Horticulture Innovation Australia

Final Report

Optimal management of pre-harvest rot in sweet cherry

Tasmanian Institute of Agriculture (TIA) -
University of Tas

Project Number: CY13001

CY13001

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Summary

This project has successfully achieved several aims which are critical for the sweet cherry industry to develop core knowledge of fruit rot pathogen identity, abundance and insights for their management.

- It was clearly established *Botrytis cinerea* was the dominant rot pathogen in the orchards surveyed in southern Tasmania and this knowledge is now widely understood by growers;
- It was found that *Alternaria alternata* was a dominant pathogen causing rot in orchards of Orange and Young in NSW and pathogenicity tests confirmed it could infect intact fruit;
- Temperature and wetness durations conducive to fruit infection (and the impact of fruit susceptibility) were modelled for *B. cinerea*, *M. laxa* and *M. fructicola;*
- Spore abundance was found to occur throughout the sweet cherry season from flowering and a new molecular method was developed to quantify spores of *B. cinerea*, *M. laxa* and *M. fructicola;*
- Latent infection was quantified for flowers and developing fruit but there was no significant relationship with rot at harvest; therefore a protocol for growers to quantify latent infection to assist prediction was not developed.

Engagement with industry during the project included multiple seminars, orchard walks and a workshop with 30 growers in NSW. Two fact sheets, several newsletter articles and scientific publications were produced.

An identification guide for common cherry rot pathogens was produced that is available to all growers.

A weather-based rot infection risk tool designed especially for sweet cherry to raise awareness of key risk times is available to growers on request.

This project has enabled development of expertise in sweet cherry pathology and collaboration between Tasmanian and NSW pathologists, which is an invaluable resource for the industry.

Recommendations arising from this project related to current disease management practices are listed in the report. Recommendations for further activities related to this project, and new activities to aid sustainable management of fruit rot are also listed.

Keywords

Sweet cherry, *Botrytis cinerea*, *Monilinia* spp., *Alternaria alternata*, rot, weather-based risk, latent infection

Introduction

Several pathogens are known to cause rot in sweet cherry fruit, primarily including *Monilinia fructicola*, *Monilinia laxa* and *Botrytis cinerea*. The *Monilinia* spp. cause brown rot while *B. cinerea* causes grey mould, however the symptoms caused by these three pathogens can be difficult to distinguish and all result in substantial losses to fruit quality. Infection of flowers or developing fruit can occur theoretically at any time in the season. Once infection of buds, flowers or young fruit has occurred, the pathogen typically exists in a latent form within the developing fruit. Rot symptoms usually only occur 1-2 weeks prior to harvest (or post-harvest), which makes early prediction of the disease's impact on the final crop difficult. Typical integrated management of brown rot relies on removal of inoculum sources, control of pests which spread spores, and use of appropriate fungicides at key times of risk. Key times of risk are not currently well understood in Australian orchards but factors such as inoculum presence and weather-based infection likelihood can be determined. Relative abundance of rot pathogens in Australian sweet cherry orchards is not well documented.

While *Monilinia fructicola* is the only species mentioned in the Australian Cherry Production Guide (James, 2011), it was not detected at all in fruit of a Tasmanian sweet cherry orchard surveyed in 2012, for which *B. cinerea* was dominant in harvest age fruit with rot symptoms (Tarbath et al. 2014). Establishing the relative importance of both pathogens is critical as implications for management are different. Also, both pathogens have slightly different optimal climatic conditions for sporulation, germination and infection. If inoculum is present, the level of infection risk to flowers or fruit can be determined based on weather. Weather-conditions that will lead to infection of spores of *M. fructicola* have been determined in cherry orchards in the USA (Biggs and Northover, 1988), while for *B. cinerea* this has not been done for cherry, but has been done on grapes (e.g. Broome et al. 1995).

This project will run controlled condition tests to determine the infection incidence across the range of temperature and wetness durations relevant to the pathogens, using local isolates of each pathogen and local, current cherry varieties. This project will develop a simple tool to develop awareness of infection risk based on weather-conditions. This tool will be particularly useful to those growers who have weather stations in their orchards (or access to local weather data), however it will not predict the amount of rot which may develop. It could help to guide decisions about fungicide application, and potentially reduce unnecessary use.

Maximum infection of fruit by rot pathogens is determined by high inoculum loads, adequate temperature and moisture to facilitate spore germination and infection, and susceptibility of the plant tissues. Assuming inoculum is present and climatic conditions are adequate, infection pathways in sweet cherry are assumed to be dominated by infection at flowering (anthesis) and at later stages of fruit development when antifungal defences are lower. Understanding these key times is important for crop management decision-making.

Methodology

This project utilized both field and laboratory studies, conducted in two states of Australia (Tasmania and NSW) over 3 seasons. The approach and methods of the major studies are provided within scientific reports in the appendices, but a brief overview is provided here also.

A. *Fruit susceptibility study*

In order to establish the susceptibility of fruit to disease at different developmental stages, symptomless fruit (from 3 varieties) were harvested from a commercial orchard and challenged with fungal pathogens in controlled laboratory tests. The treatments included a control (sterile solution), *B. cinerea* spore solution, *M. fruticola* spore solution and *M. laxa* spore solution. Fruit were incubated in moist conditions and optimal temperature for infection (20°C). Symptoms of infection were monitored and recorded. Data was analysed and infection models determined.

B. Controlled studies on influence of temperature and wetness duration on infection

A preliminary trial was conducted with cherries from one variety collected from a commercial orchard in southern Tasmania prior to harvest. Cherries were inoculated with a spore suspension of *B. cinerea*, M. laxa and *M. fructicola* and maintained at temperatures from 12.5-22.5°C (at a step of 2.5 °C) and exposed to wetness durations from 3 to 12 hours. A single incubator was used for each temperature, therefore representing only one replication in the experimental design. Infection and sporulation were assessed with a ranking scale. Results from the preliminary trial was used to design full trials which included replication of a single experimental design as above, but across three successive days. Data from trial 4 (B.cinerea and M. fructicola) were subject to regression analysis to determine the effect of temperature and wetness duration on the level of infection. Models were developed as previously by Biggs and Northover (1988) and Broome et al. (1995). These models were used to develop a simple weather-based tool that can be used for awareness of infection risk. In conjunction with information from the fruit susceptibility trial above, this risk was overlaid with developmental stages of greater host susceptibility.

C. Harvest-infection surveys

Assessment of rot at harvest was conducted in several commercial cherry orchards in Tasmania and NSW across three seasons. Site history and information about management practices undertaken that season were recorded. Fruit was surveyed and rotten fruit sampled from 10-24 trees of several cultivars. For four branches per tree, all fruit was counted and number of rotten, damaged or cracked fruit were determined. Rotten fruit was sampled and returned to the laboratory in cool storage. Isolations of fungal pathogens was made from the rotten fruit only but surface sterilization and plating of fruit tissues on to agar. Sub-cultures of fungal isolates were made for later fungal identification. Identification was based on morphological and microscopic characteristics of fungal growth in culture, as well as identification based on molecular sequencing. For sequencing, fungal material was homogenised in a buffer solution and extracted, followed by amplification by PCR and sequencing by Macrogen Pty Ltd.

D. Temporal progression surveys of latent infection

These surveys were conducted throughout the season from flowering to harvest. Sites were selected where blocks (or rows) with or without fungicide use where possible. Prior to flowering, field sites were established and signs of inoculum recorded. For selected Tasmanian sites, a weather station was installed to record temperature, relative humidity, wetness (via a leaf wetness sensor) and rainfall. Spore traps were installed at two orchards per season in Tasmania only. At the time of full bloom, surveys will included scoring visual evidence of blossom blight and removal of blossoms (with or without blight) for traditional fungal isolation. As fruit developed, 40 fruit per trees were harvested at random from selected trees and returned to the laboratory in cool storage. At the laboratory, fruit were subjected to freezing and moist incubation (the ONFIT procedure) in order for latent pathogens to be expressed. After 2-3 days incubation, fungal growth was recorded and sub-cultures made for later fungal identification. Identification was based on morphological and microscopic characteristics of fungal growth in culture, as well as identification based on molecular sequencing where necessary. Sequencing was conducted as above. Scoring of rot at commercial harvest was assessed as for part C. Relationships between latent infection at all points in the season and final rot at harvest were explored via data analysis.

E. Weather-based infection risk tool

Based on the infection models for *B. cinerea* and *M. fructicola* from part A and B above, an existing weather-based infection risk tool for grapevine was adapted for sweet cherry. This involved programming and testing in Visual Basic for Applications (VBA), within MS Excel.

Outputs

Industry resources

- An identification guide to the main rot disease pathogens has been completed. This is available on the TIA website here http://www.utas.edu.au/tia/centres/perennial-horticulture-centre/fact-sheets-and-tools/fact-sheets-and-tools
- "Cherry rot risk" predictive tool developed. This preliminary tool has been distributed to four industry representatives in Tasmania for consultation in the first instance (Peter Morrison, Roberts Ltd; Nick Owens, Reid Fruits; Ian Cover, Fruit Growers Tasmania; Bob Nisson, TIA). It will be available on request after the consultation phase. A document outlining the requirements to use the tool, features provided and how to request access, will be placed on the "TIA fact sheets and tools" webpage as above. Notification of this will be distributed via the Cherry Newsletter monthly email and appropriate industry events, including the National Cherry Development Program roadshow in 2017.

Industry information

- Newsletter article – Barry, K. 2013. Management of fruit rot in sweet cherry, prevention is better than cure. Cherry Growers Australia newsletter, December.
- Newsletter article - Barry, K. 2015. "Avoiding rotten luck in sweet cherry". Cherry Growers Australia newsletter, Summer.
- Fact sheet – Barry, K. 2013. "Brown rot"
- Fact sheet – Barry, K. 2015. "Cherry rot – what are we finding in Tasmanian and NSW orchards?"

  Fact sheets available are here http://www.utas.edu.au/tia/centres/perennial-horticulture-centre/fact-sheets-and-tools/fact-sheets-and-tools

Presentations to industry

- Barry, K. Optimal management of pre-harvest rot in sweet cherry (2013-2016): Project update. "Cherry rots and thrips" workshop. Nashdale, NSW. June 10th, 2015.
- Dodds, K. Cherry rot project: slide show of damage observed (NSW). "Cherry rots and thrips" workshop. Nashdale, NSW. June 10th, 2015.
- Tesoriero, L. NSW cherry rots 2014-15: pathology results. "Cherry rots and thrips" workshop. Nashdale, NSW. June 10th, 2015.
- Barry, K. Brown rot prevention and management. Fruit Growers Tasmania Export Registration day, Hobart, August 19th, 2015.
- Barry, K. Field day presentation. TIA/FGT Fruit Production day on 20th November 2015.
- Barry, K. Predicting weather-based risk for Botrytis and Monilinia in sweet cherry. FGT annual conference, Hobart, 17th June, 2016.

Scientific presentations

- Barry, K., Tarbath, M., Measham, P and Corkrey, R. Understanding infection pathways and tree factors for integrated disease management of brown rot and grey mould in sweet cherry. International Horticulture Congress (IHC), Brisbane, August 2014.

Scientific publications

- Barry, K, Tarbath, M, Glen, M, Measham, P and Corkrey, R. 2016. Understanding infection pathways and tree factors for integrated disease management of brown rot and grey mould in sweet cherry. Acta Horticulturae 1105, 67-72.

Theses

- Pandey, A. 2014. Studies in fungicide control of sweet cherry pathogens. M. Applied Science (Agriculture) thesis. School of Land and Food, University of Tasmania.

Outcomes

The research outcomes gained during the life of this project include:

- Incidence of fruit rot in the surveyed orchards was generally low on average over the last 3 seasons in the regions studied
- Current fungicide programs appear to be generally effective in the regions studied
- Conditions that led to more fruit rot also led to more cracking at harvest maturity in the Tasmanian sites studied
- The key rot pathogen in Tasmanian orchards was *Botrytis cinerea*, which supported a preliminary study
- The key rot pathogen in NSW orchards was *Alternaria alternata*, which was not known prior to the project
- Both flower petals and the style could host latent infections of rot pathogens
- Fruit at developing stages host latent infections but the incidence does not relate clearly to visible rot at harvest
- In controlled conditions, fruit is more susceptible to infection at early and late stages of development than middle stages around stone hardening
- Wetness periods as short as 3 hours on fruit surfaces without wounding can lead to low levels of infection by *B. cinerea* or *M. fructicola,* and the levels increase with temperature

Adoption of findings from this study:

Awareness about identification and management of rot has been raised with growers in Tasmania and NSW, as evidenced by discussion with growers following seminars and workshops. An evaluation survey following the "thrips and rots" workshop in June 2015 found that the majority of growers (approx. 30 attending) were previously unaware that *Alternaria alternata* could be a cause of production loss, and all agreed that they had learnt something new about management options for their orchards. Practice change based on the tools developed in this study (e.g. identification guide, weather-based rot risk tool) is expected to occur as growers access the tools, however further extension support is needed to ensure effective use, evaluation and adoption.

The outcomes for professional capacity:

This project has enabled three plant pathologists (Karen Barry, Morag Glen and Len Tesoriero) to conduct research in sweet cherry for the first time. They now have increased awareness of the industry, orchard management and specifically crop health issues. Two other staff (Kevin Dodds and Ross Corkrey) have also gained more expertise in the sweet cherry industry. Kevin Dodds has initiated further research on thrips in NSW orchards arising from experience gained in this project. Several technical staff have gained exposure to the industry and increased skills in pathology of sweet cherry fruit. One student (Abun Pandey) completed a Masters Applied Science thesis project related to this research and developed a key interest in the industry.

Collaboration outcomes:

This has project enabled new collaborative links to be formed between TIA and NSW DPI. Solid relationships have been developed which will enable future collaboration where opportunities arise.

Evaluation and Discussion

The research conducted in this project has met the intended aim to increase knowledge of key aspects of rot pathogens of sweet cherry. All staff involved in the project remained engaged through the 3 year duration and as such the project was effectively managed. The available resources were able to support all aspects of the project as planned.

The project has delivered the first robust data collection on cherry rot incidence and causal pathogens across 2 regions. This component of the project was well supported by molecular methods (sequencing) which enabled a high certainty of pathogen identification. While only conducted in two regions of Australia, these results provide a snapshot of the key pathogens and impact on quality of fruit at harvest. The pathogen identification guide has received very positive feedback since release.

Inoculum dynamics and infection pathways have been clarified with studies of spore abundance and latent infection across two seasons and several field sites. These results have been important to highlight the extent of infection occurring at flowering and where in the flower this occurs. Development of infection in the stylar tissue is known in some other fruit crops to lead to latent infection within the growing fruit. Based on the results, it is apparent that infection continues during the season. This is also supported by the controlled studies which showed that even though some stages of fruit development were less susceptible to infection, it was still possible to have low levels of infection occurring. The methods used for the spore abundance studies were iteratively developed during the project, using a range of preliminary trials and approached. While this is a research tool, the refinement of such methods is an important achievement alone, and has resulted in valuable data for three key pathogens.

The controlled inoculation studies with variable temperature and wetness periods were the most technically challenging aspect of this project and had to be repeated several times, but were ultimately successful. Unfortunately similar inoculation studies with flowers were not successful and would need to be approached in a different way for future attempts. These studies were not originally planned as part of project activities, but hoped to be an additional finding to support the weather-based tool.

The weather-based tool to enable situational awareness of infection risk has been developed, based on studies completed during the project. As well as being used during the season for existing orchards, the tool could be used retrospectively to review effectiveness of decision-making during the season. In addition, the weather-based tool may be useful to aid site selection of future orchards. Historical data could be run through the tool and the number of high infection risk days evaluated. It is acknowledged that this tool is not commercialized or able to integrate with automated weather data. Comparison of outputs from our tool with commercial models which include the bacchus index (used by many sweet cherry growers, but not developed with studies of sweet cherry) would be valuable. If the results are similar, support for the existing commercial models could be validated.

The key learning from the project is that multiple rot pathogens are capable of reducing quality of sweet cherry fruit and the quantity of rot which will be evident at harvest is difficult to predict. However, infection risk can now be quantified and used to increase awareness for disease management decision-making All findings in the project to date support current practices of disease management, which include reducing inoculum, understanding key pathogens present, managing the canopy to reduce wetness periods and applying appropriate fungicides at times of key infection risk.

