Alternaria apple fruit spot: New Directions

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FINAL REPORT

PROJECT AP05002 (31 October 2007)

Alternaria fruit spot: New Directions

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AP05002

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This document is the final report for the Horticulture Australia Limited funded project "Alternaria fruit spot: New directions", and as such contains the details of all scientific work carried out in this project.

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Media Summary

The scientific community does not know a great deal about Alternaria fruit spot in apples. Along with Alternaria leaf blotch, these fungal diseases have caused significant fruit losses and tree damage in apple orchards in Queensland, New South Wales and occasionally Western Australia.

This project has helped to establish a foundation to build on for future *Alternaria* research. In 2005, the project team conducted a national survey of apple orchards. The team collected over 500 samples of *Alternaria* species from diseased leaves and fruit.

These samples were lodged at the internationally recognised Department of Primary Industries and Fisheries (DPI&F) Plant Pathology Herbarium in Brisbane. Researchers from around the world can use these samples in their own research. They are also being used in the next phase of this project, which was extended in September 2006 and will run for three-and-a-half years.

After collecting and storing these samples, the project team did some preliminary experiments on the identity of the *Alternaria* species found in Australian fruit and leaves. Identifying *Alternaria* at the species level is a specialist activity, and the project team worked with Dr Michael Priest (Orange Agricultural Institute, New South Wales) and Dr Barry Pryor (University of Arizona, United States of America).

The initial results from these experiments indicate that the majority of the samples are not *Alternaria mali*, the only species currently associated with leaf and fruit spots overseas. The team will continue this testing in the next phase of the project and will investigate the implications of these results.

The project has identified some preliminary methods to manage *Alternaria* to assist growers in Queensland, New South Wales and Western Australia.

Technical Summary

Alternaria leaf blotch and fruit spot can be serious mid-late season fungal diseases affecting apple leaves and fruit, in high spring/summer rainfall production areas in Australia. Alternaria leaf blotch can cause significant premature leaf defoliation, as early as January; while the majority of Alternaria fruit spots tend to appear between four to two weeks prior to harvest. These diseases have regularly caused significant fruit losses and tree damage in Queensland, New South Wales and occasionally Western Australian apple orchards.

Work undertaken in the previous APAL/HAL funded research project "Management of Alternaria leaf and fruit spot in apples" AP02011, indicated that there might be a number of closely related pathogens causing these symptoms (Horlock *et al.* 2006). The purpose of this project was to undertake some preliminary research into the Alternaria species collected from Australian apple orchards during a national survey undertaken in 2005, and set directions for future research by developing a new project proposal.

The outcomes of this project are:

- A collection of over 500 *Alternaria* isolates, collected from disease leaves and fruit from all major apple production areas in Australia. These isolates have been lodged at the internationally recognised DPI&F Plant Pathology Herbarium (BRIP), Indooroopilly Sciences Centre, Brisbane, from where they are available for reference and use by researchers world-wide.
- Some preliminary experimental results on the identity and pathogenicity of *Alternaria* isolates infecting Australian apple leaves and fruit. Preliminary results indicate that the majority of *Alternaria* isolates causing disease symptoms on Australian apple leaves and fruit are not *Alternaria mali*.
- A review of current techniques and methodologies used to determine the identity and characteristics of *Alternaria* species infecting tree crops throughout the world.
- The extension of this project, in the form of the currently running project AP06007.

The primary aims of AP06007 include the identification of *Alternaria* species infecting Australian apple leaves and fruit; increased understanding of the lifecycles of these pathogens and improved disease management through registration of suitable fungicides and implementation of appropriate cultural control measures.

Suggestions for minimising Alternaria leaf blotch and fruit spot symptoms in New South Wales, Queensland and Western Australian orchards are presented. Minor use permits for the use of metiram and dithianon on Gala, Pink Lady and Red Delicious apple varieties in parts of New South Wales, Queensland and Western Australia were granted by the AVPMA in 2007.

Introduction

Review of relevant literature

Alternaria diseases affecting apples

In the literature there are currently three apple diseases referred to as being caused by *Alternaria* species. These are Alternaria leaf blotch (caused by *Alternaria mali*), Alternaria fruit spot (also caused by *Alternaria mali*) and Alternaria core rot (caused by *Alternaria alternata*). Alternaria core rot, or mouldy core, as it is sometimes known, usually presents as a postharvest problem, although infection most likely occurs in the field (Spotts 1990). As the symptoms of Alternaria core rot are clearly different to those of Alternaria fruit spot, and the purpose of this review is to provide background information to preharvest Alternaria field diseases, further reference to Alternaria core rot will not be made in this report.

The majority of published literature on preharvest (field) Alternaria diseases refers to leaf symptoms. Although fruit symptoms are sometimes noted as a part of other studies, there are very few studies entirely devoted to *Alternaria* field infections of fruit. Until very recently, *Alternaria* has not been a significant preharvest pathogen of apple fruit. This situation has changed markedly over the last five years, with severe *Alternaria* infection of fruit being reported from Europe (personal communication Klaus Marschall) and Australia (Queensland, and some parts of New South Wales and Western Australia). *Alternaria* infection of fruit has also been reported from the United States of America, but only as a minor problem on the varieties Indo and Ralls (Spotts, 1990).

At the beginning of the previous Apple and Pear Australia Limited/Horticulture Australia Limited project "Management of Alternaria leaf and fruit spot in apples" AP02011, a literature review focusing on *A. mali* was prepared and submitted (Milestone #2) as this was the only *Alternaria* species recorded as a pathogen of apple leaves. However, during the course of this project (AP05002) preliminary evidence has shown that there are several species of *Alternaria* that can be readily isolated from *Alternaria*-like leaf and fruit symptoms in Australia. A further complication is the issue of whether or not *Alternaria* is affecting Australian apple leaves and fruit as the primary pathogen, or as a secondary invader. Consequently, the relevance of much of the following information to the Australian *Alternaria* apple disease situation is unclear. Therefore, the information in this review should be interpreted as a background to Alternaria leaf and fruit infection research world-wide, and not as a description of the preharvest *Alternaria* diseases of apple in Australia.

Alternaria leaf blotch (caused by *Alternaria mali*)

History of detection and distribution

First described in the Netherlands in 1924 (Roberts 1924), *Alternaria mali* is found infecting apple leaves in almost every apple growing nation of the world, including North America (Filajdic and Sutton 1991), Africa (as cited in (Sawamura 1990), Europe (Bulajic *et al.* 1996; Gagkaeva and Levitin 2000; Roberts 1924), India (Gupta and R.K. 1968), Asia (Dickens and Cook 1995; Sawamura 1972) and Australia.

The information for the most recent distribution map available for *Alternaria mali* was collected in 1996 by the International Mycological Institute (Institute 1996, publ. 1997).

Nomenclature

The fungus currently termed *Alternaria mali* was first isolated from apple leaf blotches in 1914, and was formally described in 1924 (Roberts 1924). Confusingly, however, for almost two decades in the late 20th century *Alternaria mali* was referred to as *Alternaria alternata* f.sp. *mali* or *Alternaria alternata* apple pathotype (Gagkaeva and Levitin 2000; Itoh *et al.* 1998; Johnson *et al.* 2000b) by *Alternaria* toxin researchers. These name changes were very controversial at the time, and were brought about by the idea that all species of *Alternaria* that produce toxins should be called *Alternaria alternata* (Nishimura and Kohmoto 1983; Nishimura *et al.* 1978a). This work paid minimal attention to any differential characters other than toxins, and was successfully refuted on this basis (Simmons 1999), and the name *Alternaria mali* has since returned to widespread use.

The name *Alternaria mali* has also been misused to describe other diseases such as *Alternaria* core rot or mouldy core (caused by *Alternaria alternata*) (Marchionatto 1938; Ramírez-Legarreta and Jacobo-Cuéllar 1999a; b; Ramírez-Legarreta *et al.* 2000), postharvest pear decay (English 1940) and cork rot of apples (Tweedy and Powell 1962). These references, and others, can be misleading. It is therefore important to ensure that when reading literature referring to *Alternaria mali*, that it is the preharvest field disease that is being discussed.

Disease description

There is abundant information available about the symptoms produced by *Alternaria mali*, the details of its infection cycle on leaves, and its ability to overwinter in terminal buds. As most of the information in the rest of this review comes from either Japan or North America, it seemed appropriate to focus on the description of this disease from those countries.

Symptoms

Leaf lesions are first observed in late spring or early summer as small, round, blackish spots, gradually enlarging to 2-5 mm in diameter, with a brownish-purple border. Most spots undergo a secondary enlargement phase and become irregularly shaped and much darker in colour. Lesions on petioles cause the leaves to turn yellow and by mid-summer up to 50% defoliation can occur in untreated trees (Sawamura 1990).

Alternaria mali lifecycle

In Japan, researchers have found that *Alternaria mali* overwinters in dead leaves on the ground, in mechanical injuries on twigs, and in dormant buds; with spores formed in leaf lesions and swollen lenticels. Primary infection occurs in late spring, and the number of infections increases rapidly in the rainy season, with the following warm weather also contributing to increased infection. The optimal temperature for infection, symptom and spore production is 25-30^{o}C. Successful infection occurs in a few hours under laboratory conditions, and within 24-48 hours under optimal conditions in the field (Sawamura 1990).

In North Carolina (USA) infected leaves on the ground were found to be a more important overwintering site than buds, with spores on leaves germinating more readily than those in buds. Treatment of leaves with urea in the autumn had minimal effect on the amount of leaf area remaining in the following spring. Overwintering of infected leaf material on grass or bare ground, or treatment of these leaves with urea, did not reduce the number of conidia per leaf detected or their ability to germinate (Filajdic and Sutton 1995).

Pathogen dispersal

Dispersion indices, 2-dimensional distance analysis and spatial autocorrelation analysis were used to study the spatial distribution pattern of *Alternaria mali* in an apple orchard with 40 Red Delicious trees. Greater disease incidence was observed on the edges of the orchard. It is suggested that arthropods may be involved in the epidemiology of this disease and in the introduction of inoculum from outside the orchard (Filajdic and Sutton 1994).

The connection between increased levels of leaf blotch symptoms and insect damage has been noted in two major studies. European red mite (Filajdic *et al.* 1995a) and aphids (Filajdic *et al.* 1995b) in the USA, and aphids in Korea (OunHa *et al.* 1997) facilitated *Alternaria* infection, by creating wounds suitable for *Alternaria* to colonise.

Identification

This topic is covered in detail in Chapter 4 of this report.

Pathogen vs Saprophyte

Is *Alternaria* behaving as a pathogen, or a saprophyte, or both, in Australian apple orchards? This is also one of the most important questions facing current research into Alternaria leaf blotch, and particularly Alternaria fruit spot. At this stage the answer is unclear, but some valuable information on this topic has been gathered overseas.

Virulence of isolates

A wide range of pathogen virulence (ability to cause disease) levels have been reported for *Alternaria mali* isolates (infecting leaves) in several countries, including Japan (Saito *et al.* 1983), USA (Filajdic and Sutton 1992b) and eastern Asia (Dickens and Cook 1995). Virulence ranged from very aggressive to moderate on susceptible varieties, and was assessed in traditional pathogenicity tests where *Alternaria mali* spores were inoculated on to apple leaf tissue and the resulting infection rated. It is not possible to make comparisons between these types of studies, but it is interesting to note that there were significant variety x isolate effects on the levels of disease produced within each of these studies.

Disease prediction systems

Disease prediction or forecasting systems have been developed for many different plant pathogens and form an integral part of integrated pest management system. These systems vary greatly in complexity depending upon the number of factors involved in the disease cycle of particular pathogens, and range from simple ‘after rain’ application strategies (Thomas 1983), to complicated algorithms requiring accurate recording of weather data and inoculum levels (Filajdic and Sutton 1992b). Prediction systems are generally pathogen and host specific, and often require

optimisation for different climatic regions (Kim *et al.* 1986). However, a recently published model system has been shown to be useful for predicting diseases, without the detailed collection of empirical data (Magarey *et al.* 2005). The usefulness of these prediction systems will be evaluated in AP06007.

Using leaf wetness periods to determine fungicide applications for control of Alternaria leaf blight of rockmelon, Southern Texas USA. (Thomas 1983)

Using the duration of leaf wetness to determine the application of fungicides for the control of Alternaria leaf blight of rockmelon reduced the number of sprays required without increasing the incidence or severity of disease (Thomas 1983). Fungicides were only applied after leaf wetness periods of eight or more hours, resulting in one third fewer fungicide applications.

Empirical Forecasting Model for Alternaria leaf spot in apple, Korea. (Kim et al. *1986)*

This model was constructed based on modified degree day temperature and the frequency of rainfall during three growing seasons. The cumulative degree portion (CDP) was used as a parameter to determine the relationship between temperature and initial disease occurrence. CDP was defined as the time over 10^{o}C in the daily average temperature after the beginning of spring. Once the temperature threshold of ca. 160 CDP was reached, at least four subsequent rainfalls were required to initiate disease. After initiation, disease progress generally followed a pattern set by rainfall, with frequent rain events required to produce severe outbreaks. Rainfall frequency was more important than amount of rain which fell in any single event. There was also a significant relationship between disease progress and the cumulative number of air-borne *Alternaria mali* conidia. However, when used alone, the cumulative number of conidia predicted disease severity very poorly.

This model was evaluated in North Carolina (Filajdic and Sutton 1992b) over two seasons. In the first season, the model predicted the first onset of disease seven days earlier than symptoms were observed in the field, and in the second season, five days later. Due to the arbitrary nature of choosing a date for the phenophase tight cluster these predictions were considered by the authors (Filajdic and Sutton 1992b) to be within an acceptable range.

