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Rhizoctonia control on fresh market potatoes

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South Australian Research & Development Institute

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PT315

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Part C

Brassica species as potential green manure crops for control of _Rhizoctonia_

INDUSTRY SUMMARY

Survey of potato tuber seed showed that black scurf (*Rhizoctonia*) was wide spread on tubers throughout the South East of Australia. On some tuber seed lots, all the tubers were infected with *R. solani*. Although formaldehyde dips are widely used to treat tubers, there are a number of different recommendations for the strength of solutions to use and the length of dipping time. As the efficacy of each of these has been uncertain, experiments were undertaken to determine the most effective treatment for controlling tuber borne inoculum of *Rhizoctonia*.

Experiments conducted at the Lenswood Research Centre showed that a 20 minute dip in a 2% a.i. solution of formaldehyde killed all the *R. solani* on infected tubers. This dipping time is considerably longer than previous recommendations. Dipping tubers in concentrations less than this or in shorter times was not effective.

Sodium hypochloride has also been recommended as a dip to treat potato tuber seed, but our work has shown that a 20 minute dip in a 2% solution was not effective in killing sclerotes of *R. solani*. A number of other chemicals were also evaluated as tuber seed treatments and this work showed that treating tubers with either of the fungicides Rizolex, Monceren or Rovral controlled *R. solani*.

A naturally occurring fungus, *Verticillium biguttatum* was isolated from sclerotes and found to attack and kill sclerotes of *R. solani*. Experiments in the laboratory and the field showed that in some cases this fungus was as effective as chemicals in controlling sclerotes on infected tubers.

Field experiments also demonstrated the importance of both soil and seed borne inoculum in programmes used to control *R. solani*. Although fumigation with 500L/ha metham sodium reduced soil levels of *Rhizoctonia*, particular care was needed to be taken to eliminate *R. solani* from tuber seed when fumigated soil or new areas were planted.

The potential of growing *Brassica* green manure crops as a means of reducing soil levels of *R. solani* was investigated in both the laboratory and the field. While laboratory experiments showed gases from *Brassica* meal killed *R. solani*, field experiments incorporating *Brassica* plants into the soil were not conclusive.

Further experiments evaluating different cultivars, seeding rates and cultivation treatments are required before this technique can be recommended as a means of controlling the soil borne phase of *Rhizoctonia*.

TECHNICAL SUMMARY

Chemical and biological treatments for the control of tuber-borne inoculum of *R. solani* on potatoes were evaluated by testing the viability of sclerotes removed from the treated tubers. This technique showed that most sclerotes adhering to the tuber surface were devitalised when tubers were dipped for 20 minutes in a 2 % solution of formaldehyde.

Dusting tubers with tolclofos-methyl, or spraying them with fenpiclonil or pencycuron, gave control equal to formaldehyde, whereas a sodium hypochlorite dip was ineffective. A spore suspension of *V. biguttatum* applied to tubers as either a dip or spray devitalised >90% of treated sclerotes, whereas *Bacillus, Gliocladium* and *Trichoderma* were ineffective.

The importance of seed borne inoculum in the control of *R. solani* was demonstrated in field experiments where potato seed tubers treated with chemicals or biological agents were planted in soil with or without fumigation using 500 L/ha metham sodium.

Tuber treatments of either a 20 minute dip in 2% formaldehyde, sprays with pencycuron 0.15 ml a.i./10kg seed, iprodione 2 ml a.i./10kg seed or a spore suspension of 10^6 spores/ml of *V. biguttatum* or a dust with tolclofos methyl 4g a.i./10kg seed were most effective if planted in fumigated soil or soil with low levels of *R. solani*. A commercial formulation of *Trichoderma harzianmum* and *T. konigii* applied as a dust at 1.3g/10kg seed was in most cases ineffective when treated seed was planted into either fumigated or non-fumigated soil.

The incidence of progeny tubers with sclerotia varied between sites and ranged from 85% in unfumigated soil planted with infected tubers to 2% in a fumigated soil planted with Monceren treated tubers.

Except for one experiment, where tubers were harvested early, neither seed treatments nor fumigation improved total or marketable yield.

TECHNICAL REPORT

See attached articles

(a) Chemical and biological control of *Rhizoctonia solani* on potato seed tubers.

In: Australian Journal of Experimental Agriculture (1995) 35: *661-64*

and

(b) The influence of soil fumigation and tuber seed treatment on the control of *Rhizoctonia solani* on potatoes.

In: Australian Journal of Experimental Agriculture (1996) 36: *339-45*

In addition to the work documented in the above reports, studies were undertaken on several other aspects of *R. solani* control. These included the isolation and further evaluation of the mycoparasite *V. biguttatum* and evaluation of *Brassica* green manure crops as a means of biofumigation.

TECHNICAL REPORT - 1995/96

Introduction

Rhizoctonia solani is one of the most serious and widespread diseases of potatoes in Australia. The fungus attacks underground stems, roots and tubers, resulting in plant death and yield reductions of up to 30%, Hide *et al* (1992).

Rhizoctonia affects the size, shape and appearance of tubers. Skin blemishes caused by sclerotes adhering to the tuber surface are difficult to remove by washing and brushing, and are the major cause of market rejection of washed potatoes.

The disease is widespread throughout the main potato growing areas within Australia, and is particularly severe in new potato growing areas such as the Riverland and other inland growing areas. *Rhizoctonia* could be an important factor limiting the future expansion of the industry in these districts. On some properties in South Australia, *R. solani* has severely affected plant establishment and tuber quality to the extent that the livelihood of many fresh market potato growers has been threatened.

Few studies have been conducted on the control of *R. solani* on potatoes in Australia. Fungicides recently developed for control of *R. solani* (Adam and Malcolm 1988, Katari *et al* 1991), have not been critically evaluated as soil treatments in Australia.

Similarly, antagonistic fungi and bacteria that have controlled *Rhizoctonia* overseas have not been evaluated in Australia, although certain fungi and bacteria are being evaluated at the CSIRO in Adelaide specifically for the biological control of *R. solani* in nursery crops. These fungi and bacteria may have the potential for *R. solani* control in potatoes and need to be evaluated in the field.

Another method that has potential to control *R. solani* and other soil born pathogens of potatoes is the technique of injecting the soil sterilant metham sodium in a band beneath the soil surface. However the efficiency and economics of this treatment needs critical evaluation before it can be recommended to the industry.

The aim of this project was to improve potato skin quality by controlling *R. solani* and other major soil borne fungal diseases in Australia. Extensive laboratory, glasshouse and field experiments were conducted over three years to evaluate the efficacy of several antagonistic fungi and bacteria for the control of soil borne inoculum.

A bioassay technique was developed to screen potential biocontrol agents. This method was used to compare local isolates of fungi and bacteria with isolates obtained from overseas.

One of the most promising agents was a local isolate of *Verticillium biguttatum*, which killed sclerotes on treated tubers and controlled *R. solani* to levels similar to that achieved with fungicides in the field. A survey of *R. solani* infected tubers from the main potato growing areas in SA was conducted to collect further isolates of *V. biguttatum* and compare their ability to kill *R. solani* inoculum in the field.

Field experiments were undertaken to evaluate control of *R. solani* with recently developed chemicals and integrated chemical and biological control methods. Experiments comparing various concentrations of formaldehyde and fungicides such as Rizolex and Monceren were conducted for the control of *R. solani*.

Laboratory and field experiments were carried out evaluating certain *Brassica* species as green manure crops for the control of *R. solani* inoculum in the soil. This followed work by Kirkegaard, (1995) who evaluated Indian Mustard as a biocontrol of *R. solani* in broad acre crops.

Experiments were conducted in the laboratory and field evaluating Indian Mustard stems, roots and meal for control of *R. solani*. In the field, Indian and Ebony Mustard and Rangi Rape were evaluated as potential green manure crops in two main potato growing areas in South Australia.

PART A

SCREENING POTENTIAL ANTAGONISTS OF *RHIZOCTONIA*

Methods and Materials

A series of experiments were undertaken to screen potential antagonists in the laboratory for their ability to kill *R. solani* sclerotes on potatoes in comparison to the fungicide Monceren. The bioassay technique involved applying the antagonists to tubers with 10 or more sclerotes per tuber and incubating the tubers for 2 weeks at 20°C. Sclerotes were removed and the viability of the sclerotes tested by plating them onto Tap Water Agar (TWA) and checking for *R. solani* mycellial growth after 2 to 4 days incubation.

Antagonists used in these screening tests were either isolated from naturally infected sclerotes collected from the field or donated by researchers from Australia and overseas.

Nine isolates of *V. biguttatum* were isolated from naturally infected *R. solani* sclerotes on tubers collected from major potato growing regions in South Australia. The sclerotes were removed from the tuber and placed in large petri dishes lined with moist filter paper. The petri dishes were incubated for two weeks at 20^0C. After this time, spores from *V. biguttatum* colonies growing on the sclerotes were removed with a fine tipped needle and transferred on PDA plates, which were then incubated at 20^0C for 2 weeks.

Of the nine isolates detected, six were reisolated with no bacterial infections and were used in the screening tests. The isolates 1 and 2 were tested first, and were retested 12 months later with isolates 3 to 6.

An isolate of *Gliocladium roseum* obtained from naturally infected sclerotes on tubers collected from the field was tested with two samples of *Gliocladium virens* obtained from overseas.

An unknown *Bacillus* species recovered from the rhizosphere of potatoes grown in Virginia, which was shown to be inhibitory to *R. solani* in vitro (Balali unpublished 1994), was screened along with three isolates of *Bacillus subtilis* (Dr. A Harris, CSIRO, Private Mailbag 2, Glen Osmond, 5064. S.A.) and Actizyme and Kodiak, which are commercial formulations of *Bacillus subtilis* (Gustafson, Inc., 1400 Preston Rd, Suite 400, Plano, TX 75093, USA).

A commercial formulation of *Trichoderma harzianum* and *T. koningii* known as Tri-D25, (J.B. Inc., PO Box 7493, Ventura, CA 93006, USA) was compared to two isolates of *Trichoderma harzianum* obtained from overseas.

Other antagonists screened included a culture of *Pseudomonas* spp. (Dr. Y. Huang, University of Sydney, NSW, 2006), a sample of Bokashi consisting of Mk 2 (liquid) and Mk 3 (granules), (Austbloom Pty Ltd, Mt Stapylton RMB 740 Horsham, 3401, Vic) and three isolates of Binucleate *Rhizoctonia* (Dr. A Harris, CSIRO, Private Mailbag 2, Glen Osmond, 5064. S.A.).

Each antagonist was applied to 20 tubers naturally infected with ten or more sclerotes per tuber.

