## Final report

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# Epidemiology and management of fusarium basal rot in onions

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#### **Public summary**

Hort Innovation project VN20006 provides insights and strategies to reduce the impact fusarium basal rot is having on the Australian onion industry. The primary action has been developing the required knowledge for implementing a best practice, cost effective, integrated pest and disease management (IPDM) strategy. This involved developing a new diagnostic tool, improving our understanding of the pathogen and drivers of disease development; evaluating existing and new chemical, bio-stimulant, biological and cultural controls and refinement of associated rates/methods; and developing a disease risk-based decision model for management.

Fusarium basal rot has been increasing in prevalence and is the main soilborne disease of concern in some production areas, particularly in South Australia. Infection of bulbs that occurs in the field has resulted in substantive losses in storage, with incidence upwards of 30% occurring in some crops.

Fusarium oxysporum f. sp. cepae (Foc) has been confirmed as the main cause of fusarium basal rot symptoms in Australia.

Short rotations and previous occurrence of disease leading to high soil inoculum levels, prolonged lower or higher than optimum soil moisture levels, uneven crop growth, high bulb nitrogen levels, salinity issues, growing mid to late season crops, and susceptible varieties have been associated with increased incidence of bulb rots caused by Foc. This was determined by monitoring twenty crops in paddocks encompassing a range of rotation and production practices, soil moisture conditions and varieties during the 2022 and 2023 planting seasons. Incidence of bulb rot associated with *Fusarium* species ranged from nil to 55% in monitored areas.

DNA testing of root systems of barley, often grown in rotation and as a nurse crop in onion production systems indicates that it can be a host for Foc. Range of crops that Foc can infect was wider than anticipated with detections in other cereal and legume crops, as well as weeds from several families.

Infection of onion plants by Foc was often found to occur early in the crop, though disease symptoms were not visually evident until late in the crop. This indicates control measures may need to be applied at planting or early in the crop. Infection testing of individual plants from two plantings at the 8-10 leaf stage found the number of plants infected corresponded to the number that developed fusarium bulb rot at harvest, indicating most infection had already occurred by this stage of plant growth. Testing has found some infection can already be present at the 1-2 leaf stage.

In some crops, fusarium basal rot is not evident until development of rotted bulbs occurs after a period of ambient storage. DNA testing of a composite sample of the roots plus basal plates of 100 bulbs at harvest predicted in which samples fusarium basal rot would develop during ambient storage, even when observed incidence was low or nil at harvest.

Field trials that assessed efficacy of products applied as seed treatments, in furrow or post planting boom sprays have provided promising results that support progressing some treatments towards commercial availability. Trials were conducted on mid to late season brown onions at sites where incidence of bulb rot at harvest associated with Foc ranged from 6 to 37% in untreated areas.

 $Further\ information\ is\ provided\ in\ the\ fusarium\ basal\ rot\ guide\ produced\ as\ part\ of\ this\ project.$ 

#### **Technical summary**

**Issue addressed** - Fusarium basal rot has been increasing in prevalence and is the main soilborne disease of concern in some onion production areas, particularly in South Australia. Infection of bulbs that occurs in the field has resulted in substantive losses in storage, with incidence upwards of 30% occurring in some crops.

**Project approach** - Hort Innovation project VN20006 provides insights and strategies to reduce the impact fusarium basal rot is having on the Australian onion industry. The primary action has been developing the required knowledge for implementing a best practice, cost effective, integrated pest and disease management (IPDM) strategy. This involved improving our understanding of the pathogen and drivers of disease development; evaluating existing and new chemical, bio-stimulant, biological and cultural controls and refinement of associated rates/methods; and developing a disease risk-based decision model for management.

At the start of the project a review of published literature and research reports (national and international) on FBR was undertaken. The focus of the review was to gather information relevant to onion production in Australia, documenting available knowledge on epidemiology of the disease including conditions that are conducive to infection and to disease development, and the suitability and efficacy of cultural, bio-stimulant, biological and chemical control options. Findings of the review were used in consultation with the Project Reference Group to refine project research and development activities targeted at addressing gaps in knowledge required to develop an integrated strategy for disease management.

Causal agents of fusarium basal rot - Onion bulbs and plants with symptoms resembling fusarium basal rot were received from growers. Tissue samples from the basal plate, leaf scales, and roots of bulbs with typical fusarium basal rot symptoms were tested, along with samples showing atypical symptoms, such as basal plate breakdown, bulb rotting, skin discoloration and healthy bulbs. DNA was extracted and tested using Next Generation Sequencing (NGS) with SARDI's Fusarium-specific primer set, and quantitative PCR (qPCR) with the pathogen *Fusarium oxysporum* f. sp. *cepae* (Foc) DNA test developed in this project.

The pathogen Foc was the predominant species found associated with fusarium basal rot disease symptoms observed in South Australia and Queensland.

Fusarium proliferatum was associated with purple blotch on the skins of white onions and bulb rot of ambient stored onions. Fusarium oxysporum f.sp. cepae and other forma speciale of F. oxysporum, along with other Fusarium species including F. solani were found to commonly infect the roots of onions tested and may be impacting onion productivity.

**Development of new diagnostics** - A qPCR test for Foc has been developed and is available for industry and researchers to use. The assay for the specific detection and quantification of Foc was designed based on publicly available sequence data and sequencing of cultures isolated from onions with fusarium basal rot symptoms in this project. The test is modified from a published assay by Sasaki et al. (2015) targeting the identified virulence related 'Secreted In Xylem' SIX3 gene. The assay was tested for specificity using an extensive collection of DNA from the target and closely related species. The Foc assay efficiently detected pure DNA from the target and did not detect any of the other *F. oxysporum* forma speciale nor any other species assessed, indicating the assay is specific.

The Foc assay was used to assess samples of onion tissue from bulbs with and without fusarium basal rot symptoms. Results of testing aligned with the symptoms observed. Results of testing 500 g soil samples collected from paddocks after the harvest of infected crops indicates the test is not sensitive enough to detect low levels of inoculum in soil. However, when Foc was detected in the soil at monitoring sites, this was linked to a higher likelihood of fusarium basal rot occurring, when compared with sites where Foc was not detected. Detection of Foc in soil confirms the site has high risk of fusarium basal rot occurring if conditions are favourable for disease.

**Identifying drivers of disease** - To understand key drivers of disease development, crops were monitored in paddocks encompassing a range of rotation and production practices, soil moisture conditions and varieties in 20 crops during the 2022 and 2023 planting seasons. Focus was on mid to late season brown and red onion varieties grown in the Murray Mallee and South-east regions of South Australia. All crops were irrigated by centre pivot.

The incidence of rotted bulbs caused by Foc at monitoring spots varied from nil to 55%, with a high correlation between the concentration of Foc in basal plate tissue and the incidence of the disease. This indicates that Foc is the primary causal agent of fusarium basal rot. The high incidence of this disease significantly impacts productivity, as rotted bulbs are unmarketable and add considerable costs to grading and handling. Foc infection also leads to additional yield loss, with total yield reductions of up to 25% before the removal of rotted bulbs.

Testing confirmed that Foc infection was present at the 2-4 leaf stage in most crops that developed fusarium basal rot by harvest, and by the 5-7 leaf stage, infection was detected in nearly all affected crops. Many infected plants showed no obvious symptoms. DNA testing can detect Foc infection levels in crop before visual symptoms appear, helping to identify crops likely to have reduced yields and high fusarium basal rot incidence. Not all infected bulbs were rotted or had obvious symptoms of fusarium basal rot at the time of harvest. These latent infections led to further losses when kept in ambient storage. DNA testing at harvest provides a better indication of fusarium basal rot developing in storage than inspection for rotted basal plates.

Elevated total nitrogen level in tissue of harvested bulbs was associated with increased incidence of rotted bulbs caused by Foc. Lower nitrogen level in the harvested bulbs was not related to reduced yield, suggesting nitrogen level was not yield limiting within the range recorded in these monitored commercial crops. Elevated sodium and chloride levels were also associated with reduced basal plate health, indicating that salinity management might help reduce fusarium basal rot risk.

**Beneficial fungi** - Associations were found between fusarium basal rot incidence and the levels of beneficial fungi on onion bulbs at harvest. Bulbs with higher levels of Arbuscular Mycorrhiza Fungi and *Trichoderma* spp. tended to have lower incidence of fusarium basal rot.

Other soilborne diseases - In the monitored paddocks, Foc was the dominant soilborne pathogen affecting productivity, though this may not reflect the broader industry as most sites in this project were specifically selected to target monitoring of fusarium basal rot. Other diseases, such as root lesion nematodes and pink root caused by *Setophoma terrestris*, were not strongly associated with bulb yield. *Setophoma terrestris* was detected in all monitored crops, but no strong link was found between its concentration and fusarium basal rot incidence. *Sclerotium cepivorum*, the cause of white rot, was not detected, and stubby root nematode, *Paratrichodorus* spp., reduced yield in one planting. *Macrophomina phaseolina* infection early in the crop was associated with lower yields, warranting further investigation. Two of the monitored sites had substantive levels of bacterial rots, with 6% of bulbs affected by bacterial bulb rot in one area of one site and 20% recorded in a wet area at the other site. At the other sites assessed the incidence of bacterial rots recorded at harvest was 2% or less.

**Soil moisture** – Monitoring sites were set up in a centre pivot irrigated brown onion crop to study the impact of soil moisture variations on fusarium basal rot. Ten points were located between two pivot wheel tracks in three planting beds, with a nozzle creating a low moisture arc in the pivot and topography causing a gradient into a waterlogged depression. Three additional spots were established on the other side of the pivot on the low moisture arc. Both higher and lower than optimal soil moisture levels increase the risk of fusarium basal rot. Prolonged dry conditions reduce yield and increase disease risk, while high moisture conditions, if not waterlogged, can promote yield but also increase the risk of fusarium basal rot and bacterial bulb rots.

Simulated high rainfall events - In the 2022 and 2023 planting seasons, simulated high rainfall events were applied to fusarium basal rot susceptible brown onion crops. Flooding treatments, equivalent to 50 mm of rainfall, were applied once or repeatedly at various growth stages (4-5 leaf, 6-7 leaf, 50% and 80% tops down, and 2 weeks before harvest) on sandy to loamy sand soils. The 2022/23 season experienced prolonged above-average rainfall, leading to high soil moisture until the 4-5 leaf stage. In 2023/24, four significant rainfall events occurred in December and January, contributing to risk as much as the treatments. Due to the conducive natural weather conditions, none of the simulated rainfall treatments significantly changed the incidence of fusarium basal rot compared to controls.

**Nitrogen application trials** - Nitrogen was applied to four brown onion crops over the 2022 and 2023 planting seasons using sulphate of ammonium, calcium nitrate, and urea at rates from 25 to 150 kg N per ha, in addition to the grower's fertiliser program. In 2022, nitrogen was applied as a single application, while in 2023, it was split over three applications. The soil type was sand to loamy sand. Results showed that high bulb nitrogen levels were associated with higher fusarium basal rot incidence, highlighting the importance of managing crop nutrition. However, other factors influenced bulb nitrogen concentration, with site conditions having a greater impact than nitrogen application rate or the form nitrogen that was applied in each trial. Understanding nitrogen uptake pathways and its role in disease epidemiology is crucial for using crop nutrition and soil management practices to reduce fusarium basal rot risk.

**Nurse crops** - The impact of nurse crop competition was assessed by comparing onions planted within a dense barley nurse crop to those with minimal competition. Competition from the barley nurse crop reduced yield by 25% but did not significantly affect the incidence of fusarium basal rot or nitrogen concentration in bulbs. Low levels of Foc DNA in barley samples indicated some infection was present at spray-off.

Host species testing - Limited knowledge exists on whether Foc infects crops and weeds in South Australian onion production systems. Field samples from five rotation crops and 13 weed species were collected from paddocks with confirmed Foc or *S. terrestris* inoculum. Root systems were rinsed, dried, and DNA tested for Foc and *S. terrestris*, with results compared to Foc infection in onion crops. Results indicate infected onion crops would be the main contributors to Foc inoculum in the soil. qPCR testing confirmed that a wide range of crop and weed species can be naturally infected by Foc and *S. terrestris*, suggesting multiple plant families may act as reservoir hosts. Rotation crops may help maintain inoculum levels, with further research needed to determine the significance of specific rotation, cover crop, and weed species on the risk of fusarium basal rot.

**Efficacy of control treatments** - Efficacy testing of products was conducted to develop application strategies and support their future availability to the onion industry. Products were selected after consulting the Project Reference Group, reviewing literature, and discussing with companies. Controlled environment trials were used to screen potential control agents for evaluation in field efficacy trials.

Two in-crop treatment field trials tested four products (Intuity®, Luna® Experience, Brumby®, and Sergomil L60®), and three field trials assessed in-furrow and band application strategies of SYN PHI3, along with seed treatments of Evergol® Energy and SYN PHI3. Trials were conducted in paddocks with confirmed Foc inoculum and susceptible brown onion varieties. Untreated plants were tested for Foc infections during crop growth.

Results showed promising efficacy, with the best treatment reducing fusarium basal rot by 70-80% when applied infurrow at planting and followed by a band spray. Incidence of rotted bulbs in untreated controls averaged 7, 25, and 36% across three sites. The most effective in-crop spray treatment reduced fusarium basal rot at harvest by 76 and 54% at two trial sites, with similar reductions after 3 months of ambient storage. Seed treatments trialed provided little to no control of fusarium basal rot.

**Fusarium basal rot guide** - The 15-page guide incorporates knowledge and information generated in this project, as well as the best available information from local and international sources. Targeted at growers and agronomists it provides information about fusarium basal rot including its cause and symptoms, factors affecting buildup of inoculum, factors affecting infection and disease development, and strategies to reduce disease development in an integrated approach to disease management.

#### **Keywords**

Onion, Fusarium basal rot, Fusarium oxysporum, Soilborne disease, Integrated Disease Management, Pink root

#### Introduction

Project VN20006 was undertaken to reduce the impact of fusarium basal rot on the Australian onion industry, particularly in South Australia where it is most damaging. The approach involved developing a best practice integrated pest and disease management (IPDM) strategy based on a better understanding of the pathogen and its epidemiology, evaluating control strategies, and adjusting management inputs according to disease risk.

Fusarium basal rot is a disease of increasing importance in South Australia and is the main soilborne disease of concern in some production areas. Infection of bulbs that occurs in the field has resulted in substantive losses in storage from this soilborne disease, with incidence upwards of 30% reported by growers occurring in some crops.

Despite its global impact on Allium crops, there are many gaps in our knowledge of the epidemiology and management of fusarium basal rot in onions (Le et al. 2021). In Australia, not all pathogens involved have been identified and the epidemiology of the disease, including conducive factors for infection are not well characterised, limiting capability to implement a IPDM strategy.

Fusarium oxysporum f. sp. cepae (Foc) is suspected as the main causal agent of fusarium basal rot in Australia. Other species, including F. proliferatum and F. redolens have also been associated with fusarium basal rot overseas (Le et al. 2021). Both these species are known to be present in South Australia (APPD, Hollaway 2021). Varieties resistant to fusarium basal rot are not available, and varieties identified with reduced susceptibility in one region may be susceptible in another, possibly due to different causal agents or strains of causal agents being present (Taylor et al. 2013, Caligiore-Gei et al. 2020, Cramer et al. 2021). Effectiveness of crop rotations and management practices varies depending on Fusarium species (Higashida et al. 1982, Leoni et al. 2013, Cramer 2000), so confirming the causal agent or agents is important. Development of molecular tools to identify and characterise the pathogens would aid in understanding the biology of the pathogen.

Much of the loss from fusarium basal rot occurs after harvest, however infection of individual bulbs that rot in storage occurs in the field (Stadnik and Dhingra 1997). Reduction in losses requires the disease to be controlled in the field. The pathogen has long-lived survival structures that remain in the soil for many years, ready to infect onions if conditions are conducive (Le et al. 2021). Understanding when infection occurs and the factors that are conducive for infection and disease development need to be determined to underpin implementation of control methods for fusarium basal rot.

To formulate a best practice IPDM strategy, cost effective cultural, chemical and biological management options need to be identified. There are many potential biostimulant and biological products available to growers for managing soilborne diseases (<a href="https://soilwealth.com.au/2024/10/biological-products-database/">https://soilwealth.com.au/2024/10/biological-products-database/</a>). For most products, directions for use in onion production and evidence-based information on ability to reduce fusarium basal rot are lacking. No fungicides are registered for control of fusarium basal rot on onions in South Australia. Proven cultural, biological and chemical management options need to be established as part of an effective and sustainable management strategy.

The aim of this project is to address current shortcomings in fusarium basal rot control methods and conduct targeted research to inform improved disease control with best practice IPDM strategies for growers.

#### Methodology

#### Review of literature and research

At the start of the project a review of published literature and research reports (national and international) on fusarium basal rot was undertaken. The focus of the review was to gather information relevant to onion production in Australia, documenting available knowledge on epidemiology of the disease including conditions that are conducive to infection and to disease development, and the suitability and efficacy of cultural, bio-stimulant, biological and chemical control options. Knowledge gaps identified by review included insufficient information on crop nutrition, selection rotation crops, interactions with pink root, and practicality, reliability, and efficacy of control options.

Findings were used in consultation with the Project Reference Group to refine project research and development activities targeted at addressing gaps in knowledge required to develop an integrated strategy for disease management.

#### Causal agents of fusarium basal rot

Onion bulbs and plants with symptoms resembling fusarium basal rot, along with other bulb rots were received from 16 growers from production regions across Australia. Bulbs with typical fusarium basal rot symptoms, along with samples showing atypical symptoms, such as basal plate breakdown, bulb rotting, skin discoloration and healthy bulbs were selected for testing. These samples were examined, photographed, and dissected to obtain tissue samples from the basal plate, leaf scales and roots. DNA was extracted from 94 tissue samples and tested using Next Generation Sequencing (NGS) with SARDI's Fusarium-specific primer set, and quantitative PCR (qPCR) with the Foc DNA test developed in this project.

Fusarium cultures were isolated from bulbs with a range of symptom types and stored for use in other aspects of the project.

#### Development of new diagnostics

A TaqMan MGB qPCR assay for the specific detection and quantification of *Fusarium oxysporum* f. sp. *cepae* was designed based on publicly available sequence data and sequencing of cultures isolated from onions with fusarium basal rot symptoms in this project. The test is modified from a published assay by Sasaki et al. (2015) targeting the identified virulence related 'Secreted In Xylem' SIX3 gene. The primer and probe details are available to requesting third parties under MTA.

The assay was tested for specificity using an extensive collection of DNA from the target and closely related species. The Foc assay efficiently detected pure DNA from the target and did not detect any of the other *F. oxysporum* forma speciale nor any of the other species assessed, indicating the assay is specific.

#### Identifying drivers of disease

To understand key drivers of disease development, crops were monitored in paddocks encompassing a range of rotation and production practices, soil moisture conditions and varieties. Monitoring of 12 crops in the 2022 planting season and eight crops in the 2023 planting season was undertaken on mid to late season brown and red onion varieties in South Australia. All crops were irrigated by centre pivot, with four crops located in the Murray Mallee region and 16 crops located in the South-east region.

In each crop, monitoring spots were established after planting, with six monitoring spots spaced along the length of the planting in a single variety. In some crops, up to four additional spots were located on a second planting line to monitor different varieties or management practices. Monitoring spots within a crop were grouped into 2-4 zones based on differences in soil type and topography, or variety or management practice in the cases where additional spots monitored.

Measurements prior to harvest included soil pathogen DNA testing (SARDI Hort Veg panel), in-crop visual inspection and pathogen DNA testing for Foc infection at 2-4 leaf and 5-7 leaf stages. Measurements at harvest included number and

weight of bulbs, incidence and severity of fusarium basal rot, incidence of pink root, nutrient testing of bulb tissue and pathogen DNA testing of root plus basal plate samples. Bulbs were also assessed after ambient storage from selected sites. Data was analysed for association between measured parameters and the incidence of fusarium basal rot.

#### Impact of prolonged soil moisture differences on disease development

Monitoring sites were established in a centre pivot irrigated fusarium basal rot susceptible variety brown onion crop to investigate the impact of season long differences in soil moisture. Ten monitoring points were located along three planting beds. Lower than required output from a nozzle created an arc of low soil moisture in the pivot, while topography created a gradient of increasing soil moisture into a waterlogged depression that persisted throughout the season. A further three spots were established in the same three beds at the other side of the pivot, one in the dry arc of the pivot and others on either side of the dry arc. To characterise variation in soil moisture level at the 13 spots, soil water content was determined by gravimetric methods towards the end of crop growth.

Measurements at harvest included number and weight of bulbs, incidence and severity of fusarium basal rot, incidence of pink root, nutrient testing of bulb tissue and pathogen DNA testing of root plus basal plate samples. Bulbs were also assessed after period of ambient storage.

#### Impact of short-term high moisture events on disease development

Simulated high rainfall events were applied to fusarium basal rot susceptible variety brown onion crops in the 2022 and 2023 planting seasons. Flooding treatments were applied by pumping water equivalent to 50 mm of rainfall once or repeatedly onto plots with raised edges to contain water at timings of 4-5 leaf, 6-7 leaf, 50% and 80% tops down and 2 weeks before harvest. Soil type at sites were sand to loamy sands.

Incidence and severity of fusarium basal rot and the incidence of pink root were assessed at harvest and data was analysed by ANOVA using Genstat statistical software, with means compared by LSD at the 10% level.

#### Effect of nitrogen application on disease development

Nitrogen applications were applied to four brown onion crops over the 2022 and 2023 planting seasons. Nitrogen was applied in-crop using three forms of nitrogen (sulphate of ammonium, calcium nitrate and urea) to apply rates from 25 to 150 kg N per ha depending on trial in addition to the grower's fertiliser program. Nitrogen applied as a single application in the 2022 planting season and split over three applications in the 2023 planting season. Soil type at sites was a sand to loamy sand.

Measurements at harvest included number and weight of bulbs, incidence and severity of fusarium basal rot, nutrient testing of bulb tissue and pathogen DNA testing of root plus basal plate samples. Data was analysed by ANOVA using Genstat statistical software, with means compared by LSD at the 10% level.

#### Effect of nurse crop competition

Impact of nurse crop competition was assessed by comparing onions planted within a dense barley nurse crop to onions growing in between the nurse crop with minimal to no competition. Pathogen DNA testing for Foc was conducted on root and crown samples of the barley nurse crop at time of spray off.

Measurements at harvest included number and weight of bulbs, incidence and severity of fusarium basal rot, nutrient testing of bulb tissue and pathogen DNA testing of root plus basal plate samples. Data was analysed by ANOVA using Genstat statistical software, with means compared by LSD at the 10% level.

#### Host crops

There is limited knowledge on whether Foc is hosted on crops and weeds in South Australian onion production systems. Field samples of five rotation crops and 13 species of weeds were collected from areas of paddocks where inoculum of

Foc or *S. terrestris* was confirmed by DNA testing of soil. Root systems were rinsed, dried and DNA tested for Foc and *S. terrestris*. Results were compared with Foc infection detected in onion crops.

#### Efficacy of control treatments

Efficacy testing of products was conducted to investigate application strategies and provide support towards progressing products towards future availability to the onion industry. Products for testing were selected in consultation with members of the Project Reference Group, after a review of the literature, information available from overseas, discussions with companies, a scan of new products on the market and consideration to current use on onions for other purposes. Controlled environment trials were utilised to screen potential control agents to assist with selection of products for inclusion in field efficacy trials.

Two in-crop field efficacy trials of four products (Intuity®, Luna® Experience, Brumby® and Sergomil L60®) and three field efficacy trials to assess three in-furrow and band application strategies of a product in development (SYN PHI3), along with seed treatments of two products (Evergol® Energy and SYN PHI3) were conducted.

