Final Report

Internal fruit rot of capsicum

Project leader:

Dr Jenny Ekman

Report authors:

Ryan Hall

Dr Naomi Diplock

Dr Jenny Ekman

Delivery partner:

Applied Horticulture Research

Project code:

VG17012

Project:

Internal fruit rot of capsicum (VG17012)

Disclaimer:

Funding statement:

This project has been funded by Hort Innovation, using the vegetable research and development levy and contributions from the Australian Government. Hort Innovation is the grower-owned, not-for-profit research and development corporation for Australian horticulture.

Publishing details:

Published and distributed by: Hort Innovation

Level 7
141 Walker Street
North Sydney NSW 2060

Telephone: (02) 8295 2300

www.horticulture.com.au

Contents

Public summary

Internal fruit rot (IFR) of capsicums is a major issue for Australian growers. While it can occur any time, significant outbreaks are most likely to occur under warm, humid conditions – such as Central Queensland during spring, Southern Queensland during summer and autumn, Carnarvon in Western Australia during spring and Sydney during late summer. It causes economic losses, impacts marketability, and risks consumer confidence. Prior to this project, the disease's causal organisms were not well understood in Australian field-grown capsicums, creating a need for local research to identify the causes and develop effective management strategies.

This project involved several key activities to tackle IFR in capsicums. A comprehensive literature review was conducted to summarize the current global knowledge on the causal organisms of IFR. We consulted with local growers, industry representatives, and experts to understand the extent of the issue in Australia. Field and laboratory studies were performed to identify the pathogens responsible for IFR. These included collecting fruit samples, isolating organisms, and running pathogenicity trials to confirm which fungi cause IFR. These showed that *Alternaria alternata* is the most common pathogen causing IFR in Australia. However, various *Fusarium* spp. were also found to cause disease. The fact that symptoms may be caused by multiple different fungi adds to the complexity of controlling this disease.

The mechanism of infection has also been investigated. We have confirmed that infection usually occurs during flowering. We have also shown that infection can occur post-pollination, as the young fruit starts to swell. However, infection through the stigma is unlikely once fruit have set and started to expand, the stigma becoming dry and unreceptive.

Despite infection at flowering, symptoms primarily develop once capsicums turn red. IFR is rarely found in green fruit, detected occasionally in “chocolate” fruit, more frequent in largely red fruit, and most common in full red capsicums. This suggests that natural defensive compounds within the capsicum plant, which degrade as fruit mature, are key to symptom development.

We monitored seasonal changes and conducted variety trials to examine differences in susceptibility. No differences were found between any of the “blocky” and “semi-long” varieties commonly grown in open field production. There was also little effect of rain, temperature or RH. A small but significant correlation was found with wind, higher wind speeds corresponding with lower rates of IFR. Unfortunately, trials testing strategies to increase air circulation during flowering (e.g. plant spacing) were inconclusive.

Trials were conducted with a large range of chemical and biological treatments to evaluate their effectiveness in reducing IFR. A trend to lower rates of IFR was found when flowers were pre-inoculated with Trichoderma. In general, however, none of the fungicides or other biological products tested, alone or in combination, significantly reduced development of IFR in red fruit.

An explanation for this result may be the poor coverage of flowers achieved using commercial spray rigs. Standard boom sprays delivered average coverage of 32% or less. Coverage was significantly improved using an air blast system. However, issues remained, with a major decline in effectiveness from the tractor side of the boom to the outer edge. This reduced coverage from over 60% to 20%, presumably due to drops in pressure. As most fungicides have limited motility within plants, poor coverage of the floral stigma using standard spray units may explain lack of effectiveness against IFR.

This work has provided a better understanding of the main causal organisms of IFR in field-grown capsicums. The project also highlighted the limited effectiveness of chemical and biological treatments currently used, suggesting a need for optimising spray rig operation. Key outputs have been shared through the Soil Wealth Integrated Crop Protection website and can also be accessed through industry publications like Vegetables Australia.

Keywords

Capsicum; disease; *Alternaria alternata*; *Fusarium* spp.; internal fruit rot; bell pepper

Introduction

Internal fruit rot of capsicums (IFR) is a major ongoing issue for Australian growers, especially those in warm, humid growing areas such as the central Burnett region (Central Queensland) and granite belt (Southern Queensland). It has been known to affect greenhouse-grown capsicums, especially if humidity is not well controlled. Generally, the disease affects the seed and placenta inside the fruit, however it can also occur on the blossom end of the endocarp. There are often no external symptoms. The presence of disease only evident when the capsicum is cut open. Although most of the flesh remains edible, there is little tolerance for IFR by retailers and consumers. Moreover, if infection is severe, disease can spread into the edible part of the capsicum.

Infection is believed to occur at flowering. However, the disease usually does not develop until the fruit matures and ripens. As the disease does not show external symptoms at harvest, infected fruit can be packed and sent to market. This can lead to affected consignments being downgraded or destroyed. Growers may even be (temporarily) suspended from the market, losing market share.

Prior to the commissioning of this project there had been major outbreaks of internal fruit rot in capsicums in various regions across Australia, especially in the Bundaberg region. Limited pathology testing occurred suggesting the causal organism was an *Alternaria* sp., leading to a factsheet being developed as part of the VG13078 Integrated Crop Protection project.

The disease is present in international literature, noting that several fungal species have been identified as responsible since at least 1978. Over time, with improving DNA analytics, fungal species have been changed. This has resulted in some species discussed in previous literature changing name. Organisms included *Fusarium* species most notably those from the *Fusarium lactis* species complex (FLASC) (previously *Fusarium subglutinans*), but also *F. proliferatum*, *F. oxysporum* and *F. Solani* have been isolated from infected fruit and confirmed to be causing the disease. It should be noted that F*usarium* causal organism research has mostly been from greenhouse capsicum production. *Alternaria* spp. have been identified as causual organism in field crops from Hungary and Isreal. Most evidence from the literature points towards *Alternaria alternata* as the main *Alternaria* causal organism.

Other organisms have been reported to have been isolated from symptomatic fruit, these include *Botrytis cinerea* and anthracnose (*Colletotrichum* spp.). However, these isolations are few and no pathogenicity trials were conducted.

Despite research from overseas, no formal work had been conducted in Australia. Current IFR research focused almost exclusively on the FLASC, which is yet to be identified in Australia. This research and its associated outcomes also focused entirely on greenhouse capsicum production. While some greenhouse production occurs in Australia, the main areas where the disease is an issue are field capsicum production.

With limited research, a disease that is difficult to identify without destructive sampling, a low tolerance from retailers and rejection from the consumer, capsicum growers afflicted by this disease run the risk of severe impacts of their marketability. Infected fruit making its way to consumers can also negatively affect consumer attitude towards the fruit and decrease consumer decisions to purchase.

Methodology

1. Review the causes of internal rot in capsicums

Literature review

Peer reviewed publications, grey literature and industry reports were reviewed in a detailed document split into two halves. The first section summarises what is known of the causal organisms, mode of infection, environmental factors and control strategies. The second section includes a more detailed technical discussion of the science behind studies of IFR, including a comprehensive listing of all organisms associated with IFR symptoms, vectors of disease, potential toxicology and more.

Industry consultation

Project team members engaged with local grower groups, industry groups, international experts, wholesalers and experts. Growers in Bundaberg, Bowen, Stanthorpe, the Sydney Basin, and Virginia in South Australia were all engaged with the project. Consultation focused on severity, timing and conditions leading to the disease. It also sought input from stakeholders around causes of the disease.

2. Field and laboratory studies to identify the causal organisms – Survey and isolation

Disease Surveys

The objective of this task was to determine whether a single organism was responsible for IFR, or multiple pathogens were causing the disease symptoms. To obtain a wide range of fruit, notices were placed in the AUSVEG weekly update, Protected Cropping Australia magazine and Vegetables Australia asking for samples of capsicums and chillies with internal rot. The target audience were growers and market suppliers (wholesalers).

Fruit samples were obtained from a range of producers and wholesalers, sourced from a wide geographical range including Bundaberg, Bowen, Sydney and Stanthorpe. No samples were obtained from Adelaide, where IFR is uncommon. In-person sampling in Queensland, the Sydney basin and South Australia supplemented the request for samples. Sampling included field visits to assess incidence of IFR in mature fruit, as well as packhouse visits to assess incidence of IFR in the pack shed (both from reject bins and marketable fruit).

Fruit were examined for signs of fungal growth on the seed and placenta. If no fungi were visible, samples were taken of a seed, part of the placenta and the apex endocarp. Samples were plated onto ¼ PDA with 100 ppm Novobiocin.

Flowers were collected and plated onto ¼ potato dextrose agar (PDA) with 100 ppm Novobiocin to suppress bacterial growth. Three anthers, two petals, the style and the carpel were plated separately for each flower collected. Fruit infections were examined using the same media, with sections of the endocarp wall, a seed, part of the endocarp apex and the style were sectioned onto plates.

Isolation of casual organisms and pathogenicity trials

Organisms isolated from infected capsicums were singled spored onto potato carrot agar (PCA) for *Alternaria* spp., or water agar (WA) or carnation leaf agar (CLA) for *Fusarium* spp. Single spored colonies were compared with descriptions provided by Leslie and Summerell (2007) for *Fusarium* spp. and Simmons (2007) for *Alternaria* spp. Based on morphological characteristics two *Alternaria* spp. and two *Fusarium* spp. were chosen to act as inoculant for the pathogenicity trials.

Capsicum plants California Wonder (red), SV6947PB (red), Warlock (red) and SVPB8193 (yellow) were grown in greenhouses at the University of Sydney and NSW Department of Primary Industries Ourimbah. These plants were used for Koch's postulates. Inoculation occurred over five occasions, with inoculum being applied by applying a suspended spore mixture with micropipettes to flowers. Plants were covered with plastic bags for 48 hours to increase relative humidity around the flowers.

Fruit were grown until maturity, surface sterilised and plated onto ¼ PDA with 100 ppm Novobiocin. Where no fungi was visibly present, samples of the seed, placenta and endocarp apex were taken.

Where fungus was isolated, DNA samples were taken to compare with the original inoculum. During the trials DNA analysis of these species were conducted, excluding the second *Fusarium* spp. as no infection was recorded from inoculated fruit.

DNA was extracted using a DNeasy Plant Mini Kit (Qiagen Inc). Polymerase chain reaction (PCR) was used to amplify the plasma membrane ATPase gene region, allowing identification of the organisms to strain.

3. Field and laboratory studies to identify the causal organisms – Timing of infection

Infection was already widely believed to occur during flowering, remaining dormant in the ovaries until the fruit matures and ripens. In some fruit, fungal growth was visible at the apex of the endocarp, or on the remnant style remaining attached to the fruit apex (Figure 1). The project team hypothesised that the remnant style could provide a potential entry point for the causal pathogens.

Figure 1: A remnant style present on a ripe fruit. This example shows the necrotic tissue that could provide an entry of fungus into the internal parts of the fruit

Styles from ripe fruit were therefore plated onto ¼ PDA with novobiocin following two laboratory experiments conducted with ripe fruit.

Trial 1 – Inoculation of styles on mature fruit

Capsicums were inoculated with a water control or with *Alternaria alternata* inoculum (identified in the pathogenicity trials). Fruit were sealed in plastic bags for 24 hours to increase humidity and encourage infection, then at room temperature until assessment. Fruit were cut and, where no fungi was visibly present, samples of the seed, placenta and endocarp apex were taken and plated onto ¼ PDA with lactic acid.

Trial 2 – Re-inoculation of styles on mature fruit

Adjustments were made to the initial method to include a pre-soak of the styles in de-ionised water to reduce hydrophobicity during inoculation. Fruit were kept at a higher temperature for 48 hours after inoculation to improve conditions for fungal growth. Fruit was then left at room temperature until visible assessment. Fruit were cut and where no fungi was visibly present, samples of the seed, placenta and endocarp apex were taken and plated onto ¼ PDA with lactic acid.

Trial 3 – Inoculation of floral stigmas and styles retained on developing fruit

A pot trial conducted in the NSW DPI Ourimbah greenhouses examined the incidence of IFR based on the timing of inoculation of the *A. alternata* spores onto the floral stigma and style. Flowers, small fruit (<20mm diameter) and large fruit styles were inoculated with a *Alternaria alternata* inoculum. Inoculation of water was used as a control. Fruit were allowed to develop to mature red then cut and examined for symptoms of IFR. Fruit were plated onto ¼ PDA to confirm *A. alternata* presence.

4. Examine differences in varietal susceptibility

Variety Trial Richmond

A fully replicated trial was planted on 17th November 2020 at the Local Land Services Richmond trial site. Six commonly grown commercial varieties (SV6947, Aries, Ducati, Shadowfax, Warlock, and Red Jewel) were planted in a randomised block design. Flowers were spray inoculated with mould spores three times during crop development, using a mixture of *A. alternata* and *F. oxysporum*.

Variety Trials Stanthorpe

Two trials were planted in Stanthorpe with the generous assistance of Bayer-Seminis. All were red varieties, suited to the region and selected so as to include blocky as well as semi-long types. The varieties Halifax and Shadowfax were included as initial data suggested these new varieties could have some resistance. One tray (approximately 198 plants) of each variety were planted within commercial capsicum crops:

Trial 1	Trial 2
SV3255	SV3255
SV9699	SV9699
Plato	Plato
Shadowfax	Shadowfax
Halifax	Halifax
Aries	Hugo
Lapillo	Warlock
Maximinis	SV6947

The first trial was harvested on 8th February 2021. As each variety was planted in a single row rather than randomized blocks, some 'replication' was created by picking fruit in six blocks of 25 fruit (total 150 fruit) and assessing each batch separately.

Each capsicum was cut in half equatorially and examined for signs of IFR or dark seed. The severity of infection was not recorded as even a tiny lesion could develop during postharvest handling and result in retail rejection. Similarly, any discolouration of the seeds was recorded even though symptoms ranged from light to dark brown and were expressed over a small part of the placenta, the entire placenta, or only on the outer edges of the seeds themselves.

The second trial was harvested one month later on 10th March. In this case, fruit were picked in four batches of thirty fruit (total 120), each batch including 10 red, 10 part coloured and 10 full green fruit. This was to examine how early IFR / dark seed occurred during fruit development, and whether IFR correlated with dark seed.

5. Develop a model for predicting infection risk and protocols for early diagnosis

Environmental model development

Environmental effects are likely a factor in IFR incidence. Reports indicate that some regions such as around Bundaberg are more severely affected when compared to other regions such as Viriginia SA, which reports almost no incidence of the disease.

During the development of the infection risk model, various environmental variables were considered and tested. Information on disease incidence and timing of flowering was provided by wholesalers and growers, and this data was then compared with records from The Bureau of Meteorology. Insights from this analysis were then used to design trials to explore potential impact of environmental conditions.

Climatic data was collected from each trial location using Bureau of Meteorology records and field trial data where available. Climatic variables considered included total precipitation, air temperature, dew point temperature, relative humidity, average wind speed, and maximum three-second wind gusts. Weekly analyses of these variables during the flowering period were conducted to identify specific conditions that

might affect infection rates. Factors evaluated included:

- Hours of average wind speed below 5 km/h
- Hours of wind speeds greater than 15 km/h
- Hours of wind gusts exceeding 22 km/h
- Hours with temperatures within 2°C of the dew point (indicating potential leaf wetness)
- Hours with relative humidity above 95%
- Hours with relative humidity between 85% and 95%
- Total precipitation

Pearson correlation coefficients were calculated for each climatic variable against the observed infection rates, focusing on the weeks from 14 to 8 weeks prior to harvest. This allowed for identification of key climatic factors that could be linked to infection risk during the flowering period.

Evaluating the effect of increasing or decreasing air movement on IFR

A trial was conducted at the Local Land Services Richmond site to evaluate the effect of air movement on IFR. Five double rows of two blocky capsicum varieties were planted in December 2023. To increase air movement within one section of the crop, 24 solar-powered fans were installed within the crop. Fans were linked with a battery, providing 24 hour power. In two other sections windbreaks were installed to reduce air movement. As there were more than enough plants remaining to use as controls an additional treatment was added; increasing N fertilization as high N is associated with reduced uptake of calcium, so may increase risk of IFR.

Once fruit matured, weekly inspections were conducted examining fruit for signs of IFR.

Figure 2. Fans, together with a solar power supply and backup battery ,were installed within the crop (left). Fans were covered using small pieces of tarpaulin to protect them from rain and irrigation. In two other sections of the crop, blocks of capsicums were surrounded with windbreak material, reducing air movement around the plants (right).

Evaluation of wind exposure and speed on IFR

A replicated field trial was conducted at a commercial farm to evaluate the relationship between wind speed / air movement and the incidence of IFR. Anemometers and Hobo data loggers were installed at the edge row and centres of a block of the capsicums. Three randomly selected sections within the edge and centre sections were used to increase air movement further. This was done through removing every second plant. Plants were staked to ensure they did not lodge due to removal of support from neighbouring plants. In total, 2,248 red fruit were assessed across three harvest dates for symptoms of IFR.

6. Developing effective on-farm IDM – Chemical and biological sprays

Varied treatments were applied at a number of locations, including both commercial capsicum farms and a dedicated experimental field site, to test the effectiveness of chemical and biological controls. Products were

chosen based on known or expected effectiveness against the causal organisms of IFR. Methodologies varied between trials depending on capabilities of growers and staff at each location.

Trial 1 – Local Land Services site, Richmond

Planting was staggered over three dates at approximately 6 weeks= intervals. The first two plantings consisted of single rows in two separate blocks. These plants were used to model the effect of temperature and RH on IFR development, examine the effect of fruit maturity on symptom development, and test whether cold storage could reduce expression postharvest.

The final, larger planting was used to test a range of different fungicides, together with chelated calcium, and including products not currently registered for capsicums. Chemical fungicides were selected based on effectiveness against *Alternaria* and/or *Fusarium* in other crop situations. We were also provided wth three experimental products developed by Bayer. All products were applied at label rates.

Standard fungicides

1. Untreated control
2. Amistar + Miravis Duo (Group 11 and Group 3 + 7)
3. Switch (Group 9 + 12)
4. Amistar + Score + copper (Group 11 and Group 3 and protective)
5. Chelated calcium + Serenade Opti + Switch + Miravis Duo (Group 9 + 12 and Group 3 + 7

Experimental products

a. 202

b. 203

c. 204

Flowers from the first two plantings were tagged to determine the time between flowering and fruit maturation. Once red fruit started to develop they were assessed weekly for symptoms of IFR. Additional fruit were stored at either 5^{o}C or ambient for one week or 3-4 days respectively before assessment.

Trial 2 – Bundaberg trial site

This trial was conducted by Agreco Australia, with assessments conducted and report prepared by Chris Themsen and Dale Parker.

Capsicums were planted on 7 October 2022 at a rate of 30,000 plants/ha using a randomized block design. Each treatment unit was a 10m long unit planted with double rows. Irrigation was provided using sub-surface drippers. Flowers were inoculated with *A. alternata* on 21/11/22. Treatment plots received intensive fungicide and biological agent treatments every 10 days, with a total of four applications during flowering.

- Fungicides included Amistar, Miravis Duo, Switch, and Intervene
- Biological agents used included Botector, Salicylic acid, Fulzyme, Sarsil, and Calcium chelate.

Red fruit were assessed at four harvest dates, conducted at approximately two week intervals commencing 4 January 2023. It is likely that only the second harvest included fruit which had had been inoculated with *A. alternata* at flowering.

Trial 3 – Testing the effect of fungicide concentration (Bundaberg)

Discussions regarding fungicide efficacy with a commercial grower in Bundaberg led to the hypothesis that reducing the degree of dilution of the fungicide, while still applying at the label rate of active ingredient per hectare, could potentially improve effectiveness against IFR.

This was tested using the growers standard spray program (Switch plus Miravis Duo). Nine bays of capsicums were sprayed four times during the crop cycle using half the standard volume of fungicide, but keeping the

total amount of active ingredient the same. The remainder of the crop was treated as normal practice.

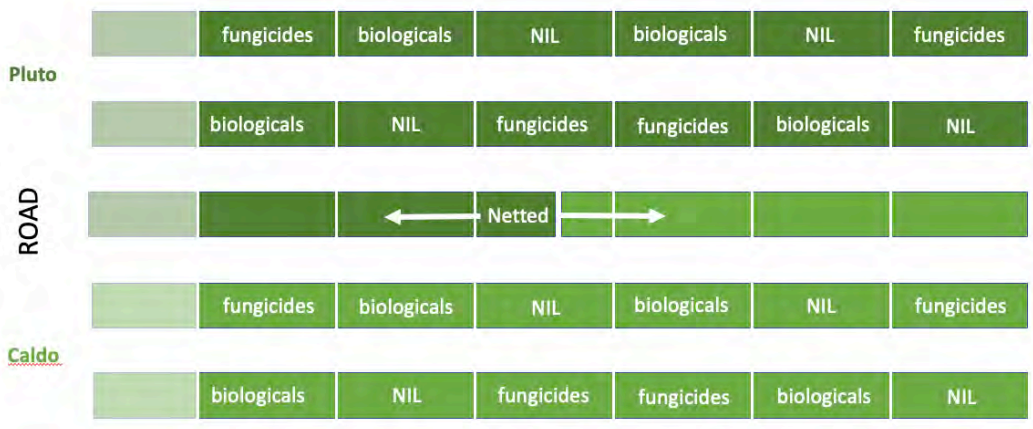
A total of 300 saleable fruit from each treatment were harvested and assessed for internal rot, with fruit graded as full red or ¾ red. Chi-square analysis was conducted to evaluate the association between treatments and internal rot incidence, as well as the correlation between fruit color grades and internal rot occurrence.

Trials 4 and 5 – Sydney Basin chemical and biological treatments

Simultaneous trials were conducted at the Local Land Services (LLS) site at Richmond and a nearby commercial farm that grows capsicums for the Sydney Market during summer months.

As previous trials of both chemical fungicides and biological products had failed to provide protection against IFR, these trials aimed to treat with as many products as possible, and as often as possible, beyond normal commercial practice. If this still failed to reduce incidence of IFR, we considered that this would effectively eliminate cover sprays as potential solutions for on-farm IDM.

The LLS site was planted with five 70m long double rows of capsicums varieties Caldo and Pluto. NNo mulch was used, and irrigation was supplied through overhead sprinklers. Treatments were allocated as shown in the plan below.



At the commercial property two 80m long beds were used for the trial, divided into 5m long blocks of two beds each. Seedlings were planted through plastic mulch and irrigated using sub-surface drippers.

Differently to the LLS site, at the commercial property all of the biological treatment units, and half of the fungicide treatment units, were treated using a hand held fogger. The remaining fungicide treatment blocks were applied using a backpack spray unit. The fogger was used to try to improve coverage of the floral stigma, which we suspected was poorly contacted using overhead sprays.

Fungicides included:

- Amistar (Group 11)
- Miravis Duo (groups 3 and 7)
- Switch (groups 9 and 12)
- Intervene (group 19)

Biologicals included:

- Botector
- Salicylic acid
- Fulzyme
- Sarsil
- Calcium chelate

At the LLS site a central area was covered with netting. This restricted both insect activity and air movement.

Fruit were harvested once mature red. Attributes recorded not only the presence of IFR, but also the number of lobes/fruit, seed colour (dark/light), presence of a hole at the fruit apex, attachment of the style, external mould and the presence of an internal black spot associated with the style.

Trial 6 – Use of Trichoderma to suppress IFR (Bundaberg)

Initial in-vitro trials examining the activity of a *Trichoderma* formulation (Nutri-Life Trichoshield, comprising *T. harzianum, T. viride*, and *T. koningii*) were followed by a field trial on a commercial property.

For the in-vitro study, subcultures of *A. alternata* and Trichoshield were inoculated either side on quarter strength PDA plates. The plates were observed regularly, examining whether *Trichoderma* overgrew the Alternaria colony, or created an inhibition zone at the colony interface.

A trial assessing the effectiveness of Trichoshield was conducted on a commercial capsicum farm. Twelve plots with approximately 400 plants were divided into four treatments: *Alternaria* inoculation, *Trichoderma* application, a control with water, and a combined *Alternaria-Trichoderma* treatment. Weekly inoculations were carried out during the flowering period, and marked flowers were monitored for development.

Fruit were assessed for symptoms of IFR once they were mature red. Overall, four harvests were conducted, aligning to the inoculation dates.

7. Developing effective on-farm IDM – Evaluation of spray coverage

Effective fungicide coverage of the floral stigma is critical for preventing infection. However, capsicum flowers often hang downwards on plants, their petals protecting the stigma and style from falling droplets. With most fungicides being purely protective, with little or no movement within the plant, good coverage is essential to prevent infection. The failure of trials using a range of fungicides to significantly reduce the risk of IFR in mature fruit suggested that lack of coverage may be a key factor.

Trial 1 – Evaluation of coverage using a standard spray unit

A field trial was conducted in a commercial capsicum crop. Three rows of plants were selected based on their position relative to the spray rig, with four plants chosen within each row. Flowers were selected randomly from different positions on the plant. A disc of water sensitive paper was pinned into the centre of each flower, covering the stigma.

Figure 3. Disc of water sensitive paper placed over the floral stigma

The plants were sprayed as per normal farm practice. Cone type nozzles were used on a standard boom spray at 120 psi, with no air assist available. The plants were left to dry for 1.5 hours, then the discs were collected and photographed. Coverage was estimated as a percentage of the disc which had stained blue. Data was analysed to determine the effects of row on coverage.

Trial 2 – Effect of pressure on spray coverage

Spray treatments were completed as per Trial 1 but with the spray pressure adjusted to 120 psi 135 psi or 150 psi. The collected discs were photographed against a white background. Adobe Photoshop was used to calculate average colour values for each disc. This was converted into a percentage change from yellow to blue for each disc. Control samples with no spray exposure provided a baseline for comparison. Differences in coverage were analysed across different pressures and row positions to determine the effectiveness of the spray treatments in achieving adequate coverage.

Trial 3 – comparison of spray coverage with and without air assist

Spray efficacy was examined at two farms with different spray rigs; Farm A had a standard boom sprayer with nozzles at 0.5m intervals. Farm B had an air blast unit, which uses pressurised air to improve penetration of spray into the crop.

Discs of water sensitive paper were inserted into flowers as previously, randomly selecting flowers in rows sprayed by the boom outer edge, centre and closest the tractor. Additional discs were used in non-sprayed control rows. After spraying with water, the discs were collected and photographed, then analysed using Adobe Photoshop.

As high RH at the time of trial resulted in slight colour change even without spraying, data was adjusted according to the control discs. This was converted to a percentage spray coverage. Results from these two units were compared to the results obtained from the spray unit tested in Trial 2.

8. Postharvest management with a near infrared grader

IFR is impossible to detect postharvest. This is one of the key issues, as retailers have low (or even zero) tolerances for IFR in capsicums. Rejections at market cost far more than rejections at the farm, as well as potentially leading to longer term loss of market share.

Near infrared (NIR) graders have been proposed as a solution to this problem. Several farms have invested in expensive NIR grading technology to avoid sending affected fruit to market, but with variable results. This activity sought to evaluate the accuracy of NIR grading systems for intercepting fruit with IFR before it is sent to market.

The trial was conducted in August 2020, using an AWETA NIR grader installed the previous season at Archies Produce, Stanthorpe. The machine represented the latest Dutch technology and was designed specifically for detection of IFR.

Figure 4. AWETA grading machine designed to detect capsicum IFR, and examination of internal quality after grading.

Due to covid travel restrictions, the trial was conducted by an AHR staff member already in Qld rather than the regular project team. Fruit was obtained through the Brisbane Markets from five different growers in Bundaberg and Bowen Gumlu. As some supplied different batches of fruit, there were nine batches in total.

To conduct an initial calibration, 30 fruit were numbered then run through the machine four times. The

grading result was recorded after each run, then the fruit cut open to examine internal quality.

Following adjustment of the machine, the nine batches of fruit were run through the machine, which classified them as “good” or “reject”. They were then cut open to examine actual internal quality.

9. Extend effective IDM options for growers

A number of extension techniques were intended to be utilised to deliver information on management of the disease. These included fact sheets, field demonstrations and farm walks, workshops, a webinar, use of the Soil Wealth Integrated Crop Protection (SWICP) (MT22004) website, and various reports and articles to be communicated through Vegetables Australia (now Australian Grower) and other electronic publications (e.g. SWICP bulletin). A video interview by AUSVEG was also used to disseminate results.

Initial results on causal organisms and mode of infection were promoted widely through articles published in Vegetables Australia and materials made available through the Soil Wealth / ICP website. The unexpected negative correlation of windy conditions with IFR has been published in Australian Grower Magazine and through a short interview video.

Other extension activities have been limited due to the lack of clear outcomes from trials. Despite numerous tests of biologicals, fungicides and different postharvest strategies, we have not determined a reliable way for growers to reduce risk from IFR.

Results and Discussion

1. Review the causes of internal rot in capsicums

Literature review

A number of different fungi are associated with development of IFR. *Fusarium lactis* complex (FLASC) is the most commonly found in Europe and the USA, along with other species of *Fusarium* and, occasionally, Alternaria. However, it should be noted that most studies are under greenhouse conditions, rather than in the field.

Many studies report that infection is likely to mainly occur during flowering, with flowers most vulnerable immediately after opening. Reports are mixed with regard to the effect of environmental conditions. There is some evidence that RH is critical to infection at low temperatures, but less important once temperature increases over 22°C. While keeping flowers dry is often cited as reducing risk of IFR, no studies were found examining differences between protected cropping and field grown plants, or those grown with drip vs overhead irrigation.

Some reports cite varietal resistance in certain greenhouse varieties, largely due to reduced symptom development during ripening. There are also some positive reports from using biological agents, although none provide full control. Fungicides have been shown effective, but only when applied at the start of flowering; as capsicums flower continually, this is extremely challenging to achieve commercially.

Numerous studies note the importance of the mycotoxins potentially produced by the organisms responsible for IFR. Simply removing diseased tissue does not necessarily remove the toxins as well. The extent to which *A. alternata* or *Fusarium* infections pose a threat to human health in commercial capsicums is unclear, however this does give further impetus to finding a solution to this difficult to control disease.

The review is attached as Appendix 1.

Industry consultation

Meetings were held with growers in capsicum growing regions including Stanthorpe, Gatton, Bundaberg and Sydney Basin. At least 20 growers have been contacted in-person and via the phone to discuss the problem over time. They represent Queensland growing regions, Sydney basin and Virginia in South Australia.

The project was also discussed with a range of other industry representatives, including Eduardo Pena (Senior agronomist, Woolworths), David Campbell (Market development lead, Monsanto Australia) and representatives from David Russos, Perfection Fresh and LaManna Premier group, these being wholesalers that are major suppliers of capsicums nationally.

Project team members collaborated with local grower groups and the VegNET officer in the Bundaberg region. Between 2021 and 2024, visits were made to multiple capsicum growers in the Bundaberg area.

The industry consultation allowed for a greater understanding the extent of the disease in Australia, for example the disease is uncommon in the protected cropping of Virigina in South Australia, but seasonally common in Bundaberg and Bowen.

2. Field and laboratory studies to identify the causal organisms – Survey and isolation

Disease Surveys

A wide range of fruit samples were collected directly from farms as well as supplied by growers and wholesalers. While no samples were obtained from Carnarvon (due to difficulty to transferring samples from Perth) or Adelaide (where IFR is not usually an issue), all other capsicum producing regions were represented. Flowers, seeds and sections of fruit placenta were also collected and plated onto potato dextrose agar.

Colonies were isolated from the initial samples through a single sporing process onto potato carrot agar, water agar or carnation leaf agar. *Alternaria* spp. were the most commonly isolated organism. For example, in the Richmond sampling all 29 infected fruit returned *Alternaria* spp.. Samples collected from Bundaberg farms were also identified as being predominantly caused by *Alternaria* spp.. A lesser number of *Fusarium* spp. were isolated, largely from fruit collected in Stanthorpe.

More details on the disease sampling is included in Appendix 2.

Isolation of casual organisms and pathogenicity trials

Four morphologically different cultures were selected for re-inoculation onto capsicum flowers, according to the dictates of Kochs postulates. The cultures were tentatively identified as *A. tenuissima*, *A. alternata*, *F. oxysporum* and *F. semitectum*. Water was used as a control.

Capsicum fruit developing from the inoculated flowers were allowed to mature then examined for symptoms of IFR. No infected fruit developed from flowers inoculated with water or *F. semitectum*. However, the other three fungal strains all caused IFR symptoms, with the fungi re-isolated from the diseased area.

These three fungi were analysed using molecular techniques, confirming their initial identification. Further DNA work conducted in 2023 by Zali Mahoney refined this result, placing both of the Alternaria strains within the *A. alternata* subspecies complex.

Isolations were also conducted on PDA plates using surface sterilised dark seeds. None of the seeds displayed fungal growth. It was concluded that “dark seed” is not the result of pathogen infection, but more likely a physiological condition.

The full report on causal organisms is included as Appendix 2.

3. Field and laboratory studies to identify the causal organisms – Timing of infection

Trial 1 – Inoculation of styles on mature fruit

Due to the progressed physiological age of the fruit, many fruit broke down quickly at room temperature. Visible symptoms of internal fruit rot were also not found in any significant numbers. A number of confounding factors such as temperature at inoculation, concentration of inoculum, the age of the fruit and time for infection to become symptomatic may have lead to this result. An additional trial was conducted to address these issues.

Trial 2 – Re-inoculation of styles on mature fruit

Despite changes to the trial design, similar issues to those faced in the initial trial occurred. This included the early rotting of fruit and lack of visible symptoms. No significant differences were found between treatments. Plating samples of internal parts of the fruit did not yield further information. It is possible that there was not enough time for infection to progress through the style.

The project team concluded that using fruit already harvested for these trials would not yield results as there was limited time to induce symptoms after inoculation before fruit started to breakdown. To address this the team setup a greenhouse trial to test the inoculation of live fruit at different stages.

Trial 3 – Inoculation of floral stigmas and styles retained on developing fruit

A. alternata was confirmed as causing IFR in 23% of fruit inoculated at flowing and 14% of fruit inoculated when they were less than 20mm diameter. No infection was detected when fruit were inoculated when more than 50mm diameter, or in the uninoculated controls.

This result confirms that infection almost always occurs at flowering. It also indicates that the style remains vulnerable to infection even after pollination has occurred and the young fruit started to swell. Based on these results it is possible that 38% of total infected fruit result from infection of the stylar end of young green fruit.

These results are largely consistent with those of Halfon-Meiri and Rylski (1983) where the greatest infection occurred at flowering and declined thereafter. What is clear from this study is that the dried attached style is an important entry point for *A. alternata*, at least until fruit develop past the 20mm diameter stage.

It was also observed that there is a high level of abortion in fruit and young flowers inoculated with *A. alternata.* This was also noted in the Israeli study and suggests that *A. alternata* infection losses are not confined to IFR in mature fruit, but may be responsible for significant yield loss in the field.

The full report on timing of infection is included as Appendix 3.

4. Examine differences in varietal susceptibility

Variety Trial Richmond

The initial assessment picked the fruit that were still intact, including red, coloured and mature green fruit. The percentage of fruit with IFR was much higher in full red than coloured fruit. No green fruit were found to have IFR. This confirms that symptoms of IFR nearly always develop as the fruit reaches full maturity, despite the fungi having been present since flowering. While there appeared to be differences between the varieties, the relatively low number of fruit assessed (65 to 159 per variety, more green than red) means that no conclusions can be drawn from this result.

Unfortunately, subsequent harvests were not possible as the crop was destroyed by a major flood event.

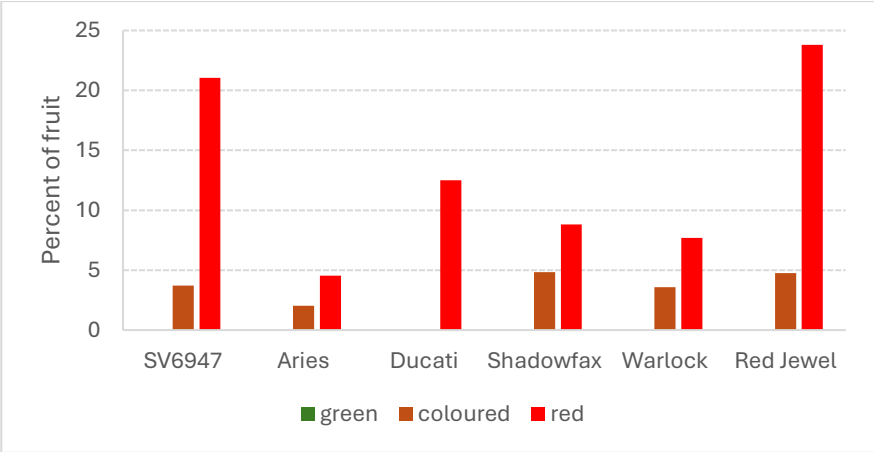


Figure 5: Initial rates of IFR in different capsicum varieties grown at the River Farm, Richmond.

Variety Trials Stanthorpe

There were no consistent, significant differences in incidence of IFR between any of the varieties examined. However, there were clear differences in rates of IFR between the two harvest dates. In February, 28% of fruit had signs of IFR, whereas in March this had fallen to 18%. In contrast, dark seed increased between the two sampling times, from 28% of fruit in February to 54% in March. However, this result should be treated with some caution, given that slight browning of the seeds can be quite subjective.

When assessing the effect of maturity, it was once more clear that IFR increases significantly as fruit develop to full red. Only 4.6% of coloured fruit had signs of mould, compared to 18% of fruit that were fully mature. This suggests that picking slightly earlier can greatly reduce the incidence of disease.

In contrast, dark seed is unrelated to fruit maturity, with green fruit not significantly different to those harvested more mature. This further supports the previous observation that no pathogens could be isolated from dark seed, and that this condition is more likely to be physiological than pathological in origin.

The full report on the variety trials is included as Appendix 4.

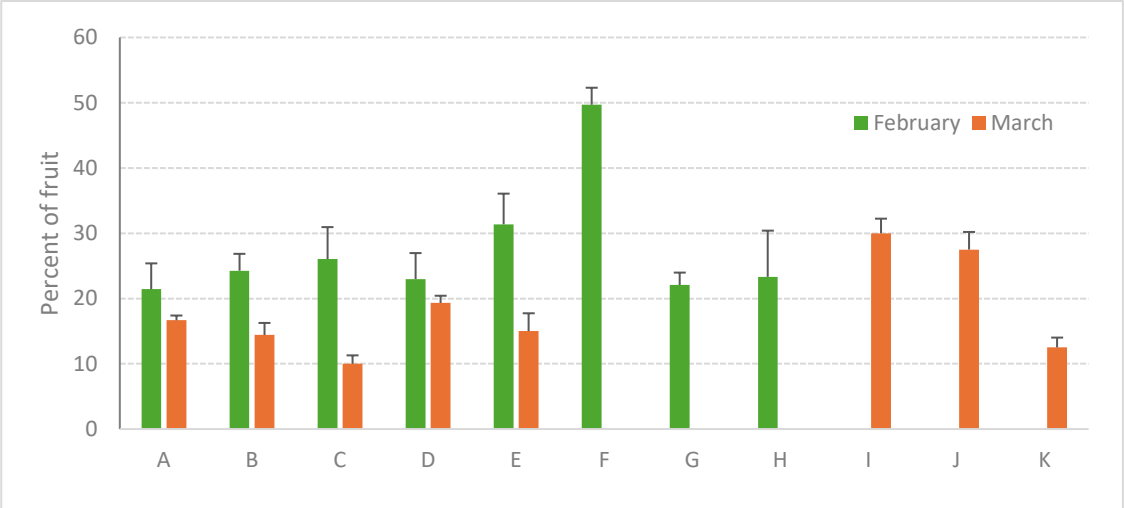


Figure 6. Percentages of red fruit with symptoms of IFR when picked in February or March in Stanthorpe. Letters indicate different varieties.

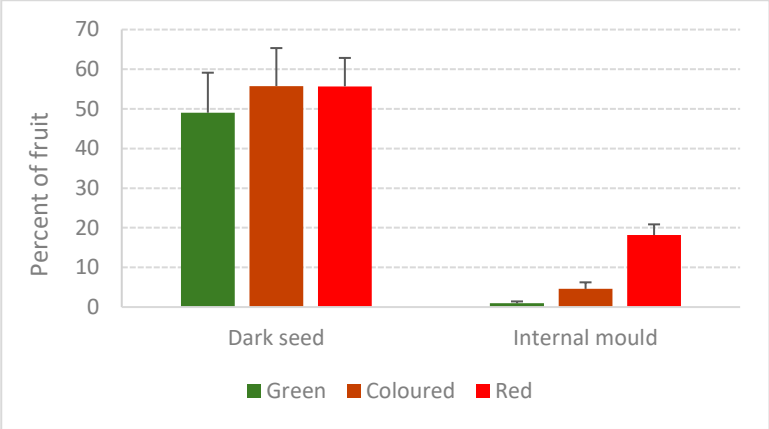


Figure 7. Percentage of fruit assessed when green, part coloured (chocolate) or full red which had signs of dark seed or IFR, fruit sampled in Stanthorpe in March.

5. Develop a model for predicting infection risk and protocols for early diagnosis

Environmental model development

No consistent correlation was found between internal rot occurrence and measures of wetness, relative humidity, or total rainfall. This finding aligns with anecdotal observations from wholesalers, who noted a (surprising) lack of internal rot during wet seasons. It seems possible that heavy rain may actually remove the spores from downward hanging flowers. The role of flower stigma exudates in enabling spore germination, regardless of environmental wetness, is also suggested as a potential contributing factor.

However, there was a significant correlation between windy conditions during flowering and subsequent infection rates. Specifically, the analysis showed that still air (wind speeds below 5 km/h) two to three months before harvest were strongly correlated with increased infection rates. This suggests that still conditions allow spores to germinate on the floral stigma. Conversely, high wind speeds (>15 km/h) and wind gusts (>22 km/h) were negatively correlated with infection (Table 1).

The findings highlight the importance of airflow in managing internal rot in capsicums.

Table 1: Correlation (r values) between infection rate and climatic variables across weeks prior to harvest

Weeks before harvest	14	13	12	11	10	9	8
Hours 2˚C or less above dew point	-0.09	-0.27	0.18	-0.63	-0.2	-0.45	-0.54
Hours > 95 % Humidity	-0.07	-0.28	0.13	-0.15	-0.28	-0.52	-0.58
Hours between 85 -95 Humidity	-0.09	-0.38	-0.07	-0.48	-0.27	0.13	0.39
Hours with wind Gusts >22 kph	-0.29	-0.76	-0.65	-0.68	-0.56	-0.54	-0.26
Total Rainfall (mm)	0.04	-0.09	0.05	-0.38	0.37	-0.03	-0.66
Hours of wind < 5 kph	0.75	0.87	0.89	0.9	0.63	0.5	0.53
Hours of wind > 15 kph	-0.49	-0.76	-0.63	-0.62	-0.67	-0.59	-0.38

Evaluating the effect of increasing or decreasing air movement on IFR

Almost no IFR occurred in the trial crop. Moreover, challenges from pest pressure and heavy rainfall resulted in early termination of the crop. Of 219 fruit assessed, only 130 were suitable for analysis.

