Reducing the impact of Black Dot on Fresh Market Potatoes

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Project Number: PT06014

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This report is published by Horticulture Australia Ltd to pass on information concerning horticultural research and development undertaken for the potato industry.

The research contained in this report was funded by Horticulture Australia Ltd with the financial support of South Australia Research & Development Institute (SARDI).

ISBN 0 7341 1951 8

Published and distributed by:
Horticulture Australia Ltd
Level 7
179 Elizabeth Street
Sydney NSW 2000
Telephone: (02) 8295 2300
Fax: (02) 8295 2399

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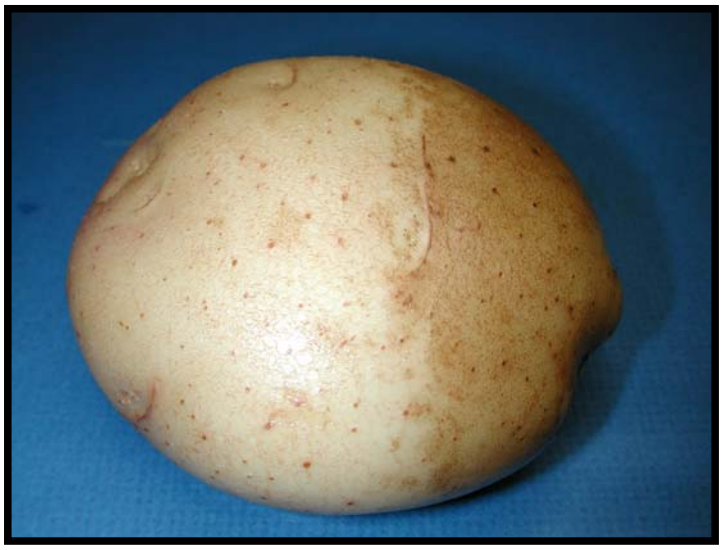
Final report

Horticulture Australia Limited

PT06014

January 2009

By Amanda Benger, Robin Harding, Barbara Hall and Trevor Wicks
South Australian Research and Development Institute



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Government of South Australia

Horticulture Australia Limited project - Final report January 2009

Project title: Reducing the impact of black dot on fresh market potatoes

HAL project number: PT06014

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This report presents results of trials evaluating a DNA based assay for the detection and quantification of *Colletotrichum coccodes* DNA in soil, and evaluating fungicides, organic or biological control agents, for the control of black dot.

This project was facilitated by HAL and funded by contributions from various companies and potato growers including: agrochemical companies (Syngenta, Nufarm Ltd, Northern Quinoa Corp.) and growers/companies (Comit Farm Produce, Golden Sunrise Fresh produce, Mondello Farms, Zerella Holdings, South Australia Potato Industry Trust and The South Australia Potato Company). The Australian Government provides matched funding for all HAL's R&D activities.

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1. MEDIA SUMMARY

Black dot (caused by the fungus *Colletotrichum coccodes*) is recognised as a disease with serious economic consequences for the fresh market potato industry in South Australia. This disease is distributed worldwide and in all Australian states that grow potatoes.

This project investigated two main themes; the first was to evaluate a DNA based assay for the detection and quantification of *C. coccodes* DNA in soil. This involved testing the ability of the assay to detect and quantify DNA in soil. It was found that the assay could detect and quantify *C. coccodes* DNA in soil at concentrations from 1.8 pg DNA/g soil to 8494 pg DNA/g soil.

The results from the molecular assay show promise for developing predictive tests to assist growers in managing the disease by determining *C. coccodes* levels in soil pre-planting.

The second theme was to evaluate fungicides, organic or biological control agents, for the control of black dot. These were applied at various rates, timings and method of application in shadehouse or field trials. The fungicides Amistar® followed by Cabrio® applied in-furrow resulted in the lowest incidence and severity on progeny tubers. There was some level of disease control observed in shadehouse trials with non chemical seed treatments of *A. ustus*, Heads Up® or acetic acid.

Currently there are no fungicides registered for the management of black dot, however the data generated from this project may assist in the registration of one or more chemicals for black dot control.

The trial results also showed that planting infected seed into infected soil resulted in high levels of disease. No treatment effectively controlled black dot infection where high disease pressure existed. Therefore to minimise disease it is recommended to plant non-infected (clean) seed into soil with low levels of *C. coccodes* DNA in soil, combined with appropriate fungicide treatments.

It is important to manage disease levels in potato crops, as it not only provides benefits for reduced tuber blemish, but also reduces soil inoculum for further crops**.**

2. TECHNICAL SUMMARY

Black dot of potatoes caused by the fungus *Colletotrichum coccodes* is a tuber blemish disease that results in significant downgrading of produce. The importance of this disease has increased in the past decade due to retailing of washed fresh market potatoes. This research addressed disease management, in particular: fungicide, biological and organic products for black dot control and the use of a molecular assay for detection of *C. coccodes* in soil. Some fungicide treatments performed well in trials whereas the results for biological and organic treatments were variable.

Major findings of this study were:

- The primers used in DNA testing (TaqMan RT-PCR) to detect *C. coccodes* were validated on 36 local and interstate isolates.
- The DNA assay detected and quantified *C. coccodes* DNA in a variety of soils in concentrations: from 1.8-8494 pg DNA/g soil.
- This assay was able to detect and quantify *C. coccodes* within the range of DNA levels found thus far in South Australian potato paddocks. Further work is required to develop disease thresholds.
- The molecular assays consistently showed a strong positive relationship between the amount of sclerotia inoculated into soil and the amount of DNA extracted.
- Larger sclerotia contained higher levels of DNA per sclerotia compared to smaller sclerotia. Further work is required to confirm this result.
- A positive relationship existed between the amount of *C. coccodes* sclerotia in soil and the final amount disease when seed was planted into soil with increasing concentrations of *C. coccodes*.
- The amount of *C. coccodes* DNA in soil in a plot (*in situ*) increased up to 24 fold over a potato-growing season when infected seed was planted into infested soil.
- The increase in soil inoculum between planting and harvest was greatest when infected seed was planted into non-infested soil.
- Planting seed with high levels of infection, or planting into heavily infested soil, resulted in high levels of disease.
- Shadehouse trials showed Amistar® (250 g/L azoxystrobin) when applied at 40 mL/100 m row to mimic in-furrow application consistently provided the highest disease control when planted in various combinations of seed and soil infection. These included: infected seed in non-infested soil and infected seed in infested soil.
- Amistar® applied at 10 or 20 mL/100 m or Cabrio® applied at 40 mL/100 m row applied to soil at planting to mimic in-furrow application also provided good disease control in some of the seed and soil combinations.
- Where low to moderate disease pressure existed in the field, in-furrow applications of some fungicides provided control.
- Where high disease pressure existed none of the treatments provided effective control of black dot.

3. INTRODUCTION

3.1 Literature review

3.1.1. Black dot disease significance and symptoms

Black dot is distributed in most parts of the world where potatoes are grown, including Africa, Australasia, Europe and North, Central and South America (Mordue 1967). This disease is also present in all potato growing states in Australia.

Disease occurs on stems, roots, stolons, foliage and tubers and is characterised by many tiny black sclerotia that present as black dots on plant tissue (Tsror & Johnson 2000) (Figure 3.1).

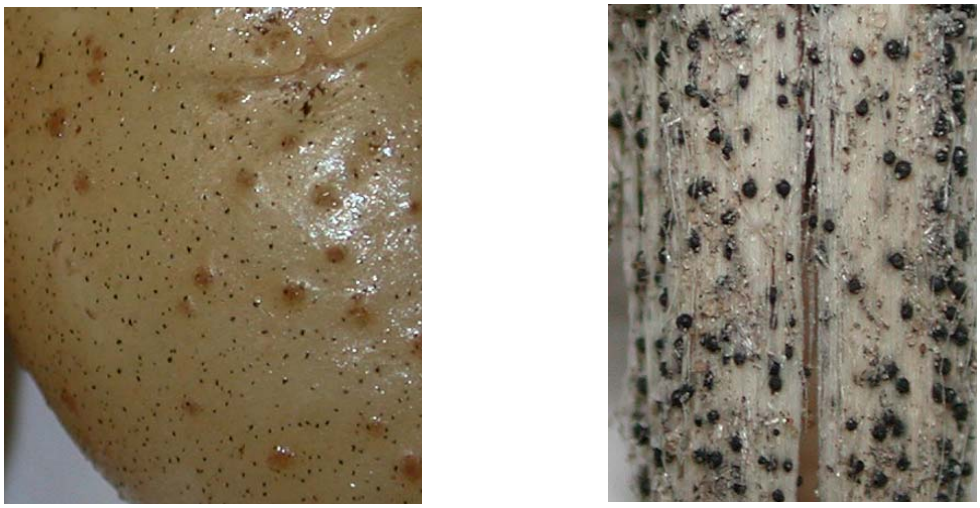

Figure 3.1: Potato tuber surface with black dot infection (L) and potato stem with black dot infection (R); sclerotia of *C. coccodes* are visible as small black dots all over the tuber surface and are found on stems both on the surface and internally.

Black dot is predominantly a tuber blemish disease causing discolouring of tuber surfaces. Due to consumers' increased demand for blemish-free tubers, black dot has emerged as a very serious economic disease (Andrivon *et al.* 1997; Carnegie *et al.* 2003; Lees & Hilton 2003 and Nitzan *et al.* 2002).

Black dot has also been associated with yield loss in Washington USA and Israel; up to 12% in the field and 43% in glasshouse trials (Johnson 1994 and Tsror *et al.* 1999) although little investigation into yield loss has occurred in Australia. Yield loss is thought to occur due to premature senescence of stem and root tissue (Mohan *et al.* 1992) and rotting of plant tissue underground (Dillard 1992). Therefore, there is less opportunity for root uptake of water and nutrients and if foliage is chlorotic and wilted, there is reduced potential for photosynthesis (Powelson & Rowe 1993).

Figure 3.2: A coliban tuber heavily infected by the blemish disease black dot.

Black dot has often been mistaken for silver scurf (*Helminthosporium solani*) due to silver lesions, similar to black dot, that can occur on tuber surfaces (Nitzan *et al*. 2002; Lees & Hilton 2003 and Gudmestad 2003). In the past this has meant that the importance of black dot has not been truly understood and therefore, targeted disease management has not occurred.

3.1.2. Characteristics of causal agent

Black dot is caused by the fungus *Colletotrichum coccodes* (Wallr.) Hughes, a Deuteromycete (Agrios 2005) that produces acervuli (up to approximately 300 µm diameter) and sclerotia (Mordue 1967). Conidia are cylindrical, hyaline with obtuse ends and have dimensions of approx. 16-24 x 2.5-4.5 µm (CMI 1967).

C. coccodes grown on potato dextrose agar (PDA) usually has evenly distributed sclerotia over the colony and sparse whitish aerial mycelium (Mordue 1967).

Recent research has identified eight Vegetative Compatibility Groups (VCG's) of *C. coccodes* (Nitzan *et al*. 2002 and Shcolnick 2007). VCG's are sub-groups within a fungal species that are identified by the ability of two or more isolates to fuse (or anastomose) and form stable heterokaryons (Leslie 1993). It is thought that genetic differences can exist between VCG's due to the retention of similar genetic information within a VCG (Leslie 1993). There have been several studies on *C. coccodes* identifying variation between different VCG's sensitivity to fungicides (Aqeel 2007), pathogenicity (Nitzan *et al*. 2002; 2006 and Aqeel *et al.* 2008) and morphology (Aqeel *et al*. 2008).

3.1.3. Epidemiology of black dot on potatoes

Sclerotia of *C. coccodes* are thought to be the primary means of survival and dissemination of the fungus (Lees & Hilton 2003). Seed-borne infection is the means of introducing this disease to previously uninfected soil (Cullen *et al.* 2002 and Lees & Hilton 2003). Sclerotia are able to survive in soil for at least 8 years (Dillard & Cobb 1998) and once soil is infected, any subsequent crop has the potential to become infected by *C. coccodes* and therefore can increase the soil inoculum level. Therefore, even with crop rotation of 8 years, it is likely that subsequent potato crops could come

into contact with sclerotia of *C. coccodes*. Many weed spp. commonly found in South Australia, including fat hen and heliotrope, have been found to host *C. coccodes* (Harding *et al.* 2004).

3.1.4. Life cycle of *C. coccodes*

The fungus is introduced to potatoes on (or in) seed (Lees & Hilton 2003) or through soil-borne inoculum (Nitzan 2006 and Johnson *et al.* 1997). After potato plants are desiccated, symptoms develop very quickly on haulms (Lees & Hilton 2003) and sclerotia can survive on crop debris and in soil (Lees & Hilton 2003). Once a host crop is planted it is thought that plant root exudates stimulate the myceliogenic (mycelial) germination of sclerotia and then infection occurs (Tu 1980). In a study on the ontogeny of sclerotia of *C. coccodes*, (Tu 1980) found that sclerotia were developed from the stroma of acervuli. In the process of this development conidia are released from acervuli and conidia may be released and washed down to progeny tubers (Harding *et al*. 2004).

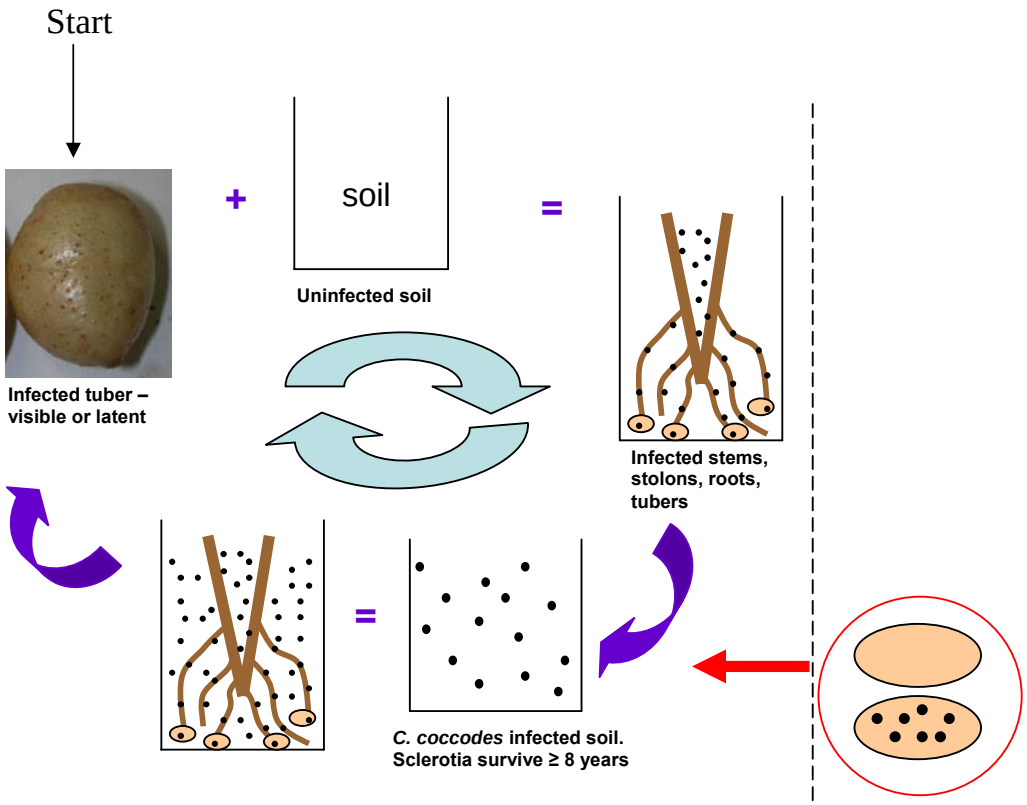

Figure 3.3: A basic diagrammatic representation of the black dot of potatoes disease cycle. Infected tubers planted into previously uninfected soil plants become infected and after harvest sclerotia remain in the soil in plant debris (and soil). Subsequent crops are infected by the fungus and inoculum builds with increasing number of crops.

Foliar infections have been reported by Johnson (1994) who demonstrated potato plants could be infected with *C. coccodes* conidia after foliage was wounded with silica sand from a sandblaster. This finding suggests that *C. coccodes* may be disseminated and infect potato foliage in a field environment where sandblasting occurs. Little research has been conducted on the importance of foliar infection in Australian conditions and it is not often observed in SA potato crops (Harding, R. 2008, pers. comm., 9th December). It is unknown how important this aspect is in management of the disease.

3.1.5. Latent infection of *C. coccodes*

Latent infections of *C. coccodes* (symptomless infection) are known in various plant species including; tomato, butternut squash, summer squash, cantaloupe, watermelon, soybean and various weed spp. (Cerkauskas 1988). Lees & Hilton (2003) speculated that when infected potato seed tubers were planted systemic infection of potato stems, stolons and roots by *C. coccodes* occurred. *C. coccodes* was confirmed within seed tubers (particularly from tuber stem end) from Washington State (Johnson *et al*. 1997) and within Australian seed sources (Harding *et al*. 2004). This suggests that *C. coccodes* can form latent or systemic infection within tubers and thus may not be visually detected prior to planting.

3.1.6. Detection of *C. coccodes* in soil

Traditionally *C. coccodes* levels in soil have been detected using soil sieves to retain and enumerate sclerotia. However, this method is very time consuming and requires significant labour (Harding, R. 2006, pers. comm. Nov). More recently, Polymerase Chain Reaction (PCR) has been used for detecting and quantifying specific pathogens in soil. Cullen *et al*. (2002) reported that two PCR primer sets were developed for sequences of the *C. coccodes* genome. These primers allowed the specific detection of *C. coccodes* isolates in naturally infested soil, potato peel and potato extract, and using TaqMan RT-PCR could detect and quantify *C. coccodes* in soil and tubers (Cullen *et al*. 2002). This is the first step in developing disease thresholds and predictions of risk, which can assist growers to make management decisions about which paddock to plant into or seed lot to use.

3.1.7. Chemical management of black dot of potatoes

Currently, there are no chemicals registered for control of black dot of potatoes in Australia. According to Lees & Hilton (2003), few chemicals are registered worldwide as fungicides have not been designed specifically to control this pathogen and other non-specific chemicals have been ineffective.

There have been some reports of some chemicals having *in vitro* and *in vivo* control of *C. coccodes* (Uribe & Loria 1994 and Read & Hide 1995). Kang *et al.* (2003), found that the growth of *C. gloeosporioides* was inhibited by acetic, oxalic, maleic and citric acids. Acetic acid completely inhibited mycelial growth when applied in media at 50 mM (Kang *et al*. 2003).

3.2 Project objectives

This project aimed to evaluate fungicides, biological or organic products at various rates, timings and methods of application for black dot control. Some particular products evaluated included: Amistar®, Cabrio®, Heads-up® and Maxim®.

In addition, this project aimed to evaluate a molecular assay for *C. coccodes* detection in soil. More specifically, to evaluate the assay over a wide range of concentrations of *C. coccodes* in soil (artificially and naturally infected) and to test if our local isolates were detected by the primers.

The benefits of this research to industry are the determination of products for registration for black dot control (currently there are none available) and therefore, improve management of black dot with associated financial benefit.

The evaluation of the molecular assay will confirm the ability of the assay to effectively detect local isolates. This will with further research in the development of this test for commercial applications, assisting growers to make management decisions about suitability of paddocks for planting potatoes.

4. TECHNICAL REPORT

4.1 General materials and methods

4.1.1. Isolates

Stems, stolons, tubers, foliage or roots from potato plants infected with *C. coccodes* were collected from South Australia, Western Australia and Tasmania.

Sections of diseased tissue were surface sterilised for 30s in 25% White King® (sodium hypochlorite 42g/L, available chlorine 4% m/v) and then plated onto V8 agar and incubated under lights (12 hour day/night) for 1 week at 22°C. After sporulation occurred on the agar 10 plugs of agar (4 mm) were placed into 0.5 ml of sterile distilled water (SDW) in Eppendorf® tubes and vortexed for 20 s. An inoculating loop was then placed into the suspension and streaked across a Petri dish of water agar (WA) (Figure 4.1).

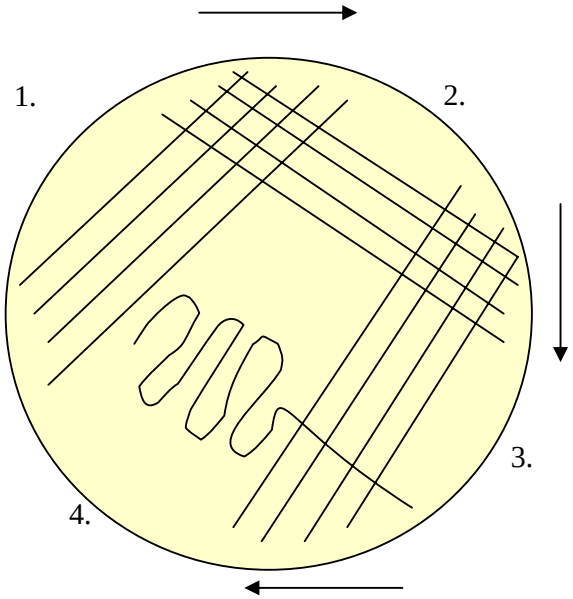

Figure 4.1: Diagram of streak plating used to separate conidia of *C. coccodes* for single-spore isolation.

Using a stereomicroscope four single conidia were removed from each plate of agar using a sterile scalpel and plated onto acidified potato dextrose agar (APDA). Petri dishes were incubated under lights (12 hour day/night) at 22°C for a further 3-7 days until colony growth. A single colony was chosen to subculture onto APDA and was given an isolate identity.

Isolates were provided as pure cultures from Victoria and mycelium was transferred from these cultures and onto NP10 media.

To confirm their identity, sclerotia of the single spored isolates of *C. coccodes* were placed into 1.5 mL plastic tubes with buffer and were tested for presence/absence of *C. coccodes* DNA using RT-PCR. A total of 29 isolates were collected from SA, 7 from Victoria, 3 from Tasmania and 2 from WA.

4.1.2. Storage of isolates

Monoconidial isolates were stored by two methods, either in sterile distilled water (SDW) or on perlite.

SDW: 1 cm x 1 cm squares of agar were cut from actively growing cultures on APDA and approximately 15 squares were added to 10 mL of SDW in McCartney bottles. The mouth of the bottle was sterilised by passing it through a flame before and after the addition of agar squares to the McCartney bottle. The lid was screwed on the bottle and further sealed with Parafilm® and appropriate labels were attached. Bottles were stored in the dark at room temperature (22°C).

Perlite: 10 cores of agar (4 mm diameter) from a sporulating culture were added to a 1.5 mL Eppendorf® tube containing 0.5 mL of SDW. Tubes were vortexed for 20 seconds to release conidia into the suspension. A 1 mL automatic pipette was used to remove the suspension from the Eppendorf® tube and add it to a McCartney bottle containing 1 g of perlite with 1 mL of potato dextrose broth (PDB). The mouth of the bottle was sterilised as described above and labelled. The bottle was shaken until suspension, PDB and perlite were combined. Bottles were stored in a dark incubator at 24°C until sclerotia were formed. Bottles were then stored in the dark at room temperature (22°C).

4.1.3. Culture of *C. coccodes*

A semi-selective media for *Verticillium dahliae* (Sorensens NP10 media: Appendix 1) was used when isolating *C. coccodes* from diseased tissue.

50% V8 broth (equal parts V8 juice and sterile distilled water) or NP10 agar were used when bulking isolates for soil or tuber inoculation. When using 50% V8 broth a Petri dish of *C. coccodes* was macerated using 2 sterile scalpels and placed into a 1 L Schott® bottle of broth containing 500 mL of broth. The mix was shaken until sclerotia were distributed evenly throughout the broth and 15 mL of mixture poured into 90 mm round Petri dishes. After 1-4 weeks of incubation in the dark at 24°C to promote the formation of sclerotia, cultures were stored in the incubator for up to 6 months until use.

When *C. coccodes* did not form sclerotia on 50% V8 broth, NP10 media was used. Single plugs of *C. coccodes* (4 mm diameter) were placed into the centre of 9 mm Petri dishes of NP10 agar. These were incubated in the dark at 24°C for 6-12 weeks or until sclerotia covered the surface of the agar and the plates were stored up to 6 months until use.

Full strength APDA or V8 agar was used when sporulation of cultures was desired (Byrne *et al.* 1997; 1998).

Water agar was used for single spore isolations.

4.1.4. Inoculation suspensions

Conidial: Conidial suspensions were prepared by blending up to 20, 90 mm plates of *C. coccodes* grown in V8 broth in a sterile kitchen blender. Excess juice was poured off before being blended. Mixture was blended in 4 bursts, first 30 s on low, second 20 s on low, third 15 s on low and fourth 15 s on high, until mixture was a grey consistency due to sclerotia being broken open. Mixture was then filtered through a sterilised muslin-lined funnel, then filtered through organza material (twice). Conidia were quantified using a haemocytometer and adjusted to 1.7×10^6 conidia/mL.

Sclerotial: *C. coccodes* was grown either in V8 broth (2007) or on NP10 agar (2008) due to poor growth in V8 broth in 2008. Sclerotia were harvested by macerating the hyphal growth in V8 broth or by scraping off sclerotia from the surface of NP10 agar. Due to the time required to scrape off the desired number of sclerotia they were prepared the day before inoculation and stored overnight in a sterile, plastic beaker (covered with aluminium foil) in a dark incubator at 24°C.

Sclerotia were mixed with SDW and filtered through a muslin-lined funnel (8 layers of muslin) to retain sclerotia and remove hyphae and remaining V8 broth or agar. Sclerotia were re-suspended in SDW and filtered first through organza mesh, then soil sieves (250 and 500 µm) to remove very small sclerotia (2007). In 2008 sclerotia were washed a second time using the muslin-lined funnel. Sclerotia were quantified by counting their number in 1 mL of suspension (in a nematode counting dish).

4.1.5. Molecular work

All molecular work was conducted by the Root Disease Testing Service (RDTS) of SARDI (Plant Research Centre, 2b Hartley Grove, Adelaide, South Australia). The TaqMan probe was used for real-time (quantitative) PCR, withC. coccodes PCR primers designed by Cullen et al. (2002) of the Scottish Crop Research Institute.

