursery and the need to develop a nursery certification scheme for the macadamia industry. There were 7 presentations:

- 1. Why the Australian Macadamia Society (AMS) is looking at Nursery accreditation.
- 2. Issues impacting nursery profitability.
- 3. Real life examples including nutritional and hardening off concerns.
- 4. NGIA Nursery Accreditation Scheme: benefits and requirements, EMS and biosecurity.
- 5. Micro-grafting technique in macadamia propagation for high throughput and profitability.
- 6. Prevention and control of important diseases.
- 7. Pest control in the macadamia nursery.

The first workshop produced two significant outcomes. Firstly, that AMS should promote the requirements, development and implementation of a nursery Accreditation Scheme in the macadamia industry. Secondly, the use of micro-grafting technique for high throughput and profitability should be further developed for adoption by nursery operators. Additional benefit of having accredited macadamia nursery scheme is that participating nurseries will offer trees for sale that are true-to-type with respect to the variety from the macadamia breeding program.

The second workshop that targeted a wider audience was held as part of the MacGroups in July 2011 in six sessions at five locations in Queensland and New South Wales macadamia production areas. The sessions focused on the control of *Phytophthora* from the nursery to field establishment. Feedback from growers revealed the desire and willingness to use the services of accredited nurseries. A workshop was also conducted on tree health during the annual industry congress in Bundaberg in 2009.

#### IV. Conclusions

Macadamia trees from different nurseries showed signs of Phytophthora canker after planting in the field. The level of disease incidence and severity varied between batches demonstrating that the occurrence may be related to respective batches of potting mix delivered to the nursery facilities.

As part of monitoring of plant health it is important that the root system be evaluated on a regular basis. Development of a vigorous root system throughout the depth of the potting bag

is critical to good tree establishment. The roots should be free of pathogens and without any 'J-roots'. Rootstock is used for its vigour in the root system so it seems logical that regular monitoring of its development be carried out. A healthy root system is the basis for a healthy tree. The choice of potting mix has a significant impact on the development of a healthy root system and attention must be given to selecting good potting mix. Since macadamia rootstock trees spend a considerable amount of time in the nursery before they are planted in the field, the longevity consistency of the mix throughout the bag is important.

Traditional approaches that rely on endpoint inspections to detect infested plants before planting are reactive and do not address unknown pathogens or pests that may occur in nurseries (Parke and Grünwald, 2012). A systems approach that is proactive and aimed at reducing the risk of infestation by correcting unsafe nursery practices for all pathogens and pests should be adopted (Parke and Grünwald, 2012). Emphasis should be placed on safe production practices that result in healthy plants. This may be achieved through a good and well-monitored accreditation scheme. One advantage of using a systems approach is that growers and nursery operators learn where their sources of contamination occur so that they can treat the "root" of the problem, not just the symptoms (Parke and Grünwald, 2012).

#### V. Communications and Extension of Research Outcomes

- Publications and Presentations
- Akinsanmi OA and Drenth A 2011 Macadamia fact sheet: Plant health- growing healthy trees from nursery to orchard. <u>Australian Macadamia Society Ltd. News</u> <u>Bulletin</u> Vol. 39 (1): 52-53. (Section 3.1 V)
- 2. Akinsanmi O & Drenth A (2010) Improving macadamia germination efficiency <u>Australian Macadamia Society Ltd. News Bulletin</u> Vol. 8 (5): 58-60.
- 3. Akinsanmi OA & Drenth A (2008) Seedling dieback in macadamia-MC07003 Australian Macadamia Society Ltd. News Bulletin Vol. 35 (1): 44-46
- 4. Akinsanmi OA and Drenth A. Case report on 2006 nursery issues in Queensland on macadamia. 14pp. Australian Macadamia Industry. June 2007

## Chapter 4 Exotic Diseases/Pathogens of Quarantine or Biosecurity Significance

### 4.1. Potential pathogens of quarantine significance

The macadamia industry is a signatory to the Emergency Plant Pest Response Deed with Plant Health Australia and has been proactively participated in producing the nut industry biosecurity plan. In order to detect and identify exotic diseases early, educational material is needed to increase awareness at the grower and processor level. At present booklets on diseases and pests of macadamias exist but they do not include exotic pests and diseases. Preparation of factsheets on a few key pests and diseases are needed to raise awareness and the acquired information will also help in case of an incursion. We also have to engage the nursery and ornamental industries as they import and trade in plants which can act as hosts for important macadamia diseases.