However, among the assessed fruit five fruit had symptoms of IFR—all from the windbreak treatment. None of the capsicums sampled in the control blocks, with constant wind provided by solar fans, or with excess nitrogen developed IFR. While these results support the model conclusions, in that low wind speeds may increase risk of IFR, fruit numbers were too low to form firm conclusions.

Evaluation of wind exposure and speed on IFR

Readings from the anemometers confirmed that wind speed was generally higher at the edge row than in the middle of the block.

In total, 2,248 fruits were assessed across three harvest dates. There was a trend to lower infection rates in the edge row where plants were half spaced, however differences were not statistically significant overall. Correlation analysis showed that wind speed was negatively correlated with infection rates in edge rows (suggesting higher wind speeds reduced rot) but the same effect was not found for middle rows. Overall, no significant link between wind speed and infection rate was found. Results may have been confounded by the limited number of harvests conducted (impacted by high rainfall), and the proximity of a waste pile. A more comprehensive study is recommended to clarify these findings.

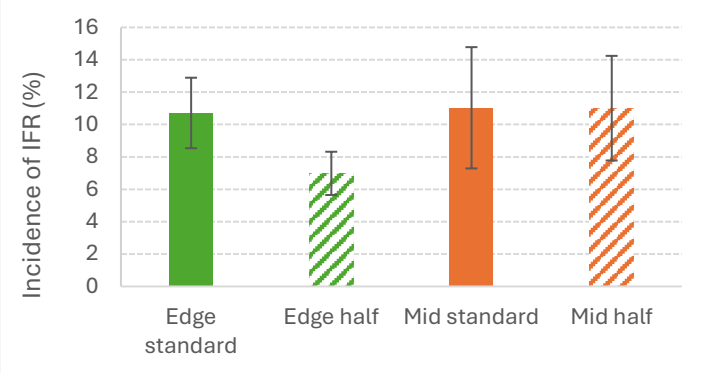


Figure 8. Incidence of IFR over three harvest dates, comparing plants and the middle and edge rows of the block, in blocks which were left as per normal commercial practice or where half of the plants had been removed to increase spacing.

Wind speed was consistently higher at the edge of the crop compared to in the middle rows (Figure 9). There was a significant correlation between wind speed and infection rate for the edge row, but not for the middle

row. Average wind speed generally fell over the flowering period corresponding with harvest dates for red capsicums. In this trial, falling average wind speeds were not reflected in increased rates of IFR. Moreover, although there was a significant difference between the middle and edge rows at the initial harvest of the different blocks (Figure 12), this was not observed at subsequent harvests.

The results of this trial are therefore inconclusive with regard to proving a correlation between wind speed and IFR.

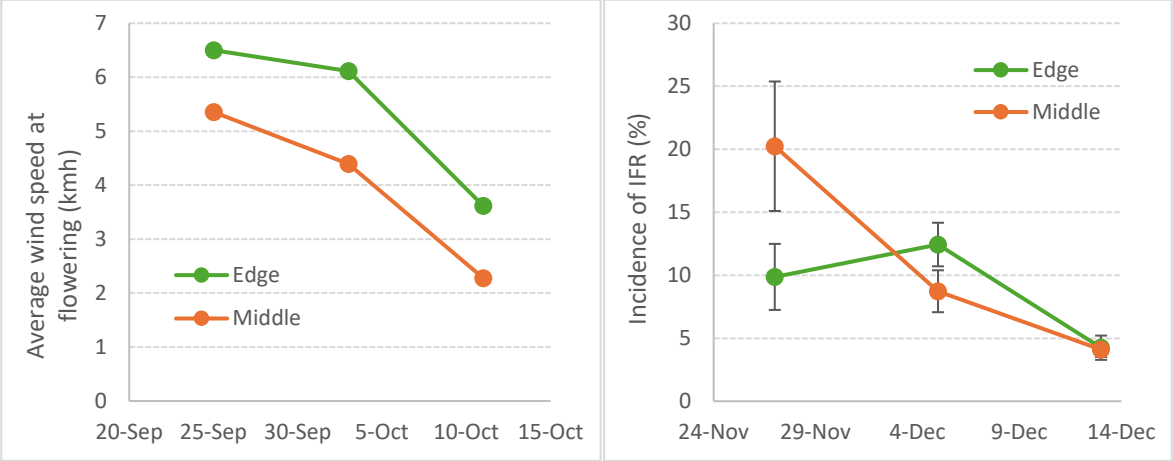


Figure 9. Average wind speed during flowering (left) and corresponding incidence of IFR in red capsicums (right); bars indicate the standard error of each mean value. The time between flowering and maturation for the three harvest dates was estimated as approximately 60 days.

A full report on environmental effects is included in Appendix 5.

6. Developing effective on-farm IDM – Chemical and biological sprays

The results across all trials consistently showed no significant differences between the treated and control groups in IFR incidence under the conditions tested despite expectations that some treatments would provide protection against the disease. The findings suggest that spray coverage may have been insufficient, prompting further investigation into factors affecting application efficacy.

Trial 1 – Local Land Services site, Richmond

Fruit tagging demonstrated that it took 60 to 65 days for fruit to mature after flowering. As previously noted, rates of IFR were far higher in full red than part red fruit, averaging 15.2% and 8.1% respectively. Rates of IFR generally fell over the four harvests conducted.

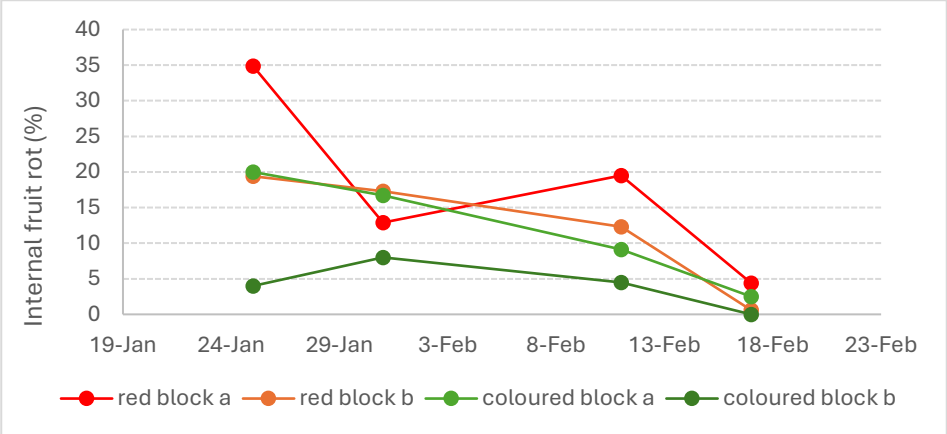


Figure 10. Rates of IFR in harvested red and part red fruit grown in two replicate blocks at the Local Land Services site, Richmond.

There was a trend to increased rates of IFR in capsicums that stored for several days under ambient conditions before assessment. Incidence of IFR did not change from initial rates when fruit were immediately transferred to a 5°C coldroom, then assessed a week later.

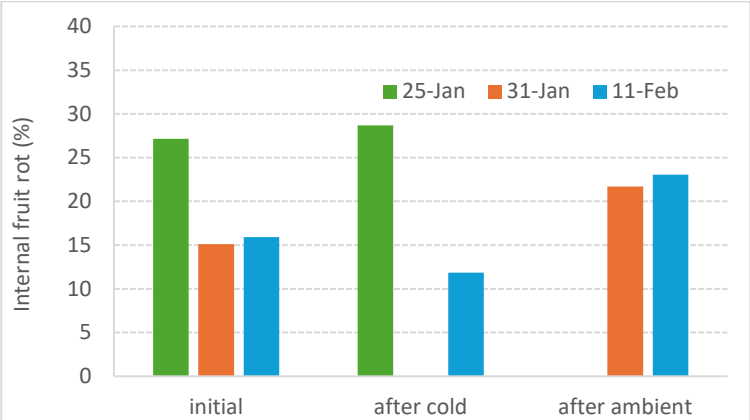


Figure 11. Rates of IFR in full red fruit, with subsamples assessed immadiately after harvest, after one week at 5oC, or after 3-4 days under ambient conditions. Data from 3 harvest dates.

Unfortunately, no data was collected from the fungicide spray trial due to major local flooding during March 2022. Only the later planting had been used for this trial, and fruit were still immature when the flood occurred.

Trial 2 – Bundaberg trial site

There were no significant reductions in observed IFR from any of the fungicide and biological products tested relative to the untreated controls, either at any of the individual harvests, or across the three harvests on average.

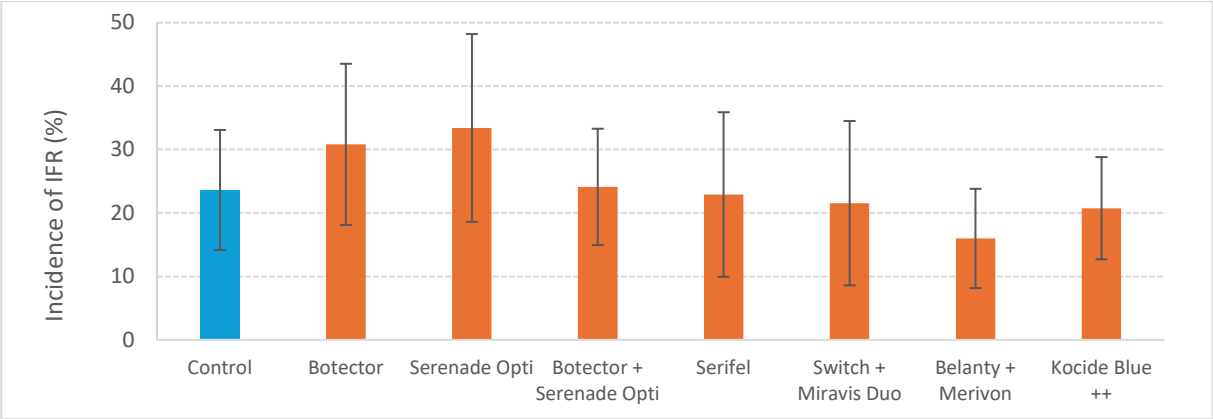


Figure 12. Recorded rates of IFR averaged across all harvest dates for control fruit compared to those treated with a range of fungicides and biological products. Bars indicate the SEM (n=3)

Trial 3 – Testing the effect of fungicide concentration (Bundaberg)

There was no effect from increasing the concentration of fungicide in solution, while still applying the same amount of active ingredient per hectare. Rates of IFR in capsicums treated with normal and concentrated fungicide were 18% and 17% respectively.

Capsicums with color grades of 5 and 4 showed internal rot incidences of 18% and 13%.

Trials 4 and 5 – Sydney Basin chemical and biological treatments

Rates of IFR were low at the LLS site, averaging 8% over four harvests. In previous years, late harvests within this region have found more than 25% of fruit infected with IFR. As results were not significantly different for Plato and Caldo, data for the two varieties was combined.

The final harvest at LLS did reveal an increase in IFR, particularly in the untreated controls. While this appears promising, the treatments were not significantly different overall.

There was also a trend to higher rates of IFR in fruit grown under netting. This was particularly apparent at the final harvest, with an average of 34% of fruit having symptoms of IFR. While there was limited replication with netting, this does support the previous observations of reduced air movement being associated with increased rates of IFR.

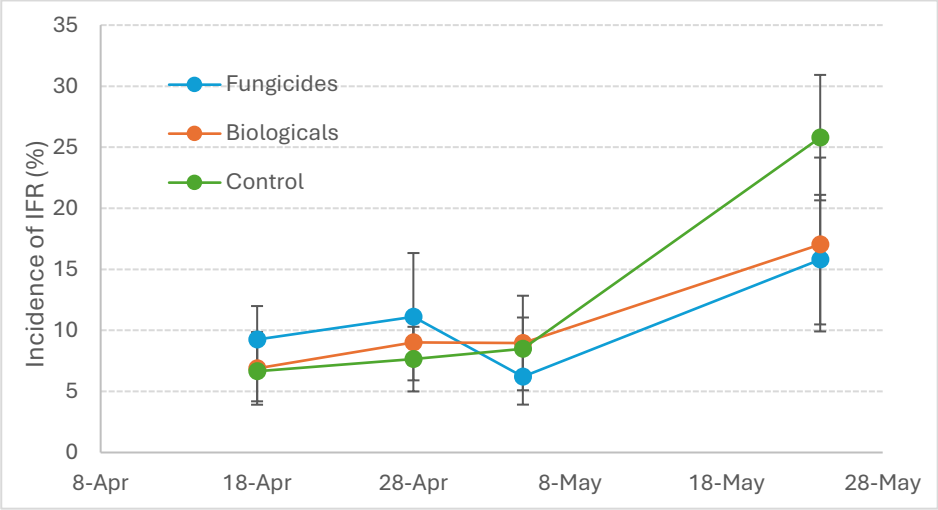


Figure 13. Average incidence of IFR across two varieties (Plato and Caldo) and four harvests. Capsicums were treated every 10 days during flowering with a range of either fungicides, biological products, or water only. Bars indicate the standard error of each mean value (n=6).

No relationship was found between symptoms of IFR and the number of lobes, presence of a hole in the apex, dark seed, style condition or the other characteristics recorded.

The exception was the presence of a dark, diseased spot at the apex of the fruit adjacent to the remnant style. Nearly 50% of fruit with IFR had this mark at the apex, compared to only 12% of fruit which did not have disease symptoms. This is consistent with the mechanism of infection through the fruit stigma and style, some disease symptoms expressing in that part of the fruit.

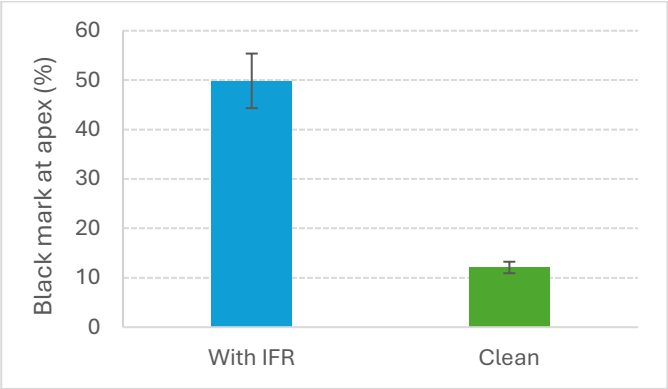


Figure 14. Percentage of fruit which had symptoms of IFR or were clean, and which also had a dark, diseased spot adjacent to the remnant style. Bars indicate the standard errors of each mean value (n=7).

Results at the commercial farm were mixed, with certain sections of the crop severely affected by *Sclerotium rolfsii,* leading to plant collapse. Moreover, the rate of IFR was low, averaging only 5.7%.

There was no evidence supporting a reduction in rates of IFR when fungicide was applied using a fogger compared to a backpack spray unit. However, this may reflect the effectiveness of the hand held unit used for the trial: a commercial air blast unit would be likely to be more effective at improving spray coverage.

Trial 6 – Use of Trichoderma to suppress IFR (Bundaberg)

The in-vitro trial showed that Nutri-Life Trichoshield was active against the *A. alternata* isolates obtained from infected capsicums. Inhibition zones were observed, together with overgrowth of the A. alternata mycelia.

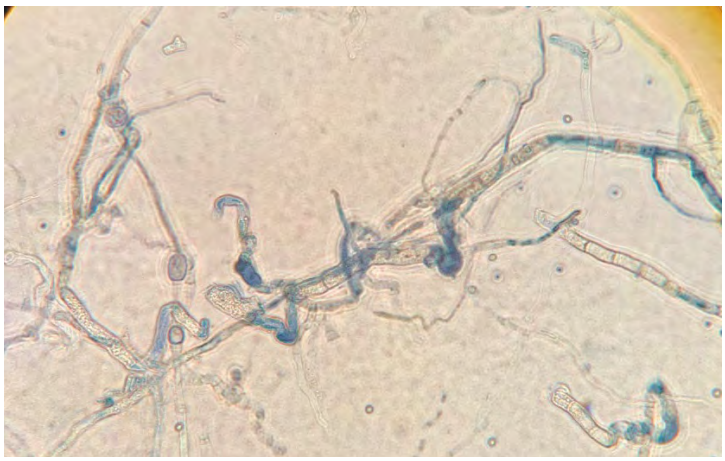


Figure 15. Microscope view highlighting blue-stained *Trichoderma* hyphae coiling around transparent *Alternaria* hyphae, indicative of parasitism

For the field trial, analysis of the data revealed no statistically significant relationship between treatment and the incidence of internal fruit rot (IFR), as determined by a Chi-square test ($\chi^2 = 3.20$, $p = 0.362$). However, a trend was observed across treatments, as flowers inoculated with *Alternaria* alone exhibited a higher incidence of IFR in mature fruit compared to other treatments, including those inoculated with both *Alternaria* and *Trichoderma*. Interestingly, treatments that included *Trichoderma* showed slightly lower incidence rates than the control. This trend suggests that *Trichoderma* may have a modest impact on reducing infection rates, warranting further investigation into its potential effects.

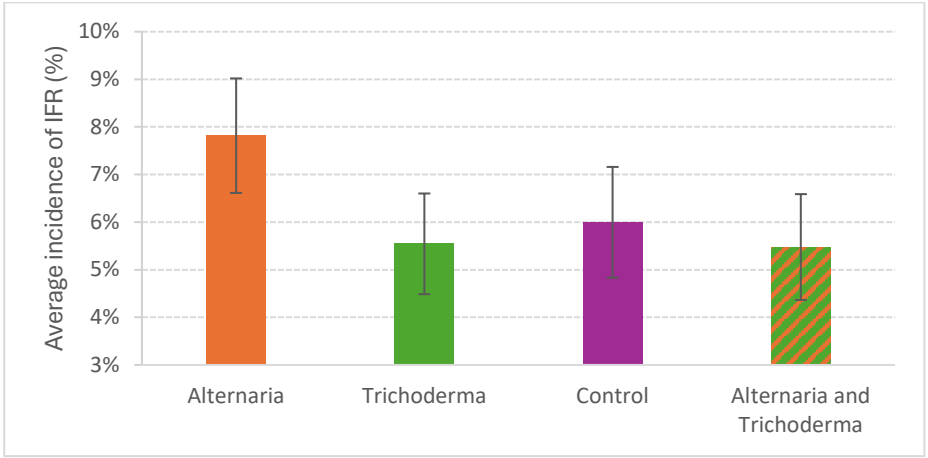


Figure 16. Incidence of IFR when flowers were inoculated with water (control), *A. alternata* spores, Nutri-Life Trichoshield or both *A. alternata* and Nutri-Life Trichoshield. Average values from four harvest dates, bars indicate the SEM of each mean value (n=4)

It should be noted that rates of IFR were low in this trial, particularly in harvests three and four, despite deliberate inoculation of flowers. It seems likely that the relatively low spore count in the inoculant used on these occasions reduced effectiveness of the treatment.

Full reports on all chemical and biological sprays applied are included in Appendix 6.

7. Developing effective on-farm IDM – Evaluation of spray coverage

Trial 1 – Evaluation of coverage using a standard spray unit

Overall, the standard spray trial indicated that the 120 psi spray treatment provided poor coverage, with variability influenced by flower position relative to the boom and within the plant canopy. On average, coverage was highest for flowers located in Row 2, which was positioned under the middle of the boom. Coverage was estimated at 65%, which is still insufficient to prevent infection of the floral stigma. Flowers located in Row 1, adjacent to the tractor edge, averaged 50% coverage, while flowers in Row 3, aligned with the outer edge of the boom, had the lowest estimated coverage of 45%.

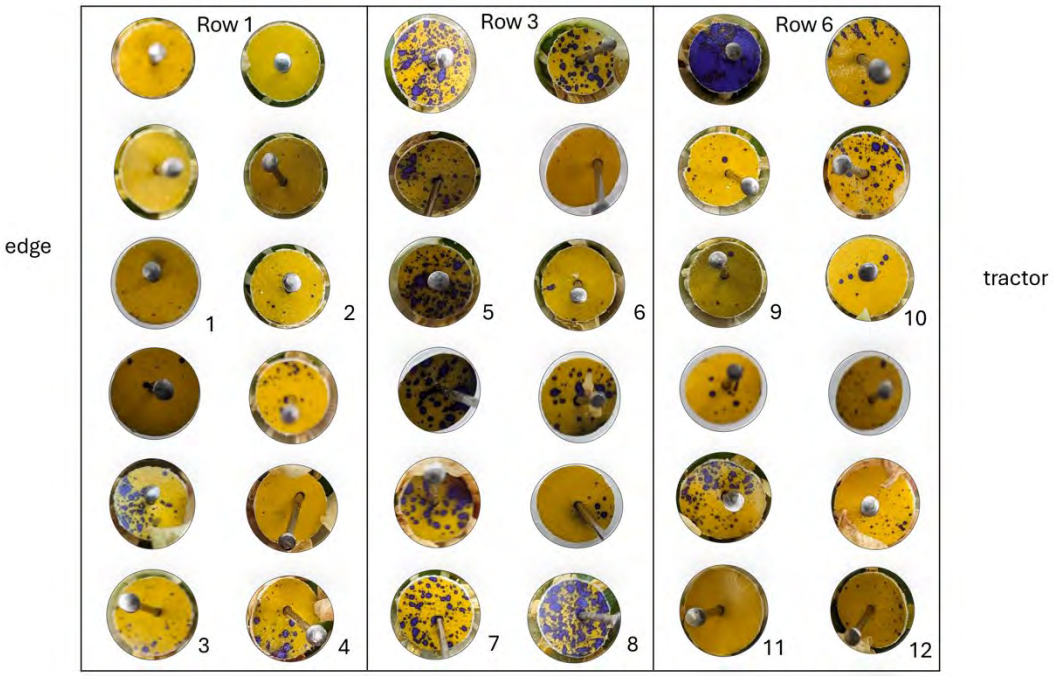


Figure 17: Spray coverage results. Blue areas indicate regions where the spray has made contact.

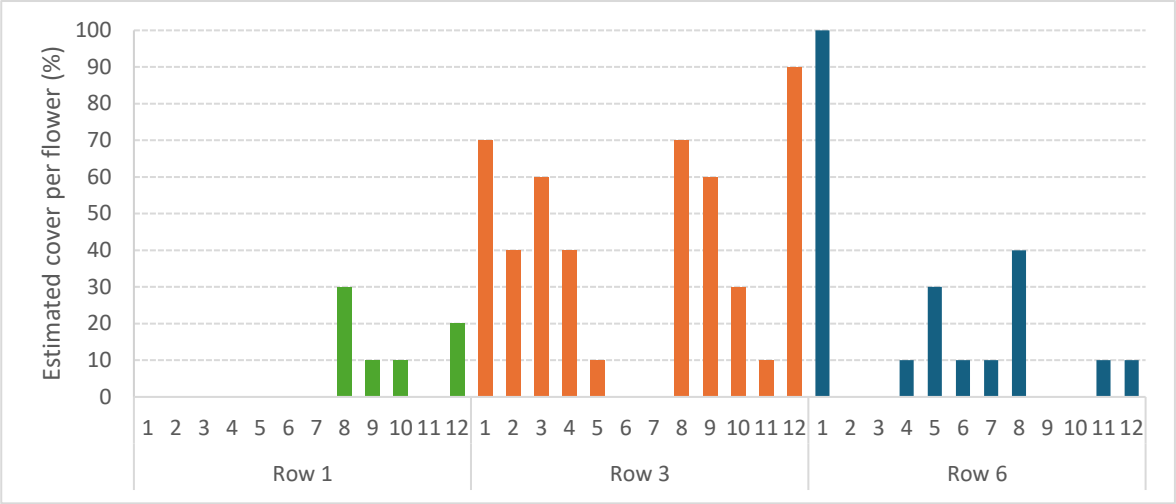
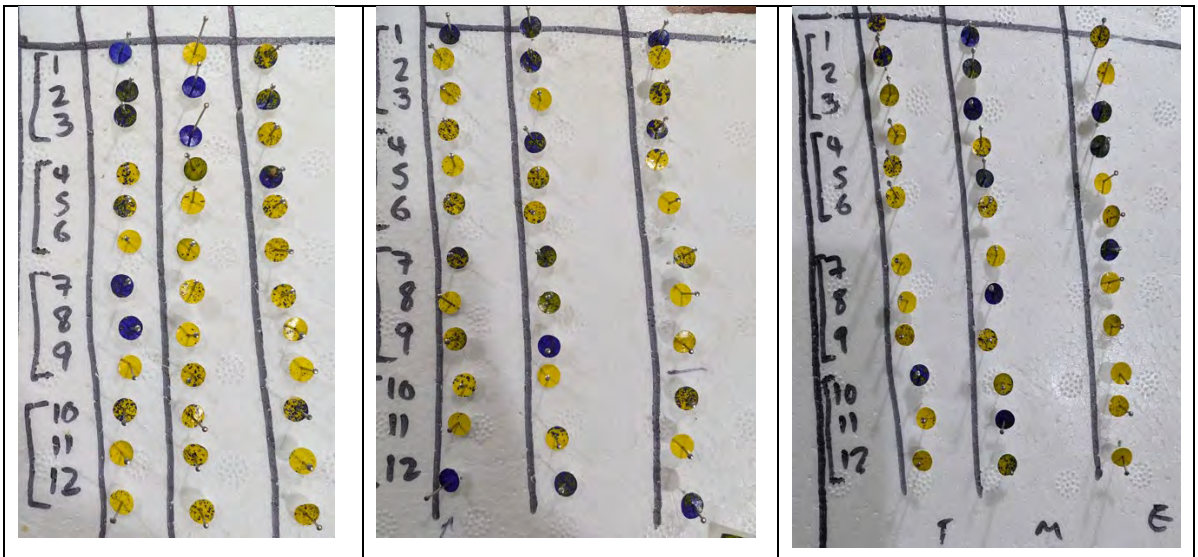


Figure 18: Estimated spray cover in each flower. Row one = edge row, row 3 = middle and row 6 = position closest to the tractor

Trial 2 – Effect of pressure on spray coverage

The second trial compared spray coverage when applied with three pressures – 120 psi, 135 psi, and 150 psi. Increasing spray pressure did not improve coverage, and was more likely to make it worse. Even at the lowest pressure tested, coverage was not optimal for preventing infections through the flower.

Coverage was also influenced by distance along the boom and tractor speed. Spray coverage was best closest to the tractor at the lowest pressure, but in the middle of the boom at higher pressures. Coverage was also better at the beginning and end of rows, likely due to slower speeds during acceleration and deceleration.



120 psi	135 psi	150psi

Figure 19: Spray cover in each flower. Blue areas indicate regions where the spray has made contact. For each pressure: First row=closet to tractor, second row = middle of boom, last row = edge of boom)

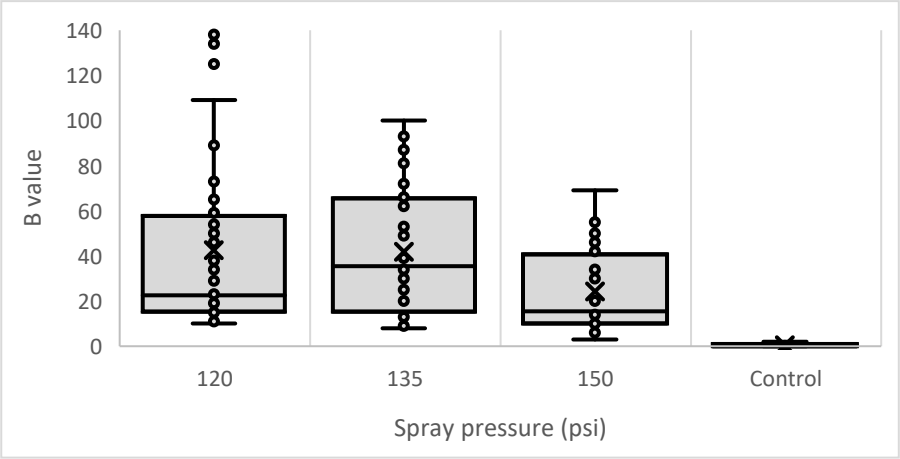


Figure 20: Box-and-whisker plot showing the distribution of B values, representing spray coverage, across different spray pressures (120, 135, and 150 psi). The plot indicates a decreasing trend in B values with increasing pressure, suggesting reduced spray cover

Trial 3 – comparison of spray coverage with and without air assist

Significant differences in spray coverage were found between rows as well as between standard boom sprays (Farm A and Farm C) and the air blast spray unit (Farm B). The number of flowers which received >50% coverage was doubled using the air blast unit, at least in the rows closest to the tractor and at the centre of the boom. Average cover was also increased using the air blast system compared to both of the boom spray units tested in these trials.

Unfortunately, however, a large decrease in efficacy was observed at the end of the air blast boom, presumably due to a pressure drop. The outer row received poor coverage regardless of spray unit used. Approximately 80% of flowers received less than 25% spray coverage. Most of these effectively received no coverage at all (<10%). While it is noted that repeated passes with spray unit can result in some overlap, with the outer edge potentially receiving >1 application, it is unclear whether this would be sufficient to make up for the poor coverage observed in these trials.

It is also noted that although Farm A and Farm C both had conventional spray booms, it appeared that the unit at Farm A was delivering finer droplets. This meant that although many flowers had only 50% coverage, this was better than the results from Farm C, where flowers mainly received either good or poor coverage.

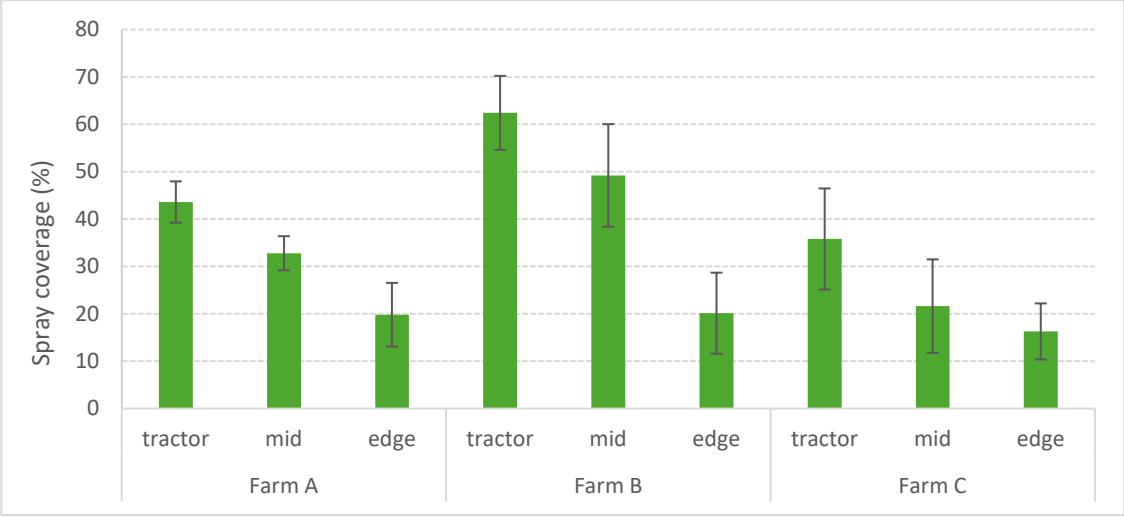


Figure 21: Average spray coverage by row, assessing a row next to the tractor, at the centre of the boom and the edge of the boom. Farm A had a conventional nozzle sprayer on an overhead boom, Farm B had an air blast sprayer, and Farm C had a spray unit with dropdown nozzles

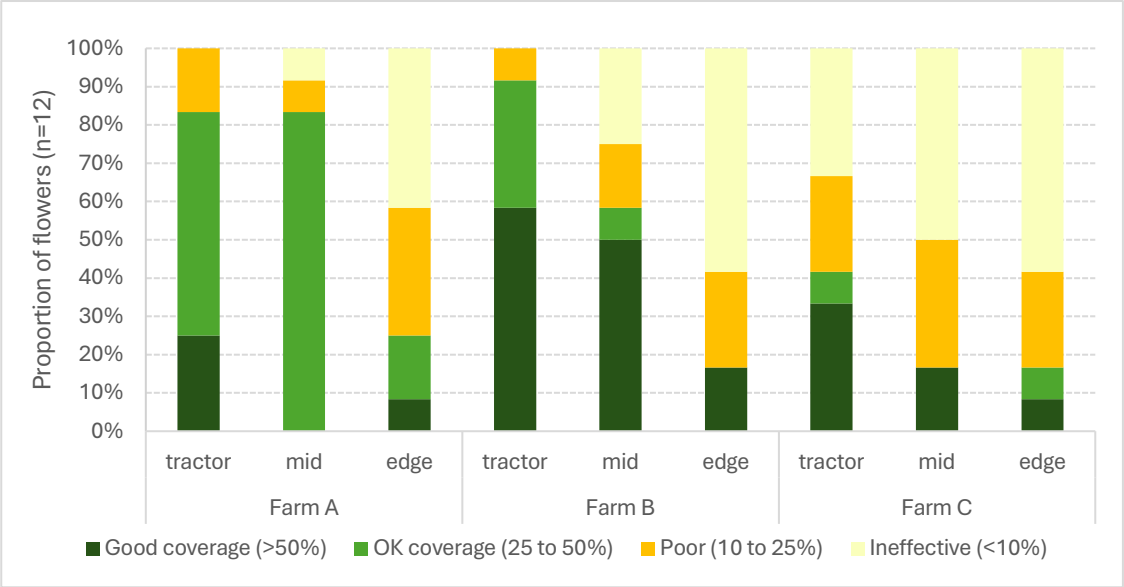


Figure 22: Proportion of flowers within each row that received good, OK, poor or ineffective coverage. Proportion of flowers is expressed as a percentage of those sampled (n=12).

For a full report on spray coverage, see Appendix 7.

8. Postharvest management with a near infrared grader

The initial setup of the machine resulted in rejection of the majority of the 30 numbered fruit passed through repeatedly, even though the true infection rate was 5 of 30 (17%);

- All (5) fruit with IFR were rejected on all four passes through the grader.
- Fruit with dark seed were rejected on 77% of occasions
- Fruit with no internal defects were rejected on 58% of occasions
- Only 11 of the 30 fruit were graded the same way each time.

The effectiveness of the grader varied greatly between batches of fruit. Setting the parameters to “reject” too high resulted in large amounts of good fruit being rejected. However, setting them too low allowed fruit with IFR to pass through to packing. For example;

- Batch C1 included 63 fruit with IFR (16%). The grader successfully removed all but five of these, making it 92% effective. However, it also rejected 187 fruit (48% of all fruit graded) which were clean or had dark seeds.

- Conversely, only 6.3% of batch C2 were rejected, of which most (8 of 13) had symptoms of IFR. However, this calibration allowed 18 fruit with internal mould to go through to packing (8.7% of total).

- The grader successfully removed all but one of the mouldy fruit from batches E1 and E2, but rejected approximately half of the remaining, good fruit.

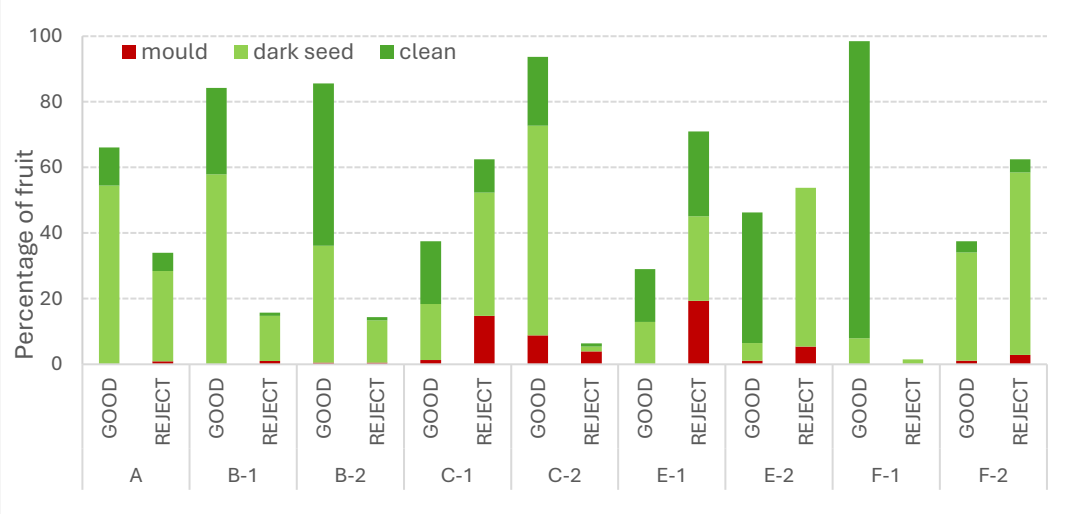


Figure 23: Output from the NIR grader; Percentage of each batch of fruit that were graded as "good" or "reject", with actual internal quality (mould / dark seed / free of defects)

Overall, it was demonstrated that the NIR grader can detect IFR. The grader reduced the rate of IFR from 6.6% across all samples to 1.5% in packed fruit. This would bring the rate of mould below the critical limit of 2% major defects imposed by the major retailers. However, internal seed discolouration proved a major issue, resulting in rejection of a large amount of good fruit. This is particularly clear when all data is combined; more than half of the rejected fruit contained dark seeds, with a further 19% having brown seeds. Conversely, only 2% of fruit passed for packing contained IFR.

A report on use of NIR to detect and reject IFR affected fruit is included in Appendix 8.

10. Extend effective IDM options for growers

https://www.youtube.com/watch?v=Ltm-WW9OYQ0

This activity has not been completed as we have not yet confirmed any effective IDM options for growers.

Outputs

Table 2: Output summary

Output	Description	Detail
Literature review	A detailed literature review was prepared that summarised and critiqued current global knowledge on the pathology, epidemiology and management of internal capsicum rot. The review was used as a basis for future project activities such as industry consultation and trial planning. It will also be used to prepare scientific papers based on this work.	The literature review will be made available upon the publishing of this final report. It can be found attached Appendix 1.
Fact sheets	A fact sheet looking a spray coverage and effectiveness for capsicum flowers is being produced and will be released prior to April 2025.	The factsheet will be promoted through the SWICP project and AUSVEG weekly updates. It will be hosted on the SWICP website and AHR website.
Webinar	A webinar was run in September 2020 to discuss the preliminary outputs of the project. This included initial findings of the disease survey and pathogenicity trials and postharvest management. It also gave an opportunity for attendees to ask questions. The webinar was released through the SWICP project on YouTube. An additional webinar is being planned for February 2025 and will discuss project outputs and findings. It will be hosted through the SWICP	The webinar has been viewed 524 times, in addition to the audience (approx. 60) that viewed it live. It is hosted on YouTube and is available on the Applied Horticultural Research and SWICP websites.
Reports and articles	Articles on the project have appeared in issues of the Vegetables Australian and Australian Grower magazines.	The articles can be viewed here: • AUSVEG article June 2020 • AUSVEG article January 2024 Vegetables Australia had a readership of around 2300, the successor publication, Australian Grower has a readership over 6000.
Video interview	A video interview produced by AUSVEG was released on the 30th of October 2023. It detailed the	The video was released on YouTube and promoted through the AUSVEG weekly update.

	progress of the project and findings from some of the trials undertaken by the project team.	The video has been viewed 57 times.

Outcomes

Table 3: Outcome Summary

Outcome	Alignment to fund outcome, strategy and KPI	Description	Evidence
Adoptable protocols for early diagnosis of the pathogens causing internal rot in capsicums	**2017-2021 SIP** Outcome 3: Improved Farm Productivity 4. Pests and Diseases • Fewer pest and disease outbreaks • Lower costs of pest and disease management for adopting growers **2022-2026 SIP** Outcome 1: Industry supply, production and sustainability 2. Identify and support opportunities to improve productivity and sustainability through effective integrated pest and disease management (IPDM), weed control, soil health and cover crops • Pest and disease management strategies are developed that mitigate crop loss in collaboration with growers • Plantings are optimised for production efficiency, sustainability and improved profitability	Adoptable protocols for early diagnosis of pathogens causing internal rot of capsicums were not able to be developed. Causal organisms were identified morphologically and genetically. Destructive sampling of green fruit can help identify whether the causal organisms are present. However, results from this project have not found this to be a good predictor of symptom development in mature red fruit. Trials demonstrated that the condition “dark seed” is NOT related to IFR. This condition is non-progressive, does not increase as fruit mature, and is likely to have physiological, not biological, origins. Non-destructive techniques are currently limited to use of NIR grading technology. While NIR grading can detect some internal mould, it also rejects good fruit and some with the non-progressive condition of “dark seed”.	Appendix 2 Appendix 8
An understanding of the transmission of each pathogen causing internal rot in capsicums	**2017-2021 SIP** Outcome 3: Improved Farm Productivity 4. Pests and Diseases • Fewer pest and disease outbreaks • Lower costs of pest and disease management for adopting growers	Ryan Hall’s honours thesis confirmed the transmission of *Alternaria alternata* and *Fusarium oxysporum* via infection through the flowers. This is consistent with international literature. Observations of infected fruit	Appendix 1 Appendix 2 Appendix 3

Outcome	Alignment to fund outcome, strategy and KPI	Description	Evidence
	2022-2026 SIP Outcome 1: Industry supply, production and sustainability 2. Identify and support opportunities to improve productivity and sustainability through effective integrated pest and disease management (IPDM), weed control, soil health and cover crops • Pest and disease management strategies are developed that mitigate crop loss in collaboration with growers • Plantings are optimised for production efficiency, sustainability and improved profitability	noted that some had holes in the apex. It was hypothesised that this would allow infection of mature fruit. However, no positive correlation was found between presence of a hole and development of IFR. We also examined whether fruit with increased numbers of lobes were more likely to develop IFR, but no correlation was found. Further studies into timing of infection found that infection could occur post flowering when fruit were small, but it did not find infection occurring in larger fruit. This shows that control strategies must target flowers and young fruit, not developing and mature fruit.	
Ground-truthed IDM options for control of internal rot in capsicums	**2017-2021 SIP** Outcome 3: Improved Farm Productivity 4. Pests and Diseases • Fewer pest and disease outbreaks • Lower costs of pest and disease management for adopting growers **2022-2026 SIP** Outcome 1: Industry supply, production and sustainability 2. Identify and support opportunities to improve productivity and sustainability through effective integrated pest and disease management (IPDM), weed control, soil health and cover crops • Pest and disease management strategies are developed that mitigate crop loss in collaboration with growers • Plantings are optimised for production efficiency, sustainability and improved profitability	Trials explored whether there were varietal differences in susceptibility to IFR. None of the commercial varieties tested were more resistant than others to development of disease. Numerous biological and chemical spray control trials were conducted. In some cases flowers were also deliberately inoculated with spore suspensions of key IFR pathogens. While a protective trend was noted when plants were pre-inoculated with Trichoderma, none of the tested products significantly reduced IFR compared to controls. While windy conditions were correlated with lower rates of IFR, trials attempting to increase air movement around plants did not significantly reduce IFR. Finally, trials examining spray coverage demonstrated that most commercial spray units do not provide good coverage of	Appendices 4, 5, 6 and 7. Note that none of these have resulted in a reliable and effective IDM strategy for control of IFR in capsicums. Linking fungicide spray coverage (and regularity of application) to actual rates of IFR in red capsicums would confirm whether this is a significant factor increasing incidence of IFR.

