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# Control of black dot in potatoes

Dr. Trevor Wicks
SA Research &
Development Institute

Project Number: PT01001

**PT01001**

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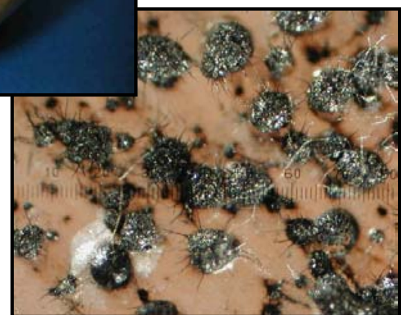
# Control of Black dot in potatoes

**FINAL REPORT**
**Horticultural Research and Development Corporation Project PT01001**

By Robin Harding, T. Wicks and B. Hall
South Australian Research and Development Institute



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# HORTICULTURAL RESEARCH AND DEVELOPMENT CORPORATION
# Final Report September 2004

**Project Title:** Control of Black dot in Potatoes

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Black dot, caused by the fungus *Colletotrichum coccodes* is a major potato disease that affects both the fresh and processing industries. It causes premature plant death, reduced yields and downgrading of produce due to blemishes. The main aim of the project is to develop management strategies to help washed potato growers and seed growers control black dot disease.

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## MEDIA SUMMARY

Over the past 20 years the fungal disease black dot caused by the fungus *Colletotrichum coccodes* has been reported throughout the main potato production areas worldwide. For many of these years, growers and researchers have considered it to be a secondary disease of low importance. However increasing demands on tuber quality in both the fresh and processing industries have shown it to be an economically important disease due to external skin blemishes, internal tissue discolouration of stem ends and yield reductions. Australian, USA and Israeli trials have shown black dot reduced yields by between 12 - 30%. The main aim of this project was to develop management strategies to control the disease for both washed potato growers and seed growers.

Trial results have shown that there is a high incidence of black dot both on and in seed potatoes and that infected seed is the primary means of introducing the disease into new regions. One trial where clean seed was planted into new ground, between 15 and 88% of the daughter tubers were infected. Seed treatments with the fungicides Maxim® and Amistar® were the most effective fungicides at reducing the impact of this disease but only where soil levels of the fungus were low. Where soil levels were high, fumigation with Metham®, Telone II® or in-furrow treatments of Amistar® reduced the impact of the disease. However if infected seed is planted under these conditions, disease incidence and severity can be higher than where no soil treatment was applied. A secondary infection cycle was shown to occur between crop maturity/haulm desiccation and harvest. Foliar application of Amistar® or withholding irrigation before harvest reduced the incidence of the disease. Less effective were the application of chemicals and slashing/removal of haulm tissue. Further studies are needed to determine optimum timing of these treatments and dose of fungicides. This will ensure that growers have a diversity of control methods and not rely solely on chemical methods that could give rise to fungicide resistant strains of fungi.

Because black dot survives in the soil for up to 8ys, it is recommended that rotations of at least this length be used. During this time non-host crops should be used and weed species such as fat hen, skeleton weed and black nightshade that are also hosts should be controlled. Planting or having crops maturing when the soil temperatures are above 24$^{o}$C increases disease incidence. Therefore growers should avoid these periods and if crops do mature during this time, lifting tubers earlier will reduce disease levels.

## TECHNICAL SUMMARY

Black dot caused by the fungus *C. coccodes* is now recognised as a major disease of potatoes worldwide. Laboratory, glasshouse and field trials have been conducted in South Australia since 2001 to develop management strategies to control the disease. The major findings for these studies were:

1. Laboratory and glasshouse studies showed that the fungus was most active around 24$^{o}$C and caused most disease around this temperature.

2. Disease development was monitored in naturally infected plants, which showed that the symptoms developed on the stem around 74 days after planting whereas tuber infection was detected 10 days later.

3. Levels of *C. coccodes* were measured on potato tuber seed used in South Australia. In 2001, 61% of seed lots were infected with black dot and 93% in 2002. In some samples all tubers were infected with *C. coccodes*. Vascular infection (internal) levels as high as 30% were also found in some samples.

4. Glasshouse studies showed that daughter seed could be infected as a result of systemic growth of the fungus from the infected mother seed. However seed tubers with external skin infection produced daughter tubers with the highest incidence and severity of *C. coccodes*.

5. Glasshouse studies were made to determine the effect of different soil inoculum levels on the development of *C. coccodes*. Plants grown in 20 microsclerotia/gm of soil senesced more rapidly than those in 10 or 15 microsclerotia/gm but there was no difference in the numbers of tubers infected or the severity of infection on tubers produced in the different inoculum levels.

6. Fourteen potato cultivars were evaluated for susceptibility to *C. coccodes* in naturally infected soil. Differences in incidence of stem colonization and severity of tuber infection were observed between different cultivars, but none were immune. Desiree was the least infected whereas Coliban was the most susceptible of the 14 cultivars evaluated.

7. Weed hosts were collected from potato fields between 2001-2003 and examined for presence of *C. coccodes* on stems and roots. The fungus was detected on common weeds such as fat hen (*Chenopodium allium*), Black night Shade (*Solanum nigrum*), Heliotrope (*Heliotropium europium*), Skeleton weed (*Chondrilla juncea*) and others.

8. Overhead irrigation applied to infected haulms just prior to harvest increased the incidence and severity of tuber infection compared to those not irrigated Experiments showed that conidia washed from infected stems or applied to the soil surface are capable of infecting tubers beneath the soil. Experiments with fluorescent dye indicated that water from overhead irrigation washes spores down the edge of the stem and along the stolon and roots to the tuber. Examination of *C. coccodes* infected tubers across a radius of a pivot showed that the highest levels of incidence and severity were at the centre of the pivot where most water is applied and lowest at the extremity where least water is applied.

9. The application of the desiccant Reglone® prior to harvest did not increase the level of *C. coccodes* infection. However the application of the fungicide Amistar® one week after Reglone® reduced both the incidence and severity of *C. coccodes* on tubers in some experiments. Other experiments confirmed the observation that the longer tubers remained in the soil after haulm destruction the more disease develops.

10. Several experiments were carried out to evaluate the potential of biofumigants from Brassica's as a means of controlling *C. coccodes*. Leaves, roots and "meal" from *B. Juncea*, *B. napus* and *R sativus* were evaluated in-vitro for their inhibitory effects on mycelial growth of *C. coccodes*. The results showed that meal of *B. Juncea* was most inhibitory, but there were variations between Brassica species and plant parts. Further experiments were carried out evaluating the efficacy of volatiles released from *B. juncea* meal in soil and an inhibiting effect was demonstrated.

11. Many fungicides and rates were evaluated in laboratory, glasshouse and field trials as tuber seed treatments for the control of *C. coccodes*. Of the 12 fungicides tested Maxim®, Amistar®, Cabrio® and Octave® were the most effective.

12. Large scale field trials were also undertaken to evaluate the efficacy of soil fumigation (Metham® and Telone®) for the control of *C. coccodes*. These trials also included the evaluation of fungicide tuber seed treatments and the use of Amistar®. The effects of soil fumigation were variable and were compromised if used in conjunction with infected tubers

## LITERATURE REVIEW

*Distribution of the disease*.
Black dot, a disease of potatoes caused by the fungus *Colletotrichum coccodes* (Wallr.) has been reported throughout the world (4, 24, 36, 53, 63) including Chile (23), Europe (2, 58), Australia (26), South Africa (15), and Israel (67).

Black dot has been reported as a minor disease (58, 60, 63), however it is now recognized as a major pathogen of potatoes, causing substantial economic losses in many countries (2, 4, 15, 16, 33, 58, 66).

*Hosts*
Overseas studies have shown that it has a wide host range that includes at least 58 species and 17 families, primarily vegetables within solanaceae (55). In addition there are a number of hosts that are classified as weeds (see appendix) (55). These include several solanaceous weed species; velvetleaf and eastern black nightshade (1, 55, 74).

Unmanaged weed populations could result in the build up of inoculum levels between rotations or may act as a source of primary inoculum for subsequent potato crops. Identifying the host plants in Australia should allow growers to develop better disease management practices through selective weed control and non-host rotational crops.

*The causal pathogen*
Overseas studies showed that there is a large degree of variability in sclerotium size, colour, aerial mycelium and virulence among isolates of *C. coccodes* (4, 40, 67). For example some isolates do not produce sclerotia (4). Variations among Australian isolates have not been studied and could show that yield losses due to *C. coccodes* infection occur without the development of sclerotia.

*Effect on yield*
Studies in the USA have shown that on poorly drained, sandy soils, *C. coccodes* causes significant yield losses (4, 33). Yield losses have also been recorded in Israel, UK and Australia (42, 65) and vary between different cultivars in both Israel and the UK (57, 66, 68). Whilst yield losses have gone mostly unrecognised in Australia, growers have observed a marked reduction in quality and packers involved in the production of washed potatoes. In an attempt to reduce the decline in quality some growers have moved production to new ground only to find similar levels of infection.

*Disease epidemiology*
In South Australia recent studies have shown that black dot is a significant problem on seed tubers, with 100% infection in some samples of certified seed tubers (14, 25). In addition the black dot fungus has been recovered internally from tubers in 75% of 54 seed lots tested (25). Similar levels of black dot on seed tubers have also been reported overseas (16, 33, 37). The effect the tuber seed infection has on the quality of daughter tubers and disease carry over has not been studied extensively.

In soil the fungus may exist as either sclerotia or conidia at undetectable levels. Sclerotia survive in the soil for up to 8 yrs (18), and while the conidia can survive for up to 1 yr in soil only a few survive longer than a few weeks (7, 22). Sclerotia survived longer and more consistently when they were free in the soil rather than associated with plant tissue (19). Recent overseas reports also show *C. coccodes* to be an air-borne pathogen (4, 34, 46) causing foliar infection (13), however it is not known how long conidia survive on foliage.

Studies have also shown that the incidence and severity of black dot increases on unwashed tubers when stored at 15°C but not at 5°C (56) and that harvesting early and storing tubers dry may prevent or reduce disease expression (48). *C. coccodes* can also exist in a synergistic relationship with one or more other pathogens such as *V. dahliae* (36, 53).

*Environmental factors that influence disease expression*

Black dot is most frequently associated with light sandy soils, low nitrogen, high temperatures, and poor water drainage (69). However latent infections in stressed plants make the effect of the abiotic and biotic factors on disease development difficult to determine. In the USA, excessive rain, irrigation and low temperatures early in the season followed by prolonged drought resulted in disease expression (63). Whilst in England irrigation reduced stem, root and tuber infection up to 18 weeks after planting, but later rotting of tubers was increased by irrigation (56).

In Israel where the crop is irrigated routinely, disease incidence and yield loss were observed when temperatures were high and soil relatively dry (69). Growth chamber studies showed that short day lengths (8:16 L: D) rather than longer day lengths resulted in higher incidence and severity of black dot on tubers (68).

*Control*

Chemical: A number of chemicals have been evaluated as seed treatments to reduce daughter tuber infection (2, 27, 43, 70), but few studies have been reported on new fungicides with different modes of action. One group, the strobilurins, are particularly effective on *Colletotrichum* sp. and evidence from the USA indicates that the use of these fungicides may be a major breakthrough in the control of black dot (34). Evaluating these new fungicides for use as seed treatments, foliar treatments or infurrow soil treatments may provide new strategies for the control of black dot.

Since seed treatments are unlikely to control tuber infection where the levels of soil inoculum are high, soil solarization or fumigation may reduce soil levels of the pathogen. However in the USA and Israel fumigation with methyl bromide, vapam or formalin sometimes failed to control *C. coccodes* (63, 68).

*Breeding:* Breeding less susceptible cultivars is another means of controlling *C. coccodes* (22, 40, 68, 69) but no studies on susceptibility of cultivars have been carried out in Australia. In the UK the commercial potato cultivars Desiree, Maris Piper, Maris Peer and Record showed significantly higher infection levels whilst Cara, Pentland Crown and Romano showed the least infection (57). In Israel the cultivars Alpha, Desiree and Agria were more susceptible than Cara and Nicola (65).

*Cultural:* Since soil levels of the fungus, environment, and varietal resistance influence the timing of infection (22, 40, 65). Sampling the crop during the growing season to determine the level of infection has been evaluated as a means to predict the appropriate harvest time of different crops to minimize tuber infection (41). Results showed that whilst this method of disease prediction worked with one cv. Estima, it was not effective with the cv.s Maris Piper, King Edward or Saxon.

*Crop Rotations:* Most growers practise crop rotation, yet *C. coccodes* still causes major disease problems, even after long breaks between potato crops. Possible reasons are that the rotations consist of other host crops (55) or the rotation intervals are to short. *C. coccodes* has been shown to survive for up to 8 years. Trials conducted in the UK found no differences on disease incidence when potatoes were grown on 2, 4 or 6 year rotations with spring barley (28). In addition the pathogen may be reintroduced on potato seed. Shorter crop intervals may be possible when combined with other cultural practices such as solarization/tarping, and mouldboard ploughing (41).

*Biofumigation:* Breakdown products released from some plants within the Brassicaceae family reduce fungal growth of *Rhizoctonia, Pythium and Fusarium in vitro* (3) and in field trials (11, 47) when grown or incorporated in the soil. These effects are attributed to glucosinolates (GSL's) released from Brassica residues into the soil and being hydrolysed by endogenous myrosinase (3). One of these breakdown products is the volatile compound isothiocyanate (ITC), which is highly biocidal to nematodes, bacteria, fungi, insects and germinating seeds (8, 54, 72). These studies suggest that biofumigant crops have potential for reducing the severity of the potato disease *C. coccodes*.

*Planting Time:* Planting dates have been shown to affect the incidence of *C. coccodes* on potatoes (49, 65). Both studies showed reduced disease incidence on potato tubers when planted at particular periods during the year. However the results of one season were variable between different years. This may be in part due to differences in environmental factors such as temperature and rainfall from year to year (63).

*Haulm Destruction:* The destruction of potato plants is primarily used to control tuber size and provide easy harvest conditions. However since the development of *C. coccodes* coincides with crop maturity/haulm senescence, it has been speculated that haulm destruction may also play a role in disease reduction. Field trials in the UK utilizing chemical destruction with root cutting showed a lower incidence of black scurf caused by *Rhizoctonia solani* (55), but the effect on black dot has not been investigated

# TECHNICAL REPORT

## Introduction

Black dot caused by the fungus *C. coccodes* is a widespread disease of potatoes in Australia. The fungus is common in soils of potato growing areas and on seed tubers. For example in some certified seed, 100% of the tubers were infected with *C. coccodes*. Because the disease is so common and develops late in the season, the significance of the disease has not always been recognised. Recent work from the USA showed that *C. coccodes* reduces yield by more than 5 tonnes/Ha (4, 34, 46). Similar yield reductions in Australia can be expected, but further losses occur with washed potatoes where infected tubers are rejected because of the skin blemishes caused by the fungus.

Previous work on this problem in Australia (42) showed that *C. coccodes* reduced yields by 12% due to plants dying prematurely. Reductions at this level may be costing the industry over $10 million annually. Additional losses due to blemished tubers may also be costing the South Australian washed potato industry over $1.2 million per year. Overall the high incidence of *C. coccodes* in seed potatoes and the detrimental effect on potato quality, now make black dot one of the most important fungal diseases of potatoes in Australia.

This project involved laboratory, greenhouse and field experiments to obtain an understanding of the disease and to develop management strategies to control or reduce the disease.

## General materials and methods

### Cultures of *C. coccodes*: -

*Selective media:* Two selective media (21, 62) were evaluated for their suitability to isolate *C. coccodes* from soil and plant samples. Sorrenson's NP10 media (62) was the most efficient at selective isolation of *C. coccodes*.

*Isolates:* 40 isolates of *C. coccodes* were obtained from naturally infected soil, potato plants and weeds within South Australia, New South Wales, Victoria, Tasmania and Western Australia. These were maintained on NP10 selective media. Three isolates from SA (77b, 58a, 17a) with different morphologies were selected to use as inoculum sources for experiments, one from soil and two from potato tubers. In addition sclerotia that formed on the plates were harvested according to previously described procedures (22), air-dried and stored at $4^{o}$C until needed.

*Storage of cultures:* Cultures were maintained on NP10 selective media at $4^{o}$C, and subcultures of each isolate stored at $-20^{o}$C.

### Detection of *C. coccodes*: -

*Plant tissue*: Stem tissue (2 - 5cm above soil line) was surface sterilized with 1% NaCL for 60 sec and rinsed in sterile water. Wafer thin sections of stem were made with a sterile scalpel blade and 5 sections plated onto a petri dish containing NP10 selective media. Sap was extracted from the remaining basal portion of each stem section by placing in a polyethylene bag and crushing the tissue with a rubber hammer. A 0.5 ml aliquot of sap was pipetted onto NP10 selective media and sealed with parafilm. After 14 days incubation at $24^{o}$C in the dark, plates were examined microscopically and scored for presence or absence of *C. coccodes* microsclerotia and the % of plants with stem infection was calculated.

*Tubers:* The stem end of tubers was sliced and where staining was obvious in the vascular tissue, 2 pieces of approx $1mm^2$ were removed with a scalpel and plated on to selective media. After 14 days incubation at $24^oC$ in the dark, plates were examined microscopically and scored for presence of *C. coccodes*.

**Assessments: -**

*Fungal variation:* Size and number/$cm^2$ of sclerotia, shape and presence of setae and size of conidia were recorded 10 days after plating the isolates onto NP10 selective media and incubated in the dark at $24^oC$.

*Microsclerotial density and size*: A $1cm^2$ grid was placed underneath each petri plate and the sclerotia numbers counted in five random squares and means calculated. The percentage inhibition of microsclerotial density was determined relative to the number of sclerotia of the control. 10 sclerotia were randomly selected at a 20mm radius from the initial inoculum source and their diameter measured using an ocular graticule at 40X magnification.

*Mycelial growth*: Fungal growth was measured as the mean of two radial dissects of the colonies at right angles to each other. Percentage of inhibition was determined relative to the growth of the control.

*Harvest tuber/stem disease assessment and yield*: At harvest all plants were carefully removed along with their tubers and washed in running water to remove most of the soil adhering to the surface. Tubers and below ground stems (2 - 5cm below soil line) were visually assessed for both the incidence and severity of *C. coccodes* using the keys shown in appendix 1. Tubers were also visually rated as a) infected with *C. coccodes* surrounding the stolon end, or b) infected with *C. coccodes* but not surrounding the stolon end. Tubers from each pot were counted and weighed into size categories of 30-80g (chats), 80-200g (small), 200-350g (medium), 350-450g (large) and >450g (oversize). Skin blemishing caused by *C. coccodes* was not used as a criterion when calculating the marketable yield.

*Stem senescence*: Plant senescence was rated using a 0 – 5 scale based on the percentage of foliage with wilting, chlorosis, necrosis, or stunting.

0= 0%,
1= 1-12%,
2= 13-25%,
3= 26-50%,
4= 51-75%
5= 76-100%

*Pot experiments:* Plants were grown in 30cm diameter pots containing 2 litres of 20mm gravel for drainage and 8 litres of pasteurised "Mt Compass sandy loam". Plants were fertilized weekly with a solution of 20-20-20 "N-P-K" (Nitrogen was NH4NO3) prepared at a concentration of 2 g/L. One seed tuber cv. Coliban, with >50% surface infection by *C. coccodes* was planted in each pot at a depth of 15cm.

*Experimental design and statistical analysis:* All trials were set up as "Completely randomised block" designs and analysed through GENSTAT V7.0®. The Least Significant Differences (LSD) were calculated by General ANOVA unless otherwise specified.

## Pathogen morphology and ecology

***Introduction:***

A number of studies investigated the morphological and ecological variations amongst isolates obtained from soil, potato plants and weed species in Australia. Some strains may be more aggressive than others or may be resistant to certain fungicides. Combining this knowledge with a better understanding of the environmental conditions affecting inoculum production by *C. coccodes* may contribute to the development of improved management strategies for the control of Black dot on potatoes.

***Fungal variation***

**Objective:** *To determine the variation in morphological characteristics among isolates of C. coccodes from different sources in Australia.*

**Materials and Methods:**

28 isolates of *C. coccodes* from potato tubers (SA), 10 from soil samples (SA, WA, Vic, Tas) and 2 from weeds (*Chenopodium album* "Fat hen", *Citrullus colocynthis* "Wild tomato") were plated onto NP10 selective media. After 10 days incubation at 24$^{o}$C sclerotia and conidia size were measured and the presence of setae and aerially or prostrate mycelia recorded.

**Results and Discussion:**

Isolates produced four distinct variations in sclerotial formation (Table 1).

1. Large and small black sclerotia, setae, mean conidia length of 23.1 and width 4.5um.
2. Small oblong black sclerotia, setae, mean conidia length of 17.5 and width 3.6um.
3. Large, round, black sclerotia, setae, mean conidia length of 21.2 and width 3.7um.
4. Small round black sclerotia, no setae, mean conidia length of 16.4 and width 3.1um.

All conidia were cylindrical with obtuse ends, hyaline and aseptate. However lengths and widths varied between isolates (15.4 um to 25.6um) and (2.5 um to 5.8um) respectively.

All isolates produced setae except those from WA. Those isolates that produced only small sclerotia (avg 125um) generally came from SA and WA, whilst isolates from Tasmania and Victoria produced both large and small sclerotia on the same plate (avg 354um). The actual role of setae is unknown (12). Similar studies in the UK (41) found no obvious differences in culture morphology between isolates of *C. coccodes*. However significant difference occurred in sensitivity to the fungicides TBZ and Fenpiclonil.

These results show that isolates of *C. coccodes* are highly variable in colony morphology, with differences in conidia size and shape, presence of setae. Overseas studies also show that significant differences occur between fungicide sensitivity, pathogenicity and optimal growth temperature (5) Research in Israel has also found four different vegetative compatibility groups (VCGs) amongst isolates collected from the Netherlands, France and Israel (51).

Two of these VCG1 and VCG2 were recovered from all three countries whereas VCG3 and VCG4 were not recovered from the sample from France. One reason why this may have occurred could be that these VCGs have adapted to specific regional climatic conditions. Further studies are required to determine if any variability exist among Australian isolates of *C. coccodes* such as VCGs, virulence, pathogenicity or fungicide resistance, so that the most effective control measures can be implemented.

**Table 1:** Morphological characteristics of *C. coccodes* collected from SA, Vic, Tasmania and WA

| Isolate # | Origin | Location | Date isolated | Morphological characteristics on NP10 media | Size of sclerotia (um) | Size of conidia L X W um | Prostrate or aerial mycelia |
|---|---|---|---|---|---|---|---|
| 45a | Potato tuber | Vic | 05-10-98 | Large & small black sclerotia, setae | 268 | 22.1 X 4.3 | P |
| 86a | Potato tuber | Vic | 05-10-98 | Large & small black sclerotia, setae | 276 | 22.3 X 4.4 | P |
| 47a | Potato tuber | Tasmania | 12-05-97 | Large & small black sclerotia, setae | 359 | 23.3 X 4.7 | P |
| 47b | Soil | Tasmania | 12-10-01 | Large & small black sclerotia, setae | 421 | 24.2 X 4.4 | P |
| 65b | Soil | Tasmania | 12-10-01 | Large & small black sclerotia, setae | 400 | 24.3 X 4.5 | P |
| 66b | Soil | Tasmania | 12-10-01 | Large & small black sclerotia, setae | 378 | 24.2 X 4.1 | P |
| 66a | Potato tuber | Tasmania | 22-03-97 | Large & small black sclerotia, setae | 382 | 24.1 X 3.2 | P |
| 12a | Potato tuber | Tasmania | 15-09-96 | Large & small black sclerotia, setae | 372 | 23.6 X 4.6 | P |
| 29b | Potato tuber | Vic | 22-07-01 | Large & small black sclerotia, setae | 356 | 19.3 X 3.5 | A |
| ***77b*** | ***Soil**** | ***SA*** | ***07/02/97*** | ***Large & small black sclerotia, setae*** | ***345*** | ***21.2 X 4.2*** | ***A*** |
| 20a | Potato tuber | SA | 07/02/97 | Large & small black sclerotia, setae | 322 | 22.0 X 4.5 | A |
| 21a | Potato tuber | SA | 01-08-01 | Large & small black sclerotia, setae | 279 | 23.4 X 4.5 | P |
| 2a | Potato tuber | SA | 01-04-97 | Large & small black sclerotia, setae | 480 | 25.6 X 5.8 | P |
| 57a | Potato tuber | SA | 01-04-97 | Large & small black sclerotia, setae | 430 | 24.3 X 4.8 | P |
| ***58a*** | ***Potato tuber**** | ***SA*** | ***01-04-97*** | ***Large & small black sclerotia, setae*** | ***387*** | ***24.1 X 4.6*** | ***P*** |
| 15a | Potato tuber | SA | 06/03/97 | Large & small black sclerotia, setae | 280 | 19.8 X 4.6 | P |
| 16a | Potato tuber | SA | 17-06-97 | Large & small black sclerotia, setae | 321 | 23.1 X 4.7 | P |
| 3c | *Chenopodium album* Fat hen, | SA | 21/04/01 | Large & small black sclerotia, setae | 326 | 23.7 X 4.6 | P |
| 4c | *Citrullus colocynthis* Wild tomato | SA | 21/04/01 | Large & small black sclerotia, setae | 342 | 24.3 X 4.8 | P |

***Isolates used for inoculum studies.***

**Table 1 cont:** Morphological characteristics of *C. coccodes* collected from SA, Vic, Tasmania and WA

| Isolate # | Origin | Location | Date isolated | Morphological characteristics on NP10 media | Size of sclerotia (um) | Size of conidia L X W um | Prostrate or aerial mycelia |
|---|---|---|---|---|---|---|---|
| 38b | Potato tuber | Vic | 18-11-01 | Small oblong black sclerotia, setae | 123 | 17.3 X 3.2 | P |
| 39a | Potato tuber | Vic | 14-02-97 | Small oblong black sclerotia, setae | 168 | 18.4 X 3.4 | P |
| 9a | Potato tuber | SA | 10-11-01 | Small oblong black sclerotia, setae | 110 | 16.2 X 2.5 | P |
| 9b | Soil | SA | 10-11-01 | Small oblong black sclerotia, setae | 130 | 17.4 X 3.9 | P |
| 81b | Soil | SA | 10-11-01 | Small oblong black sclerotia, setae | 124 | 17.3 X 3.1 | P |
| 82b | Soil | SA | 10-11-01 | Small oblong black sclerotia, setae | 132 | 17.5 X 3.4 | P |
| 60b | Soil | SA | 10-11-01 | Small oblong black sclerotia, setae | 120 | 18.1 X 3.4 | P |
| 76b | Soil | SA | 10-11-01 | Small oblong black sclerotia, setae | 138 | 17.7 X 3.8 | P |
| 63b | Soil | SA | 10-11-01 | Small oblong black sclerotia, setae | 137 | 17.5 X 4.1 | A |
| 11a | Potato tuber | SA | 19/02/97 | Small oblong black sclerotia, setae | 124 | 18.1 X 4.3 | A |
| 12a | Potato tuber | SA | 23-11-01 | Small oblong black sclerotia, setae | 96 | 17.8 X 4.6 | P |
| ***17a*** | ***Potato tuber**** | ***SA*** | ***05-09-01*** | ***Small oblong black sclerotia, setae*** | ***145*** | ***17.3 X 3.1*** | ***P*** |
| 26a | Potato tuber | SA | 14-02-97 | Small oblong black sclerotia, setae | 120 | 16.4 X 3.3 | P |
| 1a | Potato tuber | SA | 01-04-97 | Large, round, black sclerotia, setae | 360 | 23.6 X 4.2 | P |
| 22a | Potato tuber | SA | 11-04-97 | Large, round, black sclerotia, setae | 340 | 20.1 X 4.0 | P |
| 29a | Potato tuber | Vic | 14-02-97 | Large round, black sclerotia, setae | 430 | 19.0 X 3.5 | A |
| 38a | Potato tuber | Vic | 14-02-97 | Large, round, black sclerotia, setae | 342 | 22.2 X 3.2 | P |
| 53a | Potato tuber | WA | 01-06-02 | small round black sclerotia | 220 | 16.0 X 2.5 | P |
| 54a | Potato tuber | WA | 01-06-02 | small round black sclerotia | 139 | 15.4 X 3.2 | P |
| 55a | Potato tuber | WA | 01-06-02 | small round black sclerotia | 158 | 16.4 X 3.0 | P |
| 56a | Potato tuber | WA | 01-06-02 | small round black sclerotia | 126 | 17.8 X 3.5 | P |

***Isolates used for inoculum studies.***

*Effects of temperature*

**Objective:** *To determine the effect of temperatures on the growth of C. coccodes and disease development.*

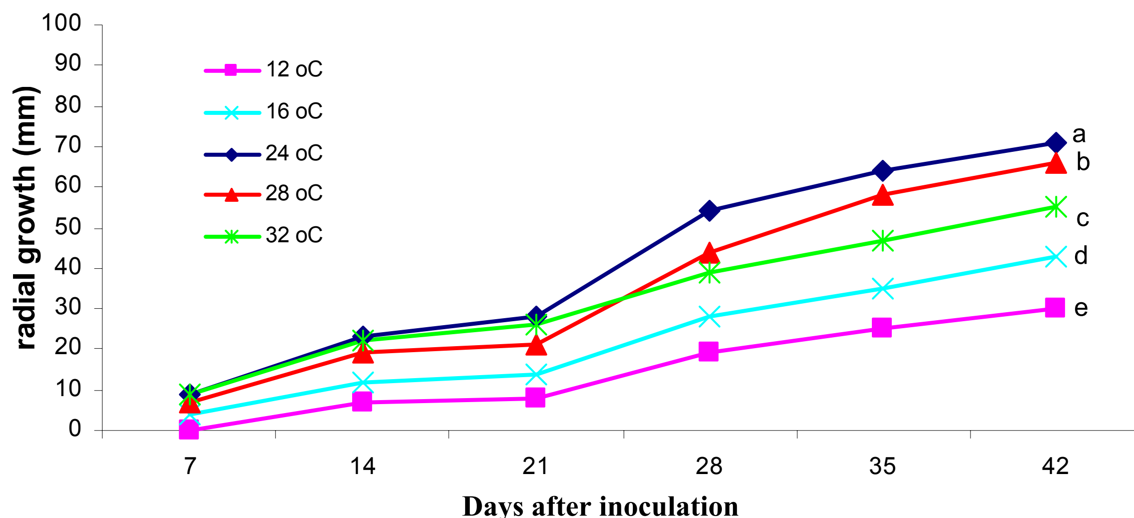
**Materials and Methods:**
***In-vitro***: Preliminary investigations of morphological characteristics of *C. coccodes* on potato plants revealed some variability among isolates from SA, WA, NSW, Tas and Vic. Three dissimilar isolates (77b, 58a, 17a) obtained from naturally infected soil or potatoes (as previously described in Table 1) were used.

