Through Chain Rot Management in Apples

Dr Robert Holmes
Victorian Department of Primary Industries (VICDPI)

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Through Chain Rot Management for Apples

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Media Summary

Postharvest rots are a significant problem in both domestic and export supply chains having an estimated annual economic impact of $16 to $25 million. Losses have been particularly high for Pink Lady™ but also excessive for Gala, Fuji, Sundowner and other varieties.

This national project diagnosed the diseases, identified the underlying causes of their increased incidence and developed the knowledge to enable industry to more effectively manage the risks. Causal factors studied included:

- inoculum sources in the field such as tree cankers and mummified fruits,
- the effectiveness of growers’ spray programs, orchard practices and postharvest practices
- the performance of sanitisers in the packing process
- potential fungicide resistance in the pathogens causing target rot and ripe spot

Also explored was the possibility of giving fruit a ‘rot risk index’ at harvest to enable fruit to be segregated and appropriately treated according to rot risk.

Examination of fruit during packout attributed most rots to the fungal pathogens *Alternaria*, *Neofabraea* (target rot) and *Penicillium* (blue mould). *Neofabraea* spp. infect fruit in the field and are dispersed by rain. The fruit is most susceptible when rain occurs over the harvest period. *Penicillium*, on the other hand, most commonly infects fruit after harvest through handling injuries such as stem punctures and scratches.

Rots were most severe in fruit that:

- was harvested wet, particularly during or within a day after sustained rain
- did not receive an effective fungicide in the three weeks before harvest
- were not drenched after harvest in a benzimidazole fungicide.

These three factors, and less attention to hygiene, were identified as the main contributors to rot risk, with the most severe rot levels occurring when all these conditions occurred. Some improvement in rot control was achieved by applying stricter hygiene practices in the field and storage facility, which limits contamination of the fruit by pathogenic fungi.

This report includes a best practice guideline for effective rot control and recommendations for further development including:

- publication of this guideline on the APAL website
- investigating regional differences in diseases and optimising controls
- assessing water sanitisation systems in fruit dump tanks
- scoping resistance issues, reviewing current fungicides and their potential replacement.
Technical Summary

Postharvest rots are a significant limitation to productivity, having an annual economic impact of $16 to $25 million. Rot incidence has been increasing and losses have been particularly high for Pink Lady™ but also excessive for Gala, Fuji, Sundowner and other varieties.

This national project diagnosed the diseases, identified the underlying causes of their increased incidence and developed the knowledge to enable industry to more effectively manage the risks. Causal factors studied included:

- inoculum sources in the field such as tree cankers and mummified fruits,
- the effectiveness of growers’ spray programs, orchard practices and postharvest practices
- the performance of sanitisers in the packing process
- potential fungicide resistance in the pathogens causing target rot and ripe spot

Also explored was the possibility of giving fruit a ‘rot risk index’ at harvest to enable fruit to be segregated and appropriately treated according to rot risk.

Examination of fruit collected during packout in five states attributed most rots to the fungal pathogens Alternaria alternata, Neofabraea alba, N. perennans and Penicillium spp. Other anthracnose diseases associated with Colletotrichum gloeosporioides and Botryosphaeria sp were diagnosed in Queensland. Also in Queensland a lenticel spot associated with a Phoma-like fungus was common on both Pink Lady and Granny Smith. The survey further supported the knowledge that Neofabraea malicorticis (Bulls-eye rot), Monilinia fructigena (Apple brown rot) and Neonectria ditissima (European canker and fruit rot) are absent from Australia. Likewise diagnoses of tree cankers in Victoria did not reveal any exotic species of concern.

Analysis of grower practices, using a rot risk model established that postharvest rots had highest incidence when fruit:

- was harvested wet, particularly during or within a day after sustained rain
- did not receive an effective fungicide in the three weeks before harvest
- were not drenched after harvest in a benzimidazole fungicide (thiabendazole is approved for domestic fruit, but not for EU exports).

These three factors, and less attention to hygiene, were the main identifiable contributors to rot risk, with the most severe rot levels occurring when all these conditions occurred. Some producers report an improvement in rot control since applying stricter hygiene practices in the field and storage facility including the use of clean debris free bins, sanitising bins and preventing contamination of bins by field soil.

Four techniques were evaluated for accelerating rot development to assess rot potential; moist incubation, overnight freezing, dipping in ethephon and paraquat. The paraquat treatment revealed the highest incidence of latent infections due to identifiable pathogens but further work is needed to validate the technique and develop a safe operating procedure, before it can be recommended to industry.

Monitoring of a fruit dump tank revealed water sanitisation systems may not be performing as well as anticipated when a line of fruit with high rot incidence is being processed. Fruit were being further contaminated with rot pathogens during packing which may cause rot development in the supply chain.

A study of in-vitro fungicide activity against Neofabraea determined that thiabendazole is still effective and its use for postharvest drenching should be supported. However DMI resistance is apparent and the agrochemical industry should be cautious about promoting DMI fungicides for Neofabraea control.
The project has discovered new information on optimising rot control and this has been incorporated with existing knowledge into a best practice guideline for effective rot management. Early drafts and subsequent updates have been published for the industry’s awareness and reference. The current document should now be made available in an accessible place such as the APAL and Horticultural Industry Network (HIN) websites.
Introduction

Since the development of the cultivar marketed as Pink Lady™, Australia has regained a significant share of the UK apple market. However, despite the fact that export returns were higher, grower confidence in the export sector has been severely undermined as a result of a number of factors including skin blemish (with losses in excess of $3 million) and reducing numbers of permissible chemicals (pre and post-harvest) which potentially limits rot control (N. Offner pers com).

Exports valued at about $19m target the premium markets in Asia and Europe. At an industry meeting in 2007 growers and exporters reported they had experienced increasing losses over the previous three seasons in major apple varieties due to postharvest rots. This had been particularly significant for Pink Lady™ for which up to 40% loss during storage was recorded.

Examination of apple rots from four states at the end of the 2007/08 storage season implicated both *Phlyctema vagabunda* (the anamorph of *Neofabraea alba*) and *Penicillium* spp. Losses were particularly significant for Pink Lady™ and excessive rot levels caused by *Phlyctema vagabunda* were also recorded in Gala, Fuji and Sundowner™. Extrapolating this exploratory work estimated the annual economic impact of postharvest rots in Australian apples as approx. $16 to $25 million.

*P. vagabunda* belongs to the *Neofabraea* genus of important branch canker and fruit rot pathogens. A more pathogenic member of the genus, *N. malicorticis* which causes severe disease in parts of North America, Europe and New Zealand is currently believed to be exotic to Australia (Cunnington, 2004). A broad survey will help establish the exotic status of *N. malicorticis* and assist with our preparedness to detect and diagnose other exotic branch canker and fruit rot pathogens such as *Neonectria ditissima* (previously known as *Nectria galligena*).

A crop loss study conducted in Victoria (Holmes, 1993) identified thirty-six species of fungi including 10 species of *Penicillium* which together caused an average of about 5% loss of apples during storage. *Phlyctema vagabunda* (the anamorph of *Neofabraea alba*) was a significant cause of postharvest rot and the incidence was highest on Red Delicious strains. Other varieties prominent at that time, Golden Delicious, Jonathan and Granny Smith, appeared to be less susceptible. Conditions in the orchard and postharvest environments influence the incidence of postharvest rots. These include:

- The amount of fungal inoculum in the orchard and postharvest and the occurrence of infection conditions
- The susceptibility of fruit to fungal infection which is influenced by fruit maturity, nutritional status, physical and physiological integrity.
- The storage and transit duration, temperature and atmosphere.
- The efficacy of fungicidal treatments applied in the field and after harvest, including sanitisers.

As a substantial proportion of infections leading to rots exist on fruit before harvest, there is an opportunity to estimate postharvest rot potential by ripening a sample of fruit taken at harvest (Spotts, 1990). Harvested fruit may carry quiescent (latent) infections of fungi such as *Alternaria, Botrytis, Fusarium* and *Neofabraea*. Infections remain quiescent while the fruit is preclimacteric, but develop into visible rots as the fruit ripen, which usually occurs several months after harvest, during storage and marketing. As apples are generally harvested in an unripe (preclimacteric) state for long term storage, this project focussed on techniques to accelerate the natural ripening of apple fruit. Some varieties such as Granny Smith require a short period of cold treatment to induce ethylene competency (Jobling et al., 1991), whereas...
other varieties such as Gala, rapidly enter their ethylene climacteric after harvest. It is expected that by applying ethylene (or ethephon) and optimal ripening temperatures a sample of fruit can be induced to reach a senescent state within 10 days after harvest, accelerating the expression of latent rots. Freezing fruit or dipping fruit in the herbicide paraquat are other techniques shown to accelerate rot expression by overcoming the fruits resistance to rots. These will also be investigated for their usefulness for estimating postharvest rot potential.

The apple industry relies on a combination of pre- and post-harvest fungicides to control fungal rots during long term storage and export. At the start of this project a range of postharvest fungicides were registered including actives with curative and protectant properties (imazalil and carbendazim) and one with mainly protectant activity (iprodione). However, the use of carbendazim was under review and imazalil was not permitted for fruit destined for Europe. Several fungicides registered for field application against postharvest rots were also not permitted or had extended withholding periods applied for some markets. The export sector in particular had a limited choice of fungicides to use, with only copper and sulphur fungicides as options within 14 days before harvest. Therefore a preliminary analysis of the available and prospective fungicides for field and postharvest use against postharvest rots was also an objective of the project.

The main objectives of this national project were to diagnose the diseases, identify the underlying causes of their increased incidence and provide industry with the knowledge and tools to more effectively and cost-efficiently manage the risks. Causal factors for detailed study include:

- inoculum sources in the field such as tree cankers and mummified fruits,
- the effectiveness of growers’ spray programs, other orchard practices and postharvest practices
- the performance of sanitisers in the packing process
- potential fungicide resistance in *Neofabraea*

The strategy used:

- Work with industry to gain a detailed understanding of the risk factors contributing to postharvest rots.
- Develop a risk assessment tool to enable industry to segregate and appropriately treat lines of fruit according to rot risk including a methodology which will establish a “rot index” for individual lines of fruit within a short time after harvest.
- Develop a through-chain disease management guideline for inclusion in the integrated pest and disease management manual written by Dr Shane Hetherington, and other industry publications.
- Publish articles in fruitgrower magazines, industry newsletters and deliver seminars.
General Materials & Methods

A project network was established for the sharing of information between the research team in each state, Pink Lady Australia, Australian Fresh Fruit Company (AFFCO), Fruitgrowers Victoria and Agrochemical manufacturers and resellers. The network identified growers and packers in 3 states who were either satisfied or dissatisfied with the effectiveness of rot control and these businesses became the case studies for analysis. An evaluation framework was designed and businesses in Queensland, Victoria and South Australia were questioned to gather information on practices to establish common problematic conditions, successful and less successful practices. A literature review was conducted to gather knowledge about 1) the epidemiology and control of Neofabraea rots in apples and 2) stimulating latent rot development to enable an early warning of rot risk.

