# Management of Alternaria leaf and fruit spot in apples

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# FINAL REPORT

**AP02011 (28 February 2006)**

# Management of *Alternaria* leaf and fruit spot in apples

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# Table of Contents

## Media Summary

Fungicide trials in New South Wales and Queensland have revealed that different *Alternaria* species are responsible for Alternaria-like leaf and fruit symptoms in each state. This has resulted in slightly different fungicide spray programs being most effective in each state.

We found that more than one species of Alternaria can cause symptoms on apple leaves and fruit in Australian orchards.

Further, Alternaria is a common, widespread fungus in Australian apple orchards, with species similar to those causing production losses in Queensland and New South Wales detected in all major production areas in Australia.

It is important to distinguish between detecting Alternaria fungi in an orchard and needing to apply chemicals to manage it. Just because Alternaria is present in an orchard does not mean it is causing enough disease to warrant management. Growers are to be alert, but not alarmed.

Alternaria species were isolated from leaf lesions in almost every variety tested in a survey of the Applethorpe Apple Germplasm Repository (apple variety collection held by the Queensland Department of Primary Industries and Fisheries). However, few varieties suffered production limiting levels of disease.

In a survey of Australian apple orchards conducted in April/May 2005, we only recorded production limiting disease levels in orchards in New South Wales (Bilpin, Picton and Orange) and Queensland (Granite Belt). Varieties commonly affected in New South Wales are currently restricted to Gala, Red Delicious, Pink Lady and Fuji in descending order of importance; while in Queensland significant losses mainly occur in Gala, Pink Lady and then Red Delicious.

Our trials have led to emergency use permits for late season use of Polyram® and Delan® in 2006 – minor use permits are currently being sought, to continue this usage, for affected areas of New South Wales and Queensland.

## Technical Summary

Alternaria leaf blotch and Alternaria fruit spot can be serious mid-late season fungal diseases affecting apple leaves and fruit respectively, in high spring / summer rainfall production areas. 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 caused significant fruit losses and tree damage in Queensland and parts of New South Wales last season, particularly in the high value varieties of Gala, Fuji and Pink Lady as well as Red Delicious.

Fungicide trials undertaken in New South Wales and Queensland, to develop a management program for *Alternaria* leaf blotch and fruit spot, lead to the suspicion that there was more than one type of *Alternaria* causing symptoms in apple orchards. The basic difference between the two programs was the success of Group I chemicals in New South Wales and Group Y chemicals in Queensland.

The discrepancy between the two sets of trial results lead to a more intensive examination of leaf blotch and fruit spot symptoms in the two states, and the discovery that more than one type of *Alternaria* can be associated with these symptoms. A wider survey of Australian apple orchards revealed that *Alternaria* is a fungus commonly isolated from apple leaf lesions in all major growing areas, and fruit spots in some areas of New South Wales and Queensland.

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, and their presence does not conclusively prove they were the initial cause of the problem.

At this point it is important to distinguish between the detection of *Alternaria* from leaf blotches or fruit spots and the presence of production limiting levels of disease, that require the application of disease management methods.

A survey of apple varieties was undertaken from the Queensland Department of Primary Industries and Fisheries, Apple Germplasm Repository at Applethorpe Research Station. This revealed that *Alternaria* species were commonly isolated from many varieties. However, very few of these varieties were experiencing production limiting levels of disease.

The survey results presented in this report 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.

Suggestions for minimising *Alternaria* leaf blotch and fruit spot symptoms in New South Wales and Queensland orchards are presented. Emergency permits for the use of metiram (Polyram®) and dithianon (Delan®) in parts of New South Wales and Queensland were granted by the AVPMA in 2006. Minor use permits to continue this usage in affected areas of New South Wales and Queensland next season, are currently being sought.

# Introduction

## *Review of relevant literature*

### *Alternaria* diseases affecting apples

In the literature there are currently three apple diseases referred too as being caused by *Alternaria* species, *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 the 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, because until very recently *Alternaria* has not been a significant pathogen of apple fruit. This situation has changed significantly over the last five years, with severe *Alternaria* infection of fruit being reported from Europe (personal communication Klaus Marschall), Asia and Australia (Queensland and some parts of New South Wales). *Alternaria* infection of fruit has also been reported from the Unites States of America, but only as a minor problem on the varieties Indo and Ralls (Spotts, 1990).

At the beginning of this project, 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 it has become clear that there may be several types 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 blotch and fruit spot research world-wide, and not 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), *A. 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, Filajdic *et al.* 1996; Gagkaeva and Levitin 2000; Roberts 1924), India (1968), Asia (Dickens and Cook 1995; Sawamura 1972) and Australia.

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

### *Nomenclature*

The fungus currently termed *A. 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 *A. mali* was referred to as *Alternaria alternata* f.sp. *mali* or *Alternaria alternata* apple pathotype (Gagkaeva and Levitin 2000; Itoh, Kiyohara *et al.* 1998; Johnson, 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, Sugihara *et al.* 1978). 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; Ramírez-Legarreta and Jacobo-Cuéllar 1999b; Ramírez-Legarreta, Jacobo-Cuéllar *et al.* 2000), postharvest pear decay (English 1940) and cork rot of apples (Tweedy and Powell 1962). Similarly the name *Alternaria alternata* f. sp. *mali* has also been used to describe mouldy core (Ramírez-Legarreta and Jacobo-Cuéllar 1999a; Ramírez-Legarreta and Jacobo-Cuéllar 1999b; Ramírez-Legarreta, Jacobo-Cuéllar *et al.* 2000). These references, and others, can be misleading; so it is 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 *A. 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 *A. 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 that those in buds. Treatment of leaves with urea in the autumn had minimal effects on amount of leaf area remaining in spring. Overwintering on grass or bare ground or treatment with urea did not affect 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 *A. 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 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; with European red mite (Filajdic, Sutton *et al.* 1995a) and aphids (Filajdic, Sutton *et al.* 1995b) in the USA, and aphids in Korea (OunHa, KyeongHi *et al.* 1997) facilitating *Alternaria* infection.

***Identification***

Trying to distinguish between pathogenic (disease initiating) and non-pathogenic or saprophytic (wound invading) forms of *A. mali* has been the cause of constant research for almost 60 years. Initially light microscopy was used, but for the last 50 years or so, researchers in Asia (particularly Japan) have focused on the production of toxins to differentiate between isolates, with some success, and a lot of controversy (see the previous section on nomenclature). More recently researchers have started to study the genes responsible for toxin production.

*Light Microscopy*

The current morphological description for *A. 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.

The technical description for the physical structures produced by *A. mali* is a subset of the description for *Alternaria alternata*, which makes using light microscopy alone for identification a specialist task. Also 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 *A. mali* cannot usually be distinguished by physical features (Sawamura 1990), and so other methods are needed.

*Toxins*

Pathogenic *A. mali* isolates are known to produce host-specific toxins (Okuno, Ishita *et al.* 1974; Sawamura 1990), with several toxins (AM toxins) reported from Japanese isolates (Kohmoto, Khan *et al.* 1976; Kohmoto, Taniguchi *et al.* 1977; Ueno, Nakshima *et al.* 1983). AM toxins showed extremely potent host-specific toxicity, and solutions of the toxins induced the same necrotic symptoms on apple leaves of susceptible cultivars, as the pathogen (Ueno 1987). The pathogenicity, and virulence, of an isolate of *A. mali* have been shown to be directly related to the forms of AM toxin the isolate produces.

A great deal of information is available about the methods of action for several of these toxins, including the effects of AM toxins on cell structures (Park, Tsuda *et al.* 1977; Ueno 1987; Ueno, Nakshima *et al.* 1983). Researchers have also used artificially derived chemicals to study the effects of specific ring structures in toxin activity (Aoyagi, Mihara *et al.* 1987; Mihara, Ikesue *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.

However the existence of a range of toxins, with variable ability to induce symptoms on apple leaves, indicates the potential for a range of *Alternaria*'s to be involved in apple diseases.

*Genetic analysis*

Some of the genes responsible (*AMT* genes) for the production of AM toxins have been cloned (Johnson, Johnson *et al.* 2000a), allowing the development of genetic tests that specifically detect strains of *A. mali* that produce those AM toxins (Johnson, Johnson *et al.* 2000b). It was also shown that disruption of *AMT* genes, lead to a loss of pathogenicity in isolates of *A. mali* from Japan (Johnson, Johnson *et al.* 2000a).

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

Other forms of genetic analysis (looking at a broader range of genes) have shown pathogenic and non-pathogenic strains of *A. mali* to be very closely related (ByungRyun, HeeWan *et al.* 1998).

***Pathogen vs Saprophyte***

Is *Alternaria* behaving as a pathogen, or a saprophyte, or both, in Australian apple orchards? As it happens, this is also one of the most important questions facing current research into *Alternaria* leaf blotch, and particularly *Alternaria* fruit spot world wide. 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 *A. mali* isolates (infecting leaves) in several countries, including Japan (Saito, Niizeki *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 *A. 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 models***

A disease prediction model was developed for *Alternaria* leaf blotch symptoms in North Carolina (Filajdic and Sutton 1992b). Similar in style to those developed for other fungal plant pathogen prediction systems, i.e. Apple scab (Black spot) warning services, the model uses environmental factors such as temperature, rainfall, leaf wetness periods and existing levels of leaf infection. As tested by the authors the system was promisingly successful, predicting six false positives (infection periods) but no false negatives (i.e. no infection periods were missed). The use of this system in Australia is being evaluated.

***Resistance***

Resistance to *A. mali* has been recorded in a number of wild Malus species including *Malus asiatica*, *M. baccata* and *M. 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). *A. 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, Nakazawa *et al.* 2001; Tabira, Shimonaka *et al.* 1998). At this time, no new *A. 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).

*Resistance of commercial varieties to Alternaria leaf blotch*

It is interesting to note that in Japan, Gala is quoted as being a variety resistant to leaf blotch (Miyashita, Nakamori et al. 2003) (Yoshioka, Ito *et al.* 2000), while in Australia we have found 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 *A. 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, Yiem *et al.* 1982) | Good |
| Chlorothalonil | Korea | (Kim, Yiem *et al.* 1982) | Good |
| Polyoxin B | Korea | (Kim, Yiem *et al.* 1982) | Good |
| Carbendazim | India | (Sharma and Sharma 1991) | Good |
| Mancozeb | Italy, China | (Ciferri 1953; CunHao, FengZhi *et al.* 2001) | Average |
| Mancozeb | China | (RuiDe, ShiJin *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, DongHyuck *et al.* 1995; RuiDe, ShiJin *et al.* 1997) | Poor |

* Although identified as *A. 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 *A. 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 *A. mali* in artificial inoculation tests (Yoon, Lee et al. 1989).

### *Managing* Alternaria mali *using antibiotics*

A number of antibiotics have been demonstrated to reduce the growth and development of *A. mali* under laboratory conditions (Cheng, Kihara *et al.* 1989; Tomiya, Uramoto *et al.* 1990; Uramoto, Itoh *et al.* 1988), and in the field in China (JinYou, MeiNa *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, Zheng *et al.* 1993; XueChi, ZhiNong *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 *A. mali* disease symptoms.

## *Alternaria* fruit spot (caused by *Alternaria mali*)

### *Disease description*

In contrast to leaf blotch, there is very little information available about fruit symptoms produced by *A. mali*; with no detailed studies of its infection cycle on fruit. Once again 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 appears on apple fruit infected in the summer (Sawamura 1990).

### *Resistance*

In studies in 1975-1976 involving 50 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 *A. mali* was not correlated with the resistance of the fruit (Saito, Takeda *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 areas 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, and their presence does not conclusively prove they were the initial cause of the problem.

## 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* based on observation of leaf lesions alone is not advisable or conclusive.

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

*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 not uncommon in some areas (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 post-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 grow significantly in size 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*.

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

**Figure 3: *Alternaria* fruit spot on Royal Gala fruit.**

## 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 Orange (New South Wales) (Figure 4), with occasional, anecdotal reports from South Australia, Victoria and Western Australia.

The survey results presented in this report 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. Be alert, but not alarmed.

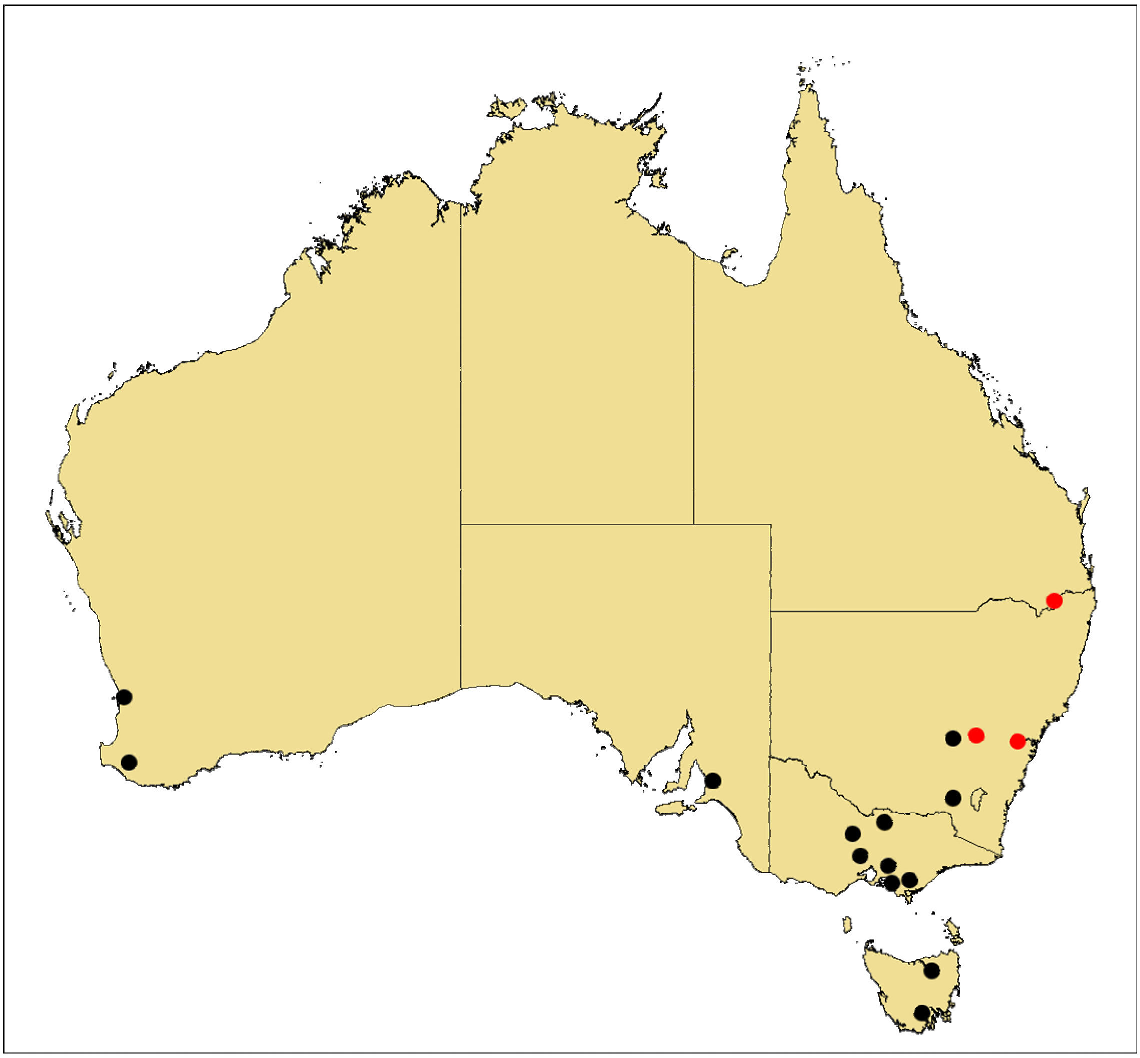

**Figure 4: Map of Major Australian apple production areas, showing areas currently affected by production limiting levels of *Alternaria* fruit spot in red.**

Finally, even if *Alternaria* 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.

# Background to Experimental work

It is important to remember that when this project began, *Alternaria*, although a serious problem for the growers involved, did not affect a large proportion of the Australian industry, and many of the decisions made about experimental work were made with this fact in mind. For instance, the decision to only trial chemicals already registered for use on apples, was made with the view at the time, that no chemical companies would pursue registration for such a small market (i.e. Granite Belt and Sydney Basin growers).

