



Through chain approach for managing brown rot in Summerfruit and Canning fruit

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Media Summary

Brown rot is the most economically damaging disease of summerfruit and canning peaches in Australia, causing an average annual loss of \$19 million despite an annual expenditure of about \$25 million on disease control. The disease also threatens market access, both as a quarantine barrier and through associated chemical residue risks.

This project developed and validated a weather-based infection risk model to support more precise fungicide timing, suitable for individual farm use. Eleven field sites were established in the Murray and Goulburn Valleys. Weather stations located at each orchard provided site specific data for estimating infection periods (IP) conducive to infection using the model and growers were notified by SMS within 12 hrs of infection conditions occurring. Disease was assessed after harvest to evaluate the success of spraying by the growers and most orchards achieved continuous improvement in rot control by following IP forecasting.

A technique was developed to assess, before harvest, the risk of development of brown rot postharvest, enabling packers and processors to appropriately segregate and treat batches of fruit according to their rot risk.

The influence of reducing *Carpophilus* beetle populations on the incidence of brown rot was demonstrated. The effectiveness of the Attract and Kill system for *Carpophilus* management was also confirmed.

Monilinia fructicola was shown to be the most important brown rot pathogen and neither of the exotic species was found in the surveys. Fungicide resistant strains of *M. fructicola* were detected, gaps in knowledge and further work has been identified to establish if this could explain the poor disease control experienced by some growers.

The industry has gained a greater understanding of brown rot risk factors and received advice on the integrated disease management of brown rot through on-farm trials, articles in the industry journals, industry seminars, and scientific publications.

A follow-on project is required to assist industry and service providers to implement the first generation of the brown rot forecasting model and the postharvest rot prediction model. Further research to add precision to the forecasting model should investigate the influence of weather on the persistence of protectant fungicides, changes in the susceptibility of different fruit types over the growing season and the impact of fungicide resistance.

Technical Summary

Brown rot caused by *Monilinia fructicola* and *M. laxa*, the most damaging disease of stonefruit, significantly impacts on fruit quality, orchard profitability and potentially jeopardises market access. Often losses are most severe in the market, which leaves consumers dissatisfied and producers' income most at risk. In recent years, disease has been so severe in the field that some crops have been abandoned before harvest. The average annual loss due to brown rot is estimated to be \$19 million nationally and the costs of control are approximately \$25 million (\$3,500/ha in the orchard and \$35/t postharvest).

Infection can be controlled with well-timed, effective fungicides; however, growers have lacked access to site-specific infection risk assessment tools to support rational spray timing. In addition, brown rot infections are typically quiescent before harvest and the likely postharvest rot incidence cannot be determined before fruit are packed for market. Fungicide resistance may be impacting on crop protection in Australia. *Monilinia* spp. resistant to MBC, Dicarboximide and DMI fungicides have been reported overseas although systematic screening has not been done in Australia. The entire supply chain would benefit greatly from a tool to predict rot levels likely to develop after harvest and through gaining a better understanding of other risk factors contributing to disease and reduced control.

Eleven field sites were established in the Murray and Goulburn Valleys. Weather stations located at each orchard provided site specific data for estimating infection risk using a weather-based model and growers were notified by SMS within 12 hrs of infection conditions occurring. Disease was assessed after harvest to evaluate the success of spraying by the growers according to the predicted rot risk. Well timed fungicides, targeting infection risk events, suppressed infections and growers demonstrated continuous improvement in rot control over 2 to 4 seasons. Moist incubating samples of fruit collected close to harvest estimated the risk of rots developed during storage, transport and marketing and identified high risk batches of fruit.

Twenty *Monilinia* isolates from peach, nectarine and plum were evaluated *in-vitro* against thiabendazole, iprodione, propiconazole and fludioxonil, representing four of the chemical families used against brown rot pre- and postharvest. Based on the EC₅₀ and MIC values, all the isolates were sensitive to fludioxonil, but showed differential responses to iprodione, propiconazole and thiabendazole, with respective mean EC₅₀ of 0.003, 0.018, 0.130, and 0.165 ug/ml. An isolate highly resistant to thiabendazole was detected in an orchard in North East Victoria.

The influence of reducing Carpophilus beetle populations on the incidence of brown rot was demonstrated, as was the effectiveness of the Attract and Kill (A&K) system for Carpophilus management. However the A&K system can only be effective if the Carpophilus population is at a medium to low level and it may take more than three seasons of trap deployment to achieve a low beetle population.

Inoculation of peach and nectarine fruit at different growth stages showed that fruit were most susceptible in the weeks up to pit hardening and in the three weeks before

harvest. This was supported by studies in the commercial orchards that determined which infection periods contributed most to postharvest rot.

The extension and communication strategy was aimed to ensure that at least 70-80% of growers and exporters on a national level were aware of the results and outputs of this project. However, maximum impact of this R&D will only occur if further resources are provided to assist industry and service providers to implement the brown rot forecasting model and the postharvest rot prediction model developed and validated in this project.

This brown rot forecasting model would be enhanced if we better understood the influence of weather and fruit surface morphology on the persistence of protectant fungicides, and if we are better able to use the knowledge of the variability of fruit susceptibility to infection over the growing season.

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1. General Introduction

1.1 Background

Summerfruit and canning fruit production are mature industries with a national gross value over \$300 million p.a. Brown rot, the most damaging disease of stonefruit, significantly impacts on fruit quality, orchard profitability and potentially jeopardises market access. Often losses are most severe in the market, which leaves consumers dissatisfied and producers' income most at risk. In recent years, disease has been so severe in the field that some crops have been abandoned before harvest. The average annual loss due to brown rot is estimated to be \$19 million nationally and the costs of control are approximately \$25 million (\$3,500/ha in the orchard and \$35/t postharvest). In 2006, peaches and nectarines put through export simulation suffered an average 21% loss (3 % to 76 % depending on the variety and the source of the fruit) due to brown rot and other rots such as botrytis grey mould. Also in 2006 and 2007 entire shipments of fresh peaches and nectarines were rejected on arrival in the UK due to brown rot, costing growers an additional \$6 per box for airfreight and disposal on top of the crop loss suffered.

There are four species of *Monilinia* recognised capable of causing brown rot of stonefruits. *M. laxa* and *M. fructicola* occur in all Australian states and the ACT. *M. fructigena* and *M. polystroma* are absent from Australia. Early literature suggested *M. polystroma* may be a variant of *M. fructigena*, but isolates of *M. fructigena* from Japan were shown to be morphologically and genetically distinct from European strains and have subsequently been reclassified as a new species *M. polystroma* (Leeuwen *et al.*, 2002).

The main causal organism of brown rot of summerfruit in Australia is *Monilinia fructicola*. This pathogen is a quarantinable pest in the European Plant Protection Zone where its status has recently changed from restricted (EPPO A1 pest status) to locally present (EPPO A2 pest status; OEPP/EPPO, 2009). *M. fructicola* is absent from South Africa (Carstens *et al.* 2010), one of our main competitors in European markets. *M. fructicola* is classified as a quarantine pest in Europe, and imports from Australia arriving after the 15th February each season, must have a declaration that they have been treated to eliminate the disease. The fact that brown rot occurs sporadically in Australian exports shows that our treatments are not eliminating the disease and this situation adds to the risks of exporting. In addition, some of the fungicides we rely on close to harvest or for postharvest rot control are under scrutiny in domestic and export markets necessitating more judicious application of the few acceptable fungicides integrated with cultural controls.

Key features of the brown rot disease include cryptic life cycle, latent infection, symptom expression during fruit ripening and the sporadic nature of disease outbreaks. As there is no objective disease risk information, orchards are sprayed irrespective of the risk of infection, yet severe losses are unexpectedly experienced. An improved management strategy is essential to minimise loss in productivity, and fruit quality through to the market. In addition, informed disease management has great potential for reducing the frequency of chemical applications and improving the efficacy of control measures applied before and after harvest.

The disease occurs in all production regions every season and is most severe when warm, damp conditions occur close to harvest. While there is some suggestion that *Carpophilus* beetle vectors the disease, no modelling has been done linking insect infestation with disease risk. It is theorised that *Carpophilus* can spread the disease when the weather conditions are not necessarily conducive to infection. The causal link needs to be understood to guide the more rational control of *Carpophilus* and other pests.

Four species of *Monilinia* (*M. fructicola*, *M. fructigena*, *M. laxa* and *M. polystroma*) cause brown rot of stonefruit, but only two species, *M. fructicola* and *M. laxa*, are known to occur in Australia. The relative abundance of the two species in an orchard, and their respective levels of fungicide resistance, impact the effectiveness of control, but little information exists to guide chemical choice. *M. fructicola*, in particular, poses a threat to market access in the EU. The exotic species *M. fructigena* and *M. polystroma* cause significant disease on stonefruit and pomefruit overseas. There is a need to extend our existing capability to identify these species for preparedness in meeting overseas market access requirements and to detect and respond to any incursion by the exotic species.

The Australian Meteorological Bureau releases brown rot warnings for growers in northern Victoria. However, these are district-wide weather forecasts based on the expected period of leaf wetness and the drying characteristics of large tree canopies, rather than modern trellised or central leader canopies. While growers find the Met Bureau warnings helpful to guide the application of protectants, the risks do not always eventuate and sprays may be applied unnecessarily. With the advent of curative fungicides there is an opportunity to wait for an infection risk before deciding to spray, taking into account any residual protection afforded by previously used protectants.

Decision support tools have been developed in USA for the management of blossom blight of peaches (an early season disease caused by the same pathogens) and for brown rot of processing prunes. Unfortunately, the prune model cannot be used in Australia because it relies on enumeration of a life cycle stage of the fungus which has not been observed under Australian conditions. In a recent project “Predicting Product Performance”, we evaluated a network of low-cost weather stations with real-time access for their suitability to determine actual infection periods in individual orchard blocks, and identified a disease risk model (G. Tate pers com) which can assess infection risks. We have also examined techniques for the determination of brown rot infection periods in different canopy types, and evaluated methods to assess latent infection in fruit (Holmes *et al.* 2007). However further R&D is required to validate these for their widespread applicability in Australian stonefruit orchards.

1.2 Objectives

The project aimed to:

- Develop and validate a weather-based infection forecasting model to support precise chemical application, suitable for individual farms.
- Develop a technique to assess brown rot risk before harvest, enabling packers and processors to appropriately segregate and treat batches of fruit according to their rot risk.

- Determine the influence of reducing *Carpophilus* beetle populations on the incidence of brown rot.
- Identify the species of *Monilinia* associated with blossom, twig and fruit phases of the disease in Australia and assess the fungicide sensitivity of some representative isolates.
- Promote a greater understanding of brown rot risk factors and advise the Summerfruit and Canning fruit industries on the integrated disease management of brown rot through articles in the industry journals, industry seminars and scientific publications

The objectives of the project were to:

1. Assist industry to achieve more efficient and cost effective brown rot control.
 - a. potentially reducing crop loss and control costs of about \$22 million p.a. after 5 years
 - b. improving the customers' and consumers' confidence in the product's integrity.
2. Provide industry with the tools to better predict and manage the performance of summerfruit through the chain to better meet market quality expectations.
3. Better prepare industry to meet the import requirements of the EU including freedom from *Monilinia fructicola* infection and through the adoption of strategic spraying, pest monitoring and improved hygiene have the potential to reduce the use of residual pesticides pre and postharvest to improve the industry's ability to meet strict MRLs in premium markets.
4. Prepare plant disease diagnosticians to identify exotic *Monilinia* species which is thought to be absent from Australia at present and which is a threat to both pome and stonefruits
5. Engage with industry through articles in the industry journals, industry seminars and scientific publications

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2 Infection risk model for improving and evaluating growers timing of brown rot fungicides in stonefruit orchards

2.1 Introduction

The brown rot fungus *Monilinia fructicola* is a major problem for summerfruit growers in Australia (Holmes et al. 2008). It causes pre and postharvest fruit losses and in severe wet seasons can cause tree damage (shoot blight).

The brown rot disease cycle starts with flower infection (blossom blight) and that leads to twig infection, green fruit rot, ripe fruit rot and shoot blight if disease pressure is high. The source of primary inoculum in Australia is believed to be mostly conidia that overwinter in mummified fruit on the ground and on trees and wood and peduncle infections (Holmes et al. 2008, Shepherd 1968). In New Zealand, ascospores discharged from apothecia arising from buried mummified fruit provide the primary inoculum for flower infection (Atkinson 1971, Tate 1979). Apothecia have also been reported as a major source of primary inoculum in South Carolina and California (Landgraf and Zehr 1982, Hong et al. 1996).

Despite repeated applications of fungicides for brown rot control, crop losses still occur regularly in summerfruit orchards in Victoria, especially during wet seasons. Examination of spray programs in three summerfruit orchards in Victoria during 2006-2007 indicated that the time of fungicide application could be greatly improved by using weather-based infection period (IP) predictions generated by the peach brown rot model (Holmes et al. 2008, Tate and Manktelow 1992). The performance of protective (pre-infection) spray programs and post-infection spraying was improved by using IP forecasting in canning peaches (Manktelow and Tate 1991, Tate and Manktelow 1992). The success in using IP predictions depends on accurate weather monitoring and IP forecasting, and knowledge of other key factors influencing disease development including disease cycles, tissue susceptibility, source of inoculum, and fungicide efficacy (Tate and Manktelow 1992).

This chapter reports the use of the peach brown rot model for evaluating spray programs applied by growers for brown rot control in several plum, nectarine and peach orchards in Victoria, Australia. The work aimed to demonstrate to industry the importance of weather stations for collecting site-specific weather data and usefulness of IP forecasting to improve the performance of preventive and post-infection treatments for brown rot. The long-term goal of the research is to integrate weather-based IP predictions with other important factors that influence brown rot development for more effective disease control.

2.2 Materials and Methods

2.2.1 Sites

Weather stations (Model-T, Western Electronics Design, Australia) were deployed in several summer-fruit orchards in Victoria to monitor temperature, leaf wetness, RH and rainfall during growing seasons between 2006 and 2011. Stations were placed within three blocks of plum cv. 'Su Plum 11', the first one at Shepparton (Goulburn Valley, 2007-2009) and the other two at Swan Hill 2 (S. Hill 2, 2008-2011) in two adjacent blocks one under netting. Stations were also set up in three blocks of nectarine cv. 'August Red' (Ardmona, 2006-2011), 'Arctic Pride' (Lake Boga 1, 2007-2009), 'August Pearl' (Swan Hill 1, 2006-2011) and in eight blocks of peach cv. 'Scarlet Snow' (N. Shepparton, 2006-2011), 'Tatura 204' (NE Shepparton, 2008-2011), 'Taylor Queen' (Cobram 2, 2008-2011), 'September Sun' (Cobram 1, 2007-2011), 'Arctic Snow' (Renmark, 2009-2011; Lake Boga 2, 2008-2009) and 'Snow King' (Warrandyte, 2010-2011). In total, five consecutive seasons were examined at three sites (N. Shepparton, Ardmona and Swan Hill 1), three seasons at another four sites (Cobram 1, NE Shepparton, Cobram 2, S. Hill 2) and one or two seasons at other sites.

Weather stations were located within tree-rows and the wetness sensor (0 = dry, 1 = wet) placed horizontally approximately 1.6 m above ground on top of the weather station with the other sensors. Weather stations recorded data at 10 min intervals and were fitted with wireless telemetry which allowed data to be remotely downloaded when required during the growing season to determine occurrence of brown rot infection periods.

2.2.2 Infection period prediction

The risk of infection by spores (IP) was determined using criteria described by the peach brown rot model (Tate pers. comm., Tate and Manktelow 1992, Tate *et al.* 1995). The model was developed for New Zealand conditions to identify periods of moisture and temperature favourable for *M. fructicola* infection. The model was developed using blossom blight infection criteria from Weaver (1950), and also fruit infection criteria data from tests conducted by Tate at UC Davis in 1984 (Tate pers. comm.). Infection risk for blossom and fruit is calculated by multiplying the hours of wetness by the mean temperature during the wet period. The intensity of the infection risk is categorised by the following disease index: Ma = marginal (90-120); L = light (121-150); M = moderate (151-180); S = severe (>181). Marginal and light disease index describes the minimum requirement for spore infection.

IP information can be used to improve performance of fungicide programs by better scheduling post-infection fungicide treatments to prevent infections if no protective cover sprays have been applied. It can also be used to improve the time of application of pre-infection spraying with protectant fungicides.

2.2.3 Spray programs and fruit rot assessment

Fungicide sprays were applied by growers according to their standard practices. They used the following fungicides with protectant activity: Chorus (cyprodinil), Bravo (chlorothalonil), Copper (copper oxychloride), Dithane (mancozeb), Thiragranz (thiram), Ziragranz (ziram), Polyram (metiram), Captan (captan), Delan (dithianon) and Goldazim (carbendazim, suspended in July 2010). They also used the following fungicides with protectant and/or post-infection activity: Tilt (propiconazole), Saprool (triforine) and Rovral (iprodione). The grower at Lake Boga 2 used only sulphur during 2009-2010. All products were applied as specified by the product label, mostly following resistance management guidelines. Fungicide applications for brown rot control are recommended during periods of known tissue susceptibility, i.e., bloom, shuck fall and pre-harvest, and may include recommendations for cover sprays during periods of green fruit. Recommendations do not take into consideration the inoculum load and severity of infection periods when deciding if a cover spray is required.

Isolations were conducted from either mummified fruit or mature fruit to determine the species of *Monilinia* present in each block. Colony morphology was compared on PDA medium against similar age cultures of reference isolates of *M. fructicola* and *M. laxa* held in the VPIR Plant Pathology Herbarium. All isolates collected from field sites were *M. fructicola*.

Incidence of fruit rot was assessed on 20 fruit collected from each of 6 adjacent 10-15 m long rows where the weather station was located. The fruit was collected at commercial harvest, placed into plastic cup trays inside single layer cartons which were then enclosed in loosely folded polyethylene bags, to increase humidity, and incubated at 20°C. Fruit rot incidence was assessed at 7 and 12 days after initial incubation as described in more detail in chapter 6.

2.2.4 Analysis of orchard data

Data collected by weather stations was converted to hourly data to calculate brown rot IP during periods of wetness associated with rainfall. Growers received IP warnings by SMS when wetness periods were conducive to moderate to severe (e.g. >150 disease index) infection periods. Growers were told to use this information as a general guide for scheduling post-infection applications of fungicides when pre-infection spraying was not adequate, if possible, but more importantly for monitoring and improving the time of application of protective sprays.

For this study, it was assumed that a protectant fungicide would provide up to 10 days of good residual activity during fruit growth and 7-10 days during bloom as specified by the product labels. Fungicides with post-infection activity were assumed to be effective (kick back activity) in controlling infections if applied within 2 days after the start of an IP. At the end of the season, the performance of growers' spray programmes were examined in relation to the time of application and type of fungicide sprays used and incidence of fruit rot recorded at harvest. The data was closely examined in relation to the number of unprotected infection periods occurring during four key stages of crop development: bloom, eight weeks after bloom (post-bloom), pit hardening and three weeks before trial harvest to determine when fungicide control failures may have occurred. The analysis assumed that inoculum (conidia) was present in the orchard

during the growing season. The weather-based brown rot model used to identify IP does not take into account host/tissue susceptibility.

Results from controlled inoculations reported in chapter 3 showed that a wetness period of 10 hrs at 20°C (e.g. disease index 150) would be sufficient for spores of *M. fructicola* to infect young nectarine fruit (53 days after bloom) and green nectarine fruit (post pit hardening or 74 days after bloom) (Figure 1). Therefore it was assumed that a disease index of 150 was adequate as a minimum requirement for spores to infect susceptible tissue if tissue was unprotected or residual activity of protectant and timing of post-infection fungicide sprays was inadequate. It was also assumed that summerfruit crops in field sites were most susceptible to infection during flowering (blossom blight), within eight weeks after bloom and three weeks before pre-harvest and least susceptible during the pit hardening stages of fruit development (Biggs and Northover, 1988; Mari et al. 2003; Chapter X).

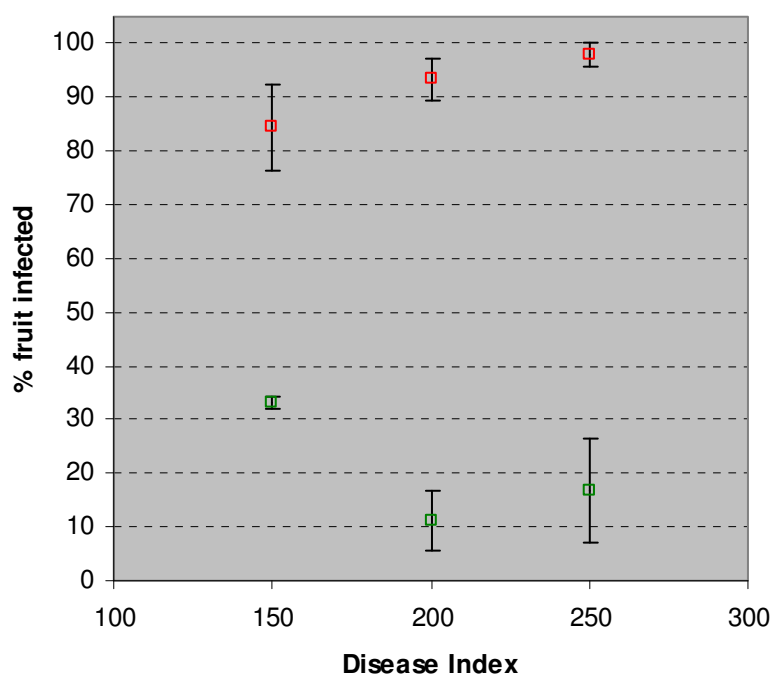


Figure 1. Percentage of nectarine fruit infected by *M. fructicola* in controlled inoculations. Nectarine fruit, picked at 53 (red symbols) and 74 days (green symbols) after bloom, were inoculated with $2-3 \times 10^5$ *M. fructicola* conidia per ml, incubated for 10 hrs at 15°C, 20°C and 25°C in wet conditions then air-dried in laminar flow cabinet and further incubated for 14 days at 20°C under high (53 days old fruit) or low (74 days old fruit) RH. Bars are SEM.

2.3 Results and Discussion

2.3.1 Plum sites

In the four seasons examined in two plum sites, fruit rot incidence at harvest ranged from 0% to 9.2% (Table 1). At Swan Hill 2, infection periods (IP) recorded during bloom and 3 weeks pre-harvest periods were properly covered with a variety of protectant and post-infection fungicides during the three growing seasons in the uncovered and netted blocks (Table 1, Figure 2). In the netted block, there was one unprotected infection period (UIP) in 2009-2010 and two in 2010-2011 during the post bloom period, while in the uncovered block there were two UIP during the same period (Table 1, Figure 3). One UIP was recorded in each of four seasons during the pit hardening period.

Control of brown rot on plums was excellent (0% fruit infected) during the first two seasons (2008-2010) in the two blocks (Table 1, Figure 2 and 3). In the 2010-2011, however, fruit rot incidence was 5.0% and 9.2% in both blocks which had two UIP during the post bloom period when susceptible fruit was probably not well protected with fungicide. A close examination of the spray program during 2010-2011 (e.g. under net) also showed that there was a period of frequent and heavy rain during the pit hardening period (14/11/2010 – 12/12/2010) when green fruit, supposed to be less susceptible to infection, was wet for long periods and not completely protected by the fungicides applied pre-infection (Figure 4). Stonefruit at pit hardening has been reported to be less susceptible to infection (Biggs and Northover, 1988, Fourie and Holz 2006, Mari et al. 2003). However, results from controlled inoculations showed that under optimal temperature and wetness conditions for infection, nectarine and peach fruit picked post pit hardening could be infected by conidia of *M. fructicola* (chapter 3). It is also possible that the residual activity of the protectant fungicide used during this time may have not lasted long enough to protect actively growing tissue against infection due to the wash-off effect from rain reducing fungicide efficacy.

At the other plum site (Shepparton), 9.2% of fruit was infected at harvest in 2007-2008 after leaving trees unprotected during IP occurring during post bloom (2 UIP), pit hardening (5 UIP) and preharvest (2 UIP) (Table 1, Figure 2 and 3). In the following season (2008-2009), fruit infections were not detected despite leaving trees unprotected during bloom (1 UIP), post bloom (1 UIP), pit hardening (5 UIP) and preharvest (2 UIP) (Table 1, Figure 2 and 3).