A predictive model for Alternaria leaf blotch infection of apple, North Carolina USA. (Filajdic and Sutton 1992b)

A predictive model for Alternaria leaf blotch of apple in the south eastern United States of America was developed using air temperature and leaf wetness. The ability of four isolates of *Alternaria mali* from Henderson County, North Carolina to infect Delicious apple seedlings was used to develop this model. The only limitation of this model is that it is based solely on environmental conditions, with no regard for inoculum availability. This weakness was demonstrated in the field trial, when no conidia were trapped in 15 out of the 34 leaf wetness periods during which the infection criteria of the model were met. The authors suggest using this model only in orchards where Alternaria has been a problem in the past.

Alter-Rater for Alternaria brown spot citrus, Florida USA. (Bhatia et al. 2003)

The Alter-Rater Model is used to predict the need for fungicide applications to control Alternaria brown spot of citrus in Florida, based on rainfall, leaf wetness and

temperature. A spray program based on this model significantly reduced disease levels in all four trial orchards, when compared to a calendar spray system; and also reduced the number of sprays applied at two of the trial sites. Previous research showed any rainfall event over 2.5 mm was enough to allow inoculum to develop, and for infection to occur, assuming sufficient duration of wetness (Timmer *et al.* 2000). This indicates that the amount of rainfall is not as important as the number of rainfall events. Some fine tuning of the Alter-Rater Model, using local knowledge of weather events and orchard disease history, will further improve the model's effectiveness. Extended periods of dew were also found to play an important role in disease development, with dew alone causing significant levels of leaf wetness. The Alter-Rate Model is available publicly on the University of Florida Automated Weather Network (FAWN) (Bhatia *et al.* 2003).

Simple generic infection model for foliar fungal plant pathogens. (Magarey et al. 2005)

This model is designed primarily for use in forecasting pathogens that do not have extensive epidemiological data. This model can use inputs based on subjective estimates of the cardinal temperatures and the wetness duration requirement, which can be obtained from the pathogen or estimated from a related pathogen. This infection model is being used to create risk maps of exotic pests for the US Department of Agriculture's Animal and Plant Health Inspection Service.

Resistance

Resistance to *Alternaria mali* has been recorded in a number of wild *Malus* species, including *Malus asiatica*, *Malus baccata* and *Malus robusta*, with resistance in these varieties controlled by a single dominant gene (Saito and Niizeki 1988). Conversely, in commercial apple varieties, resistance tends to be controlled by a single, recessive gene (Saito and Takeda 1984; Shin and Ko 1992). *Alternaria mali* resistant varieties have been an active target for Asian breeding programs for some time, using traditional techniques like crossing from resistant varieties (Saito and Takeda 1984), and the production of mutants using irradiation (Masuda and Yoshioka 1997; Saito *et al.* 2001; Tabira *et al.* 1998). At this time, no new *Alternaria mali* resistant varieties have been released onto the world market from these programs.

In Korea, resistant apple cultivars were shown to have a higher leaf hair density on the under surface compared with susceptible cultivars. Removal of leaf hairs increased the level of infection in inoculation tests (Yoon and Lee 1987a), presumably by increasing leaf injury.

Resistance of commercial varieties to Alternaria leaf blotch

It is interesting to note that in Japan, the variety 'Gala' is quoted as being resistant to leaf blotch (Miyashita *et al.* 2003; Yoshioka *et al.* 2000), while in Australia we have found 'Gala', and especially 'Royal Gala' to be extremely susceptible. This may be another indicator that we are not dealing with the same pathogen in Australia, and further research is required to establish if this is so.

Managing **Alternaria mali** *using fungicides*

A wide range of chemicals have been trialed in Europe, Asia and North America for the control of Alternaria leaf blotch, with varying levels of success (Table 1). In many papers, the authors report that an effective spray program for the control of

apple scab or black spot (caused by *Venturia inaequalis*) also significantly reduced *Alternaria mali* symptoms (Sharma and Sharma 1991).

Table 1: List of chemicals trialed for Alternaria leaf blotch* control

Product	Country	Reference	Level of Success
Iprodione	USA, Korea	(Filajdic and Sutton 1992a; Lee 1984)	Good
Captafol	Korea	(Kim *et al.* 1982)	Good
Chlorothalonil	Korea	(Kim *et al.* 1982)	Good
Polyoxin B	Korea	(Kim *et al.* 1982)	Good
Carbendazim	India	(Sharma and Sharma 1991)	Good
Mancozeb	Italy, China	(Ciferri 1953; CunHao *et al.* 2001)	Average
Mancozeb	China	(RuiDe *et al.* 1997)	Poor
Propineb	Korea	(Lee 1984)	Average
Captan	USA	(Filajdic and Sutton 1992a)	Poor
Mancozeb	USA	(Filajdic and Sutton 1992a)	Poor
Captan + Mancozeb	USA	(Filajdic and Sutton 1992a)	Poor
Bordeaux mixture	Korea, China	(JaeYoul *et al.* 1995; RuiDe *et al.* 1997)	Poor

* Although identified as *Alternaria mali* by the authors in each of the papers cited, it is not absolutely certain that each of these papers refers to the same organism that is present in Australian orchards.

Managing Alternaria mali *using nutrition*

Several researchers have also examined using nutritional supplements to reduce *Alternaria mali* symptoms. In Korea, resistant leaves showed higher levels of calcium (Yoon and Lee 1987b), while other nutrients such as N, P, K, Mg and Na did not seem to influence resistance. Foliar applications of calcium compounds inhibited leaf infection by *Alternaria mali* in artificial inoculation tests (Yoon *et al.* 1989).

Managing Alternaria mali *using antibiotics*

A number of antibiotics have been demonstrated to reduce the growth and development of *Alternaria mali* under laboratory conditions (Cheng *et al.* 1989; Tomiya *et al.* 1990; Uramoto *et al.* 1988), and in the field in China (JinYou *et al.* 1997). At this time, the Australian Pesticides and Veterinary Medicines Authority do not allow the use of antibiotics in plant production in Australia, and this situation is unlikely to change in the near future.

In a similar vein, researchers from China (Chen *et al.* 1993; XueChi *et al.* 1997) have sprayed antibiotic-producing bacteria directly onto apple trees, rather than extracting the antibiotic first, and have achieved a surprising reduction in *Alternaria mali* disease symptoms.

Alternaria fruit spot (caused by *Alternaria* species)

Disease description

In contrast to leaf blotch, there is very little information available about fruit symptoms produced by *Alternaria* species on apples; with no detailed studies of its infection cycle on fruit. Most of the available information comes from either Japan or North America, and takes the form of incidental notes and anecdotal observations.

Symptoms

Fruit infections are uncommon, except for the very susceptible variety 'Indo', and 'Ralls' under certain environmental conditions. Typically fruit infections begin in the lenticels and the pathogen does not cause fruit to rot in the field or in storage. Only scab-like spots or a dry rot appear on apple fruit infected in the summer (Sawamura 1990).

Resistance

In studies in 1975-1976 involving 50 apple varieties, 26 rootstocks and five crab-apples, all three methods of inoculating the fruit gave significant varietal differences. In most varieties, the resistance of the leaves to *Alternaria mali* was not correlated with the resistance of the fruit (Saito *et al.* 1978).

Alternaria leaf blotch and fruit spot in Australia

There is currently some confusion about the exact cause of Alternaria leaf blotch and fruit spot symptoms in Australia; namely which species of *Alternaria* are responsible for these symptoms, and in which growing regions these occur. Finding *Alternaria*-like leaf symptoms does not necessarily indicate infection by *Alternaria* fungi. Even more significantly, just because *Alternaria* is isolated from leaf or fruit lesions, does not mean that *Alternaria* was the initial cause of the problem. *Alternaria* species can be very effective secondary invaders of wounded tissues. The presence of *Alternaria* does not conclusively prove it was the initial cause of the problem, especially from old infections.

Disease description

Alternaria leaf blotch

Alternaria leaf blotch is characterised by irregular (but initially roughly circular) light brown-reddish shaped lesions, often with purple borders on leaves (Figure 1). It is important to remember that *Alternaria*-like symptoms, especially leaf symptoms, can look very similar to symptoms of physical damage, or other fungal pathogens. Therefore a diagnosis of Alternaria leaf blotch based on observation of leaf lesions alone is not advisable or conclusive.

Alternaria leaf blotch is distinguished by the fact that under conducive weather conditions the blotches will continue to grow, and leaves can drop prematurely from the tree. Tree defoliation can be severe in rainy seasons, with up to 50% defoliation

(Figure 2) as early as mid-late summer in some regions (i.e. Sydney Basin of New South Wales).

Alternaria fruit spot

Small, slightly sunken, light to medium brown spots appear on the lenticels of the fruit (Figure 3), often soon after rainfall, and usually no earlier than 6-8 weeks prior to harvest. Interestingly, fruit spots do not appear during storage, and preharvest Alternaria fruit spots do not appear to enlarge significantly during cold storage. However, once removed from cold storage existing spots can continue to grow in size, and new spots can develop, providing an excellent entry point for other secondary fruit rots.

This disease should not be confused with *Alternaria* core rot, or mouldy core, a postharvest storage rot caused by *Alternaria alternata*.

Distribution and importance

Although Alternaria leaf blotch (caused by *Alternaria* species) has been recorded in Australia for many years, the relatively new disease Alternaria fruit spot has only been consistently recorded at production limiting levels in the Granite Belt (Queensland), Sydney Basin and one property in Orange (New South Wales). There have been infrequent reports of Alternaria fruit spot from Western Australia.

The survey results previously presented (Horlock *et al.* 2006), indicate that *Alternaria* species are common fungi found in Australian apple orchards, and there is potential for further spread of this disease. How much potential, and what the real risks are of widespread, production limiting *Alternaria* infection remains unclear at this stage.

Finally, even if Alternaria leaf blotch or fruit spot is present in an orchard, environmental conditions might be such that it is not causing production limiting levels of disease. This may explain the occasional reports of symptoms from normally low summer rainfall production areas such as South Australia and some parts of Victoria.

Figure 1: Alternaria leaf blotch symptoms on Royal Gala leaves.

Figure 2: Severe premature defoliation of Royal Gala trees by Alternaria leaf blotch.

Figure 3: Alternaria fruit spot on Royal Gala fruit.

1. Preservation of *Alternaria* species collected from Australian apple leaves and fruit.

A fully catalogued, viable collection of *Alternaria* isolates from throughout Australia suitable for use in future project work by Australian and international researchers has been lodged at the internationally recognised DPI&F Plant Pathology Herbarium (BRIP), Indooroopilly Sciences Centre, Brisbane.

The collection consists of ca. 450 isolates collected from around Australia in March, April and May 2005, with an additional 50-60 isolates collected in the 2005/06 and 2006/07 seasons.

Isolates are stored as freeze dried cultures at BRIP and as an under water working collection at Applethorpe Research Station. Representative isolates have been lodged with Assoc Prof Barry Pryor, University of Arizona, Tucson, Arizona, United States of America.

This is a continuing collection with fresh isolates from affected leaves and fruit expected to be collected over the next couple of seasons.

2. Analysis of representative *Alternaria* isolates from Australian apple orchards

These studies were undertaken to provide some basic information about the isolates of *Alternaria* collected during the 2005 Australian apple orchard survey. The purpose was to find any points of difference between the populations of Alternaria species in each growing region. Of particular relevance was to identify and compare differences in the populations from Queensland and New South Wales (where production limiting levels of infection regularly occur) and the other major production areas in Australia (where production limiting levels of infection do not regularly occur). It was also anticipated that the preliminary results produced would provide an indication of areas for future research.

2.1 Identification of Alternaria *isolates*

The identification of *Alternaria* isolates to species level is a specialist activity, with a great deal of debate in the scientific literature about how to separate this group of fungi into different species (Andersen *et al.* 2002; Simmons 1999). It has recently been revealed that a number of tree crop diseases caused by *Alternaria* species actually involve several *Alternaria* species, for example Alternaria blight of *Paulownia* (Pleysier *et al.* 2006; Ray *et al.* 2005), pistachio (Pryor and Michailides 2002) and hazelnuts and walnuts (Belisario *et al.* 2004).

Alternaria mali is the only species of *Alternaria* associated with apple leaf and fruit spots currently reported in scientific literature. However, the various descriptions given for *Alternaria mali* (Bulajic *et al.* 1996; Simmons 1999) are very similar to those reported for the *Alternaria alternata* complex, and several researchers continue to refer to *Alternaria mali* as *Alternaria alternata* apple pathotype (Johnson *et al.* 2000b; Miyashita *et al.* 2003). The identification of *Alternaria mali* is commonly linked with the detection of AM-toxin, either by genetic (Johnson *et al.* 2000b) or chemical methods (Andersen and Thrane 1996; Ueno *et al.* 1975). This usage is currently being re-evaluated - refer to section on identification of *Alternaria* species using toxins in Chapter 4.

Identification of *Alternaria* isolates using morphology

A number of *Alternaria* isolates collected from New South Wales in the 2005 national orchard survey (Horlock *et al.* 2006) were sent to Dr Michael Priest, Mycologist, Orange Agricultural Institute, Orange, New South Wales for expert examination and identification.

Preliminary results have indicated that the isolates submitted were identified as *Alternaria alternata* (60%) or other *Alternaria* species (40%). Only one isolate was tentatively identified as *Alternaria mali*. Difficulties in precise identification occurred due to the changeable nature of morphological characters shown by isolates over time. It was decided to defer this laborious process until the pathogenicity of isolates had been tested first, to reduce the time involved in identification.