Experiments were conducted on Gala and Pink Lady in New South Wales and Victoria to determine if the expression of postharvest rots could be hastened to facilitate early rot risk assessment (indexing).

In the first and second seasons, fruit culled at packing showing rots were collected in Queensland, New South Wales, Victoria, South Australia and Western Australia to determine pathogens present. A limited number of isolates were tested in-vitro for resistance to postharvest fungicides.

The effectiveness and relative merits of registered and non-registered fungicides were discussed with the Agrochemical industry to identify potentially useful fungicides.

A study of a water dump on a packing line gave a useful understanding of the performance and limitations of a sanitiser system.

Project progress and outcomes were presented at several fora and published in industry magazines.

A best practice rot control guideline was produced.
Chapter 1 - Survey and Diagnostics

Introduction

A limited study examining apple rots from New South Wales, Victoria, South Australia and Western Australia at the end of the 2007/08 storage season implicated both *Phlyctema vagabunda* (the anamorph of *Neofabraea alba*) and *Penicillium* spp. Losses were particularly significant in South Australia in Pink Lady™ and excessive rot levels caused by *Phlyctema vagabunda* were also recorded in Gala, Fuji and Sundowner™. This further study was initiated to diagnose the causes of excessive levels of postharvest rot by identifying the causal fungi and analysing orchard and postharvest operations to identify the more successful and less successful control practices.

Materials and Methods

The project team identified a sample of growers and packers in Queensland, New South Wales, Victoria and South Australia who were dissatisfied with their level of postharvest rots in apples. Samples of fruit showing rots were collected from their cull bins during 2008 and 2009 and came from lines of fruit which had been in storage for 5 to 12 months. These were submitted to one of four state diagnostic laboratories for fungal identification. Fungi associated with rots were identified by direct microscopic examination, isolated aseptically from the rot margin or isolated by picking off fungal growth from rot lesions. Non-sporing fungi were identified using molecular methods and many fungi remained unidentifiable by these methods. Cultures of *Neofabraea* isolates were retained for in-vitro fungicide activity tests (Chapter 4). A questionnaire was developed to help analyse control practices and identify where infection conditions may have existed (Appendix 2). This was applied most comprehensively in South Australia where Paul James analysed 30 grower lines packed at the Lenswood Co-operative and 10 grower lines from a further 4 packers.

The project collaborated with the Pink Lady bulk shipment trial 2008 in which 6 boxes of pink Lady from each of 5 growers was set aside in simulated export and retail conditions. This fruit had a well documented history and the plan was to submit fruit which developed rot for diagnosis. However by the end of the trial no rots had developed. There were no further samples from Western Australia and no samples submitted from Tasmania.

Many fungi causing fruit rots may also occur in the orchard environment where they are associated with tree canker, twig blight and mummified fruit e.g. *Neofabraea, Botryosphaeria* and *Phomopsis*. To better understand if tree infections and mummified fruit could be contributing to postharvest rot, sampling was conducted in a Victorian orchard during bloom (October 2010). The orchard selected produced fruit with high incidences of *Neofabraea* rots. Samples were taken of limb cankers and twig blights on Granny Smith and Jonathon trees (approx 25 years old) and of mummified fruits on Pink Lady trees. Collections were diagnosed by aseptically isolating associated fungi.

Results and Discussion

Fungi associated with postharvest apple diseases

Diagnosis of diseased fruit identified 15 taxa (genera or species) of known endemic pathogens. The most frequent were *Alternaria* spp (Fig 1.8), *Penicillium* spp (Fig 1.1) and *Neofabraea* spp (Fig 1.2, 1.3 and 1.4). Five types were found only in Queensland; *Botryosphaeria* (Black Rot, Fig 1.5), *Colletotrichum gloeosporioides* (Bitter rot Fig 1.6) and *Nigrospora* sp (Fig 1.7).
associated with fruit rots; an unidentified Phoma-like fungus associated with lenticel spots (Fig 1.8) and Microsphaeropsis sp (Fig 1.9). Microsphaeropsis is not a known pathogen of apple fruit and is likely to be saprophytic or parasitic on another pathogenic fungus. A species of Microsphaeropsis commonly associated with apple, M. ochracea is a mycoparasite of the apple scab fungus Venturia inaequalis (Carisse et al.2007).

Two species of Neofabraea were identified; N. alba and N. perennans. N. alba causes a fruit rot with several common names Anthracnose, Ripe fruit rot, Lenticel rot, Target rot, Target spot (Fig 1.2). N. perennans causes the disease known as Anthracnose in Australia (Fig 1.3) and perennial canker of apple tree limbs. All fungi resembling Neofabraea in culture were sequenced to check they were not exotics eg Neofabraea malicorticis (Bull’s eye rot) and Neonectria ditissima (European canker and fruit rot). No exotic species were detected. A large proportion (20%) of isolations made from diseased fruit were not able to be identifiable with the resources available (Table 1.1).
Table 1.1 Number and frequency (%) of taxa (genera or species) of fungal isolates from diseased apple fruit. Collections were from 9 orchards in 4 states.

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<td>4 (9%)</td>
<td>1 (18%)</td>
<td>2 (18%)</td>
<td>1 (12.5%)</td>
<td>5 (50%)</td>
<td>3 (60%)</td>
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<td>1 (20%)</td>
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<td>2008</td>
<td>Pink Lady</td>
<td>1</td>
<td>1 (20%)</td>
<td>4 (18%)</td>
<td>4 (9%)</td>
<td>1 (18%)</td>
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<td>1 (12.5%)</td>
<td>5 (50%)</td>
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<td>2 (20%)</td>
<td>1</td>
<td>1 (20%)</td>
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<td>1 (20%)</td>
<td>164 (100%)</td>
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Figure 1.1 Blue mould rot caused by *Penicillium* sp. on Pink Lady (L) and Gala (R). Note lesions may not show characteristic blue spore masses.

Figure 1.2 Target rot caused by *Neofabraea alba*, Note cream coloured spore-bearing masses may be present on mature lesions.

Figure 1.3 Anthracnose rot of Granny Smith from which *Neofabraea perennans* was isolated.
Figure 1.4 Cultures of *Neofabraea perennans* (L) and *N. alba* (R). Photo R. de Boer, DPI Victoria

Figure 1.5 Rots of Granny Smith (L) and Pink Lady (R) from which *Botryosphaeria* was isolated.

Figure 1.6 Rot of Granny Smith from which *Colletotrichum gloeosporioides* was isolated.
Figure 1.7 Rot of Granny Smith apple from which *Nigrospora* sp was isolated.

Figure 1.8 Spots on Granny Smith from which a Phoma-like fungus was isolated. *Alternaria* was isolated from the larger rot (bottom right of photo).

Figure 1.9 Lesion on the side of a Granny Smith apple apparently caused by *Venturia* from which *Microsphaeropsis* sp was isolated.
Fungi associated with tree cankers, twig blights and mummified fruits

Fungi isolated from limb cankers included *Valsa malicola* (from black fruiting bodies on Granny Smith twigs and Jonathan bark, Fig 1.10); a Fusarium - like fungus from white eruptments on Jonathan bark and *Phomopsis* from fruiting bodies on mummified Pink Lady fruit. This is the first record of *Valsa malicola* in Australia. According to Jones (1990) *Valsa malicola* is a saprophytic fungus occurring on apple branches primarily diseased by other fungi. A related fungus *Valsa ceratosperma* which is not known to be present in Australia, causes Valsa canker, a serious disease of apple in Japan, China and Korea.

Tree health of the 25 year-old Granny Smith and Jonathan trees was poor with visible dieback and cankers on the major limbs and twigs. However among the fungi isolated, none represented the fruit pathogens identified causing significant crop loss in this orchard. We therefore have no evidence from this study, limited to one orchard, that tree cankers are a source of inoculum causing *Neofabraea* rots of fruit in storage.

In the younger Pink Lady block, mummified fruit were found in the trees and on the ground. Some of these fruit were showing sporulating fungi including *Phomopsis* and *Penicillium* which are known causes of postharvest rots. These fruit were actively producing infectious fungi during bloom, are potential rot hazards, and should be removed from the orchard. Fruit attached to prunings found on the ground had not broken down during winter could also be capable of producing infectious spores during fruit development.

Figure 1.10 *Valsa malicola* was isolated from black fruiting bodies on Granny Smith twigs (L) and Jonathan bark (C and R).
Figure 1.11 A Fusarium-like fungus was isolated from white eruptments on Jonathan bark.

Figure 1.12 *Phomopsis* was isolated from fruiting bodies on mummified apples (40x image on left). The larger apple in the right photograph is also showing sporulating *Penicillium*.
Evaluation of growers’ practices

*By Paul James Rural Solutions SA*

The investigation identified that many different chemical dipping and storage practices were used in the year of study (2007-08). Lines of fruit were categorised according to pre- and postharvest rot controls applied.

**Pre-harvest fungicides**

In terms of pre-harvest controls fruit which received a pre-harvest fungicide spray (usually captan) performed significantly better than fruit which were not sprayed within 3 weeks of harvest. Note: to comply with EU requirements for Pink Lady apples, captan can be used only up to 28 days before harvest; mancozeb, thiram, penconazole and hexaconazole can be used up to 14 days before harvest and copper and sulphur compounds can be used up to 7 days prior to harvest (Pink Lady Australia Grower Alert No3 April 2009). For the local market, however, captan and thiram have a 7 day withholding period whereas mancozeb has a 14 day withholding period. Most growers would not consider using copper and sulphur compounds near harvest because of the risks of visible residues, phytotoxicity and the impact on beneficials in the orchard.

Where growers had used preharvest “in field” fungicide applications the incidence of rot problems was appreciably lower than where they had not been used. One dilemma for the industry is that captan which has proven to be effective, and in the USA can be used right up to harvest for postharvest rot control (OSU 2010), is not registered in Australia for rot control but is registered for apple scab (black spot) control. To overcome problems with chemical use compliance requirements one chemical reseller is suggesting growers use captan as the last "Blackspot" spray for the season, anticipating 'coincidental' control of rots..

**Postharvest dipping and storage practices**

Fruit which were drenched in fungicide then treated with SmartFresh or drenched in fungicide and DPA performed better than fruit which did not receive a postharvest fungicide. Drench fungicides used were Carbendazim or Rovral + Fungaflor. While carbendazim was shown to be effective, it’s use was suspended in July 2010 and another fungicide in the same benzimidazole family, thiabendazole (Vorlon®), has become available. However the use of benzimidazole group fungicides is not permitted on fruit exported to the EU.

The worst case scenarios were predominantly associated with no pre harvest fungicides and no carbendazim in post harvest dips. One grower had fruit dipped and stored in 3 different coolstores with 3 distinctly different outcomes. One scenario performed very poorly and over 100 bins of fruit were lost. In this specific case the fruit had no preharvest fungicide or carbendazim applied.