Recommendations

Recommendations for current pre-harvest disease management practices in the sweet cherry industry:

1. A range of rot pathogens should be considered in disease management strategies, as a diversity of pathogens are likely to be found in all regions;

2. It can be assumed that these pathogens are present throughout the whole season, even if mummies are not evident in high numbers and even without other sources of fruit wounding;

3. Standard practices for canopy management should be adhered to in order to increase air flow and reduce canopy wetness;

4. For optimal scheduling of fungicide application, it is advised that access to high quality weather data is available which can be used to raise awareness of rot infection risk.

Recommendation for research to further the results of this project:

1. Extend the survey of rot pathogen identity and abundance to the post-harvest time period

2. Evaluate the weather-based tool outputs over several seasons and in several regions and relate to the rot abundance at harvest and post-harvest, including in tunnel-grown orchards

3. Compare the outputs of the weather-based tool, developed in this project for sweet cherry, with those of existing commercial models over several seasons and in several regions

Recommendations for future extension and evaluation activities:

1. Provide training on use of the weather-based tool for crop advisors and growers (e.g. via a webinar)

2. Evaluate how “user-friendly” the weather-based tool is and how it can be improved or integrated with automated weather data

3. Provide training on pathogen identification for crop advisors and growers

This project was designed to confirm key information on sweet cherry rot pathogens, infection pathways and infection risk, rather than evaluate efficacy of disease management options. However, this remains an important on-going need for the industry.

Recommendations for future research projects:

1. Evaluate a range of biological control options for sweet cherry and their ability to control all the major rot pathogens (e.g. bee-vectored trichoderma; bacillus products; pseudomonas products)

2. Evaluate levels of fungicide resistance in populations of key rot pathogens in order to forecast future risk of fungicide efficacy.

Scientific Refereed Publications

One article has been published based on results from activity during the CY13001 project.

- Barry, K, Tarbath, M, Glen, M, Measham, P and Corkrey, R. 2016. Understanding infection pathways and tree factors for integrated disease management of brown rot and grey mould in sweet cherry. Acta Horticulturae 1105, 67-72.

Due to the multi-season nature of trials and experiments in this project, further data was not ready early enough to enable preparation and publication prior to the project completion date. At least two journal articles will be prepared and submitted in the next 6 months, based on finding which are partially presented as appendices.

A scientific article based on the preceding related project (CY11012) was submitted and published during the course of this present project.

- Tarbath, M.P., Measham, P.F., Glen, M., Barry, K.M. 2014. Host factors related to fruit rot of sweet cherry (*Prunus avium* L.) caused by *Botrytis cinerea. Australasian Plant Pathology. DOI:* 10.1007/s13313-014-0286-7

Intellectual Property/Commercialisation

No commercial IP generated.

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Appendices

A. Survey of incidence and identity of pathogens causing rot of sweet cherry fruit in Tasmania and New South Wales

INTRODUCTION

Pre-harvest rot has had serious regional impacts in the past 10 years for cherry growers in Australia. In 2010-2011 rot incidence was so high that some orchards did not harvest whole blocks. Based on climate projections for cherry growing regions, conditions suitable for rot may become more prevalent in some regions, due to increased temperatures and more variable rainfall (Thomas et al., 2012). Until now, brown rot caused by *Monilinia* spp. has been thought to be the main type of pre-harvest rot, but rot caused by *Botrytis cinerea* looks similar and may be more prevalent than previously thought. This has implications for management.

Several pathogens are known to cause rot in sweet cherry fruit, primarily including *Monilinia fructicola*, *Monilinia laxa* and *Botrytis cinerea* (Adaskaveg et al., 2000b). The *Monilinia* spp. cause brown rot while *B. cinerea* causes grey mould, however the symptoms caused by these three pathogens can be difficult to distinguish. Infection of flowers or developing fruit can occur theoretically at any time in the season. Two other species of Monilinia are known to cause rot of sweet cherry; *M. fructigena* is widespread in Europe and *M. polystroma* has recently been found for the first time to cause rot in sweet cherry in Poland (Poniatowska et al. 2015), however neither have been reported in Australia to date and remain of biosecurity concern.

Relative abundance of rot pathogens in Australian sweet cherry orchards is not well documented. While *Monilinia fructicola* is the only species mentioned in the Australian Cherry Production Guide (James, 2011), it was not detected at all in fruit of a recently surveyed Tasmanian sweet cherry orchard, and *B. cinerea* was dominant in harvest age fruit with rot symptoms (Tarbath et al. 2014). Establishing the relative importance of both pathogens is critical as implications for management are different. While both pathogens have some similarities in infection pathways, modes of overwintering are different (which has implications for inoculum reduction) and not all fungicides currently used in Australia for brown rot target both (e.g. propiconazole is effective against *M. fructicola* but not *B. cinerea*). For those fungicides that are effective against both, different levels of fungicide resistance may develop in the populations of each pathogen. Also, both pathogens have slightly different optimal climatic conditions for sporulation, germination and infection.

Management of fruit rot is essential for high quality fruit production, but it has also become important for market access to export destinations. *M. fructicola* is a pathogen of concern for import to China and UK/EU markets. Since the commencement of fruit export to China from Tasmania in early 2013, exporters must register and conform to protocols to ensure effective management of *M. fructicola*.

This includes demonstrating freedom from *M. fructicola*.

Surveys were conducted in commercial orchards in southern Tasmania and two regions of New South Wales (Orange and Young) across three growing seasons. A range of cultivars were surveyed and in several orchards trees with and without fungicide management were compared in order to determine background presence of pathogens without active chemical control. Fruit with visible rot were counted and the causal pathogens were identified with traditional and molecular techniques. The fungal rDNA ITS region was sequenced to identify the majority of fungi recovered, while β-tubulin (reference) and G3PDH (reference) genes were required to distinguish *M. laxa* and *M. fructicola*. To identify *Alternaria* spp. four gene sequences were used (ITS, GAPDH, RPB2 and TEF1a).

SURVEYS AND PATHOGENICITY TESTS

Surveys

Several commercial sweet cherry orchards were surveyed for incidence of fruit rot in both southern Tasmania and NSW across three seasons. The surveys were conducted as close to commercial harvest date as possible (i.e. 1-2 days prior to harvest). In most cases, trials were established prior to flowering and several rows were allocated to be maintained as fungicide-free during the season, for comparative purposes. Between 10-24 trees per variety were selected, in most cases with trees from fungicide-treated rows and fungicide-free rows. For each tree, 6 branches were tagged and all fruit on those branches was assessed. Total fruit, rotten fruit, cracked fruit and other damage was recorded.

Representative fruit with symptoms of rot were removed, placed in individual bags and returned to the laboratory. Using aseptic technique, pieces of fruit were placed on to potato dextrose agar (PDA) supplemented with antibiotics to reduce bacterial contaminants. The fungi that grew from the fruit pieces were characterised (i.e. colour, texture) and sub-cultured. Several examples of each type of fungus isolated was identified with molecular methods. In short, DNA was extracted then the ITS region of DNA was amplified with primers and Polymerase Chain Reaction (PCR). The PCR products were checked with gel electrophoresis, then sent to Macrogen (South Korea) for sequencing. The sequence was then searched on the NCBI GenBank nucleotide database. For the full method, see Tarbath et al. (2015). Further genes were required to identify *Monilinia* and *Alternaria* to species level, as outlined in the results section. Information on pathogen identification was then extrapolated to all samples with rot, to enable an estimate of the incidence of each major rot type.

Pathogenicity tests - NSW

These preliminary tests were conducted to determine whether *A. alternata* (two different morphotypes) and *Aureobasidium pullulans*, all frequently isolated from rotten cherries, were able to cause rot when inoculated. Cherry fruit (cv. *Lapin*) which had not been exposed to fungicide during the growing season, were harvested from the NSW DPI Orange Agricultural Institute orchard and were placed on rectangular polystyrene sheets that had been prepared with wells onto which cherries were placed. These sheets were sat in shallow plastic trays ready for application of fungal inoculum.

A bulk inoculum was made up for each fungus from PDA cultures which were irrigated with sterile

water and carefully agitated with a sterile 'hockey stick' glass rod to release spores. Spore concentrations were estimated under a compound microscope using a haemocytometer and adjusted to 10^5 spores/mL. One set of cherries was wounded by puncturing the fruit epidermis with a sterile needle. A 50µL droplet of fungal inoculum was then placed onto cherries above the wound or at a similar position on unwounded fruit. The negative control treatment received an equivalent volume of sterile water. Trays were then sealed in plastic zip-lock bags and incubated at 20-22^{o}C on laboratory benches. As this was a preliminary experiment the treatments were pseudo-replicated with 40 fruit for each of the negative controls, 50 fruit for each of the *Aureobasidium* treatments and 80 fruit for each of the *Alternaria* treatments. Rotted fruit were assessed at 3, 8 and 14 days after inoculation.

RESULTS AND DISCUSSION

Surveys – Tasmania & NSW

The results are summarised in Table 1 and 2 below. In the Tasmanian orchards rot was detected at all sites, however in some cases it was extremely low in incidence. The 2014-15 season was associated with several heavy rainfall incidents towards harvest date which increased both cracking and rot counts. In contrast, in the 2013-14 and 2015-16 seasons rot and cracking were lower in incidence. *B. cinerea* was the dominant cause of rotten fruit in all cases, with minor detection of other species, including *M. laxa*, *Stemphyllium* sp., *Alternaria* sp., *Penicillium expansum* and Cladosporium sp. A low incidence of *M. fructicola* was detected, but not at all from fruit which was being managed with a fungicide program. ITS sequences were sufficient to distinguish most fungal pathogens to species level, but the β-tubulin and G3PDH genes were required to distinguish *M. laxa* from *M. fructicola*, which could not be distinguished morphologically.

In the NSW orchards rot incidence was typically lower than in the Tasmanian orchards surveyed and in some surveyed there was zero incidence of rot (Table 2). Similar to Tasmania, a higher incidence was detected in the 2014-15 season than the other two seasons, which was suspected to relate to hail damage, as opposed to rain events in Tasmania. In the first two seasons surveyed *Alternaria alternata* was found to be the dominant pathogen of rotten fruit sampled, while in the 2015-16 season *Monilinia* spp. was equally or more prevalent. Despite this, incidence was still low and this appearance of *Monilinia* spp. is likely to be related to the fact that fungicide was not used in any of the surveyed orchards that season.

The relationship between incidence of cracking and rot was explored and there was a weak relationship for Tasmanian sites but none for the NSW sites (Fig. A1).

Table A1. Incidence of rot and cracking detected during surveys in southern Tasmanian sweet cherry orchards, across three growing seasons.

Site	Year	Variety	Fungicide	Number of trees surveyed	% Cracking	% Rot	Main causal pathogen*	
Sth Tas 1	2013-14	Sweetheart	Y	24	4.8	0.04	B. cinerea	100%
	2013-14	Sweetheart	N	24	2.3	0.49	B. cinerea	78%
Sth Tas 2	2013-14	Simone	Y	12	2.2	0.02	B. cinerea	100%
	2013-14	Simone	N	12	1.6	0.26	B. cinerea	25%
	2013-14	Lapins	Y	24	2.7	0.10	B. cinerea	72%
Sth Tas 3	2014-15	Lapins	Y	10	9.1	1.20	B. cinerea	55%
	2014-15	Lapins	N	10	9.4	0.72	B. cinerea	60%
	2014-15	Sweetheart	N	10	15.0	12.80	B. cinerea	100%
Sth Tas 4	2014-15	Lapins	Y	10	8.2	0.80	B. cinerea	74%
	2014-15	Lapins	N	10	6.1	3.50	B. cinerea	70%
Sth Tas 5	2015-16	Simone	Y	10	0.4	0.31	B. cinerea	100%
	2015-16	Simone	N	10	0.4	0.81	B. cinerea	100%
Sth Tas 3	2015-16	Lapins	Y	10	6.1	0.34	B. cinerea	100%
	2015-16	Lapins	N	10	10.8	2.20	B. cinerea	100%
	2015-16	Simone	Y	10	5.7	1.16	B. cinerea	100%
	2015-16	Simone	N	10	2.9	1.83	B. cinerea	100%

*The main causal pathogen is that with the highest single rate of isolation recovery from rotten fruit samples. Other fungal species and nil recovery results constitute the remaining portion to 100%

Table A2. Incidence of rot and cracking detected during surveys in NSW sweet cherry orchards, across three growing seasons.

Site	Year	Variety	Fungicide	Number of trees surveyed	% cracking	% rot	Main causal pathogen*	
Orange 1	2013-14	Vans	Y	24	1.2	0.2	A. alternata	66%
		Sweetheart	Y	24	1.1	0	-	
Orange 2	2013-14	Lapins	Y	24	0.2	0	-	
		Sweetheart	Y	24	1.4	0	-	
Young 1	2013-14	Lapins	N	24	0	0	-	
		Vans	N	24	0	0	-	
Young 2	2013-14	Lapins	Y	24	0	0.2	A. alternata	17%
		Sweetheart	Y	24	0	3.0	A. alternata	60%
Orange 1	2014-15	Van	Y	24	5.5	0.4	A. alternata	56%
		Sweetheart	Y	24	11.2	0.1	A. alternata	100%
Orange 2	2014-15	Lapins	Y	24	14.4	0.5	A. alternata	100%
		Sweetheart	Y	24	4.0	0.1	A. alternata	50%
Young 1	2014-15	Lapins	N	24	1.8	0.07	A. alternata	72%
		Vans	N	24	NA	0.1	A. alternata	97%
Young 2	2014-15	Lapins	Y	24	0.3	0.7	A. alternata	72%
		Sweetheart	Y	24	0.6	6.1	A. alternata	70%
Orange 1	2015-16	Van	N	10	2.2	0.7	Monilinia spp.	100%
		Sweetheart	N	10	6.4	0.6	Monilinia spp.	100%
Young 1	2015-16	Vans	N	10	0.2	1.5	A. alternata	55%
		Lapins	N	10	0	2.6	Monilinia spp.	64%
Young 3	2015-16	Vans	N	10	0	0.7	A. alternata	51%
		Rons	N	10	0.2	0.4	Monilinia spp.	55%

*The main causal pathogen is that with the highest single rate of isolation recovery from rotten fruit samples. Other fungal species and nil recovery results constitute the remaining portion to 100%

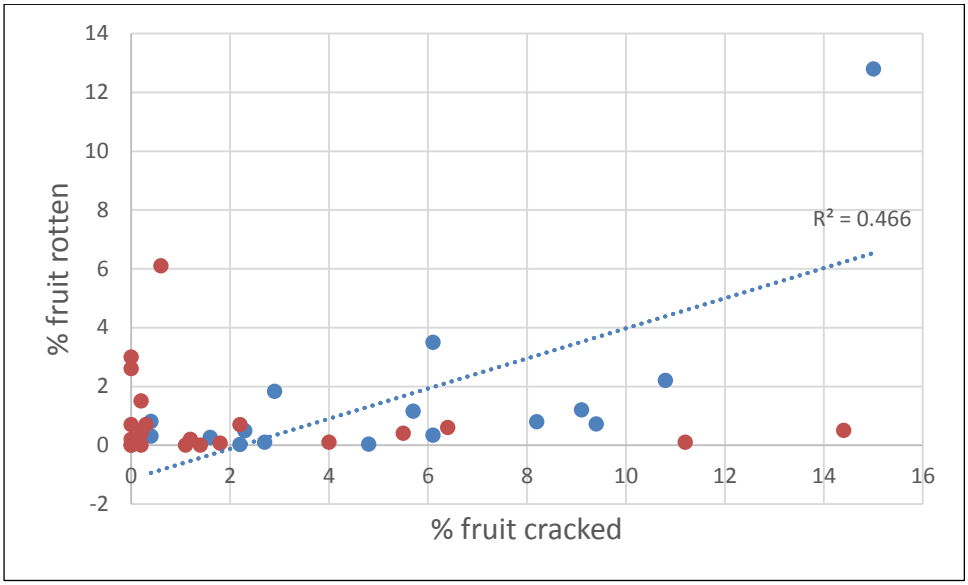


Figure A1. The relationship between percentage of rotten and cracked fruit at harvest age, from all orchards surveyed across three growing seasons. Red data points are from NSW, while blue are Tasmanian survey data. Each data point is the average of all trees surveyed for each site, variety and treatment type (i.e. fungicide or no fungicide) as shown in Table A1 and A2.