Contributions and an updated review of exotic pathogens and pests of macadamia were provided to develop an updated Nut Industry Biosecurity Plan in 2009. This was done in conjunction with the entomologists from NSW DPI. A study trip to the South African macadamia industry in September 2009 and discussions with delegates from other countries at the 4<sup>th</sup> International Macadamia Symposium in South Africa revealed the following diseases of potential quarantine significance to Australia,

## 4.2. Recent and reported new pathogens in macadamia

#### Rat tail

Rat tail (Fig. 4.1) is a disease that affects macadamia raceme in South Africa. The symptoms are similar to blossom blight or lace bug damage that occurs during flowering in Australia. It has been reported that the disease is caused by a fungal pathogen, *Cladosporium* sp. (van den Berg *et al.*, 2008). The disease is considered a minor disease that occurs during prolonged wet weather at anthesis and does not warrant any control. Blossom blight was reported to occur but the frequency and its interaction with *Cladosporium* is unclear. *Cladosporium cladosporioides* was first reported on macadamia in South Africa as the causal agent of raceme blight in 2008 (van den Berg *et al.*, 2008).



Fig. 4.1 Symptoms of Rat tail disease on macadamia raceme in South Africa

#### Trunk Blotch

Blackening along the macadamia twigs and branches with unidentified fungal structures were observed in a 12 years old orchard in South Africa. Leaves on infected twig die from the end of the twig (Fig. 4.2). The darkened blotch is visible along the trunk and more advanced symptoms showed clear advancing margin (Fig. 4.2). Death of the tissue of the bark underneath the blotch has been observed (Andrew Sheard, *Personal communications*). The pathogen was identified as an epiphytic state of *Erythricium salmonicolor* that penetrates trunks when sufficient inoculum potential has been reached (Prof. Mike Wingfield, *Personal communications*).



Fig. 4.2 Symptoms of tree blotch on (A) twig and (B) trunk of macadamia in South Africa.

#### Rosellinia-induced diseases

It was reported that extensive macadamia trees are affected by *Rosellinia* root rot caused by *R. bunodes* in Guatemala. In Colombia, the fungus *Rosellinia pepo* was reported as the causal agent of star gall causing the death of the tree while in its productive stage (Ortiz *et al.*, 2006). This pathogen is considered to be a main phytosanitary problem to the macademia tree. While in Mexico, *Rosellinia* spp. were identified as the causal agent of white rot in macadamia (Cruz B *et al.*, 1992).

The pathogen causes root and collar rot of trees, leading to a decline in the vigour of the entire plant and a general rot of the underground parts of fleshy plants. The base of the trunk at soil level can show signs of a dark, wet rot, especially if kept moist by weeds or wet weather. As the disease progresses, the infected tissue becomes rotten. Trees develop a generally unthrifty appearance with leaf yellowing, cessation of root growth and infected trees will eventually die. Occasionally the trees collapse and die rapidly and once established in orchard soils, the disease is almost impossible to control and due to its wide host range, the pathogen will attack any new trees planted in that soil.