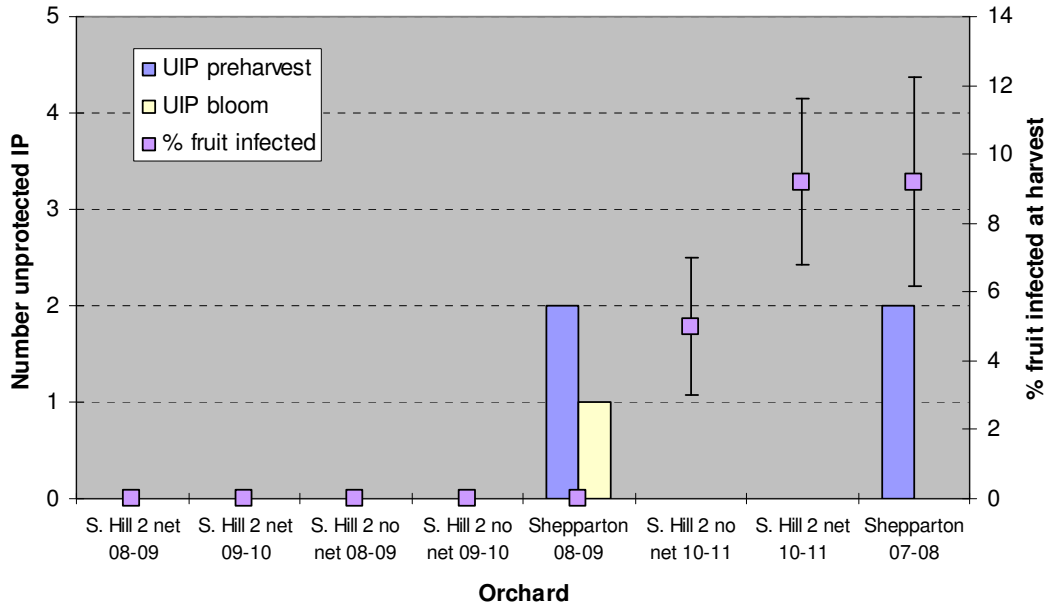


Figure 2. Number of unprotected infection periods (UIP) during bloom and preharvest at plum sites in relation to fruit brown rot incidence at harvest. Bars are SEM.

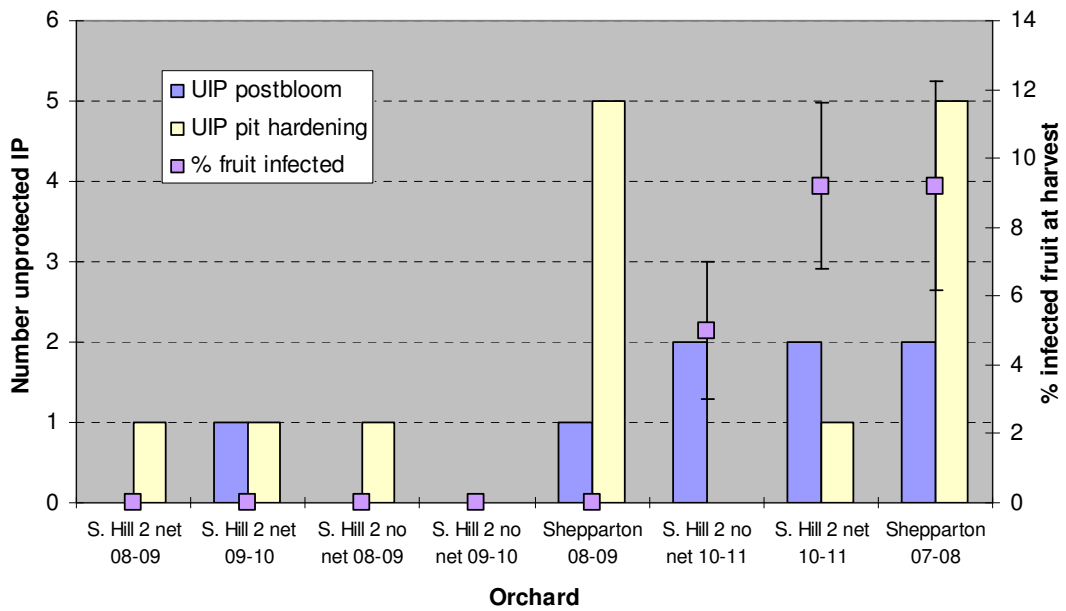


Figure 3. Number of unprotected infection periods (UIP) during post bloom and pit hardening at plum sites in relation to fruit brown rot incidence at harvest. Bars are SEM.



Figure 4. Occurrence of rain, IP and spray program implemented at S. Hill 2 plum block (netted) during 2010-2011 season. Red circles represent examples of IP left unprotected either by using unsuitable product or inadequate time of application. Rainfall from previous two seasons included for comparison.

2.3.2 Nectarine sites

In the five seasons examined at three nectarine sites, fruit rot incidence at harvest ranged from 0% to 11.7%, with the exception of Swan Hill 1 which had 90.8% of fruit rot in the wet season of 2010-2011 (Table 2, Figure 5 and 6). Fruit rot levels were low (0% - 0.9%) during four seasons at two sites. These sites had a very low number of UIP during periods of high tissue/fruit susceptibility, 1 UIP at bloom (Lake Boga 1 2008-2009 and S. Hill 1 2008-2009) and 2 UIP during preharvest (S. Hill 1 2006-2007). During post bloom, one UIP was recorded at S. Hill 1 (2008-2009), two at Lake Boga 1 (2008-2009) and S. Hill 1 (2006-2007) and three at S. Hill 1 (2009-2010). During pit hardening, three UIP were recorded at the Lake Boga 1 (2008-2009) and one at the other site.

In the other eight seasons examined, with the exception of Swan Hill 1 (2010-2011), fruit rot levels (2.5 – 11.7%) and the number of UIP during key stages of fruit development were generally higher than in sites/seasons with low disease. During these seasons, four sites had 1 or 2 UIP during preharvest (Ardmona 2006-2007, 2008-2009, 2010-2011, S. Hill 1 2007-2008) and four UIP during bloom (Ardmona 2006-2007). The same sites had 3-7 UIP during post bloom and 2-7 UIP during pit hardening. The three other sites had no UIP during bloom or preharvest (Ardmona 2009-10, 2007-2008; Lake Boga 1 2007-2008) but 3-6 UIP during post bloom and 5-8 UIP during pit hardening.

In the wet season of 2010-2011, Swan Hill 1 had only one UIP during bloom and post bloom, respectively, and three during pit hardening (Figure 5 and 6). Despite this fruit rot levels were extremely high (90.8%) and included extensive shoot blight infection damaging nectarine trees and significantly increasing inoculum for the following season. A close examination of the spray program implemented at this site showed that there were two periods of frequent and heavy rain during pit hardening (20/11/2010 – 23/12/2010) and preharvest (10/01/2011 – 15/01/2011) when the effectiveness of fungicides may have been reduced by wet weather (Figure 7). During pit hardening, the residual activity of propiconazole was probably not sufficient to protect against infection on actively growing fruit and shoot tissue during protracted wet periods. Results from controlled inoculations showed that detached nectarine fruit picked from Swan Hill 1 (2010 – 2011) at post bloom (53 days after bloom) and at pit hardening was infected by conidia of *M. fructicola* under optimal temperature and moisture conditions such as those recorded during pit hardening at S. Hill 1 (Figure 1, Chapter 3). Infections were not detected on the same fruit incubated untreated under similar conditions, indicating that fruit infection may have occurred during the wet period between 20/11/2010 and 23/12/2010 as the third test showed high levels of fruit infection 3 weeks before harvest (Figure 7). It is unknown if the first two samples of fruit tested had latent infections which could not be detected due to the immaturity of the fruit. The effect of rain on fungicide retention (wash-off) may be another factor that affected fungicide efficacy as well as inoculum load in both periods but especially preharvest.

At Swan Hill 1, the proportion of IP that were left unprotected from the total recorded during each of the four stages of fruit growth examined decreased noticeably from 2007-2008 to 2010-2011, with a corresponding reduction of fruit rot except in 2010-2011 due to high disease pressure conditions discussed earlier (Figure 8). The proportion of sprays that were in agreement with IP predictions also considerably

increased indicating that the grower used IP warnings to improve the time of application of fungicide sprays to protect trees against infection during IP. The grower at Ardmona focused on protecting the crop during bloom and preharvest which resulted only in very small reductions of fruit rot from 11.7% (2007-2008) to 3.3-2.5% (2009-2010) but rot levels increased again in 2010-2011 due to high disease pressure (Figure 9).

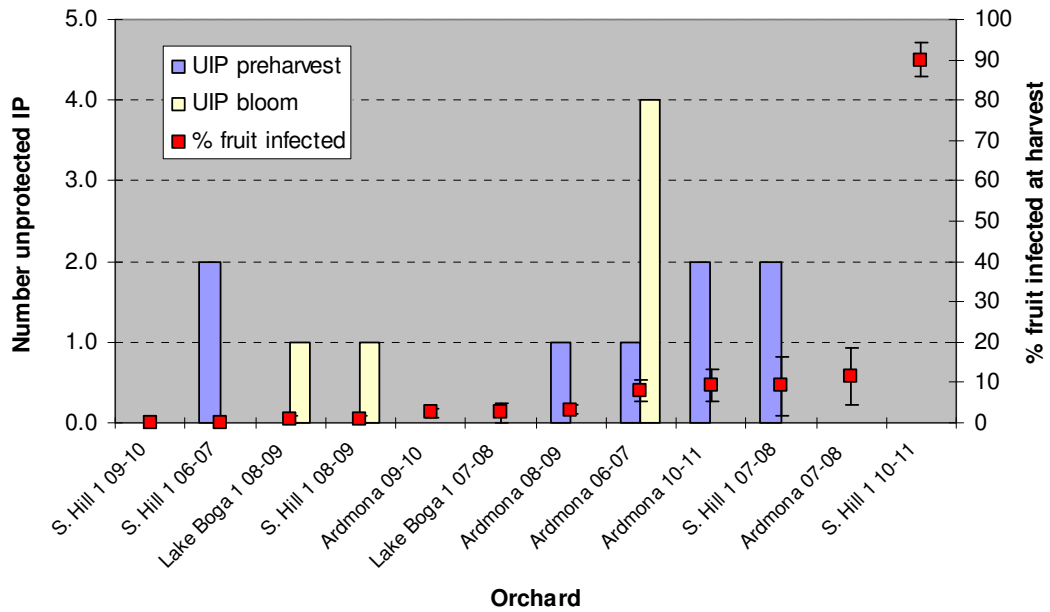


Figure 5. Number of unprotected infection periods (UIP) during bloom and preharvest at three nectarine sites in relation to incidence of fruit brown rot at harvest. Bars are SEM.

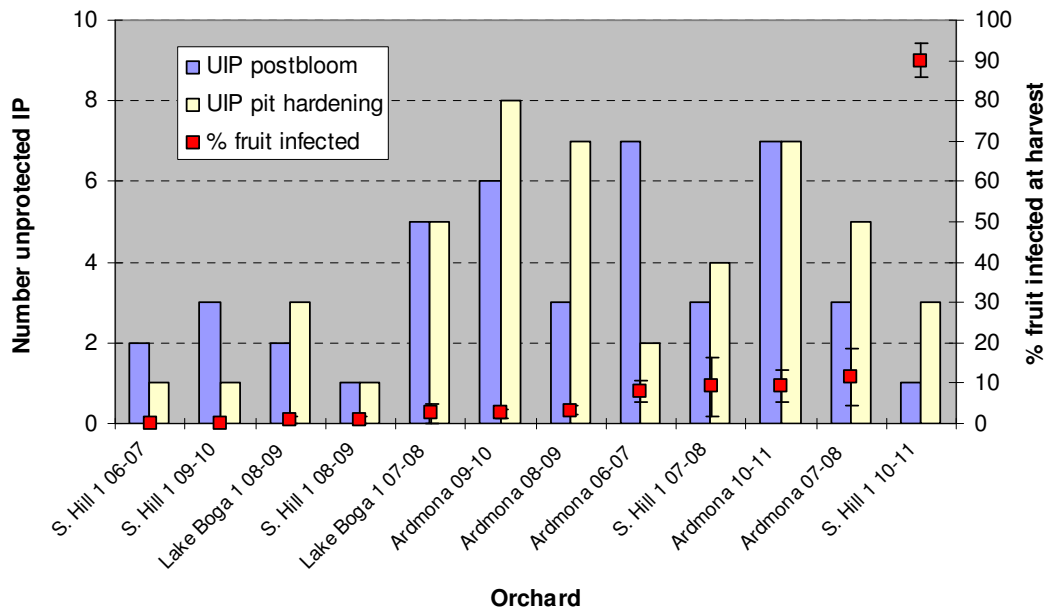


Figure 6. Number of unprotected infection periods (UIP) during post bloom and pit hardening at three nectarine sites in relation to incidence of fruit brown rot at harvest. Bars are SEM.

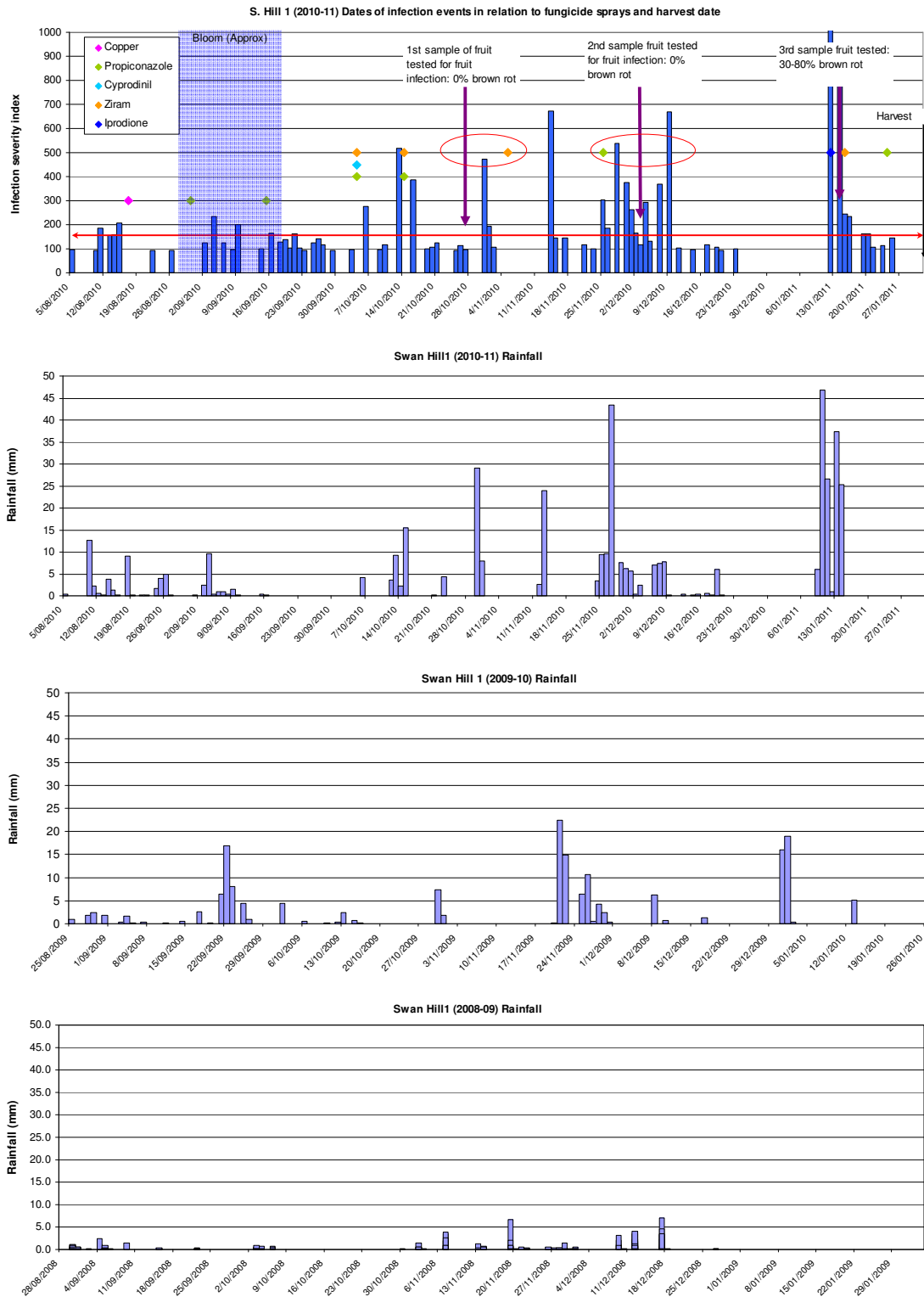


Figure 7. Occurrence of rain, IP and spray program implemented at S. Hill 1 nectarine block during 2010-2011 season. Red circles represent examples of IP left unprotected due to either use of unsuitable product or inadequate time of application and residual activity. Rainfall from previous two seasons included for comparison.

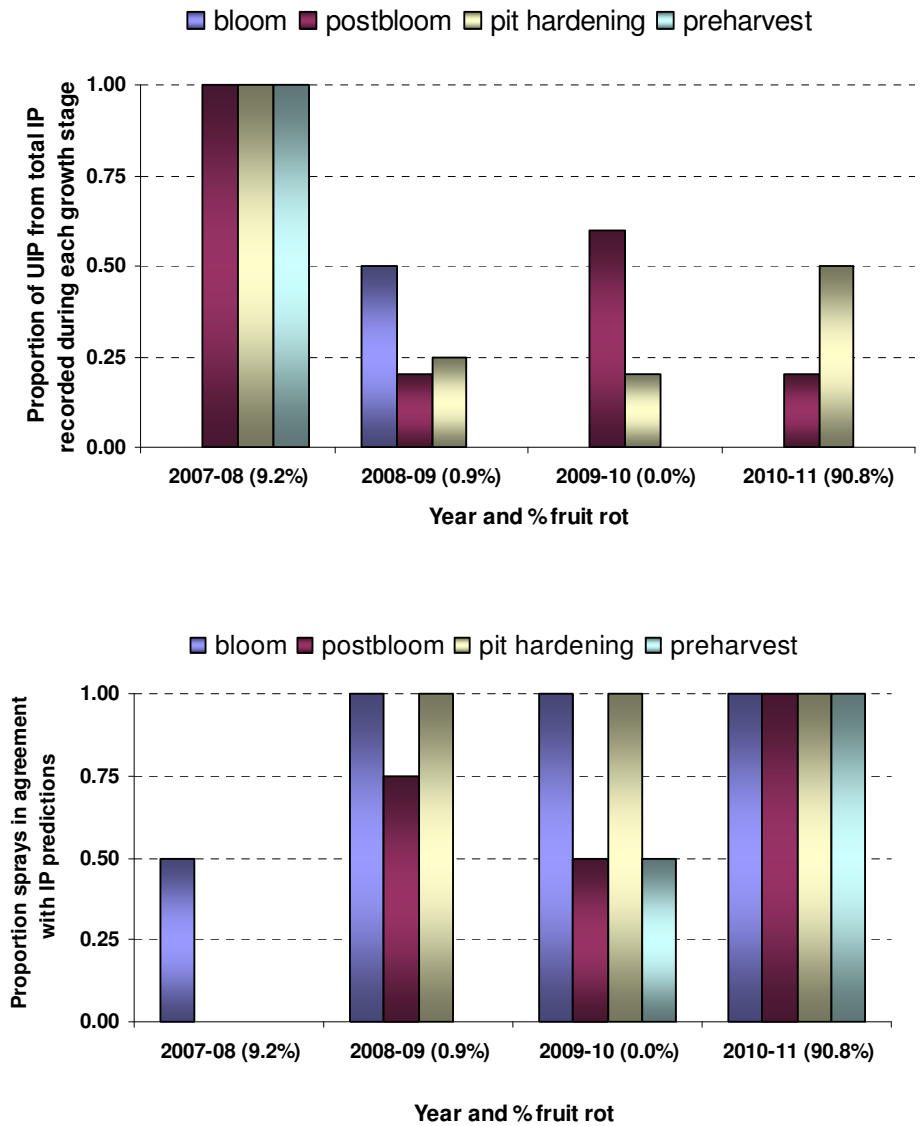


Figure 8. Proportion of UIP from total recorded at bloom, post bloom, pit hardening and preharvest (top) and proportion of sprays in agreement with IP predictions (bottom) at S. Hill 1 nectarine block during four seasons.

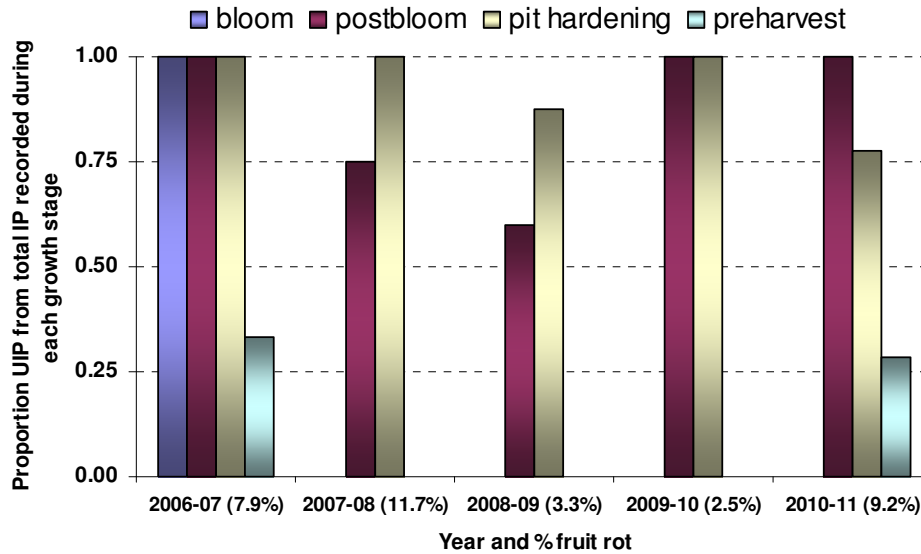


Figure 9. Proportion of UIP from total recorded at bloom, post bloom, pit hardening and preharvest at Ardmona nectarine block during five seasons.

2.3.3 Peach sites

In the seasons examined in eight peach sites, fruit rot incidence at harvest ranged from 0% to 63.37%, with most of the highest levels of fruit rot (18.3% - 63.3%) recorded during the wet season of 2010-2011 (Table 3, Figure 10 and 11).

Levels of fruit rot were low (0% to 3.3%) at five sites that had, with the exception of N. Shepparton (2008-2009), no UIP during bloom or preharvest, 1-2 UIP during post bloom (Cobram 2 2009-2010, N. Shepparton 2008-2009, Cobram 2 2008-2009), and 1-4 UIP during pit hardening except Cobram 2 (2009-2010) which had 16 UIP.

Fruit rot levels ranged from 5.0% and 17.5% in seven other sites (Table 3, Figure 10 and 11). Four of these seven sites had 1-2 UIP either during bloom or preharvest and the other three sites had 2 UIP during post bloom and 2-4 UIP during pit hardening. The rest of the sites had extremely high levels of fruit rot (28.3% - 63.3%). These sites, with the exception of Renmark, had 1-4 UIP during either bloom, post bloom or preharvest and 2-12 UIP during pit hardening, with six of these nine sites/seasons occurring during the wet season of 2010-2011 including Renmark.

At N. Shepparton, the proportion of IP that were not covered with fungicides decreased noticeably from 2007-2008 to 2010-2011, with corresponding reductions of fruit rot, except 2010-2011 due to high disease pressure (Figure 12). The proportion of sprays that were in agreement with IP predictions increased during this period indicating that the grower also used IP warnings to improve the time of application of fungicide sprays to protect trees against infection during IP.

A close examination of spray programs for two peach sites (Warrandyte and Cobram 2) with high levels of fruit rot (38.3% - 60%) during the 2010-2011 season revealed that trees at these sites were well protected with fungicides during bloom and 3 weeks preharvest when floral tissue and maturing fruit is highly susceptible to infection

(Figure 13 and 14). During the post bloom period there were only 1-2 UIP at both sites and 2 UIP during pit hardening period (19/11/2010 – 24/12/2010) at Warrandyte (38.3% fruit rot). However, 13 IP were not protected with fungicides at Cobram 2 (60% fruit rot) during the pit hardening period (25/11/2010 – 12/02/2011). The reason for potential control failures with fungicides in these peach sites could be related to susceptibility of green fruit under high disease pressure and reduced fungicide efficacy as discussed for plums and nectarines.