Trials were situated in paddocks where Foc inoculum was confirmed to be present by soil DNA testing and susceptible brown onion varieties planted. Plants from untreated areas within each of the five trial locations were tested for Foc infection at 2 to 3 crop growth stages.

At harvest, number and weight of bulbs was recorded. All treated bulbs were removed. The incidence and severity of fusarium basal rot and incidence of pink root was assessed at harvest. For some trials, fusarium basal rot was also assessed after period of ambient storage. Pathogen DNA testing for Foc and *S. terrestris* was conducted on root plus basal plate samples at harvest. Statistical analysis of trials was conducted by ANOVA using Genstat statistical software, with means compared by LSD at the 5 and 10% level.

#### Fusarium basal rot guide and involvement in industry communications, extension, and practice change programs.

A fusarium basal rot guide was prepared at the beginning of the project and then updated to incorporate knowledge and information generated in this project, as well as the best available information from local and international sources. The 15-page guide aimed at growers and agronomists provides information about the disease including its cause and symptoms, factors affecting buildup of inoculum, factors affecting infection and disease development, and strategies to reduce disease development in an integrated approach to disease management.

The project's principal investigator engaged in one-on-one discussions with growers and agronomists on soilborne disease issues, as well as presented project findings relating to fusarium basal rot at industry events, agribusiness grower nights, and technical workshops and a Masterclass. Four articles were published in the Australian Grower.

#### **Results and discussion**

#### Causal agents of fusarium basal rot

The pathogen *Fusarium oxysporum* f. sp. *cepae* (Foc) was the predominant species found associated with fusarium basal rot disease symptoms observed.

Review of next generation sequencing (NGS) data and results of specific qPCR testing for Foc on onion samples with fusarium basal rot symptoms did not identify other *Fusarium* spp. that are likely to play a significant role in causing fusarium basal rot.

Fusarium proliferatum was associated with purple blotch on the skins of white onions and bulb rot of ambient stored onions.

Fusarium oxysporum f.sp. cepae and other forma speciale of F. oxysporum, along with other Fusarium species including F. solani were found to commonly infect the roots of onions tested and may be impacting onion productivity.

#### Development of new diagnostics

A qPCR test for *Fusarium oxysporum* f. sp. *cepae* (Foc) has been developed and is available for industry and researchers to use.

The Foc assay efficiently detected pure DNA from the target and did not detect any of the other *F. oxysporum* forma speciale nor any of the other species assessed, indicating the assay is specific.

The Foc assay was used to assess samples of onion tissue from bulbs with and without fusarium basal rot symptoms. Results of testing aligned with the symptoms observed, with levels ranging from below detection to 6.3 log kDNA copies/g sample for Foc. Results of testing 500 g soil samples collected from paddocks after the harvest of infected crops indicates the test is not sensitive enough to detect low levels of inoculum in soil, with levels ranging from below detection to 1.5 log kDNA copies/g sample for Foc. However, when Foc was detected in the soil at monitoring sites, this was linked to a higher likelihood of fusarium basal rot occurring, when compared with sites where Foc was not detected. Detection of Foc in soil confirms the site has high risk of fusarium basal rot occurring if conditions are favourable for disease.

The test has been incorporated into SARDI's PREDICTA delivery platform to enable routine assessment of samples.

#### Identifying drivers of disease

Understanding when and why Foc infection occurs in onion crops is important to the development and implementation of control strategies. Monitoring of crops was undertaken to gather data on drivers of disease development in 20 crops with the following key findings.

- Incidence of rotted bulbs at monitoring spots ranged from nil to 55%. Incidence was highly correlated with the concentration of Foc in basal plate tissue of harvested bulbs indicating Foc is the main causal agent of the fusarium basal rot observed.
- The high incidence of fusarium basal rot highlights the substantive impact this disease can have on productivity. Rotted bulbs are unmarketable, and even when present at low incidence, add significant cost to grading and handling to facilitate marketing of the rest of the crop.
- Foc infection resulted in additional loss of yield to that caused by discarding of rotted bulbs. At high levels of infection, total yield was as much as 25% lower, before rotted bulbs were removed.
- Testing of plants confirmed Foc infection was present at the 2-4 leaf stage in most, but not all crops that developed fusarium basal rot by time of harvest. By the 5-7 leaf stage infection was detected in all but one of the crops that developed fusarium basal rot at harvest. Many of the infected plants had no obvious symptoms.
- DNA testing can be used to detect and assess Foc infection level in crops before symptoms are observed by visual crop inspection, identifying crops that are likely to have reduced yield and high incidence of fusarium basal rot.
- Not all infected bulbs were rotted or had obvious symptoms of fusarium basal rot at the time of harvest. These latent infections led to further losses when kept in ambient storage. DNA testing at harvest provides a better indication of fusarium basal rot developing in storage than inspection for rotted basal plates.
- Associations were found between incidence of fusarium basal rot and the levels of beneficial fungi on the root system of onion bulbs at harvest. Bulbs with higher levels of Arbuscular Mycorrhiza Fungi and *Trichoderma* spp. tended to have lower incidence of fusarium basal rot.
- Elevated total nitrogen level in tissue of harvested bulbs was associated with increased incidence of rotted bulbs
  caused by Foc. Lower nitrogen level in the harvested bulbs was not related to reduced yield, suggesting nitrogen
  level was not yield limiting within the range monitored in these commercial crops. This means there is scope to
  manage nitrogen to reduce risk of fusarium basal rot without jeopardising yield in sites where fusarium basal rot
  is expected to be an issue. When adjusting crop nutrition potential impacts on other bulb quality attributes also
  need to be considered.
- Elevated sodium and chloride level in tissue of bulbs sampled at harvest from monitoring sites were associated

with reduced health of the basal plate, suggesting salinity management may assist in reducing the risk of fusarium basal rot.

- In the paddocks monitored in this project, Foc was the dominant soilborne pathogen impacting productivity. This data may not be reflective of the broader industry, as most sites in this project were specifically selected to target monitoring of fusarium basal rot.
- Other diseases that are known to impact onion productivity in South Australia, such as root lesion nematodes and pink root, caused by *Setophoma terrestris*, were not strongly associated with bulb yield at the monitored sites.
- *S. terrestris* was detected in root systems from all 20 monitored crops. No strong linkage was found between increasing concentration of *S. terrestris* in the soil or plant roots with higher incidence of fusarium basal rot. High incidence of fusarium basal rot often occurred at sites with a low level of *S. terrestris* plant infection.
- Sclerotium cepivorum, cause of white rot was not detected at any of the sites monitored.
- Stubby root nematode, *Paratrichodorus* spp. caused a reduction in yield of large patches of onions in one of the 20 plantings monitored.
- Macrophomina phaseolina infection of plants early in the crop, which was not strongly associated with Foc infection, was associated with lower yield, including at a low yield site where Foc was not detected. Impacts of M. phaseolina on onion productivity warrant further investigation to determine if this widespread pathogen is impacting onion productivity.
- Two of the monitored sites had substantive levels of bacterial rots, with 6% of bulbs affected by bacterial bulb rot in one area of one site and 20% recorded in a wet area at the other site. At the other sites assessed the incidence of bacterial rots recorded at harvest was 2% or less.
- When onions were stored at ambient temperature for 2-3 months after harvest, multiple pathogens were sometimes involved in disease development and rotting of bulbs, including bacterial pathogens.

#### Impact of prolonged soil moisture differences on disease development

Irrigation management is a critical aspect of onion production. Both higher and lower than optimum soil moisture levels were found to increase the risk of fusarium basal rot. Prolonged dry conditions reduce yield and increase the risk of fusarium basal rot. High moisture conditions, as long as soils are not waterlogged, can promote yield, but also increase risks of both fusarium basal rot and bacterial bulb rots.

#### Impact of short-term high moisture events on disease development

The 2022/23 growing season was characterised by a prolonged period of above average rainfall that resulted in higher than desired soil moisture conditions until the 4-5 leaf stage at the trial site. In the 2023/24 season four significant rainfall events happened at the site of the trial in the December - January period, contributing to risk as much as the treatments. Due to the conducive natural weather conditions, none of the simulated rainfall treatments resulted in a significant change in the incidence of fusarium basal rot compared with the controls.

#### Effect of nitrogen application on disease development

Results from nitrogen application trials support the finding from monitoring sites that high bulb nitrogen levels are associated with higher incidence of fusarium basal rot, reinforcing the importance of managing crop nutrition. However, the results indicate factors other than nitrogen application rate or nitrogen form can impact bulb nitrogen concentration. The concentration of nitrogen in bulb tissue at harvest was influenced more by the site at which the trial was conducted than by nitrogen application rate or the form nitrogen that was applied in each trial.

A greater understanding of the uptake pathways and role nitrogen is having in disease epidemiology is required to better use crop nutrition and soil management practices to lower the risk of fusarium basal rot.

#### Effect of nurse crop competition

Competition from the barley nurse crop reduced yield by 25%, however did not significantly affect incidence of rotted bulbs caused by Foc or concentration of nitrogen in bulbs. A low detection of Foc DNA (30 kDNA copies/g sample) in root and crown samples of the barley plants indicated that some infection of the barley nurse crop was present at the time of spray off. Foc was not detected in onions (1 leaf stage) sampled at the same time as the nurse crop from areas surrounding the trial area, though Foc was detected at levels of up to 490 kDNA copies/g sample in another area of the same paddock 2 weeks later at the 1-2 leaf stage.

#### Host crops

Infected onion crops would be the main contributor to Foc inoculum levels in the soil in onion production systems.

Crops currently grown in rotation with onions may be assisting to maintain inoculum between onion crops, contributing to risk of fusarium basal rot and pink root occurring in following onion crops. qPCR testing of plant samples from onion production fields confirmed a wide range of crop and weed species can be naturally infected by Foc and *S. terrestris*, indicating plants from multiple family groups may be acting as reservoir hosts in onion production systems. Further work is required to determine the significance of specific rotation, cover crop and weed species to the risk of fusarium basal rot.

#### Efficacy of control treatments

Efficacy trials have provided promising results that support progressing some treatments towards commercial availability. The most effective treatment evaluated reduced fusarium basal rot by 70-80% when applied in-furrow at planting followed by a band spray 4 weeks later. Incidence of rotted bulbs at harvest averaged 7, 25 and 36% in the untreated controls of the three trial sites. Of the in-crop boom applied treatments assessed in two trials, the most effective chemistry reduced fusarium basal rot by 76 and 54%, with similar reductions in fusarium basal rot still attained after 3 months ambient storage, though the percentage rots had increased. Incidence of rotted bulbs at harvest averaged 8 and 22% in the untreated controls at the two trial sites respectively.

Seed treatments evaluated provided no or negligible control of fusarium basal rot.

## Outputs

A summary of the project's outputs is shown in Table 1.

Table 1: Summary of project outputs

Output	Audience	Detail
Fusarium basal rot management guide	Information targeted for growers and agronomists.	Guide available online and been promoted via links in grower/industry articles published from the project and on the onion industry extension program factsheet on fusarium basal rot. Guide incorporates project findings and current knowledge on fusarium basal rot management. Appendix 2.
Fusarium basal rot workshop	Attended by 14 participants from South Australia with an equal split of grower representatives and agribusiness agronomists/service providers.	Workshop provided an interactive forum for participants to discuss the key project findings that were presented. Workshop was organized in collaboration with the Onion Communication & Extension program, assisting with informed consistent messaging of findings.
Grower nights and industry presentations	Presentations to growers and agronomists at; 4 Agribusiness convened grower nights in South Australia; Onions Australia Conference in South Australia; Onions Australia grower night in Western Australia.	Participation in these well attended regionally held industry led events ensured growers in South Australia were kept up to date with key project findings throughout the project and could follow up specific aspects with the project researcher. Events reached greater than 80% of South Australian growers. The event in Western Australia provided awareness of the project, as well as increasing the researcher's knowledge on onion diseases impacting production in WA.
Technical forums	Soilborne Disease Masterclass attended by vegetable growers including 7 participants from the onion industry.	Michael Rettke participated in delivery of Soilborne Disease Masterclass delivered by Soil Wealth   ICP in cooperation with Onion Communication & Extension program.
	National Root Crops Agronomy Meeting.	Fusarium basal rot technical update delivered as part of presentation on DNA diagnostic testing to technical meeting held by agribusiness for their agronomists.
Sample diagnosis	Growers and agronomists.	Growers were encouraged to submit samples suspected of having fusarium basal rot. This built grower awareness of the project and the provision of testing to confirm causes of bulb rots and other soilborne diseases informed growers with diagnosis to base management decisions on. This included other diseases such as nematodes, bacterial rots, pink root, along with <i>Fusarium</i> spp. This activity provided opportunities for interstate growers to engage with the project from Queensland, Western Australia, and Tasmania.
Pathogen DNA test	Growers, agronomists, and researchers.	qPCR test for <i>Fusarium oxysporum</i> f. sp. <i>cepae</i> , cause of fusarium basal rot. Service available for industry to test soils and plant tissue.
Articles in the Australian Grower	4 articles published in Australian Grower.	Articles published in the Australian vegetable industries magazine, distributed in both digital and published forms to onion growers and industry participants across Australia, with

		articles included in the specific section on onions in each issue. Refer to Appendix 3.
		Winter 2023 - Understanding and managing fusarium basal rot in onions.
		Spring 2023 - Crop monitoring to understand the drivers of fusarium basal rot development in onions.
		Winter 2024 - Harvest of onion trials in SA continue to shed light on detection and management of onion basal rot.
		Summer 2024 - Developing an integrated pest and disease management strategy for fusarium basal rot.
Literature review	Information targeted for researchers and agronomists.	Document provided a solid basis for undertaking research in the project and a technical reference which has been provided to agronomists on request. Uploaded to Hort Innovation portal.
International researcher visit	Foster engagement of Australian growers, agronomists, and researchers with international expert.	Supported visit by Prof Lindsey du Toit from Washington State University to foster engagement with the international research community relating to bulb rots of onions and facilitated knowledge transfer from an international researcher to Australian growers through industry and individual meetings. Industry meeting organized in collaboration with the Onion Communication & Extension program, facilitating high attendance of South Australian growers.
Other media	Onion growers and agronomists.	Webinar - Presentation on reducing the impact of fusarium basal rot on the Australian onion industry as part of Onion Disease Webinar hosted by the Onion Project.
	Targeting general community awareness.	Interviews with ABC Country hour and ABC Radio Riverland/South East rural report promoted the projects work and general awareness of research and development to the broader Australian community.

#### **Outcomes**

Project VN20006 addressed Outcome 2 of the Onion Strategic Investment Plan - "The Australian onion industry has increased profitability, efficiency and sustainability through innovative Research and Development and sustainable Best Management Practice". Project activities have indirectly contributed to Outcome 3 – "Improved capability and an innovative culture in the Australian onion industry maximises investments in productivity and demand".

In the medium to long term, adopting the knowledge, management strategies, and controls developed in this project for use in an Integrated Pest and Disease Management (IPDM) strategy - based on a deeper understanding of the epidemiology of fusarium basal rot - will contribute to the economic and environmental sustainability of Australian onion production systems.

A summary of project outcomes at time of project completion is provided in Table 2.

Table 2: Summary of project outcome at the time of project completion.

Outcome	Alignment to fund outcome, strategy and KPI	Description	Evidence
Increased knowledge and awareness of fusarium basal rot management by industry stakeholders.	Outcome 2  Develop and optimise fit-for-purpose pest, weed and disease management strategies during crop growth and include postharvest quality risks.  KPI: Increase in adoption of integrated pest and disease management (IPDM) strategies and decrease in crop loss from key weeds, insect pests and diseases.	Grower guide on fusarium basal rot produced. End of project workshop delivered to growers and agronomists. Presentations delivered to growers and agronomists in South Australia, Western Australia and Queensland. Articles in Onions Australia and Australian Grower magazines.	Growers in consultation with agronomists are reviewing their crop nutrition programs in response to project findings on bulb nitrogen levels. There has been increased use of bulb nitrogen testing to inform future practice and assess onion bulb quality at harvest.  Project leader has been regularly and repeatedly invited to present at industry and agribusiness led grower information events, confirming grower interest and value of the project findings being delivered.  Greater than 80% of growers in South Australia attended one or more of the presentations, most attending multiple events.
Increased understanding of cultural, biological, bio- stimulant and chemical options in the management of fusarium basal rot through new research.	Outcome 2  Develop and optimise fit-for-purpose pest, weed and disease management strategies during crop growth and include postharvest quality risks.  KPI: Increase in adoption of integrated pest and	Crop monitoring undertaken in 20 plantings.  Twelve replicated field trials completed investigating factors including soil moisture variation, simulated high rainfall events, nitrogen application, nurse crops	Growers in South Australia aware that soil moisture, soil inoculum level, bulb nitrogen level, salinity been identified as factors associated with fusarium basal rot disease incidence.  Growers aware that plants can be infected early by Fusarium oxysporum f. sp. cepae (Foc), so may need to implement control

	disease management (IPDM) strategies and decrease in crop loss from key weeds, insect pests and diseases.  KPI: Data to support applications to the APVMA and the establishment of Maximum Residue Limits (MRLs).	and efficacy of control products and application strategies on fusarium basal rot.  Testing undertaken on potential weed and crop hosts.	measures at planting or early in crop.  Greater awareness in industry that Foc can infect wide range rotation crops and weed species. This evidenced by increased grower interest in investigating impacts of rotation crop choice on fusarium basal rot risk.  Products with efficacy on fusarium basal rot confirmed in field trials and data generated that supports process of progressing products to commercial availability.
Improved knowledge of pathogens causing fusarium basal rot in Australia.	Outcome 2  Develop and optimise fit-for-purpose pest, weed and disease management strategies during crop growth and include postharvest quality risks.  KPI: Increase in adoption of integrated pest and disease management (IPDM) strategies and decrease in crop loss from key weeds, insect pests and diseases.	Foc confirmed as main Fusarium sp. present in bulbs with typical symptoms of fusarium basal rot.  Pathogen confirmed by Next Generation Sequencing in combination with specific qPCR test for Foc, along with the sequencing of isolates cultured from infected bulbs.	Growers and agronomists aware that Foc is the main pathogen causing fusarium basal rot in Australia, with this message included in majority of project extension and communication materials.
Increased diagnostic capacity and ability to assess inoculum level of pathogens that cause fusarium basal rot in soil, root and bulb samples.	Outcome 2  Develop and optimise fit-for-purpose pest, weed and disease management strategies during crop growth and include postharvest quality risks.  KPI: Increase in adoption of integrated pest and disease management (IPDM) strategies and decrease in crop loss from key weeds, insect pests and diseases.	DNA qPCR test developed for Foc for routine testing of samples.  Specificity of test confirmed.  Strong relationships confirmed between Foc plant infection level and incidence of fusarium basal rot in surveyed commercial crops.  Capability to assess risk of fusarium basal rot developing in storage based on measuring Foc	Test added to SARDI list of PREDICTA Research tests.  Appreciation of the Foc testing capability developed in this project is evidenced by growers and agronomists continuing to submit their own samples for testing. Use of the Foc test has assisted with diagnosis on a range of plant and bulb symptoms received, with diagnosis informing the growers future management strategies.  Growers are accessing the Hort Veg soil testing service via agronomists. Testing which includes the Foc test is being used to assist in pre-plant assessment of

		infection in root plus basal plate samples at harvest confirmed.	disease risk to inform management strategies. Limitations of test sensitivity in soil samples for detecting Foc are conveyed to agronomists. When Foc detected, confirms high level inoculum present.
Enhanced collaboration with international fusarium basal rot researchers.	Outcome 2  Develop and optimise fit-for-purpose pest, weed and disease management strategies during crop growth and include postharvest quality risks.  KPI: Increase in adoption of integrated pest and disease management (IPDM) strategies and decrease in crop loss from key weeds, insect pests and diseases.  Outcome 3  Deliver extension and communication capability to create positive change in the areas of biosecurity, soil and plant health, meeting consumer expectations and trade development.	Supported visit by Lindsey du Toit from Washington State University. Findings from research on onion diseases in North America that relevant to Australian production extended to Australian growers by international expert though industry event and individual discussions. Discussions with European researchers.	Increased knowledge on findings of "Stop the Rot" project gained by Australian industry.  Growers' knowledge of production and disease management strategies in North America was expanded, which has prompted further discussion on aspects of their systems that could assist with disease management in Australia.  Visit has fostered opportunity for future research cooperation with North American researchers on fusarium basal rot.  Awareness of products and control methods being evaluated in Europe that resulted in the inclusion of product that demonstrated efficacy on fusarium basal rot in this projects trials.
Increased knowledge of soilborne pathogens and their interactions impacting onion production	Outcome 2  Develop and optimise fit-for-purpose pest, weed and disease management strategies during crop growth and include postharvest quality risks.  KPI: Increase in adoption of integrated pest and disease management (IPDM) strategies and decrease in crop loss from key weeds, insect pests and diseases.	Data generated on associations between levels of Foc and other soilborne pathogens of onions.	Data generated has assisted growers understanding of which pathogens are impacting onion productivity in paddocks where fusarium basal rot is the dominant disease issue, including that incidence of high levels pink root were not linked with a higher incidence of fusarium basal rot, with the reverse more likely.  New awareness of the widespread infection of onion roots by Macrophomina phaseolina that could be impacting productivity requires further investigation.

#### **Monitoring and evaluation**

Hort Innovation project VN20006 successfully achieved its objectives in relation to addressing the increasing prevalence of fusarium basal rot in South Australia and other warm climate production areas of Australia. The project developed foundational knowledge for implementation of best practice integrated pest and disease management (IPDM) of fusarium basal rot, which has been incorporated in a 15-page guide for growers and agronomists, providing strategies for managing FBR in an integrated approach. This was achieved by improving our understanding of the pathogen and the drivers of disease development, along with evaluating cultural practices and products to understand impact of applying these strategies and controls.

Key research and findings that underpin the IPDM strategy and address the project KPI's were the successful:

- identification of *Fusarium oxysporum* f. sp. *cepae* (Foc) as the primary causal agent of fusarium basal rot in Australia
- development of a qPCR diagnostic test specific to this pathogen that can be used to monitor infection and effectiveness of strategies.
- discovery of key drivers of disease in the field, such as high nitrogen levels in bulbs being associated with increased disease incidence.
- monitoring of other pathogens of onions to determine if contributing to increased risk, such as high levels of pink root not being associated with high incidence of fusarium basal rot.
- trials that evaluated impact of production conditions and cultural management.
- identification of products with efficacy to control the disease.

A range of strategies were used to engage with industry in the project. High industry awareness of the project and its findings was attained by the project leader delivering project updates at grower meetings organized by the industry and agribusiness, which had high participation by South Australian growers. At the beginning of the project direct interactions were fostered by offering diagnosis of bulb rot samples to growers and agronomists. This built grower awareness of the project, but also the diagnosis of causes of bulb rots and other soilborne diseases informed growers with critical information to base management decisions on. A high level of engagement occurred with growers who hosted monitoring sites or specific trials. Communication has occurred with service providers and agribusiness representatives in relation to management practices and products. Face to face engagement was assisted by publishing of four articles in the Australian Grower, with links to the fusarium basal rot guide. A fusarium basal rot workshop was conducted in South Australia towards the end of the project in collaboration with the Onion Communication & Extension program. Growers and agronomists at the workshop were highly engaged with the topics presented, evidenced by the level of questions and interactions that occurred. Discussions with growers and agronomists confirms that findings of this project are being incorporated into their thinking and practices. An example is the review of nutrition programs and adoption of nutrient analysis testing of bulb samples at harvest.

This project hosted a visit by Professor Lindsey du Toit, along with meetings with Professor Daniel Drost from the USA who visited as part of the Onion Communication & Extension program. The awareness of Australian research on fusarium basal rot built through these visits is likely to result in future interactions and cooperation with international research programs on this important disease, that is causing issues in many parts of the world.