Fungi isolated from fruit included *Neofabraea alba* (identified as *Gloeosporium album*) and *Penicillium* spp. One line of fruit was showing a black fruit rot, from which *Stemphylium* sp and a *Phoma* - like fungus were isolated.

**Other factors**

In discussing grower practices it was also clearly evident that bin hygiene varied considerably. Those growers that routinely cleaned their bins and allowed them to stand outside in sunlight for at least 2 weeks prior to use also appeared to have lower rot problems. The pick up and transport of bins with soil attached also appeared to increase the incidence of rots.
The issue of rots has been so significant within the industry that large packhouses have undertaken extensive cleaning of their coldrooms and there are plans to install a bin washing machine in at least 1 packhouse.
Chapter 2 - Rot indexing

Introduction

A substantial proportion of infections leading to postharvest rots exist in the fruit before harvest. There is therefore an opportunity to estimate postharvest rot potential by determining the amount of latent infection in a sample of fruit taken at harvest (Spotts, 1990). An assessment of the potential for postharvest rot, a 'Rot Index', could be a useful tool to assist industry determine the expected postharvest performance of individual lines of fruit.

Harvested fruit may carry quiescent (latent) infections of fungi such as Alternaria, Botrytis, Fusarium and Neofabraea. Infections remain quiescent while the fruit is preclimacteric, but develop into visible rots as the fruit ripen, which usually occurs several months after harvest, during storage and marketing. As apples are generally harvested in an unripe (preclimacteric) state for long term storage, this project focussed on techniques to accelerate the natural ripening of apple fruit. Some varieties such as Granny Smith require a short period of cold treatment to induce ethylene competency (Jobling et al., 1991), whereas other varieties such as Gala, rapidly enter their ethylene climacteric after harvest. It is expected that by applying ethylene (or ethephon) and optimal ripening temperatures a sample of fruit can be induced to reach a senescent state within 10 days after harvest, accelerating the expression of latent rots. Freezing fruit or dipping fruit in the herbicide paraquat are other techniques shown to accelerate rot expression by overcoming the fruits resistance to rots (Luo and Michailides, 2001; Biggs, 1995). These were also investigated for their usefulness for estimating postharvest rot potential.

Apple varieties which do not require a cold treatment to induce ethylene production ripen most rapidly in humid air at 25°C. The response to ethephon is also expected to be most rapid under these conditions. Rot expression will also require suitable conditions for fungal growth. Growth of Penicillium expansum is optimal at 25°C and ceases below -3°C and above 35°C (Panasenko 1967). The optimal growth temperature for Phlyctema was not found in a literature search, but a related species Neonectria grows most rapidly between 18°C and 24°C (Munson 1939).

Experiment 1. Effect of temperature and ethephon treatment on the ripening of Gala apples

Dario Stefanelli et al DPI Victoria, Knoxfield

Aim:

To measure the effect of Ethrel concentration and temperature on apple ripening and rot development.

Methods

Royal Gala apples were submerged for three minutes in batches of 50 in deionised water (control) or one of four Ethrel concentrations (100, 500, 1000 and 5000 ppm ethephon). A wetting agent (0.1% Triton X-100) was included in all solutions. All apples were left to dry at room temperature overnight. During this process apples treated with Ethrel were separated from untreated apples to minimise cross contamination.
Apples from each treatment and control were separated in groups of 5 and placed into plastic boxes containing wet paper in a Petri dish as a humidity source (Fig 2.1). Boxes were lined with paper towels and closed with a lid. Boxes were then placed in crates to separate them from the other replicates. Each crate contained a repetition of all of the Ethrel treatments plus control. Five replicates (crates) were placed in 20°C and 24°C for three weeks (Fig 2.2). Assessments were performed every other day on all apples in the trial. Firmness was measured using an Aweta non-destructive sonar instrument, with repeated firmness measured on all fruit. Measurements were performed on two sides of every fruit and repeated at the same location on the fruit, which was marked by a circled number (Fig 2.1). At the start of the experiment a sample of 25 fruit was evaluated for total soluble solids, firmness by Aweta and firmness by Effegi penetrometer. Individual fruits were weighed before and after storage.

Fruit were examined for rots and eventually removed from the trial as they attained a lesion size covering the whole side of the apple. The experiment was repeated twice; the first time with waxed apples and then with unwaxed apples.

Results

There was insufficient natural rot development during both the experiments to analyse. The waxed apples whitened due to the submersion in the treatment solutions. Pre-treatment with wax affected ripening. Firmness of the waxed fruit did not show any significant decrease during storage (Fig 2.3) which is contrary to normal development. For this reason the experiment was repeated with non-waxed apples.

**Firmness (non-waxed apples)**

Treatment with Ethrel had no effect on the firmness of the apples, they all softened at the same rate, within the given temperature. There was however an effect of temperature with both waxed and unwaxed apples softening more rapidly at 25°C than 20°C (Fig 2.4).

**Weight loss**

There was no significant difference between any treatments with respect to weight loss. This shows there was no ill effect of treatment on the ripening of the apples, as they all lost weight at the same rate.

**Total soluble solids**

There was no difference between treatments or temperature on total soluble solids. TSS did decline similarly in all treatments and this was determined to be an effect of storage and not of Ethrel concentration.

Figure 2.1. Non-destructive firmness measurement with AWETA sonar device. In the lower right hand corner a plastic box containing one experimental replicate is shown. Each apple has a spot marked where the repeated readings were taken.
Figure 2.2. Crates containing treatment replicates inside the temperature controlled chambers.

Figure 2.3. Firmness trend of waxed gala apple fruit stored at 20°C (L) and 25°C (R) as measured by Aweta non-destructive sonar instruments.

Figure 2.4 Firmness trend of non waxed gala apple fruit stored at 20°C (L) and 25°C (R) as measured by Aweta non-destructive sonar instruments.
Experiment 2. Effect of temperature and ethephon treatment on ripening and rot development in Gala apples

Christine Frisina et al. DPI Victoria, Knoxfield

Aim:
To measure the effect of ethephon and temperature on rot development in Gala apples.

Methods
Gala apples were washed in deionised water plus a wetting agent (0.1% Triton X-100), then submerged in water with or without 1000ppm ethephon (Ethrel®) for three minutes. All apples were left to dry at room temperature overnight. During this process apples treated with ethephon were separated from untreated apples to minimise cross contamination.

Apples were then placed into single layer boxes containing plastic plix trays to separate individual apples. Each tray contained 20 apples. Three trays of apples with each treatment were placed at 20°C and 25°C. At each temperature, of the three trays, two were wounded with a 3mm diameter nail stoppered to penetrate to 5mm depth. One of each of these two trays containing wounded fruit was then inoculated with *Penicillium expansum* by injecting 10µL of conidial suspension (at concentration of 1 x 10⁴ cfu/mL) into the wound with a pipette.

Firmness was measured using an Aweta non-destructive sonar instrument, with repeated firmness measured on all fruit that were not inoculated. Measurements were done on two sides of every fruit and repeated at the same location on the fruit, which was marked by a spot or cross. Individual fruit weight (g) was monitored from initial set-up and at each evaluation time. This was done on all fruit that had not been inoculated.

An initial extra sample of 20 fruit was evaluated for firmness with the Aweta non-destructive instrument and destructive firmness, using an Effegi penetrometer. Firmness by Effegi penetrometer was also measured at the end of storage. Fruit were removed from the trial as they attained a lesion size covering the whole side of the apple.

Results

Lesions

Fruit that was wound inoculated developed a rot incidence of 65-70% at day three and 100% by day seven. The trend was the same at both 20°C and 25°C. Ethephon treated fruit had a slightly lower incidence than untreated (5-10% less) at both temperatures, but this was not statistically significant.

Lesion size was not affected by treatment with ethephon. The only significant effect on lesion size was the effect of time (Fig 2.5 & 2.7). Fruit held at 20°C had similar lesion size increases as those held at 25°C.

Firmness

There was no significant difference between treatments with respect to firmness by Aweta non-destructive sonar firmness device. Trends were similar for both temperatures and with or without ethephon treatment (Fig 2.6). Treatment with Ethrel had no effect on the firmness of the apples; they all softened at the same rate, within the given temperature.
**Weight loss**

There was no significant difference between any treatments with respect to weight loss. Trends were similar for each temperatures and plus or minus ethephon.

![Graph showing lesion size (mm) of all Gala apple fruit stored at 20°C or 25°C.](image)

Figure 2.5. Lesion size (mm) of all Gala apple fruit stored at 20°C or 25°C. Dips in graphs are where fruit have been removed.

![Graph showing firmness of gala apple fruit stored at 20°C or 25°C as measured by Aweta non-destructive sonar instrument.](image)

Figure 2.6. Firmness of gala apple fruit stored at 20°C or 25°C as measured by Aweta non-destructive sonar instrument.
Figure 2.7. Gala apples after seven days incubation at 20°C or 25°C after treatment with or without ethephon plus inoculation with Penicillium expansum.
Experiment 3. Effect of ethephon treatment on ethylene production rates of postclimacteric Pink Lady apples

Dr John Golding, Dr. Shashi Satyan, Anne Harris (Biometrician), Gosford Primary Industries Institute. Industry & Investment NSW

Introduction

Experiment 1 and 2 showed that ethephon treatment did not hasten the ripening of Gala apples. The aim of this experiment is to determine if postharvest ethephon treatment promotes ethylene production in Pink Lady apples.

Materials and Methods

Pink Lady apples from a commercial orchard in Orange, NSW were randomly allocated treatments and dipped in ethephon at; 0, 100, 1,000 or 10,000 parts per million (ppm). Ten fruit were treated per concentration. Apples were dipped in 2L solution containing the assigned rate of ethephon (with 0.01% Triton X-100 surfactant) 3-4 apples at a time. Freshly prepared solution was used for each batch of 3-4 apples providing replication of treatment. The apples were submerged for 3 mins and then allowed to air dry. The fruit were stored on plastic plix trays with a plastic over-wrap to maintain high humidity and stored at 24°C for up to four weeks. Ethylene production rates were measured for each individual apple by placing each fruit into individual sealed jars, and then measuring the accumulation of ethylene in each jar by GC (Fig 2.7). Ethylene production rates were measured at treatment and 1, 2, 4, 7, 10, 14, 18, 21, 25 and 28 days after treatment.

Results

The Pink Lady apples were well into their ethylene climacteric when the ethephon was applied on 25 May 2009 at normal harvest maturity. The ethephon treatment did not affect endogenous ethylene production at 24°C in post-climacteric Pink Lady (Fig 2.8).
Experiment 4. Effect of ethephon treatment on the expression of fungal rots

Dr John Golding, Dr. Shashi Satyan, Anne Harris, Gosford Primary Industries Institute. I & I NSW

Introduction

Experiment 3 showed that ethephon treatment did not affect endogenous ethylene production at 24°C in Pink Lady which were post-climacteric at harvest. The aim of this experiment is to determine if postharvest ethephon treatment promotes rot development despite having no effect on internal ethylene levels.