Outcome	Alignment to fund outcome, strategy and KPI	Description	Evidence
		the floral stigma. This could explain the lack of efficacy in spray trials.	
Extension of findings to industry with evidence of initial uptake by growers	**2017-2021 SIP** Outcome 5: Improved Industry Capabilities for adoption and innovation 1. Communication and extension • More stakeholders accessing levy-funded research findings and outcomes • More stakeholders engaged in communication and extension events • More growers adopting practice change based on levy funded research projects • Higher economic benefits from adopting practice change, for example, cost savings or higher revenues **2022-2026 SIP** Outcome 3: Improved capability and an innovative culture that maximises investments in productivity and demand and builds a resilient Australian vegetable industry 1. Use extension and communication processes to support industry to achieve supply and demand priorities supporting profitable businesses especially in the areas of new technologies to enhance sustainable production practices, food safety, waste management, biosecurity and use of data to assist with decision-making • Establishment of a baseline and then increased share of industry (hectares) with positive change in knowledge, attitude skills aspiration and practice change and implementation of targeted high priority areas (e.g., food safety, waste management, export capability and decision-making)	The project's initial findings were communicated through a webinar, video interview and two magazine articles. Extension has been greatly limited by the lack of consistent, positive results. Without a reliable, effective IDM strategy to control IFR, there has been no purpose in conducting the planned extension activities.	

Monitoring and evaluation

Table 4: Key Evaluation Questions

Key Evaluation Question	Project performance	Continuous improvement opportunities
Information used by vegetable growers and advisors to manage internal rot of capsicums, reducing rejections by retailers and consumers	Not completed None of the trials resulted in a reliable and effective IDM strategy which could be used by growers.	Further work on spray coverage is essential. It seems extremely likely that poor coverage of the floral stigma and style is key to the lack of efficacy of the many fungicide and biological agents tested during this project. Project outcomes, with a focus on the spray coverage data, will be extended to growers through the Soil Wealth/ICP project. Information will be provided through a webinar, fact sheet, article in Australian Grower magazine and at grower workshops in Stanthorpe and Bundaberg.
Have the causal organism/s been identified?	Two casual organisms were identified using DNA techniques, these are: *Alternaria alternata* and *Fusarium oxysporum*. Initial testing also identified *Alternaria tenuissima*, but further DNA analysis has reverted this into the *A. alternata* complex. Pathogenicity testing confirmed that these organisms can cause IFR.	Further research could examine the factors that catalyse the shift from latent infection to pathogenic disease expression. It is unclear whether this is due to declines in protective compounds within the capsicum, increased availability of carbohydrates in mature fruit, or other factors that trigger changes in gene expression. Metabolomics could be an appropriate tool to examine this issue. While *F. oxysporum* has been confirmed, this group includes more than 100 forma speciales. It is unclear which of these can cause IFR in capsicums.
Has a risk model been developed?	Large amounts of environmental data correlating to expected time of infection of crops and its corresponding infection rate were collected. The data was modelled against known information on rates of IFR, assuming a development time from flower to mature fruit of approximately 2 months. High RH, temperature, rain and dew were not correlated with IFR. However, low average wind speeds were found to be associated with increased IFR.	The risk model could be refined through continuous wind measurements; at most sites only maximum and minimum readings. This could be further correlated with other environmental conditions, including temperature and humidity, as these can surely impact the ability of a spore to germinate and grow through the floral stigma.
Have (2) articles been written for Vegetables Australia?	An article was written for Vegetables Australia and another for its successor publication Australian	A final article on the spray coverage results will be prepared for publication in Australian Grower

Key Evaluation Question	Project performance	Continuous improvement opportunities
	Grower.	magazine through the Soil Wealth ICP project.
Do growers have clear, concise information on strategies to manage internal fruit rot of capsicums?	No. Despite numerous trials at commercial properties and trial sites, the project has not found any reliable and effective strategies to manage IFR in red capsicums.	The results on spray coverage offer a way forward. It seems highly likely that poor spray coverage has been a factor in both the lack of success of trials within this project, and existing commercial management. Results from this project will be communicated through the Soil Wealth ICP project. It is strongly recommended that ongoing research further examine the link between spray coverage and incidence of IFR.
Has a webinar been produced	A webinar was delivered through the Integrated crop Protection project. Additionally, a video interview with project leader Dr Jenny Ekman was conducted at Hort Connections 2023, providing a project update.	A Webinar is planned in February 2025 to provide a summary of the project's outputs and outcomes. This will be hosted through the Soil Wealth / ICP project.
Training and extension activity has increased the skills and knowledge of growers and advisors.	No. Training and extension activities have been extremely limited due to the lack of positive results.	Training and extension on effective spray coverage will be undertaken at grower workshops organised through the Soil Wealth ICP project.
Have the communication activities reached the audience	As noted in the outputs table webinars, existing video interviews and articles have reached the audience.	The new webinar, fact sheet and workshops will be promoted through the Soil Wealth ICP monthly bulletin.
Have the Factsheets, how-to-guides and reports been used	Due to the lack of effective control methods, there has been no fact sheets and how-to-guides based on the project. Reports have been limited to the milestones and associated appendices produced for Hort Innovation.	A new fact sheet on all project results, including the information on spray coverage, will be produced through the Soil Wealth ICP project.
Has new information from the research component been incorporated into delivery activities?	As stated, delivery activities have not been conducted due to the lack of positive results.	Delivery activities through the Soil Wealth ICP project will incorporate all outcomes from this project.

Recommendations

1. **Further Research into New Varieties:**
    - Further investigate capsicum varieties with potential resistance to *Alternaria* spp. and *Fusarium oxysporum*
        - Identify which *F. oxysporum* forma speciales are responsible for IFR.
    - Evaluate physiological traits that may correlate with lower susceptibility. These could include
        - Flower orientation (it may be easier to control IFR in varieties with more upward facing flowers, like some chilli varieties)
        - Style retention (varieties that shed the dead style after pollination may be less susceptible to infection)
        - Distance from retained style to the fruit placenta and seed (long varieties currently only grown in greenhouses may be more resistant than blocky types)

2. Examine agronomic and nutrition factors

- Observation suggests that crops grown with high nitrogen may be more susceptible to IFR; studies could examine the role of calcium in resistance to symptom development
- Determine the causes of “dark seed”; although this is non-progressive and does not affect fruit quality, it is sometimes confused with IFR and limits opportunities for NIR grading

2. **Improving Spray Efficacy:**
    - Conduct further studies to optimize spray parameters, including pressure, nozzle type, distance from the spray unit and application techniques, to ensure better coverage of the floral stigma both while flowering, and on young fruit.
    - Evaluate the integration of air-assisted or precision-targeting spray technologies to enhance coverage and reduce pathogen infection risks.
    - Correlate coverage with rates of IFR in red fruit.
3. **High-Risk Area Harvest Practices:**
    - Recommend growers in regions / at times with high IFR incidence harvest capsicums at half red or “chocolate” stage rather than full red to reduce the likelihood of symptomatic IFR development.
4. **Postharvest Technologies:**
    - Continue investigating Near Infrared (NIR) grading technology to improve its accuracy and calibration, reducing rejection of healthy fruit
    - Investigate novel methods to accelerate postharvest maturation of capsicums harvested when half coloured to chocolate
        - Although capsicums are classified as non-climacteric colour change may be accelerated through exposure to ethylene
        - Products that inhibit ABA (abscisic acid) production are also associated with colour development

Refereed scientific publications

11. Journal article

Journal articles identifying the causal organism in Australia and evidence of infection at early fruit stage are currently being prepared for journal submission.

References

Burgess, L.W., Summerell, B.A., Bullock, S., Gott, K.P., Backhouse, D., 1994, Laboratory Manual For *Fusarium* Research, third ed. University of Sydney, Sydney.

Halfon-Meiri, A and Rylski, I (1983). Internal mould caused in sweet pepper by *Alternaria alternata*: Fungal ingress. Phytopathology 73:67-70.

Simmons, E.G., 2007. *Alternaria*: an identification manual, first ed. CBS Fungal Biodiversity Centre, Utrecht.

Summerell, B.A., Leslie, J.F., Liew, E.C., Laurence, M.H., Bullock, S., Petrovic, T., Bentley, A.R., Howard, C.G., Peterson, S.A., Walsh, J.L., Burgess, L.W., 2011. *Fusarium* species associated with plants in Australia. Fungal Diversity. 46(1), 1-27. https://doi.org/10.1007/s13225-010-0075-8

Intellectual property

No project IP or commercialisation to report

Acknowledgements

The Project would like to acknowledge Dr Kelly Scarlett and PhD candidate Zali Mahoney who provided support in conducting DNA work on the various organisms on this project.

The project would also like to acknowledge David Healey (David Russos Wholesale) for his ongoing support, supplying samples and connections with growers in affected areas. Also Andrew Halpin and Jessy Logan, Bundaberg VegNET officers for their continued support with grower connections and trial establishment and assessment.

A special acknowledgement also to all growers who hosted trials and supplied fruit for the project. Growers in Bundaberg and Stanthorpe (particularly Archies Produce and Patanes) have been extremely generous with their time and products, facilitating trials on varieties, postharvest management and spray methodology.

Appendices

- Appendix 1: Literature review
- Appendix 2: Causes of IFR
- Appendix 3: Timing of infection
- Appendix 4: Variety trials
- Appendix 5: Environmental effects (Confidential)
- Appendix 6: Chemical and biological sprays
- Appendix 7: Spray cover efficacy
- Appendix 8: Postharvest management (Confidential)

Appendix 1 – Literature Review

Summary of the literature

Causal organisms

A number of different fungi are associated with development of internal fruit rot (IFR) in capsicums. Species of *Fusarium* are the most commonly found in studies from Europe and the USA. The main causal organism is frequently cited as *F. lactis* complex (FLASC)[1], although *F. oxysporum*, *F. proliferatum* and *F. solani* are also fairly commonly reported[2].

Alternaria alternata has also been identified as causative several times, although the most recent paper citing it was in 1989[3]. It is noted that *A. alternaria* is also often found externally on damaged parts of fruit, such blossom end rot or sunburn. *A. tenuis* was noted as causative in Israel in 1973[4] but there are no further references to this pathogen in the literature.

It seems apparent that species of *Fusarium* are the major cause of IFR in greenhouse fruit grown in cooler climates, such as Belgium, Korea and Canada. In contrast, *Alternaria* spp. are more common in hot, field environments such as New Mexico and Israel[5].

Mechanism of infection

Infection mainly occurs at flowering, with flowers most susceptible immediately before and after opening. Susceptibility then declines as the flower ages. Young fruitlets are resistant to infection by *A. alternaria*, even though the flower style is still present[4], but may remain susceptible to *Fusarium* spp.[6].

The stigma and style can be colonized by *F. lactis* within half a day of infection, the fungus spreading over the pollen grains and into the ovaries (Figure 1), allowing it to colonize the seeds as they develop[7]. This speed of infection means that removing the dying petals and stigma has no effect on disease incidence[8]. Likewise, flower morphology (large vs small, long lasting vs short-lived) has no effect on rates of IFR[9].

Infection increases flower abscission, although reports vary as to the extent of this effect; Aponyi et al[10] reported that *A. alternata* caused abscission of 8-11% of flowers, whereas Halfon-Meiri[4] reported up to 52% flower drop due to the same pathogen. Infection has been shown to

[1] Yang, J., Kharbanda, P. D., Howard, R. J., Mirza, M., 2009. Identification and pathogenicity of Fusarium lactis, causal agent of internal fruit rot of greenhouse sweet pepper in Alberta. Canadian J. of Plant Pathology. 31, 47–56.

[2] Frans, M., Aerts, R., Van Laethem, S., Ceusters, J., 2017. Environmental effects on growth and sporulation of Fusarium spp. causing internal fruit rot in bell pepper. European J. of plant pathology. 149(4), 875-883.

[3] Bruton, B.D., Chandler, L.D., Miller, M.E., Ramey, H.H., Beaton, P.G., 1989. Relationships between pepper weevil and internal mold of sweet pepper. Plant Disease. 73(2), 170-173.

[4] Halfon-Meiri, A. and Rylski, I., 1973. Penetration of Alternaria tenuis into pepper fruit. Phytoparasitica. 1, 57.

[5] Wall, M.M., Biles, C.L., 1993. Alternaria fruit rot of ripening chile peppers. Phytopathology. 83(3), 324-328.

[6] Utkhede, R.S., Mathur, S., 2004. Internal fruit rot caused by Fusarium subglutinans in greenhouse sweet peppers. Canadian J. of plant pathology. 26(3), 386-390.

[7] Yang, Y., Cao, T., Yang, J., Howard, R.J., Kharbanda, P.D., Strelkov, S.E., 2010. Histopathology of internal fruit rot of sweet pepper caused by Fusarium lactis. Canadian J. of Plant Pathology. 32(1), 86-97.

[8] Frans, M., Aerts, R., Kurt, H., Van Poucke, K. 2012. Sustainable control of internal fruit rot in bell peppers. Comm. Agric. Applied Biological Sci.

[9] Frans, M., Aerts, R., Van Calenberge, B., Van Herck, L., Ceusters, J., 2016. Influence of floral morphology and fruit development on internal fruit rot in bell pepper (Capsicum annuum). Acta Hort. 1144: 199-206.

[10] Aponyi, I., Kujáni, O., Varga, B.B.A., 1987. The ovary moulding disease of tomato-shaped pepper and control possibilities. In I International Symposium on Vegetables for Processing 220, 401-406.

destroy the pollen tubes within the flower (Figure 2), an observation which supports the previous reports that high disease loads significantly reduce yield as well as fruit quality[11].

While it is possible for infection to occur directly through lesions in the fruit surface, this is likely to result in visible damage that makes fruit unsuitable for retail sale. In the USA, there was found to be a correlation between pepper weevil damage and occurrence of IFR[3]. It is also possible for spores to transfer to the inside of the fruit if the stylar end is incompletely closed; fruit with an open stylar end has been reported to have increased incidence of IFR (M. Frans, pers. com.), which may reflect air-borne spore contamination.

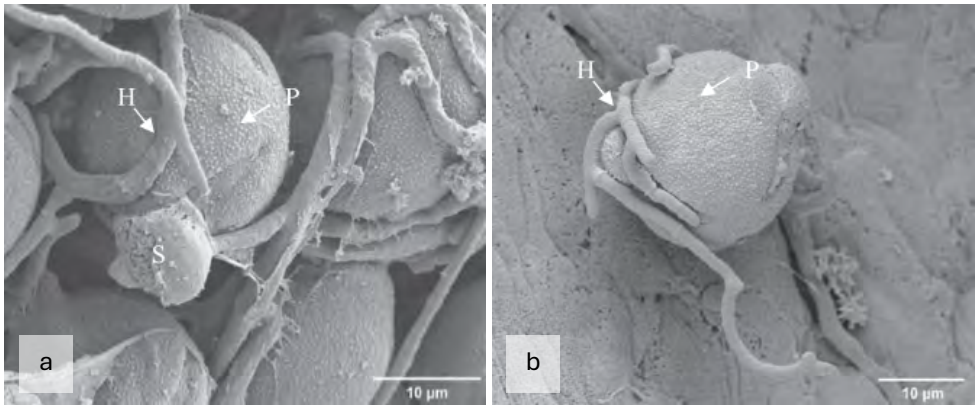


Figure 1. Scanning electron micrographs of germinating F. lactis spore (S) and extensive hyphal growth (H) on the stigma and pollen grains (P) (a), and colonisation of a pollen grain by hyphae (b). From Yang et al., 2010.

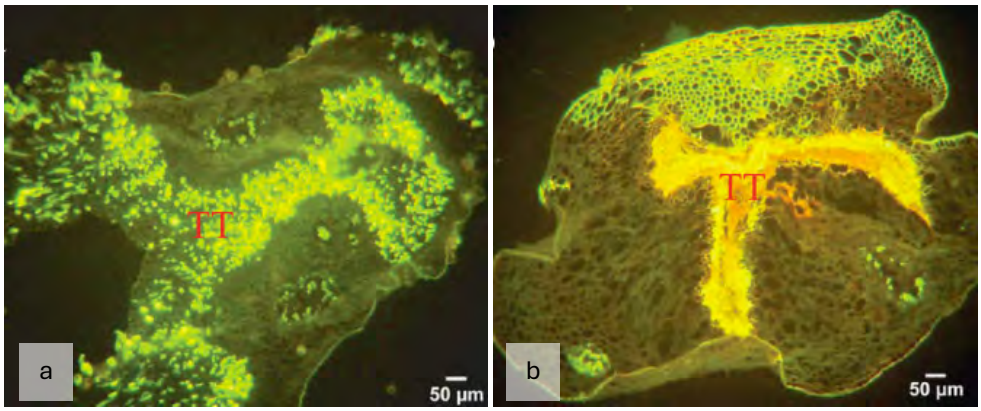


Figure 2. Fluorescence micrographs of a cross section of a style in a healthy (a) and F. lactis infected (b) flower. The green fluorescent spots indicate healthy transmitting pollen tubes, yellow staining indicates dying tissue, red indicates full necrosis. From Yang et al., 2010.

Environmental factors

Fusarium species generally grow optimally and produce most spores at around 25°C[2]. FLASC can grow well at a wide range of pH (approximately 3 to 7), with an optimum around 5. *A. alternata* tends to grow fastest at slightly higher temperatures of 25 to 30°C[12], and prefers a more neutral pH of 6.5. Like most fruit, capsicums tend to be slightly acidic during development, with pH increasing as the fruit ripen.

Moisture and humidity are key to infection. However, rainfall is not a good predictor of rates of IFR; levels of IFR were much higher in a dry season in New Jersey compared to a wet season

[11] Halfon-Meiri, A., Rylski, I., 1983. Internal mold caused in sweet pepper by Alternaria alternata: Fungal ingress. Phytopathology. 73(1).

[12] Hubballi, M., Nakkeeran, S., Raguchander, T., Anand, T., Samiyappan, R., 2010. Effect of environmental conditions on growth of Alternaria alternata causing leaf blight of noni. World J. of Agricultural Sciences. 6(2), 171-177.

with triple the rainfall[13]. This is supported by observations from Australian growers; during the 2019 season Bowen grown capsicums suffered high rates of IFR despite drought conditions, whereas fewer issues were encountered during the wetter conditions that occurred during 2020 (D. Healey, pers. com.).

High relative humidity allows rain or dew to remain longer on the flowers, favoring spore germination and, therefore, infection. Frans et al[14] found that RH was critical to infection by FLASC at lower temperatures, but had less effect at over 22°C, presumably due to the rapidity with which the fungal spores could germinate and enter the pollen tube. A similar result was reported by O'Neill and Mayne[15], who found little relationship between RH and infection when temperatures were approx. 22°C or higher. Belgian researchers have developed a greenhouse-based model predicting the likelihood of IFR in capsicums at different temperatures and RH. This strongly suggests that temperature is more important than RH, especially if average temperatures are high and infection has already occurred previously (i.e. spores are present). However, it should be noted that conditions inside a greenhouse are very different to those in the open field.

I	Temperature	Relative humidity (%)										
	(°C)	50	55	60	65	70	75	80	85	90	95	100
0	16	0	0	0	0	0	0,1	0,1	0,2	0,3	0,5	0,6
	17	0	0	0	0	0	0,1	0,1	0,2	0,4	0,5	0,7
Internal fruit rot **absent** 1 week earlier	18	0	0	0	0	0,1	0,1	0,2	0,3	0,5	0,6	0,8
	19	0	0	0	0	0,1	0,1	0,2	0,4	0,5	0,7	0,8
	20	0	0	0	0,1	0,1	0,2	0,3	0,5	0,6	0,8	0,9
	21	0	0	0	0,1	0,1	0,2	0,4	0,5	0,7	0,8	0,9
	22	0	0	0,1	0,1	0,2	0,3	0,5	0,6	0,8	0,9	0,9
	23	0	0	0,1	0,1	0,2	0,4	0,6	0,7	0,8	0,9	0,9
	24	0	0,1	0,1	0,2	0,3	0,5	0,6	0,8	0,9	0,9	1
	25	0	0,1	0,1	0,2	0,4	0,6	0,7	0,8	0,9	0,9	1
1	16	0,4	0,4	0,4	0,3	0,3	0,3	0,3	0,3	0,3	0,3	0,3
	17	0,5	0,4	0,4	0,4	0,4	0,4	0,4	0,4	0,4	0,4	0,3
Internal fruit rot **present** 1 week earlier	18	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,4	0,4	0,4
	19	0,6	0,6	0,6	0,6	0,6	0,6	0,6	0,5	0,5	0,5	0,5
	20	0,7	0,7	0,7	0,7	0,7	0,6	0,6	0,6	0,6	0,6	0,6
	21	0,8	0,8	0,8	0,7	0,7	0,7	0,7	0,7	0,7	0,7	0,7
	22	0,8	0,8	0,8	0,8	0,8	0,8	0,8	0,8	0,8	0,8	0,7
	23	0,9	0,9	0,9	0,9	0,8	0,8	0,8	0,8	0,8	0,8	0,8
	24	0,9	0,9	0,9	0,9	0,9	0,9	0,9	0,9	0,9	0,9	0,9
	25	0,9	0,9	0,9	0,9	0,9	0,9	0,9	0,9	0,9	0,9	0,9

Figure 3. Predicted probabilities of IFR in a greenhouse at varying mean weekly temperatures and RH with and without prior detection of FLASC. From Frans et al., 2018.

Control strategies

Keeping flowers dry reduces infection rates, as water activity below 0.85 inhibits fungal germination and growth. This is clearly difficult to achieve for field grown crops, where rainfall

[13] Kline, W.L. and Wyenandt, C.A. 2013. Internal fruit rot and premature seed germination of field grown colored peppers. HortScience 48:9S. S5-S6.

[14] Frans, M., Moerkens, R., Van Gool, S., Sauviller, C., Van Laethem, S., Luca, S., Aerts, R., Ceusters, J., 2018. Modelling greenhouse climate factors to constrain internal fruit rot (Fusarium spp.) in bell pepper. J. of Plant Diseases and Protection. 125(4), 425-432.

[15] O'Neill, T., Mayne, S., 2015. Sweet pepper: Aspects of the biology and control of fusarium fruit rot. Communications in agricultural and appl. biological sciences. 80(3), 569-573.

and dew are likely to provide conditions conducive to infection. While it would seem reasonable that a shift from overhead to drip irrigation could potentially provide some benefits, no information could be found in the literature examining the effect of irrigation or field management (e.g. plant spacing) on infection rates.

A range of fungicides has been tested for control of both *Fusarium* spp. and *A. alternata* on capsicum fruit and chillies. While a number have been shown to be effective, virtually all of these studies have examined the external symptoms of fruit rot, rather than IFR[16]. Nonetheless, some data is available. One study by Aponyi et al[17] studied examined the effect of fungicide applications at the start and end of flowering, determining that Sumilex 50WP and Rovral Flow provided 70-90% protection against IFR caused by *A. alternata*. Utkhede and Mathur[18] also applied fungicides at the start of flowering, in this case inoculating the flowers with *F. subglutinans* one day later. Rovral and BASF-516 both significantly reduced incidence of IFR, with Rovral also increasing average fruit weight.

Capsicums flower over a long period, and there can be flowers and fruit on plants at the same time. This can make it difficult to use fungicides effectively without increasing residues in harvested fruit[14]. Frans et al[19] screened a large number of antagonistic fungi for control of FLASC, finding two isolates of *Gliocladium roseum* which significantly inhibited infection in greenhouse trials. Utkhede and Mathur[18] instead took the approach of using commercially available biological controls; PlantShield (*Trichoderma harzianum*), SoilGard (*Gliocladium virens*), PreStop (*Gliocladium catenulatum*), Quadra-136 (*Bacillus subtilis*) and Mycostop (Stretomyces griseoviridis) were tested for control of *F. subglutinans*. PreStop and Quadra-136 provided similar control to Rovral, although it should be noted that none of the tested treatments provided full control[18]. Moreover, biological controls are usually more effective in a greenhouse situation than an open field.

One promising area for improved management appears to lie in cultivar resistance. IFR generally remains latent – but detectable through plating – in green fruit, only to develop as the fruit ripen. It seems possible that fungal inhibition and its retention at maturity may differ between varieties. A rapid screening test for such variation suggested that, for example, “Red Savina” chillies retained their resistance to FLASC as they ripened, whereas “Yellow Goronong” chillies became many times more susceptible. While several capsicum varieties were equally resistant while immature, “Sensatio” was significantly more susceptible than “Redwing” at maturity [20]. Similarly, “Bison” and “Mazurka” were less susceptible to *F. subglutinans* than “Sympathy” and “444”[6]. While this work was conducted on greenhouse varieties, it seems likely similar effects could be found for field grown fruit. Indeed, growers have consistently reported that blocky type capsicums are more likely to have IFR that thinner skinned, elongated varieties (D. Campbell, pers. com.)

---

[16] Ginoya, C.M., Gohel, N.M., 2015. Evaluation of newer fungicides against Alternaria alternata (Fr.) Keissler causing fruit rot disease of chilli. International J. of Plant Protection. 8(1), 169-173.

[17] Aponyi, I., Kujáni, O., Varga, B.B.A., 1987. The ovary moulding disease of tomato-shaped pepper and control possibilities. In I International Symposium on Vegetables for Processing 220, 401-406.

[18] Utkhede, R.S., Mathur, S., 2005. Biological and Chemical Control of Fruit Rot in Greenhouse Sweet Peppers (Capsicum annum L.) Caused by Fusarium subglutinans. J. of Biological Sciences. 5(5), 610-615.

[19] Frans, M., Moerkens, R., Van Laethem, S., Aerts, R., Ceusters, J. 2020. Biological control of Fusarium spp. in bell pepper fruit using Gliocladium species. ActaHort. 1269:59-66.

[20] Frans, M., Aerts, R., Van Laethem, S., Van Calenberge, B., Van Herck, L., Heungens, K., Van Poucke, K., Van Gool, S., Ceusters, J., 2016b. Development of a quick screening bioassay for internal fruit rot in bell pepper (Capsicum annuum L.). In Proceedings of XVIth EUCARPIA Capsicum and Eggplant Working Group Meeting 12-14 September 2016, Kecskemét, Hungary. 420-424.

Food safety

Both *Alternaria* spp. and *Fusarium* spp. can produce mycotoxins, which have been shown to have harmful effects on health as well as being possibly carcinogenic. *A. alternata* is an important source of such toxins, although human toxicity data is lacking[21]. Mycotoxins are generally stable during storage and cooking. Although some degradation can occur during drying, there has often been found to be a higher level of mycotoxin contamination in concentrated dried and ground products. Gambacorta et al found significant levels of certain *Alternaria* mycotoxins in fresh capsicums, with little or no correlation between visible mould and detection of toxins[22].

Mycotoxins can also be detected in fruit tissues that are have only very slight symptoms of infection by *F. lactis*, *F. proliferatum* or *F. verticilloides* although it is noted that more diseased samples have higher levels. Yang et al [23] found three different mycotoxins in fruit with slight symptoms of IFR collected from a commercial greenhouse. They state that simply removing the diseased tissues before the fruit is eaten will not eliminate the mycotoxins, as they are also present in symptomless tissue. It is concluded that IFR is not only an issue of quality and economics but poses a significant food safety risk.

It is unclear what threat to human health exists from the levels of mycotoxins found in fresh capsicums, given the quantities in which they are consumed. It was also unclear from the literature whether IFR that is present only on the seeds and placenta of capsicum fruit can result in presence of mycotoxins in the flesh. However, the detection of these toxins in what appears to undamaged flesh next to infected tissue certainly raises food safety concerns, and further increases the importance of controlling this disease.

Summary and Conclusions

Internal fruit rot can be caused by a number of different species of *Fusarium* and *Alternaria*. However, the key pathogens worldwide are *F. lactis* complex (FLASC) and *A. alternata*. FLASC is predominantly an issue in greenhouse production, whereas *A. alternata* appears to more frequently cause disease in field crops grown in hotter climates. Recent research has focused almost entirely on *Fusarium* spp., which is likely reflects the intensity of production.

Infection almost entirely occurs at flowering. Spores landing on the stigma of the flower rapidly germinate and grow, colonizing the pollen grains and tubes and spreading into the developing ovary. There is evidence that infection increases flower abscission, reducing yield. While moisture is critical for infection, temperature appears to be a more important determinant of infection rates, especially if spore loads are high. The disease usually remains latent in developing fruit until maturity, when it grows and spreads over the seeds, placenta and flesh.

Fungicides have limited effectiveness against IFR. Application before and during flowering is essential, which is challenging given capsicum's long flowering period and simultaneous presence of flowers and fruit. While there has been some success using biological controls,

[21] Escrivá, L., Oueslati, S., Font, G. and Manyes, L., 2017. Alternaria mycotoxins in food and feed: an overview. J. of Food Quality, 2017

[22] Gambacorta, L., Magista, D., Perrone, G. et al. 2018. Co-occurrence of toxigenic moulds, aflatoxins, ochratoxin A, Fusarium and Alternaria mycotoxins in fresh sweet peppers (Capsicum annuum) and their processed products. World Mycotoxin J. 11(1), 159-173.

[23] Yang, Y., Bouras, N., Yang, J., Howard, R.J., Strelkov, S.E. 2011. Mycotoxin production by isolates of Fusarium lactis from greenhouse sweet pepper (Capsicum annuum). Int. J. Food Microbiol. 151(2), 150-156.

these have been focused on greenhouse production and are likely to be less effective in the field. Perhaps the most promising area of control is varietal resistance. Developing capsicums are resistant to the disease; varieties that retain this resistance until full maturity may be less susceptible to mould development.

Controlling IFR is critical not only because of the effect on quality and returns to growers, but also due to potential effects on food safety. Both *Fusarium* spp. and *A. alternata* can produce mycotoxins that affect human health. These may be present in what appears to be undamaged flesh, suggesting that simply removing the mouldy part will not guarantee the edible part is toxin-free. Moreover, mycotoxins are stable during cooking and even processing. While it is not clear whether the amounts that are present in capsicums represent a threat to human health, this is certainly another important reason to find ways of controlling this disease.

Comprehensive technical review – Identification of the causal organism of internal fruit rot in capsicums (*Capsicum annum*) in Australia

By Ryan Hall, May 2020

Abstract

Internal fruit rot of capsicums (*Capsicum annum*) is a disease that causes mycelia growth on the internal parts of a capsicum with rarely any external symptoms. It has been reported around the world being caused by a range of different organisms, mainly *Fusarium lactis* and *Alternaria alternata*. While *A. alternaria* was reported earlier in the study of this disease it has not been reported since 1989. An outbreak of the disease in the early 2000s has prompted much study into *F. lactis* which has recently been identified as the main causal organism in Europe, Canada and Korea. Genetic identification methods have been developed for both *Fusarium* and *Alternaria*. There is currently no literature on the disease in Australia. Infection has been confirmed to occur via the stigma and style at flowering. The disease then enters a latent stage where no or very limited growth occurs until maturity and harvest. Mycotoxins have been associated with organisms that cause the disease, notably *Fusarium lactis* and *Fusarium proliferatum* which may pose a risk to human health. Modelling, bioassays and a range of chemical and biological controls have been developed or tested to help manage the disease. A growing understanding of the disease and its causal organism(s) is allowing for the development of more complex tools that reduce the incidence of the disease and reduce the number of consumers impacted by infected fruit.

Introduction

The capsicum (*C. annuum*) industry is a high value horticultural industry within Australia. Over 77,000 tonnes were produced in 2018/2019 with a gross farm gate value of AUD$171 million. In addition, 2,200 tonnes of chilli's valued at $9 million are also produced each year (HORT Innovation, 2019).

Capsicum crops suffer from a range of diseases; however, the IFR of mature fruits is particularly difficult to manage due to the lack of external symptoms (Utkhede and Mathur, 2004; Yang et al., 2009). Periods of bad infection have resulted in drops in consumer confidence and purchases as infected fruits are able to pass through quality assurance checks and enter the retail market (Utkhede and Mathur, 2004; Yang et al., 2009). As a result, crops may be downgraded or destroyed.

The disease presents as necrosis and sometimes mycelial growth on the internal parts of the fruit, generally the seed and placenta, with some infection also occurring on the internal parts of the pericarp (figure 1 and figure 2) (Leyendecker Jr, 1954; Halfon-Meiri and Rylski, 1983; Utkhede and Mathur, 2004). Some of the organisms that have been identified as associated with this disease are able to produce mycotoxins potentially hazardous to human health (Van Poucke et al., 2012; da Cruz Cabral et al., 2016).

Infection is thought to occur during flowering; however, the pathogen remains in the latent stage until fruit matures and ripens, often after harvest (Halfon-Meiri and Rylski, 1983; Utkhede and Mathur, 2004; Yang et al., 2010). A collection of different fungi have been found to cause the

disease, notably *Fusarium* spp. and *Alternaria* spp. (Table 1; Leyendecker Jr, 1954; Halfon-Meiri and Rylski, 1983; Utkhede and Mathur, 2004).

Research on the disease goes back as far as 1949, where both *Alternaria* and *Fusarium*, among other microbes, were identified (Leyendecker Jr, 1954). *Alternaria* spp. were identified as causing the disease in the 1970s and '80s (Halfon-Meiri and Rylski, 1973; Halfon-Meiri and Rylski, 1983; Aponyi et al., 1987). However, in the early 2000's significant outbreaks occurred in Canada. These were identified as being caused by as *F. subglutinans* (Utkhede and Mathur, 2004; Mathur and Utkhede, 2004). *Fusarium* was also found in samples in the Netherlands; F. proliferatum, F. solani and a *Fusarium* spp. similar to *F. lactis* were isolated in the Netherlands (Hubert et al., 2003 as cited by Van Poucke et al., 2012 p.g. 29). *F. lactis* was also identified as the main cause of the disease in a study conducted in 2004 to 2005 (Kharbanda et al., 2006). Utkhede and Mathur's initial findings were reclassified as *F. lactis* after molecular study of the pathogens (Yang et al., 2009). In 2007, surveys in the UK reported IFR in 1 to 37% in crops, mainly as *F. lactis* and *F. oxysporum* (O'Neill and Mayne, 2015). South Korea has recorded the disease in the form of *F. lactis* in 2010 (Choi et al., 2011). No research on the disease has been published in Australia despite the clear spread to other countries.

Growers, especially those in warm and humid growing regions that are conducive to pathogen growth (Frans et al., 2017; Hubballi et al., 2010), face a major ongoing issue. Australian growers have very limited information on the problem available to them. It is unknown what organism causes the disease in Australia, significantly limiting the ability of producers to manage the disease.

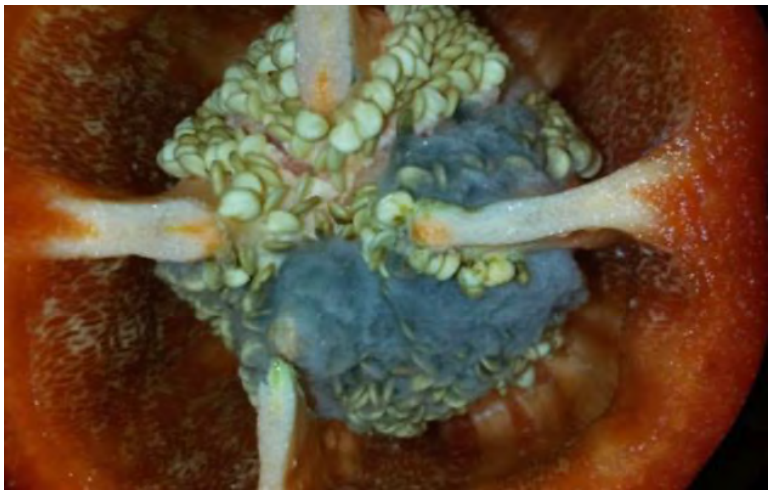


Figure 1. Internal fruit rot of C. annuum by Alternaria spp. showing infection of the placenta and seed by mycelium.

Figure 2. Internal fruit rot caused by Alternaria spp. showing infection of the blossom end of C. annuum by mycelium but with limited infection of seeds or placenta.

This review provides an overview of which organism(s) cause IFR, food safety issues associated with those microbes, what conditions favour infection, how it is thought to occur and finally different methods of control that have been proposed for the disease.

The review is broken into four parts. Causal organisms that have been isolated and reported in the literature are reviewed and compared, and then conditions for infection for the different organism are reviewed. After this how the infection is thought to occur is examined and finally, the controls that have been suggested or that are being developed are reviewed.

Known causal organisms of internal fruit rot

Fusarium spp

Fusarium spp. have been known to cause IFR in capsicum since the disease was first identified in 1948 as F. *moniliforme* and *F. roseum* (Leyendecker Jr, 1954). *Fusarium* spp. were detected in small numbers by a study conducted in Hungary from 1984 to 1986 but were not the most significant pathogen (Aponyi et al., 1987).

Researchers over the past 20 years in Europe and North America have identified a few different species of *Fusarium* that cause IFR, these being *F. proliferatum*, *F. solani*, F. *oxysporum* and *F. lactis* (Utkhede and Mathur, 2004; Mathur and Utkhede, 2004; Hubert et al., 2003 as cited by Van Poucke et al., 2012 p.g. 29; Yang et al., 2009; O'Neill and Mayne, 2015). The main causal organism among these is *F. lactis* in the form of the *F. lactis* species complex (FLASC) (Yang et al., 2009). *F. lactis* was initially isolated from milk (Pirotta and Riboni, 1879 as cited by Leslie and Summerell, 2008 p.g 188). The fungus has had other hosts, notably figs (*Ficus carica*) in the United States of America (Leslie and Summerell, 2008).

Initially identified as F. *subglutinans* it was later corrected to FLASC through genetic examination of the samples (Utkhede and Mathur, 2004; Mathur and Utkhede, 2004: Yang et al., 2009). The genes for β-tubulin, mitochondrial small subunit ribosomal DNA (mtSSU) and elongation factor 1-α were examined for their nucleotide sequences, these were then used to accurately identify various *Fusarium* spp. (Yang et al., 2009).

Some concern exists over the production of mycotoxins by FLASC. These mycotoxins could be potentially hazardous to humans if consumed (Van Poucke et al., 2012). A study on the mycotoxins produced by FLASC; found that while beauvericin and fumonisin were produced by

F. lactis not all isolates produced the mycotoxins (van Poucke et al., 2012). Another study found that *F. proliferatum* involved with internal fruit rot also produced beauvericin and fumonisins B1, B2 and B3. The beauvericin was restricted to lesion, but the fumonisins were found in the surrounding tissues meaning even if the affected area was removed, mycotoxins would still be consumed (Monbaliu et al., 2010). However, the disease can present in a range of severities, with the disease sometimes not being readily visible and detectable (Yang et al., 2010). This challenges earlier studies where the disease was not isolated from fruits without internal symptoms (Utkhede and Mathur, 2005).

Moreover, mycotoxins are generally stable with only modest reductions even if the product is cooked (Luz et al., 2017). This suggests that mycotoxins may be consumed without consumer knowledge.

Table 1. Causal organism of IFR fruit rot of capsicums showing country of infection and when infection was reported in a published piece of literature

Causal organism	Location	Year of paper	Citation
Fusarium moniliforme *Fusarium roseum* *Alternaria solani* *Alternaria tenuis* *Hormodendrum cladosporioides*	USA	1954	(Leyendecker Jr, 1954)
Alternaria tenuis	Israel	1973	(Halfon-Meiri and Rylski, 1973)
Alternaria alternata	Israel	1983	(Halfon-Meiri and Rylski, 1983)
Alternaria alternata *Botrytis cinerea* *Cladosporium spp.*	Hungary	1987	(Aponyi et al., 1987)
Alternaria alternata	USA	1989	(Bruton et al., 1989)
Fusarium subglutinans	Belgium/Netherlands	2004	(Utkhede and Mathur, 2004)
Fusarium lactis *Fusarium solani*	Canada	2006	(Kharbanda et al., 2006)
Fusarium lactis *Fusarium solani* *Fusarium proliferatum* *Fusarium oxysporum*	Canada	2009	(Yang et al., 2009)
Fusarium lactis	Canada	2010	(Yang et al., 2010)
Fusarium lactis	Korea	2010	(Choi et al., 2010)
Fusarium lactis *Fusarium oxysporum*	UK	2015	(O'Neill and Mayne, 2015)
Fusarium lactis *Fusarium oxysporum* *Fusarium proliferatum*	Belgium	2017	(Frans et al., 2017)

Of the species of *Fusarium*, *F. proliferatum*, *F. oxysporum*, *F. solani*, and even *F. subglutinans* are all present in Australia. There are no formal reports of *F. lactis* being present in Australia (Summerell et al., 2011).

Alternaria spp.

Compared to *Fusarium*, there are significantly fewer incidences of *Alternaria* spp. causing IFR in capsicums. There have been no reports of the fungus causing internal fruit rot since 1989 (table 1). The fungus is associated with many other diseases in capsicum, notably an external fruit rot caused by *A. alternata* (Wall and Biles, 1993). The first incidence of the disease was found internally in the fruit was with dried chilli peppers (C. annum) in the United States of America in 1949 (Leyendecker Jr, 1954). A range of pathogens appeared to be causing IFR in this study with two *Alternaria* spp. being identified, *A. solani* and *A. tenuis* (Leyendecker Jr, 1954). However this study had some differences with modern findings of the disease, namely the impact of frost increasing incidence of the disease and the inoculating methods focusing on mechanical injury of the fruit. The *Alternaria* spp. identified could directly penetrate the pericarp of the fruit in some cases where the surface of the fruit was in contact with moisture for long periods of time (Leyendecker Jr, 1954).

In 1973, *A. tenuis* was identified inside the fruit without external symptoms (Halfon-Meiri and Rylski, 1973). While *A. tenuis* is the species studied in this initial paper, a later survey and study by Halfon- Meiri and Rylski in 1983 identified *A. alternata* as the causal organism in Israel. In 1987, a study on tomato shaped peppers in Hungary also found that *A. alternata* caused the disease in around 76-91% of samples assessed (Aponyi et al., 1987). The disease was identified in 1989 in the USA and was thought to be spread by pepper weevil (*Anthonomus eugenii cano*) damage. However, infection of the stigma and style through airborne vectors was observed (Bruton et al., 1989). While pepper weevil may be a vector for the disease, the damage that impacts the fruit is inconsistent with other studies which all note that no external symptoms were visible. From the studies assessed by this review *A. alternata* has been identified as the main casual organism of *Alternaria* spp.