This project was the first major research initiative in Australia on fusarium basal rot of onions. Significant progress has been made in research and development, but further work is needed to effectively apply some of the identified practices. This includes broader scale evaluation of rotation and soil management strategies, refinement nutrition management strategies, and advancing the availability of identified chemical controls. The experience gained in evaluating management practices, along with critical knowledge about the pathogen and the disease, in combination with monitoring tools developed in this project places the industry in a strong position to achieve further improvements in managing fusarium basal rot.

#### Recommendations

#### Applications of project findings for growers and agronomists

- Assess risk of fusarium basal rot (and other soilborne diseases) prior to planting, based on past history, presence
  of inoculum in soil, timing of planting and environment. Manage risk accordingly. Refer to Fusarium basal rot
  guide.
- Review crop nutrition practices in relation to the association found between high bulb nitrogen level and increased incidence of fusarium basal rot. Implement best practice nutrition management.
- Utilise tissue testing of bulb nitrogen at harvest to review management practices and inform future crop nutrition strategies and assist with assessment of storage quality.
- Consider if improvements to drainage and seepage management in paddocks is justified, for example land plane and/or installation of surface and subsurface drainage infrastructure. Height requirement of raised beds.
- Implement best practice irrigation management. Risk of fusarium basal rot is lowest when optimum soil moisture maintained. Prolonged conditions that are too wet or too dry increase the risk of fusarium basal rot. Use of variable rate irrigation may assist in achieving more uniform soil moisture conditions across the paddock.
- Monitor salt levels in irrigation water, soil, and soil water to refine irrigation practices to manage excess buildup
  of soil salinity.
- Review crops grown in rotation with onions for their potential to host pathogens of onions and if unknown, use developed monitoring tools to investigate.
- Consider using practices that support populations of beneficial organisms such as Mycorrhizal fungi and *Trichoderma* spp.
- Consider using the developed in-crop infection monitoring tools to assess crop health, yield potential and risk of fusarium basal rot developing to inform management strategies.
- Consider using the developed harvest bulb infection monitoring tools to assess risk fusarium basal rot risk
  developing in storage (where infection suspected but not evident by pre and or harvest checks), informing crop
  storage and marketing.

#### Focus for future extension and communication

Key messages utilising project findings to highlight strategies that reduce risk of fusarium basal rot.

- Soil moisture Importance of maintaining optimum soil moisture levels, avoiding both too dry and too wet.
- Balanced nutrition Avoiding excess nitrogen and managing bulb nitrogen status.
- Protect the basal plate Maintain uniform growth to reduce infection risk.
- Know the risk Manage inoculum.

Information must consider impact on yield and quality of onions, as well as risk of disease.

#### Future research and development

Technologies and management practices are available and being used to implement certain components of the identified integrated pest and disease management (IPDM) strategy for basal rot, for example irrigation management. For other

components, further work is needed to effectively apply some of the identified practices. Areas where further refinement of project findings recommended are:

- Use of rotation, cover crop and soil amendment options to influence inoculum decline and build disease resilience to fusarium basal rot.
- Nutrition management strategies that lower bulb nitrogen levels to reduce fusarium basal rot risk, while not compromising onion yield and quality.
- Optimisation of use patterns of bio-stimulant compounds and biological products to maximise their efficacy against soilborne diseases of onion.

Implementation of strategies needs to be evaluated in various production systems and environmental conditions.

Efficacy trials in this project support undertaking further activity to advance commercial availability of some products.

Associated findings that warrant further investigation:

- Identifying causal organisms of bacterial bulb rots and understanding their occurrence. Two of the monitored sites had substantive levels of bacterial rots, with importance of bacterial rot confirmed by discussions with growers of onions in other regions.
- Identifying causal organism/s of symptom observed in Redwing variety onions that caused severe infection and breakdown of the basal plate, as well as root loss that generally did not extend beyond the basal plate. This symptom was not associated with Foc.
- *Macrophomina phaseolina* that was found to be infecting roots of onions warrants further investigation to determine if this widespread pathogen of other crops is also impacting onion productivity.

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#### **Intellectual property**

Intellectual property was generated in this project relating to monitoring of crops, controlled environment and field trials, including on the efficacy of products for control of fusarium basal rot. This information has been made publicly available for the benefit of the Australian onion industry.

Intellectual property was generated in this project relating to the development of a DNA test for *Fusarium oxysporum* f. sp. *cepae*. The primer and probe details are available to requesting third parties under MTA.

#### **Acknowledgements**

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The discussion and advice of agronomists and service providers throughout the project on technical matters was greatly appreciated, along with their help to provide trial materials and collect onion samples for testing.

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## **Appendix 1 – Supporting information**

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#### Causal agents of fusarium basal rot

#### Background

Fusarium oxysporum f. sp. cepae is suspected as the main causal agent of fusarium basal rot in Australia. Other species, including F. proliferatum and F. redolens have also been associated with fusarium basal rot overseas (Le et al. 2021). Both of these species are known to be present in South Australia (APPD, Hollaway 2021). Effectiveness of varietal resistance, crop rotations and management practices vary depending on the Fusarium species involved, so confirming the causal agent/s is important.

#### Methodology

Onion bulbs or plants with symptoms resembling those described as fusarium basal rot were received from eight growers across South Australia and Queensland, collected from paddocks that were exhibiting symptoms or with a history of fusarium basal rot. Samples with symptoms that could be caused by *Fusarium* spp. for inclusion in testing were received from another eight growers, including samples from Western Australia and Tasmania.

Bulbs were individually examined, photographed, and depending on symptoms and condition dissected to obtain samples of the basal plate, leaf scale tissue and sometimes roots from individual bulbs; samples were then dried at 40°C for DNA testing.

DNA was extracted from 94 tissue samples and tested by Next Generation Sequencing (NGS) using SARDI's *Fusarium*-specific primer set, as well as by quantitative PCR (qPCR) using the *Fusarium oxysporum* f. sp. *cepae* DNA test developed in this project. Tissue samples included in NGS testing comprised of 44 basal plate, 25 internal bulb leaf scale, 7 outer skin leaf scale and 19 root system samples.

Nineteen individual bulbs with symptoms typical of fusarium basal rot were included in testing. Additionally, samples of mature and semi mature bulbs from South Australia with atypical breakdown of the basal plate to that caused by fusarium basal rot (7 samples), atypical rotting of the bulb to that caused by fusarium basal rot (7 samples), pinking and or purpling of skins (6 samples) and healthy bulbs (4 samples) were included in NGS testing. Onions from Western Australia (4 samples) and Tasmania (2 samples) submitted as potentially having fusarium basal rot were included in NGS testing.

Root systems of 16 bulb samples were included in NGS testing (5 with typical fusarium basal rot symptoms, 8 with atypical symptoms and 3 healthy bulbs) along with root systems from young plants (3 composite samples) at the 3-4 leaf stage from an area with a history of fusarium basal rot.

Fusarium cultures were isolated from bulbs with a range of symptom types for use in other aspects of the project.

#### Results and discussion

Fusarium oxysporum f. sp. cepae was the predominant species found associated with fusarium basal rot of onion bulbs tested, Table 3.

qPCR testing using the DNA assay developed in this project detected high levels of *Fusarium oxysporum* f. sp. cepae in the basal plate and/or bulb leaf scale tissue of 19 bulbs with typical fusarium basal rot symptoms. Next generation sequencing (NGS) indicated *F. oxysporum* as the dominant *Fusarium* species in these samples. Other *Fusarium* species including *F. solani, F. equiseti, F. falciforme, F. acuminatum, F. brachygibbosum* and *F. proliferatum* were detected but less frequently and at lower levels than *Fusarium oxysporum*, especially in bulb leaf scale tissue.

Throughout the project the qPCR test for *F. oxysporum* f. sp. *cepae* consistently detected high levels of DNA in the infected tissue of bulbs with typical symptoms of fusarium basal rot sourced from growers, monitoring sites and trials.

Onion bulbs with symptoms other than fusarium basal rot were submitted by growers and agronomists. These symptoms that included bulb rots, root rots and basal plate breakdown were not consistent with those caused by *F. oxysporum* f. sp.

cepae, though testing did detect the pathogen at low levels in the tissue of some samples, Table 3.

Symptoms characterised by rotting of the five outer fleshy bulb leaf scales and a slightly discoloured but sound basal plate on a bulb after ambient storage was associated with *F. proliferatum*. This was determined by isolation and sequencing of cultures from the infected internal bulb leaf scale tissue, and a high reading for *F. proliferatum* detected by NGS testing. DNA of *F. oxysporum* f. sp. *cepae* was detected by qPCR at a low level in the basal plate of this bulb; *F. equiseti* sequence was also detected by NGS.

Other atypical bulb rots submitted were associated with fungal pathogens *Botrytis* sp., *Aspergillus* sp., and *Penicillium* sp. or bacterial rots, confirmed by microscopic examination for bacterial streaming. Isolation and sequencing of bacterial cultures from the submitted samples identified possible causal agents as *Burkholderia* sp. and *Enterobacter* sp..

Bulbs of the variety Redwing sampled at the early and mid bulbing stages from two growers exhibited a severe infection and breakdown of the basal plate, as well as root loss. Symptoms generally did not extend beyond the basal plate. Severe pink root was observed at both sites and confirmed by qPCR testing for *Setophoma terrestris*. This symptom was not associated with *F. oxysporum* f. sp. *cepae*, though other *Fusarium* spp. including *F. solani* may have been involved. This symptom, which is outside the scope of this project requires further investigation.

Six samples of white onions that had pink and/or purple discolouration of the outer skin were tested. *F. oxysporum* was detected in all samples by NGS testing. DNA of Foc was only detected by qPCR in two samples at low levels indicating predominance of other forma speciale of *F. oxysporum* and or saprophytic species. *F. proliferatum* was detected by NGS testing in three outer skin samples that had a distinctive purple blotch, but not in the other three samples with pink discolouration of the skin, but no distinctive purple blotch.

Testing of 19 samples of root systems by NGS detected *F. oxysporum* (15 samples), *F. solani* (10 samples), *F. equiseti* (3 samples), *F. redolens* (2 samples), *F. acuminatum* (2 samples), *F. avenaceum* (1 sample) and *F. pseudograminearum* (1 sample). *Fusarium* oxysporum was detected by NGS testing in four of five samples from bulbs with typical symptoms of fusarium basal rot, and in the roots of all five samples using *F. oxysporum* f. sp. *cepae* test developed in this project. Importance of root infection by these *Fusarium* spp. on productivity of onions was not assessed.

#### **Conclusions**

The pathogen Fusarium oxysporum f.sp. cepae is associated with fusarium basal rot disease symptoms observed in South Australia and Queensland. Review of next generation sequencing (NGS) data and results of specific qPCR testing for F. oxysporum f. sp. cepae on onion samples with fusarium basal rot symptoms did not identify other Fusarium spp. that are likely to play a significant role in causing fusarium basal rot.

Fusarium proliferatum was associated with purple blotch on the skins of white onions and bulb rot of ambient stored onions.

Fusarium oxysporum f.sp. cepae and other forma speciale of F. oxysporum, along with other Fusarium species including F. solani were found to commonly infect the roots of onions tested and may be impacting onion productivity.

Table 3: Fusarium species detected by next generation sequencing or specific qPCR test for *Fusarium oxysporum* f. sp. cepae in tissue samples of onions with fusarium basal rot and other symptoms potentially caused by *Fusarium* spp..

Description of symptom	Tissue type tested	Number samples tested	Fusarium species detected by Next Generation Sequencing with the number of detections		ysporum f. sp. cepae c DNA test)
			shown in brackets	Number of detections	Range of detections (log Kcopies DNA)
Individual bulbs with symptoms typical of fusarium basal rot	Basal plate	17	Fusarium oxysporum (17) Fusarium solani (12) Fusarium equiseti (3) Fusarium acuminatum (1) Fusarium brachygibbosum (1) Fusarium falciforme (1)	17	3.8 – 6.3
	Internal leaf scale	18	Fusarium oxysporum (18) Fusarium solani (10) Fusarium falciforme (2) Fusarium proliferatum (1)	18	3.2 – 6.2
Individual bulbs with atypical rot of the internal bulb leaf scales	Basal plate	7	Fusarium oxysporum (6) Fusarium solani (4) Fusarium proliferatum (1)	3	0 – 2.7
	Internal leaf scale	7	Fusarium oxysporum (5) Fusarium proliferatum (1)	4	0 – 2.7
Individual bulbs with atypical breakdown of the basal plate (Redwing variety)	Basal plate	7	Fusarium oxysporum (5) Fusarium solani (4)	1	0 – 2.4
Pinking of leaf scales (white onions)	Outer bulb skins	3	Fusarium oxysporum (4)	2	0-1.1
Salmon blotch of leaf scales (white onions)	Outer leaf scale skins	3	Fusarium oxysporum (3) Fusarium proliferatum (3) Fusarium solani (1)	0	0-0
Western Australian samples	Basal plate	6	Fusarium oxysporum (5) Fusarium solani (3) Fusarium equiseti (1)	2	0 – 1.8
Tasmanian samples	Basal plate	2	Fusarium oxysporum (2)	1	0-1.5
Healthy bulbs	Basal plate	4	Fusarium oxysporum (1)	0	0-0

#### **Diagnostic test development**

#### **Background**

The pathogen Fusarium oxysporum f. sp. cepae has been identified as the main causal agent of fusarium basal rot in Australia. Development of a qPCR assay was identified as an important diagnostic tool for industry, as well as for use in this project to aid understanding of the biology of the pathogen.

#### Methodology

A TaqMan MGB qPCR assay for the specific detection and quantification of *Fusarium oxysporum* f. sp. *cepae* was designed based on publicly available sequence data and sequencing of cultures isolated from onions with fusarium basal rot symptoms in this project. The test is modified from a published assay by Sasaki et al. (2015) targeting the identified virulence related 'Secreted In Xylem' SIX3 gene. The primer and probe details are available to requesting third parties under MTA.

The assay was tested for specificity using an extensive collection of DNA from the target and closely related species, including 21 distinct forma speciale of *Fusarium oxysporum* and isolates of *F. equiseti, F. proliferatum, F. solani* and *F. subglutinans*. The *F. oxysporum* f. sp. *cepae* assay efficiently detected pure DNA from the target and did not detect any of the other *F. oxysporum* forma speciale nor any other species assessed, indicating the assay is specific. Calibration standard indicated the test is linear over seven orders of magnitude and has an efficiency of 97.5%. The test was incorporated into SARDI's PREDICTA delivery platform to enable routine assessment of samples.

#### Results and discussion

To confirm the specificity observed with DNA from pure cultures, the *F. oxysporum* f. sp. *cepae* assay was used to assess field samples. Samples of onion tissue included internal bulb leaf scales, basal plate tissue and roots of infected and healthy bulbs. Results of tissue testing aligned with the symptoms observed, with levels ranging from below detection to 6.3 log kDNA copies/g sample for *F. oxysporum* f. sp. *cepae*. Results of testing 500 g soil samples collected from paddocks after the harvest of infected crops indicates the test is not sensitive enough to detect low levels of inoculum in soil, with levels ranging from below detection to 1.5 log kDNA copies/g sample for *F. oxysporum* f. sp. *cepae*.

#### **Conclusions**

A qPCR test for Fusarium oxysporum f. sp. cepae has been developed and is available for industry and researchers to use.

Sasaki, K., Nakahara, K., Shigyo, M., Tanaka, S., and Ito, S. (2015) Detection and quantification of onion isolates of *Fusarium oxysporum* f. sp. *cepae* in onion plant. Journal of General Plant Pathology 81: 232-6.

#### Monitoring of 20 crops to understand drivers of disease

#### **Background**

Understanding when and why *Fusarium oxysporum* f. sp. *cepae* (Foc) infection occurs in onion crops is important to the development and implementation of control strategies. Monitoring of crops was undertaken to gather data on drivers of disease development in 20 crops.

#### Methodology

Monitoring of 12 crops in the 2022 planting season and eight crops in the 2023 planting season was undertaken on mid to late season brown (4 OP lines, 6 hybrid lines) and red onion (3 hybrid) varieties in South Australia. All crops were irrigated by centre pivot, with four crops located in the Murray Mallee region and 16 crops located in the South-east region. In each site crop monitoring spots were established after planting and before the 2-leaf stage. Six monitoring spots, each three beds wide and 30-40 m long, were spaced along the length of the planting in a single variety. In some crops, up to 4 additional spots were located on a second planting line to monitor different varieties or management practices. Monitoring spots within a crop were grouped into 2-4 zones based on differences in soil type and topography, or variety or management practice in the cases where additional spots monitored. A composite of 30 soil cores was collected to a depth of 15 cm for pathogen DNA testing (SARDI Hort Veg panel) from each zone.

At selected monitoring spots in the 2022 planting season, 10-30 plants were sampled at the 2-4 leaf and 5-7 leaf stages for visual assessment of roots and the pathogen DNA testing of composite root plus basal plate samples. In the 2023 planting season 100 plants were sampled from selected monitoring spots at each site at the 2-4 leaf, followed by 25-50 plants at the 5-7 leaf stage for pathogen DNA testing of composite root plus basal plate samples.

Prior to the growers expected harvest date, onions were hand-picked from at least five locations within each spot, each two rows by 1 m in length, to obtain 100 bulbs for assessment. When bulbs from monitoring spots were to be assessed both at harvest and after three months storage in ambient conditions, at least 10 locations within each spot, each 2 rows by 1 m in length were harvested. Measurements included number and weight of bulbs, incidence and severity of fusarium basal rot, incidence of pink root and pathogen DNA testing of root plus basal plate samples. Pink root on each bulb was only counted if visual symptoms were easily observed on roots. Notes were made on incidence of insect damage, splitting, cracking, doubles, bolters along with presence of other bulbs rots.

Visual assessment of bulbs for fusarium basal rot was done by cutting a sliver of the basal plate off prior to assessment, and when infection was observed making subsequent cuts to determine if symptoms progressed into the bulb leaf scales. Rating scale used for visual assessment of bulbs for fusarium basal rot was 0 = Healthy, 1 = small dark spots in basal plate, 2 = slight browning or large dark spots in basal plate, 3 = infection of basal plate evident, not rotted, 4 = basal plate rotted, not spread to bulb leaf scales, 5 = basal plate rotted and extends into bulb leaf scales, 6 = breakdown of the bulb. Ratings 1 and 2 include symptoms of many potential injuries, pathogens and disorders including possible infection by Foc. Rating 3 has typical symptoms of fusarium infection that likely to develop into bulb rot if held in ambient storage. Ratings 4 to 6 are typical fusarium basal rot symptoms that have made bulbs unmarketable, and the total incidence of bulbs in these categories is the parameter reported for most trials in this report. The basal plate rating index was calculated using the formula: [1\*(number of bulbs in #1 rating category) + 2\*(number of #2 rating) + 3\*(number of #3 rating) + 4\*(number of #4 rating) + 5\*(number of #5 and #6 rating)] / (number of bulbs assessed\*5).

Nutrient testing (Eurofins PT2 analysis) was conducted on the composite bulb tissue freeze dried from 15 mm wide midsections of 15 healthy bulbs of average size for the sample (outer skin removed) from selected monitoring areas in the 2022 and 2023 planting seasons.

#### Fusarium basal rot - Incidence and impact

Incidence of fusarium basal rot (ratings 4 to 6) at monitoring spots ranged from nil to 55%. Incidence was highly correlated with the concentration of Foc detected in the root and basal plate tissue of harvested bulbs, Figure 1. This data indicates that Foc is the main causal agent of the fusarium basal rot observed.

The high incidence in some plantings highlights the substantive impact this disease can have on productivity. Rotted bulbs are unmarketable, and even when present at low incidence, add significant cost to grading and handling to facilitate marketing of the rest of the crop.

Data indicates that infection resulted in additional loss of yield to that caused by discarding of rotted bulbs. At high levels of infection, total yield was as much as 25% lower, before rotted bulbs were removed, Figure 2. When rotted bulbs are discarded this loss sometimes exceeded 50%, Figure 3. At high infection levels of Foc, data indicates that yield loss was a result of both reduced average bulb weight and reduced plant density, Figure 4. In less severely infected paddocks average bulb weight appeared to be maintained. At sites where Foc infection was low and plant density higher, the higher plant density appears to have contributed to reduced average bulb weight.

Not all infected bulbs are rotted or have obvious symptoms of fusarium basal rot at the time of harvest. These latent infections of the basal plate can result in further loss in storage and issues emerging during marketing and distribution.

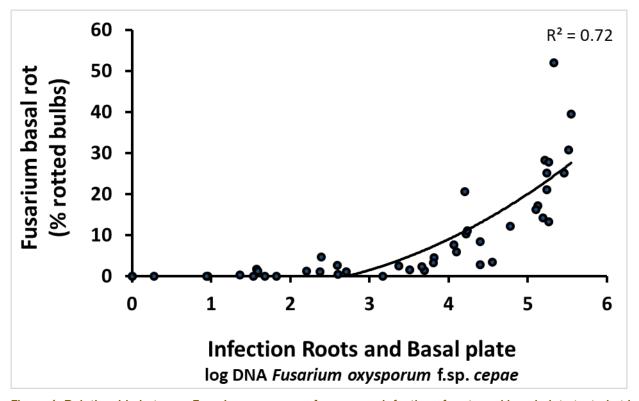


Figure 1: Relationship between *Fusarium oxysporum* f. sp. cepae infection of roots and basal plate tested at harvest with percentage of bulbs rotted by *Fusarium oxysporum* f. sp. cepae. Data from production zones located within onion plantings assessed over the 2022/23 and 2023/24 seasons.

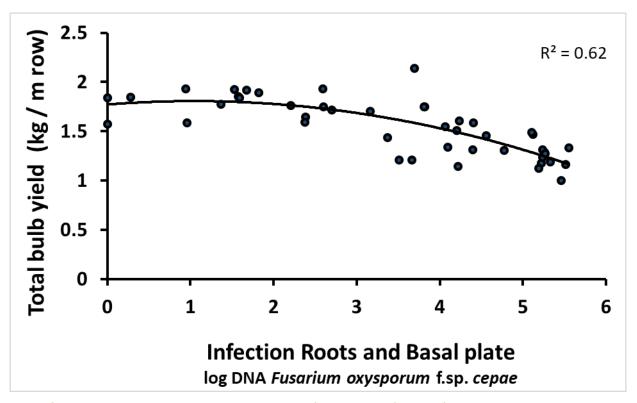


Figure 2: Relationship between *Fusarium oxysporum* f. sp. cepae infection of roots and basal plate tested at harvest with total bulb yield (Yield includes weight of rotted bulbs). Data from production zones located within onion plantings assessed over the 2022/23 and 2023/24 seasons.

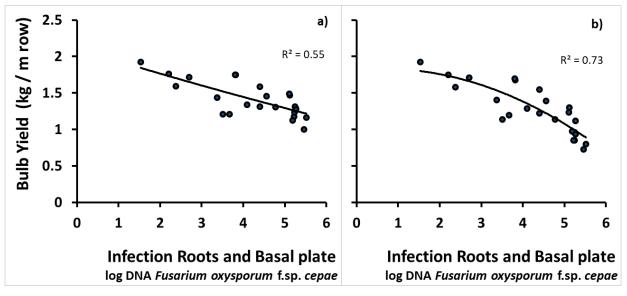


Figure 3: Relationship between *Fusarium oxysporum* f. sp. *cepae* infection of roots and basal plate tested at harvest with a) total yield and b) saleable bulbs (rotted bulbs discarded). Data from production zones located within onion plantings assessed in the 2023/24 season.