To determine the effects of temperature on the growth of *C. coccodes,* four replicated 9 cm diameter petri plates containing NP10 selective media, were inoculated with a 5mm agar plug taken from the outer edge of a 10 day old culture from each of the original three isolates. Inoculated plates were grown at 5 temperatures ranging from 12 – 32°C. Radial growth and the production of sclerotia and size of sclerotia were recorded (as previously described) at 7-day intervals over a period of 6 weeks.

***In-vivo***: Seed tubers infected with *C. coccodes* were planted into pots (as previously described) with 5 replicates per treatment and grown in growth rooms at temperatures of 15°C, 25°C and 35°C with artificial illumination (110uEm2sec1) at 16hrs light and 8 hrs dark. Plants were watered by weight every 3 days to maintain constant soil moisture content of 60% water holding capacity. At 3 weeks after complete senescence (91 days after planting), plants and tubers were harvested and visually assessed for both incidence and severity of *C. coccodes* as previously described.

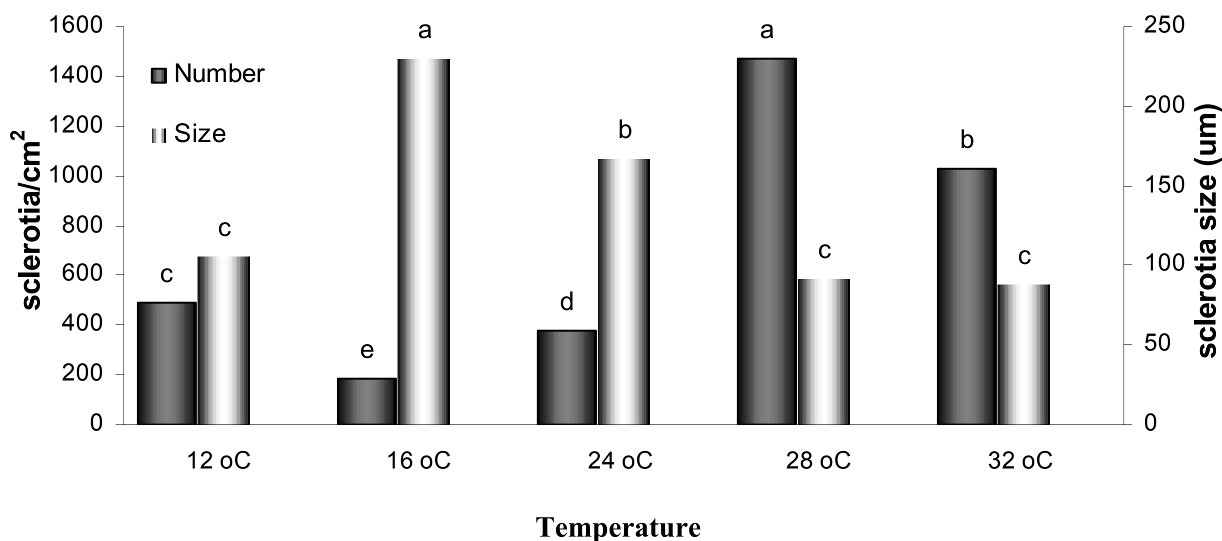
**Results and Discussion:**
*In-vitro*: The rate of growth of *C. coccodes* was highest at 24°C and lowest at 12°C (Fig 1). At every time of measurement, the relative increase of growth at different temperatures was similar, except for 32°C, where after 21 days the colony growth slowed and radial growth dropped from 2nd largest to 4th largest. Sclerotia were largest at 16°C compared to all other temperatures, whilst the sclerotial density was highest at 28°C (Fig 2).

**Figure 1:** Effect of temperature on the radial hyphal growth of *C. coccodes in-vitro.*




Treatments with the same letter are not significantly different from one another (LSD P=0.01)

**Figure 2:** Effect of temperature on the number and size of *C. coccodes in-vitro*.



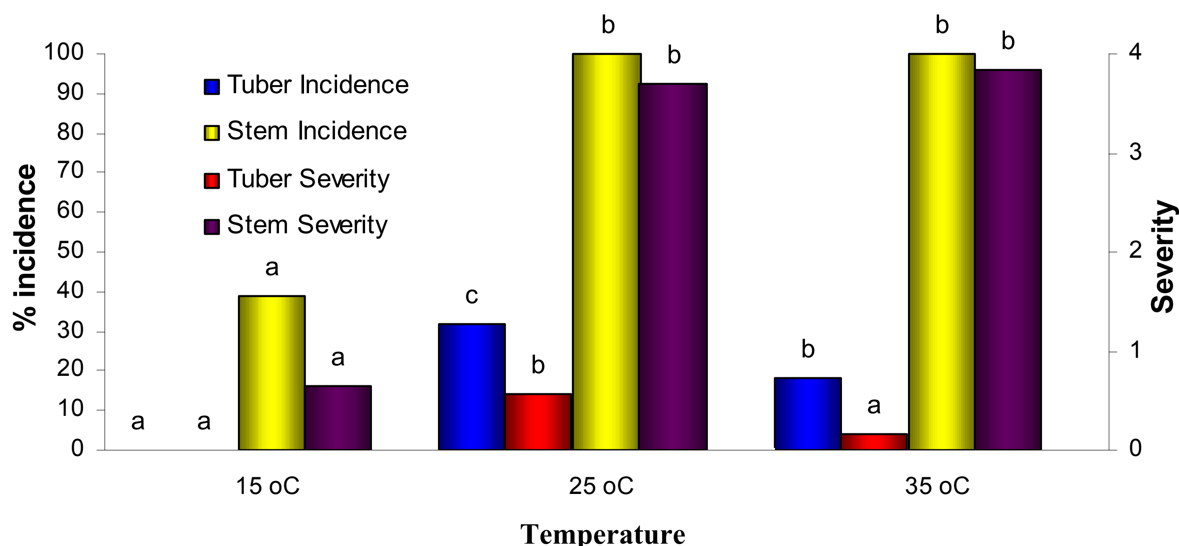

**Temperature**

Treatments with the same letter are not significantly different from one another (LSD P=0.01).

***In-vivo**: C. coccodes* did not develop on tubers grown at 15°C whereas at 25°C and 35°C. It developed on 32 and 18% of tubers respectively (Fig 3). At 15°C *C. coccodes* developed on 39% of stems whilst at 25°C and 35°C all plants were infected. Disease incidence and severity on stems was lowest at 15°C (Fig 3).

Tubers grown at 25°C had the highest level of disease, but there was no difference between amounts of stem infection at the two higher temperatures.

**Figure 3:** Effect of temperature on the incidence and severity of *C. coccodes* on tubers and stems




**Temperature**

[A]Severity rating scale 0 to 4: 0, no diseases; 1, <2%; 2, 3-10%; 3, 11-30%, 4, >30% of tuber surface affected. Treatments with the same letter are not significantly different from one another (LSD P=0.01).

Temperature had a significant affect on the development of *C. coccodes*, as the disease was most severe on tubers and stems over 25°C. This explains the field observations of increased disease incidence during summer months when soil temperatures exceed 25°C at 20cm depth (5yr avg Loxton

1995-2000, Fig 4). Also at higher temperatures there are a large numbers of small sclerotia, creating more colonies and increasing the inoculum potential.

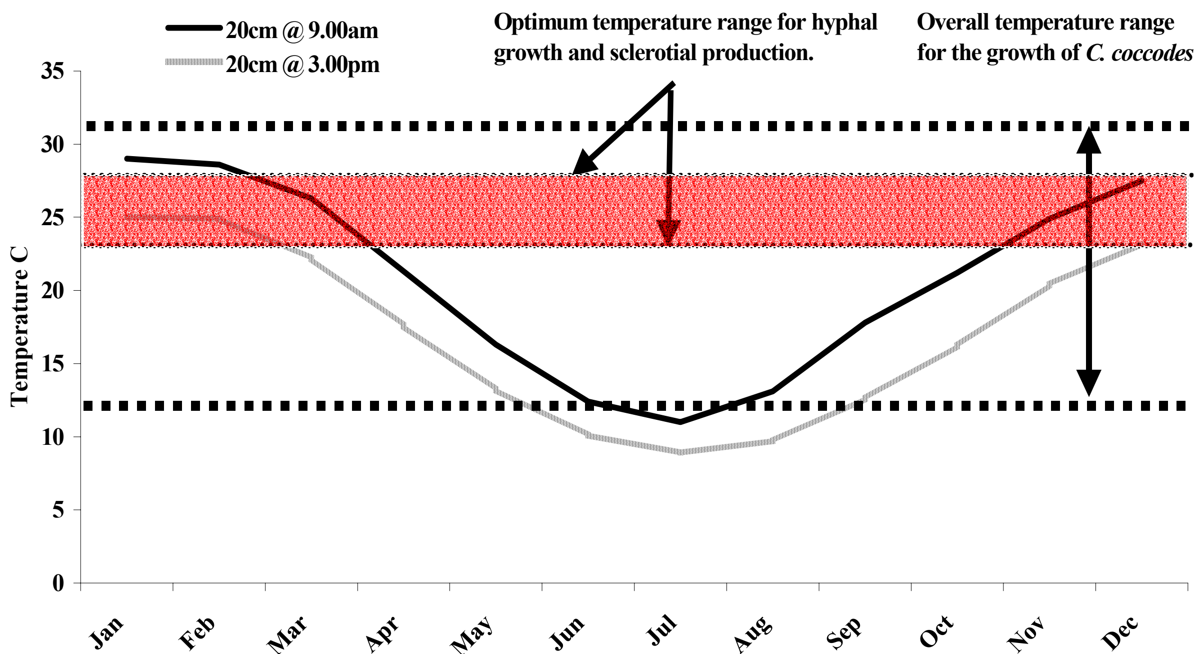
This study showed that the optimum temperature (24°C) for hyphal growth of *C. coccodes* was similar to recent findings from Israel and the UK (41, 52). The reduced activity of *C. coccodes* at lower temperatures (<15°C) may correspond with a reduction of nutrient utilisation from the host plant, so the fungus uses the colonized tissue more slowly resulting in latent infections.

The production of sclerotia and subsequent infection of plant tissue occurred over a wide range of temperatures but was greatest at 28°C and 32°C. This relationship between sclerotial density and temperature concurs with recent research in Israel (52). Whilst symptoms were not observed on tubers at 15°C a high incidence of infected stems were found at this temperature.

Whilst temperature influenced the number of sclerotial produced, other factors such as aeration and light are also likely to exert an effect on sclerotial germination. Studies have indicated that myceliogenic and sporogenic germination of sclerotia occurs over a wide range of temperatures (61, 67). However conidial and mycelial production on sclerotia appear to be more sensitive to aeration and light than to temperature (52). This suggests that conidia may be produced more abundantly on sclerotia on or above the soil surface than on sclerotia buried in soil. Sclerotia above the soil surface would be exposed to a higher concentration of sunlight and therefore a higher source of conidial inoculum readily available for dissemination by rain splash.

In conclusion, disease development was greatest at temperatures of 25°C or higher soil temperature, increasing with increasing soil temperatures. By comparing this information with the soil temperatures in a particular region, growers may be able to adjust their planting/harvesting times to reduce the risk of infection, for example in the Loxton region of SA, growers should avoid planting or harvesting crops maturing in January and February (Fig 4).

**Figure 4:** Optimum temperatures for hyphal growth and sclerotial production for the fungus *C coccodes* in relation to the 5 year average soil temperature at 10 - 20cm depths at Loxton.



***Disease development***

**Objective:** *To monitor the development of C. coccodes on potato tubers and roots.*

**Materials and Methods:**
Seed tubers cv. Coliban with 67% of tubers naturally infected with *C. coccodes* were planted on new ground (clay loam) in the Adelaide Hills of South Australia. Tubers were planted 24cm apart in rows 80cm apart in an area 100m long and 28m wide.

Every 2 weeks, commencing 7 weeks after planting, 4 plants from 25 equally spaced locations (total of 100) were dug up using a "W" sampling pattern and assessed for senescence, internal and external incidence and severity of *C. coccodes* (as previously described).

**Results and Discussion:**
*C. coccodes* was first detected in the vascular system of stems 42 days after planting, whereas external symptoms were first detected on stems 70 days after planting. The increase of sap, internal and external infections followed a similar rate of exponential development (Fig 5).

Tuber infection was not detected until 84 days after planting, but also increased exponentially to around 80% of tubers showing external symptoms of infection at harvest (Fig 5). The severity of tuber infection followed a similar rate of development, however severity of stem infection was variable (Fig 6).

*C. coccodes* was detected in stems of plants with no visible signs of infection from 56 days after planting (Fig 5). The incidence of internal infection in stems with viable *C. coccodes* was variable, and may be related to the efficacy of the surface sterilization technique on the external sclerotia, or a natural sampling variate.

While potato seed tubers can be a primary source of inoculum of *C. coccodes*, the means by which this inoculum moves from tuber seed to daughter tubers is unknown. This study shows that *C. coccodes* first develops on the stem tissue within 6 weeks after planting, before tuber skin infections are obvious around 14 weeks after planting. Similar trials have shown stems, stolons and roots developing from infected seed were infected with *C. coccodes* 6 – 8 weeks after planting (4, 41), further suggesting that this is due to systemic growth of the fungus from infected seed. As with USA studies (37), tuber infections were first observed at the stolon end suggesting that conidia produced on stem and root tissue are carried along the stolon from the mother tuber seed onto progeny tubers.

At 84 days, severity of *C. coccodes* and incidence of both external and internal (sap) increased in conjunction with the incidence of tuber infection at the stolon end. However very few tubers were infected at this date. It is not until 28 days after this initial observation that both tuber incidence and severity begin to increase (128 days). These results suggest that variations over time in the incidence of *C. coccodes* found in the belowground and above ground plant parts may reflect distinct phases of the disease (32, 33)

**Figure 5:** Incidence of *C. coccodes* on plants grown from naturally infected tubers.

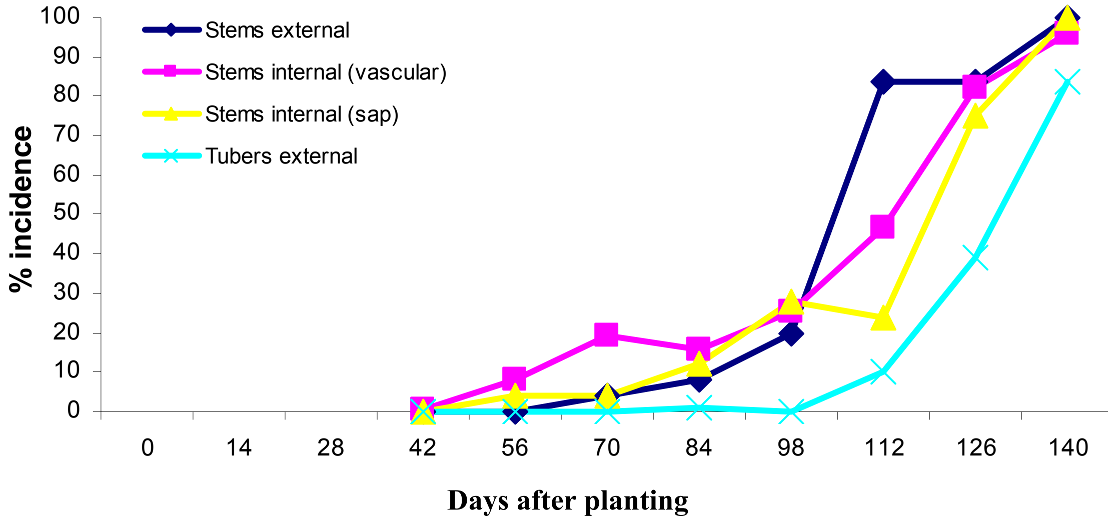



**Figure 6:** Severity of *C. coccodes* on stems and tubers of plants grown from naturally infected tubers.

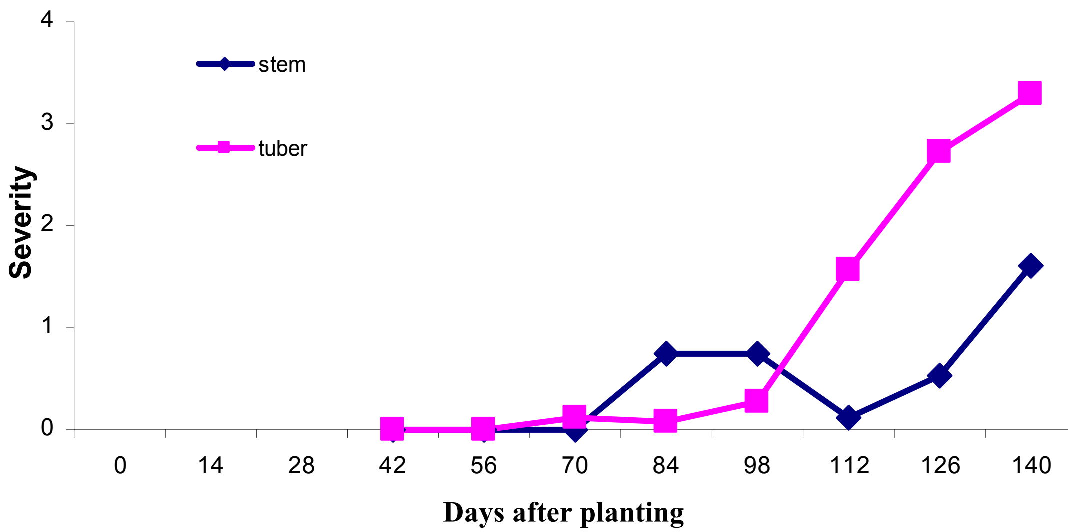
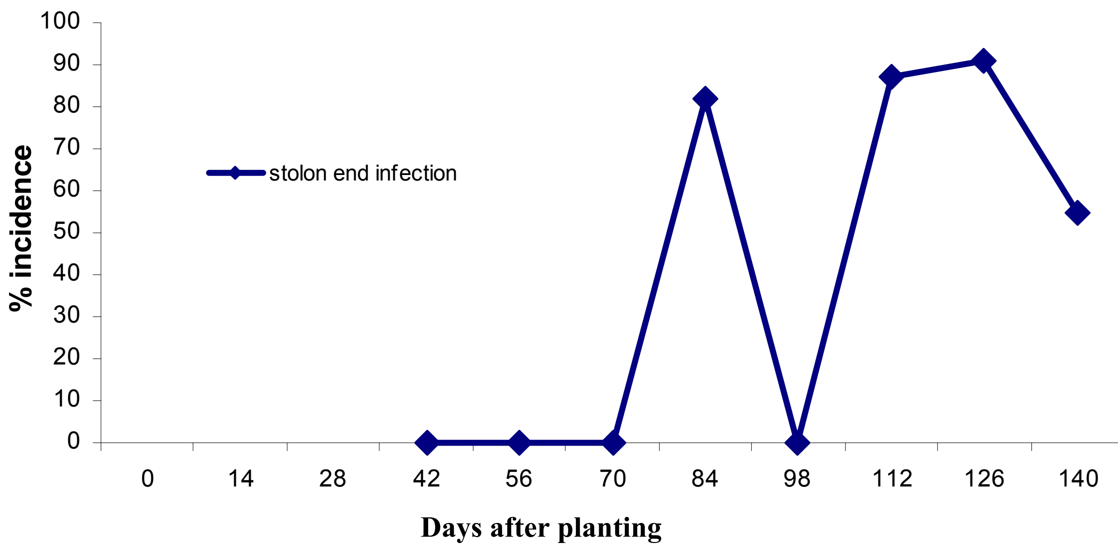



**Figure 7:** Incidence of tuber stolon infection by *C. coccodes*.



### *Tuber infection from conidia*

**Objective:** *To evaluate if C. coccodes conidia can infect mature potato tubers.*

**Materials and Methods:**
Two experiments were conducted to determine if *C. coccodes* develops on mature tubers inoculated with conidia or microsclerotia.

In experiment 1, 10 replicates of 1 tuber (cv. Coliban) with no evidence of *C. coccodes* were artificially inoculated with 5ml solutions of water containing either *C. coccodes* microsclerotia (A) or conidia (B). This was applied to the tuber surface using a 5ml syringe. Inoculum (A) was made by collecting microsclerotia from 2 month old cultures (isolate 58a) grown on NP10 selective media. Microsclerotia were scraped off the agar surface and ground in a blender with water. Inoculum (B) was prepared by soaking 200gms of stems infected with *C. coccodes* for 30 minutes in 5L water. Stems were harvested 5 days prior to soaking, from a commercial crop 5 weeks after the application of Reglone®. The concentration of both inoculums (A) and (B) were adjusted to 124cfu/ml using a haemocytometer.

In experiment 2, 10 replicates of 1 tuber (cv. Coliban) with no evidence of *C. coccodes* were planted in pots as previously described. Directly after planting tuber, each pot received 200ml of the solutions described in Table 2. The concentration of inoculums (B, C, D and E) were adjusted to 124cfu/ml using a haemocytometer. 1ml of each solution was spread out onto NP10 selective media to confirm viability.

**Table 2:** Treatments applied

| Treatment | Description |
|---|---|
| **A** | Water |
| **B** | 23 petri plates of 2 month old *C. coccodes* blended with 1L water |
| **C** | 200gms of infected stems soaked in 5L water for 30min |
| **D** | As treatment "C" but stems sprayed with Amistar®* 1 week after Reglone® was applied. |
| **E** | As per treatment "C" but the soil surface of pots was sprayed with Amistar®* |
| **F** | 50g of stems placed on top of pots and 500ml water applied per day for 7 days. |

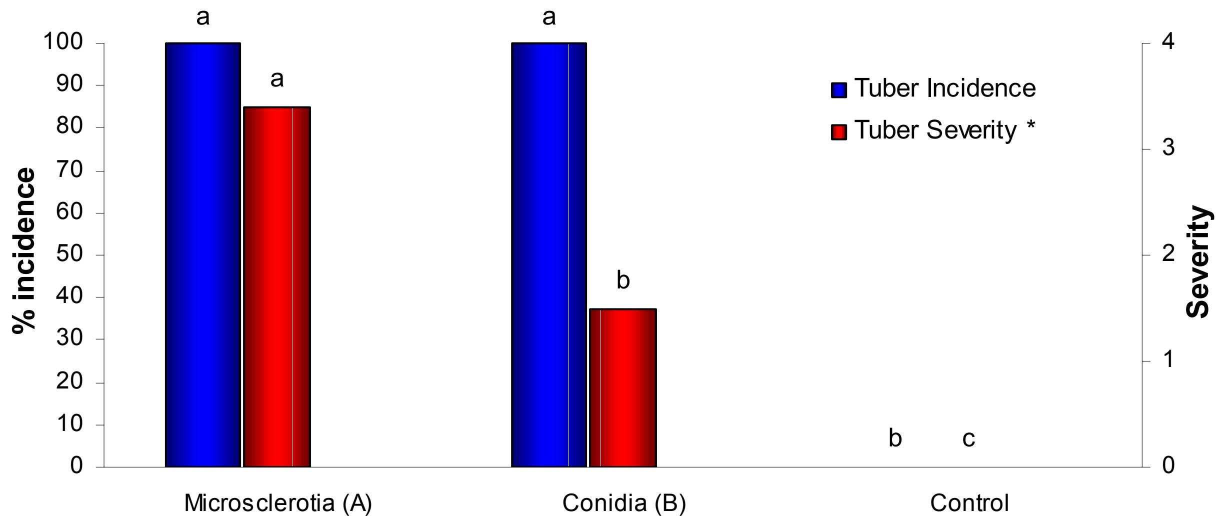
* 250g ai/Ha

For both experiments the incidence of *C. coccodes* colonies on the tuber surface was recorded at 7-day intervals for 28 days after the treatments were applied and the percentage of infection calculated.

**Results and Discussion:**
*C. coccodes* was not observed on tubers in either experiment until 4 weeks after inoculation. At this stage colonies 5 – 10mm diameter were observed on tubers inoculated with conidia or microsclerotia (Fig 8). *C. coccodes* developed on all ten tubers within each treatment except the uninoculated control. Severity of infection was less with conidial inoculation compared to microsclerotia inoculation.

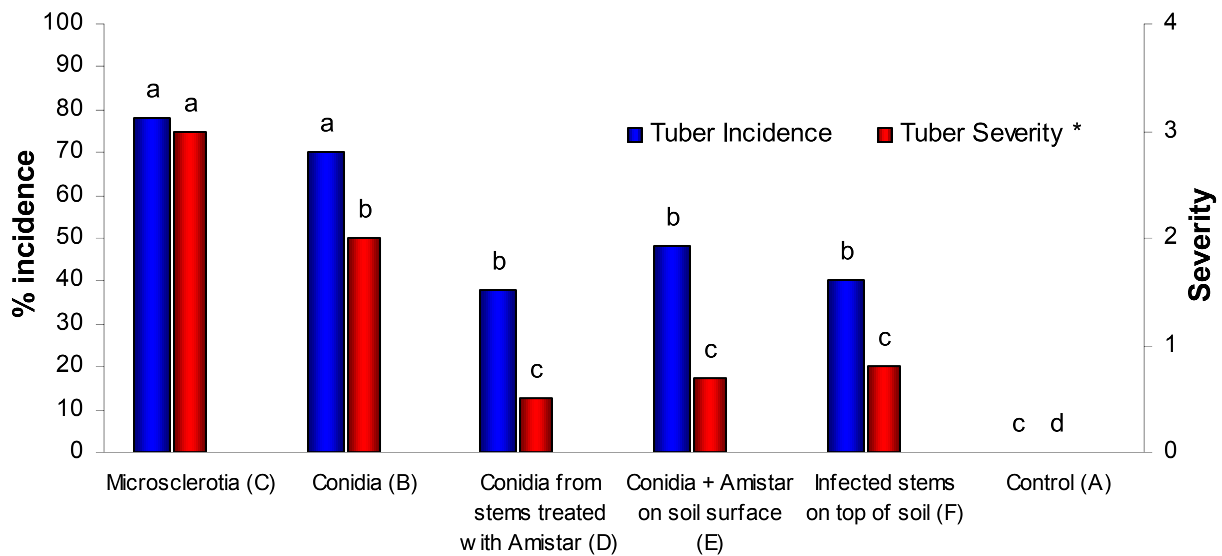
These results show that tubers can be infected with conidia of *C. coccodes* washed through the soil from the soil surface. Tubers became infected with spores applied either directly to the soil surface or washed from infected stem pieces on the soil surface. Applying Amistar® either to the stem pieces or directly onto the soil surface did not prevent tuber infection but significantly reduced the number of tubers infected and the severity of tuber infections (Fig 9). This suggests that foliar applications of Amistar® or other chemicals late in the season may be a useful technique to reduce inoculum levels from infected haulms.

**Figure 8:** Incidence and severity of *C. coccodes* on tubers inoculated with microsclerotia or conidia.




[A]Severity rating scale 0 to 4: 0, no diseases; 1, <2%; 2, 3-10%; 3, 11-30%, 4, >30% of tuber surface affected. Treatments with the same letter are not significantly different from one another (LSD P=0.01).

**Figure 9:** Incidence and severity of *C. coccodes* on tubers placed in soil where microsclerotia or conidia was applied to the soil surface.




[A]Severity rating scale 0 to 4: 0, no diseases; 1, <2%; 2, 3-10%; 3, 11-30%, 4, >30% of tuber surface affected. Treatments with the same letter are not significantly different from one another (LSD P=0.01).

***Pathogenicity of*** *C. circannis*

**Objective:** *To evaluate if C. circannis (a pathogen of onion) is pathogenic to potato.*

**Materials and Methods:**
Replicates of 10 pots per treatment (as previously described) were artificially infested with microsclerotia at concentrations of 20-microsclerotia/g soil of either *C. circannis* or *C. coccodes*. *C. circannis* inoculum was made by collecting infected onions from a commercial grower in the Riverland district of South Australia. One 5mm core of infected skin tissue was plated on NP10 selective media (X 20 plates) and incubated for 30 days at 22°C in the dark. Microsclerotia of both species was scraped off the agar surface from 30 day old cultures, ground in a blender with water, and the concentration adjusted using a haemocytometer. Microsclerotia were thoroughly mixed with air dried soil at concentrations of 1000 microsclerotia/g of soil, then diluted with the sandy loam to achieve the desired concentration.

One certified mini tuber cv. Coliban, with no obvious *C. coccodes* infection on the tuber surface, was planted in each pot at a depth of 15cm. Pots received 1.3 litres of water every 3rd day, and were fertilised as previously described.

All plants were carefully removed from the pots along with attached tubers and visually assessed for incidence and severity of *Colletotrichum sp*, and the incidence of *Colletotrichum sp* in plant tissue assessed as previously described.

Where external infection was observed, plant material was isolated onto NP10 selective media and incubated as previously described. The identity of cultures was verified by comparing sporulation and cultural characteristics typical of the species (6, 64) and with original isolates.

**Results and Discussion:**
Although *C. coccodes* did not develop on tubers grown in the control treatments, it was isolated from the stems and sap. It was also recovered from some stems, sap and roots of some tubers inoculated with *C. circannis*, indicating that either the mini tubers were infected before planting or that some other means of contamination occurred. Where mini tubers were grown in soil infected with *C. coccodes*, 60% of the daughter tubers developed black dot and the fungus was isolated from roots, stem and stem sap. On the other hand no tubers developed black dot when grown in soil infested with *C. circannis*. *C. circannis* was not recovered from tubers, stem tissue or sap of plants grown in soil infested with *C. circannis*, showing that the onion pathogen does not infect potato tubers.

**Table 3:** Incidence of *C. coccodes* or *C. circannis* on inoculated potato plants.

| Plants inoculated with: | Not inoculated | | *C. coccodes* | | *C. circannis* | |
|---|---|---|---|---|---|---|
| **Plant part** | **% infection** | | | | | |
| | *C. coccodes* | *C. circannis* | *C. coccodes* | *C. circannis* | *C. coccodes* | *C. circannis* |
| Roots | 0 | 0 | 100 | 0 | 10 | 0 |
| Tubers | 0 | 0 | 60 | 0 | 0 | 0 |
| Stem tissue | 30 | 0 | 80 | 0 | 20 | 0 |
| Stem sap | 40 | 0 | 90 | 0 | 20 | 0 |

*Weeds*

**Objective:** *To identify plants that host C. coccodes in South Australia.*

**Materials and Methods:**
Between 2001 – 2003, 10 weeds of each species shown in table 4 were collected at random from paddocks that had grown potatoes the previous year. Plants were dug up and then examined visually for the presence of *C. coccodes* on stems and roots. The sampling region included the South East, Murray Mallee and Adelaide Hills regions of South Australia.