Christine Horlock undertook some intensive training in the identification and differentiation of small-spored *Alternaria* species while visiting with Assoc Prof

Barry Pryor, University of Arizona, Tucson, USA. In particular, the differentiation of the species *alternata*, *arborescens*, *infectoria* and *tenuissima* was studied. Assoc Prof Pryor's team use a morphological scale, which assesses the spore shape and growth pattern features of isolates on low nutrient media, along with genetic analysis to identify the species. These techniques are being used in the current project (AP06007), under the supervision of Assoc Prof Pryor, to identify a larger proportion of *Alternaria* species isolated from Australian apple leaves and fruit.

Preliminary genetic analysis of *Alternaria* isolates

Analysis of eleven typical Australian isolates of *Alternaria* from apple leaves and fruit has revealed that none of these isolates are *Alternaria mali*. Partial sequences of three genes were compared to sequences of a range of *Alternaria* species in the laboratory of Assoc Prof Pryor at the University of Arizona, Tucson. Of the eleven isolates examined, seven were most similar to *Alternaria arborescens*, and three were most similar to *Alternaria tenuissima* and one was most similar to *Alternaria alternata*. This work is ongoing and is being continued in the current project AP06007.

Alternaria arborescens was originally identified from a stem canker disease of tomatoes (Simmons 1999), and is known to produce two host (tomato) specific AAL (*Alternaria arborescens lycopersci*) toxins (Simmons 1999). As the name suggests, these host specific toxins are unlikely to affect apple tissues. *Alternaria tenuissima* and *Alternaria alternata* have no known host specific toxins, but do produce a range of metabolites, some characterised and some not, that may be generally toxic to plants and animals (Andersen *et al.* 2002).

2.2 Effect of in vitro *fungicides on the* in vitro *germination and growth of* Alternaria *isolates*

The development of fungicide resistance is a relatively common phenomenon in plant agriculture worldwide. One potential difference between populations of *Alternaria* species in Queensland/northern New South Wales and the other apple production areas in Australia, is the number of fungicide applications made during a growing season. Queensland and northern New South Wales traditionally receive considerably more spring/summer rain than other Australian production areas, and subsequently apply a much larger number of fungicide sprays to control fungal diseases, including apple scab.

Alternaria species in Queensland and New South Wales may have become more resistant to commonly used fungicides, and this may explain why production limiting levels of infection occur here and not in the other states.

Aim

To compare the effect of fungicides on the mycelial growth of *Alternaria* isolates collected from Australian apple orchards.

Materials and Methods

Inoculum

Isolates of *Alternaria* were grown on ½SPDA at 25^{o}C, in darkness, for seven days. Isolates tested so far are listed in Table 2.1.

Fungicide amended media

One half strength potato dextrose agar (½PDA) was melted in a microwave, and placed in a water bath to equilibrate to ca. 50^{o}C. Fungicides were dissolved in the media suspension just prior to pouring into 90 mm plastic Petri dishes. Five fungicides were used in this study, with media amended to imitate field application rates. Fungicides and rates are listed in Table 2.2.

Inoculation

Agar discs (5 mm in diameter) were cut from the leading edge of cultures and placed at the centre of a 90 mm diameter Petri dish containing fungicide amended ½SPDA. Five replicate Petri dishes were used for each isolate/fungicide combination.

Assessment of mycelial growth

Inhibition of fungal growth was assessed by measuring the colony diameter (mm) after incubation at 25^{o}C for 7 days in darkness. By this time, colonies on the unamended control plates had reached the edge of the plate.

Table 2.1. List of *Alternaria* isolates assessed for fungicide resistance

Isolate number*	Tissue type infected	Apple variety	Location
BRIP 45469	Leaf	Pink Lady	Manjimup, WA
BRIP 46348	Leaf	Fuji	Ballykeane, NSW
BRIP 46349	Fruit	Fuji	Ballykeane, NSW
BRIP 46351	Leaf	Pink Lady	Ballykeane, NSW
BRIP 46352	Leaf	Red Delicious	Ballykeane, NSW
BRIP 46356	Fruit	Fuji	Stanthorpe, QLD
BRIP 46357	Fruit	Fuji	Stanthorpe, QLD
BRIP 46374	Leaf	Fuji	West Batlow, NSW
BRIP 46376	Leaf	Red Delicious	West Batlow, NSW
BRIP 46378	Leaf	Pink Lady	West Batlow, NSW
BRIP 46380	Fruit	Red Delicious	Bilpin / Berambing, NSW
BRIP 46387	Leaf	Granny Smith	Bilpin / Berambing, NSW
BRIP 46394	Leaf	Fuji	Bilpin / Berambing, NSW
BRIP 46399	Fruit	Unknown	Lakesland, NSW
BRIP 46414	Leaf	Braeburn	Bilpin / Berambing, NSW
BRIP 46452	Fruit	Pink Lady	Donnybrook, WA
BRIP 46464	Leaf	Pink Lady	Karragullen, WA
BRIP 46453	Leaf	Pink Lady	Donnybrook, WA
BRIP 46457	Leaf	Pink Lady	Kalamunda, WA
BRIP 46461	Leaf	Lady Williams	Kalamunda, WA
BRIP 46467	Leaf	Royal Gala	Karagullen, WA
BRIP 46477	Leaf	Granny Smith	Pickering Brook, WA
BRIP 46481	Fruit	Pink Lady	Pickering Brook, WA
BRIP 46482	Leaf	Pink Lady	Pickering Brook, WA
BRIP 46490	Leaf	Sundowner	Newlands,WA
BRIP 46492	Fruit	Sundowner	Newlands, WA
BRIP 46495	Leaf	Royal Gala	Manjimup, WA
BRIP 46507	Fruit	Pink Lady	Manjimup, WA
BRIP 46520	Leaf	Golden Delicious	Pickering Brook, WA
BRIP 46550	Fruit	Fuji	Stanthorpe, QLD
BRIP 46576	Leaf	Sundowner	Launceston, TAS
BRIP 46582	Leaf	Royal Gala	Legana, TAS
BRIP 46592	Leaf	Royal Gala	Hillwood, TAS
BRIP 46593	Leaf	Pink Lady	Hillwood, TAS
BRIP 46653	Leaf	Royal Gala	Launceston, TAS
BRIP 46656	Leaf	Fuji	Hillwood, TAS
BRIP 46857	Leaf	Fuji	Warrendale, VIC
BRIP 46884	Leaf	Royal Gala	Yarra Valley, VIC
BRIP 46909	Leaf	Pink Lady	Goulburn Valley, VIC
BRIP 46922	Leaf	Granny Smith	Ardmona, VIC
BRIP 46924	Leaf	Fuji	Ardmona, VIC
BRIP 46933	Leaf	Fuji	Shepparton, VIC
BRIP 46936	Leaf	Sundowner	Shepparton, VIC
BRIP 47177	Leaf	Pink Lady	Lenswood, SA

*Isolates referred to by BRIP numbers are available from the DPI&F, Queensland Plant Pathology Herbarium, Indooroopilly Sciences Centre, 80 Meiers Rd, Indooroopilly Queensland 4068, Australia.

Table 2.2. Fungicides for screening against *Alternaria* isolates.

Trade name	Active ingredient	Amount or product/L of media
Chorus	cyprodinil	0.40 g
Delan	diathianon	0.18 g
Dithane	mancozeb	1.80 g
Flint	trifloxystrobin	0.10 g
Polyram	metiram	1.80 g
Vision	pyrimethanil + fluquinconazole	0.75 g

Results

This testing is only partially completed and results should be considered preliminary.

No significant difference in fungicide sensitivity was detected between any of the isolates tested so far.

Discussion

Although preliminary, these results would seem to indicate that there is no substantial build up of resistance against the fungicides tested, in *Alternaria* populations from Queensland or New South Wales; when compared to *Alternaria* isolates from South Australia, Tasmania, Western Australia and Victoria.

3. Determining the pathogenicity of *Alternaria* isolates from Australian apple leaves and fruit

Alternaria is a fungal genus that is very common to agricultural crops in Australia. Many of the *Alternaria* species found in Australia are non-pathogenic or very weakly pathogenic saprobes, organisms that simply "make the most" of an existing plant wound, and feed off of already dying tissues. This kind of "infection" is significantly different to a truly pathogenic attack, where the fungus can invade healthy tissues to cause disease. Distinguishing between these two types of infection is very important;. We need to ensure that we are studying the cause of the problem (pathogenic), not a secondary issue (saprophyte).

To determine *Alternaria* isolates causing pathogenic infections on Australian apple leaves and fruit, a series of leaf and fruit inoculations was undertaken with field and glasshouse plants. *Alternaria mali* is known to be an aggressive pathogen of apple leaves and fruit (Sawamura 1990), and a strong pathogenic reaction was expected to occur on leaves and fruit inoculated with this pathogen.

3.1 In vitro *inoculation of detached apple leaves with Alternaria isolates*

Aim

To determine the pathogenicity of representative isolates of *Alternaria* against Red Delicious and Royal Gala leaves, using a detached leaf bioassay.

Materials and Methods

Isolates used

Isolates used to inoculate Red Delicious and Royal Gala leaf discs and whole leaves were collected from Australian apple orchards, and are listed in Table 3.1.

Apple variety leaves used

Leaves of Red Delicious and Royal Gala varieties were collected from field grown trees on Applethorpe Research Station, Granite Belt, Queensland.

Inoculum preparation

Isolates of *Alternaria* spp. collected during the national survey of apple orchards in 2005 were grown on half strength potato dextrose agar with streptomycin (½SPDA) at 25^{o}C under alternating 12 hour near-UV light. Spores were harvested from these isolates by washing 14 to 21 day old colonies growing on ½SPDA with sterile distilled water and dislodging the spores with a sterile glass rod. The spores were filtered through two layers of sterile gauze, and the resultant spore suspension was adjusted to a concentration of 1×10^6 spores/ml using a haemocytometer.

Table 3.1. *Alternaria* isolates used in inoculation of *in vitro* apple leaves.

Isolate number*	Tissue type infected	Apple variety	Location
BRIP 46353	Leaf	Red Delicious	Orange, NSW
BRIP 46378	Leaf	Pink Lady	Batlow, NSW
BRIP 46401	Leaf	Pink Lady	Lakesland, NSW
BRIP 46405	Leaf	Red Delicious	Lakesland, NSW
BRIP 46406	Leaf	Red Delicious	Nashdale, NSW
BRIP 46413	Leaf	Pink Lady	Thirlmere, NSW
BRIP 46575	Leaf	Golden Delicious	Spreyton, TAS
BRIP 46580	Leaf	Golden Delicious	Ranelagh, TAS
BRIP 46581	Leaf	Sundowner	Lagana, TAS
BRIP 46584	Leaf	Sundowner	Spreyton, TAS
BRIP 46847	Leaf	Jonagold	Warrendale, VIC
BRIP 46857	Leaf	Fuji	Warrendale, VIC
BRIP 46862	Leaf	Pink Lady	Warrendale, VIC
BRIP 46869	Leaf	Fuji	Yarra Valley, VIC
BRIP 46874	Leaf	Pink Lady	Yarra Valley, VIC
BRIP 46886	Leaf	Royal Gala	Warendale, VIC

*Isolates referred to by BRIP numbers are available from the DPI&F, Queensland Plant Pathology Herbarium, Indooroopilly Sciences Centre, 80 Meiers Rd, Indooroopilly Queensland 4068, Australia.

In vitro inoculations of leaf discs

Mature detached leaves were surface sterilised with 70% ethanol. Leaves were then rinsed in sterile distilled water and blotted dry with sterile tissue paper. Discs (18 mm diameter) were cut with a sterile cork borer and placed on moist filter paper in sterile Petri dishes. They were then inoculated with 20 µl of spore suspension, prepared as described above, and dotted at the centre of five replicate leaf discs. Treatment control discs were inoculated with sterile distilled water. Petri dishes were sealed with plastic wrap and incubated in darkness for 14 days, after which time, lesion development was assessed.

In vitro inoculations of whole leaves

Mature detached leaves were surface sterilised with 70% ethanol. Leaves were then rinsed in sterile distilled water and blotted dry with sterile tissue paper. Leave stems were trimmed, and whole leaves were placed on moist filter paper in sterile Petri dishes. Leaves were then inoculated with 20 µl of spore suspension, prepared as described above. Treatment control leaves were inoculated with sterile distilled water. Immediately prior to the addition of spore suspension, half of the leaves were wounded by pricking with a sterile needle. Each isolate was inoculated onto three wounded and three unwounded replicate leaves. Petri dishes were sealed with plastic wrap and incubated in darkness for 14 days, after which time, lesion development was assessed.

Lesion assessment

Lesion development was assessed 14 days after inoculation. A pathogenic infection deemed to have occurred if the lesion formed exceeded 5 mm in diameter. Isolates were deemed to be pathogenic if lesions exceeding 5 mm were formed on four out of six leaf discs, or two out of three whole leaves, on two or more occasions. These

assessments are based on the techniques of Ray *et al.* (2005) and Pegg (1966), and were selected after discussion with both senior authors.

Results and Discussion

No detailed results have been presented for these experiments.

Although this technique was attempted many times, with a variety of isolates, the production of lesions on leaf discs, and wounded or unwounded whole leaves, was not consistent within or between inoculations.