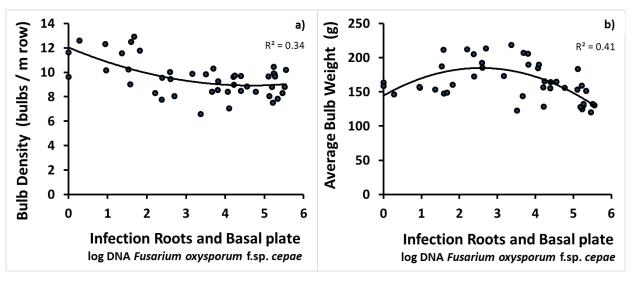


Figure 4: Relationship between *Fusarium oxysporum* f. sp. cepae infection of roots and basal plate tested at harvest with a) bulb density and b) average bulb weight. Data from production zones located within onion plantings assessed in the 2022/23 and 2023/24 seasons.

#### Timing of infection and disease development

Individual plants were sampled and tested at the 2-4 leaf and 5-7 leaf stages to monitor development of fusarium basal rot in crops from selected monitoring sites. DNA testing of the roots and basal plates confirmed Foc infection was present at the 2-4 leaf stage in most, but not all crops that developed fusarium basal rot by harvest, Figure 5. By the 5-7 leaf stage infection was detected in all but one of the crops that developed fusarium basal rot at harvest. Only a small number of plants sampled had visible symptoms indicating possible Foc infection. Many of the samples that Foc was detected in at the 2-4 and 5-7 leaf stages had no obvious symptoms. DNA testing of composite root and basal plate samples could be used to indicate crop infection and identify crops that are likely to develop a high incidence of fusarium basal rot at harvest, before symptoms would be detected by visual crop inspection. In-crop DNA testing at 5-7 leaf stage also provided an indication of at which sites yield reduction was going to occur due to fusarium basal rot, Table 6.

In addition to testing of composite samples from monitoring sites, at marked spots in three separate plantings, root and basal plate samples from individual plants were DNA tested for Foc infection, Table 4. This was undertaken at stages ranging from 8 leaf to late bulbing. Testing indicated that approximately twice as many plants sampled in-crop were infected with Foc as developed fusarium basal rot by harvest. This suggests in the sites tested, infection of the bulbs that were rotted at harvest had most likely already occurred prior to the time of in-crop sampling. Approximately half of infected bulbs had not developed bulb rot by the time of harvest, suggesting latent infections may pose risk of further losses in storage and handling.

DNA testing of two soil samples taken at or before the 2 leaf stage did not provide a reliable indication of the risk of fusarium basal rot developing in that crop, Figure 5: Relationship between the level of *Fusarium oxysporum* f. sp. *cepae* in a) soil prior to the 2 leaf stage, or root and basal plate of plants at b) 2-4 leaf stage, c) 5-7 leaf stage, d) harvest with percentage of bulbs rotted by *Fusarium oxysporum* f. sp. *cepae*. Data from onion plantings assessed over the 2022/23 and 2023/24 seasons.. Some monitored areas where Foc was below the level of test detection in soil developed high (>10%) levels of disease. In monitored areas where Foc was detected in soil, this was linked to a higher likelihood of disease. In the seven sites where soil testing detected Foc, six of these crops developed fusarium basal rot at an incidence of greater than 5%. The intensity, timing and strategy of soil sampling required to reliably assess disease risk prior to planting was not investigated.

In some crops, incidence of rotted bulbs caused by Foc does not become apparent till after a period of storage. Bulb rot can increase from low levels to unacceptable levels, for example at one site bulb rot increased from 0.6% at harvest to 8% after three months ambient storage. DNA testing of a composite sample of the roots and basal plates of 100 bulbs at

harvest was able to predict which samples would have fusarium basal rot develop during three months of ambient storage, even when observed incidence was low or nil at harvest, Figure 7. For these samples, DNA testing provided a better indication of fusarium basal rot developing in storage than observed rotted basal plates at harvest.

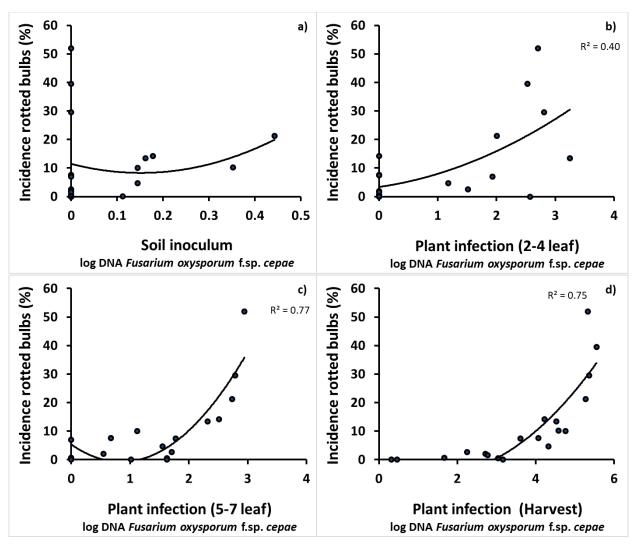


Figure 5: Relationship between the level of *Fusarium oxysporum* f. sp. cepae in a) soil prior to the 2 leaf stage, or root and basal plate of plants at b) 2-4 leaf stage, c) 5-7 leaf stage, d) harvest with percentage of bulbs rotted by *Fusarium oxysporum* f. sp. cepae. Data from onion plantings assessed over the 2022/23 and 2023/24 seasons.

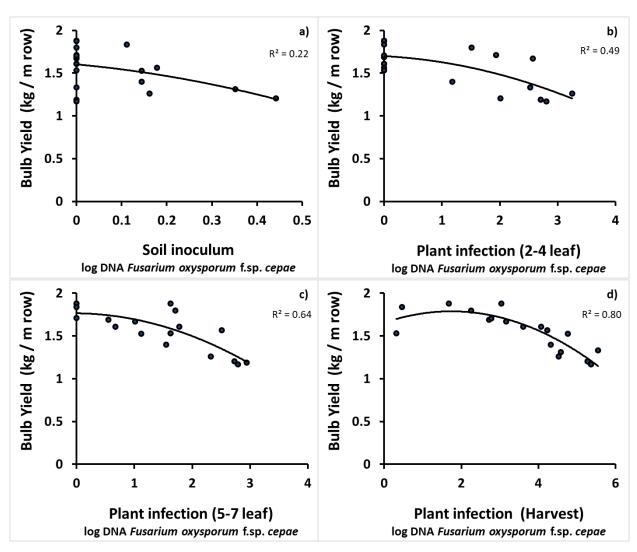


Figure 6: Relationship between the level of *Fusarium oxysporum* f. sp. cepae in a) soil prior to the 2 leaf stage, or root and basal plate of plants at b) 2-4 leaf stage, c) 5-7 leaf stage, d) harvest and the average total yield of bulbs Data from onion plantings assessed over the 2022/23 and 2023/24 seasons.

Table 4: Comparison of incidence of *Fusarium oxysporum* f. sp. cepae plant infection detected by qPCR during crop growth with the incidence of fusarium basal rot.

Site		In-crop as:	sessment			Harvest ass	essment
	Date assessed	Growth stage	Number plants tested	Incidence of plant infection (%)	Date assessed	Number bulbs assessed	Incidence rotted bulbs -Fusarium basal rot (%)
1	18 Dec 2023	9-10 leaf	40	5	8 Feb 2024	1871	3
2	15 Dec 2023	8-9 leaf	41	22	15 Feb 2024	1006	11
3	19 Jan 2024	Bulbing	45	44	1 Mar 2024	111	21

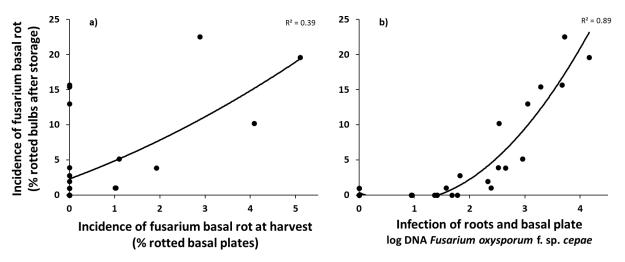


Figure 7: Comparison of visual assessment against DNA testing conducted at harvest to predict disease development after 3 months ambient storage for sites with less than 5% incidence fusarium basal rot at harvest.

#### Impact and interaction with other soilborne pathogens

In the paddocks monitored in this project, Foc was the dominant soilborne pathogen impacting productivity. This data may not be reflective of the broader industry, as most sites in this project were specifically selected to target monitoring of fusarium basal rot.

Other diseases that are known to impact onion productivity in South Australia, such as root lesion nematodes and pink root were not strongly associated with bulb yield at the monitored sites. High incidence of pink root symptoms on roots at harvest occurred in monitoring areas at a few sites. With exception of one site the populations of root lesion nematode (*Pratylenchus neglectus* and *Pratylenchus penetrans*) were not high enough to cause substantive yield loss at monitored sites, based on DNA testing of soil and root samples. *Sclerotium cepivorum*, cause of white rot was not detected at any of the sites monitored.

Based on observation of root symptoms and the reduced growth of large patches of onions at one particular planting, diagnostic testing confirmed that stubby root nematode, *Paratrichodorus* spp. was the causal agent. This nematode may been present at other sites, though obvious symptoms were not observed. Routine testing for stubby root nematode was not undertaken as part of site monitoring.

#### Setophoma terrestris

Pink root, caused by *Setophoma terrestris*, is a widespread disease of onions, with *S. terrestris* detected in root systems from all 20 monitored crops, Figure 8. No strong linkage was found between increasing concentration of *S. terrestris* in the soil prior to the 2 leaf stage, or concentration in root and basal plate samples taken at the 2-4 leaf, 5-7 leaf and harvest stages with higher incidence of rotted bulbs caused by Foc. This data indicates that a high incidence of fusarium basal rot can occur at sites with a relatively low level of *S. terrestris* inoculum and/or plant infection. In crops that were monitored, those with the highest levels of *S. terrestris* inoculum and/or plant infection were associated with crops that did not develop fusarium basal rot. These crops had high levels of visible pink root symptoms on the roots at harvest.

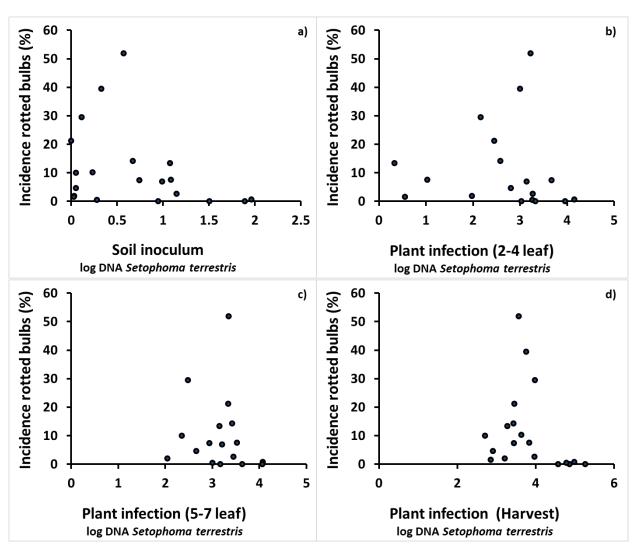


Figure 8: Relationship between the level of *Setophoma terrestris* in a) soil prior to the 2 leaf stage, or root and basal plate of plants at b) 2-4 leaf stage, c) 5-7 leaf stage, d) harvest with percentage of bulbs rotted by *Fusarium oxysporum* f. sp. cepae. Data from onion plantings assessed over the 2022/23 and 2023/24 seasons.

#### Macrophomina phaseolina

Macrophomina phaseolina, not recognised as a major pathogen of onions, was detected in root and basal plate samples taken at the 2-4 leaf stage from approximately three quarters of sites monitored, Figure 9. Increasing concentration of the *M. phaseolina* was associated with lower yield, including at a low yield site where Foc was not detected. Concentration of *M. phaseolina* DNA was not associated with the concentration of Foc in the roots and basal plate of onion plants sampled at the 2-4 leaf stage. Adding the log kDNA copies/g sample concentration of *M. phaseolina* to Foc improved the proportion of variation in yield explained by infection by Foc alone. This data suggests the impacts of *M. phaseolina* on onion productivity warrant further investigation to determine if this widespread pathogen of other crops is impacting onion productivity.

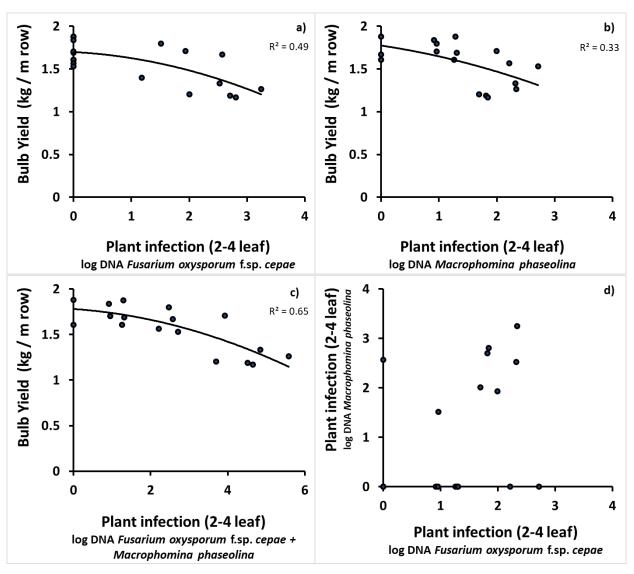


Figure 9: Relationship between level of plant infection at the 2-4 leaf stage of a) *Fusarium oxysporum* f. sp. cepae, b) *Macrophomina phaseolina*, c) combined infection level with average total yield of bulbs, along with d) relationship between level of infection of the two pathogens at the 2-4 leaf stage. Data from onion plantings assessed over the 2022/23 and 2023/24 seasons.

#### Bacterial bulb rots - field symptoms

Bulb rots caused by bacterial pathogens were observed at some sites. Field symptoms were characterised by a wet slippery rot of specific leaves of the plant, with infection of these leaves extending down into the bulb. Leaves were sometimes bleached. Rotting of plants was in a downwards direction from the source of infection on a leaf or in the neck of the bulb, as opposed to basal rot that progressed up from the basal plate of the bulb. Bacterial rots were most frequently observed in wet areas of pivots, along pivot wheel tracks and along spray tracks within crops.

At 18 of the 20 sites assessed the incidence of bacterial rots recorded at harvest ranged from nil to 2%. Two sites monitored had higher levels of bacterial rots, with 6% of bulbs affected by bacterial bulb rot in one area of one site and 20% recorded in a wet area at the other site. This data may not be reflective of the broader industry, as most sites in this project were specifically selected to target monitoring of fusarium basal rot.

When onions were stored at ambient temperature for assessment after three months, multiple pathogens were sometimes involved in disease development, including bacterial pathogens.

#### Association of disease with levels of beneficial fungi

#### Arbuscular Mycorrhiza Fungi (AMF groups)

As part of SARDI's suite of DNA tests, Arbuscular Mycorrhiza Fungi (AMF) testing is conducted for six groups. When root and basal plate samples were tested at harvest, there was a tendency for onions from sampling zones with higher levels of AMF to have lower incidence of fusarium basal rot, Figure 10.

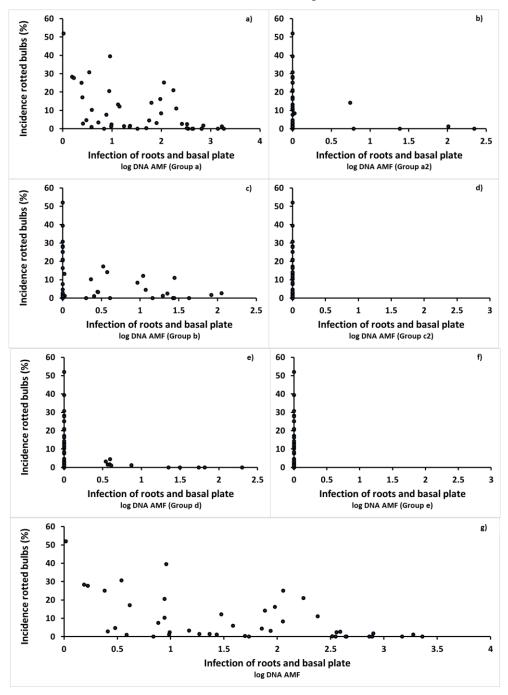


Figure 10: Relationship between the concentration of DNA in roots and basal plate sampled at harvest of Arbuscular Mycorrhiza Fungi in a) Group a, b) Group a2, c) Group b, d) Group c2, e) Group d, f) Group e, g) total six groups with percentage of bulbs rotted by *Fusarium oxysporum* f. sp. cepae. Data from production zones located within onion plantings assessed over the 2022/23 and 2023/24 seasons.

#### Trichoderma spp.

As part of SARDI's suite of DNA tests, *Trichoderma* spp. testing is conducted for two groups. When root and basal plate samples were tested at harvest, there was a tendency for onions from sampling zones with higher levels of *Trichoderma* spp. to have lower incidence of fusarium basal rot, Figure 11Figure 11: Relationship between the concentration of DNA in roots and basal plate sampled at harvest of *Trichoderma* spp. in a) Group A, b) Group B with percentage of bulbs rotted by *Fusarium oxysporum* f. sp. *cepae*. Data from production zones located within onion plantings assessed over the 2022/23 and 2023/24 seasons.

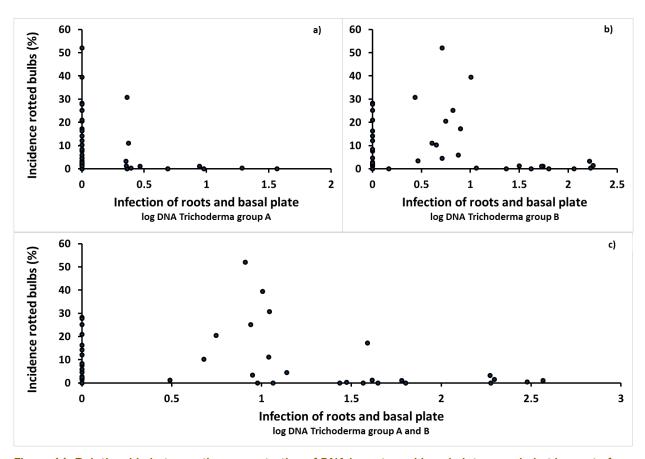


Figure 11: Relationship between the concentration of DNA in roots and basal plate sampled at harvest of *Trichoderma* spp. in a) Group A, b) Group B with percentage of bulbs rotted by *Fusarium oxysporum* f. sp. cepae. Data from production zones located within onion plantings assessed over the 2022/23 and 2023/24 seasons.

#### Association of disease with bulb nutrient levels

#### **Bulb nitrogen level**

Elevated total nitrogen level in tissue of bulbs sampled at harvest from monitoring sites was associated with increased incidence of rotted bulbs caused by Foc, Figure 12. Lower nitrogen level in the harvested bulbs was not associated with reduced yield, suggesting nitrogen level was not yield limiting within the range monitored in these commercial crops, Figure 13. It should be noted that the association of high bulb nitrogen levels with increased Foc infection would also contribute to reduced yield potential at these sites, which may be influencing the lack of a yield response. When losses to bulb rot are included, yield is substantially reduced at sites with elevated bulb nitrogen levels, Figure 14. This indicates there is scope to manage nitrogen to reduce risk of fusarium basal rot without jeopardising yield in sites where fusarium basal rot is expected to be an issue. When adjusting crop nutrition, potential impacts on other bulb quality attributes also need to be considered.

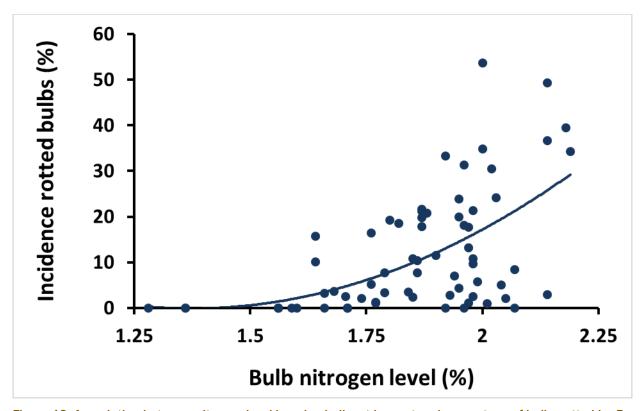


Figure 12: Association between nitrogen level in onion bulbs at harvest and percentage of bulbs rotted by *Fusarium* oxysporum f. sp. cepae. Data from monitoring spots located within onion plantings assessed over the 2022/23 and 2023/24 seasons.

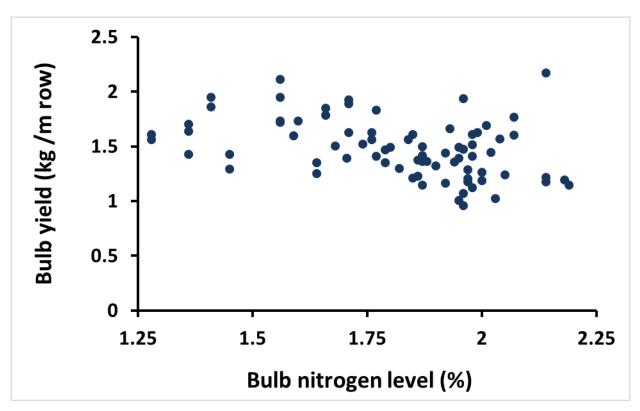


Figure 13: Relationship between nitrogen level in onion bulbs at harvest and total yield of bulbs. Data from monitoring spots located within onion plantings assessed over the 2022/23 and 2023/24 seasons.

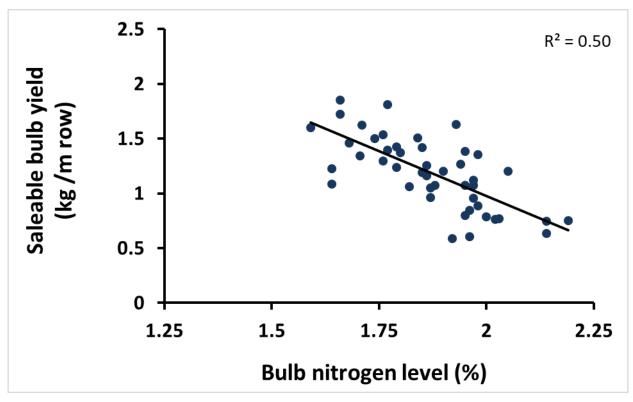


Figure 14: Relationship between nitrogen level in onion bulbs at harvest and saleable bulbs (rotted bulbs discarded). Data from monitoring spots located within onion plantings assessed in the 2023/24 season.

#### Bulb sodium and chloride levels

Elevated sodium and chloride level in tissue of bulbs sampled at harvest from monitoring sites were weakly associated with increased incidence of rotted bulbs caused by Foc, Figure 15. The association with basal plate rating was stronger than with incidence of rotted bulbs, Figure 16, indicating that the elevated levels may be linked to other damage to the basal plate. Symptoms other than those caused by Foc infection can contribute to the number of basal plates rated as 1, 2 and possibly 3. It is possible that saline conditions could contribute to damage to the basal plate and the symptoms observed.

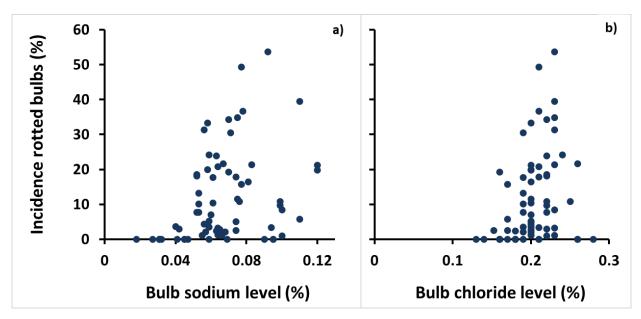


Figure 15: Association between a) sodium, b) chloride level in onion bulbs at harvest and percentage of bulbs rotted by *Fusarium oxysporum* f. sp. cepae. Data from monitoring spots located within onion plantings assessed over the 2022/23 and 2023/24 seasons.