*Rosellinia* species have been recorded all over the world. Many species occur as saprobes and only a few are well-known root pathogens (ten Hoopen and Krauss, 2006). Successful Rosellinia control usually consists of an integrated approach, in particular, the cultural control options and chemical control may supplement cultural measures (ten Hoopen and Krauss, 2006). *Rosellinia* spp. are already identified as pathogens of quarantine importance in the Nut Biosecurity Plan. *Rosellinia necatrix* causes root and wood rot of a wide range of trees that result in decline and sometimes tree death. It spreads from tree to tree and spread occurs via the fungal growth between roots and in infested soil. Due to the high level of disease severity and extensive tree death that is widespread in macadamia orchards in South America, a good understanding of the diseases caused by *Rosellinia* spp. including identity of the organisms and effective management practices for macadamia is critical for the Australian macadamia

industry. Limited information is available on how the disease is distributed across Australia and which hosts are affected.

White root rot caused by the fungus *R. necatrix* has been recorded in Queensland's Granite Belt on apples. Plant Health Australia (PHA) has declassified *R. necatrix* as an Emergency Plant Pest (EPP) under the Emergency Plant Pest Response Deed (EPPRD). Studies on the ability of *Rosellina* sp. in Australia to cause severe damage in macadamia should be investigated.

#### Pierce Disease

Researchers in Costa Rica have found that the causal agent of Pierce Disease, the bacterium *Xyllela fastidiosa* can cause problems in macadamia. *X. fastidiosa* is exotic to Australia and an important pathogen of many horticultural crops including citrus, peach, guava, avocado, coffee, and grapes as well as a number of ornamental hosts such as Bougainvillea and Hibiscus.

#### Grey kernel

A grey kernel disease complex has been reported from the USA. The main causal agent is the bacterium *Enterobacter cloacae* (Kaneshiro *et al.*, 2003). NIS appears healthy even when kernels are infected. Infected kernels emit an acrid odour and possess a cheesy, acidic flavour. It is believed that once kernels expressing foul odour reach holding bins, the odour is absorbed by healthy kernels rendering the entire batch unmarketable (Kaneshiro *et al.*, 2003).

II. Communications and Extension of Research Outcomes

• <u>Publications and presentations</u>

Femi Akinsanmi, Andre Drenth and Tom Indebitzen (2009) Pests and diseases in the South African Macadamia industry. Australian Macadamia Society News Bulletin Vol. 37 (6) pp. 38-41.

# **Chapter 5 General Discussion and Future Directions**

### 5.1. General discussion

This project has identified, developed and delivered strategic management options for key diseases affecting macadamia, in particular the two major diseases; husk spot which cause annual immediate reduction in yield and kernel quality of over \$10 million if not adequately controlled, and *Phytophthora* stem canker and tree decline which progressively cause debilitating effects thereby reducing the macadamia industries production potential. In consideration of sustainable and profitable production systems, effective disease management strategies and actions were provided. The project has increased our understanding of several macadamia disease-systems including the causal agent(s) and epidemiology of several diseases; husk rot, tree dieback, gall-canker, raceme blight and rot.

This project has delivered significant gains in the efficiency of the choice and application of chemical compounds through improved spray applications system including spray coverage and volume, concentration rates, and the number of spray applications. Benefit-cost ratio analyses were determined to underpin decisions for husk spot control and coupled with the risk assessment criteria developed, serve as vital decision making tools for growers. The integrated management approach including cultural control, choice of cultivar and decisions for fungicide spray applications were identified for husk spot in this project. Biological products were screened to assess the efficacy for husk spot control.

*Phytophthora* causes diseases with a range of symptoms within the macadamia industry. Due to the prevailing wet weather conditions, reduced soil health management in most farms, its incidence and economic impact continues to increase. This project has provided management strategies through efficient use of Phosphite to the macadamia industry. Phytophthora management must be integrated following a systems approach that includes production and sale of disease-free trees in the nurseries, enhancing root development and tree establishment in the field via planting young trees in pre-treated holes, and maintaining good soil health status in the orchards. Outcomes of the project suggest that macadamia is resilient to *Phytophthora* infection. Therefore, in order to reduce reliance on routine chemical applications to control *Phytophthora*-induced diseases, it is possible to and highly desirable to maintain good soil health that will reduce any detrimental impacts of infection. This project included extensive work with the macadamia nursery industry and stakeholders and facilitated workshops for implementing an accredited nursery scheme aimed at management of pests and diseases in nurseries.