Materials and Methods

Pink Lady apples from a commercial orchard in Orange, NSW were randomly allocated treatments. Fruit were either not dipped, or dipped in ethephon at; 0, 1, 10 or 100 ppm. There were five replicates of 20 fruit per treatment. The no dip treatment was not dipped in any solution. To ensure independence of treatments, a new fresh solution of ethephon treatment was prepared for each batch (replicate). After treatment, each replicate of 20 fruit was placed onto green plastic plix trays; these were over-wrapped in a plastic bag then placed into a plastic tub containing wet paper towel and Petri dish with water to maintain the relative humidity (Fig 2.9). The plastic tub was then over-wrapped in a plastic bag and stored at 24°C. The development of rots on each of the fruit was assessed at weekly intervals over four weeks.

For each treatment unit of 20 fruit, the total number of fruit with rots present was calculated. A generalised linear mixed model (GLMM) with logit link function and binomial errors was used to analyse the proportion of fruit affected by rots. The experiment was analysed as a factorial (treatment * storage time) split for time. Treatment effects were compared on the logit scale using least significant differences (LSD’S) at 5% where appropriate. Back-transformed proportions are also presented.
Results and Discussion

As expected more rots developed over storage time (Table 2.1), but there were no significant treatment effects, i.e., the numbers of rots were not affected by the different ethephon treatment concentrations. Nor was there an observed treatment x storage time interaction. Table 2.2 shows the proportion of rots with the different ethephon treatments over storage time at 24°C. A sample of fruit for each treatment at the end of the storage period (4 weeks) is presented in Fig 2.10. The full analysis and statistics are presented in the Appendix 1.

Table 2.1. Effect of storage time on the level of rot incidence (% total) in Pink Lady apples stored at 24°C for up to four weeks. Different letters represent significant differences.

<table>
<thead>
<tr>
<th>Storage duration</th>
<th>Rot level</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 week</td>
<td>0.02718 a</td>
</tr>
<tr>
<td>2 weeks</td>
<td>0.04885 b</td>
</tr>
<tr>
<td>3 weeks</td>
<td>0.08173 c</td>
</tr>
<tr>
<td>4 weeks</td>
<td>0.11631 d</td>
</tr>
</tbody>
</table>

Table 2.2. Effect of pre-storage dip with different ethephon concentrations (ppm) on the level of rot incidence (% total) in Pink Lady apples stored at 24°C for up to four weeks. There was no significant interaction between ethephon concentration and time.

<table>
<thead>
<tr>
<th>Storage duration</th>
<th>Ethephon concentration (ppm)</th>
<th>No Dip</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>1 week</td>
<td>0.6</td>
<td>3.8</td>
</tr>
<tr>
<td>2 weeks</td>
<td>1.2</td>
<td>4.7</td>
</tr>
<tr>
<td>3 weeks</td>
<td>2.6</td>
<td>9.7</td>
</tr>
<tr>
<td>4 weeks</td>
<td>4.8</td>
<td>11.5</td>
</tr>
</tbody>
</table>
Figure 2.10. Incidence of rots following pre-storage dip with different ethephon concentrations (ppm) in Pink Lady apples stored at 24°C for four weeks.
Experiment 5 Paraquat and ONFIT for the assessment of latent infections of Neofabraea spp. in apples

Simone Kreidl et al DPI Victoria, Knoxfield

Introduction

Infections in fruit may not be expressed until the fruit begins to ripen during storage and marketing. The ability to predict these infections could provide useful information to growers allowing them to decide how to best treat the fruit after harvest and determine an appropriate storage and supply strategy.

The overnight freezing and incubation technique (ONFIT) has been used on several types of fruit to determine the levels of latent infection. Freezing breaks down the fruit tissue and allows the infection to be expressed much more quickly than incubating to ripen the fruit. It has been used effectively to detect Monilinia fructicola on prunes (Luo and Michailides, 2001), Fusicoccum spp. on pistachio (Mila et al. 2005) and Botrytis cinerea in grapes (Michailides et al. 2005).

A similar outcome has been achieved by dipping fruit in the herbicide paraquat. As with the freezing treatment, paraquat causes the fruit tissue to break down so that pathogens can be expressed. A treatment of 2.9 g/L paraquat was used to detect latent infections of a number of apple pathogens on Nittany and Golden Delicious apples at various stages of maturity (Biggs, 1995). Northover and Cerkauskas (1994) used a dip of 6g/L paraquat to detect latent infection of Monilinia fructicola on immature plums.

The aim of this trial was to determine if paraquat or ONFIT is more effective at revealing latent infections of apple pathogens in comparison to incubation alone.

Materials and Methods

Mature Pink Lady and Granny Smith apples were collected from a commercial orchard at Launching Place, Victoria. The apples were found to be naturally infected with a number of pathogens the most common being two species of Neofabraea, Neofabraea alba and Neofabraea perennans. These are post harvest pathogens that are often not observed until after the fruit has been in storage for some time.

The harvested fruit was stored at 1°C and then given one of three treatments to determine the presence of latent infections; Paraquat 2.9g/L, ONFIT and Control (water). The Granny Smith apples were divided into 5 replicates of 18 fruit for each treatment, while the Pink Lady had 3 replicates of 20 fruit for each treatment. On the day of treatment all fruit were surface sterilized by dipping in 70% ethanol for 10 sec, followed by 0.525% NaOCl for 4min and rinsed twice with tap water for 1 minute.

The control fruit were then air dried overnight and packed in plastic trays, covered in plastic bags and moist incubated at 20°C. The ONFIT fruit were dried and packaged as for the control fruit and frozen for 24 hours at -20°C. After freezing they were placed at 20°C and moist incubated. The remaining fruit were dipped in 2.9g/L paraquat for 1 minute then dried, packaged and incubated as above. Fruit were observed 4 days after treatment and then every 2-3 days for the development of rots.
Results and Discussion

An initial assessment was carried out after 4 days storage at 20°C. At this stage the control fruit showed little change, the ONFIT fruit were evenly brown and softened and the paraquat treated fruit were partially browned and softened. Rots were becoming apparent but it was not possible at this point to identify the fungi and counts were made of number of fruit showing one or more rots (incidence). The rot incidence on Pink Lady was lowest in the ONFIT treatment and highest in the 'control' fruit. In Granny Smith rot incidence was similar (between 6 and 10%) in all treatments (Fig 2.11).

Following the 4 day assessment the ONFIT fruit were discarded as the large numbers of saprophytic fungi growing on them made further assessments difficult.

![Graph showing rot incidence after 4 days incubation](image)

Figure 2.11: Percentage of fruit infected with any rot after 4 days incubation at 20°C following treatment with paraquat, ONFIT or surface sterilized only (SS).

A final assessment was carried out after 14 days incubation when the rots were more developed and able to be identified. The fruit treated with paraquat had a much higher level of both Neofabraea spp. and total rots than the control fruit, in both apple varieties (Figure 2.12).

![Graph showing Neofabraea spp. and total rot incidence after 14 days incubation](image)

Figure 2.12: Percentage of fruit infected with Neofabraea spp. and Total rots after 14 days incubation at 20°C following treatment with paraquat or surface sterilized only (SS).
Of the three treatments investigated, paraquat revealed the highest incidence of latent infections due to identifiable pathogens. The ONFIT treatment was unsuitable for assessing the incidence of Neofabraea rots as leakage encouraged the growth of saprophytic fungi before there was sufficient incubation time to allow sporulation of Neofabraea and other common postharvest pathogens.

While the early assessments did not show much difference between the paraquat and control treatments, over time, many more infections were apparent in the paraquat treated fruit. However the unevenness of the breakdown in this treatment, thought to be due to greasiness of the apple skin restricting paraquat uptake, was a shortcoming which needs to be avoided. Selecting fruit earlier in the ripening phase, before greasiness develops should give an improved result. In addition, paraquat is highly toxic to humans and employing this technique to determine a rot index will only be possible where personnel are appropriately trained and provided with the necessary protective equipment.
Chapter 3 - Rot inoculum dynamics in a dump tank.

Simone Kreidl and Robert Holmes DPI Victoria, Knoxfield

Introduction

Rot pathogens from contaminated fruit and bins can easily be spread from one fruit to another during washing and packing. The appropriate use of sanitisers should ensure that dump tank water remains clean therefore reducing the spread of inoculum. The aim of this experiment was to look at the dynamics of rot pathogens in the dump tank over time, to determine how inoculum may change over time and the efficacy of the sanitising system in dealing with rot causing pathogens under a high inoculum load. Pears with a high rot incidence were used to provide a heavy pathogen load.

Methods

Water samples were taken from the dump tank at a commercial orchard in the Yarra Valley, Victoria, during sorting and packing pears (cv Packam’s Triumph) which had been in storage for approximately 8 months. The dump tank water was continuously filtered and dosed with approx 10ppm iodine as a sanitiser. Samples were taken at two times 10:00 am after two bins of fruit had been through the system and at 12:00 pm after 6 bins of fruit had been through. At each time three replicate samples of water were collected from the dump tank at the position were the bin is emptied (near the inlet where freshly sanitised water is delivered) and also at the opposite end of the tank where the water returns to the sanitising plant. A large beaker of water was withdrawn for each sample and a 100µL aliquot without dilution was immediately plated onto Dichloran Rose Bengal Acromycin (DRBA) agar. Diluted water samples (1:100 and 1:10,000 dilutions) were also plated. The plates were incubated at 23°C and assessed after 7 days growth. The rot incidence on fruit entering the dump tank was high (approx 5% Fig 3.1). Blue mould was the main disease present in the fruit and there was also some grey mould and target rot.

Results and Conclusion

The majority of fungi recovered were *Penicillium* spp. (Table 3.1). The numbers recovered were significantly higher (P<0.05%) at the far end of the tank where the water returned to the sanitising plant. Numbers did not increase significantly between the two sampling times (Table 3.1). While the pathogenicity of the Penicillium was not assessed, rotted fruit tissue was seen being dispersed through the dump tank and there is an assumption that Penicillium recovered from the tank was derived from the rotting fruit. Freshly sanitised water delivered to the dump tank contained very low levels of fungi indicating the sanitising plant was effective, however there was a gradient within the tank along which pathogens accumulated. Fungal concentrations surrounding fruit at the elevator end of the tank were high and at damaging levels (Holmes 1993), assuming the fungi were pathogenic. This trial indicated a need for a higher concentration of sanitiser or a higher volume of sanitised water to be delivered to the tank. The addition of a spray bar over the elevator may also be an effective means of diluting the pathogen contamination on the fruit below threshold levels.
Table 3.1 Number of colony forming units isolated per ml of dump tank water sampled at different times and locations.