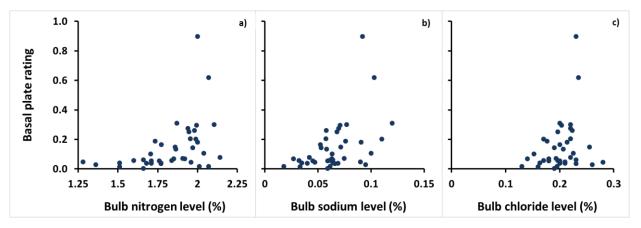


Figure 16: Association between a) nitrogen, b) sodium, c) chloride level in onion bulbs at harvest and basal plate rating. Data from monitoring spots located within onion plantings assessed over the 2022/23 and 2023/24 seasons.

#### **Conclusions**

Incidence of rotted bulbs at monitoring spots ranged from nil to 55%. Incidence was highly correlated with the concentration of Foc in basal plate tissue of harvested bulbs indicating Foc is the main causal agent of the fusarium basal rot observed.

The high incidence of fusarium basal rot highlights the substantive impact this disease can have on productivity. Rotted bulbs are unmarketable, and even when present at low incidence, add significant cost to grading and handling to facilitate marketing of the rest of the crop.

Foc infection resulted in additional loss of yield to that caused by discarding of rotted bulbs. At high levels of infection, total yield was as much as 25% lower, before rotted bulbs were removed.

Testing of plants confirmed Foc infection was present at the 2-4 leaf stage in most, but not all crops that developed fusarium basal rot by time of harvest. By the 5-7 leaf stage infection was detected in all but one of the crops that developed fusarium basal rot at harvest. Many of the infected plants had no obvious symptoms.

DNA testing can be used to detect and assess Foc infection level in crops before symptoms are observed by visual crop inspection, identifying crops that are likely to have reduced yield and high incidence of fusarium basal rot.

Not all infected bulbs were rotted or had obvious symptoms of fusarium basal rot at the time of harvest. These latent infections led to further losses when kept in ambient storage. DNA testing at harvest provides a better indication of fusarium basal rot developing in storage than inspection for rotted basal plates.

Associations were found between incidence of fusarium basal rot and the levels of beneficial fungi on the root system of onion bulbs at harvest. Bulbs with higher levels of Arbuscular Mycorrhiza Fungi and *Trichoderma* spp. tended to have lower incidence of fusarium basal rot.

Elevated total nitrogen level in tissue of harvested bulbs was associated with increased incidence of rotted bulbs caused by Foc. Lower nitrogen level in the harvested bulbs was not related to reduced yield, suggesting nitrogen level was not yield limiting within the range monitored in these commercial crops. This means there is scope to manage nitrogen to reduce risk of fusarium basal rot without jeopardising yield in sites where fusarium basal rot is expected to be an issue. When adjusting crop nutrition, potential impacts on other bulb quality attributes also need to be considered.

Elevated sodium and chloride level in tissue of bulbs sampled at harvest from monitoring sites were associated with reduced health of the basal plate, suggesting salinity management may assist in reducing the risk of fusarium basal rot.

In the paddocks monitored in this project, Foc was the dominant soilborne pathogen impacting productivity. This data may not be reflective of the broader industry, as most sites in this project were specifically selected to target monitoring of fusarium basal rot.

Other diseases that are known to impact onion productivity in South Australia, such as root lesion nematodes and pink root, caused by *Setophoma terrestris*, were not strongly associated with bulb yield at the monitored sites.

*S. terrestris* was detected in root systems from all 20 monitored crops. No strong linkage was found between increasing concentration of *S. terrestris* in the soil or plant roots with higher incidence of fusarium basal rot. High incidence of fusarium basal rot often occurred at sites with a low level of *S. terrestris* plant infection.

Sclerotium cepivorum, cause of white rot was not detected at any of the sites monitored.

Stubby root nematode, *Paratrichodorus* spp. caused a reduction in yield of large patches of onions in one of the 20 plantings monitored.

Macrophomina phaseolina infection of plants early in the crop, which was not strongly associated with Foc infection, was associated with lower yield, including at a low yield site where Foc was not detected. Impacts of *M. phaseolina* on onion productivity warrant further investigation to determine if this widespread pathogen is impacting onion productivity.

Two of the monitored sites had substantive levels of bacterial rots, with 6% of bulbs affected by bacterial bulb rot in one area of one site and 20% recorded in a wet area at the other site. At the other sites assessed the incidence of bacterial rots recorded at harvest was 2% or less.

When onions were stored at ambient temperature for 2-3 months after harvest, multiple pathogens were sometimes involved in disease development and rotting of bulbs, including bacterial pathogens.

#### Impact of prolonged soil moisture differences on disease development

#### **Background**

Variation in soil moisture often exists within irrigated onion crops, and can persist throughout the crop due to topography, seepage and uneven irrigation application rates. Monitoring of crops in the second year of the project indicated this variability was impacting development of fusarium basal rot. A trial was set up to capture the impact of persistent variation in soil moisture within an onion crop.

#### Methodology

Monitoring sites were established in a centre pivot irrigated fusarium basal rot susceptible OP variety brown onion crop to investigate the impact of season long differences in soil moisture. Ten monitoring points (Spots 1-10) were selected along three beds (replicates). Spots were located between two pivot wheel tracks. Lower than required output from a nozzle created an arc of low soil moisture in the pivot, while topography created a gradient of increasing soil moisture into a waterlogged depression that persisted throughout the season. A further three spots (Spots 11-13) were established in the same three beds at the other side of the pivot, one in the dry arc of the pivot and others on either side of the dry arc.

To characterise variation in soil moisture level at the 13 spots, soil moisture at each spot was assessed on 18 January by weighing, then drying and reweighing soil cores taken at 10cm intervals to a depth of 40cm. Cores from nine holes each 3 cm in diameter were removed from beds next to assessment beds. Sampling was done at the driest point in the irrigation cycle, with irrigation applied soon after sampling.

Onions were harvested from a 1.5 m length of each bed (10 rows) at each spot on the 14 February 2024, with the number and weight of bulbs recorded. Thirty bulbs from each plot (Plots 1-10) were placed in ambient storage for assessment after two months. Remaining bulbs (79-140 per plot) were assessed for incidence and severity of fusarium basal rot, and the incidence of pink root at the time of harvest. Notes were made on incidence of insect damage, splitting, cracking, doubles, bolters along with presence of other bulbs rots.

Nutrient testing (Eurofins PT2 analysis) was conducted on the composite bulb tissue freeze dried from 15 mm wide midsections of 15 healthy bulbs of average size for the sample (outer skin removed) at each spot (5 bulbs from each rep). Data contributed to nitrogen trials reported elsewhere.

Pathogen DNA testing for *Fusarium oxysporum* f. sp. *cepae* was conducted on composite root plus basal plate samples collected from onions assessed at harvest.

#### Results and discussion

Monitoring of crops and trials in South Australia has identified relationships between soil moisture level and the incidence of fusarium basal rot. Both higher and lower than optimum soil moisture levels can increase the risk of fusarium basal rot, Figure 17. Results were evident at harvest and became more pronounced after two months of ambient storage. Soil moisture conditions were considered close to optimal at spots 5 and 6, maximising yield and lowering incidence of fusarium basal rot, Figure 17 and Figure 18. Waterlogging which occurred in spot 9, as evidenced by the presence of surface water, was not favourable for either onions to grow or fusarium basal rot to develop. Total yield was reduced by approximately half at spot 9. At spots 8 and 10 soil moisture was high but not waterlogged. These conditions supported high yields but were also conducive to diseases such as bacterial bulb rots, as well as fusarium basal rot. Spot 10 was more elevated, effectively growing on a mound, when compared to spot 8. At the other end of the moisture spectrum, at spots 3, 4 and 12 where plants suffered prolonged water stress, total yields were reduced by around 25%, and had a higher level of fusarium basal rot when compared with spots having optimum moisture conditions. Conclusions

Irrigation management is critical aspect of onion production. Both higher and lower than optimum soil moisture levels can increase the risk of fusarium basal rot. Prolonged dry conditions reduce yield and increase the risk of fusarium basal rot. High moisture conditions, as long as soils are not waterlogged, can promote yield, but also increase risks of both fusarium basal rot and bacterial bulb rots.

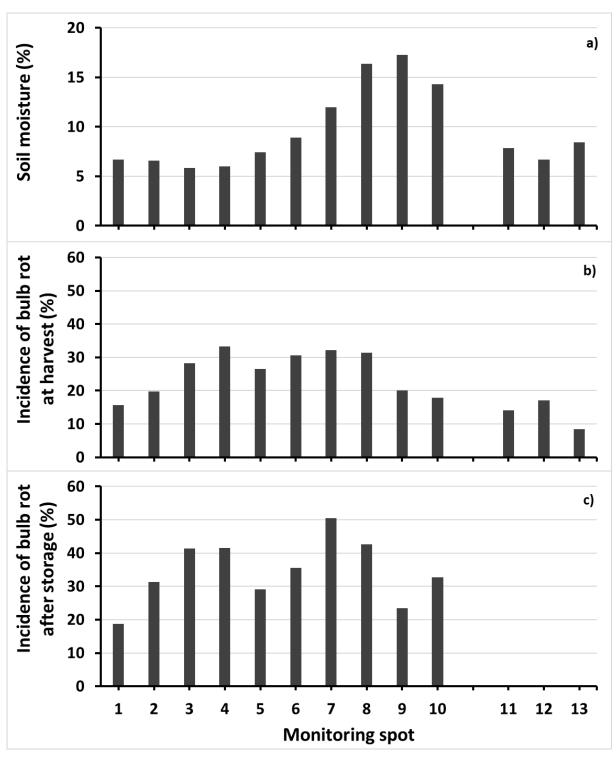


Figure 17: Impact of prolonged soil moisture variation on a) soil moisture percentage recorded on 19 January 2024, b) incidence of bulb rot at harvest, and c) incidence of bulb rot after 2 months ambient storage. Data from 13 monitoring locations along 3 planting beds in a centre pivot. (Spot 11-13 not assessed after storage).

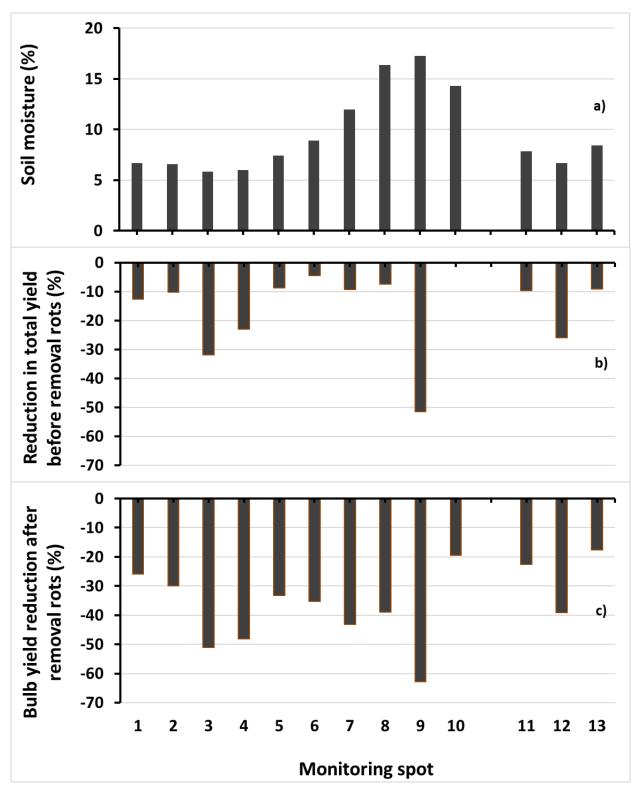


Figure 18: Impact of prolonged soil moisture variation on a) soil moisture percentage recorded on 19 January 2024, b) percent reduction in yield at harvest before removal of rots and c) after rots removed. Reduction compared to maximum total yield before rots removed obtained at monitoring spot 10. Data from 13 monitoring locations along 3 planting beds in a centre pivot.

#### Impact of short-term high moisture events on disease development

#### **Background**

Anecdotally, high incidence of fusarium basal rot has been reported to occur after major rainfall events. To investigate this, trials were set up to simulate rainfall flooding events at two sites.

#### Methodology

Simulated high rainfall events were applied to fusarium basal rot susceptible OP variety brown onion crops in the 2022 and 2023 planting seasons. Flooding treatments were applied by pumping water onto plots that had edging installed around a 2.5 m length of the centre six rows of onions in a 10-row bed. Edging was installed immediately before treatment and removed once last water treatment had soaked in. Soil type at all sites was a sand to loamy sand with the 2022 site having poor drainage at depth. Each treatment was replicated 4 times in each season.

In the first season single flooding events were applied at the 6-7 leaf (19 December), 50% tops down (8 February) and 2 weeks before harvest (27 February) stages of crop development. Each flooding treatment corresponded to approximately 50 mm of rainfall in less than 5 minutes and was applied once for each treatment.

In the second season flooding events were applied at the 4-5 leaf (30 November) and 80% tops down (1 February) stages. At the 80% tops down stage applications were either applied on 1 day or repeated on 2 consecutive days. Each flooding treatment corresponded to approximately 50 mm of rainfall in less than five minutes and was applied three times on each day for each treatment at two-hour intervals.

Timing of treatments was planned to coincide with soil temperatures of at least 15°C.

Onions were harvested from a 2 m length of six rows in each treatment plot on the 14 March 2023 and 23 February 2024, with the number and weight of bulbs recorded. Bulbs were assessed for the incidence and severity of fusarium basal rot and the incidence of pink root at the time of harvest.

Statistical analysis was conducted by ANOVA using Genstat statistical software, with means compared by LSD at the 10% level.

#### Results and discussion

The 2022/23 season was characterised by a prolonged period of above average rainfall that resulted in higher than desired soil moisture conditions until the 4-5 leaf stage at the trial site. In the 2023/24 season four significant rainfall events happened at the site of the trial in the December - January period, contributing to risk as much as the treatments. Due to the conducive natural weather conditions, none of the simulated rainfall treatments resulted in a significant change in the incidence of fusarium basal rot compared with the controls, Table 5. The incidence of pink root was significantly lower in trial 1 when the simulated water treatment was applied at the 6-7 leaf stage. This treatment resulted in a poorer basal plate rating; though not statistically significant, this treatment had the highest incidence of fusarium basal rot of the treatments applied.

#### **Conclusions**

Results of these trials are inconclusive, as other natural rainfall occurred at both trial sites that resulted in similar or higher soil moisture conditions during the growing season than the simulated flooding events.

Table 5: Effect of simulated high rainfall events on the incidence and severity of fusarium basal rot, incidence of pink root and bulb yield (including weight of rots).

Simulated Event		n basal rot at arvest	Incidence of pink root	Bulb yield (kg/m row)
_	Basal	Incidence	(%)	, 5
	plate rating	(%)		
	(0-1)			
Trial 1 (2022/23 planting)				
Control	0.36	29	9	1.21
Applied at 6-7 leaf	0.42	34	4	1.17
Applied at 50% tops down	0.31	26	11	1.23
Applied at 2 weeks before harvest	0.33	28	9	1.24
LSD (0.1)	0.071	NS	4.6	NS
Trial 2 (2023/24 planting)				
Control	0.17	16	1	1.14
Applied at 4-5 leaf	0.17	14	2	1.28
Applied at 80% tops down (1 day)	0.16	15	1	1.13
Applied at 80% tops down (2 days)	0.20	21	3	1.20
LSD (0.1)	NS	NS	NS	NS

#### Effect of nitrogen application on disease development

#### **Background**

Monitoring data in this project has found an association between higher bulb nitrogen levels at harvest with higher incidence of fusarium basal rot. Trials were conducted to investigate if application of additional nitrogen leads to increased fusarium basal rot.

#### Methodology

Nitrogen applications were applied to four brown onion crops over the 2022 and 2023 planting seasons. Nitrogen was applied as simulated fertigation treatments using a watering can and was in addition to the grower's fertiliser program. Soil type at all sites was a sand to loamy sand.

In the 2022/23 season nitrogen was applied to a fusarium basal rot susceptible OP variety at site 1 at the 5-7 leaf stage (14 December). Nitrogen (25 kg N per ha) was applied as sulphate of ammonium, calcium nitrate and urea. Treated plots were 3 m lengths of a planting bed (10 rows) with four replicates.

In the 2023/24 season nitrogen was applied in three separate applications each of sulphate of ammonium (25 kg N per ha), calcium nitrate (25 kg N per ha) and urea (25 and 50 kg N per ha) at sites 2 and 3. Applications of 25 or 50 kg N per ha were applied at two week intervals commencing at 3-4 leaf stage at site 2 (15 November) and the 2-4 leaf stage at site 3 (16 November). Site 2 was planted with a susceptible hybrid brown onion variety and site 3 with a susceptible OP brown onion variety. Plots were 3 m lengths of a single bed (10 rows) with 4 replicates.

At all sites onions were harvested from a 2 m length of bed (10 rows) in each treatment plot. Harvesting was conducted on 14 March 2023 at site 1, 8 February at site 2 and 29 February at site 3, with the number and weight of bulbs recorded. Bulbs were assessed for the incidence and severity of fusarium basal rot at the time of harvest.

Nutrient testing (Eurofins PT2 analysis) was conducted on the composite bulb tissue freeze dried from 15mm wide midsections of 15 healthy bulbs of average size for the sample (outer skin removed) from each treatment and replicate.

Pathogen DNA testing for *Fusarium oxysporum* f. sp. *cepae* was conducted on composite root plus basal plate samples collected from onions assessed at harvest from site 2 and 3 trials in the 2023/24 season.

Statistical analysis was conducted by ANOVA using Genstat statistical software, with means compared by LSD at the 10% level.

#### Results and discussion

The concentration of nitrogen in bulb tissue at harvest was influenced more by the site at which the trial was conducted than by nitrogen application rate or the form that nitrogen was applied in each trial, Table 6. Additional nitrogen application did not significantly increase nitrogen levels in bulbs at harvest in these trials; though levels in trial 3 were higher in all treatments where additional nitrogen was applied than control plots. Nitrogen applications commenced at an earlier crop growth stage in trial 3 than trial 2. Varieties were also different. The lower (25-50kg per ha) rates applied in a single application in trial 1 was less likely to influence bulb nitrogen levels than the same rates which were repeatedly applied 3 times to the crop in trials 2 and 3.

Incidence of fusarium bulb rot was highest in the site with the highest bulb nitrogen levels and lowest in the site with the lowest bulb nitrogen levels. Trials 2 and 3 were conducted on the same property in the 2023/24 season. Difference in bulb nitrogen concentration at these sites was contrary to the grower's application rates, suggesting other factors are influencing bulb nitrogen levels. While not related to application rate, when data from individual plots of these two trials was combined there was a strong relationship between Foc infection levels of composite root and basal plate samples and bulb nitrogen level measured at harvest, Figure 19. Likewise, when all available data generated in this project from trial plots was combined, increasing concentration of nitrogen in bulbs at harvest was related to increased Foc infection and incidence of fusarium basal rot, Figure 20.

#### **Conclusions**

These results support the finding from monitoring sites that high bulb nitrogen levels are associated with higher incidence of fusarium basal rot, reinforcing the importance of managing crop nutrition. However, the results indicate factors other than nitrogen application rate or nitrogen form can impact bulb nitrogen concentration. A greater understanding of the uptake pathways and role nitrogen is having in disease epidemiology is required to better use crop nutrition and soil management practices to lower the risk of fusarium basal rot.

Table 6: Effect of additional applications of three forms of nitrogen on bulb nitrogen level, incidence bulb rot caused by *Fusarium oxysporum* f. sp. cepae and bulb yield (including weight of rots).

Trial	Nitrogen treatment	Additional amount N	Bulb nitrogen	Incidence bulb rot	Bulb yield
		per ha	(%)	(%)	(kg/m row)
1	Control		2.03	25.7	1.17
	Urea	25	1.98	25.8	1.19
	Urea2	25			
	Ammonium sulphate	25	2.03	27.3	1.11
	Calcium nitrate	25	2.14	25.8	1.15
		LSD (0.1)	0.09	NS	NS
2	Control		1.71	2.5	1.39
	Urea	75	1.73	3.2	1.37
	Urea2	150	1.70	4.9	1.29
	Ammonium sulphate	75	1.74	1.8	1.36
	Calcium nitrate	75	1.70	3.0	1.31
		LSD (0.1)	NS	NS	NS
3	Control		1.86	19.4	1.11
	Urea	75	1.96	21.3	1.13
	Urea2	150	1.94	21.9	1.08
	Ammonium sulphate	75	1.94	23.3	1.07
	Calcium nitrate	75	1.91	19.9	1.11
		LSD (0.1)	NS	NS	NS

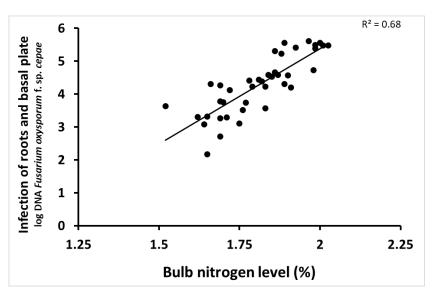


Figure 19: Relationship between nitrogen level in onion bulbs at harvest and *Fusarium oxysporum* f. sp. cepae infection of roots and basal plate tested at harvest. Data of individual plots combined from site 2 and site 3 nitrogen trials conducted in the 2023/24 season.

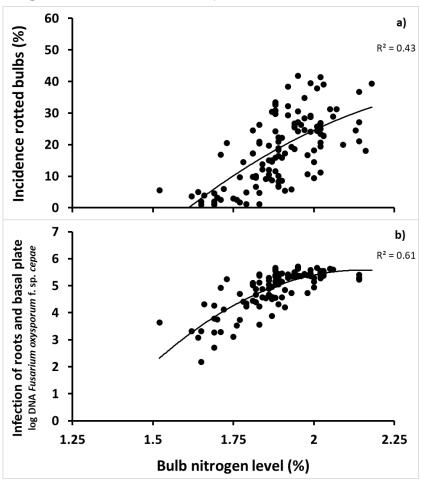


Figure 20: Relationship between nitrogen level in onion bulbs at harvest with a) percentage of bulbs rotted by *Fusarium oxysporum* f. sp. cepae and b) *Fusarium oxysporum* f. sp. cepae infection of roots and basal plate tested at harvest. Data combined from nitrogen trials, a soil moisture trial and efficacy trials conducted in the 2022/23 and 2023/24 seasons.

#### Effect of nurse crop competition

#### **Background**

Nurse crops are used to protect onions during crop establishment, however, they have the potential to compete with onions and increase disease risk by providing a pathogen bridge.

#### Methodology

Impact of nurse crop competition was assessed by comparing six rows of onions planted within a dense barley nurse crop that competed with the onions, with four rows of onions in the bed growing in between the nurse crop with minimal to no competition.

Pathogen DNA testing for *Fusarium oxysporum* f. sp. *cepae* (Foc) was conducted on a composite sample of 50 root and crowns of the barley nurse crop that were randomly sampled at time of spray off.

Onions were harvested from a 2 m length of bed in ten plots, with the nurse crop affected and unaffected rows harvested and assessed separately. Bulb number and weight were recorded before bulbs were assessed for the incidence and severity of fusarium basal rot at the time of harvest.

Nutrient testing (Eurofins PT2 analysis) was conducted on the composite bulb tissue freeze dried from 15 mm wide midsections of 15 healthy bulbs of average size for the sample (outer skin removed) for each treatment and replicate.

Statistical analysis was conducted by ANOVA using Genstat statistical software, with means compared by LSD at the 10% level.