Pathogenicity tests – NSW

The results of this preliminary trial indicated that both types of *A. alternata* could infect and rot mature cherry fruit, with and without the presence of wounds. However, infection and rot of fruit by *Aureobasidium pullulans* only occurred when the fruit was wounded.

Table A3. Rotted cherry fruit after 14 days after inoculation with *Alternaria* & *Aureobasidium* isolates

Fungal treatment	**% Rot* at 14 days +/- wounding**	
	-	**+**
Water control	3	2
Alternaria alternata (stellate)	37	33
Alternaria alternata (radial)	23	20
Aureobasidium pullulans	0	32

*Rots attributed to *Alternaria alternata* or *Aureobasidium pullulans* (respectively). There were also some minor background rots (data not shown) attributed to *Monilinia fructicola*, *Penicillium* sp. and an *Aspergillus* sp.

CONCLUSION

These surveys have revealed that in the orchards of the regions studied, rot incidence was generally low however seasonal effects were considerable.

As suggested by our earlier preliminary studies (Tarbath et al., 2014) *Monilinia* spp. were not the most prevalent pathogens associated with rot of fruit at harvest. In Tasmania grey mould caused by *B. cinerea* was the major cause of rotten fruit while in NSW *A. alternata* was established for the first time as the most dominant pathogen associated with rot and its ability to infect fruit in the absence of wounding was demonstrated. These surveys have also shown that *M. fructigena* and *M. polystroma* were not detected, as expected. The utility of molecular approaches for fungal identification was highlighted in this study and recent reports show that further refinement of methods will enable even more efficient detection in future (Papavasileiou et al. 2016; Guinet et al. 2016).

Managing pre-harvest infection and rot development is critical to ensure a high value pack out for growers, however post-harvest rot development can also present a substantial risk. Future surveys to incorporate post-harvest studies along with pre-harvest surveys would be useful to develop a full picture of the impact of rot pathogens on fruit quality along the value chain.

B. Susceptibility of sweet cherry fruit to infection during development

INTRODUCTION

Conidia of both *Monilinia* and *Botrytis* species are capable of infecting all parts of the cherry flower and fruit. Infection risk is typically thought to be highest at full bloom (Holb 2008) but secondary inoculum associated with abortion of infected fruit also poses a high infection risk later in the season (Borve et al., 2005). Floral infection may lead to blossom blight in which the blossom is killed, or may result in a non-visible (latent) infection. Conidia can also infect developing fruit and may result in invisible (latent) or visible but inactive (quiescent) infections (Adaskaveg et al. 2000). Due to non-visible infections, the amount of disease likely to occur at harvest can be difficult to predict.

Infection and disease development can be monitored in orchards with various approaches to determine when infection risk is highest and which factors have the greatest effect on risk. Given there are numerous factors (including climatic, host and pathogen) which determine the development of disease epidemics, it can be difficult to assess the relative importance of each. Laboratory studies which can control several factors and experimentally adjust only one or two factors can aid a better understanding of risk. In the studies presented in this paper the role of tree cultivar and fruit development is examined. From a field study, the temporal incidence of infection is assessed in two cultivars and expression of rot at harvest is assessed in three cultivars.

To examine these factors further when the amount of inoculum and climatic factors are controlled, a laboratory trial was conducted with three sweet cherry cultivars, three different pathogens and fruit at different developmental stages. The outcomes of these studies will be integrated with tools to inform grower decision making.

CONTROLLED INOCULATION TRIALS

In October 2013 – January 2014 an *in vitro* inoculation study was conducted to examine the relative susceptibility of sweet cherry fruit at various stages (Table B1) during ripening. Fruit was collected from two commercial orchards in Southern Tasmania; the Huon Valley (Lapins and Simone cultivars) and the Derwent Valley (Sweetheart cultivar) at three time points, from trees that had not been exposed to fungicide that season. Fruit was transported to the laboratory within 1 hour of collection, surface sterilized and dried. A sub-sample of fruit was measured to characterize the fruit development. Fruit was placed into plastic containers on a rubber grid and then inoculated with a spore solution of one of three different fungal pathogens.

Table B1. Average size of sweet cherry fruit used for the *in vitro* inoculation tests at each stage of development.

	Sweetheart			Simone			Lapins		
	Width (mm)	Length (mm)	Days pre-harvest	Width (mm)	Length (mm)	Days pre-harvest	Width (mm)	Length (mm)	Days pre-harvest
Stage 1 (19th Nov)	8.3	9.8	61	9.1	9.4	57	13.1	13.7	57
Stage 2 (16th Dec)	18.9	19.4	34	18.6	17.9	30	17.8	17.1	30
Stage 3 (7th Jan)	27.5	23.8	7	26.8	22.1	3	26.9	22.9	3

Botrytis cinerea (isolate R4T3C5 obtained from the field study above), *Monilinia laxa* (reference culture 32346 from VPRI Herbarium, Department of Environment and Primary Industries, Victoria) and

Monilinia fructicola (isolate #81, Department of Environment and Primary Industries, Victoria, Knoxfield) were used in this study. The latter two cultures were inoculated on surface sterilized white peach fruit, incubated and reisolated prior to use in the study, to ensure pathogenicity. All isolates were cultured on potato dextrose agar and incubated at 25°C in the dark. When cultures were 7-10 days old a spore suspension was prepared by shaking culture pieces in sterile water, filtering through sterile muslin and then vacuum filtration through 8 µm Millipore membranes to collect spores. The concentration was adjusted to approx. 4 x 10^4 per ml in sterile water after spore counting with a haemocytometer. A 30 µl drop of spore solution was used to inoculate fruit (unwounded). Fruit were then incubated in plastic containers held at 98% relative humidity and 20°C for up to 14 days (Figure B1). Assessments of symptom development were made every 3-4 days and if sporulation occurred, fruit were removed from the container.

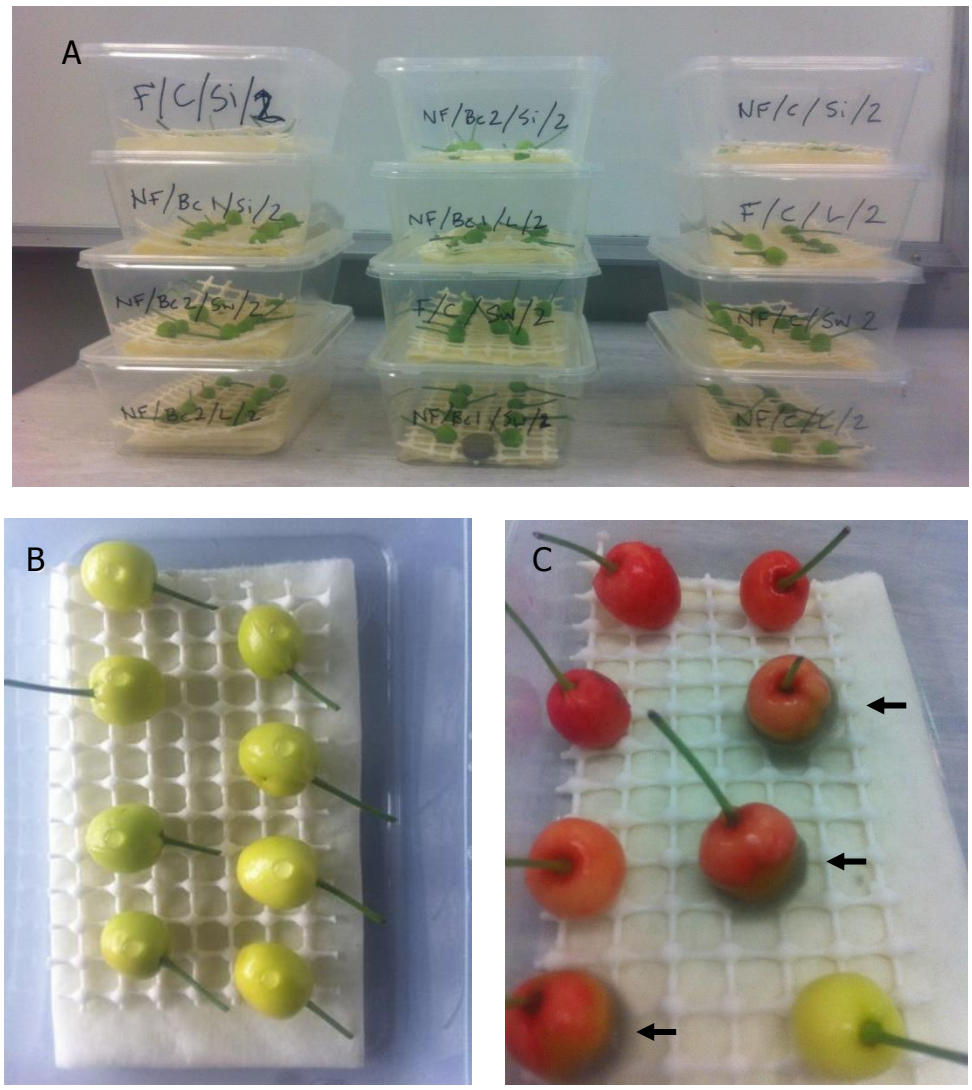


Figure B.1. A) A selection of containers with fruit of "stage 1" prior to incubation; note the silver thermocron sensor inside the lower middle container; B) A container with fruit of "stage 2" with droplets of inoculum, prior to incubation. C) A container with fruit of "stage 2" after incubation, showing 3 fruit (arrows) with rotting from the base and *Botrytis cinerea* growth visible.

For each cherry cultivar and each pathogen, 8 replicate fruit were assessed in each of 5 experimental blocks. Each block consisted of 5 treatments total, of which 4 treatments used fruit with no fungicide (*B. cinerea*, *M. laxa*, *M. fructicola* and a control consisting of sterile water inoculation) and a final treatment used fruit with fungicide applied commercially for an additional control. This later treatment revealed little difference in results to the control with fruit without fungicide and was not further analysed.

A logistic regression approach was calculated using Proc Logistic in SAS version 9.3 in which cultivar was included as a categorical effect, time as a continuous effect, and their interaction. The interaction was dropped if not significant as determined by type III wald tests. From the final model odds ratios (ORs) were determined to compare the relative odds of one fruit stage developing rot compared to another. Where the interaction was significant the ORs were calculated for day 9.

RESULTS AND DISCUSSION

Plots of rot development (over time since inoculation) for fruit of each stage were created and an example for the Sweetheart cultivar is shown in Fig. B2. Typical of other cultivars also, the number of fruit which developed rot when inoculated with *M. fructicola* was greater than for fruit inoculated with the other two pathogens. Comparison of odds ratio quantifies whether rot is more likely to occur or not for each fruit stage (Table B2). Analysis shows that only for Simone fruit (inoculated with *B. cinerea* or *M. laxa*) is there greater odds that stage 2 fruit will become rotten compared to stage 1. However, fruit stage 3 of all cultivars (except Lapins inoculated with *B. cinerea*, which is an anomaly) has much greater odds of developing rot than fruit of stage 1. For example, in the case of Sweetheart fruit inoculated with *M. laxa*, the odds ratio is 107, indicating the much greater risk of fruit at stage 3 developing rot compared to stage 1 fruit. As expected, in all cases, fruit at stage 2 has lower odds (all values below 1) of developing rot than fruit stage 3 (Table B2). Therefore, the difference in rot susceptibility is greater between stage 2 and 3 fruit than between stage 1 and 2 fruit. This highlights that infection risk increases non-linearly as fruit develops and the risk increases at a greater rate towards harvest. This agrees with findings from the latent infection field study and with similar studies elsewhere (Xu et al., 2007).

Table B2. Chance (odds ratio) of rot developing when fruit stages are compared for each cultivar and pathogen. Odds ratios >1 indicate higher chance while <1 indicate reduced chance of rot.

		Stage 2 vs Stage 1	Stage 3 vs Stage 1	Stage 2 vs Stage 3	P value (fruit stage)
Sweetheart	Bc	0.61	3.17	0.19	<0.0001
	Ml	1.00	107.17	0.01	<0.0001
	Mf	0.05	5.52	0.01	<0.0001
Simone	Bc	1.94	3.83	0.51	<0.0033
	Ml	3.54	7.77	0.46	<0.0055
	Mf*	6.31	190.8	0.072	0.0078
Lapins	Bc	0.13	0.76	0.17	<0.0001
	Ml	0.46	3.43	0.13	<0.0001
	Mf	0.48	7.52	0.06	<0.0001

*Due to a significant time x fruit stage interaction, odds ratios were determined for day 9.
Bc = *B. cinerea*, Ml = *M. laxa*, Mf = *M. fructicola*.

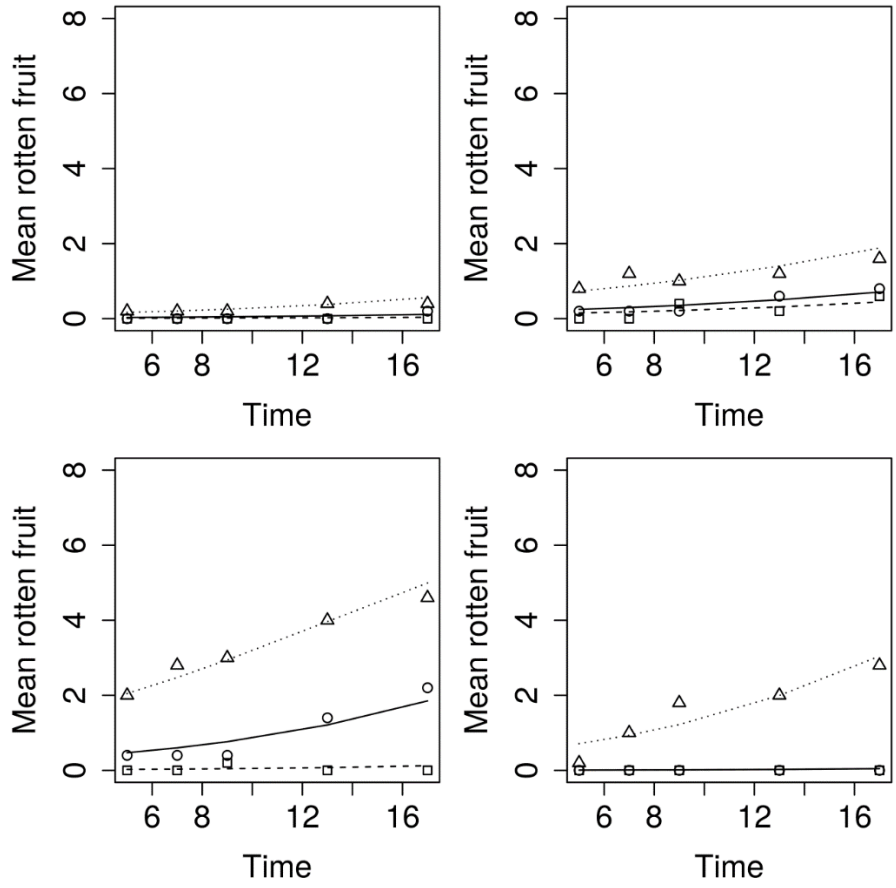


Fig. B2. Mean observed counts and predictions for number of rotted Sweetheart fruit (of 8 per block) after inoculation with three different pathogens in an *in vitro* test, where time is the number of days after inoculation. Fruit stages: triangles = 3 (7 days pre-harvest,), circles = 2 (34 days pre-harvest), and squares = 1 (61 days pre-harvest). Similar trends were found for Lapins and Simone cultivars.

From this and future studies related to weather-based infection risk, models can be developed and field validated to provide grower tools which can aid assessment of infection risk and decision making (e.g. fungicide scheduling). Where this study could be improved is in the inclusion of flowers as the first part of the developmental stage. As flowers are fragile and cannot be surface sterilized, a comparative procedure would have to be developed for fruit to enable controlled conditions that can be replicated for fruit of each stage. In terms of understanding relative susceptibility, exactly how much more or less flowers are susceptible to infection compared to fruit would be helpful.

C. Modelling the effect of temperature and wetness period on infection risk for intact, mature sweet cherry fruit inoculated with Botrytis cinerea *and* Monilinia *spp.*

INTRODUCTION

Various weather-based disease risk models exist for both *Botrytis cinerea* [e.g. Bacchus index developed for grapes (Kim et al. 2007)] and *Monilinia* species [e.g. peaches and nectarines (Holmes *et al.* 2008)], which have both been utilized within field tools by a range of commercial applications. However, there are no field-validated models which have been specifically developed for or applied to sweet cherry. In addition, the relative risk comparison between *B. cinerea* and *Monilinia* spp. have not been compared on sweet cherry in the same study.