While most identification of *Alternaria* spp. has been conducted by morphological studies, more recent studies have developed genetic methods for identification. The use of the plasma membrane ATPase as a method of genetically identifying *Alternaria* spp. has been established as a useful tool, future study of *Alternaria* spp. causing internal fruit rot should be identified using this system (Eflar et al., 2019).

Although no research has been conducted on internal fruit rot causing *Alternaria* spp. pathogens, a study has been done on fruit mould *Alternaria* spp. notably *A. alternata*. The organism can produce tenuazonic acid, alternariol, alternariol monomeathyl ether, altenuene and tentoxin (de Cruz Cabral et al., 2016). Tenuazonic acid has minimal degradation with heat over a period of 30 to 90 minutes however; both alternariol and alternariol monomeathyl do not degrade with heat unless pressure is applied (Combina et al., 1999). Dry baking has shown to cause some degradation of alternariol, alternariol monomeathyl ether and altenuene, while wet baking does not cause any degradation (Siegel et al., 2010).

Unfortunately limited research has been done on the degradation of mycotoxins produced by Alternaria spp. due to the effects of heat treatments that imitate cooking conditions (Escrivá et al., 2017). There is reasonable concern that unknown consumption of mycotoxins by

consumers would occur in both cooked and uncooked capsicums that are infected by *A. alternata*.

A. alternata has been reported for some time by both grains and horticultural industries in Australia (Webley et al., 1997: Ash and Lanoiselet, 2001). *A. solani* is present in Australia, notably on potatoes; another solanaceae crop (Horsfield et al., 2010).

Other potential pathogens

Botrytis cinerea has been isolated from the trials conducted in Hungary, mostly alongside *Alternaria* spp. (Aponyi et al., 1987). This study also found small amounts of *Cladosporium* spp. and various saprophyte bacteria (Aponyi et al., 1987).

Leyendecker Jr (1954) isolated 21 different organisms. Most were *Fusarium*, *Alternaria*, and notably *Hormodendrum cladospoarioides* however; given this range of organisms isolated it can be assumed that many are secondary pathogens not responsible for the disease. Conditions for the trial were also different to modern studies, with a focus being placed on the impact of frost and inoculation conducted via the penetration of the pericarp (Leyendecker Jr, 1954). It should be noted that these species, other than *A. tenuis*, have rarely been isolated in subsequent pathology trials on IFR of *C. annuum*.

Conditions for the disease

Environmental conditions favouring Fusarium spp. growth

Some research has been conducted on the conditions for the disease when *Fusarium* is the causal organism; however less information is available for *Alternaria* or other causal organisms.

The disease has been known to increase when heavy rainfalls occurred before harvest (Kline and Wyenandt, 2014). In addition, F. *oxysporum*, *F. proliferatum* and FLASC grow optimally at 25°C. This temperature has also been shown to produce the highest number of spores for FLASC which may assist in its spread through greenhouses or fields (Frans et al., 2017).

FLASC has a broad pH range of 3 to 7 in which it can grow. *F. oxysporum* preferred pH 4-7 and F. proliferatum grew at only pH 5-8, both more concise ranges. However, for all *Fusarium* spp. mentioned here the optimal pH was found to be 5 (Frans et al., 2017).

Reductions in oxygen below 20% impacted on the growth rate of FLASC and *F. oxysporum* but lead to a slight increase in *F. proliferatum* (Frans et al., 2017). The authors suggest that using modified atmospheres to reduce the O_2 concentration inside the fruit could inhibit development of postharvest IFR. However, capsicums have limited gas permeability and, therefore, variable responses to modified atmospheres. It is likely to be difficult to avoid development of internally anaerobic conditions, which would negatively impact on flavour and quality.

Environmental conditions favouring Alternaria alternata growth

Some research has been done on *A. alternata* and the conditions that influence its growth. Similar to the Fusarium species, temperatures that are most productive for mycelial growth range from 25°C to 30°C with the optimal temperature being 27°C (Misaghi et al., 1978; Hubballi et al., 2010). The optimal pH differed from that of Fusarium, with *A. alternata* achieving optimal

mycelia growth at 6.5 pH (Hubballi et al., 2010). Unlike the *Fusarium* spp., *A. alternata* only saw limitation to mycelia growth when there was no oxygen present, with this also reduced sporulation (Misaghi et al., 1978).

Capsicum variety

When initially studied in Canada a range of *C. annuum* cultivars were inoculated with what was thought to be *F. subglutinans*. The results of that study showed some varieties were less vulnerable to internal fruit rot (Utkhede and Mathur, 2004: Kharbanda et al., 2006).

A study of the impacts of flower morphology and fruit development on disease found that flowers with larger ovaries, a greater number of petals and exposed anther crowns did not have higher incidences of the disease. Longer wilting periods for the reproductive parts of the flower and petals also had no effect on incidence (Frans et al., 2016a).

Low fruit loads on one of the varieties appeared to increase disease incidence but this was not confirmed by the other variety (Frans et al., 2016a). This result could suggest that some flowers abscise after infection as observed in previous studies (Halfon-Meiri and Rylski, 1983); however more research is required to determine this.

The results of this study confirm there are differences in susceptibility between the varieties, however it was unable to confirm that floral morphology, longevity or fruit development had any effect on susceptibility (Frans et al., 2016a). This area of the literature is very limited; an understanding of why different varieties are more or less susceptible has currently not been determined.

How infection occurs

Where infection occurs

Infection of flowers, as a mode of entry for *A. alternata*, has been known since the early 1980's (Halfon-Meiri and Rylski, 1983). This differed from previous studies on the subject, where it was believed that infection occurred through the pericarp (Leyendecker Jr, 1954). Infection via flowers has been challenged in the USA where it is thought that the pepper beetle (*Anthonomus eugenii cano*) causes damage to the pericarp allowing entry of pathogens into the fruit (Bruton et al., 1989).

A study comparing mechanical insertion of *Fusarium* inocula through the fruit and inoculation of flowers revealed that more fruit developed IFR when infection occurred at flowering (Utkhede and Mathur, 2004). Further research into flower inoculation led to the hypothesis that specialised gynoecial entry was being used by the fungus to infect the fruit (Yang et al., 2010).

The use of scanning electron microscopy found that the stigma and style can be colonised by F. lactis in as little as 12 hours after infection (Yang et al., 2010). One day after inoculation, spores were produced and began colonising pollen grains in the inoculated flower (Yang et al., 2010). This is supportive of the initial symptoms observed by Halfon-Meiri and Rylski (1983) who detected browning and blackening of stigma tissue 1 to 2 days after inoculation. These results are also similar to the observations of *A. alternata* gaining entry into the fruit via the stigma and style (Aponyi et al., 1987). After 5 to 6 days, growth inside the style and the ovary becomes

apparent, confirming that infection occurs through the micropyle following pollen tubes of the plant at anthesis (Yang et al., 2010).

Potential vectors of disease

Internal infection of seeds has been observed, including from asymptomatic fruits. It is likely that infected seed has been a vector for the disease (Kharbanda et al., 2006; Yang et al., 2010). Scanning electron microscopy has identified fungal hyphae being transported with infected pollen by bees, showing that pollinating insects are also a likely vector of the disease (Kharbanda et al., 2006).

Fusarium spores have also been found in the air of greenhouses affected by the disease, suggesting that airborne infection is also possible (Kharbanda et al., 2006).

Controls

Cultural controls and modelling

Cultural controls can be used to keep water activity below 0.85 on the flowers, as water activity below this value is not supportive for *F. lactis* growth (Frans et al., 2017). This can most easily be accomplished by keeping flowers dry, however for field capsicums this may prove difficult to control unless crops are grown in seasons or regions where rainfall is expected to be low. A prediction model as been developed for Belgian and Dutch greenhouse conditions that could successfully interpret temperature and relative humidity data and suggest if infection were occurring (Frans et al., 2018). Field grown capsicum models do not currently exist and would be an area where further research could occur.

Chemical and biological applications

A range of biological and chemical controls for what was thought to be *F. subglutinans* were studied, showing that preventative fungicides and biological treatments applied to flowers were able to lessen the occurrence of the disease (Utkhede and Mathur, 2005). The bacteria Bacillus subtilis and the fungus *Gliocladium catenulatum*, in Quadra-137 and PreStop® respectively, were somewhat effective in reducing disease incidence when added to the flowers (Utkhede and Mathur, 2005).

Rovral® (Iprodione) and BASF-516 (boscalid and pyraclostrobin) were effective chemical controls of the disease, (Utkhede and Mathur, 2005). None of the controls studied by Utkhede and Mathur (2005) have been registered for use in capsicums in Australia (APVMA, 2020). The confusion over *F. subglutinans* and *F. lactis* may require a study to confirm the effectiveness of the treatments, and these chemicals would need to be registered for use on capsicums in Australia before they would provide any benefit to Australian capsicum growers.

A range of fungicides have been tested for their effectiveness of controlling fruit rot caused by A. alternata (Aponyi et al., 1987; Ginoya and Gohel, 2015). Tebuconazole (50%) + Trifloxystrobin (25%) (75 WP) was able to control over 80% of the fruit rot while Hexaconazole (5 EC) was able to control 72% (Ginoya and Gohel, 2015). It should be noted that these were applied at first sign of disease and not as preventative fungicides (Ginoya and Gohel, 2015). Procymidone, iprodione and polyoxin B have been found to effectively decrease the incidence of the disease

caused by *A. alternata* and *B. cinerea* if applied at the start of flowering (Aponyi et al., 1987). None of these fungicides are currently registered for use in Australia on capsicums (APVMA, 2020).

Variety Tolerance

There is some evidence that there are differences in varietal susceptibility (Utkhede and Mathur, 2004: Kharbanda et al., 2006; Frans et al., 2014; Frans et al., 2016a). A quick screening bioassay has been developed in Belgium that will allow for new cultivars to be quickly tested for susceptibility to the disease caused by *Fusarium* spp. (Frans et al., 2016b). This is an area where research could be conducted after the identification or confirmation of the causal organism(s) in Australia.

Conclusions

The main causal organisms of IFR are *F. lactis,* and *A. alternata*. Other organism such as *F. proliferatum*, *F. oxysporum*, *F. solani* and *F. subglutinans* can be responsible for the disease but do not make up the single largest cause. There appears to be a 30 year gap since the last report of *A. alternata* caused internal fruit rot of *C. annum*. This is surprising considering how confidently it was isolated and determined as the causal organism in Israel, Hungary and the USA. Good genetic assessment methods have been developed to allow for accurate identification of isolates for both *Fusarium* spp. and *Alternaria* spp.

Infection of the fruit occurs through the style and stigma, the pathogens follow the pollen tube into the ovary of the fruit and stay dormant until the fruit has matured in most cases, however internal symptoms can be present in immature fruit. While spread of the disease has been attributed to infected seed further study should be undertaken to understand the spread of the disease between regions and countries.

The literature mostly agrees on how infection occurs with any disagreements coming from an earlier lack of understanding. There is a split in findings in the literature between *Fusarium* and *Alternaria* as causal organism which may be due to geographic or field versus greenhouse divides. Other organism with smaller incidences such as *B. cinerea* appear fleetingly in the literature and are never isolated again, possibly suggesting some type of secondary or rare version of the disease.

More research is needed to be conducted on the function of tolerance or resistance to the disease; this is required so the process of producing more resistant cultivars can occur at a faster rate.

There is currently no literature that specifically looks at mycotoxin production of *Alternaria* spp. involved with internal fruit rot. While comparisons can be made to *Alternaria* spp. in other crops more specific research is required to understand the potential hazard to human health from mycotoxins in capsicums.

While some research has been conducted on controls of *A. alternata* it is now over 30 years old and new studies should be undertaken to develop controls if *A. alternata* or another *Alternaria* spp. becomes apparent as a causal organism of the disease.

References

Aponyi, I., Kujáni, O., Varga, B.B.A., 1987. The ovary moulding disease of tomato-shaped pepper and control possibilities. In I International Symposium on Vegetables for Processing 220, 401-406. https://doi.org/10.17660/ActaHortic.1988.220.56

Ash, G.J., Lanoiselet, V.M., 2001. First report of Alternaria alternata causing late blight of pistachio (Pistacia vera) in Australia. Plant Pathology. 50(6). https://doi.org/10.1046/j.1365-3059.2001.00626.x

Australian Pesticides and Veterinary Medicines Authority (APVMA), 2020, APVMA PubCRIS database search. https://portal.apvma.gov.au/pubcris (accessed 1 May 2020)

Bruton, B.D., Chandler, L.D., Miller, M.E., Ramey, H.H., Beaton, P.G., 1989. Relationships between pepper weevil and internal mold of sweet pepper. Plant disease. 73(2), 170-173. https://doi.org/10.4141/cjss89-010

Combina, M., Dalcero, A., Varsavsky, E., Torres, A., Etcheverry, M., Rodriguez, M., Gonzalez, Q.H., 1999. Effect of heat treatments on stability of alternariol, alternariol monomethyl ether and tenuazonic acid in sunflower flour. Mycotoxin Res. 15(1), 33-38. https://doi.org/10.1007/BF02945212

da Cruz Cabral, L., Terminiello, L., Pinto, V.F., Nielsen, K.F., Patriarca, A., 2016. Natural occurrence of mycotoxins and toxigenic capacity of Alternaria strains from mouldy peppers. International J. of food microbiology. 236, 155-160. https://doi.org/10.1016/j.ijfoodmicro.2016.08.0052

Escrivá, L., Oueslati, S., Font, G. and Manyes, L., 2017. Alternaria mycotoxins in food and feed: an overview. J. of Food Quality, 2017. https://doi.org/10.1155/2017/1569748

Frans, M., Aerts, R., Van Calenberge, B., Van Herck, L., Ceusters, J., 2016a. Influence of floral morphology and fruit development on internal fruit rot in bell pepper (Capsicum annuum). In III International Symposium on Postharvest Pathology: Using Science to Increase Food Availability. 1144, 199-206. https://doi.org/10.17660/ActaHortic.2016.1144.29

Frans, M., Aerts, R., Van Laethem, S., Ceusters, J., 2017. Environmental effects on growth and sporulation of Fusarium spp. causing internal fruit rot in bell pepper. European J. of plant pathology. 149(4), 875-883. https://doi.org/10.1007/s10658-017-1235-4

Frans, M., Aerts, R., Van Laethem, S., Van Calenberge, B., Van Herck, L., Heungens, K., Van Poucke, K., Van Gool, S., Ceusters, J., 2016b. Development of a quick screening bioassay for internal fruit rot in bell pepper (Capsicum annuum L.). In Proceedings of XVIth EUCARPIA Capsicum and Eggplant Working Group Meeting in memoriam Dr. Alain Palloix, 12-14 September 2016, Kecskemét, Hungary. 420-424.

Frans, M., Moerkens, R., Van Gool, S., Sauviller, C., Van Laethem, S., Luca, S., Aerts, R., Ceusters, J., 2018. Modelling greenhouse climate factors to constrain internal fruit rot (Fusarium spp.) in bell pepper. J. of Plant Diseases and Protection. 125(4), 425-432. https://doi.org/10.1007/s41348-018-0159-3

Frans, M., Sauviller, C., Van Mechelen, M., Heungens, K., Van Poucke, K., Van Calenberge, B., Van Herck, L., Aerts, R., 2014. Sustainable control of internal fruit rot in bell pepper: a multidisciplinary approach. Communications in Agricultural and Appl. Biological Sciences. 79(3), 373-373.

Ginoya, C.M., Gohel, N.M., 2015. Evaluation of newer fungicides against Alternaria alternata (Fr.) Keissler causing fruit rot disease of chilli. International J. of Plant Protection. 8(1), 169-173. https://doi.org/10.15740/HAS/IJPP/8.1/169-173

Halfon-Meiri, A. and Rylski, I., 1973. Penetration of Alternaria tenuis into pepper fruit. Phytoparasitica. 1, 57. https://doi-org.ezproxy1.library.usyd.edu.au/10.1007/BF02980307

Halfon-Meiri, A., Rylski, I., 1983. Internal mold caused in sweet pepper by Alternaria alternata: Fungal ingress. Phytopathology. 73(1). https://doi.org/67-70. 10.1094/phyto-73-67AGR:IND83029045

Horsfield, A., Wicks, T., Davies, K., Wilson, D., Paton, S., 2010. Effect of fungicide use strategies on the control of early blight (Alternaria solani) and potato yield. Australasian Plant Pathology. 39(4), 368-375. https://doi.org/10.1071/AP09090

HORT Innovation, 2019. Australian Horticulture Statistics Handbook 2018/19. https://www.horticulture.com.au/growers/help-your-business-grow/research-reports-publications- fact-sheets-and-more/grower-resources/ha18002-assets/australian-horticulture-statistics- handbook

Hubballi, M., Nakkeeran, S., Raguchander, T., Anand, T., Samiyappan, R., 2010. Effect of environmental conditions on growth of Alternaria alternata causing leaf blight of noni. World J. of Agricultural Sciences. 6(2), 171-177.

Kharbanda, P.D., Yang, J., Howard, R.J., Mirza, M., 2006. Internal fruit rot of greenhouse peppers caused by Fusarium lactis–a new disease. The Greenh. Bus. 5, 11–16

Kline, L.W., Wyenandt, C.A., 2014. Internal fruit rot and premature seed germination of field grown colored peppers. In Proceedings the 22nd International Pepper Conference. Vina Del Mar, Chili. 17-20.

Leslie, J.F., Summerell, B.A., 2008. The Fusarium laboratory manual. John Wiley & Sons, Ames

Luz, C., Saladino, F., Luciano, F.B., Mañes, J., Meca, G., 2017. Occurrence, toxicity, bioaccessibility and mitigation strategies of beauvericin, a minor Fusarium mycotoxin. Food and Chemical Toxicology. 107, 430-439. https://doi.org/10.1016/j.fct.2017.07.032

Mathur, S., Utkhede, R., 2004. Molecular detection of Fusarium subglutinans, the causal organism of internal fruit rot in greenhouse peppers. Canadian J. of plant pathology. 26(4), 514-521. https://doi.org/10.1080/07060660409507172

Misaghi, I.J., Grogan, R.G., Duniway, J.M., Kimble, K.A., 1978. Influence of environment and culture media on spore morphology of Alternaria alternata. Phytopathology. 68(1), 29-34. https://doi.org/10.1094/Phyto-68-29

O'Neill, T., Mayne, S., 2015. Sweet pepper: Aspects of the biology and control of fusarium fruit rot. Communications in agricultural and appl. biological sciences. 80(3), 569-573.

Siegel, D., Feist, M., Proske, M., Koch, M., Nehls, I., 2010. Degradation of the Alternaria mycotoxins alternariol, alternariol monomethyl ether, and altenuene upon bread baking. J. of agricultural and food chemistry. 58(17), 9622-9630. https://doi.org/10.1021/jf102156w

Summerell, B.A., Leslie, J.F., Liew, E.C., Laurence, M.H., Bullock, S., Petrovic, T., Bentley, A.R., Howard, C.G., Peterson, S.A., Walsh, J.L., Burgess, L.W., 2011. Fusarium species associated with plants in Australia. Fungal Diversity. 46(1), 1-27. https://doi.org/10.1007/s13225-010-0075-8

Utkhede, R.S., Mathur, S., 2004. Internal fruit rot caused by Fusarium subglutinans in greenhouse sweet peppers. Canadian J. of plant pathology. 26(3), 386-390. https://doi.org/10.1080/07060660409507157

Utkhede, R.S., Mathur, S., 2005. Biological and Chemical Control of Fruit Rot in Greenhouse Sweet Peppers(Capsicum annum L.) Caused by Fusarium subglutinans. J. of Biological Sciences. 5(5), 610-615. https://doi.org/ 10.3923/jbs.2005.610.615

Van Poucke, K., Monbaliu, S., Munaut, F., Heungens, K., De Saeger, S., Van Hove, F., 2012. Genetic diversity and mycotoxin production of Fusarium lactis species complex isolates from sweet pepper. International J. of Food Microbiology. 153, 28–37. https://doi.org/10.1016/j.ijfoodmicro.2011.10.011

Wall, M.M., Biles, C.L., 1993. Alternaria fruit rot of ripening chile peppers. Phytopathology. 83(3), 324-328. https://doi.org/10.1094/Phyto-83-324

Webley, D.J., Jackson, K.L., Mullins, J.D., Hocking, A.D., Pitt, J.I., 1997. Alternaria toxins in weather-damaged wheat and sorghum in the 1995-1996 Australian harvest. Australian J. of Agricultural Res. 48(8), 1249- 1256. https://doi.org/10.1071/a97005

Yang, J., Kharbanda, P. D., Howard, R. J., Mirza, M., 2009. Identification and pathogenicity of Fusarium lactis, causal agent of internal fruit rot of greenhouse sweet pepper in Alberta. Canadian J. of Plant Pathology. 31, 47–56. https://doi.org/10.1080/07060660909507571

Yang, Y., 2009. Infection and mycotoxin production by Fusarium lactis, causal agent of internal fruit rot of sweet pepper. https://doi.org/10.7939/R3S01C

Yang, Y., Cao, T., Yang, J., Howard, R.J., Kharbanda, P.D., Strelkov, S.E., 2010. Histopathology of internal fruit rot of sweet pepper caused by Fusarium lactis. Canadian J. of Plant Pathology. 32(1), 86-97. https://doi.org/10.1080/07060660903503681

Appendix 2 – Identification of the organism/s causing internal fruit rot in capsicums (*Capsicum annuum*) in Australia

Ryan William Hall

Bachelor of Science in Agriculture

AFNR4102 Research Project B / Research paper

2020

Number of words:	6222
Number of references:	41
Number of tables:	8
Number of figures:	13

Supervisor:	Dr Gordon Rogers
Assoc Supervisor:	Dr Jenny Ekman
Assoc Supervisor:	Dr Len Tesoriero
Other participant:	Dr Kelly Scarlett

Table of Contents

Abstract

In 2015 a severe outbreak of internal fruit rot of capsicums (*Capsicum annuum*) occurred, particularly impacting Queensland producers. The disease presented as mycelial growth on the seed and placenta of fruit but did not have any external symptoms. This led to a large number of rejections by retailers and a drop in consumer confidence. Internal fruit rot of capsicums is a well-known disease internationally where it has had significant impacts on greenhouse grown crops. However, it was unknown if the causal organism found in recent European studies was the same organism causing the disease locally. The purpose of this study was to identify the cause of internal rot of capsicums in Australia.

A disease survey was carried out to obtain organisms causing disease and included samples sent from producers and wholesalers. A Koch's postulates trial was conducted using the four main fungal species isolated and four varieties of capsicum. Interactions between variety and treatment showed there were distinct varietal susceptibilities. The three fungal species re-isolated from infected fruit underwent PCR analysis using the plasma membrane ATPase gene region for *Alternaria* sp. and ITS, TEF1 and RPB2 regions for *Fusarium* sp.. The organisms were identified as *Alternaria alternata, Alternaria tenuissima and Fusarium oxysporum*. While these species of fungi are consistent with those known to cause the disease in Europe North America and the Middle East, this is the first time these specific species have been demonstrated to cause the disease in Australia. *Fusarium lactis,* the main causal organism internationally, was not recovered from the disease survey.

This study successfully identified three fungal species that cause internal rot of capsicum in Australia. As a result, control methods can start to be explored.

1. Introduction

1.1 Objectives of study

This study aims to determine the major causal fungi for an internal fruit rot of capsicum (*Capsicum annuum*) in Australia. It also aims to characterise the organism(s) associated with internal fruit rot in Australia to species level through morphological and DNA analysis, and determine their causal nature by completing Koch's postulates. Organism(s) found to cause the disease in Australia will be compared with those found in North America, Europe and the Middle East. The pathogenicity assay will also determine if infection occurs at flowering.

1.2 Background

C. annuum, known as capsicum, bell pepper, sweet pepper, chilli pepper and pepper, is a significant global industry, produced both in greenhouses and in field. Over 36 million tonnes of chillies and capsicums and over 4 million tonnes of dried chillies and capsicums were produced worldwide in 2018 (FAO, 2018). Australian capsicum production has been trending upwards with 77,000 tonnes being produced in the 2018/2019 financial year. This crop had an estimated production value of AU$171 million, around 3-4% of Australia's total vegetable production value. Most capsicums in Australia are grown in field conditions. Queensland is the main state of production. There has been a recent increase in greenhouse production in some of Australia's southern states (HORT Innovation, 2019).

A large number of diseases exist for *C. annuum*; however internal fruit rot is of particular importance due to the difficulty of detecting symptoms before the fruit reaches the consumer. Internal fruit rot's main symptom is fungal mycelial growth on the interior parts of the fruit, primarily on the seeds and placenta (Halfon-Meiri and Rylski, 1983; Utkhede and Mathur, 2004;

Fig. 1). Infection is known to occur during flowering. Infection is more likely soon after anthesis and pollination has occurred, and decreases in the days after anthesis (Halfon-Meiri and Rylski, 1983). Spores germinate and mycelial threads follow the pollen tube down to the ovary, where the fungus lies dormant until maturity (Yang et al., 2010). External symptoms are rarely present by the time the consumer purchases fruit (Utkhede and Mathur, 2004; Yang et al., 2009). When external symptoms of soft discoloured areas of necrosis on the exocarp near the calyx appear, internal rot is severe (Utkhede and Mathur, 2004).

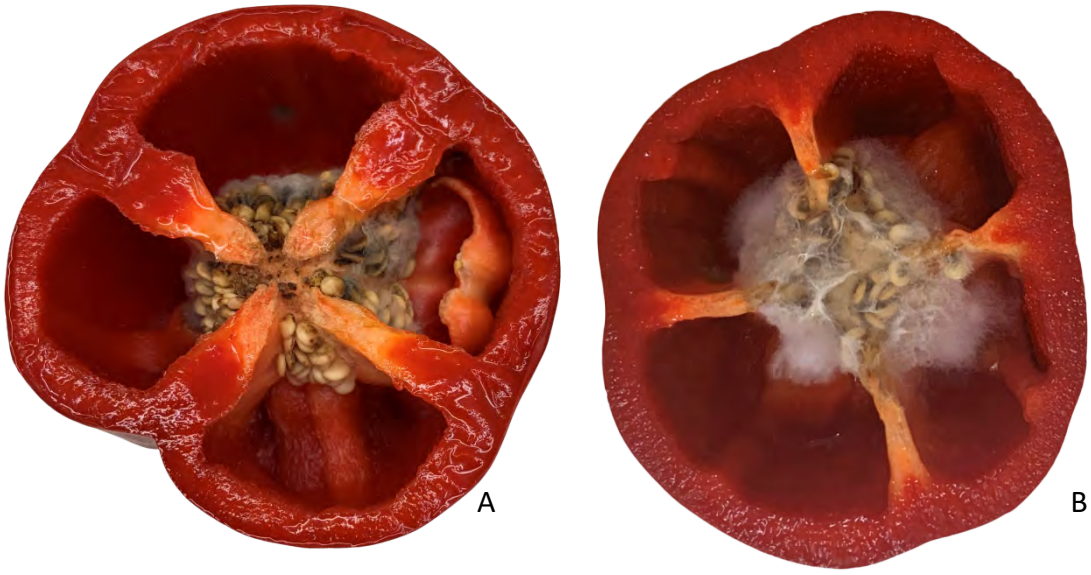


Fig. 1. Symptoms of internal fruit rot of C. annuum, showing mycelial growth on seeds and placenta, A presents as a darker fungi and was identified as A. alternata B presents as a white fungi and was identified as F. oxysporum

First identified in 1954 in the USA by Leyendecker Jr, the disease has been attributed to different fungi in a range of geographical locations over the past 65 years. Two major groups of fungi have emerged as the main causal pathogen; species of *Alternaria* and *Fusarium*. *Alternaria alternata* has been identified as the primary causal organism from the genus *Alternaria* (Halfon-

Meiri and Rylski, 1983; Aponyi et al., 1987; Bruton et al., 1989). The main causal organism of the most recent outbreaks in greenhouses over the last 16 years has been found to be *Fusarium lactis* (Utkhede and Mathur, 2004; Kharbanda et al., 2006; Yang et al., 2009; Van Poucke et al., 2012; O'Neill and Mayne, 2015; Frans et al., 2017). The disease has also been found to occur in South Korea and China where large greenhouse growing industries operate (Choi et al., 2011; Wang et al., 2019).

Alternaria tenuis, a synonym name for *A. alternata,* as well as *Alternaria solani* were originally identified as causing the disease (Leyendecker Jr, 1954; Halfon-Meiri and Rylski, 1973). A greater range of *Fusarium* spp. have been found causing the disease including: *Fusarium proliferatum, Fusarium oxysporum, Fusarium lactis, Fusarium equiseti* and *Fusarium fujikuroi* (Kharbanda et al., 2006; Yang et al., 2009; Van Poucke et al., 2012; Wang et al., 2019). *Fusarium subglutinans* was originally named as a causal organism of the disease but has since been reclassified as *F. lactis* through subsequent DNA analysis (Utkhede and Mathur, 2004; Mathur and Utkhede, 2004; Kharbanda et al., 2006; Yang et al., 2009).

Other organisms have been associated with the disease; however, there has been limited research on these organisms and their ability to cause infection has not been confirmed. These include *Fusarium moniliforme* and *Fusarium roseum*, since reclassified as *Fusarium verticillioides* and *Fusarium graminearum* (Leyendecker Jr, 1954). *Botrytis cinerea* was identified in one study in Hungary; however, it has not been identified before or since (Aponyi et al., 1987).

Both *A. alternata* and *F. lactis* grow best in conditions where the temperature is around 25-30°C and humidity is high (Misaghi et al., 1978; Hubballi et al., 2010; Frans et al., 2017). These temperatures are typical of those experienced by the major capsicum growing regions in

Queensland, Bundaberg (average max temp 25.7 to 30.4ºC) and Bowen (average max temp 25.1 to 29.9ºC) during their capsicum production seasons (Bureau of Meteorology, 2020). Moreover, limited air exchange between the external air and internal cavity suggests that the internal atmosphere is likely to be above 90% relative humidity (Jennifer Ekman, pers. comm.).

Fig. 2. *C. annuum* crops growing in field and greenhouse conditions, A shows the Sydney basin farm where sampling took place whereas B shows a greenhouse in Southern Queensland used to grow capsicum minis (not sampled), images show differences in conditions of growth. The field crop uses plastic soils coverings and drip irrigation where the greenhouse crop used coco coir growth media and fertigation.

Recent research on the disease in Europe, Canada and China has focused entirely on the greenhouse industry (Utkhede and Mathur, 2004; Kharbanda et al., 2006; Yang et al., 2009; Wang et al., 2019). Trials were conducted on field crops during the 1980s; however, there appears to have been little research undertaken since (Halfon-Meiri and Rylski, 1983; Aponyi et al., 1987; Bruton et al., 1989). Field environments may share general trends in terms of

temperature with greenhouse production, but overall the environmental conditions are very different. Field crops are more exposed to the wet and windy weather that favours fungal sporulation and dispersal.

1.3 Impact on Australian industry

Very little research into the disease has been conducted in Australia; as of this paper no published literature on the disease in Australia exists. A factsheet was produced in 2015 by Applied Horticultural Research together with Hortus Technical Services. This suggested that *A. alternata* was the most common organism associated with internal rot. Field crops were believed more likely to be infected by fungi than those grown under protected cropping structures. However, *A. alternata* was not confirmed using Koch's postulates on the isolates, and the information was not peer-reviewed (Soil Wealth, 2015).

Internal fruit rot is one of the most common causes of customer complaints. To reduce the probability of infected fruit reaching the customer, retailers require that 40 randomly selected capsicums per batch are cut to check for internal fruit rot. If one of these 40 fruit is found to have internal mould, this will exceed the limit of 2% major defects. In this case, the entire consignment is rejected. Some batches of fruit have been found to have up to 25% infection (Cally Chng, pers. comm.).

The lack of research in Australia restricts the ability of producers to control the disease, especially as it is still unclear in Australia whether internal mould is caused by one fungal organism or several. Understanding the causal organism(s), and confirming the mechanism of infection, is critical to allow development of effective control measures.

2. Materials and methods

2.1 Collection of samples and disease survey of internal rot in Australia

Collection of fungal isolates occurred through a variety of means. A more structured sampling process based on Elfar et al., (2019) was planned to be used at multiple locations, however due to the impact of COVID-19 on travel restrictions this was only able to be completed once at a location within the Sydney basin (Fig. 2A). A paddock used to grow *Capsicum annuum* (var *Red Jewel*, Clause Seeds) was sampled. Flowers were collected and plated onto ¼ potato dextrose agar (PDA) with 100 ppm Novobiocin to suppress bacterial growth. Three anthers, two petals, the style and the carpel were plated separately for each flower collected. Fruit infections were examined using the same media, with sections of the endocarp wall, a seed, part of the endocarp apex and the style were sectioned onto plates.

Fruit samples were also received from a range of producers and wholesalers to allow for a wider geographical range of diseased fruit across Australia. Samples were provided from the Bowen and Bundaberg regions of Queensland which are major centres of capsicum production (HORT Innovation, 2019). Although both whole and cut fruit were received, preference was placed on whole fruit as cut fruit were susceptible to contamination during initial cutting and transport.

All fruit were examined for signs of internal fungi, with visible mycelia sampled. If no fungi were visible samples were taken of a seed, part of the placenta and the apex endocarp. Samples were plated onto ¼ PDA with 100 ppm Novobiocin.

Simple comparisons of percentages were conducted to analyse the data produced in the disease survey. This was due to the large range of locations and considerable number of uncontrolled variables.

2.2 Morphological characteristics

Colonies were isolated from the initial samples through a single sporing process onto potato carrot agar (PCA) for *Alternaria spp*., with water agar (WA) and carnation leaf agar (CLA) used as media for *Fusarium spp*.

The processes for obtaining single spored colonies suitable for microscopic characterisation for *Alternaria* spp. followed the method set out by Simmons in 2007. For *Fusarium* spp. obtaining similarly pure colonies was achieved using methods outlined by Burgess et al., in 1994.

Morphological characteristics were compared with descriptions provided by Leslie and Summerell (2007) for *Fusarium* spp. and Simmons (2007) for *Alternaria* spp.

2.3 Koch's postulates

Fifty plants each of four varieties of *C. annuum* were grown from seed, these being: *California Wonder* (red), *SV6947PB* (red), *Warlock* (red) and *SVPB8193* (yellow). Seeds were treated with a Thiram fungicide coating to reduce the likelihood of any seed-borne disease. Potting equipment was sterilised using 0.01% hyper-chlorite for 1 minute before being rinsed in water. Plants were raised from seed in greenhouse conditions of 30°C / 22°C day / night temperatures. Irrigation was initially provided by overhead misters and then transitioned to a drip system. Plants were divided with 10 plants per treatment in two blocks (five plants per block).

A selection of two morphologically representative cultures of both *Alternaria* and *Fusarium* spp. isolated from the disease survey were grown on ¼ PDA with 100 ppm Novobiocin. For each treatment, the dish was filled with sterile water and lightly brushed with a spreader to release spores. This spore filled water was collected into 50 mL falcon tube and made up to 50 mL for

storage. A haemocytometer was used to estimate spore concentrations. The second inoculum was made by macerating a whole plate of a fungal culture with a stick blender and straining through a single layer of cheesecloth.

As flowering was not uniform over all the varieties, inocula were applied on five separate occasions in order to achieve a minimum of three flowers inoculated per plant.

Inoculation was conducted by dispensing 100 µL of inocula onto the stigma, style and petals using a micropipette (Fig. 3). Prior to inoculation, inoculum was inverted to ensure mixture of spores in the water. Plastic bags were then placed over the plants that were inoculated for 48 hours to simulate higher humidity. Later into the trial the flowers were inoculated using a small paint brush that was used to brush the reproductive parts of the flower along with the petals and sepals. Inoculation occurred once or twice a week until three to four fruit were established on the plant and new flowers began to consistently abort.

Fig. 3. Inoculation of capsicum flower with micropipette dispensing 100 µL spore suspension

When fruit were ripe, (yellow for *SVPB8193* and red for other varieties), they were harvested using surface sterilised secateurs (hand pruners) and labelled corresponding to their variety, treatment, block, pot, and fruit number. Observations of external symptoms such as wounds, lesions and rots were made. Fruit were then surface sterilised using 70% ethanol and cut equatorially using a scalpel in a sterile laminar flow cabinet. If visible, fungal growth was taken and plated onto ¼ PDA with 100 ppm Novobiocin, where no fungi was visibly present, samples of the seed, placenta and endocarp apex were taken.

Table 1
Spore counts, as determined by a haemocytometer, used to inoculate flowers during Koch's postulates trial

Treatment	Spore count (per mL) 1st inoculum	Spore count (per mL) 2nd inoculum
A. tenuissima (treatment A)	3×10^4	2×10^4
A. alternata (treatment B)	3×10^4	5×10^4
F. oxysporum (treatment C)	5×10^5	9×10^5
*F semitectum** (treatment D)	8×10^4	2×10^5

*isolate identified through morphological characteristics only

Analysis of the proportions of infected fruit were completed by creating a generalised linear model (GLM). Interaction between variety and treatment were included in this model. To complete a Tukey's honestly significant difference (HSD) test the GLM model was converted to an analysis of variance (ANOVA) model. Similar GLM models were created, and HSD tests were also run on the variety and treatment variables to determine any general trends.

2.4 Molecular biology

Two isolates of *Alternaria spp.* and one isolate of *Fusarium* sp. were grown on ¼ PDA with 100 ppm Novobiocin for seven to 10 days at 25°C. Mycelium was then collected for DNA extraction by scraping the surface with a sterile scalpel, avoiding collecting media. DNA was extracted using a DNeasy Plant Mini Kit (Qiagen Inc). The *Alternaria* samples underwent a polymerase chain reaction (PCR), amplifying the plasma membrane ATPase gene region, with the use of ATPDF1/ATPDR1 primers (Lawrence et al., 2013). Samples underwent an initial denaturation at 95°C for one minute, 35 cycles of denaturation at 95°C for 15 seconds, annealing at 60°C for 15 seconds and elongation at 72°C for 15 seconds. A final elongation at 72°C went for five minutes.

Table 2
Primers pair details used in PCR to identify to species the isolates used as innocula, Gene region, direction of primer, primer name its 5' 3' sequence and its reference are shown.

Region	Direction	Primer	5' 3'	Reference
Plasma membrane	F	ATPDF1	ATCGTCTCCATGACCGAGTTCG	Lawrence
ATPase	R	ATPDR1	TCCGATGGAGTTCATGATAGCC	et al., 2013
Internal transcriber	F	ITS1	TCCGTAGGTGAACCTGCGG	White et
spacer (ITS)	R	ITS4	TCCTCCGCTTATTGATATGC	al., 1990
Translation elongation	F	EF1	ATGGGTAAGGA(A/G)GACAAGACG	O'Donnell
factor 1-α (TEF1)	R	EF2	GGA(G/A)GTACCAGT(G/C)ATCATGTT	et al., 1998
DNA-directed RNA	F	5F2	GGGGWGAYCAGAAGAAGGC	O'Donnell
polymerase II second	R	7cR	CCCATRGCTTGYTTRCCCAT	et al., 2007
largest subunit (RPB2)				

The *Fusarium* sample underwent PCR for three different gene regions, Internal transcriber spacer (ITS) (White et al., 1990), Translation elongation factor 1-α (TEF1) (O'Donnell et al.,

1998) and DNA-directed RNA polymerase II second largest subunit (RPB2) (O'Donnell et al., 2007). The PCR process did not change except for annealing temperature, with ITS and TEF1 annealing at 55°C. The RPB2 primers underwent a gradient PCR from 49 to 57°C to determine the best annealing temperature. This temperature was found to be 56°C. Further details about the different primers can be found in table 2. PCR products were run on 1% agarose gel in Tris-acetate-EDTA (TAE) buffer and stained with thiazole orange to determine successful PCRs.

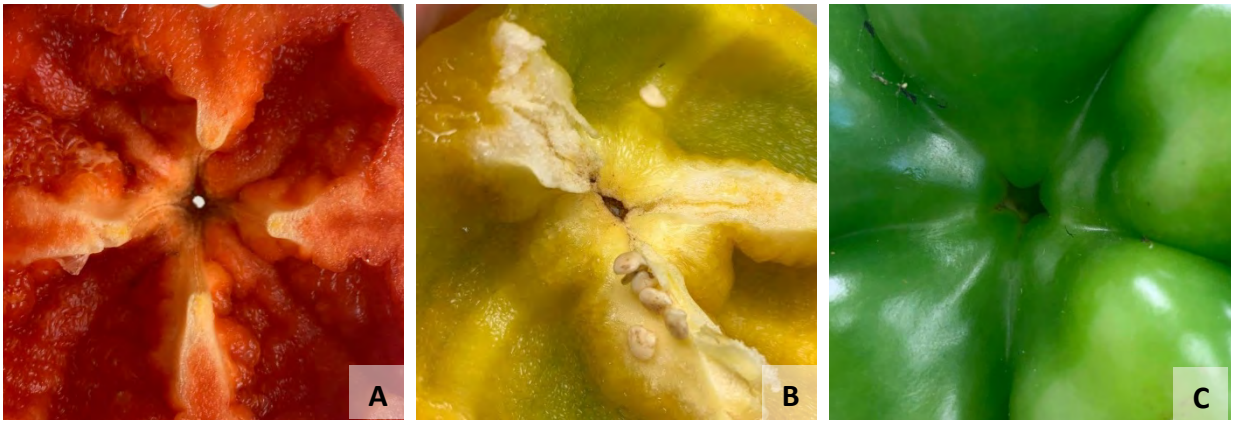


Fig. 4. Holes in the pericarp of fruit at the location of the style, A and B show internal views of holes while C shows an external view

DNA purification was conducted with an isolate II PCR and gel kit and its associated method from BIOLINE. DNA concentration was assessed using a Thermo Scientific NANODROP 2000c Spectrophotometer. Samples were made up to 30 ng of DNA with 0.8 pmol/µL of the respective forward or reverse primer for the gene region that had undergone PCR. Purified PCR products underwent sanger sequencing in both directions carried out by the Australian Genome Research Facility (AGRF), Australia.

Sequence data was edited and aligned using Geneious Prime 2016.11.0.4 (https://www.geneious.com) to obtain clean sequences. A standard nucleotide BLAST was

conducted on the consensus sequences comparing the sequences to reference sequences in the GenBank (https://www.ncbi.nlm.nih.gov/) database.

3. Results

3.1 Disease survey results

The collection of flowers at the Sydney basin property yielded a high level of *Alternaria* spp., 93% of the flowers collected contained the fungi of the genus. *Fusarium* spp. were isolated at a lower rate but were still present on 27% of the flowers collected. Table 3 displays the percentage incidence of isolation and where the infection was found to occur on the flower.