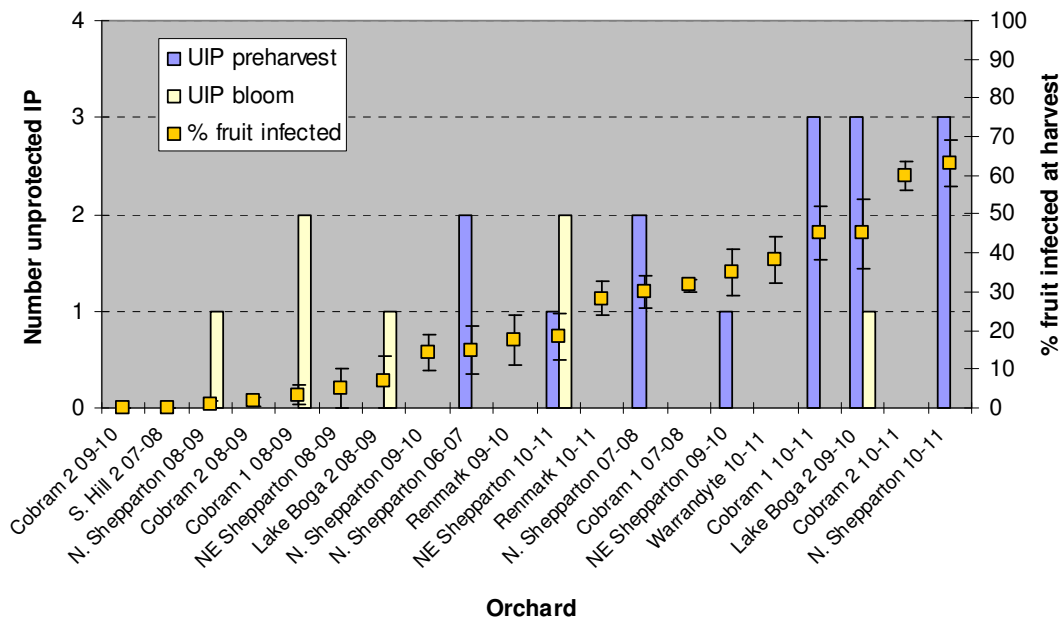


Figure 10. Number of unprotected infection periods (UIP) during bloom and preharvest at peach sites in relation to incidence of fruit brown rot at harvest. Bars are SEM.

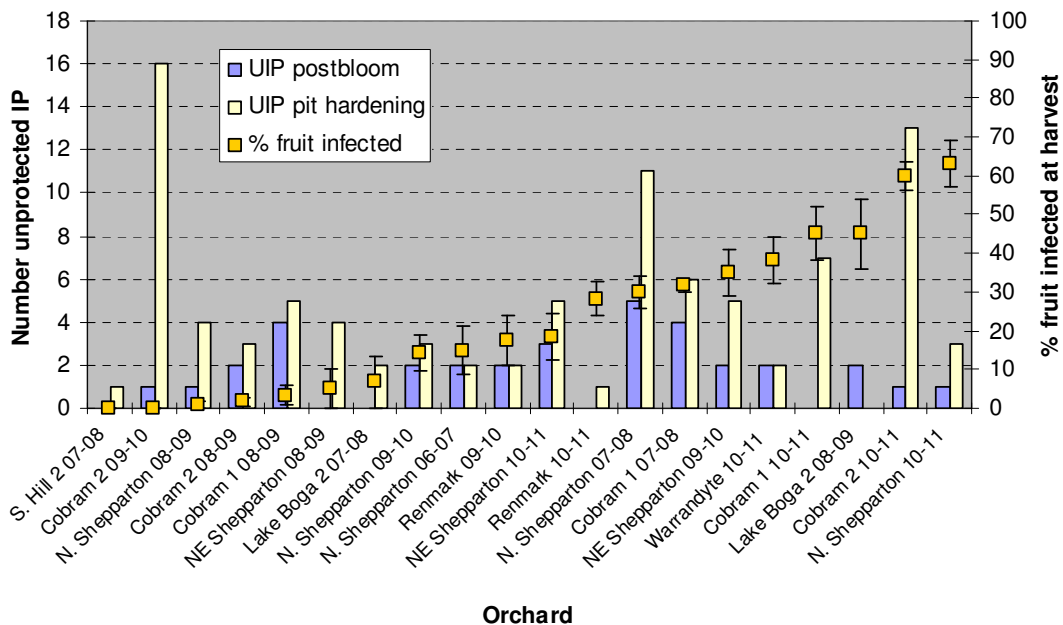


Figure 11. Number of unprotected infection periods (UIP) during post bloom and pit hardening at peach sites in relation to incidence of fruit brown rot at harvest. Bars are SEM.

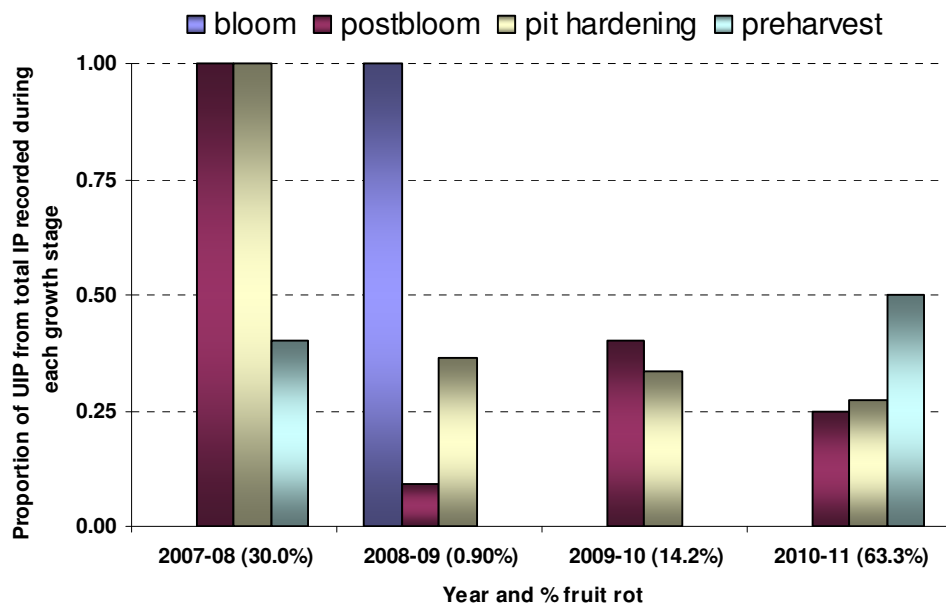


Figure 12. Proportion of UIP from total recorded at bloom, post bloom, pit hardening and preharvest (top) and proportion of sprays in agreement with IP predictions (bottom) at N. Shepparton peach block during four seasons.

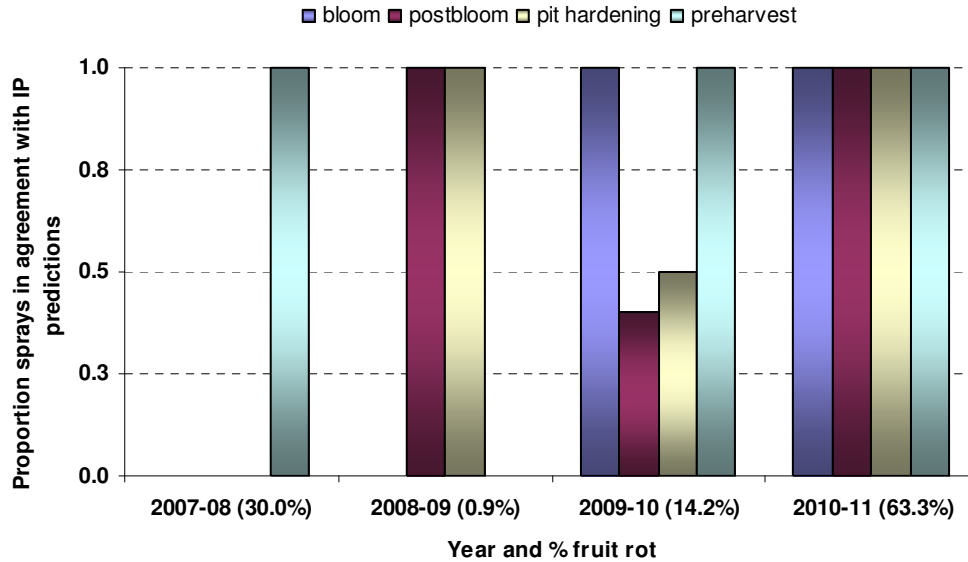


Figure 13. Occurrence of rain, IP and spray program implemented at Warrandyte peach block during 2010-2011 season. Red circles represent examples of IP left unprotected either by inadequate time of application or residual activity.

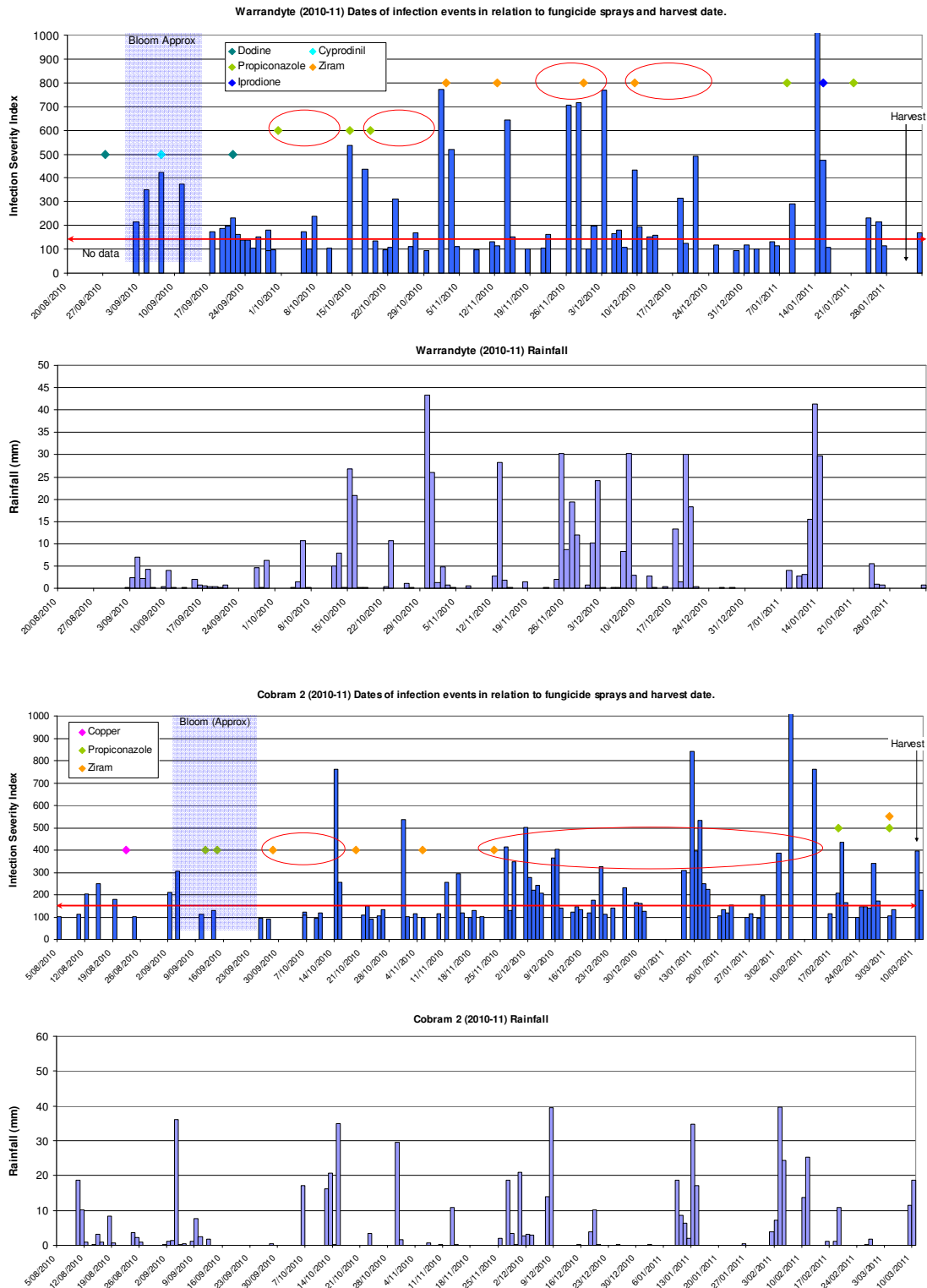


Figure 14. Occurrence of rain, IP and spray program implemented at Cobram 2 peach block during 2010-2011 season. Red circles represent examples of IP left unprotected either by inadequate time of application and residual activity or lack of sprays.

2 4 Conclusions

The network of weather stations used was reliable and provided site-specific weather data needed for estimating infection periods using the peach brown rot model. Grower collaborators reported that infection period (IP) forecasting provided by SMS helped to better schedule post-infection fungicide spraying for control of brown rot in stonefruit orchards in Victoria. IP warnings also allowed growers to monitor and better time application of protectant fungicides.

This study also used IP predictions to examine the performance of spray programs applied by grower collaborators. The peach brown rot model was very useful for identifying periods of wetness that were conducive to floral tissue (blossom blight) and fruit infection. The typical control program used by growers involved a series of protectant (cover) sprays during bloom and fruit development stages (e.g. post bloom and pit hardening stages), combined with post-infection sprays applied soon after suspected IP using products with curative activity. Products with both curative and some protectant activity were used mainly preharvest.

In general, analysis of spray programs in relation to fruit rot at harvest showed that growers left trees unprotected during several to many weather related (IP) events conducive to infection. Results showed that control of brown rot was more effective in sites/seasons where trees were well protected with fungicide during IP occurring through the periods of bloom and 3 weeks before harvest with only one or no IP left unprotected during the post bloom period (eight weeks after bloom) when fruit is highly susceptible to infection. For instance, good disease control was obtained at S. Hill 1 2009-2010 (nectarine), S. Hill 2 2008-2010 (plum) and Cobram 2 2008-2009 (peach) by protecting trees against infection during all IP during bloom and preharvest and leaving only a few IP unprotected during post bloom and pit hardening periods. In contrast, sites with high levels of fruit rot had greater number of IP unprotected during the three key periods of stonefruit growth when floral tissue and/or fruit is reported to be highly susceptible to infection by *M. fructicola*. Good control of blossom blight is required to reduce inoculum available for infection later in the season. However, it was assumed that conidia are available from other inoculum sources in the orchard for infections during the growing season.

Examination of three sites where IP and spray programs were monitored in the same block over 3-4 years showed that IP forecasting contributed to better scheduling of fungicide sprays resulting in a reduction of IP left unprotected during key stages of fruit growth, which corresponded well with some reduction of fruit rot at harvest. Successful spray timing requires accurate prediction of IP the application of the fungicide just before the wet event (protectant) and within the kick-back period to optimise fungicide efficacy.

Some sites with low disease at harvest had a few unprotected IP during the pit hardening period (e.g. from end post bloom period to 3 weeks before harvest), suggesting that fruit may be less susceptible to infection during this period as reported by overseas researchers. However, disease pressure conditions were different during the seasons examined. 2010-2011 was the most challenging season for growers with some fungicide programs failing to control brown rot despite growers doing a good job

covering most of the extremely large number of IP recorded during the growing season. Fungicide failures could have been due to the effect of rainfall quantity and intensity on fungicide persistence (residue) and efficacy, especially for protectants. However, data on fungicide residue is not available to verify this theory. Contact fungicides are sprayed over canopies to provide a protective layer and thus prevent the establishment of fungal infections. Repeated applications are required to maintain the protective layer on the surface of expanding foliage or enlarging fruit. It is well known that rain removes large portions (wash-off) of agrochemicals deposited on plant surfaces with the main factors involved in the wash-off being rain intensity, rain quantity, time between application of sprays and rainfall onset, pesticide formulation, water solubility of the active and type of crop (Cabras et al. 2001, Hunsche et al. 2007). Sizes and surfaces of stonefruit also vary greatly at different growth stages probably influencing the retention of fungicides under different moisture conditions. More work is required to determine the efficacy of current protectant and curative fungicides under frequent and heavy rain for different stonefruit including evaluation of new fungicides not available to the stonefruit industry in Australia. This is needed to develop a better strategy for controlling brown rot during such extreme wet events.

It is unknown whether populations of *M. fructicola* in the field sites have become tolerant to field rates of eradicant fungicides regularly used such as propiconazole and iprodione. Overseas *Monilinia* spp. resistant to iprodione and propiconazole have been reported on stonefruit (Schnabel and Bryson 2004, Yoshimaru et al. 2004), however, a systematic study on sensitivity of *Monilinia* spp. to dicarboximide and DMI fungicide groups is lacking in Australia. Limited *in vitro* work showed that some of the nineteen isolates of *M. fructicola* tested from stonefruit orchards in Victoria varied in their tolerance to iprodione and propiconazole (chapter 5). More data is required to determine the extent of reduced sensitivity to iprodione and overall sensitivity to propiconazole in orchards for establishing baseline activities for future monitoring and develop strategies for the use of these two fungicides within brown rot control programs.

Failures could also have been due to the effect of long periods of wetness in warm temperatures on susceptibility of green fruit during the pit hardening period when a large number of IP were not covered with fungicides. Our controlled inoculations have clearly shown that detached green fruit (nectarine and peaches) at pit or post hardening can be infected by *M. fructicola* under continuous moisture conditions at optimal temperatures for infection. More data is required to determine susceptibility of different plum, nectarine and fresh and canning peach cultivars at different stages of fruit maturity to *M. fructicola*, especially during the pit hardening period under different inoculum and moisture levels. This work should include determining the minimum temperature and moisture requirement for spore infection to enhance the accuracy of the peach brownrot model for IP forecasting. The precision of the predictive model can be improved through better understanding of the interactions of tissue/fruit characteristics, environmental conditions and fungicide activity. The influence of IP severity and dew events on fruit rot at harvest also needs to be considered in relation to other orchard factors influencing disease development. While dew can provide the necessary moisture for the infection process, the duration of wetness is not usually long enough at low temperatures in the morning to qualify for IP.

The success of using IP forecasting for improving brown rot control depends on accurate monitoring of weather variables and knowledge of source of inoculum, disease

cycles, susceptibility of stonefruit cultivars and efficacy of fungicides (Tate and Manktelow 1992). Conidia of *M. fructicola* appear to be the main source of inoculum for infections in the sites investigated. However, further work is required to determine this is the only source of inoculum across stonefruit growing regions including low chill fruit. Good control of brown rot and orchard sanitation would reduce the amount of inoculum available for future infections. All these factors and practices including historical information at the block level must be integrated into a disease management system for more effective management of brown rot in stonefruit with potential associated reduction in fungicide applications and risks of development of fungicide resistance.

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Table 1. Summary of IP occurrence, number of sprays, unprotected infection periods (UIP) during four stages of fruit development and incidence of fruit rot at harvest at two plum sites over three seasons in Victoria.

Orchard	Year	Net	Total infection periods	Total number of sprays	Number of infection periods (IP), unprotected infection periods (UIP) and sprays applied during four key stage of crop development												Fruit rot
					Bloom			Post-bloom			Pit-hardening			Pre-harvest			
					IP	UIP	Sprays	IP	UIP	Sprays	IP	UIP	Sprays	IP	UIP	Sprays	
S. Hill 2	08-09	yes	13	10	2	0	2	4	0	3	5	1	3	2	0	2	0.0
		no	13	10	2	0	2	4	0	3	5	1	3	2	0	2	0.0
	09-10	yes	15	13	3	0	3	6	1	5	4	1	2	2	0	2	0.0
		no	12	12	3	0	2	4	0	6	3	0	2	2	0	1	0.0
	10-11	yes	18	12	3	0	3	5	2	5	6	1	3	1	0	1	9.2
		no	17	11	3	0	3	6	2	3	7	0	4	1	0	1	5.0
Shepparton	07-08	-	9	5	0	0	3	2	2	2	5	5	0	2	2	0	9.2
	08-09	-	11	3	1	1	1	2	1	1	6	5	1	2	2	0	0.0

Table 2. Summary of IP occurrence, number of sprays, unprotected infection periods (UIP) during four stages of fruit development and incidence of fruit rot at harvest at three nectarine sites over many seasons in Victoria.

Orchard	Year	Total infection periods	Total number of sprays	Number of infection periods (IP), unprotected infection periods (UIP) and sprays applied during four key stage of crop development												Fruit rot
				Bloom			Post-bloom			Pit-hardening			Pre-harvest			
				IP	UIP	Sprays	IP	UIP	Sprays	IP	UIP	Sprays	IP	UIP	Sprays	
Ardmona	06-07	16	2	4	4	0	7	7	0	2	2	0	3	1	1	7.90
	07-08	11	6	0	0	2	4	3	1	5	5	0	2	0	3	11.7
	08-09	14	8	1	0	3	5	3	3	8	7	0	2	1	2	3.33
	09-10	17	5	2	0	2	6	6	1	8	8	0	1	0	1	2.50
	10-11	26	6	3	0	2	7	7	0	9	7	2	7	2	2	9.20
Swan Hill 1	06-07*	8	5	2	0	2	3	2	3	1	1	0	2	2	0	0.00
	07-08	10	3	1	0	2	3	3	0	4	4	0	2	2	1	9.20
	08-09	11	6	2	1	1	5	1	4	4	1	1	0	0	0	0.90
	09-10	12	7	2	0	2	5	3	2	5	1	2	0	0	1	0.00
	10-11	17	10	3	0	2	5	1	3	6	3	1	3	0	3	90.8
Lake Boga 1	07-08	12	7	0	0	2	5	5	0	5	5	0	2	0	4	2.50
	08-09	10	5	1	1	0	4	2	1	5	3	2	0	0	2	0.83

* different block used in the first year.

Table 3. Summary of IP occurrence, number of sprays, unprotected infection periods (UIP) during four stages of fruit development and incidence of fruit rot at harvest at eight nectarine sites over many seasons in Victoria.

Orchard	Year	Total infection periods	Total number of sprays	Number of infection periods (IP), unprotected infection periods (UIP) and sprays applied during four key stage of crop development												Fruit rot	
				Bloom			Post-bloom			Pit-hardening			Pre-harvest				%
				IP	UIP	Sprays	IP	UIP	Sprays	IP	UIP	Sprays	IP	UIP	Sprays		
N. Shepparton	06-07*	9	5	2	0	3	2	2	1	2	2	0	3	2	1	14.9	
	07-08	21	11	0	0	3	5	5	2	11	11	0	5	2	4	30.0	
	08-09	27	10	1	1	1	11	1	5	11	4	3	4	0	1	0.90	
	09-10	13	13	1	0	2	5	2	5	9	3	4	3	0	2	14.2	
	10-11	23	13	2	0	3	4	1	3	11	3	3	6	3	2	63.3	
Cobram 1	07-08	17	7	0	0	2	4	4	0	7	6	1	4	0	4	31.7	
	08-09	13	5	2	2	0	4	4	0	5	5	0	2	0	5	3.33	
	10-11*	24	13	4	0	4	6	0	5	8	7	2	7	3	2	45.0	
NE Shepparton	08-09	15	9	1	0	1	3	0	3	10	4	3	1	0	1	5.00	
	09-10	19	8	2	0	2	5	2	2	8	5	1	4	1	2	35.0	
	10-11	19	10	3	2	1	6	3	4	7	5	3	3	1	3	18.3	
Cobram 2	08-09	19	9	1	0	2	8	2	4	6	3	2	3	0	1	1.70	
	09-10	28	9	3	0	3	5	1	3	16	16	0	4	0	3	0.00	
	10-11	25	9	2	0	3	4	1	3	16	13	1	3	0	3	60.0	
Lake Boga 2	07-08	13	10	1	1	2	5	0	4	6	2	3	1	0	1	6.70	
	08-09**	14	11	1	1	0	4	2	4	6	0	5	3	3	2	45.0	
Renmark	09-10	10	9	2	0	3	3	2	2	3	2	1	2	0	3	17.5	
	10-11	18	18	3	0	3	4	0	4	8	1	5	3	0	6	28.3	
Warrandyte	10-11	30	13	4	0	2	12	2	6	10	2	2	4	0	3	38.3	
S. Hill 2	07-08	13	10	0	0	3	6	0	4	7	1	2	0	0	0	0.0	

* different block used.

** = only sulphur sprays used.

3. Susceptibility of stonefruit to infection by *Monilinia fructicola* during different stages of fruit maturity

3.1 Introduction

Brown rot, caused by *Monilinia fructicola*, continues to be a major field and postharvest disease problem for stone fruit growers in Australia. In Victoria, brown rot is the most serious disease of fresh and canning stone fruit varieties, particularly nectarines and peaches in most fruit growing districts (Holmes et al. 2008). The industry relies on fungicide applications during fruit development and postharvest, in combination with low temperature storage for brown rot control. Despite these practices, heavy losses still occur especially when wet weather conditions prevailed during spring and summer of 2010-2011. Some of the reasons for poor disease control in the field may include high levels of disease carry over, inadequate timing of fungicide applications, lack of information on fungicide efficacy and new effective fungicide treatments, and the possibility of fungicide resistance. The development of new tools to aid disease management and control strategies is therefore urgently needed for improving brown rot control and reducing current losses in the field and postharvest.