**Results and Discussion:**
*C. coccodes* was found on either the stems or roots of eight species shown in table 1.

**Table 4:** Presence of *C. coccodes* on 28 weed species in SA 2001 – 2003.

| Family | Scientific Name | Common Name | Presence of Black dot |
|---|---|---|---|
| Poaceae | *Lolium rigidium* | Annual ryegrass | No |
| Poaceae | *Hordeum leporinum* | Barley grass | No |
| Cucurbitaceae | *Citrullus lanatus* | Bastard melon | No |
| **Solanaceae** | ***Solanum nigrum*** | **Black nightshade** | **Yes** |
| Zygophyllaceae | *Tribulus terrestris* | Caltrop | No |
| Asteraceae | *Arctotheca calendula* | Capeweed | No |
| **Chenopodiaceae** | ***Chenopodium album*** | **Fat hen** | **Yes** |
| Asteraceae | *Conyza bonariensis* | Flaxleaf fleabane | No |
| **Boraginaceae** | ***Heliotropium europaeum*** | **Heliotrope** | **Yes** |
| Lamiaceae | *Marrubium vulgare* | Horehound | No |
| Caryophyllaceae | *Silene apetala* | Mallee catchfly | No |
| Asphodelaceae | *Asphodelus fistulosus* | Onion Weed | No |
| Cucurbitaceae | *Cucumis myriocarpus* | Paddy melon | No |
| Onagraceae | *Oenthera glazioviana* | Primrose | No |
| **Solanaceae** | ***Solanum esuriale*** | **Quena** | **Yes** |
| Brassicaceae | *Diplotaxis tenuifolia* | Sand rocket | No |
| **Brassicaceae** | ***Capsella bursa-pastoris*** | **Shepherds purse** | **Yes** |
| **Asteraceae** | ***Chondrilla juncea*** | **Skeleton weed** | **Yes** |
| Chenopodiaceae | *Salsola kali* | Soft roly poly | No |
| Asteraceae | *Dittrichia graveolens* | Stinkwort | No |
| Polygonaceae | *Emex australis* | Three cornered jack | No |
| Brassicaceae | *Sinapsis arvenis* | Wild mustard | No |
| Poaceae | *Avena fatua* | Wild oats | No |
| Brassicaceae | *Raphanus raphanistrum* | Wild radish | No |
| **Cucurbitaceae** | ***Citrullus colocynthis*** | **Wild tomato** | **Yes** |
| Brassicaceae | *Brassica tournefortii* | Wild turnip | No |
| Brassicaceae | *Rapistrum rugosum* | Wild turnip | No |
| **Polygonaceae** | ***Polygonum aviculare*** | **Wire weed** | **Yes** |

This survey has shown that *C. coccodes* survives on the stems and/or roots of eight common weeds in SA. Several of the species are also listed as hosts in the USA (55) eg *Solanum nigrum* and *Capsella bursa-pastoris.* Although the pathogenicity of *C. coccodes* isolated from weeds was not tested on potatoes, it is possible that uncontrolled weed populations could support and increase inoculum levels between rotations or may act as a source of primary inoculum for subsequent potato crops. Growers should be able to develop better disease management practices through selective weed control and non-host rotational crops.

Two of the species identified are from the same family "solanaceae" whilst the remaining came from different families. In several cases, *C. coccodes* was not observed on other species within the same family.

This survival of *C. coccodes* inoculum in soil is not just restricted to weed hosts but also occurs with volunteer potatoes left over from harvest. These sprout the following year and then serve as host for the pathogen and other diseases. In a recent survey of potato paddocks in South Australia, up to 50,000 volunteer plants/ha were found one year after production (data not shown) and in the USA 237,500 tubers/ha remained in the field after harvest (20). The Australian survey also showed that volunteer tubers remain viable for up to 4 years after the initial crop. The control of these volunteers and of weed hosts is crucial to reducing the primary inoculum for subsequent potato crops.

## Pathogen and plant interaction

***Introduction:***

These studies were undertaken to gain a better understanding the pathogen infection cycle and how it interacts with the host plant. The aim was to help growers and researchers to improve chemical and/or cultural methods of control by specifically targeting susceptible stages of the disease.

***General materials and methods:***

*Internal infection*: The stem end of tubers was sliced and where staining was obvious in the vascular tissue, 2 pieces of approx $1mm^2$ were removed with a scalpel and plated onto NP10 selective media. After 14 days incubation at $24^oC$, plates were examined microscopically and scored for the presence or absence of *C. coccodes*.

*Mini seed tubers:* These were stored in moist vermiculite for approximately 3 mo at $22^oC$ until dormancy was broken. Tubers were kept under the above conditions until sprouts 0.5-1cm in length had developed. Sprouted tubers were then stored at $5^oC$ until 2 - 5 days before planting when they were again warmed to $22^oC$.

*Tuber infection points*

**Objective:** *To determine the incidence of internal (vascular) and external (skin) infections of C. coccodes on tubers.*

**Materials and Methods:**
In 2001 and 2002, a sample of 100 potato tubers was collected at random from bins of 28 commercial potato properties. Properties were selected at random with no particular bias in respect to seed source or variety. However, tubers were tested only where the origin of the seed lot could be identified and was accompanied with a certificate of registration.

All tubers were assessed for both the incidence and severity of *C. coccodes* as previously described. However only tubers from the 2002 sampling were assessed for internal incidence of *C. coccodes*. Data was analysed by Spearman's rank correlation coefficient.

**Results and Discussion:**
Seed lots collected in 2001 showed a range of infection levels. While 61% of the lots were infected, most (28%) had less than 5% infection and none had more than 30% (Table 5). In 2002 there was a dramatic increase, with 93% of seed lots infected and all had over 30% of tubers with *C. coccodes*.

No correlation was found between the infection on the tuber surface and internal infection (r = 0.18, P = 0.001) in 2002 (Table 7). However all tubers with internal infections also had external infections (Tables 7), and a strong correlation exists between the individual severity ratings and the % of tubers infected internally (r = 0.70, P = 0.001). Although the importance of vascular infection of *C. coccodes* in seed tubers has not been determined this aspect is of concern as vascular infections are unlikely to be controlled by treating infected tubers with fungicides applied to the tuber surface.

**Table 5:** Detection of *C. coccodes* on seed tubers during 2001 and 2002.

| % of tubers in which *C. coccodes* was detected. | # of seed lots 2001 | % of seed lots 2001 | # of seed lots 2002 | % of seed lots 2002 |
|---|---|---|---|---|
| 0 | 11 | 39.2 | 2 | 7.1 |
| 1-5 | 8 | 28.5 | 0 | 0 |
| 6-10 | 4 | 14.2 | 0 | 0 |
| 11-20 | 3 | 10.7 | 0 | 0 |
| 21-30 | 2 | 7.1 | 0 | 0 |
| 31-100 | 0 | 0 | 26 | 92.9 |
| **Total % infected** | | **60.8** | | **92.9** |

Similar surveys conducted in the USA (37) and in the UK (58) showed that the incidence of *C. coccodes* in certified seed tubers ranged from 0 – 90% and 0 – 75% respectively. These studies confirm that infected tuber seed is the main means of introducing *C. coccodes* into potato production areas.

South Australia produces 25% of national production making it the major potato producing state within Australia. Approximately 30,000 tonnes of imported seed produces 334,697 tonnes of potatoes (Australia Bureau of statistics 2002). Improved understanding of the extent of this disease on seed tubers from different seed producing regions may help in developing more efficient disease management strategies. Results could be used to compare cultural practices and environmental factors in areas that exhibit different levels of the disease or the planting of seed tubers from farms with relatively low levels of infection.

**Table 6:** Certified seed survey (2001) % tubers infected with *C. coccodes* externally and the average severity.

| Variety | % External *C. coccodes* | % Severity *C. coccodes* | % Internal *C. coccodes* |
|---|---|---|---|
| Coliban | 0 | 0 | |
| Coliban | 4 | 0.3 | |
| Coliban | 0 | 0 | |
| Coliban | 0 | 0 | |
| Coliban | 18 | 0.8 | |
| Coliban | 9 | 0.5 | |
| Desiree | 28 | 1.2 | |
| Desiree | 5 | 0.4 | |
| Desiree | 0 | 0 | |
| Coliban | 0 | 0 | |
| Coliban | 29 | 1.2 | |
| Coliban | 0 | 0 | |
| Coliban | 9 | 0.6 | |
| Atlantic | 0 | 0 | |
| Atlantic | 2 | 0.3 | **Not Assessed** |
| Desiree | 7 | 0.5 | |
| Coliban | 0 | 0 | |
| Atlantic | 3 | 0.2 | |
| Atlantic | 4 | 0.3 | |
| Atlantic | 10 | 0.6 | |
| Atlantic | 0 | 0 | |
| Coliban | 0 | 0 | |
| Coliban | 16 | 0.8 | |
| Coliban | 5 | 0.3 | |
| Coliban | 20 | 1.0 | |
| Coliban | 0 | 0 | |
| Coliban | 1 | 0.2 | |
| Coliban | 2 | 0.2 | |
| **Avg.** | **6.1** | **0.3** | |

**Table 7:** Certified seed survey (2002) % tubers infected with *C. coccodes* either externally/internally and the average severity.

| Variety | % External *C. coccodes* | % Severity *C. coccodes* | % Internal *C. coccodes* |
|---|---|---|---|
| Coliban | 96 | 1.5 | 18 |
| Coliban | 98 | 2.6 | 25.6 |
| Coliban | 100 | 2.6 | 33 |
| Desiree | 100 | 1.9 | 0 |
| Coliban | 100 | 3.0 | 0 |
| Bison | 85 | 1.5 | 32.4 |
| Kennebec | 96 | 1.9 | 15.3 |
| Coliban | 89 | 1.9 | 7 |
| Desiree | 100 | 2.6 | 7 |
| Coliban | 100 | 1.6 | 16 |
| Coliban | 100 | 2.6 | 0 |
| Coliban | 100 | 2.4 | 1 |
| Coliban | 83 | 1.4 | 5 |
| Desiree | 100 | 2.9 | 1 |
| Desiree | 100 | 2.5 | 0 |
| Atlantic | 99 | 2.4 | 1 |
| Coliban | 99 | 2.1 | 0 |
| Atlantic | 100 | 2.6 | 1 |
| Ruby Lue | 100 | 2.5 | 1 |
| Ruby Lue | 100 | 2.0 | 0 |
| Coliban | 100 | 1.8 | 0 |
| Coliban | 98 | 2.2 | 1 |
| Coliban | 0 | 0.0 | 0 |
| Coliban | 100 | 0.0 | 0 |
| Coliban | 88 | 1.5 | 0 |
| Coliban | 92 | 1.8 | 0 |
| Desiree | 100 | 2.0 | 0 |
| Coliban | 0 | 0 | 0 |
| **Avg.** | **90.1** | **1.9** | **5.9** |

***Light on C. coccodes seed***

**Objective:** *To evaluate the effects of light on the viability of C. coccodes on seed tubers.*

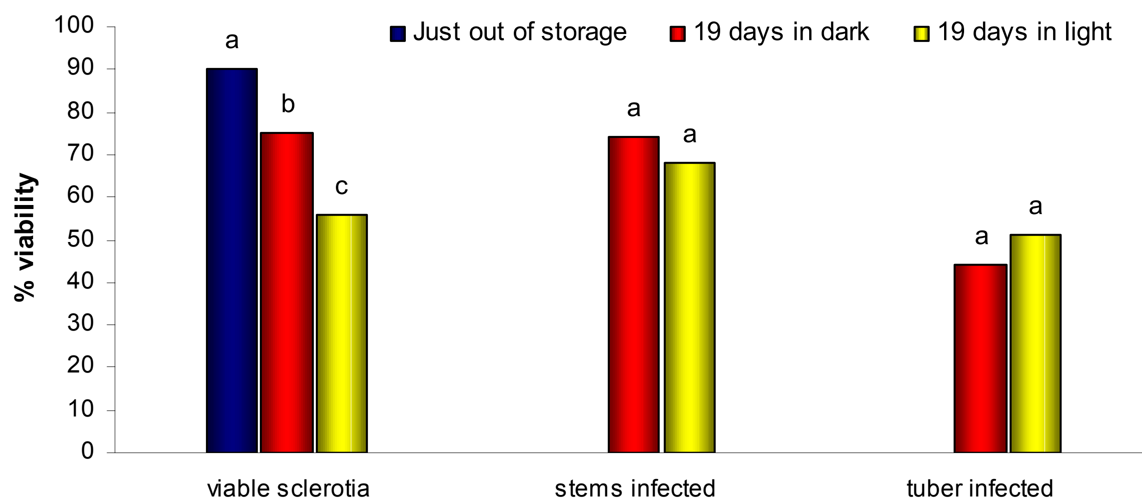
**Materials and Methods:**
40 seed tubers with more than 75% of their surface area infected with *C. coccodes* were removed from cold storage after 2 months at 4.5°C. Tubers were then placed in the dark at room temperature ranging from (16 – 24°C) for 3 days. After this time two 5mm cores were removed from the stolon end of each potato and plated onto NP10 selective media.

20 of these tubers were then exposed to natural sunlight at ambient temperatures (16 – 24 °C) whilst the remaining 20 tubers were kept in the dark in similar temperatures. After 19 days, two 5mm cores containing *C. coccodes* microsclerotia were removed from each potato and plated onto NP10 selective media. All 40 tubers were then planted into pots, watered and fertilized as previously described. All plates were incubated for 14 days at 22°C in the dark, after which the sclerotial viability was determined as previously described. After 3 months growth, all daughter tubers and stems from each pot were assessed for incidence of *C. coccodes* as previously described.

**Results and Discussions:**
The exposure of potato seed tubers to light induces the formation of a green pigmentation on the surface of the potato. In this experiment all tubers that were exposed to light became green, indicating an increase in the presence of glycoalkaloids. Ninety percent of sclerotia were viable on tubers removed from cold storage. The viability was reduced to 75% and 54% when tubers were kept for a period of 19 days in the dark or light respectively. These differences had little influence on disease development on daughter tubers as both the incidence of infected stems and tubers were similar on potato plants grown from tubers subjected to either the light or dark treatments.

Whilst the exposure of seed tubers to light showed no effect on the viability of *C. coccodes* it may be beneficial to seed growers. Planting seed that has been exposed to light hardens sprouts and advances the physiological stage giving an earlier emergence, and earlier maturity. This is advantageous where a larger number of smaller tubers are desirable. In addition this early maturity reduces the time where a crop is susceptible to virus infections. Since greening of tubers is strongly affected by the quality, duration, and the light intensity as well as temperature, further trials need to conducted to fully evaluate the effects of greening on tuber borne disease

**Figure 10:** Effect of light on the viability of *C. coccodes* recovered from tubers exposed to light.




Treatments with the same letter are not significantly different from one another (LSD P=0.01).

### *Systemic infection*

**Objective:** *To establish if seed borne C. coccodes is systemic.*

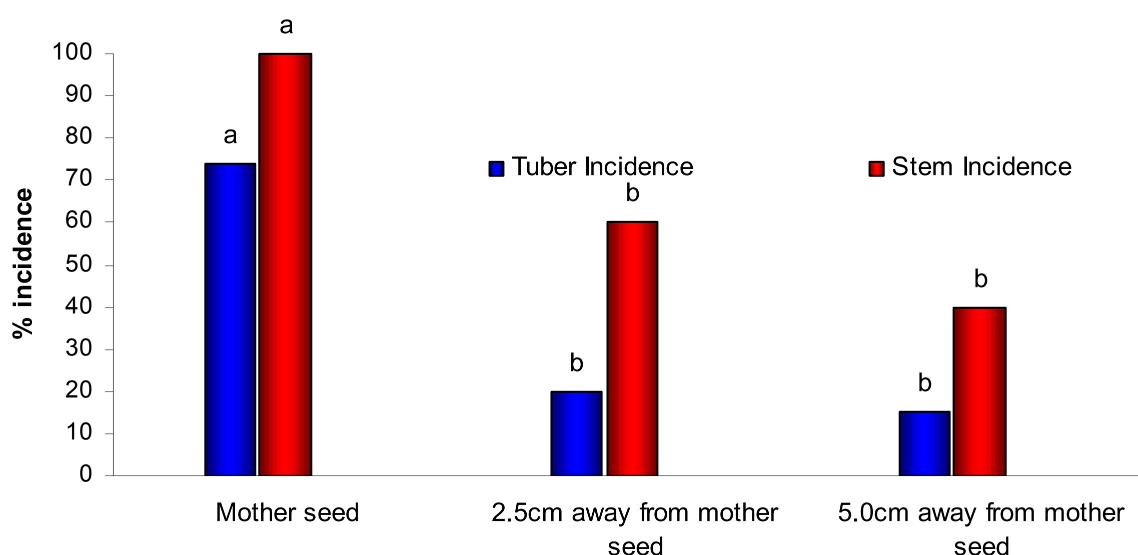
**Materials and methods:**
30 tubers, cv Coliban, with more than 30% of their surface affected by *C. coccodes* were planted into pots as previously described (1st August 2003), 1 tuber per pot. Eighteen days after planting (19th August 2003) 20 pots were selected at random and the plants carefully dug up. Half the stems from each plant were cut at 2.5cm from the mother seed tuber, and the other half cut at 5cm.

Stems were then replanted into new pots containing sterile soil and the mother seeds discarded. All pots were watered and fertilized as previously described. Two months after planting stems and daughter tubers of all treatments were assessed for the external presence of *C. coccodes*. In addition, plants that were not separated from the mother seed 18 days after planting, had internal isolations from the main stolon at 1, 2, 3, 4 and 5cm from the mother seed tuber and at 1, 2, 3, 4, and 5cm from soil line (green tissue) and sap extracted from stem tissue at 1, 2, 3, 4 and 5cm from the soil line.

**Results and Discussion:**
The incidence of *C. coccodes* on tubers was significantly reduced when the main root system was cut either 2.5 or 5.0 cm above the mother seed compared to uncut root systems (60%, 40% and 100% respectively). Disease incidence in stems followed similar pattern, with 20%, 5% and 74% respectively (Fig 11). Stem and stolon infection reduced with increasing distance from the infection source, but was still detectable. The incidence of *C. coccodes* in stolon tissue significantly decreased from 40% at 1 and 2cm to 20% at 3 and 4cm and 0% at 5cm away from the mother seed. *C. coccodes* was recovered from the sap of all stems collected from 1 to 5 cm above the soil line. No *C. coccodes* was isolated from internal stem tissue in any of the treatments. In other experiments *C. coccodes* was recorded from tissue before sap, and there was no apparent reason for the nil detected in tissue in this experiment. It is unlikely that external growth of *C. coccodes* exceeded 2.5 or 5.0 cm within 18 days after planting. It is more likely that the infections of *C. coccodes* developed from tissue colonized internally.

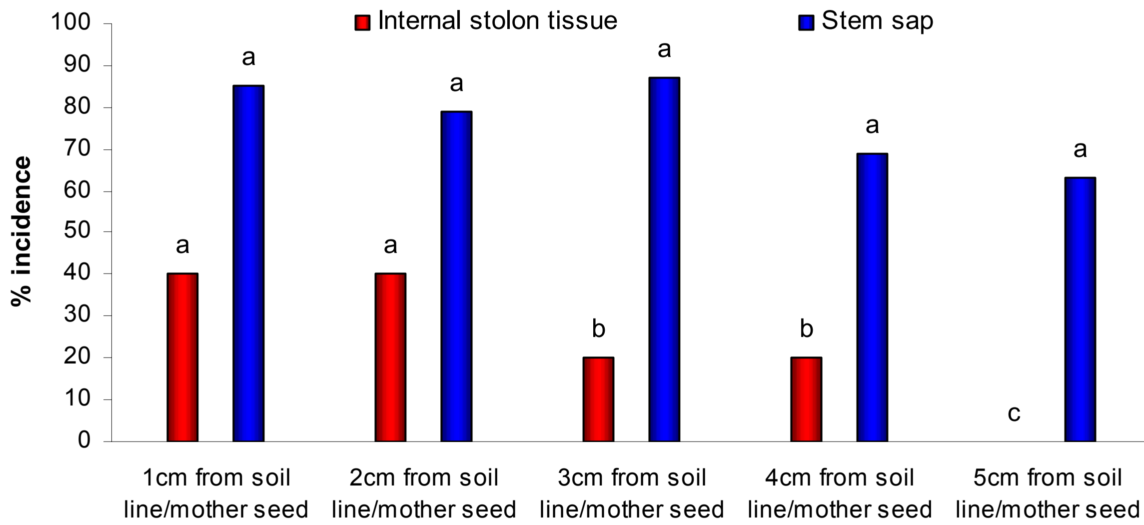
These results show that infection from the mother seed exerted a continual influence on the infection of progeny, and that infection from the mother seed starts soon after planting. The early infection of plant tissue suggests that this is due to systemic growth of the fungus from the infected seed piece.

**Figure 11:** Incidence of *C. coccodes* on tubers and stems grown from infected tubers or plant tissue that was isolated from stem tissue either 2.5cm or 5.0cm above the mother tuber.




Treatments with the same letter are not significantly different from one another (LSD P=0.05).

**Figure 12:** Incidence of *C. coccodes* in stem sap and stolon tissue at 1 – 5cm away from either the mother seed or soil line (cv. Coliban).




Treatments with the same letter are not significantly different from one another (LSD P=0.05).

***Systemic carry over and disease transfer through soil.***

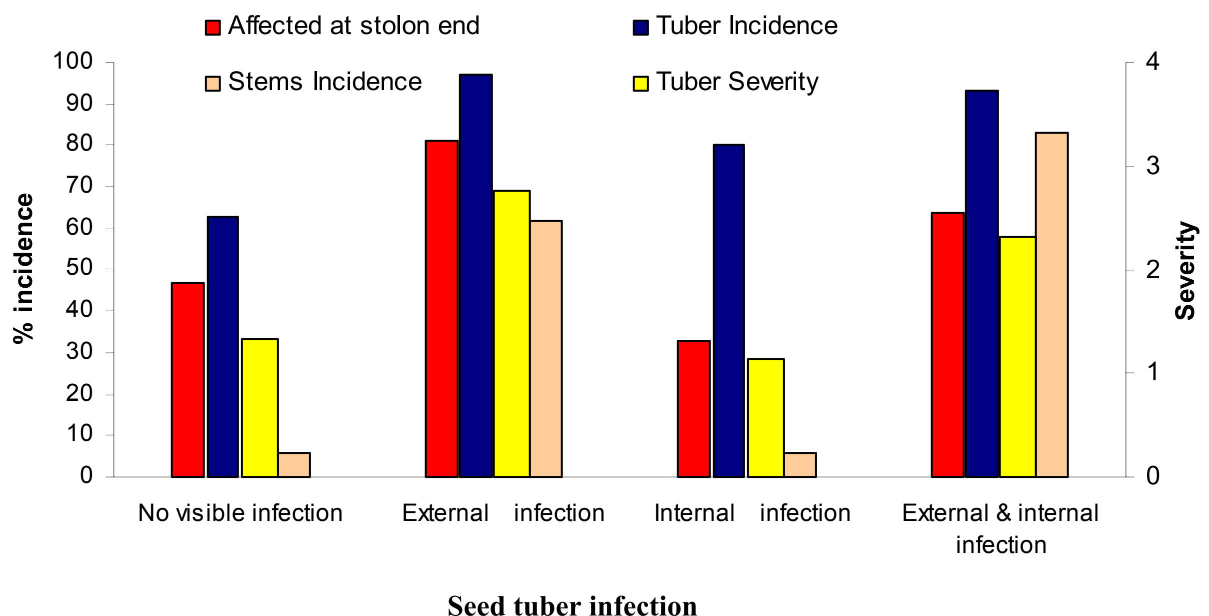
**Objective:** *To evaluate the development of C. coccodes on daughter tubers grown from tubers infected either externally, internally or both and to determine if tubers can be infected if planted adjacent to infected tubers.*

**Materials and Methods:**
Two experiments were conducted using certified seed tubers, cv. Coliban. Tubers were assessed for the incidence of *C. coccodes* both externally and internally as previously described. They were then placed into the categories of external, internal, external + internal or no visible external infection. All tubers in the external incidence category had greater than 50% of their surface affected by *C. coccodes*. In the first experiment, 10 tubers within each category described above were planted into pots on the 17$^{th}$ November.

In the second experiment, 10 tubers within each category described above (except those with internal and external infection) were planted on the same day into trays (45cm x 30cm x 25cm), placed 25cm apart from tubers with no visible sign of *C. coccodes*. All plants were watered and fertilized as previously described. After 3 months growth, all daughter tubers and one stem selected at random from each pot/tray was visually assessed for both incidence and severity of *C. coccodes* as previously described. Tubers assessed in the second experiment were those produced from the clean mother seed.

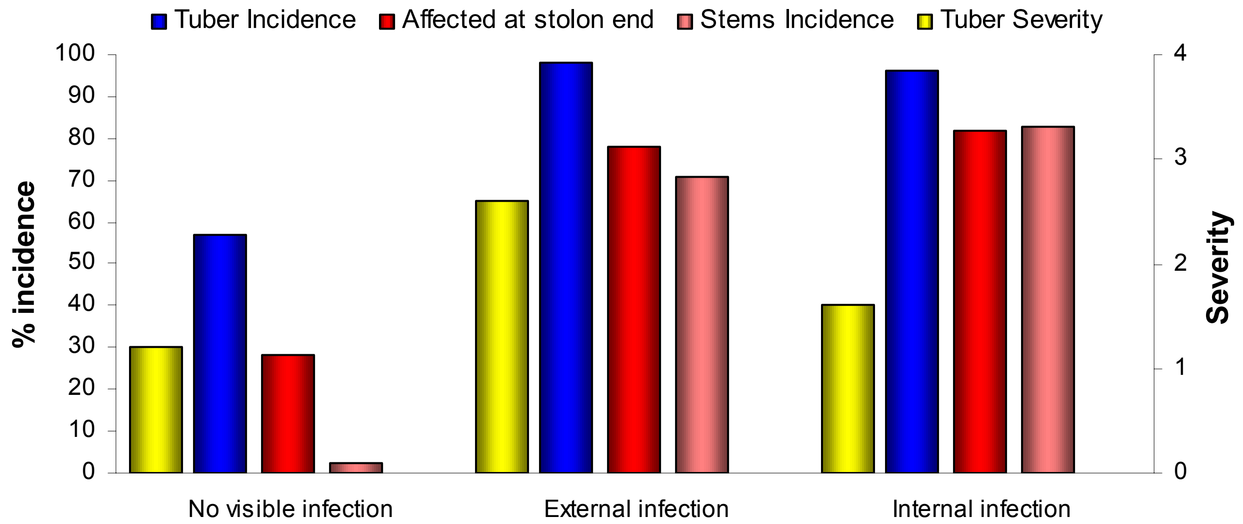
**Results and Discussion:**
The incidence of daughter tubers with external infection was lowest where mother seed had no visible signs of *C. coccodes*. However the level of infection was above 60%, suggesting that the mother seed was infected with *C. coccodes* but not detected visually (Fig 13). The incidence of stem infection by *C. coccodes* was significantly lower where mother seed had either no visible sign of *C. coccodes* or only internal *C. coccodes* (both 5%) compared to those with either external or external plus internal incidence of *C. coccodes* (61% & 82% respectively). Observations of greater stem infections developing in plants grown from seed tubers in which *C. coccodes* was detected compared to those grown from seed tubers with no *C. coccodes* has previously been observed.(37). Seed tubers with external infection produced daughter tubers with the highest incidence and severity of infection, as well as stem infection and number of tubers affected at the stolon end. This indicates that while internal infections cause disease in daughter tubers, external skin infections are of more significance, and have a greater effect on the total disease levels at harvest.

**Figure 13:** Effect of external or internal infection of *C. coccodes* on the development of disease in daughter tubers. Shadehouse trial, Lenswood 2002 (Expt 1)



The incidence of *C. coccodes* on daughter tubers, percentage affected at stolon end and number of stems affected was significantly lower when seed tubers were planted alongside seed with no visible sign of *C. coccodes* compared to those planted alongside infected seed (Fig 14). Similar levels of disease developed on tubers and stems of plants grown near seed tubers with internal or external infection. However the severity of tuber infection was less with internally infected tubers. These results show that propagules of *C. coccodes* move through the soil from infected seed tubers to infect daughter tubers of adjacent plants.

**Figure 14:** Development of *C. coccodes* on tubers and stems of plants planted 25cm away from infected tubers Shadehouse trial, Lenswood 2002. (Expt 2)



***Potato cultivars***

**Objective:** *To evaluate cultivar susceptibility to C. coccodes.*

**Materials and Methods:**
Fourteen commercial potato cultivars were grown on a commercial property in the Murray Mallee region of SA (8 fresh market, 3 crisp and 3 french fry varieties). The trial was planted on 14/02/01, plots were double rows by 5 m long, replicated 5X, rows 86cm apart and set spacing 28cm. 2 months after complete senescence tubers from all treatments were hand dug from randomly chosen 2m sections in both rows. These were placed into cold storage for 28 days after which sub samples of 100 tubers from each replicate were assessed for both incidence and severity of *C. coccodes* as previously described.

**Results and Discussion:**
All cultivars were susceptible to *C. coccodes*, with Coliban, the most widely used cultivar for washed potatoes, one of the most susceptible (Table 8). Overseas studies have shown that thin-skinned cultivars, such as many chipping cultivars, are more susceptible to *C. coccodes* than thicker-skinned ones (30). These results indicate that the tuber infection was lower in the crisping and frying varieties, but stem infection was not similarly aligned.

Significant differences were observed in the incidence of stem colonization between cultivars and the severity of tuber surface infection (Table 8). Differences between stem and tuber infection was observed in some cultivars, eg Desiree had the lowest level of stem infection, but one of the highest levels of tuber infection.

Research from the UK has shown that there are significant differences between cultivars for resistance to *C. coccodes*. Severity of infection was greater in the early cultivars such as “Maris Bard” compared to the main crop cultivars such as “ Romano” and “Shelagh”. This was attributed to the fact that the early cultivars set tubers before those of the main crop cultivars which means that tubers are in contact with soil inoculum for a longer period. In addition, variation did occur within both early and late cultivars, suggesting that there is a genetic influence. Further trials on Australian varieties are required to establish these differences and assist in producing cultivars with improved disease resistance. If a resistance gene is identified then it may be possible to incorporate this into those cultivars suitable for the fresh market.

**Table 8:** Incidence of *C. coccodes* colonization on fourteen cultivars of potato grown in the Murray Mallee district of SA, 2002

| Variety | % of infected stems | % of infected tubers | Disease severity | Skin type | Use |
|---|---|---|---|---|---|
| Desiree | 20 e | 76 e | 3.7 a | thin | Fresh |
| Sonic * | 50 cd | 34 a | 2.5 b | thick | Crisp |
| Ruby Lou | 50 cd | 68 d | 3.6 a | thin | Fresh |
| Winter Gem | 60 bc | 45 b | 2.1 b | thin | Fresh |
| Nadine | 60 bc | 79 e | 3.1 ab | thin | Fresh |
| Dawmor | 70 b | 55 c | 2.5 b | thick | Crisp |
| Shine | 70 b | 59 c | 2.6 b | thick | Fresh |
| Fontenot | 80 ab | 69 d | 3.2 a | thin | Fresh |
| Atlantic | 80 ab | 36 a | 2.1 b | thick | Crisp |
| Riverina Russet | 80 ab | 30 a | 1.8 b | netted | Fry |
| Ida Rose | 80 ab | 59 c | 2.8 b | thin | Fresh |
| Russet Burbank(CS) | 90 a | 32 a | 2.0 b | netted | Fry |
| Shepody | 90 a | 30 a | 2.2 b | netted | Fry |
| Coliban | 100 a | 76 e | 3.3 a | thin | Fresh |

[A]Severity rating scale 0 to 4: 0, no diseases; 1, <2%; 2, 3-10%; 3, 11-30%, 4, >30% of tuber surface affected. Treatments with the same letter are not significantly different from one another.