4.1.6. Pot trials planting and maintenance

Plants were grown in 4 - 4.2 L of pasteurised 'University of California' (UC) soil mix (SARDI Plant Growth Services, Plant Research Centre, 2b Hartley Grove, Adelaide, South Australia) using pots 20 cm diameter with a capacity of 4.7 L. Before pots were filled with soil, paper towel (40 cm x 19 cm doubled over lengthwise) was placed at the bottom to prevent soil from leaking through the drainage holes.

Coliban seed tubers used in these trials were either G2 or G4 seed, visually free of black dot, or infected commercially available seed. Seed tubers visually free of black dot were surface sterilised by washing then in 1% available chlorine for 5 mins, then rinsing twice in clean water for 5 mins each.

Tubers were planted at a depth of approximately 10 cm and watered directly after planting then every 3-4 days before emergence and every 2-3 days post-emergence. Yates Thrive® soluble all-purpose plant food was applied (as per instructions on box) weekly until harvest unless otherwise stated.

Plants were desiccated between 98-110 days post-sowing with the herbicide Reglone® at the rate of 3.5 L/ha in 200 L water/ha. Plants remained in pots for several weeks after desiccation to allow for disease development (pots were watered approximately once a week). After this period tubers and stems were removed from pots and placed into plastic snap-lock bags (stems and tubers were separated by paper towel) and stored at 4°C in the dark. Unless specified otherwise, in the days following harvest tubers and stems were washed under gently running water to remove adhering soil. Tubers and stems were then visually assessed for presence of black dot up to 2 weeks post-harvest.

4.1.7. Tuber inoculation

Surface-sterilised Coliban tubers were placed so that they covered the base of a sterile 52 L storage container. The conidial suspension was sprayed onto the tubers using a sterile 500 mL hand atomiser, the lid of the container was replaced and the container gently agitated so that all tuber surfaces were covered in suspension. Each batch of approximately 200 tubers was split into thirds so that tubers were in 1 layer in the container. Tubers were then placed into another 52 L container (previously sterilised with 70% ethanol) with the lid resting on top of the container but allowing air circulation. Sterile moist paper towel was placed amongst tubers to contribute to humidity and to remove some excess moisture from tubers when they breathe. This process was repeated until the number of required tubers was inoculated. Tubers were sprayed with sterile distilled water every 3-4 days to maintain humidity and sclerotia were evident on tubers 2 weeks after inoculation.

4.1.8. Soil inoculation

In 2007 the weight of soil per volume was calculated for UC soil and the required amount of inoculum (isolate Cc156/06) added as a sclerotial suspension. Several methods were used to mix the inoculum into the soil in 500 L bins. Initially 100 L of soil was placed into the bin and the inoculum poured evenly over the surface and was mixed in by hand using spades and forks. To improve the mixing, the sclerotial suspension was mixed with half the soil (50 L) first and then the remaining 50 L of soil added and mixed by hand as previously described.

In 2008 a large electric mixer at the Plant Research Centre (SARDI Plant Growth Services, Plant Research Centre, 2b Hartley Grove, Adelaide, South Australia) was used to mix the inoculum with the UC soil in batches of 600-700 kg. Inoculum (isolate Cc24/07K) was added to water to a total of 6 L and was mixed into the soil for 22 minutes.

For small amounts of soil a small electric cement mixer was used to mix 40 kg of UC soil with sclerotial suspension in 2 L of water.

After mixing, smaller amounts of soil were immediately put into 4.7 L capacity, 20 cm diameter pots with paper towel covering drainage holes until use (up to 7 days). Soil mixed in larger quantities was stored in 500 L bins for up to 14 days and placed into pots just before planting.

Soil was sampled in 200-500 g samples and taken to the RDTS of SARDI for detection and quantification of *C. coccodes* DNA in soil.

4.1.9. Products and rates used in trials

The active ingredient, rates used in the trial and the application methods have been outlined for all fungicide, biological or organic products used in trials (Table 4.1).

4.1.10. Application of products

Tuber treatments. For small batches, tubers were placed into plastic bags (30 x 20 cm) and each treatment was applied at equivalent rates per tonne of seed by a pipette. The bag was then twisted so that air was captured and tubers were gently tossed around the bag to completely coat the entire surface. Tubers were removed from bags and air dried for two days in covered plastic trays previously sterilised with 70% ethanol.

Larger batches of tubers were treated by spraying the required amount of chemical over tubers in either buckets or potato crates and agitating tubers until full coverage of tubers was obtained. Where all tubers in a field trial were treated with Maxim®, the growers applied the chemical as per standard commercial practice.

Shadehouse soil treatments. In-furrow treatments were simulated by making a well for the tuber and spraying the desired amount of chemical into the well and onto the soil surrounding the well. The tuber was then placed into the well and treated soil surrounding the well was carefully folded back over the tuber.

Surface treatments were applied directly to the soil surface of each pot once tubers had been planted.

4.1.11. Soil sampling

Where indicated, shadehouse trials had a large handful of soil removed from each pot at harvest. This was stored in the dark at 4^{o}C (to reduce pathogen replication) for up to 6 weeks but usually for 1-7 days until a 50g sample was removed from each replicate of a treatment, bulked together and shaken for 20-30 seconds until well mixed and then taken to the RDTS at Waite (2008). In 2007 a 200-500g sample of each replicate of a treatment was sampled and sent to the RDTS at Waite.

Unless indicated otherwise, field soils were sampled using an AccuCore soil sampler (Spurr Soil Probes). 40 cores (1cm diameter x 15cm length) were taken in a "W" shaped pattern over the designated sampling area and placed into a clear, labelled snap-lock bag. Samples were stored at 4^{o}C in the dark for up to 5 days until being taken to the RDTS at Waite.

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Table 4.1: Fungicides, biological and organic products and active ingredients used in shadehouse and field trials throughout the duration of the project.

Treatment name	Product name	Active ingredient	Trial rate	Application
Maxim® seed	Maxim®	250 g/L fludioxonil	250 mL/tonne seed	Seed
Amistar® seed	Amistar®	250g/L azoxystrobin	13.32 mL/tonne seed	Seed
Heads up® seed	Heads-up®	extract of *Chenopodium quinoa* 49.65%	1g/L (1 L treats 100 kg seed)	Seed
Aspergillus ustus seed	*Aspergillus ustus*	Spore suspension of *Aspergillus ustus* (5x106 spores/mL)	2 L/tonne seed	Seed
Cabrio® seed	Cabrio®	250 g/L pyraclostrobin	13.32 mL/tonne seed	Seed
Acetic acid seed	Acetic acid (50 mM)	50 mM acetic acid	2 L/tonne seed	Seed
Amistar® rate 1 in-furrow	Amistar®	250g/L azoxystrobin	10 or 20 mL/100 m row	In-furrow
Amistar® rate 2 in-furrow	Amistar®	250g/L azoxystrobin	20 or 40 mL/100 m row	In-furrow
Amistar® rate 3 in-furrow	Amistar®	250g/L azoxystrobin	40 mL/100 m row	In-furrow
Amistar® rate 1 surface	Amistar®	250g/L azoxystrobin	500 mL/ha	Soil surface
Amistar® rate 2 surface	Amistar®	250g/L azoxystrobin	1000 mL/ha	Soil surface
Cabrio® rate 1 in-furrow	Cabrio®	250 g/L pyraclostrobin	10 or 20 mL/100 m row	In-furrow
Cabrio® rate 2 in-furrow	Cabrio®	250 g/L pyraclostrobin	40 mL/100 m row	In-furrow
Cabrio® rate 1 surface	Cabrio®	250 g/L pyraclostrobin	500 mL/ha	Soil surface
Cabrio® rate 2 surface	Cabrio®	250 g/L pyraclostrobin	1000 mL/ha	Soil surface
Aspergillus ustus high (surface)	*Aspergillus ustus*	Spore suspension of *Aspergillus ustus* (5x106 spores/mL)	4320 L/ha	Soil surface
Tea tree oil extract (10%) in-furrow	Tea tree oil extract (10%)	Diluted tea tree oil extract (10%)	70 mL/100 m row	In-furrow
Acetic acid high (in-furrow)	Acetic acid (50 mM)	50 mM acetic acid	690 mL/100 m row	In-furrow
Voom® surface (5% in water)	Voom®	mustard oil, 15-20% w/v allyl isothiocyanites	0.9720 L/ha	Surface

Table 4.2 (cont): Fungicides, biological and organic products and active ingredients used in shadehouse and field trials throughout the duration of the project.

Treatment name	Product name	Active ingredient	Trial rate	Application
Octave® in-furrow	Octave®	462 g/kg prochloraz	10 g/100 m row	In-furrow
Amistar® foliar (350 ml/ha)	Amistar®	250g/L azoxystrobin	350 mL/ha	Foliage or haulms (post-desiccation)
Heads up® foliar (1g/L)	Heads-up®	Extract of *Chenopodium quinoa* 49.65%	1 g/L, 130 L/ha	Foliage
Octave® foliar (175 g/ha)	Octave®	462 g/kg prochloraz	175 g/ha	Foliage
Acetic acid foliar	Acetic acid (50 mM)	50 mM acetic acid	50 mM, 230 L/ha	Foliage or haulms (post-desiccation)
Pine oil	Organic Interceptor™ contact weed spray	Pine oil	20% dilution of product	Desiccation - foliage
Reglone®	Reglone® herbicide	200 g/L diquat	3.5 L/ha or as grower practice	Desiccation - foliage

4.1.12. Assessment of disease

Tuber blemishing was assessed using a 0-4 scale where:

0 = no blemishing due to black dot
1 = < 25 % of the tuber blemished
2 = 25-49 % of the tuber blemished
3 = 50-74 % of the tuber blemished
4 = > 75% of the tuber blemished

Below ground stem symptoms were assessed using the following scale:

0 = no disease
1 = single lesion, <25mm
2 = single lesion 26-50 mm (or a composite of small lesions totalling less than 50 mm)
3 = single lesion 51-75 mm (or a composite of small lesions totalling less than 75 mm)
4 = single lesion > 76 mm (or a composite of small lesions totalling more than 76 mm)

Root symptoms were assessed by estimating the percentage of root tissue with black dot symptoms.

4.1.13. Tuber size categories

Tubers harvested from the field

Cocktails (0-50 g)
Chats (50-100 g)
Small (100-200 g)
Medium (200-350 g)
Large (350-450 g)
Oversize (>450 g)

4.1.14. Statistical design and analysis

Shadehouse and field trials were arranged in Randomised Complete Block Designs (RCBD) (all pot trials and some field) or split-plot designs.

Experiments were analysed using GenStat V. 9 or 10.1 for PC/Windows (Rothamsted Experimental Station). ANOVA were used for the pot and field experiments. Data was transformed (log function) when GenStat showed the raw data to be skewed (and requiring transformation). Transformed data was presented back-transformed by the initial function before being presented. Where significant differences were observed ($P<0.05$) treatment means were separated by least significant differences (lsd's). If data was transformed the lsd value refers to that of the transformed data. Some of the 2008 pot trial data was analysed by Dr Chris Dyson, SARDI statistician using GenStat V. 11.

4.2 In vitro trials and molecular test development

4.2.1. Comparison of acervuli/sclerotia grown on various media

Fungal sclerotia can occur in different sizes, and this could impact upon the results of DNA assays. Therefore, tests were undertaken to determine media which could effectively produce various sizes of sclerotia.

Aim

To determine whether different media influences the size of sclerotia.

Materials and Methods

One 5 mm plug of *C. coccodes* (isolate Cc156/06) was plated onto either a Petri dish of a selective media (NP10), Acidified Potato Dextrose Agar (APDA) and ½ APDA (one replicate plate per media). Cultures were grown in the dark at 24°C for 43 days.

Ten random sclerotia were removed from each agar plate and placed into 2 drops of sterile water on a glass slide and covered with a glass coverslip. Sclerotia were measured for width and length at 200 x magnification with an Olympus BH-2 compound microscope.

Results

Sclerotia grown on NP10 media had the shortest length (241 µm) and width (213 µm) than those grown on APDA or ½ APDA (Table 4.3). Sclerotia grown on ½ APDA were shorter in length but wider than those on APDA.

Table 4.3: Size of sclerotia of *C. coccodes* grown on NP10, APDA or ½ APDA. ±SEM.

	Growth media		
	NP10	**APDA**	**½ APDA**
Mean size (µm)	241.4 x 213.1	423.2 x 355.5	412.1 x 367.6
SEM	11.5 x 13.9	27.6 x 35	16.4 x 14.3
Length:width Ratio	1.13	1.19	1.12

SEM = standard error of the mean.

Discussion

These results showed that varying the media used could effectively produce sclerotia of different sizes. NP10 media is a semi-selective media for enumeration of *Verticillium* spp. from soil (Sorrensen *et al*. 1991). It has no added carbohydrates or sugar compared to the APDA and ½ APDA which has 4 g potato starch (carbohydrate) and 20 g dextrose (sugar) per 1 L of agar. This may have contributed to the difference in size of sclerotia however, the reason was not tested in this study. A future study may investigate potential causes of sclerotial size differences, including organic matter

content and nutrient status of soil. Studies are also needed to confirm whether sclerotia found in various naturally infected soils are similar in size or vary within the same paddock.

The importance of larger vs. smaller sclerotia and the impact on disease expression has not yet been established but should be in the future to assist with disease prediction tools.

4.2.2. Germination of sclerotia over time

This was a preliminary study to obtain an indication of the time period of one form of sclerotial germination in controlled conditions.

Aim

To assess the germination of *C. coccodes* sclerotia over time.

Materials and methods

Sclerotia, grown in 50% V8 broth for 40 days as previously described, were dried in an open Petri dish overnight in an operating laminar flow. The following day 300 individual sclerotia were aseptically removed from a single plate of *C. coccodes* using sterile forceps. Sclerotia were surface sterilised in 70% ethanol for 90 seconds then air dried in the laminar flow on paper towel (Huang & Erickson 2002).

Five replicates of thirty surface-sterilised sclerotia were placed onto 2 layers of Whatman® filter paper moistened with 3 mL of SDW in the lids of 90 mm diameter plastic Petri dishes (Sanogo & Pennypacker 1997). The bottom of the Petri dish was placed in the lid and sealed with Parafilm®. Plates of sclerotia were placed into a dark incubator at 24^{o}C in a completely random design and were removed for assessment.

Germination of 20 sclerotia selected at random was assessed, after 0, 1, 2, 4, 6, 24, 30, 48, 54 and 192 hours of incubation at 24^{o}C in the dark. Sclerotia were considered germinated (myceliogenically) when hyphae had emerged from the sclerotia.

Results

Germination of surface sterilised sclerotia was not evident until > 50 hours after plating and 53% had germinated after 192 hours of incubation (Figure 4.2).

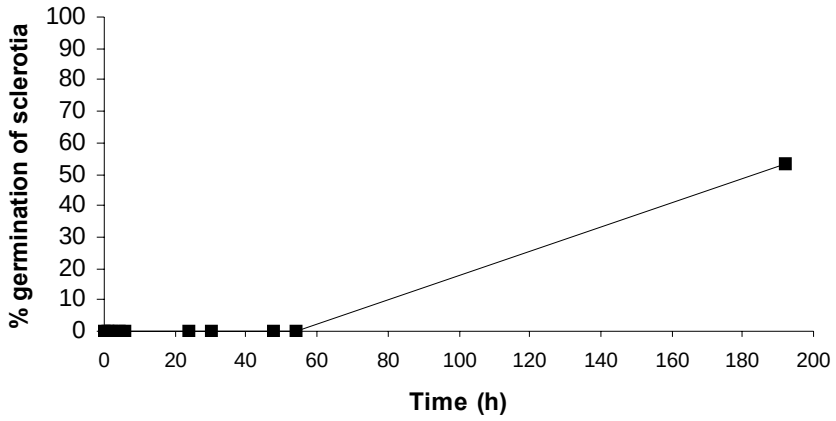


Figure 4.2: The germination of surface sterilised sclerotia of *C. coccodes* over time.

Discussion

C. coccodes sclerotia did not start germination until at least 50 hours after incubation. This delay in germination may have implications in the field, particularly for the timing of chemical application. If chemicals applied at planting rapidly loose activity, the actively growing mycelium of the germinating sclerotia may have no effective contact and therefore, have limited activity.

This study was a preliminary study and only looked at one of the two forms of sclerotial germination (see Sanogo & Pennypacker 1997) and we do not know which form occurs in the field. The type and timing of sclerotial germination most commonly found in the field is an area requiring further investigation.

Many factors may accelerate the germination of sclerotia including: release of host exudates and which may be used as an energy source (Willetts 1971), temperature and irrigation factors. These factors (except irrigation) could also be looked at *in vitro* in future experiments once the method of germination in the field is established.

4.2.3. Amount of DNA in sclerotia grown on different culture media

This study was undertaken to determine whether there is a relationship between the size of sclerotia and the amount of DNA detected.

Aim

To compare the amount of *C. coccodes* DNA in various sizes of sclerotia.

Materials and methods

100 sclerotia of the isolate Cc156/06 were removed using either sterile forceps or a sterile needle from 43 day old cultures grown on 50% V8 broth, NP10, PDA or ½ PDA. The amount of *C. coccodes* DNA was extracted and quantified as previously described. There was no replication. Measurements of sclerotial size were not taken

during this experiment but were considered similar to the previous experiment (see 4.2.2).

Results

The smaller sclerotia grown on NP10 media had lower amounts of DNA per sclerotium (2670 pg) compared to the sclerotia grown on PDA (18320 pg), ½ PDA (11270 pg) or 50% V8 broth (5500 pg) (Figure 4.3).

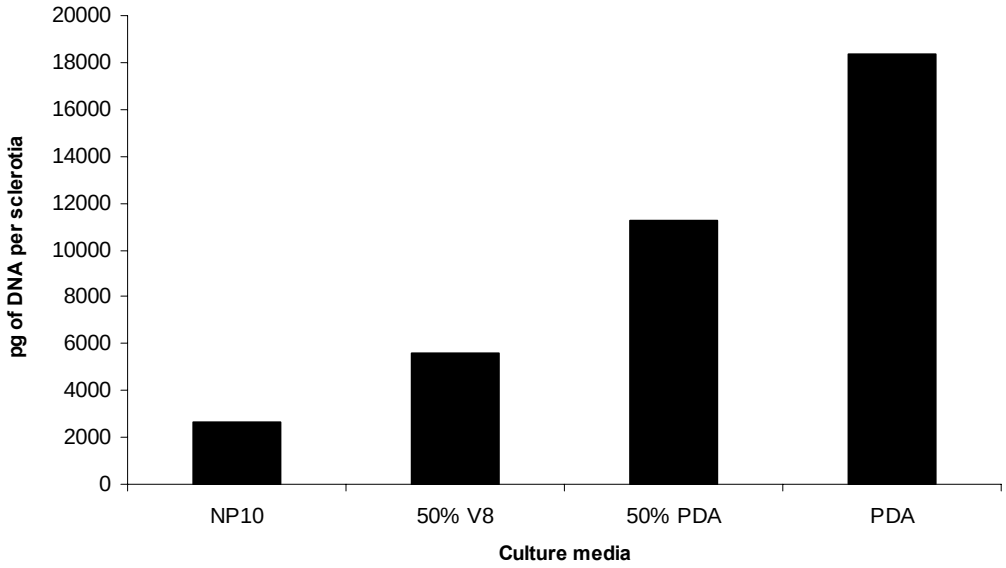


Figure 4.3: The (estimated) amount of DNA of sclerotia (picogram (pg) DNA/sclerotia) grown on different culture media.

Discussion

The results show that the amount of DNA detected is related to the size of the sclerotia found in a previous study (4.2.1). A replicated repeat of this experiment in the future would provide more evidence of this preliminary result.

One implication of this sclerotial size difference (and related DNA contents) is in the interpretation of soil DNA test results. For example, there may be two soils with similar amounts of DNA per g of soil but different sized sclerotia. This result would not allow an understanding of the distribution or size range of sclerotia in soil and subsequently how this may relate to plant disease expression. For example, would a soil with smaller sclerotia (therefore, more sclerotia throughout soil) result in greater disease than a soil with larger sclerotia but less sclerotia in soil?

Alternatively, would the larger sclerotia be able to produce more mycelia or conidia when germinating and thus infect a larger area of soil?

And is there sclerotial size variation in naturally infected field soils? These are important areas for future work for improved understanding and interpretation of results from DNA based assays.

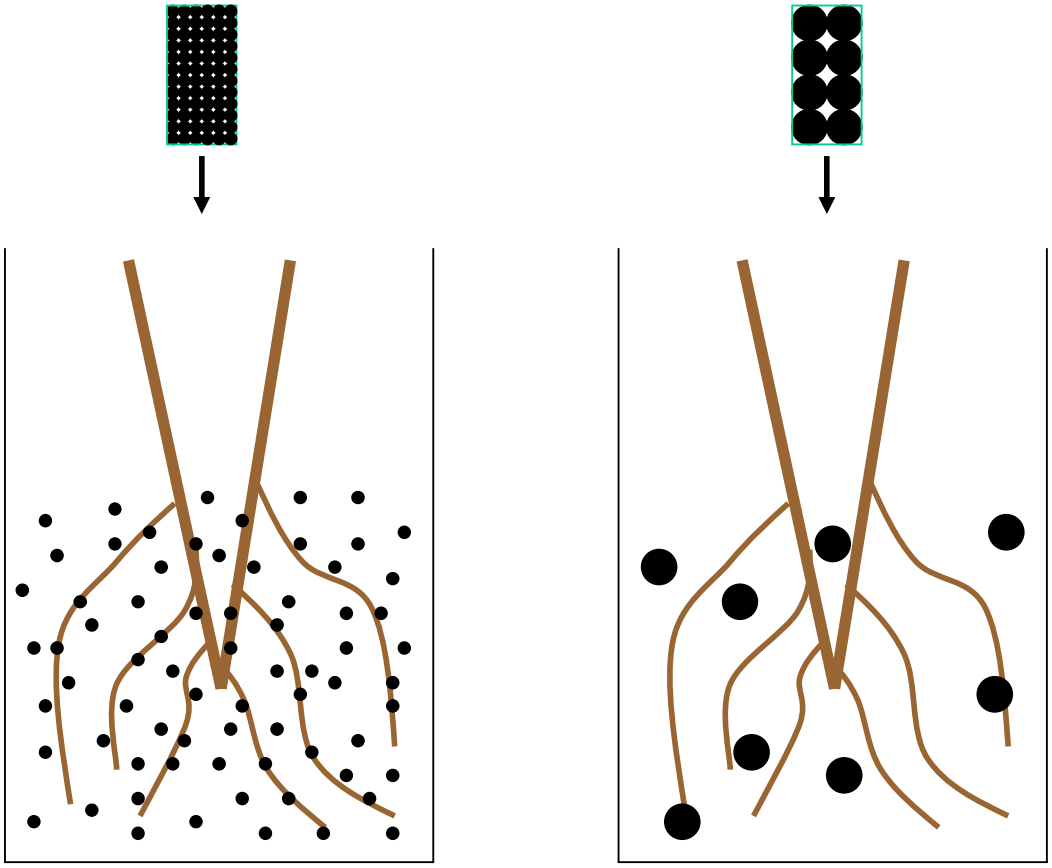


Figure 4.4: Diagram of the potential distribution of sclerotia of different sizes (same DNA content) in soil. The diagram of soil on the left has smaller sclerotia therefore, a more intense distribution of sclerotia in soil and the diagram on the right shows soil which has larger sclerotia and a less-intense distribution of sclerotia in soil.

4.2.4. *C. coccodes* DNA quantification in artificially inoculated soils

These studies were undertaken to contribute to the development of a molecular assay for the detection of *C. coccodes* in soil. The experiments were designed to test the assay when increasing amounts of sclerotia were added to different soils: Mt Compass sand, Riverland sand or UC soil mix.

Aim

To observe the relationship between the number of C. coccodes sclerotia (grown on NP10 media and in 50% V8 broth) inoculated into soil and the amount of DNA extracted using RT-PCR (TaqMan).

Materials and methods

Experiments 1 and 2: Mount Compass sand or UC soil was autoclaved for 15 minutes at 121ºC, cooled and 200-500 g was placed into clear plastic snap lock bags (15 x 25 cm). Various amounts of *C. coccodes* sclerotia (isolate Cc59/07T) harvested from NP10 media as previously described were added to the bags of sterilised sand or soil in a total of 34 mL (UC soil) or 20 mL (Mt Compass sand) of water to provide final concentrations of 0, 0.5, 1, 2, 4, 8, 16 and 32 sclerotia/g sand.

Experiments 3 and 4: Sclerotia grown on NP10 (Cc156/06) were harvested by removing the required number of sclerotia in agar and macerating the agar (and sclerotia). Sclerotia were harvested from 50% V8 broth (Cc156/06) as previously described and mixed with water.

The sclerotia grown from NP10 media or 50% V8 broth were used to inoculate Riverland sand to provide final concentrations of 0, 0.002, 0.01, 0.02, 0.01, 0.2 and 1 sclerotia/g sand (not autoclaved) or 0, 0.5, 1, 2, 4, 8, 16 and 32 sclerotia/g sand (autoclaved).

C. coccodes DNA was quantified by SARDI RDTS as previously described.

Results

In all experiments but experiment 4 (V8 broth), there was a strong positive relationship between the number of sclerotia added to the sand or soil and the amount of DNA extracted (Figures 4.5, 4.6, 4.7 and 4.8).

Amounts of DNA extracted per sclerotial concentration was similar for all sand and soil with sclerotia harvested from NP10. However, DNA from sclerotia harvested from 50% V8 broth was more variable. In experiment 3 (Figure 4.7), sclerotia from 50% V8 broth yielded the highest amount of DNA, with approximately 9000 pg *C. coccodes* DNA/g soil at the rate of 1 sclerotia/g soil. However, in experiment 4 (Figure 4.8) the amount of DNA extracted from Riverland sand inoculated with sclerotia grown in 50% V8 broth was much lower than in all other experiments, with 8.8 pg DNA/ g sand.

Sclerotia in all experiments at the inoculation rate of 1 sclerotia/g soil grown on NP10 media varied from 141.75 pg DNA/g soil in experiment 3 to 326.4 pg DNA/g soil in experiment 2.