#### Results and discussion

Competition from the barley nurse crop reduced yield by 25%, however did not significantly affect incidence of rotted bulbs caused by Foc or concentration of nitrogen in bulbs, Table 7. A low detection of Foc DNA (30 kDNA copies/g sample) in root and crown samples of the barley plants indicated that some infection of the barley nurse crop was present at the time of spray off. Foc was not detected in onions (1 leaf stage) sampled at the same time as the nurse crop from areas surrounding the trial area, though Foc was detected at levels of up to 490 kDNA copies/g sample in another area of the same paddock 2 weeks later at the 1-2 leaf stage.

#### Conclusion

In this trial conducted at a site with high Foc disease pressure, the incidence of bulb rots caused by Foc was not increased by severe competition from a barley nurse crop.

Table 7: Effect of nurse crop competition on bulb nitrogen level, incidence of bulb rot caused by *Fusarium* oxysporum f. sp. cepae and bulb yield (including weight of rotted bulbs).

Nurse crop treatment	Bulb nitrogen	Incidence bulb rot	Bulb yield (kg/m row)
	(%)	(%)	(8,)
Limited competition	1.94	29	1.20
Competition	1.96	24	0.89
LSD (0.1)	NS	NS	0.08

#### **Host crops**

#### **Background**

Management of crops and the control of host weeds in rotations is an important part of reducing inoculum between onion crops. There is limited knowledge on whether Foc is hosted on crops and weeds in South Australian onion production systems.

#### Methodology

Samples of five rotation crops were collected from paddocks where fusarium basal rot had occurred in the past. Weeds of 13 species growing in areas of onion crops where fusarium basal rot was present were also sampled. Soil was collected from the locations where plants were dug up or that fell off root systems, with soil combined and mixed before 500 g subsamples were taken. These soil samples were DNA tested to confirm inoculum of Foc and *S. terrestris* present in soil at each area from which samples collected, before plant samples were included in the results.

Root systems from the sampled crop and weed plants were removed, rinsed, dried and DNA tested for Foc and *S. terrestris*. All reported detections in plant root systems were higher than the level detected in the soil from the area they were collected from.

Ten crop species were grown for six weeks in a controlled environment room at  $26^{\circ}$ C Day /  $16^{\circ}$ C Night temperatures and a 16-hour daylength. Ten plants of each crop and variety were grown in a sand media in individual 50 mm tubes with water supplied from the bottom. Tubes were inoculated at seeding with 1 mL of  $1*10^{6}$  Foc spores per mL (Isolate 14-19-2) injected in the seeding hole before covering the seed. At six weeks root systems were removed from the sand media, rinsed, dried and DNA tested for Foc in two lots of five plants. Composite 500 g samples of the sand media removed from root systems were tested from each lot.

#### Results and discussion

#### Field samples

Detection of Foc by qPCR testing indicated root infection of barley, lucerne and faba beans was present and these species may be acting as reservoir hosts of the pathogen, Table 8. Lower incidence and concentration of Foc detection in canola samples suggest it is a weaker host compared to the other species tested.

Detection of *S. terrestris* indicates barley, lucerne, canola and faba beans were also infected by this pathogen and these crops could assist to maintain inoculum of *S. terrestris* between onion crops.

Of the 13 weed species tested, Foc was detected by qPCR testing in annual ryegrass, marshmallow, sow thistle, wireweed and cutleaf nightshade, Table 8. Infection levels were higher in sow thistle than other species.

Average infection levels of root and basal plates of infected onions at harvest are several orders of magnitude higher than the amount detected in root systems of the other crops and weeds tested. Onion crops would be the main contributor to inoculum levels in the soil in onion production systems, with infected bulbs producing high numbers of spores that remain in the soil after the crop is harvested. In this preliminary study aimed at determining other plant species that may be infected by Foc, it was not determined if the Foc infected roots of crops and weeds were producing spores.

#### Controlled environment samples

Roots of the four cereal crops (barley, wheat, triticale and oats), five pulse crops (lentils, lupins, field peas, faba beans and chickpeas) and one brassica crop (canola) were infected by Foc when inoculated with Foc spores at planting and grown under optimum temperatures for the pathogen in a controlled environment room. This indicates these species can be infected by Foc with potential to be hosts of the pathogen, but may not reflect likelihood of infection or their potential to maintain inoculum under normal field conditions between onion crops.

Root systems of plants had obvious symptoms of infection, such as brown discolouration, compared with healthy roots of uninoculated plants. Foc levels in soil from tubes that plants had been grown in ranged from below detection to 6 kDNA copies per gram of soil. This indicates the high levels detected in root tissue was from infection of the plants.

Many of these species are winter crops, grown when temperature conditions are not favourable for Foc infection and growth. Levels of root infection were much higher for the same species grown in the controlled environment room than when sampled from field conditions. For example, Foc concentration in roots of faba beans sampled in the field ranged from 27 to 86 kDNA copies per gram dry sample compared with 4,307 to 19,888 kDNA copies per gram dry sample when grown in the controlled environment room. These results suggest that if these crops are grown out of season, for example as summer cover crops, they may be more susceptible to infection by Foc.

These results indicate, that while assays in a controlled environment room may demonstrate that a plant species can be infected by Foc, the results do not provide a good indication of what infection levels are likely under field conditions on a grower's property.

#### **Conclusions**

Infected onion crops would be the main contributor to Foc inoculum levels in the soil in onion production systems.

Crops currently grown in rotation with onions may be assisting to maintain inoculum between onion crops, contributing to risk of fusarium basal rot and pink root occurring in following onion crops. qPCR testing of plant samples from onion production fields confirmed a wide range of crop and weed species can be naturally infected by Foc and *S. terrestris*, indicating plants from multiple family groups may be acting as reservoir hosts in onion production systems. Further work is required to determine the significance of specific rotation, cover crop and weed species to the risk of fusarium basal rot.

Table 8: Detection of *Fusarium oxysporum* f. sp. cepae and *Setophoma terrestris* in the roots of crops and weeds collected from paddocks used for onion production where inoculum of these pathogens confirmed to be present in the soil by DNA testing.

					Fusarium oxysporum f. sp. cepae				Setophoma terrestris					
Situation Common name	Scientific name Family	Number plants sampled	Sample lots tested	Detections		NA	in values copies/g mple)	Sample lots tested	Detections		NA	in values copies/g nple)		
Crop	Barley	Hordeum vulgare	Poaceae	100	4	4	17	-	33	4	4	125	-	208
	Lucerne	Medicago sativa	Fabaceae	60	4	4	14	-	56	8	7	0	-	4,714
	Canola	Pisum sativum	Brassicaceae	80	8	3	0	-	12	8	8	26	-	1,089
	Faba beans	Vicia faba	Fabaceae	90	5	5	27	-	86	5	5	88	-	539
	Peas	Pisum sativum	Fabaceae	61	2	0	0	-	0	2	2	61	-	281
	Onion (Harvest)	Allium cepa	Alliaceae	~ 10,000	98	90	0	-	425,000	98	98	34	-	507,000
Nurse crop	Barley	Hordeum vulgare	Poaceae	174	6	3	0	-	445	6	4	0	-	518
Weed	Annual ryegrass	Lolium rigidum	Poaceae	34	5	3	0	-	312					
	Marshmallow	Malva parviflora	Malvaceae	12	12	1	0	-	389					
	Sow thistle	Sonchus oleraceus	Asteraceae	43	9	5	0	-	2,541					
	Wireweed	Polygonum aviculare	Polygonaceae	23	4	1	0	-	11					
	Cutleaf nightshade	Solanum triflorum	Solanaceae	5	1	1			31					

Table 9: Concentration of *Fusarium oxysporum* f. sp. cepae DNA in the root systems of crop species when grown in controlled environment room. Inoculated at seeding with 1 mL of 1\*10<sup>6</sup> Foc spores per mL (Isolate 14-19-2). Plants assessed 6 weeks after planting.

			Fusarium oxyspo	rum f. sp. cepae			
Crop	Variety	Family	(kDNA copies/g sample)				
			Lot 1	Lot 2			
Barley	Spartacus	Poaceae	682	5,070			
			41,865	18,463			
Wheat	Mace	Poaceae	4,011	2,873			
Triticale	Tahara	Poaceae	4,066	2,151			
Oats	Yallara	Poaceae	11,699	3,648			
Canola	Enforcer	Brassicaceae	517	10,395			
	Stingray	Brassicaceae	4,744	14,544			
Lentils	PBA Blitz	Fabaceae	1,029	527			
	PBA Jumbo	Fabaceae	1,895	6,913			
Lupins	Bateman	Fabaceae	656	10,915			
Field peas	PBA Gunyah	Fabaceae	12,131	2,747			
	PBA Taylor	Fabaceae	14,376	7,848			
Faba beans	PBA Amberley	Fabaceae	4,307	8,019			
	PBA Samira	Fabaceae	19,888	6,265			
Chickpea	Genesis 090	Fabaceae	50,047	8,028			
	Kyabra	Fabaceae	24,808	14,123			

#### **Efficacy of control treatments**

#### **Background**

There are currently no registered chemical products, with exception of soil fumigants, for the control of fusarium basal rot of onions in Australia. Efficacy testing of products was conducted to investigate application strategies and provide support towards progressing products towards future availability to the onion industry. Products for testing were selected in consultation with members of the Project Reference Group, after a review of the literature, discussions with companies, information available from overseas, a scan of new products on the market and consideration to current use on onions for other purposes. Trials were then used to refine the list of products selected for field trials. A list products used in efficacy trials is provided in Table 10.

Table 10: List of products used in efficacy trials and their active ingredients.

Product	Ac	tive ingredients (a.i)
Belanty <sup>®</sup>	75 g/L	mefentrifluconazole
Brumby®480 SC	480 g/L	prothioconazole
Evergol® Energy	76.8 g/L	prothioconazole
Seed Treatment	61.4 g/L	metalaxyl
	38.4 g/L	penflufen
Intuity®	250 g/L	mandestrobin
Luna® Experience	200 g/L	fluopyram
	200 g/L	tebuconazole
Sergomil L60®	5.5 %w/w	copper
Sprayseal®	430 g/L	tebuconazole
Switch®	375 g/kg	cyprodinil
	250 g/kg	fludioxonil
SYN PHI3	450 g/L	
Velum® Prime	400 g/L	fluopyram
Wilchem BioTeam plus BiopHilm	Min 1 x 10 <sup>9</sup> CFUs per mL	Bacillus subtilus, Azospirillum brasilense, Bacillus megaterium, Bacillus mucilaginosus

#### Controlled environment trials

#### Methodology

Controlled environment trials were utilised to screen potential control agents to assist with selection of products for inclusion in field efficacy trials.

#### Trial A.

Efficacy of six chemistries and a biological product were evaluated with two untreated controls (inoculated and uninoculated) in randomized complete block experiment conducted in controlled environment trial, with three replicates. Onion seeds of a fusarium basal rot susceptible OP variety were planted in sand media in individual 50 mm tubes with water supplied from a tray at the bottom. Each replicate of each treatment consisted of 25 tubes, each planted with two plants. Tubes were inoculated at seeding with 1 mL of 5\*10³ Foc spores per mL (Isolate 14-19-2) injected in the seeding hole before covering. Treatments were applied with a hand spray and incorporated by a single light watering to wet the sand media to below the seed depth. Treatment and equivalent rates per ha were Velum® Prime at 1,000 mL/ha, Luna® Experience at 1,000 mL/ha and 2,000 mL/ha, Belanty® at 1,250 mL/ha, Switch® at 800 g/ha and Wilchem BioTeam 100 mL/ha plus Wilchem BiopHilm.

Plants were grown for 10 weeks in a controlled environment room at 26°C Day / 16°C Night temperatures and a 16-hour daylength. Plants were then removed from the sand media, root systems rinsed, plants weighed, and average plant weight calculated. No plant death of emerged seedlings occurred. Root systems of all plants were removed by cutting off 1 cm above the basal plate, dried and the composite sample for each replicate and treatment qPCR tested for Foc.

#### Trial B.

Efficacy of three chemistries were evaluated with two untreated controls (inoculated and uninoculated) in a in complete block experiment conducted in a controlled environment trial, with three replicates. Onion seeds of a fusarium basal rot susceptible OP variety were planted in sand media in individual 50 mm tubes with water supplied from a tray at the bottom. Each replicate of each treatment consisted of 25 tubes, each planted with one plant. Tubes were inoculated at seeding with 1 mL of 5\*10<sup>6</sup> Foc spores per mL (Isolate 14-19-2) injected in the seeding hole before covering. Treatments were applied with a hand spray and incorporated by a single light watering to wet the sand media to below the seed depth. Treatment and equivalent rates per ha were Velum Prime at 1,000 mL/ha, Sprayseal at 780 mL/ha) and Switch at 800 g/ha and SYN PH3 at 550 mL/ha.

Plants were grown for four weeks in a controlled environment room at 26°C Day / 16°C Night temperatures and a 16-hour daylength before counting the number of healthy plants present.

Statistical analysis of trials was conducted by ANOVA using Genstat statistical software, with means compared by LSD at the 10% level.

#### Results and discussion

Testing of chemicals in Trial A identified SYN PHI3 for inclusion in field trials, based on the significant reduction in Foc infection achieved and maintenance of a similar average plant weight to the uninoculated control, Table 11. Luna Experience also significantly reduced Foc infection and was selected for inclusion in field trials, though average weight of plants treated with this product tended to be lower than the uninoculated control. Further testing found that both active ingredients (fluopyram, tebuconazole) in this product reduced seedling losses, Table 12. Plants treated with tebuconazole were stunted and this contributed to plant loss in this treatment.

Switch significantly reduced Foc infection in Trial A and reduced seedling losses in Trial B.

The biological product BioTeam and chemical product Belanty both tended to reduce Foc infection, though the difference was not statistically significant.

#### **Conclusions**

SYN PHI3 and Luna Experience were selected for inclusion in field trials. Switch was also found to reduce Foc infection of seedlings.

Table 11: Efficacy of chemicals and a biological product to reduce Foc root and basal plate infection of onion plants grown in a controlled environment room. Inoculated at seeding with 1 mL of 5\*10³ Foc spores per mL (Isolate 14-19-2). Plants assessed 10 weeks after planting.

Treatment	Average Plant	Infection of roots
Trial A	Weight	Fusarium oxysporum f. sp. cepae
	(g)	(kDNA copies / g dry root)
Control - Not inoculated	3.21	0
Control - Inoculated	2.57	18,073
Velum® Prime	2.05	10,142
Luna® Experience	2.81	2,643
Luna® Experience * 2	2.81	3,034
Intuity®	2.79	10,850
Switch®	3.51	4,942
SYN PHI3	3.28	129
BioTeam plus BiopHilm	3.07	10,396
LS	D (0.1) 0.41	8,362

Table 12: Efficacy of chemicals to reduce seedling loss of onions grown in a controlled environment room. Inoculated at seeding with 1 mL of  $1*10^6$  Foc spores per mL (Isolate 14-19-2). Plants assessed 4 weeks after planting.

Treatment	Healthy plants
Trial B	(Number from 25 tubes)
Control - Not inoculated	22
Control - Inoculated	7
Velum® Prime	15
Sprayseal®	10
Switch®	14
LSD (0.1)	2.6

#### **Field trials**

#### Methodology

In the 2023 planting season five field efficacy trials were conducted (Trials 1-5) in onion crops. Sites were selected based on the past history of disease and pathogen DNA testing of soil samples to confirm the presence of Foc inoculum.

Two in-crop treatment field efficacy trials (referred to as Trial 1 and 2) of the same four products plus an untreated control were conducted in a randomized complete block design with six replicates. Treated plots were 3 m long by 1 bed width (10 rows). Trial 1 was planted on 28 August 2023 with a susceptible hybrid brown onion variety and Trial 2 was planted on 13 September 2023 with a susceptible OP brown onion variety. Treatments were Intuity boom applied at 6 and 10 weeks after planting at rate of 1,200 mL/ha, Luna Experience boom applied at 6 and 10 weeks after planting at rate of 1,000 mL/ha, Brumby boom applied at 6, 10 and 14 weeks after planting at rate of 400 mL/ha, and Sergomil L60 applied with a watering can at 6, 10 and 14 weeks after planting at a rate of 5,000 mL/ha in 10,000 L of water/ha.

Onions were harvested from a 2 m length of bed (10 rows) in the centre of each treatment plot. All treated bulbs were removed. Trial 1 was harvested on 7 February 2024 and Trial 2 on 6 March 2024, with the number and weight of bulbs recorded. 50 bulbs were removed and placed in ambient storage for 3 months prior to assessment. Remaining onions (80-156 bulbs per replicate plot) were assessed for the incidence and severity of fusarium basal rot and the incidence of pink root at the time of harvest.

Three field efficacy trials (referred to as Trials 3, 4, and 5) were conducted to assess in-furrow and band treatments of 1 product and seed treatment with two products. Trials were planted with a single bed nine row planter, using the centre three rows to compare seed treatments and the outer three rows on each side to compare in-furrow and band treatments. Untreated controls were included in each bed. Treated strips were 30 to 40 m long and set up for harvesting five replicates in Trial 3, six replicates in Trial 4 and four replicates in Trial 5. All trials were planted with the same susceptible OP brown onion variety. Trial 3 was planted on 12 September 2023, Trial 4 on 13 September 2023 and Trial 5 on 29 September 2023. Seed treatments were lightly coated with neat SYN PHI3 and Evergol Energy. Seed was precoated prior to application of seed treatments. In-furrow and band treatments were SYN PHI3 applied in-furrow at planting, SYN PHI3 applied in-furrow at planting plus as band spray four weeks after planting and watered in, and SYN PHI3 applied as band spray four days after planting and watered in. All applications were applied at rate of 550 mL/ha.

Onions were harvested from a 4 m length strip of three rows in each replicate. All treated bulbs were removed. Trial 3 was harvested on 23 February 2024, Trial 4 on 29 February 2024 and Trial 5 on 7 March 2024, with the number and weight of bulbs recorded. Harvested onions (47-185 bulbs per replicate plot) were assessed for the incidence and severity of fusarium basal rot and the incidence of pink root at the time of harvest.

Plants from untreated areas within each of the five trial locations were sampled and composite root and basal plate tissue pathogen DNA tested for *Fusarium oxysporum* f. sp. *cepae* at two or three times during crop growth.

Pathogen DNA testing for Foc and *Setophoma terrestris* was conducted on composite root plus basal plate samples of the harvested bulbs that assessed for fusarium basal rot from each trial, treatment, and replicate.

Statistical analysis of trials was conducted by ANOVA using Genstat statistical software, with means compared by LSD at the 5 and 10% level.

#### Results and discussion

Infection by Foc was monitored by qPCR testing of root and bulb/basal plate samples collected from buffer areas of trial sites, Table 13. In three of the five trial sites some infection was confirmed to have occurred at the 1-2 leaf stage, including in the two in-crop trials just prior to the first treatments being applied. Testing was not conducted again in the remaining two sites until the 6-7 leaf stage, when infection was found to be present.

#### **In-crop treatments**

All treatments (Intuity, Luna Experience, Brumby and Sergomil) reduced the incidence of bulb rots caused by Foc compared to untreated controls (at 10%, but not 5% level of significance) when bulbs were assessed at harvest or after three months ambient storage in Trial 1, Table 14. Brumby reduced the incidence of bulb rots significantly more than Intuity and Sergomil L60, but not Luna Experience. When compared at the 5% level of significance, only Brumby and Luna Experience treatments reduced incidence of bulb rot compared to untreated controls when assessed after three months storage, with no significant differences between treatments and the untreated control when assessed at harvest.

Disease pressure was higher in Trial 2, with the incidence of bulb rot in the untreated control (21.9%) almost 3 times as high as in Trial 1 (7.6%). When bulbs were assessed at harvest Luna Experience and Brumby but not Intuity and Sergomil L60 reduced the incidence of bulb rots caused by Foc compared with the untreated control. When bulbs were assessed after 3 months of ambient storage, Brumby® significantly reduced the incidence of bulb rots compared with the untreated control and all other treatments.

There were no significant differences in bulb yield between treatments or when compared with the untreated control, Table 14.

Incidence of pink root on root systems of bulbs at harvest was not observed in Trial 1 and was very low in Trial 2, with infection level observed unlikely to have had any direct impact on crop productivity, Table 14. Though the incidence of pink root in Trial 2 was very low, the incidence was greater following the Intuity treatment than in the untreated control when compared at the 10% level of significance.

#### In-furrow and band application strategies for SYN PHI3

Application of SYN PHI3 significantly reduced incidence of bulb rots caused by Foc when applied as an in-furrow spray at planting, a band application four days after planting or in-furrow spray at planting plus a band application four weeks after planting, Table 15. Trial 4 was the highest disease pressure site, having higher levels of bulb rots in the untreated controls when compared with the other two trial sites. In Trial 4 application in-furrow plus a band spray four weeks after planting was more effective at reducing bulb rots than the single application strategies. In Trials 3 and 5, there were no significant differences between the three strategies.

qPCR testing of Foc infection in root and basal plate samples at harvest found all SYN PHI3 treatments lowered infection levels compared to the untreated control in Trial 5, and when applied as an in-furrow spray at planting or in-furrow spray at planting plus a band application four weeks after planting, but not a band application four days after planting in Trial 3, Table 16. In Trial 4, Foc infection was only significantly lower in the treatment where SYN PHI3 was applied in-furrow plus a band spray four weeks after planting when compared at the 10% level of significance, and not when single applications of SYN PHI3 had been applied.

Impact of application of SYN PHI3 on bulb yield were not consistent, Table 15. Yields of onions treated with SYN PHI3 were as high or higher than in the untreated control, except for the in-furrow treatment in Trial 3 which had a lower yield than the untreated control. Averaged over the three trials yield was highest when SYN PHI3 was applied as a band four days after planting.

Incidence of pink root on root systems of bulbs at harvest was very low, with infection level observed unlikely to have had any direct impact on crop productivity, Table 15. Though the incidence of pink root was very low, SYN PH13 applied as an infurrow spray at planting, a band application four days after planting or in-furrow spray at planting plus a band application four weeks after planting reduced pink root incidence in Trials 3 and 4. This result is supported by all SYN PHI3 treatments significantly reducing *S. terrestris* infection measured by qPCR of root and basal plate samples at harvest in each of the three trials, Table 16.

#### **Seed treatments**

Seed treatments of Evergol Energy and SYN PHI3 were not effective at reducing the incidence of fusarium basal rot caused by Foc, nor did they have any significant effect on bulb yield or the incidence of pink root, Table 17. Only exception was in Trial 3; SYN PHI3 significantly reducing the incidence of bulb rots caused by Foc from 21.3 to 16.5%.

#### **Conclusions**

SYN PHI3 applied as an in-furrow treatment at planting, band application four days after planting, or combination of an infurrow treatment at planting and band application four weeks later all reduced incidence of bulb rots caused by Foc assessed at harvest.

Brumby applied as an in-crop spray three times during crop growth (6, 10 and 14 weeks after planting) reduced incidence of bulb rots caused by Foc when assessed at harvest of after three months of ambient storage.

Compared with SYN PHI3 and Brumby, other treatments evaluated were less effective at reducing bulb rots caused by Foc, especially if bulbs were ambient stored after harvest for three months.

Seed treatments evaluated provided no or negligible control of fusarium basal rot.

Table 13: Concentration of *Fusarium oxysporum* f. sp. cepae DNA in the root and basal plate sections of onion plants sampled from the untreated buffer areas at five efficacy trial sites during crop growth.