***Inoculum levels***

**Objective:** *To evaluate the effect of different soil inoculum levels on the development of C. coccodes on potato tubers and roots*

**Materials and Methods:**
Replicates of 30 pots per treatment were artificially infested with *C. coccodes* microsclerotia at concentrations of 0, 5, 10 and 20 microsclerotia/g soil. Inoculum was made by collecting microsclerotia from 1 month old cultures (isolate 58a) grown on NP10 selective media. Microsclerotia were scraped off the agar surface, ground in a blender with water, and the concentration adjusted using a haemocytometer. Microsclerotia were thoroughly mixed with air dried soil at concentrations of 1000 microsclerotia/g of soil, then diluted with the sandy loam to achieve the desired concentrations.

One certified mini tuber cv. Coliban, with no obvious *C. coccodes* infection on the tuber surface, was planted in each pot at a depth of 15cm. Pots received 1.3 litres of water every 3$^{rd}$ day and were fertilised as previously described. 5 plants selected at random from each treatment were visually assessed for senescence every 2 weeks starting 7 weeks after planting.

All plants that were assessed for senescence were carefully removed from the pots along with attached tubers and visually assessed for both the incidence and severity of *C. coccodes* on tubers and stems and the incidence of *C. coccodes* in plant tissue (as previously described).

**Results and Discussion:**
Senescence developed at similar rates in all treatments until 56 days after planting where it increased in all inoculated treatments (Fig 15). Plants grown in 20-microsclerotia/g soil senesced more rapidly than the plants grown in the lower levels of inoculum. At 98 days after planting, *C. coccodes* had developed on 47% - 64% of tubers and 100% of stems on plants grown in inoculated soil (Fig 15).

Whilst overseas studies (10,15) showed *C. coccodes* severity on tubers increased as inoculum increased, there were no significant differences on the levels of *C. coccodes* infection on tubers grown in soil with different levels of inoculum. However both the internal stem incidence and severity increased with increasing inoculum levels. Plants grown in soil with 5 and 10cfu/g soil had significantly lower levels of *C. coccodes* in sap (0 and 2cfu's respectively) compared to those grown in 20cfu/g (458 cfu's/ml) (data not shown).

**Figure 15:** Rate of senescence in Coliban potato plants grown in soil with different inoculum levels.

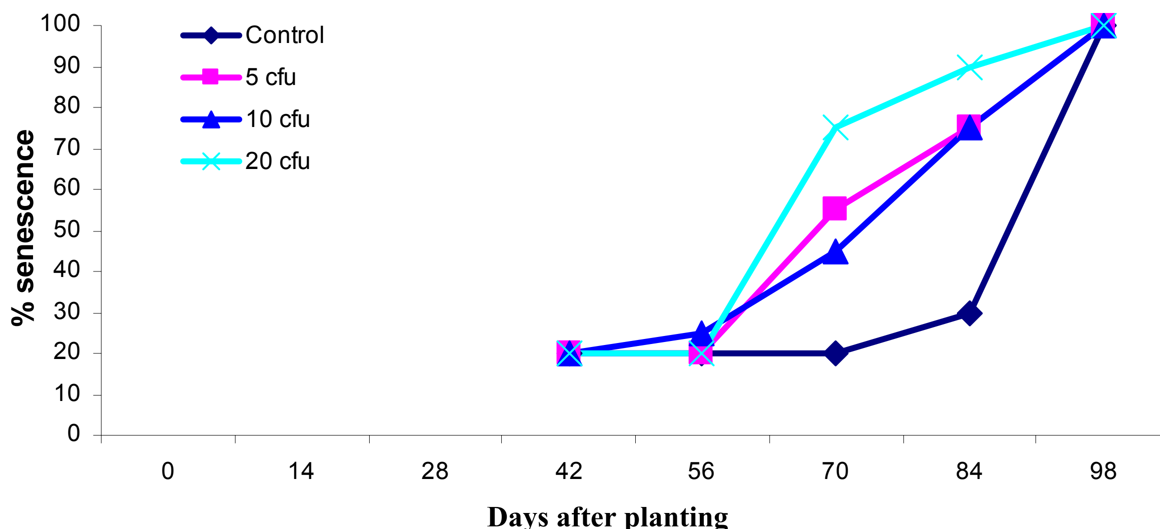
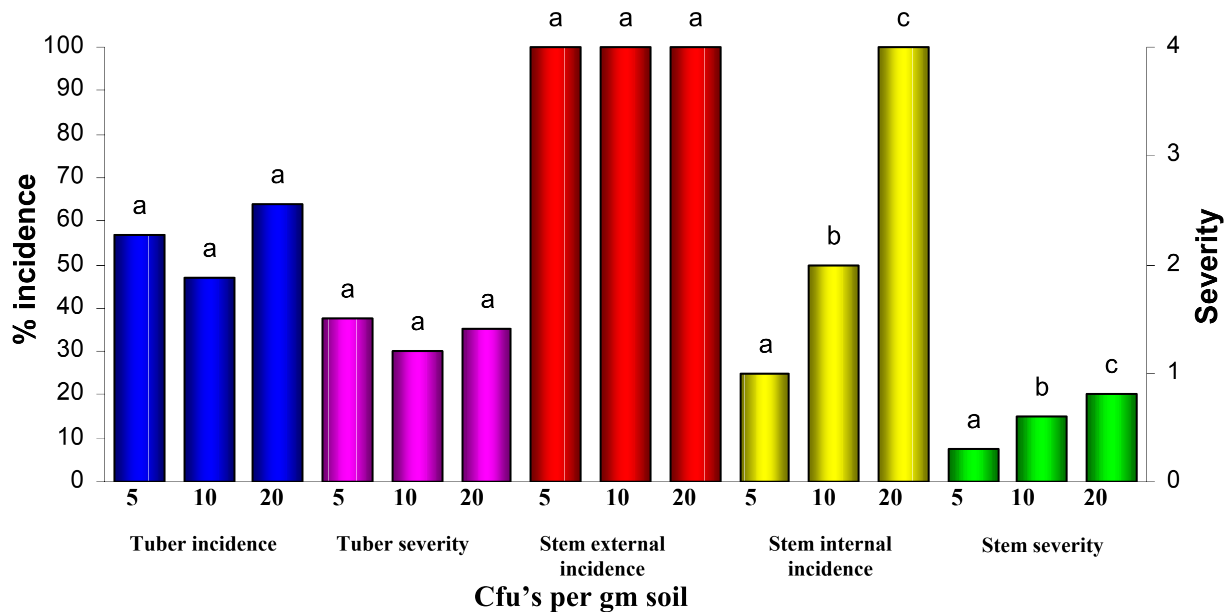



**Figure 16:** Effect of soil inoculum level on the incidence and severity of *C. coccodes* on tubers and stems (external and internal) at 96 days.




Treatments with the same letter are not significantly different from one another (LSD P=0.05).

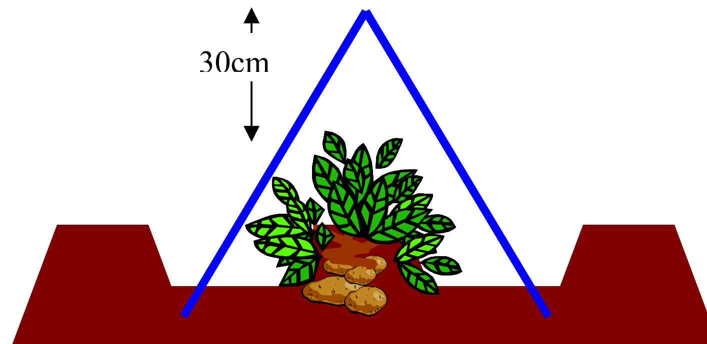
In this study the range of inoculum densities of *C. coccodes* used was similar to those found in soils where potatoes have been grown in the Mallee region of South Australia. Whilst a study in the USA (4) has shown that infected tubers are highly correlated with colony forming units (cfu's) found in the soil, no differences in incidence or severity of tubers or external stem infection were detected in the plants grown at different inoculum levels in this experiment. Internal vascular infection of stems and disease severity on stems were the only factors to vary with different levels of inoculum. Previous studies using populations ranging from (0 – 10 microsclerotia/gm dry soil) found significant differences in infection levels between the different densities (9, 10). Further studies need to be done as 10 microsclerotia/g soil may be above the threshold for causing significant levels of disease. Therefore inoculation with more of the lower concentrations may show the threshold where disease will occur.

***Water and haulm disease development.***

**Objective:** *To evaluate the effects of overhead irrigation after haulm senescence on the incidence of C. coccodes.*

**Materials and Methods:**
Certified seed tubers cv. Coliban naturally infected with *C. coccodes* were planted at two sites on 12th December 2003 in the Mallee region of SA. At site one, tubers had 48% incidence and 1.45 severity and at site two 56% incidence and 1.56 severity. Reglone® was applied (3.5L/Ha) to site one (15th March) and site two (7th April). Two days later plastic tents were placed over single rows covering 6 plants (Fig 17). These were placed in a randomised block design and replicated 7 times. Plastic was supported at least 30cm above plants to allow air movement and to reduce any greenhouse like effects. In addition the sides of the tents were buried at least 30cm below the soil line so that any run off water was diverted away from the tubers. Overhead irrigation via a centre pivot occurred on average twice a week with approx 4mm applied at each watering. Six weeks after the application of Reglone the middle two plants from each tent and their tubers were harvested and then assessed for incidence and severity of *C. coccodes* (as previously described).

**Figure 17:**

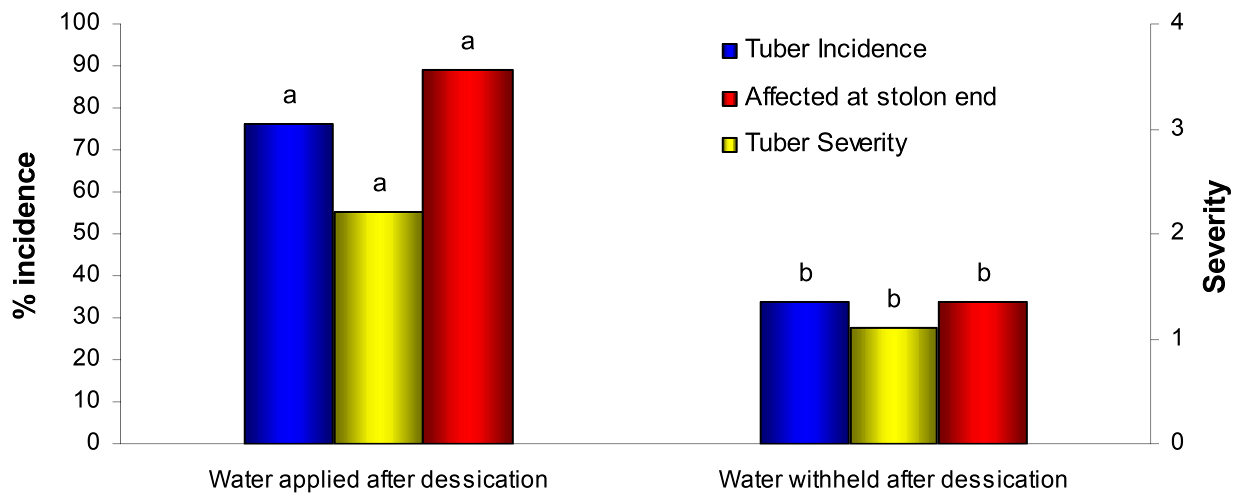



**Results and Discussion:**
In the Mallee area of SA, potato plants are often irrigated after "haulm desiccation" to reduce skin blemishes caused from excessive hot, dry soils and to assist with the harvesting process. In this trial reducing exposure to the irrigation significantly reduced incidence and severity of *C. coccodes* developing on tubers (Figs 18, 19). At both sites, *C. coccodes* developed on more than 70% of tubers watered after desiccation whereas around 30% were infected in the covered treatment. These results further confirm the role of overhead watering dispersing conidia from infected tissue through the soil onto daughter tubers.

The application of water at this stage also increases the potential infection levels as free water or a relative humidity of 100% (6) is needed for conidial germination. The high incidence of stolon end infection compared to bud end infection in this experiment has also been observed in several other experiments in this report and by overseas researches (37). Conidia are stimulated to adhere to the plant tissue (tubers/roots) in response to host leachates. Since they do not function as survival structures their viability declines rapidly if water is withheld or high temperatures occur (6, 45). Once the conidia penetrate the host tissue, the fungus will survive unseen between the wax layer and the cuticle structures (latent infection).

**Figure 18:** Incidence of *C. coccodes* on daughter tubers (Site 1).

**Figure 19:** Incidence of *C. coccodes* on daughter tubers (Site2).




Treatments with the same letter are not significantly different from one another (LSD P=0.05).

***Reglone/Dye***

**Objective:** *To evaluate if water applied to desiccated plant stems above ground can transfer spores on to the main root system.*

**Materials and Methods:**
A commercial crop of potatoes cv. Coliban grown in the Murray Mallee was sprayed with the desiccant Reglone® (15/12/03). Eight days later, different rates of water containing a 5% solution of florescent dye was applied to individual plants and to the base of plants using a watering can (Table9), with 5 replicates of each treatment (1 plant = 1 replicate).

**Table 9:**Rates of water and zone of application.

| Treatment | Application zone | Dye rates | Irrigation rates |
|---|---|---|---|
| A | Plant foliage | 500L/Ha | - |
| B | Plant foliage | 500L/Ha | 5000ml per m2 |
| C | Plant foliage | 2000L/Ha | - |
| D | Plant foliage | 2000L/Ha | 5000ml per m2 |
| E | Soil at plant base | 500L/Ha | - |
| F | Soil at plant base | 500L/Ha | 5000ml per m2 |
| G | Soil at plant base | 2000L/Ha | - |
| H | Soil at plant base | 2000L/Ha | 5000ml per m2 |

**Results and Discussion:**
Fluorescent dye was detected on the roots of potato plants in all treatments. Where water volumes of 2000L/ha were applied, most of the roots were contaminated with fluorescent dye. In the treatment where the lowest rate 500L/ha of water was applied only 40% of the roots were detected with fluorescence.

These results show that high volumes of water applied to the soil penetrates the soil along the edge of the stem and moves along the stem to the roots rather than perculate through the soil to the roots. The usual amount of water applied by centre pivot irrigation is approx 4 – 5mm (4000 - 5000ml/m2). This would wet the soil to approx 2.5 – 3.75cm in depth, whilst a second irrigation cycle would wet the soil to approx 10cm in depth. These volumes of water are applied frequently to crops that have matured during the summer months of South Australia.

These results suggest that high volume of water applied to potato crops post desiccation or near harvest is likely to wash conidia of *C. coccodes* from infected stems and move them along the stem and roots to infect stolons and daughter tubers prior to harvest.

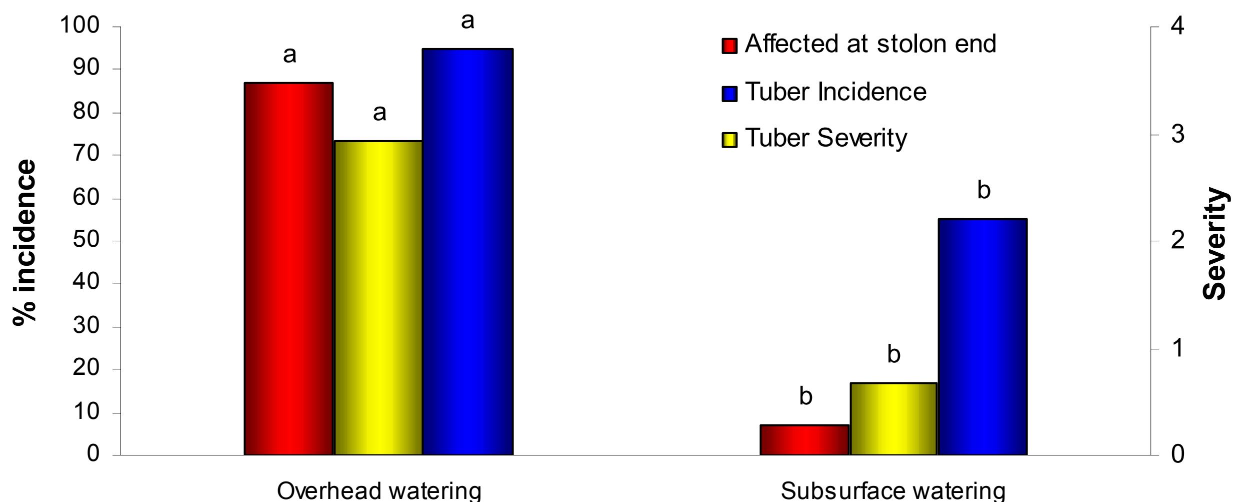
***Irrigation***

**Objective:** *To evaluate the effects of overhead and subsurface irrigation on the incidence of C. coccodes on daughter tubers.*

**Materials and Methods:**
Seed tubers, cv. Coliban, with more than 50% of their surface affected by *C. coccodes* were selected from certified seed. One tuber was planted in each pot at a depth of 15cm on the $1^{st}$ August 2003. Replicates of 5 pots were placed into a growth room at a temperature of $25^{oC}$ with artificial illumination (110uEm2sec1) at 14hrs light and 8 hrs dark photoperiod. All pots received 1.3 litres of water every $3^{rd}$ day and were fertilized as previously described. 5 pots received overhead irrigation via a watering can and 5 pots received subsurface irrigation by placing the water in a tray at the base of each pot. After 3 months, all daughter tubers from each pot were assessed for incidence and severity of *C. coccodes* as previously described.

**Results and Discussion:**
The incidence and severity of stolon end infection were significantly less on plants watered from the base of pots compared to overhead watering (Fig 20). More than 90% of tubers irrigated with overhead watering developed black dot compared to 55% where plants were watered subsurface. Similarly the severity of the tuber infection and the number of tubers affected at their stolon end was significantly higher in tubers grown from plants watered overhead compared to those watered subsurface. Since inoculum was only present on the original seed piece these results suggest that water moving downwards through the soil plays a significant role in moving inoculum from the infected tuber seed to the daughter tubers.

**Figure 20:** Effect of overhead or subsurface watering on the incidence and severity of *C. coccodes* on daughter tubers.




Treatments with the same letter are not significantly different from one another (LSD P=0.05).

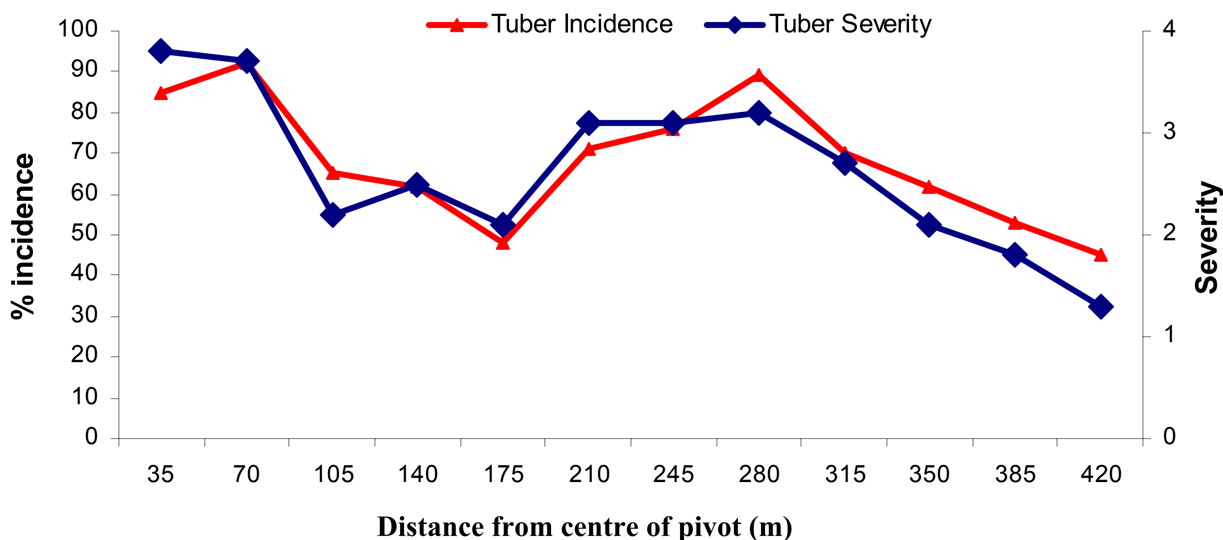
### *Pivot variations*

**Objective:** *To measure the incidence and severity of C. coccodes on tubers collected along the radius of a centre pivot irrigation system.*

**Materials and Methods:**
Certified seed tubers cv. Coliban naturally infected with *C. coccodes* (48% incidence & 1.45 severity) were planted at 4ton/Ha on 12th December 2003 in the Mallee region of SA. Reglone® was applied (3.5L/Ha) on 15th March. The trial site consisted of 12 plots (35m X 20 rows) running from the centre point to the outer point. Six weeks after the application of Reglone®, 20 plants and their tubers were harvested from each plot using a "W" sampling pattern across 20 rows within each plot. These were then visually assessed for both incidence and severity of *C. coccodes* (as previously described).

**Results and Discussion:**
These results show that both the incidence and severity of *C. coccodes* infection was highest at the centre of the pivot where most water is applied and lowest at the extremity of the pivot where the least water is applied to the crop (Fig 21). A similar study on the incidence of "Late Blight Tuber Rot" showed a similar pattern of infection in that the highest level of disease developed close to the pivot centre where most water was applied (35).

**Figure 21:** Incidence and severity of *C. coccodes* on tubers from plants grown at various distances from the pivot centre.



## MANAGEMENT

***Introduction:***

Surveys of certified seed planted in SA showed a high incidence of *C. coccodes*. This infected seed is the primary source of introducing the disease into new potato growing areas. There is a need to develop effective treatments to minimise this primary spread of the disease from seed to progeny tubers.

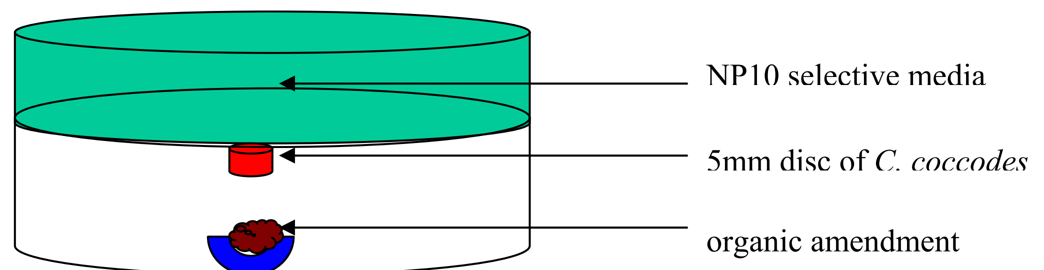
***General materials and methods:***

*Source of test material:* Cold pressed defatted mustard seed meal or pellets containing 25% meal of the cultivar *Brassica juncea* were obtained from commercial outlets and used as the source of glucosinolates.

*Soil fumigation***:** Fumigants were applied using a Rumstead, 5 bladed injection unit (Pictures 4 and 5 Page 73-74) set at a depth of 25cm. Metham Sodium (32.7% ai of sodium methyldithiocarbamate) was applied at 700L/ha and Telone II 200L/ha (1140g ai of 1,3-dichloropropene). Within 4 hours after chemical application, 5mm of water was applied to the trial areas.

*Organic amendments***:** Amendments of seed meal, pellets and freeze dried plant samples were placed in 2cm diameter vessels and hydrated with water on a v/w basis (500ul/0.1g). Controls were exposed to 200ul of water alone. These were placed in inverted petri dishes, which each contained a 5mm disc of mycelium taken from the margin of a 5 – 7 day old fungal colony of *C. coccodes* (58a). The petri dishes were sealed with Gladwrap to contain any volatiles produced (Fig 22).

**Figure 22:**




*Seed treatment methods*. Seed tubers, cv. Coliban, with more than 50% of their surface covered by *C. coccodes* sclerotia were selected from a commercial grower. Batches of ten tubers were either sprayed with a fungicide using a commercial "Controlled Droplet Applicator" or dipped in a chemical solution (Table 10).

*Irrigation*: Pots were watered with an automatic irrigation system that applied 4 litres of water every 3$^{rd}$ day.

*Sampling and detection of C. coccodes in soil:* Soil samples were collected from trial sites using a "W" shaped sampling pattern with a minimum of 25 equally spaced assessment sites per field. The size of each sample area varied due to the different size of trial sites. Soil was sampled with a 2.5cm diameter corer used to remove at least a 100g-soil sample. Samples were bulked and mixed by rotating the soil in the plastic bag in which samples were collected. A 500g-soil sample was air dried at room temperature (15 to 25°C) for 2 weeks to eliminate short-lived propagules such as conidia and mycelial fragments. After drying, the soil was homogenised and sieved through 850, 500 and 250 micron sieves. The resulting soil sample was analysed for the presence of *C. coccodes* by either of two methods:

**Method 1**: Plating out five sub samples of 0.01mg onto separate plates of NP10 selective media, using an Anderson Sampler (9).

**Method 2:** 4 gm of dried soil was added to 40ml of 0.2% water agar and 1ml of the solution spread over the surface of 5 plates of NP10 selective media.

Plates from both methods were incubated at 24°C in the dark for 7 – 14 days and then examined under a bifocal microscope for colonies typical of *C. coccodes*. The number of colony forming units (CFU) g of unseived soil was calculated using the formula (X/0.01) x Y/200, where.

| | |
|---|---|
| Total weight of unseived soil | = 500gms |
| Total weight of soil plated out | = 0.01gms |
| Total weight of sieved soil | = Y |
| Total number of Cfu's on plate | = X |

*Assessments*: Tubers from each pot or the centre 2m of both rows in each the plot were hand dug, washed, counted and weighed into size categories of 30-80g (chats), 80-200g (small), 200-350g (medium), 350-450g (large) and >450g (oversize). Skin blemishing caused by *C. coccodes* was not used as a criterion when calculating the marketable yield. Disease levels were assessed as previously described.

**Table 10:** Fungicides evaluated as tuber seed treatments

| Treatment | Active ingredient | Concentration (%) | Rate of product/1000kg tubers |
|---|---|---|---|
| **Seed sprayed*** | | | |
| Amistar® | 500g/kg azoxystrobin | 0.04 | 1.6 gm |
| Filan® | 500g/Kg Boscalid | 0.04 | 1.6 gm |
| Cabrio® | 250g/kg Pyraclorostrobin | 0.04 | 3.4 gm |
| Dithane® | 750g/kg mancozeb | 0.11 | 3.0 gm |
| Fungaflor® | 750g/kg imazil | 0.75 | 20 gm |
| Maxim® | 100g/L fludioxonil | 1.25 | 250 ml |
| Octave® | 462g/Kg prochloraz | 0.14 | 6.0 gm |
| Tecto® | 500g/L thiabendazole | 2.25 | 91 ml |
| **Seed dipped**** | | | |
| Formalin® | 400g/L formaldehyde | 4.0 | |
| Sporekill® | 120g/L didecyl dimethyl NH4CI | 1.00 | |
| Chlordox® | 2% chlorine dioxide<br>85% phosphoric acid | 0.05 and 0.1 | |

* Applied in 2L water
** Dipped for 15 minutes and rinsed with water once
Untreated tubers were dipped in water for 15 min.

*Biofumigation*

**Inhibition from volatiles released from Brassica sp**

**Objective:** *To evaluate the antifungal properties of volatiles released from three different Brassica species, mustard meal, mustard pellets and one oat species against C. coccodes.*

**Materials and Methods:**

***Sampling green manure crops***: Seed of *B. juncea, B. napus, Raphanus sativus* and Oat *cv* Drummond were purchased from a commercial supplier and planted in a commercial potato field at Woodside, South Australia in Autumn. At 10% flowering one hundred plants were dug up at random from each cruciferous plot to a depth of 15cm. Oat plants were collected from five $1m^2$ areas when kernels were at milky stage (Feekes scale 11.1). Soil was washed from the roots of all plants and a sub sample of 5 plants from each sample separated into leaf, stem and root tissue and immediately freeze-dried. Dried material was weighed, ground and stored in sealed vials at 20 $^{o}$C.

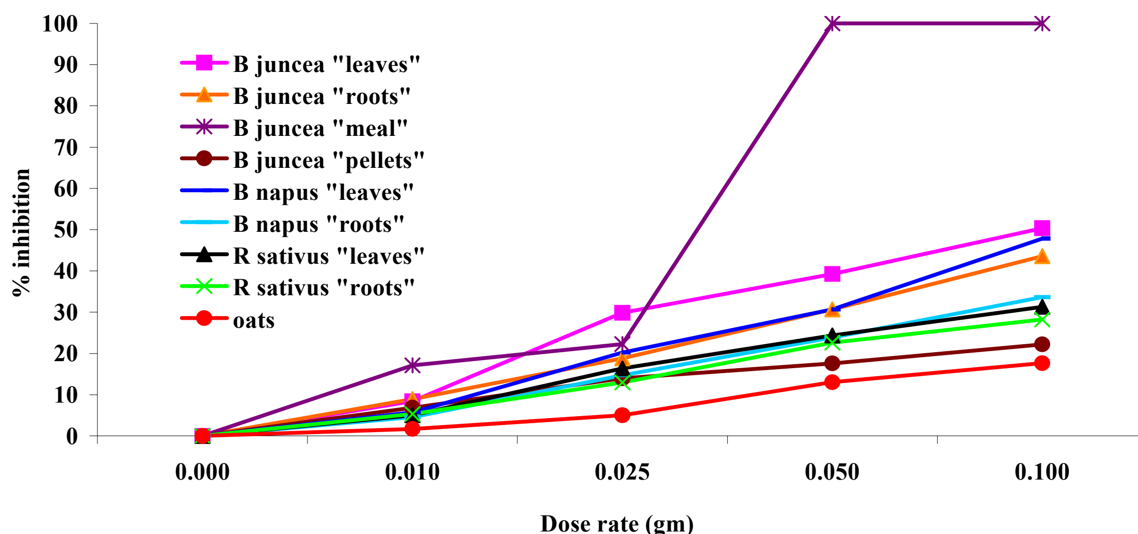
***Analysis of glucosinolates***: A 300mg sample of freeze-dried *Brassica* material obtained from root, stems or leaves. Levels of glucosinolates (GLS's) were analysed using a gradient HPLC method by Dr J Kirkegaard (CSIRO Plant Industry Canberra).

***Mycelial growth in the presence of volatiles released from organic amendments***: Seed meal, pellets (as previously described) and freeze dried plant samples ranging from 0 to 0.1gm were hydrated and placed in petri dishes, containing *C. coccodes* isolate 58a (as previously described) and replicated 7X. Plates were incubated at 22 $^{o}$C in the dark for up to 11 days and the percentage inhibition of fungi calculated as previously described.