## *Survey of Australian apple production areas*

During the course of the project, it became clear that *Alternaria* symptoms were being observed on a broader number of apple varieties and distributed over a larger number of apple orchards both within Queensland and New South Wales, as well as overseas (mainly Europe and Asia). In order to determine the magnitude of the problem, an Australia-wide survey of Australian apple orchards was undertaken. Due to the wide range of apple varieties grown in Australia, surveyors concentrated their attention on the four varieties exhibiting the most severe symptoms in Queensland and New South Wales at the time, namely Gala, Fuji, Pink Lady and Red Delicious.

## *Chemical management of Alternaria leaf blotch and fruit spot*

Field trials testing chemicals registered for use on apples (usually for apple scab/black spot) were carried out in the Granite Belt, Queensland over two seasons (02/03 and 04/05) under the direction of Shane Dullahide and Christine Horlock. Similar trials were undertaken by Dr Shane Hetherington, in the Berambing (02/03) and Thirlmere (01/02) districts of New South Wales.

In the interests of brevity, only brief descriptions of this trial work are provided in the main text of this report. Full experimental details, including spray timetables and chemical rates, are provided in Appendix A: Queensland Field Trial, Appendix B: New South Wales Field Trial Picton 01/02 and Appendix C: New South Wales Field Trial Berambing 02/03.

### Queensland Field Trials

Preliminary trial work was undertaken in the Granite Belt over two seasons (2001/02 and 2002/03). A larger more detailed trial was undertaken in 2004/05. Prior to the start of this project in July 2002, a small field trial was undertaken on a commercial Royal Gala (the predominant variety affected at the time) orchard, looking at several fungicides currently registered for use against apple scab/black spot. This trial showed Delan® (dithianon) a group Y fungicide, to be more effective in reducing *Alternaria* leaf symptoms, than Bogard® (difenoconazole), Spin® (carbendazim), and Vision® (fluquinoconazole + pyrimethanil). In 2002/03 two field trials repeated this work, with one trial focusing on fungicides, and the other trial on the number and timing of fungicide applications.

A larger more extensive trial comparing calendar and post-rainfall spray programs was undertaken in the same orchard as the previous trials in 2004/05. For the first time, this trial also looked at the application of chemicals up until two weeks prior to harvest (mid-January), and specifically rated fruit symptoms as well as leaf symptoms.

## New South Wales Field Trials

### *Picton, 2001/02*

*Alternaria* leaf spot has been a significant disease of coastal apples since its first appearance five to six (and possibly 10) years ago. The areas affected in New South Wales are the Camden/Picton region and the Berambing/Bilpin region, which can be broadly described as belonging to the Sydney basin.

In 2001 a fungicide trial was established at Thirlmere in collaboration with representatives of the fungicide companies Aventis and Syngenta and under the supervision of NSW Agriculture (now NSW DPI) District Horticulturist, Lawrence Ullio. This orchard had a relatively long history of *Alternaria* leaf spot epidemics and had been severely infected during the previous two seasons (1999/2000 and 2000/01)

### *Berambing, 2002/03*

In 2002 a fungicide trial was established at Berambing in the Bilpin region to further test successful chemicals from the previous trial in early and late block (three applications per block) applications. This orchard had a relatively long history of *Alternaria* leaf blotch epidemics and had been severely affected during the previous two seasons (2000/01 and 2001/02).

# Materials and Methods

## *Survey of Australian apple production areas*

The distribution and importance of *Alternaria* leaf blotch and fruit spot diseases in Australian orchards was assessed by an Australia-wide survey of apple production areas. Contact with local plant pathologists and industry representatives, combined with direct contact with growers, assisted in assessing the level of grower concern and actual production losses directly attributable to *Alternaria*-like symptoms.

The surveys were undertaken in April and May 2005, at the very end of the 04/05 season, to maximise the chances of finding symptoms. All major apple production areas in New South Wales, South Australia, Tasmania, Victoria and Western Australia were surveyed. Surveys were undertaken in Queensland over the course of the 03/04, 04/5 and 05/06 seasons, with many isolates obtained from DPI&F trial sites throughout the Granite Belt.

Fruit and leaf material displaying any symptoms that could possibly be *Alternaria*-like were sampled; initial isolations were made in the state of origin with minimal transfer of leaf material interstate. Pure cultures were sent directly to the DPI&F Plant Pathology Herbarium (BRIP) Indooroopilly, Brisbane for preliminary identification and long term storage. Western Australian cultures have also been lodged in the Department of Agriculture Western Australia Plant Pathogen Collection (WAC). A selection of isolates has been sent to Dr Michael Priest (Mycologist, DPI NSW) for identification to species level.

## *Rating major commercial apple varieties in Australia for susceptibility*

In attempting to determine the susceptibility of commercially grown apple varieties in Australia, a two pronged approach was taken. Firstly to note the varieties naturally infected by *Alternaria* symptoms in each of the major growing areas, and secondly to note the apple varieties infected in the Apple and Pear Germplasm Repository at Applethorpe Research Station, Queensland.

Initially we proposed inoculating varieties with isolates of *Alternaria*, but once it became clear that there was potentially more than one species of *Alternaria* involved this idea was postponed. It was felt to be of greater importance to try and determine how many *Alternaria* species were involved first, and then determine their individual host range later. Also as *Alternaria* species are very good secondary invaders, effectively colonising any wounds on plant tissues, it was felt that artificial inoculation procedures might skew infectivity studies; resulting in false positives. Therefore it was decided to observe natural infections as much as possible.

### Australian apple orchard survey

During the course of the *Alternaria* survey of Australian apple orchards, apple varieties affected by *Alternaria* leaf and fruit symptoms were noted. Also noted were varieties near affected trees with no symptoms.

*Alternaria* leaf symptoms can look quite similar to symptoms of other secondary infecting plant pathogens and to physical damage. Hence, observation of *Alternaria*-

like symptoms is not sufficient to determine the cause of the problem. Also as a range of *Alternaria's* can cause similar symptoms, and *Alternaria* can be both pathogenic and saprophytic on apple tissues, it is important to isolate the pathogen from the symptom, have the pathogen identified and perform pathogenicity testing in order to be really sure that *Alternaria* is the initial cause of the problem.

### Applethorpe Apple and Pear Germplasm Repository survey

The survey conducted at the Applethorpe Apple and Pear Germplasm Repository involved observing all scion varieties for leaf symptoms in mid-December 2005, and leaf and fruit symptoms in early February 2006. Symptomatic leaves and fruit of apple varieties were sampled to enable isolation, and *Alternaria* isolates stored at BRIP for future examination.

## *Chemical management of Alternaria leaf blotch and fruit spot*

### Queensland Field Trials

#### *Granite Belt, 2002/03*

Two preliminary chemical spray trials (Trial One: Active Ingredients and Trial Two: Active ingredient and spray timing) were undertaken in a commercial orchard of eight year old Royal Gala trees, which had been severely affected by *Alternaria* leaf blotch and fruit spot the previous season. Trial One evaluated the ability of each chemical to manage *Alternaria*, without the complicating factors of the number of applications applied or their timing; while Trial Two was designed to see if the number or timing of applications contributed to the success of the chemical in reducing *Alternaria* symptoms.

The trial consisted of two adjacent blocks of trees with 75 m long rows of 33 trees, running ca. north to south. Treatment trees were selected on the basis of similar levels of size and vigour, with buffer (untreated) trees in between each treated tree. Treatments were randomised over the two rows, with five replications used for Trial One and four replications for Trial Two.

Treatments were applied on a calendar basis approximately seven to 14 days apart, from late September until the end of November 2002, to cover the period from pink bud to mid-season. Products trialled included: Bogard® (difenoconazole), Chorus® (cyprodinil), Delan® (diathianon); Nustar® (flusilazole), Spin® (carbendazim) Stroby® (kresoxim-methyl) and Vision® (fluquinconazole + pyrimethanil).

#### *Granite Belt, 2004-2005.*

This trial was undertaken as a joint activity with Serve-Ag Stanthorpe over the 2004/05 season, on the same stand of mature Royal Gala trees used previously on 2002/03. The spray schedule was devised by Shane Dullahide (DPI&F) and Stephen Tancred (Serve-Ag Stanthorpe); treatments were applied by Serve-Ag and ratings made by DPI&F, Applethorpe staff. Full details are provided in Appendix A.

Given the previous success of Delan, and the poor results from the other chemicals, it was decided to trial Delan at two rates. This was achieved by using a lower chemical concentration (dilute) than the standard label rate, at two water volumes (standard and low), resulting in trees being effectively treated with either the label rate (standard volume) or half the label rate (low volume) of chemical.

In this trial, chemical application was continued right up until two weeks prior to fruit harvest, six-eight weeks longer than the previous trials. Chemical applications resumed after the final fruit harvest, in an attempt to further reduce premature defoliation.

*Treatments*

Delan®, Polyram®, Ziram Granuflo® (ziram) and four experimental (unregistered on apple) chemicals were used in either a calendar based, or post-rainfall spray program. Delan® was also applied at the standard volume and at a low volume rate, explained above. Applications started at the end of October 2004, and finished two weeks prior to harvest in mid-January 2005.

After harvest, three further calendar and two further post-rainfall sprays were made to evaluate the abilities of these chemicals to reduce defoliation after fruit harvest.

*Assessment of disease*

Leaf infection assessments

Leaf assessments were made approximately every 10 days after the onset of symptoms, on the 21 December 2004. The number of spots per leaf, and the number of spotted leaves were counted for the 20 terminal leaves on 40 branches (20 on the north and 20 on the south side) of each tree.

Leaf defoliation assessments

Leaf infection and defoliation assessments were made at roughly monthly intervals after fruit harvest. The first after harvest assessment on the 21 February 2005 consisted of the usual leaf spot and infected leaf counts used prior to fruit harvest. Due to the level of defoliation demonstrated by the trees the two subsequent ratings were made by subjectively assessing the amount of fallen leaves under the trees (16 March 2006) and the amount of leaves left in the tree canopy (21 April 2006).

Fruit assessments

Fruit were harvested randomly (independent of maturity) on the 31 January 2005, the first harvest of the crop. Fruit were assessed by counting the number of spots per fruit, and the number of spotted fruit, from 50 fruit (25 from the north and 25 from the south side) of each tree. All disease lesions, insect and physical damage were marked onto the 25 ripest fruit from each tree, which were then cold stored at 2-4°C for six months before being rated again.

## New South Wales Field Trials

### *Picton, 2001/02*

Treatments were applied to blocks of mature Royal Gala trees, comprised of several consecutive rows, running ca. west to east. The trial was grower sprayed with a commercial air blast sprayer at the high volume (dilute) rate of 200 L/ha. Full details are provided in Appendix B.

*Treatments*

Applications of treatment chemicals were made at roughly 10 day intervals from late September to mid December 2001, exact details are provided in Appendix B. Chemicals trialed included Bogard®, Chorus®, Experimental D (dodine), Nustar®, Rovral® (iprodione), Stroby®, Thiram® (tetramethylthiuram disulfide) and Vision®.

*Assessment of disease*

Infection levels within the trees were assessed in two ways during the course of the trial. Namely, the number of infected leaves in the tree canopy (between 1.5-2.5 m above the orchard floor) and the weight of defoliated leaves (trialed as a quantitative measure of leaf loss). Randomly selected trees within the site were chosen for these measurements.

***Berambing, 2002/03***

Following on from the previous season's trial, a range of schedules was trialed focusing on the successful Vision®, Chorus® and Bogard® treatments from the 2001 trial. The trial was conducted in a commercial block of six year old Hi-early Delicious apples. Chemicals were applied by the grower using an air blast sprayer. Chemical applications occurred according to the schedule given in Appendix C.

The theory behind the design of treatments was to compare Vision® or Chorus® as "early" season sprays, with Vision® or Chorus® as "middle" season sprays, with Bogard® to finish. Experimental D and Nimrod® (bupirimate) sprays were also applied during this trial to control powdery mildew and apple scab, neither of these chemicals has been shown to have any effect on *Alternaria*. Dipel® (*Bacillus thuringensis* v. *kurstaki*) was also applied late in the season for insect control.

Disease assessments commenced when *Alternaria*-like symptoms were first noticed in the trial, 91 days after the application of the first fungicides.

***Leaf infection***: Trial design was, five replicates per treatment, where a replicate is one tree. For each replicate 100 leaves were assessed for the presence of disease around the circumference of the tree. Measurements were made at chest height. Leaves were counted from the outside surface, and within, the canopy. This measurement was repeated at >2 m of height. There were eight assessments carried out during the season

***Leaf fall***: Hessian bags supported by a circular wire frame were suspended under the canopies of 3 trees per treatment. The dry weight of apple leaves caught in these traps was measured.

# Results

## *Survey of Australian apple production areas*

A survey of apple orchards undertaken in April and May 2005, demonstrated the presence of *Alternaria*-like symptoms on apple leaves from all major apple growing regions in Australia. *Alternaria*-like symptoms were also observed on the fruit of some varieties in Queensland, New South Wales (Sydney Basin and Orange) and Victoria (Yarra Valley). *Alternaria* isolates were obtained from leaves and some fruit displaying *Alternaria*-like symptoms, from all of the states surveyed (Table 2).

**Table 2: Number of *Alternaria* isolates collected from each of the major apple production areas in Australia**

| **Production Area** | **State** | **Number of *Alternaria* isolates*** |
|---|---|---|
| Bilpin / Berambing | NSW | 40 |
| Central West NSW - Orange | NSW | 3 |
| South Western NSW - Batlow | NSW | 14 |
| Thirlmere / Lakesland | NSW | 14 |
| Granite Belt | QLD | 26 |
| Adelaide Hills | SA | 10 |
| Huon Valley | TAS | 4 |
| Spreyton | TAS | 8 |
| Tamar Valley | TAS | 12 |
| Goulburn Valley | VIC | 78 |
| Melbourne suburbs | VIC | 45 |
| Yarra Valley / Dandenongs | VIC | 21 |
| Donnybrook | WA | 11 |
| Manjimup | WA | 33 |
| Perth Hills | WA | 31 |

* As these isolates have not been identified to species level, it is not clear how many of these isolates are the same species of *Alternaria*. It is anticipated that many of these isolates are the same species.

Due to the large number of isolates, only preliminary (genus level) identifications are available at the time of writing. It is hoped to report species level identification results in the continuing project "*Alternaria* fruit spot: New Directions" APO5002.

It is interesting to note that *Alternaria* has only been recorded as a significant problem (i.e. causing production limiting losses) on fruit and leaves in Queensland and New South Wales (Sydney Basin and Orange only). Reports of severe defoliation, possibly due to *Alternaria* leaf infection were reported in 1994 from Donnybrook and 2002 from Manjimup, Western Australia; along with occasional reports of *Alternaria*-like symptoms being observed in South Australia and Victoria. However, *Alternaria* fruit spot continues to be reported (by growers and researchers alike) to only be a production limiting problem in Queensland and some part of New South Wales.

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. 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, it does not mean that *Alternaria* was the initial cause of the problem.

*Alternaria* species can be very effective secondary invaders of wounded tissues, and their presence does not conclusively prove they were the initial cause.

Finally, even if *Alternaria* 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.

## *Rating major commercial apple varieties in Australia for susceptibility*

### Australian apple orchard survey

*Alternaria*-like symptoms were observed on leaves in all of the apple production areas surveyed; however, fruit symptoms were observed only in Queensland and New South Wales (Sydney Basin and Orange). A summary of the main varieties found to have *Alternaria*-like symptoms and from which *Alternaria* cultures were obtained is listed in Table 3.

**Table 3: *Alternaria* cultures isolated from *Alternaria*-like symptoms observed on apple leaves or fruit**

| **Variety** | **Number of orchards where *Alternaria* species were isolated / Number of orchards where that variety was surveyed*** | | | | | |
|---|---|---|---|---|---|---|
| | NSW | QLD | SA | TAS | VIC | WA |
| Red Delicious | 9/11 | 3/3 | 2/2 | 2/2 | 1/1 | |
| Fuji | 5/6 | 1/1 | 6/6 | 3/3 | 5/5 | |
| Gala | 7/7 | 5/5 | 11/11 | 5/5 | 3/3 | 2/2 |
| Golden Delicious | | 2/2 | 1/1 | 3/3 | | 1/1 |
| Granny Smith | 6/7 | | | | 2/2 | |
| Rootstock | 1/1 | | | | | 1/1 |
| Other | 2/2 | 1/1 | | | 2/2 | 1/1 |
| Pink Lady | 7/9 | | 3/3 | 2/2 | 5/5 | 8/8 |
| Sundowner | | | 3/3 | 3/3 | 2/2 | 1/1 |

* It should be noted that this table does not indicate the level of infection, only presence or absence of *Alternaria* in leaf lesions.