Information on the susceptibility of different stone fruit crops is vital for optimising the time of application of disease control strategies (i.e. fungicides, biocontrols) during periods favourable for *Monilinia* spp. infection. The effect of fruit maturity on the susceptibility to *Monilinia* spp. was investigated in peaches and apricots during most of the fruit development stages in Canada and Italy (Biggs and Northover 1988, Mari et al. 2003). Biggs and Northover (1988) reported that peach fruit inoculated with *M. fructicola* were highly susceptible to infection for approximately 2 to 3 weeks beginning the week after the period of abscission of non-pollinated or aborted fruit and 2 weeks before full ripeness but resistant at pit hardening. Mari et al. (2003) showed that inoculated apricots and peaches also had similar susceptibilities to *M. laxa*, with green fruit at the pit hardening stage being the most resistant to infection. Wounded and unwounded fruit were shown to have the same susceptibility to *M. laxa*.

Little information is available on the precise susceptibility of canning peaches and nectarine at different growth stages in Australian orchards to *Monilinia* spp. Attempts in the 2008-2010 seasons by the project team to investigate the susceptibility of canning peaches by inoculating in the field peach cv. 'Tatura 204' and 'Taylor Queen' yielded variable data. It was felt too many influences in the field, i.e. high levels of brown rot towards harvest; damage caused by storm or Carpophilus beetles near harvest; uneven retention of surface wetness or build up of high temperatures on bagged fruit after inoculation; had possibly masked differences between disease levels on fruit inoculated at different growth stages. It was necessary to conduct investigations under controlled environmental conditions where the impacts of some of these variables can be minimised.

The objectives of this study were therefore to investigate the susceptibility of a canning peach cv. 'Tatura 204' and fresh market nectarine cv. 'August Pearl' to brown rot, by inoculating fruit at various growth stages with conidia of *M. fructicola*, and incubating the inoculated fruit under controlled environmental conditions.

3.2 Materials and Methods

3.2.1 Sampling of peaches

Canning peach fruit, cv. 'Tatura 204' were collected from a commercial stonefruit orchard located in Toolamba, Goulburn Valley, Northern Victoria. The orchard has had brown rot in the previous season but had been sprayed to control the disease. Fruit were collected from six rows of trees from early fruit set to preharvest. Five fruit were collected from each of nine randomly selected trees per row. Each replicate consisted of ninety fruit from two adjacent rows, and fruit from two other separate rows made up a different replicate.

Fruit were harvested at four stages of fruit development in the 2010-2011 season. They were: small fruitlets at around shuck fall (7th October, 14 days after full bloom), pre-pit hardening (4th November, 52 days after bloom), after pit hardening (2nd December, 80 days after bloom) and preharvest (19th January, 128 days after bloom or 10 days before harvest). Fruit were transported overnight in chilled cartons and the treatments were applied the following day. An additional twenty fruit were collected until December, they were cut to check whether they were at pre or post pit hardening growth stage.

3.2.2 Inoculation and incubation of peaches

Ninety fruit in each replicate were divided into groups of 15 for application of three inoculation treatments and two storage temperatures. Three replicates of fruit were used for each inoculation and incubation combination. The inoculation treatments were: (1) control (uninoculated, unwounded), (2) inoculated and wounded, (3) inoculated but unwounded. The fruit were then incubated at two temperatures, 15°C and 20°C, in growth cabinets.

All of the fruit were washed in three changes of deionised water to remove possible carry over of brown rot spores from the field. The control fruit were dried in a laminar flow cabinet so that they are not wet when placed into incubation. Wounding to simulate wind rub was accomplished by rubbing the fruit surface with a folded piece of filter paper, to remove some of the fruit trichomes and slightly bruise the fruit surface.

The wounded and unwounded fruit were dip-inoculated for one minute in a suspension containing $1-2 \times 10^5$ *M. fructicola* conidia per ml. Conidia were washed from eight *M. fructicola* isolates grown on Potato Dextrose Agar (PDA) using deionised water, and the conidia concentration adjusted after counting using a haemocytometer. Fresh 1-2 week old colonies on PDA were used for harvesting the conidia. The eight *M. fructicola* isolates were obtained from naturally occurring fruit infections from several orchards in the Goulburn and Murray Valley regions. Isolates were collected over three seasons and, grown on PDA media, and then stored at 4°C in sterile water.

Fruit from each replicate, whilst still moist from the inoculation, was placed in individual cups of a plastic tray, the tray was laid inside a plastic box (6 x 18 cm), which was covered with a lid (Figure 1). Wet paper towels were placed below the tray of fruit, to maintain high humidity and to facilitate moisture retention and infection. The larger fruit collected near harvest date were individually placed in the cups of plastic

fruit tray liners, which fitted in single layer inside lidded cardboard fruit boxes. The boxes of fruit were then put inside large plastic bags to encourage build up of humidity inside the trays.

Both the inoculated and control fruit were incubated at 15 and 20°C, they were assessed for brown rot infections after 7 and 13 days.

3.2.3 Sampling of nectarines

Nectarine fruit cv. 'August Pearl' were collected from an orchard with a history of low incidence of brown rot. Fruit were collected from a section of the block (2 rows of 50 m long each) located in a summerfruit orchard in Swan Hill, north-west Victoria. Fruit were collected at three stages of fruit development on the 26th October (53 days after bloom), 2nd December (post pit hardening or 90 days after bloom) and on the 14th January 2010 (133 days after full bloom or around two weeks before commercial harvest). Fruit were stored in an 'esky' overnight at room temperatures and inoculated within 24 hours of harvest.

3.2.4 Inoculation and incubation of nectarines

Fruit were dip-inoculated in a suspension of $2-3 \times 10^5$ *M. fructicola* conidia per ml. The conidia preparation and *M. fructicola* culture collection procedures were as described in the above section for peaches. The inoculation treatments consisted of: (1) control (uninoculated, not wetted i.e. 'dry'), (2) control (uninoculated, wetted), (3) inoculated, wetted. For the uninoculated, wetted treatment, fruit was dipped in distilled water and incubated wet to determine if any natural infection on the fruit would develop after storage.

The inoculation and preparation of fruit in trays for incubation were similar to the procedures described for peaches.

Boxes containing the inoculated and control fruit were placed inside plastic bags, they were then incubated in growth cabinets, at 15, 20 or 25°C, for 10 hours with a relative humidity (RH) of 80-90% and under lights. Temperatures and RH. were monitored during the incubation period with TinyTag Hasting Data Loggers. Generally, after initial incubation, the boxes were removed from the growth cabinets, the wet paper removed, and the fruit allowed to dry inside a laminar flow cabinet for 30 minutes. The trays of fruit were then stored on the shelves with the lids on (simulating high humidity, >80% RH) or off (simulating low humidity, $\pm 40\%$ RH) in a controlled temperature room at 20°C. The fruit were then assessed for *M. fructicola* infection at 7 and 14 days after inoculation and the incidence of brown rot for each treatment was analysed.

There were three replicate boxes for each of the three treatments (inoculated, untreated dry and wet) with each box containing 15 (post bloom) or six fruit (pit hardening and preharvest). Fruit inoculated after bloom was incubated wet at 15°C, 20°C and 25°C for 10 hours initially, followed by drying for 30 minutes in a laminar flow cabinet and then incubated in the controlled temperature room at 20°C for 14 days under high humidity (lids on). Fruit from pit hardening were also incubated wet under the same temperatures and RH regimes, but the final incubation at 20°C was under low humidity (lids off), and included one treatment incubated initially wet at 20°C for 10 hours then a final

incubation under high humidity (lids on). Fruit collected before harvest were subjected only to initial wet incubation at 20°C, with the secondary incubation at low and high RH.

3.2.5 Data analysis

Peach data were analysed with general analysis of variance (ANOVA, Genstat, Lawes Agricultural Trust, Rothamstead Experimental Station, Harpenden, UK). Data for similar treatments were analysed across the different fruit stages. Treatment means were compared with Fischer protected least significant difference test (LSD, $P \leq 0.05$). Nectarine data were analysed with two-way analysis of variance (ANOVA, Genstat, Lawes Agricultural Trust, Rothamstead Experimental Station, Harpenden, UK). For the nectarine data (14 days assessment data), treatment (inoculation) and temperature (post-bloom and pit hardening only), and treatment and moisture (pre-harvest) were the factors in the ANOVA. Data for similar temperature (20°C) and moisture regime (high) across the three fruit stages were analysed with one-way analysis of variance. Treatment means were compared with Fischer protected least significant difference test (LSD, $P \leq 0.05$).

3.3 Results and Discussion

3.3.1 Susceptibility of peaches at different growth stages

Analysis of the percentage of fruit infected with brown rot after seven and 13-day incubation are presented for the four sampling dates preharvest (Tables 1-2). Fruit rots were more severe with longer incubation period, but other fungal and yeast infections also occurred, particularly when the fruit were incubated at the higher temperature of 20°C. For fruit at the shuck-fall, pre- and post pit hardening stages, data from longer incubation period of 13 days (Figure 3, Table 2) are presented because fruit rots for these growth stages were more evident than the seven day incubation period (Figure 2, Table 1).

Overall, peach fruit from the four growth stages differed in their susceptibility to brown rot, with the highest proportions of fruit showing disease symptoms near harvest (Table 1-2).

The control (uninoculated, unwounded) fruit had low levels of natural infection, with only 2% or less of the fruit developed brown rot (Table 1). This low background infection has allowed comparison of differences in the susceptibility of fruit at various growth stages, at incubation temperatures tested. Had high levels of natural infection occurred, the effects of inoculation would have been masked, as found in the field inoculation experiments.

Inoculated fruit incubated at 20°C developed between 17 to 84% brown rot. The lowest disease incidence was on fruit from the post pit-hardening stage (Table 1, Figure 2). The fruit from the preharvest stage developed significantly more brown rot than fruit from any other stages of growth. The unwounded fruit appeared to be less susceptible to infection at the post pit-hardening stage than at shuck-fall (Table 1).

The data showed increased susceptibility to brown rot with increased fruit maturity close to harvest (Figure 2). The trend in increased susceptibility was also observed for inoculated fruit incubated at 15 °C (Table 1). The data support previous reports ((Biggs and Northover 1988, Fourie and Holz 2003, Mari et al. 2003) that fruit after pit-hardening stage were less susceptible to brown rot infection, and fruit closer to maturity were highly susceptible to brown rot infection. Fourie and Holz (2003) showed that reduced disease incidence on nectarine fruit at the pit hardening stage was associated with resistance to *M. laxa* mycelial penetration and disease expression. Similar mechanisms might have occurred with canning peaches.



Figure 1: Peach fruitlets (post shuck-fall) in plastic trays after inoculation with conidia of *M. fructicola* (right). Peach fruitlet with *M. fructicola* sporulation (top left), an uninfected peach fruitlet (bottom left).

3.3.2 Effect of incubation temperatures on rot development

The fruit incubated at 15°C developed rots more slowly and had lower rot incidence than those incubated at 20°C (Table 1).

At 15°C, brown rot incidence ranged from 0 to 67%, with the highest incidence once again on fruit at preharvest growth stage; whilst the lowest incidence was after pit hardening for the unwounded fruit and before pit hardening for the wounded fruit.

The wounded fruit did not develop any more rots than the unwounded fruit (Table 1). The data is in agreement with the findings of Mari et al. (2003). This suggests that removal of surface hairs and slight bruising without breaking the fruit surface on canning fruit variety such as ‘Tatura 204’ had little influence in promoting infection by *M. fructicola*. It may also be possible that the exposed fruit surface did not contain stomata, considered to be the site of penetration for germinating conidia. Whether this suggests mechanical resistance rather than biochemical resistance had little influence on fruit susceptibility needs further evaluation because previous reports had conflicting conclusions (Jerome 1958, Mari et al. 2003). More studies on the germination and penetration of conidia on fruit surfaces may aid the understanding on the influence of wounding and fruit physiology on development of brown rot on various fruit varieties.

Table 1: Mean percentage of fruit infected by *Monilinia fructicola*, on inoculated and uninoculated fruit of four different growth stages, incubated for 7 days at 15 and 20°C.

Inoculation and incubation treatments	% fruit infected with brown rot after 7-day incubation				LSD (≤ 0.05)
	Fruit growth stage (days after full bloom)				
	24	52	80	128	
Inoculated, unwounded 20°C	44.4b	40.0ab	19.1a	84.4c	20.95
Inoculated+wounded, 20°C	40.0a	28.9a	16.7a	84.4b	37.29
Control Uninoculated, unwounded, 20°C	0	2.2	0.0	0	
Inoculated, unwounded 15°C	22.2ab	11.1a	7.1a	60.0b	38.19
Inoculated+wounded, 15°C	0.0a	0.0a	11.9a	66.7b	23.03
Control Uninoculated, unwounded 15°C	0	0	0	2.2	

LSD: Least significant difference at $P \leq 0.05$. Means (%) followed by different letters across the row indicate significant differences between fruit maturity stages.

Table 2: Mean percentage of fruit infected by *Monilinia fructicola*, on inoculated and uninoculated fruit of four different growth stages, incubated for 13 days at 15 and 20°C.

Inoculation and incubation treatments	% fruit infected with brown rot after 13-day incubation				LSD ($P \leq 0.05$)
	Fruit growth stage (days after full bloom)				
	24	52	80	128	
Inoculated, unwounded, 20°C	37.8 a	77.8 a	52.4 a	na	72.39
Inoculated+wounded, 20°C	48.9 a	77.8 b	52.4 a	na	20.60
Control Uninoculated, unwounded, 20°C	4.4 a	2.2 a	0 a	17.7 b	13.05
Inoculated, unwounded 15°C	24.4 a	33.3 a	21.4 a	na	45.97
Inoculated+wounded, 15°C	24.4 a	28.9 a	23.8 a	na	27.04
Control Uninoculated, unwounded 15°C	0	0	0	2.22	

LSD: Least significant difference at $P \leq 0.05$. Means (%) followed by different letters across the row indicate significant differences between fruit maturity stages. na – Fruit were discarded because all fruit were rotted in the inoculated treatments.

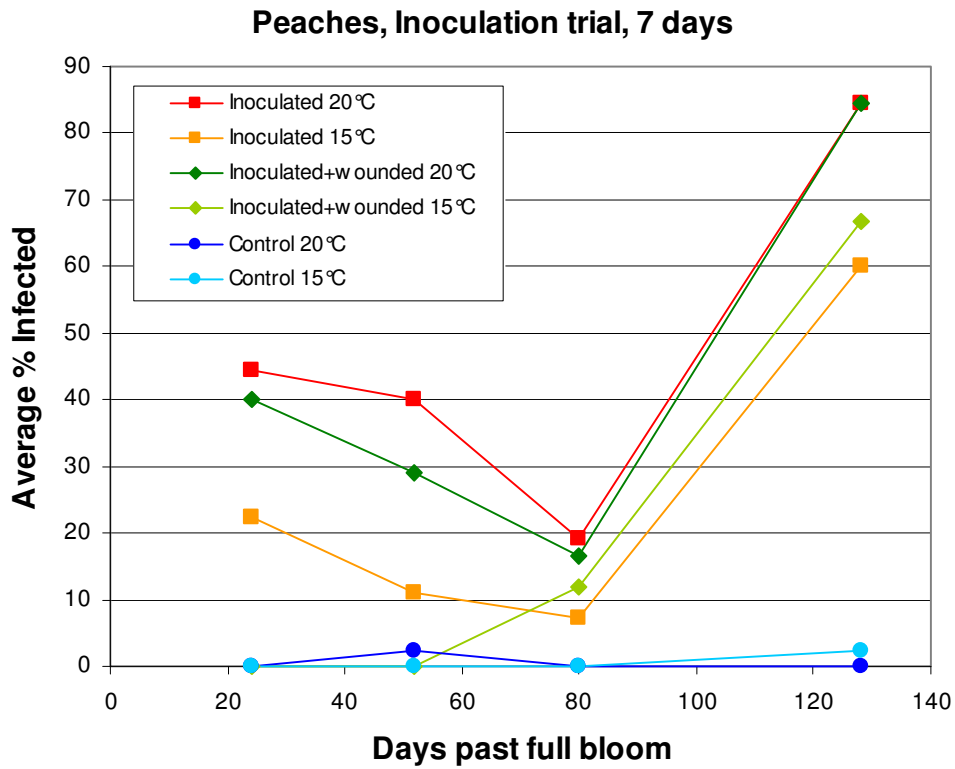


Figure 2: Incidence of brown rot infection by *Monilinia fructicola* on inoculated and control fruit of four different growth stages past full bloom, incubated for 7 days at 15 and 20°C.

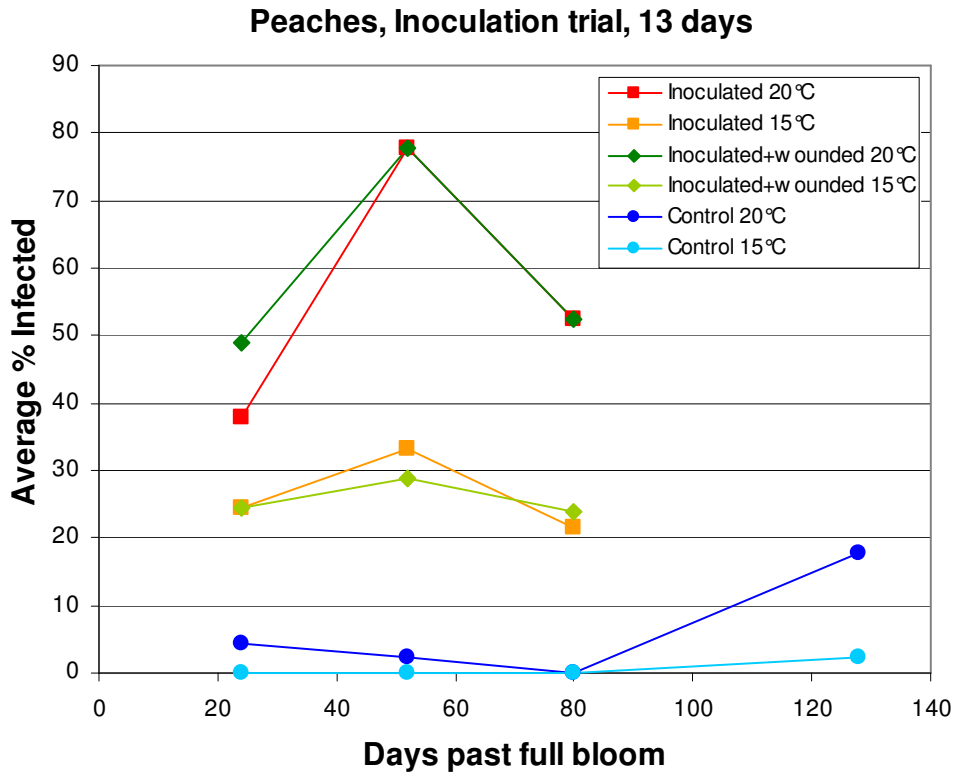


Figure 3: Incidence of brown rot infection by *Monilinia fructicola* on inoculated and control fruit harvested at four different growth stages after 13 days incubation at 15 and 20°C.

3.3.3 Effect of nectarine fruit maturity on infection

Nectarine fruit from each of the three stages of maturity inoculated under similar conditions (i.e. 20°C and high humidity) were highly susceptible to *M. fructicola* infection (Figure 4, Table 3). The percentage of fruit with brown rot infection was 93.3% at 53 days after bloom, 77.8% at pit hardening and 87.5% about 2 weeks before harvest, these values were not significantly different. Inoculated fruit from pit hardening incubated at 20 °C and low humidity had significantly less rot infection (11.1%) than the respective fruit incubated at high humidity (77.8%).

Uninoculated fruit from post-bloom and pit hardening did not develop brown rot infection after incubation under dry or wet conditions and different temperatures. However, uninoculated fruit picked two weeks preharvest was already infected by *M. fructicola* in the field because high levels of brown rot developed after incubation (Table 1). Fruit infection levels were significantly greater on untreated fruit that was incubated for 14 days under high RH after an initial period of 10 hrs either wet or dry (66.7% - 83.3%) than similar fruit incubated at low RH (29.2% - 33.3%). The data indicate lack of natural brown rot infection earlier in the growing season up to pit hardening, but the disease levels increased close to harvest.

Inoculated fruit from preharvest that was incubated in high humidity had significantly higher levels of rots than inoculated fruit incubated in low humidity. However, there were no significant differences in rot levels between inoculated and uninoculated fruit when comparing fruit incubated in each of the humidity conditions.



Figure 4: Green fruit of nectarine picked soon after pit hardening in plastic trays 14 days after inoculation with conidia of *M. fructicola*.

Table 3: Mean percentage of nectarine fruit (cv. August Pearl) that developed brown rot infection by *M. fructicola* at three stages of fruit maturity after 14 days incubation in different temperatures and moisture conditions.

Incubation conditions		Inoculation treatment ^A		
Temperatures, 10 hrs wetness	RH over 14 days at 20 °C	Uninoculated dry	Uninoculated wet	Inoculated wet
Fruit picked 53 days after bloom				
15 °C	high	0.0 c ^B	0.0 c	84.4 b
20 °C	high	0.0 c	0.0 c	93.3 ab A
25 °C	high	0.0 c	0.0 c	97.8 a
Treatment $P = <0.001$ Temperature $P = 0.226$ Inoculation x temperature $P = 0.214$				
Fruit picked after pit hardening				
15 °C	low	0.0 d	0.0 d	33.3 b
20 °C	low	0.0 d	0.0 d	11.1 c
20 °C	high	0.0 d	0.0 d	77.8 a A
25 °C	low	0.0 d	0.0 d	16.7 c
Treatment $P = <0.001$ Temperature $P = <0.001$ Inoculation x temperature $P = <0.001$				
Fruit picked 2 weeks before harvest				
20 °C	low	33.3 b	29.2 b	33.3 b
20 °C	high	83.3 a	66.7 a	87.5 a A
Humidity $P = <0.001$ Treatment $P = 0.322$ Humidity x inoculation $P = 0.609$				

^A Fruit were dipped in a spore (conidia) solution and incubated wet inside plastic containers in controlled temperature cabinets for 10 hours followed by incubation at 20°C in low ($\pm 40\%$ RH) or high humidity ($>80\%$ RH) conditions. Untreated fruit was incubated dry or dipped in distilled water only.

^B Means (%) followed by different letters indicate significant differences between treatments within each stage of fruit maturity, whereas capital letters indicate differences between stages of growth (within inoculated column only) for inoculation at 20°C in high humidity only.

The results of this preliminary study with nectarines are similar to those obtained by Biggs and Northover (1988) and Mari et al. (2003) which showed that peaches and apricots were most susceptible to *Monilinia* spp. infection shortly after bloom and before harvest. However, our results with nectarine fruit from the post pit hardening stage differed from these overseas studies which showed peaches and apricots after pit hardening were resistant to *Monilinia* spp. infection, but this does not appear to occur in nectarines.

The resistance of young, green stonefruit (peach, plum, and apricot) to infection by *Monilinia* spp. has been previously reported in other studies in Australia but precise information on fruit maturity and inoculum concentration is lacking (Jenkins and Reinganum 1965; Jerome 1958; Wade 1951 and 1956). Work by Biggs and Northover

(1988) and Mari et al. (2003) showed that green apricot and peach fruit were resistant to *Monilinia* spp. infection around the pit hardening stage under inoculation conditions similar to those tested in this study. In our study, peach and nectarine fruit were inoculated with high levels of inoculum ($1-4 \times 10^5$ spores per ml) and subjected to incubation under optimal temperature and moisture conditions for infection (i.e. nectarines 10 hrs wetness and several days of high relative humidity). Biggs and Northover (1988) inoculated peach fruit with inoculum concentrations ranging from 10^4 to 10^6 spores per ml, and incubation in the dark for 22 hours at 20°C and 95% RH, followed by drying for two hours at 20°C and 60% RH, then incubated for a further 6 days at 20°C and 95% RH. Mari *et al* (2003) inoculated peach and apricot fruit with 10^5 conidia per ml and incubated fruit at 20 °C and 95% RH for 7 days.

In our work, brown rot infection levels were significantly greater in green nectarine fruit (post pit hardening stage) inoculated and incubated for 14 days at 20°C in high humidity conditions than similar fruit incubated at low humidity after the initial period of 10 hrs of wetness. It is possible that differences in susceptibility observed in green nectarine fruit could be due to the length of the incubation period in constant wet and high humidity which may have allowed conidia of *M. fructicola* to infect more green fruit. In the field, similar periods of long wetness followed by high RH are not uncommon like during the late spring – early summer period of 2010, which occurred after the fruit for the pit hardening stage inoculation (90 days after bloom) was collected.