A series of regular workshops, industry news bulletin articles, presentations at various industry meetings, annual conferences, MacGroup and best practice group meetings and field days in parallel with the research and development throughout the project duration, served as effective platform for maintaining consistent contact with growers. This ensured effective delivery of information and transfer of knowledge. This contributed to the high initial adoption rate of 35% that was recorded for adoption of the new fungicide (Cabrio®) for husk spot in the project.

### 5.2. Future directions

This project has recognized the need for further research to improve the decision making tools for husk spot control including selection of resistant cultivar, disease forecasting to fine tune decision making, and development of cultural practices that will reduce inoculum density and minimise the impact of the disease. Future research should focus on integrated management of husk spot through improved cultural control practices that include removal and rapid degradation of sticktights from the tree canopy using mechanical and/or biological approaches. Alternative chemicals to replace carbendazim that contribute to existing chemical arsenals should be provided along with simple-to-use management decision making tools. The levels of the economic returns that are achieved through fungicide spray applications to control husk spot are very high. If the current reliance on chemical applications as the sole control measure in mature orchards is to be reduced, other control options, including use of biological products should achieve a high similar or better benefitcost-ratio than the chemical applications. The acceptable 'economic threshold' needs to be identified. Screening and selection of husk spot resistant varieties within the MC09021 and future breeding programs should continue using phenotypic traits and molecular-assisted markers.

Future R&D should assess the effects of improved soil health management on reduction of disease incidence and severity; investigate different soil health treatments and management options for improving productivity in declining trees; assess macadamia species and varieties for selection of Phytophthora disease resistant rootstock; and develop an improved integrated management strategy for Phytophthora with the aim of reducing reliance on phosphorus acid, including resistant rootstock selection. The frequency of application and effective concentration of phosphite in macadamia roots for control of Phytophthora diseases should be determined. An improved understanding of the economic impacts of Phytophthora diseases to individual growers is required to accelerate adoption of research outcomes.

Assessment and control of other endemic diseases should be continued. Importance of these diseases may shift from minor to major if not adequately controlled. For instance, husk rot and flower blight have the potential to cause significant yield loss under disease conducive conditions. Although this project MC07003 has identified the causal agent of husk rot, information on the timing of infection and the conditions that predispose nut to infection still remain unresolved. The information on the timing and the conditions required for infection is essential before any meaningful and effective disease management options are provided. Seasonal poor fruit set often attributed to flower blight caused by *Botrytis cinerea* is an ongoing concern. This is due to poor understanding of symptoms and the nature of flower blight caused by this pathogen and disease control measures at flowering. The routine diagnosis of diseased materials in macadamia that enabled early detection and prompt management of emerging diseases in the industry in the MC07003 project should be continued.

## **Chapter 6 Technology Transfer**

### 6.1. Publications

#### 6.1.1. Books

 Akinsanmi OA, Miles AK, and Drenth A. (2009) Macadamia. In: Diseases of fruit crops in Australia, 3<sup>rd</sup> edition. Cooke, T., Persely D. and House S. (Eds.). Department of Primary Industries and Fisheries, Queensland, Australia.