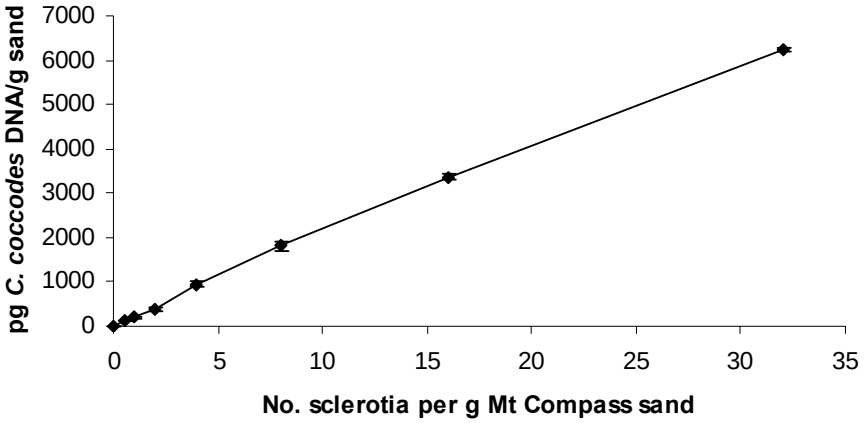


Figure 4.5, Experiment 1: The relationship between the number of *C. coccodes* sclerotia (isolate Cc59/07T) grown on NP10 media and inoculated into Mount Compass sand and the amount of DNA extracted (pg DNA/ g soil). The r^2 value for the relationship (raw data) was 0.994 (three outliers removed). Bars = ± SEM.

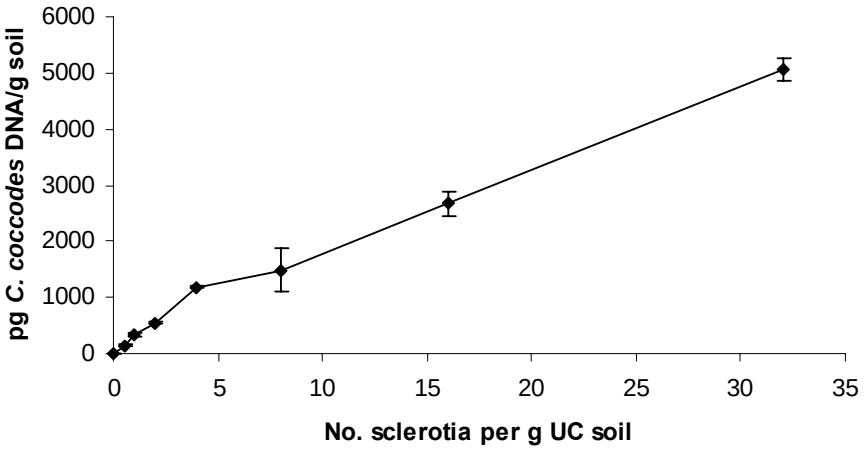


Figure 4.6, Experiment 2: The relationship between the number of *C. coccodes* sclerotia (isolate Cc59/07T, grown on NP10 media) and inoculated into UC soil and the amount of DNA extracted (pg DNA/g soil). The r^2 value for the relationship (raw data) was 0.9474 (two outliers removed). Bars = ± SEM.

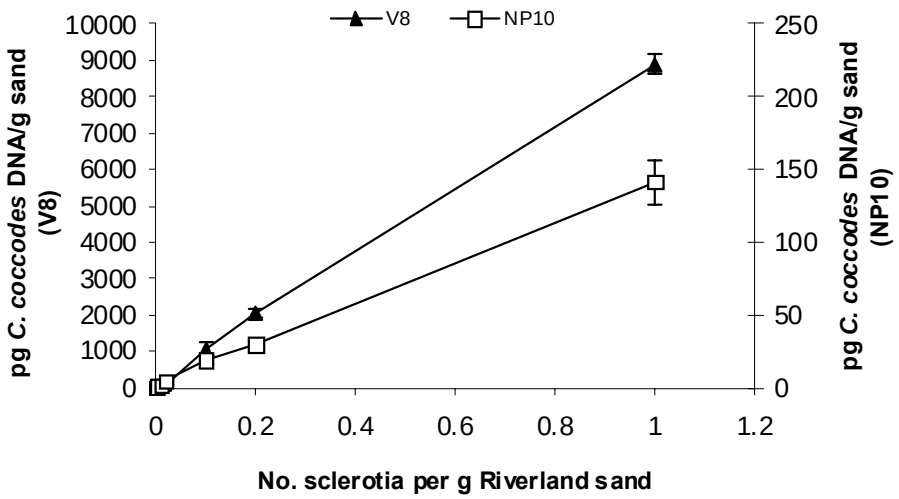


Figure 4.7, Experiment 3: The relationship between the number of C. coccodes sclerotia (isolate Cc156/06, grown on NP10 media or in 50% V8 broth) inoculated into Riverland sand and the amount of DNA extracted (pg DNA/ g soil). The r2 value for the relationship (raw data) for the sclerotia grown on NP10 was 0.9532. The r2 value for the relationship (raw data) for the sclerotia grown in 50% V8 broth was 0.9926. Bars = ± SEM

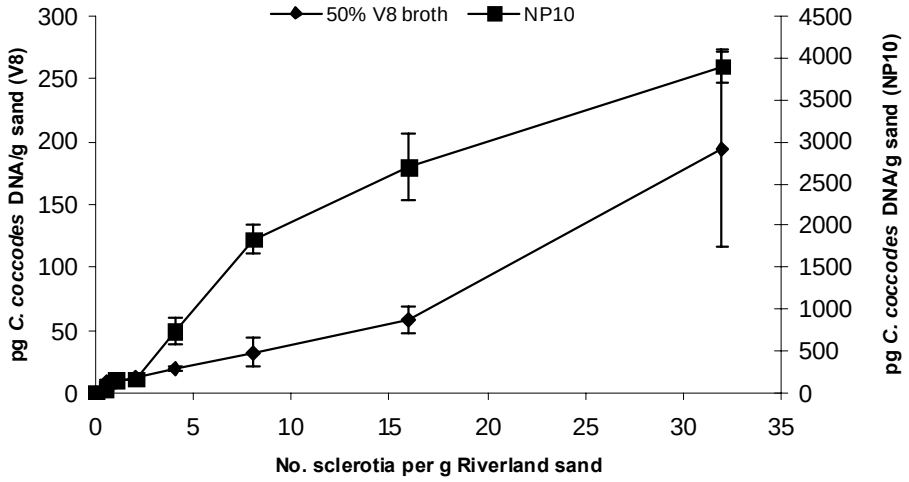


Figure 4.8, Experiment 4: The relationship between the number of *C. coccodes* sclerotia (isolate Cc156/06, grown on NP10 media or in 50% V8 broth) inoculated into Riverland sand and the amount of DNA extracted (pg DNA/ g soil). The r^2 value for the relationship (raw data) for the sclerotia grown on NP10 was 0.8692 (two outliers removed). The r^2 value for the relationship (raw data) for the sclerotia grown in 50% V8 broth was 0.5095 (two outliers removed). Bars = ± SEM

Discussion

These results show that the qPCR test detected and quantified *C. coccodes* DNA over a large range of sclerotial concentrations in soil. During these studies 1-8494 pg *C. coccodes* DNA/g soil was detected, which is within the range of DNA levels previously detected in SA field soils (15-1200 pg DNA/g soil, data not shown). This indicates that the qPCR assay is able to reliably detect *C. coccodes* DNA in a linear manner within the DNA range found thus far in SA soils.

However, in Experiment 3 the difference between the yield of DNA between the sclerotia grown on NP10 media and in 50% V8 broth was higher than expected. Results from the previous experiment comparing the DNA content of sclerotia grown on different media show that sclerotia grown in 50% V8 broth had more than double the amount of DNA per sclerotia compared to those grown on NP10 media (section 4.2.3). However, results from this work showed more than a 60 fold increase in DNA content between sclerotia grown on NP10 media or in 50% V8 broth at the highest inoculation rate. This variation indicates that to develop an effective prediction tool, more work is needed in the area of sclerotial size and the corresponding DNA extracted.

The result for sclerotia grown in 50% V8 broth and inoculated into Riverland sand at 0-32 sclerotia per g sand (Experiment 4, Figure 4.8) were not expected to be so low and may have been due to an error in the rate of inoculation although this is unlikely.

4.2.5. Disease expression studies

A series of pot and field studies were undertaken to assess the amounts of *C. coccodes* in soil and *in planta* during a potato-growing season and to assess disease expression with varying levels of soil inoculum.

Experiment 1. To monitor the amount of *C. coccodes* in the soil throughout a potato growing season and to assess development of the pathogen in soil and the amount of disease on tubers after 100 days growth.

Experiment 2. To detect *C. coccodes* in potato plants and soil during a potato growing season and assess final disease at harvest (after 100 days growth).

General materials and methods

Seed naturally infected with black dot (cv. Coliban, 95% incidence, 2.86 severity) was cut and treated with builders lime dust (as per commercial practice to manage soft rot) 4 days prior to planting and left to suberise in the dark. Seed was cut so that at least one eye remained on a seed piece. Experiments 1 and 2 were hand planted into the soil of a field plot at Lenswood Research Centre on the 19th December 2007 at 20 cm spacing with rows (2.5 m long) spaced 70 cm apart. Replicates were planted at 70 cm spacing.

Plots were desiccated 100 days post-sowing as previously described and were left in the ground for approx. 5 weeks (35 days) until they were hand harvested, washed and stored before at 4°C before assessment.

A pre-planting soil sample (40 cores in a "W" shape) was taken of the area of both experimental plots on 30/11/07 and showed that the area had an average of 57 pg *C. coccodes* DNA/g soil.

Experiment 1: Markers were placed every 40 cm (5 in each replicate) to give a set point for soil sampling throughout the season. Soil was sampled five times during the season: at emergence, flowering, in-season, 10 days post-desiccation and at harvest. A sample of four cores at each marker or 20 per row were taken for each replicate at each sampling period as previously described (methods adapted from Department for Environment 2002). Tuber number, yield, disease incidence and severity were measured.

Experiment 2: At plant emergence (20 days post-sowing) a soil sample was taken in a 'W' shaped pattern (40 cores) over the planted area.

At 51 days post-sowing a single plant from each replicate was removed from the soil and taken back to the laboratory. Soil samples were taken along each replicate row of potatoes (20 cores). Once in the laboratory, sections of plant tissue (0.5- 1 cm long) were removed using a sterile scalpel from roots, stolons, stem (at soil line) and the below surface stem. Tissue samples were surface-sterilised in 25% White King® (sodium hypochlorite 42 g/L, available chlorine 4% m/v) for 30 s and then rinsed twice in sterile distilled water for 30 s each. Tissue was dried on sterile paper towel in a laminar flow cabinet, and pieces were aseptically placed onto NP10 agar (four pseudo-replicate tissue pieces on each of the plates). Once fungal growth and sclerotial production had occurred plates were examined for *C. coccodes* recovery from tissue. Tuber number, yield, disease incidence and severity were measured.

Results

Experiment 1: The amount of DNA in soil increased 25 fold over the potato-growing season, from 57 pg DNA/g soil pre-planting to 1406 pg DNA/g soil at harvest. The greatest increase was observed between 83 and 110 days post-sowing.

Table 4.4: Soil sampling and quantity of *C. coccodes* DNA in soil of the plant growth plots over the potato growing season at Lenswood Research Centre 2008.

Days post-sowing	Growth stage	Amount of C. coccodes DNA at sampling (pgDNA/g soil)
20	Emergence (8/1/08)	93
61	Flowering (18/2/08)	366
85	In season (13/3/08)	572
110	10 DPD (7/4/08)	1347
134	Harvest (1/5/08)	1406

Experiment 2: At emergence there was 131 pg of *C. coccodes* DNA/g soil.

C. coccodes was recovered from 30% of roots, 30 % of stolons, 50% of stems at the soil line and 55% of stems under the soil at 51 days post-sowing (Figure 4.9). At the time of sampling there were no visual signs of black dot on tubers, stolons, roots, stems or foliage.

A mean of 773 pg DNA/ g soil was detected in soil samples at harvest. This was an increase of 716 pg DNA/g soil from pre-planting to post-planting.

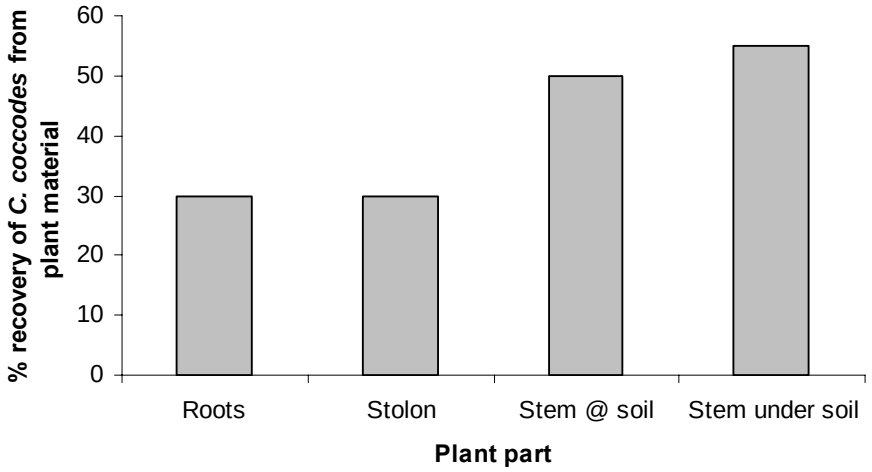


Figure 4.9: The recovery (%) of *C. coccodes* from plants 51 days post-sowing grown from infested seed in soil infested with *C. coccodes*.

Discussion

The results show that *C. coccodes* is able to increase significantly in soil over a potato growing season when infected seed is planted.

C. coccodes was detected in plant tissue at 51 days post-sowing even though no visual symptoms were observed. This confirms observations elsewhere that *C. coccodes* can establish latent infections in plant tissue (Cerkauskas 1988 and Harding *et al*. 2004).

The large increase observed in the amount of *C. coccodes* DNA between sampling at 85 days and 110 days post-sowing could have been due to the activation of this latent infection of *C. coccodes* (Cerkauskas 1988), as plants were desiccated at 100 days post-sowing and had begun to senesce prior to desiccation.

Although the importance of soil-borne versus seed-borne inoculum was not established in this trial it has shown that planting infected seed into infected soil can result in a 25 fold increase in *C. coccodes* DNA in soil.

Further work is required to establish the effect planting non-infected seed has on the amount of *C. coccodes* in soil over time; this will help to establish the importance of seed vs. soil inoculum.

4.2.6. Increasing concentration of infected field soil

This pot trial was undertaken to evaluate disease expression on progeny tubers grown in naturally infected soil at various concentrations.

Aim

To evaluate the effect of increasing concentrations of naturally infected soil has on black dot disease expression on potatoes.

Materials and methods

Soil naturally infected with *C. coccodes* was collected from a field at Parilla in the Mallee of South Australia.

The field soil was mixed with UC soil to attain field soil concentrations of 0, 0.39, 0.78, 1.56, 3.13, 6.25, 12.50, 25, 50 and 100% using a sterilised cement mixer as previously described. The cement mixer was washed out between soil treatments and soil was mixed from the control to the 100% field soil (increasing concentration of field soil) soil samples were taken before soil was put into pots. Ten replicate pots (20 cm diameter, 4.7 L capacity) were each filled with 4 L of the mixed soil and planted with one surface-sterilised seed potato (cv. Coliban) with no visible symptoms of black dot. One replicate pot of soil for each treatment was kept in the growth room for the duration of the trial.

Pots were randomised and placed into a Controlled Environment Room (CER) set at 30^{o}C with 12-hour day/night cycle.

Plants were maintained as previously described and were desiccated approximately 85 days post-sowing with Roundup® (glyphosate 360).

Tubers were harvested approximately 2 weeks after desiccation and washed the same day before storage and assessed for: emergence, disease incidence and severity on tubers, stems and roots, yield (weight of progeny tubers) and DNA in soil post planting. Soil was taken from each pot within each treatment were bulked together subject to whether 1. tubers had germinated and grown in the soil, 2. tubers had not germinated or 3. soil was not planted to potatoes but left in the same environment for the duration of the trial.

Results

Tuber emergence was variable (Figure 4.10) with no linear dose response. Tubers planted into pots with a higher proportion of field soil generally had lower emergence than those with a lower proportion of field soil.

Black dot was not observed on tubers after harvest within any of the treatments, however, black dot was observed on stems and roots of treatments with higher concentrations of field soil. For example, the treatment of 0.39% field soil had a stem disease rating of 1 and the treatment of 25% had a stem disease rating of 3.2 and the 100% field soil treatment had a rating of 4 although only 1 stem emerged in this

treatment (data not shown). The 0% field soil treatment did not have disease on stems (data not shown).

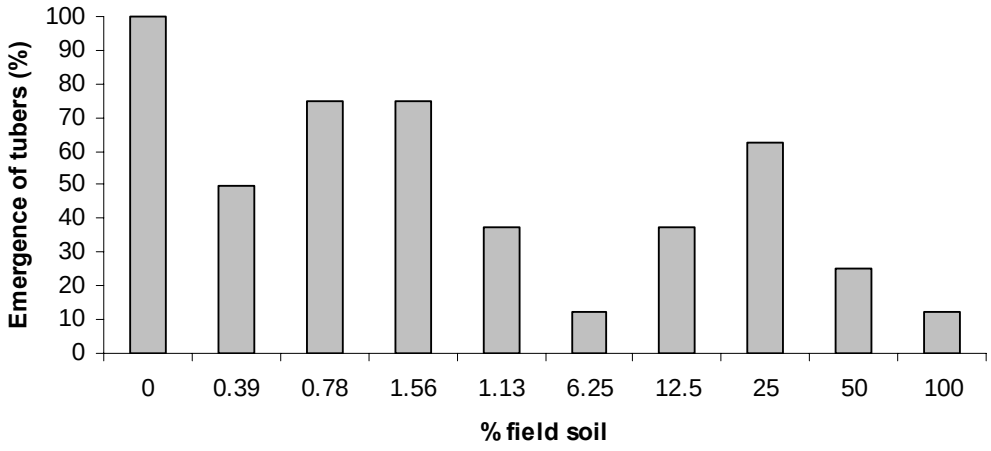


Figure 4.10: Emergence (%) of tubers when grown in infected field soil at increasing concentrations.

A general decline in yield was observed as the concentration of field soil increased (Table 4.5).

Table 4.5: Yield of progeny tubers grown in infected field soil at increasing concentrations.

% field soil	Yield (g/plant)
0	55.76
0.39	56.67
0.78	47.88
1.56	36.06
3.13	27.88
6.25	25.45
12.5	27.42
25	51.52
50	33.64
100	11.21

The amount of DNA appeared to relate well with the percentage of field soil pre-planting and for the soil left unplanted in pots in the controlled environment room (Table 4.6). The soil in which tubers did not emerge generally had a similar amount of DNA as the soil pre-planting. The amount of DNA in soil planted to potatoes increased over the growing season in all but two treatments (6.25 and 100% field soil) which had the lowest rates of germination.

Table 4.6: Amount of *C. coccodes* DNA in soil diluted with various amounts of UC soil pre-planting in soil with increasing concentrations of infected field soil.

		***C. coccodes* (pg DNA/ g soil) post-planting**		
% field soil	***C. coccodes* (pg DNA/ g soil) pre-planting**	**Soil not planted**	**Soil no emergence**	**Soil with plants growing**
0	0	0	-	42
0.39	1	0	0	1,559
0.78	3	28	0	147
1.56	14	19	8	189
3.13	12	11	11	456
6.25	62	41	38	20
12.5	64	79	62	1,624
25	164	125	640	444
50	349	199	358	919
100	687	686	704	426

Discussion

The seed used in this trial had unexpectedly poor germination in some treatments compared to the control (UC soil only). For example, the addition of only 0.39% field soil (1 pg *C. coccodes* DNA at planting) resulted in only 50% emergence.

Prior to planting, large sprouts were removed from tubers, leaving at least 1 sprout of 2-10 mm on each tuber with the expectation that each tuber would successfully germinate. Poor germination was also noted in other pot trials (in the shadehouse) when the same seed was used, therefore it is most probable that some of the poor germination was due to inherent seed characteristics and not a treatment effect.

Black dot disease symptoms were not observed on progeny tubers. This may have been due to the high temperature in the Controlled Environment Room (30°C) which can inhibit the growth of *C. coccodes* (Harding *et al.* 2004). Another possibility is that the *C. coccodes* isolate in the field soil was non-pathogenic to potatoes, however, this is unlikely due to tubers in another trial presenting with disease after being grown in the same soil. Another possible reason may be the presence of other organisms in the soil. These organisms may have become more active at higher temperatures and antagonised *C. coccodes*, reducing its ability to infect tubers.

The use of diluted field soil was effective in providing decreasing amounts of black dot in soil for experimental purposes.

The soil-borne DNA results show that the amount of C. coccodes DNA in soil generally increased, in the presence of a host plant, i.e. potato. In the absence of a growing plant, the amount of DNA in soil in this experiment was relatively stable. The treatment of 100% field soil had less C. coccodes DNA in it at harvest than pre-

planting, which was not expected. Due to only 1 replicate plant germinating in this treatment the soil result was based upon a soil sample of 50 g, whereas most other treatments had >200 g of soil for the test. The other exception was the treatment of 6.25% field soil, which had 1 replicate potato germinate and also had a low amount of DNA detected post-harvest.

4.2.7. Soil inoculum

Four experiments were undertaken over the 2007 and 2008 seasons evaluating disease levels in soil and on tubers when infected or uninfected seed tubers were planted into infected soil.

Aim

To investigate the effect soil inoculation rate has on the disease incidence and severity of progeny tubers from either an uninfected or infected seed tuber.

Materials and methods

UC soil was inoculated at various rates (Table 4.7) with sclerotia as previously described and immediately put into labelled plastic pots, using uninoculated soil as a control. Soil was sampled after mixing and sent to the SARDI RDTS for *C. coccodes* DNA quantification. In 2007 a single bulked sample was collected for each treatment and in 2008 samples from each replicate of each treatment were collected and bulked into their respective treatments.

Tubers were planted approx. 10 cm deep into 5 (2007) or 10 (2008) replicate pots (Table 4.7). Pots were watered directly after planting and were maintained as previously described.

Table 4.7: Details of inoculation rate, seed source, time of planting, desiccation or harvest, and number of replicates for the soil inoculum experiments.

Experiment no.	Inoculation rate (UC mix)	Tubers	Planting (days after inoculation)	Desiccation (Days after planting)	Harvest (Days after planting)
1	0, 0.01, 0.1, 1 & 10	Coliban, surface-sterilised, visually free of disease	1	102	127
2	0, 0.01, 0.1, 1 & 10	Coliban, artificially inoculated, 95% incidence, severity rating 2	1	102	127
3	0, 0.01, 0.1 & 1	Coliban, surface-sterilised, visually free of disease	5	99	135
4	0, 0.01, 0.1 & 1	Coliban, naturally infested, 100% incidence, severity 4	5	100	135

Plants were desiccated 127-135 days post-sowing (Table 4.6) and progeny tubers were harvested from each pot. After harvest, soil from each replicate of each treatment was collected to test for DNA content. In 2007 each replicate was tested and in 2008 replicates of each treatment were bulked and tested.

Severity data in Experiment 1 and Experiment 4 required transformation by log(+0.1) and back-transformed data has been presented.

Results and discussion

An overview of results is presented in Table 4.8. The DNA in the soil post planting was extremely variable, and did not always increase with the increasing inoculum rate. However the amount of DNA in soil post-harvest was much higher in all treatments compared to the amount of DNA pre-planting, indicating that the growth of the potato plant allowed for pathogen replication.

It was also observed that the lower the initial inoculum the greater the replication of DNA in the soil when measured at the end of the season. For example, in 2008 when infected seed was planted the amount of DNA in soil for the 0.01 treatment increased by 1403 times but the DNA only increased 41 times in the 1 sclerotia/g soil treatment. This may be due to the pathogen reaching saturation in the soil quicker due to the higher amount of DNA in soil at the beginning of the season. Further research is required to investigate this.

The results in Experiments 3 and 4 showed that when infected seed was planted into infested soil (at increasing rates) the final amount of disease and DNA in soil post-harvest was higher (in all but 1 treatment) than when uninfected seed was planted (Experiment 3). This was not seen in Experiment 1 and 2, where post harvest DNA levels were generally higher and more variable.

The soil DNA result pre-planting in Experiments 1 and 2 for the treatment of 0.01 showed 0 pg of DNA/g soil, however, infection occurred and *C. coccodes* DNA was detected at harvest. Infection most likely was introduced during the season through infection within the seed or through water splash with adjacent pots while watering. Another possibility is that complete mixing did not occur and the soil sample tested was not representative of the whole soil.

A high amount of DNA was detected in the Experiment 3 control treatment post-harvest. The soil sample may have had some infected root tissue in it, which would have exaggerated the actual amount of DNA. Although care was taken to remove roots from soil samples it was difficult to ensure all small roots were removed.

The trend was that both disease incidence and severity on tubers generally increased as the number of sclerotia in soil increased, with the lowest disease at 0.01 and the highest at 1 sclerotia/g soil. Significant differences in disease incidence and severity were observed between treatments in Experiment 1 and 4 only.

The yield was variable, and no significant differences were observed between the treatments in any of the experiments, and there was no correlation between yield and either disease levels or DNA levels.

These results showed that planting seed whether visually free of disease or infested/inoculated with black dot, into soil artificially inoculated with increasing numbers of *C. coccodes* sclerotia resulted in an increase in disease at the end of the season. This indicates that there is a positive relationship between increased soil inoculum and disease within the range of 1-159 pg *C. coccodes* DNA/g soil.

To minimise disease it is recommended to plant non-infested (clean) seed into soil with low amounts of *C. coccodes* DNA in soil.

Table 4.8: Overview of results of trials investigating the effect of planting infected or non-infected seed into soil with increasing amounts of *C. coccodes* sclerotia. Results show the amount of DNA in soil pre and post planting for each experiment and the disease incidence and severity of progeny tubers. Treatments with the same letters are not significantly different from each other. ns = no significant differences were detected between the treatments.