<table>
<thead>
<tr>
<th>Time and Location</th>
<th>Penicillium</th>
<th>Other</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>10am Inlet</td>
<td>0 a</td>
<td>3</td>
<td>3 a</td>
</tr>
<tr>
<td>10am Elevator</td>
<td>117 b</td>
<td>0</td>
<td>117 b</td>
</tr>
<tr>
<td>12am Inlet</td>
<td>3 a</td>
<td>17</td>
<td>20 a</td>
</tr>
<tr>
<td>12am Elevator</td>
<td>127 b</td>
<td>7</td>
<td>134 b</td>
</tr>
<tr>
<td>LSD (P&lt;0.05%)</td>
<td>59</td>
<td>66</td>
<td></td>
</tr>
</tbody>
</table>

Figure 3.1 Approximately 5% of fruit entering the dump tank had visible rot.
Chapter 4 - Effectiveness of four fungicides against Neofabraea spp in-vitro

Simone Kreidl et al DPI Victoria, Knoxfield

Introduction

Target rot, caused by Neofabraea spp was the third most common postharvest disease recorded in the survey and was responsible for heavy losses in South Australia during 2008. Losses due to this pathogen have increased over recent years and it is important to understand why this is and to develop better strategies for control. Well timed application of efficacious fungicides during the growing period and postharvest would be expected to offer economic control, but as with any pathogen, overuse of fungicide from certain chemical families may lead to reduced fungicide sensitivity in the fungal population.

Some work has been done overseas examining the potential resistance of this pathogen to the Benzimidazole fungicide thiophanate methyl (Weber 2009, Weber and Palm 2010), where it was found that the population contained three distinct strains, sensitive, intermediate and highly resistant. However there is a scarcity of information on the sensitivity of Neofabraea spp. to other commonly used fungicides.

The aim of this trial was to test the sensitivity of some Australian strains of N. perennans and N. alba to four fungicides from different activity groups representing the currently registered fungicides and fungicides registered overseas which could potentially be registered in Australia for control of these fungi.

The fungicides selected were thiabendazole and iprodione used for over 20 years for postharvest drenching, fludioxonil newly registered in 2010 for postharvest drenching and belonging to a fungicide group which has not been used previously in Australia, and propiconazole a representative of the DMI fungicides which have been used in apple orchards for over 20 years for scab control.

Materials and Methods

Two isolates of Neofabraea alba from Pink Lady apples and two isolates of N. perennans from Granny Smith apples were collected in a commercial orchard in the Yarra Valley, Victoria. An additional isolate of N. alba was obtained from Fuji apples from Harcourt, Victoria.

The effectiveness of varying concentrations of 4 fungicides was tested against these isolates. The fungicides used were propiconazole (Tilt, Syngenta), iprodione (Rovral Bayer), thiabendazole (Tecto, Syngenta), and fludioxonil (Scholar, Syngenta). Concentrations used were; propiconazole, seven concentrations between 0.015 and 0.35µg/ml; fludioxonil eight concentrations between 0.0025 and 0.15µg/ml; iprodione, six concentrations between 0.005 and 1.62µg/ml and thiabendazole, twelve concentrations between 0.05 and 10µg/ml.

The appropriate amount of fungicide to make these concentrations was introduced into molten potato dextrose agar and plates containing the fungicides were poured the day before setting up the trial. Stock plates of the five isolates were grown at 20°C in darkness for approximately two weeks before beginning the trial. On the day of the trial 5mm plugs of mycelia were cut from these freshly growing culture plates with a cork borer and placed at the centre of the fungicide amended plates. Three replicate plates were made for each isolate and each concentration. These were then cultured at 20°C in darkness.
The diameters of the resulting fungal colonies, minus the 5mm plug, were measured after 6 and 10 days growth. The percentage inhibition of growth due to the fungicide was calculated relative to control colonies grown on un-amended PDA.

Results and Discussion

An initial trial using concentrations of thiabendazole above 0.5µg/ml resulted in 100% inhibition of fungal growth. The trial was repeated using lower concentrations (Fig 4.1) where it was found that the growth of our 5 isolates was inhibited by 50% at concentrations between 0.1 and 0.21 µg/ml. All of the isolates showed some sensitivity to fludioxonil and Iprodione (Figs 4.2 and 4.3), however only one, an *N. alba* strain, reached inhibition of 50% at the concentrations used. There was a wide spread in the reaction of these isolates to propiconazole. The two *N. perennans* isolates were the most sensitive reaching 50% inhibition at 0.04 and 0.15µg/ml respectively (Figure 4.5).

![Graph](image1)

*Figure 4.1: Growth inhibition of *Neofabraea* spp. by thiabendazole after 10 days growth at 20°C*

![Graph](image2)

*Figure 4.2: Growth inhibition of *Neofabraea* spp. by fludioxonil after 10 days growth at 20°C*
Through Chain Rot Management for Apples

Figure 4.3: Growth inhibition of *Neofabraea* spp. by iprodione after 10 days growth at 20°C

Figure 4.4: Five isolates of *Neofabraea* spp. after 10 days growth on media amended with iprodione
Discussion

Of the five isolates tested all were sensitive to thiabendazole at the concentrations tested with 50% inhibition occurring between 0.1 and 0.2 µg/ml. These were within the sensitive range or less than the intermediate resistance values published by Weber and Palm (2010). This study supports the continued use of thiabendazole for postharvest drenching. While there was some sensitivity demonstrated to iprodione and fludioxonil, the concentrations used gave less than 50% inhibition. Further work is therefore required to determine the degree of sensitivity to these chemicals and to relate in-vitro inhibition to field efficacy. The N. alba strains were most sensitive to thiabendazole, iprodione and fludioxonil but N. perennans isolates were most sensitive to the DMI fungicide propiconazole. The wide spread of effective concentrations for propiconazole may indicate a lessening of sensitivity in some strains, and further testing is important before any consideration is given to registering a DMI fungicide for the control of these fungi. While no DMI fungicide is currently registered or has been registered for the control of Neofabraea in Australia, several DMIs have been used for many years in apple orchards against other diseases and it is not unexpected that mixed sensitivity exists in Neofabraea. DMI fungicides provide fair to good control of Neofabraea malicorticis in USA (OSU, 2010)

This testing used a small number of isolates from Victoria and provided an idea of effective concentrations in-vitro. Testing more isolates from more regions is suggested for the purpose of reviewing fungicide efficacy.
Chapter 5 - General Discussion

Features of the main diseases

The purpose of the survey reported in Chapter 1 was to identify and correct the postharvest rot diseases with the highest significance through sampling fruit from growers and packers reporting severe losses. Features and controls for the diseases with the highest incidences are discussed below.

New data from this survey has been used to revise the common postharvest disease table published by Holmes (1993) (Table 5.1). The survey did not attempt to provide a definitive analysis of the fungi involved in apple fruit diseases throughout Australia. A definitive study would require more comprehensive and representative sampling.

Table 5.1 Scientific names of the anamorphic and teleomorphic states of fungi commonly causing postharvest diseases of apple fruit in Australia (Revised from Holmes 1993).

<table>
<thead>
<tr>
<th>Accepted name of Anamorph</th>
<th>Synonyms of Anamorph</th>
<th>Teleomorphs</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alternaria alternata</em> (Fr.) Keissler</td>
<td><em>Alternaria tenuis</em> Nees</td>
<td><em>Botryotinia fuckeliana</em> (de Bary) Whetzel</td>
<td><em>Alternaria</em> rot</td>
</tr>
<tr>
<td><em>Alternaria mali</em> Roberts</td>
<td></td>
<td></td>
<td><em>Alternaria</em> blotch</td>
</tr>
<tr>
<td><em>Botrytis cinerea</em> Pers</td>
<td></td>
<td><em>Gloeosporium cingulatum</em> (S.) S.&amp; von S.</td>
<td><em>Grey mould</em> rot</td>
</tr>
<tr>
<td><em>Cladosporium herbarum</em> (Pers.) Link</td>
<td></td>
<td></td>
<td><em>Cladosporium</em> rot</td>
</tr>
<tr>
<td><em>Colletotrichum gloeosporioides</em> (Penzig) Sacc.</td>
<td><em>Colletotrichum fructigenum</em> (Berk.) Vasiljevsky</td>
<td><em>Glomerella cingulata</em> (S.) S.</td>
<td><em>Anthracnose, Bitter</em> rot</td>
</tr>
<tr>
<td><em>Fusarium</em> spp</td>
<td><em>Fusarium solitum</em> West</td>
<td></td>
<td><em>Fusarium</em> rot</td>
</tr>
<tr>
<td><em>Fusicoccum</em> spp</td>
<td></td>
<td></td>
<td><em>White</em> rot</td>
</tr>
<tr>
<td><em>Mucor piriformis</em> Fisher</td>
<td></td>
<td></td>
<td><em>Mucor</em> rot</td>
</tr>
<tr>
<td><em>Penicillium expansum</em> Link ex Gray</td>
<td></td>
<td></td>
<td><em>Blue mould</em> rot</td>
</tr>
<tr>
<td><em>Penicillium solitum</em> West</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Phytophthora cactorum</em> (Leb. &amp; Cohn) Schroet.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Sphaeropsis</em> spp</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Stemphylium botryosum</em> Wallr.</td>
<td></td>
<td></td>
<td><em>Black</em> rot</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>(canker)</em></td>
</tr>
</tbody>
</table>
| | | | *Black spot* |}

Chapter 5 - General Discussion
1. Alternaria rot

Alternaria rot is caused by several species of Alternaria and related genera, but mainly *A. alternata*. The disease is distinctly different from Alternaria blotch which is caused by *A. mali* and which does not develop postharvest. Infections occur in apples and pears where the epidermis is injured, e.g. wounding and sunburn, or commonly in the core tissue of apple cultivars with open calycine sinuses. Decays are slow growing, circular, dark brown to black and firmer than blue mould rot. The inoculum is ubiquitous being found on dead organic material in the orchard and on fruit storage bin surfaces. Although it is a weak parasite of fruits, Alternaria is insensitive to benzimidazole fungicides eg thiabendazole commonly used post-harvest and this favours its development postharvest. Thorough mulching or removal of prunings and prevention of fruit injury will help prevent this disease. There are no fungicides registered in Australia to control Alternaria rot in apples, however several fungicides for example the multisite protectant fungicides used in the orchard for other diseases will assist. Pristine® registered on apples for scab and powdery mildew control has activity against this group of fungi and is labelled in the USA for control of *A. mali*.

2. Blue mould rot

Blue mould rot is caused by several species of Penicillium and is generally considered the most damaging post-harvest disease of pome fruits in Australia (Little and Holmes 2000). Penicillium expansum is the most common and virulent species affecting pome fruit however slower growing species cause significant disease. Formerly less important species, *P. verrucosum* and *P. solitum* are more tolerant of some postharvest fungicides than *P. expansum*. Maintaining hygienic orchard and postharvest environments and following fungicide resistance management guidelines are important control strategies.

Infections establish through wounds and bruises, and lenticels (only in very ripe fruit). Spotts et al. (1988) found blue mould spores were abundant on fruit bins, in post-harvest drenches and in water flumes. However there are no studies which establish the comparative importance of each source in the epidemiology of blue mould.

Apple and pear tissue decayed by *P. expansum* and other Penicillium species may contain the mycotoxin patulin. Therefore fruit with blue mould is unsuitable for processing.