The level of inconsistency in results is so great, that it would seem to indicate a critical failure or basic flaw in some part of the protocol. The nature of this basic flaw has not been determined. Some factors which could have contributed to this experiments failure include environmental conditions during the inoculation process, the need for wounding of leaf tissues prior to inoculation and age/maturity of spores used for inoculation.

In order to reduce the potential for problems with this technique, it was decided to simplify the process and attempt further inoculations on attached whole leaves.

3.2 Inoculation of attached apple leaves with Alternaria *isolates*

Aim

To determine the pathogenicity of representative isolates of *Alternaria* collected from Australian apple orchards against attached Red Delicious and Royal Gala leaves.

Materials and Methods

It was decided to use a smaller number of isolates in this trial and perform a larger number of inoculations under a wider range of conditions to try and determine optimal conditions for leaf infection. The most effective inoculation conditions are reported below.

Isolates used

Isolates used to inoculate attached Red Delicious and Royal Gala leaves are listed in Table 3.2. Isolates BRIP 46847 and 46857 were selected as they had demonstrated the ability to produced lesions on unwounded leaves in the previous experiments. BRIP 46378 was chosen as it had not produced any signs of infection in the previous experiments.

Table 3.2. *Alternaria* isolates used in inoculation of attached apple leaves.

Isolate number*	Tissue type infected	Apple variety	Location
BRIP 46378	Leaf	Pink Lady	Batlow, NSW
BRIP 46847	Leaf	Jonagold	Warrendale, VIC
BRIP 46857	Leaf	Fuji	Warrendale, VIC
BRIP 46922	Leaf	Granny Smith	Ardmona, VIC

*Isolates referred to by BRIP numbers are available from the DPI&F, Queensland Plant Pathology Herbarium, Indooroopilly Sciences Centre, 80 Meiers Rd, Indooroopilly Queensland 4068, Australia.

Apple variety leaves used

Potted trees of Red Delicious and Royal Gala varieties were grown in the Plant Pathology Glasshouse at the Applethorpe Research Station, Granite Belt, Queensland.

Inoculum preparation

As described in section 3.1.

Glasshouse inoculations

The spore suspension was sprayed onto potted apple plants using a hand atomiser until all leaves were covered and runoff achieved. Treatment control plants were inoculated with sterile distilled water. The potted apples were then covered with a large polyethylene bag to maintain a high level of humidity (>95%). After seven days the polyethylene bag was removed, and symptoms left to develop for a further 14 days. Lesion development was assessed 21 days after inoculation.

Results and Discussion

No detailed results have been provided for these experiments. Although the level of consistency demonstrated by this technique was substantially better than that demonstrated by the *in vitro* method/s, it still requires a significant level of improvement.

These experiments are ongoing, and new methods of inoculation, different isolates and a broader range of apple varieties are currently being examined.

3.3 Inoculation of mature apple fruit in vitro with Alternaria isolates

Aim

To determine the pathogenicity of representative isolates of *Alternaria* collected from Australian apple orchards against mature Red Delicious fruit.

Materials and Methods

Isolates used

Table 3.3. *Alternaria* isolates used in *in vitro* inoculation of mature apple fruit.

Isolate number*	Tissue type infected	Apple variety	Location
BRIP 46348	Leaf	Fuji	Ballykeane, NSW
BRIP 46349	Fruit	Fuji	Ballykeane, NSW
BRIP 46351	Leaf	Pink Lady	Ballykeane, NSW
BRIP 46550	Fruit	Fuji	Granite Belt, QLD
BRIP 46656	Leaves	Fuji	Hillwood, TAS
BRIP 46924	Leaves	Fuji	Ardmona, VIC
BRIP 47177	Leaf	Pink Lady	Lenswood, SA

*Isolates referred to by BRIP numbers are available from the DPI&F, Queensland Plant Pathology Herbarium, Indooroopilly Sciences Centre, 80 Meiers Rd, Indooroopilly Queensland 4068, Australia.

Apple varieties used

Fully mature Red Delicious fruit from field trees were sourced from Applethorpe Research Station, Granite Belt, Queensland.

Inoculum preparation

As described in section 3.1.

In-vitro inoculations

Mature detached fruit were surface sterilised with 70% ethanol, rinsed in sterile distilled water and blotted dry with sterile tissue paper. Circles (ca. 25 mm in diameter) were drawn onto the surface of fruit with a permanent marker pen, and 25 µl of spore suspension placed in the centre of each circle. Treatment control fruit were inoculated with sterile distilled water. Fruit were then placed into sterile plastic boxes and stored at 100% relative humidity for 48 h at 24-25^{o}C. Fruit were then held at 80-85% relative humidity and 24-25^{o}C for a further 10 days, after which lesion development and size of lesions was recorded.

Results and Discussion

Despite several attempts using a range of slightly different techniques, no lesions were observed on any of the inoculated detached fruit.

Fruit may have been too mature for infection or wounding may have been required. Further experiments are underway, using immature fruit, and fruit still attached to the tree.

4. Methods for investigating *Alternaria* diseases of tree crops

The purpose of this review is to identify appropriate methods to use in the identification and characterisation of *Alternaria* species causing disease symptoms on apple leaves and fruit in Australia.

Methods used to study Alternaria diseases of citrus and Australian grown *Paulownia* trees are also considered. Alternaria diseases of citrus and *Paulownia* are of particular relevance as they both involve a number of different small-spored *Alternaria* species within the *Alternaria alternata* grouping; a situation that appears similar to Alternaria diseases on Australian apples.

The genus Alternaria

The genus *Alternaria* Nees. includes a diverse assemblage of species that occur worldwide in a variety of habitats (Simmons 1992). Many species are common saprobes and are ubiquitous agents of decay (Rotem 1994). As plant pathogens, more than 4000 Alternaria-host associations are recorded in the USDA Fungal Host Index, and the genus ranks 10th among 2000 fungal genera listed, based on the total number of host records (Farr *et al.* 1989).

Alternaria species are some of the most prodigious producers of toxic secondary metabolites, and more than 70 compounds of varying toxicity have been reported to date (Montemurro and Visconti 1992b). *Alternaria* species and related taxa are becoming increasingly important as human pathogens, especially in immunocompromised patients (Anaissie *et al.* 1989; Rossmann *et al.* 1996; Vartivarian *et al.* 1993). *Alternaria* species are also some of the most common and potent airborne allergens (Aukrust *et al.* 1984; Karlsson-Borgå *et al.* 1989).

Alternata-*like small-spored* Alternaria *species*

The taxonomy of small-spored *Alternaria* species is constantly under review (Andersen *et al.* 2005; Andersen *et al.* 2002; Andersen and Thrane 1996; Cooke *et al.* 1998; Hong *et al.* 2005; Peever *et al.* 2005; Peever *et al.* 2004; Pryor and Gilbertson 2000; Roberts *et al.* 2000; Simmons 1999). *Alternaria alternata* is fundamentally a saprophytic fungus, and the toxin-producing pathogens (including *Alternaria mali*) can usually only be differentiated from one another and *Alternaria alternata* by pathogenicity or toxin analysis (Nishimura *et al.* 1982; Nishimura *et al.* 1978b). Some authors have, however, shown careful morphological examination to reveal differences between some of these fungi and *Alternaria alternata* (Simmons 1999).

Recent literature suggests that *Alternaria alternata* is a species grouping and that more than one taxon (species) is contained within this group (Simmons 1990; 1992; 1994). Many of the difficulties in taxonomic classification of species within the genus *Alternaria* are due to the lack of a sexual stage (Rotem 1994; Simmons 1992; Yu 1992). The frequent absence of a sexual stage presents two problems for the traditional classification of the *Alternaria* genus: not only is there an absence of sexual structure morphology as a taxonomic character, but also it is not possible to assess species boundaries on the basis of sexual incompatibility. In addition,

convergent evolution of spore forms may bias existing taxonomic schemes (Cooke *et al.* 1998).

As a result, the species of *Alternaria* causing blotches on apple leaves have been referred to in several different ways in scientific literature, including *Alternaria mali*, *Alternaria alternata* pv *mali* and the apple pathotype of *Alternaria alternata*.

There are several other *Alternaria* fungi which have been referred to in different ways, including Alternaria diseases of citrus, potato, Japanese pear, tobacco and strawberry. Similarly, the criteria used to justify the different ways of naming these species also varies, with different authors attributing more or less relevance to the techniques used.

Identification of small-spored Alternaria *species*

Some authors use only one form of identification, while others argue over which identification method is the most correct, i.e. morphology vs genetics vs pathogenicity vs toxin or metabolite production. For this reason the most logical approach to the identification of small-spored *Alternaria* species would seem to require at least two or three of the abovementioned methods.

Using morphological characters

Morphology remains the most reliable single method for identification, but requires a skilled specialist and takes diligence and time (Andersen *et al.* 2006b). *Alternaria* species are conidial fungi, most of which have no known sexual stage. The taxonomy of *Alternaria* is based primarily on the morphology and development of conidia and conidiophores, and, to a lesser degree, on host plant association and colony morphology (Simmons 1967).

Alternaria species are differentiated primarily by conidium characteristics including size, septation, presence and/or size of a beak and pattern of catenation (Simmons 1992). Based on morphological features, the genus *Alternaria* has been described as having several subgeneric groups, containing species with similar conidium characteristics. The *Alternata* group includes species with small catenate spores, such as *Alternata alternata* and *Alternata mali* (Simmons 1995).

The current morphological description for *Alternaria mali* is as follows. The hyphae are hyaline to dark gray or dark olive green. Conidia (13-50 x 6-20 µm) are dark olive or blackish brown and obclavate, ovate, or round; they form in long chains of five to 13 (usually five to eight). Their outer walls are usually smooth, but occasionally verrucose. They have very short beaks or are beakless (Simmons 1999).

The technical description for the physical structures produced by *Alternaria mali* can be considered a subset of the description for *Alternaria alternata*. This means identification by light microscopy alone is a specialist task. The physical features of *Alternaria* species are very susceptible to growth conditions, and when artificially cultured, will develop different physical characters with even very slight variations in temperature, humidity and light conditions. Compounding the problem is the fact that pathogenic and non-pathogenic strains of *Alternaria mali* cannot usually be distinguished by physical features (Sawamura 1990), and so other methods of identification are needed.

The physical features (morphology) of *Alternaria* species can be highly variable, especially when growth conditions are not consistent. This can make identification by comparison of mycelia difficult when comparing large numbers of isolates that cannot all be grown at once, and when comparing isolates between different laboratories and research groups.

Morphological plasticity in response to non-standard growth conditions is a commonly observed phenomenon in fungi (Roberts *et al.* 2000). Diagnostic growth media, temperature and light conditions have been developed for *Alternaria* (Simmons 1992), but their application has been inconsistent.

In particular, Andersen *et al.* (2001) noted that many authors simply used two dimensional spore measurements to identify *Alternaria* species, because the squash mounts they were using for examining conidia did not preserve any of the three dimensional structures of conidia and conidiophores which can be observed at 50 x magnification. It should also be noted that several authors (Andersen *et al.* 2001; Pryor and Michailides 2002; Simmons 1999) felt that *Alternaria* cultures should not be grown in the dark on high nutrient media as this results in the production of excess aerial mycelia that prevents the development of three dimensional sporulation patterns.

Alternaria alternata, *Alternaria gaisen* (Alternaria black spot of Japanese pear) and *Alternaria longipes* were able to be separately identified morphologically by growing cultures under standardized and carefully controlled conditions. Consistent differences between species were observed in three dimensional structures at x 50 magnification, and distinct differences in conidia observed in tape preparations at x 400 magnification (Andersen *et al.* 2001). These results were confirmed by analysis of the chemical compounds produced by the isolates and by the genetic analysis of Roberts *et al.* (2000). These authors were also able to distinguish between these three species using growth characteristics at different temperatures on DRYES medium (Frisvard 1983). This method requires standardised growth conditions and a sufficient number of isolates in order to build a reliable system (Andersen *et al.* 2001).

The *Alternaria alternata* complex infecting Australian *Paulownia* trees consists of the small-spored catenulate species-groups of *Alternaria alternata*, *Alternaria tenuissima* and *Alternaria arborescens*. These species are difficult to distinguish, but under controlled conditions, morphological examination of the arrangement of conidia on conidiophores, number of conidia in chains, and pattern of chain branching can be used to separate the three species from each other as well as from *Alternaria infectora* (Ray *et al.* 2005).

Using chemical (toxin or metabolite) production

Alternaria species are able to produce a variety of chemically different compounds. Some of these are toxic to mammals and birds, and some to plants. Different species of *Alternaria* produce different compounds, and most of the known compounds or secondary metabolites have been reported from fungi identified as *Alternaria alternata* (King and Schade 1984).

Toxins

The study of small-spored *Alternaria* species in recent years has been highly focused on toxin production, especially host specific toxin (HST) production (Akamatsu *et al.* 1999; Andersen *et al.* 2001; Andersen *et al.* 2006b; Markham and Hille 2001; Miyashita *et al.* 2001).

Pathogenic *Alternaria mali* isolates are known to produce host-specific toxins AM-toxins I, II and III (Kohmoto *et al.* 1976; Kohmoto *et al.* 1977; Okuno *et al.* 1974; Sawamura 1990; Ueno *et al.* 1983) and tentoxin (Montemurro and Visconti 1992a). AM-toxins have shown extremely potent host-specific toxicity, and solutions of the toxins induce the same necrotic symptoms on the apple leaves of susceptible cultivars, as the pathogen itself. The pathogenicity, and virulence, of an isolate of *Alternaria mali* has been shown to be directly related to the forms of AM-toxin the isolate produces (Ueno 1987).