Inoculation rate (no. sclerotia/g soil)	**Experiment 1. Non-infected seed 2007**		**Experiment 2. Infected seed 2007**		**Experiment 3. Non-infected seed 2008**		**Experiment 4. Infected seed 2008**	
	***C. coccodes* DNA in soil**		***C. coccodes* DNA in soil**		***C. coccodes* DNA in soil**		***C. coccodes* DNA in soil**	
	Pre	***Post***	***Pre***	***Post***	***Pre***	***Post***	***Pre***	***Post***
0	3	2385	3	3643.6	4	3,476	0	1,892
0.01	0	2960.2	0	6746.4	7	964	1	1,403
0.1	23	7524	23	2971	13	124	17	2,270
1	192	6419.4	192	4979	220	734	159	6,665
10	1899	6150.8	1899	6423.4	-	-	-	-
	Incidence	***Severity***	***Incidence***	***Severity***	***Incidence***	***Severity***	***Incidence***	***Severity***
0	10^{a}	0.1^{a}	70.8	1.12	0	0	1.5^{a}	0.024^{a}
0.01	4^{a}	0.037^{a}	42.9	0.55	0	0	9.9^{ab}	0.059^{ab}
0.1	33^{a}	0.65^{ab}	41.5	0.56	4.42	0.04	32.1^{b}	0.211^{b}
1	69.8^{b}	1.09^{b}	62	1	35.96	0.436	62.9^{c}	0.65^{c}
10	67.2^{b}	1.54^{b}	73	1.04	-	-	-	-
LSD	**29.61**	**0.2052**					**23.87**	**0.3217**
P value	<0.001	=0.003	ns	ns	ns	ns	<0.001	<0.001
	Yield (g/plant)		***Yield (g/plant)***		***Yield (g/plant)***		***Yield (g/plant)***	
0	151.3		131.4		92.58		106.5	
0.01	151.7		148.2		125.45		133.2	
0.1	169.9		97		97.27		133.3	
1	152		147.9		121.36		133.3	
10	103		111.1		-		-	
	ns		ns		ns		ns	

4.3 Management of black dot – shadehouse trials

Shadehouse trials were undertaken to investigate the relationship between the amount of inoculum in soil and the disease on progeny tubers, to assess the efficacy of fungicides, biological controls and organic products to reduce black dot disease when applied at various rates, timings and methods of application. These trials were designed to complement the field trials.

4.3.1. Seed treatments

Two pot trials in a shadehouse at Lenswood Research Centre were undertaken in 2007 and 2008 to evaluate a range of potential seed treatment strategies to reduce the level of black dot on progeny tubers. Seed treatments are a useful and often effective method of controlling soil-borne diseases. Currently, there are no seed treatments recommended for the control of black dot of potatoes.

Aim

To evaluate the efficacy of various fungicide, biological and organic tuber seed treatments on the control of black dot.

Experiment 1 - 2007

Materials and methods

Either surface-sterilised, visually disease-free tubers or inoculated tubers were planted in inoculated or non-inoculated soil after being treated with the various chemical and biological treatments shown in Table 4.9. The black dot severity rating of inoculated tubers ranged from 25-50% (severity rating 2) with an incidence of 90%. Single, treated tubers were planted into 5 replicate pots (20 cm diameter, 4.7 L capacity) into inoculated or non-inoculated soil.

Three combinations were used to test products: (a) planting non-infected seed into soil inoculated with C. coccodes; (b) planting inoculated seed into non-inoculated soil and (c) planting inoculated seed into infested soil. Each seed/soil combination was analysed as a separate trial.

Seed treatments were applied as previously described (section 4.1.10) and the Heads up® foliar treatment was applied at 42 days post-sowing at the equivalent rate of 230 g of product in 230.4 L/ha using a hand-held sprayer.

At 83 days post-sowing plants were desiccated as previously described and 28-30 days post-sowing tubers were removed from the pots and stored and washed as previously described. Assessments included: tuber disease incidence, severity, stem and root disease and yield.

The severity data required transformation by log (+0.1) and back-transformed data have been presented.

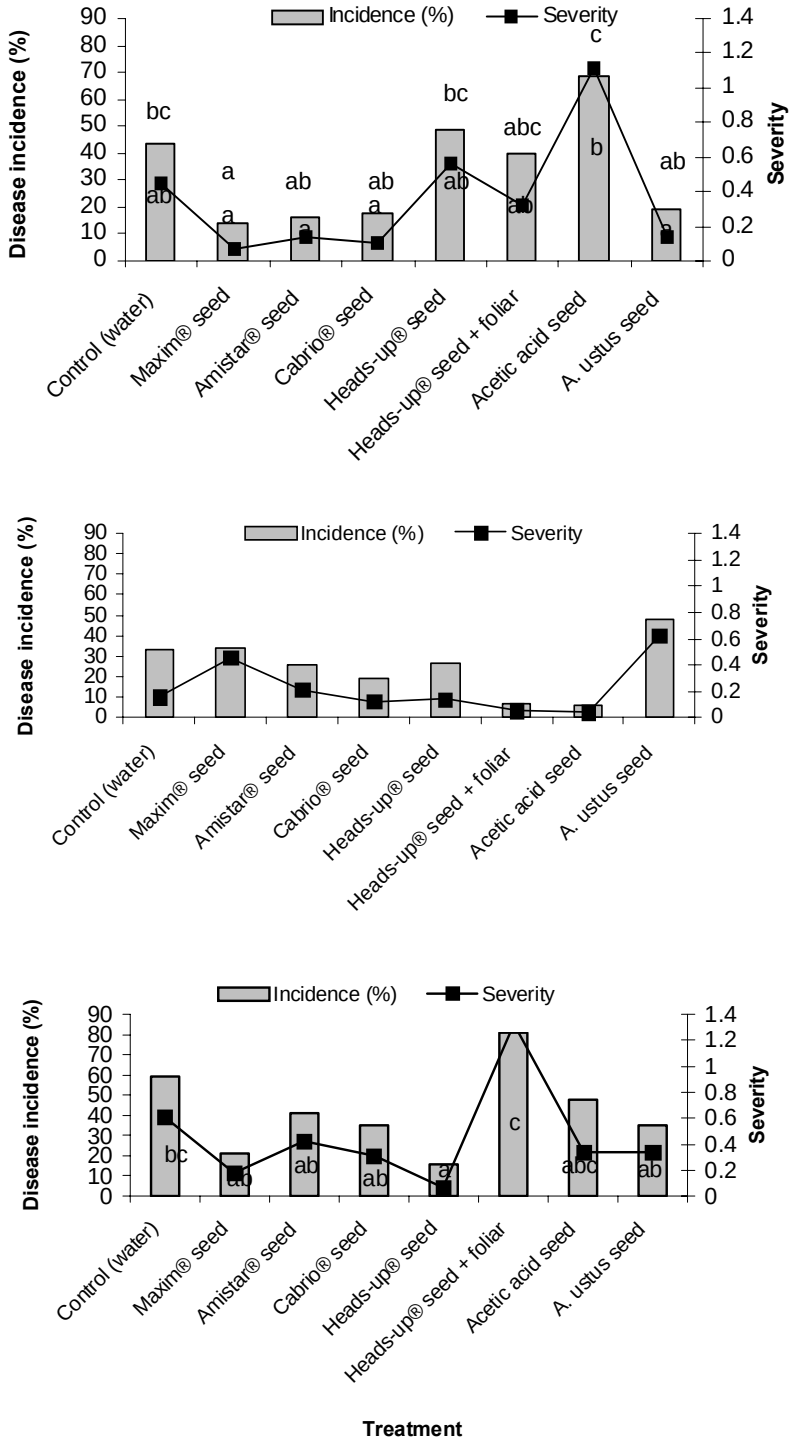
Table 4.9: Active ingredient of seed treatments, application rate of product per tonne of seed and total amount of water and product per tonne of seed.

Treatment	mL of product per tonne seed	Amount of water per tonne seed (L)
Maxim® (250 g/L fludioxonil)	250	1.75
Amistar® (250 g/L azoxystrobin)	13.32	1.75
Heads-up® (extract of *Chenopodium quinoa* 49.65%)	1g/L (1 L treats 100 kg seed)	10
Heads-up® foliar (extract of *Chenopodium quinoa* 49.65%)	1 g/L with 230.40 L/ha	230.4 L/ha
Aspergillus ustus (5 x 106)	2 L/tonne	2
Cabrio® (250 g/L pyraclostrobin)	13.32	1.75
Acetic acid (50 mM)	2 L/tonne	2
Heads-up® seed + foliar (extract of *Chenopodium quinoa* 49.65%)	1g/L (1 L treats 100 kg seed)	10
Untreated control (water)	2 L/tonne	2

Results

In the untreated controls, 33.2% of progeny tubers developed black dot when inoculated tubers were planted into non-inoculated soil (Figure 4.11a), compared to 43.8% when non-inoculated tubers were planted into inoculated soil (Figure 4.11b). The highest disease occurred (59%) on untreated tubers when inoculated seed was planted into inoculated soil (Figure 4.11c).

Cabrio® seed treatment gave similar results when both clean seed and infected seed was planted into infested soil. Maxim®, Amistar® and *A. ustus* also reduced disease levels when applied to non-inoculated seed and planted into inoculated soil. When inoculated seed was planted into inoculated soil, Maxim®, Heads up®, Cabrio® Amistar®, acetic acid and *A. ustus* were the most effective treatment at reducing the disease incidence on progeny tubers. The addition of the foliar Heads up® to the Heads up® seed treatment actually increased disease incidence from 15.5%-81.1%.



(a) Non-inoculated seed planted into inoculated soil (significant differences in disease incidence and severity between

(b) Inoculated seed planted into non-inoculated soil (no significant differences in disease incidence and severity between treatments).

(c) Inoculated seed in inoculated soil (significant differences in disease incidence between treatments but not in disease severity).

Figure 4.11. Disease incidence (%) and severity of progeny tubers grown from (a) inoculated seed and planted into non-inoculated soil, (b) non-inoculated seed and planted into inoculated soil and (c) from inoculated seed and planted into inoculated soil. Treatments with the same letter are not significantly different from each other (P<0.05). Letters in bars represent significant differences of disease incidence, letters above bars represent significant differences of disease incidence.

No significant differences in yield were observed between treatments (Table 4.10). Tuber yields were variable and there were no strong trends between treatments of different seed/soil combinations. Similarly, there were no consistent trends between treatments which lowered disease and yield results. Results are to be interpreted with caution and are presented to give an indication of yield.

Table 4.10: Yield (g/plant) of progeny tubers grown from treated, black dot inoculated or non-inoculated seed and planted into black dot inoculated or non-inoculated soil.

Seed/soil infection	Inoculated seed, non-inoculated soil	Non-inoculated seed, inoculated soil	Inoculated seed, inoculated soil
Treatment	**Tuber yield (g/plant)**		
Control (water)	92.9	109.0	112.2
Maxim® seed	120.9	56.1	97.2
Amistar® seed	69.4	116.8	93.0
Cabrio® seed	108.3	132.6	114.3
Heads-up® seed	109.2	81.0	98.4
Heads-up® seed + foliar	91.9	120.7	125.9
Acetic acid seed	97.8	114.0	98.0
***Aspergillus ustus* seed**	104.0	101.7	113.9

Discussion

No single treatment provided consistent control under the 3 difference seed/soil combinations. However, Maxim® and Cabrio® applied to seed tubers showed good control when either infested or non-infested seed was planted into infested soil.

Experiment 2. 2008

Materials and methods

Seed tubers (cv. Coliban) infected with black dot (severity rating 4, 100% incidence) were treated with Maxim® or Heads up® seed treatments (as described in 4.1.7) 2 days before planting (Table 4.11).

Soil and sclerotia were mixed in a large mixer as previously described and soil was sampled after mixing.

Single treated tubers were planted into 10 replicate pots (20 cm diameter, 4.7 L capacity) containing non-infested UC soil. Plants were desiccated 103 days post-sowing (DPS) and harvested 138 days post-sowing as previously described. Assessments of tuber disease incidence, severity and yield and stem and root disease were made.

Table 4.11: Seed treatment product and water rates applied to tubers before planting into UC soil.

Treatment	Product rate	Water rate (L/tonne seed)
Maxim®	250 mL/tonne seed	2 L
Heads-up®	1g/L (1 L treats 100 kg seed)	10 L
Control	-	2 L

Results and discussion

Poor germination occurred in this trial, with 70% of the Heads Up® treated tubers emerged, 80% of the Maxim® and 30% of the control. Only 3 replicate tubers emerged in the control of which 2 replicates had disease; this was too few to be able to statistically analyse disease incidence and severity of the control (Dyson, C. 2008, pers. comm., 5th Nov). Disease incidence and severity of the Maxim® seed treatment was significantly lower than that of the Heads Up® seed treatment ($P<0.05$). Severity data was transformed by log+0.1 and mean data has been presented for incidence and severity (Figure 4.12).

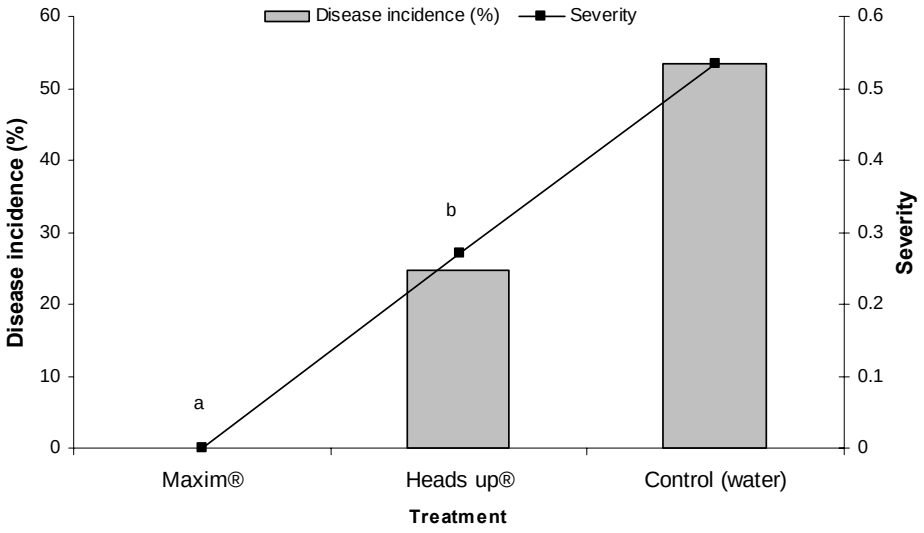


Figure 4.12: Disease incidence and severity of progeny tubers grown from infested seed which was treated with either Maxim® or Heads up®.

The yield was significantly higher in the tubers treated with Heads up® or Maxim® (Table 4.12). The amount of DNA in soil post-harvest was much higher for the Heads up® treatment than for the Maxim® or the untreated control (Table 4.12).

Table 4.12: *C. coccodes* DNA content in soil post-harvest or yield of progeny tubers grown from infested seed which was treated with either Maxim® or Heads up® to seed and planted into non-infested soil. Treatments with the same letter are not significantly different from each other.

Treatment	Yield (g/plant)	pg *C. coccodes* DNA/g soil post-harvest
Heads up®	190.0[a]	2136
Maxim®	157.9[a]	365
Control (water)	37.1[b]	167
L.S.D (P<0.05)	2.75	-

Discussion

Maxim® had significantly lower disease incidence and severity than Heads Up® applied to seed although no significant differences in yield were observed between these treatments. The poor germination of tubers in this trial was likely due to partial shrivelling of tubers prior to treatment. The high amount of DNA in the soil of the Heads up® treatment was surprising due to the lower disease levels observed. The Heads up® treatment may have protected the plant from disease but not prevented the replication of the pathogen in soil.

4.3.2. Soil treatments

Pot trials were undertaken in the shadehouse at the Lenswood Research Centre to compare soil treatments. In 2007 treatments were applied to the soil surface or to mimic in-furrow application and in 2008 treatments were only applied to mimic in-furrow application.

Aim

To evaluate the efficacy of fungicide, biological and organic soil treatments for the control of black dot applied either in-furrow or to the soil surface.

Materials and methods

2007

Artificially inoculated tubers (section 4.1.7) or surface-sterilised tubers visually free of disease were planted into pots (20 cm diameter, 4.7 L capacity) containing either non-inoculated or inoculated soil at a rate of 0.2 cfu/g soil as previously described. Treatments (Table 4.13) were applied either in-furrow or to the soil surface as previously describes using 5 replicate pots per treatment and maintained in the shadehouse as previously described.

Table 4.13: The treatments and their corresponding product rates in either amount of product per hectare (mL) for surface applied treatments, or amount of product per 100 m row (in-furrow).

Treatment	Amount of product per ha (mL) (2007)	Amount of product per 100 m row (mL) (2007)	Amount of product per 100 m row (mL) (2008)	Water rate (L/100 m row)
Amistar® rate 1 in furrow	-	10	20	0.891
Amistar® rate 2 in furrow	-	40	40	0.960
Amistar® rate 1 surface	500	-	-	
Amistar® rate 2 surface	3000	-	-	
Cabrio® rate 1 in furrow	-	10	20	0.966
Cabrio® rate 2 in furrow	-	40	40	0.903
Cabrio® rate 1 surface	500	-	-	
Cabrio® rate 2 surface	3000	-	-	
A. ustus high (surface)	-	4320000	-	
Tea tree oil extract** (10%) in furrow	-	70	-	
Acetic acid high (in furrow)	-	690	-	
Voom® surface	2.77	-	-	
Octave®			10(g)	0.966
Water	1.38	-		0.900

**Tea tree oil extract only applied to pots with infected seed and non-infected soil

Three combinations were used to test products: (a) planting non-infected seed into soil inoculated with *C. coccodes*; (b) planting inoculated seed into non-inoculated soil and (c) planting inoculated seed into infested soil. Each seed/soil combination was analysed as a separate trial.

Plants were desiccated 82 days post-sowing and tubers and stems were removed from the pots 28-30 days post-desiccation and assessed for disease incidence, severity and yield as previously described.

2008

Non-infected seed (cv. Coliban) or *C. coccodes* naturally infected seed were planted into either non-inoculated UC soil or soil artificially inoculated with *C. coccodes* at a rate of 0.2 cfu/g soil (or Trial A 37 pg *C. coccodes* DNA/g soil and Trial B 41.5 pg *C. coccodes* DNA/g soil). Treatments (Table 4.13) were applied either to the soil in-furrow or to foliage (foliage results have been placed into section 4.3.3). Seed was planted into plastic pots (20 cm diameter, 4.7 L capacity) and products applied as previously described (section 4.1.2). Plants were maintained as previously described.

Three combinations were used to test products: (a) planting non-infected seed into soil inoculated with *C. coccodes*; (b) planting inoculated seed into non-inoculated soil and (c) planting inoculated seed into inoculated soil. Each seed/soil combination was a separate trial.

In the 2008 trials, plants were desiccated 98 and 110 days post-sowing and tubers were harvested between 135-143 days post-sowing and assessed for tuber disease incidence, severity and yield and stem and root disease as previously described. Due to the size of trials, planting or spraying was staggered hence, not all trials were desiccated or harvested in the exact time period.

Results and discussion

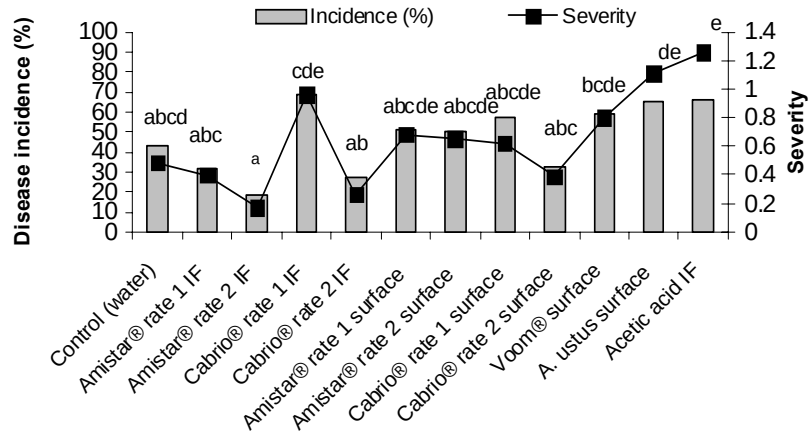
2007

The amount of disease on progeny tubers for the control was similar in each seed and soil combination, varying between 43.3% incidence when non-inoculated seed was planted into inoculated soil and up to 50.9% incidence when inoculated seed was planted into non-inoculated soil (Figure 4.13).

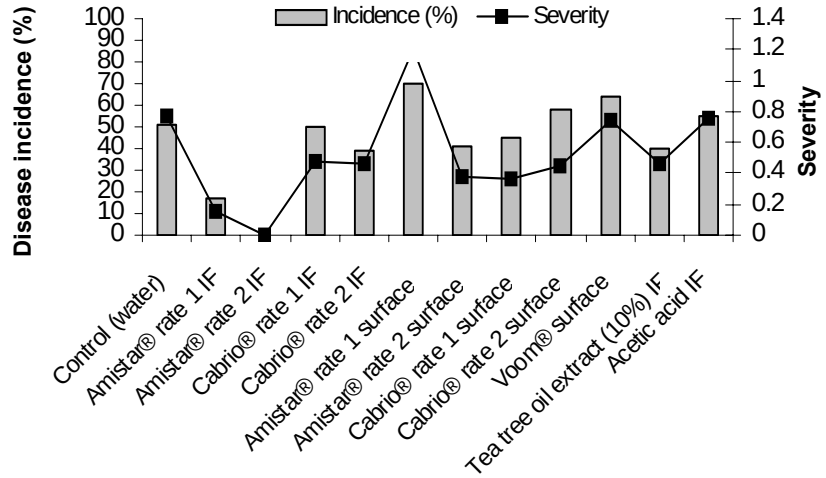
Overall, the in-furrow treatments gave better control of black dot than soil surface treatments, with Amistar® applied at the higher rate of 40 mL/100 m row giving the best control in two soil and seed combinations (a and c).

In all combinations many of the treatments had more disease than the control. Cabrio® at 40 mL/100 m row (a) and Amistar® at 10 mL/100 m row (c) also provided some control.

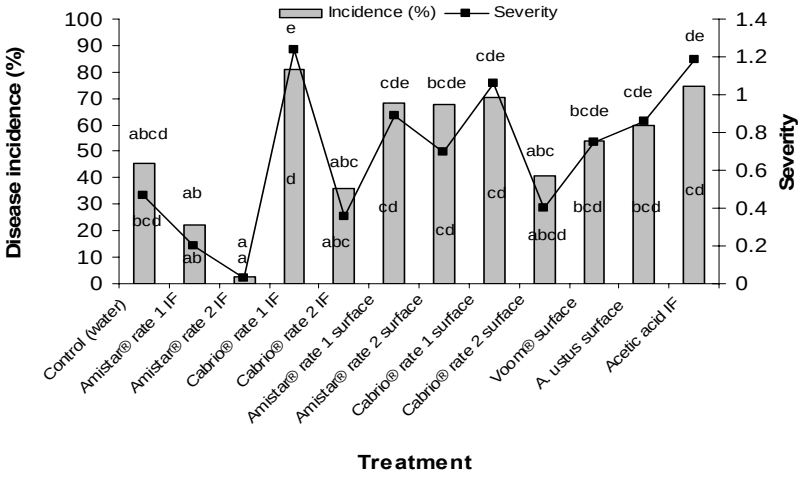
Data for ANOVA of disease severity required transformation (log+1) in each of the three seed and soil combinations and back-transformed data have been plotted. When inoculated seed was planted in non-infested soil (2007) the Amistar® applied in-furrow 40 mL/100 m row was tested against other treatments by multiplying the LSD of disease incidence (37.02) or severity (0.1812) by 1/square root of 2. It was found that the disease incidence and severity of Amistar® in-furrow rate 2 was not significantly different from Amistar® in-furrow rate 1.



(a) Non-inoculated seed into inoculated soil (significant differences in disease severity between treatments but



(b) Inoculated seed into non-inoculated soil (no significant differences between treatments)



(c) Inoculated seed into inoculated soil (significant differences in disease incidence and severity between treatments)

Figure 4.13: Disease incidence and severity of progeny tubers treated with various fungicide, biological or organic products at different rates and timings and planted with (a) non-inoculated seed into inoculated soil (treatments with the same letters above bars are not statistically significantly different from each other for disease severity (P= 0.019, LSD=0.1618)) (b) inoculated seed into non-inoculated soil (no significant differences were found) and (c) inoculated seed into inoculated soil (treatments with the same letter are not significantly different from each other for disease incidence (%) (P=0.008, LSD=39.90) and severity (P=0.008, LSD=0.1819)).

There were no significant differences in yield between treatments (Table 4.14).

Table 4.14: Yield of tubers planted into artificially infested seed in artificially infested soil and treated with various fungicide, biological or organic soil treatments applied either in-furrow or to the soil surface.

Treatment	Tuber yield (g/plant)		
	Non-inoculated seed, inoculated soil	Inoculated seed non-inoculated soil	Inoculated seed inoculated soil
Control (water)	101.9	65.7	114.1
Amistar® rate 1 in-furrow	131.4	102.8	105.3
Amistar® rate 2 in-furrow	134.2	110.6	130.9
Cabrio® rate 1 in-furrow	114.2	88.4	112.3
Cabrio® rate 2 in-furrow	134.1	112.9	100.3
Amistar® rate 1 surface	123.9	111	96.4
Amistar® rate 2 surface	97.5	84.5	139.7
Cabrio® rate 1 surface	101.7	76.8	100.8
Cabrio® rate 2 surface	129.5	94.7	119.4
Voom® surface	122	90.5	120.2
Aspergillus ustus surface	117.4	75.4*	112.3
Acetic acid in-furrow	98.4	107.4	95.4

*Tea tree oil extract (10%) in-furrow

Discussion

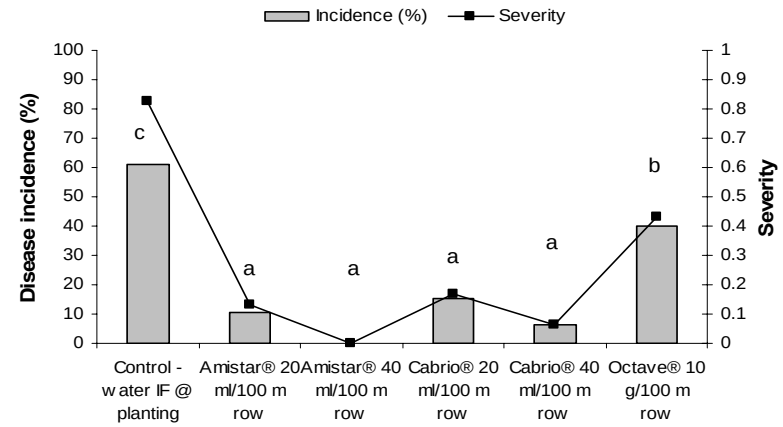
Amistar® applied in-furrow at 40 mL/100 m row resulted in the lowest disease incidence and severity. This suggests that Amistar® was able to protect plants from yield loss, possibly through protecting the plant from early senescence (Mohan *et al*. 1992).