Fruit from the post pit hardening stage was not infected with brown rot, however, the fruit collected two weeks before harvest was infected by *M. fructicola*. It is uncertain if fruit infections (latent) might have occurred before this wet period, or between post pit hardening and preharvest.

Previous field work by Jerome (1958) suggested that the low incidence of brown rot in green fruit of stone fruits was due to low spore concentration in the field and not to a higher resistance of fruit to infection by *M. fructicola*. Our inoculation study with peaches and nectarines clearly showed differential susceptibility, particularly after pit hardening under optimal infection conditions. Further investigations including repeating the experiment with additional peach and nectarine cultivars in controlled and field conditions would be useful to determine if differences in green fruit (post pit hardening) susceptibility to *M. fructicola* infection are due to fruit characteristics and/or temperature/wetness conditions during infection. It would also be useful to have more understanding of fruit susceptibility during the early stages of fruitlet development.

3.4 Conclusions

Peach fruit from the four growth stages (shuck fall, pre and post pit hardening, preharvest) differed in their susceptibility to brown rot. Fruit close to maturity were highly susceptible to brown rot. The levels of brown rot were lowest on fruit at the post pit hardening stage. The data support overseas studies that fruit growth stages have a marked influence on susceptibility to brown rot, but the mechanisms for peach fruit resistance are still not clearly understood.

Results also showed that nectarine fruit picked approximately seven weeks after bloom and two weeks before harvest were highly susceptible to *M. fructicola* infection as

reported by overseas studies. Results also indicated that temperature and duration of wetness/humidity may also influence brown rot development, especially on green fruit from the post pit hardening stage. A high RH (>80%) for 14 days after an initial 10 hr wetness period allowed spores of *M. fructicola* to infect more green nectarine fruit at 20°C.

A higher incubation temperature of 20°C allows brown rot to develop more rapidly on peaches, hence a shorter incubation period of seven days is adequate for determining disease risks, particularly for fruit at or close to maturity. This will shorten the time required for quality control check of rot levels in the pack-house. Sub-samples of fruit may be retrieved from cold storage, kept at room temperatures (in the absence of controlled temperature room), and assessed for rot development after a week. If checking brown rot infections on fruit at other growth stages with lesser susceptibility and possibly lower inoculum levels, a longer incubation period of up to 14 days may be required, particularly if the fruit is held at cooler temperatures, e.g. 15°C (or at room temperatures in cooler seasons).

The lack of brown rot infections on uninoculated nectarine fruit (wet and dry) from the post bloom and pit hardening stages suggest that the fruit may have not been infected by latent infection of *Monilinia* spp. However, this fruit was still green and several weeks away from reaching maturity. Studies by Fourie and Holz (2003a and 2003b) indicate that on nectarines and plums, infection by *M. laxa* was already established when fruits approached maturity. The possibility that high levels of natural *M. fructicola* infections observed on nectarine fruit collected 2 weeks before harvest could be related to the formation of latent infections; or driven by high inoculum levels and rapid shoot/fruit growth during extremely wet weather conditions observed in 2010-2011 season after the post pit hardening stage; or physical damage of fruit skins warrants further investigation. The information is required to develop appropriate fungicide control strategies during extreme wet conditions.

3.5 References

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4. Control of Carpophilus beetle and it's effect on incidence of brown rot

4.1 Introduction

Carpophilus beetles have been implicated as vectors of *Monilinia fructicola* spores. Brown rot is a major problem for fruit growers and also manifests later in the value chain either in the market or once consumers have purchased the produce. In southern Australia, *Carpophilus* spp. are major pests of stone fruit including cherries (James *et al.* 1997, Hossain 2006, Hossain *et al.* 2007). Economic losses of up to 30% have been reported (Hossain *et al.* 2000) at harvest due to direct consequence of Carpophilus damage, as they chew and penetrate fruit near the stem end. The beetles also can cause indirect damage by serving as a vector of brown rot (*Monilinia fructicola*), carrying spores (Kable 1969, Chang and Jensen 1974, Tate and Ogawa 1975) that germinate to cause an infection resulting in rapid fruit breakdown (Hely *et al.* 1982) both on the tree and also during the post harvest storage period. Development of an attract-and-kill (A&K) system using synthetic aggregation pheromone plus food-attractant (Bartelt and Hossain 2006, Hossain *et al.* 2007), provided effective protection of ripening crops, when deployed 6-8 weeks before onset of fruit colour change (Hossain *et al.* 2006, 2007). Anecdotal evidence suggests that controlling Carpophilus reduces the brown rot incidence in fruit. The aim of this work was to understand how A&K traps can reduce Carpophilus populations in stone fruit and thus reduce the incidence of brown rot.

4.2 Materials and Methods

Field trials were established during the 2008/09 fruit growing season in four 1 ha peach (var. T204) blocks in the Goulburn Valley area and continued to 2009/10 seasons on two properties (property-1 and property-2). All four blocks used were almost similar in terms of their agronomic practices, tree training, and the age of the trees and fungicide program. The A&K system has been used for the last 6-7 years in properties used in the 2008/09 season and on property-1 used in 2009/10 season, consequently Carpophilus populations gradually reduced to a very low level before the experiment was commenced. Two blocks were treated with A&K system (one in each property) and the other two blocks were used as control with the grower's normal practice of pesticide treatment as required. On property-1, both treated and control blocks were used for both seasons, whereas on property-2 the grower decided not to continue and we used another property in the same district in the 2009/10 season. On this new property, the treated block was known to have very high Carpophilus populations and high fruit damage, and A&K system had never been used.

The beetle populations were monitored in both A&K treated and control blocks using funnel traps baited with synthetic food attractant. A diagonal transect with three monitoring traps was established in both control and treated blocks, starting from the upwind (North-West) corner with traps placed approximately 30m, 60m and 100m away from the corner of the plots. The monitoring traps were placed one week prior to the deployment of attract-and-kill traps to measure the initial pre-treatment Carpophilus populations. Standard MagnetTM funnel traps (Agrisense, Pontypridd, Glamorgan, UK)

were used as monitoring traps and were hung at a height of 1.5 m on tree branches. The monitoring traps were baited with approximately 150 ml synthetic food attractant in a 250 mL glass jar covered with mosquito netting, and a small piece (1 cm x 1 cm) of dichlorvos impregnated insecticide strip (Killmaster zero, Barmac Industries Pty. Ltd., Queensland, Australia) as killing agent.

The A&K system also uses the synergistic attractiveness of synthetic *Carpophilus* spp. pheromone and synthetic co-attractant (food-attractant) developed through chemical analysis of ripening peaches, which is considered to be very successful in protecting the crop (Hossain *et al.* 2007, 2008). The A&K traps were placed at the upwind (North-West) corner of the treated blocks 8-9 weeks before the projected harvest date. The traps were placed about 50 m apart from one another and were held in a metal ring fixed to a metal fence picket at a height of 1.5 m from the ground. Funnel trap (23 x 17 cm, Bioglobal, Queensland, Australia) was used as the A&K trap, baited with 300 mL of synthetic co-attractant in a plastic container covered with mosquito netting to prevent beetle entry, a pheromone septum hung from the lid of the trap with a metal wire and a piece (3 x 2 cm) of dichlorvos impregnated insecticide strip to kill captured beetles. The pheromone septum was impregnated with the three-species blend (5-ethyl-3-methyl-2,4,6-nonatriene, 6-ethyl-4-methyl-3,5,7-decatriene, 5-ethyl-7-methyl-3,5,7-undecatriene, 3,5,7-trimethyl-2,4,6,8-decatetraene, 7-ethyl-3,5-dimethyl-2,4,6,8-decatetraene, 3,5,7-trimethyl-2,4,6,8-undecatetraene, 7-ethyl-3,5-dimethyl-2,4,6,8-undecatetraene) described by Bartelt (1999) and used by Hossain *et al.* (2007). The pheromone septa are commercially available from IPDM-DSP, Great Lakes IPM, Vestaburg, MI, USA. The synthetic co-attractant (food attractant) solution was an aqueous solution with ethanol as the main constituent and acetaldehyde, ethyl acetate, 2-methyl-1-propanol, 3-methyl-1-butanol, and 2-methyl-1-butanol as minor components (Bartelt & Hossain, 2006).

The control blocks were selected at least 2 km away from the A&K treated blocks to avoid any interference of pheromone from the treated plots. The control blocks had no A&K traps (with monitoring traps only), but received insecticide sprays of parathion-methyl which is part of the usual grower practice for *Carpophilus* control.

The A&K and monitoring traps were serviced weekly until two/three weeks after fruit was picked for brown rot testing. During servicing the synthetic co-attractant was replaced weekly and new pheromone septa in A&K traps were added every two weeks. The *Carpophilus* spp. caught in A&K and monitoring traps every week were collected and transported to the laboratory for identification and estimation of the number of beetles as described by Hossain *et al.* (2006, 2009b).

In both years fruit was picked for brown rot testing on 15 January. 120 fruit were harvested from both the A&K and control blocks at each orchard. Twenty fruit were picked from each of 6 replicates, consisting of 6 rows of trees located in the centre of the block. Within the replicate 2 fruit were picked from each of 10 adjacent trees, one from high on the tree and one low. Fruit were harvested into 43cm x 36cm single layer cardboard fruit trays containing plastic inserts with individual cups for 20 fruit. After transport the individual trays were enclosed in large plastic bags (drawstring rubbish bags) to maintain high humidity, and stored at 20°C. The incidence of brown rot and other rots was assessed after 7 and 12 days incubation.

4.3 Results and Discussion

Most of the *Carpophilus* spp. (>98%) caught in traps were *C. davidsoni* and the results described hereafter refer to this species.

Monitoring data showed that the number of *Carpophilus* beetles caught on both properties in the 2008/09 season, both in control and A&K treated blocks, were similar during the first week (Fig 1). Trap catches in control blocks increased in the second week on both properties and remained high throughout the season compared to catches in the A&K treated blocks. Trap catches sharply dropped in the treated blocks after A&K trap deployment and remained low until the end of the season. Trap catches in both control blocks fluctuated over time. The fluctuations were greater on property-2 and the average number of *Carpophilus* beetles caught was more than 500 beetles/trap/week on most of the sampling days until early January. However on property-1 after a sharp drop on week 3 the number of *Carpophilus* beetles caught in monitoring traps was only greater than 500 beetles/trap/week on 2 December.

The A&K traps caught very high numbers of *Carpophilus* beetles on property-2, especially during the first 5 weeks after A&K traps were deployed (Fig. 2). After that, the number of *Carpophilus* in A&K traps were low on both properties.

In the 2009/10 season, monitoring data showed that *Carpophilus* caught on both properties, in both control and A&K treated blocks, were similar especially in the first week (Fig. 3). In the second week the number of *Carpophilus* caught in monitoring traps on property-1 was the highest (1033/trap/week) in the treated block and for the rest of the season populations were almost identical in both control and treated blocks. On property-2, *Carpophilus* caught in monitoring traps increased in week 4 in both treated (8833/trap/week) and control (1217/trap/week) blocks. The following week trap catch in the control block dropped to a very low level, trap catches remained high in treated blocks until the end of December.

The number of *Carpophilus* beetles caught in A&K traps was very high on property-2, especially in the first 6 weeks (Fig. 2). The highest number of *Carpophilus* (178500/block) was caught on property-2 on 11 December. Even in late December trap catches on property-2 were very high compared to that on property-1. From early January *Carpophilus* numbers were similar in both properties and remained similar for the rest of the season.

The A&K system was developed to protect ripening stone fruit from *Carpophilus* damage. The beetles generally attack the fruit immediately before harvest and at this stage spraying of insecticide is restricted in order to follow the prescribed withholding periods. The growers were very keen to use the A&K system as it would replace the use of pesticide sprays to protect the crop from *Carpophilus* damage. Moreover, spraying insecticides can be detrimental to the predatory insect populations thus creating a potential for pest 'flare ups'.

The laboratory assessment of the incidence of brown rot in fruits revealed that in 2008-09 brown rot incidences in both properties were significantly higher in control blocks than in A&K treated blocks. (Fig 4a,b).

In the following season (2009-10) brown rot incidences on property-1 and property-2 were low and not significantly different between A&K and control (Fig 4a, b). With such low incidences the impact of the A&K traps was not demonstrated. Rot after 12 days incubation occurred only in the A&K fruit however the A&K traps removed very high numbers of *Carpophilus* until early January. It is possible that *Carpophilus* populations remained high after that but the beetles were more attracted to the ripening fruit than the traps. During mid-January T204 start to ripen and the aroma coming from the huge amount of fruit may have reduced the effectiveness of the A&K and monitoring traps. Studies conducted in stone fruits and figs showed that *Carpophilus* catches in food-based baits decreased as the season progressed and the fruit started ripening (Hossain *et al.* 2006, Simmons *et al.* 1931, Smilanick and Ehler 1976, Smilanick 1979). This may be because competition from fruit volatiles reduces trap effectiveness, especially when beetles infesting the crops begin to emit their own aggregation pheromone.

As we discussed earlier property-2 had a history of high *Carpophilus* population and damage and A&K traps had not been previously used. In contrast on property-1 A&K traps had been used over the last 6/7 years. We presume A&K traps used for one season was not sufficient to reduce *Carpophilus* populations to a low level well before fruit ripening. From our previous experience we found that if a property has a high *Carpophilus* population, it generally takes at least 3 to 4 seasons of treatment to bring populations to a medium to low level using A&K traps (M. Hossain unpublished data). When we started using A&K system on property 1 the *Carpophilus* population was also high, and it took more than three seasons to reduce the population to the current (low) level.

From these experiments we can conclude that *Carpophilus* populations could be kept under control using the A&K system thus reducing the incidence of brown rot. However the A&K system can only be effective if the *Carpophilus* population is at medium to low levels and it may take more than three seasons of trap deployment to achieve a low beetle population.

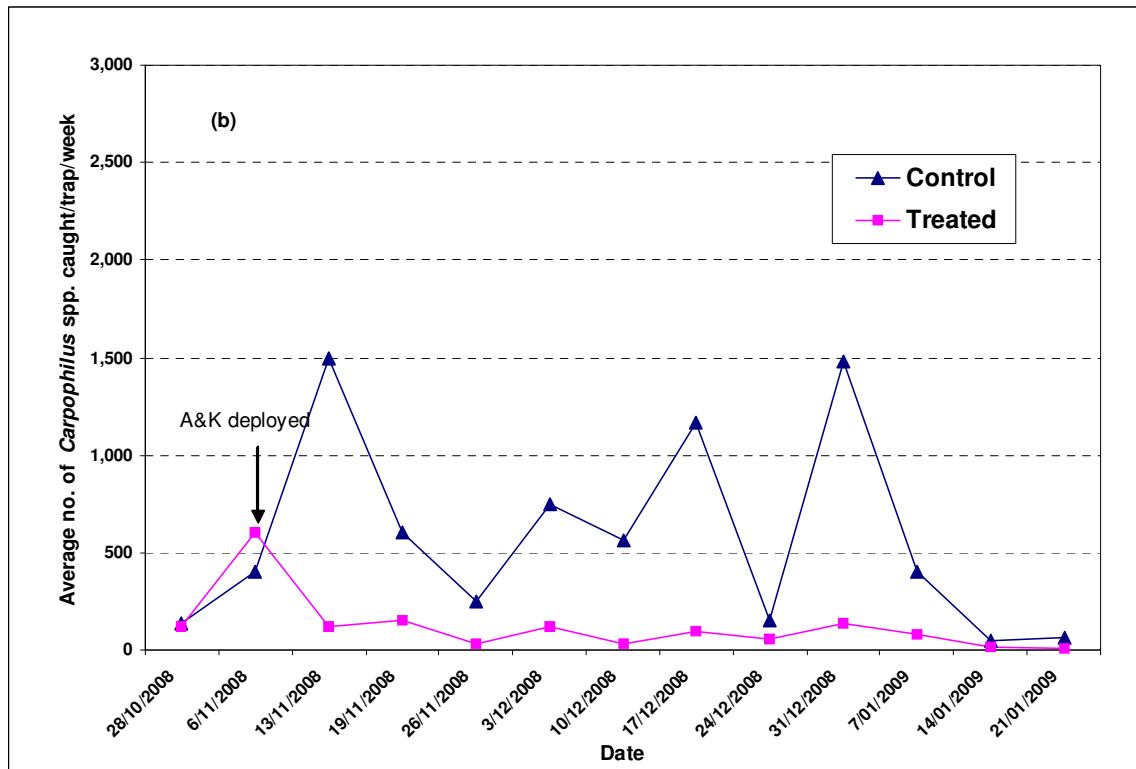
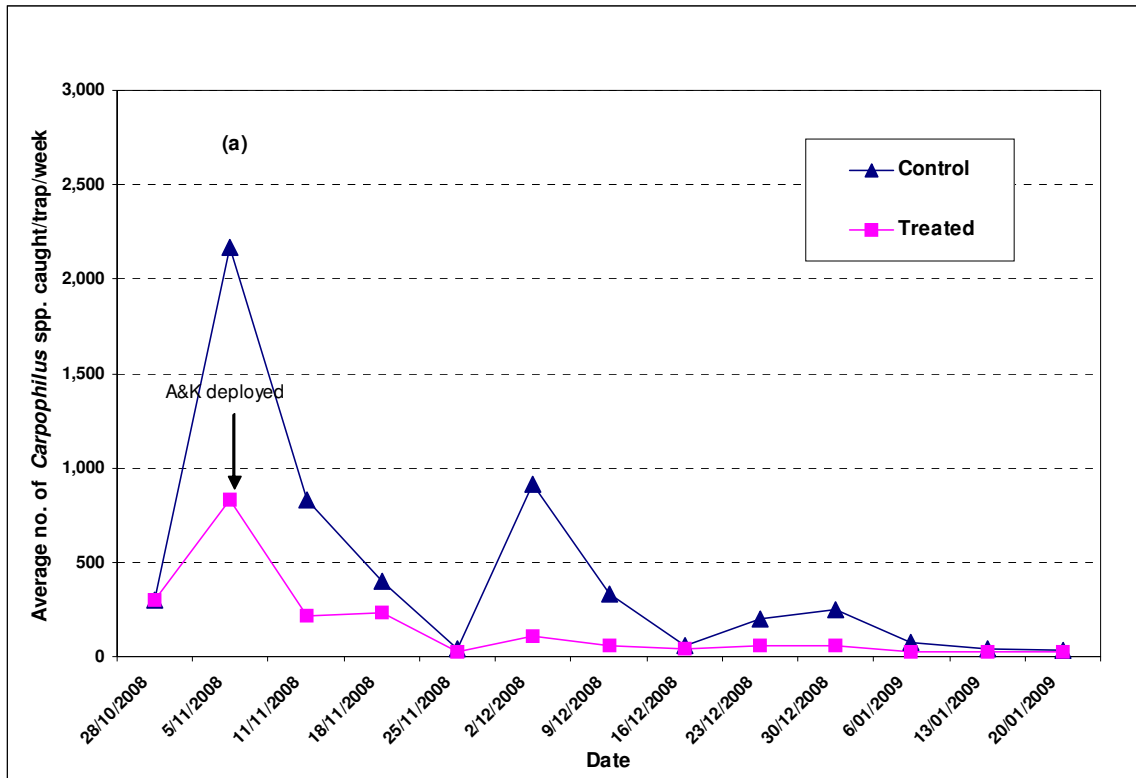


Figure 1. Monitoring data with synthetic co-attractant during 2008/09 season on property-1 (a) and property-2 (b).

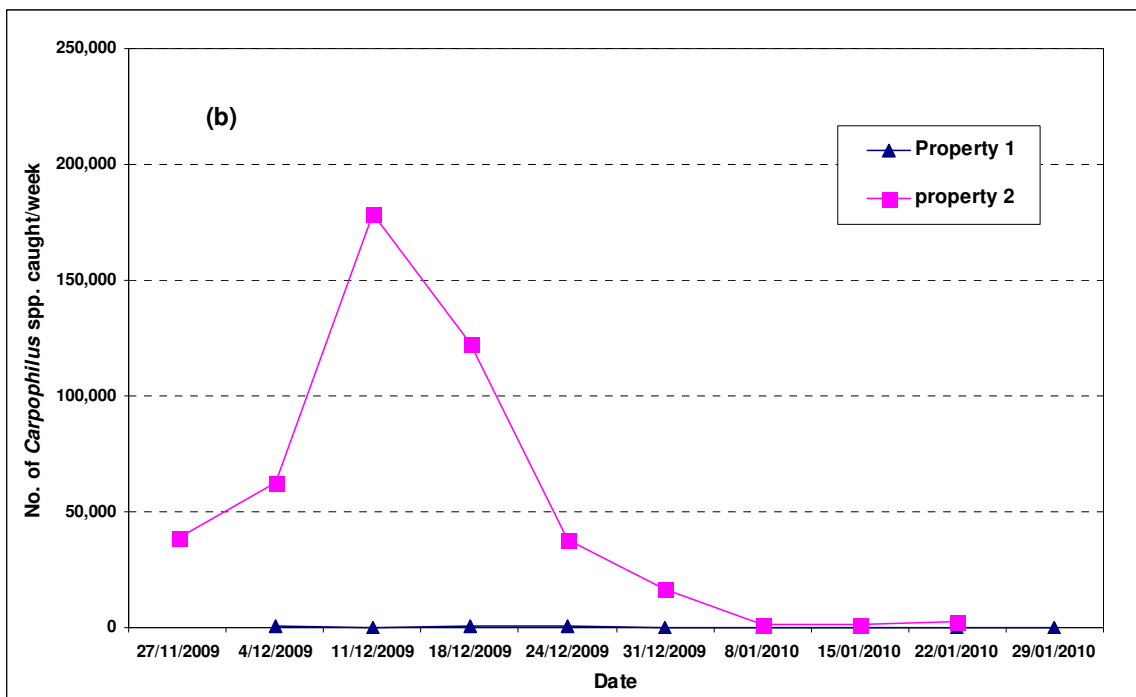
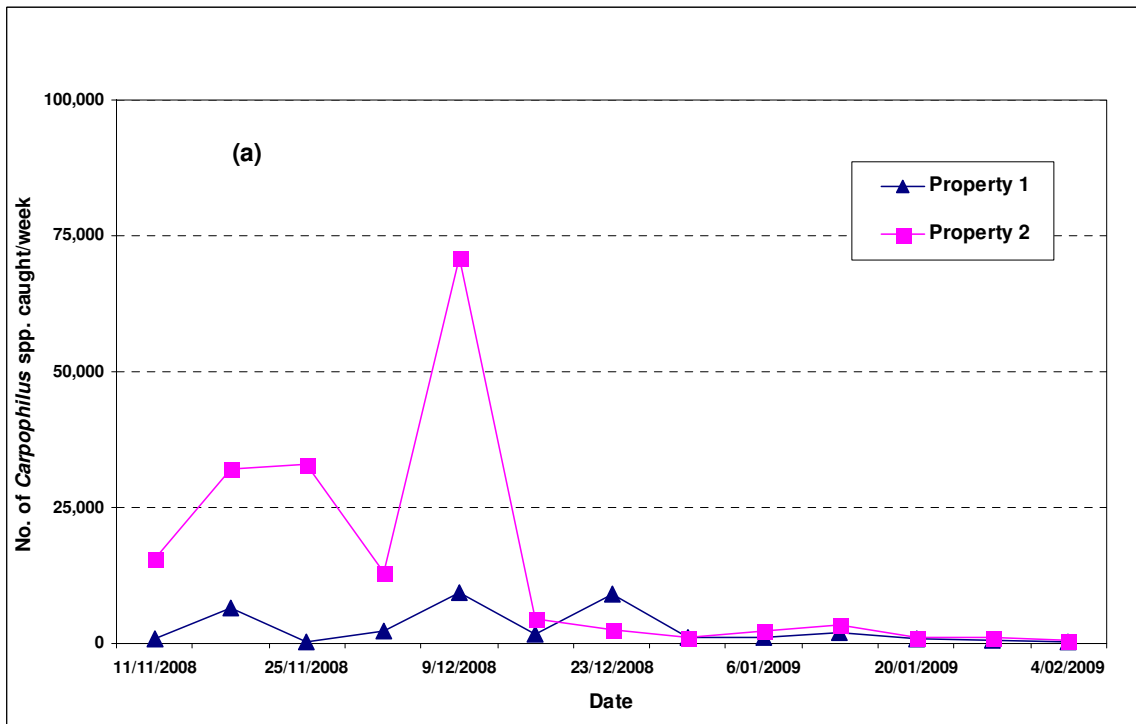


Figure 2. Total number of *Carpophilus* spp. caught in attract & kill traps during 2008/09 (a) and 2009/10 (b) seasons.