A great deal of information is available about the modes of action for several of these toxins, including the effects of AM-toxins on cell structures (Park *et al.* 1977; Ueno 1987; Ueno *et al.* 1983). Researchers have also used artificially derived chemicals to study the effects of specific ring structures in toxin activity (Aoyagi *et al.* 1987; Mihara *et al.* 1986). Although interesting from a pure science perspective, and useful in variety susceptibility screening, this area of research has yet to produce any immediately useful applied results for the apple industry.

Recently, a PCR test has been developed which can detect AM toxin I genes from apple tissues and pure *Alternaria* cultures. Interestingly, the morphological studies associated with this new test indicate that AM toxin I genes could be found in other closely related species of *Alternaria*, such as the *Alternaria tenuissima* species group (Andersen *et al.* 2006a). Several studies have shown host specific toxins produced by *Alternaria* species to be located on conditionally dispensable chromosomes (Hatta *et al.* 2002; Johnson *et al.* 2001; Masunaka *et al.* 2005; Tsuge *et al.* 2005).

In *Alternaria mali* the loss of one 1.1-Mb chromosome containing the *Alternaria mali toxin (AMT)* genes resulted in a non-pathogenic strain of the fungus (Johnson *et al.* 2001). In citrus, the transfer of host specific toxin production ability has been suggested, via the exchange of such chromosomes (Masunaka *et al.* 2005), although the mechanism for such an exchange was not postulated.

This poses some interesting taxonomic questions, as some studies have identified apple leaf pathogens as *Alternaria mali* based on the production of AM toxin I (Andersen *et al.* 2002). If AM toxin is not specific to *Alternaria mali*, then the presence of this toxin alone is not sufficient for identification of *Alternaria* species as *mali*. This will have serious implications for diagnostic tests based on the presence of AM-toxin I genes, and for all previous diagnoses based on presence of AM-toxins alone.

Metabolites

The production of secondary metabolites on standardised laboratory media has been used to distinguish between morphologically similar species, including small spored *Alternaria* species (Andersen *et al.* 2005; Andersen *et al.* 2001; Andersen *et al.* 2002; Andersen *et al.* 2006b; Andersen and Thrane 1996). A profile of metabolites can be

visualised using chromatographic methods such as thin layer chromatography and ultra-violet light, or high performance liquid chromatography and diode array detection (Andersen *et al.* 2002; Smedsgaard 1997).

This technique shows some promise for the rapid identification of moderate numbers of isolates, once standard profiles have been developed for the relevant species.

Using genetic characters

Some of the genes responsible (*AMT* genes) for the production of AM toxins have been cloned (Johnson *et al.* 2000a), allowing the development of genetic tests that specifically detect strains of *Alternaria mali* that produce AM toxin I (Johnson *et al.* 2000b). Disruption of *AMT* genes, led to a loss of pathogenicity in isolates of *Alternaria mali* from Japan (Johnson *et al.* 2000a).

Work has also shown that these genes are located on non-essential gene segments, known as conditionally dispensable chromosomes (Tsuge *et al.* 2005). This means that the production of toxins is not essential for the survival of *Alternaria mali*, and there may be some potential for the development of mild strains. Mild strains of *Alternaria mali* may be able to fill the niches normally taken by pathogenic strains, thereby reducing symptoms and reducing production losses.

The Internal Transcribed Spacer (ITS) region has been used to differentiate fungi at or below the species level, and to examine phylogenetic relationships between *Alternaria* species that produce host specific toxins. The inability of Kusaba and Tsuge (1995) to distinguish between HST producing and non-HST producing isolates of *Alternaria alternata* suggests that these two groups are closely related. In fact they support the idea that HST producing small spored *Alternaria* species are intraspecific variants of a single variable species *Alternaria alternata* (Kusaba and Tsuge 1995). Similarly, restriction fragment length polymorphism (RFLP) analysis of a broader range of genes, has shown pathogenic and non-pathogenic strains of HST and non-HST producing *Alternaria* species, including *Alternaria mali,* to be very closely related (ByungRyun *et al.* 1998).

Subsequent analysis of *Alternaria mali* sequences by RFLP-based analysis of the ITS region did not provide sufficient sequence variability to distinguish between the *Alternaria* species studied (Roberts *et al.* 2000). These authors agreed with previous reports (Simmons 1999) that the ITS region is not suitable on its own to distinguish small-spored *Alternaria* species, and suggested that more variable genes need to be found to distinguish these pathogens by sequence analysis. This work indicates the artificial nature of the pathotype nomenclature system for small-spored *Alternaria* species (Roberts *et al.* 2000).

Genetic analysis using isozyme variations

Hwang *et al.* (1987) analysed genetic variation in populations of 500 *Alternaria mali* isolates collected from five areas in Korea, using the genetically determined isozyme system for esterases. Horizontal starch gel electrophoresis (Poulik 1957) was performed on mycelia taken from the growing edge of a five day old culture. Gels were then stained for esterases. More than 40 different esterase patterns were observed in the 500 isolates tested. Esterase patterns were qualitatively and quantitatively different among isolates. Divergence values in esterase isozymes of

Alternaria mali were not correlated to the geographic distances among locations. Environmental differences during *in vitro* culture, including temperature and culture age, did not influence isozyme patterns. The main conclusion drawn was that the esterase isozymes were heterogeneously distributed in the geographic locations. Such genetic diversity in a population of *Alternaria mali* may be important for adaptation to heterogeneous environments (Hwang *et al.* 1987).

Using multiple methods

It is important to use more than one method of identification when determining the relatedness of small-spored *Alternarias* from the same host. It is also vital when reading the results of *Alternaria* studies to keep in mind that the isolates being referred to as specific species may have been misidentified, especially if only one method of identification was originally used.

A data matrix based on a combination of 86 characters/variables (chemical, morphological and cultural) was used to successfully separate *Alternaria* isolates into two groups *Alternaria infectoria* and *Alternaria alternata*. The separation also allowed isolates from each of these groups to be further separated into three subgroups. The *Alternaria alternata* group produced only the HST alternariol and alternariol monomethyl ether, while the *Alternaria infectoria* group produced a range of unidentified, unique metabolites (Andersen and Thrane 1996). The authors also suggest that identification should not be made on chemical and cultural characters alone, and that this data should be used in conjunction with genetic analysis for accurate diagnoses (Andersen and Thrane 1996).

Some authors have taken identification to a new level by developing automated and unbiased (without human interpretation) image analysis systems based on the phenotype for use in *Alternaria* species classification (Andersen *et al.* 2005). Six small spored *Alternaria* species were used, *Alternaria gaisen* (Japanese pear), *Alternaria longipes* (tobacco), *Alternaria alternata*, *Alternaria limoniasperae* (citrus), *Alternaria tangelonis* (citrus) and *Alternaria turkisafria* (citrus). Most species segregated easily using different combinations of colony colour and texture characters; but *Alternaria gaisen* was less homogeneous species in this study and clustered with a lower level of similarity. Growth rates, expressed as colony diameters at different temperatures and times, proved to have high taxonomic value. The production of metabolites/toxins confirmed the cultural character results. The authors recommend the use of reference isolates, and multiple means of identification (Andersen *et al.* 2005).

A separate group of researchers have also developed a multiple identification method technique for identifying small-spored *Alternaria* species, by comparing new isolates to a known group of isolates from the species *Alternaria arborescens, Alternaria alternata, Alternaria tenuissima* and *Alternaria infectoria* (Belisario *et al.* 2004; Hong *et al.* 2005; Hong *et al.* 2006; Pryor and Gilbertson 2000; Pryor and Michailides 2002; Teviotdale *et al.* 2001). This method uses various combinations of morphological characters, genetic analysis and pathogenicity tests to identify the causal pathogens of Alternaria diseases and relate the identity of isolates to known/defined type species.

DNA analysis includes sequencing and random amplified polymorphism DNA and polymerase chain reaction RFLP analysis of the ITS and intergenic spacer (IGS)

regions of nuclear rDNA. These methods provide a reasonably rapid means for identifying which isolates are causing disease symptoms, and to which known species of *Alternaria* the isolates are most closely related. This technique has been used successfully on a number of crops with Alternaria diseases including pistachio (Belisario *et al.* 2004; Pryor and Michailides 2002), hazelnut and walnut (Belisario *et al.* 2004; Hong *et al.* 2006), *Paulownia* (Ray *et al.* 2005) and almond (Teviotdale *et al.* 2001).

This system, using multiple identification methods, is currently being used to identify a representative panel of *Alternaria* species collected from Australian apple orchards, as a part of AP06007.

Practical techniques for use in AP06007

Pathogenicity tests

Determining the pathogenicity of saprophytic pathogens can be a very difficult process, especially when the organism/s involved are only weakly pathogenic. The methods attempted so far in this project (AP05002) have not been successful in consistently demonstrating pathogenicity of apple *Alternaria* isolates. The following methods have been selected for experimental evaluation in AP06007 as they include the broadest range of successful methods currently reported.

In vitro

Detached leaves

Filajdic and Sutton (1992b)

Detached leaves from one-month-old Delicious seedlings were inoculated with *Alternaria mali* spores suspensions to determine the influence of leaf wetness and temperature on infection of apple leaves. Twenty leaves, with petioles attached and their adaxial surfaces facing upwards were placed in each humid chamber. Spore suspensions were then applied until the point of run off, with an artist's airbrush. Humid chambers were then sealed and incubated for seven days. Virulence of the isolates was assessed by the percentage of leaf surface area covered by lesions.

Dickens and Cook (1995)

Leaves from field apple and Asian pear (*Pyrus pyrifolia*) trees were used in a detached leaf bioassay to determine the pathogenicity of isolates of *Alternaria mali* and *Alternaria gaisen* respectively. Leaves were inoculated by placing mycelial plugs (3 mm in diameter) of Alternaria cultures onto each leaf, and covering the leaf with cotton wool moistened with distilled water. Leaves were placed, 10 per isolate per host, in plastic boxes lined with damp, absorbent paper (damp chambers), and incubated in a growth room. Leaves were examined after 7-10 days. The results from these isolations matched those from attached leaves inoculated in the same manner (refer to next section).

Canihos et al. (1999)

A detached leaf assay was used by Canihos *et al.* (1999) to study the effect of temperature and leaf wetness on infection of immature Minneola tangelo leaves by isolates of *Alternaria citri*. Isolates differed in aggressiveness, but there was no

significant difference between isolates in their response to temperature and leaf wetness duration. Washed conidia were used at a concentration of 10^4/ml, which provided a scorable number of lesions on each leaf. Petioles of immature leaves were placed into test tubes containing water, and each leaf held in place with parafilm around the top of the tube. Leaf tubes were then placed into tube holding racks and put into a moist incubation chamber. Leaves were misted with the conidial suspension, and placed at various temperatures. After different time periods, leaves were removed from the humid chambers, dried with a fan and kept at room temperature (21-24^{o}C) and humidity (40-70%). Lesion numbers were scored 36 h after removal from the humid chambers (Canihos *et al.* 1999), and correlated well with field infection results (Solel *et al.* 1997).

Miyashita et al. (2001)

Leaves cultured from apple meristem cells were used to evaluate resistance to AM-toxins I, II and III (Miyashita *et al.* 2001). These leaves were more sensitive to the toxins and gave more reliable responses than from field grown leaves or glasshouse grown trees, due to the homogenous nature of the cultured leaves and the method of toxin application. Leaves were cultured from meristemmatic bud tissues, excised and placed in 96 well plates. Toxin solution (20 µl) was placed on leaves and incubated for 48 h at 25^{o}C in the dark. Necrotic reactions were characterised by leaves turning dark brown in colour, with discolouration starting in the stem and moving into the leaf tissue. Three leaves were used to evaluate each variety/toxin concentration, and a positive response was recorded when more than two leaves became necrotic.

Vicent et al. (2004)

Leaf bioassay techniques were used by Vicent *et al.* (2004) to determine the susceptibility of a range of citrus cultivars to *Alternaria alternata*. Leaves were inoculated by placing droplets of conidial suspensions on the underside of young leaves. Inoculated leaves were then incubated in a warm (27^{o}C), humid environment for 48 h, and rated according to the development of necrotic lesions.

Leaf discs

Ray *et al.* (2005) assessed the pathogenicity of *Alternaria* isolates using discs cut from young, fully expanded, *Paulownia* leaves. Discs were surface sterilised with 2% NAOCl for 10 s and then washed three times in sterile water. Discs were submerged in water for 2 h prior to inoculation. Poor lesion development occurred on undamaged leaves, so discs were wounded by lightly pressing a glass rod onto the leaf surface to cause a bruise. After bruising, leaf discs were immediately inoculated with 5 µl of spore suspension. Seven replicate discs were used for each isolate. Controls were inoculated with sterile water. Leaf discs were placed in individual petri dishes, wrapped and left in the dark for two days before transferring to the light for five days. Lesion diameter was recorded seven days after inoculation. Lesions were significantly larger than the controls for all *Alternaria* isolates.

Fruit

Dickens and Cook (1995)

Dickens and Cook (1995) also inoculated apple fruit and Asian pear fruit to determine pathogenicity of *Alternaria mali* and *gaisen* isolates. Half-grown fruit were inoculated with rectangular pieces (10 x 12 mm in size) of mycelia cut radially from

the leading edges of colonies. Mycelial plugs were placed on wounded sites on the sides of the fruit and covered with cotton wool moistened with distilled water. Six replicates of each isolate were used, and each fruit had up to six different inoculum points. Wounding was by either steel wool abrasion or superficial scalpel cuts. Fruit were observed for symptoms after 10 days. Pathogenic isolates produced black spots on the fruit surface, with one isolate from Portugal also producing internal rotting.