The control pots often had less disease than the treatments, which may have been due to uneven mixing of soil (in treatments with inoculated soil).

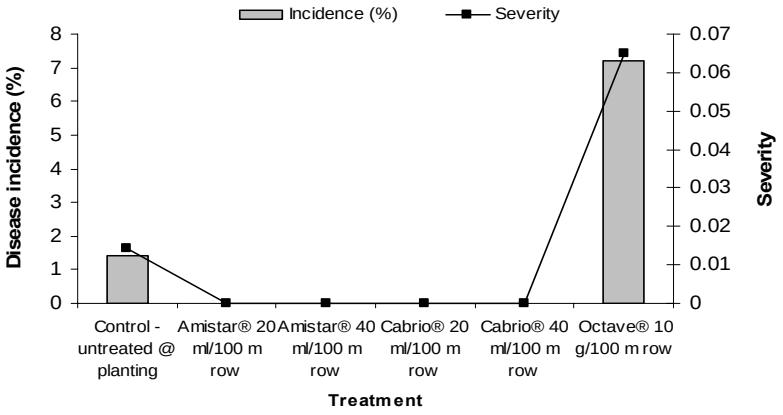
2008

When non-infested seed was treated and planted into inoculated soil all treatments significantly reduced the incidence (%) ($P<0.001$) and severity ($P<0.001$) of black dot on progeny tubers compared to the control (Figure 4.14a). When infected seed was planted into inoculated soil Amistar® applied at 20 or 40 mL/100 m row and Octave® applied at 10 g/100 m row had significantly less disease severity than the other treatments (Figure 4.14c).

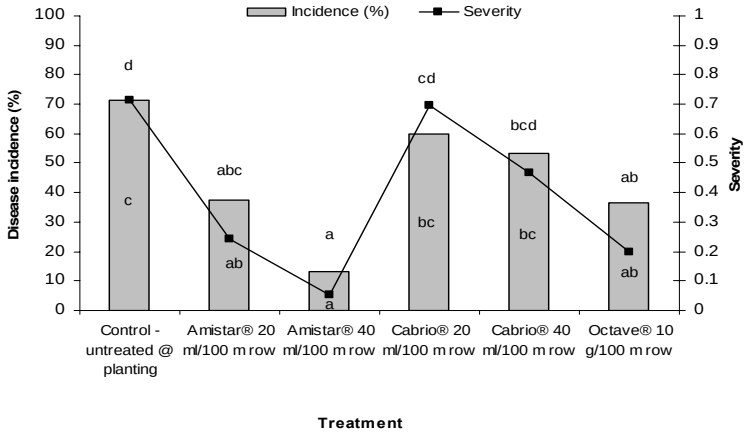
The severity data in trial C required transformation by the log(+0.1) function and back-transformed data has been presented.



(a) Non-infected seed in inoculated soil (significant differences in disease incidence and severity between treatments).



(b) Naturally infected seed in non-inoculated soil (no significant differences between treatments)



(c) Naturally infected seed in inoculated soil (significant differences in disease incidence and severity between treatments).

Figure 4.14: Disease incidence (%) and severity of progeny tubers grown from (a) non-infected seed tuber in inoculated soil (treatments with the same letter are not significantly different from each other when measuring disease incidence (P<0.001, LSD=19.09) or disease severity (P<0.001, LSD=0.2444)), (b) infected seed tubers in non-inoculated soil and (c) an infected seed tuber grown in inoculated soil (treatments with the same letter are not significantly different from each other - letters within bars refer to disease incidence (P = 0.007, LSD=30.59) and letters above bars refer to severity (P = 0.001, LSD=0.3689)).

The yield results were variable however, significant differences were observed between treatments when infected seed was planted into inoculated soil (Table 4.15).

Table 4.15: Yield (g/plant) of progeny tubers grown from either an infected or non-infected seed tuber planted in infested or non-infested soil.

	Tuber yield (g/plant)		
Treatment	**Non infected seed in inoculated soil**	**Infected seed in non-inoculated soil**	**Infected seed in inoculated soil**
Control - water IF @ planting	150.6	124.7[abc]	134.6
Amistar® 20 ml/100 m row	121.4	142.1[a]	111.1
Amistar® 40 ml/100 m row	121.4	108.3[bc]	117.3
Cabrio® 20 ml/100 m row	148.5	124.9[abc]	119.1
Cabrio® 40 ml/100 m row	120.5	135.2[ab]	125.5
Octave® 10 g/100 m row	134.1	99.1[c]	118.6

At planting the soil had an average of 37 pg *C. coccodes* DNA/g soil. After harvest the treatment with the lowest amount of *C. coccodes* DNA in each of the seed and soil combinations was Amistar® applied in-furrow at 40 ml/100 m row whereas the control had the highest amount of DNA in two of the three seed and soil combinations (Table 4.16). There were only low levels of disease when infected seed was planted into non-inoculated soil therefore, these yield and DNA results need to be interpreted with caution.

Table 4.16: The amount of *C. coccodes* DNA/g soil post-harvest after various treatments were applied to soil at planting.

	***Colletotrichum coccodes* pg DNA/g soil post harvest**		
Treatment	**Non-infected seed in inoculated soil**	**Infected seed in non-inoculated soil**	**Infected seed in inoculated soil**
Control - water IF @ planting	2,442	8,624	2,814
Amistar® 20 ml/100 m row	469	145	1,470
Amistar® 40 ml/100 m row	76	1	601
Cabrio® 20 ml/100 m row	713	131	2,077
Cabrio® 40 ml/100 m row	654	51	2,486
Octave® 10 g/100 m row	1,280	529	2,873

Discussion

The control treatment when planted with infected seed into non-inoculated soil had the highest amount of DNA in it post-harvest. This may have been due to the fungus from the infected seed replicating rapidly due to absence of competition from the non-

inoculated soil. The DNA result may have also been exaggerated by the presence of infected plant tissue in the soil sample.

The treatment which provided the best disease control in each seed/soil combination (Amistar® 40 mL/100 m row) also had the lowest amount of *C. coccodes* DNA in soil post-harvest. This shows that the application of Amistar® to the soil at the beginning of the season reduced the amount of DNA in soil post-harvest. This may have been due to direct reduction of the fungal inoculum or through protecting the plant from infection by the fungus.

The results also show that when infected seed was planted into inoculated soil, disease and soil DNA levels were the highest. Therefore, it is not recommended to plant infected seed into infested soil.

General discussion

Results show that Amistar® at 40 mL/ha in-furrow applied at planting consistently provided the best disease control (incidence and/or severity) in two of the seed and soil combinations (a and c) and b in 2008. Amistar® 10-20 mL/100 m row and Cabrio® 40 mL/100 m row applied in-furrow also provided good disease control.

Amistar® (active ingredient azoxystrobin) and Cabrio® (active ingredient pyraclostrobin) are both strobilurin fungicides. Strobilurin fungicides disrupt the energy system of fungal spores and hyphae by inhibiting mitochondrial respiration (Bartlett *et al.* 2002). Therefore, the fungicide applied in-furrow may have inhibited conidial or sclerotial germination of inoculum near the potato seed. This may have reduced/inhibited infection on the mother tuber. *C. coccodes* has been found to travel systemically through a potato plant from an infected mother tuber (Harding *et al*. 2004). Therefore, by the azoxystrobin preventing inoculum contacting tubers at planting it may have prevented the systemic infection of the plant and hence progeny tubers.

Amistar® applied in-furrow at 40 mL/100 m row also had the lowest levels of DNA at the end of the season. This suggests that Amistar® prevents growth of the fungus when applied early in the season, possibly preventing/reducing infection of the emerging plant. Combined with the reduction in infection on progeny tubers, the fungicide appears to reduce the replication of the fungus in the soil.

The surface applied treatments and the acetic acid applied in-furrow did not lower disease incidence and severity compared to most of the in-furrow treatments and the untreated control in each of the seed/soil combinations. Possible reasons for this may have been due to the fungicide sitting on the surface of the soil and not being able to move down the soil profile (Wicks, T. 2007, pers. comm.) to where the seed and *C. coccodes* inoculum was.

The results in 2007 were much more variable than those in 2008. Several reasons may have contributed to this difference including: the method of soil mixing in 2008 potentially provided a more consistent distribution of sclerotia in soil; naturally infected seed was used in 2008 (opposed to artificially inoculated in 2007); different

isolates were used to inoculate soil and tubers; and different spray bottles were also used to apply chemicals in 2008 and these appeared to give a finer spray.

4.3.3. Foliar and haulm treatments

As little is known about the effect of foliar applied fungicides on black dot control they were trialled in these experiments. Previously, post-desiccation haulm application of Amistar® was trialled with some success and was included as a treatment in these trials.

Aim

To investigate the efficacy of various fungicide or organic in-season foliar or post-desiccation haulm treatments on black dot control on progeny tubers.

Materials and methods

2007

Naturally infected tubers (cv. Coliban) with black dot incidence 100% and severity rating 2 were planted into pots (20 cm diameter, 4.7 L capacity) of non-inoculated UC soil (8/1/07). Each treatment was replicated 4 times with a single tuber in a pot considered a replicate. Plants were maintained as previously described. Foliar applications were applied 39 days post-sowing using a 500 mL Hills sprayer (Table 4.17).

All plants were desiccated 101 days post-sowing (DPS) as previously described and 7 days post-desiccation (DPD) Amistar® or acetic acid were applied to the remaining haulms.

Table 4.17: Foliar treatment product and water rates applied foliage 39 days post-planting or to desiccated haulms 7 days post-desiccation.

Treatment and rate	Applied to	2007 product rate	2007 water rate
Amistar® foliar (350 mL/ha)	Foliage 39 days post sowing or to desiccated haulms 7 days post-desiccation	350 mL/ha	200 L/ha
Acetic acid (50 mM)	Foliage 39 days post sowing or to desiccated haulms 7 days post-desiccation	50 mM	230 L/ha
Control foliar (water)	Foliage 39 days post-sowing	-	230 L/ha

Tubers were removed from the pots 119 days post sowing.

Assessments of progeny tuber disease incidence, severity and yield and stem and root disease were made.

2008

Non-infected seed (cv. Coliban) or *C. coccodes* naturally infected seed (cv. Coliban) were planted into either non-inoculated UC soil or soil artificially inoculated with *C. coccodes* in three combinations. These were: (a) non-infected seed/inoculated soil, (b) infected seed tuber in non-inoculated soil and (c) infected seed in inoculated soil.

Foliar treatments (Table 4.18) were applied to foliage 41 (a) or 37 (b & c) days post-sowing using a 350 mL hand sprayer. Two squirts of the applicator were applied so that as much foliage as possible was covered. The difference in the time of application in the foliar trials was due to the large size of trials.

Plants were desiccated 98-110 days post-sowing and tubers were harvested 143 (a), 135 (b) or 136 (c) days post-sowing.

Assessments of progeny tuber disease incidence, severity and yield and stem and root disease were made.

Table 4.18: Foliar treatment product and water rates applied to foliage 37-41 days post-sowing.

Treatment	Product rate	Water rate
Amistar®	350 mL/ha	130 L/ha
Heads up®	1 g/L	130 L/ha
Octave®	175 g/ha	127 L/ha
Control	-	117 L/ha

Results

2007

No significant differences in disease incidence or severity were observed between treatments (Figure 4.15).

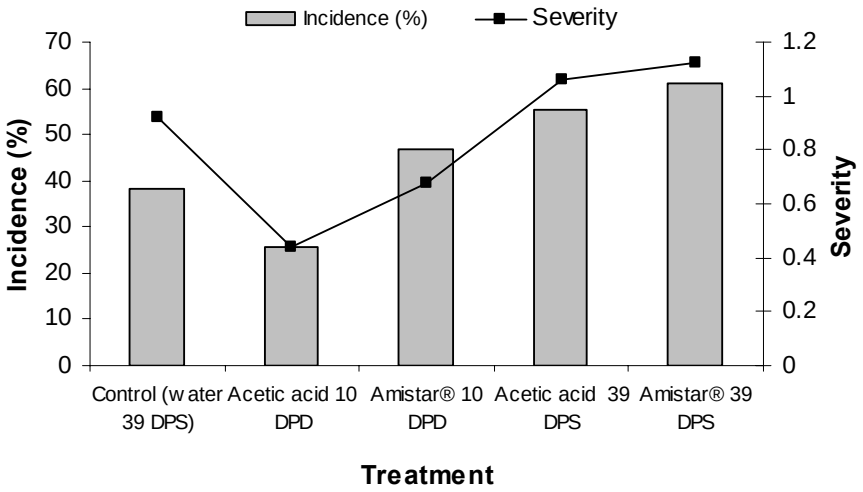


Figure 4.15: Disease incidence (%) and severity of progeny tubers grown from an infested mother tuber grown in non-infested soil. DPS = days post-sowing and DPD = days post-desiccation.

Yield results were variable and no significant differences were observed (Table 4.19).

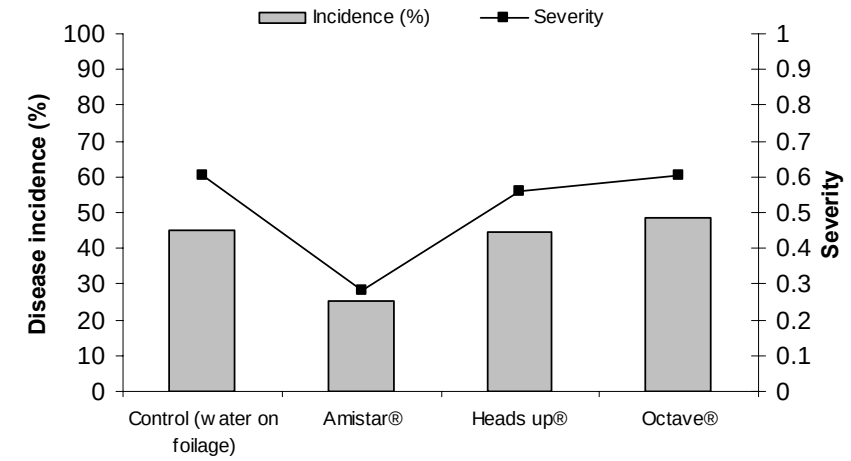
Table 4.19: Yield of progeny tubers grown from a naturally infected seed tuber and planted into non-inoculated soil and treated with foliar applied chemicals 39 days post-sowing or 10 days post-desiccation.

Treatment	g/plant
Control (water 39 DPS)	137.7
Acetic acid 10 DPD	117.7
Amistar® 39 DPS	110.7
Acetic acid 39 DPS	103.6
Amistar® 10 DPD	90.7

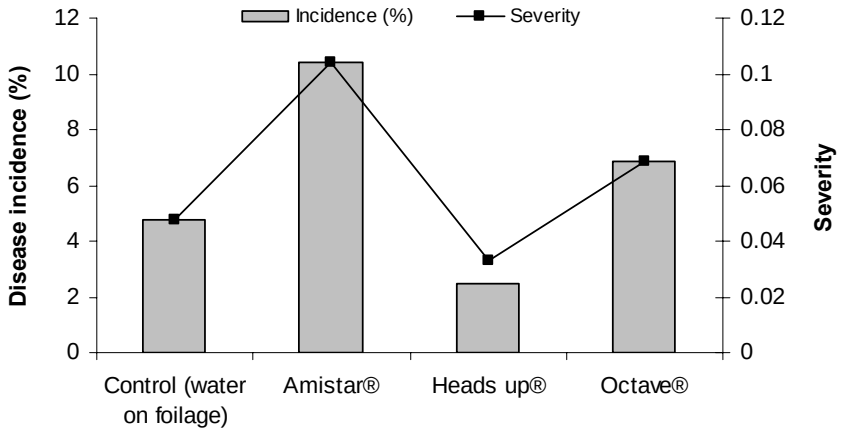
2008

No significant differences were observed in disease incidence or severity between foliar treatments (Figure 4.16).

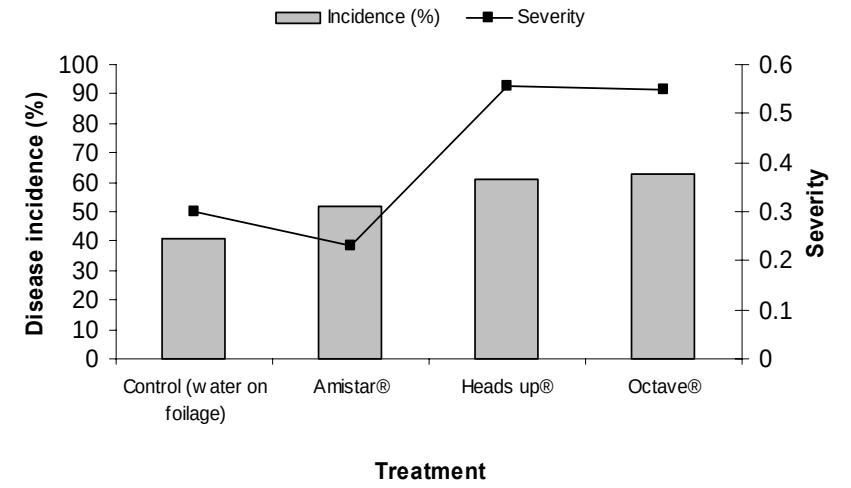
However there was a trend for the incidence of tuber infection to be higher in the untreated control (mean 40.7-44.9%) when inoculated soil (Figure 4.16, a and c) was used, compared to non-inoculated soil (mean 4.75%) (Figure 4.16b). The severity data in trial C required transformation by the log (+0.1) function and back-transformed data has been presented.



(a) A non-infected seed tuber grown in inoculated soil (no significant differences between treatments).



(b) Infected seed tuber grown in non-inoculated soil (no significant differences between treatments).



(c) Infected seed in inoculated soil (no significant differences between treatments).

Figure 4.16: Disease incidence (%) and severity of progeny tubers grown from (a) a non-infected seed tuber grown in inoculated soil, (b) infected seed tuber and grown in non-inoculated soil and (c) infected seed in inoculated soil with treatments applied as foliar applications 39-41 days after sowing.

Yield results were variable (Table 4.20). While there was a significant difference between treatments in tuber yield per plant where infected seed was planted into non-inoculated soil, no trends were observed over all seed/soil combinations.

Table 4.20: Yield (g/plant) of tubers grown from a non-infected seed tuber in soil inoculated with *C. coccodes* sclerotia.

	Tuber yield (g/plant)		
Treatment	**Non-infested seed, inoculated soil**	**Infested seed in non-inoculated soil**	**Infested seed in inoculated soil**
Control foliar (water)	107.2	138.94[ab]	143.6
Amistar® foliar (350 ml/ha)	95.5	115.45[b]	130.4
Heads up® foliar (1g/L)	121.4	145.00[a]	127.6
Octave® foliar (175 g/ha)	147.1	155.91[a]	80.6

All treatments within each trial had similar amounts of DNA present post-harvest (Table 4.21). It is noted that when infected seed was planted into inoculated soil the DNA content in soil post-harvest was the highest.

Table 4.21: The amount of *C. coccodes* DNA in soil after harvest after various foliar treatments were applied to plants in three trials of different seed/soil combinations.

	pg *C. coccodes* DNA/g soil post-harvest		
Treatment	**Non-infected seed, inoculated soil**	**Infected seed, non-inoculated soil**	**Infected seed, inoculated soil**
Control foliar (water)	2,008	3,144	3,903
Amistar® foliar (350 ml/ha)	2,394	1,781	4,736
Heads up® foliar (1g/L)	2,108	1,046	8,517
Octave® foliar (175 g/ha)	2,641	1,426	3,030

Discussion

Results of these trials show that fungicides applied to plant foliage 39-40 days post-sowing did not reduce disease on progeny tubers at the end of the season compared to the control plants. These results suggest that foliar applied fungicides at 40 days post-sowing are not effective for the control of black dot on potatoes.

4.3.4. Desiccation treatments

Aim

To evaluate the effect of desiccation methods on disease incidence and severity of tubers grown from naturally infected tubers.

Materials and methods

Naturally infected tubers (cv. Coliban) with black dot incidence 100% and severity rating 2 (between 25-50 % of tuber surface covered with black dot) were planted into pots (20 cm diameter, 4.7 L capacity). Plants were maintained as previously described and desiccation treatments were applied 101 days post-sowing. Treatments included pine oil (20%), flaming and Reglone® (3.5 L/ha in 200 L water/ha). The Reglone® treatment is the industry standard and was used as the control treatment.

To flame potato plants a "Bernzomatic" propane burner was used. The flame was run over the leaves until they wilted, then back again. Tubers were harvested 27 days after desiccation.

Results

There were no significant differences in incidence and severity between treatments (Figure 4.17).

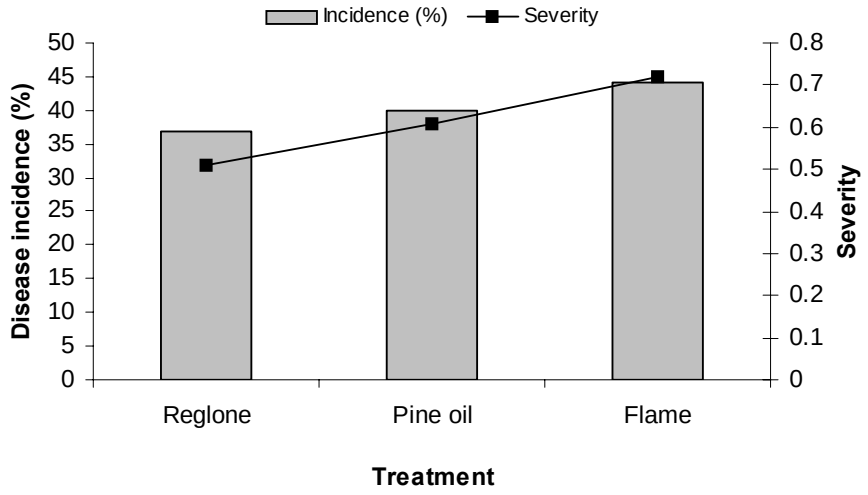


Figure 4.17: Incidence and severity of black dot on progeny tubers grown from an infected mother tuber in non-inoculated soil and desiccated with either the herbicide (Reglone®), pine oil or flaming.

Discussion

All treatments resulted in similar amounts of disease, indicating that desiccation with Pine oil or flaming warrant further investigation, particularly when they may assist with providing an alternative to chemical application.

4.3.5. Combination trial

During pot trials in 2007 and 2008 some treatments, timings and methods of application performed well and resulted in lower disease. The following trial was

undertaken to assess some of those treatments that performed well in combination with each other.

Aim

To evaluate the efficacy of various seed, soil and foliar treatments on black dot disease incidence and severity of tubers grown from naturally infected seed.

Materials and methods

Naturally infected seed cv. Coliban (severity rating 1, incidence 100%) was planted into pots with soil artificially inoculated with *C. coccodes* (0.2 cfu/g soil, or 37 pg *C. coccodes*/g soil). The day prior to planting seed treatments were applied (Table 4.22) and seed was left to dry in plastic trays covered with paper towel. Pots were watered at planting and maintained as previously described (section 4.1.6). Seed and foliar treatments (Table 4.22) were applied as previously described

Plants were desiccated 101 days post-sowing and tubers were harvested from pots 124-125 days post-sowing and washed, stored and assessed for tuber disease incidence, severity, stem disease and tuber yield as previously described.

Table 4.22: Combinations of seed, soil and foliar treatments applied pre-planting, at planting or 40 days post-sowing.

Combination trial			
Treatment code	**Seed**	**Soil (in-furrow)**	**Foliar**
1	Maxim® (250 mL/tonne seed in 2 L water)	Amistar® (20 mL/100 m row, in 0.891 L water)	
2	Maxim® (250 mL/tonne seed in 2 L water)	Cabrio® (20 mL/100 m row, in 0.966 L water)	
3	Maxim® (250 mL/tonne seed in 2 L water)		Amistar® 350 mL/ha in 130 L water/ha
4	Control (untreated)		

Results

No significant differences were observed between treatments due to difficulty in obtaining viable estimates of standard error of treatment means (Dyson, C. 2008, pers. comm., 5th November) (Figure 4.18).

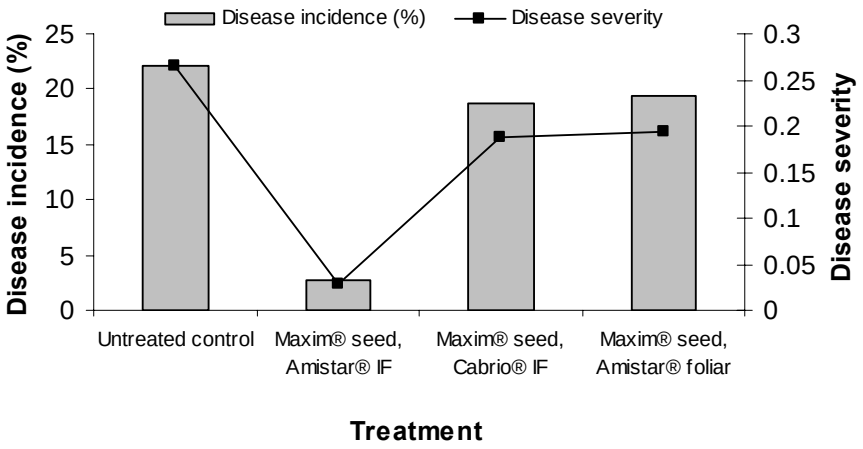


Figure 4.18: Disease incidence (%) and severity of progeny tubers grown from naturally infected progeny tubers in soil artificially inoculated with *C. coccodes*.

No significant differences were observed for tuber yield (Table 4.23).

Table 4.23: Yield of progeny tubers planted into inoculated soil with naturally infected seed and treated with various combinations of seed, soil or foliar applications.