3. Anthracnose diseases

Anthracnose rots are a group of diseases with several common names. *Neofabraea alba* (see table 5.1) causes target rot or target spot, *Neofabraea perennans* causes ripe spot and perennial canker on branches, *Colletotrichum gloeosporioides* causes bitter rot, *Botryosphaeria obtusa* causes black rot *Botryosphaeria dothidea* causes white rot. Infection is mainly pre-harvest, by direct penetration of lenticels and parasitism of injuries, russet, growth cracks and through the calycine sinus. Heavy rains near harvest cause enlarged lenticels and growth cracks thereby increasing fruit susceptibility. Spores are also dispersed by rain and require free moisture to germinate. Infections remain quiescent until fruit become less resistant to disease during ripening. Lesions are brown, moderately firm and sunken, enlarging during storage and marketing to cover a large proportion of the fruit surface. Lesions may develop concentric rings of light and dark decay and produce copious quantities of conidia in acervuli; pink coloured in C. gloeosporioides, grey in *N. perennans* and cream in *N. alba*.

In this survey and associated analysis these diseases were worse in fruit harvested wet, particularly during or within a day after sustained rain; in fruit which did not receive an effective multisite protectant fungicide within the 3 weeks before harvest; in fruit which were not drenched after harvest in a Benzimidazole fungicide (products containing either carbendazim or thiabendazole). Note residues of these fungicides are not permitted in fruit exported to the UK and carbendazim use was suspended locally from July 2010. At the time of writing a single product containing thiabendazole was registered and available from resellers in Australia (see chemical control below). We did not find evidence of the tree canker phases of these diseases.
4. Lenticel spot associated with a Phoma-like fungus

This was a frequent occurrence in Granny Smith and Pink Lady apples grown in the Granite Belt, Queensland, only. Very little is known about this disease. The main feature is the location of the spots centred on lenticels, more on the exposed side of the fruit and distributed from the stem to the calyx end, rather than localised as is the case with bitter pit. Spots developed in storage and occurred on fruit from orchards with incomplete fungicide protection, ie where the interval between protectants was 3 weeks or more during February and March rather than 2 weeks or less.

Through chain rot management guidelines

Orchard hygiene

Careful attention to the likely sources of fungal spores in the orchard will assist in the prevention of infections and place less pressure on postharvest fungicides. Many pathogens causing postharvest rots (e.g. Mucor and Phytophthora) are soil borne. It follows that fruit and handling equipment should be isolated from the soil. When pruning, remove low branches which might set fruit in contact with grass or the ground. Fruit contacting the ground or grass and fallen fruit should not be mixed with ‘clean’ fruit at harvest. Contamination of bins with soil, plant debris or dropped fruit can be prevented by keeping them on trailers or laying them on sawdust or wood chips while in the orchard. Efforts should be made on receival at the storage facility to prevent the movement of orchard soil from the delivery apron into the storage area. This strategy is most important, and more difficult to achieve, in wet harvest seasons.

Anthracnose fungi such as Neofabraea and Colletotrichum may over-winter in cankers, in mummified fruit or on dead wood. Mummified fruits and affected limbs should be removed during pruning and disposed of. It has been shown in Oregon USA that dropped WBC (Bartlett) pears causes a build-up of Mucor in the soil which may contaminate the later maturing varieties. Where there is a possibility for cross contamination, dropped fruit should also be collected and disposed of. Extraneous plant material such as leaves, twigs and spurs should be kept out of fruit bins as these may injure fruit or contribute to the build up of fungal spores.

Packing shed hygiene

The reduction of fungal populations in the packing shed is achieved through isolation of decayed fruit and sanitation of surfaces, drenches and water flumes. Fruit which rot during bulk storage are a source of fungal inoculum which may infect fruit during the sorting and packing operation. In bulk handling systems it is not convenient to remove rotted fruit before it enters the water flotation tank or the bin tipping chute. Fruit decayed by the soft rots Mucor and Penicillium often sink to the bottom of the flotation tanks and macerate over fruit and conveyor systems. Decayed tissue releases infectious fragments of the pathogens. Whole and macerated decayed fruit are partially removed from flotation tanks, with leaf skimmers. High capacity sand filtration systems give more effective removal. Using a disinfectant at the correct concentration in the flotation water and in a fruit wash after the sorting table will minimise inoculum on the fruit. Decayed fruit removed at the sorting table and debris from the flotation tank should not be allowed to contaminate containers used for good fruit and should be disposed of carefully. Daily burial with a covering of earth at a distance downwind from the packing shed or regular collection by a waste contractor are recommended disposal methods.

Fruit which rot during storage also contaminate storage bins. Decay fungi can survive for many months on wooden bins especially if they are stacked in cool, dark and humid places. Contaminated bins contribute greatly to the build up of fungal inoculum in fungicide drenches and in water flotation tanks. Sanitising contaminated bins before each season can be achieved in a number of ways.
The level of infection after dipping depends on the concentration of the fungal inoculum, the fungicide used and the level of resistance. The accumulation of spores including fungicide resistant spores in dipping and drench tanks is reduced if fruit are pre-washed with non-recycled fresh water and/or sanitised water before drenching with fungicide/DPA (see below).

**Pre-season treatment of bins**
In the survey of grower practices presented in Chapter 1, it was clearly evident that bin hygiene varied considerably. Those growers that routinely cleaned their bins and allowed them to stand outside in sunlight for at least 2 weeks prior to use had less rot problems. The pick up and transport of bins with soil attached also appeared to increase the incidence of rots and was a particular issue when the harvest period was wet.

Bins can be freed of fungal contamination with a high pressure water cleaner (10,000 – 14,000 Kpa) using either hot water (70°C+) or an approved disinfectant. Disinfectants approved for use in food handling areas or hypochlorites at 50 to 100 mg/L (ppm) free available chlorine (or Nylate®) are suitable. Disinfectant or fungicide dips are ineffective unless pressure washing or scrubbing is first used to remove fruit residues which harbour fungi.

An alternative sanitation treatment evaluated on wooden bins involves heating bins at 50°C for a period of 24 h in air humidified to near saturation. Exposure of bins to intense sunlight is an effective sanitation treatment, however, bins must be turned to expose all surfaces. Exposure to intense summer sun for 8-10 hours is sufficient to kill both *Mucor* and *Penicillium*.

**Pre-washing fruit before dipping:**
Fruit can be washed with non-recycled fresh water and recycled water treated with Nylate® or hypochlorite (10 mg/L free available chlorine equivalents) to reduce spores entering the DPA fungicide drench. Pre-washers can be installed before the DPA drencher if the conveyor is lengthened sufficiently to allow the washing solution to run off before DPA and fungicide is applied. Pre-washing has an added benefit of prolonging the life of the DPA and fungicide solution, although if prewashing is used, more thought needs to go into calculating top-up rates in the drencher, due to possible dilution (see Holmes and Washington 2011 for more detail). Bins of fruit treated with chlorine before DPA drenching should be well drained, as chlorine is incompatible with DPA.

**Treating flotation tank and washing water:**
Chlorine, bromine and iodine compounds and dissolved ozone kill fungal spores on contact and are effective at low levels provided the concentration is monitored continually and maintained to meet the demand. It is also important that the water being treated has sufficient retention time in contact with the sanitiser. The study presented in Chapter 3 showed that under high inoculum loads, the sanitising plant may not be able to meet the demand for sanitiser, as sanitiser is consumed by organic material. Fresh sawn timber bins, soil and organic debris reduce the effectiveness and increase the need for more chemical. At high pH, hypochlorites are less active; at low pH they become unstable, create odour problems and cause corrosion of metals. The recommended pH for washing and dump tank water is 6.0 to 7.5. Automated pH control is provided by some sanitiser systems notably the Wobelea Nylate® system. A concentration of 10 mg/L free available chlorine is sufficient provided the monitoring and dispensing system is quick enough to react to sudden changes in demand. Hypochlorite concentrations of 50 to 100 mg/L are sometimes recommended where control of the level is poor. A high capacity sand filter will minimise the amount of organic material in the flotation tank and facilitate sanitiser control. Hypochlorites and BCDMH are compatible with sodium sulphate for pear flotation but not with sodium silicate or sodium carbonate. The penetrating ability of chlorine solutions can be
improved with the addition of Triton A.G.98 wetter, however, this consumes chlorine and additional chlorine needs to be added. The frequency of water changes in flotation tanks depends on the throughput of fruit, the amount of rotten fruit and other debris entering the system, the efficiency of filtration and the volume of the system. If filtration and chlorine control is poor it is recommended not to exceed 50 bins throughput per 1 000 litres of dump water as this is likely to give pathogen counts in excess of 300 spores/mL.

**Cleansing equipment and coldrooms:**

Rollers, brushes and belts must not become contaminated with rotten fruit residue or grit and should be cleaned as necessary. Coldstore walls, floors and refrigeration evaporators may also harbour moulds. Steam, pressurised hot water and non corrosive sanitising chemicals are suitable for application to grading lines and floors. Sanitisers containing thickeners or foaming agents are most convenient to use on coldstore walls. Products chosen should be registered for food handling areas and used as directed to avoid residual odours.

**Handling considerations**

To minimise postharvest decays it is essential to avoid harvest and postharvest injuries. Innovative machinery developed over the last century has enabled higher capacity and less labour intensive fruit handling, however cuts, punctures and abrasions may still be frequent problems.

Pickers may need to be carefully supervised and instructed to avoid pulled, torn and broken stems. Any unnecessary drop or rolling movement increases the incidence of injuries especially if foreign materials such as twigs are present. Although slight bruising may not increase rotting it indicates impacts which also result in stem punctures. New pickers should be instructed in picking technique, correct adjustment of picking bags and ladder handling to avoid bruising fruit. Pickers’ finger nails should be kept short or cotton gloves worn. To avoid cuts during stacking, do not overfill fruit bins. Tracks should be graded pre-season and tractors should be driven at slow speed. Avoid immersion dipping of open tube apple varieties such as Red Delicious strains if there has been a history of core rots.

**Chemical control**

Maintaining cover with multisite protectants is the main strategy for the prevention of fungal infections in the orchard which can lead to postharvest rots. Table 5.2 lists the fungicides registered for application to fruiting trees to control postharvest rots. These fungicides are all old-generation multi-site protectants which can be used within the label directions to achieve good rot control. Orchardists supplying the export markets in Europe in particular need to be very aware that some of these are not permitted or have lengthened withholding periods. Complying with export requirements can restrict complete rot protection during a very critical phase, close to harvest, when fruit are highly susceptible and weather conditions may be more conducive to disease. Pink Lady Australia publishes 'Grower Alerts' to keep their members well informed about permitted chemicals.