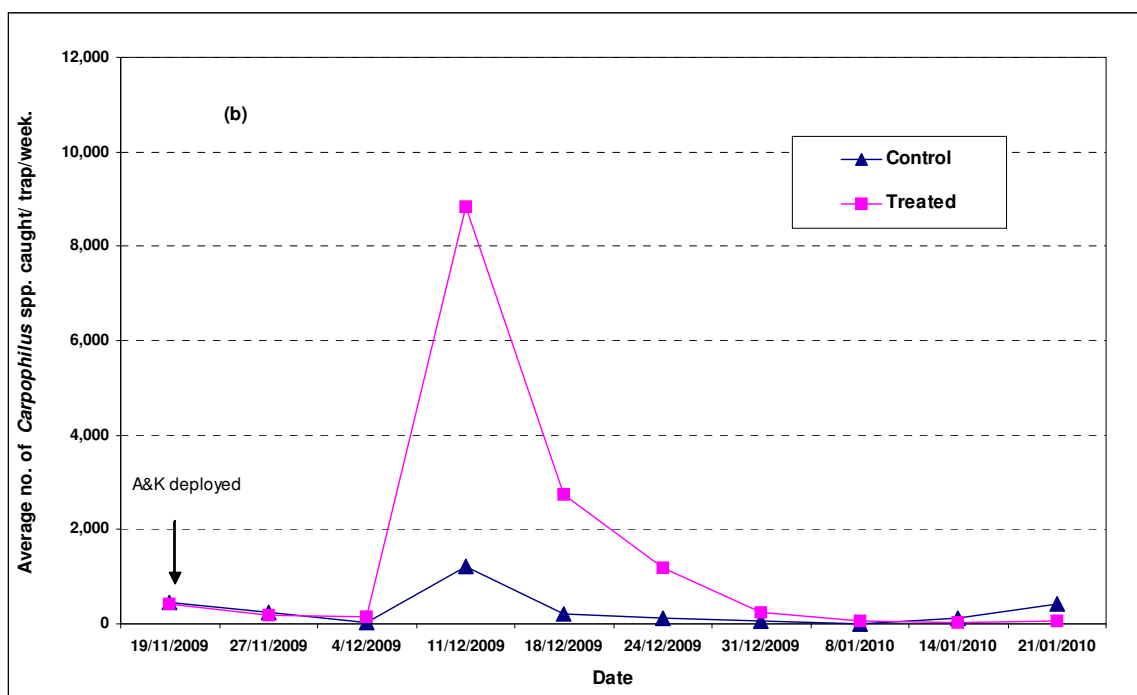
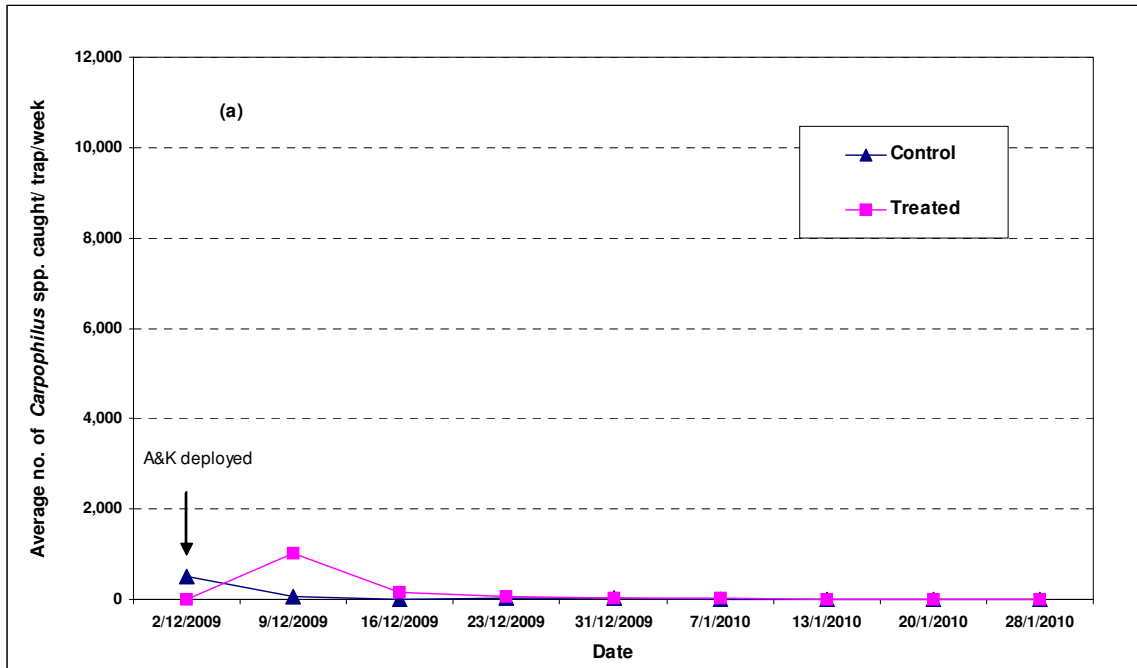


Figure 3. Monitoring data with synthetic co-attractant during 2009/10 season on property-1 (a) and property-2 (b).

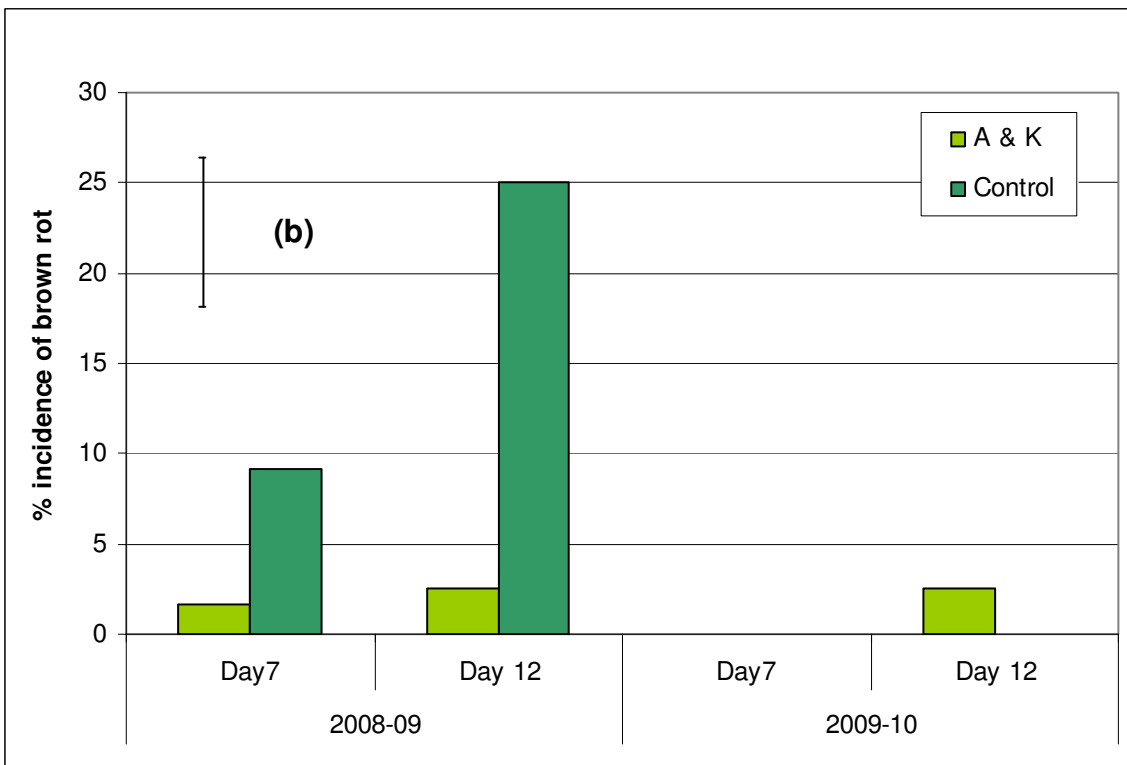
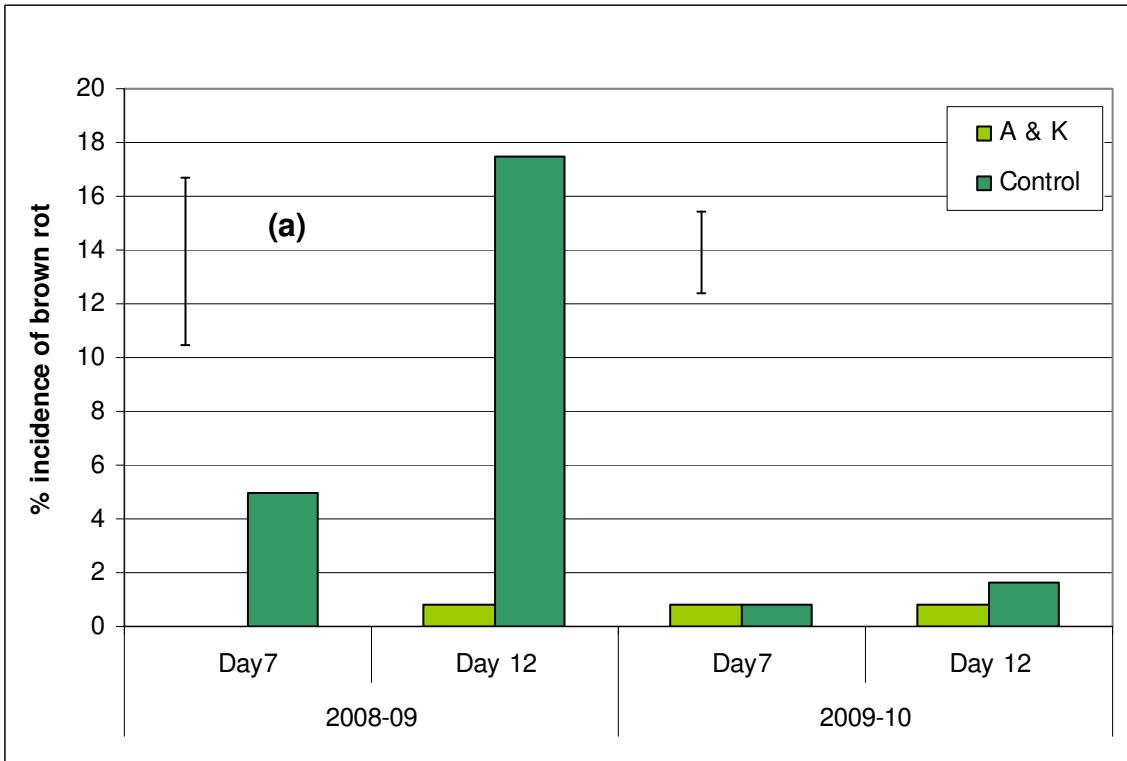


Figure 4. Effect of attract and kill beetle traps on total rot incidence on property-1 (a) and property-2 (b). Two different properties were used for property-2 in 08-09 and 09-10.

4.4 References

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5. Sensitivity of *Monilinia* species from stonefruit to pre and postharvest fungicides

5.1 Introduction

Control of brown rot relies heavily on the application of protectant and eradicant fungicides during key crop growth stages. Well timed application of efficacious fungicides during the growing period is usually expected to offer economic control. However, crop losses due to blossom blight and fruit rots had occurred despite the application of fungicides (Chapter 2), and further losses had been recorded on fruit postharvest.

A number of factors may contribute towards loss of disease control. These may be: (i) use of a calendar spray program rather than well-timed application of fungicides; (ii) heavy inoculum pressure in the orchard; (iii) presence of resistant strains from long term, successive application of the main fungicides.

A range of protectant and eradicant fungicides are currently registered for control of brown rot of stonefruit (Table 1). These include the protectants captan, mancozeb, ziram, thiram, and the protectants/eradicants triforine and propiconazole, all of which have been in use for many years. The dimethylation inhibitor (DMI) fungicide activity group for example has been used in stonefruit orchards for over 25 years for rust and brown rot control. Two fungicides registered for postharvest drenching of stonefruit, triforine and iprodione, have also been used for over 25 years. In contrast, fludioxonil, which is from a new chemical family, phenylpyrrols and first registered for drenching in 2010, has not previously been used in Australian orchards.

Long term and intensive use of fungicides often leads to reduced fungicide sensitivity in fungal populations, however, resistance can also develop within a few years of fungicide application (Penrose 1990, Zehr et al. 1999). Benomyl-tolerant isolates of *M. fructicola* were obtained from a stone-fruit orchard in New South Wales, as early as 1976 (Penrose 1990). A programme of six benomyl sprays over one season (1988-89) on peach and nectarine trees had resulted in the build-up of benomyl-tolerant populations of *M. fructicola*. Overseas, *Monilinia* spp. resistant to iprodione, propiconazole, fenbuconazole and thiophanate-methyl have been reported on stonefruit (Elmer and Gaunt 1993, Zehr et al. 1999, Schnabel et al. 2004, Yoshimura et al. 2004, Cox et al. 2009). The situation in Australia is unclear because there has been no previous publication on studies of systematic screening or detection of *Monilinia* spp. from stonefruit that are tolerant to dicarboximide and DMI fungicide groups.

Development of resistance to one active ingredient in a fungal pathogen may render reduced effectiveness of other fungicides belonging to the same chemical group. In Australia, a numerical coding system for fungicides of different chemical groups is implemented to help fungicide users to avoid spraying fungicides from the same chemical group in multiple, consecutive applications. Fungicide products are classified into chemical activity groups based on their active ingredients (CropLife Australia 2010).

Table 1: Active ingredients of fungicides registered in Australia for brown rot and blossom blight of peaches and nectarines as at 1st July 2010.

Active ingredients	Fungicide activity group*	Crop & Situation																			
		Dormant		Fruiting Trees							P/Harvest Dip										
		Nectarine - Dormant Brown rot	Peach - Dormant Brown rot	Canning Peach - Brown rot	Nectarine - Blossom blight (M. laxa)	Nectarine - Brown fruit ro (M. fructicola)	Nectarine - Brown fruit ro (M. spp)	Nectarine - Brown fruit ro (M. laxa)	Peach - Blossom blight (M. laxa)	Peach - Brown fruit ro (M. fructicola)	Peach - Brown fruit ro (M. spp)	Peach - Brown fruit ro (M. laxa)	Stone fruits - Blossom blight (M. laxa)	Stone fruit brown fruit ro (M. fructicola)	Stone fruit brown fruit ro (M. spp)	Stone fruit brown fruit ro	Nectarine - Postharvest dip	Peach - Postharvest dip	Stone fruit postharvest dip		
Iprodione	2																				
Procymidone	2																				
Fenbuconazole	3																				
Propiconazole	3																				
Triforine	3																				
Cyprodinil	9																				
Fludioxonil	12																				
Captan	M4																				
Chlorothalonil	M5																				
Copper oxychloride	M1																				
Dithianon	M9																				
Dodine	M7																				
Mancozeb	M3																				
Sulphur, Elemental	M2																				
Lime Sulphur, (Polysulfide)	M2																				
Wettable Sulfur	M2																				
Thiram	M3																				
Ziram	M3																				

Dormant
 Bloom
 Growing season
 Postharvest dip

* CropLife Australia fungicide activity group codes, 23rd November 2010.

The spray programs outlined in Chapter 2 indicate surveyed growers tended to apply propiconazole consecutively without alternating with a fungicide from another chemical group. Reports of brown rot infection despite application of a full fungicide program suggested the need to investigate if the fungal populations had developed resistance to the fungicides currently in use.

The objectives of this study were to evaluate the sensitivity of *M. fructicola* strains collected from stonefruit orchards in Victoria and South Australia, to ascertain the risks of reduced fungicide efficacy. Four fungicides from different activity groups were selected for the evaluation. They consisted of propiconazole, a protectant and curative fungicide registered for brown rot control during the growing season; iprodione, a protectant and curative fungicide registered for postharvest drenching and field applications; and fludioxonil a protectant, registered in 2010 for post-harvest treatment against brown rot. A fourth fungicide, thiabendazole, is currently registered for postharvest dip of pomefruit but not for stonefruit. It is of interest as a potential alternative to carbendazim, which belongs to the same fungicide activity group and had a major use for both field and postharvest disease control, but its registration has been suspended since the review by the Australian Pesticide and Veterinary Registration Authority (APVMA) in 2007.

5.2 Materials and Methods

Monilinia isolates were collected from peach, nectarine and plum orchards and home gardens in Victoria and South Australia for over three seasons. The cultures were grown on potato dextrose agar medium (PDA), and agar plugs containing the fungal cultures were stored in sterile distilled water at 4°C. Cultures were refreshed by subbing on PDA, and 1-2 week old colonies were used for the sensitivity assays.

Nineteen *M. fructicola* and one *M. laxa* isolates were selected for the assays, to represent different production regions, hosts and seasons. The effectiveness of varying concentrations of four fungicide products against these isolates was determined. Three of the fungicides: thiabendazole 'Tecto', propiconazole 'Tilt' and fludioxonil 'Scholar', are marketed by Syngenta. The fourth fungicide, iprodione 'Rovral', is marketed by Bayer CropScience. These fungicides belong to the, benzimidazole, triazole (DMI), phenylpyrrole, and dicarboximide chemical groups respectively.

The range of concentrations tested was: propiconazole, five concentrations between 0.015 and 0.125 µg/ml; iprodione, six concentrations between 0.005 and 1.62µg/ml, fludioxonil, five concentrations between 0.0025 and 0.005µg/ml; and thiabendazole, twelve concentrations tested in two batches; first batch at high concentrations of 0.05-10 µg/ml, and the second batch at lower concentration range of 0.06-0.24 µg/ml. The controls consisted of un-amended PDA plates, ie. zero fungicide concentration.

The required fungicide concentrations were prepared by adding the appropriate volumes of the fungicide products to sterilised, molten PDA. The plates amended with fungicides were poured the day before screening was conducted. Fresh cultures of the 19 isolates were grown at 20°C in darkness for approximately one week prior to fungicide assay. On the day of the assay, 5 mm plugs of mycelia were cut from the culture plates with a cork borer and placed in the centre of the fungicide amended and control plates. Two replicate plates were prepared for each combination of isolate and fungicide concentration. These plates were then incubated at 20°C in darkness.

Two colony diameters perpendicular to each other were measured after incubation for six days for each fungal isolate and the 5 mm mycelial plug diameter was subtracted (Figure 1). The percentage inhibition of fungal growth due to the fungicide relative to control colonies grown on un-amended PDA was calculated using the formula: $(\text{diameter}_{\text{control}} - \text{diameter}_{\text{fungicide}}) * (\text{diameter}_{\text{control}}^{-1}) * 100$. The 50% effective concentration (EC₅₀) and the minimal inhibitive concentrations (MIC) were estimated from the graph obtained for each isolate at respective fungicide concentrations. The EC₅₀ of each replicate was used to derive the Fisher's Least Significant Difference (LSD) of the means between isolates in one-way analysis of variance (ANOVA).

5.3 Results and Discussion

All the *Monilinia* isolates showed differential sensitivity to each fungicide tested (P<0.05, Table 2). An example of the variation in fungal growth, ie. colony sizes, on propiconazole amended PDA is shown (Figure 1). The variation in fungal growth

responses is not unexpected, particularly when most of the isolates had been collected from different regions and seasons, and from orchards with different fungicide use regimes and history.

The growth of *Monilinia* isolates was inhibited to different levels by the four fungicides. Low EC₅₀ and MIC values were obtained for fludioxonil, indicating all the isolates were sensitive to this new fungicide (Figure 2, Table 2). The baseline sensitivity of *M. fructicola* to fludioxonil was able to be determined, at a low EC₅₀ range of 0.012-0.006 ug/ml (Table 2), since this fungicide has not been applied in the orchards. It was not possible to determine the baseline sensitivity of the isolates to the other three fungicides since these products or those from the same fungicide groups have been in used in orchards for many years.

The comparatively higher MIC and wider EC₅₀ ranges amongst the isolates tested on iprodione and propiconazole amended media indicates that the isolates showed differential response to these fungicides (Figures 3-4, Table 2). The EC₅₀ ranges for iprodione and propiconazole (Table 2) were lower than those reported overseas (Zehr et al. 1999, Schnabel et al. 2004, Cox et al. 2009). The baseline EC₅₀ value of *M. fructicola* in South Carolina peach orchards prior to exposure to propiconazole sprays was approximately 0.03 ug/ml (Zehr et al. 1999). This is comparable to the low EC₅₀ range of 0.012-0.029 ug/ml for propiconazole found in this study. However, large mean MIC values of more than 0.2 ug/ml were needed to totally inhibit the growth of these isolates.

The EC₅₀ and MIC values for iprodione and thiabendazole were comparatively higher than those obtained with propiconazole (Table 2), and isolates resistant to iprodione and thiabendazole have been detected. The EC₅₀ concentrations of iprodione for resistant isolates were up to 8.5 times higher than those of the sensitive isolates. Iprodione fungicides have been applied in stonefruit orchards for a longer duration than propiconazole. The longer term exposure is probably a contributing factor for detection of iprodione resistant isolates. This could potentially pose a risk for ineffective protection with iprodione as a post-harvest dip if some of the infections are caused by iprodione resistant *M. fructicola*.

Isolate number 2, cultured from mummified fruit collected from an orchard in the Goulburn Valley, was resistant to thiabendazole at 0.25-10.0 ug/ml, this range of concentrations was highly inhibitory to the other isolates. The highest rate of 10µg/ml is 61 times higher than the mean EC₅₀ value (Table 2, Figure 5). Whilst thiabendazole is not currently registered for stonefruit, its use as a postharvest dip of pomefruit raises an interest in investigating the possibility of its use as an alternative to carbendazim for control of brown rot. It would be useful to have more information on possible existence of high level resistance in more strains, if this fungicide is to be considered for postharvest control of brown rot.

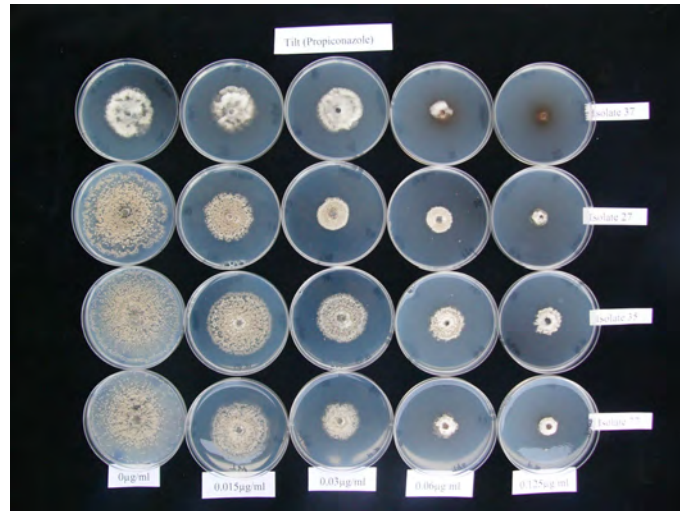


Figure 1: One *Monilinia laxa* isolate (top row) and three *Monilinia fructicola* isolates showing varying sensitivity, generally with reducing colony diameters at increasing concentrations of propiconazole (left to right: 0, 0.015, 0.03, 0.06, 0.125 ug/ml).

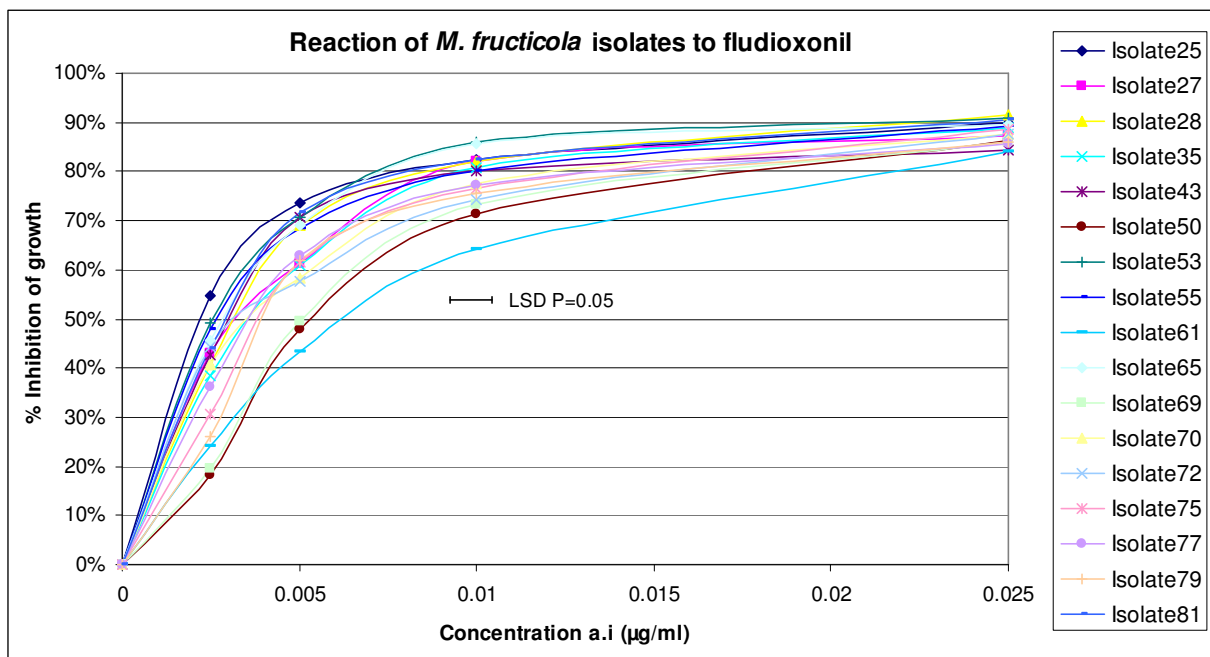


Figure 2: Percentage inhibition of mycelial growth of *Monilinia fructicola* isolates by a range of fluidoxonil concentrations, relative to growth on unamended PDA. Values for the isolates are the means of four colony diameters from two replicates. LSD refers to the EC50 of each isolate.