#### **6.1.2.** Peer-refereed Journal

- 1. Akinsanmi OA and Drenth A (2012) Economic returns from fungicide application to control husk spot of macadamia in Australia is influenced by spray efficiency, rates and costs of application. *Crop Protection* Accepted April 2012
- 2. Akinsanmi OA, Topp B and Drenth A (2012) Pericarps retained in the tree canopy and stomatal abundance are components of resistance to husk spot caused by *Pseudocercospora macadamiae* in macadamia. *Euphytica* 185:313-323
- 3. \*\*Miles AK, Akinsanmi OA, Aitken EAB and Drenth A. (2010) Source of *Pseudocercospora macadamiae* inoculum in macadamia trees and its use for characterising husk spot susceptibility in the field. *Crop Protection* 29: 1347-1353.
- 4. \*\*Miles AK, Akinsanmi OA, Aitken EAB, and Drenth A. (2010) Timing of infection of macadamia fruit by *Pseudocercospora macadamiae*, and climatic effects on growth and spore germination. *Australasian Plant Pathology* 39: 453-462.
- 5. Akinsanmi OA and Drenth A. (2010) Spatial pattern and the effects of climatic factors on husk spot disease in macadamia. *Australasian Plant Pathology* 39: 125-131.
- 6. \*\*Miles AK, Akinsanmi OA, Sutherland PW, Aitken EAB, and Drenth A. (2009) Infection, colonisation and sporulation by *Pseudocercospora macadamiae* on macadamia fruit. *Australasian Plant Pathology* 38: 36-43.
- 7. \*\*Akinsanmi OA, Miles AK and Drenth A. (2008) Alternative fungicides for reducing *Pseudocercospora macadamiae* infection in macadamia. *Australasian Plant Pathology* 37: 141-147.
- 8. \*\*Akinsanmi OA, Miles AK and Drenth, A. (2007). Timing of fungicide application for control of husk spot caused by *Pseudocercospora macadamiae* in macadamia. *Plant Disease* 91: 1675-1681.

\*\* Indicates inclusive of output of MC03007 project

### 6.1.3. Industry News Bulletins and E-newsletters

- Akinsanmi OA, with John Pretorius, Kevin Quinlan, Paul O'Hare and Andre Drenth 2011. Husk spot control with fungicide spray applications: Is it profitable? <u>Australian</u> <u>Macadamia Society Ltd. News Bulletin</u> Vol. 39 (6): 62-66.
- 2. Akinsanmi OA and Drenth A 2011 Macadamia fact sheet: Plant health- growing healthy trees from nursery to orchard. <u>Australian Macadamia Society Ltd. News</u> <u>Bulletin</u> Vol. 39 (1): 52-53.
- 3. Akinsanmi OA & AMS Crop protection IRG- June 2011 Husk spot management information sheet <u>Australian Macadamia Society Ltd. News Bulletin</u> Vol 39 (3).
- 4. Akinsanmi OA & AMS Crop protection IRG. April, 2011 Essential Facts and Principles of Husk Spot Management in Australian Macadamia Society Ltd.\_e-newsletter.
- Femi Akinsanmi, Andre Drenth and Tom Indebitzen (2009) Pests and diseases in the South African Macadamia industry. Australian Macadamia Society News Bulletin Vol. 37 (6) pp. 38-41.
- 6. Akinsanmi OA and Drenth A (2009) *Phytophthora* diseases management <u>Australian</u> <u>Macadamia Society Ltd. News Bulletin</u> Vol. 36 (1): 32-34.
- 7. Akinsanmi OA and Drenth A (2008) *Phytophthora* in macadamia. <u>Australian</u> <u>Macadamia Society Ltd. News Bulletin</u> Vol. 35 (4): 49-50.
- 8. Akinsanmi OA and Drenth A (2008) Challenges and tools for husk spot control <u>Australian Macadamia Society Ltd. News Bulletin</u> Vol. 35 (2): 53-55.
- 9. Akinsanmi OA and Drenth A (2008) Seedling dieback in macadamia-MC07003 Australian Macadamia Society Ltd. News Bulletin Vol. 35 (1): 44-46.
- 10. Akinsanmi OA and Drenth A (2007) Update on macadamia diseases management program. <u>Australian Macadamia Society Ltd. News Bulletin</u> Vol. 34 (6): 52-55.