**Results and Discussion:**

Glucosinolates (GSL's) released from *Brassica* residues are hydrolized by endogenous myrosinase (3) to produce breakdown products, one of which is the volatile compound isothiocyanate (ITC). These are highly biocidal to a diverse range of organisms including nematodes, bacteria, fungi, insects and germinating seeds (8,38,54,72). With all isolates of *C. coccodes*, volatiles from leaf and root tissue were more suppressive than those from stem tissue and leaf tissue alone, *B. juncea* and *B. napus* were more inhibiting than *R. sativus* (Fig 23).

**Figure 23:** Inhibition of mycelial growth after exposure to volatiles released from *Brassica* residues at different rates and concentrations of ITC liberating GLS.



GLS levels were highest in the meal, where 48 umol/g were released (Table 11). The next highest levels of 38 umol/g were released from the leaves of *B. juncea* and the lowest 2.1 umol/g from *R. sativus* stem tissue. GLS levels were highest in leaf tissue compared to root or stem tissue from *B. juncea*, however levels were higher in root in all other species. The lowest levels in all plants were found in stems with levels ranging from 2 to 6 umol/g.

**Table 11:** Concentrations of ITC liberating GLS.

| | ***Organic amendment*** | | | | | |
|---|---|---|---|---|---|---|
| | *B. juncea* Meal | *B. juncea* Pellets | *B. juncea* leaf, (root) | *B. napus* leaf, (root) | *R. sativus* leaf, (root) | Oats leaf |
| ***GLS (umol/g)*** | ***48*** | ***10*** | ***38 (16)*** | ***25 (36)*** | ***6 (12)*** | ***0*** |

This work shows that volatiles emanating from *B. juncea* meal or *B. juncea* and *B. napus* leaves and roots are inhibitory to *C. coccodes*. All *Brassica* plant materials tested suppressed fungal growth, but the response varied between plant parts. For example *B. juncea* meal completely inhibited growth of *C. coccodes* whereas leaf tissue only inhibited fungi between 23 – 52%.

Suppression generally increased with increasing levels of amendment. The higher level of suppression caused by the *B. juncea* meal extracts compared to the pelletised formulations are consistent with the higher ITC concentrations found in the meal.

This study and others (59) show that glucosinolates vary among Brassica species and even cultivars within the same species. They also vary between plant parts, growth stage and also change with environment. In addition Rosa (59) also showed that time of exposure needed to control pathogen growth differs between fresh and dried amendments. Fresh cabbage required an extra 10 days to achieve the same degree of control of *F. oxysporum* f. sp. *conglutinans* compared with dried cabbage.

**Hydration effects on volatiles released from *B. juncea***

**Objective:** *To evaluate the effects of varying hydration periods on the anti fungal properties of volatiles released from defatted mustard seed meal against C. coccodes.*

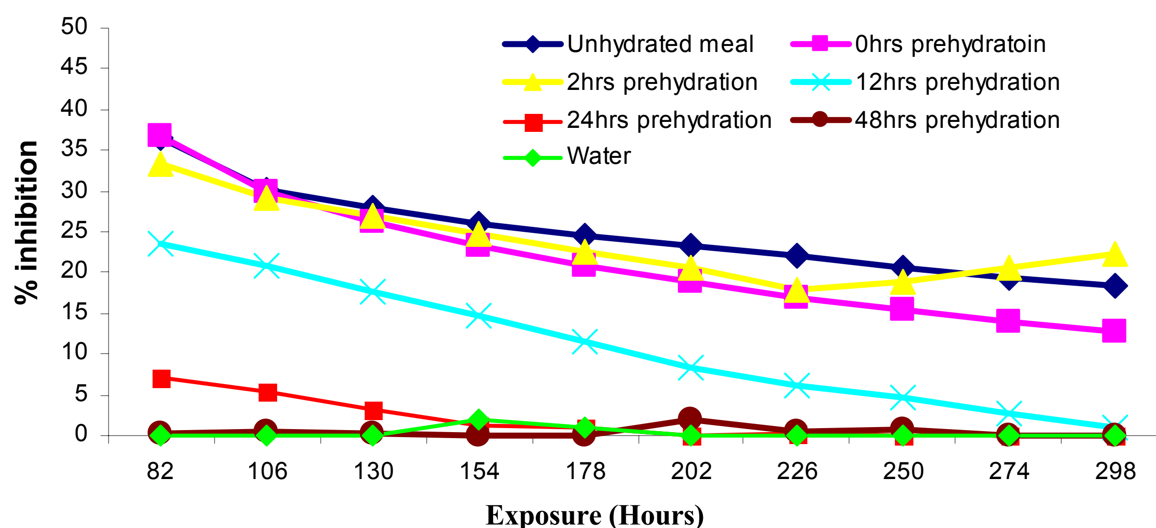
**Materials and Methods:**
Amendments of 0.1gm mustard seed meal were hydrated for 0, 2, 12, 24 or 48hrs before being placed in petri dishes containing *C. coccodes* isolates (77b, 58a, 17a), as previously described. Each plate was replicated 6 times. Plates were incubated at 22 °C in the dark for up to 11 days and the percentage inhibition of fungi was calculated.

To determine if the treatments were fungitoxic, amendments were removed after 298 hrs and 5 days later were inspected to see if hyphal growth had increased.

**Results and Discussion:**
Volatiles released from *B. juncea* seed meal were inhibitory to the growth of *C. coccodes* (Fig 24), but not lethally, as hyphal growth recommenced once the meal was removed.

The effect of the seed meal on hyphal growth decreased over time, and with increasing prehydration time. A possible reason for this is that once certain isothiocyanates become hydrolized they dissipate more readily into the atmosphere, reducing the concentration of any allelopathic compounds.

**Figure 24:** Inhibition of *C. coccodes* after exposure to volatiles released from *B juncea* seed meal.



**Efficacy of volatiles released from *B. juncea* seed meal in soil**

**Objective:** *To evaluate the efficacy of volatiles released from B. juncea seed meal on C. coccodes at varying depths in potting soil.*

**Materials and Methods:**
Mt Compass sandy loam mixed with or without mustard seed meal at the equivalent rates of 1, 2 and 3 ton/ha was placed into PVC cylinders (10cm diameter by 38cm height), 6 cylinders per meal concentration. Six individual muslin bags each one containing 5gm of inoculated vermiculite were placed at 7, 14 and 21cm depths, in each tube. Inoculant was prepared by grounding 1 month old *C. coccodes* cultures (isolate 77b) on NP10 selective media with water and the concentration adjusted to 200 cfu/ml using a haemocytometer. 200 ml of the resulting solution was then added to 600 gms of vermiculite and hand mixed to provide a uniform distribution of the inoculum. Cylinders were then placed upright onto drainage racks and 800mls of water added to hydrate the meal. The tops of the cylinders remained open. After 192 hrs the muslin bags were removed and 20 pieces of Vermiculite from each bag plated onto NP10 selective media. Inhibition of the individual fungi was determined by the percentage of vermiculite pieces that grew *C. coccodes*.

**Results and Discussion:**
Both 2 and 3 ton/ha of seed meal had significantly higher levels of inhibition at 14 and 21cm than all other treatments or depths (Table 12). Inhibition increased with increasing depth, and with increasing concentration of meal.

One possible reason inhibition increased as the depth increased is that GLS (mainly 2-Propenyl and 3-Butenyl) in the upper profile dissipated into the atmosphere more readily whilst GLS in lower levels were confined for longer periods due to the increased soil matter above acting as a seal. The availability of isothiocyanates in soil has been shown to depend on the nature of the soil (59). Thus, soils with higher levels of clay or organic matter are likely to benefit less from Brassica amendment than lighter soils.

**Table 12** Effect of leachates from Mustard Meal, on the viability of *C. coccodes* buried at different depths after 192hrs exposure.

| | **% Inhibition** | | | |
|---|---|---|---|---|
| | **Control** | **1 ton** | **2 ton** | **3 ton** |
| **7cm** | 0 | 0.9 a | 3.7 a | 4.6 a |
| **14cm** | 0 | 3.7 b | 6.7 b | 14.7 b |
| **21cm** | 0 | 3.7 b | 8.8 c | 15.1 b |
| | | **LSD 2.2** | **LSD 1.9** | **LSD 2.1** |
| **Mean (LSD = 3.5)** | 0 a | 2.8 a | 6.4 b | 11.5 c |

Treatments with the same letter are not significantly different from one another (LSD P=0.05).

*Chemical control*

**Invitro assessment of tuber seed treatments**

**Objective:** *To evaluate in vitro various fungicides applied as potato tuber seed treatments for the control of C. coccodes.*

**Materials and Methods:**
Two laboratory experiments were conducted to evaluate the efficacy of various fungicides as tuber seed treatments. Seed tubers were treated with fungicides at rates as previously described. Tubers were air dried for 15 – 20 minutes and then placed on plastic trays. After incubation for 7 days in the dark at room temperature, five 5mm cores containing *C. coccodes* microsclerotia were removed from each potato and plated onto NP10 selective media. After 14 days incubation at 22$^{o}$C in the dark the percentage inhibition of radial growth and microsclerotia density were determined (as previously described).

In the second experiment, the more effective chemicals were re-examined at 3 different rates (Table 13) on a different seed source but with a similar incidence of *C. coccodes*. Tubers were also incubated for 7 days in the light.

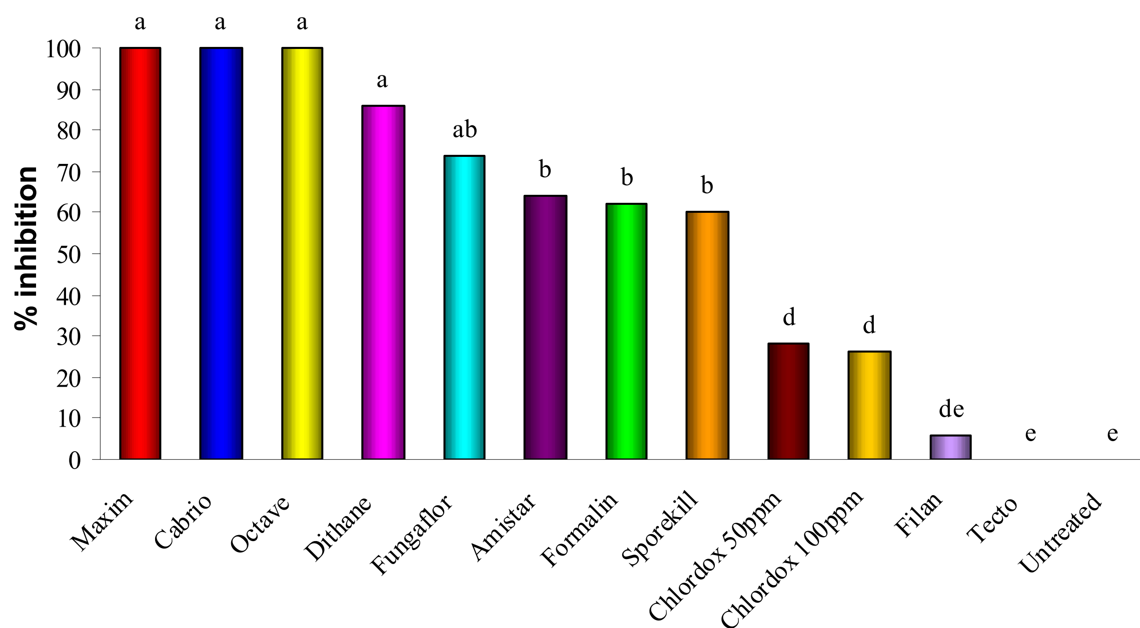
**Table 13**: Chemicals and rates applied

| Treatment | Active ingredient | Concentration (%) | Rate of product/ha/ 1000kg tubers |
|---|---|---|---|
| **Seed sprayed*** | | | |
| Amistar® | 500g/kg azoxystrobin | 0.048 | 2.0gm |
| | | 0.04 | 1.6gm |
| | | 0.02 | 0.8gm |
| Cabrio® | 250g/kg Pyraclorostrobin | 0.04 | 3.4gm |
| | | 0.03 | 2.4gm |
| | | 0.02 | 1.6gm |
| Dithane® | 750g/kg mancozeb | 0.22 | 6.0gm |
| | | 0.17 | 4.5gm |
| | | 0.11 | 3.0gm |
| Fungaflor® | 750g/kg imazil | 1.50 | 40gm |
| | | 1.25 | 30gm |
| | | 0.75 | 20gm |
| Maxim® | 100g/L fludioxonil | 1.25 | 250ml |
| | | 0.937 | 187.5ml |
| | | 0.625 | 125ml |
| Octave® | 462g/Kg prochloraz | 0.14 | 6.0gm |
| | | 0.105 | 4.5gm |
| | | 0.07 | 3.0gm |
| **Seed dipped**** | | | |
| Formalin® | 400g/L formaldehyde | 5.0 | |
| | | 4.0 | |
| Chlordox® | 2% chlorine dioxide<br>85% phosphoric acid | 2.0 | 2000ppm |
| | | 1.0 | 1000ppm |
| | | 0.1 | 100ppm |

* Applied in 2L water
** Dipped for 15 minutes and rinsed with water once
Untreated tubers were dipped in water for 15 min.

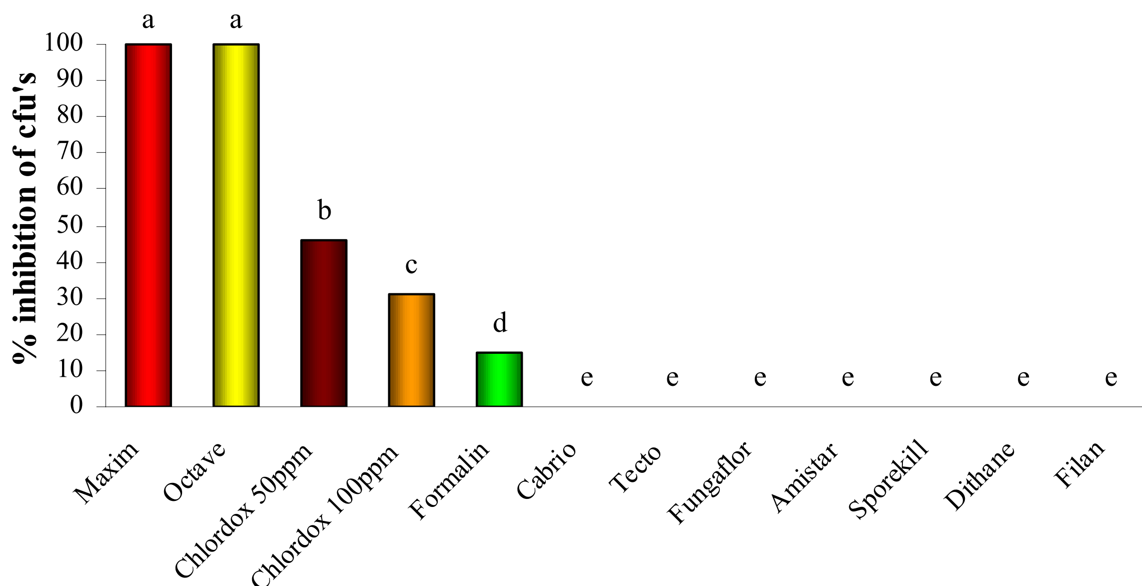
**Results and Discussion:**
In experiment one, tuber seed treatments of Maxim®, Octave® and Cabrio® completely inhibited mycelial growth of *C. coccodes* (Fig 25). Growth was inhibited to various degrees by other treatments but *C. coccodes* was not inhibited with Tecto® treated tubers. Amistar® was effective at much lower doses than all other chemicals in reducing hyphal growth. Maxim® and Octave® were the most effective at inhibiting microsclerotia whilst inhibition of microsclerotia was 50% or less in all other treatments (Fig 26).

**Figure 25:** Inhibition of mycelial growth of *C. coccodes* from cores of skin taken from tubers treated with fungicides (Expt 1).




Treatments with the same letter are not significantly different from one another (LSD P=0.05).

**Figure 26:** Inhibition of *C. coccodes* microsclerotia density produced from cores of skin taken from tubers treated with fungicides on selective media, (Expt 1).




Treatments with the same letter are not significantly different from one another (LSD P=0.05).

The overall inhibition was less in experiment 2 (Figs 27, 28) than experiment one, possibly due to a different isolate of *C. coccodes* (eg. different seed source) or that incubation period took place in high humidity trays under light.

**Figure 27:** Inhibition of mycelial growth of *C. coccodes* from cores of skin taken from tubers treated with fungicides, (Expt 2).

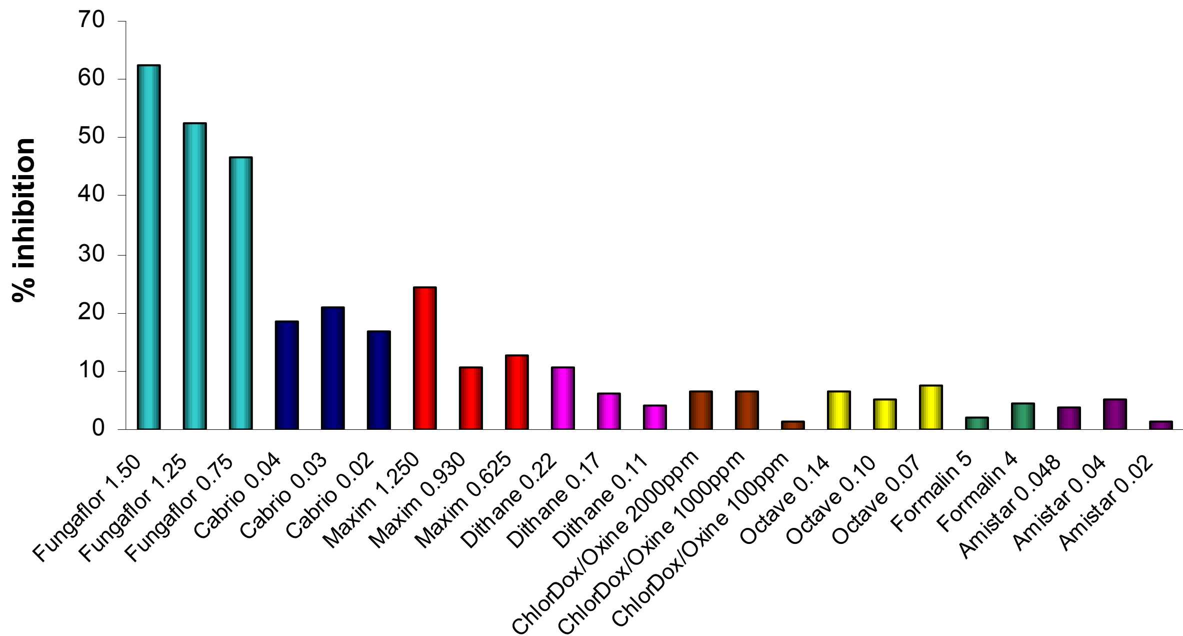
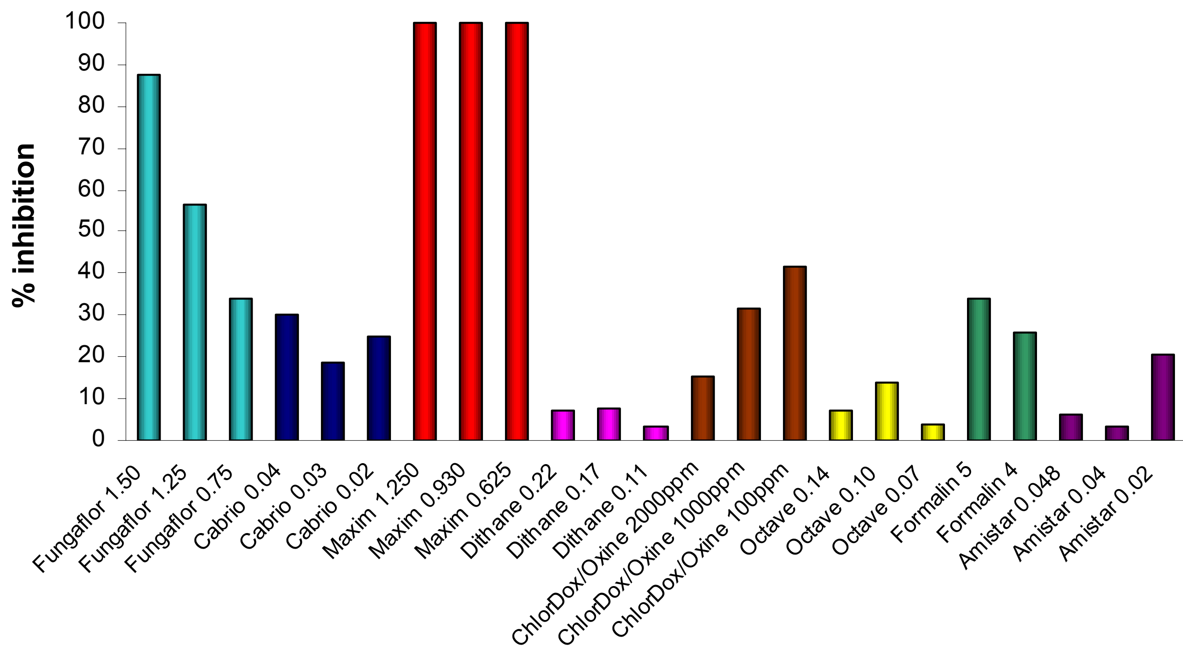



**Figure 28:** Inhibition of *C. coccodes* microsclerotia density produced from cores of skin taken from tubers treated with fungicides on selective media, (Expt 2).




As dose rates of Fungaflor®, Maxim® and Dithane® increased, mycelial growth decreased. All remaining treatments did not show a strong dose response. Only Fungaflor® showed a dose response to microsclerotia production. As in experiment 1, Maxim® inhibited microsclerotia production completely at all rates whilst inhibition increased when dose rates decreased with both Chlordox® and Amistar®. This may have been due to the fungicides initiating a change in the fungus so that it became more tolerant.

## Effect of fungicides on transmission in storage

**Objective:** *To determine if chemical treatments applied to the mother seed influenced the levels of tuber infection by C. coccodes on the daughter tubers during cold storage.*

**Materials and Methods:**
Daughter tubers from the previous experiment, grown from treated seed, were assessed for the incidence and severity of *C. coccodes* (as previously described) prior to being placed in storage at 4.5$^o$ C. After a period of five months the level of *C. coccodes* on tubers was reassessed.

**Results and Discussion:**
The incidence or severity of *C. coccodes* did not change significantly within any of the treatments after 5 months storage at 4.5C (Figs 29, 30). However a significant decrease in the incidence and severity occurred within the control tubers. This was possibly due to an increase in the incidence of Silver Scurf, which can mask regions affected by *C. coccodes* when visually inspected. In all treatments Silver scurf incidence increased by 79% or more; eg: untreated tubers increased from 21% to 100% (data not presented).

**Figure 29:** Effect of chemical treatments on the incidence (% tubers affected) of *C. coccodes* on the cultivar Coliban at harvest and 5months after cold storage, Lenswood 2002-03

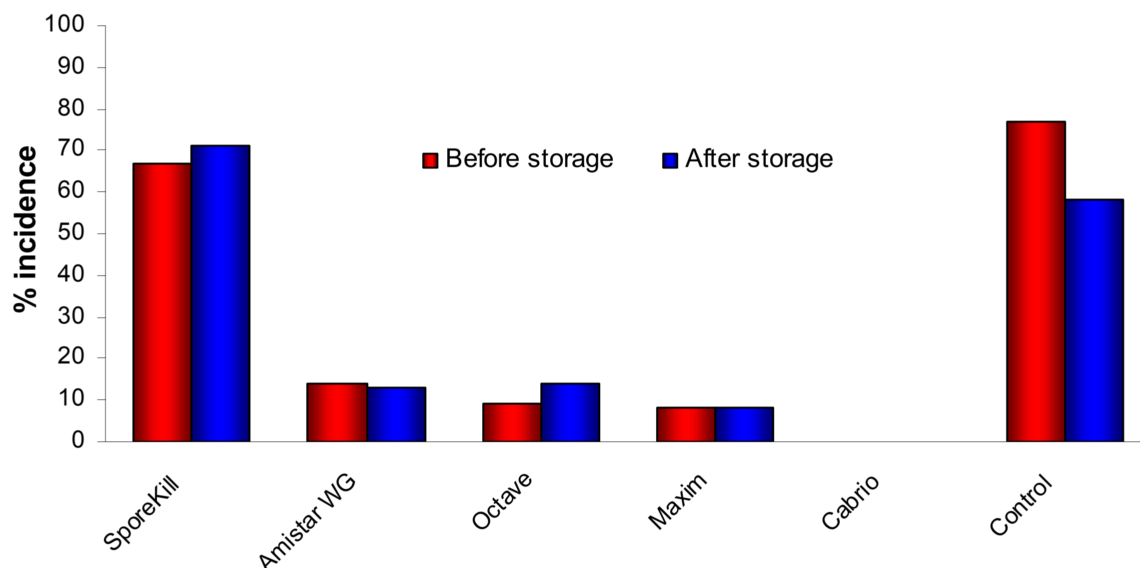
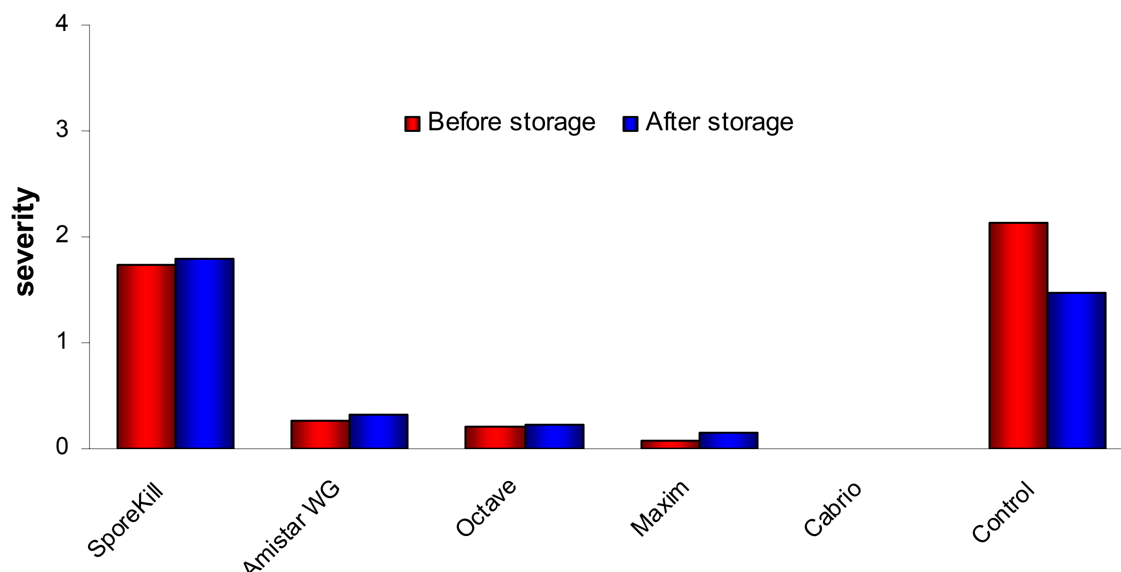



**Figure 30:** Effect of chemical treatments on the severity of *C. coccodes* on Coliban at harvest and 5 months after cold storage, Lenswood 2002-03



**Effect of fungicides on carry over of seed.**

**Objective:** *To demonstrate the introduction of C. coccodes into the soil on tuber seed and to determine the survival in soil.*

**Materials and Methods:**
In August 2002, seed tubers cv. Coliban, with more than 50% of their surface affected by *C. coccodes* were treated with fungicides at rates as previously described. Tubers were left to air dry for 15 – 20 minutes before planting.

Infected tubers were planted one per pot and 15cm deep into pots with each treatment replicated 10X. Pots were watered and plants fertilized as described in general materials and methods. Plants were grown for 2 months, after this time, plants and tubers from each pot were harvested and assessed for both incidence and severity of *C. coccodes* as previously described. The soil was left fallow in the pots for 12 months with no water or fertilizer treatments.

In November 2003, certified mini tubers of the variety Kennebec were planted into the same pots. After 3 months growth, daughter tubers were assessed for both incidence and severity of *C. coccodes* as previously described.

**Results and Discussion:**
In most treatments, the incidence and severity of infection was highest in the second crop grown from untreated mini tubers (Figs 31, 32), even where good control was achieved with the initial treatments of Maxim® and Octave®. These results show that infected seed tubers, whether treated or not are a primary source of introducing inoculum into the soil, where it establishes itself and eventually serves as soil-borne inoculum for future crops (31, 37, 56). Cabrio® significantly reduced the levels of *C. coccodes* in the following year, with only 5% infection. Only Amistar® completely inhibited infection developing in the 2nd planting. Results also confer with UK studies in that sclerotia can survive for periods of one year or more in soil (22). More work needs to be undertaken to determine the most appropriate treatments to reduce soil inoculum to acceptable levels and hence rotation scheduling.