Vicent et al. (2004)

Fruit bioassay techniques were used by Vicent *et al.* (2004) to determine the susceptibility of a range of citrus cultivars to *Alternaria alternata*. Fruit were inoculated by placing pieces of filter paper soaked in conidial suspensions on to the skin of the fruit. Inoculations were incubated in a warm (27^{o}C), humid environment for 48 h, and then rated according to the development of necrotic lesions. A polynomial equation was then used to develop a disease severity index, which successfully demonstrated a relationship between the level of infection and the size/developmental stage of inoculated fruit.

In vivo

Attached leaves

Dickens and Cook (1995)

The success of detached leaf bioassays performed by Dickens and Cook (1995) were verified by inoculation of attached apple and Asian pear leaves with *Alternaria mali* and *Alternaria gaisen*, respectively. Mycelial plugs (3 mm in diameter) were taken from *Alternaria* cultures, and placed onto leaves attached to field trees. Plugs were covered with cotton wool moistened with distilled water, and the leaves were enclosed in individual polythene bags to maintain high humidity. Six leaves per isolate per host were inoculated, and leaves examined 7-10 days after inoculation. Infected leaves were shed 7-10 days after inoculation. Reduced rates of leaf fall were observed when isolates were kept in culture for 12 months, indicating a loss in pathogenicity during isolate storage. For some isolates, lesions only developed on some leaves, however, no specific explanation for this was given.

Everts and Lacy (1996)

Dry conidia of *Alternaria porri* were used by Everts and Lacy (1996) to inoculate attached onion leaves. Conidia were harvested from culture plates by suspension is sterile distilled water, and filtration on a 0.8 μm-pore-size membrane filter. Spores were dried for 24 h over anhydrous CaSO4 before use. Dried spores were weighed and 2.5 g was dispersed in a settling tower over each onion plant. Plants were then carefully transferred to a dew chamber at 24^{o}C for 24 h, and then to a growth chamber for four days at 24^{o}C. Symptoms, flecks or lesions, were rated at various times after inoculation.

Tree trunk inoculations

Ray *et al.* (2005) inoculated thin stems of *Paulownia* trees with *Alternaria* isolates by inserting mycelial plugs under the bark, then grew the plants in a controlled environment of 30^{o}C days and 17^{o}C nights. The resulting lesions were measured. Control plants were inoculated with sterile agar plugs, and after 18 days the wound had completely healed.

Whole plants

Pegg (1966)

Rapidly expanding leaf tissue and young fruit of Emperor mandarins were inoculated by Pegg (1966) with *Alternaria alternata*. Aqueous spore suspensions were sprayed onto seedlings under saturated humidity, and leaf spots were produced within 16 h of inoculation. *Alternaria* was reisolated from the leaf spots. In field inoculations many of the young fruit dropped within three days of inoculation. Squares of mycelia taken from the leading edge of colonies were placed on young leaves under saturated humidity. Lesions were observed within 24 h of inoculation. Mature leaves were resistant to infection. As fruit developed there was a decrease in susceptibility, particularly after the stage where the oil glands became prominent.

Filajdic and Sutton (1992b)

Whole potted Delicious seedlings were inoculated with *Alternaria mali* spores, by spraying adaxial and abaxial leaf surfaces to the point of run off with a $1x10^5$ suspension of conidia. After inoculation, whole plants were placed into plastic bags that contained wet paper towels to maintain leaf wetness. The seedlings were then placed into controlled environment chambers for seven days at 24^oC under continuous light. Infection levels were assessed by using the lower portion of the Horsfall-Barratt scale.

5. Project extension application

The project AP06007 "Alternaria fruit spot: New Directions – Extension" began on the 1 September 2006. Some project proposal details are included below.

Project Method

1. Epidemiological studies.

a. How many species of *Alternaria* are involved?
b. Source of inoculum.
c. Infection process.
d. Infection conditions.
e. Symptom development and susceptibility differences between apple varieties.
f. Alternative hosts.

2. Development of an orchard disease management plan

a. Assessing a broader range of chemicals.
b. Application and timing of fungicides.
c. Disease surveillance.
d. Integrated management.

3 Overseas travel

It is anticipated that exchange of ideas and development of future collaborations with overseas scientists will be greatly enhanced by overseas travel. An initial trip is planned to the United States of America, to visit Professor Turner Sutton (University of North Carolina), and Associate Professor Barry M. Pryor (University of Arizona). Prof Sutton is the premier researcher in the field management of Alternaria leaf blotch in North America. Assoc Prof Pryor has been using new technologies to investigate the lifecycles of *Alternaria* species, and molecular analysis to clarify *Alternaria* species taxonomy.

Project Outcomes

1. Identification of *Alternaria* species causing fruit spot and leaf blotch symptoms on Australian apples.
2. A reasonable understanding of the disease cycle of *Alternaria* on Australian apples, including sources of inoculum, environmental conditions needed for infection, the infection process and disease development from infection to sporulation.
3. The ability to accurately assess levels of disease incidence and the severity of Alternaria diseases in apple.
4. The most effective fungicides against each species of *Alternaria*, and when is the most appropriate (effectiveness and economic) part of the lifecycle to apply them.
5. Australian apple growers will be familiar with an integrated approach to *Alternaria* management in apple orchards, combining chemical, physical and cultural control methods.
6. The Australian apple industry and broader scientific community will be familiar with the outcomes of our work.

Conclusions

The *Alternaria* species collection has been preserved as freeze dried cultures at the DPI&F Plant Pathology Herbarium, Indooroopilly Sciences Centre, Brisbane. This collection is now ready for continued use by our research team and available to other *Alternaria* researchers world-wide.

Preliminary investigations into the identity of *Alternaria* species causing Alternaria fruit spot and leaf blotch on Australian apples have revealed that many of the *Alternaria* isolates obtained are not *Alternaria mali*. Further studies to reveal the identity of a larger proportion of the isolates are currently underway.

Pathogenicity testing of *Alternaria* isolates continues to be difficult, with inconsistent results from a variety of inoculation techniques. Some of these difficulties may be because many of the isolates tested may not be *Alternaria mali*, and consequently may not be strongly pathogenic on apple tissues. This work is ongoing in AP06007, and it is hoped that new techniques outlined in the literature review presented in this report will finally solve the problem.

Technology Transfer

Grower meetings

- 31 January 2006 - Project progress was presented to Granite Belt apple growers and the APAL Industry Advisory Committee at Applethorpe Research Station.
- 4 April 2006 - Information about all pathology projects was presented to Mr Tim Mulherin, Minister for Primary Industries and Fisheries at Applethorpe Research Station.
- 22 May 2007 – Project results were presented to growers at a NSW DPI Field day at Bilpin, NSW. Approximately 20 local growers observed the results of the NSW field trial, and discussed *Alternaria* management with Dr Shane Hetherington and Deidre Gunning of NSW DPI.
- 19 June 2007 - Presentation of results of Queensland field trials to Queensland growers at a Growcom sponsored field day at Applethorpe Research Station.

Industry magazine articles

Horlock, CM. (2006). Managing late season Alternaria leaf blotch and fruit spot infections. Tree Fruit magazine, December 2005/January 2006 edition.

Horlock, CM. (2007). Alternaria Fruit Spot: New Directions. 2006-07 Apple and Pear Australia Limited industry report, p 22.

Recommendations – Scientific

The level of uncertainty surrounding the identity and role of *Alternaria* species in Alternaria leaf blotch and fruit spot symptoms in Australian apple orchards warrants further investigation. The potential for further spread of these symptoms was demonstrated by the detection of significant levels of Alternaria fruit spot symptoms in Orange (NSW) for the first time during the 2005/06 season, and the intermittent nature of outbreaks in Western Australia.

Further supporting the need for more research into the causes of these symptoms is the fact that Alternaria leaf blotch and fruit spot disease in Australia appears to be caused by a different species of *Alternaria* than recorded overseas.

The next logical steps to be undertaken in Australian *Alternaria* research are:

1. To determine the identity, to species level, and pathogenicity of isolates collected in the Australian apple orchard survey.
2. To determine if different species of *Alternaria* are causing the same disease symptoms in commercial apple orchards throughout Australia.
3. To determine if the same species of *Alternaria* is responsible for Alternaria leaf blotch and Alternaria fruit spot.
4. To determine which *Alternaria* species are primary and secondary pathogens of apple leaves and fruit in Australian orchards.
5. To pinpoint any physical, chemical or genetic differences between pathogenic isolates of *Alternaria* from Australian orchards; and use these differences to develop an accurate detection system.
6. To develop a program (or programs) for the effective management of Alternaria leaf blotch and fruit spot in Australia.
7. To compare isolates of *Alternaria* infecting apple leaves and fruit in Australia with overseas isolates.
8. To determine the symptoms produced by different species of *Alternaria* on a range of commercial apple varieties.

Recommendations – Industry

Alternaria disease – points for growers to remember

- Alternaria leaf blotch and fruit spot may not be caused by the same species of *Alternaria* in all growing areas, so management methods effective in one region may not be as effective in another.
- Not all leaf blotches are caused by *Alternaria*. Physical damage can cause symptoms which are very similar. Isolation and analysis is the only definite means of identifying Alternaria leaf blotch.
- If *Alternaria* is isolated from leaves or fruit in your orchard, it does not always mean that production-limiting levels of infection are occurring. Isolation of *Alternaria* species from your orchard, without production-limiting levels of infection, may not require fungicide application.
- The use of Rovral® (iprodione) for field management of *Alternaria* is strongly discouraged, for several significant reasons, including:
    - Iprodione is not registered for field use in Australia.
    - The risk of fungicide resistance developing in field and postharvest apple pathogen populations will be significantly increased by field use.
    - There is no scientific evidence to suggest that iprodione reduces Alternaria fruit spot on apple.

Alternaria management suggestions

General preventative disease management techniques

- Reduce over-wintering inoculum by ensuring that infected leaves are completely broken down over winter, and do not survive to initiate another disease cycle in the spring.
- Reduce physical damage to leaves and fruit as much as possible throughout the season by careful use of orchard equipment.
- Also reduce the physical injuries by effectively manage insect pests, and other diseases.
- Maintain good tree nutrition, especially calcium. The bitter pit symptom can creates significant fruit surface wounds, which can subsequently become invaded by *Alternaria*.

Current fungicide permits for *Alternaria* management

#PER9787 Polyram / apples / Alternaria control
Effective from 8 June 2007 to 30 June 2009.
For use in NSW, QLD & WA only

#PER9788 Delan 700 WG/ apples / Alternaria control
Effective from 8 June 2007 to 30 June 2009.
For use in NSW, QLD & WA only

Queensland

- Growers who experienced significant *Alternaria* infection last season are encouraged to use preventative measures against the disease this season.

- Use of the broad spectrum fungicides (metiram, dithianon or mancozeb) several times during the early-mid growing season, as a part of a regular black spot management program, will assist in reducing levels of *Alternaria* inoculum in the orchard during the growing season.
- After the black spot season has finished, growers should make further fungicide applications (especially during the period from 8 weeks to 7 days prior to harvest) to manage Alternaria fruit spot in Gala, Pink Lady and Red Delicious varieties.
- Please refer to the previously mentioned permits for instructions on late season applications of metiram (PER9787) and dithianon (PER9788).

New South Wales

- The inclusion of several applications of Vision® (or similar Group I fungicide) early in the season, is suggested, as a part of a regular apple scab/black spot spray program.
- The use of the broad spectrum fungicides (metiram, dithianon or mancozeb) several times during the early-mid growing season, as a part of a regular black spot management program, will assist in reducing levels of *Alternaria* inoculum in the orchard during the growing season.
- After the black spot season has finished growers should make further fungicide applications (especially during the period from 8 weeks to 7 days prior to harvest) to manage Alternaria fruit spot in Gala, Pink Lady and Red Delicious varieties.
- Please refer to the previously mentioned permits for instructions on late season applications of metiram (PER9787) and dithianon (PER9788).

Western Australia

- Outbreaks of *Alternaria* in Western Australia have often been associated with higher than average spring/summer rainfall in the past, so growers are advised to consider carefully their need for late season fungicide applications.
- Growers who experienced significant *Alternaria* infection last season, and who have had significantly greater than average rainfall this season, are encouraged to use the preventative measures described above this season.
- Growers experiencing average or lower than average levels of rainfall should monitor leaves carefully over the summer and apply fungicides as soon as moderate-high levels of leaf infection are observed.

Other states

- At the time of printing no fungicides are registered for use.
- If following suggestions for Queensland, New South Wales or Western Australia, remember that no trials have been undertaken in your region.
- At this stage only very limited information is available about the number or type of *Alternaria* species present in other states.

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References

Akamatsu H, Otani H, Taga M, Kodama M, Johnson R, Kohmoto K (1999) Molecular karyotypes for Alternaria plant pathogens known to produce host-specific toxins. *Current Genetics* **35**, 647-656.

Anaissie EJ, Bodey GP, Rinaldi MG (1989) Emerging fungal pathogens. *European Journal of Clinical Microbiology & Infectious Diseases* **8**, 323-330.

Andersen B, Hansen ME, Smedsgaard J (2005) Automated and unbiased image analyses as tools in phenotypic classification of small-spored *Alternaria* spp. *Phytopathology* **95**, 1021-1029.

Andersen B, KrØger E, Roberts RG (2001) Chemical and morphological segregation of *Alternaria alternata*, *A. gaisen* and *A. longipes*. *Mycological Research* **105**, 291-299.