				Fus	arium oxyspor	um f. sp	o. cep	ае
Efficacy trial	Date sampled	Growth stage (leaf number)	Sample Detection	Detections	(kDN	_	values pies/g le)	
	18/10/2023	1-2	101	4	1	0	-	56
Trial 1	15/11/2023	4-5	99	4	2	0	-	48
	15/12/2023	8-10	50	2	1	0	-	84
	27/10/2023	1-2	104	4	1	0	-	490
Trial 2	16/11/2023	3-4	103	4	2	0	-	337
	10/01/2024	8-10	51	2	2	116	-	678
	18/10/2023	1	175	2	1	0	-	276
Trial 3	16/11/2023	3-4	96	2	2	109	-	1,169
	1/12/2023	6-7	147	3	3	449	-	697
Trial 4	18/10/2023	1	153	2	0	0	-	0
111d1 <del>4</del>	1/12/2023	6-7	131	3	3	179	-	656
	10/1/2024	8-10	85	2	2	518	-	1,280
Trial 5	16/11/2024	1-2	104	2	0	0	-	0
	16/12/2023	6-7	149	3	3	36	-	814

Table 14: Efficacy of four chemicals applied in-crop to reduce the incidence of bulb rot caused by *Fusarium oxysporum* f. sp. *cepae* at harvest and after 3 months ambient storage, along with the incidence of pink root assessed at harvest in three field trials in commercial plantings. Bulb yield includes weight of rotted bulbs.

Trial	Treatment	Incidence of pink root (%)	Incidence bulb rot at harvest (%)	Incidence bulb rot after storage (%)	Bulb yield (kg/m row)
Trial 1	Control	0	7.6	14.6	1.31
	Intuity <sup>®</sup>	0	4.2	9.7	1.36
	Luna® Experience	0	3.6	8.1	1.39
	Brumby®	0	1.8	4.8	1.38
	Sergomil L60®	0	4.0	9.8	1.37
	LSD (0.05)		NS	5.7	
	LSD (0.1)	NS	3.3	4.7	NS
Trial 2	Control	0.1	21.9	24.2	1.24
	Intuity®	0.6	19.6	24.5	1.22
	Luna® Experience	0.2	16.9	21.9	1.25
	Brumby®	0	10.0	12.7	1.22
	Sergomil L60®	0.2	17.9	24.7	1.21
	LSD (0.05)	NS	4.7	6.8	
	LSD (0.1)	0.4	3.9	5.7	NS

Table 15: Efficacy of three application strategies to reduce the incidence of bulb rot caused by *Fusarium oxysporum* f. sp. cepae and pink root assessed at harvest using SYN PHI3 in three field trials in onion crops. Bulb yield includes weight of rotted bulbs.

Trial	Treatment	Incidence of pink root	Incidence bulb rot at harvest	Bulb yield
		(%)	(%)	(kg/m row)
Trial 3	Control	1.3	25.0	1.58
Trial 3	SYN PHI3 In-furrow	0	5.4	1.40
	SYN PHI3 Band	0.2	8.7	1.61
	SYN PHI3 In-furrow + Band	0.1	5.4	1.64
	LSD (0.05)	0.6	3.5	0.09
Trial 4	Control	2.9	36.3	1.11
	SYN PHI3 In-furrow	0	17.7	1.21
	SYN PHI3 Band	0.3	14.8	1.44
	SYN PHI3 In-furrow + Band	0	7.1	1.04
	LSD (0.05)	1.4	5.3	0.09
Trial 5	Control	0.1	6.8	1.02
	SYN PHI3 In-furrow	0	1.1	1.19
	SYN PHI3 Band	0	1.6	1.42
	SYN PHI3 In-furrow + Band	0	2.1	1.05
	LSD (0.05)	NS	3.2	NS

Table 16: Efficacy of three application strategies using SYN PHI3 in three field trials to reduce the infection level of pathogens that cause fusarium basal rot and pink root measured on roots and basal plate of harvested onions. Bulb yield includes weight of rotted bulbs.

Treatment	Infection of roots								
	Fusarium	oxysporum f	sp. cepae	Seto	phoma terre	stris			
	(kDNA	copies / g dr	y root)	(kDNA	copies / g dr	y root)			
	Trial 3	Trial 4	Trial 5	Trial 3	Trial 4	Trial 5			
Control	88,724	212,893	85,172	2,916	10,916	539			
SYN PHI3 In-furrow	35,486	202,078	5,278	153	1,368	36			
SYN PHI3 Band	59,198	159,899	11,788	507	670	47			
SYN PHI3 In-furrow + Band	20,287	120,994	19,860	445	170	24			
LSD (0.05)	32,305	NS	47,204	1,135	4,967	117			
LSD (0.1)		58,428							

Table 17: Efficacy of two seed treatments to reduce the incidence of bulb rot caused by *Fusarium oxysporum* f. sp. cepae and pink root assessed at harvest in three field trials in commercial plantings. Bulb yield includes weight of rotted bulbs.

Trial	Seed Treatment	Incidence of pink root	Incidence bulb rot at harvest	Bulb yield (kg/m row)
		(%)	(%)	
Trial 3	Control	0.4	21.3	1.49
	SYN PHI3	0.2	16.5	1.50
	Evergol® Energy	0.4	19.1	1.40
	LSD (0.05)	NS	3.1	NS
Trial 4	Control	1.1	24.9	1.06
	SYN PHI3	0.4	22.2	1.00
	Evergol® Energy	1.6	25.3	1.04
	LSD (0.05)	NS	NS	NS
Trial 5	Control	0	5.2	1.24
	SYN PHI3	0	3.8	1.34
	Evergol® Energy	0	6.6	1.30
	LSD (0.05)	NS	NS	NS

# Appendix 2 – Guide to fusarium basal rot management

# Fusarium basal rot

Onions









#### **Fusarium basal rot of onions**

This document focuses on the disease Fusarium basal rot, providing information on the biology, drivers of disease development and possible management strategies available to onion growers.

It is intended to provide information to assist best practice control of Fusarium basal rot using integrated disease management strategies, known as IDM.

All integrated disease management strategies aim to determine the cause of the problem and apply the most appropriate management solutions to limit unacceptable loss in marketable yield. The solutions should:

- utilise a range of cost-effective methods to achieve disease control in crops
- be effective for as long as possible
- minimise adverse effects on users, the environment and other crop management systems.

There are a range of issues to consider when deciding what solution is the most appropriate.

#### These include:

- correctly identifying the pest or disease
- understanding the threat posed to production
- identifying the possible actions needed to minimise the impact
- undertaking the most appropriate management strategies
- assessing the effectiveness of the management strategies undertaken.

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### **Fusarium basal rot**

Fusarium basal rot (FBR) is a major disease of onions throughout the world. Records of onions with symptoms of this disease in Australia go back as far as the early 1900s. The disease has become of greater concern in recent years, particularly in South Australia, but also in other regions of Australia where onions are grown in hot conditions. FBR may be evident in the field, however losses continue to occur in storage.

Losses of crop can exceed 50%. Rotted bulbs are unmarketable, and even when present at low incidence, add significant cost to grading and handling to facilitate marketing of the rest of the crop.

## Pathogens that cause Fusarium basal rot

Fusaria are a type of fungi that inhabits most agricultural soils. The majority of Fusarium spp. are non-pathogenic, posing no risk to crops including onions. A small number of Fusarium spp. can infect onions and cause disease.

Fusarium oxysporum forma specialis (f. sp.) cepae (abbreviated to FOC) is the most common cause of FBR in Australia. The pathogen can also cause damping off and root rot of onions. There are many other forms, known as formae speciales, of Fusarium oxysporum that cause disease of other crops in Australia, however, none of these are documented to cause basal rot of onions.

Other species of *Fusarium* cause disease of onions in Australia. *Fusarium proliferatum* has been associated with bulb rot in storage and purple blotch symptoms on the skin of white onions.

Fusarium solani has been found to commonly infect root systems of onions in Australia. The importance of *F. solani* and other pathogenic *Fusarium* spp. found to be infecting roots is not well understood.

FBR occurs in other Allium species including garlic, leek, and shallot.

\*Fusarium oxysporum f. sp. cepae (FOC) is the fungal **pathogen**, whereas Fusarium basal rot (FBR) is the **plant disease**, most commonly caused by FOC.

# **Inoculum and how it spreads**

Soilborne inoculum is considered the main source of infection risk in direct seeded crops.

FOC produces long lived spores, called chlamydospores, which can survive in soil for more than four years, waiting to infect onions when they are planted.

FOC can survive in the soil on organic residues, and on roots of non-symptomatic host species.

Seed is a possible source of inoculum if adequate controls are not in place to prevent and manage seed infection or contamination and could introduce the pathogen to new locations.

Water movement and machinery can carry infected soil, spreading the pathogen. It is suspected that manure of animals that have consumed infected bulbs could assist dispersal.

Overseas, the use of sets for planting, rather than direct seeding, is a common way new paddocks are infected.

# Infection by Fusarium oxysporum f. sp. cepae (FOC)

Infection by FOC can occur at any stage of crop development, including prior to emergence. While infection is possible at any time, the development of symptoms is most likely early (damping off before or soon after emergence) or during bulb maturation and storage (basal rot).

Fungus living in the soil infects roots or basal plates. It may also infect lower parts of the bulb scales. Wounding of tissue, such as cracking caused by rapid growth, mechanical or pest damage, increases the risk of infection. Enzymes released by the pathogen assist with infection and in the disintegration of root and basal plate tissue as the disease progresses.

Infection of the basal plate does not immediately result in infection of the bulb scales. Depending on conditions and variety this can take many months, if infection of the bulb scales occurs at all. In some varieties there is a stronger barrier to progression of infection from the basal plate to the bulb scales.

Development of FBR in storage has mostly been linked to infection of the basal plate that occurred in the field, with minimal spread between bulbs in storage.

Plants may be infected in the field, without the development of obvious symptoms. If conducive conditions occur as bulbs mature or after harvest, these infections may develop into FBR of bulbs. Monitoring of paddocks that developed FBR by time of harvest has found that FOC infection of some plants had usually occurred by the 2-4 leaf stage, with infection in these paddocks highly likely by the 5-7 leaf stage. Infection was not visually obvious during field inspection of these crops.

Early infections in the field, even if they do not progress to cause bulb rot, can reduce plant growth and increase susceptibility of infected plants to other diseases. High levels of FOC infection have been associated with reduced yield which is in addition to losses from rotted bulbs.

# Symptoms of Fusarium basal rot

Basal rot is most obvious late in the crop or in storage, particularly ambient storage. Field infection by pathogenic *Fusarium* spp. can occur at any stage from germination to harvest.

#### **Mature bulbs**

Basal rot, as the name suggests, starts from the base of the bulb. Symptoms at harvest may go unnoticed. When cut, basal plates of infected bulbs will usually have a brown discolouration. Bulbs with cracked or damaged basal plates are more likely to be infected than other bulbs.

As disease advances, basal plate tissue breaks down (due to the action of enzymes released by the pathogen) taking on a soft texture and light tan to brown colour. White cobweb-like growth (fungal hyphae) may be present around and on the basal plate. Infected bulb scales are light tan to brown in colour, flesh is broken down with a slightly watery appearance. Infection of bulb scales extends from the base of the bulb and may infect the entire bulb. Infected bulb scales may have pink to purple discolouration, most often observed towards the base when outer scales are removed. On white onions this can be obvious on the outer scales.

External symptoms at onset of bulb rot are shrinking and darkening of leaf scales around the basal plate. Eventually the onion bulb shrinks and collapses within the intact outer dry leaf scales.

Secondary infection by bacterial pathogens often contributes to breakdown of infected bulbs. Severely rotted bulbs attract infestation of the basal end by maggots.



Figure 1. Fusarium basal rot of mature bulbs

## Field symptoms in maturing crops

In the field, severely diseased plants can be seen from a distance. Diseased plants show dieback or yellowing of the leaf tips, which curl down or collapse. Leaves wilt and in severe cases plants die. Considerable decay of the roots and basal plate will have occurred by the time above-ground symptoms are visible.

When pulled up, infected plants may have poor root health (reduced number of roots, and roots that are browned and shrunken) or no roots. Closer observation will reveal discolouration and rot of the basal plate. In plants with advanced symptoms, the basal plate will detach easily from the bulb. Rot may extend into the base of the bulb leaf scales.

Depending on the time of infection, bulbs may not be fully formed at harvest, having an elongated shape.

Disease may occur on isolated plants or small patches of plants, which in turn are usually grouped in larger areas within a paddock. Under conducive conditions in heavily infested paddocks these areas can extend over large portions of the paddock.

FBR is more likely in areas with poor drainage but can also occur in well drained areas and drier areas of fields. Monitoring or scouting should focus on poorly drained areas in the first instance. Basal rot is most likely along wheel tracks where drainage is impeded, and soil movement occurs from the passing of machinery or irrigator.

Bulbs that do not rot until put in storage can have healthy appearing root systems at harvest.







Figure 2. Fusarium basal rot of young plants and developing bulbs.

## Damping off seedlings

Fusarium spp. can infect germinating seeds and seedlings, killing them before or soon after emergence. Other pathogens including *Pythium* spp. may cause similar symptoms. Plants with infected roots and basal plates that survive will lack vigour.

## Other possible causes of bulb rot

Correct diagnosis of the cause of bulb rots is important. Decay may not be the result of *Fusarium* spp. Rotting of the base of the bulb may also be caused by white rot (*Sclerotium cepivorum*). If bulbs are not exhibiting typical FBR symptoms (particularly rotting of the basal plate, which then extends into the bottom bulb leaf scales) other possible causes should also be investigated. If bulb rotting starts from the stem end, other diseases such as Botrytis neck rot (*Botrytis* spp.) or bacterial rots (Pectobacterium, *Burkholderia* spp., *Pantoea* spp.) are more likely causes. Decayed bulbs are frequently infected by more than one pathogen.

If unsure, consult with a plant diagnostic laboratory.

## Factors affecting F. oxysporum f. sp. cepae inoculum build-up

## History of Allium crops

Risk of FBR increases with the number of infected onion crops grown on a site and the shortness of rotations.

Allium crops including onions and shallots (*Allium cepa*), garlic (*A. sativum*) and leeks (*A. porrum*) are hosts of FOC.

## **Alternate hosts**

## **Crops**

FOC has been shown to persist on non-Allium crop species, even though they do not show any symptoms. These crops are sometimes referred to as 'reservoir hosts' or 'non-symptomatic hosts', assisting the pathogen to survive, and include: corn (Zea mays), black bean (Phaseolus vulgaris), oat (Avena sativa) and Sudan grass (Sorghum x drummondi). FOC infection of barley (Hordeum vulgare), lucerne (Medicago sativa), and faba beans (Vicia faba) has been detected in South Australian onion paddocks.

Contribution to FOC inoculum and disease risk by rotation crops used in Australian onion growing systems is not well understood.

#### Weeds

Oxalis corniculata (creeping oxalis) and Oxalis pes-caprae (soursob) are reported to host FOC and would assist in maintaining inoculum levels between onion crops. It is likely that other weeds host the pathogen. For example, FOC infection has been detected in roots of annual ryegrass (*Lolium rigidum*) and sow thistle (*Sonchus oleraceus*) plants in South Australia.

## **Factors affecting disease development**

## **Onion variety**

Onion varieties vary in their susceptibility to FBR, with plant breeding companies actively selecting varieties for their level of resistance. For further information refer to your seed suppliers.

## Soil type

FBR can occur across a wide range soil types. However, drainage is the main factor influencing disease development. Therefore, heavier soils or soils with subsoil constraints such as compaction are likely to have greater FBR problems.

Saline soil conditions also appear to favour disease.

### Soil moisture

Higher and lower than optimum soil moisture levels can increase the risk of Fusarium basal rot, especially when they persist for long periods and are accompanied with soil temperatures optimal for pathogen infection.

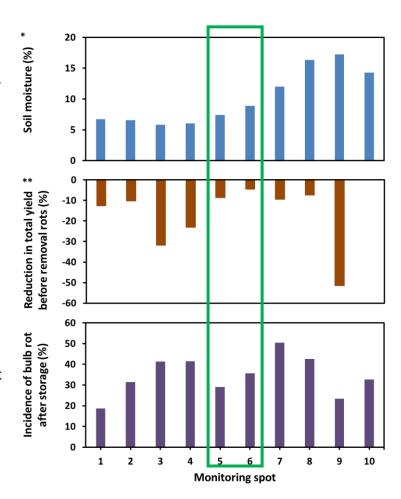
A trial in South Australia (figure adjacent) highlighted the impact of prolonged soil moisture differences in a centre pivot irrigated onion crop on the incidence of FBR.

Soil moisture conditions were considered close to optimal at monitoring locations 5 and 6, maximising yield and lowering incidence of FBR (green box).

Prolonged waterlogging which occurred in location 9 (low spot in pivot) was not favourable for either onions or FBR to develop.

High moisture but not waterlogged conditions such as in locations 8 and 10 supported high yields but were also conducive to FBR and bacterial bulb rots.

At the other end of the moisture spectrum, at locations 3 and 4 where plants suffered prolonged water stress, total yields were reduced by around 25%, and had a higher level of FBR when compared with locations having adequate moisture conditions.



- \* Sampling to characterise soil moisture differences undertaken at the driest point in the irrigation cycle.

  Irrigation was applied soon after sampling.
- \*\* Reduction compared to maximum total yield obtained at monitoring spot 10 (yield includes weight of rots).

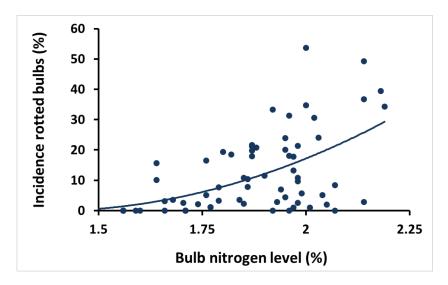
## Soil temperature

Infection and disease development is possible within the range 15-32°C but is favoured by higher temperatures within this range. FOC grows most rapidly at 26-28°C (optimal temperature). The pathogen has minimal activity at temperatures below 15°C. Infection and disease development can still occur above 32°C but is less likely.

Risk of FBR is greater for late plantings in spring when soil temperatures are higher. Under these conditions seedling damping off may also occur in paddocks if high levels of inoculum are present. Early infection (even without obvious symptoms) increases the risk of FBR developing later or in storage.

#### Plant nutrition

High nitrogen levels increase the risk of FBR.



Crop monitoring in South Australia found higher levels of nitrogen in tissue of harvested bulbs is associated with increased incidence of FBR. In the monitored paddocks lower nitrogen level in the harvested bulbs was not related to reduced yield. This suggests nitrogen application could be reduced in some situations to assist manage risk of FBR. When adjusting crop nutrition, potential impacts on yield and bulb quality need to be considered.

Note: Factors other than nitrogen application rate influence bulb nitrogen concentration.

Balanced nutrition that promotes even growth and maintains adequate calcium levels in the bulbs is important to producing onions with long storage potential.

#### Damage to roots and basal plate

Basal plates that have been damaged are more susceptible to infection. Root and basal plate damage can be caused by soil dwelling insects (particularly onion maggots) or nematodes. Cracking and splitting of the basal plate, such as that caused by fluctuating soil moisture levels, excessive nitrogen or weather conditions increases risk of FBR.

## **Summer thunderstorms**

Summer thunderstorms can produce ideal conditions for infection; that is, warm wet conditions coinciding with potential for cracking of the basal plate.

Table 1: Factors affecting development and impact of fusarium basal rot

Driver of disease	Decreased disease risk
high pathogen inoculum level	low pathogen inoculum level
susceptible variety	tolerant variety
root and basal plate damage	absence of pests
other soil pathogens present	absence of other soil pathogens
	<ul> <li>healthy soil biota</li> </ul>
<ul> <li>planting into warm soils</li> </ul>	<ul> <li>cool conditions during early growth</li> </ul>
<ul> <li>soil temperature &gt; 26°C</li> </ul>	<ul> <li>soil temperature &lt; 15°C</li> </ul>
excess nitrogen	optimum nutrition
excess moisture, poor drainage	<ul> <li>uniform adequate moisture availability</li> </ul>
<ul> <li>prolonged moisture stress</li> </ul>	availability
high salinity or sodicity	<ul> <li>low salinity and sodicity</li> </ul>
summer thunderstorms	mild consistent weather
ambient storage	cool storage

# Strategies to reduce disease development

Management strategies for FBR can be separated into three main areas:

- management of the paddock between onion crops
- management of the onion crop
- management of the harvested bulbs.

## Reduce inoculum - management of the paddock between onion crops

Paddock management is aimed at reducing inoculum build up prior to planting onions.

### **Crop rotation**

Infected onion crops are the main contributor to increases in soil inoculum levels of FOC. Effectiveness of rotations is compromised by the pathogen's ability to survive in soil without a host. Rotation lengths of four or more years are considered good practice to reduce the risk of FBR. To be of benefit, an even longer rotation may be required in paddocks with a high incidence of disease in the previous crop. Because FOC persists between hosts on organic matter, the efficacy of break crops will depend on the breakdown of suitable organic residues.

Selection of suitable non-host crops is limited by a lack of comprehensive information on which crops host FOC. Rotation and cover crops, even if potential hosts, are less likely to be infected by FOC when grown in cool conditions. Weeds in non-host rotations may act as a reservoir of inoculum and need to be managed effectively. It is critical to control *Allium* spp. and *Oxalis* spp. between onion crops, as they are known to host FOC.

## Manage risk – onion crop

## Timing of planting

Infection of seedlings is favoured under hot moist conditions. Paddocks that are known to be at high risk of FBR may be better suited to sowing in timeslots when soil temperatures are cooler (for example autumn-winter as opposed to spring plantings in South Australia). Risk of other diseases favoured by cool conditions at planting, such as onion stunt, need to be considered.

#### Plant nutrition

Onion plants with above optimum levels of nitrogen are more vulnerable to FBR. Ensure adequate nitrogen nutrition throughout the crop.

## Irrigation and drainage

Risk of FBR is lowest when optimum soil moisture maintained. Over irrigation should be avoided as FBR is favoured by wet soil conditions. Prolonged low soil moisture reduces yield and also increases risk of FBR. Use best practice irrigation technologies, such as soil moisture monitoring linked to crop water use and predicted weather conditions. Onions grown on raised beds may have a lower incidence of FBR.

Improvement of drainage using land planning and/or installation of surface and subsurface drainage infrastructure can improve soil moisture and water seepage management. This can result in reduced risk of FBR, as well as increased uniformity leading to higher overall productivity and quality of onions produced.

Soil amendments should be applied to improve uniformity of soil moisture and irrigation efficiency at sites with non-wetting soils, inadequate infiltration rates or sodicity.

Depending on the soil types and topography, use of variable rate irrigation may assist in achieving more uniform soil moisture conditions across the paddock.

Monitor salt levels in irrigation water, soil and soil water to refine irrigation practices to manage excess buildup of soil salinity.

## **Biological control**

Several biological control organisms have demonstrated ability to reduce the incidence of FBR including strains of:

- Trichoderma harzianum
- Trichoderma viride
- Bacillus subtilis
- Pseudomonas fluorescens.

Arbuscular mycorrhizal fungi populations which can be beneficial to onions may also assist the plant's resilience to disease.

Achieving meaningful disease control can depend on the suitability of each biocontrol strain to sitespecific field conditions and the level of disease pressure. Information on products containing these organisms relating to control or suppression of FOC in onions under Australian conditions is limited.

#### Chemical control

Correctly applied fumigation is effective at reducing *Fusarium* spp. inoculum in the soil. If fumigation is used, it is advisable to replace the beneficial soil biota as soon as possible. There are many commercial products available; however, none have been tested for this purpose in onions.

Growers need to be aware that the introduction of pathogens back into sterilised soils can result in higher levels of disease, due to the lack of competition.

There are currently no fungicides registered in Australia for the control of FBR in onions.

## Manage risk – harvested crop

## Harvesting practices

Minimise mechanical damage to bulbs during harvest.

Do not dispose of infected (cull) bulbs in paddocks used for onion production.

## Storage conditions

Ambient storage conditions can favour progression of basal rot, with rapid progression of symptoms at temperatures between 25 to 32°C.