A 200ml spore suspension, at 10^6 spores/ml, of each *V. biguttatum, Trichoderma, Gliocladium*, *Bacillus* and *Pseudomonas* isolate was sprayed onto the tubers. The spore suspension of each isolate was prepared by placing 10mls of sterile water onto the colonies growing on PDA plates, and then shaking the plates to loosen the spores into the water, which was then poured off the plates. The suspension was sprayed onto the infected tubers using a hand held sprayer.

The Actizyme was prepared by placing 6gms of pellets into 100ml of water and left to stand for 30 minutes to allow the pellets to dissolve. The mixture was filtered through muslin and then sprayed onto the infected tubers with a hand held sprayer. The Kodiak was dissolved in sterilised water at the recommended rate of 0.62gms/L. The infected tubers were rolled around in plastic bags containing the Kodiak mixture.

The tubers treated with Bokashi were first sprayed with 200mls of the Mk2 liquid and then rolled in the Mk 3 granules until completely covered. With the Binucleate *Rhizoctonia,* ten 5mm PDA plugs were placed next to sclerotes on the tubers.

The Monceren powder (12.5% a.i.) was applied by shaking tubers and the powder in a plastic bag at a rate of 20gm/10kg of tubers. Tubers treated with a combination of Monceren and *V. biguttatum*, were initially dusted with 1/4 recommended concentration of Monceren and then sprayed with 200 mls of the *V. biguttatum* spore suspension. The untreated tubers were sprayed with 200ml of sterile water.

After treatment the tubers were placed into trays lined with a moist cloth which were then sealed in a plastic bag to create humid conditions. The trays were incubated for 2-3 weeks at 20^0C.

After incubation, ten sclerotes were removed from each tuber and transferred onto TWA plates. The plates were incubated at 20^{0}C for 24 hours. The viability of each sclerote was checked after 24 hours by examining the plates for mycellial growth from each sclerote. The sclerotes were scored as viable provided typical *R. solani* mycellial growth occurred.

The tubers sprayed with each *V. biguttatum* isolate were incubated and then ten sclerotes and ten pieces of hyphae next to the sclerotes were transferred onto TWA plates, incubated and examined as above.

Results

The results of several experiments have been summarised in tables 1 & 2.

Table 1: The percentage of viable sclerotes recovered from tubers after treatment with antagonists or chemicals and incubated at 20^{0}C for 2-3 weeks - 1994.

TREATMENT	TYPE	PERCENTAGE OF VIABLE SCLEROTES
Control	no treatment	99
V. biguttatum - isolate 1	fungus	17
Verticillium + Monceren	fungus + chemical	0
Trichoderma *	fungus	99
Bacillus (Kodiak)	bacteria	92
Bacillus (Actizyme)	bacteria	87
Monceren	chemical	10

*** Several isolates tested**

Table 2: The percentage of viable sclerotes recovered from tubers after treatment with antagonists or chemicals and incubated at 20°C for 2-3 weeks - 1995.

TREATMENT	TYPE	PERCENTAGE OF VIABLE SCLEROTES
Control	no treatment	85-100
V. biguttatum - isolate 1	fungus	74
V. biguttatum * isolates 2 to 6	fungus	56-77
Trichoderma *	fungus	99-100
Gliocladium *	fungus	69-79
Binucleate *Rhizoctonia**	fungus	40-65
Bacillus *	bacteria	58-95
Bacillus (Kodiak)	bacteria	69-95
Pseudomonas ssp.	bacteria	95
Bokashi	bacteria/yeast base	57-100
Monceren	chemical	0-10

*** Several isolates tested**

These show that 85-100% of the sclerotes on the untreated tubers were viable in all experiments. The combination of *V. biguttatum* and Monceren was the most effective treatment as none of the sclerotes were viable after 2 to 3 weeks incubation.

The effect of *V. biguttatum* on *Rhizoctonia* was extremely variable between different isolates and between the same isolate in different experiments. For example, isolate 5 killed 44% of sclerotes, whereas the other isolates killed 23-31%. In 1994, isolate 1 killed 83% of the sclerotes but in 1995 only killed 26%.

Between 15-39% of the hyphal strands taken from *V. biguttatum* treated tubers were viable, compared to 63% from untreated tubers. Isolate 5 killed 86% of the hyphal strands, and isolates 2, 3, 4 and 6 killed 71-78%, whereas isolate 1 killed 61%.

Isolates of *Trichoderma* and *Pseudomonas* were ineffective as 95-100% of the sclerotes were viable after three weeks incubation. *Bacillus, Gliocladium* and Bokashi were also ineffective as the number of sclerotes viable after treatment ranging from 57-100% (Tables 1 & 2).

Discussion

The results show that of the organisms tested, *V. biguttatum* was most effective in reducing the viability of *R. solani* sclerotes although there was considerable variability in the antagonistic activity between isolates. Of particular concern is the reduced viability of isolate 1 which occurred after storage and culture over 12 months. Further work is required on the activity of this antagonist as field experiments showed *V. biguttatum* to be as effective as chemicals in some situations. *V. biguttatum* has controlled *Rhizoctonia* on potato in the Netherlands (van der Boogert, 1992) and further collaboration with these workers is required if the fungus is to be developed into a commercial product for use in Australia. Other antagonists such as *Bacillus, Gliocladium*, Bokashi and binucleate *Rhizoctonia* showed little activity in these experiments and do not warrant further investigation.

Overall, treating sclerotes with organisms and checking for viability was a simple and effective means of screening potential biocontrol agents for *Rhizoctonia*.

Table 3: Summary of antagonistic activity against *R. solani* sclerotia

ORGANISM	TYPE	ACTIVITY
Verticillium * (1994)	fungus	++++
Verticillium * (1995)	fungus	+
Trichoderma *	fungus	+
Gliocladium *	fungus	+
Bacillus *	bacteria	+
Pseudomonas ssp.	bacteria	+
Bokashi	bacteria/yeast base	+
Binucleate *Rhizoctonia* *	fungus	+
Monceren	chemical	++++

*** Several isolates tested**

PART B

VERTICILLIUM BIGUTTATUM AS POTENTIAL BIOCONTROL OF *RHIZOCTONIA*

Laboratory and Field Experiments

(a) Survey

A survey was undertaken to determine the incidence of *V. biguttatum* in the main potato growing areas of South Australia. Potatoes infected with *R. solani* sclerotes were collected from Northern Adelaide Plains, Adelaide Hills, The Lakes, Riverland, Mallee and South East regions of South Australia. Sclerotes were removed from potatoes placed in large petri dishes lined with moist filter paper and incubated at 20^0C for 2-3 weeks. After this period the sclerotes were examined for the presence of *V. biguttatum* growing on the surface.

A fine tipped needle was used to remove spores of *V. biguttatum* and transfer them onto PDA. These were then incubated for 2 weeks at 20^0C.

(b) Screening *Verticillium biguttatum* isolates

Laboratory experiments were conducted to evaluate the antagonistic activity of six *V. biguttatum* isolates against *R. solani*. The isolates were obtained from naturally infected

sclerotes on tubers collected from the Northern Adelaide Plains, The Lakes, Riverland and South East regions of South Australia. The method used to screen these isolates are described in part (A).

(c) Growth Rates and spore production of *V. biguttatum* isolates.

Laboratory experiments were conducted to measure the growth rate of the six *V. biguttatum* isolates at various temperatures, and to compare the growth rates to an Ag 3 isolate of *R. solani*. This was done to see if Australian isolates of *V. biguttatum* behaved similarly to those from the Netherlands and the U.K.

The growth rates of the *R. solani* and *V. biguttatum* isolates were determined by placing 5mm plugs of each isolate onto 5 PDA plates and then incubating the plates at 5, 10, 15, 17, 20, 22.5, 25, 30 and 35^{0}C for two weeks. The radial growth of the *V. biguttatum* isolates were measured weekly, and the *R. solani* isolate every two days due to the faster growth rate.

After 2 weeks incubation a spore suspension of each *V. biguttatum* isolate grown at each temperature was prepared as described in Part A. Spore production was determined by counting spores using a neubauer haemocytometer and calculating the number of spores in 1ml of the suspension. The length and width of ten spores from each solution were measured under the 40x objective on the compound microscope.

(d) Antagonistic activity of *V. biguttatum* during Cool Room Storage

This experiment was conducted to evaluate if *V. biguttatum* applied before cool storage was effective in controlling *R. solani* on seed potatoes.

Sixty cured Atlantic minitubers infected with ten or more sclerotes were placed in onion bags for each treatment.

Spore solutions of *V. biguttatum* were prepared from the six isolates used in the previous laboratory experiments. Colonies of *V. biguttatum* were cut out from PDA plates and macerated with sterile water in a food blender. The blended mix was strained through muslin and then diluted to produced a 10^6spores/ml suspension. Treated tubers were dipped in the suspension for 60 seconds and either stored normally or in high humidity.

After treatment, the tubers were placed on a pallet and stored in a cool room at 4°C for three weeks. Half of the bags of tubers were kept moist during storage by covering them with damp hessian bags.

After cool room storage, 20 tubers from each treatment were removed to test the viability of the sclerotes. Ten sclerotes from each tuber were transferred onto TWA plates and incubated at 20^0C, for 24 hours. Sclerotes with typical *Rhizoctonia* mycellial growth on the TWA plates were scored as viable.

Field Experiments

(e) Evaluation of *V. biguttatum* and commercial chemical and biological products for the control of seed borne and soil borne *R. solani* on potatoes.

A field experiment was conducted on a commercial potato growing property at Virginia, 50km north of Adelaide, South Australia. The soil, which is predominantly red brown earths, had been fallowed for three years. Potatoes grown at the site in 1992 were heavily infected with *R. solani*. Sequoia seed tubers with 5% *R. solani* infection were used in this experiment.

Seed treatments of *V. biguttatum* and Rizolex, and soil drench treatments of *V. biguttatum* and Tri-D25 (as described in Part A) applied at emergence and four weeks later, were evaluated for the control of *R. solani*..

The seed treatments included untreated tubers, tubers sprayed with *V. biguttatum* spore suspension, and tubers dusted with Rizolex. A 10^6 spores/ml suspension of *V. biguttatum* was prepared in the laboratory using the method as described in (d). The spore suspension was applied to the tubers on the day of planting with a Mantis Mist Sprayer. The seed was evenly covered with the suspension as the tubers rolled thought the spray unit on a conveyor belt system. Rizolex was applied by the grower.

The seed was planted at 45cm intervals within 30cm wide rows. The number of plants that emerged in each treatment was recorded in at least a 100m of each treated row at 45 and 56 days after planting.