Fungicide distributors may be able to provide information on the level of 'coincidental' control provided by fungicides applied to target other orchard diseases. A good amount of information on 'coincidental' control is available on the internet. For instance Pristine® registered in Australia for apple scab and mildew control is labelled in the USA for Alternaria blotch, bitter rot, black rot, and white rot control. Likewise captan which is labelled for rot control in USA and registered in Australia for scab control, but not rot control, could be providing 'coincidental' rot control. To overcome problems with chemical use compliance requirements one chemical reseller is suggesting growers use captan as the last "Blackspot" spray for the season.
Postharvest dipping and storage practices

It is important that fungicides registered for postharvest drenching are reserved for that purpose and not used in the orchard before harvest. Fungicides approved for postharvest drenching (fluoxi donor, thiabendazole, iprodione and imazalil) belong to activity groups prone to resistance. Their overuse increases the risk of storage rots developing resistance. The fungicide resistance management strategy for postharvest chemicals is firstly to use sanitisation practices to reduce the pathogen population and secondly to use a combination of complimentary chemicals to achieve broad spectrum and more complete control. Combinations are advisable when disease pressure is highest, for example in wet harvest seasons.

Table 5.2 Active ingredients of fungicides registered in Australia for field and postharvest use against various rot diseases of apples and other pome fruit

<table>
<thead>
<tr>
<th>Active Ingredients</th>
<th>Fungicide activity group</th>
<th>Fruiting Trees</th>
<th>P/Harvest Dip</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Bitter Rot (Colletotrichum gloeosporioides, Glomerella cingulata)</td>
<td>Anthracnose (Pezicula penicillia, Neofabraea perennans, Gloeosporium penicillia)</td>
</tr>
<tr>
<td>Thiabendazole</td>
<td>1</td>
<td></td>
<td>Blue</td>
</tr>
<tr>
<td>Iprodione</td>
<td>2</td>
<td></td>
<td>Blue</td>
</tr>
<tr>
<td>Imazalil</td>
<td>3</td>
<td></td>
<td>Blue</td>
</tr>
<tr>
<td>Fludioxonil</td>
<td>12</td>
<td></td>
<td>Blue</td>
</tr>
<tr>
<td>Copper oxychloride</td>
<td>M1</td>
<td></td>
<td>Blue</td>
</tr>
<tr>
<td>Dithianon</td>
<td>M9</td>
<td></td>
<td>Blue</td>
</tr>
<tr>
<td>Mancozeb</td>
<td>M3</td>
<td></td>
<td>Blue</td>
</tr>
<tr>
<td>Metiram</td>
<td>M3</td>
<td></td>
<td>Blue</td>
</tr>
<tr>
<td>Thiram</td>
<td>M3</td>
<td></td>
<td>Blue</td>
</tr>
<tr>
<td>Zineb</td>
<td>M3</td>
<td></td>
<td>Blue</td>
</tr>
<tr>
<td>Ziram</td>
<td>M3</td>
<td></td>
<td>Blue</td>
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</tbody>
</table>

Always read the label and consult with your supplier about the suitability of products

Rot indexing

Four techniques were evaluated for accelerating rot development to assess rot potential. Unexpectedly all ethephon treatments failed to accelerate ripening and failed to accelerate the expression of latent rots. This lack of response may have been due to the varieties chosen for study. Judging by their degree of starch hydrolysis at normal harvest maturity Gala and Pink Lady may be well into their climacteric phase and unresponsive to externally applied ethylene. Ethylene production rates indicated the Pink Lady used in experiment 3 (Chapter 2) were in their climacteric stage.

Of the other three treatments, paraquat revealed the highest incidence of latent infections due to identifiable pathogens. The ONFIT treatment was unsuitable for assessing the incidence of Neofabraea rots as leakage encouraged the growth of saprophytic fungi before there was sufficient incubation time to allow sporulation of Neofabraea and other common postharvest pathogens.
Unfortunately, paraquat is highly toxic to humans and employing this technique to determine a rot index will only be possible where personnel are appropriately trained and provided with the necessary protective equipment.

Review of project objectives

The main objectives of this national project were to diagnose the diseases, identify the underlying causes of their increased incidence and provide industry with the knowledge and tools to more effectively and cost-efficiently manage the risks.

There was comprehensive national involvement in surveys and diagnoses to determine the causal organisms and the management and environmental factors associated with high and low rot incidences. Fruit rots from Queensland, New South Wales, Victoria and South Australia were diagnosed and while there were similarities in pathogens identified, several types of pathogens were isolated only from Queensland. Notably a Phoma-like fungus which could not be positively identified to species level was associated with a lenticel spot disease. Only two samples were received from Western Australia where rots were not a significant problem. No exotic fruit diseases were found in the survey further supporting the knowledge that *Neofabraea malicorticis* (Bulls-eye rot), *Monilinia fructigena* (Apple brown rot) and *Neonectria ditissima* (European canker and fruit rot) are absent from Australia. Likewise diagnoses of tree cankers in Victoria did not reveal any exotic species of concern.

Causal factors for detailed study included:

- inoculum sources in the field such as tree cankers and mummified fruits,
- the effectiveness of growers’ spray programs, other orchard practices and postharvest practices
- the performance of sanitisers in the packing process
- potential fungicide resistance in *Neofabraea*

The highest rot risks occurred where fruit were picked while wet or within one day of heavy rain, where in-field fungicides were not used in the three weeks before harvest and where no postharvest fungicide was used. Fruit which received a pre-harvest fungicide spray (usually captan) performed significantly better than fruit which were not sprayed within 3 weeks of harvest. Fruit which were drenched in fungicide then treated with SmartFresh or drenched in fungicide and DPA performed better than fruit which did not receive a postharvest fungicide. Those growers that routinely cleaned their bins kept bins free from orchard soil during harvest also generally achieved better rot control.

Diagnoses of tree cankers did not show a link with fruit diseases, however mummified fruit were found in trees during bloom which harboured fruit rot pathogens.

Monitoring of a fruit dump tank revealed water sanitisation systems may not be performing as well as anticipated when a line of fruit with high rot incidence is being processed. Fruit were being further contaminated with rot pathogens during packing which may cause rot development in the supply chain.

A study of in-vitro fungicide activity against *Neofabraea* determined that thiabendazole is still effective and its use for postharvest drenching should be supported. However DMI resistance is apparent and the agrochemical industry should be cautious about promoting DMI fungicides for *Neofabraea* control.

These findings and other known risk factors from prior work have been incorporated into a through-chain disease management guideline. An early draft of this was forwarded to Shane
Hetherington for inclusion in the integrated pest and disease management manual and updated information has been distributed in awareness articles for industry.

The project also endeavoured to develop a risk assessment tool to enable industry to segregate and appropriately treat lines of fruit according to rot risk “rot index”. This was partially achieved. The most successful method of promoting fruit senescence to enable an assessment of the amount of latent disease was dipping fruit in paraquat followed by 7 days incubation. Further validation work is needed to establish if the rot index correlates with actual rot development after long-term storage and ripening. In addition, paraquat is hazardous to humans and a safe operating procedure would need to be developed and approved before any use is recommended.
Chapter 6 - Technology Transfer

Adoption was a key objective of this project and was facilitated by a communication strategy managed by the project team and PLA/AFFCO. The project cross referenced project AP07048 - Assessment of bulk export shipments of Pink Lady apples to the UK, which will be collecting rot incidence data. The principal target audiences were growers, exporters and chemical resellers.

The extension and communication strategy aimed to ensure that at least 70-80% of growers/exporters on a national level will be aware of the results and outputs of this project.

Publications


WS Washington and Holmes R (2010) Mucor Rot of Pome Fruit, Agriculture Note No AG0166, Department of Primary Industries Victoria

Presentations


Chapter 7 - Recommendations

The project has discovered new information on optimising rot control and this has been incorporated with existing knowledge into a best practice guideline for effective rot management. While early drafts and subsequent updates have been published for the industry’s awareness and reference, a more lasting site for access to this information is needed. It is suggested that APAL commission publication of this guideline on the APAL website and link this information to other information providers such as the Horticultural Industry Network (HIN).

There is a strong indication of regional differences in types of fruit diseases. It wasn’t an intention of this project to understand these differences, however for cost effective control local situations need to be well understood. Regional associations should discuss the need for local research and development to optimise rot control.

Monitoring of a fruit dump tank revealed water sanitisation systems may not be performing as well as anticipated when a line of fruit with high rot incidence is being processed. Individual packers who are concerned about prevention of postharvest rots should have their sanitising systems assessed by a diagnostic laboratory for effectiveness and re-engineer equipment as required.

A study of in-vitro fungicide activity on a small sample of *Neofabraea* isolates suggested that thiabendazole is still effective as a postharvest drench and that iprodione may not be against this pathogen. In addition, DMI resistance is apparent and the agrochemical industry should be cautious about promoting DMI fungicides for in-field *Neofabraea* control. There may be substantial productivity loss if resistance is a significant limitation to control. The agrochemical industry in partnership with the apple industry should scope this possibility and review current fungicides and their potential replacement.

The project endeavoured to develop a risk assessment tool to enable industry to segregate and appropriately treat lines of fruit according to rot risk “rot index”. Further validation work is needed to establish if the rot index correlates with actual rot development after long-term storage and ripening. In addition, paraquat is hazardous to humans and a safe operating procedure would need to be developed and approved before any use is recommended.

Further engagement between the apple and agrochemical industry is needed to devise alternative late season and postharvest rot control practices to meet the requirements of export markets. New fungicides which are classed as “reduced risk” and have short or no withholding periods are now in use by many trading partners but have not been registered in Australia.
Bibliography of literature cited


Jones AL. (1990) Leucostoma canker, p40 In Compendium of Apple and Pear Diseases, American Phytopathological Society Press St Paul Minnesota


**Dr John Golding, Dr. Shashi Satyan, Anne Harris (Biometrician), Gosford Primary Industries Institute. Industry & Investment NSW**

For each treatment unit of 20 fruit, the total number of fruit with rots present was calculated. A generalised linear mixed model (GLMM) with logit link function and binomial errors was used to analyse the proportion of fruit affected by rots. The experiment was analysed as a factorial (treatment * storage time) split for time. Treatment effects are compared on the logit scale using least significant differences (LSD’S) at 5% where appropriate. Back-transformed proportions are also presented.

The treatment effect on the proportion of fruit with rots was not significant. Nor was there an observed treatment*storage time interaction. Storage was significant with more rots developing over time.