A study on effect of temperature and wetness duration on *Monilinia fructicola* infection of sweet cherry (Biggs & Northover 1988) has been conducted but not trialed in the field to our knowledge. The study involved inoculation of sweet cherry fruit with a conidial suspension of *M. fructicola* at temperatures of 15 – 30° C and between 6 – 18 hours wetness periods. The optimum temperature was observed to be between 20-22°C and a logistic equation as below was found to be the best regression model to describe the infection incidence in sweet cherry, as below.

$\text{Ln}\,[Y/(1-Y)] = b_0 + b_1W + b_2T + b_3WT + b_4T^3$ (Biggs & Northover 1988)

Y = infection of ripe fruit, W = wetness period, T = temperature

The Bacchus index for predicting risk of infection by *B. cinerea* in grapes for each hour of surface wetness is:

$I = 1 / [84.37 – (7.238 * \text{hourly average temp}) + 0.1856 * \text{hourly average temp}^2]$ (After Kim et al. 2007)

The aim of this series of studies was to model infection probability when mature sweet cherry fruit are inoculated with spores of either *B. cinerea* or *M. fructicola* and incubated at a range of temperature and wetness periods. Fruit were not wounded prior to inoculation as previous studies have shown that for *M. fructicola* inoculated on wounded stone fruit the infection will be 100% for any temperature or wetness duration (Kreidl et al. 2015) – therefore any treatment effects would be masked. While wounding is often considered important for *B. cinerea*, we refrained from wounding also in order to enable a comparison with the *Monilinia* spp.

CONTROLLED INOCULATION TRIALS

Several trials were conducted to determine the influence of temperature and fruit wetness on fruit infection (Table C1.), using fruit that were within 4 weeks of harvest maturity. Fruit were obtained from the orchard within 24 hours of inoculation. Fruit was surface sterilized and dried, then placed into plastic containers on a rubber grid (8 fruit per container, moist paper towel added to create humidity). All fungal isolates used were cultured on potato dextrose agar and incubated at 25°C in the dark. When cultures were 7-10 days old a spore (conidial) suspension was prepared by shaking culture pieces in sterile water, filtering through sterile muslin and then vacuum filtration through 8 µm Millipore membranes to collect spores. The concentration was adjusted to approx. 4×10^4 per ml in

sterile water after spore counting with a haemocytometer. The spore solution was made 6-24 hours before inoculation and kept at 4°C. For trial 1-3 a 30 µl drop of spore solution was used to inoculate fruit (unwounded). For trial 4 sterilised paper discs (made from Whatman No. 1 filter paper) were soaked in the spore solution and placed on the fruit, as for the procedure by Kreidl et al. (2015) and shown in Figure C1. Control treatments were inoculated with sterile water in the same way.

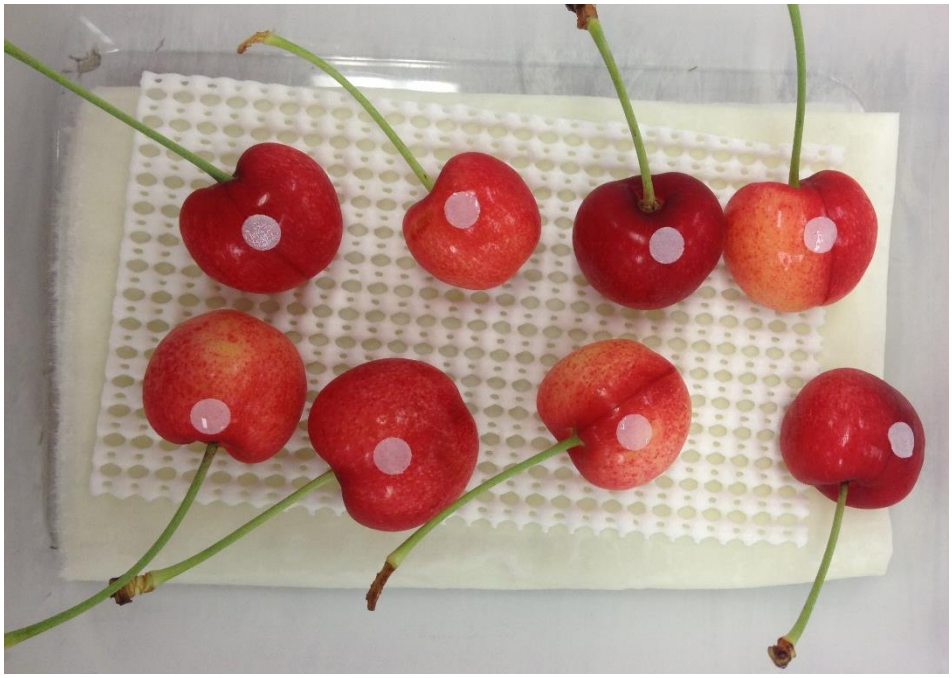


Figure C1. Inoculation of freshly collected sweet cherries in a laboratory trial (trial 4).

Containers of inoculated fruit were labelled and then placed at the appropriate treatment temperature in incubators, in the dark. As incubators are not always able to hold the desired temperature, a temperature and RH sensor (thermocron sensor, OnSolution, Australia) was placed inside one container of each cabinet to record actual temperature. Data were later downloaded and results reported against actual temperature. After the correct elapsed wetness period, fruit were dried off completely and containers were then placed at 20°C for incubation to enable any infection to develop symptoms. For trial 3 & 4 the moist paper was removed from the container at that stage to ensure the no further infection took place with any residual spores at high relative humidity. For experiment 2-4, two containers (16 fruit total) for each wetness period x temperature combination were prepared for each treatment (pathogens and control). This was repeated three days in a row with exactly the same methodology. Therefore, the data from each day of the trial can be considered an independent replicate.

Incubated fruit were observed at 7 and 14 days and the presence of lesions, rot and sporulation of the inoculated pathogen were recorded. When fruit started to sporulate and infection was confirmed, the fruit was removed from the container to reduce chance of cross-contamination. Given rot arising from non-inoculated controls was extremely low, background expression of latent infections could be dismissed (NB. fruit was kept "fresh" in these trials, not frozen as for the ONFIT procedure in which latent infection expression was promoted).

Data from each trial were analysed to determine if there were statistically significant effects of temperature and wetness period. Data from trial 4 were used to develop logistic regression models

for both pathogens in mature cherries, with the following formula:

Logit(*rot*) = *f*(T,W)

Where *rot* is the proportion of rotten cherries, W is the wetness period, T is temperature, *f*( ) is a linear combination of W, T, W^2, T^2, W^3, T^3 and their interactions, with coefficients to be estimated for each term. A fixed block effect was also included.

Table C1. Summary of the controlled inoculation trials conducted between 2013 – 2015.

Trial	Cultivar	Pathogens	Target temperature (°C)	Wetness periods	Inoculation method	Design
1 (2013) Preliminary trial	Sweetheart (no fungicide)	*B. cinerea* *M. laxa* *M. fructicola*	12.5, 15.0, 17.5, 20.0, 22.5	3, 6, 9 and 12	Droplets	Multiple randomised replication in one cabinet per temperature, conducted over 1 day
2 (2014)	Sweetheart (possible fungicide residue issue)	*B. cinerea* *M. laxa*	12.5, 15.0, 17.5, 20.0, 22.5	1, 3, 5 and 10	Droplets	Randomised and replicated over 3 successive days
3 (2015a)	Sweet Georgia (no fungicide)	*B. cinerea* *M. laxa*	12.5, 15.0, 17.5, 20.0, 22.5	3, 6, 9 and 24	Droplets	Randomised and replicated over 3 successive days
4 (2015b)	Simone (no fungicide)	*B. cinerea* *M. fructicola*	12.5, 15.0, 17.5, 20.0, 22.5	3, 6, 9 and 24	Soaked paper discs	Randomised and replicated over 3 successive days

Two inoculation trials were attempted with excised flowers in vitro (Figure C2), based on a protocol demonstrated by Prof. Jim Adaskaveg (University of California, USA) using sand to support flowers during inoculation. However the method was not successful for sweet cherry flowers with this experimental design as they senesced quickly unless maintained at high relative humidity, which would have provided constant infection conditions. Therefore, while this may be an appropriate method for testing pathogenicity of various fungal isolates, to quantify effects of temperature and wetness the method was not suitable. It is noted that inoculation of intact flowers may provide a

more realistic trial (see Kreidl et al. 2015), however ability to control conditions in the field is problematic.

Figure C2. Inoculation of freshly collected sweet cherry flowers in a laboratory trial.

RESULTS

Controlled inoculation trials were challenging to manage and despite infection occurring at various levels in experiment 1-3, the results did not show any significant differences between temperature or wetness treatments. This may have been for a range of reasons, including inoculation method (i.e. the droplet method was not ideal), pathogenicity of isolates, continued infection after the wetness period theoretically ended. With each successive experiment new variables were altered to obtain higher infection rates when fruit were subject to optimal conditions (i.e. at 20°C and 24 hour wetness, it would be expected that 100% infection would be obtained). The final experiment (trial 4) led to greatly improved results, probably due to the change in inoculation method (use of a soaked paper disc) which ensured the inoculum retained contact with the fruit throughout the wetness period. *M. laxa* was not included in this final trial as infection rates always proved to be very low compared to *B. cinerea* and *M. fructicola*.

The data from trial 4 were analysed to fit a logistic regression which could model the probability of infection based on temperature and wetness period as linear predictors. For *Botrytis cinerea* the fitted and observed values are shown in Figure C3 – C5 and for *M. fructicola* shown in Figure C6-C7. Analysis of maximum likelihood estimates showed that temperature and wetness duration were both high significant factors to determine infection by *B. cinerea* ($P = 0.0002$, $P<0.0001$ respectively) and *M. fructicola* ($P<0.0001$ for both). The highest probability of infection for both pathogens occurred at the 20°C temperature treatment with 24 hour wetness period.

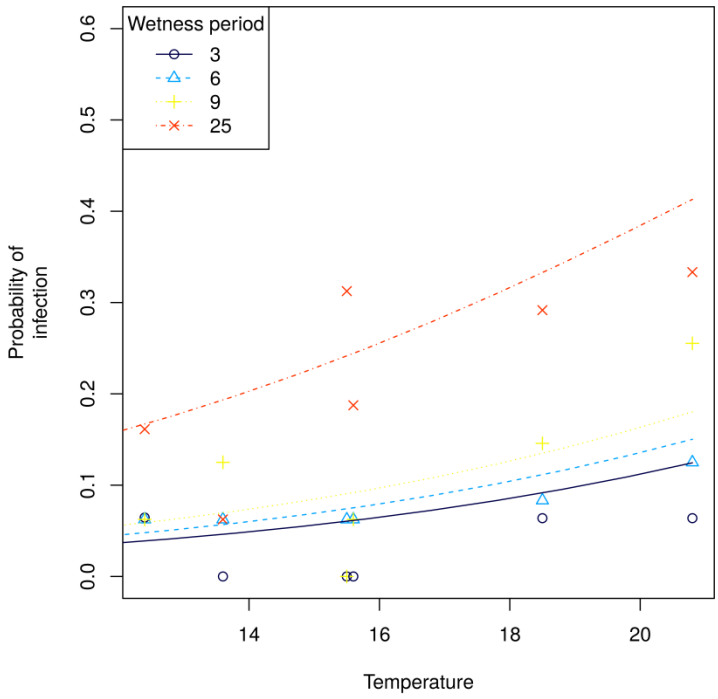


Fig C3. Fitted and observed values of infection probability plotted against temperature for different wetness periods (experiment 4, *B. cinerea*).

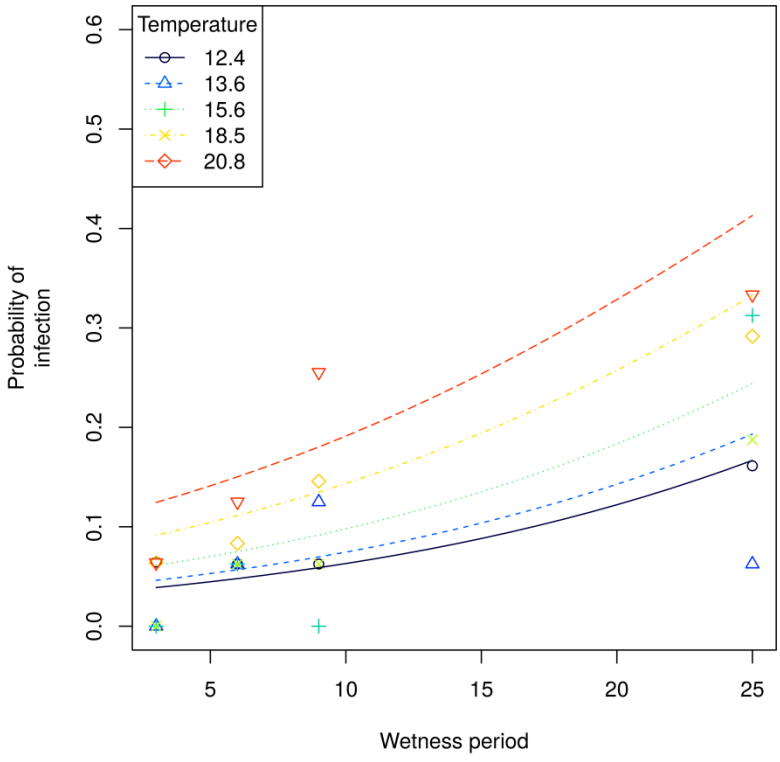


Fig C4. Fitted and observed values of infection probability plotted against wetness periods for different temperatures (experiment 4, *B. cinerea*).

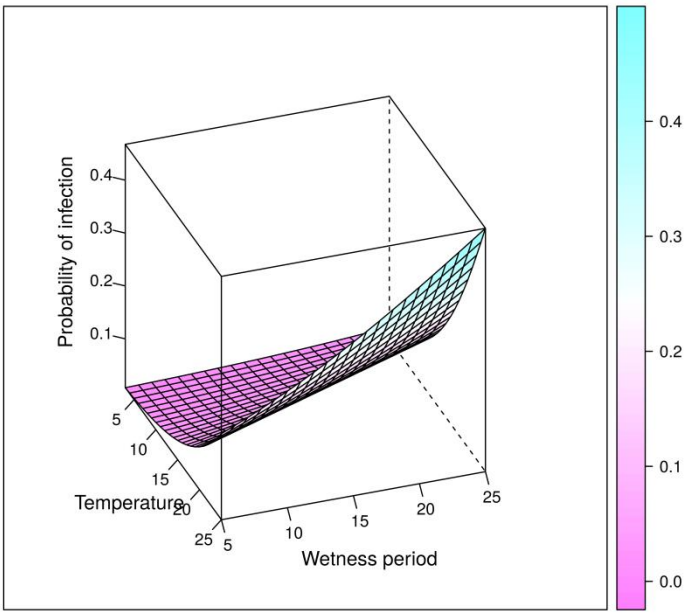


Fig. C5. Fitted values of infection probability against temperature and wetness period (experiment 4, *B. cinerea*).