Andersen B, Krøger E, Roberts RG (2002) Chemical and morphological segregation of *Alternaria arborescens*, *A. infectoria* and *A. tenuissima* species-groups. *Mycological Research* **106**, 170-182.

Andersen B, Smedsgaard J, Jorring I, Skouboe P, Pedersen LH (2006a) Real-time PCR quantification of the AM-toxin gene and HPLC qualification of toxigenic metabolites from *Alternaria* species from apples. *International Journal of Food Microbiology* **111**, 105-111.

Andersen B, Smedsgaard J, Jorring I, Skouboe P, Pedersen LH (2006b) Real-time PCR quantification of the AM-toxin gene and HPLC qualification of toxigenic metabolites from *Alternaria* species from apples. *International Journal of Food Microbiology* **111**, 105-111.

Andersen B, Thrane U (1996) Differentiation of *Alternaria infectoria* and *Alternaria alternata* based on morphology, metabolite profiles, and cultural characteristics. *Canadian Journal of Microbiology* **42**, 685-689.

Aoyagi H, Mihara H, Kato T, Yamada M, Ueno T (1987) Synthesis of enantio AM-toxin I. *Agricultural and Biological Chemistry* **51**, 1707-1709.

Aukrust L, Beaumont F, *et al.* (1984) 'Atlas of moulds in Europe causing respiratory allergy.' (ASK Publishing: Copenhagen Denmark).

Belisario A, Maccaroni M, Coramusi A, Corazza L, Pryor BM, Figuli P (2004) First report of *Alternaria* species groups involved in disease complexes of hazelnut and walnut fruit. *Plant Disease* **88**, 426.

Bhatia A, Roberts PD, Timmer LW (2003) Evaluation of the Alter-Rater model for timing of fungicide applications for control of alternaria brown spot of citrus. *Plant Disease* **87**, 1089-1093.

Bulajic A, Filajdic N, Babovic M, Sutton TB (1996) First report of *Alternaria mali* on apples in Yugoslavia. *Plant Disease* **80**, 709.

ByungRyun K, HeeWan K, SeungHun Y, Yasuo I, Keisuke K (1998) RAPD analysis of host-specific toxin (HST) producing *Alternaria* species. *Korean Journal of Plant Pathology* **14**, 92-98.

Canihos Y, Peever TL, Timmer LW (1999) Temperature, leaf wetness and isolate effects on infection of Minneola tangelo leaves by *Alternaria* sp. *Plant Disease* **83**, 429-433.

Chen GY, Zheng TM, Mao QY (1993) Experiments on the control of apple leaf and fruit diseases with fluorescent *Pseudomonas* sp. *Chinese Journal of Biological Control* **9**, 163-166.

Cheng XC, Kihara T, *et al.* (1989) A new antibiotic, tautomycetin. *Journal of Antibiotics* **42**, 141-144.

Ciferri R (1953) Five years of experiments with fungicides with a zinc ethylene bisdithiocarbamate base. *Notiz. Malatt. Piante, 1953.* **23**, 3-19 pp.

Cooke DEL, Forster JW, Jenkins PD, Jones DG, Lewis DM (1998) Analysis of intraspecific and interspecific variation in the genus *Alternaria* by the use of RAPD-PCR. *Annals of Applied Biology* **132**, 197-209.

CunHao C, FengZhi L, ChangCun W, PeiHua C, MingXiang F (2001) Techniques for the control of the main diseases of apple variety Fuji in the western part of Liaoning province. *China Fruits*, 33-34.

Dickens JSW, Cook RTA (1995) Japanese pear black spot and apple *Alternaria* blotch. *Bulletin OEPP* **25**, 651-659.

English WH (1940) Taxonomic and pathogenicity studies of the fungi which cause decay of Pears in Washington. *Res. Stud. St. Coll. Wash.* **8**, 127-128 pp.

Everts KL, Lacy ML (1996) Factors influencing infection of onion leaves by *Alternaria porri* and subsequent lesion expansion. *Plant Disease* **80**, 276-280.

Farr DF, Bills GF, Chamuris GP, Rossman AY (1989) 'Fungi on plants and plant products in the United States.' (APS Press: St. Paul, Minnesota USA).

Filajdic N, Sutton TB (1991) Identification and distribution of *Alternaria mali* on apples in North Carolina and susceptibility of different varieties of apples to *Alternaria* blotch. *Plant Disease* **75**, 1045-1048.

Filajdic N, Sutton TB (1992a) Chemical control of *Alternaria* blotch of apples caused by *Alternaria mali*. *Plant Disease* **76**, 126-130.

Filajdic N, Sutton TB (1992b) Influence of temperature and wetness duration on infection of apple leaves and virulence of different isolates of *Alternaria mali*. *Phytopathology* **82**, 1279-1283.

Filajdic N, Sutton TB (1994) Spacial pattern of *Alternaria mali*, the causal agent of *Alternaria* blotch of apples. *Zatita Bilja* **45**, 311-319.

Filajdic N, Sutton TB (1995) Overwintering of *Alternaria mali*, the causal agent of *Alternaria* blotch of apple. *Plant Disease* **79**, 695-698.

Filajdic N, Sutton TB, Walgenbach JF, Unrath CR (1995a) The influence of European red mites on intensity of *Alternaria* blotch of apple and fruit quality and yield. *Plant Disease* **79**, 683-690.

Filajdic N, Sutton TB, Walgenbach JF, Unrath CR (1995b) The influence of the apple aphid/spirea aphid complex on intensity of *Alternaria* blotch of apple and fruit quality characteristics and yield. *Plant Disease* **79**, 691-694.

Frisvard JC (1983) A selective and indicative medium for groups of *Penicillium viridicatum* producing different mycotoxins on cereals. *Journal of Applied Bacteriology* **54**, 409-416.

Gagkaeva TY, Levitin MM (2000) Identification of the pathogen causing leaf spots on apple trees in Krasnodar province orchards. *Mikologiya i Fitopatologiya* **34**, 58-62.

Gupta GK, R.K. A (1968) Outbreaks and new records. *Pl. Prot. Bull. F.A.O.* **16**, 32-33.

Hatta R, Ito K, Hosaki Y, Tanaka T, Tanaka A, Yamamoto M, Akimitsu K, Tsuge T (2002) A conditionally dispensable chromosome controls host-specific pathogenicity in the fungal plant pathogen *Alternaria alternata*. *Genetics* **161**, 59-70.

Hong SG, Liu D, Pryor BM (2005) Restriction mapping of the IGS region in Alternaria spp. reveals variable and conserved domains. *Mycological Research* **109**, 87-95.

Hong SG, Maccaroni M, Figuli PJ, Pryor BM, Belisario A (2006) Polyphasic classification of *Alternaria* isolated from hazelnut and walnut fruit in Europe. *Mycological Research* **110**, 1290-1300.

Horlock CM, Hetherington SD, Dullahide SR (2006) Management of Alternaria leaf and fruit spot in apples. Department of Primary Industries and Fisheries, Queensland, Apple and Pear Australia Limited/HAL Funded Project Final Report AP02011 No.

Hwang BK, Yun JH, Kim ZS (1987) Geographic variation of esterase isozymes in populations of *Alternaria mali* on apple leaves. *Journal of Phytopathology* **119**, 225-231.

Institute IM (1996, publ. 1997) IMI Distribution Maps of Plant Diseases. In 'IMI Distribution Maps of Plant Diseases'. Wallingford UK. (CAB INTERNATIONAL).

Itoh Y, Kiyohara R, Kawamoto Y, Kodama M, Otani H, Walton JD, Kohmoto K (1998) A catalytic domain of a cyclic peptide synthetase that is specific for the apple pathotype of *Alternaria alternata* and its possible involvement in host-specific AM-toxin production. In 'Molecular genetics of host-specific toxins in plant disease. Proceedings of the 3rd Tottori International Symposium Daisen, Tottori, Japan, 24-29 August, 1997.' (Eds K Kohmoto, OC Yoder) pp. 53-61.

JaeYoul U, DongHyuck L, SangKye L (1995) Development of fungicide spray program for the apples to be exported to the United States of America. *Korean Journal of Plant Pathology* **11**, 17-29.

JinYou W, MeiNa L, Zhuang Q (1997) Experiment on suitable timing of applying special germicide for control of apple leaf spot disease. *China Fruits*, 29-30.

Johnson LJ, Johnson RD, Akamatsu H, Salamiah A, Otani H, Kohmoto K, Kodama M (2001) Spontaneous loss of a conditionally dispensable chromosome from the Alternaria alternata apple pathotype leads to loss of toxin production and pathogenicity. *Current Genetics* **40**, 65-72.

Johnson RD, Johnson L, Itoh Y, Kodama M, Otani H, Kohmoto K (2000a) Cloning and characterization of a cyclic peptide synthetase gene from *Alternaria alternata* apple pathotype whose product is involved in AM-toxin synthesis and pathogenicity. *Molecular Plant-Microbe Interactions* **13**, 742-753.

Johnson RD, Johnson L, Kohmoto K, Otani H, Lane CR, Kodama M (2000b) A polymerase chain reaction-based method to specifically detect *Alternaria alternata* apple pathotype (*A. mali*), the causal agent of Alternaria blotch of apple. *Phytopathology* **90**, 973-976.

Karlsson-Borgå Å, Jonsson P, Rolfsen W (1989) Specific IgE antibodies to 16 widespread mold genera in patients with suspected mold allergy. *Annals of Allergy* **63**, 521-526.

Kim CH, Cho WD, Kim SC (1986) An empirical model for forecasting Alternaria leaf spot in apple. *Korean Journal of Plant Protection* **25**, 221-228.

Kim SB, Yiem MS, Jang HI (1982) Studies on the chemical control of *Alternaria* leaf spot in apple trees. *Research Reports, Office of Rural Development, S. Korea, Horticulture* **24**, 77-82.

King ADJ, Schade JE (1984) *Alternaria* toxins and their importance in food. *Journal of Food Protection* **47**, 886-901.

Kohmoto K, Khan ID, Renbutsu Y, Taniguchi T, Nishimura S (1976) Multiple host-specific toxins of *Alternaria mali* and their effect on the permeability of host cells. *Physiological Plant Pathology* **8**, 141-153.

Kohmoto K, Taniguchi T, Nishimura S (1977) Correlation between the susceptibility of apple cultivars to *Alternaria mali* and their sensitivity to AM-toxin I. *Annals of the Phytopathological Society of Japan (Nihon Shokubutsubyori Gakkai Ho)* **43**, 65-68.

Kusaba M, Tsuge T (1995) Phylogeny of *Alternaria* fungi known to produce host specific toxins on the basis of variation in internal transcribed spacers of ribosomal DNA. *Current Genetics* **28**, 491-498.

Lee CU (1984) Tolerance of *Alternaria mali* to various fungicides. *Korean Journal of Plant Protection* **24**, 19-24.

Magarey RD, Sutton TB, Thayer CL (2005) A simple generic infection model for foliar fungal plant pathogens. *Phytopathology* **95**, 92-100.

Marchionatto JB (1938) 'Mouldy core' of the Apple. Preliminary note. *Rev. argent. Agron.* **5**, 179-186 pp.

Markham JE, Hille J (2001) Host-selective toxins as agents of cell death in plant-fungus interactions. *Molecular Plant Pathology* **2**, 229-239.

Masuda T, Yoshioka T (1997) *In vitro* selection of a mutant resistant to *Alternaria* blotch disease in 'Indo' apple. In 'Technical News - Institute of Radiation Breeding' p. 2 pp.

Masunaka A, Ohtani K, Yamamoto H, Akimitsu K, Peever TL, Timmer LW, Tsuge T, Yamamoto M (2005) An isolate of Alternaria alternata that is pathogenic to both tangerines and rough lemon and produces two host-selective toxins, ACT- and ACR-toxins. *Phytopathology* **95**, 241-247.

Mihara H, Ikesue K, Lee S, Aoyagi H, Kato T, Ueno T, Izumiya N (1986) Cyclic peptides: SSI. Syntheses of *Alternaria mali* toxin I analogs containing L-lactic acid or L-2-hydroxy-4-methylpentanoic acid residue. *International Journal of Peptide and Protein Research* **28**, 141-145.

Miyashita M, Nakamori T, Miyagawa H, Akamatsu M, Ueno T (2003) Inhibitory activity of analogs of AM-toxin, a host-specific phytotoxin from the *Alternaria alternata* apple pathotype, on photosynthetic O_2 evolution in apple leaves. *Bioscience, Biotechnology and Biochemistry* **67**, 635-638.

Miyashita M, Nakamori T, Murai T, Yonemoto T, Miyagawa H, Akamatsu M, Ueno T (2001) Structure-activity relationship study of host-specific phytotoxins (AM-toxin analogs) using a new assay method with leaves from apple meristem culture. *Zeitschrift Fur Naturforschung C-a Journal of Biosciences* **56**, 1029-1037.

Montemurro N, Visconti A (1992a) *Alternaria* metabolites - chemical and biological data. In '*Alternaria*: Biology, Plant Diseases and Metabolites'. (Eds J Chelkowski, A Visconti) pp. 449-557. (Elsevier: Amsterdam).

Montemurro N, Visconti A (1992b) *Alternaria* metabolites: chemical and biological data. In '*Alternaria*: biology, plant diseases and metabolites.' (Eds J Chelkowski, A Visconti) pp. 449-557. (Elsevier Science Publishers: Amsterdam Netherlands).

Nishimura M, Kohmoto K (1983) Host-specific toxins and chemical structures from *Alternaria* species. *Annual Review of Phytopathology* **21**, 87-116.