Activity of the pathogen is reduced at temperatures of 8-15°C, with disease progression inhibited at long term storage temperatures of 4°C and below. Storage facilities require adequate ventilation and low (65-70%) relative humidity.

Check for disease and infected basal plates prior to harvest and avoid storing bulbs from paddocks where field infection detected. qPCR testing can assist risk assessment if non symptomatic infection suspected.

# Fusarium basal rot – Summary of management strategies

## Know your risk

- Conduct pre-plant disease risk assessment of paddocks (history, environment).
- Manage paddocks using best available practices and according to risk.

## Pre-crop

- extend rotation length in paddocks with known risk: > 4 years
- control host weeds: Oxalis spp., Allium volunteers
- improve surface and subsurface drainage if inadequate
- grow non-host cover crop and incorporate prior to onions
- in fumigated soil, follow with soil amendments to replace beneficial biota and improve suppression

### Crop

- select less susceptible varieties
- manage other pests and pathogens that cause root damage, such as onion maggot
- promote even growth to protect integrity of basal plate
- optimise nutrition for balanced growth and bulb quality: avoid excess nitrogen
- · avoid over irrigation and development of wet spots
- · avoid prolonged periods of low soil moisture
- plant into cooler soils where possible (<20°C)</li>

## Storage

- avoid storing bulbs with high incidence of field infection
- store crop in cool storage at 4°C or lower, with low humidity and ventilation management to avoid condensation

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## Appendix 3 – Articles published in the Australian Grower

R&D | BASAL ROT IN ONIONS





Basal rot symptoms in onions. Below, Michael Rettke of SARDI in the field.

## UPDATE

# Understanding and managing fusarium basal rot in onions

The project, Epidemiology and management of fusarium basal rot in onions (VN20006) is well underway with researchers undertaking field trials and testing to understand the role of fusarium in basal rot and develop management strategies for this major disease for the onion industry in Australia.

The aim of the three year project is to develop an integrated pest and disease management strategy to reduce the impact of fusarium basal rot in onions. Infection can cause seedling losses in the field, but is more problematic late in the crop and during storage.

Fusarium is a soilborne fungus with many species, primarily existing as spores that can remain in the soil for a number of years. To date, the role of Fusarium species and their relationships in the Australian onion industry are not well understood.

Overseas, fusarium basal rot has been a problem for many years including in the USA and Netherlands, and also in shallot onions grown in Asia. It is now becoming more apparent in South Australia, and to a lesser extent in other hot climate production areas of Australia. In cooler climate production areas such as Tasmania, fusarium basal rot is less of a problem.

Project lead, Michael Rettke, of SARDI says that most researchers believe the pathogen to be a specific strain of Fusarium oxysporum, but there have been reports of other Fusarium species/ strains causing basal rot of onions. Part of the project initial aims was to determine which Fusarium species is the primary cause of basal rot in Australia.

"We have undertaken a great deal of sampling of onions with basal rot symptoms and used DNA testing of infected tissue, as well as isolation of the pathogens and sequencing to determine which Fusarium species is the cause. Fusarium oxysporum f. sp. cepae has been confirmed as the main species associated with basal rot of onions in Australia. Through the testing capability developed we can now routinely test for and monitor levels of the main species causing fusarium basal rot in Australia," said Michael.

Infection can occur at any time during growth. The tests and trials conducted show that the basal plate normally becomes infected before the bulb scales. It then may take months for the bulb scales to become affected, bulb rot

often revealing itself during storage.
The infection of the basal plate at harvest is usually visibly obvious, however the tests that the SARDI researchers have developed can be used to detect the presence of the pathogen.

"The preliminary results also showed that it is slightly more complicated than we anticipated. High levels of another closely related strain of Fusarium oxysporum are sometimes present in the roots and basal plate of onions and may impact crop health and increase likelihood of basal rot caused by Fusarium oxysporum f. sp. cepae occurring. However, this other strain by itself does not usually seem capable of entering the onion bulb beyond the basal plate."

Impact on disease and productivity by this other strain is being further investigated.

"Our field trials have been conducted in the Murray Mallee and SE of South Australia" Michael said, adding that he

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prefers to have trials done in the field, to better understand what happens with natural variations in environmental. conditions, and crop management in commercial production systems.

"We conduct assessments soon after planting, during the growth phases, and at harvest. We have also put onions in storage for three months to monitor for basal rot occurrence. In addition, we use moisture probes in the soil, conduct nutritional analysis and assess agronomic inputs to see what is going on. This will give us a better understanding of the drivers of the disease, how it progresses and where, and why some paddocks and regions have a greater problem than others."

#### Michael suspects that moisture conditions and drainage are part of the reason for the pathogen's prevalence in certain areas.

It is also known that some onion varieties are less susceptible to fusarium basal rot. In some cases, pink root has also been found together with basal rot; this is under further investigation to see if the two diseases are interacting. That said, high levels of fusarium basal rot have been observed in paddocks with low pink root pressure.

Right. Basal rot in onions.

#### The next phase of research

"As we progress through and understand the pathogen better, we have gained greater clarity on what strategies can be used to combat the fungus.

\*Beside investigating the effect of irrigation practices, crop rotation and host crops, we will be looking at testing the effectiveness of biological control and chemical treatments. There are no registered chemical products for fusarium basal rot control in onions currently available."

With grower input and irrigation practices varying considerably, field trials will be used to assess how varying inputs such as irrigation can affect risk and progression of disease. It is also known from other crops that higher N levels encourages fusarium to flourish - there have been reports of both ammonia and nitrate forms of N increasing disease incidence.

Crop rotations will be important as the fungus can survive for around four years in the soil, but host plants are yet to be properly investigated. Research from South Africa has shown that the weed oxalis, is an alternative host.



This project has been funded by Hort Innovation using the onion research and development levy and funds from the Australian Government





"Now that we have an understanding of the research that has been done to date overseas, and identified the major cause of the disease here in South Australia, we can conduct field trials, with a focus on crop rotations, and adding the use of biologicals, bio-stimulants and chemicals where we can to develop management strategies that may help the grower."





# Crop monitoring to understand the drivers of fusarium basal rot development in onions

The project Epidemiology and management of fusarium basal rot in onions (VN20006) is developing an integrated pest and disease management strategy to reduce the impact of fusarium basal rot in onions. This requires an understanding of the pathogen, disease development and impacts of grower management.

To gain insights into the drivers of disease development twelve centre pivot irrigated crops were monitored in the 2022 planting season in South Australia. Incidence of fusarium basal rot in these mid to late season crops ranged from nil to 55% at harvest. Drivers investigated included inoculum, soil moisture, nutrition, soil biology and variety and there are some characteristics in common from the sampling areas with high levels of fusarium basal rot (Table 1).

In the monitored crops, the level of infection at harvest was measured with SARDI's DNA test for Fusarium oxysporum f. sp. cepae, and was strongly related to the incidence of fusarium basal rot observed. This indicates that this is the dominant pathogen causing the disease (Figure 1).

In some crops, incidence of rotted bulbs further increased when placed in ambient storage conditions for three months; disease progression under these circumstances can be slowed by cool storage below 4°C. DNA testing of a composite sample of the roots and basal plates of 100 bulbs at harvest was able to predict which samples would have fusarium basal rot develop during ambient storage, even when observed incidence was low or nil at harvest (*Figure 2*). For these samples, DNA testing provided a better indication of fusarium basal rot developing in storage than observed rotted basal plates at harvest.

Top. Typical rot development caused by Fusanium oxysporum f. sp. cepae in the field. Image courtesy Michael Rettke, SARDI.

Hort ONION Innovation FUND

This project is funded by Hort Innovation using the onion research and development levy and funds from the Australian Government.

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TABLE 1 Associations found between incidence of fusarium basal rot and monitored parameters in 12 crops.\*

CROPS WITH LOW INCIDENCE	CROPS WITH HIGH INCIDENCE
Inoculum	
Fusarium basal rot not observed in previous crops     Furnigated prior to planting	Known history of fusarium basal rot     Confirmed infection early in crop

Prior occurrence of fusarium basal rot in a paddock indicates a disease risk for the next crop, especially if it occurred less than 4 years ago. Disease risk is likely related to the level of inoculum in the soil. However, using the soil sampling and testing procedures investigated so far, the DNA test developed for *Pusarium oxysporum* f. sp. cepae is not sensitive enough in soil to provide an accurate indication of disease risk. Some paddocks have a higher disease risk than expected from knowledge of previous crops.

Soil Moisture	
Good drainage	Periods of high/excess soil moisture

Soil moisture probes were installed to compare locations within and between crops. Prolonged excessive soil moisture early in the crop was associated with high levels of infection in crops grown in pivots where inoculum was present. This high soil moisture early in the crop was due to unseasonably high rainfall and site-specific drainage characteristics, rather than over-irrigation.

Infection can occur early in crops. Last season, in sites where high levels of disease developed, infection was present in composite plant samples tested at the 5-7 leaf stage. In some crops where disease developed, infection of plants was present prior to the 3-4 leaf stage. Infection was not visually apparent at these stages of crop development in the composite samples tested. Infection by Fusarium oxysporum f. sp. cepae was also confirmed in selected individual plants that were showing leaf dieback and browning around the basal plate at the 3-4 leaf stage.

Monitoring within crops showed the incidence of fusarium basal rot was generally elevated by high soil moisture levels in the upper soil profile compared to areas where soil moisture was closer to optimum. High rainfall/storm events have been associated with high incidence of disease, though this was not observed in monitoring sites where this occurred last season and requires further investigation

Nutrition	
↑ Calcium levels     ↑ Soil effective cation exchange capacity (ECEC)	

Soil nutrient analysis was conducted on samples taken soon after planting and tissue nutrient analysis performed on bulb samples after harvest. These associations indicated above between fusarium and plant nutrition are not unexpected. They, along with weaker associations found with other nutrient levels, warrant further investigation of their importance to management of infection and disease development.

Soil Biology	
Arbuscular mycorrhizal fungi (AMF) detected on roots at harvest	Levels of AMF low or below detection

Biologicals are commonly applied to onlon crops with the aim of promoting soil and root health. DNA testing was used to quantify levels of six groups of AMF and two groups of Trichoderma on composite root tissue samples at the 3-4 leaf, 5-7 leaf and harvest growth stages. The tests utilised do not identify specific species present. AMF populations which can be beneficial to onlons may also assist the plant's resilience to disease. Our results support these associations. Trichoderma group A and group B were detected in some samples. Species of Trichoderma including strains of T. harzianum (group A) and T. viride (group B) have been reported elsewhere to reduce the incidence of fusarium basal rot

Variety	
Lower susceptibility	Susceptible

Development of disease was consistent with expectations of the disease susceptibility of varieties planted. Direct comparisons were not made between varieties in the same plantings.

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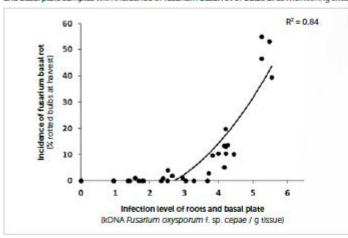
<sup>\*</sup> These observations and associations are based on monitoring 12 crops in the 2022 planning season in South Australia. The findings require further investigation to establish if cause and effect relationships exist between these parameters and the incidence and severity of fusarium basal rot.



CONTINUED FROM PAGE 79

Targeted monitoring of crops will be undertaken in the 2023 planting season in South Australia to build on these first year findings, along with causeand-effect trials to investigate source and level of nitrogen application, and simulated high rainfall events to investigate soil moisture impacts.

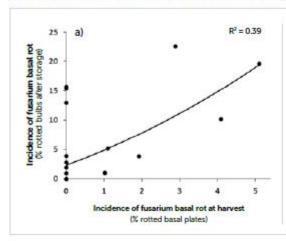
FIGURE 1. A Relationship between Fusarium oxysporum f. sp. cepae infection levels of root and basal plate samples with incidence of fusarium basal rot of bulbs at 12 monitoring sites.

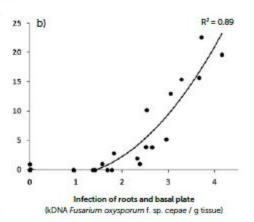


Basal rot bulb cut open

Image courtesy Michael Rettke, SARDI.

FIGURE 2. Comparison of (a) visual assessment with (b) DNA testing conducted at harvest to predict disease development after 3 months' ambient storage. Sites had less than 5% incidence fusarium basal rot at harvest.







# Harvest of onion trials in SA continue to shed light on detection and management of onion basal rot



Leaf tipping due to basal rot.

The project Epidemiology and management of fusarium basal rot in onions (VN20006) aims to develop an integrated pest and disease management (IPDM) strategy to reduce the impact of fusarium basal rot in onions.

Infection of onion bulbs in the field by soil borne fusarium basal rot is known to result in substantial losses before harvest and in storage, however disease epidemiology was not well understood, which has limited the development of an appropriate management strategy.

In order to develop a best practice, cost-effective IPDM strategy, the project is working toward understanding of the pathogen and its epidemiology, and evaluate the use of cultural, biological and chemical controls.

Now in its final year, the project has identified Fusarium oxysporum f. sp. cepae (Foc) as the main cause of fusarium basal rot symptoms of onions in Australia.

During the 2022 and 2023 planting seasons, to understand key drivers of disease development, crops were monitored in paddocks that had a range of rotation and production practices, soil moisture conditions, and varieties. Incidence of bulb rot associated with Foc species ranged from nil to 55 per cent in monitored areas.

Testing of samples in the 2022 season at the five to seven leaf stage indicated that Foc infection can already have occurred, though may not be visually evident until late in the crop or storage. Subsequent testing this season has found some infection is already present at the one to two leaf stage, indicating control options may need to be applied at planting or early in the crop.

One key outcome of the research is that managing soil moisture to limit prolonged high or low soil moisture is required to optimise yield and reduce the risk of fusarium basal rot. Results from the first season of monitoring highlighted the impact that high and prolonged soil moisture can have on increased incidence of bulb rots caused by Foc and bacterial infection.

Trials and monitoring in the second season found that development of basal rot can also be favoured by persistent lower than optimum soil moisture through the duration of the growing season, that is at soil moisture levels at which some yield reduction occurs. In addition, while high and prolonged soil moisture supports basal rot development, continuously waterlogged conditions appear to be too wet for high levels of disease. Such conditions dramatically reduce yield and can favour bacterial rots.



The aggressiveness of Fusarium spp., including multiple Foc isolates, was assessed using three techniques (basal plate inoculation, injection into bulbs and injection in leaf scales) in controlled environment room studies. Results confirmed that inoculation with Foc produced similar symptoms to those visually identified in the field. Aggressive isolates were then used to screen chemistries for their ability to inhibit Foc infection of seedlings under controlled conditions. This assisted in selection of chemistries for field evaluation.

FIND OUT MORE

Fusarium Basal Rot guide horticulture.com.au/contentasse ts/32b961e9d8a947618188cdc83f832dfd/fusarium-basalrot-guide-june-2022.pdf

This project has been funded by Hort Innovation, using the orion 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. Project Number: VM20006

Hort ONION FUND

This season, field trials were set up to assess efficacy of products applied as seed treatments, in furrow or post planting band or boom sprays. Trials were conducted on mid to late season brown onions in centre pivot irrigated paddocks where inoculum of Foc had been confirmed in the soil by pre-plant DNA testing. These trials have provided promising results with some treatments that support progressing towards commercial availability.

The final phase of the project is to complete the assessments of the 2024 harvest samples, including after ambient storage of bulbs. Project findings including on soil moisture, nitrogen nutrition, rotation management, timing of management strategies will be incorporated into a new version of the fusarium basal rot management guide.

Above. Monitoring efficacy field trials



With no fungicides currently registered in Australia for control of fusarium basal rot in onions, controlling soil inoculum levels is crucial for effective management.

The project Epidemiology and management of fusarium basal rot in onions (VN20006) aims to develop an integrated pest and disease management (IPDM) strategy to reduce the impact of fusarium basal rot in onions.

Fusarium basal rot is one of the most significant soilborne disease threats to onion production in warm production regions of Australia. Management of fusarium basal rot requires an integrated approach targeted at reducing the buildup of disease risk in the soil (soil inoculum) and managing the crop to reduce likelihood of disease infection and development.

Conditions that have been associated with increased disease risk of fusarium basal rot include short rotations and previous occurrence of disease, prolonged lower or higher than optimum soil moisture levels, uneven crop growth, high bulb nitrogen levels, salinity issues, growing mid to late season crops, and susceptible varieties.

#### Soil inoculum - disease risk

Fusarium oxysporum f. sp. cepae (FOC) has been identified as the main pathogen that causes fusarium basal rot of onions in Australia. Spores of FOC produced on infected onions can remain in the soil for at least four years, with the potential to infect the next onion crop. Extending rotation length beyond four years is beneficial to reducing the risk of fusarium basal rot. In paddocks with a high incidence of disease in the previous crop, rotations longer than four years may be required to be of benefit.

Testing as part of this project found root infection by FOC can occur on crops that are grown in rotation with onions (such as cereals and legumes), along with weeds (including annual ryegrass and sow thistle). However, based on quantitative DNA testing of roots, cereal and legume rotation crops do not have the potential to cause the large increase in soil inoculum that occurs when an infected onion crop is grown.

They are sometimes referred to as reservoir hosts, as they help maintain inoculum levels in the soil, rather than build them up. Rotation crops infected by FOC do not show obvious symptoms. These rotation crops are usually, but not always, grown in winter when conditions are less favourable for infection and growth of FOC. Temperatures of 26-28°C are considered optimum for growth of FOC, with minimal growth below 15°C.

Barley used as a nurse crop for late season onions showed higher levels of infection than winter grown crops, and this is likely due to that higher soil temperature. When crops such as barley and faba beans were inoculated with FOC and grown in controlled environment rooms at 26°C, higher levels of root infection were detected than on roots of the same crops collected from winter grown crops in naturally infected fields.

Limited testing of winter grown canola from an infected field site suggested it is a poor host of FOC. However, canola is a good host of the nematode Pratylenchus neglectus, which poses a significant yield risk to onion production. Additionally, canola does not support arbuscular mycorrhizal populations that are already present in the soil of some onion production systems and can be beneficial to onions.

Choosing rotation and cover crops that have positive effects on soil condition and microbiology is important prior to planting onions, and this should be considered along with their potential impact on inoculum levels of soilborne diseases. Other aspects to be considered include their suitability for conditions and time of year to be established, compatibility with weed control strategies and ability to provide ground cover.

Long-term studies are required if we are to more thoroughly understand the impact that specific rotation and cover crop strategies have on the risk of soilborne diseases, including fusarium basal rot, on soil health and on onion productivity more generally.

#### Reducing crop risk

Project results indicate the importance of managing irrigation and plant nutrition as part of an integrated program to reduce the risk of fusarium basal rot.

Monitoring of crops and trials in South Australia has identified relationships between soil moisture level and the incidence of fusarium basal rot. Both higher and lower than optimum soil moisture levels can increase the risk of fusarium basal rot, with an example of findings presented in Figure 1.

Soil moisture conditions were considered close to optimal at spots five and six, maximising yield and lowering incidence of fusarium basal rot. Prolonged waterlogging which occurred in spot nine was not favourable for either onions to grow or fusarium basal rot to develop. Total yield was reduced by approximately half at spot nine. At spots eight and 10, soil moisture was high but not waterlogged.

These conditions supported high yields but were also conducive to diseases such as bacterial bulb rots, as well as fusarium basal rot. At the other end of the moisture spectrum at spots three and four, where plants suffered prolonged water stress, total yields were reduced by around 25 percent, and had a higher level of fusarium basal rot when compared with spots having optimum moisture conditions. Management of both irrigation and nitrogen are a critical part of maintaining even growth of the onion crop, helping protect the basal plate from infection.

Crop monitoring has revealed an association between higher bulb nitrogen level at harvest and higher incidence of fusarium basal rot, as presented in Figure 2. Lower nitrogen level in the harvested bulbs was not related to reduced yield, suggesting nitrogen level was not yield limiting within the range monitored in these commercial crops

This indicates there is scope to manage nitrogen to reduce risk of fusarium basal rot without jeopardising yield, i.e. applying sufficient but not excessive nitrogen will reduce fusarium basal rot without limiting yield potential.

Other bulb quality attributes need to be considered when adjusting fertiliser programs. The level of nitrogen in the bulb was not solely influenced by the nitrogen application rate, which indicates that other crop management practices and soil conditions are important drivers of bulb nitrogen status and the crops susceptibility to fusarium basal rot.

Management of both irrigation and nitrogen are a critical part of maintaining even growth of the onion crop, helping protect the basal plate from infection. Sudden growth spurts or plant stress may increase risk of fusarium basal rot developing if the pathogen is present and environmental conditions are suitable.

#### Treatment options

There are currently no fungicides registered in Australia for the control of fusarium basal rot in onion crops. Several active ingredients and formulations have been demonstrated to suppress the disease in trials conducted as part of this project.

These results provide support for progressing some treatments towards commercial availability.

A number of biological products that are available to growers in Australia contain micro-organisms (e.g. Trichoderma harzianum, T. viride, Bacillus subtilis, Pseudomonas fluorescens. Funneliformis mosseae) for which overseas studies have demonstrated their ability to reduce the incidence of fusarium basal rot in field trials. Achieving meaningful disease reduction depends on the suitability of each biocontrol strain to site-specific field conditions and the level of disease pressure

Monitoring of crops has shown that infection can be present at the seedling stage, even though symptoms may not be visible until close to harvest. Monitoring in mid to late season grown onions has shown that most infection of plants in the crop appears to have already occurred by the time of bulb initiation. This indicates implementation of control strategies needs to start early in the crop, if not at or prior to planting.

Where inoculum levels of FOC in the soil are high and conditions are favourable for disease development, fusarium basal rot can lead to substantial losses or even crop failure. In these situations, fumigation is an option to reduce the level of inoculum in the soil prior to planting. Such treatments should be followed up with practices to re-establish a favourable soil biology for onions and the implementation of other management practices as part of an integrated strategy to reduce disease risk.

development, including

The pathogen can also cause

seedling damping off, root infection and bulb rot early

in the crop. Monitoring data collected from 20 crops

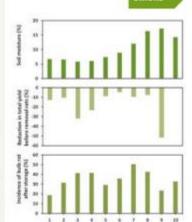
indicates that high infection

levels of FOC are associated

with yield losses of up to 25

ercent, over and above the alb rots observed at harves

prior to emergence.



and topography on, a) soil moisture as record prior to harvest, bil reduction in total bulb yield at harvest (before removal of rots) compared to highest yield recorded a monitoring spot 10 and, c) the cumulative incidence of rotted bulbs caused by fusarium basal rot after two months ambient. storage. (Spot nine was waterlogged for prolonged periods, each monitoring spot had three replicates).

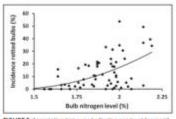


FIGURE 2. Association between bulb nitrogen is measured at harvest and the incidence of fusar rot at monitoring locations assessed within 20 crops in

Effective management of fusarium basal rot involves reducing the buildup of inoculum or its reduction before planting, coupled with managing the crop in ways that reduce likelihood of disease infection and development, including irrigation, nutrition and soil health management.



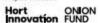
Scan QR code for access to the Fusarium basal rot guide by Michael Rettke.



#### LEARN MORE

Find out more in the Fusarium Basal Rot guide: horticulture.com.au/contentassets/32b961e9d8a947 618188cdc83f832dfd/fusarium-basal-rot-guide-

Epidemiology and manage ent of fusarium basal rot in onions has been funded by Hort Innovation using the onion research and development levy and contributions from the Australian Governme Project VN20006



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Fusarium basal rot (as the name suggests) is typified by the rott-ing of bulbs starting from the basal plate. Bulb rotting usually starts to become obvious late in the crop, with leaf tipping an indicator of diseased plants. In other cases, bulb rots may only develop after harvest during storage.

Infection of onions by FOC