It is important to note that sometimes more than one type of *Alternaria* was isolated from an orchard or variety; and conversely that similar appearing isolates of *Alternaria* were sometimes obtained from different varieties, orchards or states. It should also be remembered that sampling was skewed towards the varieties of Fuji, Gala, Pink Lady and Red Delicious.

The question now is how many of these isolates are truly pathogenic? And how many of these isolates from different orchards or regions are effectively the same?

### Apple Germplasm Repository survey

Fungal cultures isolated from symptomatic apple leaf and fruit tissue, collected from the Applethorpe Apple and Pear Germplasm Repository, were identified to genus level (i.e.

*Alternaria* or not) and stored for future use. Varieties exhibiting leaf symptoms in December 2005, from which an *Alternaria* isolate was obtained, are listed in Table 4.

**Table 4: Applethorpe Apple and Pear Germplasm Repository apple varieties from which *Alternaria* cultures were isolated from *Alternaria*-like symptomatic leaves (December 2005 survey).**

| | | | |
|---|---|---|---|
| 21-75 | Delicious type | M7 VIC | Red Dougherty |
| 23-153 | Earlidel | Mayspur | Ruby Gem |
| 62-92 | Early Machintosh | Mill | Senator |
| 66-103 | Early Strawberry | MM105 | Shizuka |
| 71-16 (seedling) | Fuji Nagafu 6 | MM109 | Spartan |
| 86-1 | Fuji Nagafu 12 | MM25 | Spitzenberg |
| 96-82 | Geeveston fanny | M zumi Caleocarpa | Splendour |
| Abas | G3 NPK/SPY | Nebuta | Sumac seedling |
| Alice | Goodwin Sdg | Nedswitskiana | Summerdel |
| Bachetti 2 | Granny Smith (Sorbello) | NY 66305-103 | Takane |
| Caldicott | Jonared | NY 674 | Tasman Pride |
| Commercial Legal Telder | Jonathon A | NY 7428-12 | Trenordan Red |
| Condo Pippin | Kogetsu | NY 74828-12 | Tsugaru |
| Crew's Delicious | Lalla | Priam | Winter Banana |
| Crimson Crofton | Legana | Pritchard | Yarlington Mill |
| Crofton | London Pippin | PSER 11 T27 | |
| Dearman's Red Delicious | M 429 | Red Braeburn | |

## *Chemical management of Alternaria leaf blotch and fruit spot*

### Queensland Field Trials

#### *Preliminary trials 2002/03*

The two trials performed in 2002/03 demonstrated the group Y fungicide, Delan® to continue to have the most potential for *Alternaria* disease management, with a fortnightly spray schedule starting at petal fall being the most effective at reducing leaf symptoms.

*Trial 1: Chemical active ingredient trial*

Delan® was the most effective at reducing *Alternaria* symptoms, resulting in both the lowest number of spots per leaf, as well as the lowest number of leaves with spots; when assessed in mid-February and at the end of March.

*Trial 2: 2002 Chemicals and spray timings trial*

In this trial, Delan® applied eight times (in a 6-10 day schedule from mid-October to the end of November) during the early part of the season was the most effective. As the other chemicals in this trial were only applied on four or five occasions during the same period, the question now becomes is it the chemical or the consistent application which is providing the protection?

***Granite Belt, 2004/05***

Once again Delan®, along with Polyram® and Experimental A were the most effective at reducing *Alternaria*-like leaf blotch symptoms, as well as fruit spot. Infection levels had remained high in the block since the previous trial (2002/03), with good rainfall during the intervening summer providing excellent conditions for disease development and the crisp winter conditions leading to good carryover of inoculum on leaf litter.

There was no significant difference between fungicide treatments applied in a post-rainfall or calendar manner, so in this report only the post-rainfall application data will be reported for each fungicide used. There was also no significant difference between the high volume or low volume sprays of Delan®, so only the results from the low volume spray will be reported here. Similarly, there was no significant difference between the two concentrations of Experimental D (100 ml or 120 ml/100 L) so data is presented only for the post-rainfall application of the lower concentration (100 ml/100 L).

The decision to present the data in this manner was made on the basis that a post-rainfall spray regime (which usually results in fewer applications), reduced spray volumes, and the use of the lowest effective concentration of active ingredient are the cornerstones of integrated pest management principles. Full details of all treatments are in Appendix A.

*Weather*

The weather for the 2004/05 season in the Granite Belt was unusual compared to long term average rainfall records; with a reasonably dry October and November, good rain in December, and dry rest of the summer, with very little rain in January and February 2005.

The relatively low January/February rainfall is interesting as most of the literature, and growers anecdotal experience, indicates that rain is required for successful infection and expression of *Alternaria* disease symptoms. However, leaf wetness data recorded for the same period shows high levels of overnight leaf wetness (dew) and relative humidity within the tree canopy throughout the season from September 2004 until March 2005 (Table 5). Was this increased moisture sufficient to sustain an infection (or symptom initiation) period started by heavy rain in early December (3 weeks prior to the first observation of symptoms)?

**Table 5: Rainfall, leaf wetness and relative humidity readings for the Granite Belt 2004/05.**

| Month | Total rainfall (mm) | Average number of hours per day over 95% leaf wetness | Average maximum leaf wetness (%) | Average number of hours per day over 99% relative humidity | Average maximum overnight relative humidity (%) |
|---|---|---|---|---|---|
| September | 3.0 | 2.7 | 80.4 | 8.2 | 99.7 |
| October | 55.4 | 4.2 | 77.9 | 7.4 | 98.2 |
| November | 87.0 | 4.8 | 94.6 | 8.8 | 99.8 |
| December | 127.4 | 5.3 | 93.3 | 9.7 | 99.3 |
| January | 43.8 | 3.7 | 88.5 | 8.7 | 99.9 |
| February | 55.6 | 4.4 | 90.2 | 9.0 | 99.4 |
| March | 27.0 | 4.7 | 95.3 | 10.2 | 100.0 |
| Whole season | 399.2 | 4.3 | 88.68 | 8.9 | 99.4 |

*Alternaria leaf blotch*

Leaf assessments were performed every 10 days from the first observation of symptoms in late December 2004, until the first fruit harvest on the 31 January 2005. Three leaf ratings were made after fruit harvest in late February and March of 2005, to assess premature defoliation.

Number of blotches per leaf

This form of rating was not particularly useful in this trial for determining differences between the efficacies of the different chemicals, with only one rating (leaf assessment #5, 10 February 2005) demonstrating any significant differences between the treatments. It is worth noting, however, that this rating demonstrated Delan®, Polyram® and Experimental A treated trees to have significantly reduced blotch numbers. Data provided in Appendix A.

Number of infected leaves

In comparison, Delan® and Polyram® treatments were significantly different from the untreated controls by the third rating (20 January 2005); with these two chemicals remaining consistently more effective at reducing *Alternaria*-like leaf symptoms for the remainder of the trial (Figure 5).

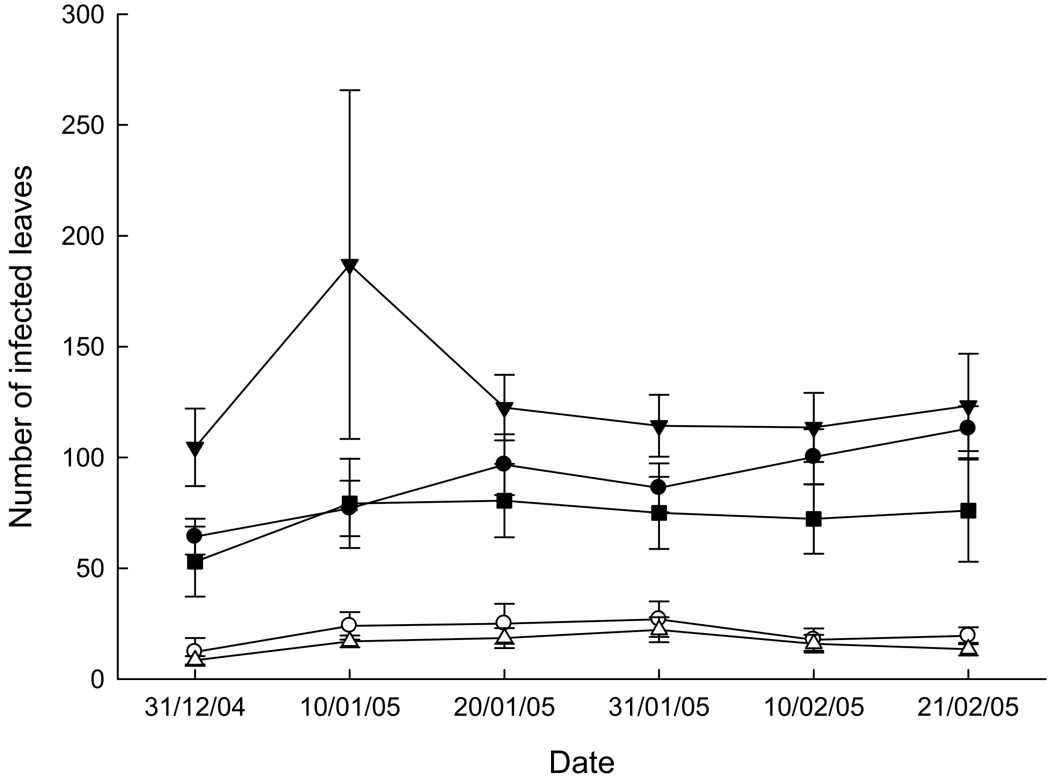


**Figure 5: Granite Belt 04/05 trial – Number of infected leaves.**

Changes in the number of infected leaves for untreated apple trees (●) and those treated with the following fungicides; Delan (○); Experimental D (▼); Polyram (△) and Ziram (■). Data presented are means ± s.e. (*n*=4).

The after harvest infected leaf assessments continued the previous trends, with the total number of leaf spot counts continuing to show no differences between any of the treatments; and the number of infected leaf counts demonstrating Polyram®, Delan® and Experimental A to be the most effective at reducing the total number of infected leaves (Appendix A).

*Late season defoliation assessments*

The first subjective leaf fall assessment demonstrated no significant difference between any of the assessments, except for Delan®, which was slightly better than the untreated control. The second (and final) defoliation assessment undertaken in April, showed Polyram® was the most effective chemical at reducing premature defoliation, with Polyram® treated trees retaining the most leaves at the end of the trial (Figure 2). It should also be noted that the use of any of the fungicides trialed, except Experimental D 100 and Experimental D 120 (calendar), significantly reduced premature defoliation when compared to the untreated control (Appendix A).

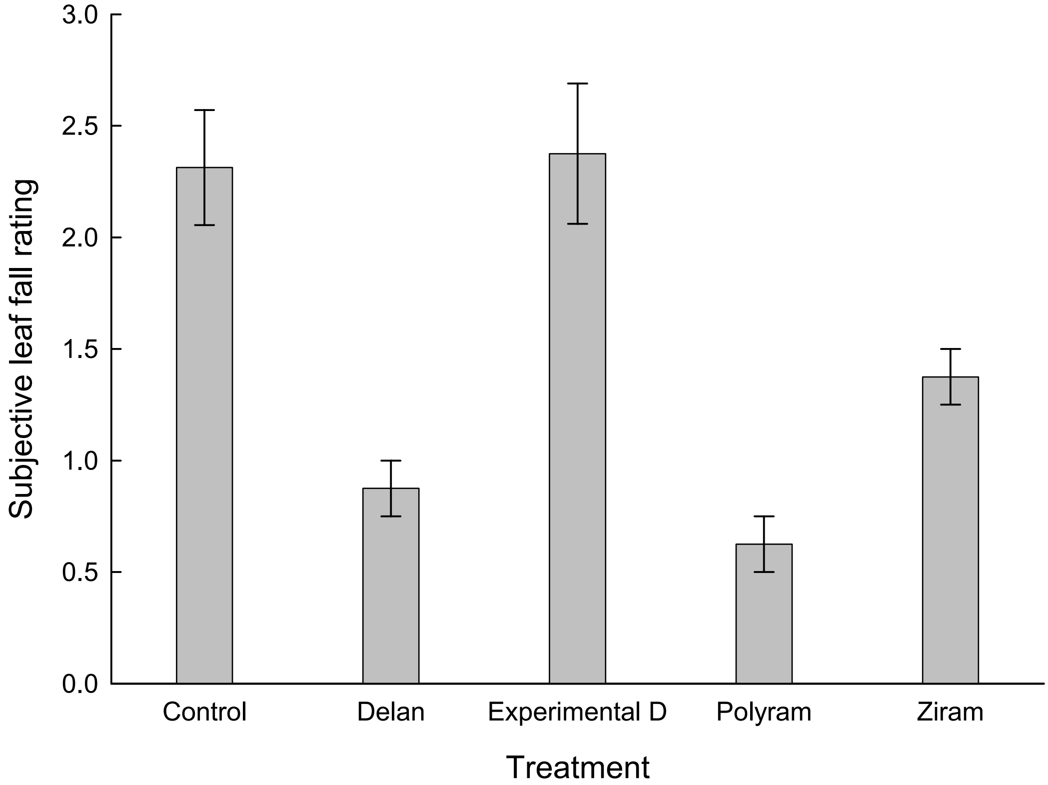


**Figure 6: Granite Belt 04/05 – Subjective rating of leaf fall, 11 weeks after fruit harvest**
Subjective rating of leaf fall, measured 11 weeks after fruit harvest for untreated apple trees and those treated with the following fungicides; Delan, Experimental D, Polyram and Ziram. Data presented are means ± s.e. (*n*=8).

*Alternaria fruit spot*

Fruit from the trial was rated on two occasions, at the time of harvest (2-4 February 2005), and six months later after storage at 4$^{o}$C. For the first, harvest rating, 50 fruit were randomly picked from each tree irrespective of maturity, and rated for symptoms of insect, disease and physical damage. The 25 most mature fruit from each tree were stored at 4$^{o}$C; after all physical symptoms were marked on the fruit and recorded.

The rating performed immediately after harvest showed that there were significant differences between the treatments with regard to the total number of leaf spots, and the number of infected fruit. After storage, however, when symptoms that developed during storage were rated, there were no significant differences between any of the treatments.

Measurement of the number of spotted fruit was considered to be the more meaningful of the two assessments below, as current market practise is to reject fruit with a single *Alternaria* spot. Full details and data are provided in Appendix A.

<u>Number of spots per treatment</u>

At harvest, Polyram® (calendar and post-rainfall), Delan® (post-rainfall), Experimental A and Experimental C significantly reduced the total number of spots produced per treatment (Appendix A). Interestingly, there were two treatments, Experimental D 120 (calendar) and Experimental D 100 (post-rainfall), which produced significantly more symptoms than the untreated control. It is unclear why this occurred, but an increased level of fruit injury could be a possibility.

Number of spotted fruit

Polyram®, Delan®, Experimental A and Experimental C were the most successful treatments at reducing the number of affected fruit, with Experimental B (post-rainfall), Ziram® (calendar) and Experimental D 120 (post-rainfall) also showing some positive effects (Figure 7). Once again there were two treatments that produced significantly more symptoms than the untreated control, namely Experimental D 100 (post-rainfall) and Experimental D 120 (calendar). The reason for this result is similarly unclear.