The drench treatments included an application of either Tri D25 or a suspension of *V. biguttatum* to the soil at emergence only and at emergence and four weeks later. The Tri-D25 was applied at a rate of 1gm/20L and the *V. biguttatum* was applied at $4x10^6$ spores/ml. The drench treatments were replicated twice in each seed treatment area, with 20L of the suspension being applied to 15m x 0.3m plots. The treatments were applied to the soil using a knapsack sprayer, with the spray nozzle removed. Drench treatments were timed so that the applied inoculum was leached into the soil by rain or irrigation.

Eight weeks after emergence, 8 plants were collected from each plot and assess for the presence of stem lesions caused by *R. solani*. The lesions were assessed using a rating systems of 0-5, similar to the system described by Frank *et al* (1976). The severity of the lesions on the stems was converted to a scale of 0-100 described by Wicks *et al* (1994).

At 18 weeks after emergence, 25 tubers from each plot were harvested by hand. The tubers were weighed and then assessed for the incidence and severity of *R. solani* and Powdery Scab. The diseases were rated on a scale of 0-4, similar to the system described by Dijst, (1985).

(f) Comparison of *V. biguttatum* seed and soil drench treatments for the control of *R. solani* on potatoes

A field experiment was conducted to evaluate *V. biguttatum* as a seed treatment and soil drench for the control of *R. solani* on potatoes at the Lenswood Research Centre, in the Adelaide Hills.

The experimental site, which is predominantly sandy loam soils, had been planted to potatoes in the previous year. The soil had not been fumigated in the previous experiment, but one third of the area had been incorporated with Indian Mustard.

Due to low levels of *R. solani* observed on the bioassay plants grown in soil collected from the site and volunteer plants, the experimental site was inoculated with a mycellial suspension of *R. solani.* This was done by macerating 30 culture plates (90 mm) of *R. solani* in a blender and then diluting the suspension with 20L of sterile water. The suspension was applied to the soil at approximately 30 ml/m row using a knapsack sprayer with the nozzle removed.

The area was planted with Coliban potatoes which were examined during storage and found to have 4% of the tubers infected with *R. solani.*

The treatments included untreated seed, *V. biguttatum* treated seed, soil drenched with a *V. biguttatum* spore suspension at emergence only, drenched with *V. biguttatum* suspension at emergence and again four weeks later, and planting seed treated with Monceren and then drenching the soil with a *V. biguttatum* suspension at emergence.

A 10^6ml/spore suspension of *V. biguttatum* was applied to the tubers on the day of planting using a hand held spray gun. The suspension was prepared in the laboratory, using the method mentioned in experiment (d). Monceren was applied to tubers at 20gms/10kg, by shaking tubers in a plastic bag containing the 12.5% a.i. formulated powder.

Each treatment was replicated 12 times, in 15m x 0.75m plots. The tubers were planted at 16cm intervals. Plant emergence was recorded at 3 and 6 weeks after planting.

A 10^6spores/ml *V. biguttatum* suspension was applied to the drench treatment plots at a rate of 20L/plot, using a knapsack sprayer with the spray nozzle removed. Immediately after applying the spore suspension the area was irrigated for at least an hour.

Eight weeks after emergence, eight plants were collected from each plot and assessed for the presence of *R. solani* lesions on the stems. The lesions were assessed using the same rating system as described in experiment (e).

The potatoes from each plot were harvested 18 weeks after emergence, using a single row harvester. The potatoes from each plot were weighed and 100 tubers randomly selected were assessed for the incidence and severity of *R. solani*, Black Dot and Powdery Scab. The diseases were assessed using the same rating system as described in experiment (e).

(g) Evaluation of chemical and biological products for the control of *R. solani* on potatoes in fumigated and unfumigated soil

This field experiment was conducted to evaluate chemical and biological control of *R. solani* on potatoes grown in fumigated and unfumigated soil, on a commercial potato growing property at Virginia, about 50km north of Adelaide. The soil type was predominantly red brown earths.

The experimental site was planted to potatoes in the previous year, and in fallow for the three years prior to the potato crop. The experimental site was divided into two sections; one half fumigated with Vapam 330L/ha four weeks before planting, and the other half unfumigated. Coliban seed was used in the experiment, of which 100% were infected with *R. solani* and 38% with Silver Scurf.

Tuber seed treatments included untreated tubers, tubers dipped in Formaldehyde, and tubers sprayed with either Monceren, *V. biguttatum* or 1/4 recommended concentration of Monceren plus *V. biguttatum.*

The Formaldehyde treatments were applied by placing the tubers in onion bags and dipping them in a 60L vat containing 2% a.i. Formaldehyde solution for 20 minutes. The tubers were rinsed with water 3 times and planted five days later.

Monceren (25% a.i.) at a rate of 60ml/100kg of seed, was diluted in 200ml of water and applied to the seed with a hand held sprayer as the tubers were rotated in a hand driven cement mixer. A 2 x 10^6spores/ml suspension of *V. biguttatum* was prepared in the laboratory using the method mentioned in experiment (d). Approximately 200ml of suspension was used to cover 10kg of seed using the cement mixer. Tubers treated with *V. biguttatum* and 1/4 concentration of Monceren were placed in the cement mixer and sprayed with each agent. The treated tubers were then placed in moist hessian bags and kept in humid conditions for two days before planting.

Eight replicates of each treatment was planted in the fumigated and unfumigated areas. The tubers were planted at 14cm intervals in 25m x 0.86m plots. The number of plants that emerged in each treatment was recorded at 15, 22, 36 and 43 days after planting.

At eight weeks after planting, 20 plants were collected from the control plots and the stems assessed for the incidence and severity of *R. solani* lesions using the rating system described in experiment (e) and the incidence of pruned stolons.

At 18 weeks after planting, 8 potato plants in each plot were assessed for the presence of Black Dot on the stems.

The potatoes were harvested 19 weeks after planting with a single row harvester. The tubers from each plot were weighed and 100 tubers selected at random assessed for the incidence and severity of *R. solani*, Black Dot and Silver Scurf using the rating system described in experiment (e).

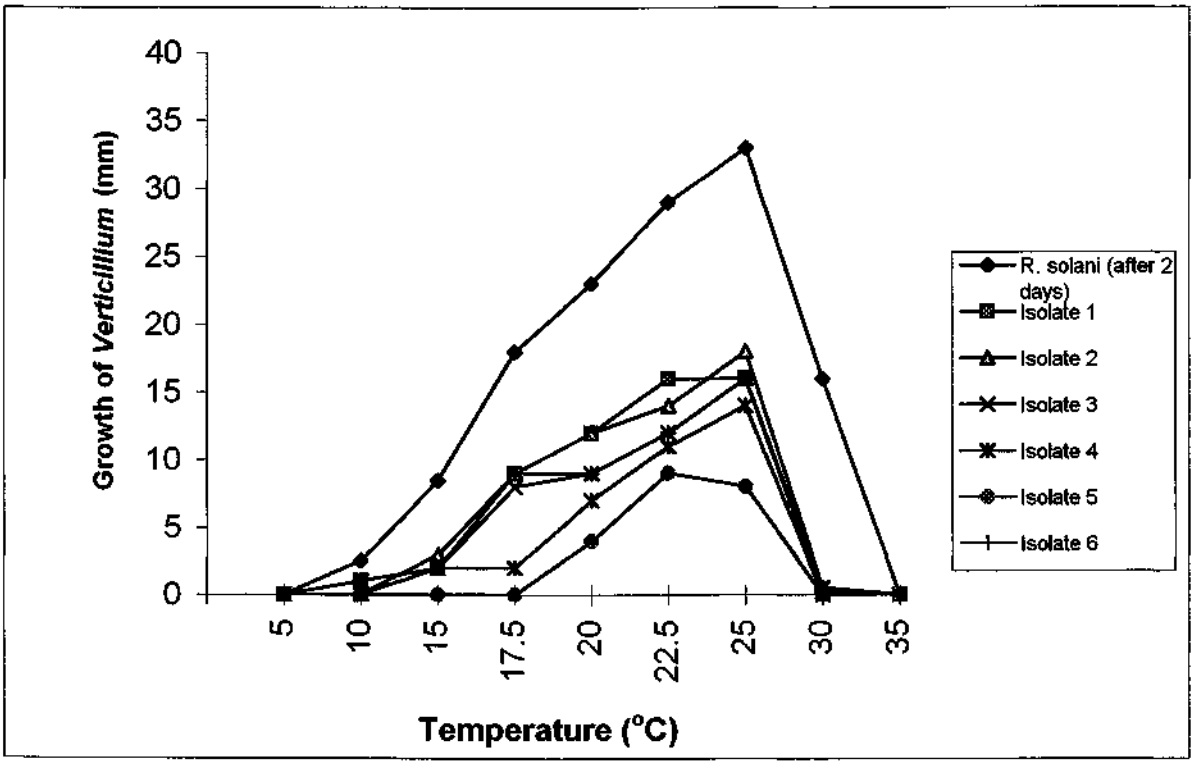
Results

Laboratory Experiments

(a) Survey

Nine isolates of *V. biguttatum* were obtained from sclerotes on naturally infected tubers collected from each major potato growing area in South Australia. Six of the isolates were reisolated with no bacterial contamination.

Figure 1. Growth Rate of *V. biguttatum* isolates 1-6 after 2 weeks and an Ag 3 *R. solani* isolate after 2 days at temperatures ranging from 5-35^{0}C.



(b) Screening *V. biguttatum* isolates

In this experiment all six isolates of *V. biguttatum* had only a slight inhibitory effect on the viability of *R. solani* sclerotes as between 56% and 77% of the sclerotes produced mycellium compared to 93% in the control.

(c) Growth rates and spore production of *V. biguttatum* isolates

These results show that all *V. biguttatum* isolates as well as the *R. solani* isolate have similar optimum growing temperature of 22.5 to 25^{0}C (Figure 1). *R. solani* grew over a wider temperature range (10 - 30^{0}C) compared to that of the *V. biguttatum* isolates which were most active between 17.5-25^{0}C. The growth rate of *R. solani* between 17.5-25^{0}C was significantly greater than that of *V. biguttatum* isolates. For example, *R. solani* grew to the edge of the 9 cm PDA plate in 3-4 days, whereas the *V. biguttatum* colonies grew 2-3cm in diameter after 2 weeks.

Each isolate produced the most spores at 22.5°C with numbers ranging from 500-2000 spores/ml, except isolate 2 which produced 1400 spores/ml at 20^{0}C and only 600 spores at 25^{0}C. Spore production of isolates 2, 3 and 4 ranged from 600-2000 spores/ml, compared to isolates 1 and 5, which produced 200-600 spores/ml. Isolate 6 produced 1500 spores/ml at the optimum temperature of 22.5^{0}C, and 600 spores/ml or less at the other temperatures.