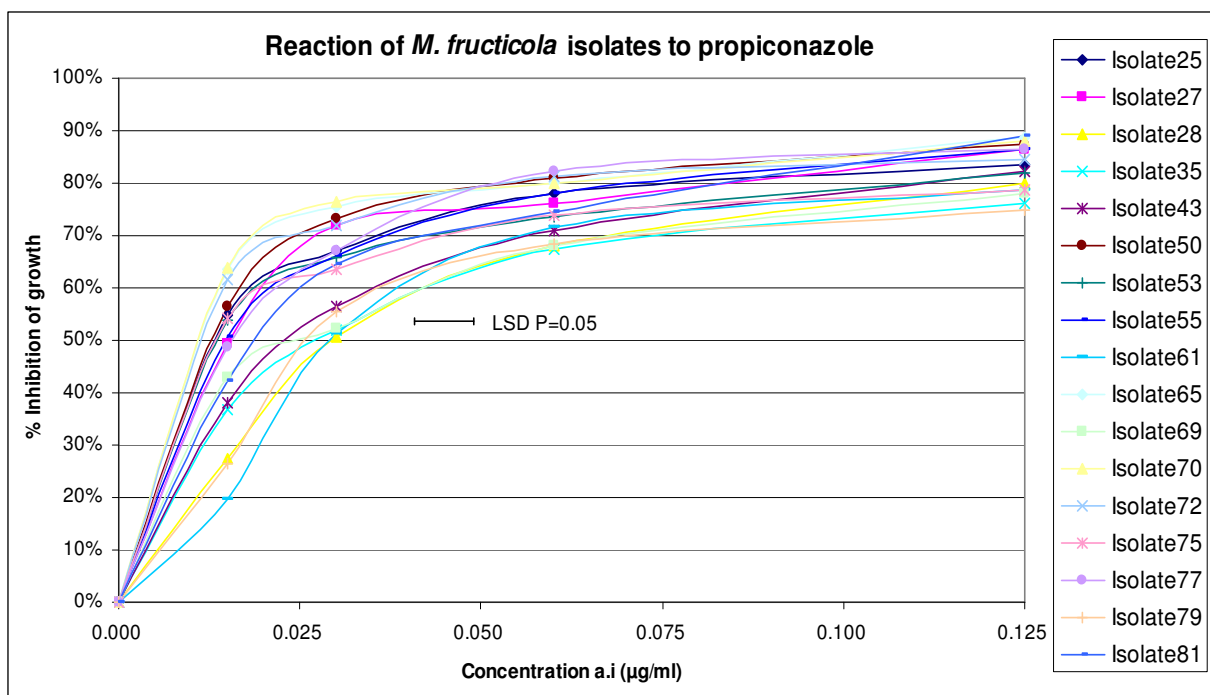


Figure 3: Percentage inhibition of mycelial growth of *Monilinia fructicola* isolates by a range of propiconazole concentrations, relative to growth on unamended PDA. Values for the isolates are the means of four colony diameters from two replicates. LSD refers to the EC50 of each isolate.

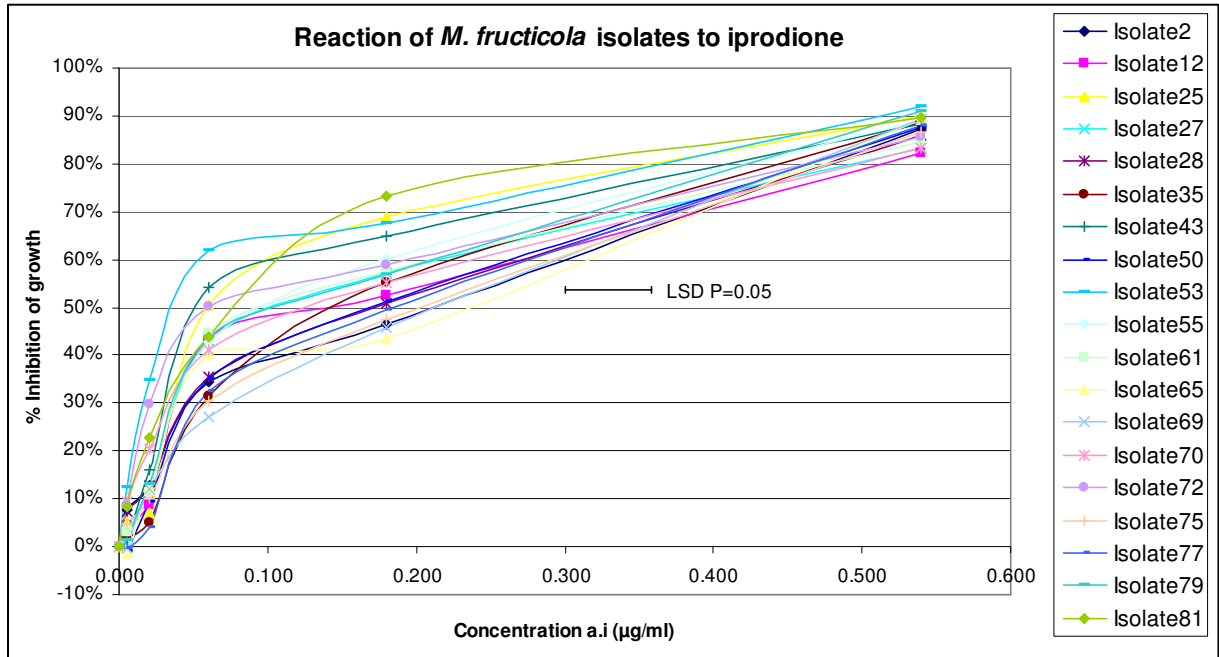


Figure 4: Percentage inhibition of mycelial growth of *Monilinia fructicola* isolates by a range of iprodione concentrations, relative to growth on unamended PDA. Values for the isolates are the means of four colony diameters from two replicates. LSD refers to the EC50 of each isolate.

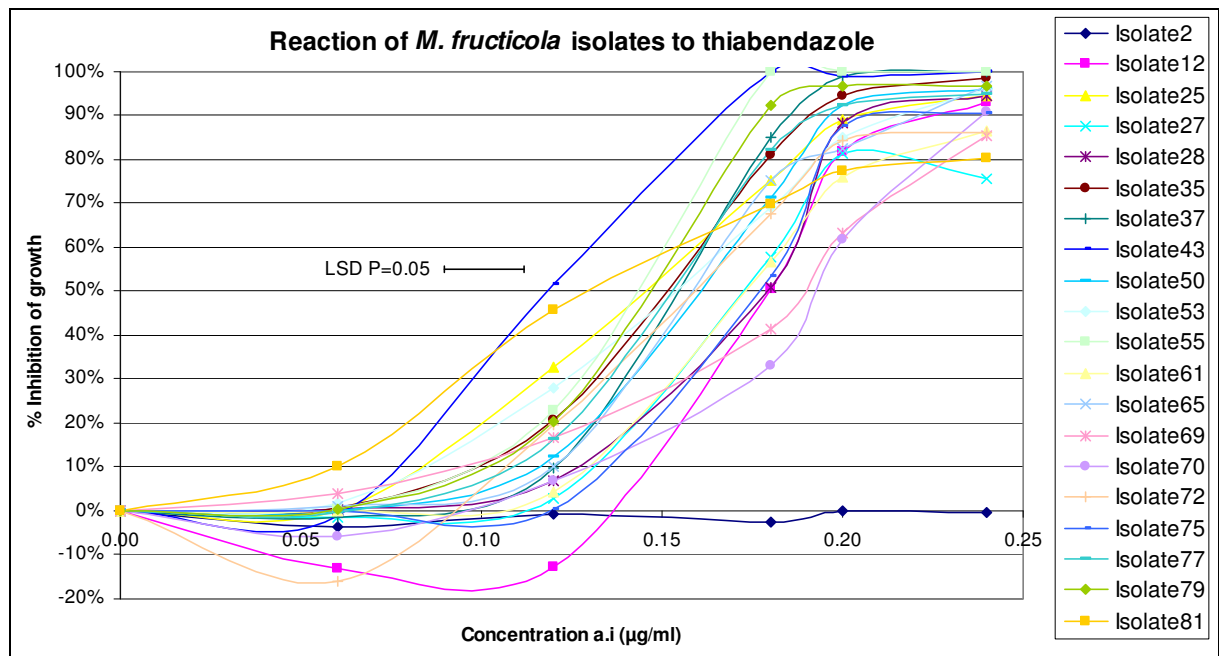


Figure 5: Percentage inhibition of mycelial growth of *Monilinia fructicola* isolates by a range of thiabendazole concentrations, relative to growth on unamended PDA. Values for the isolates are the means of four colony diameters from two replicates. LSD refers to the EC50 of each isolate.

Table 2: Statistical differences (P<0.05) between the EC₅₀ values (ug/ml) of 19 isolates of *Monilinia fruticicola* grown on media amended with fungicides, and the minimum inhibitory concentrations of the fungicides.

Isolate	EC ₅₀ [*] Propiconazole	EC ₅₀ Fludioxonil	EC ₅₀ Thiabendazole	EC ₅₀ Iprodione				
2	ND	ND	HR	0.2110	gh			
12	ND	ND	0.1745	0.1515	def			
25	0.01335	a**	0.00222	a	0.1465	bc	0.0585	ab
27	0.0152	ab	0.00312	abcd	0.17285	de	0.1015	bcd
28	0.02935	d	0.00309	abcd	0.1744	e	0.17225	efg
35	0.0275	d	0.00352	bcd	0.1512	cd	0.13765	de
43	0.0226	bcd	0.002985	abc	0.11965	a	0.0507	ab
50	0.01275	a	0.00526	e	0.16005	cde	0.17055	efg
53	0.0139	a	0.00256	ab	0.1549	cde	0.035	a
55	0.01475	ab	0.002655	abc	0.1415	abc	0.096	bcd
61	0.02885	d	0.006470	f	0.17310	de	0.10075	bcd
65	0.0109	a	0.002875	abc	0.15845	cde	0.236	h
69	0.0273	d	0.00506	e	0.19675	f	0.2197	gh
70	0.01085	a	0.00352	bcd	0.1999	f	0.132	cde
72	0.01125	a	0.003095	abcd	0.1591	cde	0.06675	ab
75	0.01325	a	0.00380	cd	0.17305	de	0.2005	fgh
77	0.01555	ab	0.00352	bcd	0.1514	cd	0.184	efgh
79	0.0257	cd	0.004255	de	0.14765	bc	0.1025	bcd
81	0.01855	abc	0.0029	abc	0.12625	ab	0.077	abc
Mean								
EC ₅₀ (ug/ml)	0.018	0.003	0.165	0.130				
EC ₅₀ range (ug/ml)	0.026- 0.029	0.0012- 0.006	0.118- 0.180	0.026- 0.220				
MIC*** (ug/ml)	>0.20	0.050	>0.50	>1.60				
LSD ****	0.008183	0.001186	0.02218	0.05889				

*EC₅₀ values for each isolate were calculated from the respective graphs for the four fungicides based on 50% inhibition of mycelia colony diameters (Figures 2-5).

** Values within each column for each fungicide with the same letter are not significantly different based on ANOVA (P < 0.05).

***MIC: Mean minimum inhibitory concentrations were based on 100% growth inhibition of isolates.

**** LSD: Least Significant Difference (P<0.05) for the EC₅₀ values between isolates for each fungicide was calculated in one-way ANOVA for the total number of isolates tested, using the mean colony diameter of each replicate.

ND: No data.

HR: Not inhibited at the range of concentration.

5.4 Conclusions

The study has provided some insights on the potential risks of fungicide resistance in brown rot based on a limited number of fungal isolates sourced from a small number of orchards. *Monilinia* isolates tolerant of higher concentrations of iprodione and thiabendazole have been detected in stonefruit orchards in Victoria. An isolate from an orchard in the Goulburn Valley was shown to be highly resistant to thiabendazole. A newly registered fungicide, fludioxonil, was effective in suppressing the growth of *M. fructicola* *in vitro*, even when used at low fungicide concentrations.

The use of *in vitro* assays to test a small number of isolates from Victoria has provided a relatively quick method for determining the effective concentrations and sensitivity baseline of fungicides. Testing a larger number of isolates from different stonefruit production regions will provide better indications of risks of fungicide resistance industry-wide, under different disease and selection pressures.

Further tests of the effectiveness of the three fungicides in preventing fruit rots caused by resistant isolates using inoculated fruit are needed, to more realistically correlate with the reduced efficacy of protectant and eradicant fungicides for control of brown rot on the fruit. The knowledge of fungicide failure in stonefruit orchards has so far been anecdotal or speculative.

Loss of efficacy in disease control due to fungicide resistance is generally expected to manifest slowly rather than as sudden, complete failure in brown rot control. The presence of a small number of resistant isolates in the population of *M. fructicola* is probably less likely to result in ineffective control of brown rot. Control failures will occur when the resistant strains eventually dominate the pathogen population in the orchard. A detailed study of larger *M. fructicola* populations within individual orchards will be needed to determine the relative abundance of resistant and sensitive populations, in order to more accurately ascertain the risks of control failure in the field situation.

While this project studied fungicides from all but one of the approved activity groups with specific modes of action, further investigations are warranted to establish baseline activities for new actives under consideration. In addition, an industry-wide survey is needed due to the gaps in knowledge of the existence of fungicide resistance and associated effectiveness of disease control. The information gained will allow clearer guidelines to be developed to better inform growers on selection or mixing of fungicides in order to prevent or delay onset of fungicide resistance.

Until further information is gained, the strategy for growers to manage the possible existence of resistant populations or minimise loss of fungicide sensitivity is to (i) ensure well-timed spray application through utilising brown rot prediction tools or predictive advice; (ii) alternating different chemical groups in the brown rot spray program; (iii) monitor for resistant brown rot populations if feasible, or monitor for hot-spots with control failures, so that more targeted studies can be undertaken to aid management of fungicide resistance, (iv) ensure thorough spray coverage to avoid localised deposits with low fungicide concentration.

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6 Prediction of postharvest fruit rot potential with pre harvest assessment methods

6.1 Introduction

Many fruit diseases such as brown rot of stone fruit have a latent, expressionless infection phase. Disease symptoms are expressed only after the fruit reach the necessary stage of ripeness or senescence, usually during storage or marketing. In order for growers to determine the storage and market potential for their fruit, it is necessary for them to know the risk of postharvest fruit rot.

Several methods have been developed to accelerate fruit ripening and senescence in order to estimate the amount of latent infection during fruit development and at harvest. These include freezing the green fruit (Overnight Freezing and Incubation Test or ONFIT) or treating with herbicide in order to induce tissue senescence, followed by incubation at room temperature to allow disease expression. Another method is a simple moist incubation test conducted shortly before harvest using ripening fruit incubated under market (warm) conditions to accelerate disease expression. The ONFIT has been used successfully to determine latent infection of *Monilinia fructicola* on prunes (Luo and Michailides, 2001) and summer fruit (Wood et al 2004), *Fusicoccum* spp. on pistachio (Mila et al. 2005) and *Botrytis cinerea* in grapes (Michailides et al. 2005). The ONFIT and moist incubation methods were evaluated in this study for their potential to identify latent infection of *M. fructicola* in stone fruit.

6.2 Materials and Methods

6.2.1 Moist incubation method

Fruit was collected from stone fruit trials established in commercial orchards in the Murray and Goulburn Valley districts of South Eastern Australia. The trials sites, weather and control programs implemented at the sites are described in more detail in chapter 2. In brief, this study used nectarine, peach (fresh market and canning) and plum fruit from several cultivars and orchards during 2006-2011 to evaluate the moist incubation method. The cultivars used were plum cv. 'Su Plum 11' (Shepparton, 2007-2009; Swan Hill 2, 2008-2011) nectarine cv. 'August Red' (Ardmona, 2006-2011), 'Arctic Pride' (Lake Boga 1, 2007-2009), 'August Pearl' (Swan Hill 1, 2006-2011) and peach cv. 'Scarlet Snow' (N. Shepparton, 2006-2011), 'Tatura 204' (NE Shepparton, 2008-2011), 'Taylor Queen' (Cobram 2, 2008-2011), 'September Sun' (Cobram 1, 2007-2011), 'Arctic Snow' (Renmark, 2009-2011; Lake Boga 2, 2008-2009) and 'Snow King' (Warrandyte, 2010-2011). Fruit was collected from the same sites over 3-5 seasons in 9 of the 12 locations.

Twenty fruit were picked from each of 6 rows (replicates) of trees, 10-15 m long, with 2 fruit picked from each of 10 adjacent trees, one picked from the top and one from the bottom section of each tree. A total of 120 fruit were harvested from each orchard during the last week before commercial harvest. Fruit were harvested into 43cm x 36cm single layer cardboard fruit trays containing plastic inserts with individual cups for 20 fruit. Fruit were transported and stored at room temperature overnight after which individual trays were enclosed in large plastic bags (drawstring rubbish bags) to

maintain high humidity, and stored at 20°C to simulate market conditions. The incidence of brown rot and other rots was assessed after 7 and 12 days incubation.

6.2.2 ONFIT

Immature and mature peaches and nectarines were harvested after pit hardening and just prior to commercial harvest from five of the orchards studied during the 2006-07 season. Twenty fruit were harvested from each of 6 plots at each orchard as described earlier. In addition, fruit samples of several varieties of canning peaches (Tatura 204, 211 and 222; Golden Queen and Taylor Queen) were harvested from trial plots at DPI Tatura during the 2009-10 season. Fruit were picked at five stages of maturity: shuck fall, pre-pit hardening, post-pit hardening, 2 weeks pre harvest and at harvest. Twenty fruit from each of 5 varieties were tested at each stage.

Batches of 20 immature fruit were surface sterilised in a solution containing 15200 ppm ethanol; 8.4 ppm sodium hypochlorite (NaOCl) and 5µL of Tween20/L (Luo and Michailides 2003). Fruit were dipped into 1.5 L of solution and washed for 20 minutes with constant, but gentle agitation and then rinsed 3 times with deionised water for 1 minute each and allowed to dry in a laminar flow cabinet. Fruit were then placed in plastic fruit trays with cups each tray holding 20 fruit each which were placed on an upturned sterilised seedling tray within plastic crates (43cm x 34cm x 16cm) then sealed and frozen for 24 hours at -20°C. Crates were then moved to a controlled temperature room at 21°C. Approximately 500 mL of water was added to the bottom of each crate to maintain high humidity. Fruit were observed each day and assessed for rot development after 7-8 and 10-11 days of incubation.

The mature fruit were washed in 4 L of surface sterilising solution as described above. Mature peaches from an initial test appeared to be not properly surface sterilised with the normal solution, therefore fruit from the main harvest were treated with a double strength sterilising solution (30400ppm ethanol and 16.8ppm NaOCl). Fruit was frozen and stored as described earlier with the exception that water was not added to the bottom of containers as the fruit contained enough moisture to maintain high humidity. Rot development was assessed after 6 days.

The canning peaches were sterilised by dipping into two solutions the first containing 70% ethanol for 10 seconds and then 0.5% NaOCl for 4 minutes followed by rinsing twice for 1 minute in deionised water. Following freezing for 24 hours they were incubated in sealed plastic containers, lined with moistened paper towel to maintain humidity, for 1 week at 20°C.

6.3 Results and discussion

6.3.1 Moist incubation test

For nectarine, plum and peach, rot incidences after 7 days incubation were similar to those after 12 days incubation when the incidence of fruit rot was below 12% (Figures 1, 2 and 3). For peaches however rot incidence increased between the 7 day and 12 day assessments (Figure 4). SEM values indicated that a 120 fruit sample was sufficient to estimate post harvest fruit rot potential from latent infections in a batch of fruit at the research block level (approximately 300-400 m²).

Results from a nectarine and a peach site monitored over five consecutive seasons showed that fruit rot incidences varied from year-to-year (Figure 5). Rot incidence in fruit from these sites increased linearly over the 12 day incubation period, with a higher incidence after 12 days incubation.

In the nectarine and peach sites in seasons with low disease (1.7-3.3% fruit rot), the fruit rot levels detected after 7 days incubation were similar to rot levels detected after 12 days incubation. In seasons with higher disease (4.2-11.67%), rot levels detected at 12 days incubation were within the range of the values detected after 7 days incubation. Therefore, in low disease sites it is likely that a 7 days incubation period would be sufficient to give a good estimate the postharvest rot potential. When peach fruit rot level was above 10% at the 7 day incubation period, incidence increased further after 12 days incubation, therefore when the 7 day incidence is above 10% further incubation is required for full rot expression.

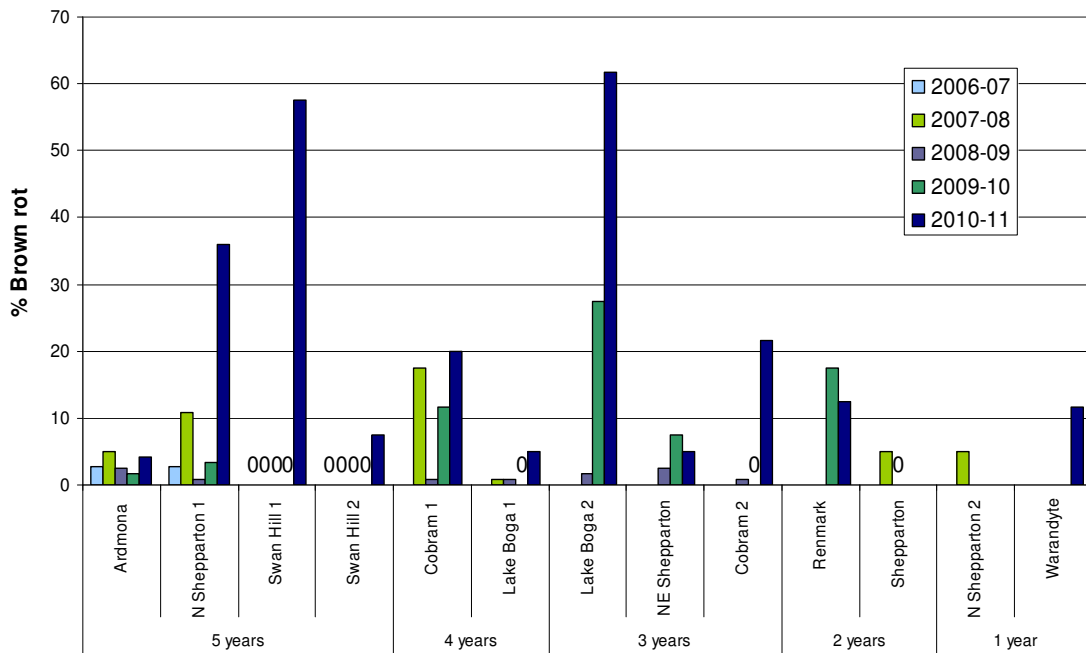


Figure 1. Mean incidence of brown rot on fruit harvested just prior to the commercial harvest at each field site over 5 years of sampling, after 7 days moist incubation at 20°C.