Nishimura S, Kohmoto K, Otani H, Ramachandran P, Tamura F (1982) Pathological and epidemiological aspects of *Alternaria alternata* infection depending upon a host-specific toxin. In 'Plant infection: the physiological and biochemical basis.' (Eds Y Asada, WR Bushnell, S Ouchi, CP Vance) pp. 199-214. (Springer-Verlag: Tokyo).

Nishimura S, Sugihara M, Kohmoto K, Otani H (1978a) Two different phases in pathogenicity of the Alternaria pathogen causing black spot disease of Japanese pear. *Journal of the Faculty of Agriculture, Tottori University* **13**, 1-10.

Nishimura S, Sugihara M, Kohmoto K, Otani H (1978b) Two different phases in pathogenicity of the Alternaria pathogen causing black spot disease of Japanese pear. *J Fac Agric Tottori University* **13**, 1-10.

Okuno T, Ishita Y, Nakayama S, Sawai K, Fijita T, Sawamura K (1974) Isolation of a host-specific toxin produced by *Alternaria mali* Roberts. *Annals of the Phytopathological Society of Japan* **40**, 375-376.

OunHa R, KyeongHi C, SoonWon L, JongSeung C, YongIn L (1997) Influence of the different density of *Aphis citricola* on the shoot growth, fruit quality and disease occurrence in 'Fuji' apples. *Journal of the Korean Society for Horticultural Science* **38**, 66-70.

Park P, Tsuda M, Hayashi Y, Ueno T (1977) Effect of a host-specific toxin (AM-toxin I) produced by *Alternaria mali*, an apple pathogen, on the ultrastructure of plasma membrane of cells in apple and Japanese pear leaves. *Canadian Journal of Botany* **55**, 2383-2393.

Peever TL, Carpenter-Boggs L, Carris LM, Timmer LW, Bhatia A (2005) Citrus black rot is caused by phylogenetically distinct lineages of *Alternaria alternata*. *Phytopathology* **95**, 512-518.

Peever TL, Su G, Carpenter-Boggs L, Timmer LW (2004) Molecular systematics of citrus-associated *Alternaria* species. *Mycologia* **96**, 119-134.

Pegg KG (1966) Studies of a strain of *Alternaria citri* Pierce, the causal organism of brown spot of Emperor Mandarin. *Queensland Journal of Agricultural and Animal Sciences* **23**, 15-28.

Pleysier CE, Bayliss KL, Dell B, Hardy GESJ (2006) Temperature, humidity, wounding and leaf age influence the development of Alternaria alternata lesions on leaves of Paulownia fortunei. *Australasian Plant Pathology* **35**, 329-333.

Pryor BM, Gilbertson RL (2000) Molecular phylogenetic relationships amongst *Alternaria* species and related fungi based upon analysis of nuclear ITS and mt SSU rDNA sequences. *Mycological Research* **104**, 1312-1321.

Pryor BM, Michailides TJ (2002) Morphological, pathogenic, and molecular characterization of *Alternaria* isolates associated with alternaria late blight of pistachio. *Phytopathology* **92**, 406-416.

Ramírez-Legarreta MR, Jacobo-Cuéllar JL (1999a) Economic importance, epidemic development and spatial distribution of mouldy core (*Alternaria alternata* f. sp. *mali*) on Red Delicious apples. *Revista Mexicana de Fitopatología* **17**, 108-112.

Ramírez-Legarreta MR, Jacobo-Cuéllar JL (1999b) Structure of a model for mouldy core (*Alternaria alternata* f. sp. mali) of Red Delicious apple. *Revista Mexicana de Fitopatología* **17**, 29-36.

Ramírez-Legarreta MR, Jacobo-Cuéllar JL, Sánchez-Chávez E, Soto-Parra JM (2000) Mouldy core, its economic importance in several types of technical management of orchards and its relation to the production of Red Delicious apple in the state of Chihuahua. *Revista Mexicana de Fitopatología* **18**, 10-14.

Ray JD, Burgess T, Malajczuk N, Hardy GESJ (2005) First report of *Alternaria* blight of Paulownia spp. *Australasian Plant Pathology* **34**, 107-109.

Roberts JW (1924) Morphological characters of *Alternaria mali* Roberts. *Journ. Agric. Res.* **27**, 699-708 pp.

Roberts RG, Reymond ST, Andersen B (2000) RAPD fragment pattern analysis and morphological segregation of small-spored *Alternaria* species and species groups. *Mycological Research* **104**, 151-160.

Rossmann SN, Cernoch PL, Davis JR (1996) Dematiaceous fungi are an increasing cause of human disease. *Clinical Infectious Diseases* **22**, 73-80.

Rotem J (1994) 'The genus *Alternaria*: biology, epidemiology, and pathogenicity.' (American Phytopathological Society: St Paul, Minnesota, USA.).

RuiDe J, ShiJin L, PeiSheng W, JiQing W, Qing L (1997) Control of apple leaf spot diseases by spraying. *China Fruits*, 51.

Saito A, Nakazawa N, Suzuki M (2001) Selection of mutants resistant to Alternaria blotch from in vitro-cultured apple shoots irradiated with X- and gamma-rays. *Journal of Plant Physiology* **158**, 391-400.

Saito K, Niizeki M, Wake M, Hidano Y (1983) Fundamental studies on breeding of the apple. IX. Variation of pathogenicity and chemical tolerance to polyoxin in *Alternaria mali* Roberts. *Bulletin of the Faculty of Agriculture Hirosaki University*, 23-30.

Saito K, Takeda K (1984) Genetic analysis of resistance to *Alternaria* blotch (*Alternaria mali* Roberts) in apple. (Studies on the breeding of the apple. VIII). *Japanese Journal of Breeding* **34**, 197-209.

Saito K, Takeda K, Nakayama R (1978) Fundamental studies on breeding apple by means of hybridization. V. Correlation of resistance in leaf blade and fruit to *Alternaria mali*. *Bulletin of the Faculty of Agriculture, Hirosaki University (Hirosaki Daigaku Nogakubu Gakujutsu Hokuku)*, 42-49.

Saito KI, Niizeki M (1988) Fundamental studies on breeding of the apple. XI. Genetic analysis of resistance to *Alternaria* blotch (*Alternaria mali* Roberts) in the interspecific crosses. *Bulletin of the Faculty of Agriculture, Hirosaki University*, 27-34.

Sawamura K (1972) Studies on apple Alternaria blotch caused by *Alternaria mali* Roberts. *Bulletin of the Faculty of Agriculture, Hirosaki University*, 152-235.

Sawamura K (1990) Alternaria blotch. In 'Compendium of Apple and Pear Diseases'. (Eds AL Jones, HS Aldwinckle) p. 100. (APS Press: St Paul, Minnesota).

Sharma VP, Sharma SK (1991) Effect of apple scab spray schedule on powdery mildew and Alternaria leaf spot. *Indian Journal of Mycology and Plant Pathology* **21**, 190-191.

Shin YU, Ko KC (1992) Studies on Alternaria leaf spot (*A. mali* Roberts) in apple trees (*Malus domestica* Borkh): epiphytotics, screening methods and inheritance of resistance. III. Inheritance of resistance. *Journal of the Korean Society for Horticultural Science* **33**, 372-378.

Simmons EG (1967) Typification of *Alternaria*, *Stemphylium*, and *Ulocladium*. *Mycologia* **59**, 67-92.

Simmons EG (1990) *Alternaria* themes and variations (27-53). *Mycotaxon* **37**, 79-119.

Simmons EG (1992) *Alternaria* taxonomy: Current status, viewpoint, challenge. In '*Alternaria*: biology, plant diseases and metabolites.' (Eds J Chelkowski, A Visconti) pp. 1-36. (Elsevier Science Publishers: Amsterdam
Netherlands).

Simmons EG (1994) *Alternaria* themes and variations (106-111). *Mycotaxon* **50**, 409-427.

Simmons EG (1995) *Alternaria* themes and variations (112-144). *Mycotaxon* **55**, 55-163.

Simmons EG (1999) Alternaria themes and variations (236-243) host-specific toxin producers. *Mycotaxon* **70**, 325-369.

Smedsgaard J (1997) Micro-scale extraction procedure for standardization screening of fungal metabolite production in cultures. *Journal of Chromatography A* **760**, 264-270.

Solel Z, Oren Y, Kimchi M (1997) Control of Alternaria brown spot of Minneola tangelo with fungicides. *Crop Protection* **16**, 659-664.

Spotts RA (1990) Alternaria rot. In 'Compendium of Apple and Pear Diseases'. (Eds AL Jones, HS Aldwinckle). (APS Press: St Paul, Minnesota).

Tabira H, Shimonaka M, Kohmoto K, Otani H, Banno K (1998) Selection of a resistant mutant to *Alternaria* blotch in apple and analysis of proteins associated with the susceptibility by 2-D gel electrophoresis. In 'Molecular genetics of host-specific toxins in plant disease. Proceedings of the 3rd Tottori International Symposium Daisen, Tottori, Japan, 24-29 August, 1997.' (Eds K Kohmoto, OC Yoder) pp. 387-397.

Teviotdale BL, Viveros M, Pryor B, Adaskaveg JE (2001) First report of alternaria leaf spot of almond caused by species in the *Alternaria alternata* complex in California. *Plant Disease* **85**, 558.

Thomas CE (1983) Fungicide applications based on duration of leaf wetness periods to control Alternaria leaf blight of cantaloup in South Texas. *Plant Disease* **67**, 145-147.

Timmer LW, Darhower HM, Zitko SE, Peever TL, Ibanez AM, Bushong PM (2000) Environmental factors affecting the severity of Alternaria brown spot of citrus and their potential use in timing fungicide applications. *Plant Disease* **84**, 638-643.

Tomiya T, Uramoto M, Isono K (1990) Isolation and structure of phosphazomycin C. *Journal of Antibiotics* **43**, 118-121.

Tsuge T, Hatta R, *et al.* (2005) Conditionally dispensable chromosomes controlling pathogenicity of *Alternaria alternata*. In 'Genomic and genetic analysis of plant parasitism and defense. Proceedings of the 9th Japan-US Science Seminar on Plant-Pathogen Interactions, Shizuoka, Japan, 2-7 November 2003'. (Eds S Tsuyumu, JE Leach, T Shiraishi, T Wolpert) pp. 50-60.

Tweedy BG, Powell D (1962) Cork rot of Apples and its causal organism, a pathogenic strain of *Alternaria mali*. *Phytopathology.* **52**, 1073-1079 pp.

Ueno T (1987) Chemistry and biological function of host-specific phytotoxins. In 'Pesticide Science and Biotechnology. Proceedings of the Sixth International Congress of Pesticide Chemistry held in Ottawa, Canada, 10-15 August, 1986.' Oxford UK. (Eds R Greenhalgh, TR Roberts) pp. 89-96. (Blackwell Scientific Publications Ltd).

Ueno T, Hayashi Y, Nakashima T, Fukami H, Nishimura S, Kohmoto K, Sekiguchi A (1975) Isolation of AM-toxin I, a new phytotoxic metabolite from Alternaria mali. *Phytopathology* **65**, 82-83.

Ueno T, Nakshima T, Fukami H (1983) Chemical basis of host recognition of plant pathogenic fungi. *Memoirs of the College of Agriculture, Kyoto University* **122**, 93-129.

Uramoto M, Itoh Y, Sekiguchi R, Shin-ya K, Kusakabe H, Isono K (1988) A new antifungal antibiotic, cystargin: fermentation, isolation, and characterization. *Journal of Antibiotics* **41**, 1763-1768.

Vartivarian SE, Anaissie EJ, Bodey GP (1993) Emerging fungal pathogens in immunocompromised patients: classification, diagnosis, and management. *Clinical Infectious Diseases* **17**, S487-S491.

Vicent A, Badal J, Asensi MJ, Sanz N, Armengol J, García-Jiménez J (2004) Laboratory evaluation of citrus cultivars susceptibility and influence of fruit size on Fortune mandarin to infection by *Alternaria alternata* pv. *citri*. *European Journal of Plant Pathology* **110**, 245-251.

XueChi F, ZhiNong Y, WeiMin X, RuiHong M, Chai C (1997) Study on the biological control of *Alternaria solani* f.sp. *mali* and *Botryosphaeria berengeriana* of apple trees. *China Fruits*, 7-10.

Yoon JT, Lee JT (1987a) Effect of apple leaf hair density on the occurrence and infection of Alternaria leaf spot caused by *Alternaria mali* Roberts. *Korean Journal of Plant Protection* **26**, 245-249.

Yoon JT, Lee JT (1987b) Effect of calcium on the apple varieties resistance to Alternaria leaf spot and mycelial growth of`*Alternaria mali* Roberts. *Korean Journal of Plant Protection* **26**, 239-244.

Yoon JT, Lee JT, Choi DU, Shon SG (1989) Inhibitory effects of calcium compounds on the outbreak of *Alternaria* leaf spot caused by *Alternaria alternata* f.sp. *mali*. *Korean Journal of Plant Pathology* **5**, 306-311.

Yoshioka T, Ito Y, Masuda T (2000) Induction of mutant resistant to *Alternaria blotch* of apple by gamma-ray irradiation. In 'Gamma Field Symposia' pp. 69-79.

Yu SH (1992) Occurence of *Alternaria* species in countries of the Far East and taxonomy. In '*Alternaria*: biology, plant diseases and metabolites'. (Eds J Chelkowski, A Visconti) pp. 37-62. (Elsevier: Amsterdam).