Dimensions of *V. biguttatum* spores ranged in length between 3.5-4um ± 0.05 and width 1.5um ± 0.08 and were within the range of spore sizes described for this fungus.

In addition all the isolates were identified as *V. biguttatum* by M. Priest - NSW Department of Agriculture.

(d) Antagonistic activity of *V. biguttatum* during Cool Room storage

V. biguttatum applied to tubers before cool storage was ineffective as less than 5% of the sclerotes were killed in most treatments.

Field Experiments

(e) Evaluation of *V. biguttatum* and chemical and biological products.

At 45 days after planting, 56% of the plants emerged in the *V. biguttatum* seed treated area, 69% in the Rizolex area, and 89% in the untreated area. By day 56, 73-77% of the plants had emerged in the *V. biguttatum* and Rizolex treatments, whereas 92% had emerged in the untreated plots. Plants from tubers treated with *V. biguttatum* were smaller and had less foliage than the plants grown in the other treatments.

Plants from the plots drenched with *V. biguttatum* had a lower incidence of stem lesions than plants from the undrenched plots (Table 4). One exception was the treatment drenched with *V. biguttatum* at emergence on untreated seed, where 52% of the plants were infected in comparison to 48% in the undrenched plots. Plants from the plots treated with Tri-D25 had in most cases a higher percentage of stem lesions than the plants from the untreated areas.

The incidence of lesions on plants collected from each drench treatment in the area planted with *V. biguttatum* treated seed ranged from 16-44%, in comparison to Rizolex treated seed 24-54%, and untreated seed 33-57% (Table 4).

Table 4. The incidence of *R. solani* stems lesions on potato plants from drenched and undrenched soil treatments, Virginia, 1995

Drench Treatments	Percentage of Stems with *R. solani* Lesions		
	Seed Treatments		
	Untreated	*V. biguttatum*	Rizolex
V. biguttatum emergence + 4 weeks	33	16	30
V. biguttatum emergence only	52	29	24
Tri-D25 emergence + 4 weeks	57	44	46
Tri-D25 emergence only	46	38	54
Undrenched	48	35	39

Disease assessment at harvest showed that the incidence and in most cases the severity of *R. solani* sclerotes on tubers was extremely variable and because of this there were no significant effects of the seed treatments (Table 5).

No significant differences were detected in the level of disease between the main drench treatments when the data was analysed ignoring seed treatment effects.

Table 5. **The incidence and severity of *R. solani* on tubers harvested from each seed and drench treatment, Virginia, 1996.**

Drench	Mean number of tubers with sclerotes			LSD (P = 0.05)	Severity of Infection			LSD (P = 0.05)
	Seed Treatments				Seed Treatments			
	Untreated	*V. biguttatum*	Rizolex		Untreated	*V. biguttatum*	Rizolex	
V.biguttatum E + 4wks	2	20	16	25	0.5	8.5	5	8
V.biguttatum E only	24	28	10	20	11	12	3	11
Tri-D25 E + 4wks	12	24	5	38	6	10	2	17
Tri-D25 E only	10	16	4	12	5	9	3	7
Untreated	35	42	16	39	21	20*	5*	20

Note:- E + 4wks = drenched at emergence and 4 weeks later

E only = drenched at emergence only

*** significantly different with t -test (P = 0.05)**

Due to a lack of replication of each treatment, the results were also analysed using t-tests. The same results were obtained as in the LSD analysis, except for one case where the t-test results showed that the severity of sclerotes on tubers from the Rizolex seed treatment was significantly lower than that of tubers from the *V. biguttatum* seed treatment in the undrenched plots.

In this experiment large differences in the incidence and severity of sclerotes on the tubers were obvious between some treatments but statistical analysis indicated that these differences were not significant.

Powdery Scab was obvious at harvest and had developed in up to 5% of the tubers in some treatments although there was no significant difference in either the incidence or severity of scab between any treatment. Black Dot was not found on the tubers.

The average tuber weight at harvest was 93, 104 and 109 gm/tuber, for the untreated, *V. biguttatum* and Rizolex treated seed respectively.

The average tuber weight from the drench treatments ranged from 101 to 105 gm/tuber, except the tubers from the plots drenched with Tri-D25 at emergence only where tubers weighed 96 gm/tuber.

There was no significant difference in tuber weight between seed or drench treatments.

(f) Comparison of *V. biguttatum* seed and soil drench treatments.

Three weeks after planting the percentage of plants that emerged in each treatment ranged from 23-32% per plot, except for Monceren treated tubers where 15% emerged. Six weeks after planting 72-80% of plants had emerged in all treatments.

The incidence of *R. solani* stems lesions ranged from 34% on the control plants to 26% on those planted with *V. biguttatum* treated seed (Table 6). However, there was no significant difference between treatments with either the incidence or severity of stem lesions

Table 6. **Incidence and severity *R. solani* stems lesions on plants collected from each treatment at eight weeks after emergence, Block C, Lenswood, 1996**

Treatment	Percentage of Stem Lesions	Severity of Stem Lesions
Control	34	10
V. biguttatum Seed Treatment	26	10
1 x *V. biguttatum* Drench	34	12
2 x *V. biguttatum* Drench	31	14
Mon + *V. biguttatum* Drench	35	14
LSD	17	10

The incidence and severity of sclerotes was slightly higher on the tubers harvested from the control plots than tubers from the other treatments (Table 7).

Table 7. **Results (logged data) of incidence and severity of *R. solani*, Black Dot and Powdery Scab on potatoes harvested from *V. biguttatum* seed and drench treatments, Block C, Lenswood, 1996**

Treatment	*Rhizoctonia*		Black Dot		Powdery Scab		Weight per Tuber
	Incidence*	Severity	Incidence	Severity	Incidence	Severity	(gms)
Control	14 (2.24)	83 (3.78)	63 (4.03)	29 (3.24)	13 (2.22)	4 (1.34)	246 (5.5)
V. biguttatum Seed treatment	13 (1.94)	73 (3.15)	71 (4.21)	35 (3.50)	7 (1.79)	3 (1.09)	240 (5.5)
1 x *V. biguttatum* Drench	8 (1.58)	54 (2.91)	54 (3.86)	27 (3.19)	7 (1.54)	3 (0.84)	242 (5.5)
2 x *V. biguttatum* Drench	12 (1.89)	73 (3.18)	57 (3.84)	25 (3.05)	11 (2.09)	4 (1.27)	243 (5.5)
Monceren + *V. biguttatum* Drench	7 (1.29)	37 (2.29)	59 (3.98)	27 (3.21)	8 (1.88)	3 (1.12)	231 (5.4)
LSD	(0.6)	(0.95)	(0.46)	(0.5)	(0.65)	(0.44)	(0.1)

*** transformed data in brackets**

Data was transformed to log (x + 1) as initial analysis showed the data was not normally distributed. Analysis of the transformed data showed that the incidence and severity of *R. solani* was significantly lower on the tubers grown in the plots drenched with either one application of *V. biguttatum*, or Monceren plus *V. biguttatum*, in comparison to the other treatments (Table 7).

The severity of *R. solani* was lower on tubers from the area where Indian Mustard had been incorporated into the soil in the previous experiment.

The incidence and severity of Black Dot and Powdery Scab on the untreated tubers and tubers grown in the *V. biguttatum* treatments were similar. The incidence of Powdery Scab was higher on the untreated tubers in comparison to the other treatments, but the results were not significantly different (Table 7).

The mean tuber weight of untreated potatoes, 246 gms, was not significantly different to the weight of tubers from the *V. biguttatum* treatments, which ranged from 231-242gms per tuber.

(g) Evaluation of chemical and biological treatments in fumigated and unfumigated soil

The percentage of potatoes that emerged in each treatment in the unfumigated and fumigated soil were similar at both 15 and 43 days after planting. More plants emerged in the untreated plots in both the unfumigated and fumigated areas, except in the Monceren treatment in the unfumigated soil (Figures 2 & 3).

Fewer plants emerged in the formaldehyde treatments than other treatments in both unfumigated and fumigated soil (Figures 2 & 3).

Figure 2. **The emergence rate of plants during 15-43 days after planting, in each seed treatment in the unfumigated soil, Virginia, 1995.**