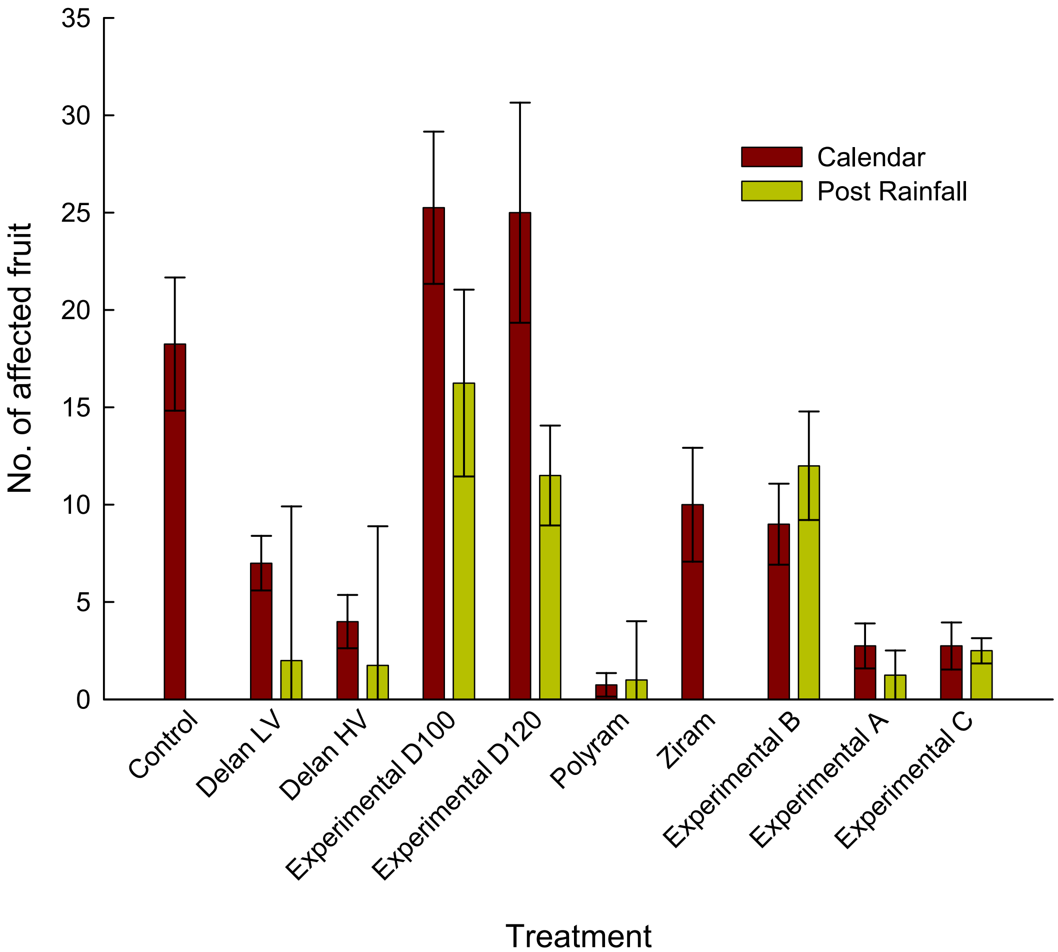


**Figure 7: Granite Belt 04/05– Number of *Alternaria* affected fruit at harvest**

Number of affected fruit for untreated apple trees and those treated with the following fungicides; Delan LV, Delan HV, Experimental D100, Experimental D120, Polyram, Ziram, Experimental A, B and C. Data presented are means ± s.e. (n=4).

## New South Wales Field Trials

### *Picton, 2001/02*

*Weather*

The weather in 2001/02 was unusual, compared to long term averages. Spring was very dry. In early February the orchard received approximately 400 mm of rain over a three week period. The orchard manager first observed *Alternaria* leaf symptoms on the control block on February 3rd 2002.

*Assessment of leaf infection*

Leaf infection assessments for this trial were carried out on the 15 February 2002 (Figure 8).

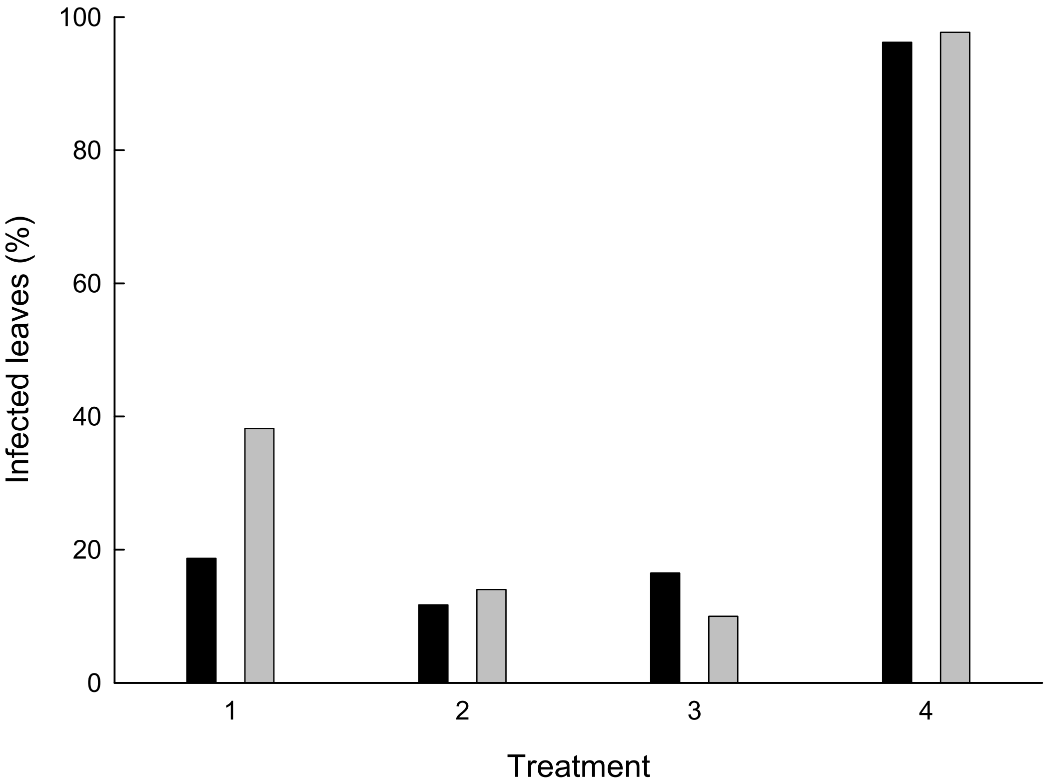


**Figure 8: Picton 01/02 – Number of infected leaves (%)**

| Treatment | Brief description |
|---|---|
| 1 | Chorus®, Chorus®, Chorus®, Bogard®, Bogard®, Bogard® |
| 2 | Vision®, Vision®, Vision®, Vision®, Vision®, Bogard® |
| 3 | Nustar®, Syllit®/Rovral®, Syllit®/Rovral®, Nustar®, Stroby®, Bogard® |
| 4 | Syllit®, Syllit®/Thiram®, Syllit®, Nustar®, Stroby®, Bogard® |

This trial shows the relative effectiveness of early (relative to the onset of symptoms) applications of Vision® and Chorus® in early spring. However it is hard to draw any comparisons with the Queensland field work as the final application of product occurred almost two months prior to the final rating (mid-December), whereas Queensland treatments were continued through the fruit maturation period to harvest (at the end of January).

***Berambing, 2002/03***

This block had been infected in previous seasons and as a result early defoliation had occurred. Despite high levels of infection during this season – up to 90% for individual trees – very little premature defoliation occurred.

*Weather*

Drought affected Bilpin through the 2003/04 season. Irrigation to the trees was limited, particularly during December 2002 and January 2003. Bilpin was also hit by a hail storm in February which caused a lot of leaf and fruit damage, creating many potential entry points for *Alternaria* infection.

*Assessment of leaf infection*

Eight assessments were undertaken but the last two (May 1 and May 15) were affected by natural leaf fall. As leaves senesced and fell from the trees, infected leaves dropped first. This led to a lower value/level of leaf infection recorded on trees. Consequently data for the final two readings are not presented in Figure 9.

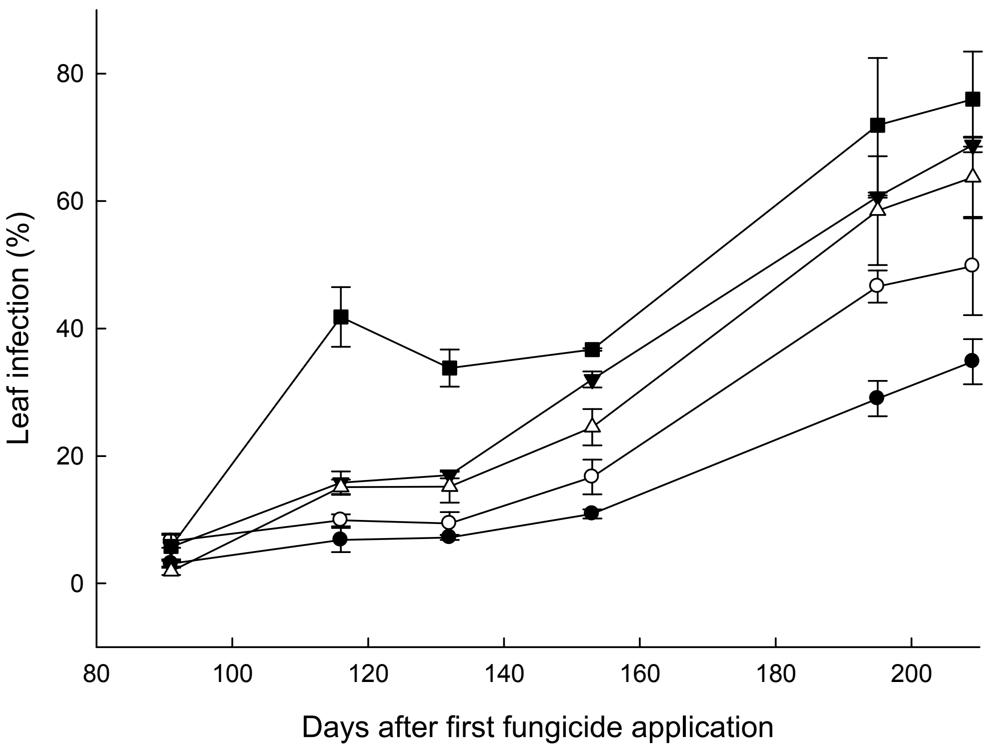


**Figure 9: Berambing 02/03 – Leaf infection (%) observed during spray trial**

Changes in leaf infection (%) for untreated apple trees (■) and those treated with the following fungicides; Vision Early (●); Vision Late (○); Chorus Early (▼) and Chorus Late (△), recorded 91, 116, 132, 153, 195 and 209 days after the first fungicide application. Data presented are means ± s.e. ($n$=5).

The lowest level of leaf infection was recorded where the trees had been given three consecutive applications of Vision® as the first sprays of the season. This treatment reduced leaf infection when compared to the control treatment throughout the season. It also provided significantly ($p<0.05$) better control of leaf infection than Chorus® for the majority of assessment times. Early application also gave better control of leaf infection, particularly later in the season.

*Assessment of leaf fall*

Six assessments were carried out (153, 195, 209, 223, 237 and 258 days after first fungicide application), to assess the level of defoliation experienced by the trial trees (Figure 10).

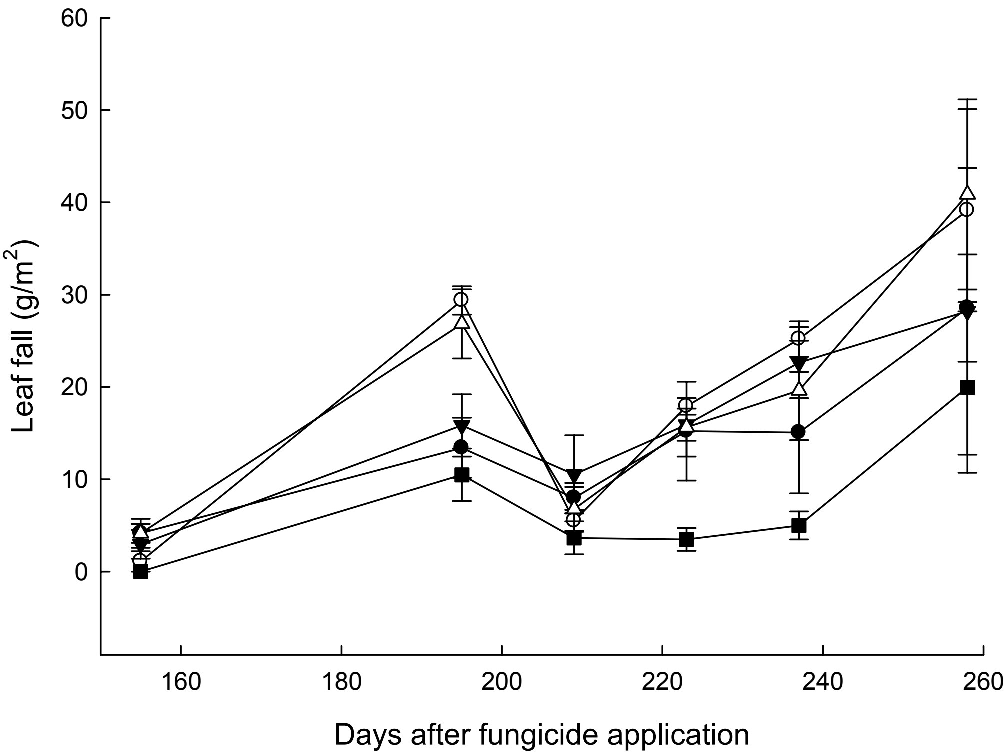


**Figure 10: Berambing 02/03 – Leaf fall levels (g/m²) observed over the course of the trial**

Changes in leaf fall (g/m²) for untreated apple trees (■) and those treated with the following fungicides; Vision Early (●); Vision Late (○); Chorus Early (▼) and Chorus Late (△), recorded 155, 195, 209, 223, 237 and 258 days after fungicide application. Data presented are means ± s.e. ($n$=3).

Despite consistently carrying the heaviest leaf infection, the control treatment suffered the lowest leaf loss. The reason for this result remains unclear.

In this trial infected leaves tended to be retained by the trees; with early application of Chorus® or Vision® resulting in significantly lower rates of defoliation than later applications.

# Discussion

## *Survey of Australian apple production areas and rating of major apple varieties in Australia for susceptibility*

The April/May 2005 survey showed *Alternaria* to be a fungus commonly found on the leaves and fruit of apples in Australian orchards. As the specific identity and pathogenic nature of many of the isolates collected is yet to be determined it is difficult to draw too many conclusions from this work.

The survey of Australian apple orchards and rating of apple scion varieties from the Applethorpe Apple and Pear Germplasm Repository, have demonstrated the ability of *Alternaria* species to infect a wide range of commercial apple varieties in Australia. Once again as the specific identity and pathogenic nature of many of these species is yet to be determined, the significance of these results remains unclear.

However, just because *Alternaria* species were widespread, and easily found, in all of the orchards surveyed; this does not mean that *Alternaria* leaf blotch and fruit spot are causing production limiting levels of disease in these areas. The presence of *Alternaria* alone is not sufficient reason for concern in areas where production limiting levels of *Alternaria* symptoms have not been found.

## *Fungicide trials and development of integrated Alternaria management*

Interestingly, although initially using similar chemicals, trials in Queensland and New South Wales ultimately determined two different groups of chemicals to be most effective against *Alternaria* leaf blotch and fruit spot. Initially designed to be used as part of a regular apple scab/black spot (caused by *Venturia inaequalis*) spray program, both of these sets of trials were undertaken using chemicals currently permitted for use against apple scab/black spot in Queensland and New South Wales, and focused on relatively early season applications (when apple scab sprays are typically applied).

Preliminary fungicide trials in the Granite Belt (Queensland) production area over the 2000/01, 2001/02 and 2002/03 seasons, indicated that the broad spectrum Group Y chemicals (in particular Delan®) were the most successful at reducing *Alternaria* leaf blotch symptoms in Royal Gala apples.

Meanwhile similar trials undertaken in the Picton (2001/02) and Berambing (2002/03) districts of the Sydney Basin (New South Wales) showed the Group I fungicides (in particular Vision®) to be the most effective at reducing leaf symptoms.

***Granite Belt, 2004/05***

The final trial undertaken in the Granite Belt (2004/05), used chemicals previously shown to be effective in Queensland, over the whole season from October 2004 until two weeks prior to harvest at the end of January 2005.

***Leaf blotch***

Polyram®, Delan® and Experimental A were consistently (although at times only slightly) better than the other treatments at reducing the number of infected leaves during the later part of the season, prior to and just after fruit harvest. The significance of reduced leaf infection, and its relationship with premature defoliation, was not studied in detail in this trial, but it is a reasonable assumption to conclude that reduced leaf infection earlier in the season, would lead to reduced premature defoliation at the end.

The application of fungicides to trees after fruit harvest, was also shown to significantly reduce the level of premature defoliation. Polyram® was the most effective chemical at reducing premature defoliation, with Polyram® treated trees retaining the most leaves at the end of the trial. The previous trend was continued with Delan® and Experimental A being the next most effective treatments. It should also be noted that the use of any of the fungicides trialed, except Experimental D 100 and Experimental D 120 (calendar), significantly reduced premature defoliation when compared to the untreated control.

***Fruit spot***

Interestingly, the results from the fruit ratings were quite different to those of the leaf ratings; with Polyram®, Delan®, Experimental A and Experimental C significantly reducing the total number of spots produced per treatment. Even more interesting are the results from the number of spotted fruit assessment, which shows Polyram®, Delan®, Experimental A and Experimental C to be the most successful; but that Experimental B (post-rainfall), Ziram® (calendar) and Experimental D 120 (post-rainfall) were also effective. Once again there were treatments (Experimental D 100 post-rainfall and Experimental D 120 calendar) that produced significantly more fruit spots than the untreated trees. Why this occurred is not clear.