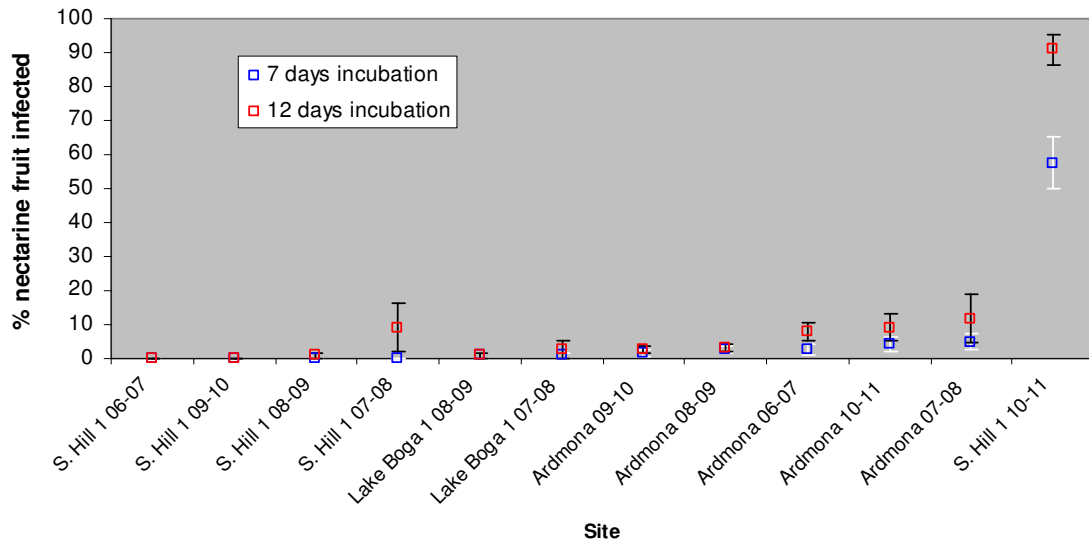


Figure 2. Mean incidence of brown rot of nectarine fruit after 7 and 12 days moist incubation. Bars are SEM.

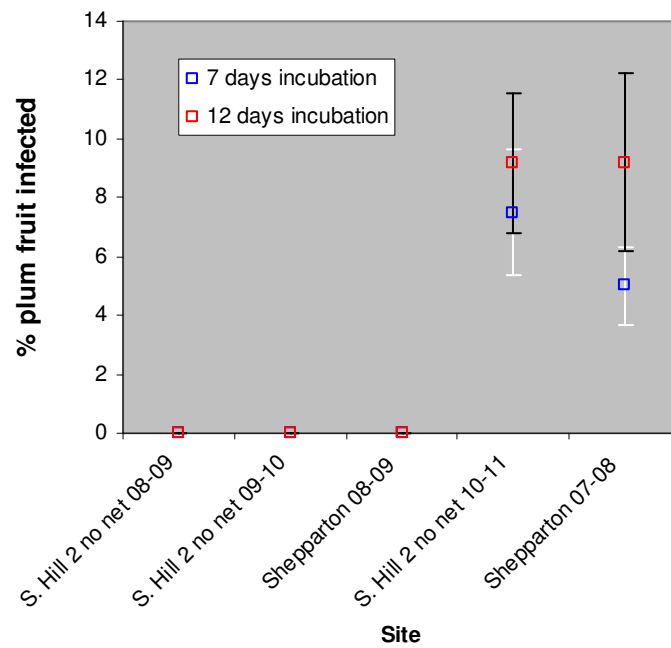


Figure 3. Mean incidence of brown rot of plum fruit after 7 and 12 days moist incubation. Bars are SEM.



Figure 6: Peach fruit after moist incubation for 7days at 20°C.

6.3.2 ONFIT incubation test

Immature peach and nectarine fruit treated with ONFIT had low levels of fruit rot, with only one fruit from Swan Hill East found to be infected with *M. fructicola*. Mature fruit treated with ONFIT were infected with a number of fungal pathogens. The majority of fruit were severely infected with *Rhizopus stolonifer* which grows very vigorously and may have limited the expression and/or identification of other fungi. *M. fructicola* was detected only in one fruit from Ardmona following ONFIT. Even using a stronger concentration of surface sterilising solution did not completely eliminate interference by other fungi known to be more saprophytic.

On canning peaches, ONFIT was an effective method for identifying latent infections in immature fruit without much interference from other fungi. A different solution was used to surface sterilise the fruit which may have been more effective in reducing superficial contamination. Only one out of 100 fruit picked at the post pit hardening stage developed rot caused by *M. fructicola* (Table 1). From the three cultivars of peaches picked 2 weeks pre-harvest and treated with ONFIT, only Tatura 222 developed rot caused by *M. fructicola* (Table 1).

Mature fruit treated only with the moist incubation method were heavily infected with *M. fructicola*, and moist incubation was sufficient to detect latent infections (Table 1).

Table 1. Incidence of brown rot on immature peaches treated with ONFIT (unshaded table cells) and mature fruit moist incubated at 20°C for 7 days (shaded table cells).

Variety	Shuck fall	Pre-pit hardening	Post-pit hardening	2weeks Pre-harvest	Harvest*
Tatura 204	0	0	0	0	0
Tatura 211	0	0	0	0	100
Tatura 222	0	0	0	60	90
Golden Queen	0	0	5	100	100
Taylor Queen	0	0	0	57.9	100

6.4 Conclusions

The moist incubation method was very effective for determining latent infections in mature plum, nectarine and peach fruit within 12 days of incubation at 20°C. This method could be useful for predicting potential risk of fruit rots post harvest using 120 fruit samples harvested shortly before (i.e. 7 days) or at commercial harvest. The method requires commercial validation including correlating detected rot levels pre harvest with post harvest rot levels to determine its accuracy for larger block sizes.

The pre harvest test used alone will allow growers and packers to make more informed decisions about the marketing of fruit especially if fruit rot levels are high. Results showed that when latent infections were low (<12%) at 7 days, extending the assessment to 12 days is not required for estimating fruit rot potential, but when latent infections were higher (>12%), a 12 days incubation period would give a better estimate of fruit rot potential. However, because low levels of fruit rot are sufficient to spoil the quality of a fruit batch, the 7 day incubation period would provide a good estimate of fruit rot potential.

In our experiments the immature fruit tested with ONFIT had very low levels of *Monilinia* latent infections and therefore we did not find ONFIT to be a valuable tool at this growth stage. In addition, the amount of interference by saprophytic fungi in the ONFIT method made assessment of brown rot incidence too difficult on immature and mature peach and nectarine fruit. ONFIT was developed to induce disease expression from latent infections in immature fruit by killing fruit tissue by freezing to allow fungal latent infection to resume growth. Wood et al (2004) reported that the use of ONFIT 14 days from harvest can provide good prediction of brown rot post harvest losses, especially when integrated with other relevant orchard information. However, during the 2003-04 season ONFIT was not as reliable as expected in providing prediction of post harvest brown rot and several factors may have been responsible for inaccuracy including interference by *Rhizopus* and *Mucor* spp.

Our work indicates that moist incubation at 20°C was a better method to allow expression of latent brown rot in mature fruit picked close to harvest. The moist incubation method is a simple, reliable and inexpensive tool for predicting post-harvest brown rot potential to assist supply chain managers to determine storage potential, and safe distribution conditions. Export suppliers in particular would find this a valuable

quality assurance tool. The usefulness of this method can be enhanced by integrating additional relevant information such as spray diary and Carpophilus beetle trapping data at the block level as demonstrated by work conducted by Wood et al. (2004). This system needs to be investigated over a wider range of block sizes to confirm it is robust enough to predict postharvest rot levels with different seasons and summerfruit crops.

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7 Technology Transfer

The extension and communication strategy aimed to ensure that at least 70-80% of growers / exporters on a national level were aware of the results and outputs of this project. The main conduit was the Australian Fruit Grower distributed free-of-charge to all Summerfruit Australia Ltd and Apple and Pear Australia Ltd members.

On-farm trials were a focus of the model development and discussions were held annually and at key decision points with the 13 collaborating growers. All growers used the infection risk information provided by the on-farm weather stations interpreted through the model to review their protectant program and to make decisions on strategic curative applications in response to infection risk.

Several meetings were held with chemical reseller organisations, horticultural consultants, the Bureau of Meteorology and with HortPlus New Zealand. These groups were identified as potential service providers for the Brown Rot Predictive Model. Results were presented at industry and scientific conferences.

7.1 Publications

Gouk S C, Holmes R, Kreidl S, Wu W, and Villalta O. 2011. Sensitivity of *Monilinia* species from stonefruit to propiconazole, iprodione and fludioxonil fungicides Poster in *Joint Conference of the Asian Confederation of Plant Pathologists/ Australasian Plant Pathology Society*, April 26-29 2011, Darwin.

Gouk S C, Holmes R, Kreidl S, Wu W.J, Villalta O. 2011. Sensitivity of *Monilinia* Species to Pre and Post Harvest Fungicides Abstracts: *Joint Conference Australasian Postharvest Horticulture Committee (APHC), the Australian Society for Horticultural Science (AuSHS) and the New Zealand Institute of Agriculture and Horticulture Science (NZIAHS)* 18-22 Sept. 2011, Lorne.

Holmes, R., Villalta, O., Kreidl, S., Partington, D., Hodson, A. and. Atkins T A. 2007. Weather-based Model Implemented in HortPlus MetWatch with Potential to Forecast Brown Rot Infection Risk in Stone Fruit. *Acta Horticulturae*. 803:19-27.

Holmes R, Kreidl S, and Villalta, O. 2007. Developing Decision Support Tools for the Integrated Management of Brown Rot in Summerfruit p26-27 *Australian Fruitgrower* December 2007.

Holmes R, Kreidl S, Lorimer S, Hossain M, Gouk C and Villalta O. 2009 Decision support for the management of brown rot in summerfruit *Swan Hill Premium Pickings* Vol 18 (March 2009) p 14-15.

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Kreidl S, Holmes R, Villalta O, Gouk C, Hossain M, Thomson F and Riches D. 2011. Development of Decision Support Tools for the Management of Brown Rot in Stonefruit Abstracts: *Joint Conference Australasian Postharvest Horticulture Committee (APHC), the Australian Society for Horticultural Science (AuSHS) and the New Zealand Institute of Agriculture and Horticulture Science (NZIAHS)* 18-22 Sept. 2011, Lorne

7.2 Presentations

Gouk S C, Holmes R, Kreidl S, Wu W.J, Villalta O. 2011. Sensitivity of *Monilinia* Species to Pre and Post Harvest Fungicides Presented Poster: *Joint Conference Australasian Postharvest Horticulture Committee (APHC), the Australian Society for Horticultural Science (AuSHS) and the New Zealand Institute of Agriculture and Horticulture Science (NZIAHS)* 18-22 Sept. 2011, Lorne.

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Kreidl S, Holmes R, Villalta O, Gouk C, Hossain M, Thomson F and Riches D. 2011. Development of Decision Support Tools for the Management of Brown Rot in Stonefruit Presented Poster: *Joint Conference Australasian Postharvest Horticulture Committee (APHC), the Australian Society for Horticultural Science (AuSHS) and the New Zealand Institute of Agriculture and Horticulture Science (NZIAHS)* 18-22 Sept. 2011, Lorne.

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8 Conclusion

This project developed and validated a weather-based infection forecasting model to support more precise fungicide timing, suitable for individual farm use. A technique was developed to assess, before harvest, the risk of development of brown rot postharvest, enabling packers and processors to appropriately segregate and treat batches of fruit according to their rot risk. These two new predictive tools will assist industry to improve the management of brown rot pre and post harvest.

The benefit of reducing *Carpophilus* beetle populations on the incidence of brown rot was demonstrated as well as the effectiveness of the Attract and Kill system for *Carpophilus* management.

Monilinia fructicola was shown to be the most important brown rot pathogen and neither of the exotic species were found in the surveys. Susceptibility of peach and nectarine to *M. fructicola* was determined at different fruit stages to better target fungicide spraying supported by weather based infection forecasting. Fungicide resistant strains of *M. fructicola* were detected and further work is needed to establish if this could explain the poor disease control experienced by some growers.

The industry gained a greater understanding of brown rot risk factors and received advice on the integrated disease management of brown rot through on-farm trials, articles in the industry journals, industry seminars, and scientific publications.

8.1 Disease risk modelling

The network of weather stations used was reliable and provided site-specific weather data needed for estimating infection periods using the peach brown rot model. Grower collaborators reported that infection period (IP) forecasting provided by SMS helped to better schedule post-infection fungicide spraying for control of brown rot in stonefruit orchards in Victoria. IP warnings also allowed growers to monitor and assess the timing of protectant fungicides. Examination of three sites where IP and spray programs were monitored in the same block over 3-4 years showed that IP forecasting contributed to better scheduling of fungicide sprays resulting in a reduction of postharvest brown rot.

This study also used IP predictions to examine the effectiveness of growers' spray programs. By analysing IP inadvertently left unprotected, we gained a better understanding of the fruit growth stages which are most susceptible to infection. Prevention of postharvest brown rot was most effective when IP that occurred during bloom, in the period up to pit hardening and in the 3 weeks before harvest were fully protected.

During the extremely wet final season (2010/11), effective rot control was not achieved in some orchards and we speculate that the persistence of the protectant fungicides was compromised by the extreme rainfall. A better understanding of the influence of rain and fruit growth on the persistence of fungicides is needed to develop a strategy for controlling brown rot in wetter climates and in wetter seasons.

8.2 The susceptible growth stages

Inoculation of peach and nectarine fruit at different growth stages showed that fruit were most susceptible in the weeks up to pit hardening and in the three weeks before harvest. This is supported by the IP studies in commercial orchards described above. The implication is that chemical and cultural controls should be intensified during the most susceptible growth stages and potentially relaxed during the more resistant phase after pit hardening and before the preharvest maturation phase.

8.3 The influence of *Carpophilus*

The experiments demonstrated that *Carpophilus* populations could be kept under control using the A&K system thus reducing the incidence of brown rot. However the A&K system can only be effective if the *Carpophilus* population is at medium to low levels and it may take more than three seasons of trap deployment to achieve a low beetle population.

8.4 Sensitivity of *M. fructicola* to field and postharvest fungicides

The study using *in vitro* bioassays has provided some insights on the potential risks of fungicide resistance in brown rot based on a limited number of fungal isolates sourced from a small number of orchards. *Monilinia* isolates resistant to higher concentrations of iprodione and thiabendazole have been detected in stonefruit orchards in Victoria. An isolate from an orchard in the Goulburn Valley was shown to be highly resistant to thiabendazole. A newly registered fungicide, fludioxonil, was effective in suppressing the growth of *M. fructicola*, even when used at low fungicide concentrations.

While this project studied fungicides from all but one of the approved activity groups with specific modes of action, further investigations are warranted to establish baseline activities for new actives under consideration. In addition, an industry-wide survey is needed to determine the extent of fungicide resistance and associated breakdown of disease control. The information gained will allow clearer guidelines to be developed to better inform growers on selection or mixing of fungicides in order to prevent or delay onset of fungicide resistance.

8.5 Predicting postharvest rot incidence

The moist incubation method using a 120 fruit sample from each orchard block was very effective for determining the amount of latent infection in mature plum, nectarine and peach fruit. When latent infections were low (<12%) after 7 days moist incubation, extending the assessment to 12 days was not required, but when latent infections were higher (>12%), a 12 day incubation period gave a truer estimate of fruit rot potential. The moist incubation method is a simple, reliable and inexpensive tool for predicting post-harvest brown rot potential to assist supply chain managers to determine storage potential and safe distribution conditions. Export suppliers in particular would find this a valuable quality assurance tool. The usefulness of this method could be enhanced by integrating additional relevant information such as spray diary and *Carpophilus* beetle

trapping data at the block level as demonstrated by work conducted by Wood et al. (2004). This system needs to be investigated over a wider range of block sizes to confirm if it is robust enough to predict postharvest rot levels with different seasons and summerfruit crops.

8.6 Technology Transfer

The extension and communication strategy aimed to ensure that at least 70-80% of growers / exporters on a national level were aware of the results and outputs of this project. However, maximum impact of this R&D will only occur with the enhancements outlined below and an on-going knowledge support to the Summerfruit and Canning fruit industries, the agrochemical industry and other service providers.

8.7 Recommendations for further research and development

The project has developed and validated two assessment tools (models) to predict the risk of brown rot, which can be used by industry to determine:

- When weather-related infection periods occur during fruit development so application of post-infection (curative) fungicides can be applied to support a protectant program for improved disease control,
- The risk of postharvest rot development with sufficient notice pre-harvest to determine an appropriate marketing strategy for each line of fruit (eg divert high risk lines to short supply chains).

We have had discussions with several organisations interested in providing services to growers using disease risk models. However, in order to provide the most value to industry, the weather-based infection risk model needs to include in addition to temperature and moisture thresholds for spore germination, information on fungicide (protectant) persistence on trees under different rainfall amounts and to factor-in changes in host susceptibility over the growing season. Improved application of fungicides using disease forecasting in combination with orchard sanitation practices have the potential to provide sustainable control of brown rot.

Experience in the 2010/11 season where several spray programs failed, suggested that fungicide persistence (wash-off) and residual activity under high rainfall was shorter than that achieved by the recommended frequency of application on the product labels. Another possible reason was the vigorous growth of fruit trees which resulted in rapid growth of new tissues for infection between fungicide applications, as indicated by extensive shoot blight infection as the season progressed. However, no residue data before and after rainfall are available to support this hypothesis.

Fruit sizes and surfaces of peaches, nectarines and cherry vary greatly at different growth stages. In addition to the influence of fruit physiology on the susceptibility of fruits to brown rot infection, physical differences in fruit surfaces have an influence on the retention of fungicides, under different moisture conditions. The precision of a predictive model for control of brown rot of Summerfruit can be improved through better understanding of the interactions of fungicide activity, fruit characteristics and environmental conditions. DPI has the capability to undertake in-depth investigation to

fill this knowledge gap, with expertise gained from the current and previous research projects on integrated management of fruit diseases, namely disease prediction, weather monitoring, determination of fruit susceptibility and fungicide activity/resistance, and development of Integrated Pest and Disease Management (IPDM) strategies.

We are proposing three years of validation work to add precision to the two predictive models, publish outcomes and assist industry and their service providers with their implementation.

The industry has access to a range of broad-spectrum protectant fungicides which are highly effective when coverage is maintained, however several of these fungicides are under review and may either be withdrawn or have their withholding periods extended. Likewise, the available curatives belong to just four chemical activity groups and they could become more restricted or lose some effectiveness due to pathogen resistance. The agrochemical and horticultural industries need to give more consideration to the highly effective, but less toxic, brown rot and blossom blight fungicides which can be used close to harvest when the fruit are most susceptible to infection.

There is scope for DPI's expertise to assist the industry with more specific disease/fungicide interaction research, to better support the precision of the predictive model, and to explain why there may be lack of control. It is crucial if a predictive model is to recommend timing of a specific fungicide, that we have good scientific basis and confidence of fungicide performance under given environmental parameters.

DPI Victoria is well placed to provide advice to the agrochemical industry on current grower practice including the benchmark fungicides; application timing and application volume for use in comparative efficacy trials. DPI Victoria also has established R&D and extension capabilities that may be utilised for further improvement of brown rot control, through development and refinement of: a disease predictive model to determine the optimal timing of the trial fungicide; techniques to estimate disease pressure and tissue susceptibility at each growth stage; techniques to assess the actual levels of latent infection at all stages of fruit development including a postharvest rot risk assessment; determination of the interactions of fungicide activity, fruit characteristics and environmental conditions to better support the predictive model and brown rot control; determination of the baseline activity of new fungicides against local brown rot isolates to facilitate the industry's on-going vigilance for fungicide resistance.

Appendix 1. Rot Hazard Management – Peaches and Nectarines

Control Point / Hazard	Control	Verification
Dormancy		
Brown Rot / Blossom Blight inoculum carryover	Maintain hygienic orchard environment – <ul style="list-style-type: none"> • Remove dropped fruit • Remove mummified fruit by pruning off shoots to which they are attached. Burn or bury mummified fruit and diseased shoots • Prune-out dead and diseased twigs • Mulch or remove other prunings 	Inspect orchard pre-bloom
Carpophilus beetle overwintering	Maintain hygienic orchard environment – <ul style="list-style-type: none"> • Remove dropped fruit 	Inspect orchard pre-bloom
Crowded tree canopy	Prune and shape trees to achieve an open canopy <ul style="list-style-type: none"> • to allow good spray penetration and more rapid drying after rain and dew • to reduce fruit-to-fruit contact which harbours pests and diseases • to reduce fruit rubbing in windy conditions 	Inspect orchard pre-bloom
Budswell to Harvest		
Infected flowers and shoots	Protect at budswell <ul style="list-style-type: none"> • Use approved protectants eg, Copper oxychloride or chlorothalonil 	

Control Point / Hazard	Control	Verification
	Forecast blossom infection <ul style="list-style-type: none"> • Model for prunes by Luo, Morgan and Michailides (2001) also applies to peaches and nectarines Protect against and eradicate infection <ul style="list-style-type: none"> • Several protective and eradicant fungicides are approved 	Inspect flowers
Fruit infections – Brown Rot, Botrytis and other fungi	Maintain a spray program using both protectant and curative fungicides from different activity groups (to avoid resistance). <ul style="list-style-type: none"> • Use Met Bureau Brown Rot warnings to forecast risk and apply a protective spray before conditions conducive to disease occur (Warnings are supplied from January to March for Northern Victorian regions only) • Use a weather station to determine when conducive conditions have occurred and apply a curative fungicide within the period specified on the label. • Assess rot risk, by incubating a sample of fruit approx 10 days before harvest. • Segregate and treat fruit at harvest according to rot risk 	If concerned about fungicide efficacy, consult a R&D agency for fungicide resistance testing. Assess retained sample and packout records. Customer satisfaction.
Fruit infections – Mucor, Fusarium and other soilborne pathogens	Discard fruit which have contacted the soil or grass. Train trees and manage grass to avoid fruit contact.	Inspection of trees prior to harvest

Control Point / Hazard	Control	Verification
Wounding - Punctures and scratches	Control chewing insects eg Carpophilus beetle, Light-Brown Apple Moth, European Earwigs	Insect monitoring eg trapping Visual inspection of fruit including survey of reject bins
Wounding - Punctures and scratches	Prevent bird attack	Visual inspection of fruit including survey of reject bins
Harvesting Equipment		
Wounding - Punctures and scratches	Ensure bins are smooth, without protrusions and free from debris	Visual inspection
Picking bags are contaminated with rot fungi	Clean bags and buckets by pressure washing or scrubbing with sanitised water	Visual inspection and swab test
Bins are contaminated with rot fungi	Clean bins to remove all signs of fruit residue and other debris. Disinfect bins.	Visual inspection and swab test
Harvest		
Bins become contaminated with rot fungi	Keep bins off wet ground (using trailers, or placing on wood shavings etc.)	
Bins and drench become contaminated with rot fungi	Prevent machinery carrying orchard soil onto unloading apron and handling areas	
Fruit are contaminated by rot fungi	Reject fallen fruit and fruit with obvious rot (eg bird damaged fruit)	Supervision of harvest
Wounding – Punctures and scratches	Train pickers	Supervision of harvest
Wounding – Punctures and scratches	Train tractor drivers, grade tracks, use low trailer tyre pressure	
Wet fruit highly susceptible to rot infection	Allow fruit to dry before harvest	
Postharvest		
Wounding – Punctures and scratches	Minimise the number of handling steps eg	

Control Point / Hazard	Control	Verification
	number of times bins are lifted and placed down	
<i>Fungicide application – on-line or bulk drench</i>		
Fruit are contaminated by rot fungi	Rinse fruit in sanitised water prior to drenching or grading	ORP Probe readings or regular manual testing of sanitiser concentration
Fruit are contaminated by rot fungi	Replace drench frequently – according to label directions Clean application equipment often	Swab test
Postharvest fungicides ineffective	Strictly follow label directions eg. Application method, rate and timing (within specified period after harvest)	
Postharvest fungicides ineffective	Follow label directions for the prevention and management of fungicide resistance	Fungicide resistance testing
<i>Cooling and storage</i>		
Fruit lose condition during storage	Maintain strict temperature tolerances, especially avoiding chilling zone	Temperature and atmosphere logging
<i>Packing</i>		
Fruit are contaminated by rot fungi during sorting and grading	Regularly replace and sanitise dump tank water	ORP Probe readings or regular manual testing of sanitiser concentration
Fruit are contaminated by rot fungi during sorting and grading	Remove rotting fruit and debris from dump tank	
Fruit are contaminated by rot fungi during sorting and grading	Spray fruit with fresh or sanitised water immediately after inspection table.	

Control Point / Hazard	Control	Verification
Fruit are contaminated by rot fungi during sorting and grading	Wash down sorting equipment daily	
Fruit are contaminated by rot fungi during sorting and grading	Empty culled fruit container two-hourly and cover culled fruit (bury or covered dumpmaster) Control Vinegar flies in the packing shed	
Wounding during sorting and grading packing– Punctures and scratches	Correct set up and maintenance of grading line	Instrumented sphere analysis, Quality audit
<i>Ripening</i>		
Fruit are contaminated by rot fungi	Ripen in new or sanitised containers Clean ripening room	Swab test Plate exposure test
Infection conditions occur	Avoid free moisture (dew) formation on fruit	Observation
Fruit become over-ripe or remain too long at room temperature	Control ripening appropriate to fruit condition	Firmness testing

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