**Figure 31:** Effect of chemical treatments on the incidence (% tubers affected) of *C. coccodes*, on the cv. Coliban Shadehouse trial, Lenswood 2002/03

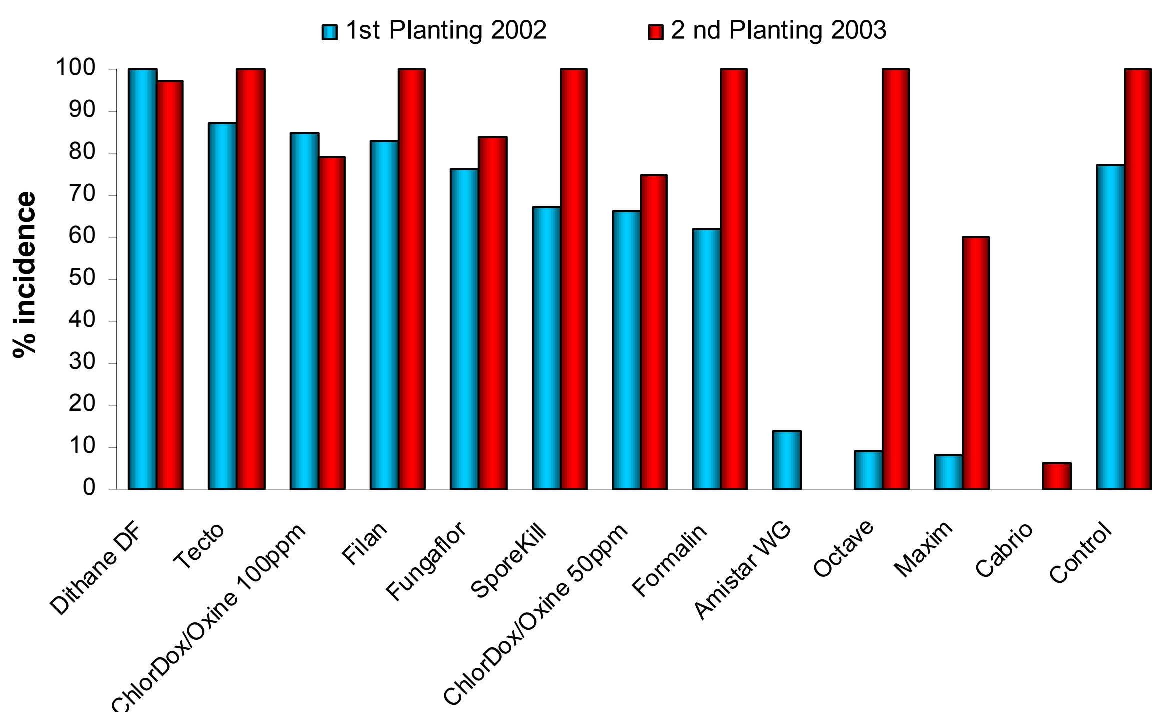
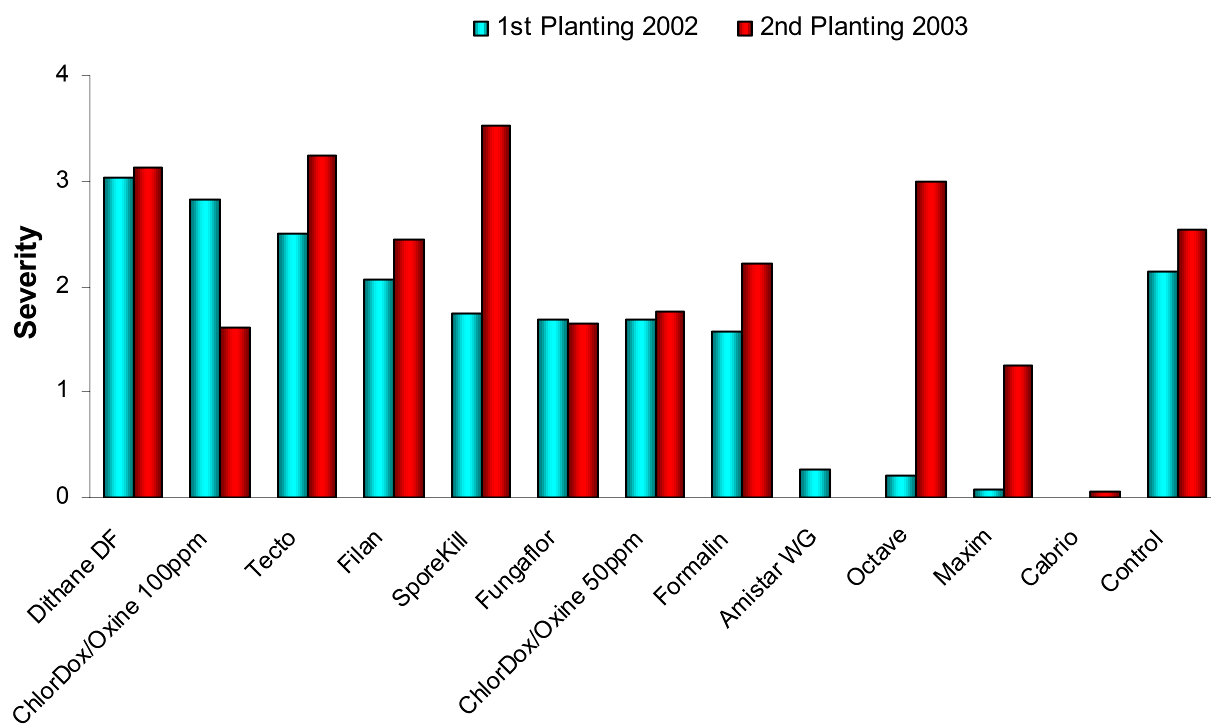



**Figure 32:** Effect of chemical treatments on the severity of C. *coccodes*, on the cv. Coliban Shadehouse trial, Lenswood 2002/03


1st Planting 2002
2nd Planting 2003
Severity
4
3
2
1
0
Dithane DF
ChlorDox/Oxine 100ppm
Tecto
Filan
SporeKill
Fungaflor
ChlorDox/Oxine 50ppm
Formalin
Amistar WG
Octave
Maxim
Cabrio
Control

### Greenhouse screening of fungicides

**Objective:** *To evaluate the effect of fungicides applied as tuber seed treatments for the control of C. coccodes on stems/tubers.*

**Materials and Methods:**
Experiments were conducted in the greenhouse to evaluate various fungicides as tuber seed treatments. Seed tubers were treated with various fungicides as previously described. Tubers were left to air dry for 15 – 20 minutes before planting.

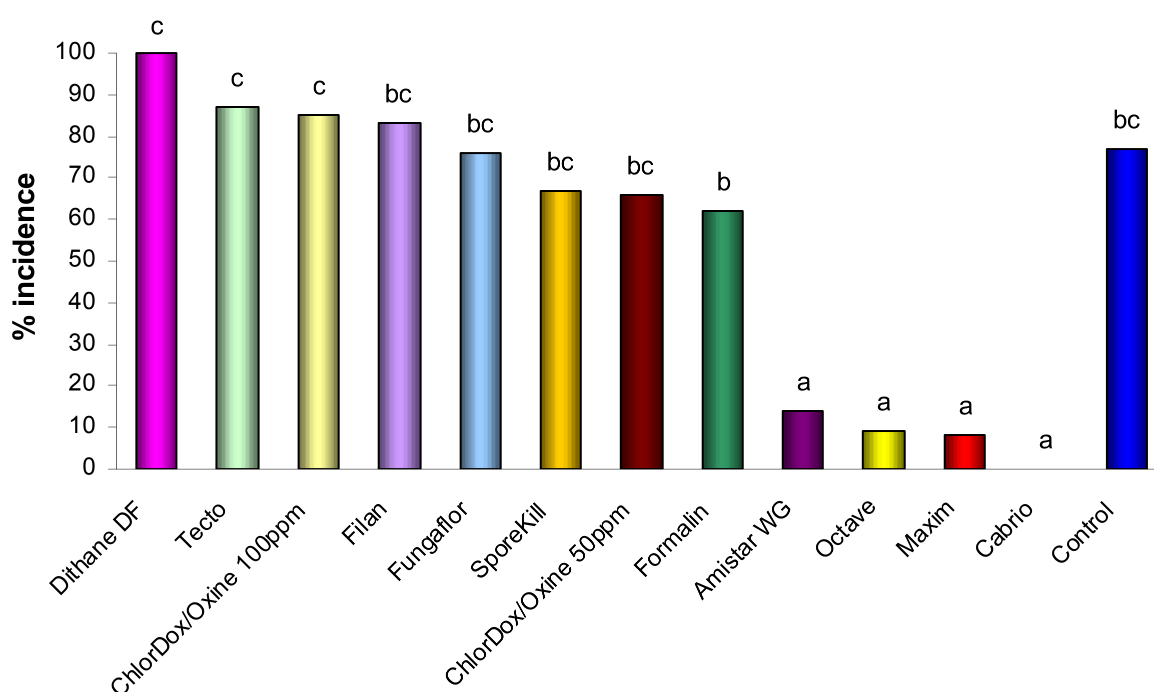
One seed tuber infected with *C. coccodes* was planted at 15cm depth into pots, replicated 10X. Pots were watered and plants fertilized as described in general materials and methods. Insecticides were applied as needed to control whitefly and thrips. Plants were grown for 2 months in a greenhouse where the mean maximum temperature was 23.2C and minimum 15.4C.

After this time, plants and tubers from each pot were harvested and visually assessed for both incidence and severity of *C. coccodes* as previously described.

**Results and Discussion:**
77% of tubers from plants grown from untreated tubers were infected with *C. coccodes* (Fig 33). Amistar®, Octave®, Maxim® and Cabrio® were the only treatments that reduced both the incidence (14, 9, 8, & 0 % respectively) and severity (0.3, 0.2, 0.3 & 0.0 respectively) of *C. coccodes* (Figs 33, 34). All other treatments had levels of infection similar to the untreated control.

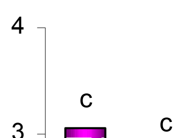
Amistar®, Maxim® and Cabrio® were the only treatments with significantly less stem infection compared to the untreated control (Figs 35, 36).

**Figure 33:** Effect of chemical seed treatments on the incidence of *C. coccodes* on daughter tubers cv. Coliban.



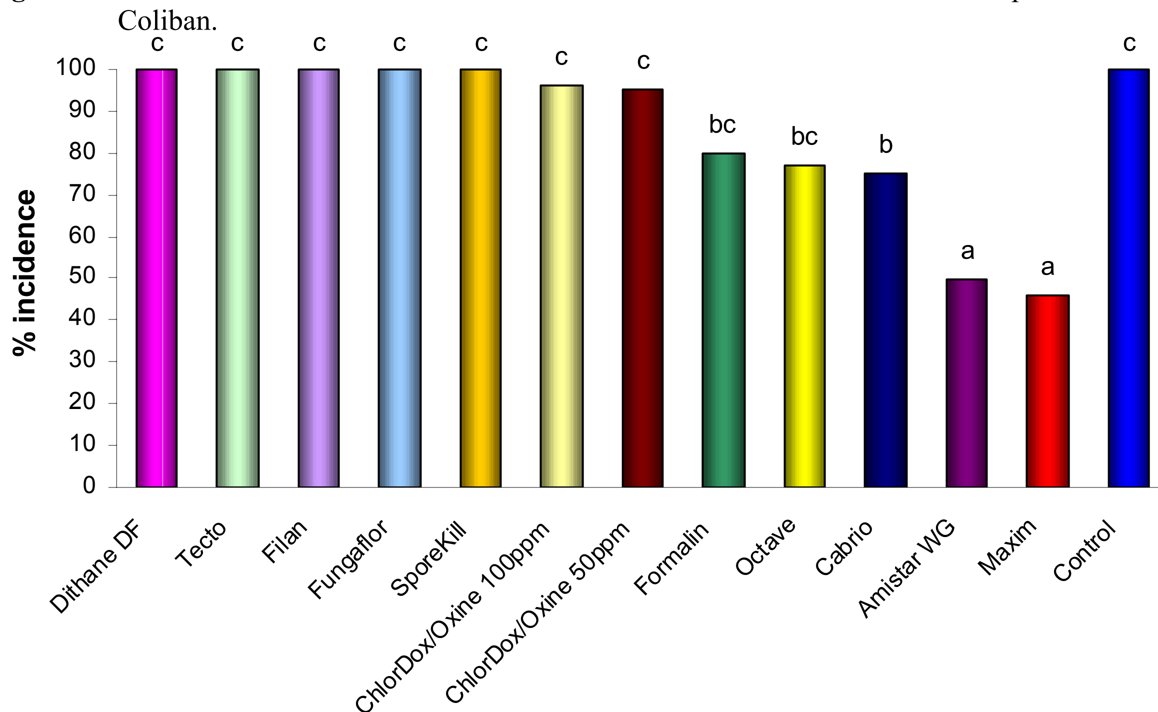

Treatments with the same letter are not significantly different from one another (LSD P=0.01).

**Figure 34:** Effect of chemical seed treatments on the severity of *C. coccodes* on daughter tubers cv. Coliban.



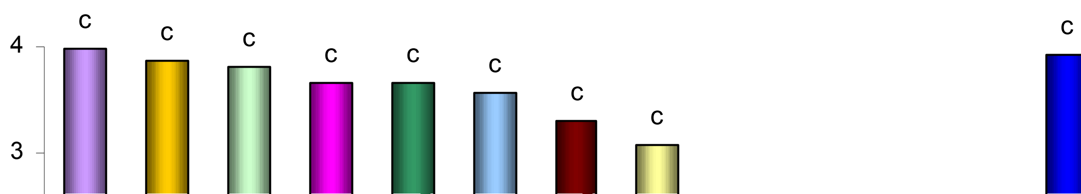
Treatments with the same letter are not significantly different from one another (LSD P=0.01).

**Figure 35:** Effect of chemical seed treatments on the incidence of *C. coccodes* on plant stems cv. Coliban.




Treatments with the same letter are not significantly different from one another (LSD P=0.01).

**Figure 36:** Effect of chemical seed treatments on the severity of *C. coccodes* on plant stems cv. Coliban.



Treatments with the same letter are not significantly different from one another (LSD P=0.01).

**Field and greenhouse evaluation of seed and soil treatments.**

**Trial 1.**
**Objective:** *To evaluate in Greenhouse and in vivo, fungicides and biological products for the control of C. coccodes on stems/tubers.*

**Materials and Methods:**
Seed tubers were treated with fungicides as previously described, at rates shown in Table 14. Tubers were left to air dry for 15 – 20 minutes and then five 5mm cores containing *C. coccodes* microsclerotia were removed from each potato and plated onto NP10 selective media and incubated at 22°C in the dark for 4 weeks. Percentage inhibition of radial growth and sclerotial density were determined after 28 days (as previously described).

One seed tuber was planted into a pot on 17/11/02 (replicated 10X) and plants watered and fertilized as previously described. Plants were grown for 3 months in a glasshouse where the mean maximum temperature was 26.4 °C and minimum 16.7 °C. At 2 weeks after complete senescence; plants and tubers were harvested from each pot and visually assessed for both incidence and severity of *C. coccodes* (as previously described).

**Table 14:** Chemicals/biologicals and rates applied.

| Treatment | Active ingredient | Concentration (%) | Rate of product/1000kg tubers |
|---|---|---|---|
| **Seed sprayed*** | | | |
| Amistar® | 500g/kg azoxystrobin | 0.04 | 1.6 gm |
| Cabrio® | 250g/kg Pyraclorostrobin | 0.02 | 1.2 gm |
| Fungaflor® | 750g/kg imazil | 0.75 | 20 gm |
| Maxim® | 100g/L fludioxonil | 1.25 | 250 ml |
| Octave® | 462g/Kg prochloraz | 0.14 | 6.0 gm |
| Voom 25® | (20 % 2-propenyl-ITC) | 5.0 | 500ml in 1.5L water |
| Voom 25® | (20 % 2-propenyl-ITC) | 2.5 | 250ml in 1.75L water |
| **Seed dipped**** | | | |
| Formalin® | 400g/L formaldehyde | 4.0 | |
| **Soil treated** | | | |
| Voom 25® | (20 % 2-propenyl-ITC) | 1.0 | 10L in 190L water/ha |
| Trichogrow® | 1.5kg/ha as a drench at planting (500L water) and then every 28 days | | |
| Mustard Meal | | 1 ton/ha100gm/m2 (7.1gm/pot | |
| Mustard Meal | | 2 ton/ha200gm/m2 (14.2gm/pot | |

* Applied in 2L water
** Dipped for 15 minutes and rinsed with water once
Untreated tubers were dipped in water for 15 min.

**Results and Discussion:**
Maxim® and Octave® were the most inhibitory to the production of *C. coccodes* sclerotia (Fig 37), however inhibition of mycelial growth was under half that of sclerotial density. Maxim®, Amistar®, Cabrio®, Octave® and the 5% rate of Voom® provided the best control of tuber incidence and severity of *C. coccodes*. (Fig 38) as well as inhibiting the development of *C. coccodes* at the stolon ends of tubers and on stems (Fig 39).

**Figure 37:** *In-vitro* inhibition of *C. coccodes* 28 days after treating tubers with different chemicals. Shadehouse, Lenswood, 2002

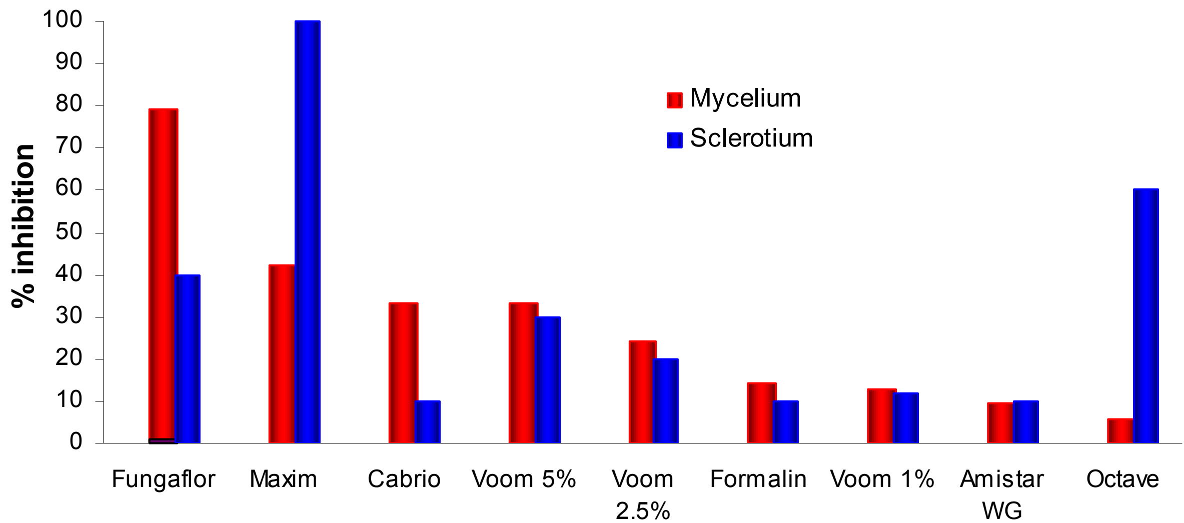
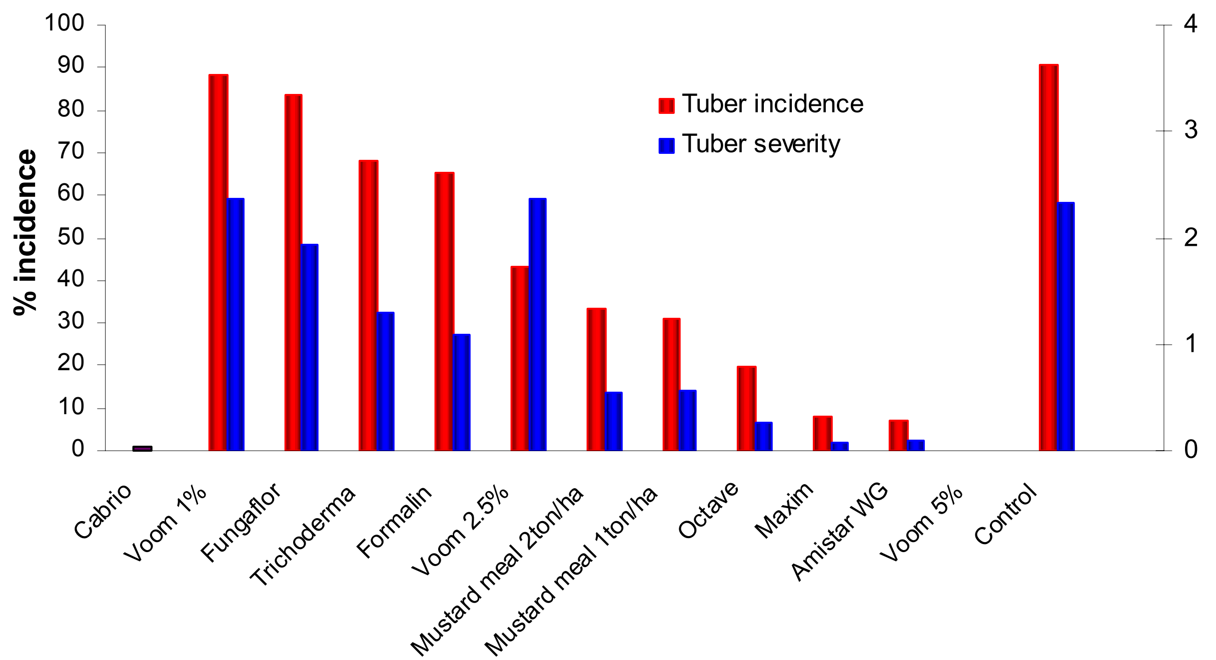



**Figure 38:** Effect of seed treatments on the incidence and severity of *C. coccodes* on daughter tubers (cv. Coliban), Lenswood 2002



**Trial 2.**
**Objective:** *To evaluate in-furrow application of Amistar® and seed tuber treatments of Amistar®, Maxim® and Octave® for the control of C. coccodes in potatoes.*

**Materials and methods:**
Two trials were conducted on consecutive years at the Lenswood Centre situated approximately 50km east of Adelaide on ground that had previously been planted to potatoes and was known to be infected with *C. coccodes*. At site one the level of *C. coccodes* in the soil was 36 CFU (colony forming units)/gm of dry soil, but levels were not measured preplanting at site two. At both sites tubers cv. Coliban naturally infected with *C. coccodes* were used. No tuber disease analysis was conducted at site one whilst at site two 43% of tubers were infected with *C. coccodes*. 10kg batches of tubers were sprayed with fungicides (shown in table 15) using a hand sprayer whilst being rotated in a hand-operated cement mixer. Tubers were left to air dry 1 day prior to planting.

Amistar® was applied using motorised back-back sprayers to the soil in-furrow at planting just prior (Site one) and just following (site two) placement of the seed tuber. The rates of application are outlined in Table 14.

Site one was planted on 11th December 2002, whilst site two was planted on the 24th November 2003.

Both trials were planted in plots 2 rows wide with 5 replicates of each treatment. Plots were 6m long (23 seed pieces at 26cm spacing) at site one and 8m long (32 seed pieces at 25cm spacing) at site two. The treated rows were separated by 2 and 1 buffer rows of tubers cv. Coliban (site one and site two respectively), with 2 barrier rows on either edge of the treated rows. The rows were banked at site one on 17th January 2003 and at site two on the 12th January 2004 and irrigated as required.

The trials were inspected regularly for foliar diseases, but levels of infection were low and did not warrant spraying. At site one the trial was allowed to senesce naturally and was harvested on 23rd April. Site two was sprayed off with Reglone® on the 5th March and harvested on 16th March. Assessment of tubers was as previously described.

**Table 15:** Chemicals and rates applied

| Treatment | Active ingredient | Concentration (%) | Rate of product/ha | Rate of product/1000kg tubers |
|---|---|---|---|---|
| **Seed sprayed*** | | | | |
| Maxim® | 100g/L fludioxonil | 1.25 | | 250 ml |
| Octave® **(site one only)** | 462g/Kg prochloraz | 0.14 | | 6.0 gm |
| Amistar® **(site two only)** | 500g/kg azoxystrobin | 0.04 | | 1.6 gm |
| **Soil treated** | | | | |
| Amistar SC®** **(site one only)** | 250g/kg azoxystrobin | 0.106 | 1.7L | |
| Amistar SC®** **(site two only)** | 250g/kg azoxystrobin | 0.156 | 2.5L | |

* Applied in 2L water
** Applied at 400L water/ha

*Emergence*: The number of plants emerged per plot were counted at 40 and 36 days after planting at site one and site two respectively.

*Harvest disease assessments and yield*: Tubers were harvested and the yield calculated at both sites (as previously described) except that site two where 6m was dug. They were then visually assessed for both incidence and severity of *C. coccodes* (as previously described).

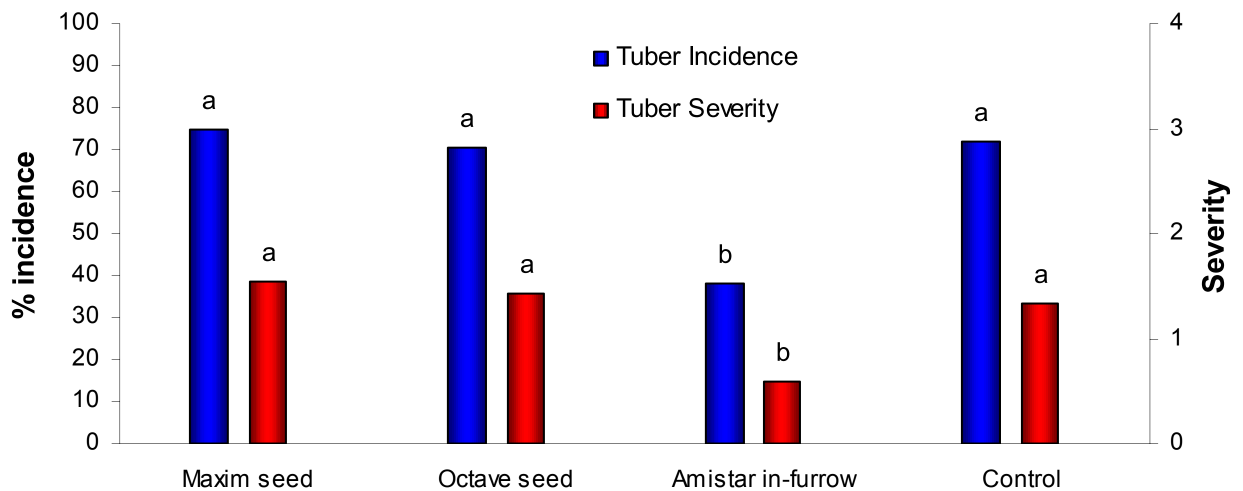
**Results and Discussion:**

*Emergence:* Between 73 and 80% of plants emerged at site one and between 90 to 96% of plants at site two. At each site there was no significant difference in emergence levels between treatments.

*Disease assessments:* At site one, *C. coccodes* developed on 72% of tubers in the control plots. Whilst Amistar® applied in furrow (drench) significantly lowered levels of *C. coccodes* tuber infection compared to the control, the Maxim® and Octave® seed treatments had no effect (Fig 39). At site two *C. coccodes* developed on more than 96% of tubers in the control. Maxim® treatment had significantly higher levels of tuber infection compared to Amistar® applied as either seed or in furrow treatments (Fig 40). The lowest level of *C. coccodes* (43%) was found in the 250g a.i./Ha rate of Amistar® applied as a seed treatment. The severity of *C. coccodes* on the tubers followed a similar trend as the incidence, in that the severity was highest (>2) in the controls and lowest (0.7) in the 250g a.i./Ha rate of Amistar® applied in furrow.

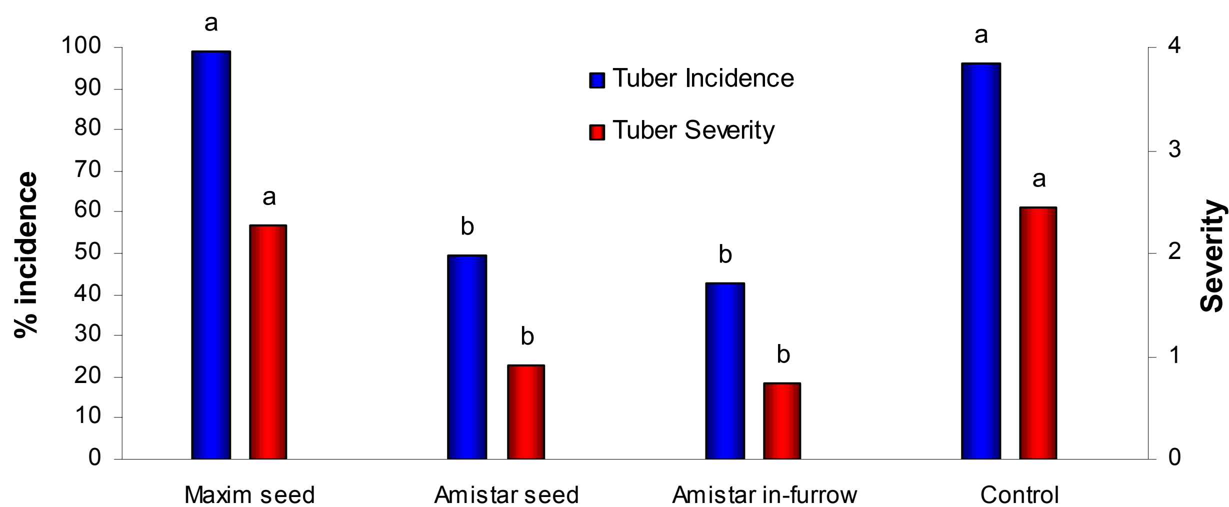
The trial showed that Amistar® provides good control of *C. coccodes* whether applied to the tubers before planting or to soil in furrow at planting. In both of these trials Amistar® was more effective than Maxim® when applied as a tuber seed treatment.

**Figure 39:** Effect of in furrow (drench) fungicide treatment and seed treatment on the incidence and severity of *C. coccodes* on daughter tubers (Site 1).




Treatments with the same letter are not significantly different from one another (LSD P=0.01).

**Figure 40:** Effect of in furrow (drench) fungicide treatment and seed treatment on the incidence and severity of *C. coccodes* on daughter tubers (Site 2).




Treatments with the same letter are not significantly different from one another (LSD P=0.01).

*Yield:* At site one yields were not significantly different between treatments compared to the control (37.4 ton/ha). However yields were highest where Amistar® was applied in furrow (39.5 ton/ha) and lowest in the Octave® and Maxim® treatments (35.6 & 33.9 ton/ha respectively). At site two all yields were less than the control (25.6 ton/ha), Maxim® was the only treatment that reduced yields significantly (14.3 ton/ha). Amistar® in furrow and on seed were 21.0 and 17 ton/ha respectively.

**Trial 3.**
**Objective:** *To evaluate tuber seed treatments and infurrow soil applications for the control of C. coccodes on naturally infected potatoes.*

**Materials and Methods:**
This trial was planted on a commercial property in Mallee region of SA at 3.5ton/Ha. Seed tubers were treated with fungicides as previously described (Table 10). Tubers were air dried for 1- 2 days before planting. Plots were 10 m long by 2 rows wide with 6 replicates of each treatment. Treated rows were separated by 2 buffer rows. The grower maintained the site as per commercial practice. Two additional treatments were included, where fungicides were applied to the soil in-furrow (drench) at planting in a band (< 15cm) just prior to placement of seed tuber (Table 16). Motorised back-back sprayers were used to apply both products.

At 3 weeks after complete senescence tubers were harvested, the yield calculated and tubers visually assessed for both the incidence and severity of *C. coccodes* (as previously described).