It is reassuring to see the same treatments having a similar trend on symptom expression in fruit as in leaves; but as this is only a single trial, it would be premature to draw too many conclusions from this work.

***Calendar versus post-rainfall treatments***

The majority of results from this showed no significant differences between the effectiveness of fungicides applied using a post-rainfall or calendar schedule. This is a very odd result. It simply does not seem logical that fungicides with very little systemic or curative capacity could work when applied in a curative (i.e. post-rainfall) manner. Possible explanations are that:

- The exact timing of fungicide application has a reduced importance, if the population of *Alternaria* fungi on the leaves and fruit is kept low through continued applications during the season.
- *Alternaria* is not the original cause of infection, and the fungicides are somehow preventing the initial infection/injury/problem, rather than directly reducing symptom development.
- Perhaps the infection process is much longer than suggested by the current literature; although this would seem unlikely.

Finally, with regard to the Granite Belt 2004/05 trial, as we found in the initial trials the fungicide remains the most important factor in symptom reduction.

So what does all of this mean? Current research indicates that the use of Polyram® or Delan® as part of a season long spray program will result in significant reductions of *Alternaria* leaf blotch and fruit spot in Queensland; and the use of Group I fungicides (in particular Vision®) during the first half of the season has been shown to be similarly effective in New South Wales.

The development of such different, yet effective, spray programs for each region is unusual. There are several possibilities which might explain these results. The most likely, however, is that there are different populations of *Alternaria* infecting the different regions, and the difference in climatic conditions between New South Wales and Queensland is also having an effect.

*Extras*

It is also worth remembering that just because a pathogen has been detected in an area, does not mean it is a problem. In our opinion finding the pathogen is only a problem if it is causing production limiting levels of disease.

# Technology Transfer

The research results generated during this project were communicated to a variety of different audiences, by a number of different methods including:

- Articles in Industry publications
    - Two articles were published about this project in the APAL Levy magazine. The first in 2005 "*Managing Alternaria blotch in apples"* and the second "Managing *Alternaria* infection of apple leaves and fruit" in 2006.
    - An article was published in the December / January 2005 / 2006 Edition of Tree Fruit magazine entitled "Managing late season *Alternaria* leaf blotch and fruit spot infections".
- Poster presentation at the 2004 APAL industry conference entitled "Management of *Alternaria* leaf and fruit spot in apples".
- Regular milestone reports, providing information on experimental results, were provided to HAL as part of this project.
- During the survey of Australian Apple orchards, undertaken throughout Australia in April and May 2005, contact was made with orchardists, industry representatives, plant pathologists and agriculture department extension officers from each of the apple growing states in Australia.
- Experimental results produced during the course of this project were used to obatin two Emergency permits, one for metiram and one for dithianon (details below), in Queensland and New South Wales for the late season management of *Alternaria* leaf blotch and fruit spot.
    - PER9060 - Polyram (metiram) / apples/ *Alternaria*<br>Valid 23/12/05 to 30/06/06<br>Emergency permit<br>Valid for NSW & Qld only
    - Emergency permit<br>PER9075 - Delan 700 WG (dithianon) / apples/ *Alternaria*<br>Valid 10/01/06 to 30/06/06<br>Valid for NSW & Qld only

## 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 last season.

Further supporting the need for more research into the causes of these symptoms is the fact that *Alternaria* leaf blotch and fruit spot disease appears to be significantly different from *A. mali* infections 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 the identity, to species level, and pathogenicity of isolates collected from the Applethorpe Apple Germplasm Repository.
3. To determine if different species of *Alternaria* are causing the same disease symptoms in commercial apple orchards throughout Australia.
4. To determine if the same species of *Alternaria* is responsible for *Alternaria* leaf blotch and *Alternaria* fruit spot.
5. To determine if *Alternaria* is a primary or secondary pathogen (or both) of apple leaves and fruit in Australian orchards.
6. To pinpoint any physical, chemical or genetic differences between pathogenic isolates of *Alternaria* from Australian orchards; and use these differences to develop a high through-put, accurate detection system.
7. To develop a program (or programs) for the effective management of *Alternaria* leaf blotch and fruit spot in Australia.
8. To compare isolates of Australian apple infecting *Alternaria*, with overseas isolates.
9. To determine the effect on symptoms of different isolates of *Alternaria* on a range of commercial apple varieties.
10. To determine if isolates of Alternaria obtained from Australian pear leaves are the same organism, or group of organisms, causing infection on Australian apple leaves.

# 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 area may not be as effective in another area
- 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 preharvest field management of *Alternaria* is strongly discouraged, for several significant reasons, including
    - Iprodione is not registered for preharvest use in Australia.
    - The risk of fungicide resistance developing in field and postharvest apple pathogen populations.
    - There is no scientific evidence to suggest that iprodione reduces *Alternaria* fruit spot on apple.

  See Appendix D for further details.

## *Alternaria management suggestions*

### General 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, and effectively managing insect pests, and other diseases.
- Maintain good tree nutrition, especially calcium, as bitter pit creates significant fruit surface wounds, which can subsequently become invaded by *Alternaria*.

### Queensland

- The inclusion of several applications of Polyram® or Delan® as a part of a regular apple scab/black spot spray program.
- If permits are approved, use Polyram® until three weeks prior to harvest and Delan® until seven days prior to harvest for the highly susceptible varieties Fuji, Gala, Pink Lady and Red Delicious.
- Remember that some of the treatments in the Queensland trials, Experimental D in particular, produced more symptoms than no treatment.

### New South Wales

- The inclusion of several applications of Vision® (or similar Group I fungicide) early in the season as a part of a regular apple scab/black spot spray program.

- If permits are approved, use Polyram® until three weeks prior to harvest and Delan® until seven days prior to harvest for the highly susceptible varieties Fuji, Gala, Pink Lady and Red Delicious.

## Other states

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

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## NSW Field Trial Collaborators

Field trial coordinator – Dr Shane Hetherington, NSW DPI.

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# Appendix A: Queensland Field Trial

Queensland Government
Department of Primary Industries and Fisheries

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## *Applethorpe Research Station, Plant Pathology Experiment Report*

**Alternaria leaf blotch and fruit spot fungicide trial**
**Granite Belt, Queensland 2004/05**

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## Introduction

Preliminary trial work was undertaken in the Granite Belt over two seasons (2001/02 and 2002/03). A larger more detailed trial was undertaken in 2004/05. Prior to the start of this project in July 2002, a small field trial was undertaken on a commercial Royal Gala (the predominant variety affected at the time) orchard, looking at several fungicides currently registered for use against apple scab/black spot. This trial showed Delan® (dithianon) a group Y fungicide, to be the more effective in reducing Alternaria leaf symptoms, than Bogard® (difenoconazole), Spin® (carbendazim), and Vision® (fluquinoconazole + pyrimethanil). In 2002/03 two field trials repeated this work, with one trial focusing on the difference between fungicides, while the other trial focused on the number and timing of fungicide applications.

A larger more intensive trial comparing calendar (preventative) and after rain (curative) spray programs was undertaken in the same orchard as the previous trials in 2004/05. For the first time, this trial also looked at the application of chemicals up until two weeks prior to harvest (mid-January), and specifically rated fruit symptoms as well as leaf symptoms.

## Materials and Methods

This trial was undertaken as a joint activity with Serve-Ag Stanthorpe over the 2004/05 season, on the same stand of mature Royal Gala trees used previously on 2002/03. The spray schedule was devised by Shane Dullahide (DPI&F) and Stephen Tancred (Serve-Ag Stanthorpe); treatments were applied by Serve-Ag and ratings made by DPI&F, Applethorpe staff.

Given the previous success of Delan, and the poor results from the other chemicals, it was decided to trial Delan at two rates. This was achieved by using a lower chemical concentration (dilute) than the standard label rate, at two water volumes (standard and low), resulting in trees being effectively treated with either the label rate (standard volume) or half the label rate (low volume) of chemical.

In this trial, chemical application was continued right up until two weeks prior to fruit harvest, six-eight weeks longer than the previous trials. Chemical applications resumed after the final fruit harvest, in an attempt to further reduce premature defoliation.

### *Treatments*

Delan®, Polyram®, Ziram Granuflo® (ziram) and four experimental unregistered (on apple) chemicals were used in either a calendar based "preventative" program, or a spray after rain "curative" program. Delan was also applied at the standard volume and at a low volume rate, explained above. Applications started at the end of October 2004, and finished two weeks prior to harvest in mid-January 2005.

After harvest, three further protectant and two further curative sprays were made to evaluate the abilities of these chemicals to reduce defoliation after fruit harvest.

**Table 1: List of chemical products used**

| Product Name | Active Ingredient (ai) | Concentration of Active Ingredient | Formulation | Batch Number |
|---|---|---|---|---|
| Delan 700 WG | dithianon | 700 g/kg | Water Dispersible Granule | 70-770 |
| Polyram 700 DF | metiram | 700 g/kg | Dry Flowable | 37-1290 |
| Ziram Granuflo 760 DF | ziram | 760 g/kg | Dry Flowable | G360027498 |
| Experimental A 300 SC | - | 300 g/L | Suspension Concentrate | - |
| Experimental B 300 SC | - | 300 g/L | Suspension Concentrate | - |
| Experimental C 400 SC | - | 400 g/L | Suspension Concentrate | - |
| Experimental D 500 WG | - | 500 g/kg | Water Dispersible Granule | - |

**Table 2: List of chemical treatments**

| No. | Treatment | Program | Rate | |
|---|---|---|---|---|
| | | | Product (g or ml/100 L) | Active Ingredient (g ai/100 L) |
| 1 | Untreated control | nil | nil | nil |
| 2 | Delan 700 WG @ 12.6 g ai/100 L | Calendar | 18 g | 12.6 |
| 4 | Delan 700 WG @ 12.6 g ai/100 L LV* | Calendar | 18 g | 12.6 |
| 6 | Polyram 700 DF @ 122.5 g ai/100 L | Calendar | 175 g | 122.5 |
| 8 | Ziram 760 DF @ 114 g ai/100 L | Calendar | 150 g | 114 |
| 9 | Experimental D 500 WG @ 5 g ai/100 L | Calendar | 10 g | 5 |
| 11 | Experimental A 300 SC @ 15 g ai/100 L | Calendar | 50 ml | 15 |
| 13 | Experimental B 300 SC @ 15 g ai/100 L | Calendar | 50 ml | 15 |
| 15 | Experimental C 400 SC @ 40 g ai/100 L | Calendar | 100 ml | 40 |
| 17 | Experimental C 400 SC @ 48 g ai/100 L | Calendar | 120 ml | 48 |
| 3 | Delan 700 WG @ 12.6 g ai/100 L | After rain | 18 g | 12.6 |
| 5 | Delan 700 WG @ 12.6 g ai/100 L LV* | After rain | 18 g | 12.6 |
| 7 | Polyram 700 DF @ 122.5 g ai/100 L | After rain | 175 g | 122.5 |
| 10 | Experimental D 500 WG @ 5 g ai/100 L | After rain | 10 g | 5 |
| 12 | Experimental A 300 SC @ 15 g ai/100 L | After rain | 50 ml | 15 |
| 14 | Experimental B 300 SC @ 15 g ai/100 L | After rain | 50 ml | 15 |
| 16 | Experimental C 400 SC @ 40 g ai/100 L | After rain | 100 ml | 40 |
| 18 | Experimental C 400 SC @ 48 g ai/100 L | After rain | 120 ml | 48 |

* LV = Low volume

**Table 3: Spray application and disease assessment schedule**

| Date | Calendar program | After rain program | Disease Assessment |
|---|---|---|---|
| 28/10/04 | ✓ | | |
| 08/11/04 | | ✓ | |
| 11/11/04 | ✓ | ✓ | |
| 23/11/04 | | ✓ | |
| 30/11/04 | ✓ | | |
| 06/12/04 | | ✓ | |
| 12/12/04 | ✓ | ✓ | |
| 20/12/04 | ✓ | | |
| 21/12/04 | | | Preliminary leaf rating |
| 28/12/04 | | ✓ | |
| 31/12/04 | | | First leaf assessment |
| 04/01/05 | ✓ | | |
| 10/01/05 | | | Second leaf assessment |
| 18/01/05 | ✓ | ✓ | |
| 20/01/05 | | | Third leaf assessment |
| 31/01/05 | | | Fourth leaf assessment and Harvest fruit assessment |
| 10/02/05 | | | Fifth leaf assessment |
| 21/02/05 | | | Sixth leaf assessment |
| 26/02/05 | ✓ | ✓ | |
| 11/03/05 | ✓ | | |
| 16/03/05 | | | Seventh leaf assessment |
| 22/03/05 | ✓ | ✓ | |
| 21/04/05 | | | Eighth leaf assessment |
| 11/07/05 | | | Stored fruit assessment |

***Assessment of disease***

*Leaf infection assessments*

The number of *Alternaria* spots per leaf, and the number of spotted leaves, was counted for the 20 terminal leaves on 40 branches (20 on the north and 20 on the south side) of each tree. On the summer growth branches, leaves are counted from the dark green fully mature leaves; which were 20-50 cm from the end of the branches. On the fruiting spurs the terminal five leaves are counted.

Full leaves were rated if possible, but half leaves (ie: where part of the leaf is missing due to insect or other damage) are used if necessary, with two half leaves equalling one whole leaf.

Before harvest

Leaf assessments were made approximately every 10 days after the onset of symptoms, on the 21 December 2004, through until fruit harvest on 31 January 2005.

After harvest

Three leaf assessments were performed after fruit harvest, with the sixth assessment made three weeks after harvest (21 February), and the two subsequent assessments performed after a further three weeks (16 March) and eight weeks (21 April). Leaf infection counts, as detailed above were performed for the 21 February and 16 March ratings, but not the final rating.

*Defoliation assessments*

Leaf defoliation assessments were made at roughly monthly intervals after fruit harvest. The after-harvest defoliation ratings occurred on the 16 March and 21 April 2005, and consisted of subjective ratings assessing the amount of fallen leaves under the trees (16 March 2006) and the amount of leaves left in the tree canopy (21 April 2006).

*Fruit assessments*

At harvest

Fruit were harvested randomly (independent of maturity) on the 31 January 2005, the first harvest of the crop. Fruit were assessed by counting the number of spots per fruit, and the number of spotted fruit, from 50 fruit (25 from the north and 25 from the south side) of each tree. All disease lesions, insect and physical damage were marked onto the 25 ripest fruit from each tree, which were then cold stored at 2-4$^{o}$C for six months before being rated again.

After six months cold storage

The 25 most mature fruit, as described above, were stored at 4$^{o}$C (in a cold room) in cardboard packing boxes with plastic liners and rated again after 6 months of storage.

## Results*

*Detailed data is only provided where there are significant differences between the treatments.

Delan®, along with Polyram® and Experimental A were the most effective chemicals at reducing Alternaria-like leaf blotch and fruit spot symptoms. Infection levels had remained high in the block since the previous trial (2002/03), with good rainfall during the intervening summer providing excellent conditions for disease development and the crisp winter conditions leading to good carryover of inoculum.

There was no significant difference between fungicide treatments applied in a post-rainfall or calendar manner. There was also no significant difference between the high volume or low volume sprays of Delan®, or between the two concentrations of Experimental D (100 ml or 120 ml / 100 L).

***Weather***

The weather for the 2004/05 season in the Granite Belt was unusual with a reasonably dry October and November, good rain in December, and a dry summer. There was very little rain in January and February 2005.

The relatively low January/February rainfall is interesting as most of the literature, and growers anecdotal experience, indicates that rain is required for successful infection and expression of Alternaria disease symptoms. However, leaf wetness data recorded for the same period shows high levels of overnight leaf wetness (dew) and relative humidity within the tree canopy throughout the season from September 2004 until March 2005 (Table 4). Was this increased moisture sufficient to sustain an infection, or symptom initiation, period started by heavy rain in early December (3 weeks prior to the observation of the first symptoms on the 21 December)?

**Table 4: Rainfall, leaf wetness and relative humidity readings - Granite Belt 2004/05.**

| Month | Total rainfall (mm) | Average number of hours per day over 95% leaf wetness | Average maximum leaf wetness (%) | Average number of hours per day over 99% relative humidity | Average maximum overnight relative humidity (%) |
|---|---|---|---|---|---|
| September | 3.0 | 2.7 | 80.4 | 8.2 | 99.7 |
| October | 55.4 | 4.2 | 77.9 | 7.4 | 98.2 |
| November | 87.0 | 4.8 | 94.6 | 8.8 | 99.8 |
| December | 127.4 | 5.3 | 93.3 | 9.7 | 99.3 |
| January | 43.8 | 3.7 | 88.5 | 8.7 | 99.9 |
| February | 55.6 | 4.4 | 90.2 | 9.0 | 99.4 |
| March | 27.0 | 4.7 | 95.3 | 10.2 | 100.0 |
| Whole season | 399.2 | 4.3 | 88.68 | 8.9 | 99.4 |

***Leaf infection assessments***

Leaf assessments were performed every 10 days from the first observation of symptoms in late December 2004, until the first fruit harvest on the 31 January 2005. Three leaf ratings were made after fruit harvest in late February and March of 2005, to assess premature defoliation.