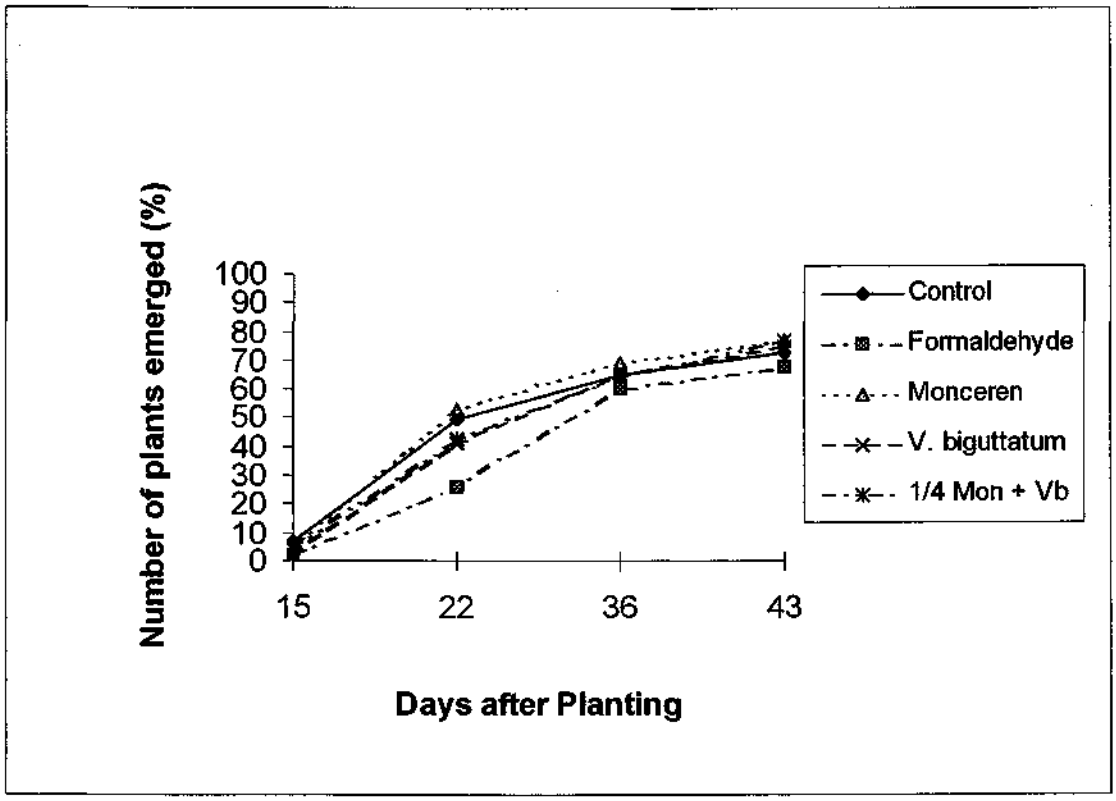
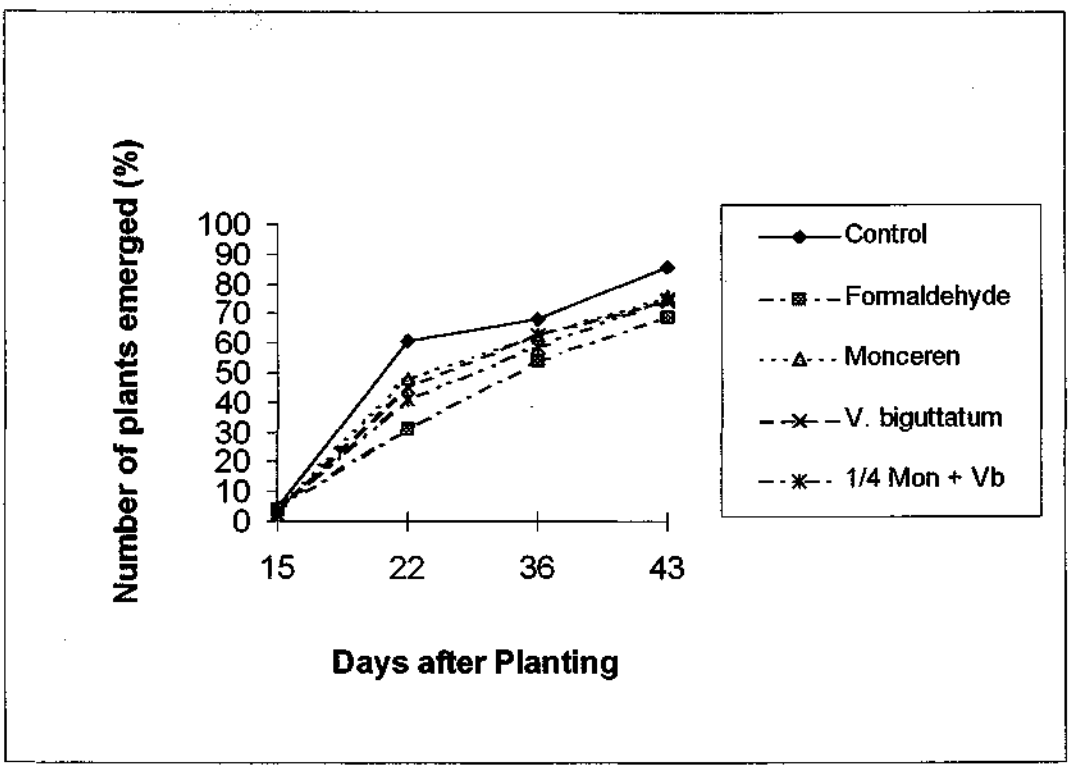


Figure 3. The emergence rate of plants during 15-43 days after planting, in each seed treatment in the fumigated soil, Virginia, 1995.



The results also show the rate of emergence of plants in the *V. biguttatum* and Monceren treatments in the fumigated soil were very similar, whereas the number of plants that emerged in the Monceren treatment in the non fumigated soil was higher (Figures 2 & 3).

The incidence and severity of *R. solani* stem lesions was higher in the unfumigated control area compared to that in the fumigated area, however the incidence of stolon infection was the same (Table 8).

Table 8. **Incidence and severity of *R. solani* lesions on stems and incidence of pruned stolons on potato plants from the untreated seed treatments in the unfumigated and fumigated soil, Virginia, 1995**

Soil Treatment	*R. solani* lesions on stems		Stolons infected by *R. solani*
	Incidence (%)	Severity	Incidence (%)
Unfumigated	41	15	5
Fumigated	28	11	5

The incidence of Black Dot on the base of the potato stems ranged from 75 to 100% in all treatments in either the unfumigated or fumigated soil and there was no significant difference between the treatments.

At harvest the incidence and severity of *R. solani* was similar in both the fumigated and unfumigated areas (Table 9) and no significant differences were detected between treatments. The incidence of *R. solani* was generally low ranging from 7% in the formaldehyde treated seed grown in unfumigated soil to 18% in the *V. biguttatum* treated seed also in unfumigated soil.

Table 9. **Incidence and severity of *R. solani* and Black Dot on potatoes grown in fumigated and unfumigated soil, Virginia, 1995**

Seed Treatments	*RHIZOCTONIA*				BLACK DOT			
	Unfumigated Soil		Fumigated Soil		Unfumigated Soil		Fumigated Soil	
	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity
Control	13	5	16	5	60	32	50	24
Formaldehyde	7	2	16	6	69	35	54	29
Monceren	15	6	17	6	67	37	60	37
V. biguttatum	18	7	17	6	63	35	63	32
1/4Mon +Vb	17	7	12	4	69	35	80	38
LSD	12	6	13	5	24	19	24	17

On the other hand the incidence of Black Dot was much higher and occurred on between 50 to 80% of tubers in most treatments (Table 9). No treatment reduced the incidence or the severity of Black Dot compared to the control.

Mean tuber weights were not significantly different between treatments except for the 1/4 Monceren plus *Verticillium* which was significantly less than the control in the unfumigated soil, and the formaldehyde treatment which was significantly greater than the control in the fumigated soil (Table 10).

Table 10. Mean tuber weight and total yield of potatoes harvested from each seed treatment in fumigated and unfumigated soil, Virginia, 1995

Soil Treatment	Average Tuber Weight (gm)		Average Total Yield (kg)	
	Unfumigated Soil	Fumigated Soil	Unfumigated Soil	Fumigated Soil
Control	133	121	91	97
Formaldehyde	132	148	85	80
Monceren	125	123	92	92
V. biguttatum	117	133	93	101
1/4 Mon +Vb	115	126	82	86
LSD	17	20	17	14

Discussion

V. biguttatum appears to be widespread in the main potato growing areas of South Australia. Whether similar distribution occurs in other areas of Australia needs to be investigated as this fungus has a significant effect on the viability of *R. solani* sclerotes. All isolates of *V. biguttatum* were inactive at temperatures of below 17.5°C which suggests they are likely to have little activity on soil populations of *R. solani* in winter crops or in areas where potatoes are grown in predominantly cool climates. The inactivity of *V. biguttatum* at low temperatures also explains why there was little antagonistic activity when treated tubers were stored at cool room temperatures of 4°C for 3 weeks. If *V. biguttatum* is to be used as a tuber seed treatment it would need to be applied after tubers have been removed from cool storage and allowed to "warm up" to temperatures approaching 20°C. The variation in the antagonistic activity between isolates and the loss of antagonistic activity of isolate 1 over 12 months of subculturing and storage suggest that careful and regular monitoring of activity should be undertaken with future control studies. Also a range of isolates or a range of the most antagonistic isolates should be used in the development of any commercial product.

Initial field experiments showed *V. biguttatum* to be as effective as chemical tuber seed treatments for the control of seed borne inoculum of *R. solani* (Wicks *et al* 1995, 1996). However in subsequent field experiments the evidence for antagonistic activity was not as clear cut. There was a trend for less *Rhizoctonia* to develop on the stems of plants grown in soil drenched with *V. biguttatum* but this effect was not always reflected in the control of *Rhizoctonia* on daughter tubers.

Despite this it would seem that the application of *V. biguttatum* spores to the soil surface followed by an overhead irrigation or rain may be a feasible technique of increasing soil levels of the fungus and ultimately a means of reducing the level of *Rhizoctonia* inoculum in the soil. Levels of *V. biguttatum* and their relation to *Rhizoctonia* inoculum in soils from potato fields have not been measured in Australia and need to be investigated. This may lead to a better understanding of the populations of *V. biguttatum* in soils and how these can be manipulated to reduce the levels of *Rhizoctonia* in soils and to reduce the incidence of disease caused by *Rhizoctonia*.

Further work with *V. biguttatum* is warranted as the fungus is an aggressive mycoparasite, it survives for relatively long periods in the soil and did not appear to have any detrimental effects on potato yield. *V. biguttatum* had no marked effect on other diseases such as Powdery Scab and Black Dot and was compatible with low rates of the fungicide Monceren. The use of an integrated chemical and biological treatment of seed in fumigated or virgin soil followed by artificial inoculation of the soil with the antagonist should be evaluated as a means of developing and maintaining soil with low or nil levels of *Rhizoctonia*.

PART C

BRASSICA SPECIES AS POTENTIAL GREEN MANURE CROPS FOR CONTROL OF *RHIZOCTONIA*

Introduction

Brassica species, such as canolas and mustards, have proven to be successful rotation crops in broad acre farming. As well as being important cash crops, researchers are discovering that certain *Brassica* species have the ability to reduce some pests and diseases when incorporated into the soil as a green manure crop. The *Brassicas* lower levels of soil borne diseases such as *Gaeumannomyces* and *Rhizoctonia* (Kirkegaard *et al,* 1993), kill insect larvae and nematodes, as well as improve soil structure to increase nutrient uptake (Dick, 1994).

Some *Brassica* species have a high concentration of naturally occurring volatile isothiocyanates (ITC's), which are breakdown products from the non volatile glucosinolates (Matthiessen *et al,* 1995). One form of ITC's, methyl isothiocyanate, is the active ingredient of the synthetically produced chemical metham sodium, which is commercially used as a soil fumigant.

Researchers at CSIRO, Canberra, are breeding *Brassicas* with higher levels of glucosinolates and testing the biocidal properties of various *Brassica* crops (Kirkegaard *et al,* 1993).

Experiments were undertaken in South Australia to determine if soil borne diseases of potatoes could be reduced by using *Brassicas* as green manure crops. Several experiments were conducted to determine the effect of Indian Mustard on the control of *R. solani*, in laboratory and field

conditions. Other field experiments compared the biocide properties of Indian Mustard, Ebony Mustard and Rangi Rape in reducing *R. solani*, Black Dot, Powdery Scab and Silver Scurf which are common soil born diseases in potato crops that cause the reduction in yields and tuber quality.