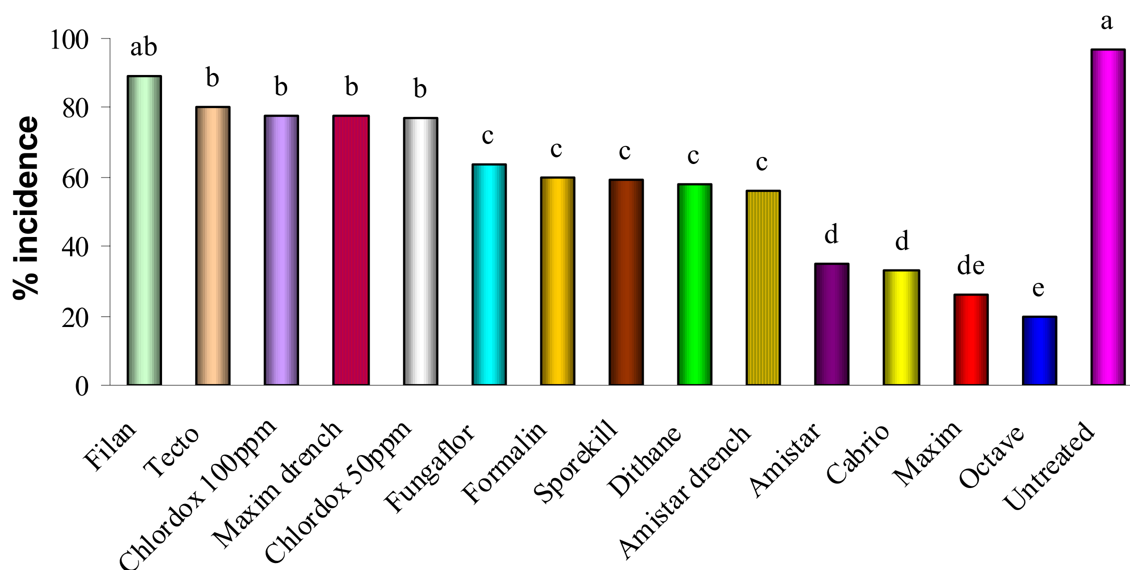
**Table 16**: Application method, rate and active ingredient of fungicide evaluated.

| Treatment | Active ingredient | Concentration (%) | Rate of product/ha/1000kg tubers |
|---|---|---|---|
| **Soil sprayed***** | | | |
| Amistar® | 500g/kg azoxystrobin | 0.062 | 500 gm |
| Maxim® | 100g/L fludioxonil | 0.029 | 1.18 L |

*** Applied in 400L/ha

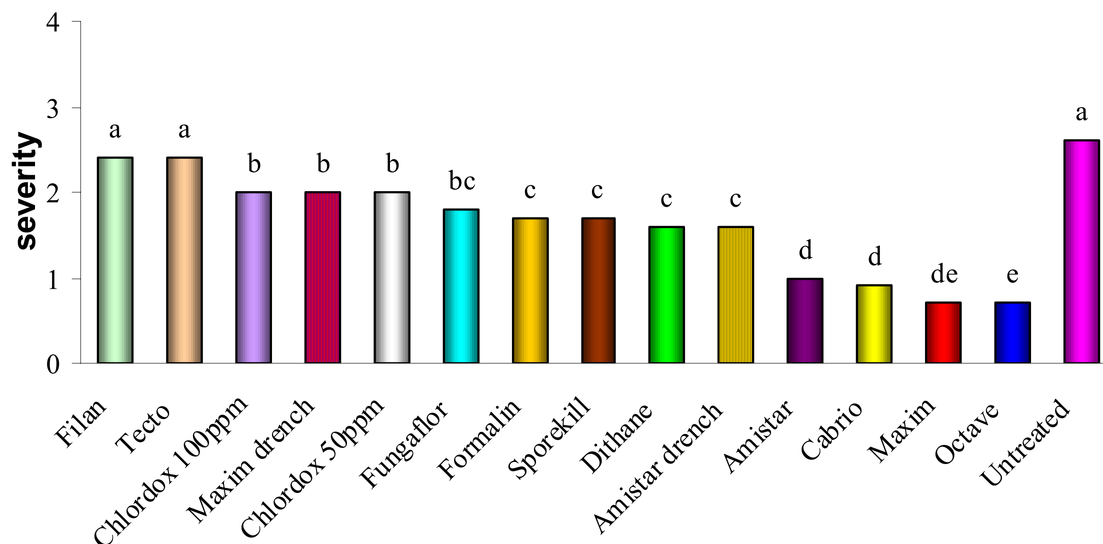
**Results and Discussion:**
Nearly all of tubers grown from untreated seed were infected with *C. coccodes* at harvest (Fig 41). Significantly less infected tubers developed in most treatments where the mother seed was treated with fungicides. The most effective of these treatments were Maxim®, Octave®, Amistar® and Cabrio® where 40% or less of tubers were infected with *C. coccodes.*

**Figure 41:** Effect of fungicide seed tuber treatments on the incidence of *C. coccodes* on daughter tubers at harvest.




Treatments with the same letter are not significantly different from one another (LSD P=0.01).

**Figure 42:** Effect of fungicide seed tuber treatments on the severity of *C. coccodes* on daughter tubers at harvest.




Treatments with the same letter are not significantly different from one another (LSD P=0.01).

In-furrow (drench) treatments of Maxim® and Amistar® were less effective than seed treatment in reducing disease incidence or severity (Fig 41, 42). However one overseas study has reported that the use of azoxystrobin as an in-furrow drench were effective (Severity reduced by 60 – 80% and incidence reduced by 50 –100%) (50). One possible reason may be that the majority of daughter tuber infections were from seed borne rather than soil inoculum. Also the application rates used in our trials may have been too low. For example in the UK Amistar® is applied at higher rates 750 – 1500 gm Ha (personal comm. S.Wale 2004) compared to the rate 250 gm Ha used in our trials.

**Trial 4.**

**Objective:** *To evaluate applications of Amistar®, Maxim®, Cabrio® and Octave® for the control of C. coccodes.*

**Materials and methods:**
Certified seed tubers cv. Coliban naturally infected with *C. coccodes* (100% incidence and 3 severity) were used in this trial. One tonne batches of tubers were passed through a grader resulting in approx 30% of tubers being cut to size. Tubers were treated with fungicides at rates as outlined in Table 17. In addition tubers were coated after fungicides were applied in mixture of 50% cement and 50% fur bark. Tubers were air dried for 2 days before planting.

20 tubers from each treatment were collected at random and two *C. coccodes* microsclerotia were removed from each potato and plated onto NP10 selective media. Plates were incubated at 22$^{o}$C in the dark for 2 weeks before the viability of sclerotia was determined.

10 tubers from each treatment were evaluated in a greenhouse trial. One seed tuber was planted into a pot on 24th March 2003, replicated 10 times (as previously described). Pots were watered and plants fertilized as previously described. Plants were grown for 3 months in a greenhouse.

Remaining tubers were planted on 26$^{th}$ March 2003 in the Mallee region of SA at 4 ton/Ha. 25 samples of soil randomly collected from across the trial site before planting indicated a level of *C. coccodes* in the soil of 2 CFU (colony forming units)/gm of dry soil. The trial was planted in plots 4 rows wide and 100m long in a randomised block design, with 5 replicates of each treatment.

The number of plants emerged per plot were counted at 40 days after planting.

On the 7$^{th}$ July, 4 weeks after complete senescence, plants and tubers were harvested from each pot and plot and yields calculated as described in general materials and methods in this section. They were then visually assessed for both incidence and severity of *C. coccodes* (as previously described).

**Table 17:** *Chemicals and rates applied*

| Treatment | Active ingredient | Concentration (%) | Rate of product/1000kg tubers |
|---|---|---|---|
| **Seed sprayed*** | | | |
| Maxim® | 100g/L fludioxonil | 1.25 | 250 ml |
| Amistar® | 500g/kg azoxystrobin | 0.08 | 3.3 gm |
| Octave® | 462g/Kg prochloraz | 0.28 | 12.0 gm |
| Cabrio® | 250g/kg Pyraclorostrobin | 0.08 | 6.6 gm |

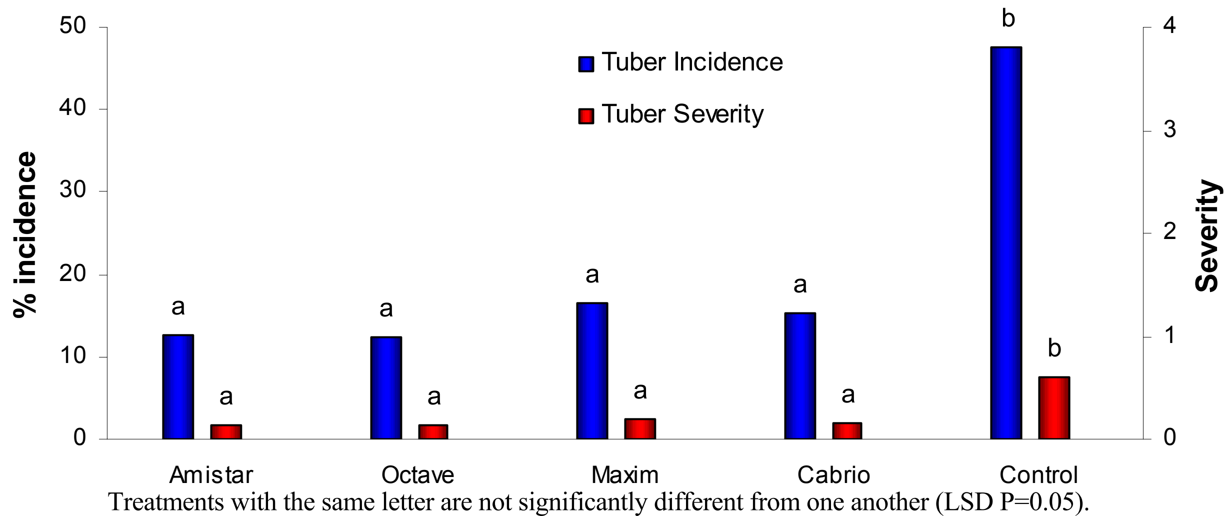
* Applied in 2L water

**Results and Discussion:**
Assessments 40 days after planting showed that Maxim® applied to tuber seed significantly reduced tuber emergence compared to the untreated tubers in both the field and greenhouse trials by 14% & 13% respectively. Emergence in all other treatments was around 90% and showed no significant differences compared to the control or between each other.

Maxim® was the only treatment that significantly inhibited the germination of sclerotia after treatment compared to the untreated tubers (0% & 50% respectively). All other treatments were not significantly different to the untreated tubers.

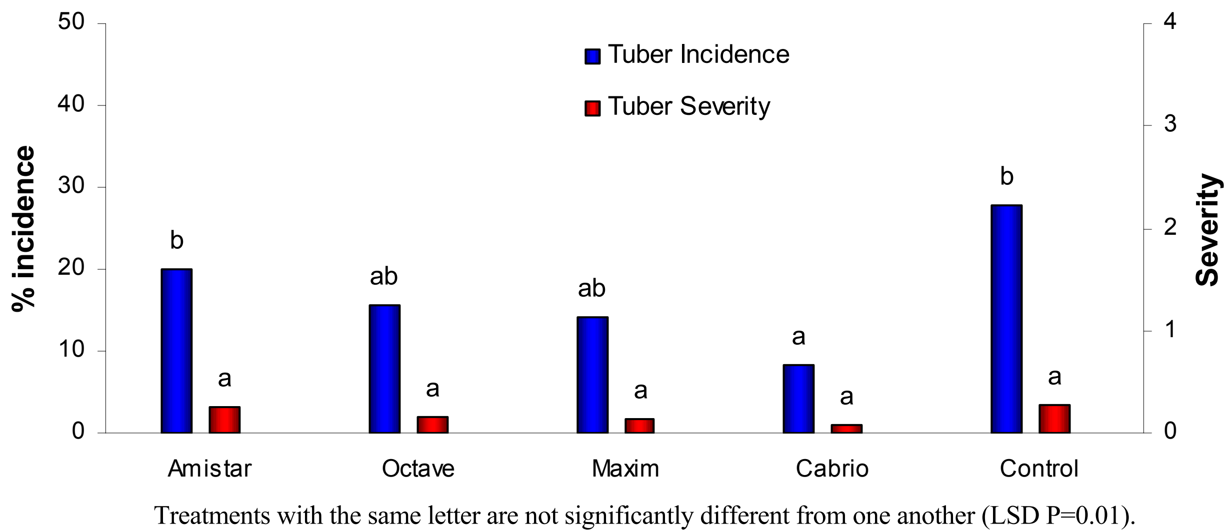
All treatments significantly reduced the incidence and severity of tuber infection compared to the control in greenhouse trials (Fig 43), but only Cabrio® significantly reduced the incidence of disease in the field trial (Fig 44). This is possibly due to the level of soil infection already present in the field. Amistar® and Maxim® were the only treatments that significantly increased the yield (Fig 45).

**Figure: 43:** Effect of Amistar®, Maxim®, Cabrio® and Octave® seed treatment on the incidence and severity of *C. coccodes* on daughter tubers grown in the green house.




Treatments with the same letter are not significantly different from one another (LSD P=0.05).

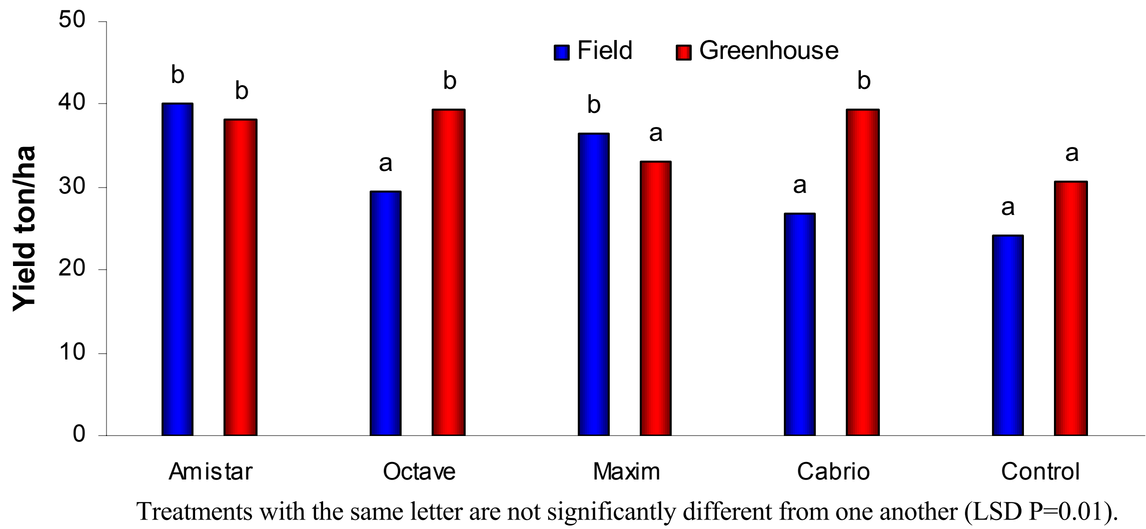
**Table 44:** Effect of Amistar®, Maxim®, Cabrio® and Octave® tuber seed treatments on the incidence and severity of *C. coccodes* on daughter tubers grown in the field.




Treatments with the same letter are not significantly different from one another (LSD P=0.01).

**Yield.**

**Figure 45:** Effect of Amistar®, Maxim®, Cabrio® and Octave® seed treatment on the yield of daughter tubers.




Treatments with the same letter are not significantly different from one another (LSD P=0.01).

**Evaluation of fumigation**

**Trial 1.**
**Objective:** *To evaluate the effects of various fungicides applied as either tuber seed treatments or in furrow soil treatments, for the control of C. coccodes in soil fumigated with and without Metham Sodium®.*

**Materials and Methods:**
Trials were conducted on two commercial grower properties in the Mallee region approximately 250km northeast of Adelaide. At both sites soil was fumigated with Metham Sodium® (as previously described), site one on 7th November and site two on 8th November 2002 (Pic 1,2,3,4).

Seed tubers, cv. Coliban were used at both sites. At site one, 25% of the tubers were infected with *C. coccodes* and an average severity rating of 1.1 whereas site two all of the tubers were infected with *C. coccodes* and an average severity rating of 2.7.

At both sites chemicals were applied to whole tubers (as previously described) at rates outlined in Table 18, and allowed to dry before storing overnight. At site one, cut seed weighing approx 42 – 94gms/each were treated in batches of 110kg the day before planting (22nd November) whilst at site two, whole seed weighing approx 70 – 120gms/each were treated in batches of 25kg two days prior to planting (11th December). Filan®, Tecto® and Chlordox® at 0.05% were not included; Dithane® was included at a higher rate and additional treatments included (Table 18). In-furrow sprays were applied at planting just prior to placement of the seed tuber using motorised sprayers.

Both sites had previously been planted to potatoes approx 5yrs ago, but site one was planted to canola last year and site two was under pasture. Soil samples randomly collected from the trial sites before planting indicated that the levels of *C. coccodes* at site one were 4.6 cfu's (colony forming units)/gm of dry soil and 29.6 cfu's/gm dry soil at site two.

Site one was sprayed off on the 12th March and harvested on 14th April, whilst site two was sprayed off on the 18th March and harvested on 23rd April. Plots at both trials were 4 rows wide and 10m long with a set spacing of 17.5cm and row spacing of 85cm at site one and 80cm site two. Design was as previously described, with 4 replicates of each treatment. The treated rows were separated by 2 buffer rows of tubers. The trial site was maintained as per the commercial practices of the grower.

**Table 18:** Rates of fungicides.

| Treatment | Active ingredient | Concentration (%) | Rate of product/ha/1000kg tubers |
|---|---|---|---|
| **Seed sprayed*** | | | |
| Cabrio® | 250g/kg Pyraclorostrobin | 0.08 | 6.8 gm |
| Dithane® | 750g/kg mancozeb | 0.49 | 13.5 gm |
| Maxim® + Sporekill® | only at site two | 1.25 + 1.00 | 250 ml + 20 ml |
| **Soil sprayed***** | | | |
| Amistar® | 500g/kg azoxystrobin | 0.04 | 250 gm |
| Maxim® | 100g/L fludioxonil | 0.29 | 1175 ml |

* Applied in 2L water
** Dipped for 15 minutes and rinsed with water once
*** Applied in 400L/ha
Untreated tubers were dipped in water for 15 min.
All in furrow treatments are applied using Solo® motorised backpack sprayers in a volume of 400L/Ha.

At 3 weeks after complete senescence, tubers were harvested and the yields calculated (as previously described). Tubers and stems were then assessed for both incidence and severity of *C. coccodes* as previously described.

**Results and Discussion:**

*Disease assessments:* At site 1, *C. coccodes* developed on 44% of tubers in the unfumigated plots compared to 29% in the fumigated (Table 19). Maxim® and Octave® provided the best control in the unfumigated plots, reducing the incidence of disease to 18 and 16% respectively. The Amistar® in-furrow (drench) treatment significantly increased both the incidence and severity of disease compared to control in the unfumigated areas, with the highest levels of disease found in this treatment (68% incidence and 1.0 severity). In the fumigated areas, Fungaflor® was the only treatment to markedly reduce disease incidence, although due to the variability the differences between treatments were not significant.

In trial 2, where high inoculum levels were present, the results were extremely variable, and disease levels were generally lower than in site one with lower soil inoculum. Low levels of disease were found in the control, with 14% and 10% of tubers infected in the unfumigated and fumigated plots respectively. Maxim® again provided good control with 9% of tubers infected in the unfumigated treatments. The sanitizers (Sporekill®, Chlordox®/oxine and Formalin®) also had lower levels of disease compared to some other treatments, possibly due to controlling the high level of seed infection.

Amistar® in-furrow (drench) had high levels of disease, 40% incidence compared to 14% in the control and 9% with Maxim®. In the fumigated area, many treatments reduced disease levels however Maxim® was not effective, with the highest levels of disease (26%) observed.

Overall, in soil with high levels of inoculum, fumigation reduced these levels and the seed treatments provided little additional benefit. Where low levels of soil inoculum occur, the seed treatments were more effective in unfumigated soil.

**Picture 1:** Incorporation of green manure crop (Brassica), four weeks prior to fumigation, (Site 1).

**Picture 2:** Fumigation, (Site 1).

**Picture 3:** Incorporation of pasture, four weeks prior to fumigation, (Site 2).

**Picture 4:** Fumigation, (Site 2).

**Table 19**: Effect of ... erity of *C. coccodes*, on tubers cv ... m.

| Treatment | ... noculum (Site 2) |
| --- | --- |

| | % tubers affected | | Severity [A] | | % tubers affected | | Severity [A] | |
|---|---|---|---|---|---|---|---|---|
| | *Unfume* | *Fumigated* | *Unfume* | *Fumigated* | *Unfume* | *Fumigated* | *Unfume* | *Fumigated* |
| Maxim® | 18 a | 31 b | 0.2 a | 0.3 | 9a | 26 b | 0.2 a | 0.6 b |
| Maxim®/Sporekill® | | | | | 34 b | 8 a | 0.8 ab | 0.2 a |
| Amistar ®WG | 35 ab | 32 b | 0.4 a | 0.5 | 19 ab | 10 ab | 0.5 ab | 0.2 a |
| Cabrio® (full) | 23 ab | 25 b | 0.3 a | 0.3 | 44 bc | 5 a | 1.0 b | 0.1 a |
| Cabrio® (half) | 39 b | 25 b | 0.5 a | 0.3 | 26 ab | 10 ab | 0.5 ab | 0.2 a |
| Octave® | 16 a | 23 b | 0.2 a | 0.4 | 32 b | 7 a | 0.7 ab | 0.1 a |
| Dithane® DF | 29 ab | 40 b | 0.4 a | 0.6 | 39 bc | 6 a | 0.8 b | 0.1 a |
| SporeKill® | 32 ab | 42 b | 0.4 a | 0.5 | 12 ab | 5 a | 0.2 a | 0.1 a |
| ChlorDox®/Oxine | 43 b | 24 ab | 0.5 a | 0.6 | 11 ab | 9 a | 0.2 a | 0.2 a |
| Formalin® | 29 ab | 21 ab | 0.4 a | 0.3 | 13 ab | 6 a | 0.2 a | 0.1 a |
| Fungaflor® | 40 b | 12 a | 0.5 a | 0.2 | 22 ab | 4 a | 0.3 ab | 0.1 a |
| Drench Maxim® | 32 ab | 23 ab | 0.4 a | 0.5 | 44 bc | 19 b | 1.0 b | 0.4 b |
| Drench Amistar® | 68 c | 29 ab | 1.0 b | 0.4 | 40 bc | 10 ab | 1.0 b | 0.2 ab |
| Control | 44 b | 29 ab | 0.5 a | 0.6 | 14 ab | 10 ab | 0.3 b | 0.2 a |
| **LSD (P = 0.05)** | **20.49** | **17.59** | **0.358** | **ns** | **21.21** | **9.34** | **0.56** | **0.19** |

[A]Severity rating scale 0 to 4: 0, no diseases; 1, <2%; 2, 3-10%; 3, 11-30%, 4, >30% of tuber surface affected
Treatments with the same letter are not significantly different from one another (LDS P = 0.005)

**Table 20:** Effects of different chemical treatments on the number and yield of tubers/plot of the cultivar Coliban (Marketable tubers: 80-450g)

| **Treatment** | **low inoculum (Site 1)** Total yield of tubers (ton/ha) | | **high inoculum (Site 2)** Total yield of tubers (ton/ha) | |
|---|---|---|---|---|
| | *Unfume* | *Fumigated* | *Unfume* | *Fumigated* |
| Maxim® | 42 b | 33 ab | 57 | 60 |
| Maxim® + Sporekill® | | | 62 | 54 |
| Amistar® WG | 40 b | 47 b | 61 | 60 |
| Cabrio® (full) | 43 b | 43 b | 60 | 59 |
| Cabrio® (half) | 46 b | 50 b | 61 | 64 |
| Octave® | 47 b | 51 b | 59 | 64 |
| Dithane® DF | 46 b | 57 b | 60 | 58 |
| SporeKill® | 29 a | 40 ab | 55 | 62 |
| ChlorDox®/Oxine | 40 b | 54 b | 60 | 59 |
| Formalin® | 35 b | 41 ab | 62 | 55 |
| Fungaflor® | 42 b | 39 ab | 57 | 53 |
| Drench Maxim® | 40 b | 49 b | 61 | 59 |
| Drench Amistar® | 47 b | 50 b | 64 | 59 |
| Control | 22 a | 28 a | 60 | 57 |
| **LSD (P = 0.05)** | **12.49** | **14.28** | **ns** | **ns** |

Treatments with the same letter are not significantly different from one another (LSD P=0.05.

Yields were extremely variable, however despite the varying level of disease control. In soil with low levels of disease most treatments increased yield significantly in both fumigated and unfumigated soil compared to the control (Table 20). There was also a slight increase in yield in the fumigated soils, with only Fungaflor® and Maxim® having lower total yields in fumigated soils. In the trial site with high levels of disease, there was no significant difference in yields between any of the treatments, with fumigation having little effect. Whilst the fumigant reduced soil borne levels of *C. coccodes* and improved yield, they are increasingly

becoming restricted in their use due to their expense and toxicity. All of these issues have resulted in research on more environmentally friendly alternatives such as bio-fumigation. Whilst our laboratory results and others (39, 54) on *Brassica sp* as biofumigants show promise for controlling *C. coccodes*, more research on their effectiveness in the field is required.

**Trial 2.**

**Objective:** *To evaluate fungicides applied to tuber seed and infurrow soil treatments, both with and without Metham Sodium® and Telone II® for the control of C. coccodes using seed with low and high incidence of C. coccodes.*

**Materials and Methods:**

Prior to planting, soil was fumigated with either Metham sodium® or Telone II® (1,3-dichloropropene) as previously described, at Lenswood Centre situated approximately 50km east of Adelaide, on the 19th November. Two different lots of certified seed tubers cv. Coliban were used, one batch were all infected externally and 16% internally ("diseased seed") whilst no disease was detected externally or internally on the other batch of tubers ("healthy seed"). Planting occurred on the 19th December.

The trial area had previously been planted to potatoes and was known to be infected with *C. coccodes*. 25 samples of soil were randomly collected from the trial area prior to fumigation and 9 weeks after fumigation. The mean *C. coccodes* levels prior to fumigation were 83, 112 and 56 cfu's/gm dry soil and 63, 98 and 78 cfu's/gm after fumigation in the untreated, Metham® and Telone® plots respectively.

10kg batches of tubers were sprayed with fungicides (Table 21) using a hand sprayer whilst being rotated in a hand-operated cement mixer. Tubers were treated the day before planting, and allowed to dry before storing overnight.

In-furrow treatments were applied at planting just prior to placement of the seed tuber using a motorised sprayer.

The trial was planted in plots 4 rows wide and 5m long (set spacing 35cm spacing, row spacing 76cm). The design was as previously described, with 4 replicates of each treatment. Whole seed weighing approx 100 – 180gms/each was used. The treated rows were separated by 2 buffer rows of tubers. The trial was inspected regularly for Target spot, but levels of infection were low and did not warrant spraying. The trial was sprayed off on the 22nd March and harvested on 30th April.

**Table 21:** Rates of Fungicides.

| Treatment | Active ingredient | Concentration (%) | Rate of product/ha/1000kg tubers |
|---|---|---|---|
| **Seed sprayed*** | | | |
| Amistar® | 500g/kg azoxystrobin | 0.04 | 1.6 gm |
| Maxim® | 100g/L fludioxonil | 1.25 | 250 ml |
| Octave® | 462g/Kg prochloraz | 0.14 | 6.0 gm |
| **Soil sprayed***** | | | |
| Amistar® | 500g/kg azoxystrobin | 0.04 | 250 gm |
| Maxim® | 100g/L fludioxonil | 0.29 | 1175 ml |

* Applied in 2L water
** Dipped for 15 minutes and rinsed with water once
*** Applied in 400L/ha
Untreated tubers were dipped in water for 15 min.

At 3 weeks after complete senescence, tubers were harvested and yields calculated (as previously described). Tubers were then assessed for both incidence and severity of *C. coccodes* as previously described.

**Results and Discussion:**

Fumigation had little effect on the levels of disease at harvest (Table 22), with untreated tubers in the Telone® fumigated area having higher levels of disease than in the control (79% and 59% ("healthy seed") and 88 and 76% ("diseased seed") respectively).

Only the Metham® area planted to untreated "diseased" seed had less disease than the non-fumigated area (67% and 76% respectively) (Table21). This is not unexpected, as the soil inoculum levels were not reduced significantly by fumigation. Levels were higher after Telone® fumigation (56% to 78% cfu/gm dry soil) and Metham® only reduced the levels slightly (112 to 98 cfu/gm dry soil).

Where "healthy" seed was planted, the control achieved by treating seed was variable with Amistar® in-furrow (drench) performing best in the unfumigated soil (4% incidence and 0.16 severity), Octave® in the Metham® treated soil (6% incidence and 0.2 severity) and Amistar® in the Telone® treated soil (3% and 0.03 severity). Most treatments reduced disease, with the exception of Maxim® drench in the unfumigated area (which had significantly higher disease than the control) and both Maxim® and Amistar® in-furrow drench in the Metham® treated soil. A similar trend was observed in the treatments where "diseased" seed was used, however Amistar® seed treatment performed better than the drench in unfumigated soil (1% incidence, 0.01 severity and 52% incidence, 0.55 severity). Amistar® was one of the best treatments in all fumigated areas, with 5% and 8% incidence in Metham® and Telone® respectively. Overall the drench treatments were not as effective as the seed treatments except when applied to Telone® treated soil.

In this trial Metham® did not significantly improve disease control, however it significantly increased overall yield when "healthy" tubers were planted. Where "diseased" tubers were planted, fumigation was ineffective at controlling the disease and often resulted in decreased yields. The combination of Telone® fumigation and Amistar® seed treatments or drench gave the best yield result (Table 23) when "diseased" seed was planted.