*Number of spots per leaf*

This form of rating was not particularly useful in this trial in determining differences between the efficacies of the different chemicals, with only one rating (leaf assessment #5, 10 February 2005) demonstrating any significant differences between the treatments (Table 5). It is worth noting, however, that this rating demonstrated Delan®, Polyram® and Experimental A treated trees to have significantly reduced total spot numbers.

*Number of infected leaves*

In comparison, Delan® and Polyram® treatments were significantly different from the untreated controls by the third rating (20 January 2005); with these two chemicals remaining consistently more effective at reducing Alternaria-like leaf symptoms for the remainder of the trial (Figure 1, Tables 6-8).

*After harvest*

The after harvest (21 February) infected leaf assessments continued the previous trends, with the total number of leaf spot counts continuing to show no differences between any of the treatments; and the number of infected leaf counts demonstrating Polyram®, Delan®, Experimental A and Experimental C to be the most effective at reducing the total number of infected leaves (Table 9). In the final infection count, performed in March (Table 10), the trend of Polyram®, Delan® and Experimental A producing the least number of symptoms returned. Several other treatments significantly reduced the number of infected leaves when compared with the untreated trees, but were not as effective as Polyram®, Delan® or Experimental A.

**Table 5: Leaf Assessment 5 – Total number of leaf spots per tree**

| Fungicide* | Application Schedule | Number of infected leaves # |
|---|---|---|
| Experimental A | Post-rainfall | 15.0 a |
| Polyram® | Post-rainfall | 19.5 a |
| Delan® LV | Post-rainfall | 26.8 a |
| Delan® HV | Calendar | 29.2 a |
| Polyram® | Calendar | 29.5 a |
| Delan® HV | Post-rainfall | 40.5 a |
| Experimental A | Calendar | 45.5 a |
| Delan® LV | Calendar | 62.5 ab |
| Experimental C | Calendar | 86.0 abc |
| Experimental C | Post-rainfall | 87.2 abc |
| Experimental B | Calendar | 158.8 abc |
| Experimental B | Post-rainfall | 219.2 abc |
| Experimental D 120 | Calendar | 237.8 abc |
| Ziram® | Calendar | 259.2 abc |
| Experimental D 100 | Post-rainfall | 344.0 bcd |
| UTC | Untreated | 368.0 cd |
| Experimental D 100 | Calendar | 574.2 d |
| Experimental D 120 | Calendar | 621.2 d |

* HV = High volume; LV = Low Volume
# Numbers with same letter after them are not significantly different.
Shaded treatments are not significantly different to the untreated control

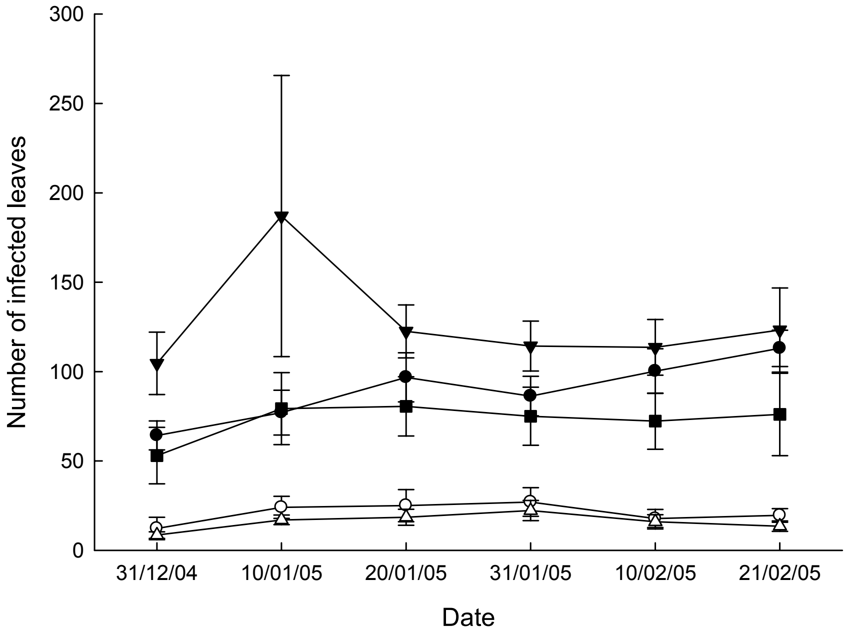


**Figure 1: Granite Belt 04/05 trial – Number of infected leaves.**

Changes in the number of infected leaves for untreated apple trees (●) and those treated with the following fungicides; Delan (○); Experimental D (▼); Polyram (△) and Ziram (■). Data presented are means ± s.e. (*n*=4).

**Table 6: Leaf Assessment 3 – Number of infected leaves**

| Fungicide* | Application Schedule | Number of infected leaves # |
|---|---|---|
| Experimental A | Post-rainfall | 12.50 a |
| Delan® HV | Calendar | 15.00 ab |
| Polyram® | Calendar | 17.00 ab |
| Delan® HV | Post-rainfall | 17.25 ab |
| Polyram® | Post-rainfall | 18.50 abc |
| Delan® LV | Post-rainfall | 25.00 abc |
| Experimental A | Calendar | 35.75 abcd |
| Delan® LV | Calendar | 40.50 abcd |
| Experimental C | Calendar | 48.00 bcde |
| Experimental C | Post-rainfall | 53.75 cdef |
| Experimental B | Calendar | 61.25 defg |
| Experimental D | Post-rainfall | 77.25 efgh |
| Ziram® | Calendar | 80.50 efgh |
| Experimental D 120 | Post-rainfall | 85.50 fgh |
| Experimental B | Post-rainfall | 89.25 ghi |
| UTC | Untreated | 96.75 hi |
| Experimental D 120 | Calendar | 105.25 hi |
| Experimental D 100 | Calendar | 122.50 i |

* HV = High volume; LV = Low Volume

\# Numbers with same letter after them are not significantly different.

Shaded treatments are not significantly different to the untreated control

**Table 7: Leaf Assessment 4 – Number of infected leaves**

| Fungicide* | Application Schedule | Number of infected leaves [#] |
|---|---|---|
| Delan® HV | Calendar | 16.00 a |
| Experimental A | Post-rainfall | 16.50 a |
| Polyram® | Calendar | 17.25 a |
| Delan® | Post-rainfall | 19.25 a |
| Polyram® | Post-rainfall | 22.25 a |
| Delan® | Post-rainfall | 27.00 a |
| Delan® | Calendar | 32.75 a |
| Experimental A | Calendar | 38.50 ab |
| Experimental C | Calendar | 44.75 abc |
| Experimental C | Post-rainfall | 50.75 abcd |
| Experimental B | Calendar | 70.00 bcde |
| Experimental D 120 | Post-rainfall | 71.75 bcde |
| Ziram® | Calendar | 75.00 cde |
| UTC | Untreated | 86.25 def |
| Experimental B | Post-rainfall | 88.00 ef |
| Experimental D 100 | Post-rainfall | 90.00 ef |
| Experimental D 100 | Calendar | 114.25 f |
| Experimental D 120 | Calendar | 116.50 f |

* HV = High volume; LV = Low Volume
[#] Numbers with same letter after them are not significantly different.
Shaded treatments are not significantly different to the untreated control

**Table 8: Leaf Assessment 5 – Number of infected leaves**

| Fungicide* | Application Schedule | Number of infected leaves[#] |
|---|---|---|
| Experimental A | Post-rainfall | 10.75 a |
| Polyram® | Post-rainfall | 16.00 ab |
| Delan® HV | Calendar | 16.25 ab |
| Delan® LV | Post-rainfall | 17.75 ab |
| Delan® HV | Post-rainfall | 17.75 ab |
| Polyram | Calendar | 18.00 abc |
| Experimental A | Calendar | 28.25 abc |
| Delan® LV | Calendar | 30.00 abc |
| Experimental C | Post-rainfall | 46.75 bcd |
| Experimental C | Calendar | 49.00 cde |
| Experimental B | Calendar | 70.25 def |
| Ziram® | Calendar | 72.25 def |
| Experimental D 120 | Calendar | 79.75 ef |
| Experimental B | Post-rainfall | 80.00 ef |
| Experimental D 100 | Post-rainfall | 90.00 fg |
| UTC | Untreated | 100.2 fg |
| Experimental D 100 | Calendar | 113.50 g |
| Experimental D 120 | Calendar | 115.25 g |

* HV = High volume; LV = Low Volume
[#] Numbers with same letter after them are not significantly different.
Shaded treatments are not significantly different to the untreated control

**Table 9: Leaf Assessment 6 – Number of infected leaves**

| Fungicide* | Application Schedule | Number of infected leaves# |
|---|---|---|
| Polyram® | Calendar | 12.50 a |
| Experimental A | Post-rainfall | 13.25 a |
| Polyram® | Post-rainfall | 13.50 a |
| Delan® HV | Post-rainfall | 14.25 a |
| Delan® HV | Calendar | 15.75 a |
| Delan® LV | Post-rainfall | 19.50 a |
| Delan® LV | Calendar | 26.25 ab |
| Experimental A | Calendar | 29.00 ab |
| Experimental C | Calendar | 36.50 ab |
| Experimental C | Post-rainfall | 37.50 ab |
| Experimental B | Calendar | 59.00 bc |
| Ziram® | Calendar | 76.00 cd |
| Experimental D120 | Post-rainfall | 78.50 cde |
| Experimental B | Post-rainfall | 86.75 cdef |
| Experimental D 120 | Post-rainfall | 103.25 defg |
| UTC | Untreated | 113.00 efg |
| Experimental D100 | Calendar | 116.50 fg |
| Experimental D100 | Calendar | 123.25 g |

* HV = High volume; LV = Low Volume
# Numbers with same letter after them are not significantly different.
Shaded treatments are not significantly different to the untreated control

**Table 10: Leaf Assessment 7 – Number of infected leaves**

| Fungicide* | Application Schedule | Number of infected leaves# |
|---|---|---|
| Delan® HV | Post-rainfall | 13.50 a |
| Experimental A | Post-rainfall | 14.25 a |
| Delan® HV | Calendar | 22.25 ab |
| Delan® LV | Post-rainfall | 25.25 ab |
| Experimental A | Calendar | 27.00 ab |
| Polyram® | Post-rainfall | 30.50 abc |
| Polyram® | Calendar | 38.50 abcd |
| Delan LV | Calendar | 50.50 bcd |
| Experimental C | Calendar | 62.75 cd |
| Experimental C | Post-rainfall | 67.50 de |
| Experimental B | Calendar | 99.50 ef |
| Ziram® | Calendar | 132.50 fg |
| Experimental B | Post-rainfall | 149.50 gh |
| Experimental D120 | Post-rainfall | 159.75 gh |
| Experimental D120 | Post-rainfall | 170.75 h |
| UTC | Untreated | 177.75 h |
| Experimental D100 | Calendar | 182.25 h |
| Experimental D100 | Calendar | 182.75 h |

* HV = High volume; LV = Low Volume
# Numbers with same letter after them are not significantly different.
Shaded treatments are not significantly different to the untreated control

***Late season defoliation assessments***

The first subjective leaf fall assessment demonstrated no significant difference between any of the assessments, except for Delan®, which was slightly better than the untreated control. The second (and final) defoliation assessment undertaken in April, showed Polyram® was the most effective chemical at reducing premature defoliation, with Polyram® treated trees retaining the most leaves at the end of the trial (Table 11). It is worth noting that Delan® and Experimental A were almost as effective as Polyram® (Figure 2 and Table 11). It should also be noted that the use of any of the fungicides trialed, except Experimental D 100 and Experimental D 120 (calendar), significantly reduced premature defoliation when compared to the untreated control (Table 11).

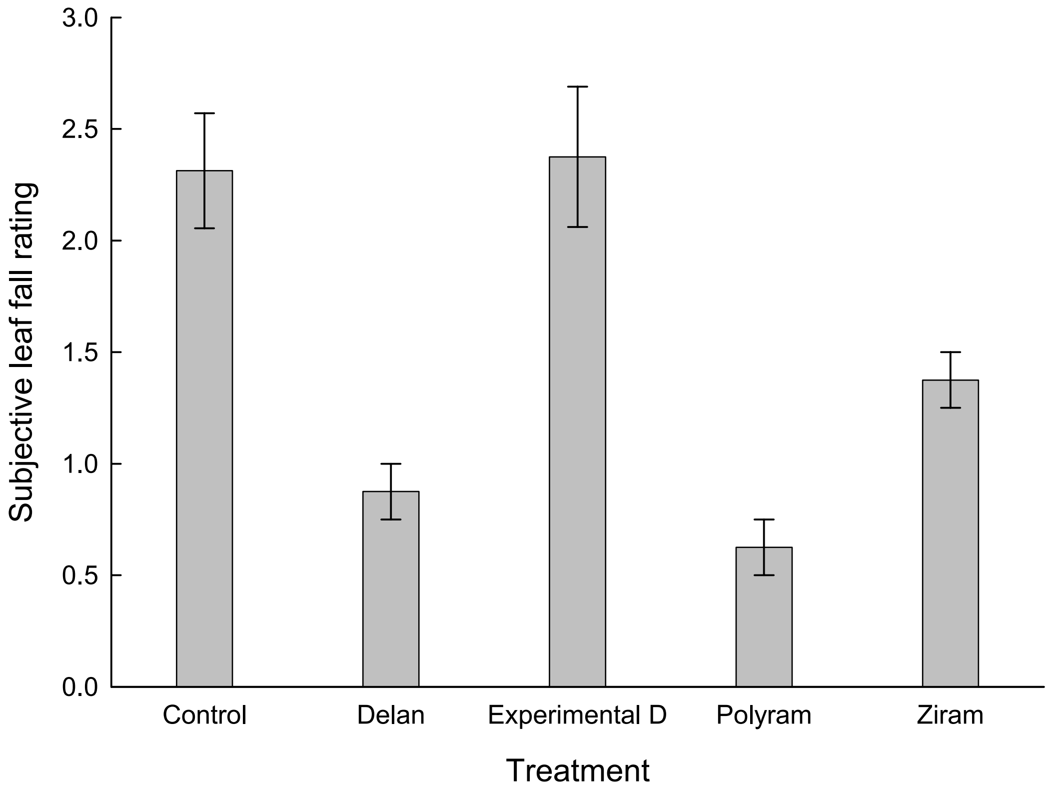


**Figure 2: Granite Belt 04/05 – Subjective rating of leaf fall, 11 weeks after fruit harvest**

Subjective rating of leaf fall, measured 11 weeks after fruit harvest for untreated apple trees and those treated with the following fungicides; Delan, Experimental D, Polyram and Ziram. Data presented are means ± s.e. (*n*=8).

**Table 11: Defoliation assessment 2 – Subjective rating of foliage remaining on the tree**

| Fungicide* | Application Schedule | Number of infected leaves# |
|---|---|---|
| Polyram® | Post-rain | 0.62 a |
| Delan® LV | Calendar | 0.87 ab |
| Experimental A | Post-rain | 0.87 ab |
| Delan® LV | Calendar | 1.00 ab |
| Delan® HV | Post-rain | 1.00 ab |
| Delan® HV | Calendar | 1.12 ab |
| Polyram® | Calendar | 1.25 ab |
| Experimental A | Calendar | 1.25 ab |
| Experimental D120 | Post-rain | 1.31 b |
| Ziram® | Calendar | 1.37 bc |
| Experimental C | Post-rain | 1.37 bc |
| Experimental B | Calendar | 1.50 bcd |
| Experimental C | Calendar | 1.50 bcd |
| Experimental D100 | Calendar | 2.00 cde |
| Experimental B | Post-rain | 2.06 de |
| Experimental D120 | Calendar | 2.25 e |
| UTC | Untreated | 2.31 e |
| Experimental D100 | Post-rain | 2.37 e |

* HV = High volume; LV = Low Volume
# Numbers with same letter after them are not significantly different.
Shaded treatments are not significantly different to the untreated control

***Fruit assessments***

Fruit from the trial was rated on two occasions, at the time of harvest (2-4 February 2005), and six months later after storage at 4°C. For the first, harvest rating, 50 fruit were randomly picked from each tree irrespective of maturity, and rated for symptoms of insect, disease and physical damage. The 25 most mature fruit from each tree were stored at 4°C; after all physical symptoms were marked on the fruit and recorded.