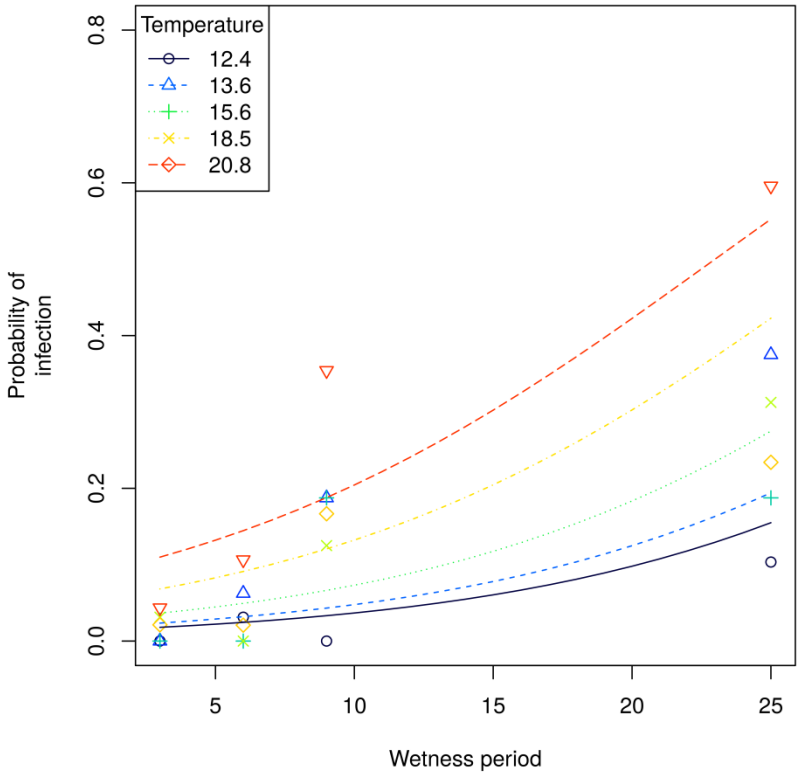


Fig C6. Fitted and observed values of infection probability plotted against temperature for different wetness periods (experiment 4, *M. fructicola*).

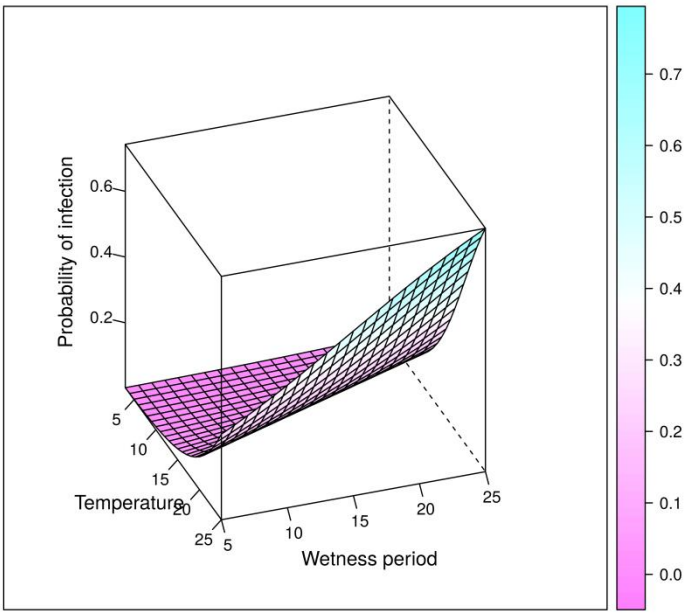


Fig. C7. Fitted values of infection probability against temperature and wetness period (experiment 4, *M. fructicola*).

DISCUSSION

These studies have revealed that the effect of temperature and wetness duration led to similar trends in infection probability found in previous studies (Biggs and Northover, 1987; Broome et al. 1995; Kim et al. 2007; Kreidl et al. 2015) with these pathogens, with likelihood of infection increasing with temperature (up to 20°C tested here in trial 4) and wetness period. How similar these trends are to other models is important to explore in order to demonstrate which existing models and weather-based infection tools could be equally applicable.

Weather-based risk models can predict likelihood of high infection occurrence, but are not able to quantify how that may translate into the exact amount of rot at harvest. A complete integrated orchard model is required to forecast disease progress accurately. Such a model would require knowledge of how inoculum loads, susceptibility of cherry cultivars, canopy microclimate and disease management (i.e. fungicides or other treatments) all interact with weather across the season to determine the amount of rot. Data in this project will contribute to the development of such a model, which could be developed in the future if the industry considers it a priority.

Maximum infection of fruit by rot pathogens is determined by high inoculum loads, adequate temperature and moisture to facilitate spore germination and infection, and susceptibility of the plant tissues. Assuming inoculum is present and climatic conditions are adequate, infection pathways in sweet cherry are assumed to be dominated by infection at flowering (anthesis) and at later stages of fruit development when antifungal defences are lower. In a study of rot infection of sweet cherry in Washington, USA, there was a positive relationship between weeks after petal fall and fungal colonization, with between 60-80% of fruit colonized by week 5-6 and between 80-100% colonized

by week 12 (Dugan and Roberts, 1994). While this study has only presented results from trials with fruit, the role of flower infection is acknowledged as highly important. Unfortunately inoculation trials with flowers were not successful within this project.

This research demonstrates that infection can occur with relatively short periods of fruit wetness and therefore strongly reinforces the current best practice crop management to increase air flow in canopies. The current increase of tunnel grown orchards (to reduce rainfall-induced splitting damage) holds promise of reduced wetness, however relative humidity may increase and if sufficiently high infection could still take place without fruit wetness.

D. Development and preliminary testing of a weather-based tool to predict rot pathogen infection risk in sweet cherry.

INTRODUCTION

The practical application of knowing which factors most influence fruit infection is that tools can be developed to aid awareness and decision-making in disease management. Tools to examine how weather and other factors impact disease incidence can be used to retrospectively interpret disease outcomes and use this information to plan strategically for future seasons. Alternatively, these tools can be use tactically to make decisions related to optimal management timing (e.g. fungicide application or frequency) during the season.

One major aim of this project was to use the information gained in the two studies reported above (report B and C) to develop and test a model specific to sweet cherry infection by those pathogens implicated as predominant based on the surveys conducted (report A). While fully validated weather-based disease prediction tools require substantial development and testing over several years, we aimed to develop a preliminary tool which could raise awareness of risk and also serve as a proof of principle research tool.

TOOL DEVELOPMENT

A non-commercialized weather-based infection risk tool ("version 1") developed within MS Excel for *Botrytis cinerea* in grapes was made available by David Riches (LaTrobe University). This was developed as a research tool to both process weather data into different time intervals and to apply the Bacchus index (see report C) to predict grape infection risk. To adapt the tool for our purposes ("version 2") and also make it user-friendly for crop advisors or growers, the following key changes were made:

Function	Version 1	Version 2
Weather data input	Temp, RH, leaf wetness and rainfall data need to be manually edited and integrated to one file (located only on C:/) with data arranged in a particular column order and saved as a .txt file below being used by the program. This step would be prohibitive for a busy crop consultant or orchard manager.	A graphic user interface added with file selection function. Either of two files formats ('ANFIC' or 'Tiny Tags') from commonly used weather station data loggers can be selected, from any drive or folder and with any file name. The user is given dialog box style prompts to select each weather file type (wetness, temp & RH, rainfall) in .xls format. NB. Prior to use, each weather file will have to be converted to .xls by a simple import step and ensure all data sets start at the same date and time.
Infection risk model	The Bacchus model determines the output of infection risk (the Bacchus index).	The logistic regression models developed in our study (see report C) for each of Botrytis and Monilinia replaced the Bacchus model. A function was added to the graphical user interface so that users can switch the infection risk output between the two pathogens based on the models developed for

		each in our studies.
Crop stage dates can be added specific to the season	Stages were related to grapevine growth (5% cap fall, 80% capfall, pre-bunch closure, veraison, harvest)	Stages were adapted for relevance to sweet cherry (1=10% bloom, 2=50% bloom, 3=100% bloom, 4=pea size, 5=stone hardened, 6=harvest)
Infection risk output considers phenological change in fruit susceptibility	Not considered in this version.	A function was added whereby the infection risk was reduced around the time of stone hardening (i.e. reduced by half for dates related to stage 4.5 to 5.5). This is based on our findings and those with other stone fruit that fruit at this stage is less susceptible to new infection and the early and late stages of fruit development are most susceptible.
The critical threshold of the infection index	When the Bacchus index reaches a value of 0.1 or more, conditions for an infection event are predicted.	User can vary the wetness threshold and critical probability of infection.

INPUT REQUIREMENTS AND ADVICE

To obtain the most accurate situational awareness of infection-risk, weather data should be collected from as close to the orchard block as possible. Weather stations should be placed outside the spray zone of the crop so that leaf wetness sensors do not record sprays as wetness. Temperature and relative humidity sensors should be placed within a Stevenson Screen or similar.

The weather-based infection risk tool requires data with 10 minute intervals. Weather data can be input for a whole season with historical data (up to about 20 years), or with selected periods of data.

The format of the "ANFIC" files should be as below. Header text should be in all other columns per standard output (columns 5-9, 12-18) to enable the model to function, but the data cells can be empty.

Column 1	Date (header row); format as 1/01/2016
Column 2	Time (header row); 24 hour format including seconds, 00:00:00
Column 3	Ave AirTemp (degC)
Column 4	Ave Humidity (%)
Column 10	Ave LeafWet (%)
Column 11	Total Rain (mm)

The format of the three "Tiny Tag" files should be as below. If the start time of each set of files is slightly offset, this does not need to be corrected.

Rainfall

	Time	1			
S/N		364335			
Type		Tinytag Plus Rainfall			
Description		styx cherrys			
Property		Rainfall			
1	21/09/2015 16:01	0.0 mm per interval			
2	21/09/2015 16:11	0.0 mm per interval			
3	21/09/2015 16:21	0.0 mm per interval			

Temperature and RH

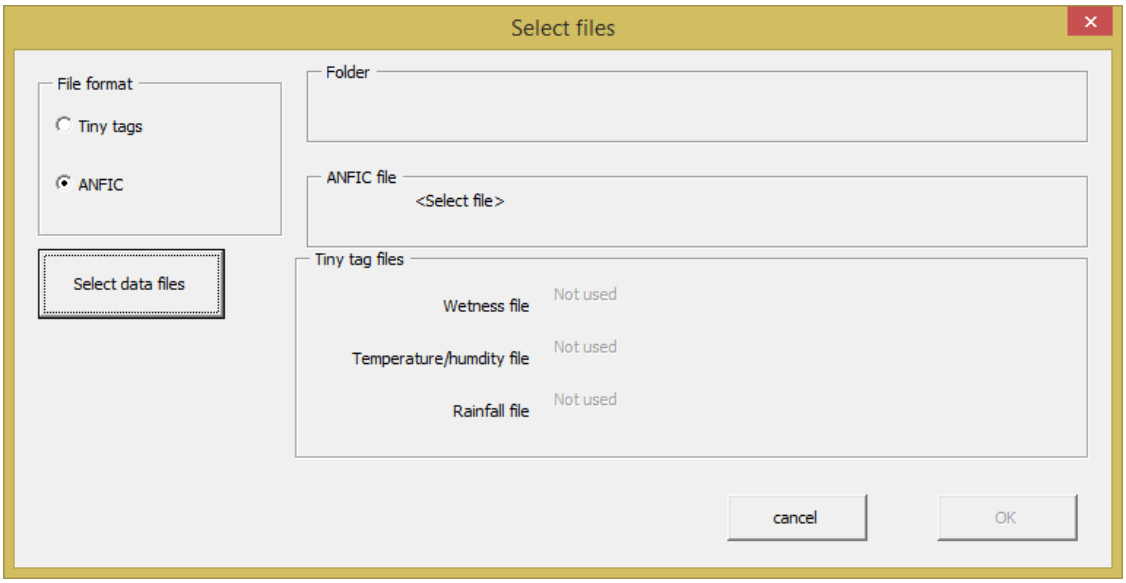
	Time	1	2		
S/N		366119	366119		
Type		TGU-4500	TGU-4500		
Description		Styx cherrys	Styx cherrys		
Property		Temperature	Humidity		
1	21/09/2015 15:57	22.932 °C	40.0 %RH		
2	21/09/2015 16:07	22.700 °C	39.5 %RH		
3	21/09/2015 16:17	22.616 °C	38.1 %RH		

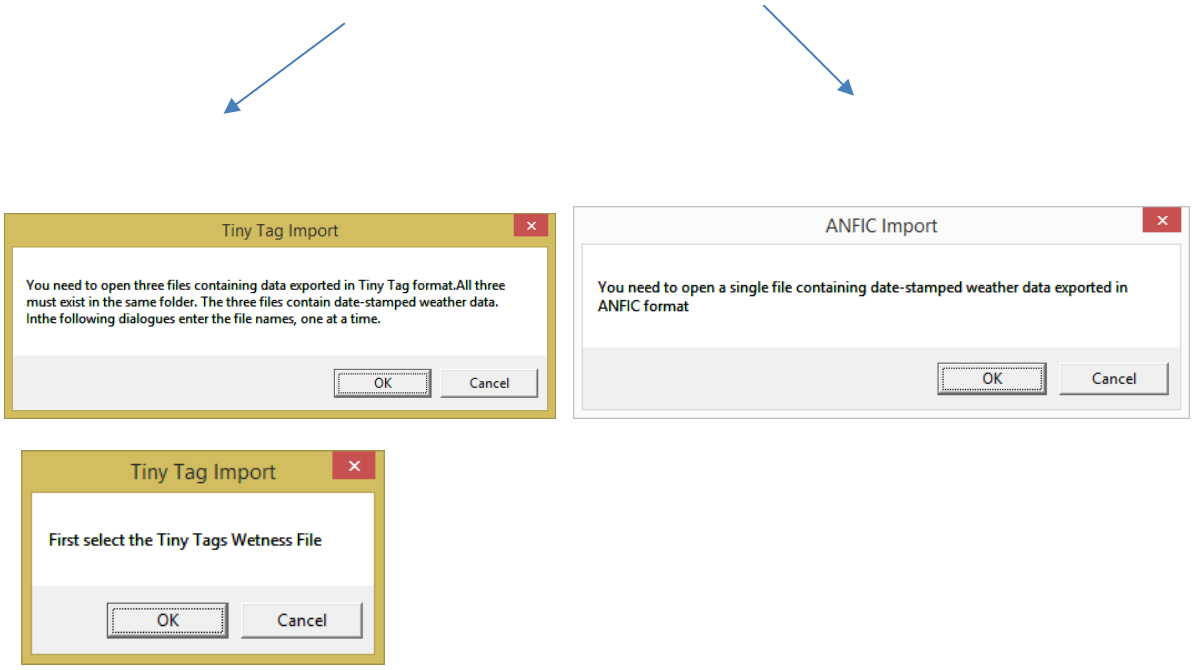
Leaf wetness

	Time	1			
S/N		305669			
Type		Tinytag+ Leaf Wetness			
Description		styx cherrys			
Property		Wetness			
1	21/09/2015 16:00	0.0 %%			
2	21/09/2015 16:10	0.0 %%			
3	21/09/2015 16:20	0.0 %%			

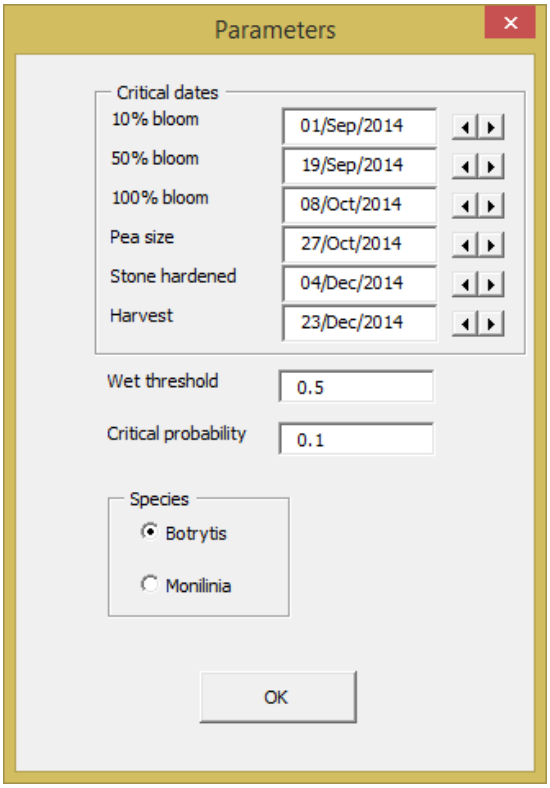
USER STEPS

1. Export weather data to the appropriate format and check for any data errors.
2. Select the file format and then hit “select data files”