### 6.1.4. Conferences and Symposia Abstracts

- 1. Akinsanmi OA and Drenth A. 2011. Husk spot in macadamia- Poster presented at the Annual Australian Macadamia Industry Conference, Noosaville 9-12 November, 2011.
- 2. Akinsanmi OA and Drenth A. 2011. Phytophthora in macadamia- Poster presented at the Annual Australian Macadamia Industry Conference, Noosaville 9-12 November, 2011.
- 3. Akinsanmi OA and Drenth A Stress predispose macadamia roots to *Phytophthora* infection. Submitted to the 6th Australasian Soilborne Diseases Symposium (9 11 August 2010).
- 4. Drenth A, Akinsanmi OA and Miles AK. 2009. Macadamia diseases in Australia. Southern African Macadamia Growers' Association Yearbook 17. 48-52.
- 5. Akinsanmi OA and Drenth A (2009) Pyraclostrobin is effective against husk spot. Southern African Macadamia Growers' Association Yearbook 17. 53-56.
- 6. Akinsanmi OA, Miles AK and Drenth A (2008) Unravelling the evolutionary dynamics of a novel cercosporoid fungus. 9th International Mycological Congress, Torino, Italy 24-29 August. *Journal of Plant Pathology* 90:S2.81 supplement.
- Akinsanmi OA &Drenth A. 2008. Review of husk spot control and diseases affecting macadamia. Australian Macadamia Society Annual Conference. 30 October- 1 November 2008. Ballina, New South Wales.

- 8. Akinsanmi OA, Grose M and Drenth A. (2007) Cross-pathogenicity of *Nectria pseudotrichia*. Proceedings of the 16th Biennial Australasian Plant Pathology Society Conference, 24-27 September, Adelaide, Australia. p.224.
- 9. Miles AK, Akinsanmi OA and Drenth A. (2007) The disease cycle of *Pseudocercospora macadamiae* on macadamia. Proceedings of the 16th Biennial Australasian Plant Pathology Society Conference, 24-27 September, Adelaide, Australia. p. 235.

### 6.2. Presentations

### 6.2.1. Industry Workshops and Field Days

- 1. Akinsanmi OA (Annual presentation every July from 2007 to date). Macadamia diseases- Annual research updates: Presented at the Macadamia Pest Scout and Consultant meeting, Brisbane.
- 2. Akinsanmi OA (2007, 2008, 2010, 2011) Best Practice group (growers quarterly meetings). Location: several in Queensland and New South Wales.
- 3. Akinsanmi OA 2009 Unravelling husk spot in macadamia. Presented at DPI&F/APPS Seminar day, Brisbane.
- 4. Akinsanmi OA 2009 Integrated management of husk spot. Field day. Beerwah Sunshine Coast, Alstonville, NSW.
- 5. Akinsanmi OA & Drenth A 2009 Study tour South African macadamia industry and 4th International Macadamia Symposium, South Africa. September, 10-17, 2009.
- 6. Akinsanmi OA & Drenth A 2009 Nursery workshop, DPI Indooroopilly Science Centre, June, 2, 2009.
- 7. Akinsanmi OA (2011) Husk spot management Australian macadamia industry MacGroups at several in Queensland and New South Wales.
- 8. Akinsanmi OA and Drenth A (2012) Phytophthora management Australian macadamia industry MacGroups at several in Queensland and New South Wales.

### **6.2.2. Technical/Special Reports**

- 1. Akinsanmi OA. Husk spot: estimate of value to the Australian Macadamia Industry – Special Report June 2011.
- 2. Akinsanmi OA. Status of macadamia diseases and sudden tree dieback in Atherton Tableland. Australian Macadamia Industry December 2011.
- 3. Akinsanmi OA and Drenth A. Status and putative macadamia diseases in Mackay district. Australian Macadamia Industry December 2011.
- 4. Akinsanmi OA and Drenth A. Case report on 2006 nursery issues in Queensland on macadamia. 14pp. Australian Macadamia Industry. June 2007
- 5. Akinsanmi OA and Drenth A. Research on pyraclostrobin in macadamia. 18pp. Special Report to Nufarm Australia Ltd. August 2007

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