Generalized linear mixed model analysis

**Method:** c.f. Schall (1991) Biometrika

**Response variate:** totalrots

**Binomial totals:** totalfruit

**Distribution:** binomial

**Link function:** logit


**Fixed model:** Constant + Treatment + Storage + (Treatment.Storage)

**Tests for fixed effects**

**Analysis of variance**

<table>
<thead>
<tr>
<th>Fixed term</th>
<th>Wald statistic</th>
<th>n.d.f.</th>
<th>F statistic</th>
<th>d.d.f.</th>
<th>F pr</th>
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</thead>
<tbody>
<tr>
<td>Treatment</td>
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<td>4</td>
<td>1.90</td>
<td>17.7</td>
<td>0.155 ns</td>
</tr>
<tr>
<td>Storage</td>
<td>99.16</td>
<td>3</td>
<td>33.05</td>
<td>58.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Treatment.Storage</td>
<td>11.81</td>
<td>12</td>
<td>0.98</td>
<td>58.0</td>
<td>0.474 ns</td>
</tr>
</tbody>
</table>

**Table of predicted means for Treatment**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0 ppm</th>
<th>100 ppm</th>
<th>1000 ppm</th>
<th>10000 ppm</th>
<th>No dip</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-3.996</td>
<td>-2.625</td>
<td>-2.623</td>
<td>-1.811</td>
<td>-2.686</td>
</tr>
</tbody>
</table>

**Standard errors of differences**

- Average: 0.6852
- Maximum: 0.7135
- Minimum: 0.6636

**Table of predicted means for Storage**

<table>
<thead>
<tr>
<th>Storage</th>
<th>1 week</th>
<th>2 weeks</th>
<th>3 weeks</th>
<th>4 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-3.578</td>
<td>-2.969</td>
<td>-2.419</td>
<td>-2.028</td>
</tr>
</tbody>
</table>

**Standard errors of differences**

- Average: 0.1752
- Maximum: 0.2140
- Minimum: 0.1310
Through Chain Rot Management for Apples

Table of predicted means for Treatment.Storage

<table>
<thead>
<tr>
<th>Storage 1 week</th>
<th>2 weeks</th>
<th>3 weeks</th>
<th>4 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 ppm</td>
<td>-5.049</td>
<td>-4.338</td>
<td>-3.608</td>
</tr>
<tr>
<td>100 ppm</td>
<td>-3.213</td>
<td>-3.016</td>
<td>-2.229</td>
</tr>
<tr>
<td>1000 ppm</td>
<td>-3.520</td>
<td>-2.773</td>
<td>-2.522</td>
</tr>
<tr>
<td>10000 ppm</td>
<td>-2.731</td>
<td>-1.777</td>
<td>-1.367</td>
</tr>
<tr>
<td>No dip</td>
<td>-3.377</td>
<td>-2.941</td>
<td>-2.369</td>
</tr>
</tbody>
</table>

Standard errors of differences

Average: 0.7020
Maximum: 0.9682
Minimum: 0.2157

Standard error of differences for same level of factor:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average:</td>
<td>0.3734</td>
</tr>
<tr>
<td>Maximum:</td>
<td>0.7638</td>
</tr>
<tr>
<td>Minimum:</td>
<td>0.2157</td>
</tr>
</tbody>
</table>

Back-transformed Means (on the original scale) – Proportion of fruit with rots

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Storage 1 week</th>
<th>2 weeks</th>
<th>3 weeks</th>
<th>4 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 ppm</td>
<td>0.01805</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 ppm</td>
<td>0.06754</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1000 ppm</td>
<td>0.06766</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10000 ppm</td>
<td>0.14055</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No dip</td>
<td>0.06378</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: means are probabilities not expected values.
2. Framework for rot hazard assessment containing entries by one participating grower.

**Rot Hazard Assessment – Apples**
Objective: Identify control steps and practices which may contribute to improved rot control. Complete one form for each grower or batch

| Orchard Name and Locality: Grower 1 | Reference to diagnostic samples - P1 G1 |
| Storage Name and Locality: Packer 1 | |
| Apple Variety: GRANNY SMITH | |
| Batch or Harvest Date: 24.03.09 | |

<table>
<thead>
<tr>
<th>Hazard</th>
<th>Control step</th>
<th>Actual practice</th>
<th>Verification</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Preharvest</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Inoculum carryover</strong></td>
<td>Maintain hygienic orchard environment – Remove cankers on tree limbs Mulch dropped fruit and twigs or remove from orchard</td>
<td>Slash branches after pruning. Mulch applied to rows. Dropped fruit and twigs not removed.</td>
<td>Inspection pre-bloom</td>
</tr>
<tr>
<td><strong>Fruit infections – Gloeosporium, Botrytis and other fungi</strong></td>
<td>Maintain a protectant spray program ensuring final spray close to harvest. Apply curative fungicides following risk periods (eg sustained leaf wetness period more than 2-3 weeks after the last protectant spray)</td>
<td><strong>Fungicide</strong> myclobutani + zineb ziram mancozeb mancozeb ziram ziram ziram</td>
<td><strong>Date</strong> 10.11.08 26.11.08 12.12.08 28.12.08 14.01.09 02.02.09 16.02.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Sprayer output</strong> eg HV, L.V., ULV 970L/ha = HV</td>
<td><strong>Sprayer output</strong> eg HV, L.V., ULV 970L/ha = HV</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HV</td>
<td>HV</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HV</td>
<td>HV</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HV</td>
<td>HV</td>
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<tr>
<td></td>
<td></td>
<td>HV</td>
<td>HV</td>
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<tr>
<td></td>
<td></td>
<td>HV</td>
<td>HV</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Error! Unknown document property name.
<table>
<thead>
<tr>
<th>Fruit infections – Mucor and other fungi</th>
<th>Discard fruit which have contacted the soil or grass. Train trees and manage grass to avoid fruit contact.</th>
<th>Trees are pruned so that fruit does not touch the ground.</th>
<th>Inspection prior to harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wounding - Punctures and scratches</td>
<td>Control chewing insects eg Codling moth and LBAM</td>
<td>Sprays for codling moth, woolly aphid.</td>
<td>Pheromone trapping Visual inspection including survey of reject bins</td>
</tr>
<tr>
<td>Wounding - Punctures and scratches</td>
<td>Prevent bird attack</td>
<td>Shoots birds if possible. Uses a bird scarer.</td>
<td>Visual inspection including survey of reject bins</td>
</tr>
<tr>
<td>Wounding - Punctures and scratches</td>
<td>Ensure bins are smooth, without protrusions and free from debris</td>
<td>Bins are planed or plastic.</td>
<td>Visual inspection</td>
</tr>
<tr>
<td>Bins are contaminated with rot fungi</td>
<td>Clean bins to remove all signs of fruit residue and other debris. Disinfect bins.</td>
<td>Bins are disinfected.</td>
<td>Visual inspection and swab test</td>
</tr>
<tr>
<td>Harvest</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bins become contaminated with rot fungi</td>
<td>Keep bins off wet ground (using trailers, or placing on wood shavings etc.)</td>
<td>Bins are on trailers.</td>
<td></td>
</tr>
<tr>
<td>Bins and drench become contaminated with rot fungi</td>
<td>Prevent machinery carrying orchard soil onto unloading apron and handling areas</td>
<td>Yes this is done. Picking is almost always done in dry weather and so soil is not attached to tyres.</td>
<td></td>
</tr>
<tr>
<td>Fruit are contaminated by rot fungi</td>
<td>Reject fallen fruit and fruit with obvious rot (eg bird damaged fruit)</td>
<td>Yes. No fruit is collected off the ground.</td>
<td>Supervision of harvest</td>
</tr>
<tr>
<td>Fruit are contaminated by rot fungi</td>
<td>Do not pick fruit which is wet</td>
<td>Agreed.</td>
<td></td>
</tr>
<tr>
<td>Fruit overly turgid and easily damaged</td>
<td>Do not pick fruit which is wet</td>
<td>Agreed.</td>
<td></td>
</tr>
<tr>
<td>Wounding – Punctures and scratches</td>
<td>Train pickers</td>
<td>Endeavour to train pickers.</td>
<td></td>
</tr>
<tr>
<td>Wounding – Punctures and scratches</td>
<td>Train tractor drivers, grade tracks, use low trailer tyre pressure</td>
<td>Train tractor drivers.</td>
<td></td>
</tr>
</tbody>
</table>

**Postharvest**
<table>
<thead>
<tr>
<th>Wounding – Punctures and scratches</th>
<th>Minimise the number of handling steps eg number of times bins are lifted and placed down</th>
<th>Fruit is not pre-sized, but goes straight into cold store and then direct from cold store for packing.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drenching</td>
<td>Rinse fruit in sanitised water prior to drenching</td>
<td>Not rinsed</td>
</tr>
<tr>
<td></td>
<td>Follow industry code of practice for postharvest drenching (see APAL website)</td>
<td>If fruit is being packed immediately then it is not drenched. Rest goes to commercial cold store and grower/packer does not know what happens there.</td>
</tr>
<tr>
<td></td>
<td>Replace drench frequently – according to label directions</td>
<td>Use DPA test kit to verify concentration</td>
</tr>
<tr>
<td>Postharvest fungicides ineffective</td>
<td>Strictly follow label directions eg. Rate and timing (within specified period after harvest)</td>
<td>Fungicide</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Not Known</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Time applied ie hours after harvest</td>
</tr>
<tr>
<td>Postharvest fungicides ineffective</td>
<td>Follow label directions for the prevention and management of fungicide resistance</td>
<td>Fungicide resistance testing</td>
</tr>
<tr>
<td>Touch burn on fruit susceptible to</td>
<td>Allow fruit to drain after drenching</td>
<td></td>
</tr>
<tr>
<td>rots</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cooling and storage</td>
<td>Allow wounds to seal by delaying cooling 6 hours (shed cooling)</td>
<td>Fruit not cooled until end of day or next day – could be 6 to 12 hours before cooling.</td>
</tr>
<tr>
<td></td>
<td>Maintain strict CA and temperature tolerances</td>
<td>Stored in a commercial cold store. Practices not known.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Temperature and atmosphere logging</td>
</tr>
<tr>
<td>Packing</td>
<td>Regularly replace and sanitise dump tank water</td>
<td>Rotting fruit removed early before packing.</td>
</tr>
<tr>
<td></td>
<td>Rotting fruit removed early before packing</td>
<td>ORP Probe readings or regular manual testing of sanitiser concentration</td>
</tr>
<tr>
<td></td>
<td>Remove rotting fruit and debris from dump tank</td>
<td>Rotting fruit removed early before packing</td>
</tr>
<tr>
<td></td>
<td>Spray fruit with fresh or sanitised water</td>
<td>Yes, fruit is washed but rotten fruit has been</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Issue</td>
<td>Action</td>
<td>Result</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>---------------------------------------------</td>
<td>-----------------------------------------</td>
</tr>
<tr>
<td>During sorting and grading</td>
<td>Wash down sorting equipment daily</td>
<td>Removed daily.</td>
</tr>
<tr>
<td>Fruit are contaminated by rot fungi during sorting and grading</td>
<td>Empty culled fruit container two-hourly and cover culled fruit (bury or use covered bin)</td>
<td>Dumped at end of day.</td>
</tr>
<tr>
<td>Wounding during sorting and grading – Punctures and scratches</td>
<td>Correct set up and maintenance of grading line</td>
<td>Tries to do this.</td>
</tr>
<tr>
<td>Transit</td>
<td>Refrigerated transport and holding rooms</td>
<td>Fruit does not lose condition during transport, but it is not kept well at the markets – often sitting out in hot weather.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Instrumented sphere analysis, Quality audit</td>
</tr>
</tbody>
</table>