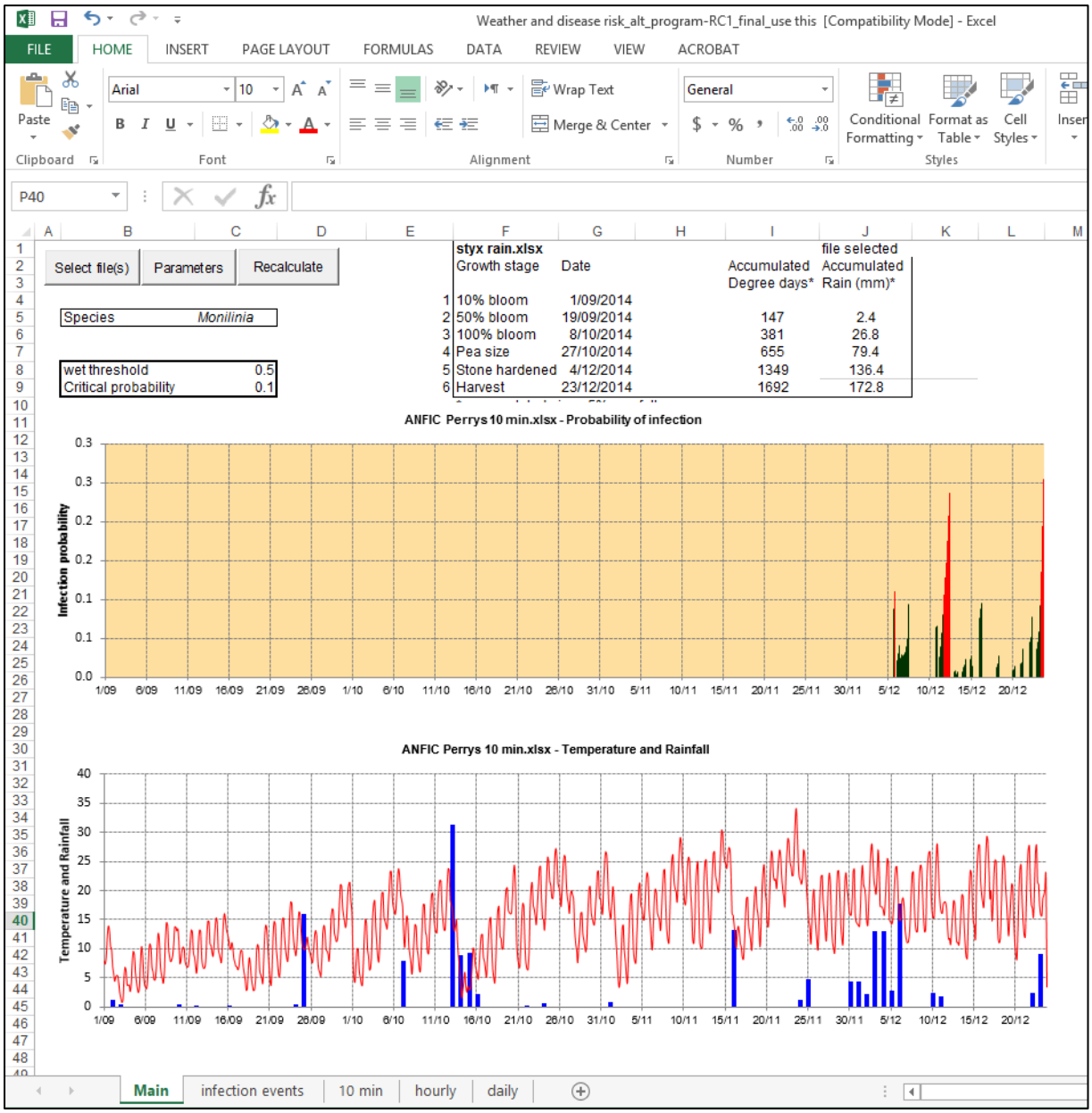




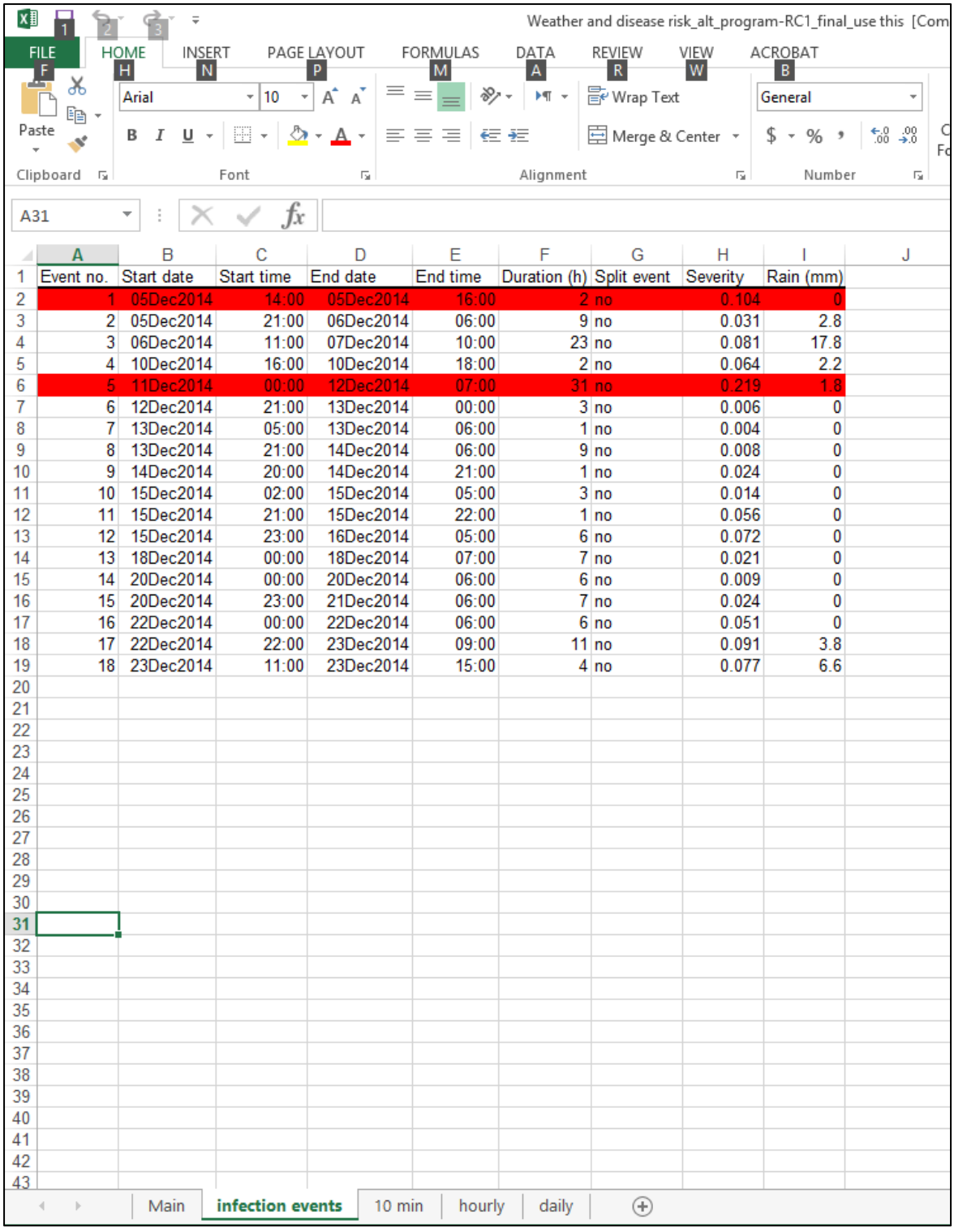
3. Upload files from any drive and folder on your computer.
4. The display on the “main” tab should be refreshed with the data you have selected.
5. Now alter parameters to suit your data – first the critical dates, then species of rot pathogen. Keep wetness threshold at 0.5 and critical probability at 0.1. Close the dialog box.



6. Now hit “recalculate” to refresh the display with the new parameters.



7. Investigate time and duration of infection dates in more detail in the “infection events” tab.



Event no.	Start date	Start time	End date	End time	Duration (h)	Split event	Severity	Rain (mm)
1	05Dec2014	14:00	05Dec2014	16:00	2	no	0.104	0
2	05Dec2014	21:00	06Dec2014	06:00	9	no	0.031	2.8
3	06Dec2014	11:00	07Dec2014	10:00	23	no	0.081	17.8
4	10Dec2014	16:00	10Dec2014	18:00	2	no	0.064	2.2
5	11Dec2014	00:00	12Dec2014	07:00	31	no	0.219	1.8
6	12Dec2014	21:00	13Dec2014	00:00	3	no	0.006	0
7	13Dec2014	05:00	13Dec2014	06:00	1	no	0.004	0
8	13Dec2014	21:00	14Dec2014	06:00	9	no	0.008	0
9	14Dec2014	20:00	14Dec2014	21:00	1	no	0.024	0
10	15Dec2014	02:00	15Dec2014	05:00	3	no	0.014	0
11	15Dec2014	21:00	15Dec2014	22:00	1	no	0.056	0
12	15Dec2014	23:00	16Dec2014	05:00	6	no	0.072	0
13	18Dec2014	00:00	18Dec2014	07:00	7	no	0.021	0
14	20Dec2014	00:00	20Dec2014	06:00	6	no	0.009	0
15	20Dec2014	23:00	21Dec2014	06:00	7	no	0.024	0
16	22Dec2014	00:00	22Dec2014	06:00	6	no	0.051	0
17	22Dec2014	22:00	23Dec2014	09:00	11	no	0.091	3.8
18	23Dec2014	11:00	23Dec2014	15:00	4	no	0.077	6.6

FUTURE USE AND VALIDATION

The adapted tool is a preliminary version which has been developed with limited testing to date. Before wide distribution it will be trialed with selected sweet cherry crop advisors to check for system "bugs" and functionality. The industry will then be made more broadly aware that the tool is ready to trial – this will be via invitation to contact Dr. Karen Barry to request a copy and user guide. This will ensure that user evaluation can be incorporated with any future development of the tool. Future

research will be required to field validate the tool to a standard that is reliable for the industry. However, in the meantime this presents a tool that will support growers to develop "situational awareness" of exactly how weather conditions may relate to infection risk in their orchard.

E. Assessment of inoculum and latent infection levels during the growing season in sweet cherry

INTRODUCTION

Fruit rot pathogens typically cause latent infections during flowering and fruit development, which then present a risk for disease expression at harvest or post-harvest. Previous investigations of latent infection in sweet cherry showed that infections could be detected at high levels early in fruit development and increased over time (Tarbath et al. 2014). That study did not examine floral infection and was only conducted in one orchard. It is assumed that floral infection is a key time for infection and as many fungicides are not registered for use past petal fall, this is a common time for preventative and/or curative spray applications in sweet cherry. Determining whether there are common trends in time of infection across a variety of trials is useful to support (or not) that practice.

Monitoring latent infection levels may be useful as an indication of rot levels at harvest. Presumably, very low levels of latent infection during the season would translate to low levels of visible rot at harvest, and vice versa. However, few studies have demonstrated such relationships, possibly due to the complex range of factors that determine whether latent infection does express as visible rot.

In both NSW and Tasmanian orchard sites, latent infection of key pathogens was measured over time and relationship with visible rot at harvest was assessed. To gauge whether latent levels of infection may have been related to inoculum load, mummified fruit were counted prior to bud burst and spore traps were set up in Tasmanian orchards and a new molecular approach for relative spore load assessment was developed.

STUDY DESIGN AND METHODS

Tasmania

Field trials were established at two orchards for both the 2014-15 and 2015-16 season. Ten trees per variety were tagged in a single row and fungicide was removed from the spray schedule for the entire season (including flowering). Prior to flowering, trees were surveyed for the presence of mummified fruit (mummies) in the canopy from the previous season. The total number of mummies were counted for the whole tree. While mummified fruit were often visible on the orchard floor, these were not included in the count. At the onset of flowering, six branches per tree were tagged and symptoms for browning or blossom blight were assessed. Flowers were sampled and returned to the laboratory for dissection, surface sterilization and incubation to determine the percentage of infection by rot pathogens. As the season progressed, fruit were randomly selected from all the tagged branches of each tree, to result in 40 fruit per tree. This was done at four time points in the season.
For the 40 fruit that were selected, these were returned to the laboratory and frozen overnight (using the "overnight freezing and incubation" protocol, ONFIT) to kill the fruit tissue. Fruit were then surface sterilized and placed into moist containers (8 fruit per container) and incubated in the dark at 20 C for 7-14 days. Incubated fruit were assessed and the presence of common target pathogens was determined based on the morphology of the fungal growth.

A weather station was installed at each site to record hourly temperature, relative humidity and wetness (via a leaf wetness sensor) and also a rainfall gauge. Wind vanes were also installed in the

2015-16 season. A volumetric spore trap (Quest, Melpat International) was installed at each site to operate throughout the season. This was powered by a solar panel to ensure continuous field operation. Spore trap discs were coated with a spray-on Vaseline onto which spores would adhere. The discs were removed every 7 days and replaced. The relative abundance of spores of *B. cinerea*, *M. laxa* and *M. fructicola* was determined with a purpose-designed molecular protocol with species-specific DNA primers, which is not further described here. Results for *M. fructicola* are pending and not shown here.

NSW

Field trials were established at two orchards for the 2014-15 season, and for one orchard in the 2015-16 season. Ten trees were selected as above. Counts of mummified fruit was made prior to flowering, and flower symptoms were recorded as above. The same flower and fruit sampling method (the ONFIT protocol) as above was conducted.

RESULTS

NSW

Studies of latent infection in sweet cherry flowers and fruit revealed that a range of fungal pathogens could be isolated from non-symptomatic samples. In the 2014-15 season *Sclerotinia* sp. was dominant in both flowers and fruit of trees of the orchard at Young and present in the flowers in trees of the orchard at Orange (Figure E1). In the 2015-16 season a change of orchard management occurred and no fungicides were applied. In the orchard at Orange, no *Sclerotinia* was detected, however *Alternaria* was present and *Monilinia* sp. was detected in all fruit stages.

The percentage of fruit with latent infections of *Alternaria* decreased at fruit stage 2 and then increased again at fruit stage 3 in all orchards, varieties and both seasons, except for Sweetheart in the 15-16 season (Table E1). In the latter case, a higher percentage of fruit had latent infections of *Alternaria* in stage 2 fruit than 1 and 3. Although *Alternaria* was dominant at flowering in that case, none of the rotten fruit at harvest time were infected by *Alternaria*.

Table E1. Percentage of cherry flowers and fruit with latent infection by *A. alternata* and visible rot at harvest for fruit in NSW sites. Note that both sites had conventional fungicide programs in 2014-15, but in 2015-16 fungicide was not used at the Orange 1 site.