The rating performed immediately after harvest showed that there were significant differences between the treatments with regard to the total number of leaf spots, and the number of infected fruit. After storage, however, when symptoms that developed during storage were rated, there were no significant differences between any of the treatments.

*Number of spots per treatment*

At harvest, Polyram® (calendar and post-rainfall), Delan® (post-rainfall), Experimental A and Experimental C significantly reduced the total number of spots produced per treatment (Table 12). Interestingly, there were two treatments, Experimental D 120 (calendar) and Experimental D 100 (post-rainfall), which produced significantly more symptoms than the untreated control. It is unclear why this occurred, but an increased level of fruit injury could be a possibility.

*Number of spotted fruit*

Polyram®, Delan®, Experimental A and Experimental C were the most successful treatments at reducing the number of affected fruit, with Experimental B (post-rainfall), Ziram® (calendar) and Experimental D 120 (post-rainfall) also showing some positive effects (Figure 3 and Table 13). Once again there were two treatments that produced significantly more symptoms than the untreated control, namely Experimental D 100 (post-rainfall) and Experimental D 120 (calendar). The reason for this result is also unclear. This measurement of efficacy was considered to be the more meaningful of the two, as current market practise is to reject fruit with a single *Alternaria* spot.

**Table 12: Fruit assessment 1 – Total number of fruit spots per treatment at harvest**

| Fungicide* | Application Schedule | Number of infected leaves# |
|---|---|---|
| Polyram® | Calendar | 1.00 a |
| Polyram® | Post-rainfall | 1.00 a |
| Experimental A | Post-rainfall | 1.25 a |
| Delan® HV | Post-rainfall | 2.75 a |
| Experimental A | Calendar | 2.75 a |
| Experimental C | Calendar | 3.25 a |
| Experimental C | Post-rainfall | 3.50 a |
| Delan® LV | Post-rainfall | 4.75 ab |
| Delan® HV | Calendar | 6.00 abc |
| Delan® LV | Calendar | 10.00 abc |
| Experimental B | Calendar | 11.25 abc |
| Experimental B | Post-rainfall | 19.25 abcd |
| Ziram® | Calendar | 21.25 abcd |
| UTC | Untreated | 29.50 bcd |
| Experimental D100 | Calendar | 31.50 cde |
| Experimental D120 | Post-rainfall | 39.25 de |
| Experimental D100 | Post-rainfall | 56.75 ef |
| Experimental D120 | Calendar | 70.25 f |

* HV = High volume; LV = Low Volume
# Numbers with same letter after them are not significantly different.
Shaded treatments are not significantly different to the untreated control

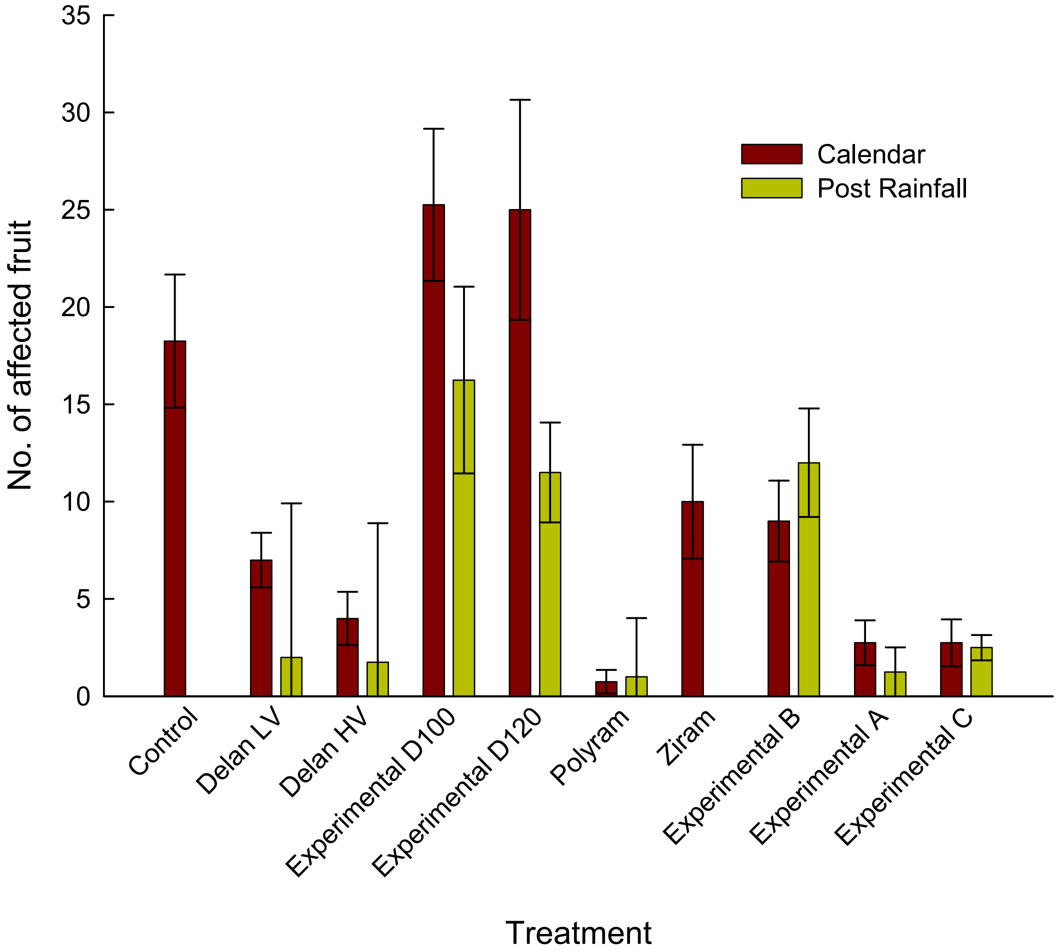


**Figure 3: Granite Belt 04/05– Number of Alternaria affected fruit at harvest**

Number of affected fruit, measured at harvest, for untreated apple trees and those treated with the following fungicides; Delan LV, Delan HV, Experimental D100, Experimental D120, Polyram, Ziram, Experimental A, B and C. Data presented are means ± s.e. ($n$=4).

**Table 13: Fruit assessment at harvest – Total number of affected fruit per treatment at harvest**

| Fungicide* | Application Schedule | Number of infected leaves# |
|---|---|---|
| Polyram® | Calendar | 0.75 a |
| Polyram® | Post-rainfall | 1.00 a |
| Experimental A | Post-rainfall | 1.25 a |
| Delan® HV | Post-rainfall | 1.75 a |
| Delan® LV | Post-rainfall | 2.00 a |
| Experimental C | Post-rainfall | 2.50 a |
| Experimental A | Calendar | 2.75 ab |
| Experimental C | Calendar | 2.75 ab |
| Delan® HV | Calendar | 4.00 abc |
| Delan® LV | Calendar | 7.00 abcd |
| Experimental B | Calendar | 9.00 bcd |
| Ziram® | Calendar | 10.00 cde |
| Experimental D120 | Post-rainfall | 11.50 de |
| Experimental B | Post-rainfall | 12.00 def |
| Experimental D100 | Calendar | 16.25 ef |
| UTC | Untreated | 18.25 f |
| Experimental D120 | Calendar | 25.00 g |
| Experimental D100 | Post-rainfall | 25.25 g |

* HV = High volume; LV = Low Volume
\# Numbers with same letter after them are not significantly different.
Shaded treatments are not significantly different to the untreated control

## Conclusions

### *Leaf blotch*

- Polyram®, Delan® and Experimental A were consistently, significantly better than the other treatments at reducing the number of infected leaves during the later part of the season, when symptoms were observed (Figure 1 and Tables 6-10) .
- Polyram® was the most effective chemical at reducing premature defoliation, with Polyram® treated trees retaining the most leaves at the end of the trial (Table 11). It is worth noting that Delan® and Experimental A were almost as effective as Polyram®.
- The application of fungicides to trees after fruit harvest can significantly reduce the level of premature defoliation. As demonstrated by the fact that the use of any of the fungicides trialed, except Experimental D 100 and Experimental D 120 calendar, significantly reduced premature defoliation when compared to the untreated control (Table 11).

### *Fruit spot*

- Interestingly the results from the fruit ratings were quite different to those of the leaf ratings.
- As well as Polyram®, Delan® and Experimental A, Experimental C was also effective at significantly reducing the total number of spots produced per treatment (Table 12).

- Even more interesting are the results from the number of spotted fruit assessment (Table 13), which shows Polyram®, Delan®, Experimental A and Experimental C to be the most successful; but that Experimental B (post-rainfall), Ziram® (calendar) and Experimental D 120 (post-rainfall) were also effective.
- Once again there were treatments that produced significantly more fruit spots than the untreated trees. Why this occurred is not clear.

***Calendar vs post-rainfall treatments***

- Results from this trial suggest that application of fungicides after rain is just as, if not more, effective than using preventative or calendar sprays.
- Unsure why fungicides with very little systemic / curative capacity appeared to work when applied in a curative (i.e. post-rainfall) manner. Possible explanations include:
    - Maybe it doesn't matter when in the Alternaria lifecycle the fungicide is applied, as long at there are regular applications throughout the season.
    - Perhaps the Alternaria is not the original cause of infection, and the fungicides are simply reducing symptom development.
    - Perhaps the infection process is much longer than suggested by the current literature.

# Appendix B:  New South Wales Field Trial Picton 01/02

NSW Agriculture

**Deciduous Fruits Pathology Laboratory**

**Experiment Fruitpath-007**

**Alternaria Leaf Spot Fungicide Trial**

**Picton 2001/02**

# Background

Alternaria leaf spot has been a significant disease of coastal apples since its first appearance five to six (and possibly 10) years ago. The areas affected in NSW are the Camden/Picton region and the Kurrajong / Bilpin region.

Overseas (primarily Japan and Korea; though recently also USA) control has relied on fungicide treatments, primarily based on Captan. Similar treatments in Australia, both in NSW and affected regions in Queensland (Shane Dullahide, personal communication) have proven ineffective.

In 2001 a fungicide trial was established at the Cedar Creek Orchard at Picton in collaboration with Aventis and Syngenta and under the supervision of NSW DPI District Horticulturist, Lawrence Ullio.

This orchard had a relatively long history of *Alternaria* leaf spot epidemics and had been severely infected during the previous two seasons (2000/01 & 1999/2000)

## *Collaborator Details*

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# Materials and Methods

## *Trial Site Details*

The trial was undertaken in a stand of mature Gala trees (Figure 1), with rows running approximately east-west.

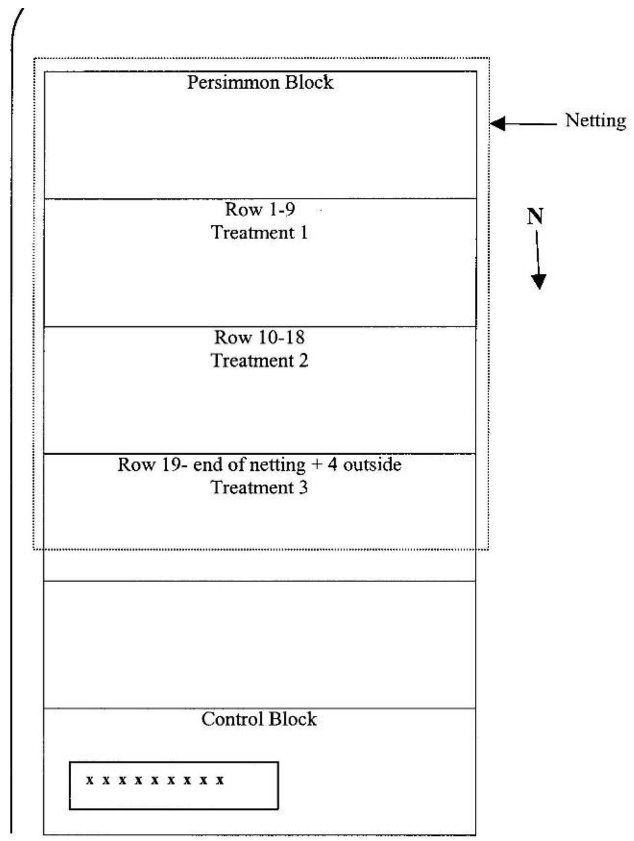


**Figure 1: Map of the Gala trial block used**

## Weather

The weather in 2001/02 was unusual. Spring was very dry. In early February the orchard received approximately 400mm of rain over a three week period. The manager first observed symptoms on the control block on February 3$^{rd}$ 2002.

## *Treatments*

Chemical applications were made by the orchard manager, using a commercial air blast sprayer at the high volume (dilute) rate of 200 L per hectare.

**Table 1: Schedule of treatment applications**

| Treatment | Early spur burst | Pink | Full bloom | Fruit development | | |
|---|---|---|---|---|---|---|
| | 26/9/2001 | 5/10/2001 | 15/10/2001 | 25/10/2001 | 14/11/2001 | 19/12/2001 |
| 1 | Chorus® | Chorus® | Chorus® | Bogard® | Bogard® | Bogard® |
| 2 | Vision® | Vision® | Vision® | Vision® | Vision® | Bogard® |
| 3 | Nustar® | Syllit® Rovral® | Syllit®® Rovral® | Nustar® | Stroby® | Bogard® |
| 4 | Syllit® | Syllit® Thiram® | Syllit® | Nustar® | Stroby® | Bogard® |

Bogard® = 35 g/100 L (25/10/2001 and 14/11/2001), 40 g/100 L (19/12/2001)
Chorus® = 40 g/100 L (All dates)
Nustar® =10 g/100 L (All dates)
Rovral® = 120 g/100 L (All dates)
Stroby® =10 g/100 L (All dates)
Syllit® = 50 ml/100 L (All dates)
Thiram® = 50 ml/100 L (All dates)
Vision® =75 ml/100 L (All dates)

## *Experimental design*

### *Percentage of leaves infected*

Treatments were applied to 10 replicate trees (one tree = one replicate) within the sample assessment area, and disease status (diseased or healthy) of 250 leaves at chest-height and 250 leaves at 2.5 m above the ground was recorded. Initially leaves were to be assessed lower than this but the orchard floor had been sprayed with Basta (herbicide) and damage to lower leaves looked very similar to disease symptoms.

# Results

The number of infected leaves was assessed on the 15 February 2002 (Figure 2), with treatments two and three producing the lowest numbers of infected leaves.

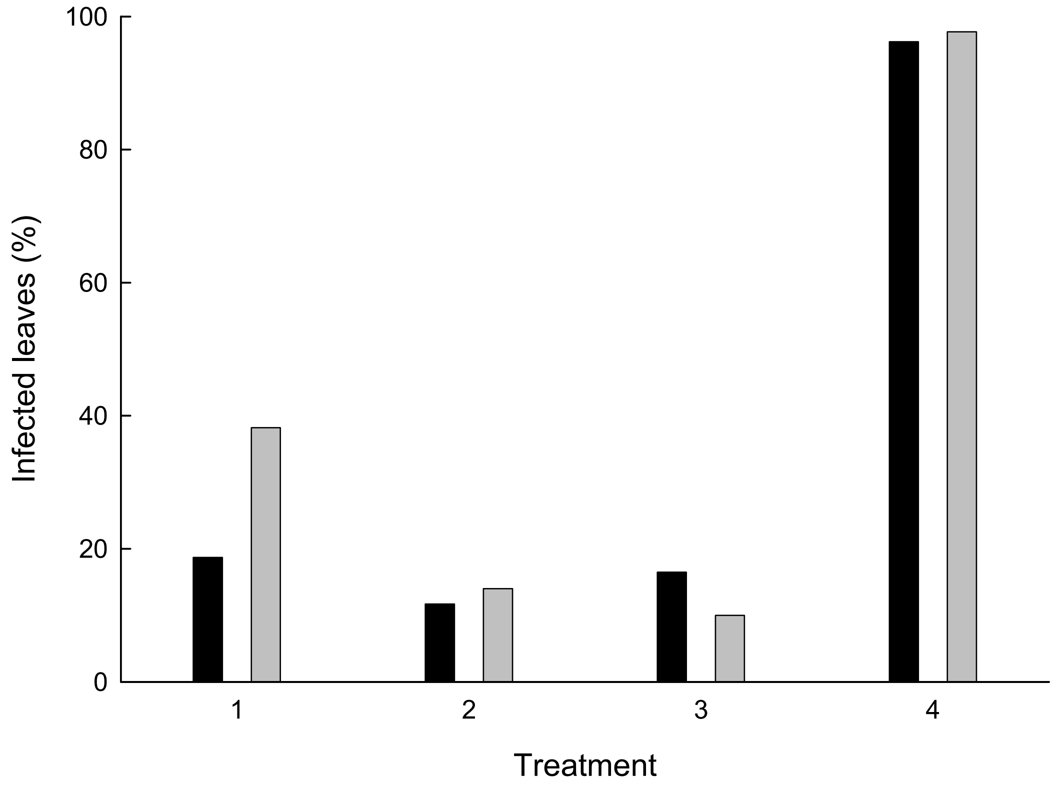


**Figure 2: Picton 01/02 – Number of infected leaves (%)**

| Treatment | Brief description |
|---|---|
| **1** | Chorus®, Chorus®, Chorus®, Bogard®, Bogard®, Bogard® |
| **2** | Vision®, Vision®, Vision®, Vision®, Vision®, Bogard® |
| **3** | Nustar®, Syllit®/Rovral®, Syllit®/Rovral®, Nustar®, Stroby®, Bogard® |
| **4** | Syllit®, Syllit®/Thiram®, Syllit®, Nustar®, Stroby®, Bogard® |

# Appendix C: New South Wales Field Trial Berambing 02/03

NSW Agriculture

## Deciduous Fruits Pathology Laboratory

## Experiment Fruitpath-016

**HAL project APO2011: Management of Alternaria leaf and fruit spot in apples.**

**NSW Agriculture Results**

**2002/03**

# BACKGROUND

A trial in the Picton region during 2001/02 (FRUITPATH-007) found that fungicides containing a Group I component provided good control of the disease. The fungicides tested in this trial were Chorus (Cyprodinil) and Vision (fluquinoconazole + pyrimethanil)

In 2002 a fungicide trial was established at Berambing in the Bilpin region (Orchardist, Brian Hungerford) to test Chorus and Vision. Both fungicides were tested in early and late block (3 applications per block) applications and compared to a control treatment sprayed largely with guanidine (Syllit, dodine)

This experiment is part of project APO2011 – Management of Alternaria leaf and fruit spot in apples

This Orchard had a relatively long history of Alternaria Leaf Spot epidemics and had been severely affected during the previous two seasons (2000/01 & 1999/2000).