Treatment	Tuber yield (g/plant)
Untreated control	106.6
Maxim® seed, Amistar® IF	131.4
Maxim® seed, Cabrio® IF	120.1
Maxim® seed, Amistar® foliar	107.9

The amount of DNA in soil post-harvest varied, with the control and Maxim® seed + Amistar® foliar treatments having more *C. coccodes* DNA in soil than the other treatments (Table 4.24).

Table 4.24: The amount of *C. coccodes* DNA in soil for each treatment in the combination trial planted with naturally infected seed into inoculated soil.

Treatment	pg *C. coccodes* DNA/g soil post-harvest
Untreated control	2,371
Maxim® seed, Amistar® IF	1,572
Maxim® seed, Cabrio® IF	2,727
Maxim® seed, Amistar® foliar	1,468

Discussion

Although no significant differences were observed between treatments in this trial, some interesting possible trends were observed. The use of Amistar® applied in furrow again provided an observed reduction in mean disease levels, and future studies are warranted to establish whether the use of combination treatments improve control over in furrow alone.

4.3.6. Heads-up® trial

Aim

To evaluate the efficacy of Heads up® plant protectant when applied to seed, soil or foliage on the control of black dot.

Materials and methods

Naturally infected seed (cv. Coliban, severity rating 1, 100% incidence) were planted into pots (20 cm diameter, 4.7 L capacity) containing 50% field soil diluted with UC soil. Soil contained an average of 411 pg *C. coccodes* DNA/g soil pre-planting. Maxim® or Heads up® were applied to tubers one day before planting as previously described (Table 4.25).

Pots were randomised, watered and maintained as previously described.

Plants were desiccated at 101 days post-sowing and tubers were harvested 30 days after desiccation and washed, stored and assessed for tuber disease incidence, severity, yield and stem disease as previously described.

Table 4.25: Treatments and rates of Heads up® used in the Heads up® trial.

Treatment	Product rate	Water rate
Maxim®	250 ml/tonne seed	2 L water/tonne
Control (water)	-	2 L water/tonne seed
Heads up® seed	1g/L water (1 L treats 100 kg seed)	1 L treats 100 kg seed
Heads up® seed and foliar	1g/L (1 L treats 100 kg seed) 1 g/L in 200 L/ha	1 L treats 100 kg seed, foliar: 200 L water/ha
Heads up® seed + Amistar® in-furrow	1g/L (1 L treats 100 kg seed). Amistar® 20 ml/100 m row in 0.891 L water	1 L treats 100 kg seed/ 0.891 L water/100 m row
Heads up® seed, Amistar® in-furrow and Heads up® foliar	1g/L (1 L treats 100 kg seed). Amistar® 20 ml/100 m row in 0.891 L water. Heads up® foliar 1 g/L in 130 L water/ha	1 L treats 100 kg seed/0.891 L water/100 m row/130 L water/ha

Results and discussion

No significant differences were observed in this trial due to the difficulty in obtaining viable estimates of standard error of mean as many replicates had no visible disease (0% incidence, 0 severity) (Dyson, C. 2008, pers. comm., 5th November). However,

the combinations which included Amistar® in-furrow had the lowest levels of disease, with 0 and 3.4% incidence compared to 22% in the untreated control (Figure 4.19).

The treatments with Amistar® applied in-furrow at planting had the lowest level of DNA detected in soil post-harvest (Table 4.26). The greatest increase in DNA was in the control treatment. This indicates that managing disease levels not only provided benefits for reduced tuber blemish, but also reduced soil inoculum for further crops**.**

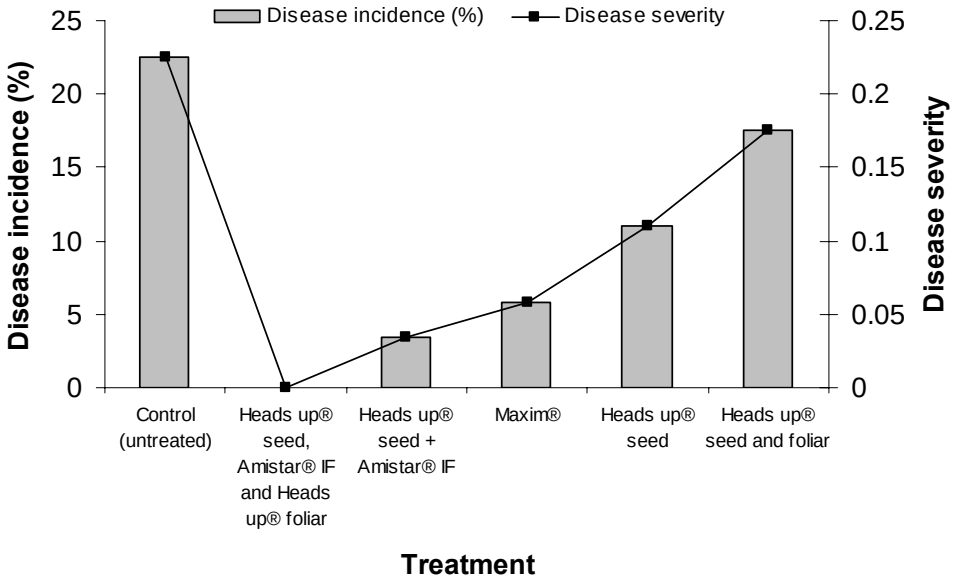


Figure 4.19: Disease incidence and severity of progeny tubers grown from infected seed tubers planted into soil naturally infested with black dot (and diluted with UC soil) and treated with Heads up®, Amistar® or Maxim® in various combinations and methods of application.

Table 4.26: Yield of progeny tubers treated with Heads up®, Amistar® or Maxim® in various combinations and methods of application and the amount of *C. coccodes* DNA in soil post-harvest.

Treatment	Yield (g/plant)	pg *C. coccodes* DNA/g soil post-harvest
Control (untreated)	111.9	4,258
Heads up® seed and foliar	99.1	2,107
Heads up® seed	88.8	3,592
Maxim®	84.7	2,151
Heads up® seed + Amistar® in-furrow	82.0	708
Heads up® seed, Amistar® in-furrow and Heads up® foliar	69.1	649

It was expected (but not seen) that using infected field soil would result in a high proportion of disease on tubers of the untreated control. Pre-plant soil tests showed

411 pg *C. coccodes* DNA/g soil which in other trials has resulted in high disease on progeny tubers. A possible reason for these results may be that the isolate in the soil was less pathogenic than isolates in other trials. This trial has demonstrated the use of field soil in pot trials is not always effective, even though disease resulted when the soil was in the field.

Although significant differences were not observed these results suggest that the combination of Heads up® (seed/and foliar) and Amistar® applied in-furrow were effective at reducing disease. Heads up® can inhibit viral, fungal and bacterial growth (Reilly *et al*., no date) and therefore, may have contributed to a reduction in black dot disease. According to Heads up® Plant Protectants Inc. (no date) Heads up® works by stimulating plant protective chemical pathways in the plant before pathogen attack.

4.4 Management of black dot - field trials

Eleven field trials at five sites were undertaken on growers' properties at Waikerie, Parilla and Peebinga and on the Lenswood Research Centre to assess the efficacy of various fungicide, biological and organic treatments on black dot control in naturally infected soil.

4.4.1. Parilla (2007)

Aim

Two field trials were undertaken on a grower's property at Parilla in 2007 to assess the efficacy of various fungicide, biological and organic treatments on black dot.

Materials and methods

Coliban seed (not assessed for black dot) was planted into a single row (0.75 m wide) of 20 m in length on the 13th of February 2007 in a random complete block design (5 replicates). A 6-row planter was used to plant seed, with four single rows used per block in this trial and 2 as buffer rows. Amistar® and Cabrio® were applied in-furrow using a Team Sprayers® applicator attached to the growers' planter. The four sprayers were used to apply separate treatments to individual rows. Seed treatments (Table 4.27) were applied the afternoon before planting as previously described.

Foliar/surface sprays were applied to growing plants on Thursday 29th of March (45 days post-sowing) using Hills® 5-7 L hand-held applicators.

The plants in this trial died from target spot infection approximately 100 days post-sowing, therefore Reglone® desiccation was not applied. Pine oil and flame (as described in 4.4.2) were applied at 100 days post-sowing.

3 m of each replicate was hand dug on 2-3rd July 2007 (approximately 100 tubers). Tubers were taken back to Lenswood Research Centre, washed, weighed in size category and stored until assessment of progeny tubers for disease incidence and severity.

Table 4.27: Treatments and rates used in the Amistar® and combination trial at the Parilla field trials 2007.

Treatment	Amount of active ingredient (a.i.)	Trial rate (product)	Water trial rate
Untreated control			
Maxim® (seed) (control)	100g/L fludioxonil	250 mL/tonne seed	2.5 L/tonne seed
Amistar® in-furrow rate 1 (low) (maxim seed)	250g/L azoxystrobin	10 mL/100 m row	1 L/100 m of row
Amistar® in-furrow rate 1 (low) (untreated seed)	250g/L azoxystrobin	10 mL/100 m row	1 L/100 m of row
Amistar® in-furrow rate 2 (medium) (untreated seed)	250g/L azoxystrobin	20 mL/100 m row	1 L/100 m of row
Amistar® in-furrow rate 3 (high) (untreated seed)	250g/L azoxystrobin	40 mL/100 m row	1 L/100 m of row
Amistar® in-furrow rate 1 (low) + foliar (40 DPS)	250 g/L azoxystrobin	10mL/100m of row + 350 mL/ha	1 L/100 m of row + 200L water/ha
Amistar® foliar (45 DPS)	250 g/L azoxystrobin	350 mL/ha	200L water/ha
Amistar® surface high (45 DPS)	250 g/L azoxystrobin	1000 mL/ha	200 L water/ha
Amistar® seed	250 g/L azoxystrobin	13.32 g/tonne seed	2 L water/tonne seed
Heads up® seed	1 g/L	10 L/tonne	10 L/tonne
Heads up® foliar (45 days post planting)	1 g/L extracts of *Chenopodium quinoa*	In 200 L water/ha	200 L/ha
Heads up® seed + foliar (45 days post planting)	1 g/L extracts of *Chenopodium quinoa*	10 L/tonne/in 200 L water/ha	10 L/tonne/in 200 L water/ha
Plantmate® soil drench	*Trichoderma harzianum*	1.5 kg/ha in 200 L water/ha	200 L water/ha
Plantmate® granules (at planting)	*Trichoderma harzianum*	25 kg/ha	-
Pine oil desiccation Organic Interceptor™ contact weed spray	Pine oil (9.7-11.7%)	20% mixture	
Flame treatment			

Table 4.28: Treatments and rates used in the Cabrio® trial at the Parilla field trials 2007.

Treatments	Amount of active ingredient (a.i.)	Trial rate (product)	Water trial rate
Maxim® (seed) (control)	100g/L fludioxonil	250 mL/tonne seed	2.5 L/tonne seed
Cabrio® in-furrow rate 1 (low) (maxim seed)	250g/L azoxystrobin	10 mL/100 m row	1 L/100 m of row
Cabrio® in-furrow rate 1 (low) (untreated seed)	250g/L azoxystrobin	10 mL/100 m row	1 L/100 m of row
Cabrio® in-furrow rate 2 (medium) (untreated seed)	250g/L azoxystrobin	20 mL/100 m row	1 L/100 m of row
Cabrio® in-furrow rate 3 (high) (untreated seed)	250g/L azoxystrobin	40 mL/100 m row	1 L/100 m of row
Cabrio® in-furrow rate 1 (low) + foliar 40 DPS	250 g/L pyraclostrobin	10mL/100m of row + 350 mL/ha	1 L/100 m of row + 200 L water/ha
Cabrio® foliar (40 -60 DPS)	250 g/L pyraclostrobin	350 mL/ha	200L water/ha

Results Trial 1.

Low levels of disease were observed in this trial, with the untreated control treatment having less than 1% incidence (Figure 4.20). There were no significant differences between treatments.

Incidence and severity data were transformed by log (+1) and log (+0.01) respectively and back-transformed data have been presented.

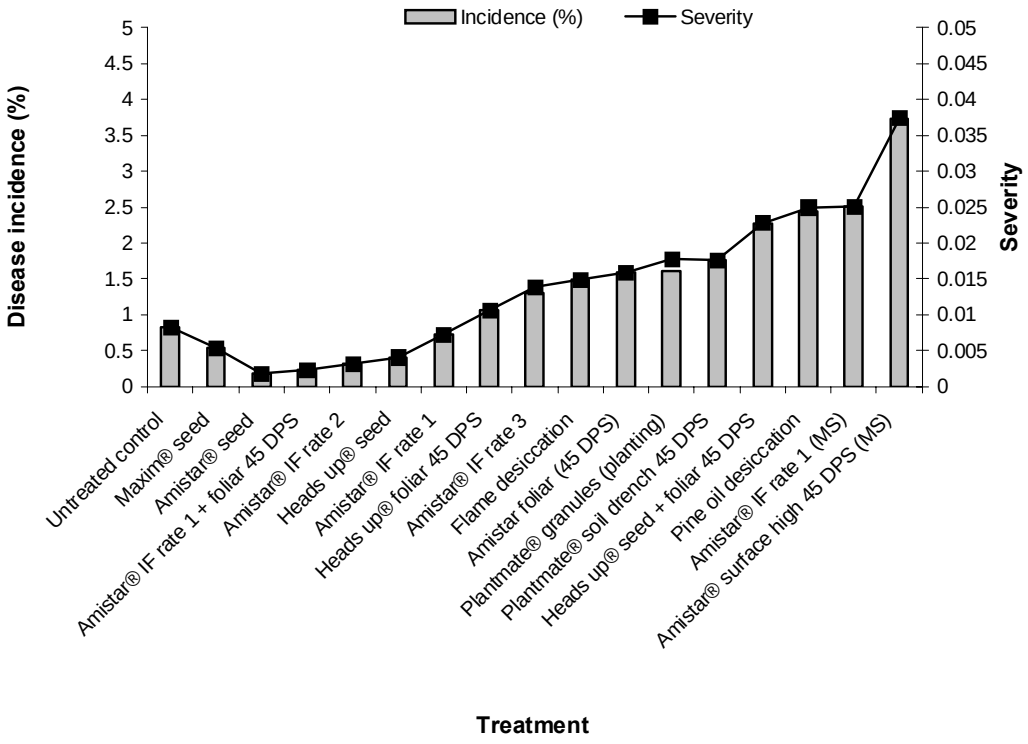


Figure 4.20: Disease incidence (%) and severity of progeny tubers treated with Amistar® and Heads-up® at various rates and application methods.

Significant differences in yield (P=0.030) were observed between treatments (Table 4.29). The treatment with the highest yield was Heads up® seed + foliar 45 days post-sowing (25.38 t/ha). The treatment with the lowest yield was Amistar® in-furrow rate 1 (MS) (13.45 t/ha).

Table 4.29: The yield of tubers (t/ha) harvested from 3 meters of row. Treatments with the same letter are not statistically significantly different from each other. DPS = days post-sowing.

Description	Yield (t/ha)	
Untreated control	22.39	ab
Maxim® seed	14.55	cd
Heads up® seed + foliar 45 DPS	25.38	a
Amistar® foliar 45 DPS	23.93	ab
Heads up® foliar 45 DPS	23.52	ab
Plantmate® granules (planting)	22.92	ab
Amistar® surface high 45 DPS (Maxim® seed)	22.76	ab
Flame desiccation	22.55	ab
Pine oil desiccation	20.86	ab
Amistar® in-furrow rate 1	20.75	abc
Amistar® in-furrow rate 2	20.55	abc
Amistar® seed	20.47	abc
Amistar® in-furrow rate 3	20.35	abc
Heads up® seed	20.14	abc
Plantmate® soil drench 45 DPS	19.43	abcd
Amistar® in-furrow rate 1 + foliar 45 DPS	18.12	bcd
Amistar® in-furrow rate 1 (Maxim® seed)	13.45	d
l.s.d	6.3	

Results Trial 2

Again, very low levels of disease were detected and no significant differences were found between treatments (Figure 4.21).

Incidence and severity data were transformed by log (+1) and log (+0.01) respectively and back-transformed data have been presented.

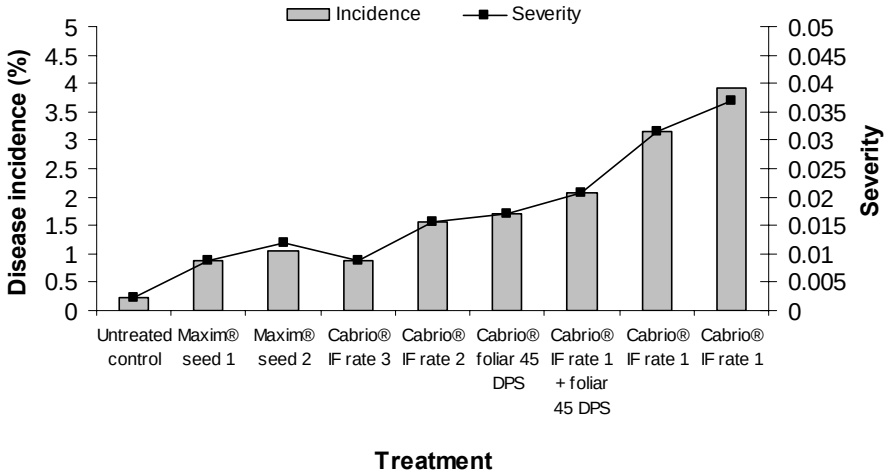


Figure 4.21: Disease incidence (%) and severity of progeny tubers treated with Cabrio® at various rates and application methods.

There were statistically significant differences in yield (t/ha) between treatments (Table 4.30). The treatment with the highest yield was Cabrio® foliar 45 days post-sowing (32.33 t/ha). The lowest yield was observed for the Maxim® seed treatment (19.05 t/ha).

Table 4.30: The yield (t/ha) of tubers harvested from 3 meters of row. Treatments with the same letter are not statistically significantly different from each other. DPS = days post-sowing.

Description	Yield (t/ha)	
Untreated control	26.4	ab
Maxim® seed	23.6	bc
Cabrio® foliar 45 DPS	32.3	a
Cabrio® in-furrow rate 1	29.3	ab
Cabrio® in-furrow rate 1 + foliar 45 DPS	28.2	ab
Cabrio® in-furrow rate 3	28.0	ab
Cabrio® in-furrow rate 1	25.8	b
Cabrio® in-furrow rate 2	22.6	bc
Maxim® seed	19.1	c
l.s.d.	6.5	

Discussion

Very low disease levels were seen in trials and any trends of promising fungicide or application method were not evident.

The significant differences in yield between treatments may reflect the variation in soil or irrigation characteristics. With such low disease levels, the differences in yield are unlikely to be due to the effect of black dot.

However, Maxim® seed treatment in both trials had significantly less yield than most other treatments. The reason for this may require elucidation through further field trials if the trend continues.

4.4.2. Waikerie (2007)

General aim To assess efficacy of various fungicide, biological and organic treatments on black dot control in naturally infected soil.

Three trials were conducted at Waikerie in 2007. Trial 1 investigated various Amistar® and Cabrio® treatments, Trial 2 investigated various foliar and post-desiccation treatments and Trial 3 examined various desiccation treatments.

TRIAL 1.

Aim

To assess the efficacy of Amistar® and Cabrio® applied in-furrow, to the soil surface and to foliage on black dot control.

Materials and methods

The grower supplied seed treated with Maxim® as per commercial practice and hence, tubers were unable to be assessed for black dot. The following day tubers were planted using a 6-row planter, with the middle rows used for the trials and the outer two on either side used as a buffer. Plots were 20 m long and 4 rows wide with 75 cm spacing between rows.

A Team-sprayers® spray unit was attached to the potato planter, with applicators attached to the tines of the grower's planter. Amistar® and Cabrio® were applied in-furrow through the applicator. Treatments (as outlined in Table 4.31) were applied to 4 replicate blocks in a split-plot design.

56 days post-planting foliar and surface applications were applied to the two middle rows. Hills® hand-held 5-7 L applicators were used to apply chemicals and the desired amount of chemical was applied to the unit area.

Sites were maintained as per commercial practices of the grower. Plants were sprayed off with Reglone® and tubers from a total of 3 metres of the treated rows were harvested by hand on 21-22 June 07 (141-142 DPS) in 3 meter strips (desiccation trial) or two strips of 1.5 meters (due to two rows being treated) this totalled approx. 100 tubers each replicate. Tubers were taken to Lenswood Research Centre, washed, weighed in size category, stored and then assessed for disease incidence and severity on progeny tubers.

Table 4.31: Treatments and rates used in the Waikerie field trials 2007.

Treatment	Amount of active ingredient (a.i.)	Trial rate (product)	Rate of water application
Amistar® in-furrow rate 1 (low)	250 g/L azoxystrobin	10 mL/100 m of row	In 1 L/100 m of row
Cabrio® in-furrow rate 1 (low)	250 g/L pyraclostrobin	10 mL/100 m of row	In 1 L/100 m of row
Amistar® in-furrow rate 2 (medium)	250 g/L azoxystrobin	20 mL/100 m of row	In 1 L/100 m of row
Cabrio® in-furrow rate 2 (medium)	250 g/L pyraclostrobin	20 mL/100 m of row	In 1 L/100 m of row
Amistar® in-furrow rate 3 (high)	250 g/L azoxystrobin	40 mL/100 m of row	In 1 L/100 m of row
Cabrio® in-furrow rate 3 (high)	250 g/L pyraclostrobin	40 mL/100 m of row	In 1 L/100 m of row
Amistar® in-furrow rate 1 (low) + foliar 56 DPS	250 g/L azoxystrobin	10mL/100m of row + 350 mL/ha	In 1 L/100 m of row + 200L water/ha
Cabrio® in-furrow rate 1 (low) + foliar 56 DPS	250 g/L pyraclostrobin	10mL/100m of row + 350 mL/ha	In 1 L/100 m of row + 200L water/ha
Amistar® foliar (56 DPS)	250 g/L azoxystrobin	350 mL/ha	In 200 L water/ha
Cabrio® foliar (56 DPS)	250 g/L pyraclostrobin	350 mL/ha	In 200 L water/ha
Amistar® surface (high) 56 DPS	250 g/L azoxystrobin	1000 mL/ha	In 200 L water/ha
Cabrio® surface (high) 56 DPS	250 g/L pyraclostrobin	1000 mL/ha	In 200 L water/ha
Amistar® surface (low) 56 DPS	250 g/L azoxystrobin	350 mL/ha	In 200 L water/ha
Cabrio® surface (low) 56 DPS	250 g/L pyraclostrobin	350 mL/ha	In 200 L water/ha
Maxim Seed (control)	100 g/L fludioxonil	250 mL/tonne seed	2.5 L/tonne of seed

Results and discussion

Only low levels of disease were found in this trial with the two control plots having 1% and 5.5% incidence (Table 4.32). Cabrio® applied in-furrow at rate 2 had a significantly higher disease incidence than the other treatments except for Amistar applied in-furrow at rate 3.

Disease severity data ranged from scores of 0.0027 to 0.33 and no significant differences were observed between treatments (data not shown).

Incidence and severity data required transformation by log (+1) and log (+0.01) respectively and back-transformed data have been presented.

Table 4.32: Disease incidence of progeny tubers planted into infected soil and treated with Amistar® and Cabrio® at various rates and timings. Treatments with the same letter are not significantly different from each other.

Treatment description	Disease incidence (%)	
Amistar® control (Maxim® seed)	5.5	f
Cabrio® control (Maxim® seed)	1.1	abcde
Amistar® surface low 56 DPS	0.2	a
Amistar® surface high 56 DPS	0.3	ab
Cabrio® IF rate 1 + foliar 56 DPS	0.3	ab
Cabrio® surface low 56 DPS	0.8	abc
Cabrio® foliar 56 DPS	0.9	abcd
Cabrio® surface high 56 DPS	1.2	abcde
Amistar® IF rate 1	1.7	abcdef
Cabrio® IF rate 1	2.2	abcdef
Amistar® IF rate 1 + foliar 56 DPS	2.5	bcdef
Cabrio® IF rate 3	2.7	cdef
Amistar® foliar 56 DPS	4.0	def
Amistar® IF rate 2	5.0	ef
Amistar® IF rate 3	6.5	fg
Cabrio® IF rate 2	18.8	g
LSD (P=0.008)	**0.45**	

Yields ranged from 24 t/ha to 32 t/ha, (data not shown) but there were no significant differences between treatments or any observed relationship with disease levels. The soil DNA levels were also low (data not shown), varying from 2 to 135 pg *C. coccodes* DNA/g soil between whole plots.

Discussion

The treatment with the highest disease (Cabrio® in-furrow 40 mL/100 m row) was one of the more effective treatments in the pot trials. However, two of the four replicates in this treatment had much higher disease levels than the other replicates, which also relate with high levels of *C. coccodes* DNA at harvest (data not shown). Unlike the pot trials, there was no observable trend for in-furrow treatments to be more effective. Disease levels in all treatments were very low and also varied between replicates within the same treatments.

Although significant differences were observed in disease incidence it is difficult to establish any trends of particular fungicides or application method lowering disease. The above mentioned variation in soil DNA may indicate that the pattern of natural soil infection was not uniform and this contributed to the variability in disease incidence and severity.

TRIAL 2.

Aim To assess the efficacy of different foliar and post-desiccation treatments on black dot control.

Materials and methods

Tubers were planted and maintained as in trial 1. 56 days post-planting foliar and surface applications as outlined in Table 4.33 were applied to the two middle rows. Hills® hand-held 5-7 L applicators were used to apply chemicals and the desired amount of chemical was applied to the unit area to the 4 replicates.

Tubers were harvested from 3 meters over the two rows and taken to Lenswood Research Centre where they were washed and graded (weighed) and assessed for disease incidence and severity.

Table 4.33: Rates of product and water for the foliar and desiccation trial planted at Waikerie 2007.