Laboratory and Field Experiments

Methods

(a) Effect of volatile gases from dry frozen Indian Mustard foliage, roots and meal on the growth of *R. solani* in vitro

A laboratory experiment was conducted to evaluate the effect of dry frozen stems, foliage and roots of Indian Mustard as well as meal on the *in vitro* growth of *R. solani* isolates from Anastomosis Groups 3 and 8. The meal is mostly crushed seed, a by-product of the oil extraction process, and was supplied by J. Kirkegaard, CSIRO, Canberra. Either stems, roots or meal of Indian Mustard, at rates of 0 to 1gm, were placed in 2.5cm wide plastic lids. The lids were placed in petri dishes, with a 5mm plug of *R. solani* on PDA placed immediately above the lid containing the plant material. The petri dishes were sealed with parafilm to contain any volatiles produced, and then incubated at 21^{0}C in darkness. After 72 hours, the radial growth of *R. solani* colonies were measured.

(b) Comparison of Indian Mustard foliage, roots and meal incorporated into the soil to control of *R. solani* on potatoes

An initial field experiment was conducted at Lenswood Research Centre, in the Adelaide Hills, to evaluate whether foliage or roots of the *Brassica* plants were effective in controlling *R. solani*. The experiment site had been planted to potatoes in the previous two years.

Treatments included untreated soil, whole plants incorporated into the soil and either left bare or covered with plastic, or a combination of foliage and stems or roots incorporated into the soil with or without Mustard meal.

The Indian Mustard was incorporated into the soil when 80% of the plants were flowering, as this is thought to be at minimum pod production. The glucosinolates concentrate within the pods, which break down in the soil at a much slower rate than the foliage.

To test the efficiency of each treatment after the Indian Mustard was incorporated into the soil, potato tubers naturally infected with ten or more sclerotes were planted in each treatment area. Five weeks later the tubers were removed and the viability of the 10 sclerotes per tuber were tested by removing the sclerotes from the tubers, transferring them onto TWA plates and incubating at 20^0C. After 24 hours the number of sclerotes which produced typical *R. solani* mycellial growth on TWA was recorded.

(c) Effect of Indian Mustard meal on the control of *R. solani* on previously infected tubers.

Field trials were conducted at the Lenswood Research Centre, to evaluate whether Indian Mustard meal planted with the seed potatoes was effective in controlling *R. solani*. The experimental site had been planted to potatoes in the previous two years.

Indian Mustard was grown and incorporated into the soil at the experimental site before the seed potatoes were planted. In this experiment, Pontiac seed potatoes with 32% *R. solani* and 100% Silver Scurf infection was used. The seed potatoes were cut and then dusted with Mancozeb a week before planting.

Three aspects were investigated:-

(i) different rates of Indian Mustard meal planted with the seed tubers

(ii) one rate of meal planted with seed tubers which had been dipped in Formaldehyde

(iii) large amounts of meal surrounding the seed potatoes at planting

(i) Potato seed were planted with either 0.5, 1 and 2 kg of Indian Mustard meal per 5m. The seed planted in the control plots had been dipped in 2% a.i. Formaldehyde for 20 mins and rinsed three times in water a week before planting.

Each treatment was replicated 6 times in 5m x 0.3m plots. The tubers were planted by hand at 20cm intervals, with the meal placed in the hole beneath each seed piece. The emergence of potatoes in each treatment was recorded at 10, 16 and 23 days after planting.

The potatoes were harvested 21 weeks after planting with a single row harvester. The potatoes from each plot were weighed and then assessed for the incidence and severity of *R. solani*. The surface area of the tubers covered by sclerotia was rated on a scale of 0-4, similar to the one described by Dijst (1985). The severity of sclerotia on the tubers was converted to a 1-100 scale as described by Wicks *et al.* (1994).

(ii) The treatments included untreated seed and Formaldehyde treated seed planted with 20ml of meal per 4m. The seed potatoes treated with formaldehyde were dipped in the same method mentioned in (i). The untreated seed was treated with Dithane when the tubers were cut.

Formaldehyde treated seed was planted in four rows (30m x 0.3m) and two half rows (15m x 0.3m) and the untreated seed in two half rows. The seed potatoes were planted with a two row seeder at 20cm intervals with the meal applied at 20 ml of meal per 4 m, with the seed through the fertiliser box.

The potatoes were harvested 21 weeks after planting with a single row harvester. The tubers were weighed and then assessed for the incidence and severity of *R. solani* using the same scale as described above.

(iii) The seed potatoes in this trial were hand planted with either 20 or 40g of meal surrounding each seed piece. In the control plot the seed was planted without meal. In each treatment, ten tubers naturally infected with *R. solani* were planted in a row at 20cm intervals.

The potatoes were harvest by hand eight weeks after planting. The viability of the sclerotes from each tuber were tested using the same method mentioned in experiment (1).

(d) Comparison of three *Brassica* species to control *R. solani*

Two field experiments were conducted to evaluate *Brassica* species as green manure crops on commercial potato-growing crops in the Virginia, 50km north of Adelaide, and Monteith, 150km south east of Adelaide, South Australia. Soils were predominantly red brown earths at Virginia and loamy sands at Monteith.

Both experimental sites were sown to potatoes two years previously, and have since been sown to either carrots, onions or barley.

At Montieth the treatments included Indian and Ebony Mustard, Rangi Rape and untreated soil. Ten replicates of each treatment were planted randomly through the experimental site on 4th October, 1995. The plots were 50m x 3.75m in size.

At Virginia the treatments were Indian and Ebony Mustard and untreated soil, planted in 145m x 3.74m plots on 12th September 1995. Two replicates of Indian Mustard and untreated soil were

planted in the southern end of the experimental site and two replicates of Ebony Mustard and untreated soil were planted in the northern end.

At both sites, each *Brassica* species was sown by hand at a rate of 8kg/hectare. The emergence rate of each *Brassica* species at Virginia was determined by counting the number of plants that had emerged within a $1m^2$ quadrant, at ten metre intervals in each plot.

The *Brassica* plants at Monteith were slow to establish due to the cabbage moth damage, but grew rapidly after the moth population was controlled with one application of Baldock 25.

The *Brassica* plants were incorporated into the soil when 80% of the plants were flowering. At Virginia this was November 13th and at Monteith December 5th.

A glasshouse bioassay experiment was conducted to evaluate the levels of *R. solani* in the soil after the *Brassicas* were incorporated. Soil samples were collected from each plot at both experimental sites at 1 day after and 6 and 12 weeks after incorporation. One kilogram of soil from each plot was separated into 30cm high pots. Five Sebago minitubers were planted 25cm deep in each pot to produce long stemmed plants. This created a greater surface area of plant tissue for *R. solani* infections. The stems were harvested 4-5 weeks after planting and assessed for *R. solani* lesions. The stem lesions were rated on a scale of 0-5, similar to the one described by Frank *et al.* (1976). The severity of the stem lesions was converted to a 0-100 scale described by Wicks *et al.* (1994).

At Virginia, a bioassay experiment was conducted to determine the mortality rate of *R. solani* sclerotes on tubers planted in either moist or dry *Brassica* treated soil. Naturally infected tubers, with ten or more sclerotes, were buried at ten metre intervals in each plot the day after the *Brassica* plants were incorporated into the soil. Every alternate tuber site was watered with 1L of water every week.

Tensiometers were placed at each tuber site to record soil moisture each week. A 100gm soil sample was also collected from each site, oven dried and then weighed to determine the soil moisture content. The tubers were removed after 5 weeks and the viability of ten sclerotes per tuber were tested using the same method as mentioned in the previous experiment.

At around 12 weeks after the *Brassicas* were incorporated into the soil seed tubers were planted at both sites. At Virginia, Coliban seed potatoes were planted at 45cm intervals in 30cm wide rows. At Montieth, half the experimental site was planted to Colibans and the other half to Ottoways, both planted at 30cm intervals in 30cm wide rows. Approximately 5% of the Coliban seed and 98% of the Ottoway seed potatoes were infected with *R. solani*. Ninety-three percent of the Ottoway seed was infected with Silver Scurf.

The emergence of the potato plants in each plot was determined by counting the number plants that emerged within a 10m x 1row quadrant. At Virginia, ten quadrants were counted in each plot and were randomly selected from the middle two rows. An emergence count was not recorded at Montieth.

Twelve weeks after planting, eight potato plants were harvested from each plot and the stems assessed for *R. solani* lesions using the same methods as previously described.

Potatoes were harvested by hand 18-20 weeks after planting and 100 tubers were selected at random from each plot, weighed and assessed for the incidence and severity of skin diseases on the tuber surface. The diseases were rated using the scales described above in (c).

Results

Laboratory and Field Experiment

(a) Volatile gases from dry frozen *Brassica* material

All rates of the Indian Mustard Meal inhibited both Ag 3 and Ag 8 isolates completely, except for the Ag 8 isolate at 0.01gms of meal, which was inhibited by 61% (Table 11). The plant foliage and stems inhibited both *R. solani* isolates completely at above 0.25g whereas the plant roots did not completely inhibit until 1.0gms.

The inhibition of the Ag 3 isolate was similar between 0.1gms to 0.5gms of roots, ranging from 83-90%, whereas a higher level of inhibition of the Ag 8 isolate was observed as the amount of roots increased. The inhibition of the Ag 8 isolate ranged from 34% at 0.1gms to 76% at 0.5gms.

Table 11. **Inhibition of *R. solani* growth by volatiles from dry frozen Indian Mustard tops, roots, and meal.**