Table 3
Location of fungi on flowers plated from samples from Sydney basin farm and their incidence in percentage

	% Incidence			
Species isolated	**Ovary**	**Style**	**Stamens**	**Petals**
Alternaria spp.	36	29	40	48
Fusarium spp.	7	7	7	9

Of the 100 fruit sampled, 29 had symptoms of internal fruit rot. Only four fruit contained *Fusarium* spp., however, *Alternaria* spp. were found in all infected fruit. The number of fruits infected with *Fusarium* spp. reflected the lower presence found on flowers. *Fusarium* appeared to be evenly distributed in the fruits sampled at the Sydney basin farm. *Alternaria* spp. appeared to be more variable with slightly less infection occurring at the endocarp apex (Table 4).

In total, 104 of 227 fruit in the disease survey had symptoms of internal rot. A large number of fruit were sent pre-cut by producers and suppliers. A range of fungal genera were isolated from

red, yellow, and green fruit (Table 5). Fruit which had lost the external style resulting in a hole at the apex were commonly infected by *Alternaria*, *Fusarium* and other air-borne fungal species (Fig. 4). This was also the case for much of the *Cladosporium* spp. and *Rhizopus* spp. isolated from the fruit. Lepidopteran insect larvae and thrips nymphs were rarely observed in fruit with holes.

Table 4
Recovery of fungal species (%) from a Sydney basin farm and location in the fruit

	% Incidence			
Species isolated	**Endocarp wall**	**Seed**	**Endocarp apex**	**style**
Alternaria spp.	18	23	11	18
Fusarium spp.	1	2	2	2
Penicillium spp.	0	0	1	0

Although green fruit rarely showed symptoms of internal fruit infection, 13 out of 16 sampled fruit were found to contain both *Alternaria* spp. and *Fusarium* spp. when plated onto media. Only one of these fruit displayed clear symptoms of internal rot where the rest did not or showed symptoms of other disease such as yeasty rot. Only two of the infected green fruit did not have a hole at the apex end of the fruit.

Within this survey, several fruit with “dark seed” were observed (Fig. 5). Seeds ranged from slight to severe discolouration. Discolouration presented as a dark brown or black colour on the entire seed, along the edge of the seed or in the centre of the seed with the edge of the seed remaining a normal colour. 30 fruit with discolouration in the seed and no visible mycelium were plated onto ¼ PDA with 100 ppm Novobiocin. All were negative for fungi.

Table 5
Organisms isolated and the frequency in which they were found in fruit collected or received in the disease survey that had symptoms of internal rot

Species isolated	Number isolated from surveyed fruit	% of fruit with fungi from disease fruit
Alternaria spp.	80	76.9
Fusarium spp.	31	29.8
Cladosporium spp.	7	6.7
Rhizopus spp.	3	2.9
Penicillium spp.	1	1.0

Fig. 5. Examples of seed discolouration, A = minor, B = moderate, and C= severe discolouration, these examples show discolouration around the edge of the seed; the other main type is the inverse of this, with the centre of the seed being discoloured and the edge being normal.

Two *Alternaria* spp. and two *Fusarium* spp. were commonly found during the survey (Fig. 6; Fig. 7; Fig. 8; Fig. 9). Under light microscopy studies of the fungi the *Alternaria* spp. had small conidia produced in chains consistent with a group of species referred to as *A. alternata*. One *Fusarium* sp. produces large falcate microconidia (also referred to as mesoconidia) and is morphologically and culturally similar to *Fusarium semitectum* (Leslie and Summerell, 2007).

Colony growth produced an initial yellow beige pigment in the media which later tuned into a brown colour.

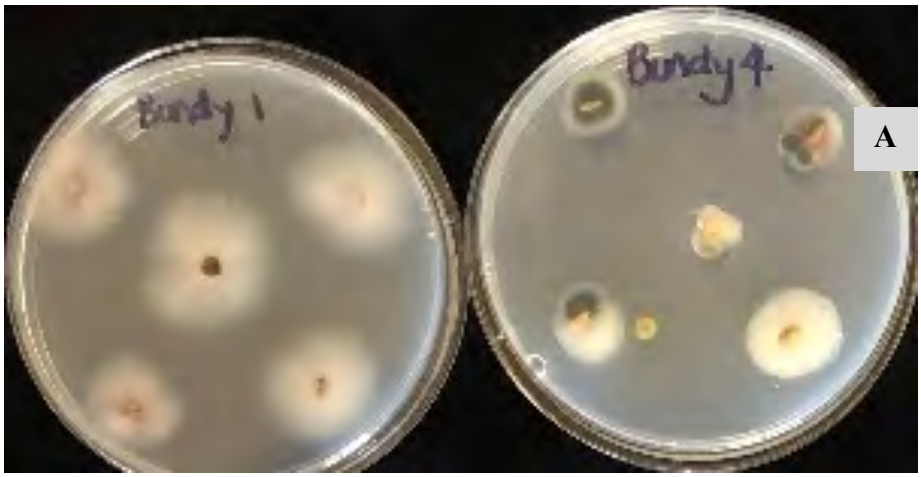


Fig. 6. ¼ PDA and novobiocin plates of the initial isolation of Fusarium spp. used for inoculum, bundy 1 was used for treatment C and bundy 4 was used for treatment D. Bundy 4 presented with a Fusarium morphologically similar to Bundy 1 (A), green Cladosporium spp. and 1 Fusarium colony that turned a yellow white colour.

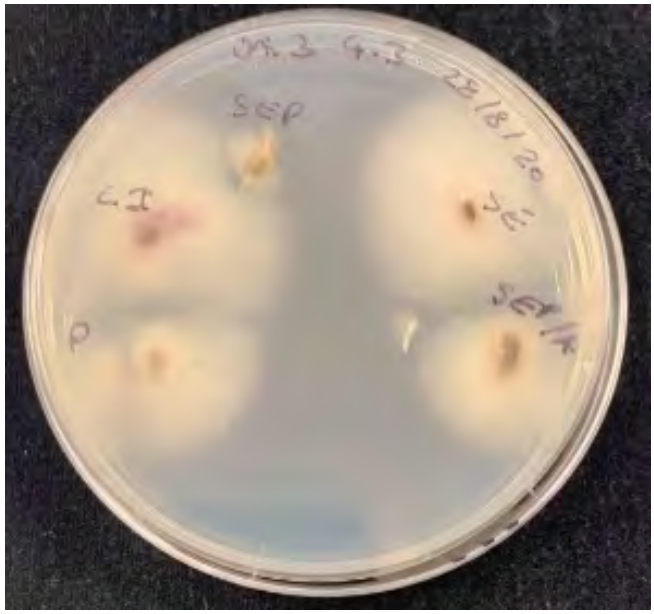


Fig. 7. Re-isolate from treatment C showing pink *Fusarium* spp. present on multiple parts of the fruit, a key to the labels on the agar plate is as follows, p = placenta, LI = lesion endocarp, SEP = septa, SE = seed, SEP/A = septa/apex endocarp.

The other most common *Fusarium* isolates have small microconidia formed on pseudophialides in agar colonies with a pink pigmentation (Fig. 7; Fig. 10c). Morphologically similar species of

these fungi were isolated from flower parts at anthesis. This fungi is similar to *Fusarium oxysporum* (Leslie and Summerell, 2007).None of the *Fusarium* spp. were consistent with *F. lactis* which has a characteristic zig-zag chain formation of microconidia (Leslie and Summerell, 2007).

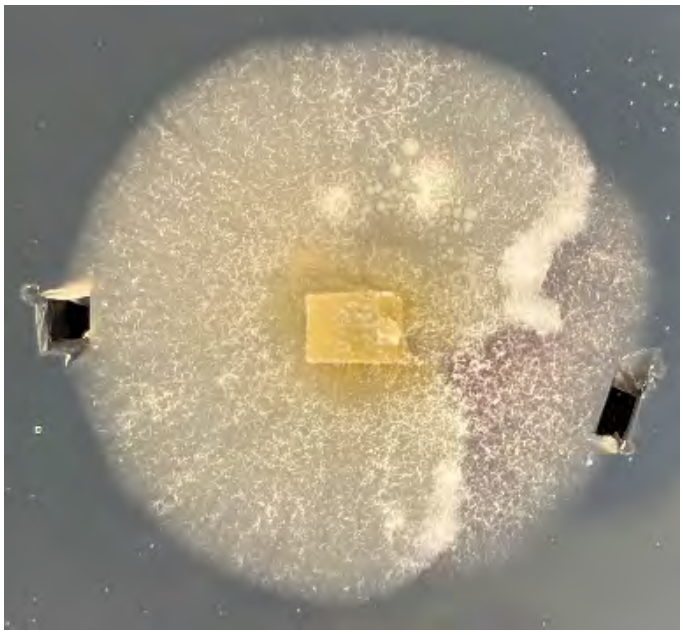


Fig. 8. An example of the Yellow-white *Fusarium* spp. used for treatment D, with a pink *Fusarium* spp. contaminating the bottom right side of the colony.

While the two *Alternaria* spp. were more distinct, further examination of the two *Fusarium* spp. grown on a carnation leaf agar was undertaken. The *Fusarium* spp. used for *F. oxysporum* produced both macroconidia and chlamydospores. The macroconidia were curved and relatively wide when compared to the other *Fusarium* sp. macroconidia (Fig. 11; Fig. 12). The chlamydospores produced by *F. oxysporum* were either as individual spores or in short chains, this combined with the macroconidia was consistent with the morphological descriptions of *F. oxysporum* (Leslie and Summerell, 2007).

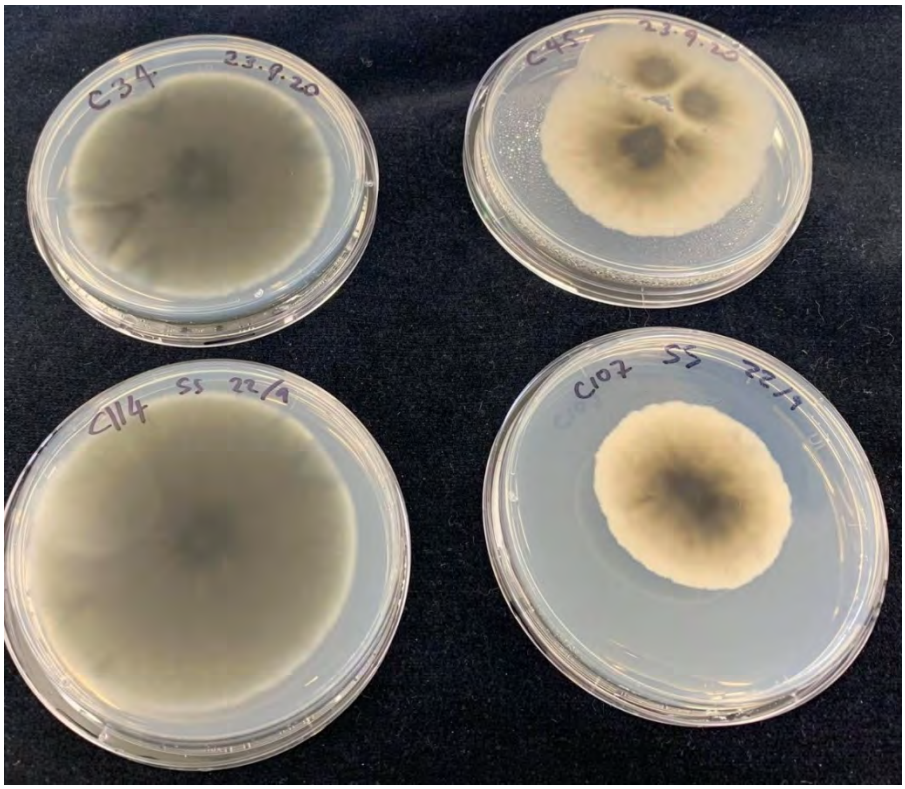


Fig. 9. Example of how colony morphology was used to confirm re-isolation after infection. Inoculum are shown as the top two plates (C34, treatment A, and C45, treatment B) with their respective re-isolates below.

The macroconidia produced by *F. semitectum* had a spindle-shape with curved ends (Fig. 12). This combined with the rabbit ears formation of mesoconidia and a beige-brown pigmentation on PDA suggests that this organism is very consistent with the descriptions of *F. semitectum* (Leslie and Summerell, 2007; Fig. 10).

3.2 Koch's postulates results

Over the course of the project 520 fruit were grown and assessed, with 41 returning fungal isolates, or an overall 7.9% infection rate. Infection within the varieties varied from 3.9% to 12.0% (Table 6). When assessed by treatment, infection ranged from zero to 17% (Table 7).

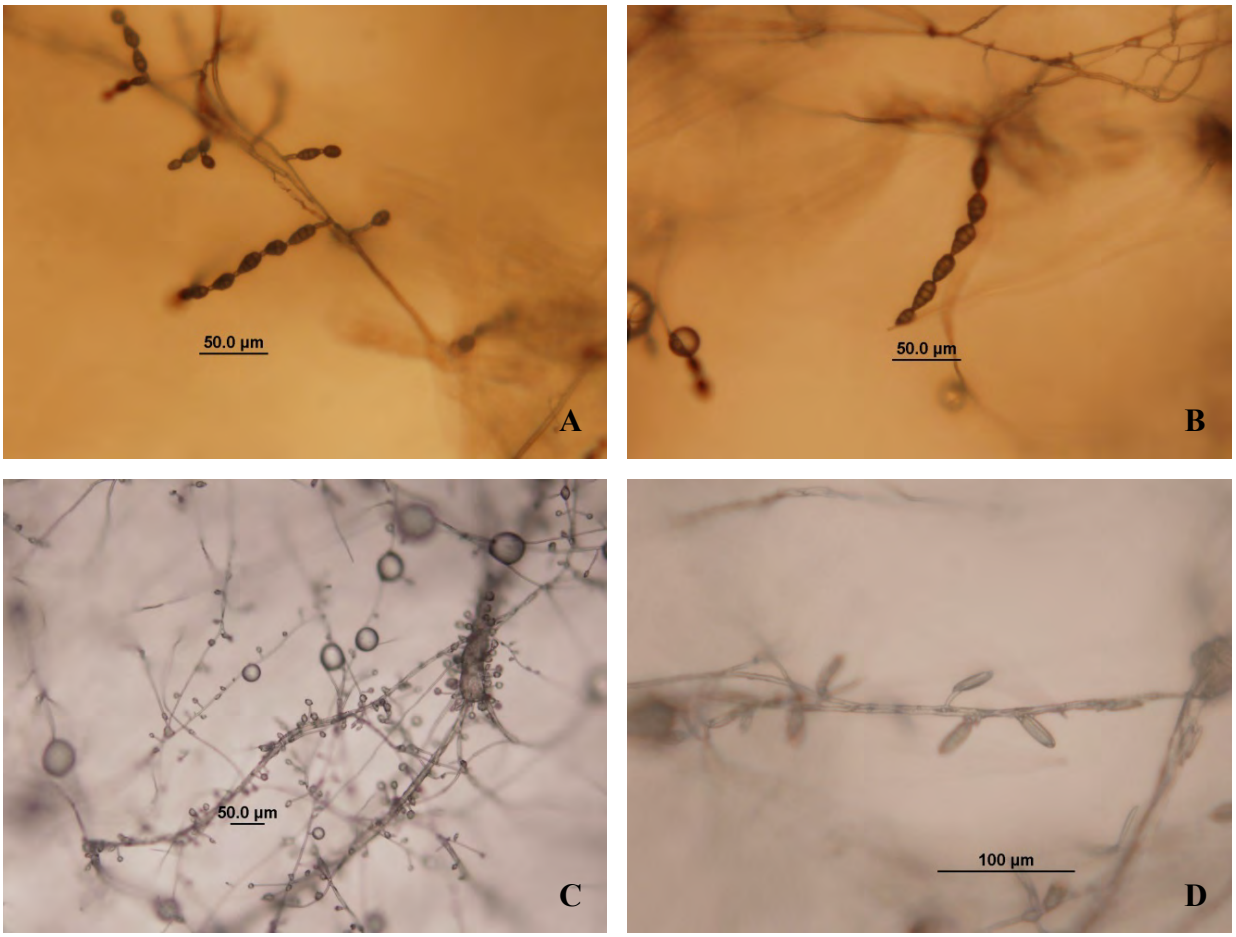


Fig. 10. Microscopic images of spore morphology of inoculum, A and B show clubbed shaped spores in chains consistent with *A. alternata* on PCA, C shows microconidia consistent with many *Fusarium spp.* on WA, and D shows mesoconidia similar to *F. semitectum* on WA.

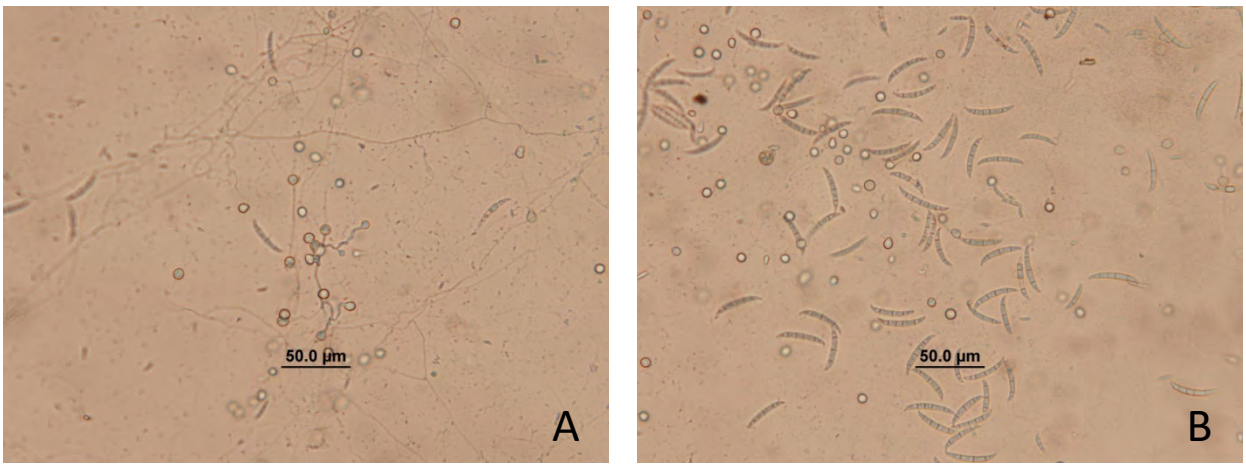


Fig. 11. Further images of spore morphology for treatment C grown on carnation leaf agar (CLF). Image A shows round chlamydospores. Image B shows Chlamydospores and a significant number of macroconidia.

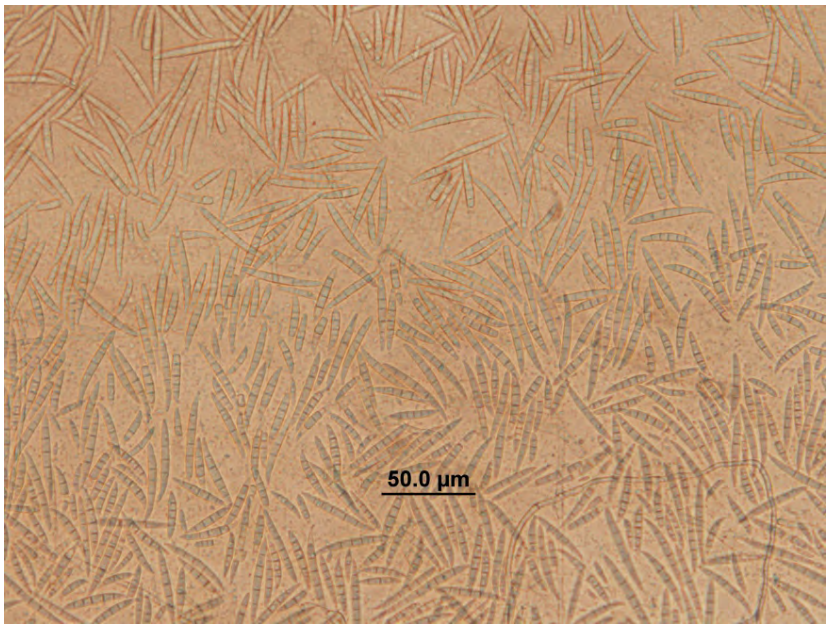


Fig. 12. Macroconidia morphologically consistent with *F. semitectum* produced by treatment D when grown on carnation leaf agar.

Statistical analysis was conducted through the use of a generalised linear model (GLM) and a Tukey's HSD test in the R statistical program. No significant difference was found between the two blocks used in this experiment. No difference was found between varieties (Table 6). Differences were found between treatments that recorded infection and those that did not (Table 7).

The interaction model between treatment and variety was converted to an ANOVA model. The summary of the ANOVA model returned p-values <0.05 for all predicting variables. A Tukey's HSD test was then applied and found that *F. oxysporum* in *California wonder* was significantly different to other varieties and treatment interactions. All other treatments that returned an infection were not significantly different with each other in terms of their susceptibility. Interactions that returned infection were significantly different to those that did not.

Table 6
Summary of Koch's postulate trial showing the number of fruits per variety and the percentage infection each variety

Variety	Number of fruits	Number of fruits with fungal re-isolation	% infection
SV6947PB (PB)	130	5	3.85a
SVPB8193 (8913)	159	9	5.66a
Warlock	156	18	11.54a
California wonder	75	9	12.00a

* Letters represent differences as described by a Tukey's HSD test

This can be visualised by looking at Fig. 13 which shows over 50% of inoculated fruit for *F. oxysporum* in *California wonder* were infected. No instances of *A. alternata* were present in *California wonder* and no incidences of *F. oxysporum* were present in *SV6947PB*.

Colony morphology was used to confirm re-isolation from fruit (Fig. 9). Some instances of *Cladosporium* spp. were also found growing on the plates after isolation. *Cladosporium* is a known environmental microbe and in the few instances where it was found there was no visible fungal infection inside the fruit leading to these results being discarded from the main data as contaminants.

Two control fruit also returned visible fungal infections when isolated and grown on ¼ PDA with 100 ppm Novobiocin. The fungi appeared to be similar to *F. oxysporum*. These results were considered likely to be due to cross contamination, as one fruit was suffering from blossom-end rot and the other had a cracked exocarp allowing for pathogen entry.

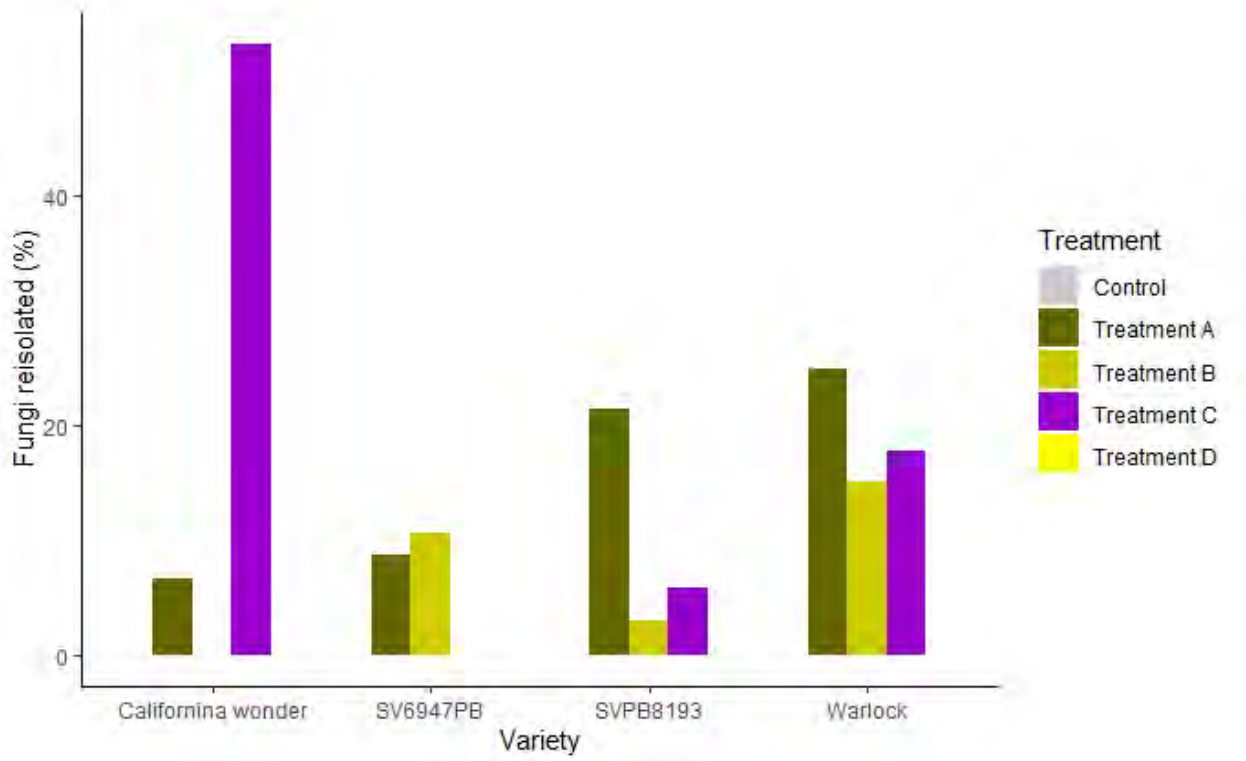


Fig. 13. Percentage of mature fruit from four varieties that were inoculated at flowering with one of four spore solutions. Treatment A = *A. tenuissima*, Treatment B = *A. alternata*, Treatment C = *F. oxysporum*, Treatment D = *F. semitectum*.

3.3 Results from DNA

The BLAST analysis returned confident results of the organisms. Treatment A was found to be *A. tenuissima,* treatment B was identified as *A. alternata* and treatment C as *F. oxysporum*. The percentage identity was greater than 99% for all isolates except for *A. tenuissima* which had a value of 98.97% (Table 8).

4. Discussion

4.1 Discussion of the disease survey

All fungi isolated in table 5 are consistent with other studies (Leyendecker Jr, 1954; Aponyi et al., 1987, Van Poucke et al., 2012). The disease survey confirmed that *Alternaria* spp. and

Fusarium spp. are associated with capsicum internal fruit rot in Australia. A larger proportion of affected fruit yielded *Alternaria* spp. compared with *Fusarium* spp. These results are consistent with several early studies where *Alternaria* spp. were recovered from symptomatic fruit growing in field conditions (Halfon-Meiri and Rylski, 1983; Aponyi et al., 1987; Bruton et al., 1989).

Table 7
Summary of Koch's postulates trial showing percentage re-infection by different treatments

Treatment	Number of fruits	Number of fruits from which fungus was re-isolated	% infection
Control	103	0	0 a
A (*A. tenuissima)*	98	17	17 b
B (*A. alternata)*	109	9	8 ab
C (*F. oxysporum)*	104	15	14 b
D (*Fusarium* spp.)	106	0	0 a

* Letters indicate groups which are significantly different

This contrasts with more recent literature published in the northern hemisphere which has focused heavily on *Fusarium* spp., especially *F. lactis* (Utkhede and Mathur 2004; Kharbanda et al., 2006; Yang et al., 2009; Van Poucke et al., 2012). The capsicum internal rot disease in the northern hemisphere mostly affects plants grown under greenhouse conditions (Yang et al., 2009; Frans et al., 2014; Wang et al., 2019). *F. lactis* has not been reported in Australia and there have been no significant product rejections in the market chain for greenhouse capsicums with internal rot symptoms (Summerell et al., 2011; Phillip Ritchie, pers comms). *F.lactis* was not detected in this survey.

The results suggest that environmental field conditions may be more favourable to infection by *Alternaria* spp. than *Fusarium* spp., however this has not been confirmed and it should be noted

that conditions for growth of *A. alternata* and *Fusarium* spp. are similar (Misaghi et al., 1978; Hubballi et al., 2010; Frans et al., 2017). Differences in the abundance of organisms may account for the differences in greenhouse and field conditions. As saprophytes, *Alternaria* spp. reproduce on decaying materials, in field conditions these materials are readably available. Sun scalded fruit or fruit affected by blossom-end rot (an environmental disorder caused by lack of sufficient calcium in growing plant tissue) can be sources of inoculum in field, these fruit defects were readily observed in the Sydney basin survey location. Greenhouses are much cleaner and provide less material for saprophyte reproduction (Fig. 2).

Table 8
Species identification of treatments using the plasma membrane ATPase gene region for treatments A and B, and the ITS, TEF1 and RPB2 gene regions for treatment C. The GenBank accession number most closely associated with the isolates analysed is included.

Treatment	Organism	Percentage identity (%)	GenBank Number
Treatment A	*Alternaria tenuissima*	98.97	MH492705.1
Treatment B	*Alternaria alternata*	99.67	CP061880.1
		99.83 (ITS)	MH575293.1
Treatment C	*Fusarium oxysporum*	100 (TEF1)	CP052043.1
		100 (RPG2)	MN892310.1

The morphological analysis of the organisms using light microscopy suggested that the *Alternaria* spp. were part of the *Alternaria alternata* species complex. This species group consists of a few similar organisms including *A. alternata* and *A. tenuissima* (Armitage et al. 2015). *A. alternata* has also been known as *A. tenuis* but has been renamed (Von Keißler 1912). All incidences of *Alternaria* spp. causing internal rot have been confirmed as *A. alternata* or *A. tenuis,* which is consistent with the morphological findings of this study (Leyendecker Jr, 1954;

Halfon-Meiri and Rylski, 1973; Halfon-Meiri and Rylski, 1983; Aponyi et al., 1987; Bruton et al., 1989).

The *Fusarium* spp. analysed suggested that treatment C was likely *F. oxysporum* and that treatment D was likely *F. semitectum*. *F. oxysporum* is a widely known causal organism of internal fruit rot of capsicums and its presence is unsurprising (Yang et al., 2009; O'Neill and Mayne, 2015; Frans et al., 2017). *F. semitectum* however, is not a known causal organism and its presence is surprising. The organism is known to cause root rot in *C. annuum* grown in greenhouse conditions in China (Li et al., 2018).

The complete absence of fungal species in discoloured seed is consistent with the industry understanding of internal rot and its symptoms; dark seeds are not considered a major defect on retail quality specifications. Despite this, it is likely that the discolouration has been mistaken for internal fruit rot; if not by industry workers then definitely by consumers. This may, in turn, inflate the number of complaints by the general public or rejected consignments of fruit by retail, especially when discolouration is severe (Fig. 5).

4.2 Discussion of Koch's postulates

Three out of the four fungal inoculants were re-isolated from infected fruit, suggesting that three organisms cause symptoms of internal rot. The white yellow *Fusarium* with mesoconidia resembling *F. semitectum* was not re-isolated. The lack of infection in all cultivars for *F. semitectum* suggests that it was not the cultivar that restricted infection.

There is a range of possible reasons for the lack of infection. The methods of infection may have been ineffective for this treatment, although this seems unlikely as infection occurred with other treatments using this method of infection. During the trial there were difficulties in maintaining

the temperatures in the greenhouse. It is possible that this may have impacted on infection. A low spore count for *F. semitectum* in comparison to the other *Fusarium* treatment may also have reduced infection (Table 1).

However, perhaps the two most likely reasons for this result were that the organism was a secondary infection or contaminant or there was an issue with the spore type ratios. The source of the *F. semitectum* was from a fruit that was sent pre-cut. There is a possibility that the organism was contaminated during its initial assessment to determine if the disease was present. This is supported by the observation that a collection of different organisms were present when the fruit was plated out (Fig. 6a).

Another possible explanation is the spore type ratio. It is well known that media has a large influence on the macro and microconidia ratios (Chittem and Kulkarni, 2008). ¼ PDA with 100 ppm Novobiocin was the media used to produce inoculum for the trial. *F. semitectum* can form different conidial bodies called mesoconidia, and when plated on the media used it only produced this type of conidia. The mesoconidia may be incompatible with the floral method of infection and provides a possible reason for the lack of infection.

It should be noted that the control and *F. semitectum* treatment both returned infection from a *Fusarium* morphologically similar to *F. oxysporum,* used for treatment C. However, the fruit that returned this result also showed external symptoms, soft rot or a split exocarp. *Fusarium* spores from a similar *Fusarium* sp. that causes the disease have been found to be present in greenhouse air, and are likely the source of the infection (Kharbanda et al., 2006).

Infection of fruit with no obvious external symptoms, and that were inoculated via the flower strongly suggests that infection occurs during flowering. This is consistent with other literature,

with both *Alternaria* spp. and *Fusarium* spp. being found to infect via the floral parts of the fruit (Halfon-Meiri and Rylski, 1983; Aponyi et al., 1987; Yang et al., 2010). Infected fruit with holes in the apex suggests that the flower may not be the only way infection occurs, however holes in that part of the fruit are only sometimes associated with internal rot and are therefore not the primary mode of infection.

Infection rates of inoculated fruit were lower than other trials (Halfon-Meiri and Rylski, 1983; Yang et al., 2009). The results are within the five to 20% infection rates reported by northern hemisphere greenhouse producers, but were lower than expected for this trial (Frans et al., 2016a).

Fungi were isolated in some symptomless fruit. It is known that the disease remains latent, or grows only slowly while fruit is immature (Halfon-Meiri and Rylski, 1983; Yang et al., 2009; Yang et al., 2010). During the disease survey this was observed on green fruit, however, this also occurred in some of the ripe fruit in the Koch's postulates trial. It is possible that fruit were not picked when fully ripe, or that some fruit resist the development of symptoms associated with infection. It is also possible that fungal infection develops significantly during postharvest storage and transport to retail.

The statistical analysis of the variety variable alone suggested there was no difference between varieties in terms of number of infected fruit (Fig. 7). This contrasted with results that have shown there can be a difference between varieties (Utkhede and Mathur, 2004: Kharbanda et al., 2006; Frans et al., 2014; Frans et al., 2016a). The difference between varieties was better observed with the interaction of specific treatments and varieties (Fig. 13).

Analysis of treatment and variety interaction yielded more informative results. No internal rot as caused by *A. alternata* was found in *California wonder* despite being found in the other varieties. A similar scenario occurred with *F. oxysporum* in *SV6947PB*. Of the infection that did occur, only *F. oxysporum* was significantly more common than the other infections caused by different treatments in the other varieties (Fig. 13). It seems likely that there is a difference in susceptibility between varieties, an observation that is consistent with other trials (Utkhede and Mathur, 2004: Kharbanda et al., 2006; Frans et al., 2014; Frans et al., 2016a).

The mechanisms of varietal susceptibility are currently unknown. It has been suggested that fruit load can impact infection, with lower fruit loads resulting in higher rates of infection (Frans et al., 2016a). This was not observed in this trial as the relatively low number of fruit in the *California wonder* variety shared a very similar proportion of infection as the more numerous fruit bearing variety of *warlock* (Table 6).

4.3 Discussion of DNA and similarity to world-wide results.

F. oxysporum is a commonly identified causal organism of capsicum internal rot in Europe and Canada (Yang et al., 2009; O'Neill and Mayne, 2015; Frans et al., 2017). It has been found at higher frequencies than *F. proliferatum* and found at a similar rate to *F. solani* (Kharbanda et al., 2006; Yang et al., 2009; Frans et al., 2017; Wang et al., 2019). While usually found to cause wilting through root infections, *F. oxysporum* f.sp. *niveum* has been known to infect watermelons seeds via inoculation of the flower. However, this did not cause internal rot symptoms similar to that of capsicums in terms of internal mycelial growth and was limited to seeds (Petkar and Ji, 2017). It is likely that other internal rot *Fusarium* spp. are present in Australia. Restriction in travel due to COVID-19 and its impact on the disease survey may have resulted in the absence of

some species; further surveys may reveal more species. Both *F. proliferatum* and *F. solani* are commonly found in Australia and are known to cause the internal fruit rot overseas (Yang et al., 2009; Summerell et al., 2011).

The absence of *F. lactis* in the disease survey is unsurprising as it has not yet been identified as present in Australia (Summerell et al., 2011). It has so far only been associated with studies focused on greenhouse grown capsicums and not on field crops (Utkhede and Mathur, 2005; Kharbanda et al., 2006; yang et al., 2009; Van Poucke et al., 2012).

A. alternata has been identified causing the disease multiple times as either *A. tenuis* or *A. alternata* (Leyendecker Jr, 1954; Halfon-Meiri and Rylski, 1983; Aponyi et al., 1987; Bruton et al., 1989). Absence of *A. alternata* in the form of treatment B in the *California wonder* variety contrasts with earlier studies conducted (Halfon-Meiri and Rylski, 1983). This may suggest that spore concentrations were too low for infection (Table 1). A spore concentration of 2×10^5/ mL was used by Halfon-Meiri and Rylski in 1983 and is higher than the spore concentration used by this study. Despite low spore concentration when compared to Halfon-Meiri and Rylski (1983) infection still occurred in the other varieties (Fig. 13). It is also possible that environmental factors were not properly controlled, or some other exogenous factor had an impact on infection.

Internal infection of fruit by *Alternaria* spp. is well known across multiple commodities. *A. alternata* has been reported as causing internal fruit rots in other species such as the disease, black rot in citrus which has a similar internal infection and lack of external symptoms. (Akimitsu et al., 2003). Both *A. alternata* and *A. tenuissima* have been isolated from mouldy core of apples. (Elfar et al., 2019). Infection of apples was demonstrated to occur through flowers.

Application of fungicides at flowering has been shown to reduce apple infection (Reuveni et al., 2002).

The most interesting reported organism at first glance is *A. tenuissima* which, as of yet, has not been reported as causing the disease. However, *A. tenuissima* is part of the *Alternaria alternata* species group (Armitage et al. 2015). Returned species are consistent with international research except for *A. tenuissima* which has not been identified as a casual organism (Halfon-Meiri and Rylski, 1983; Utkhede and Mathur, 2004; Kharbanda et al., 2006; Yang et al., 2009; Frans et al, 2017).

4.4 Future directions and potential controls Australia can implement

With the causal organisms in Australia known it is imperative that control methods be introduced to reduce incidences of the disease. During the *F. lactis* outbreak in Canada a range of controls were proposed. These included good sanitation, reducing humidity, maintaining good air circulation and preventing wounding to the stem and fruit. However, these controls were devised for a greenhouse situation and may be limited for Australian field production (Kharbanda, 2006). It would be possible to implement some rough strategies to reduce internal rot in field crops by reducing the use of overhead irrigation to maintain flower dryness (Soil Wealth, 2015).

Varietal susceptibility is a well reported factor of internal fruit rot in capsicums, both in this paper and the literature (Kharbanda, 2006; Fig. 13). However, the mechanism of resistance is currently unknown. Studies on floral morphology yielded inconclusive results about resistance due to flower size and shape (Frans et al., 2016a). The creation of a bioassay to test susceptibility of capsicum varieties provides a method where new cultivars could quickly and efficiently be tested for internal rot resistance (Frans et al., 2016b).

A range of chemical controls have been proposed for both *A. alternata* and *Fusarium* spp., However currently none of these are approved for use in capsicum production in Australia (Utkhede and Mathur, 2005; Aponyi et al., 1987). Application of a *Bacillus subtilis* biological treatment has seen success in reducing incidence of *F. lactis* when applied to flowers, the effectiveness of this bacterium on other *Fusarium* spp. and *A. alternata* is currently unknown (Utkhede and Mathur, 2005). Control trials are currently being planned to investigate potential controls for Australian field production.

5. Conclusion

The internal fruit rot of *C. annuum* has a range of causal organisms, of those found in Australia it can be confirmed that *A. tenuissima*, *A. alternata*, and *F. oxysporum*, cause the disease. With the exception of *A. tenuissima* these are well known causal organisms.

Internal fruit rot can be caused by infection through the flower; however this may not be the only method of entry. The presence of holes at the style of the fruit, blossom end rot and cracks on the exocarp may also allow for entry of pathogens.

No variety in this trial was able to reduce the incidence of disease. Varieties did however reduce incidence of specific organisms causing disease. Differing varietal susceptibility was consistent with international literature.

6. References

Akimitsu, K., Peever, T.L., Timmer, L.W., 2003. Molecular, ecological and evolutionary approaches to understanding Alternaria diseases of citrus. Molecular Plant Pathology. 4(6), 435-446. https://doi.org/10.1046/j.1364-3703.2003.00189.x

Armitage, A.D., Barbara, D.J., Harrison, R.J., Lane, C.R., Sreenivasaprasad, S., Woodhall, J.W., Clarkson, J.P., 2015. Discrete lineages within Alternaria alternata species group: identification using new highly variable loci and support from morphological characters. Fungal biology. 119(11), 994-1006. https://doi.org/10.1016/j.funbio.2015.06.012

Aponyi, I., Kujáni, O., Varga, B.B.A., 1987. The ovary moulding disease of tomato-shaped pepper and control possibilities. In I International Symposium on Vegetables for Processing 220, 401-406. https://doi.org/10.17660/ActaHortic.1988.220.56

Bruton, B.D., Chandler, L.D., Miller, M.E., Ramey, H.H., Beaton, P.G., 1989. Relationships between pepper weevil and internal mold of sweet pepper. Plant dis. 73(2), 170-173. https://doi.org/10.1094/PD-73-0170

Bureau of Meteorology, 2020. Climate Data Online. http://www.bom.gov.au/climate/data/index.shtml?bookmark=200 (Accessed 11 November 2020).

Burgess, L.W., Summerell, B.A., Bullock, S., Gott, K.P., Backhouse, D., 1994, Laboratory Manual For Fusarium Research, third ed. University of Sydney, Sydney.

Chittem, K., Kulkarni, S., 2008. Effect of Media on the growth of Fusarium oxusporum f. sp. gerberae and Fusarium oxysporum f. sp. dianthi. Karnataka. J. of Agricultural Sciences. 21. 303-304.

Choi, H.W., Hong, S.K., Kim, W.G., Lee, Y.K., 2011. First report of internal fruit rot of sweet pepper in Korea caused by Fusarium lactis. Plant dis. 95(11), 1476-1476. https://doi.org/10.1094/PDIS-03-11-0157

Elfar, K., Zoffoli, J.P., Latorre, B.A., 2019. Alternaria spp. on apparently healthy apples as a potential inoculum source for moldy core development and the effect of resistant and susceptible apple cultivars. European J. of Plant Pathology. 155(3), 743-754. https://doi.org/10.1007/s10658-019-01802-2

Food and Agriculture Organisation of the United Nations (FAO), 2018. FAOSTAT. http://www.fao.org/faostat/en/#data/QC/visualize (Accessed 11 November 2020).

Frans, M., Aerts, R., Van Calenberge, B., Van Herck, L., Ceusters, J., 2016a. Influence of floral morphology and fruit development on internal fruit rot in bell pepper (Capsicum annuum). In III International Symposium on Postharvest Pathology: Using Science to Increase Food Availability. 1144, 199-206. https://doi.org/10.17660/ActaHortic.2016.1144.29

Frans, M., Aerts, R., Van Laethem, S., Ceusters, J., 2017. Environmental effects on growth and sporulation of Fusarium spp. causing internal fruit rot in bell pepper. European J. of plant pathology. 149(4), 875-883. https://doi.org/10.1007/s10658-017-1235-4

Frans, M., Aerts, R., Van Laethem, S., Van Calenberge, B., Van Herck, L., Heungens, K., Van Poucke, K., Van Gool, S., Ceusters, J., 2016b. Development of a quick screening bioassay for

internal fruit rot in bell pepper (Capsicum annuum L.). In Proceedings of XVIth EUCARPIA Capsicum and Eggplant Working Group Meeting in memoriam Dr. Alain Palloix, 12-14 September 2016, Kecskemét, Hungary. 420-424.