**Table 22:** Effect of chemical treatments on the incidence (% tubers affected) and severity of *C. coccodes* on the cultivar Coliban in a field trial. ("Healthy seed" lot one and "Diseased seed" lot two) Lenswood

| Treatment | "Healthy" seed (Lot one) | | | "Diseased" seed (Lot two) | | |
|---|---|---|---|---|---|---|
| | % tubers affected | | Severity [A] | % tubers affected | | Severity [A] |
| ***Unfumigated*** | | | | | | |
| Amistar® | 16 | a | 0.35 | 1 | a | 0.01 |
| Drench Amistar® | 4 | a | 0.16 | 52 | c | 0.55 |
| Maxim® | 23 | a | 0.66 | 28 | b | 0.13 |
| Drench Maxim® | 83 | c | 1.11 | 81 | d | 0.57 |
| Octave® | 23 | a | 0.30 | 51 | c | 0.34 |
| Control | 59 | b | 0.39 | 76 | d | 1.12 |
| ***Metham***® | | | | | | |
| Amistar® | 45 | b | 0.46 | 15 | a | 0.19 |
| Drench Amistar® | 75 | c | 0.58 | 42 | b | 0.44 |
| Maxim® | 22 | a | 0.31 | 54 | bc | 0.61 |
| Drench Maxim® | 98 | d | 0.83 | 58 | bc | 0.42 |
| Octave® | 6 | a | 0.20 | 16 | a | 0.15 |
| Control | 90 | cd | 1.08 | 67 | c | 0.28 |
| ***Telone***® | | | | | | |
| Amistar® | 3 | a | 0.03 | 8 | ab | 0.12 |
| Drench Amistar® | 15 | a | 1.06 | 26 | b | 0.32 |
| Maxim® | 53 | b | 0.53 | 25 | b | 0.18 |
| Drench Maxim® | 10 | a | 0.27 | 3 | a | 0.03 |
| Octave® | 39 | b | 0.34 | 30 | b | 0.93 |
| Control | 79 | c | 0.69 | 88 | c | 0.63 |
| **LSD (P = 0.01)** | **20.93** | | **NS** | **21.41** | | **NS** |

[A]Severity rating scale 0 to 4: 0, no diseases; 1, <2%; 2, 3-10%; 3, 11-30%, 4, >30% of tuber surface affected
Treatments with the same letter are not significantly different from one another (LDS P = 0.005)

**Table 23:** Effects of different chemical treatments on the number and yield of tubers/plot of the cultivar Coliban in a field trail at Lenswood, ("Healthy seed" lot one and "Diseased seed" lot two) Lenswood

| **Treatment** | **"Healthy" seed (lot one) Total yield of tubers (ton/ha)** | | **"Diseased" seed (lot two) Total yield of tubers (ton/ha)** | |
|---|---|---|---|---|
| ***Unfumigated*** | | | | |
| Amistar® | 26 | ab | 62 | ns |
| Drench Amistar® | 47 | c | 65 | ns |
| Maxim® | 26 | ab | 61 | ns |
| Drench Maxim® | 36 | b | 61 | ns |
| Octave® | 18 | a | 61 | ns |
| Control | 31 | b | 66 | ns |
| ***Metham***® | | | | |
| Amistar® | 43 | bc | 63 | b |
| Drench Amistar® | 39 | bc | 44 | a |
| Maxim® | 58 | d | 51 | a |
| Drench Maxim® | 44 | c | 49 | a |
| Octave® | 35 | b | 49 | a |
| Control | 22 | a | 65 | b |
| ***Telone***® | | | | |
| Amistar® | 35 | ab | 62 | bc |
| Drench Amistar® | 36 | ab | 66 | c |
| Maxim® | 41 | b | 57 | b |
| Drench Maxim® | 30 | a | 54 | ab |
| Octave® | 35 | ab | 52 | ab |
| Control | 37 | ab | 48 | a |
| **LSD (P = 0.01)** | **8.52** | | **8.08** | |

Treatments with the same letter are not significantly different from one another (LDS P = 0.005)

**Haulm desiccation**

**Objective:** *To evaluate the effect of pre-harvest treatments of either removing potato haulms, spraying Reglone® desiccant, or applying Amistar® on potato haulms on the control of C. coccodes.*

**Materials and Methods:**
Two trials were conducted on new ground (sandy loam) in the Mallee region of South Australia. Seed tubers cv. Coliban were used at both sites. At site one 44% of tubers planted were infected with *C. coccodes*, whereas at site two no *C. coccodes* was detected on seed tubers. Tubers at site one were treated with the fungicide Maxim® 100 FS (100g/L Fludioxonil) prior to planting. At both sites tubers were planted 24cm apart in rows 80cm apart. Plots were 12m long by 16m wide, arranged in a randomised block design and replicated five times. At 100 days post planting the following treatments were applied to the crop, Reglone at (3.5L/ha), Reglone® followed by Amistar® at (250g ai/ha) one week later, complete removal of haulms and roots or natural senescence.

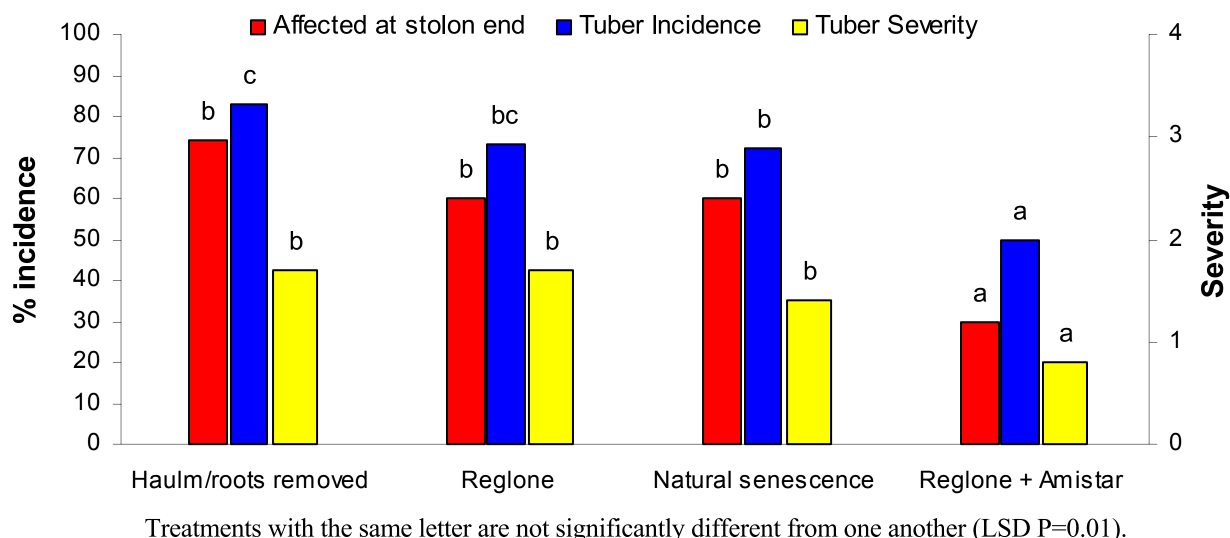
Nine weeks after the treatments, 40 plants were harvested from each plot and yields calculated as previously described. A subsample of 100 tubers was washed and visually assessed for both incidence and severity of *C. coccodes* as previously described. In addition the incidence of infection by *C. coccodes* at the stolon end was recorded.

**Results and Discussion:**
At both sites more than 70% of tubers from naturally senesced crops or those sprayed with Reglone® were infected with *C. coccodes.* Significantly less tuber infection developed where Amistar® was applied (Fig 46, 47) at both sites, and the haulm/root removal reduced infection at one site (Fig 47). At both sites the numbers of tubers with stolon end infections was significantly lower where Amistar® was applied.

Total yields ranged from 51.6 – 62.0 ton/ha at site one and 29.5 – 32.2 ton/ha at site two, but at each site did not significantly differ between treatments (P=0.05).

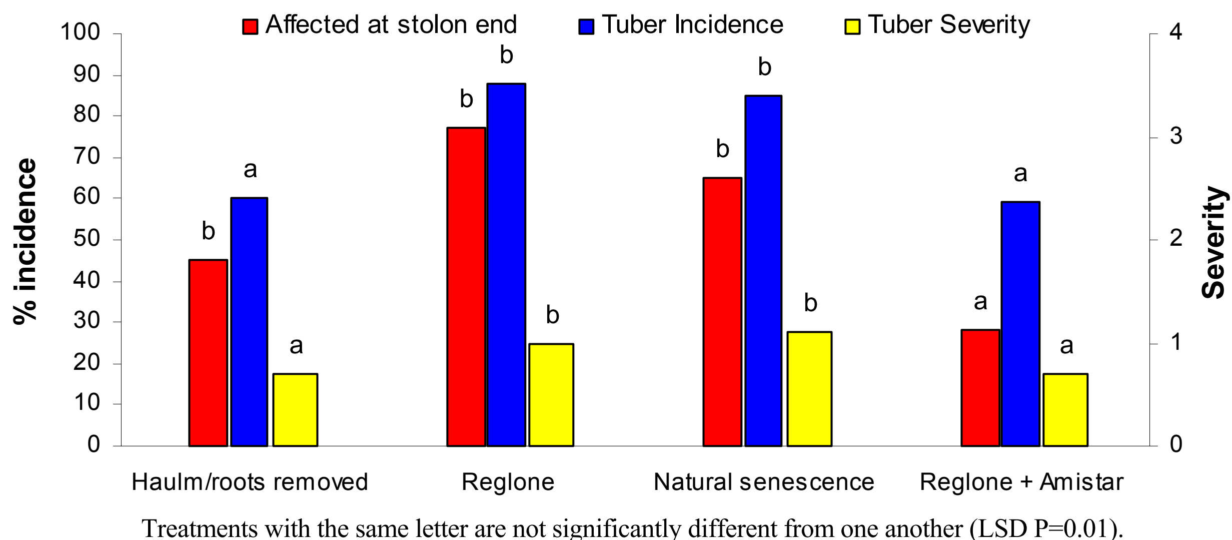
Marketable yields ranged from 47.5 – 58.5ton/ha at site one and also did not significantly differ between treatments, however marketable yields at site two were significantly higher (P=0.05) where Amistar® was applied or haulms/roots were removed (29.5 and 25.2ton/ha respectively) compared to Reglone® or natural senescence (22.6 and 22.5ton/ha respectively).

**Figure 46:** The incidence and severity of *C. coccodes* on tubers 9 weeks after (A) removal of haulms and roots, (B) applying Reglone®, (C) natural senescence, or (D) applying Reglone® followed by Amistar® (Site one).




Treatments with the same letter are not significantly different from one another (LSD P=0.01).

**Figure 47:** The incidence and severity of *C. coccodes* on tubers 9 weeks after (A) removal of haulms and roots, (B) applying Reglone®, (C) natural senescence, or (D) applying Reglone® followed by Amistar® (Site two).




Treatments with the same letter are not significantly different from one another (LSD P=0.01).

A large percentage of potato crops grown in Australia are sprayed off with the desiccant herbicide Reglone® prior to harvest to reduce interference at harvest, to improve skin-set and to control tuber size. These trials have shown that Reglone® did not increase the incidence or severity of *C. coccodes* compared to plants that senescenced naturally.

Where Amistar® was applied the total and marketable yields were similar to those where Reglone® was applied, except at site two where marketable yields were increased by 31%.

Removal of haulms and root tissue reduced the level of *C. coccodes* infection compared to Reglone® at only one site. A possible reason for this was the combination of lower disease incidence on seed used and the variation of temperature at plant maturity (2), resulting in a reduced disease pressure (4, 7). Site one matured during the warm summer months, which are more conducive to the growth of *C. coccodes*, whilst site two matured during the cooler Autumn months which are less favourable to the growth of *C. coccodes*. On the other hand the fungicide Amistar® applied one week after Reglone® had a major effect on reducing both incidence and severity of *C. coccodes* on tubers. The reasons for this are unclear. It may be that spores, which are washed down from the haulm through the soil to the tubers (10), were killed by Amistar® residues on the soil surface. It is unlikely that Amistar® either inhibited the spore production on the stems or restricted systemic movement of the fungus from the haulms.

The high incidence of tubers with infections at the stolon end suggests that the primary infection of daughter tubers is a result of the pathogen being introduced via the stolons. If the primary source of inoculum were the soil then it would be expected that the development of microsclerotia on the tubers would have been at random over the tubers surface (13). However with the majority of infections confined to the stolon end of tubers, it is highly possible that the primary source of inoculum was the original seed piece.

These results reinforce previous observations that seed without visible signs of *C. coccodes* may still be infected. Trial 2 was planted with clean seed into new ground, but between 15 and 88% of daughter tubers were infected in the different treatments. Further trials need to be done before the use of late season applications of fungicides can be recommended for the control of black dot.

**Trial 3.**

**Objective:** *To evaluate the effect of removing potato haulms or spraying Reglone® desiccant or Amistar® on potato haulms just before harvest for the control of C. coccodes.*

**Materials and Methods:**
A trial was conducted on new ground (sandy loam) in the Riverland of South Australia. Seed tubers cv. Coliban with 23% of tubers infected with *C. coccodes* were planted without any fungicides treatments. Tubers were planted 23.5cm apart in rows 80cm apart. Plots were 12m long by 16m wide, arranged in a randomised block design and replicated seven times. At 106 days post planting 17th April 2003 the following treatments were applied to the crop.

A Remove Haulm/roots completely
B Remove Haulm/roots completely (apply Trichoderma * “1.5kg/ha” 1 week later)
C Remove Haulm/roots completely (apply Amistar® “250g a.i/ha” 1 week later)
D Remove Haulms only
E Remove Haulms only (apply Trichoderma * “1.5kg/ha” 1 week latter)
F Remove Haulms only (apply Amistar® “250g a.i/ha”1 week later)
G Reglone® (3.5L/ha)
H Reglone® (Slash 1 or 2 weeks later)
I Reglone® (apply Trichoderma* “1.5kg/ha”1 week later)
J Reglone® (apply Amistar® “250g a.i/ha”1 week later)
K Amistar® 2 days prior to Reglone®
L Reglone® + Bordeaux (copper sulphate and hydrated lime 10:10:100) at 1 week

* “Trichogrow®” (25million cfu/gm) Agrimm technologies LTD

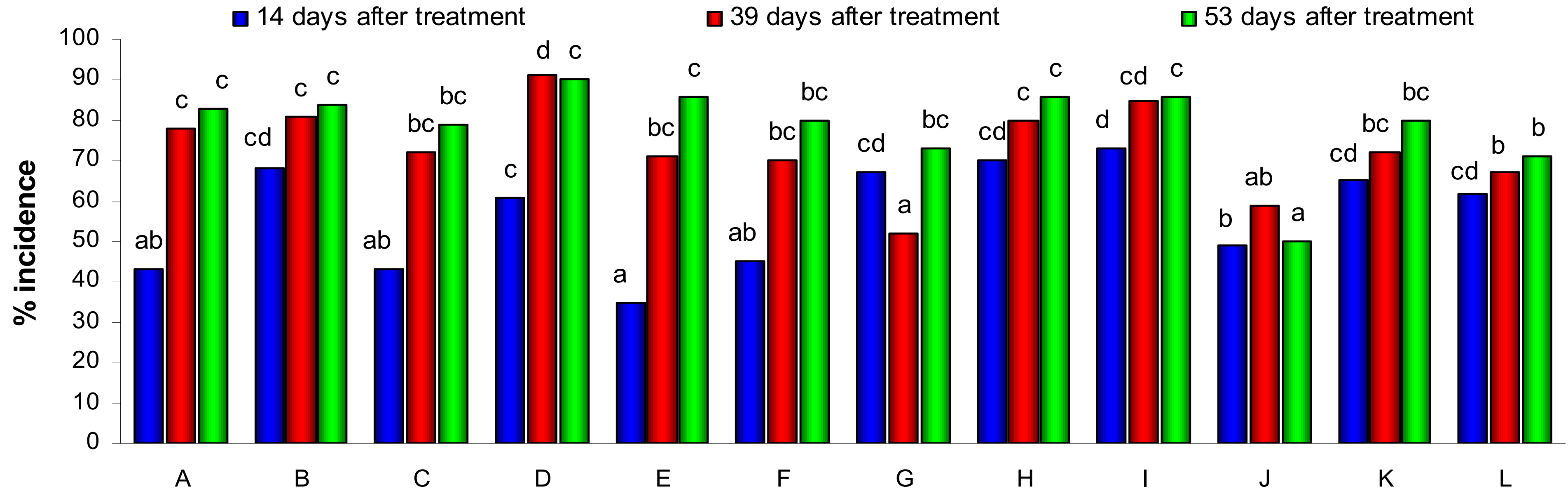
25 plants were harvested from each plot at 120, 145 and 159 after planting and yields calculated as previously described. A subsample of 100 tubers was washed and visually assessed for both incidence and severity of *C. coccodes* as previously described. In addition the incidence of infection by *C. coccodes* at the stolon end was recorded. Irrigation was applied approx every 7 days between 120 and 159 days.

**Results and Discussions:**
In nearly all treatments, except some where Reglone® was applied, harvesting early reduced the incidence of diseased tubers (Fig 48). The best treatments for early harvest (14 days after treatment) were removal of haulms and roots, or removal of haulms (+/- roots) and applying Amistar® or Trichoderma. At 39 days after treatment, Reglone® alone or Reglone® + Amistar® provided the best control. By 53 days after treatment, Reglone® + Amistar® one week later was the most effective treatment.

The severity of infection was more variable, at different times of harvest (Fig 49), Reglone® + Amistar® was again the most effective treatment at harvest time. The application of Amistar® after Reglone® also reduced the incidence of stolon infection on tubers (Fig 50).

These results show that the longer tubers remain in the ground after crop destruction, the more disease can occur. Crops that are destroyed earlier to suit market size may need to be ground stored for longer to allow tubers skins to harden before digging (71). This can increase disease levels if the conditions are suitable. However depending on the time of year, later harvesting may mean tubers are growing when the soil is warmer so may still have more disease. Further research is needed to develop a model that will be able to predict probable disease levels to assist in timing of destruction and subsequent harvest.

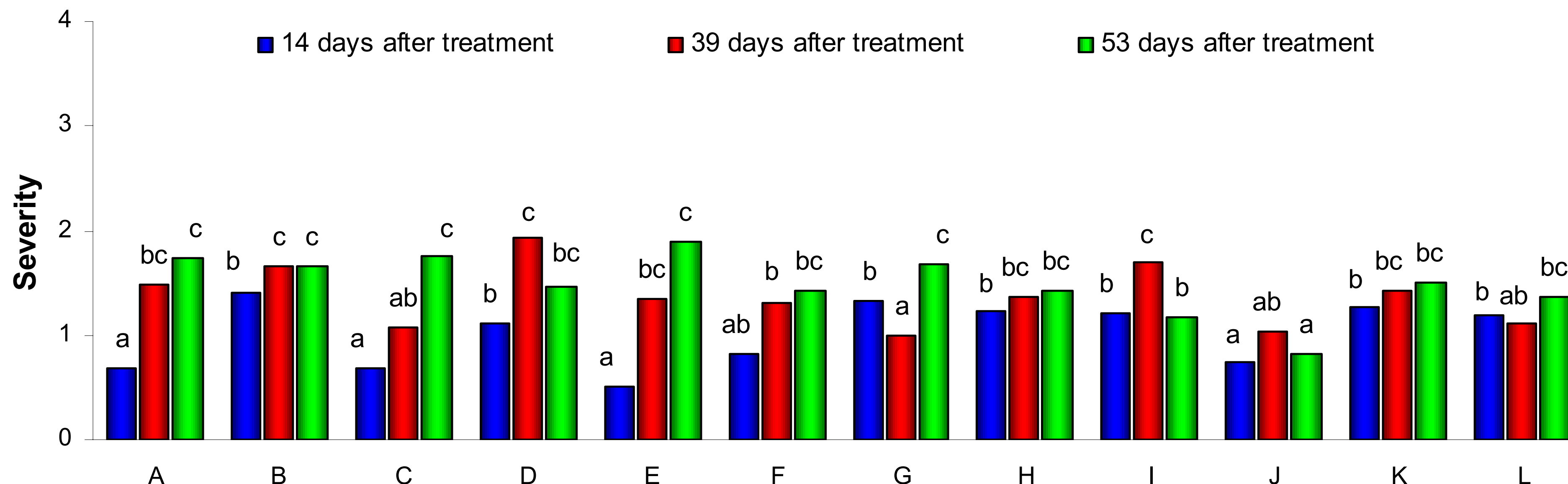
**Figure 49:** Effect of removing plant matter and or applying Amistar at maturity on the incidence of *C. coccodes* on daughter tubers when harvested at 3 times after treatments.




Treatments with the same letter are not significantly different from one another (LSD P=0.01).

| | | | |
|---|---|---|---|
| A | Remove Haulm/roots completely | G | Reglone® |
| B | Remove Haulm/roots completely (apply Tricho 1 week later) | H | Reglone® (Slash 1 or 2 weeks later) |
| C | Remove Haulm/roots completely (apply Amistar® 1 week later) | I | Reglone® (apply Tricho 1 week later) |
| D | Remove Haulms only | J | Reglone® (apply Amistar® 1 week later) |
| E | Remove Haulms only (apply Tricho 1 week latter) | K | Amistar® 2 days prior to Reglone® |
| F | Remove Haulms only (apply Amistar® 1 week later) | L | Reglone® + Bordeaux (copper sulphate and hydrated lime 10:10:100) at 1 week |

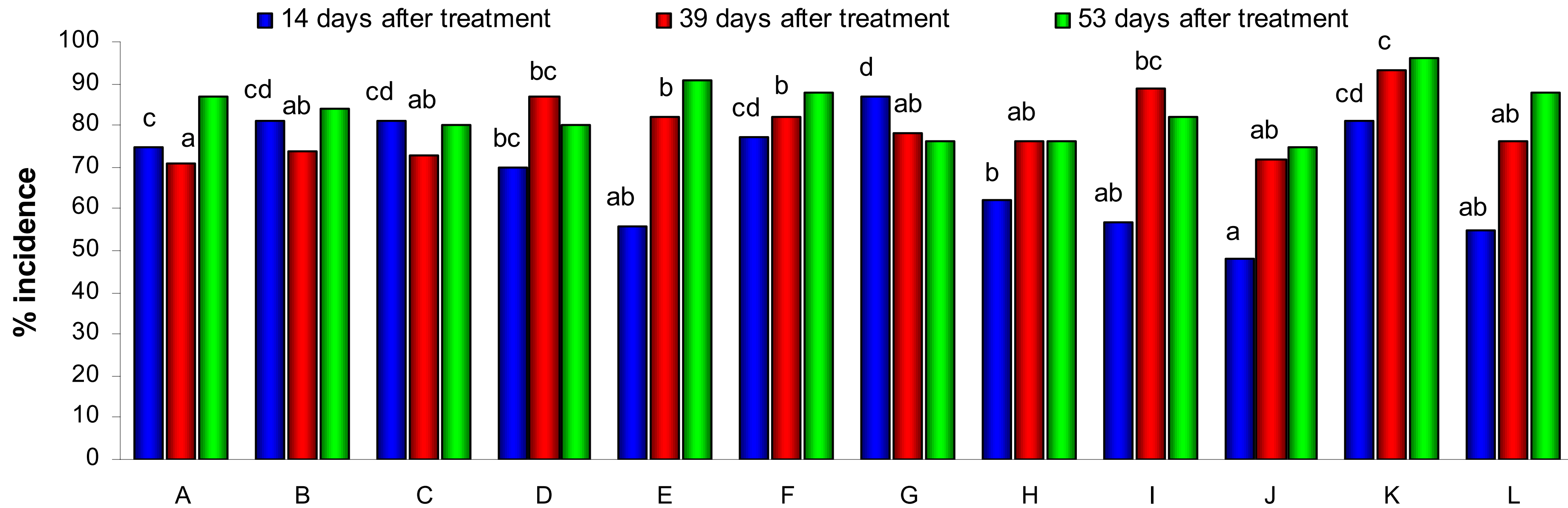
**Figure 50:** Effect of removing plant matter and or applying Amistar at maturity on the severity of *C. coccodes* on daughter tubers when harvested at 3 times after treatments.




Treatments with the same letter are not significantly different from one another (LSD P=0.01).

| | | | |
|---|---|---|---|
| A | Remove Haulm/roots completely | G | Reglone® |
| B | Remove Haulm/roots completely (apply Tricho 1 week later) | H | Reglone® (Slash 1 or 2 weeks later) |
| C | Remove Haulm/roots completely (apply Amistar® 1 week later) | I | Reglone® (apply Tricho 1 week later) |
| D | Remove Haulms only | J | Reglone® (apply Amistar® 1 week later) |
| E | Remove Haulms only (apply Tricho 1 week latter) | K | Amistar® 2 days prior to Reglone® |
| F | Remove Haulms only (apply Amistar® 1 week later) | L | Reglone® + Bordeaux (copper sulphate and hydrated lime 10:10:100) at 1 week |

**Figure 51:** Effect of removing plant matter and or applying Amistar at maturity on the incidence of *C. coccodes* infection at the stolon end of daughter tubers when harvested at 3 times after treatments.




Treatments with the same letter are not significantly different from one another (LSD P=0.01).

| | | | |
|---|---|---|---|
| A | Remove Haulm/roots completely | G | Reglone® |
| B | Remove Haulm/roots completely (apply Tricho 1 week later) | H | Reglone® (Slash 1 or 2 weeks later) |
| C | Remove Haulm/roots completely (apply Amistar® 1 week later) | I | Reglone® (apply Tricho 1 week later) |
| D | Remove Haulms only | J | Reglone® (apply Amistar 1 week later) |
| E | Remove Haulms only (apply Tricho 1 week latter) | K | Amistar® 2 days prior to Reglone® |
| F | Remove Haulms only (apply Amistar® 1 week later) | L | Reglone® + Bordeaux (copper sulphate and hydrated lime 10:10:100) at 1 week |

## TECHNOLOGY TRANSFER

Presentations of research findings contained in this report have been carried out via direct contact with growers, processors and other industry representatives and discussions with other researchers. Articles have been included in the Grower Magazine, Potato Australia and the Vegetable Platter (Appendix 1)

***Workshops:***

- Grower's workshops Pebinga (SA). June 2002.
- Biofumigation workshop (WA). September 2002
- Potato industry workshop in Mannum (SA). November 2003.
- Growers workshop Mannum (SA). February 2004
- Growers workshop Parilla (SA). February 2004
- Growers workshop Penola (SA). February 2004

Research outcomes on the impact of black dot and possible management strategies were presented to growers of fresh and processing potatoes, along with industry representatives from both packers and processors.

***Articles***

- Black dot in potatoes. Potato Australia. (2002). September, Volume 13, pp 38 – 39.
- Controlling Black dot on Potatoes. Veg Link. (2002). September.
- Black dot an underrated disease of potatoes in Australia. Potato Australia. (2003). September, Volume 14, pp 46 – 48.
- Black dot research. A4 newsletter (2004). July. (Received by 228 growers).
- Black dot research results. Potato Australia. (2004). September, Volume 15, pp
- Black dot…a major disease of potatoes. South Australian Growers Magazine (2004). April, pp 15 - 16.

***Conference presentations:***

- Effects of haulm removal, Reglone® desiccant and Amistar® on the levels of black dot (*Colletotrichum coccodes*) on potatoes. (Presentation) 3rd Australasian Soil borne Disease Symposium. (2004). February

- Disease progress of black dot *Colletotrichum coccodes* on potato tubers and roots. (Poster) 3rd Australasian Soil borne Disease Symposium. (2004). February

- Evaluation of fungicides for the control of *Colletotrichum coccodes* (black dot) on potatoes. (Presentation). 3rd Australasian Soil borne Disease Symposium. (2004). February

- The effect of temperature on the viability and growth rate of black dot (*Colletotrichum coccodes)* in-vitro. (Poster) 3rd Australasian Soil borne Disease Symposium. (2004). February

- History of black dot. 3rd Biennial Seed Potato Industry Workshop and Trade Display. (2003). August.

- Black dot Research Results. 3rd Biennial Seed Potato Industry Workshop and Trade Display. (2003). August.

- Black dot Alternative Hosts & Cultivar susceptibility. 3rd Biennial Seed Potato Industry Workshop and Trade Display. (2003). August.

- Evaluation of fungicides as a potato seed tuber treatment for the control of black dot. (Presentation/Poster), 8th International Congress of Plant Pathology in Christchurch, New Zealand, incorporating the 14th Australasian Plant Pathology Conference. (2003). February.

## RECOMMENDATIONS – SCIENTIFIC AND INDUSTRY

**The following recommendations for the management of black dot have been made based on the results of this report.**

**Cultural:**

- Reduce host weed populations during the rotation period.
- Delay plantings that result in crops maturing when soil temperatures (20cm depth) are at or above 24$^{o}$C.
- Use clean certified seed from established seed growers and inspect seed in the field or in storage prior to purchase.
- Where possible harvest the crop early.
- Reducing the amount of water between spray off and harvesting.
- Haulm removal

**Seed treatments:**

- Where disease or soil is present on tubers, treat seed with Maxim® and Amistar®.
- Use fungicide treated seed only where soil inoculum is low or absent.

**Chemical:**

- Apply an in furrow treatments of Amistar® (250g ai/ha ) at planting.*
- Apply a foliar application of Amistar® (250g ai/ha) 1 week after haulm desiccation.*

  *NB: **Amistar® is not registered for this use on potatoes*

*fumigation*

- Biofumigation may be affective, but further research is required to develop techniques and products that provide consistent control

- Where soil levels of black dot are high chemical fumigation may be useful but is not always effective.

**These studies have identified several new chemical and cultural methods to control the disease black dot. However there are a number of areas in which further research is recommended so that growers can have a greater range of integrated pest management options available.**

- Test certified seed tubers for *C. coccodes* to determine seed lots to be treated with fungicides.
- Test *C. coccodes* isolates from weed host for pathogenicity on potatoes.
- Testing of isolates obtained from potatoes and weed hosts from different regions for analysis of their DNA and VCG's.
- Measure soil populations of *C. coccodes* in different regions and soil types to determine economical threshold levels.
- Test Australian potato cultivars for resistance to *C. coccodes*
- Further monitor disease progress to develop a model to predict the potential incidence and severity of the disease on tubers at harvest.
- Evaluate bioassays and DNA probes to detect *C. coccodes* in soil and tubers.
- Trials to evaluate the timing/dose and fungicide type.
- Evaluate different *C. coccodes* isolates for resistance to chemicals.
- Further evaluate strobilurins fungicides as seed or in-furrow treatments
- Evaluate alternative techniques such as the application of water several months before planting potatoes or the application of small amounts of glucose (22) or starch to stimulate the germination and sporulation of inoculum before planting.
- Development of products that will disrupt the formation of appressoria on tubers. These are stimulated to adhere to the host surface in response to host leachates and by the presence of bacteria, which cause nutrient stress. They are however inhibited by high temperatures (45).

## ACKNOWLEDGMENTS

We wish to thank potato growers of South Australia for their co-operation in allowing field trials to be conducted on their properties. We also gratefully acknowledge the Horticultural Australia LTD, Australian government and Potato industry for financially supporting this work and Miss Carol Horsman for field studies and technical assistance. In addition, we are grateful to the staff at the Plant Research Centre and Lenswood Research Centre for their help in undertaking this project.

## DISCLAIMER

The results in this report are submitted on the basis that the tests were conducted by SARDI in accordance with the protocol requested by or agreed to by your company, and using test chemicals provided by your company, based on scientific information currently available to SARDI.

Neither SARDI nor its officers accept any liability resulting from the interpretation or use of the information contained herein. Use of information is at the risk of the user to the extent permissible by law.

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## APPENDICES

Scientific and common names of weeds that host *C. coccodes* in the USA (55)

| Family | Scientific name | Common name |
|---|---|---|
| *Amaranthaceae* | *Amaranthus albus* | Tumble pigweed |
| | *A. retroflexus* | Redroot pigweed |
| *Chenopodiaceae* | *Chenopodium album* | Common lambs- quarters |
| *Compositae* | *Cirsium arvense* | Canada thistle |
| *Convolvulaceae* | *Convolvulus arvensis* | Field bindweed |
| *Cruciferae* | *Capsella bursa-pastoria* | Shepherds purse |
| *Gramineae* | *Agropyron repens* | Quackgrass |
| | *Digitaria sanguinalis* | Large crabgrass |
| | *Panicum dichotomiflorum* | Fall panicum |
| | *Setaria lutescens* | Yellow foxtail |
| *Malvaceae* | *Abutilon theophrasti* | Velvetleaf |
| *Oxalidaceae* | *Oxalis stricta* | Common yellow wood sorrel |
| *Polygonaceae* | *Polygonum pennsylvanicum* | Pennsylvania smartweed |
| *Solanaceae* | *Solanum dulcamara* | Bitter nightshade |
| | *S. nigrum* | Black nightshade |
| Unknowns | | Common chickweed |
| | | Saltwort |
| | | Bermudagrass |
| | | Hemp |
| | | jimsonweed |

**Black dot on below ground stem tissue**

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

**Black dot and silver scurf.**

1= <1/4 tuber surface area covered
2= 1/4 - 1/2 tuber surface area covered
3= 1/2 - 3/4 tuber surface area covered
4= >3/4 tuber surface area covered