Foliar and desiccation	**Amount of active ingredient (a.i.)**	**Trial rate (product)**	**Rate of water application**
Heads up® foliar (56 DPS)	Extracts of *Chenopodium quinoa*	1 g/L of water	200 L of water/ha
Maxim® seed (control)	100 g/L fludioxonil	250 ml/tonne of seed	2.5 L/tonne of seed
Plantmate® surface 56 DPS	*Trichoderma harzianum*	1.5 kg/ha	
Foliar acetic acid 56 DPS	50 mM acetic acid		In 200 L water/ha
Amistar® in-furrow and foliar acetic acid 56 DPS	250 g/L azoxystrobin + 50 mM acetic acid	10 ml/100m of row	In 1 L /100 m row
Reglone® desiccation	200 g/L diquat	As grower practice	
Pine oil desiccation	Pine oil	20 % mixture	
Organic Interceptor™ contact weed spray	Pine oil (9.7-11.7%)	20% pine oil mixture	
Reglone®	200 g/L diquat	As grower practice	
Flame			

Results

Incidence of disease in this trial was also low and no significant differences were found between treatments (Figure 4.22).

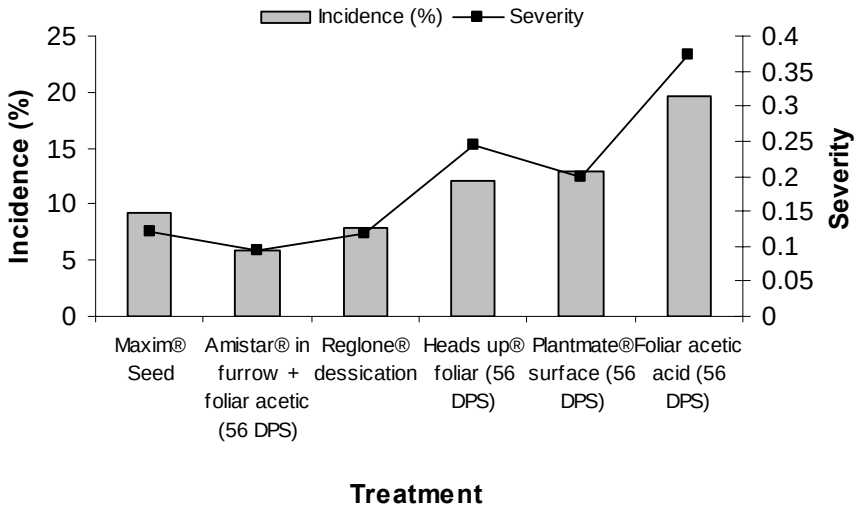


Figure 4.22: Disease incidence and severity of progeny tubers planted into infected soil and treated with various foliar or desiccation treatments.

No significant differences in yield (t/ha) were recorded between treatments (Table 4.34).

Table 4.34: Yield of tubers (t/ha) treated various foliar or surface applications. DPS = days post-sowing.

Treatment	Yield (t/ha)
Maxim® Seed	41.9
Reglone® desiccation	44.2
Foliar acetic acid (56 DPS)	44.0
Amistar® IF + foliar acetic acid (56 DPS)	43.4
Plantmate® surface (56 DPS)	38.0
Heads up® foliar (56 DPD)	34.4

The amount of *C. coccodes* soil DNA at harvest (data not shown) were very low, ranging from 15 to 45 pg *C. coccodes* DNA/g soil.

Discussion

The difference in disease was not large enough to provide good comparisons between treatments, although there was a trend for Amistar® in-furrow to again provide the best control of disease.

TRIAL 3.

Aim

To assess the efficacy of different desiccation treatments on black dot control.

Materials and methods

Potatoes were planted and maintained as in trial 1. 112 days post-sowing actively growing potatoes were desiccated with either a gas burner or pine oil. The gas burner was used to desiccate potato haulms by flaming. The burner was supplied by Elgas Ltd and contained burners spreading 1.5 m in width and was pulled behind a tractor at 1.5-3 km/h. 5 replicates of each treatment were applied.

Results

Once the burner was passed over haulms wilting occurred immediately and plants were dead within several hours.

No disease was detected on any tubers. There were no significant differences in yield between each of the three treatments (Table 4.35).

Table 4.35: Yield of tubers desiccated in various ways including burning, application of pine oil and application of Reglone® herbicide.

Treatment	Yield (t/ha)
Control (Reglone®)	45.7
Pine oil	39.8
Burn	38.2

The levels of *C. coccodes* soil DNA at harvest (data not shown) were very low, ranging from 1 to 4 pg *C. coccodes* DNA/g soil.

Discussion

Further trials are required to assess the efficacy of desiccating plants by flame as the disease pressure was too low to assess for black dot.

4.4.3. Mallee 1 (2008)

Aim

To investigate the effectiveness of various seed, soil and foliar and treatments on black dot control (general trial). To assess the effectiveness of various foliar and post-desiccation haulm treatments on black dot control (foliar trial).

Trial 1. A comparison of Amistar® and Cabrio® applied at various rates and timings.

Trial 2. A smaller trial comparing foliar treatments applied at 40 days post-sowing.

Materials and methods

Trial 1

Visually clean Coliban seed (0% incidence, 0 severity) was used in this trial. Most seed was left untreated however, the treatments of Maxim® or Heads up® seed were applied the evening before planting with (Trial 1, Trial 2, Table 4.36). On the 7th of February 2008 the trial was planted using a 4-row planter (middle two rows were treated). Plots were 9 m in length, with 85 cm row spacing and potato spacing of 21 cm.

Treatments applied, application methods and rates applied are in Table 4.36. Various seed and in-furrow treatments at different rates and placement were applied at planting. The growers own (modified) applicator was used to apply Amistar® and Cabrio® in-furrow treatments. All other treatments were applied using Hills® 5-7 L garden sprayers.

Foliar sprays were applied to plant foliage 42 days post-sowing using the previously mentioned sprayers.

Desiccation sprays were not applied due to the plants naturally dying off due to target spot and frost. Post-desiccation sprays were applied 139 days post-sowing using the Hills® sprayers.

Tubers were harvested 210-211 days post sowing and 2 m were harvested from each Amistar® plot and 4 m from each Cabrio® plot. Tubers were then taken to Lenswood Research Centre for washing, sorting, weighing and assessment of up to 100 tubers for disease incidence and severity. At harvest a 40 core soil sample was collected for each block of the split-plot and foliar trials combined and sent to the RDTS at SARDI for analysis.

Trial 2

Foliar sprays were applied to plant foliage 42 days post-sowing using Hills® 5-7 L sprayers (Table 4.37).

Desiccation sprays were not applied due to the plants naturally dying off due to target spot and frost. Post-desiccation sprays were applied 139 days post-sowing using the Hills® sprayers.

Tubers were harvested 210-211 days post sowing and 4 m were harvested. Tubers were then taken to Lenswood Research Centre for washing, sorting, weighing and assessment of up to 100 tubers for disease incidence and severity.

Table 4.36: Trial 1 treatments and their rates applied at the Mallee site 1 (2008). DPS = days post-sowing and PD = post-desiccation.

Treatment	Amount of active ingredient (a.i.)	Trial rate (product)	Water Application rate
Maxim® seed	100 g/L fludioxonil	250 mL/tonne seed	2.5 L/tonne seed
Amistar® IF rate 1	250 g/L azoxystrobin	15.5 mL/100 m row	71.4 L/ha
Amistar® IF rate 2	250 g/L azoxystrobin	30 mL/100 m row	71.4 L/ha
Amistar® IF rate 1 + Amistar® PD spray	250 g/L azoxystrobin	15.5 mL/100 m row	71.4 L/ha
Amistar® rate 1 + foliar 42 DPS	250 g/L azoxystrobin	15.5 mL/100 m row	71.4 L/ha
Amistar® IF rate 2 + foliar 42 DPS	250 g/L azoxystrobin	30 mL/100 m row	71.4 L/ha
Cabrio® IF rate 1	250 g/L pyraclostrobin	15.5 mL/100 m row	71.4 L/ha
Cabrio® IF rate 2	250 g/L pyraclostrobin	30 mL/100 m row	71.4 L/ha
Cabrio® IF rate 1 + PD spray	250 g/L pyraclostrobin	15.5 mL/100 m row	71.4 L/ha
Cabrio® IF rate 1 + foliar 42 DPS	250 g/L pyraclostrobin	15.5 mL/100 m row	71.4 L/ha
Cabrio® IF rate 2 + foliar 42 DPS	250 g/L pyraclostrobin	30 mL/100 m row	71.4 L/ha

Table 4.37: Trial 2 treatments and their rates applied at the Mallee site 1 (2008). DPS = days post-sowing and PD = post-desiccation.

Treatment	Amount of active ingredient (a.i.)	Trial rate (product)	Water Application rate
Amistar® 42 DPS	250 g/L azoxystrobin	350 mL/ha	200 L/ha
Cabrio® 42 DPS	250 g/L pyraclostrobin	350 mL/ha	200 L/ha
Heads up® 42 DPS	Extracts of *Chenopodium quinoa*	1 g/L	200 L/ha
Amistar® PD	250 g/L azoxystrobin	350 mL/ha	200 L/ha
Cabrio® PD	250 g/L pyraclostrobin	350 mL/ha	200 L/ha
Untreated control			

Results

Trial 1.

Overall, disease levels were low in this trial and no significant differences were observed (Figure 4.23). Two treatments of seed treated with Maxim® were used as the control treatment (one in each split-plot) and disease incidence was 1% in one plot (Maxim® A) and 4% in the second (Maxim® C). Incidence and severity data required transformation by log (+1) log (+0.01) respectively. Back-transformed data have been presented.

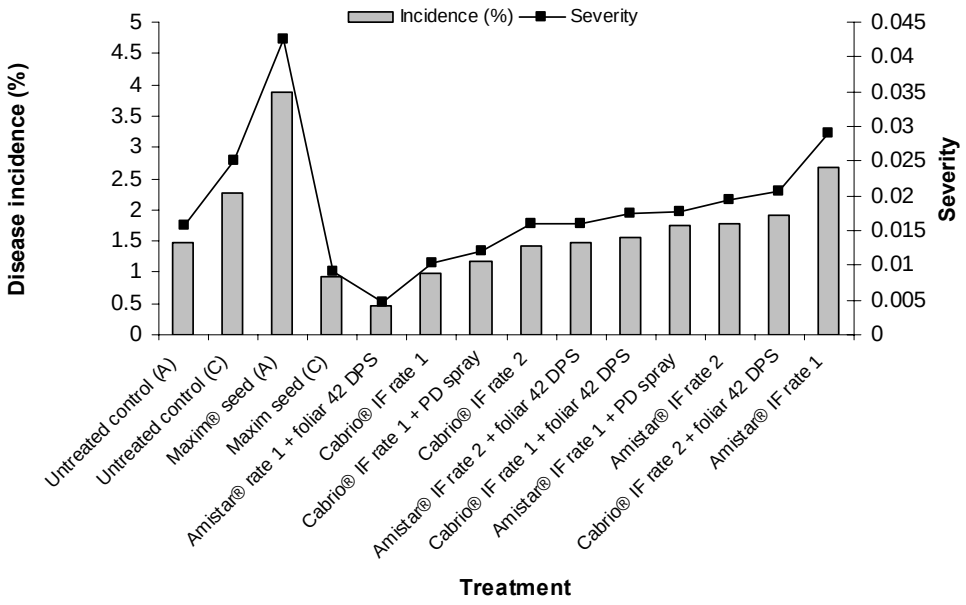


Figure 4.23. Disease incidence and severity of progeny tubers grown in naturally infected soil and treated with either Amistar® or Cabrio® at various rates, placement and timings. DPS = days post-sowing and PD = post-desiccation.

Yield varied from 31.5 t/ha in Maxim® C control treatment, to 48.6 t/ha in the Amistar® in-furrow 30 mL/100 m row plus foliar 42 days post-sowing treatment (Table 4.38). No significant difference in yield was observed between treatments.

Table 4.38. Yield of progeny tubers grown in infected soil and treated with either Amistar® or Cabrio® at various rates, placement and timings. The weight of tubers from 2 m of plot has been used as the basis to calculate yield in t/ha.

Treatment	Yield (t/ha)
Untreated control (A)	43.1
Untreated control (C)	40.7
Maxim® seed (A)	46.4
Maxim® seed (C)	31.5
Amistar® IF rate 1 + PD spray	51.0
Amistar® IF rate 2 + foliar 42 DPS	48.6
Amistar® rate 1 + foliar 42 DPS	47.3
Cabrio® IF rate 1	46.7
Cabrio® IF rate 2 + foliar 42 DPS	44.6
Cabrio® IF rate 2	44.0
Cabrio® IF rate 1 + PD spray	43.9
Cabrio® IF rate 1 + foliar 42 DPS	42.7
Amistar® IF rate 1	42.3
Amistar® IF rate 2	35.2

The levels of *C. coccodes* soil DNA at harvest were variable (data not shown), ranging from 0 to 234 pg *C. coccodes* DNA/g soil.

Trial 2.

The untreated control had the highest disease incidence and severity of all treatments (3.5% and 0.014 respectively), however there were no significant differences in disease incidence or severity between the treatments. Incidence and severity data were transformed by log +1 and log +0.001 respectively and back-transformed data have been presented.

The levels of *C. coccodes* soil DNA at harvest (data not shown) varied, ranging from 0 to 234 pg *C. coccodes* DNA/g soil.

No significant differences were observed between treatments when measuring yield.

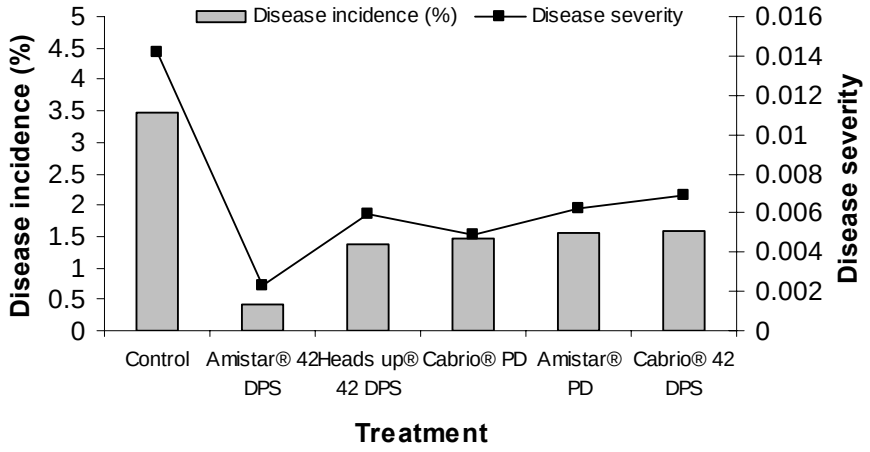


Figure 4.24: Disease incidence (%) and severity of progeny tubers grown in infected soil and treated with applications of various treatments to foliage during the growing season or haulms applied post-desiccation. DPS = days post-sowing and PD = post-desiccation.

Table 4.39: Yield of progeny tubers grown in naturally infected soil and treated with various foliar and post-desiccation applications of fungicides or organic products.

Treatment	Yield (t/ha)
Control	46
Cabrio® 42 DPS	46.8
Heads up® 42 DPS	42.6
Amistar® PD	41.9
Amistar® 42 DPS	41.6
Cabrio® PD	36.6

Discussion

Disease incidence and severity were low in both trials, and the yield results were variable. In the second trial the trend was for treatments to have lower disease than the control, however further testing is required to establish statistically significant differences.

4.4.4. Mallee 2 (2008)

Aim

Trial 1. To investigate the effectiveness of various seed, soil and foliar and treatments on black dot control.

Trial 2. To assess the effectiveness of various foliar and post-desiccation haulm treatments on black dot control.

Materials and methods

Coliban seed (12% incidence, severity 0.84) was treated the evening before planting with Maxim® or Heads up® (Table 4.36). On the 6th of February 2008 the trial was planted using a 6 row planter (middle two rows were treated). Plots were 10 m in length except the Maxim® treatments which were 8 m in length. Row spacing was 85 cm and potatoes were spaced 19 cm apart.

In trial 1, various seed and in-furrow treatments at different rates and placement (Table 4.40) were applied at planting, with foliar sprays applied to plant foliage 42 days post-sowing as previously described. A Team Sprayers® applicator was attached to the growers own planter and was used to apply Amistar® in-furrow. All other soil treatments were applied after planting (by digging soil to reveal the furrow, applying the chemical, and covering the furrow again). All chemicals excepting Amistar® in-furrow were applied using Hills® 5-7 L garden sprayers.

In trial 2, foliar sprays (Table 4.41) were applied to plant foliage 42 days post-sowing as previously described.

Desiccation sprays were not applied due to the plants naturally dying from target spot infection. Post-desiccation sprays were applied 139 days post-sowing using the Hills® sprayers.

6 m of tubers were harvested from plots 209-210 days post-sowing and tubers were taken to Lenswood Research Centre for washing, sorting, weighing and assessment of up to 100 tubers for disease incidence and severity. At harvest a 40 core soil sample was collected for each block and sent to the RDTS of SARDI for analysis.

Table 4.40: Trial 1 treatments and their rates applied to the Mallee site 2 (2008).

Treatment	Amount of active ingredient (a.i.)	Trial rate (product)	Water Application rate	Plot rate (water) mL
Amistar® in-furrow rate 1	250 g/L azoxystrobin	15 mL/100 m row	50 L/ha	85
Amistar® in-furrow rate 2	250 g/L azoxystrobin	30 mL/100 m row	50 L/ha	85
Amistar® in-furrow (rate 1) + foliar (42 DPS)	250 g/L azoxystrobin	15 mL/100 m row	50 L/ha	85
Cabrio® in-furrow rate 1	250 g/L pyraclostrobin	15 mL/100 m row	50 L/ha	85
Cabrio® in-furrow rate 2	250 g/L pyraclostrobin	30 mL/100 m row	50 L/ha	85
Cabrio® in-furrow (rate 1) + foliar (42 DPS)	250 g/L pyraclostrobin	15 mL/100 m row	50 L/ha	85
Heads up® seed	Extracts of *Chenopodium quinoa*	1 g/L 1 L treats 100 kg seed		
Heads up® seed + foliar	Extracts of *Chenopodium quinoa*	1 g/L 1 L treats 100 kg seed		
Maxim® seed	100 g/L fludioxonil	250 mL/tonne seed	2.5 L/tonne seed	52.75
Untreated seed				

Table 4.41: Trial 2 treatments and their rates applied to the Mallee site 2 (2008).

Treatment	Amount of active ingredient (a.i.)	Trial rate (product)	Water Application rate
Amistar® 42 DPS	250 g/L azoxystrobin	350 mL/ha	200 L/ha
Cabrio® 42 DPS	250 g/L pyraclostrobin	350 mL/ha	200 L/ha
Heads up® 42 DPS	Extracts of *Chenopodium quinoa*	1 g/L	200 L/ha
Amistar® PD	250 g/L azoxystrobin	350 mL/ha	200 L/ha
Cabrio® PD	250 g/L pyraclostrobin	350 mL/ha	200 L/ha
Untreated control			

Trial 1

Results

Disease levels were higher in this trial than previous field trials, with the untreated control having 25% of progeny tubers infected (Figure 4.25). Amistar® applied in-furrow at planting combined with a foliar application at 42 days post-sowing had significantly lower disease incidence (5.5%) than all other treatments. The untreated control had very similar disease incidence to the Maxim® seed treatment.

Disease severity data required transformation by the log+0.01 function and back-transformed data have been plotted.

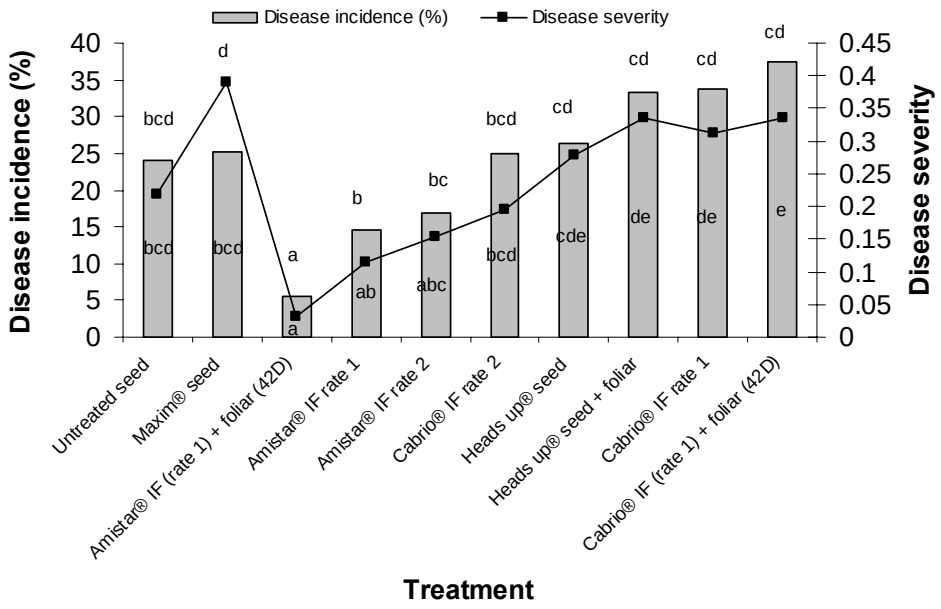


Figure 4.25: Disease incidence and severity of progeny tubers grown in infected soil and treated with various fungicide or organic products at various rates and timings of application. Treatments with the same letter within bars are not significantly different from each other for disease incidence (LSD = 11.56) and treatments with letters above bars are not significantly different from each other for disease severity (LSD = 0.3745).

Significant differences between treatments were observed in the yield data (Table 4.42). Cabrio® in-furrow (15 mL/100 m row) + foliar (42 days post-sowing) had the highest yield of 23 t/ha, but also had the highest incidence of diseased tubers (35%).

The levels of *C. coccodes* soil DNA at harvest were variable (data not shown), ranging from 1 to 667 pg *C. coccodes* DNA/g soil.

Table 4.42: Yield of progeny tubers grown in infected soil and treated with various fungicide or organic products at various rates and timings of application. Treatments with the same letter are not significantly different from each other (LSD = 5.115).

Treatment	Yield (t/ha)	
Untreated seed	15.5	bc
Maxim® seed	12.4	c
Cabrio® in-furrow (rate 1) + foliar (42 DPS)	23.1	a
Amistar® in-furrow rate 1	20.3	ab
Cabrio® in-furrow rate 1	20.1	ab
Cabrio® in-furrow rate 2	19.7	ab
Heads up® seed	18.6	ab
Heads up® seed + foliar	17.9	b
Amistar® in-furrow (rate 1) + foliar (42 DPS)	17.6	b
Amistar® in-furrow rate 2	16.6	bc

Discussion

Two treatments with Amistar® applied in-furrow provided the best reduction in disease, however this was not reflected with an increase in yield. The lower disease levels suggest that the fungicide can protect the plant from infection by *C. coccodes*, which could reduce yield by causing premature senescence of stem and root tissue (Mohan *et al.* 1992) and/or rotting of plant tissue underground (Dillard 1992). This suggests that Amistar® could result in higher yields as the plant is able to photosynthesise for longer and add carbon to tubers.

Trial 2

Results

No significant differences were observed between treatments in either disease levels (Figure 4.26) or yield (Table 4.43).

The amount of *C. coccodes* soil DNA at harvest varied (data not shown), ranging from 5 to 343 pg *C. coccodes* DNA/g soil.

Discussion

These results show that no treatment reduced disease incidence or severity compared to the control, suggesting that foliar application of fungicides were not effective in this trial. However there was a trend for Amistar® to lower disease incidence and severity, which requires further investigation.

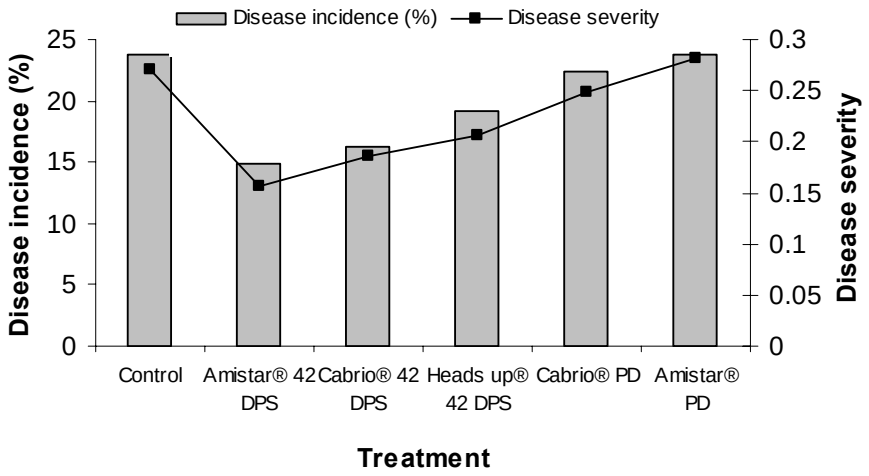


Figure 4.26: Disease incidence and severity of progeny tubers grown in infected soil and treated with various fungicide or organic products at 40 days post-sowing or post-desiccation.

Table 4.43: Yield of progeny tubers grown in infected soil and treated with various fungicide or organic products at 40 days post-sowing or post-desiccation.

Treatment	Yield (t/ha)
Control	17.24
Heads up® 42 DPS	20.52
Cabrio® 42 DPS	20.01
Cabrio® PD	18.24
Amistar® PD	17.57
Amistar® 42 DPS	17.53

4.4.5. Lenswood (2008) - Seed treatments

Due to low levels of disease in commercial field trials, 2 small field trials were undertaken at the Lenswood Research Centre under more controlled conditions to evaluate seed treatments and post-desiccation trials.

Aim

To investigate the efficacy of Amistar® applied in-furrow and Maxim® seed treatment on black dot control.

Materials and methods

Seed naturally infected with black dot (cv. Coliban 95% incidence, 2.86 severity), and either left untreated or treated with Maxim® was planted on Friday 21/12/07 at Lenswood Research Centre. Tubers were planted at a depth of approx. 10 cm and

spaced 30 cm apart (row spacing was 70 cm) and mounded. A pre-planting soil test indicated that the soil contained 76 pg of *C. coccodes* DNA/g soil.

Maxim® (Table 4.44) was applied the day before planting by pipetting the mixed chemical to the desired number of tubers in a green plastic garbage bag (Glad®). The bag was then twisted to trap air and was shaken thoroughly to completely coat tubers in chemical. Tubers were left to dry in plastic crates and covered with hessian bags overnight before planting.

The Amistar® was applied to the furrow approx 2-5 mins before tubers were planted using a 7 L Hills® garden sprayer.