Frans, M., Sauviller, C., Van Mechelen, M., Heungens, K., Van Poucke, K., Van Calenberge, B., Van Herck, L., Aerts, R., 2014. Sustainable control of internal fruit rot in bell pepper: a multidisciplinary approach. Communications in Agricultural and Appl. Biological Sciences. 79(3), 373-373.

Halfon-Meiri, A., Rylski, I., 1973. Penetration of Alternaria tenuis into pepper fruit. Phytoparasitica. 1, 57. https://doiorg.ezproxy1.library.usyd.edu.au/10.1007/BF02980307

Halfon-Meiri, A., Rylski, I., 1983. Internal mold caused in sweet pepper by Alternaria alternata: Fungal ingress. Phytopathology. 73(1).

HORT Innovation, 2019. Australian Horticulture Statistics Handbook 2018/19. https://www.horticulture.com.au/growers/help-your-business-grow/research-reports-publications-fact-sheets-and-more/grower-resources/ha18002-assets/australian-horticulture-statistics-handbook/?__FormGuid=a2cd5e51-ecbe-4c53-ae51-a870fc5dfc75&__FormLanguage=en&__FormSubmissionId=c354c440-662c-491c-bf0f-c157977a60d2#PDF (accessed 17 October 2020).

Hubballi, M., Nakkeeran, S., Raguchander, T., Anand, T., Samiyappan, R., 2010. Effect of environmental conditions on growth of Alternaria alternata causing leaf blight of noni. World J. of Agricultural Sciences. 6(2), 171-177.

Kharbanda, P.D., Yang, J., Howard, R.J., Mirza, M., 2006. Internal fruit rot of greenhouse peppers caused by Fusarium lactis–a new disease. The Greenh. Bus. 5, 11–16

Lawrence, D.P., Gannibal, P.B., Peever, T.L., Pryor, B.M., 2013. The sections of Alternaria: formalizing species-group concepts. Mycologia. 105(3), 530-546. https://doi.org/10.3852/12-249

Leslie, JF, Summerell, B.A., 2007, The Fusarium Laboratory Manual, Blackwell Publishing, Ames, Iowa, USA. https://doi.org/10.1002/9780470278376

Leyendecker Jr, P.J., 1954. Fungi associated with internal contamination of sun-dried chile in New Mexico. Bulletin of the Torrey Botanical Club. 400-404. https://doi.org/10.2307/2482281

Li, H.Y., Guo, W., Liu, D., Li, M.Q., 2018. First Report of Fusarium semitectum Causing Root Rot of Greenhouse Pepper (Capsicum annuum) in China. Plant Dis. 102(10), 2032-2032. https://doi.org/10.1094/PDIS-11-17-1704-PDN

Misaghi, I.J., Grogan, R.G., Duniway, J.M., Kimble, K.A., 1978. Influence of environment and culture media on spore morphology of Alternaria alternata. Phytopathology. 68(1), 29-34. https://doi.org/10.1094/Phyto-68-29

O'Donnell, K., Kistler, H.C., Cigelnik, E., Ploetz, R.C., 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. Proceedings of the National Academy of Sciences. 95(5), 2044-2049. https://doi.org/10.1073/pnas.95.5.2044

O'Donnell, K., Sarver, B.A., Brandt, M., Chang, D.C., Noble-Wang, J., Park, B.J., Sutton, D.A., Benjamin, L., Lindsley, M., Padhye, A., Geiser, D.M., 2007. Phylogenetic diversity and microsphere array-based genotyping of human pathogenic fusaria, including isolates from the

multistate contact lens-associated US keratitis outbreaks of 2005 and 2006. J. of clinical microbiology. 45(7), 2235-2248. https://doi.org/10.1128/JCM.00533-07

O'Neill, T., Mayne, S., 2015. Sweet pepper: Aspects of the biology and control of fusarium fruit rot. Communications in agricultural and appl. biological sciences. 80(3), 569-573.

Petkar, A., Ji, P., 2017. Infection courts in watermelon plants leading to seed infestation by Fusarium oxysporum f. sp. niveum. Phytopathology. 107(7), 828-833. https://doi.org/10.1094/PHYTO-12-16-0429-R

Reuveni, M., Sheglov, D., Sheglov, N., Ben-Arie, R., Prusky, D., 2002. Sensitivity of Red Delicious apple fruit at various phenologic stages to infection by Alternaria alternata and moldy-core control. European j. of plant pathology. 108(5), 421-427. https://doi.org/10.1023/A:1016063626633

Simmons, E.G., 2007. Alternaria: an identification manual, first ed. CBS Fungal Biodiversity Centre, Utrecht.

Soil Wealth, 2015. Internal rot in capsicums causes and controls https://www.soilwealth.com.au/imagesDB/news/ICP_CapsicumRot_FINAL_Email.pdf (accessed 11 November 2020).

Summerell, B.A., Leslie, J.F., Liew, E.C., Laurence, M.H., Bullock, S., Petrovic, T., Bentley, A.R., Howard, C.G., Peterson, S.A., Walsh, J.L., Burgess, L.W., 2011. Fusarium species associated with plants in Australia. Fungal Diversity. 46(1), 1-27. https://doi.org/10.1007/s13225-010-0075-8

Utkhede, R.S., Mathur, S., 2004. Internal fruit rot caused by Fusarium subglutinans in greenhouse sweet peppers. Canadian J. of plant pathology. 26(3), 386-390. https://doi.org/10.1080/07060660409507157

Utkhede, R.S., Mathur, S., 2005. Biological and Chemical Control of Fruit Rot in Greenhouse Sweet Peppers(Capsicum annum L.) Caused by Fusarium subglutinans. J. of Biological Sciences. 5(5), 610-615. https://doi.org/10.3923/jbs.2005.610.615

Van Poucke, K., Monbaliu, S., Munaut, F., Heungens, K., De Saeger, S., Van Hove, F., 2012. Genetic diversity and mycotoxin production of Fusarium lactis species complex isolates from sweet pepper. International J. of Food Microbiology. 153, 28–37. https://doi.org/10.1016/j.ijfoodmicro.2011.10.011

Von Keißler, K., 1912. Zur Kenntnis der Pilzflora Krains. Beih Bot Centralbl 29:395–440.

Wang, A., Islam, M. N., Johansen, A., Haapalainen, M., Latvala, S., Edelenbos, M., 2019. Pathogenic Fusarium oxysporum f. sp. cepae growing inside onion bulbs emits volatile organic compounds that correlate with the extent of infection. Postharvest Biology and Technology. 152, 19-28. https://doi.org/10.1016/j.postharvbio.2019.02.010

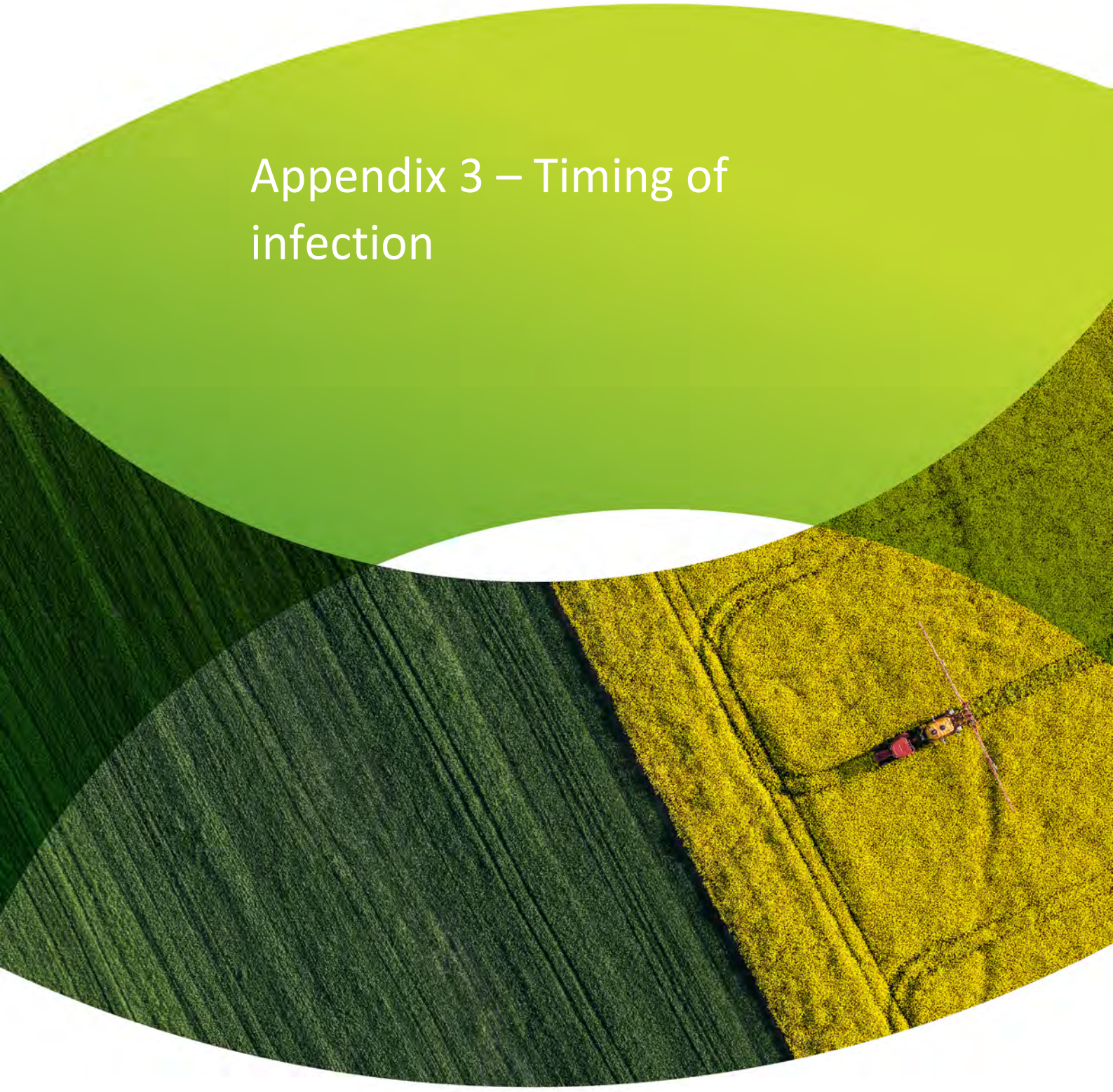
Wang, J., Wang, S., Zhao, Z., Lin, S., Van Hove, F., Wu, A., 2019. Species Composition and Toxigenic Potential of Fusarium Isolates Causing Fruit Rot of Sweet Pepper in China. Toxins. 11(12), 690. https://doi.org/10.3390/toxins11120690

White, T.J., Bruns, T., Lee, S.J.W.T., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications. 18(1), 315-322.

Yang, J., Kharbanda, P. D., Howard, R. J., Mirza, M., 2009. Identification and pathogenicity of Fusarium lactis, causal agent of internal fruit rot of greenhouse sweet pepper in Alberta. Canadian J. of Plant Pathology. 31, 47–56. https://doi.org/10.1080/07060660909507571

Yang, Y., Cao, T., Yang, J., Howard, R.J., Kharbanda, P.D., Strelkov, S.E., 2010. Histopathology of internal fruit rot of sweet pepper caused by Fusarium lactis. Canadian J. of Plant Pathology. 32(1), 86-97. https://doi.org/10.1080/07060660903503681

Appendix 3 – Timing of infection



APPLIED HORTICULTURAL RESEARCH ABN 58 134 308 982
Suite 340, Biomedical Building, 1 Central Avenue, Australian Technology Park, Eveleigh NSW 2015
info@ahr.com.au | +612 8627 1040 | www.ahr.com.au

Authors: **Ryan Hall, Len Tesoriero**

Mobile: **+61 406 994 509**

Introduction

These lab-based trials aimed to determine if internal fruit rot of capsicum could be induced via inoculation of the remnant style of harvested ripe fruit. This could influence both selection of varieties with improved resistance to IFR (for example, varieties that shed the floral style) and the timing of control strategies, including fungicides as well as biological controls.

Trial 1: Fruit style inoculation

Why look at the style on fruit?

Throughout the project, team members have noted that while infection can occur during flowering, it could also occur after flowering. The style could provide an entry point for the causal organisms that could be exploited to cause internal fruit rot symptoms (Figure 1). If infection can occur after flowering, it poses severe challenges to control of internal fruit rot of capsicums.

Figure 1: A remnant style present on a ripe fruit. This example shows the necrotic tissue that could provide an entry of fungus into the internal parts of the fruit

Treatments

Three treatments of control (no treatment), water control (water inoculation) and treated (inoculated with causal organism) were set up. Fifty ripe fruit for each treatment (150 in total) were obtained from Sydney markets.

Inoculum was prepared on ¼ PDA agar plates, and suspended in sterile, de-ionized water. Using a paint brush, inoculum was applied to the style. Fruit were then placed into sealed plastic bags for 24 hours to increase the humidity to encourage infection.

The water control conducted the same method but without the inoculum. The control had no treatment except for being placed into plastic bags to increase the humidity.

Results

Due to the progressed physiological age of the fruit, many fruit broke down quickly while at room temperature. Visible symptoms of internal fruit rot were also not found in any significant number.

A number of confounding factors such as temperature at inoculation, concentration of inoculum, the age of the fruit and time for infection to become symptomatic may have lead to this result. An additional trial was conducted to address these issues.

Trial 2: Fruit Style Inoculation

Treatments

Adjustments were made to the original trial to increase the likelihood of obtaining higher quality data. these included

- Pre-soaking the styles in deionized water to reduced hydrophobic forces from the style during inoculation (Figure 2)
- Fruit kept at increased temperature (25*C) for 48 hours after inoculation to improve conditions for fungal growth
- A higher concentration of inoculum was prepared (Figure 3)

Assessment was conducted both visually and by plating samples from internal parts of the fruit

Figure 2: fruit from Sydney markets pre-soaking in sterile deionized water to reduce the hydrophobic nature of the remnant styles

Figure 3: project team preparing inoculum

Results and Discussion

Despite changes to the trial design, similar issues to those faced in the initial trial occurred. This included the early rotting of fruit and lack of visible symptoms. No significant differences were found between treatments. Plating samples of internal parts of the fruit did not yield further information. It is possible that there was not enough time for infection to progress through the style.

The project team concluded that using fruit already harvested for these trials would not yield results as there was limited time to induce symptoms after inoculation before fruit started to breakdown. To address this the team has setup a greenhouse trial to test the inoculation of live fruit at different stages.

Trial 3 – Infection timing of *Alternaria alternata* and development of internal fruit rot of capsicums

By Len Tesoriero

Specialist Plant Pathologist

Crop Doc Consulting Pty Ltd

Introduction

We have previously demonstrated the internal fruit rot (IFR) of field capsicums in Australia is mostly caused by the fungus *Alternaria alternata.* A similar disease on greenhouse and field capsicums in the Northern Hemisphere is mostly caused by a different fungus, *Fusarium lactis* (Kline & Wyenandt, 2014; Frans *et al*., 2017), which was not detected in surveys and diagnostics of Australian field capsicums.

One older report from Israel (Halfon-Meiri & Rylski, 1983) described an IFR of capsicums caused by *A. alternata*. They demonstrated that infection occurred at the flowering stage, with the greatest infection occurring in fruit that were inoculated one day before or on the day of anthesis. In the variety California Wonder, disease incidence decreased when flowers were inoculated 5-6 days after anthesis, and no disease was observed when fruit were inoculated 20 days after anthesis. In another variety, Yellow Wonder, disease incidence was higher in fruit developing from flowers inoculated 2 days after anthesis compared with flowers inoculated after 7 days. Their results appear to be consistent with studies of *F. lactis* demonstrating that infection occurs when fungal spores germinate on the style and follow the pollen tube down and into the ovaries (Yang *et al*., 2010; Frans *et al*., 2017).

Understanding the period when fruit are most susceptible to infection is important when considering management options using agrichemicals or biological products.

This experiment was designed to determine if IFR of red fruit only results when *A. alternata* infects vestigial ovaries during flowering or if entry can occur via the dried remains of styles on developing fruit given they commonly remain attached to until and after maturity.

Methods

A greenhouse trial was designed to compare the following treatments:

1. Nil inoculum
2. Inoculation of styles at flowering
3. Inoculation of styles on fruit <20 mm diameter
4. Inoculation of large green fruit

Commercial capsicum seedlings (var Plato) were individually transplanted into 4 litre plastic pots (14x14x20cm) and attached to 60 cm stakes. They were grown together in a glasshouse bay (20-30^{o}C diurnal temperature range) until flowering when they were moved to a larger house with 5

suspended rows on which pots were placed in treatment groups (Figure 1). Each set of 8-9 pots was arranged in randomised treatment units down each row which formed 5 replicated blocks. The greenhouse was maintained at a similar temperature range to the glasshouse bay.

An *A. alternata* isolate (#20-39), originally from a capsicum affected by IFR, was single-spored, and incubated on ¼-strength potato dextrose agar medium for 14 days at 25°C. Inoculum was prepared by macerating cultures and filtering through 2 layers of cheesecloth. The inoculum concentration was estimated with a haemocytometer under a light microscope and adjusted to 10^6 mL^{-1}. Inoculum was kept refrigerated in a set of six 50 mL Falcon tubes. Plants were inoculated three times over the course of the trial. A plastic pipette was used to apply up to 5 mL of inoculum dropped directly to the styles of flowers and fruit. During the first inoculum application, we observed that styles were quite hydrophobic so 40 µL Tween 20 was added to each inoculum vial and subsequently used.

Plants received fertilisers as required via drippers placed at the base of each plant. Red fruit were harvested on 4 occasions and were cut and assessed in a laboratory for visible infection, colour (1-5 scale), number of lobes, and if a hole at the apex was visible. The trial timeline of activities is summarised in Table 1.

Affected and symptomless control fruit were sampled from each treatment and cultured to ¼ PDA medium confirming an *A. alternata* was associated with disease symptoms.

Table 1. Trial timeline and key activities

Date	Activity
6/9/2023	Seedlings potted up
22/9/2023	First flowers observed
13/10/2023	First inoculation
2/11/2023	Pots moved into large greenhouse & 2nd inoculation
13/12/2023	1st harvest of red fruit
18/12/2023	2nd harvest + 3rd inoculation
22/12/2023	3rd harvest
9/01/2024	4th harvest and trial terminated

Results and Discussion

Alternaria alternata was confirmed causing an IFR in 23% of fruit inoculated at flowing and 14% of fruit inoculated when they were <20 mm diameter (Table 2). No infection was detected on red fruit after inoculation when they were large and green, or in uninoculated controls. This result confirms that a majority of the IFR infection occurs at flowering but also shows that approximately 38% of the total infected fruit resulted from infection of the stylar end of young green fruit.

These results are largely consistent with those of Halfon-Meiri and Rylski (1983) where the greatest infection occurred at flowering and declined thereafter. Unfortunately, the Israeli study didn't fully describe the appearance or size of developing fruit at 5-, 7- or 20-days post anthesis so it is difficult compare their results with those obtained here. What is clear from this study is that the dried

attached style is an important entry point for *A. alternata*, at least until fruit develop past the 20 cm diameter stage. Another observation that several flowers and young fruit aborted after inoculation was also noted in the Israeli study and suggests that *A. alternata* infection losses are not confined to internal fruit rots.

In addition to the *Alternaria* infected fruit there were a total of 6 fruit in which the fungus *Cladosporium* sp. was identified. A hole at their stylar ends on 4 of these fruit was detected and suggests a likely entry point for infections. *Cladosporium* species are common aerially borne fungi which have also been described associated with IFR and black mould diseases of capsicums in our earlier diagnostic studies.

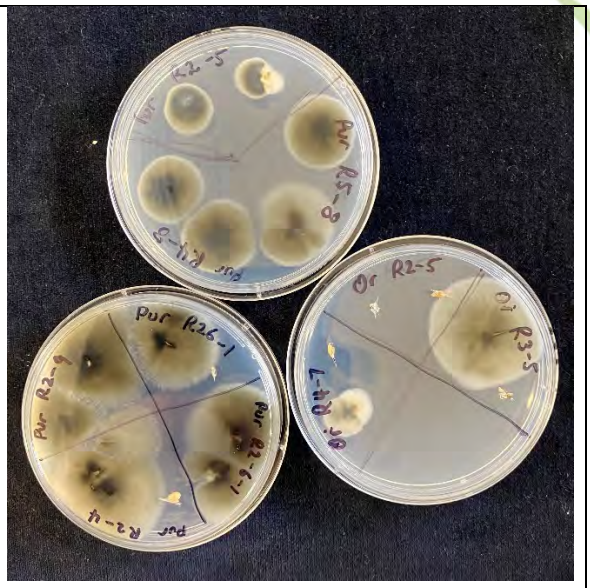
Table 2. Proportions of red fruit with IFR of total fruit

Replicate	**1**	**2**	**3**	**4**	**5**	**Total**	**%**
Inoculation time							
Nil	0/23	0/25	0/24	0/26	0/23	0/121	0
Flowering	3/27	7/22	7/18	6/23	5/34	28/124	23
Small fruit	0/20	3/17	2/25	5/23	4/18	14/103	14
Large green	0/16	0/19	0/15	0/22	0/20	0/97	0

Capsicum plants arranged into 5 rows (replicates) each consisting of 4 randomised treatments units of 8-9 pots

Capsicum trial plants with the first red fruit

Typical *A. alternata* mould on ovary and seeds associated with capsicum IFR



Recovery of typical olive-grey cultures of *A. alternata* growing from infected tissue on agar plate

Figure 4. Images from inoculation trial 3.

References

Frans, M, Aerts, R, van Laethem, S and Ceusters, J (2017). Environmental effects on growth and sporulation of *Fusarium* spp. Causing internal fruit rot in bell pepper. European Journal of Plant Pathology 149:875-883.

Halfon-Meiri, A and Rylski, I (1983). IFR caused in sweet pepper by *Alternaria alternata*: Fungal ingress. Phytopathology 73:67-70.

Kline L and Wyenandt, C (2014). Internal fruit rot and premature seed germination of field grown coloured peppers. In *Proceedings of the 22nd International Pepper Conference*, Vina del Mar, Chili, 17-20.

Yang, Y, Tieesen, C, Yang, J, Howard, R, Kharbanda, P, and Stelkov, S (2010). Histopathology of internal fruit rot of sweet pepper caused by Fusarium lactis. Canadian Journal of Plant Pathology 32:86-97.

Appendix 4 – Examine differences in varietal susceptibility

APPLIED HORTICULTURAL RESEARCH ABN 58 134 308 982
Suite 340, Biomedical Building, 1 Central Avenue, Australian Technology Park, Eveleigh NSW 2015
info@ahr.com.au | +612 8627 1040 | www.ahr.com.au

Author: **Jenny Ekman**

Mobile: **+61 407 384 285**

Email: **jenny.ekman@ahr.com.au**

Disclaimer:

Applied Horticultural Research Pty Ltd (AHR) makes no representations and expressly disclaims all warranties (to the extent permitted by law) about the accuracy, completeness, or currency of information in this report.

Users of this material should take independent action before relying on its accuracy in any way. Reliance on any information provided by AHR is entirely at your own risk.

AHR is not responsible for, and will not be liable for any loss, damage, claim, expense, cost (including legal costs) or other liability arising in any way (including from AHR or any other person's negligence or otherwise) from your use or non-use of information in this report.

Table of Contents

Introduction

One of the questions asked by growers relates to differences in susceptibility to internal fruit rot (IFR) of different varieties. In part, this was triggered by observations of very low levels of IFR in capsicums from SA when compared to field grown fruit from NSW and Queensland.

There was discussion as to whether varieties that were more prone to retaining the stigma throughout fruit growth and development would be more susceptible to infection. There was also a hypothesis that long capsicum varieties may be less affected than blocky types due to the physical distance that develops between the infected stigma and the placental seeds.

Variety trials to examine potential differences between key red capsicum varieties were therefore planted in Sydney and Stanthorpe, Qld.

Sydney Basin Trials

Method

A fully replicated trial was planted on 17th November 2020 at the Richmond Local Land Services trial site. Six varieties were planted; SV6947, Aries, Ducati, Shadowfax, Warlock and Red Jewel, as shown in the trial layout below.

Flowers were spray inoculated with mould spores three times during crop development, using a mixture of *A. alternata* and *F. oxysporum*.

Unfortunately, just as the first fruit were turning red, the crop was attacked by Queensland fruit fly. Within a week, large numbers of fruit were found to be infested with larvae. The crop was treated with insecticide, however significant numbers of maggots were still present at the first crop assessment on 15th March, reducing the number of fruit which could be assessed for IFR.

Results

The initial assessment picked the fruit that were still intact, including red, coloured and mature green fruit. The percentage of fruit with internal fruit rot was much higher in red than coloured fruit, while no green fruit were found to have IFR. While there appeared to be differences between the varieties, (Figure 1) the relatively low number of fruit assessed (65 to 159 per variety, with more green than red) unfortunately means that no conclusions can be drawn from this result.

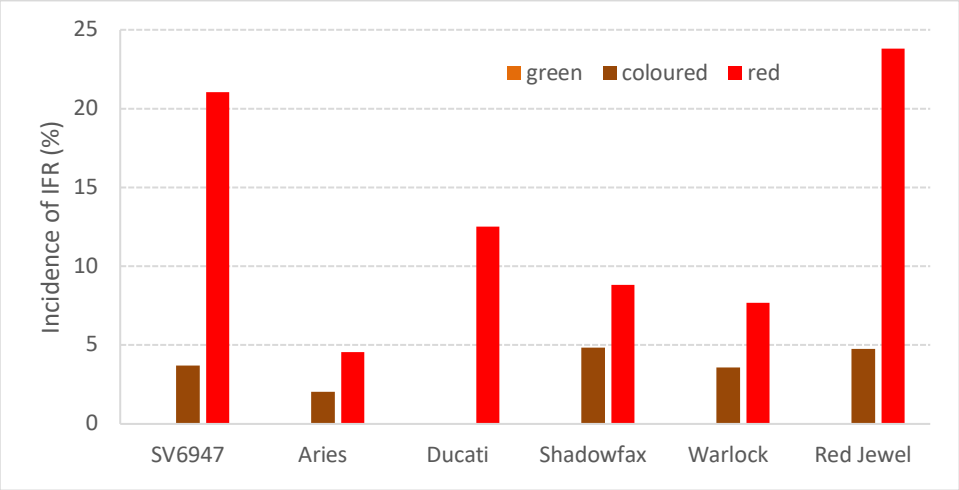


Figure 1. Initial results from the variety trial at the River Farm, Richmond NSW

Two days after this assessment heavy rain started to fall. Major flooding occurred within the Hawkesbury, completely submerging the crop, which remained under water for at least six days. The site could not be accessed again until 30th March, when the total crop loss was apparent (*Figure 2*).

Figure 2. Richmond River Farm field site on 15th March (left) and 30th March (right)

Stanthorpe Trials

Method

Two trials were planted in Stanthorpe, with the generous assistance of Bayer-Seminis. All were red varieties, suited to the region and selected so as to include blocky as well as semi-long types. The varieties Halifax and Shadowfax were included as initial data suggested these new varieties could have some resistance. One tray (approximately 198 plants) of each variety were planted within commercial capsicum crops:

Trial 1	Trial 2
SV3255	SV3255
SV9699	SV9699
Plato	Plato
Shadowfax	Shadowfax
Halifax	Halifax
Aries	Hugo
Lapillo	Warlock
Maximinis	SV6947

The first trial was harvested on 8th February 2021. As each variety was planted in a single row rather than randomized blocks, some 'replication' was created by picking fruit in six blocks of 25 fruit (total 150 fruit) and assessing each batch separately.

Each capsicum was cut in half equatorially and examined for signs of IFR or dark seed (Figure 3). The severity of infection was not recorded as even a tiny lesion could develop during postharvest handling and result in retail rejection. Similarly, **any** discolouration of the seeds was recorded even though symptoms ranged from light to dark brown and were expressed over a small part of the placenta, the entire placenta, or only on the outer edges of the seeds themselves.

Figure 3. Capsicum assessment trial 1, showing mould (cv. Lapillo), a group of mouldy fruit (cv. Aries) and Camila Campos (Bayer-Seminis) conducting the assessments

The second trial was harvested one month later on 10th March. In this case, fruit were picked in four batches of thirty fruit (total 120), each batch including 10 red, 10 part coloured and 10 full green fruit. This was to examine how early mould / dark seed occurred during fruit development.

Results

To avoid unwarranted conclusions regarding susceptibility to mould and dark seed, Bayer-Seminis has requested that the names of the different varieties be deleted from the results.

There were no consistent, significant differences in incidence of IFR between any of the varieties examined in both trials one and two. One variety (F) had significantly higher rates of IFR than others in trial 1. However as this variety had not been planted in trial 2, this cannot be regarded as a reliable, replicated result. Similarly, rates of dark seed for different varieties were not consistent between trials one and two.

There was a clear difference in rates of IFR between the two harvest dates. In February, 28% of fruit had signs of IFR, whereas in March this had fallen to 18% (*Figure 4*). In contrast, dark seed increased between the two sampling times, from 28% of fruit in February to 54% in March. However, this result should be treated with some caution, given that slight browning of the seeds can be quite subjective. Moreover, if fruit had both dark seed and IFR, they were classified as mouldy only.

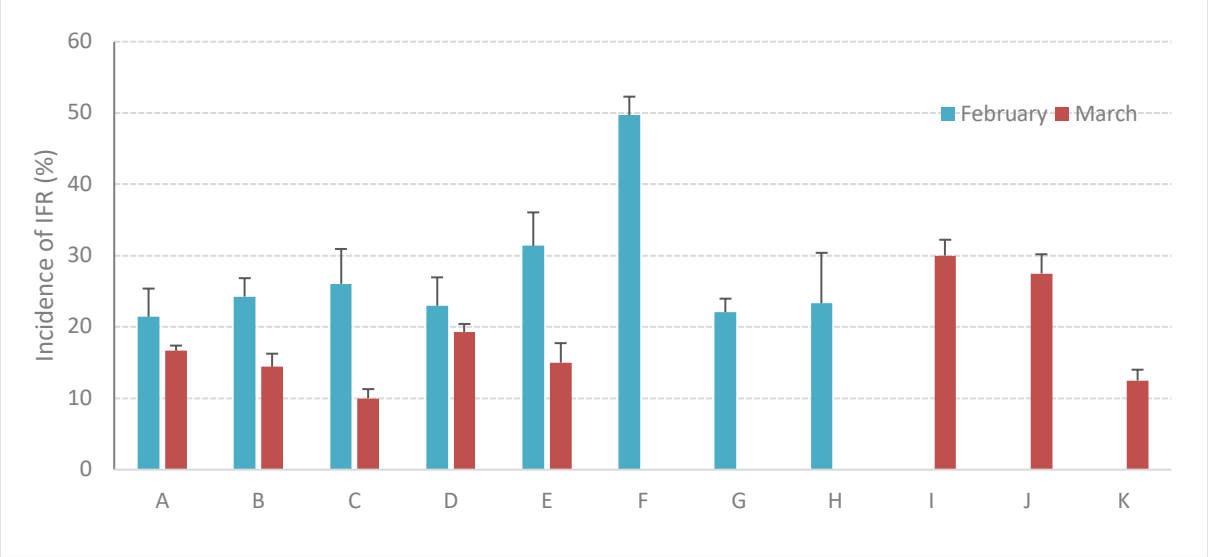


Figure 4. Percentage of red fruit with signs of IFR at the February (trial 1) and March (trial 2) assessments. Bars indicate the standard error of each mean value.

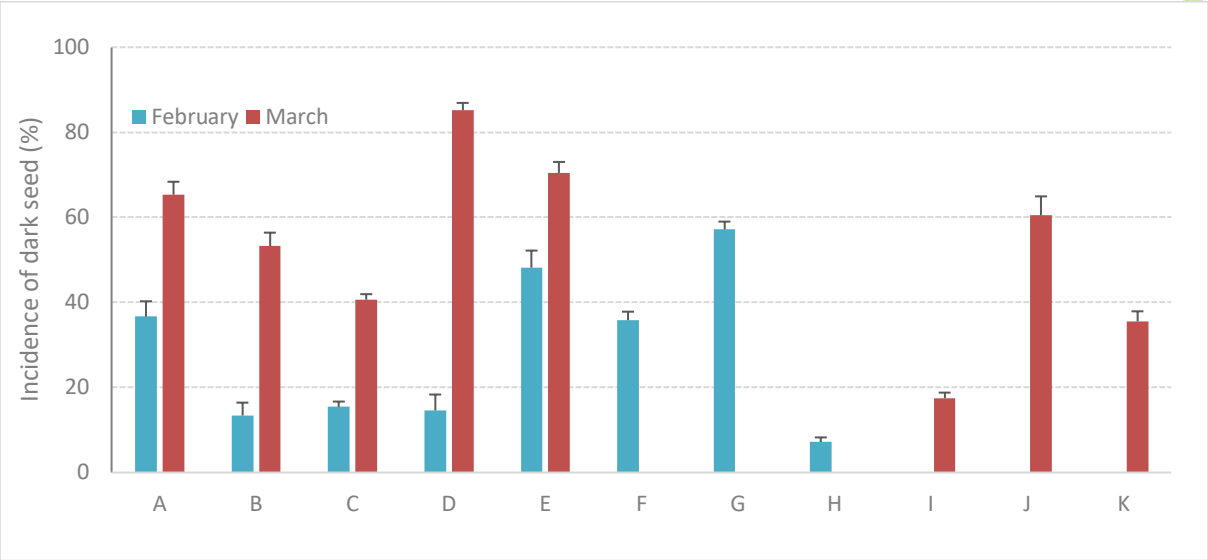


Figure 5. Percentage of red fruit classified as having dark seed at the February (trial 1) and March (trial 2) assessments. Bars indicate the standard error of each mean value.

When assessing the effect of maturity, it is clear that IFR increases significantly as fruit become fully red. Only 4.6% of coloured fruit had signs of IFR, compared to 18% of fruit that were fully red. This suggests that picking slightly earlier can greatly reduce the incidence of disease.

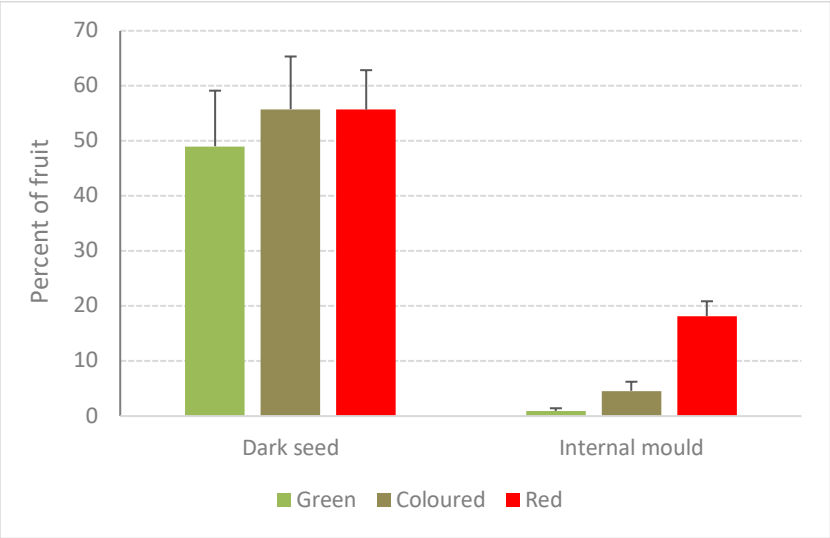
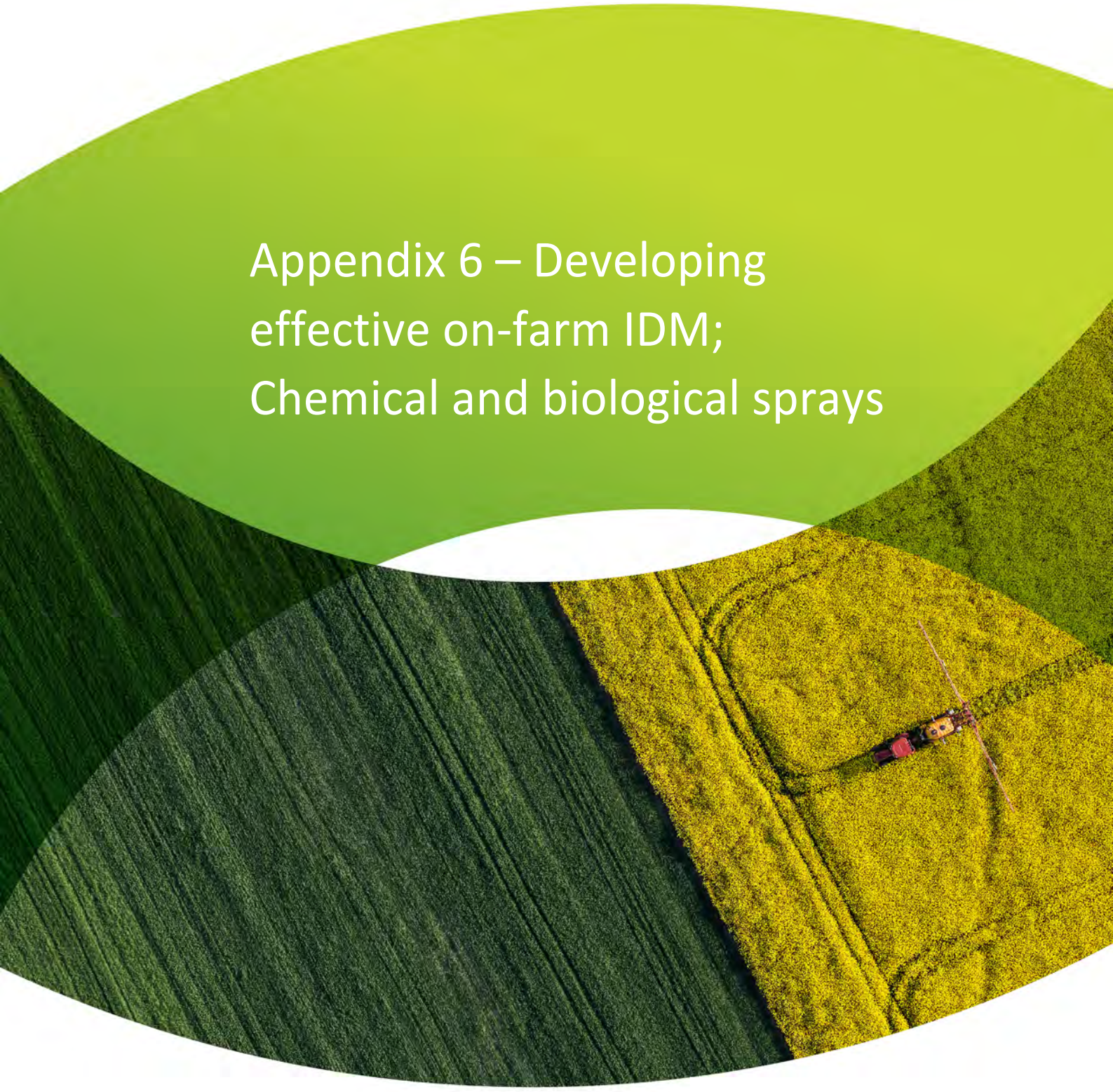


Figure 6. Percentage of fruit assessed when green, part coloured, or full red which had signs of dark seed or IFR at the March assessment.

In contrast, dark seed is unrelated to fruit maturity. Rates of dark seed in green fruit were not significantly different to those harvested when more mature.

It has previously been observed that no pathogens could be isolated from dark seed. It seems highly likely that this condition has physiological, rather than pathological, origins.

Appendix 6 – Developing effective on-farm IDM; Chemical and biological sprays



Authors: **Naomi Diplock and Ryan Hall**

Mobile: **+61 403 740 123**

Email: **naomi.diplock@ahr.com.au**

Contents

Introduction and Background

Internal fruit rot (IFR) in capsicums, primarily caused by *Alternaria alternata* and *Fusarium* species, significantly impacts yield and fruit quality. This report summarizes findings from six trials conducted in Sydney and Bundaberg, aimed at evaluating management strategies for IFR, including fungicides, Trichoderma-based biocontrol agents, and variations in spray application methods. Trials were conducted under both research and commercial farm conditions, addressing the role of environmental factors, application techniques, and post-harvest practices.

Key findings include:

- **Fungicide Treatments**: Fungicide applications showed limited efficacy in reducing IFR incidence, with no statistically significant differences observed between treated and control groups in multiple trials.
- **Trichoderma Biocontrol**: Trichoderma-based treatments, including Nutri-Life Trichoshield™, demonstrated potential in slightly reducing IFR incidence. However, overall IFR rates were low and these reductions were not statistically significant.

The overall low incidence of IFR across trials may have reduced the ability to detect treatment effects, emphasizing the importance of refining inoculation protocols and environmental monitoring in future studies. Despite the lack of statistically significant results, Trichoderma-based treatments and other integrated approaches show promise and warrant further exploration under varied conditions to enhance sustainable capsicum production.

1 Trial 1: Local Land Services site Richmond

1.1 Trial setup

Planting at the Richmond Lowlands trial site was staggered over three dates at approx. 6 week intervals so as to cover the full growing period for this district.

Rows of capsicums were planted over two blocks on 1/10/2021 and 17/11/2021 and 11/1/2022. Hobo temperature + RH loggers were installed within the crop in both blocks. Flowering began in late November.

Plantings 1 and 2 (1 row each in the top and bottom blocks at the trial site) were to be used to model the effect of temperature and humidity on IFR development, examine the effect of fruit maturity on IFR and test whether cold storage could reduce expression of IFR symptoms postharvest.