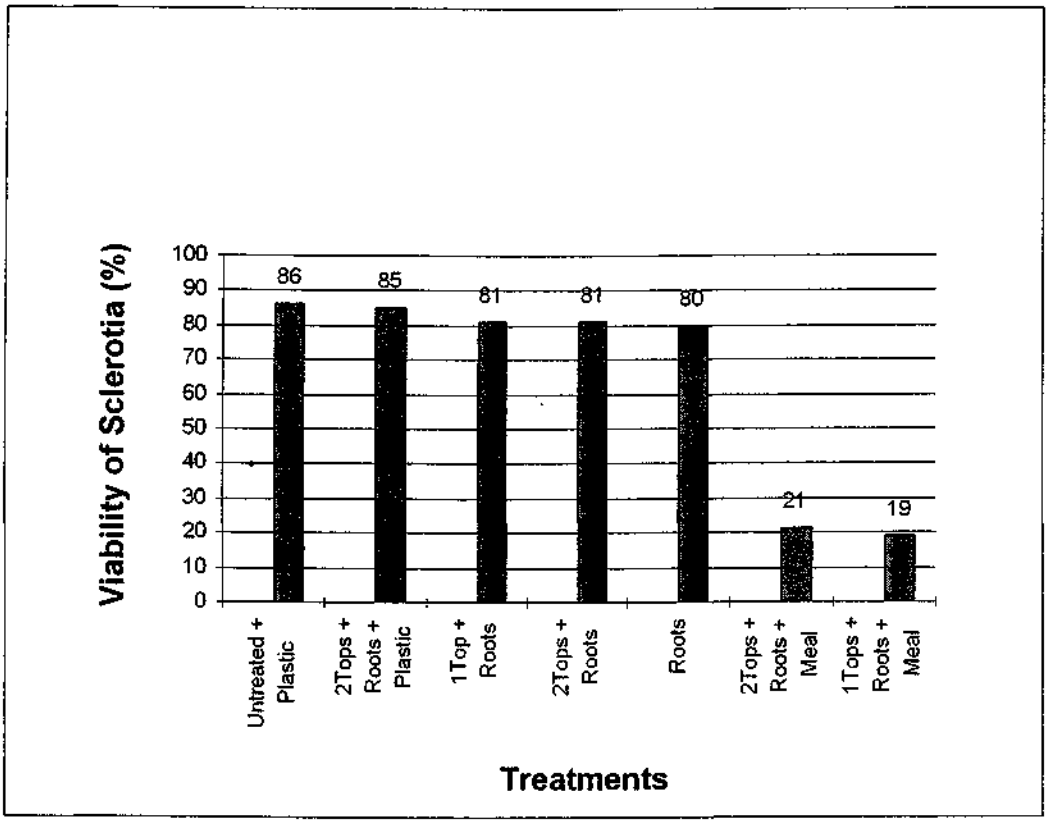
TREATMENTS	INHIBITION (%)															
Indian Mustard (gms)	0.0		0.01		0.025		0.05		0.1		0.25		0.5		1.0	
Anastomosis Group	3	8	3	8	3	8	3	8	3	8	3	8	3	8	3	8
TOPS	0	0	-	-	-	-	-	-	85	37	100	100	100	100	100	100
ROOTS	0	0	-	-	-	-	-	-	83	34	89	47	90	76	100	100
MEAL	0	0	100	61	100	100	100	100	100	100	-	-	-	-	-	-

- = not tested

(b) **<u>Comparison of Indian Mustard foliage, roots and meal incorporated into soil</u>**

The results in Figure 4 show around 20% of the sclerotia from tubers buried in soil treated with Mustard meal were viable. In comparison, up to 86% of sclerotia were viable in treatments with untreated soil or where *Brassica* roots or foliage were incorporated into the soil.

Figure 4. **Incidence of viable *R. solani* sclerotes on bioassay tubers after being buried for four weeks in the Indian Mustard Soil Treatments, Lenswood, 1994**



(c) Effect of Indian Mustard meal on previously infected tubers

(i) The plants grown in the presence of meal emerged at a much slower rate than the plants in the control plots. For example, 16 days after planting 80% of the plants had emerged in the control plots compared to 5-17% in the meal treatments (Table 12). By day 23, most of the plants in the control plots had emerged, but only 25-57% had emerged in the meal treated plots.

Table 12: Emergence of potatoes planted with varying rates of Indian Mustard meal, Block C, Lenswood, 1995

Treatments	Plants Emerged (%)		
	Day 10	Day 16	Day 23
Control	0	80	96
0.5kg Meal/5m	0	17	57
1kg Meal/5m	0	11	39
2kg Meal/5m	0	5	25

In this experiment, the incidence and severity of *R. solani* on tubers was low (Table 13), and there was no significant differences between treatments. However, tuber weights from the control plots were significantly higher than those from the meal treatments.

Table 13: Incidence and severity *R. solani* on tubers planted with varying rates of Indian Mustard Meal, Block C, Lenswood, 1995

Treatment	*Rhizoctonia*		Tuber weight (gm)
	Incidence (%)	Severity	Incidence (%)
Control (Formaldehyde)	2	0.42	142
0.5kg Meal/5m	0	0	114
1kg Meal/5m	0.2	0.04	121
2kg Meal/5m	0	0	115
LSD	3	0.60	23

(ii) *R. solani* was not found on tubers from the plots treated with meal only (Table 14), and on tubers in the other treatment the incidence was 1%. Tubers weights from the control plots were less than those from the meal treated soil (Table 14).

Table 14: Incidence and severity of *R. solani* on tubers treated with Formaldehyde and planted with Indian Mustard meal, Block C, Lenswood, 1995

Treatment	*Rhizoctonia*		Weight per Tuber
	Incidence (%)	Severity	Percentage
Control (meal)	0	0	114
Form + Meal	1.0	0.23	138

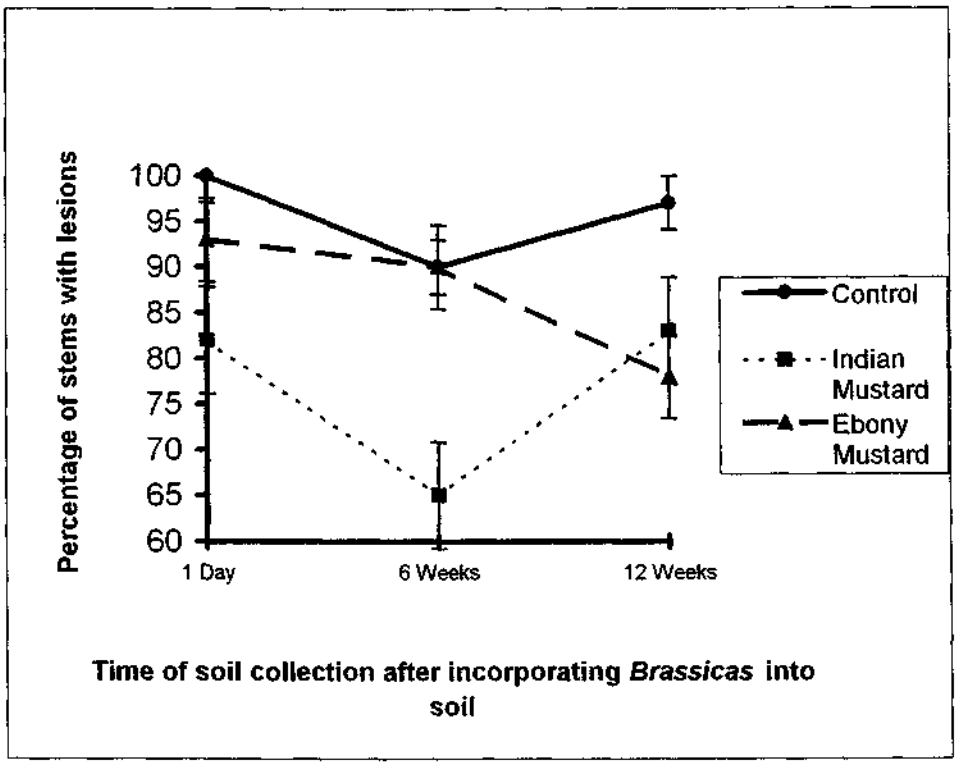
(iii) At harvest, the potatoes were difficult to remove from the soil as the meal had set in a 1-2cm thick hard band around each tuber. Large amounts of meal buried with the seed potato did not kill *R. solani*, as 95-100% of the sclerotes from the treated and untreated tubers were viable.

(d) Comparison of three *Brassica* species for the control of *R. solani*

Two weeks after sowing, 100 *Brassica* plants/m^2 had emerged at Virginia. However at both sites the emergence was uneven causing patchy areas of plants throughout each plot.

Assessment of stem lesions on the minitubers grown in soil collected at various dates after incorporation showed no significant differences between treatments. The results in Figure 5 show a decrease in number of *R. solani* lesions on the stems from plants grown in soil collected 6 weeks after incorporating the Indian Mustard. However the number of lesions increased to the original level after a further 6 weeks. Lesions numbers on plants grown in Ebony Mustard treated soil gradually decreased during the 12 weeks.

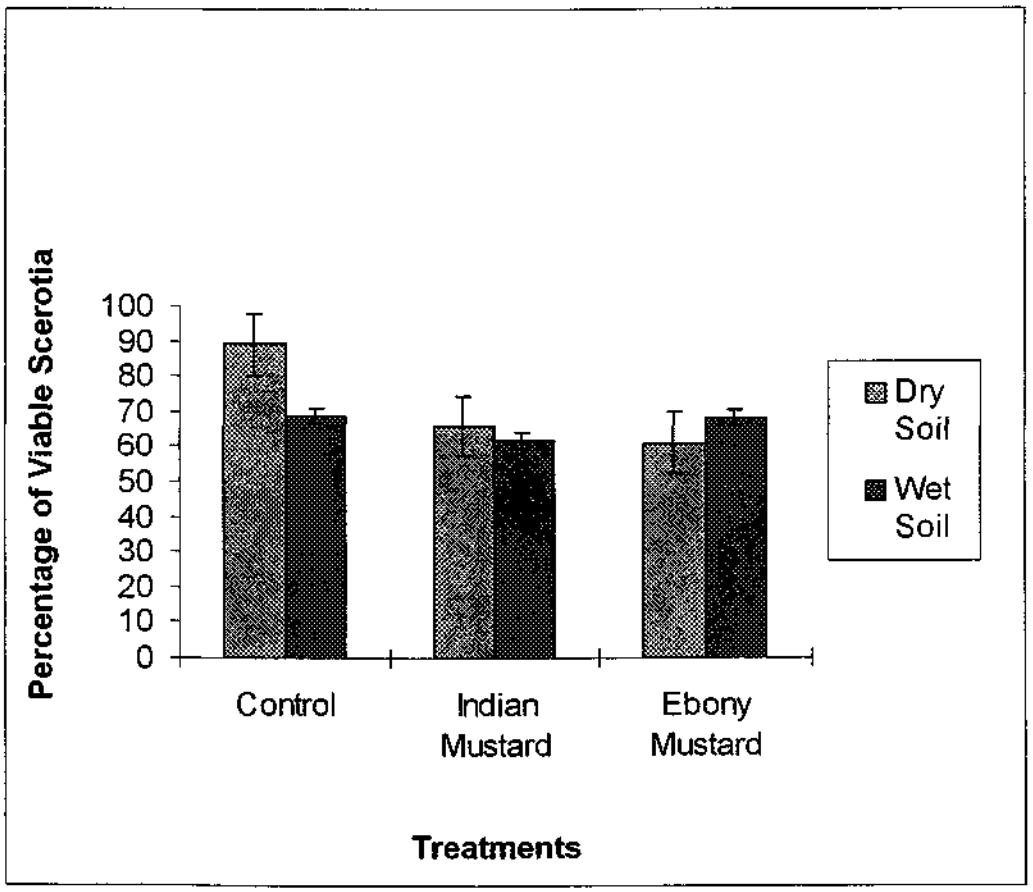
Figure 5. **Incidence of *R. solani* stem lesions on minitubers grown in soil collected twelve weeks after incorporating Indian and Ebony Mustard and Rangi Rape into the soil, Virginia 1996.**



Assessment of the number of viable sclerotes on tubers buried for five weeks after incorporation showed that both the *Brassica* treatments reduced the number of viable sclerotes compared to the control in dry but not wet soil (Figure 6).