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# Materials and Methods

## *Trial Site Details*

The trial was conducted in a block of 6 year old Hi-early Delicious apples. A drought affected Bilpin through the 2003/04 season. Irrigation to the trees was limited, particularly during December 2002 and January 2003.


**Figure 1: Map of northern Sydney Basin district including Berambing**

## *Treatments*

Chemicals were purchased from project funds and applied by Mr Hungerford. He was given a copy of the trial plan (Table 1) and a rough timetable of when applications should be made. This timetable was modified due to wind and rain delays and the actual application dates are listed below (Table 2).

**Table 1: Layout of trial trees**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Row 6 | G | G | G | G | G | G | G | G | G | G | G |
| Row 5 | X | X | ❺ | ❹ | ❸$_b$ | X | ❷$_b$ | X | ❶$_b$ | X | X |
| Row 4 | X | ❺ | ❹$_b$ | X | ❸$_b$ | X | ❷ | $X_b$ | ❶ | X | X |
| Row 3 | ❺ | $X_b$ | ❹ | X | ❸$_b$ | X | ❷ | $X_b$ | ❶ | X | X |
| Row 2 | ❺ | X | ❹$_b$ | X | ❸$_b$ | X | ❷$_b$ | X | ❶ | X | X |
| Row 1 | ❺ | X | ❹ | $X_b$ | ❸ | $X_b$ | ❷ | $X_b$ | ❶ | X | X |

X Unmonitored
❶,❷,❸,❹,❺ Percentage of leaves infected monitored
b Leaf fall monitored with bags
G Gala

Additionally Mr Hungerford asked whether he could apply Nimrod® (bupirimate) to control powdery mildew. I agreed to this as there is no record of group H fungicides having activity against *Alternaria mali*. Dipel® (*Bacillus thuringensis* v. *kurstaki*)was also applied late in the season.

The basic structure of the treatments included:

- Treatment 1: Vision® early = a block of three 'Visions' early in the season followed by Syllit® (Dodine; with no known activity against *Alternaria*) and finished with Bogard®.
- Treatment 2 : Vision® late = a block of three 'Visions®' applied as the 4th, 5th and 6th fungicide applications.
- Treatment 3: Chorus® early = as per treatment 1 except Chorus® replaces Vision®.
- Treatment 4: Chorus® late = as per treatment 2 except Chorus® replaces Vision®.
- Treatment 5: Control = no fungicide with recorded activity against Alternaria.

**Table2: Treatment application schedule**

| Row | Day (Date) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 20.9.02 | 1.10.02 | 12.10.02 | 27.10.02 | 5.11.02 | 17.11.02 | 27.11.02 | 8.12.02 | 25.12.02 | 7.1.03 |
| 1 | V N | V N | V | S N | S N | S | B | B | B | B D |
| 2 | S N | S N | S N | V N | V | V | B | B | B | B D |
| 3 | C N | C N | C N | S N | S N | S N | B | B | B | B D |
| 4 | S N | S N | S N | C N | C N | C | B | B | B | B D |
| 5 | S N | S N | S N | S N | S N | S | S | S | S | S D |

B = Bogard® (difenoconazole) @ 35 g / 100L
C = Chorus® (cyprodinil) @ 40g / 100L
D = Dipel® (*Bacillus thuringensis* v. *kurstaki*)
N = Nimrod® (bupirimate)
S = Syllit® (dodine) @ 80mL / 100L
V = Vision® (fluquinconazole + pyrimethanil) @ 75 mL / 100L

## *Assessment*

Assessments commenced when disease was first noticed in the trial 91 days after the application of the first fungicides.

### Leaf infection

Five replicates per treatment, where a replicate = 1 tree. For each replicate 100 leaves were assessed for the presence of disease around the circumference of the tree. Measurements were made at chest height. Leaves were counted from the surface and within the canopy. This measurement was repeated at >2 m of height. There were eight assessments carried out during the season

### Leaf fall

Hessian bags supported by a circular wire frame were suspended under the canopies of 3 trees per treatment. The dry weight of apple leaves caught in these traps was measured.

# Results

## *Leaf infection*

Eight assessments were undertaken but the last two (May 1 and May 15) were affected by natural leaf fall. As leaves senesce and fall, infected leaves dropped first. This led to a decline in the leaf infection recorded on trees.

The lowest level of leaf infection was recorded where the trees had been given 3 consecutive applications of Vision (fluquinconazole + pyrimethanil) as the first sprays of the season. This treatment reduced leaf infection when compared to the control treatment throughout the season. It also provided significantly ($p<0.05$) better control of leaf infection than Chorus (cyprodinil) for the majority of assessment times. Early application also gave better control of leaf infection, particularly later in the season.

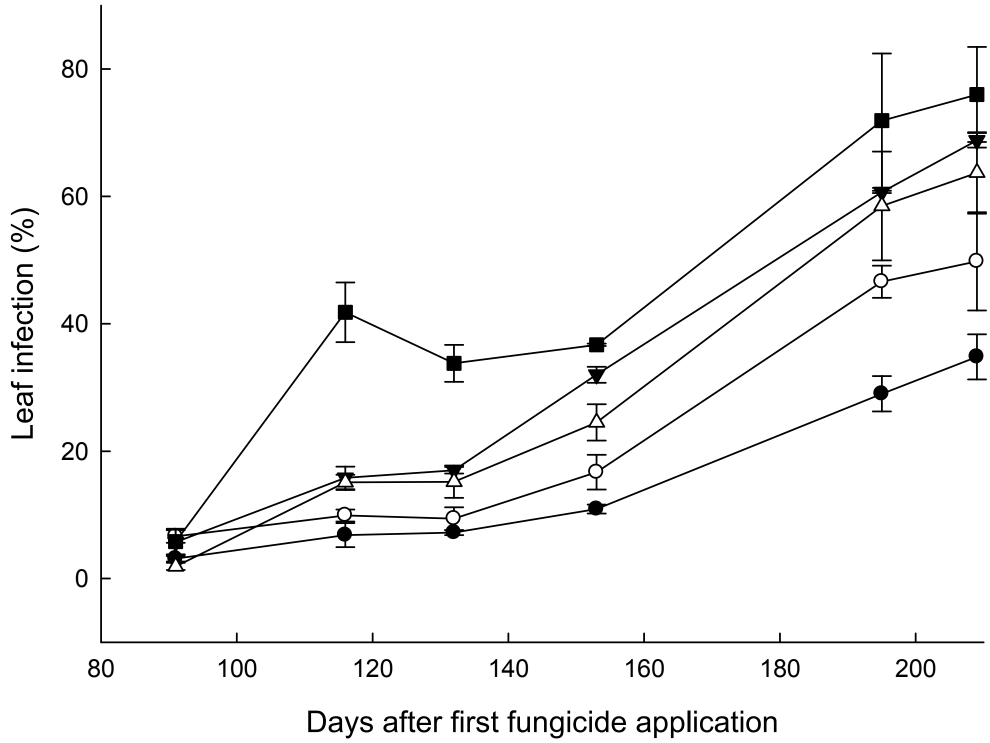


**Figure 2:** Changes in leaf infection (%) for untreated apple trees (■) and those treated with the following fungicides; Vision Early (●); Vision Late (○); Chorus Early (▼) and Chorus Late (△), recorded 91, 116, 132, 153, 195 and 209 days after the first fungicide application. Data presented are means ± s.e. ($n$=5).

## *Observations*

This block had been infected in previous seasons and as a result early defoliation had occurred. Despite high levels of infection during this season – up to 90% for individual trees – very little premature defoliation occurred.

Bilpin was also hit by a hail storm in February which caused a lot of leaf and fruit damage.

Graham Nicol (Bayer Cropsciences) supplied Mr Hungerford with a quantity of Flint (trifloxystrobin) which they claim to have activity against *A. mali*. This was applied to a block of Pink Ladies and Royal Gala trees which had been severely infected last season. Although there were no control (unsprayed) trees there were very low levels of disease in these two blocks. While there was a significant reduction in disease on blocks sprayed with Vision®, this result was not as convincing as that obtained at Picton 2001/02.

Throughout the season all treatments resulted in significant reductions in leaf infection, with an early application of vision producing the greatest reduction in leaf infection

## Leaf fall

Six assessments were carried out (153, 195, 209, 223, 237 and 258 days after first fungicide application)

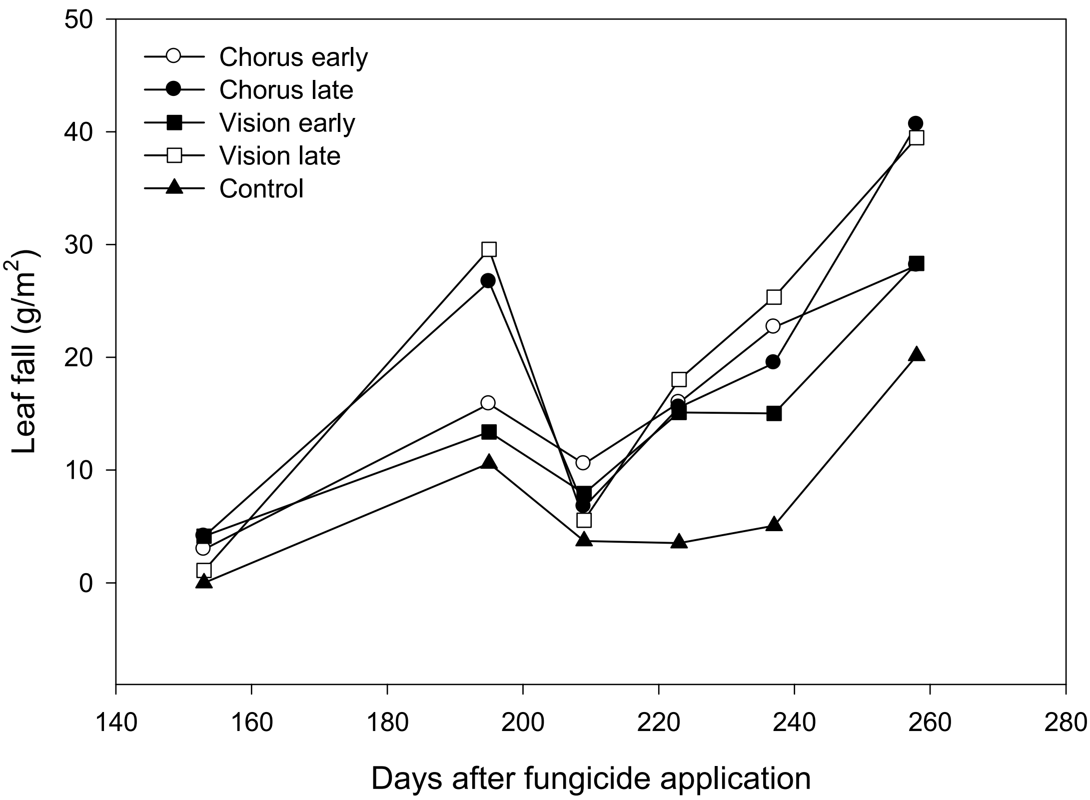


**Figure 3:** Changes in leaf fall (g/m2) for untreated apple trees (■) and those treated with the following fungicides; Vision Early (●); Vision Late (○); Chorus Early (▼) and Chorus Late (△), recorded 155, 195, 209, 223, 237 and 258 days after fungicide application. Data presented are means ± s.e. (n=3).

Despite consistently carrying the heaviest leaf infection, the control treatment suffered the lowest leaf loss.  In this trial infected leaves tended to be retained by the trees. Early application of Chorus or Vision resulted in significantly lower rates of leaf loss than late applications.

# Appendix D:

## *Reasons not to use Iprodione (Rovral®) for preharvest management of* **Alternaria** *leaf blotch or fruit spot*

### 1. Iprodione (Rovral®) is not registered for preharvest use on Australian apples.

### 2. Development of fungicide resistant pathogen populations.

Iprodione is an "at risk" chemical, meaning that it is particularly susceptible to resistance development in pathogen populations, and must be used extra carefully to avoid this happening. Once fungicide resistance develops in a pathogen population it is impossible eliminate, and will significantly reduce the effectiveness of that product.

The preharvest use of iprodione will significantly increase the chances of resistance developing in Australian apple-pathogen populations. Iprodione resistance has already been recorded in Japan, Korea and China (Asari and Takahashi 1988; Kim and Lee 1987; Lee 1984; Tanaka, Shimomura *et al.* 1989). Of particular note is an example in Korea where 70% of leaf isolates collected were resistant to iprodione, with the majority of these isolates producing significant numbers of large spots when inoculated onto apple fruit sprayed with iprodione (Kim and Lee 1987).

Preharvest applications of iprodione may also lead to resistance developing in postharvest pathogen populations; with resistance to iprodione already recorded in several important postharvest pathogens, of most relevance are grey mould (*Botrytis cinerea*) in apples in the Netherlands (Sansone, Rezza *et al.* 2005); blue mould (*Penicillium expansum)* in USA apples (Rosenberger and Meyer 1981) and Alternaria rot (postharvest fruit spot caused by *Alternaria alternata*, a different Alternaria to Alternaria leaf blotch and preharvest fruit spot – *Alternaria mali*) in West Virginia, USA (Biggs 1994).

Also in the US, where iprodione was found to be effective at reducing the number of Alternaria blotches per leaf (but not the total number of blotched leaves) (Sutton, 2005), iprodione is not registered as a preharvest spray due to the risks associated with resistance development in postharvest pathogens. With the eminent postharvest pathologist Alan Biggs (University of West Virginia) expressly warning in his 1994 paper (Biggs 1994) that preharvest application of Iprodione should not occur for exactly this reason.

Finally, iprodione is currently the only effective postharvest fungicide available for use in apples that is able to control a broad range of fungal diseases; if resistance to this product develops there are no other products available to take its place. There are other chemicals with the ability to reduce Alternaria leaf blotch and preharvest fruit spot.

**3. No scientific evidence that iprodione significantly reduces Alternaria fruit spot on apple.**

There is currently no field data available which demonstrates the ability of iprodione to reduce either the number of Alternaria spots per fruit, or the total number of spotted fruit. The only data currently available is for use against Alternaria leaf blotch.

As iprodione only reduces the number of blotches per leaf, not the total number of blotched leaves (Sutton 2005), it is unlikely to reduce the total number of spotted fruit. And as one spot per fruit is sufficient to make the fruit unacceptable to most markets, this would not increase the number of saleable fruit harvested.

It is highly unlikely that a permit would be given for the use of iprodione considering the aforementioned lack of scientific evidence, and the high risk of resistance.

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