The third, larger planting (3 rows in each block) was designed to be used to test a range of different fungicides (3 replicates/treatment) plus three experimental products supplied by Bayer (1 replicate/treatment) and the addition of chelated calcium. Capsicum crops in the Hawkesbury region mainly suffer IFR late in the season, hence why the later planting was used for this part of the research. Treatments were:

Standard fungicides

1. Untreated control
2. Amistar + Miravis Duo (Group 11 and Group 3 + 7)
3. Switch (Group 9 + 12)
4. Amistar + Score + copper (Group 11 and Group 3 and protective)
5. Chelated calcium + Serenade Opti + Switch + Miravis Duo (Group 9 + 12 and Group 3 + 7

Experimental products

a. 202

b. 203

c. 204

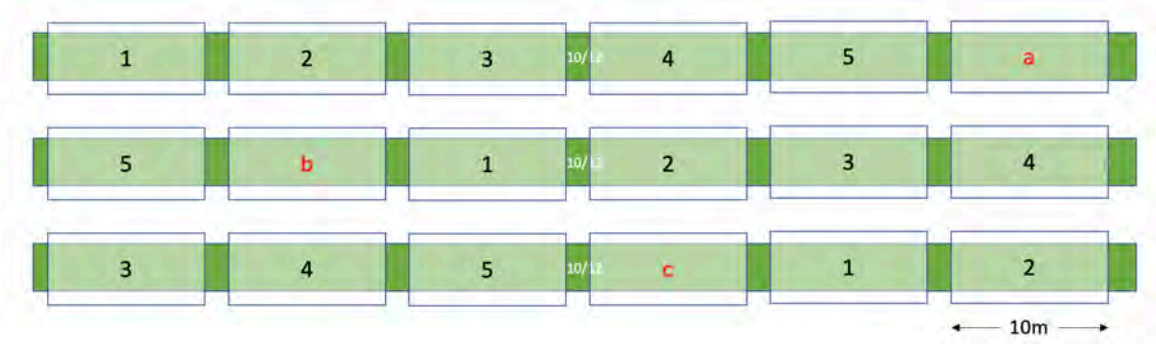


Figure 1-1. Fungicide application trial plan 2021-2022.

To monitor the time from flowering to fruit maturation, 100 randomly selected flowers were tagged each week. As the early fruit matured, red capsicums were harvested and cut open to examine for signs of IFR..

Figure 1-2: Capsicums planted on 1 October 2021

Figure 1-3: Initial 1/10/2021 planting with supporting string lines, plus seedlings planted on 17/11/2021

Figure 1-4. Tagged flowers (left) and a temperature and RH logger installed in the crop

A third activity examined the extent to which IFR develops postharvest. Our hypothesis is that rapid cooling after harvest may reduce development of IFR within supply chains. A small cold room was previously constructed on-site specifically for this purpose. Capsicums that are not required for assessment at harvest will be stored at either 5°C or 20°C for up to a week before assessment to determine the influence of temperature on IFR expression.

1.2 Results

Note: this trial was cut short due to local flooding during March 2022. Although some initial data had already been collected, the numbers of fruit are relatively low. this data should therefore be considered as indicative of trends rather than as a complete data set.

Figure 1-5: March 2022 trial site showing flood damage and crop loss

Four harvests were conducted before the flood occurred. These were almost entirely fruit derived from the first planting date on 1/10/2021, with only a very few coloured and red fruit recovered from the second planting.

The third planting was still immature, so no results were obtained from the different fungicide applications.

Tagging demonstrated that it took 60 to 65 days for fruit to develop to maturity. This did not change over the period examined (harvests from 25 January to 17 February).

The percentage of fruit with internal fruit rot generally declined over the four weeks of harvest. Internal mould nearly twice as common in fully red fruit as those which had developed to coloured / half red stage, averaging 15.2% and 8.1% respectively. This is consistent with previous observations that IFR is most common in fully mature, red capsicums, with incidence reduced in semi coloured fruit. IFR is rarely found in green capsicums.

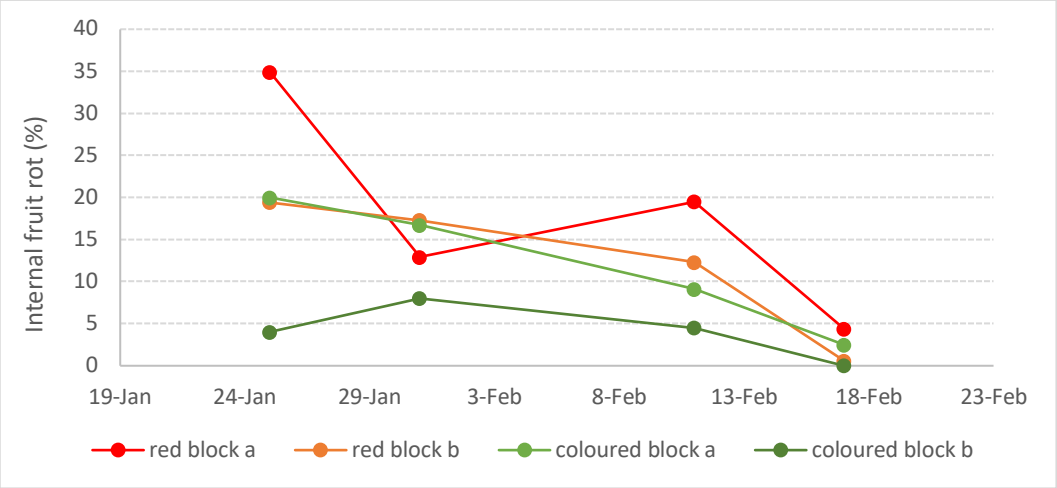


Figure 1-6: Rates of internal fruit rot in harvested full red and part red fruit, grown in two replicate blocks, between 25/1 and 17/2

It was also noted that IFR was somewhat higher in block a fruit (top of the slope) compared to those in block b (bottom of the slope). No differences in temperature or RH were found between these blocks, so it is unclear why this effect appears to have occurred. Potential causes could be spore load within the soil at the different locations, or the amount of wind exposure on each block.

Subsampled fruit from two harvests were stored under either ambient conditions for 3-4 days, or inside a cold room at 5°C for approximately one week. They were then cut open to examine for IFR as previously.

There was a small trend to increased rates of IFR in the capsicums which had been stored under ambient conditions. However, this change was not statistically significant. Capsicums stored cold did not differ from the assessment at harvest.

It had been hypothesised that IFR continues to develop during storage and transport, especially if fruit are not kept well refrigerated. Even though the infection is already present at harvest, poor postharvest management could permit greater expression of symptoms of disease. While this result is somewhat supportive of this theory, only two of four cohorts of fruit stored at ambient registered a numerical increase in rates of IFR.

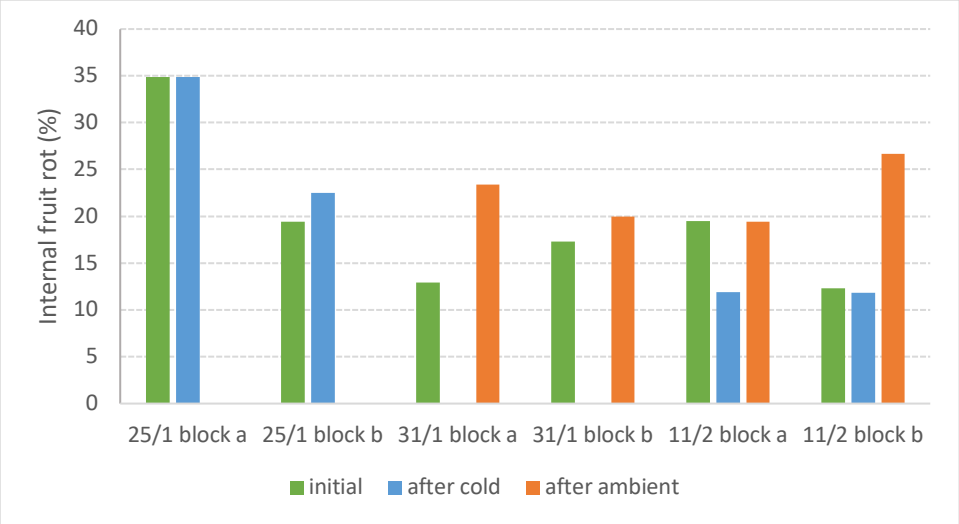


Figure 1-7: Rates of internal fruit rot in harvested full red fruit, grown in two replicate blocks, and then either stored in a cold room for approximately one week or left under ambient conditions for 3-4 days, before assessment.

Future work should re-examine this using larger numbers of capsicums. This work should also record disease severity, rather than presence alone; a tiny amount of fungal growth may be undetectable to consumers, whereas fluffy mycelium covering the surface of the seed mass is both obvious and more likely to result in complaints to the retailer.

2 Trial 2: Bundaberg chemical trial

2.1 Background

A replicated trial was established at a research farm to compare the infection rate of internal fruit rot (IFR) in harvested capsicums between spray treatments following artificially induced flower infection.

2.2 Methodology

Halifax were planted on 7th October 2022 at a rate of 29,630 plants/ha in a randomised complete block design (Figure 2-1). Each treatment is 10 m long, with a plant spacing of 0.5 m.

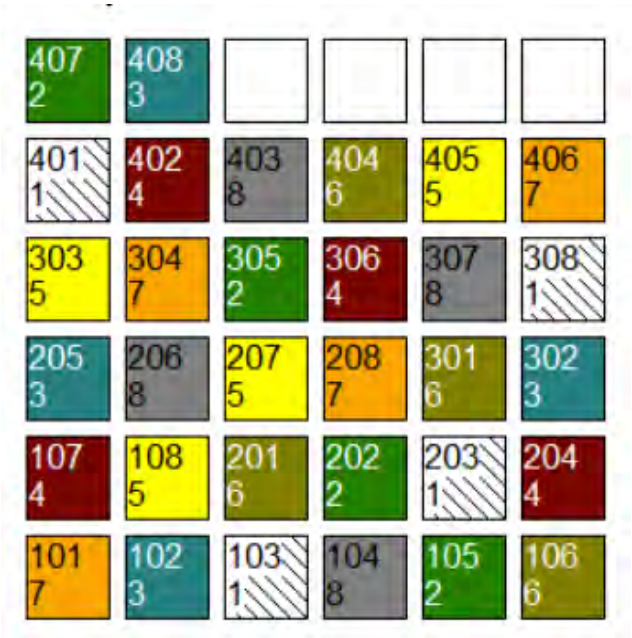


Figure 2-1: Trial plan

Plants were foliar inoculated with a mix of *Alternaria alternata* and *Alternaria tenuissima* on 21/11/22 at flowing. This was achieved by blending 6 plates of each fungal culture with 1 L of water followed by dilution in 6 L of water.

Eight spray treatments have been applied as per Table 2-1 and Table 2-3

Table 2-1: Treatment list

No.	Product	Rate	Application Code	Application schedule
		Product (mL or g/ha L)		
1	Untreated control	- -	- -	- -
2	Botector	1000	A B C D	Applied as four dilute applications in a water volume of 418 L/ha, commencing at BBCH 53.
3	Serenade Opti	2000	A B C D	
4	Botector fb Serenade Opti	1000 fb 2000	A C fb B D	
5	Serifel	500	A B C D	
6	Switch fb Miravis Duo	1000 fb 1000	A C fb B D	
7	Belanty fb Merivon	1250 fb 240	A C fb B D	
8	Kocide Blue Xtra + Manic WG Fungicide Plus fb BravoWeatherstik	1650 + 3600 fb 2300	A B C D + A B fb C D	

Table 2-2: Treatment product details

Product name	Active ingredient	Concentration	Formulation	Batch number
Botector	Aureobasidium pullulans (strains DSM 14940 and DSM 14941)	5 x 10^9 cfu/g	Water Dispersible Granule	2020-YCF035-09
Serenade Opti	Bacillus amyloliquefaciens strain QST 713	1.3 x 1010 CFU/g	Wettable Powder	LJDP0012
Serifel	Bacillus amyloliquifaciens strain MBI600	110g/kg (>5.5 x 1010 CFU/g)	Wettable Powder	25723675
Switch WG	cyprodinil + fludioxonil	375 g/kg + 250 g/kg	Water Dispersible Granule	SM06D0877
Miravis Duo	difenoconazole + pydiflumetofen	125 g/L + 75 g/L	Suspension Concentrate	Not provided
Belanty	Mefentrifluconazole	75 g/L	Suspension Concentrate	0020950310
Merivon	pyraclostrobin + fluxapyroxad	250 g/L + 250 g/L	Suspension Concentrate	0020290448
Kocide Blue Xtra	Copper present as cupric Hydroxide	350 g/kg	Powder	MAR19HG022
Manic WG Fungicide Plus	Mancozeb	625 g/kg	Wettable granule	KS3MZE7501
Bravo Weatherstik	chlorothalonil	720 g/L	Suspension Concentrate	AAC6JED1C

Table 2-3: Treatment application and harvest assessment dates

Date	Event
17/11/2022	Treatment application A
21/11/2022	Disease inoculation
24/11/2022	Treatment application B Crop safety assessment
02/12/2022	Treatment application C Crop safety assessment
08/12/2022	Treatment application D Crop safety assessment
18/01/2023	Harvest assessment
01/02/2023	Harvest assessment
03/03/2023	Harvest assessment

2.2.1 Fruit assessment and Analysis

Fruit was assessed on 3 harvest dates from 18 January 2023 to 3 March 2023 for incidence of internal fruit rot. Analysis of variance tests and Tukey's HSD tests were conducted using ARM2022.5 to determine any treatment affect in incidence of IFR for each harvest date.

2.3 Results and Discussion

There were no significant differences between treatments at any of the harvest dates (p= 0.45 (18 Jan), p= 0.15 (1 Feb), p= 0.86 (3 Mar). There was no reduction in IFR from any of the fungicides and biologicals tested when averaged across the three harvests conducted.

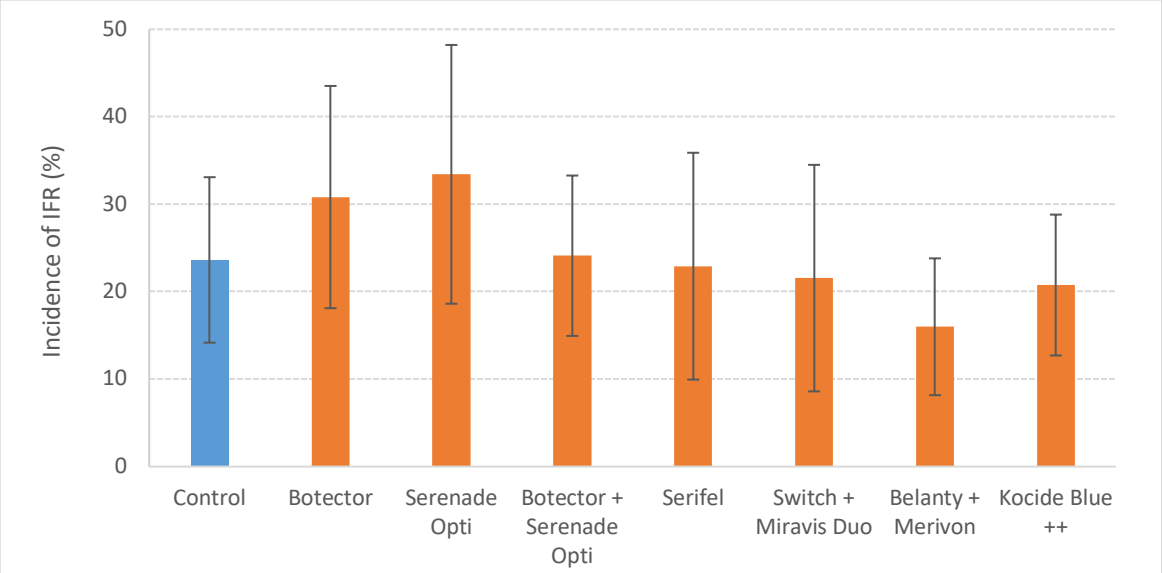


Figure 2-2: Recorded rates of IFR averaged across all harvest dates for control fruit (sprayed with water only) compared to a range of different fungicides and biologicals. Bars indicate the standard error of each mean value (n=3).

3 Trial 3: Testing the effect of fungicide concentration (Bundaberg)

3.1 Background

Discussions with a Bundaberg grower regarding spray efficacy led to the hypothesis that reducing the spray volume would reduce runoff, potentially increasing efficacy. This can be achieved by using the label product rate per hectare, but halving the amount of water used to dilute it. Spray volume and speed are then adjusted accordingly.

3.2 Methodology

Seminis 6947 were planted on 18/08/22. Nine bays of plants have received treatment 2, with the remainder of the farm receiving treatment 1. One bay comprises 9 beds of 90 m length.

All capsicums have been sprayed according to the existing spray regime with two observational treatments:

During applications of Switch and Miravis Duo

1. Standard application volume
2. Roughly half the standard application volume, using same chemical rate as standard (half the water)

Dates of Switch and Miravis Duo applications:

Switch	4/10/22
Switch	13/10/22
Miravis Duo	21/10/22
Switch	29/10/22

Three hundred saleable fruit from each treatment (standard control program and the more concentrated fungicide mix) were harvested and assessed on December 12. Fruit were graded by colour and assessed for presence or absence of IFR. Fruit that were a full red were assessed as 5, those that were chocolate or part red were rated as 4.

Figure 3-1. Capsicums harvested for assessment, and an example of IFR

Chi-square analysis was conducted to examine the association between treatments 1 and 2 and the occurrence of IFR. A chi-square analysis was also conducted to examine the association between colour grade 5 and colour grade 4 treatments and the incidence of IFR.

3.3 Results and Discussion

Internal fruit rot was found at relatively high rates in both normal and concentrated fungicide application treatments at 18% and 17% respectively. Capsicums with colour ratings of 5 and 4 had IFR incidences of 18% and 13% respectively.

3.3.1 Chi square results:

The results of chi-square analysis revealed a non-significant association between the half water treatment and control in the incidence of IFR (chi2 = 0.0, p = 1.0). The application of the fungicide at recommended label rate, but with half the amount of water, had no impact on the incidence of IFR when compared to the control treatment using the recommended amount of water.

Chi-square analysis was conducted to examine the association between colour grade 5 and colour grade 4 treatments. The chi-square test statistic was calculated to be 1.321. Despite a 5% difference in incidence of IFR, high variability within the data meant that this was not statistically significant (p = 0.517).

Despite this, the lower incidence of IFR in slightly immature fruit is consistent with previous observations. Early harvests offer a potential strategy for marketing fruit at high risk of development of IFR symptoms.

4 Trials 4 and 5: Sydney Basin chemical and biological treatments

4.1 Methods

The trial aimed to test the effects of an intensive fungicide program and an intensive program using biological agents, including ones that have been claimed to stimulate root growth or trigger defence responses within plants. Although this is not according to label directions, no positive results had been gained using individual fungicides. It was therefore considered that this would provide the final test of whether fungicides had any chance of controlling internal fruit rot (IFR).

Field trials were established the Local Land Services site on Richmond Lowlands as well as a nearby (but higher) commercial farm at Freemans Reach.

4.1.1 Trial layout

The LLS site used a 70m long block with five double rows of capsicums. The two commercial varieties planted were Caldo and Pluto. The treatments were randomly allocated to 10m blocks. The plants were irrigated using overhead sprinklers and regularly treated with insecticide to control fruit fly.

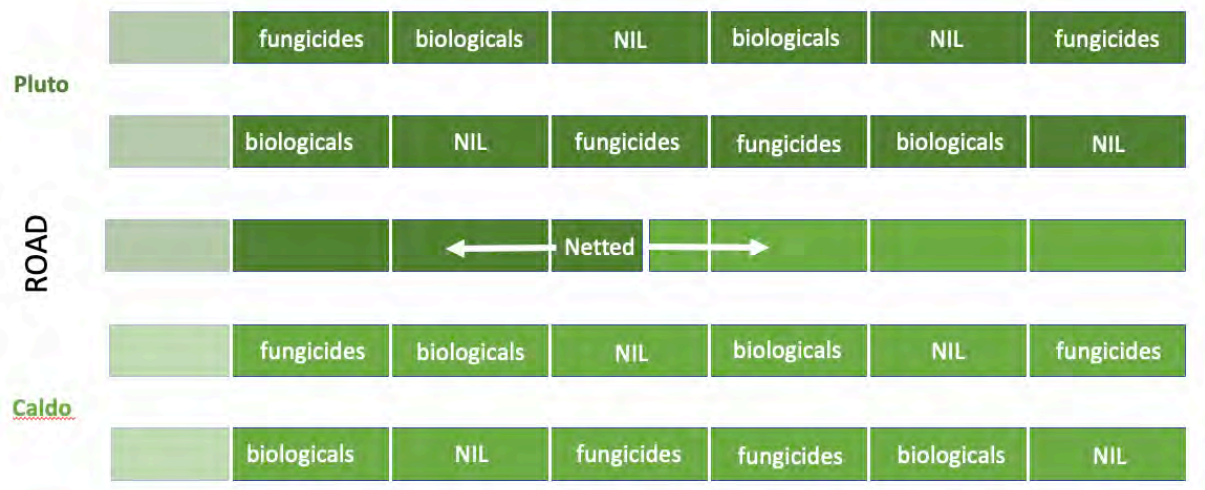


Figure 4-1. Layout of the LLS field trial

At the commercial, Freemans Reach farm, two 80m long beds were used for the trial. These were divided into 5m long blocks, each with two beds side by side. The treatments were allocated along the beds as shown in Figure 4. The hilled up beds were covered with plastic mulch, with capsicums planted in double rows on each. Irrigation was supplied by sub-surface drippers.

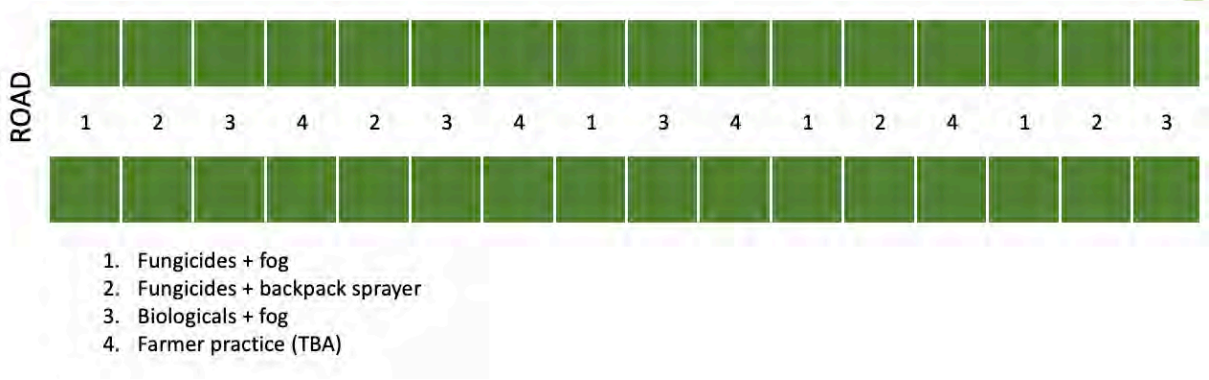


Figure 4-2. Layout of trial at commercial farm.

4.1.2 Treatments

At the commercial site, the control was the farmer's own normal practice, whereas at the LLS site the control was nil fungicide applications.

The objective was to apply the selected products every 10 days during flowering. Wet weather meant some dates were changed, however, either four or five sprays in total were applied to the plants at each site. At the commercial site an additional treatment compared the effects of fungicide application using a fogger (Ryobi ONE+ fogger)with fungicide applied using a normal, battery powered backpack spray unit (Ryobi ONE+ 15L backpack sprayer). It was hypothesised that the foggers would increase coverage of the flowers, ensuring all downward-facing surfaces were adequately covered.

Fungicides included:

- Amistar (Group 11)
- Miravis Duo (groups 3 and 7)
- Switch (groups 9 and 12)
- Intervene (group 19)

Biologicals included:

- Botector
- Salicylic acid
- Fulzyme
- Sarsil
- Calcium chelate

At the LLS site a central section was covered with netting. This was to restrict movement of insects, which were suspected of potentially spreading infection between flowers. The nets were applied to an untreated section (control) of the crop on 24/02/23.

All plants were sprayed to runoff at each treatment application.

Table 4-1. Treatment applications at LLS site.

Date of spray	Fungicide and rate (fungicides)	Biological and rate (Biologicals)
24/02/2023	Miravis Duo (1mL/L)	Sarsil (2mL/L)
6/03/2023	Switch (1g/L) + Intervene (0.4g/L)	Botector (1g/L) + Salicylic acid (0.5g/L)
17/03/2023	Amistar (1g/L)	Sarsil (2mL/L)
30/03/2023	Miravis Duo (1mL/L)	Botector (1g/L) + Salicylic acid (0.5g/L)

Table 4-2. Treatment applications at commercial farm site.

Date of spray	Fungicide and rate	Biological and rate
15/02/2023	Amistar (1g/L)	Botector (1g/L) + Salicylic acid (0.5g/L)
24/02/2023	Miravis Duo (1mL/L)	Sarsil (2mL/L)
9/03/2023	Switch (1g/L) + Intervene (0.4g/L)	Botector (1g/L) + Salicylic acid (0.5g/L)
17/03/2023	Amistar (1g/L)	Sarsil (2mL/L)
30/03/2023	Miravis Duo (1mL/L)	Botector (1g/L) + Salicylic acid (0.5g/L)

Red fruit were harvested five times at each site. The initial harvest was on 18 April with the final harvest on 24 May. At this time the first frosts had hit the region, so the plants were starting to die back. At each harvest, individual fruit were examined for the following attributes:

- Number of lobes (3, 4 or 5)
- Presence of dark seed
- Presence of external mould
- Presence of IFR – black
- Presence of IFR – white
- Presence / absence of a hole at the fruit apex
- Attachment of the style
- Mould present on the style
- Internal black spot associated with style

4.2 Results and Discussion

4.2.1 Local Land Services site

The plants grew well at the LLS site. The major disadvantage of growing without mulch was decay of fruit contacting the soil. While this limited the number of commercial quality red fruit, sufficient fruit were harvested to obtain good estimates of IFR.

Figure 4-3. Capsicums growing at the LLS site, Richmond Lowlands.

Rates of IFR were low at the LLS site, averaging 8% over four harvests. In previous years, harvests at this time and within this region have found more than 25% of fruit infected with IFR. As results were not significantly different for Plato and Caldo, data for the two varieties was combined.

The final harvest at LLS did reveal an increase in IFR, particularly in the untreated controls. While this appears promising, the treatments were not significantly different overall.

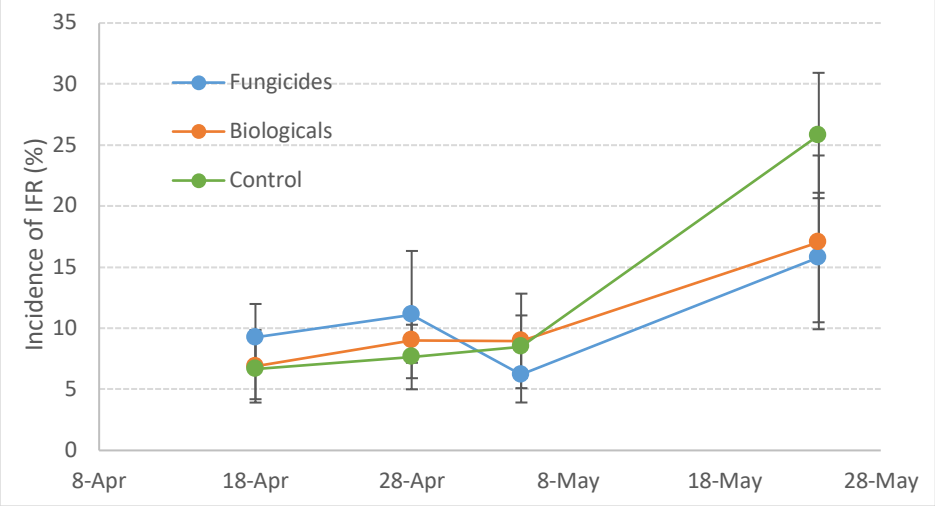


Figure 4-4. Average incidence of IFR across two varieties (Plato and Caldo) and four harvests. Capsicums were treated every 10 days during flowering with a range of either fungicides, biological products, or water only. Bars indicate the standard error of each mean value (n=6)

Only two blocks of plants were netted, so replication was limited. However, there was a trend to higher rates of IFR in fruit grown under netting; at the final harvest on 24 May, 47% and 22% of capsicums grown under the two blocks of netting had symptoms of IFR. While high variability in the data means this increase was not statistically significant, the trend is consistent with the findings detailed in Appendix 7. That is, that strategies that reduce air movement around plants potentially increase IFR.

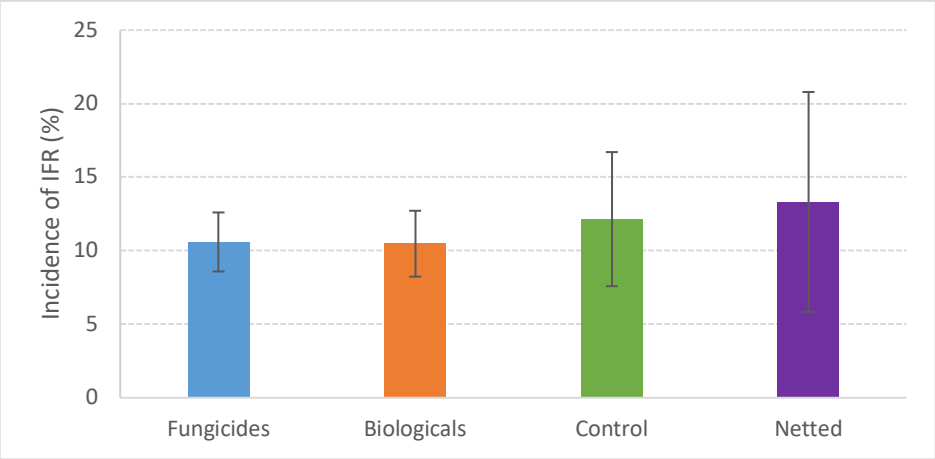


Figure 4-5. Average incidence of IFR across four harvests of two varieties (Plato and Caldo). Plants were treated at 10 day intervals with a range of fungicides, biologicals or water only, or covered with netting during the course of fruit development.

No relationship was found between symptoms of IFR and the number of lobes, presence of a hole in the apex, dark seed, style condition or the other characteristics recorded.

The exception was the presence of a dark, diseased spot at the apex of the fruit adjacent to the remnant style. Nearly 50% of fruit with IFR had this mark at the apex, compared to only 12% of fruit which did not have disease symptoms. This is consistent with the mechanism of infection through the fruit stigma and style, some disease symptoms expressing in that part of the fruit.

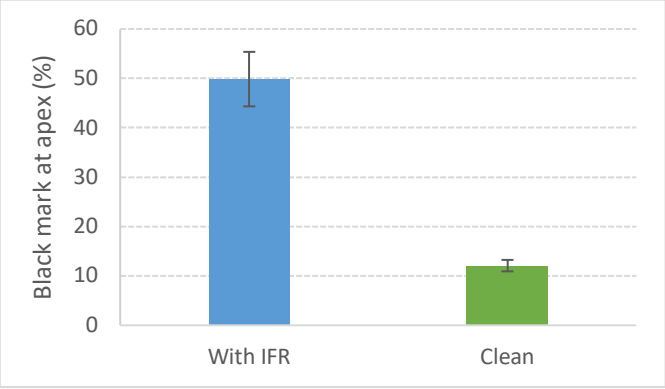


Figure 4-6. Percentage of fruit which had symptoms of IFR or were clean, which also had a dark, diseased spot adjacent to the remnant style. Bars indicate the standard errors of each mean value (n=7)

4.2.2 Freemans Reach commercial operation

The results at the commercial farm were less positive. The grower has extensive experience growing capsicums, usually producing a bumper crop in late summer. However, this year sections of the crop were badly affected by *Sclerotium rolfsii.* This devastating disease is very difficult to manage, with the soil borne sclerotia highly resistant to fungicides.

Figure 4-7. Capsicums growing at the commercial farm site in Freemans Reach. Image at right shows plants which have collapsed due to infection by S. rolfsii.

Only a small number of fruit was suitable for assessment: in total, 122 red fruit were picked over two harvests. In addition, the rate of IFR was low, averaging only 5.7%. This makes it impossible to draw any meaningful conclusions from the results.

Despite these issues, the existing data suggests that infection rates were not reduced when fungicides were applied with a fogger instead of a standard backpack spray unit. Of all fruit harvested from the replicate blocks, three fogged fruit had symptoms of IFR, compared to two fruit sprayed using the backpack.

The fogger used was a hand-held, battery pack unit. Although it did produce finer droplets than the backpack spray unit, it did not replicate the power and volume of a commercial air blast unit. It was therefore resolved to repeat this trial with a commercial unit on farm if possible.

5 Trial 6: Use of *Trichoderma* to suppress incidence of IFR (Bundaberg)

5.1 Background

Internal fruit rot (IFR) in capsicums, primarily caused by *Alternaria alternata* and *Fusarium* species, poses a significant threat to crop yield and fruit quality. The disease typically establishes itself during flowering or early fruit development. Spores travel down the pollen tube to infect the developing fruit tissues or gain entry through the necrotic style of young fruits. Symptoms often remain dormant until fruit ripening, making early detection and effective management challenging.

Currently, disease management relies heavily on chemical fungicides. These have demonstrated limited effectiveness against IFR. This has driven interest in exploring biocontrol alternatives, particularly those involving *Trichoderma* species, which are widely recognized for their potential in suppressing fungal pathogens.

Research by Begum et al. (2007) demonstrated that various *Trichoderma* species effectively controlled *Alternaria* fruit rot in chili under field conditions. Their findings revealed that *Trichoderma harzianum* not only significantly reduced disease severity but also enhanced plant growth and yield. Additionally, a combined treatment of *T. harzianum* and *Alternaria tenuis* reduced disease incidence by 72%, showcasing the ability of *Trichoderma* to deliver sustainable and effective disease control while minimizing reliance on chemical fungicides.

Building on this foundation, the current trial evaluates the efficacy of Nutri-Life Trichoshield™, a commercial Trichoderma formulation *containing T. harzianum, T. viride*, and *T. koningii*, in suppressing IFR under field conditions.

5.2 Invitro studies of *Alternaria* and *Trichoderma*

An in vitro observational trial was conducted to evaluate the antagonistic potential of Nutri-Life Trichoshield™, a commercial *Trichoderma* formulation, against *Alternaria alternata*. The study aimed to observe both the direct interaction between the fungal species and the mechanisms by which *Trichoderma* suppressed *Alternaria* growth.

5.2.1 Experimental Setup

Alternaria alternata and Nutri-Life Trichoshield™ (comprising *T. harzianum, T. viride*, and *T. koningii*) were cultivated separately on quarter-strength PDA (potato dextrose agar) plates. Lactic acid was added to the medium to suppress bacterial growth, ensuring a clear fungal interaction. Plates were incubated under controlled conditions of temperature (25°C ± 2°C) and light (12-hour photoperiod) to promote consistent and vigorous growth of both fungal colonies.

Once optimal growth was achieved, subcultures of *A. alternata* and Nutri-Life Trichoshield were inoculated on opposite sides of the same PDA plates. The fungal colonies were allowed to grow toward each other over a period of nine days. Observations were made periodically to monitor interaction dynamics and progression.

5.2.2 Macroscopic observations

After nine days of growth, clear antagonistic behaviour by *Trichoderma* was evident (Figure 5-1).

Visible changes included:

- Inhibition Zones: A distinct inhibition zone, where *Alternaria* growth slowed or halted entirely, was observed at the interface of the two colonies.
- Overgrowth: *Trichoderma* hyphae overgrew the *Alternaria* colony, covering and suppressing its growth.

5.2.3 Microscopic observations

Microscopic examination revealed key insights into the mechanisms of antagonism (Figure 2). One notable observation was hyphal coiling, where *Trichoderma* hyphae wrapped around the hyphae of *Alternaria*, a behaviour commonly associated with parasitism.

5.2.4 Significance of findings

The observed interactions highlight the potential of Nutri-Life Trichoshield™ as a biocontrol agent. The combination of macroscopic suppression and microscopic hyphal interactions demonstrates that Nutri-Life Trichoshield can effectively inhibit *Alternaria alternata* growth through direct antagonism. These findings provide a solid foundation for further field trials to evaluate the practical applications of Nutri-Life Trichoshield™ in managing internal fruit rot in capsicums.

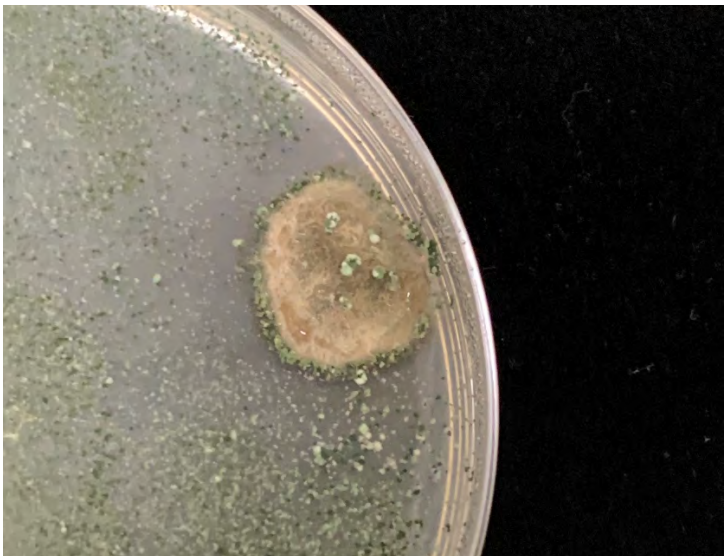


Figure 5-1: Trichoderma actively attacking Alternaria on a PDA plate, showing inhibition zones and overgrowth.

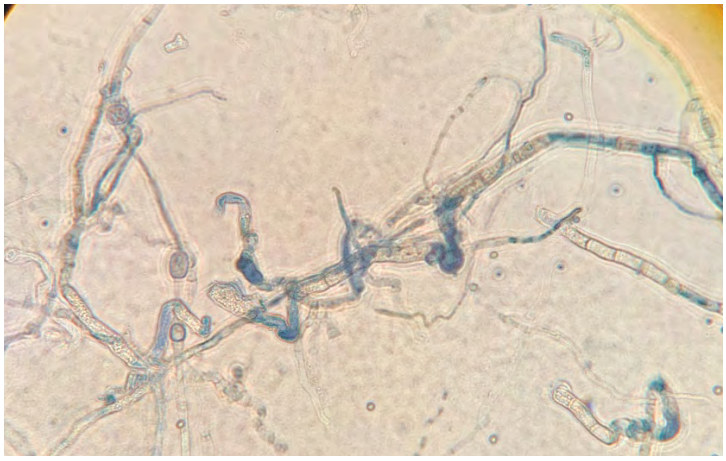


Figure 5-2: Microscopic view highlighting blue-stained Trichoderma hyphae coiling around transparent Alternaria hyphae, indicative of parasitism.

5.3 Field trial

A field trial was established to evaluate the efficacy of Nutri-Life Trichoshield™ in managing internal fruit rot (IFR) under commercial growing conditions. This trial aimed to assess the biocontrol agent's ability to suppress disease symptoms caused by *Alternaria alternata*.

5.3.1 Experimental Design

The trial was carried out on a commercial capsicum farm, maintaining standard practices for irrigation, fertilization, and pest management to ensure consistency with typical farming conditions. Four treatments were implemented in a randomized block design, with three replicates per treatment, resulting in a total of 12 plots and approximately 400 plants. Each replicate consisted of 30 plants, with two buffer plants placed between plots to minimize the risk of cross-contamination.

The treatments included:

- A: *Alternaria alternata* inoculation
- B: Trichoderma (Nutri-Life Trichoshield™)
- C: Control (dechlorinated water with soap)
- D: Combined *Alternaria alternata* and Nutri-Life Trichoshield

5.3.2 Trial Establishment

Inoculations were carried out weekly, on Tuesdays or Thursdays, depending on weather conditions and on farm spray schedules. Over four weeks during flowering, two flowers per plot were marked with flagging tape above a fully fertilized open flower to align flowering dates with the timing of fruit harvest. Photographs of two flowers or fruits per plot were taken each week to document development.

5.3.3 Inoculation Preparation

All treatment solutions were prepared using tap water dechlorinated by standing in open containers for at least 24 hours. A standard soapy water solution (two drops of dishwashing liquid per liter) was prepared for use in all spray applications to ensure uniform dispersion of treatments.

Figure 5-3: In field inoculation preparation

Control Treatment

For the control, five liters of dechlorinated water were mixed with 200 milliliters of soapy water in the spray tank. Plants were sprayed thoroughly, and the remaining volume was measured to refine estimates for subsequent treatments.

***Trichoderma* Preparation**

For the *Trichoderma* treatment, five grams of Nutri-Life Trichoshield powder were weighed per liter of solution. The powder was mixed with 200 milliliters of soapy water, shaken thoroughly, and 100 milliliters of this solution were added to the spray tank. An additional 100 milliliters of soapy water and the required volume of dechlorinated water were added. The remaining Trichoderma solution was stored in an insulated container, and plants were sprayed thoroughly.

***Alternaria* Preparation**

Alternaria spores were prepared from eight fungal plates per spray date. Each plate was rinsed with a small amount of soapy water, and a glass spreader was used to lift the spores into the liquid. The spore suspension was combined into a jar and adjusted to the required volume.

Combined *Trichoderma* and *Alternaria* Treatment

For the combined treatment, 100 milliliters of *Alternaria* suspension were added to the spray tank, with an additional 100 milliliters reserved for the *Alternaria*-only treatment. The *Trichoderma* solution was then added, followed by dechlorinated water to reach the required volume.

***Alternaria*-Only Treatment**

For the *Alternaria*-only treatment, the reserved suspension was added to the spray tank along with 100 milliliters of soapy water and dechlorinated water. After spraying, the tank was cleaned with detergent and triple-rinsed to avoid cross-contamination. Alternaria spores were counted at approximately 300 spores/ml in the spray application.

The order of application was as follows: Control (C), *Trichoderma* (B), *Alternaria* and *Trichoderma* (D), and *Alternaria* (A). Spraying was conducted from both sides of the plants, with emphasis on flowers and young fruit.

Fruit Assessment Protocol

Harvesting began with the ripening of the king fruit and was completed at the end of the commercial harvest of the crop (four weeks of harvest at weekly intervals). Fully red fruit were harvested, while unripe fruit were left for subsequent collection.

Each fruit was bisected below the seeds to preserve seed integrity, and internal rot was recorded as a binary measure (presence/absence). All fruit were discarded following farm management protocols.

Photographs of each batch of ten fruits were taken to document each assessment.

5.3.4 Results and Discussion

Fruits were collected with flowering date tags recorded. Fruit tagging confirmed that it took approximately 50 to 60 days for flowers to develop into mature red fruit.

Overall, rates of IFR declined over the four harvests conducted. This could be due to relatively low spore concentrations within the suspensions used to inoculate the flowers at later flowering dates. However, it could also relate to environmental conditions at the time of inoculation or even to plant health during the trial period.