2014-15	**Fungicide**	**Latent infection (% with *A. alternata*)**				**Harvest rot**	
Variety/Site		*Flowers*	*Fruit stage 1*	*Fruit stage 2*	*Fruit stage 3*	*% rot*	*%rot (A. alternata)*
Lapins (Young 2)	Y	0	4.8	0.5	0.8	0.7	72
Sweetheart (Young 2)	Y	0	0.3	0.3	1.0	6.1	70
2014-15	**Fungicide**	**Latent infection (% with *A. alternata*)**				**Harvest rot**	
Variety/Site		*Flowers*	*Fruit stage 1*	*Fruit stage 2*	*Fruit stage 3*	*% rot*	*%rot (A. alternata)*

Van (Orange 1)	Y	15.0	10.5	0	5.5	0.4	56
Sweetheart (Orange 1)	Y	7.5	8.5	0	8.5	0.1	100
2015-16	**Fungicide**	**Latent infection (% with *A. alternata*)**				**Harvest rot**	
Variety/Site		*Flowers*	*Fruit stage 1*	*Fruit stage 2*	*Fruit stage 3*	*% rot*	*%rot (A. alternata)*
Van (Orange 1)	N	63	13	2	6.5	0.7	0
Sweetheart (Orange 1)	N	62	12	20	12.2	0.6	0

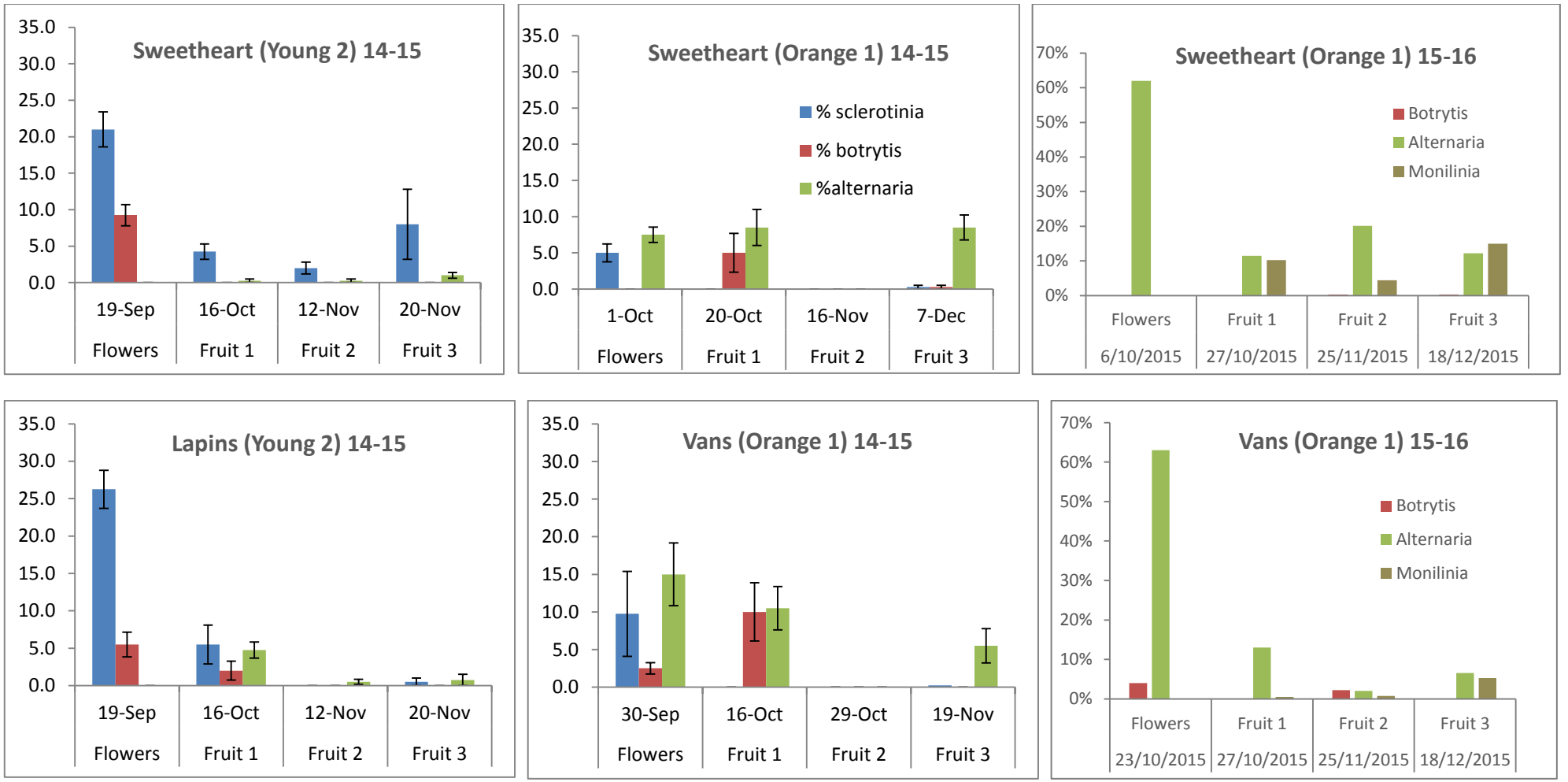


Figure E1. Percentage of flowers and fruit with latent infection caused by the 3 most prevalent pathogens, for two orchards in the 2014-15 season, and one orchard in the 2015-16 season.

Tasmania

Studies of latent infection in sweet cherry flowers and fruit revealed that *B. cinerea* was the dominant pathogen present and therefore abundance of this alone was summed and presented here. Studies in the 2014-15 season revealed considerably less latent infection in fruit of trees with fungicide applied, which would be expected, therefore latent infection was studied in the trees without fungicide only for the 2015-16 season (Table E2). Dissection of flowers revealed that latent infection of *B. cinerea* was present in both petals and styles (Table E2). Trends in percentage of fruit with *B. cinerea* latent infection were highly variable as fruit developed. There were no statistically significant relationships between percentages of latent infection at respective stages with percentage of visible rot at harvest.

Table E2. Percentage of cherry flowers and fruit with *B. cinerea* latent infection and the visible rot at harvest date, for several sites in Southern Tasmania.

Lapins (Sth Tas 4) 2014-15	**Mummies (mean count per tree)**	**Latent infection (% of fruit with *B. cinerea*)**				**Harvest rot**	
		*Stage 1 (27/10)**	*Stage 2 (10/11)*	*Stage 3 (24/11)*	*Stage 4 (8/12)*	*Total incidence (%)*	*Rot due to B. cinerea*
No Fungicide	0	NA	16.3 ±2.4	11.1 ± 1.8	38.7 ± 4.2	3.5 ± 0.9	2.5 ± 0.6
Fungicide	0	NA	2.8 ± 2.2	1.5 ± 1.3	3.4 ± 1.2	0.8 ± 0.2	0.4 ± 0.1
Lapins (Sth Tas 3) 2014-15	**Mummies (mean count per tree)**	**Latent infection (% of fruit with *B. cinerea*)**				**Harvest rot**	
		Stage 1 (3/11)	*Stage 2 (17/11)*	*Stage 3 (1/12)*	*Stage 4 (15/12)*	*Total incidence (%)*	*Rot due to B. cinerea*
No Fungicide	0.6	23.3 ± 4.1	20.3 ± 2.9	23.2 ± 4.0	68.4 ± 6.0	0.7 ± 0.2	0.4 ± 0.1
Fungicide	2.6	7.2 ± 2.2	13.8 ± 3.1	6.5 ± 3.6	20.7 ± 5.0	1.2 ± 0.2	1.1 ± 0.1

Simone (Sth Tas 5) 2015-16	**Mummies (mean count per tree)**	**Latent infection (% with *B. cinerea*)**					**Harvest rot**	
		Flowers	*Stage 1 (3/11)*	*Stage 2 (18/11)*	*Stage 3 (1/12)*	*Stage 4 (14/12)*	*Total incidence (%)*	*Rot due to B. cinerea*
No Fungicide	0.7	25% petals, 7% styles	17	18	37	8	0.81	0.81
Fungicide	3.2	NA	NA	NA	NA	NA	0.31	0.31
Simone and Lapins (Sth Tas 3)	**Mummies (mean count per tree)**	**Latent infection (% with *B. cinerea*)**					**Harvest rot**	
		Flowers	*Stage 1 (10/11)*	*Stage 2 (26/11)*	*Stage 3 (8/12)*	*Stage 4 (6/1)*	*Total incidence (%)*	*Rot due to B. cinerea*
Simone - No Fungicide	8	30% petals, 27% styles	35	36	16.5	17.3	1.8	1.8
Simone - Fungicide	6.2	NA	NA	NA	NA	NA	1.2	1.2
Lapins – No fungicide	28.9	19% petals, 10% styles	17	14	14	44	2.2	2.2
Lapins - fungicide	3.4	NA	NA	NA	NA	NA	0.3	0.3

NA, not assessed; * date of sampling

Figure E2. Pre-trial preparation to allocate fungicide-free rows and counting mummified fruit prior to flowering, in a sweet cherry orchard (Sth Tas 5) during the 2015-16 season.

The molecular detection methods developed were able to quantify relative "spore load" for the selected target species from the spore traps discs (Figure E3 – E6). Data shows that both *B. cinerea* and *M. laxa* were present as airborne spores in all orchards where the spore trap was positioned. In the 2015-16 season, spore traps were placed into the field approx. 3-4 weeks earlier than in 2014-15 and it was demonstrated that spores were present from early in October, around the time of flowering (Figures E5-6).

Given quantification of each pathogen species was determined against a species-specific standard curve, the scales of spore load for each pathogen cannot be compared to each other. However, comparison of daily trends in spore abundance reveal that apart from Sth Tas 4 (Figure E3), there were similar trends in spore release for both *B. cinerea* and *M. laxa* for the other orchards (Figures E4-6).

Figure E3. Left: A Quest spore trap within sweet cherry orchard (Sth Tas 5) during the 2015-16 season. Right: Mummified fruit remaining within a tree after flowering.

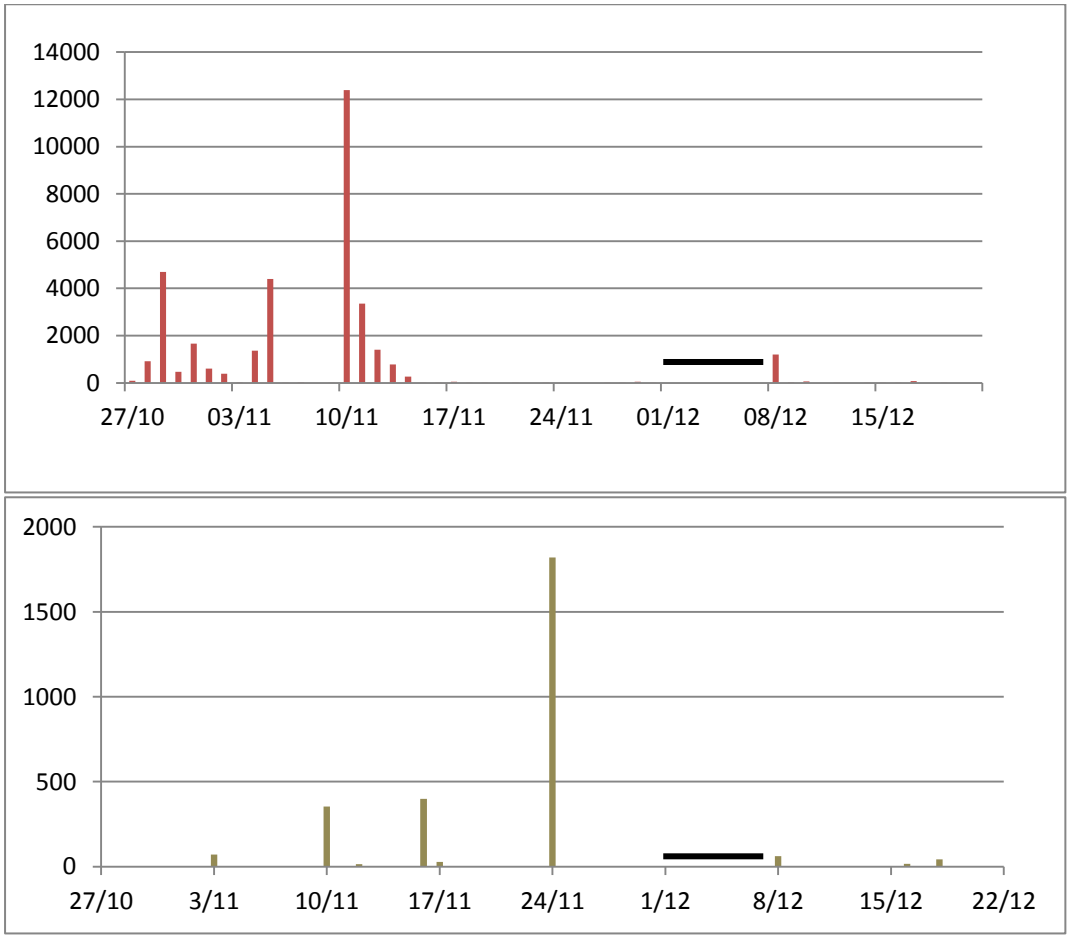


Figure E4. Relative spore load (unitless) based on spore trap samples from an orchard (Sth Tas 4) in the 2014-15 season. Upper chart = *B. cinerea*, lower chart = *M. laxa*.

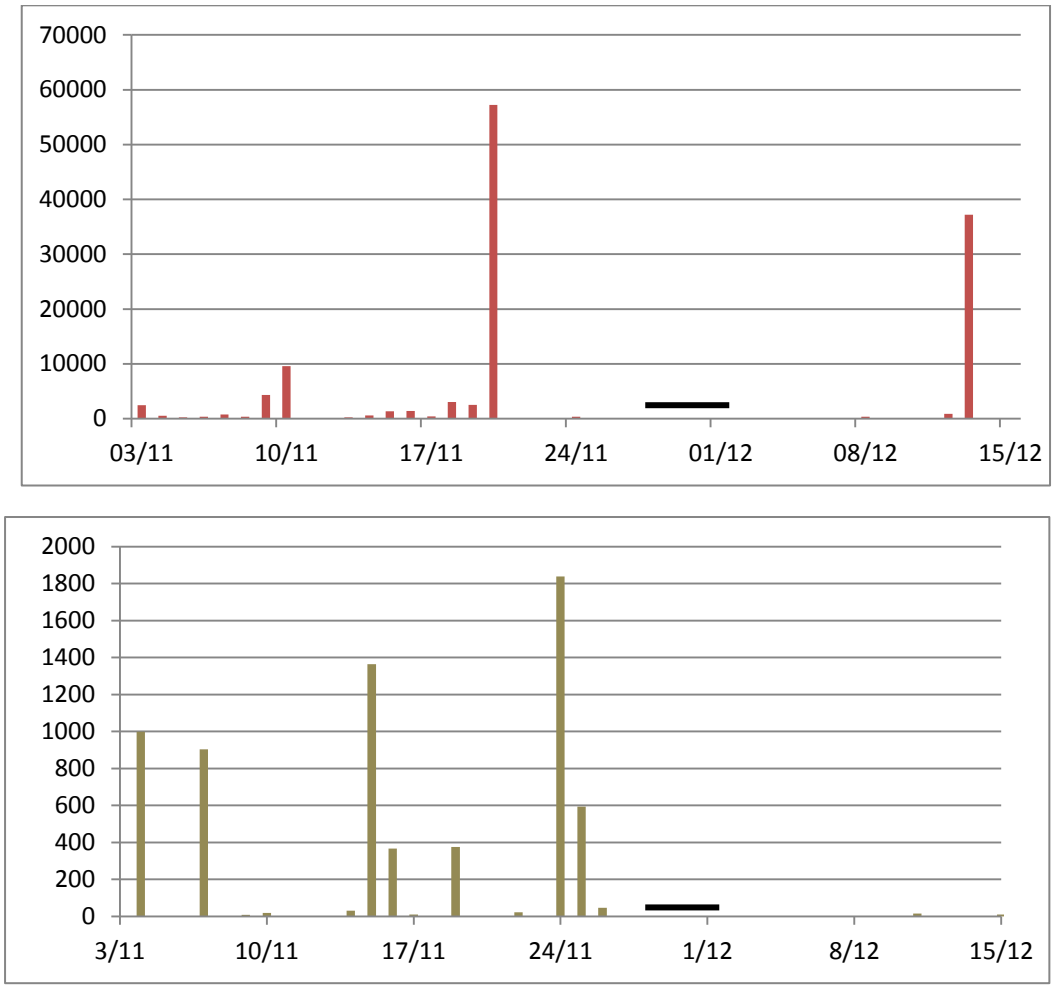


Figure E5. Relative spore load (unitless) based on spore trap samples from an orchard (Sth Tas 3) in the 2014-15 season. Upper chart = *B. cinerea*, lower chart = *M. laxa*. Black line = missing data.