Table 4.44. Seed and soil treatments applied in the seed treatment trial at Lenswood Research Centre.

Treatment	Product rate	Water rate
Maxim®	250 ml/tonne seed	2.5 L water/tonne seed
Maxim® + Amistar® IF	250 ml/tonne seed + Amistar® 10 ml/100 m row	in 2.5 L water/tonne seed + 2.860 L/100 m row
Control (untreated)	-	2.75 L water/tonne seed

The plot was watered 7 days after planting and then every 2-3 days for approximately 30 minutes.

At plant emergence and at harvest soil samples were taken of each replicate in a 'W' shaped pattern of 40 cores.

Plants were desiccated with Reglone® (3.5 L/ha in 200 L of water/ha) 97 DPS. Potatoes were harvested 33 days post-desiccation and in the following days were washed, sorted into sizes and stored at 4°C before assessment.

All tubers in a replicate were harvested and were washed, weighed and tubers assessed for disease incidence and severity.

Results

High levels of disease were observed, with 90% of tubers in the Maxim® treatment infected with black dot (Figure 4.27). Although the incidence and severity of disease was slightly reduced with the Amistar® in-furrow treatment, no significant differences were observed. There were also no significant differences in yield between the treatments (Table 4.45).

The mean DNA in soil of all treatments at plant emergence was 92 pg *C. coccodes* DNA/g soil, and this had risen to 621 pg DNA/g soil at harvest (data not shown).

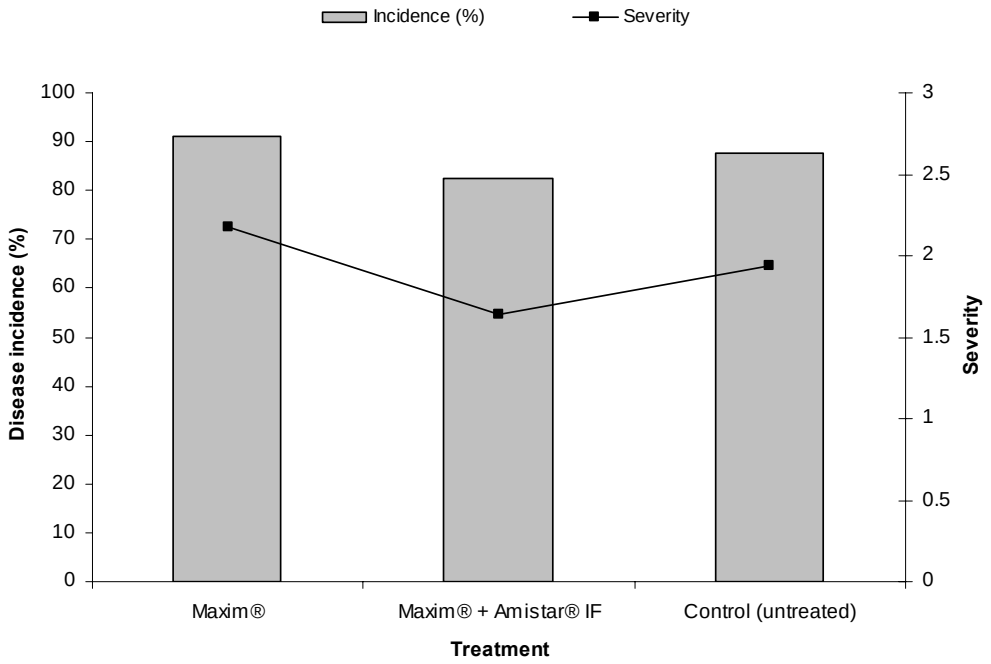


Figure 4.27: Disease incidence and severity of progeny tubers grown from an infected mother tuber and treated with either Maxim® seed treatment, Maxim® plus Amistar® in-furrow or untreated (water only).

Table 4.45: Yield of progeny tubers planted from an infected mother tuber treated with Maxim®, Maxim® and Amistar® in-furrow or untreated.

Treatment	Yield (t/ha)
Control (untreated)	25.2
Maxim®	23.9
Maxim® + Amistar® in-furrow	22.0

Discussion

All treatments had similar amounts of disease after 100 days growth, indicating that the fungicides were not effective when there were high levels of disease on seed.

4.4.6. Lenswood (2008) - Desiccation/post-desiccation applications

Aim

To investigate the efficacy of various desiccation and post-desiccation treatments on black dot control.

Materials and methods

Infected seed (cv. Coliban 95% incidence, 2.86 severity) was cut and treated with builders lime dust 4 days prior to planting and left to suberise in a dark environment.

Seed was cut so that at least one eye was remaining on a seed piece. Seed pieces were hand planted into a disease plot at Lenswood Research Centre on the 19th December 2007 at 20 cm spacing with rows spaced 70 cm apart and a space of 140 cm between replicates.

Plants were desiccated 100 days post-sowing according to their respective treatments (Table 4.46) and post-desiccation treatments were applied 108 days post-sowing using Hills® 5-7 L hand-held sprayers.

Table 4.46: Chemical rates for desiccation and post-desiccation treatments for a trial conducted at Lenswood Research Centre 2008.

Treatment	Amount of active ingredient (a.i.)	Trial rate (product)	Water Application rate
Reglone®	200 g/L diquat present as diquat dibromide monohydrate	3.5 L/ha	200 L water/ha
Pine oil	Pine oil desiccation Organic Interceptor™ contact weed spray	Pine oil (9.7-11.7%)	20% mixture
Reglone® + Amistar® 7 DPD	Reglone® as above, Amistar® 250 mL/L azoxystrobin	350 mL/ha	200 L water/ha
Natural (no treatment)	-	-	-

The natural treatment was left to senesce and die after watering was stopped at desiccation. When the Reglone® and pine oil treatments were being sprayed the natural treatment plots were covered with plastic to avoid contamination by the desiccants.

The pine oil desiccation spray was applied at a rate of 2% pine oil, not the recommended 20%. Therefore, a second spray was applied four days later at the correct rate. However, plants did not show signs of senescence and another spray was applied eight days after the initial application (at the time that Amistar® was applied).

All potatoes in a replicate were harvested 35 days post-desiccation, washed, sorted into sizes, weighed and assessed for disease incidence and severity.

Soil from the trial area was sampled at pre-planting two samples of 40 cores. A soil sample was taken over each replicate post-harvest.

Results

High levels of disease were observed in this trial (70-77%). There were no significant differences found between treatments in either disease incidence or severity (Figure 4.28) or yield (Table 4.47).

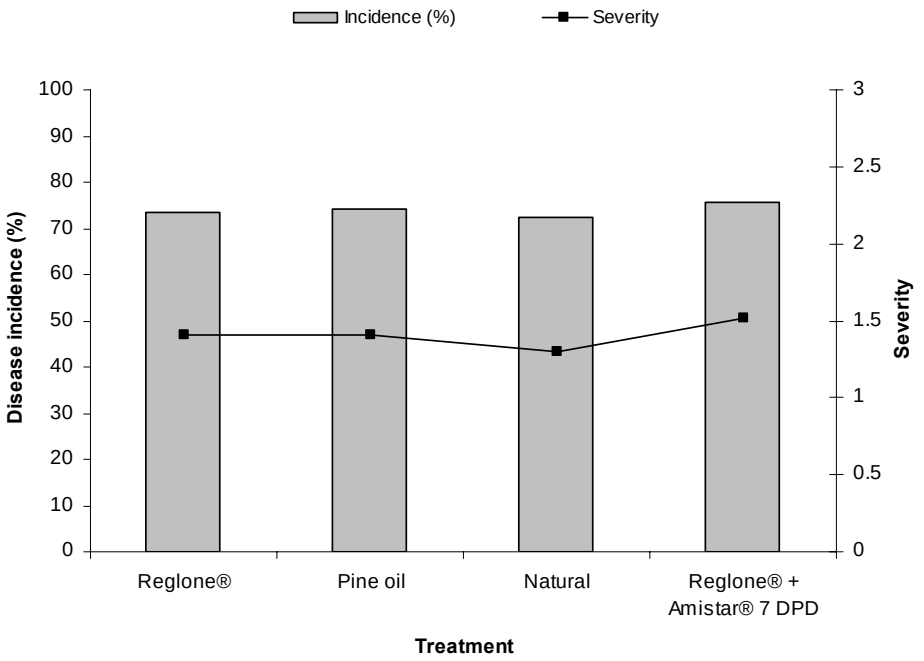


Figure 4.28: Disease incidence and severity of progeny tubers grown from an infected seed piece and desiccated with Reglone® or pine oil or desiccated with Reglone® and treated with Amistar® 7 DPD or left to die naturally.

At harvest the plots treated with Reglone® were desiccated, however the other treatments were still yellow and had not completely desiccated.

The mean amount of *C. coccodes* DNA increased from 52 pg DNA/ g soil at emergence to 773 pg DNA/ g soil at harvest.

Table 4.47: Yield of progeny tubers grown from infested seed and desiccated using various products.

Treatment	Yield (t/ha)
Pine oil	32.2
Natural	29.2
Reglone®	25.4
Reglone® + Amistar® 7 DPD	24.7

Discussion

Very little difference was observed between treatments when measuring disease incidence (%) and severity. This indicates that desiccation treatments are not effective at reducing black dot when planting seed with high levels of infection.

It is noted that as in previous trials, the amount of *C. coccodes* DNA in soil increased considerably over the growing season.

4.5 Conclusions from management trials

Amistar® applied in-furrow provided good disease control in pot trials, and this was also evident in one field study. While many of the field studies had very low disease and no significant differences were observed between treatments, the trend was for in-furrow treatment of Amistar® from 10-40 mL/100 m row to have lower levels of disease. This was not seen in the field trial with high disease levels, which indicated that while Amistar® is effective at reducing disease on progeny tubers, it may not provide economic control in all situations. It may be beneficial to undertake more large-scale field trials on commercial properties using Amistar® applied in-furrow.

Cabrio® applied in-furrow reduced disease in some shadehouse trials. Cabrio® and Amistar® are both strobilurin fungicides with similar modes of action. Therefore while the results from most field trials were unclear, further trials are warranted to test the results found in shadehouse trials in a field situation.

Several of the treatments evaluated worked well in the more controlled pot trials compared to field trials, and may warrant further investigation. For example Maxim® applied as a seed treatment significantly reduced disease incidence and severity in most shadehouse trials but not in field trials. Maxim® is commonly used as a seed treatment to control *Rhizoctonia*, therefore it would be of value to undertake further field testing of Maxim® for the control of black dot, particularly if it can enhance disease protection when used in combination with another treatment.

Similarly non chemical treatments which showed some level of disease reduction should be investigated further, as alternatives to chemical treatments will benefit the environment and appeal to the consumer. These include Heads Up®, acetic acid, *A. ustus*, Plantmate®, Voom® and tea-tree oil extract.

It is important to continue to evaluate other fungicides (of a different class), biological controls or organic products to use in rotation to control black dot. Strobilurin fungicides are considered to carry a high risk of pathogens developing resistance to its mode of action. Although it is an effective fungicide and there is evidence for its suitability for use in the management of black dot, there must not be over-reliance on this fungicide group. By rotating fungicides in different groups and seeking biological control or organic control measures, there can be effective and sustained use of azoxystrobin in managing black dot of potatoes.

5. TECHNOLOGY TRANSFER

Research findings contained in this report have been presented to growers by one-to-one contact, at grower meetings, through newsletters and magazine articles. A seminar and poster presentation was presented to the scientific community.

Newsletters:

Black Dot Research, Issue 1, May 2007
Black Dot Research, Issue 2, Jan/Feb 2008

Industry magazines:

'Reducing the impact of black dot on fresh market potatoes' Potatoes Australia (June 2008), pp. 36-37.
'What is black dot?' Potatoes Australia (February 2007), pp. 28-29.

Conference proceedings/posters:

'Quantification of *C. coccodes* DNA in artificially inoculated sand', 16th biennial Australasian Plant Pathology Society conference, Adelaide 24-27 September 2007.
'Vegetative compatibility groups in *Colletotrichum coccodes*, the causal agent of black dot on potato', 16th biennial Australasian Plant Pathology Society conference, Adelaide 24-27 September 2007.

Scientific seminar:

'Reducing the impact of black dot on fresh market potatoes' seminar presented at the SARDI Waite Seminar Series 2008, May 22nd 2008.

Industry/grower updates:

Research update on seed/soil borne disease and their management strategies. Landmark, Murray Bridge 13th February 2008.
Potato disease seminar, Murray Bridge, 22nd October 2008.
'DNA monitoring tools for soil borne diseases of potatoes', Vegetable Industry Conference, Sydney, 29th May – 1st of June 2007.

Overseas study tour:

91st Annual meeting of the Potato Association of America, tours of private and government research facilities in mid/upper England and in Holland. September 2007.

6. MAIN OUTCOMES

6.1 Recommendations – scientific and industry

- To minimise disease it is recommended to plant non-infected (clean) seed into soil with low amounts of *C. coccodes* DNA in soil.
- No treatment effectively controls black dot infection with high disease pressure. It is recommended not to use seed with high levels of infection or plant into heavily infected soil.
- Where moderate to low disease pressure exists use in-furrow applications of fungicide.
- Amistar® applied in-furrow at 40 mL/100 m row provided the best disease control and Amistar® and Cabrio® at 20 or 40 mL/100 m row also provided control. It is recommended that the industry support registration of these products for control of black dot.
- That work be continued to develop a commercial test for prediction of risk from black dot.

6.2 Recommended further work

Studies need to continue on the development of a molecular test as a prediction tool for growers. The following will require further work:

- Whether sclerotia found in various naturally infected soils are similar in size or vary within the same paddock. This will aid in understanding what the soil test result means.
- The importance of larger vs. smaller sclerotia and the impact on disease expression.
- Potential causes of sclerotial size differences, including organic matter content and nutrient status of soil. This may help in predicting sizes of sclerotia in a soil depending on local environmental conditions.
- To establish the importance of seed vs. soil-borne inoculum in the field to understand how much either source contributes to final disease.

Fungicides:

- Further testing of products to find suitable rotation fungicides, biological or organic products to reduce black dot on progeny tubers at harvest.
- Further testing of combinations of seed, in-furrow and foliar treatments for black dot control.
- Large scale field trials to test Amistar® in-furrow.
- Investigating the type and timing of sclerotial germination most commonly found in the field, to determine the most effective timing of fungicide applications and to investigate factors that may accelerate this germination, including release of host exudates (Willetts 1971) and temperature.

7. ACKNOWLEDGEMENTS

We wish to thank and acknowledge potato growers of South Australia for their co-operation in allowing field trials to be conducted on their properties.

We gratefully acknowledge funding from the Commonwealth of Australia through Horticulture Australia Limited and from various companies and potato growers (voluntary contributors). These include: agrochemical companies (Syngenta, Nufarm Ltd, Northern Quinoa Corp.) and growers/companies (Comit Farm Produce, Golden Sunrise Fresh produce, Mondello Farms, Zerella Holdings, South Australia Potato Industry Trust and The South Australia Potato Company).

Many people have been involved in undertaking this project we wish to thank them for their valuable help. Thank you to Brett Malic, Tiffany Barlow, Alex Walter for technical assistance and to the farm staff at Lenswood Research Centre and staff of the Plant Research Centre for their help. Thank you also to the Root Disease Testing Service of SARDI and to Chris Dyson, SARDI statistician.

8. REFERENCE LIST

Agrios, G. N. (2005) Plant Pathology, Ed 5th. Elseviar Academic Press, Amsterdam; Boston

Andrivon, D., Ramage, K., Guérin, C., Lucas, J. M. & Jouan, B. (1997). Distribution and fungicide sensitivity of *Colletotrichum coccodes* in French potato-producing areas. *Plant Pathology*, 46:722-728

Aqeel, A. M. (2007). Response of *Colletotrichum coccodes* to QoI fungicide *in vitro*. *Plant Pathology Journal*, 6:283-290

Aqeel, A. M., Pasche, J. S. & Gudmestad, N. C. (2008). Variability in morphology and aggressiveness among North American vegetative compatibility groups of *Colletotrichum coccodes*. *Phytopathology*, 98:901-909

Bartlett, D. W., Clough, J. M., Godwin, J. R., Hall, A. A., Hamer, M. & Parr-Dobrzanski, B. (2002). The strobilurin fungicides. *Pest Management Science*, 58:649-662

Byrne, J. M., Hausbeck, M. K. & Hammerschmidt, R. (1997). Conidial germination and appressorium formation of *Colletotrichum coccodes* on tomato foilage. *Plant Disease*, 81:715-718

Byrne, J. M., Hausbeck, M. K., Meloche, C. & Jarosz, A. M. (1998). Influence of dew period and temperature on foliar infection of greenhouse-grown tomato by *Colletotrichum coccodes*. *Plant Disease*, 82:639-641

Carnegie, S. F., Choiseul, J. W. & Roberts, A. M. I. (2003). Detection of *Colletotrichum coccodes* and *Helminthosporium solani* in soils by bioassay. *Plant Pathology*, 52:13-21

Cerkauskas, R. F. (1988). Latent colonization by *Colletotrichum* spp.: Epidemiological considerations and implications for mycoherbicides. *Canadian Journal of Plant Pathology*, 10:297-310

Cullen, D. W., Lees, A. K., Toth, I. K. & Duncan, J. M. (2002). Detection of *Colletotrichum coccodes* from soil and potato tubers by conventional and quantitative real-time PCR. *Plant Pathology*, 51:281-292

Department for Environment, Food and Rural Affairs (2002). Epidemiology of the soil-borne phase of potato tuber blemish diseases. *Final Project Report*,

Dillard, H. R. (1992) *Colletotrichum coccodes*: the pathogen and its hosts. *In* JA Baily, MJ Jeger, eds, Colletotrichum: Biology, Pathology and Control. C.A.B. International

Dillard, H. R. & Cobb, A. C. (1998). Survival of *Colletotrichum coccodes* in infected tomato tissue and in soil. *Plant Disease*, 82:235-238

Gudmestad, N. (2003). NDSU plant pathologists focus on black dot [Online: accessed 18th September 2008] URL: http://www.ext.nodak.edu/extnews/newsrelease/2003/041003/08ndsupl.htm).

Harding, R., Wicks, T. & Hall, B. (2004) Control of black dot in potatoes, Final report Horticultural Research and Development Corporation Project PTO1001. South Australian Research and Development Institute

Heads Up Plant Protectants (no date) HeadsUp© Brochure for Soybeans. Online, accessed 4th December 2008. URL: http://www.sar-headsup.com/trials/soybeans/HeadsUp-Pamphlet.pdf

Huang, H. C. & Erickson, R. S. (2002). Overwintering of *Coniothyrium minitans*, a mycoparasite of *Sclerotinia sclerotiorum*, on the Canadian Prairies. *Australasian Journal of Plant Pathology*, 31:291-293

Johnson, D. A. (1994). Effect of foliar infection caused by *Colletotrichum coccodes* on yield of russet burbank potato. *Plant Disease*, 78:1075-1078

Johnson, D. A., Rowe, R. C. & Cummings, T. F. (1997). Incidence of *Colletotrichum coccodes* in certified potato seed tubers planted in Washington State. *Plant Disease*, 81:1199-1202

Kang, H.-C., Park, Y.-H. & Go, S.-J. (2003). Growth inhibition of a phytopathogenic fungus, *Colletotrichum* species by acetic acid. *Microbiological Research*, 158:321-326

Lees, A. K. & Hilton, A. J. (2003). Black dot (*Colletotrichum coccodes*): an increasingly important disease of potatoes. *Plant Pathology*, 52:3-12

Leslie, J. F. (1993). Fungal vegetative compatibility. *Annual Review of Phytopathology*, 31:127-150

Mohan, S. K., Davis, J. R., Sorensen, L. H. & Schneider, A. T. (1992). Infection of aerial parts of potato plants by *Colletotrichum coccodes* and its effects on premature vine death and yield. *American Journal of Potato Research*, 69:Abst

Mordue, J. E. M. (1967) *Colletotrichum coccodes*. *In* C.M.I. Descriptions of Pathogenic Fungi and Bacteria, Vol No. 131. Commonwealth Agricultural Bureaux

Nitzan, N. (2006). Colonization of potato plants after aerial infection by *Colletotrichum coccodes*, causal agent of potato black dot. *Plant Disease*, 90:999-1003

Nitzan, N., Hazanovsky, M., Tal, M. & Tsror (Lahkim), L. (2002). Vegetative compatibility groups in *Colletotrichum coccodes*, the causal agent of black dot on potato. *Phytopathology*, 92:827-832

Nitzan, N., Tsror (Lahkim), L. & Johnson, D. A. (2006). Vegetative compatibility groups and aggressiveness of North Americal isolates of *Colletotrichum coccodes*, the causal agent of potato black dot. *Plant Disease*, 90:1287-1292

Powelson, M. L. & Rowe, R. C. (1993). Biology and management of early dying of potatoes. *Annual Review of Phytopathology*, 31:111-126

Read, P.J. & Hide, G.A. (1995). Effects of fungicides on the growth and conidial germination of *Colletotrichum coccodes* and on the development of black dot disease of potatoes. *Annals of Applied Biology*, 126:437-447.

Reilly, S.K., Hollis, L., Jones, R.S., Peterson, T.A., Greenway, D. & King, R. (no date). Saponins of *Chenopodium quinoa* Biopesticides Registration Action Document. U.S. Environmental Protection Agency Office of Pesticide Programs Biopesticides and Pollution Prevention Division. Online, accessed 4th December 2008. URL: http://www.epa.gov/opp00001/biopesticides/ingredients/tech_docs/brad_097094.pdf

Sanogo, S. & Pennypacker, S. P. (1997). Factors affecting sporogenic and myceliogenic germination of sclerotia of *Colletotrichum coccodes*. *Plant Disease*, 81:333-336

Shcolnick, S. (2007). Additional vegetative compatibility groups in *Colletotrichum coccodes* subpopulations from Europe and Israel. *Plant Disease*, 91:805-808

Sorensen, L. H., Schneider, A. T. & Davis, J. R. (1991). Influence of sodium polygalacturonate sources and improved recovery of *Verticillium* spp from soil. *Phytopathology*, 81:1347

Tsror , L., Erlich, O. & Hazanovsky, M. (1999). Effect of *Colletotrichum coccodes* on potato yield, tuber quality, and stem colonisation during spring and autumn. *Plant Disease*, 83:561-565

Tsror , L. & Johnson, D. A. (2000) *Colletotrichum coccodes* on potato. *In* D Prusky, S Freeman, M Dickman, eds, *Colletotrichum*. Host specificity, pathology and host-pathogen interaction. The American Phytopathological Society, St. Paul, Minnesota, pp 362-373.

Tu, J. C. (1980). The ontogeny of the sclerotia of *Colletotrichum coccodes*. *Canadian Journal of Botany*, 58:631-636

Uribe, E. & Loria, R. (1994). Response of *Colletotrichum coccodes* to fungicides *in vitro*. *American Journal of Potato Research*, 71:455-465.

Willetts, H. J. (1971). The survival of fungal sclerotia under adverse environmental conditions. *Biological Reviews*, 46:387-407.

9. APPENDIX

9.1 Culture media and recipes

NP10 agar (modified from (Sorensen *et al.* 1991))

Part 1.

500 ml distilled water
5 g polygalacturonic acid, Na salt
Autoclave for 15 mins at 15 psi, cool to 50°C

Part 2.

500 ml distilled water
1 g Difco® bacto agar
1 g KNO_3
1 g KH_2PO_4
0.5 g KCL
0.5 g $MgSO_4*7H_2O$
0.5 ml Tergitol NP-10
Autoclave for 15 mins at 15 psi, cool to 50°C

Then add:
0.05 g Streptomycin sulphate
0.05 g chlortetracycline HCL
0.05 g chloramphenicol

Combine parts 1 and 2 (and antibiotics), stir, and pour into plates immediately

V8 juice agar

300 ml V8 juice® (Campbell's original formula)
2% agar
700 ml distilled water

Add all ingredients, mix and autoclave for 15 minutes at 121°C

(Byrne *et al.* 1997; Byrne *et al.* 1998).

Tap water agar (2%)

Bacto agar 20 g
1000ml distilled water

Add ingredients, mix and autoclave for 15 minutes at 121°C

Acidified full strength Potato Dextrose Agar (APDA)

39 g PDA (Difco ™ Becton, Dickinson and Company, USA).
1000 ml distilled water
1 ml lactic acid

Mix PDA and distilled water, then dissolve PDA in water by heating mix in a microwave for 10 mins on high, stirring once after 5 mins. Autoclave dissolved PDA for 15 mins at 121ºC, allow to cool to approx. 71°C then add 1ml of lactic acid to 1000ml of PDA and pour plates immediately. Full strength PDA follows the same recipe but lactic acid is not added.

½ strength PDA

19.5 g PDA (Difco ™ Becton, Dickinson and Company, USA).
100 ml distilled water

Mix PDA and distilled water, then dissolve PDA in water. Autoclave dissolved PDA in water for 15 mins at 121°C.

¼ strength PDA

9.75 g PDA (Difco ™ Becton, Dickinson and Company, USA).
100 ml distilled water

Mix PDA and distilled water, then dissolve PDA in water. Autoclave dissolved PDA in water for 15 mins at 121°C.

PDA + streptomycin

39 g PDA (Difco ™ Becton, Dickinson and Company, USA).
1000 ml distilled water

Autoclave agar then once cooled to approx. 50°C add 1 ml of stock concentration of Streptomycin sulphate (100 mg/ml) per 1000 ml of agar.

V8 broth (50%) (300ml)

270ml V8 juice® (Campbell's original formula)
30ml distilled water

Mix water and V8 juice, measure pH to ensure broth is in the range of pH 4.5-5.5, and then autoclave for 15 mins at 121ºC.

9.2 Abbreviations used in this report

DNA = deoxyribonucleic acid
DPS = days post-sowing
DPD = days post-desiccation
PDA = potato dextrose agar
APDA = acidified potato dextrose agar
PCR = polymerase chain reaction
RT-PCR = real time polymerase chain reaction
cfu = colony forming unit (a sclerotium for the purposes of this report)
UC = University of California soil
ml = milli litre
km/h = kilometres per hour
SEM = standard error of the mean
LRC = Lenswood Research Centre
SDW = sterile distilled water
Pg = picogram(s)