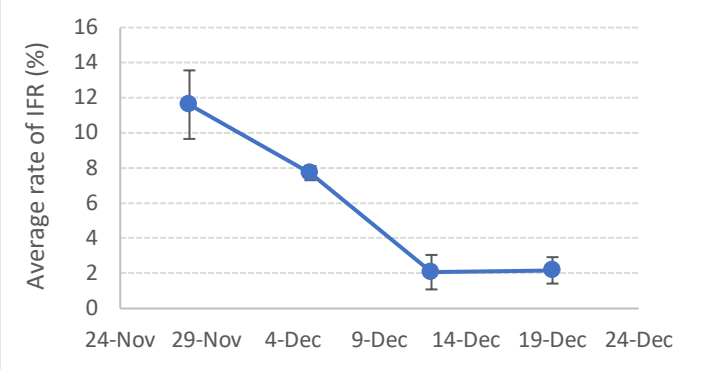


Figure 5-4: Average incidence of Internal fruit rot over four harvest dates. Bars indicate the SEM of each value.

Analysis of the data revealed no statistically significant relationship between treatment and the incidence of internal fruit rot (IFR), as determined by a Chi-square test ($\chi^2 = 3.20$, $p = 0.362$). While the mean incidence rates varied slightly among treatments, these differences were not statistically significant. Additionally, a correlation analysis showed a very weak negative relationship ($r = -0.035$) between treatment and IFR incidence.

Despite the lack of statistical significance, a trend was observed across treatments (Figure 5-5) Treatments inoculated with *Alternaria* alone exhibited a higher incidence of IFR compared to other treatments, including those inoculated with both *Alternaria* and *Trichoderma*. Interestingly, treatments that included *Trichoderma* showed slightly lower incidence rates than the control. This trend suggests that *Trichoderma* may have a modest impact on reducing infection rates, warranting further investigation into its potential effects.

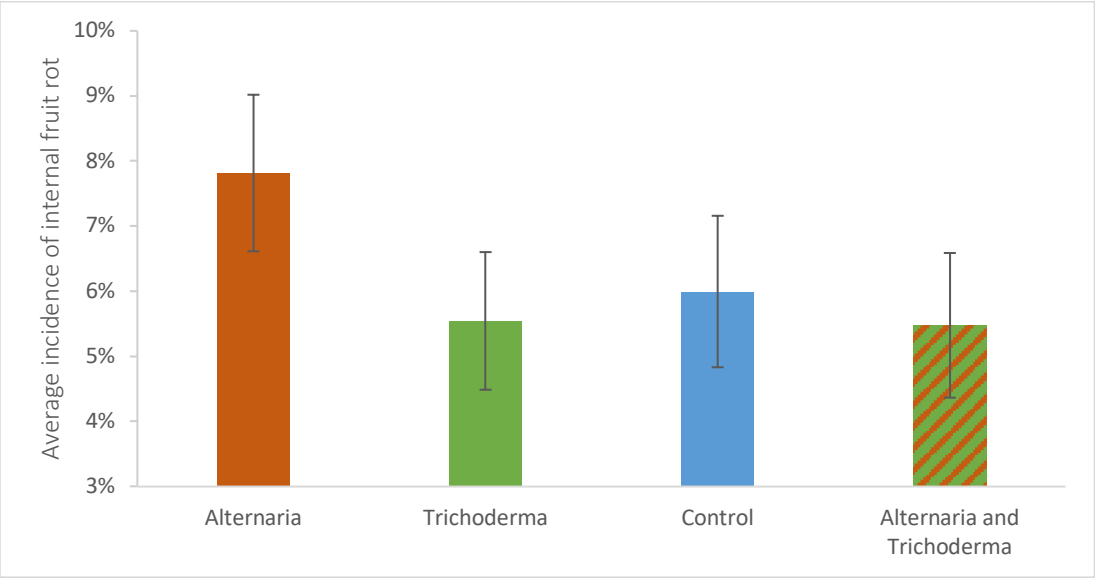


Figure 5-5: Average incidence of Internal fruit rot when flowers were inoculated with water (control), A. alternata spores, Nutri-Life Trichoshield or both A. alternata and Nutri-Life Trichoshield. Average values from four harvest dates, bars indicate the SEM of each mean value (n=4)

The lack of significant differences indicates that, under the conditions of this trial, the application of *Trichoderma* (Nutri-Life Trichoshield™) did not significantly reduce the incidence of IFR in capsicum fruits compared to the untreated control or the *Alternaria*-only treatment. Several factors may have influenced these results:

- **Low Baseline Incidence**: The overall low incidence of IFR in the trial may have reduced the ability to detect treatment effects. This may have been due to lower-than-ideal spore counts in the *Alternaria* treatments.
- **Environmental Conditions**: Unseasonably high temperatures and other environmental stressors, such as sunburn and blossom end rot, may have affected fruit development and masked potential treatment effects.
- **Pathogen Dynamics**: The timing of inoculation and the interaction between the pathogens and *Trichoderma* may not have been optimal to suppress mould development. It is also noted that some of the inoculum solutions had lower than optimal spore suspension concentrations, which may also have affected the results. Further studies under varying environmental conditions, with optimized inoculation protocols and pathogen pressures, may help clarify the role of *Trichoderma* in managing IFR.

5.4 Trial 6 photos

King fruit at first inoculation 15/10/24

Plants at first inoculation 15/10/24

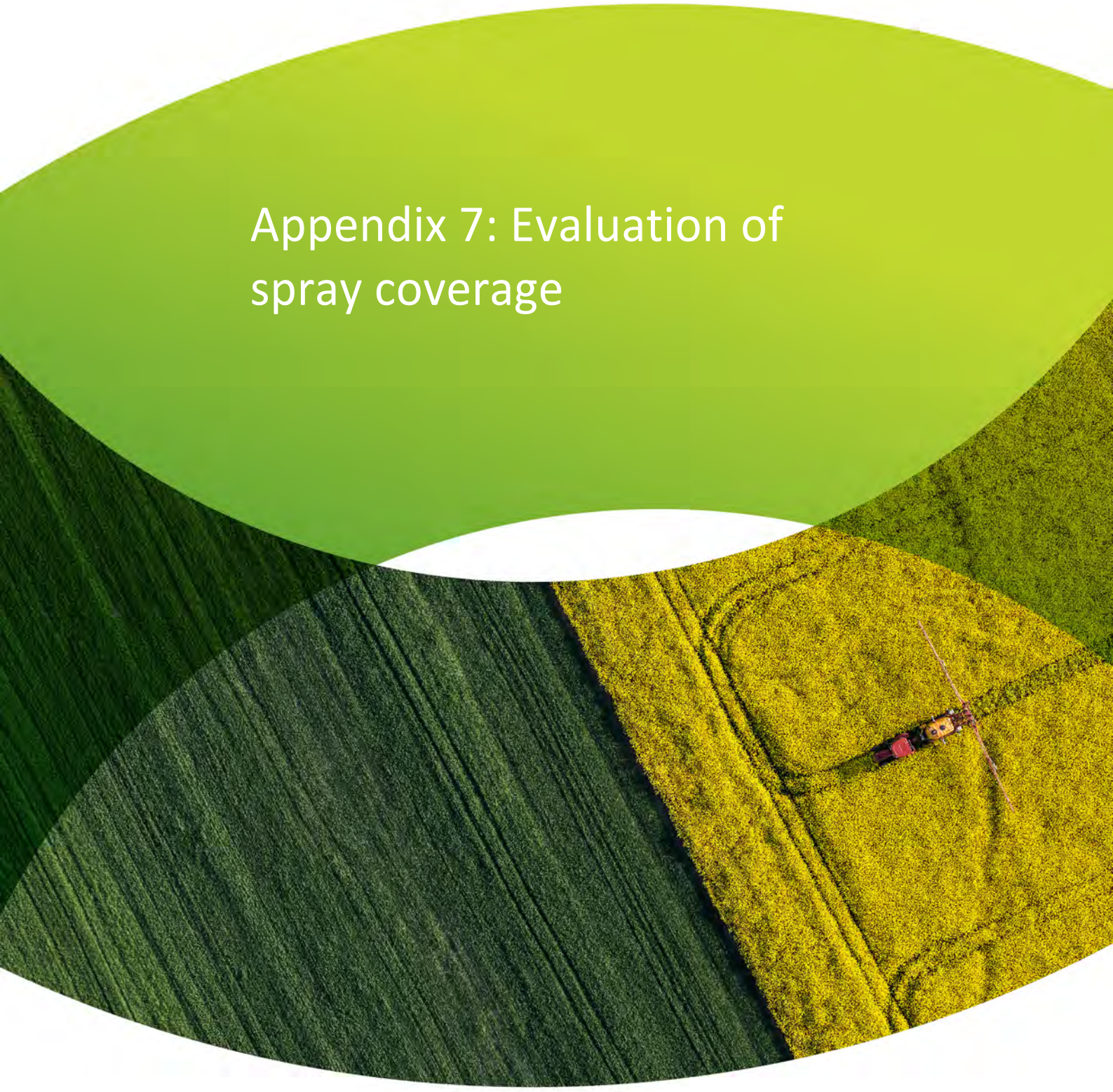
Plants 21/11/24- fruit is now close to first harvest

Fruit 21/11/24- close to first harvest of red capsicum

21/11/24. Some plants showing collapse due to infection by Sclerotium rolfsii. These plants are being rouged as they are found throughout the crop.

28/11/24 First assessment of ripe fruit

Appendix 7: Evaluation of spray coverage



APPLIED HORTICULTURAL RESEARCH ABN 58 134 308 982
Suite 340, Biomedical Building, 1 Central Avenue, Australian Technology Park, Eveleigh NSW 2015
info@ahr.com.au | +612 8627 1040 | www.ahr.com.au

Authors: **Naomi Diplock and Jenny Ekman**

Mobile: **+61 403740123**

Email: **naomi.diplock@ahr.com.au**

CONTENTS

1 Evaluation of coverage using a standard spray unit

1.1 Background

Internal fruit rot (IFR) of capsicums, caused by *Alternaria alternata* and *Fusarium* spp., infects the fruit during flowering, with spores traveling down the pollen tube and causing infection, typically after ripening.

A spray trial was conducted in a commercial capsicum crop to evaluate the efficacy of spray treatments, with a particular focus on achieving effective coverage of the floral stigma, this being where the initial infection occurs.

1.2 Methods

Water-sensitive paper was cut into small pieces using a hole punch and was strategically pinned within flowers to cover the stigma and adjacent floral structures prior to spraying.

Figure 1: Placement of water sensitive paper inside flowers.

Three rows were selected based on their placement relative to the spray rig (next to the tractor edge, middle of the boom, and edge of the boom). Four plants per row were chosen at approximately 10-meter intervals, with three flowers per plant. The flowers were randomly selected to include various positions, stages of openness, and orientations.

Spraying was conducted by the operator following standard commercial procedures. Cone type nozzles were used on a standard boom spray at 120psi, with no air assist available. Sprays included Copper, Switch, Movento, Tipster and a wetter.

After spraying was completed, the plants were left to dry for 1.5 hours. Once dry, all water-sensitive paper was collected and photographed, with details recorded for the row, plant number, and flower position.

1.3 Results

Spray coverage was generally poorer than expected (Figure 1), with the edge row and the row closest to the tractor receiving less coverage compared to the middle row (Figure 4, Figure 3). Statistical

analysis (Two way ANOVA) revealed a significant difference in coverage between rows (P= 0.012), with a notable disparity between the edge row and the middle row.

Figure 2: Spray coverage inside flowers. Blue indicates areas where spray has made contact.

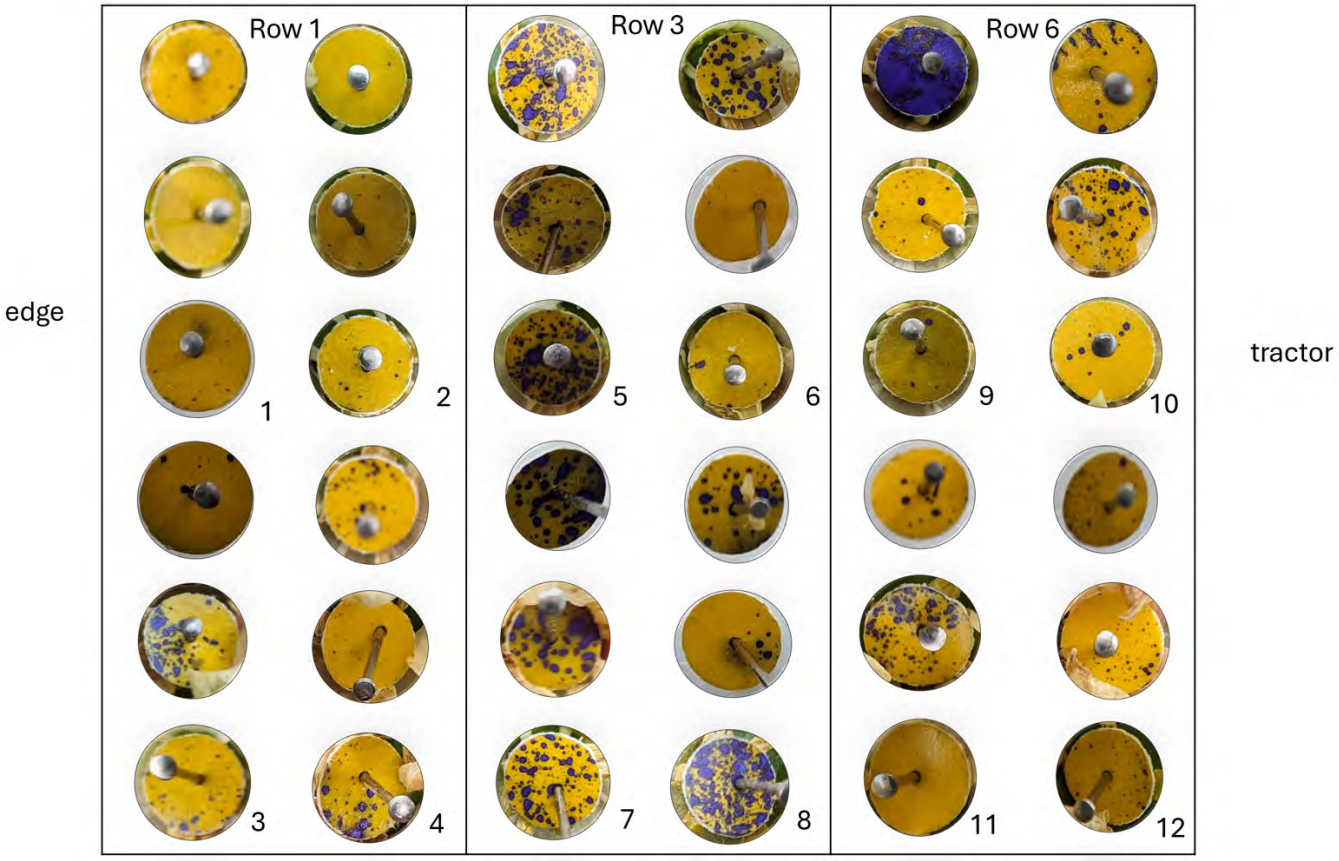


Figure 3: Spray Coverage Results. Blue areas indicate regions where the spray has made contact.

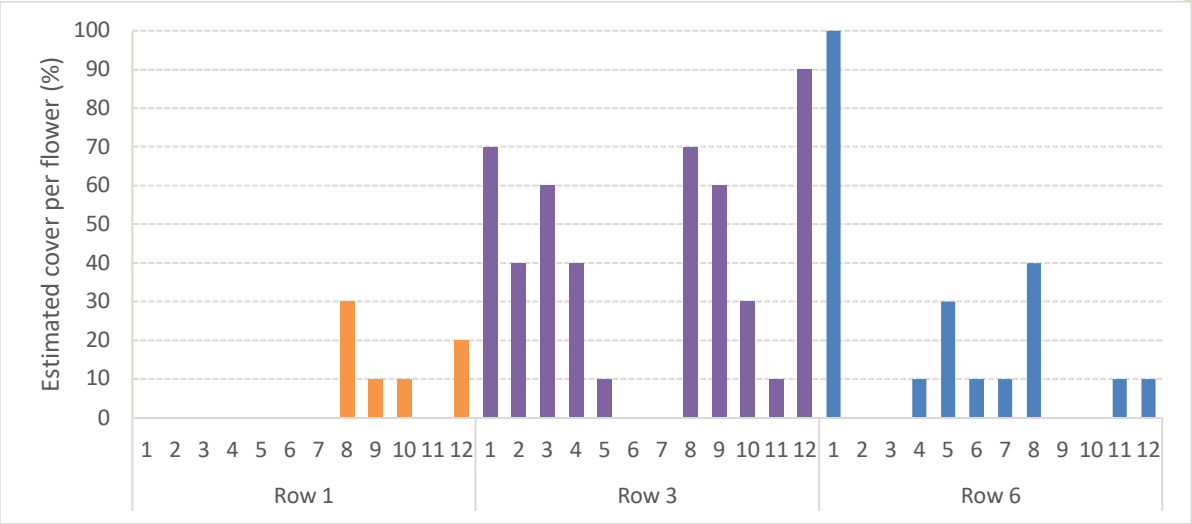


Figure 4: Estimated spray cover in each flower. Row one = edge row, row 3 = middle and row 6 = position closest to the tractor

1.4 Discussion

Spray coverage was suboptimal, with numerous flowers receiving minimal to no spray contact. Although certain fungicides possess systemic properties, their efficacy in penetrating the internal floral structures, particularly the stigma where A. alternata infiltrates via the pollen tube, remains undocumented. This inadequate coverage may elucidate the findings of prior studies, which reported no significant impact of fungicide application on the incidence of internal mold.

Further research is planned to conduct spray trials investigating methods to improve coverage within capsicum flowers by manipulating parameters such as spray pressure, tractor speed, and water volume. Additionally, evaluating the effectiveness of air-assist spray rigs in enhancing coverage inside capsicum flowers warrants further investigation.

2 Effect of pressure on spray coverage

2.1 Background

Internal fruit rot (IFR) of capsicums, primarily caused by the fungal pathogens Alternaria alternata and Fusarium spp., poses a significant challenge to growers. These pathogens infect the fruit during flowering, with spores traveling down the pollen tube to establish infection. Symptoms typically manifest post-ripening, reducing marketable yield and leading to economic losses.

Managing IFR requires precise and effective application of fungicides to target the infection site early. The initial infection predominantly occurs at the centre of the flower, making this region a critical target for spray treatments. Achieving uniform coverage within the intricate floral structures, such as the stigma and adjacent areas, is particularly challenging due to the small, enclosed morphology of capsicum flowers.

To address this issue, a spray trial was conducted in a commercial capsicum crop to evaluate the efficacy of spray treatments under varying pressures. The trial aimed to identify whether optimizing spray parameters, such as pressure, could enhance coverage at the infection-prone floral centre. This study provides valuable insights into improving application techniques to minimize IFR infections and safeguard crop productivity.

2.2 Methods

To evaluate the efficacy of spray coverage, a field trial was conducted in a commercial capsicum crop. The experiment focused on assessing spray coverage at the floral centre, the critical site for infection by *Alternaria alternata* and *Fusarium* spp.

2.2.1 Placement of water-sensitive paper

Water-sensitive paper, cut into small circular pieces using a hole punch, was strategically pinned within flowers to cover the stigma and adjacent floral structures. This placement was chosen to simulate the areas most susceptible to fungal infection and assess the effectiveness of spray treatments at reaching these locations.

Figure 5: Placement of water sensitive paper inside flowers.

2.2.2 Row and Flower Selection

Three rows of plants were selected based on their proximity to different positions along the spray rig:

- Row 1: Adjacent to the tractor edge.
- Row 2: Positioned under the middle of the boom.
- Row 3: Aligned with the outer edge of the boom.

Within each row, four plants were chosen at approximately 10-meter intervals. Each plant had three flowers selected at random, ensuring diversity in:

- Position on the plant (e.g., top, middle, bottom branches),
- Stage of flower development (open and partially open), and
- Orientation relative to the spray direction.

Flowers were selected to account for the variability in structural characteristics that could influence spray deposition.

2.2.3 Spray Treatments

Spraying was conducted using a standard boom sprayer equipped with cone-type nozzles, operated under commercial practices. The trial evaluated three spray pressures:

- 120 psi,
- 135 psi, and
- 150 psi.

The sprays included a combination of Copper, Switch, Movento, Tipster, and a wetter to enhance adherence and distribution. No air-assist technology was used during the trial.

Figure 6. Commercial spray rig before lowering into the crop

2.2.4 Post-Spray Collection

After spraying, the plants were left to air-dry for 1.5 hours to allow the water-sensitive paper to dry. Once dry, the paper was carefully removed, ensuring no damage to the samples. Each piece was labelled with details of:

- The row number,
- Plant number, and
- Flower position.

Samples were photographed under consistent lighting conditions for subsequent analysis.

2.2.5 Analysis of spray coverage

Spray coverage was determined by assessing water-sensitive paper where areas that were wet from the spray turned blue (Figure 7). This was analysed using digital image processing techniques. The photographs of water-sensitive paper were processed using Adobe Photoshop, where the coverage area was isolated, and RGB (Red-Green-Blue) colour values were recorded. The B value (blue channel) served as the primary metric for spray coverage, with higher B values indicating greater deposition.

Control samples, which had no spray exposure, exhibited a B value of zero (entirely yellow). This established a baseline against which coverage in treated samples was compared. Variability in coverage was analysed across the different pressures and row positions to identify patterns of deposition and effectiveness.

Figure 7: Spray coverage inside flowers. Blue indicates areas where spray has made contact.

2.3 Results

Spray coverage varied significantly with pressure settings. Analysis of B values (blue channel) revealed a decreasing trend in coverage as spray pressure increased, with the highest coverage

observed at 120 psi and the lowest at 150 psi. A box-and-whisker plot (Figure 8) illustrates the distribution of B values for each pressure setting, showing both median differences and variability within treatments.

Pearson's correlation analysis indicated a weak but statistically significant negative correlation between spray pressure and B values ($r = -0.25$, $p = 0.008$), suggesting that higher pressures were associated with reduced spray deposition.

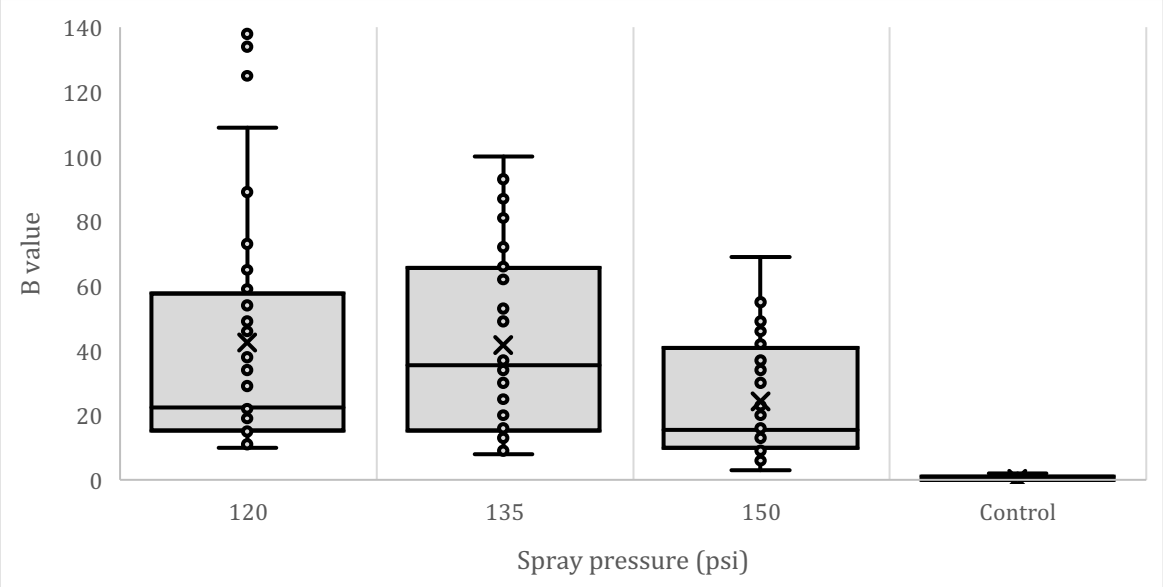
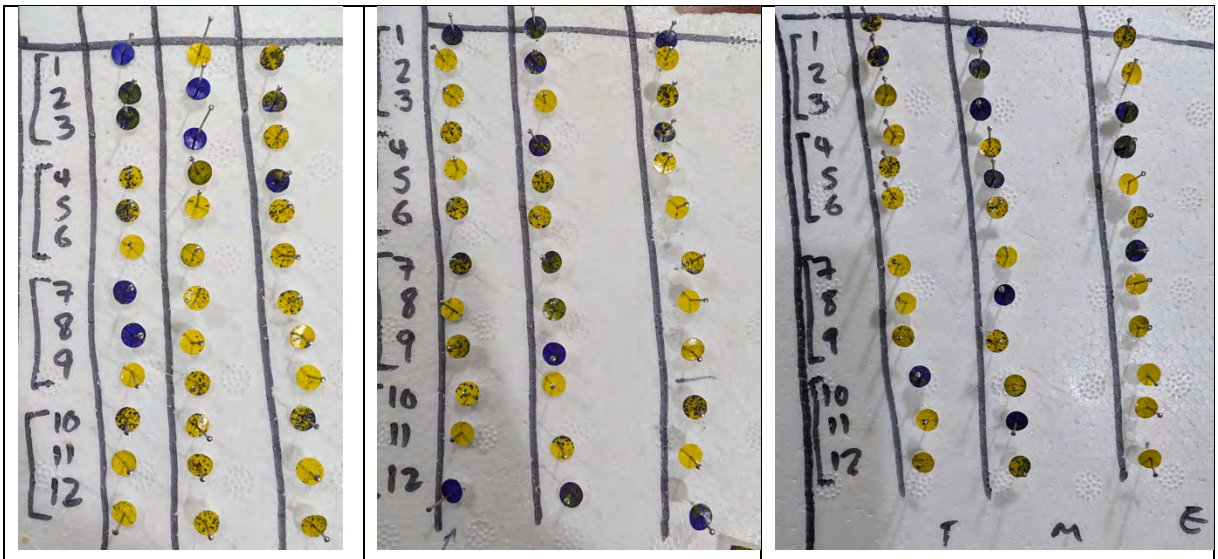


Figure 8: Box-and-whisker plot showing the distribution of B values, representing spray coverage, across different spray pressures (120, 135, and 150 psi). The plot indicates a decreasing trend in B values with increasing pressure, suggesting reduced spray coverage at higher pressures. Outliers are marked and may represent localized variations in spray application.

2.3.1 Spatial Variability Along Rows

Visual inspection highlighted improved spray coverage on plants located at the start of the row (plants 1–4) for 120 psi, 135 psi, and 150 psi treatments (Figure 4). This localized increase in coverage is likely due to the tractor's slower speed during initial acceleration. Similarly, enhanced deposition was observed in plants 1–4 at the end of rows in the 135 psi treatment, attributed to tractor deceleration prior to turning.



120 psi	135 psi	150psi

Figure 9: Spray cover in each flower. Blue areas indicate regions where the spray has made contact. For each pressure: First row=closet to tractor, second row = middle of boom, last row = edge of boom)

2.4 Discussion

This study investigated the efficacy of spray coverage in capsicum flowers across different spray pressures and positions relative to the boom. Contrary to expectations, increasing spray pressure resulted in reduced coverage, as indicated by a weak but statistically significant negative correlation between pressure and B values. This finding challenges the assumption that higher pressures, which produce finer droplets, enhance coverage through improved distribution and penetration into complex floral structures.

One potential explanation for this trend is overspray or drift. Higher pressures may generate finer droplets that are more prone to being carried away by air currents, particularly in an open-field environment, reducing deposition on target areas. Additionally, the increased velocity of droplets at higher pressures may lead to inconsistent patterns of deposition, resulting in gaps or uneven coverage.

No statistically significant differences in coverage were detected between positions along the boom (tractor edge, middle, or boom edge). This uniformity suggests that the spray rig maintained consistent delivery across the width of the boom, highlighting the efficacy of the nozzle configuration and boom height used in this trial. However, localized variability in deposition, such as outliers observed in the box-and-whisker plots, may be attributed to flower morphology, orientation, or environmental conditions, such as wind.

An interesting observation was the enhanced spray coverage on plants located at the start of the row across all pressures. This improvement is likely due to the tractor's slower speed during acceleration, which increased the duration of exposure to the spray as the tractor reached ideal speed. Similarly, improved coverage on plants 1–4 during the 135 psi treatment was observed near the end of the row as the tractor decelerated to make a turn. These findings underscore the influence of tractor speed dynamics on spray application efficiency and suggest that speed control could be an important factor in optimizing coverage.

The results of this trial emphasize the need for further investigations into spray application methods. Future studies could explore the role of air-assist sprayers, which may mitigate drift and improve penetration into complex floral structures.

In conclusion, while this trial demonstrated the impact of pressure and tractor speed on spray coverage, it also revealed areas where improvements in technology and methodology could enhance the precision and effectiveness of fungicide applications. The findings suggest that inconsistent coverage, particularly under higher pressures and varying tractor speeds, may contribute to the poor control of IFR observed in capsicum crops despite regular spray applications. Further assessments of different spray rigs, including air assisted applications and different nozzle types are required to fully understand the factors influencing coverage, particularly for achieving effective deposition in complex floral structures.

3 Comparison of spray coverage with and without air assist

3.1 Background

Internal fruit rot of capsicums remains problematic despite growers regularly applying fungicide to crops. Trials during this project have found no effect of a range of different fungicides and biological products on rates of internal mould development in red capsicums. This result is unexpected as the causes of internal fruit rot are known (Alternaria alternata and Fusarium spp.), with several commercial fungicides and biologicals known to have action against these pathogens.

Trials have also demonstrated that infection occurs during flowering, or shortly after fruit set. Capsicum flowers often hang downwards on the plant. If sprays are applied from above, it seemed possible that the flower petals could prevent the fungicide from reaching the stigma, this being the site of infection.

The previous two trials demonstrated that the boom type spray units with downward facing nozzles commonly used on capsicum farms do not provide adequate coverage of the flowers. While a few received full coverage, many received little or none.

In this third trial we examined the effectiveness of two other types of spray units, used at two different farms.

3.2 Methods

3.2.1 Trial setup and location

Trials were conducted in two commercial capsicum crops located in Stanthorpe, Queensland.

- The crop at Farm A was undergoing its first flush of flowering, with only small fruits starting to form.
- The crop at Farm B was slightly further advanced, with larger green fruits already well developed

Farm A was using a standard spray boom with nozzles at 0.5m intervals. The unit was calibrated to deliver 1,050 L/Ha along a 9m boom while travelling at 6km/h.

Farm B was using an air blast system on a shorter (4 row, instead of 6 row) boom. As this system uses air to volatilize and the spray as well as “ruffle” the plant leaves, it was expected that this would improve penetration into the capsicum flowers.

As previously, small, circular discs of water sensitive paper water sensitive paper were strategically pinned within flowers, covering the stigma and style. In both cases, plants were selected at four different locations within the rows nearest the tractor, in the middle of the boom, and at the outer edge of the boom. Three randomly selected flowers on each plant were used to assess coverage, with each marked with flagging tape below the flower (so as not to affect coverage).

Figure 10. As previously, discs of water sensitive paper were pinned into the centres of randomly selected flowers along three different rows.

An additional four discs per trial were placed on non-sprayed plants as untreated controls. This proved extremely important, as the high relative humidity (RH) at the time of the trial resulted in all discs undergoing a slight colour change, even before water was applied.

Figure 11. High relative humidity at the time of the trial meant that the water sensitive discs started to darken even before the spray had been applied

Both of the sprayers followed their normal spray practices but used water only for the purpose of the study.

Figure 12. Spray rig used at Farm A.

Figure 13. Air blast system used at Farm B.

Following spraying, the discs were collected and pinned onto a Styrofoam board labelled according to their plant position and row number.

3.2.2 Analysis of spray coverage

The discs were initially photographed in situ immediately after collection. On return to the lab, the discs were re-photographed using an SLR camera equipped with macro lens and under controlled lighting conditions. As little or no further colour change had occurred over the intervening period, the higher quality images could be used for analysis.

The photographs were processed using Adobe Photoshop. After adjusting brightness and removing the pin head from the image, the coverage area was isolated and averaged. The colour values for this area could then be recorded manually from the Photoshop Color Picker dialogue box. This describes colour using a number of different systems, including Adobe Lab; RGB (an additive system used for screen displays, where high values = lighter) and CMYK (a subtractive system used for printing, where high values = darker).

In this case, colour change was not simply from yellow to blue, but had to take into account darkening due to high RH. Whereas the “B” value (Blue, from RGB) had proven the best measure of colour change previously, the best way to calibrate against RH-induced darkening proved to be a combination of the “C” (cyan) and “Y” (yellow) values from CMYK. As this is a subtractive system, it was less influenced by overall lightness/brightness than RGB and clearly differentiated between sprayed and unsprayed discs.

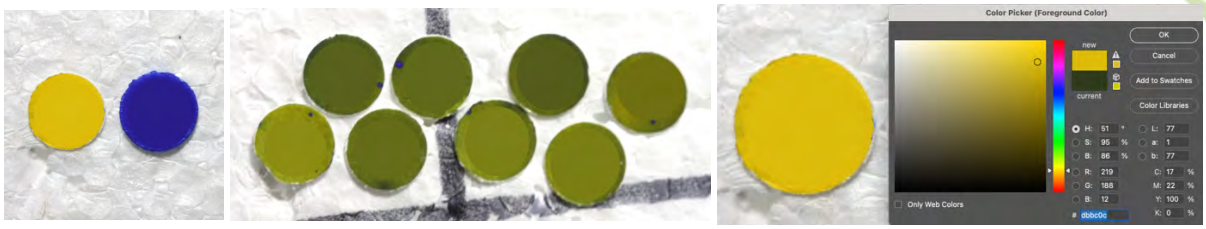


Figure 14. A dry (yellow) and wet (purple) disc shown at left. Controls used in this trial are shown at centre. Image at right shows the Adobe dialogue box with various systems used to express colour. All discs have been processed so as to remove the pin and average the central area of each disc.

Discs which had little or no spray exposure returned a value of Y = 100%. As spray exposure increased, and discs changed from yellow to purple, the percentage yellow fell. Although some discs which had had a very small amount of spray exposure still returned Y = 100%, these had elevated values for C.

Control discs all returned Y = 100%, and had an average of C = 57%.

The overall colour change for each disc could therefore be calculated as:

(100 – Y value) + (C value – 57).

This returned a value of **approximately 0** for discs with no spray coverage, and a **maximum of 135** for discs fully wetted.

To change this value to a percentage coverage, all results were multiplied by (100/135) = 0.74.

The same process was carried out on the data from trial 2, using the data from spray applied at 120psi (Farm C). As the results of this trial were not affected by high RH, the difference was calculated as purely the change between a fully dry disc and a fully wet one, with no need to adjust for controls. Again, the results were converted to an estimated percentage coverage for each disc.

Average spray coverage was calculated by row and spray system. In addition, the number of flowers within each row (n=12) that received good coverage (>50%); OK coverage (25 to 50%); poor coverage (10 to 25%) or effectively zero coverage (<10%) was recorded. The proportion of flowers in each category was graphed as a percentage of the whole.

3.3 Results

There were clear differences in spray coverage between the row at the outer edge of the boom and the row closest to the tractor. Coverage declined significantly with distance along the boom, falling by approximately half for the conventional spray units and two-thirds for the air blast unit. While variability was high, especially for Farm C, this difference was statistically significant.

Overall spray coverage was highest for the air blast unit (Farm B), which averaged 44% across all rows. In contrast, Farm A averaged 32% coverage and Farm C only 24% coverage.

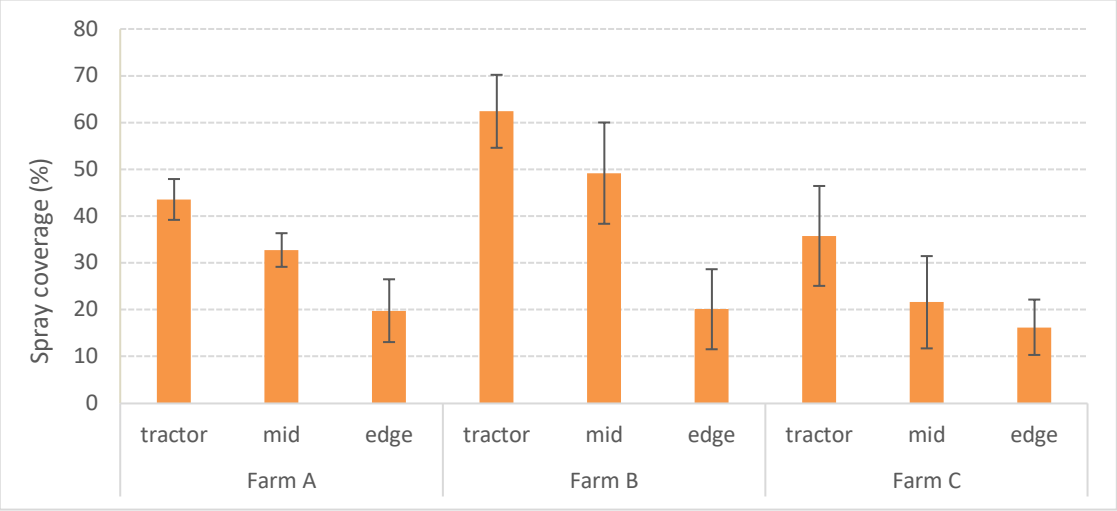


Figure 15. Average spray coverage by row, assessing a row next to the tractor, at the centre of the boom and the edge of the boom. Farm A had a conventional nozzle sprayer on an overhead boom, Farm B had an air blast sprayer, and Farm C had a spray unit with dropdown nozzles

While the averages for Farms A and C were similar, this conceals large differences in the type of coverage flowers received. At farm A, 10 of 12 flowers in rows 1 and 4 received at least 25% coverage. This fell to 5 of 12 flowers in row 1 and 2 of 12 flowers in row 3 at Farm C. At both farms, only 2 or 3 flowers in the outermost row (row 6) received at least 25% coverage.

These results suggest that the nozzles used at Farm C may have delivered relatively large droplet sizes. Flowers either received a high dose, or almost none. In contrast, Farm A appears to have been delivering a smaller droplet size, which may have been further dispersed under the high RH conditions at the time. At Farm A, more flowers received at least some spray, even if it appeared inadequate to provide good protection.

The airblast sprayer at Farm B achieved excellent coverage in the row next to the tractor, 11 of 12 flowers receiving at least 25% coverage. However, this dropped rapidly with distance from the tractor; only 2 of 12 flowers had good coverage, with the remaining 10 flowers less than 25% covered.

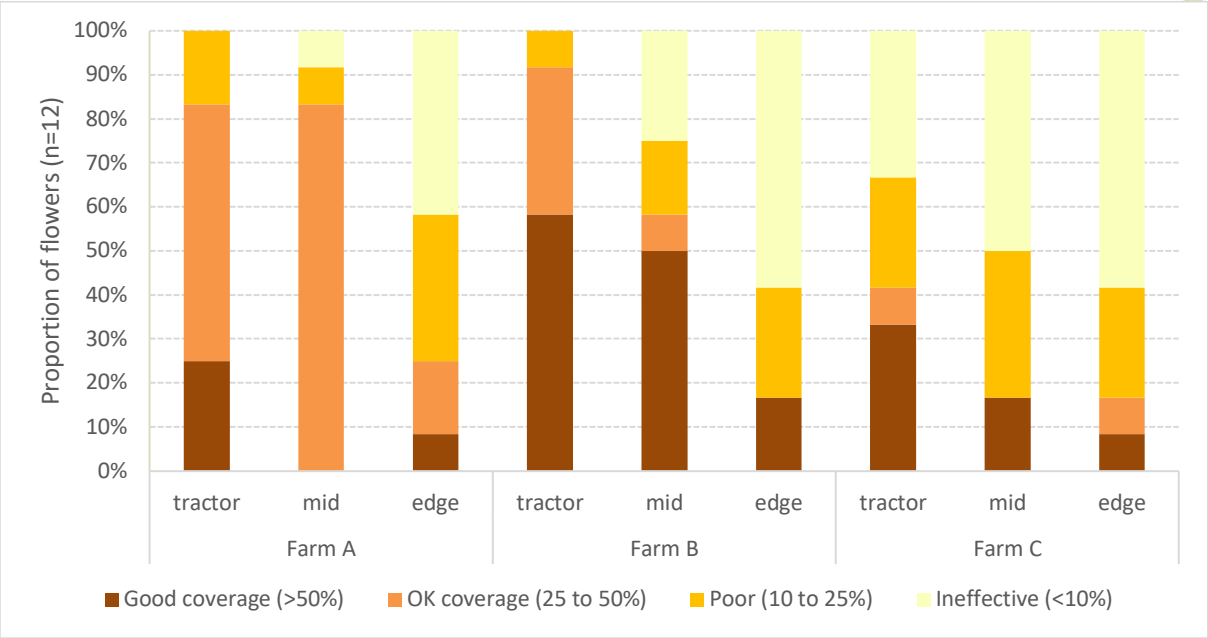


Figure 16. Proportion of flowers within each row that received good, OK, poor or ineffective coverage. Number of flowers is expressed as a percentage of those sampled (n=12).

3.4 Conclusions and Discussion

Capsicum flowers often orient downwards on the plant. This means that the stigma and style – the infection point for pathogens that cause internal mould – are protected by the flower petals from sprays applied above the plant. We hypothesised that this could provide some explanation for the lack of effectiveness of fungicides against these pathogens.

These trials have confirmed that normal commercial spray rigs do not provide good coverage of capsicum flowers. As fungicides have very limited motility in the plant, thorough coverage is essential in order to prevent infection. Once infection has occurred, the fungal spore germinating and growing into the flower ovaries, protective fungicides will be powerless against it.

We had further hypothesised that using an air assist system would provide better coverage. While improved coverage had been previously attempted using a hand held fogger (Appendix 6), in that trial it did not improve results. However, a handheld unit does not match the effectiveness of a commercial air blast spray unit.

This trial confirmed that an air blast sprayer has the potential to achieve far better spray coverage of capsicum flowers than conventional boom sprayers. However, a large drop in efficacy at the end of the boom remains a concern. There is likely to be some overlap when spraying each group of four rows, so that the outer row may effectively be treated 1.5 to 2 times. However, it is unclear whether this overlap is sufficient to achieve good coverage.

While the boom sprayer used at Farm A was superior to the spray unit previously assessed at Farm C, coverage was still far below optimal. Significant numbers of flowers remained unprotected, and therefore susceptible to infection by the pathogens that cause internal fruit rot.

Most fungicides are protective only, with little or no mobility within plant tissues. Achieving good coverage of the flower style is essential to reduce infection risk. None of the spray units in this trial achieved satisfactory coverage of flowers.

Future work should:

- Repeat the trials with larger numbers of flowers/row
- Examine whether there is significant crossover spray at the outer edge of the boom when the tractor runs down the next block, reducing the effect of low coverage on this row
- Determine whether the effectiveness of spray coverage relates to actual rates of internal fruit rot in red capsicums