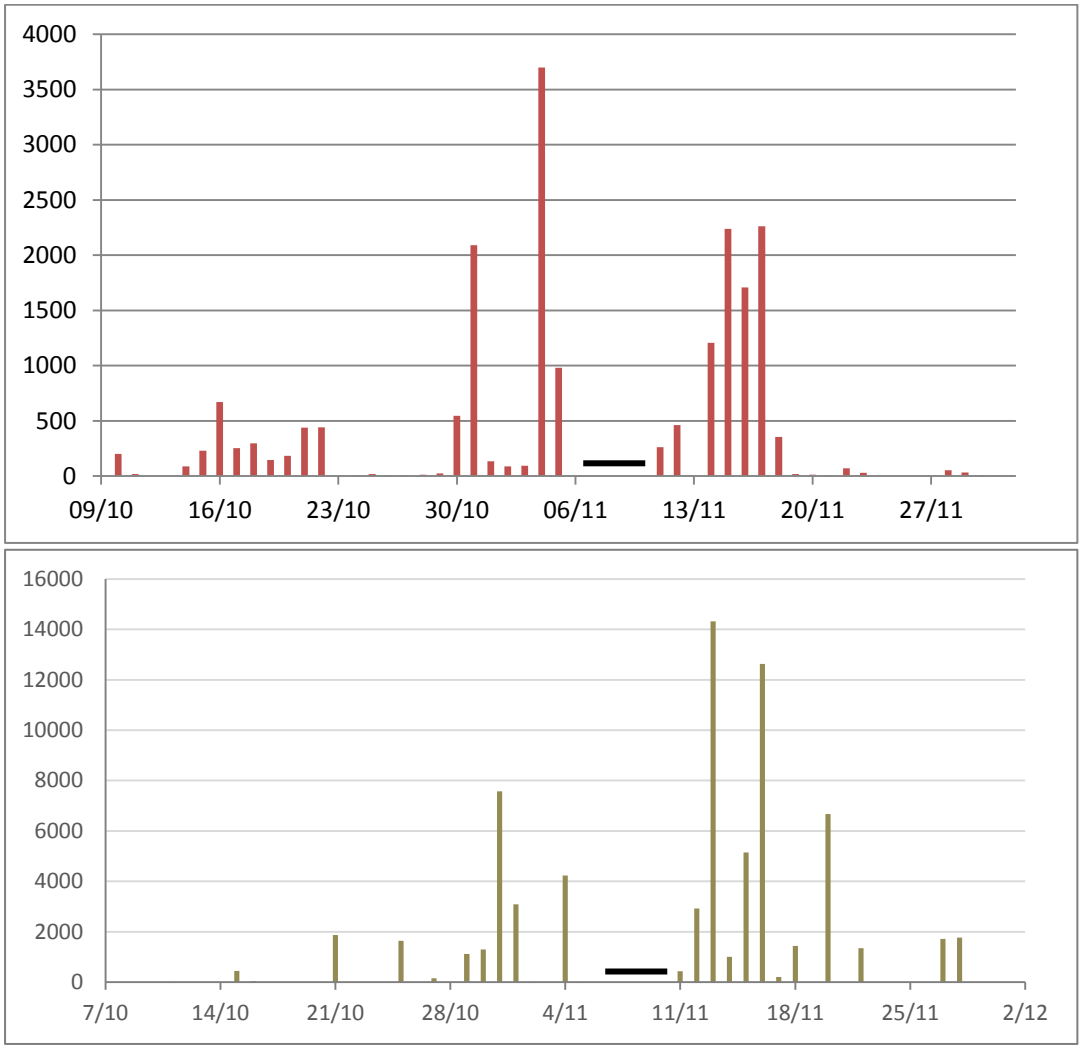


Figure E6. Relative spore load (unitless) based on spore trap samples from an orchard (Sth Tas 3) in the 2015-16 season. Upper chart = *B. cinerea*, lower chart = *M. laxa*. Black line = missing data.

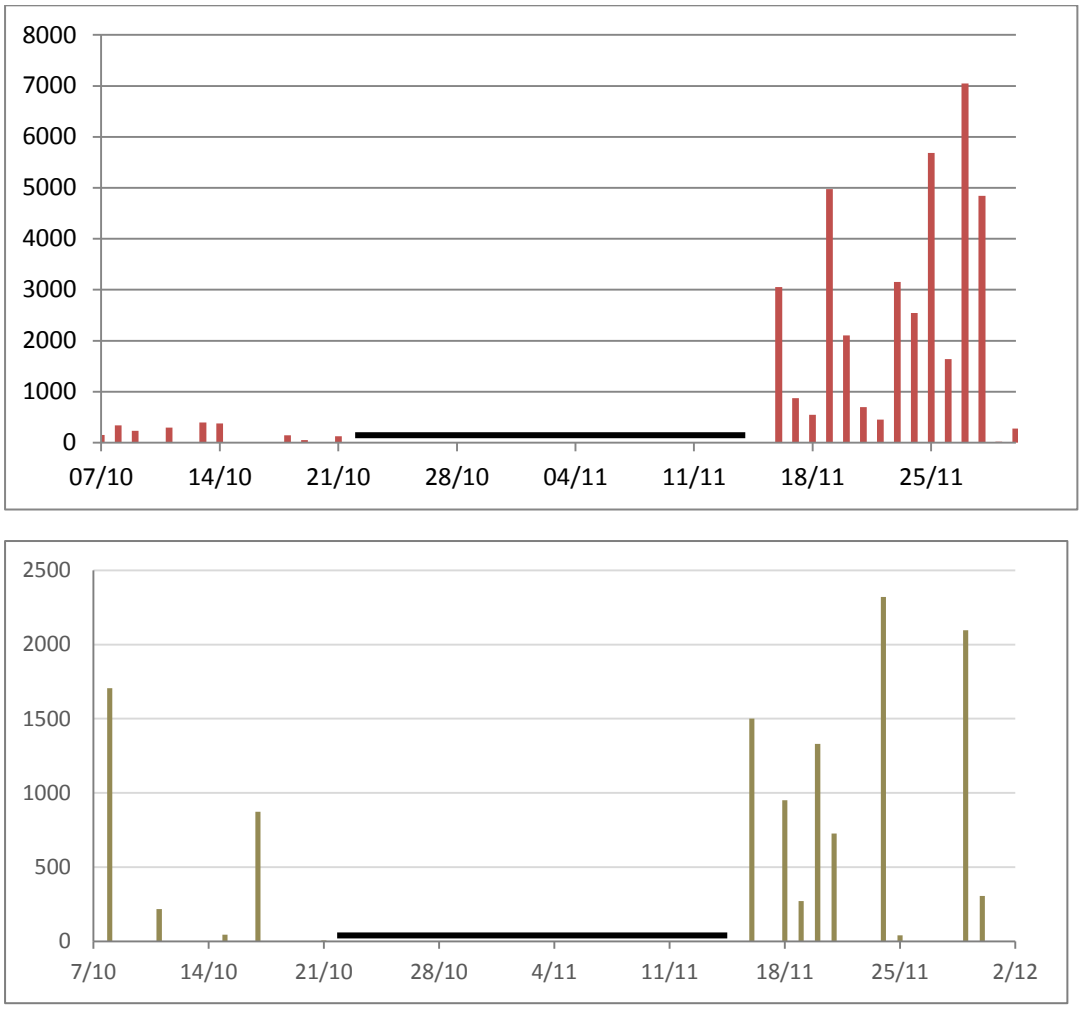


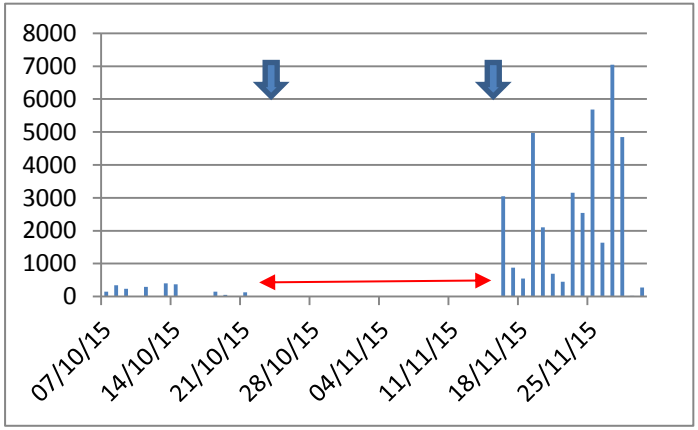
Figure E7. Relative spore load (unitless) based on spore trap samples from an orchard (Sth Tas 5) in the 2015-16 season. Upper chart = *B. cinerea*, lower chart = *M. laxa*. Black line = missing data.

The latent infection, relative spore load, weather data and highest infection risk periods were compiled for two sites (over page) in order to cross-reference findings. In case study A, latent infection of *B. cinerea* was low for stage 1 and 2 fruit and then increased substantially in stage 3. This may relate to timing of rainfall in the presence of spore load. The lower levels of latent infection in stage 4 fruit could be related to drop of infected fruit towards the harvest date. For the Simone cultivar in case study B, the levels of latent infection decreased by approx. half for the stage 3 and 4 fruit compared to stage 1 and 2. The stage 3 and 4 fruit sampling time related to less rainfall and much lower relative spore load in the air, which may explain why latent infections did not continue to rise. However, the opposite trends was true for the Lapins cultivar.

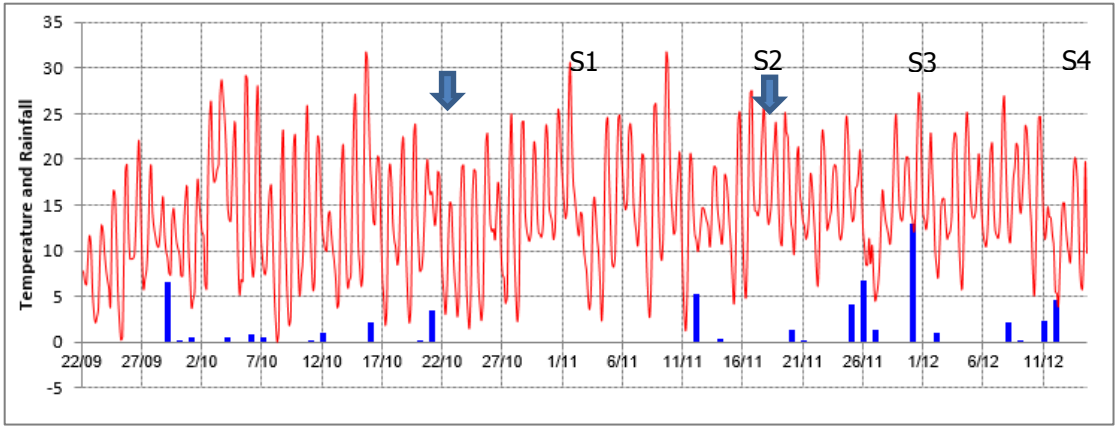
Case study A: Season 2015-16, Site 5, Tasmania, Cultivar Simone

Incidence of mummies, presence of Botrytis cinerea as fruit develop, and total rot at harvest date.

Fungicide	**Mummies (mean count per tree)**	**Latent infection (% with *B. cinerea*)**					**Harvest rot**	
		Flowers	*Stage 1 (3/11)*	*Stage 2 (18/11)*	*Stage 3 (1/12)*	*Stage 4 (14/12)*	*Total incidence (%)*	*Rot due to B. cinerea*
No Fungicide	0.7	25% petals, 7% styles	17	18	37	8	0.81	0.81
Fungicide	3.2	NA	NA	NA	NA	NA	0.31	0.31



Botrytis cinerea spore abundance over time, as determined by species-specific qPCR. Values are relative and unitless. Red arrow denotes missing data. Blue arrows denote highest infection risk based on weather-based tool.

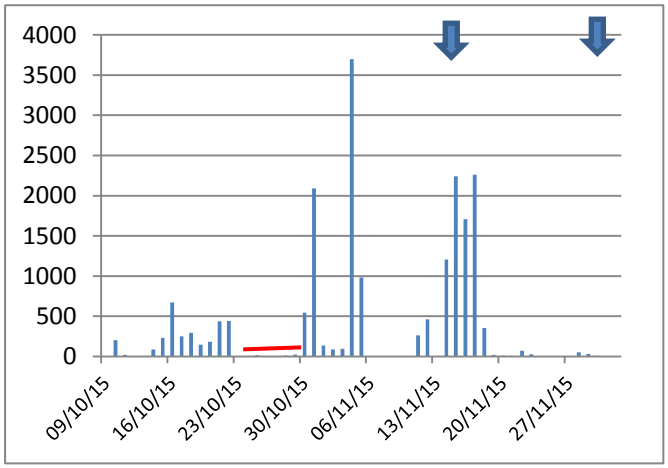


Temperature (10 min intervals) and total daily rainfall data for the site, with fruit sampling dates of each fruit stage. Blue arrows denote highest infection risk based on weather-based tool.

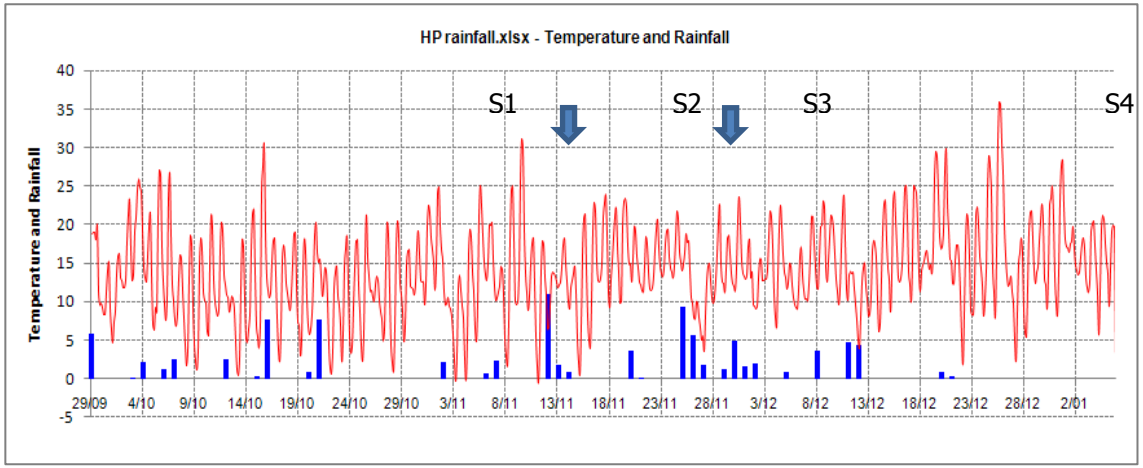
Case study B: Season 2015-16, Site 3, Tasmania, Cultivar Simone and Lapins

Incidence of mummies, presence of Botrytis cinerea as fruit develop, and total rot at harvest date.

Fungicide	**Mummies (mean count per tree)**	**Brown flowers (%)**	**Latent infection (% with *B. cinerea*)**					**Harvest rot**	
			Flowers	*Stage 1 (10/11)*	*Stage 2 (26/11)*	*Stage 3 (8/12)*	*Stage 4 (6/1)*	*Total incidence (%)*	*Rot due to B. cinerea*
Simone - No Fungicide	8	0	30% petals, 27% styles	35	36	16.5	17.3	1.8	100%
Simone - Fungicide	6.2	0	NA	NA	NA	NA	NA	1.2	100%
Lapins – No fungicide	28.9	0	19% petals, 10% styles	17	14	14	44	2.2	100%
Lapins - fungicide	3.4	0	NA	NA	NA	NA	NA	0.3	100%



Botrytis cinerea spore abundance over time, as determined by species-specific qPCR. Red line denotes missing data. Blue arrows denote high infection risk based on weather-based tool.



Temperature (10 min intervals) and total daily rainfall data for the site, with fruit sampling dates of each fruit stage.

DISCUSSION

This study revealed that latent infection of several pathogens occurred from the flowering stage in all sweet cherry orchards and that it was considerably lower when a fungicide program was in place. *B. cinerea* was the dominant pathogen in the Tasmanian samples, as previously found by Tarbath et al (2014) and this is clearly linked to the dominance of *B. cinerea* as the cause of rotten fruit at harvest. Latent infection generally increased over time as fruit developed, but not always, and there was no clear relationship between amount of latent infection at any stage of flower or fruit development and the expression of rot at harvest. Latent infection due to *A. alternata* in fruit from NSW orchards was much lower than latent infection due to *B. cinerea* and again there were no clear trends between amount of latent infection and that found as visible rot at harvest date.

While the amount of latent infection could not be related to visible rot at harvest, it would be worthwhile to explore whether pre-harvest latent infection levels could predict rot found post-harvest. High latent infection "loads" may enable assessment of rot risk for fruit that requires prolonged storage and shipping (Dugan and Roberts, 1998). What is not clear is the mechanism by which latent infection develops in to visible rot. In stone fruit such as apricot, volatiles of ripening fruit have been implicated as having a major role in the activation of latent infections (Cruickshank and Wade, 1992). Cracking has been associated with rot in harvest fruit (see section A) and non-visible cuticular fractures may also promote rot development (Borve et al. 2000). While fruit maturity is likely to be a major factor, ways to predict this would be valuable in future studies.

Monitoring and quantification of *B. cinerea* and *M. laxa* spores in the Tasmanian orchards revealed that spores were detectable by the time of flowering and spore load was variable across the season. Rather than constant detection of spores at similar levels every day, spore load was variable between negligible and extremely high. While data at this stage is shown as relative, further analysis will convert this data to an estimate of spore numbers. However, the trends demonstrate that given spore load varies dramatically, weather conditions conducive to infection cannot be considered alone to determine the amount of infection that occurs, as in the absence of spores the risk will be nil.

In summary these studies have revealed the dynamic nature of spore release by rot pathogens and the presence of latent infection throughout the season.

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