A similar number of viable sclerotes were collected from tubers which were buried in treated and untreated soil and watered during the five weeks (Figure 6).

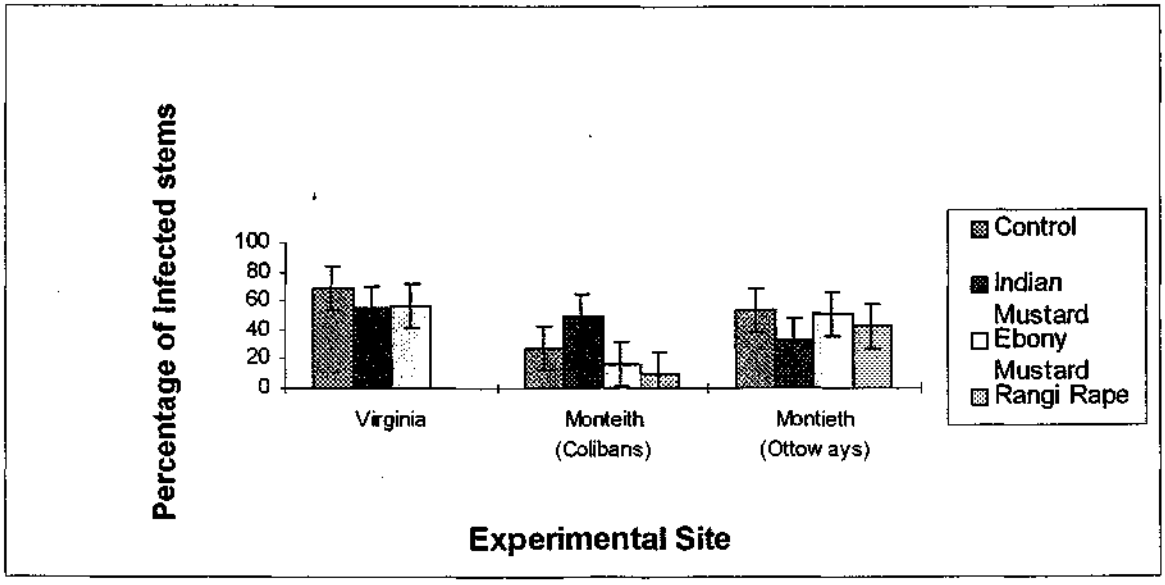
Figure 6. Viability of *R. solani* sclerotes collected from naturally infected tubers buried in soil for five weeks after incorporating Indian and Ebony Mustard, Virginia, 1996



The rate of emergence was similar for all treatments five weeks after planting.

At both experimental sites there was no significant difference in the number of lesions found on potato stems collected from each treatment, despite the higher number of lesions found on stems from control plots at Virginia (Figure 7).

Figure 7. Incidence of potato stems infected with *R. solani* 15 weeks after planting tubers in soil incorporated with Indian and Ebony Mustard and Rangi Rape, 1996.



Tuber assessments at both the Virginia and Monteith sites showed no significant difference between treatments in either the incidence or severity of *R. solani* on tubers (Tables 15 & 16).

Table 15. **Incidence and severity of skin diseases on potatoes planted in soil, following the incorporation of green manure *Brassica* crops at Monteith, 1996**

Treatment	*Rhizoctonia*		Powdery Scab		Weight per tuber
	Incidence	Severity	Incidence	Severity	(gms)
Control	28	19	2	0.8	166
Indian Mustard	22	15	1	0.2	184
Ebony Mustard	24	17	5	2.0	168
Rangi Rape	24	17	1	0.2	175
LSD	8	6	4	2	19

Table 16. **Incidence and severity of skin diseases on potatoes planted in soil, following the incorporation of green manure *Brassica* crops at Virginia, 1996**

Treatment	*Rhizoctonia*		Black Dot		Weight per tuber
	Incidence	Severity	Incidence	Severity	(gms)
Control	53	28	22	8	182
Indian Mustard	48	25	15	6	177
Control	39	17	23	10	187
Ebony Mustard	47	25	23	8	203
LSD	11	7	10	3	19

Powdery Scab was only found on tubers grown at Montieth and Black Dot on tubers grown at Virginia.

Although the incidence of Black Dot was lower on tubers grown in Indian Mustard treated soil, the results were not significantly different (Tables 15 & 16).

At both experimental sites, higher yields were obtained from plants grown in some of the *Brassica* treated soils. However these yields were not significantly different at these sites.

Discussion

Volatiles from Indian Mustard killed *Rhizoctonia* mycellium in closed petri dishes, demonstrating the potential of this plant material to control *Rhizoctonia* when incorporated into soil as a green manure crop. However this was not achieved in field experiments where green manure crops of both Indian Mustard and Ebony Mustard failed to significantly reduce soil levels of *Rhizoctonia* or to control *Rhizoctonia* on potato tubers buried in treated soil. The reasons for this were unclear although it may be associated with the biomass of green manure incorporated into the soil. In our experiments a seeding rate of 8 Kg/Ha resulted in approximately 100 plants/m^2 at emergence. Plant development was not optimal in our experimental sites and plants in some areas suffered initial deficiencies and were subject to insect attack. Although we attempted to rectify the nutritional and insect problems, these factors may have influenced the production of glucosinolates. In any case further work needs to be done to fine tune the use of *Brassica* crops, particularly in determining the

most appropriate seeding rate in different soil types and climates as well as the ideal planting times for various *Brassica* lines. In our experiments the *Brassicas* were sown in spring, but planting at other times may produce plants with higher levels of glucosinolates or greater biomass per unit area.

Indian Mustard meal was shown to be a highly potent source of volatiles that significantly inhibited plant emergence when the meal was applied with potato tuber seed at planting. These volatiles were not sufficient to kill or reduce the viability of sclerotes on seed tubers, in the one experiment where tubers were planted with meal. However further experiments along these lines need to be conducted, particularly the use of pelletised mustard meal. Mustard meal in this form could be readily applied at planting with fertiliser and seed and may be a useful biofumigant. Overall, these experiments have shown that considerable work needs to be done before *Brassicas* can be recommended as a reliable means of reducing soil borne levels of *Rhizoctonia.*

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RECOMMENDATIONS

Extension/Adoption

This work has confirmed other studies showing that *Rhizoctonia*, Black scurf, is common on most seed potatoes grown in Australia. As a result, *R. solani* is widespread in all potato growing areas of Australia. Potato growers have therefore been advised to treat seed tubers by either dipping the tubers in a 2% a.i. solution of formaldehyde or to treat the tubers with other suitable fungicides, such as Rizolex, Rovral or Monceren, before planting in new areas or fumigated soil. Details of this project have been presented to at least 5 grower meetings in South Australia, Victoria and NSW and documented in the potato Australia magazine.

DIRECTIONS FOR FUTURE RESEARCH

Future work is needed to exploit the use of the mycoparasite *Verticillium biguttatum* as a seed treatment for the control of *Rhizoctonia* on potatoes. Commercial development of a formulated product needs to be encouraged in Australia and this compared with a similar agent imported from Holland.

Integrated chemical and biological treatments need to be evaluated as seed and furrow treatments for *R. solani* and other diseases. In particular a critical evaluation of in furrow chemical treatments at planting needs to be undertaken as this practice is widespread in many potato growing areas and it is not known if these treatments are effective.

Further studies need to be done to fine tune the use of *Brassica* green manure crops in rotation with potatoes. Factors such as the most appropriate *Brassica* cultivar, planting time, seeding rate, and frequency of rotation need to be investigated as well as the use of pelletised *Brassica* meal as a means of biofumigating soil.

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Chemical and biological control of *Rhizoctonia solani* on potato seed tubers

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Summary. Chemical and biological treatments for the control of tuber-borne inoculum of *R. solani* on potatoes were evaluated by testing the viability of sclerotes removed from treated tubers. This technique showed that most sclerotes adhering to the tuber surface were devitalised when tubers were dipped for 20 min in a 2% solution of formaldehyde. Dusting tubers with tolclofos-methyl, or spraying them with fenpiclonil or pencycuron, gave control equal to formaldehyde, whereas a sodium hypochlorite dip was ineffective. A spore suspension of *Verticillium biguttatum* applied to tubers as either a dip or a spray devitalised >90% of treated sclerotes, whereas similar treatments of other organisms such as *Bacillus*, *Gliocladium*, and *Trichoderma* were ineffective.

Introduction

Black scurf caused by *Rhizoctonia solani* Kühn is a serious disease of potatoes worldwide. On potatoes grown for the fresh market, the development of sclerotes on the tuber surface can severely reduce marketability. Reducing tuber-borne inoculum is considered crucial in the control of this disease, and treatments both chemical (Hide and Cayley 1982; Cother 1983; Adam and Malcolm 1988; Harris *et al.* 1988) and biological (Jager and Velvis 1985, 1986, 1988; Jager *et al.* 1991) have been evaluated as seed tuber treatments.

Apart from the work by Cother (1983) there are few reports on the evaluation of chemical tuber seed treatments in Australia. Similarly, the use of fungal antagonists to control *R. solani* on potatoes has been reported from Holland (Jager *et al.* 1991) and elsewhere (Escande and Echandi 1991) but little work has been conducted on this topic in Australia.

Investigations were therefore undertaken to compare a range of chemicals and to evaluate potential antagonists as tuber seed treatments for the control of *R. solani* on potatoes.

Materials and methods

Tubers of cv. Atlantic naturally infected with *R. solani* and with ≥5 visible sclerotes/tuber were used in most experiments. Tubers were either collected from the field at harvest and stored at 4°C before use or selected from commercial seedlots that had been stored at 4°C. Before treatments were applied, most batches of tubers were dipped in tap water and gently agitated to remove excess soil from the tuber. Tubers were drained and air-dried before use.

Depending on the treatment, tubers were dusted, sprayed, or dipped, with care taken to ensure that the treatment was applied evenly over the surface. Dusts were applied by placing tubers inside a plastic bag, sprinkling them with sufficient material to coat, then gently rotating the tubers in the closed bag. Sprays were applied with a hand-operated, fine misting spray that used about 2 mL to cover evenly each tuber per treatment. In the dip treatments, tubers were immersed in a 4-L suspension or solution of the test material for the times indicated.

Batches of tubers were drained after treatment and then placed in plastic food trays that were enclosed in plastic bags to maintain high humidity. These were incubated at 20°C for 14 days before individual sclerotes varying in size from 1 to 4 mm diameter were removed from the tuber and gently pressed onto the surface of tapwater agar (TWA) plates. Sclerotes were considered viable if typical rhizoctonia-like growth was detected microscopically (x100) after 48 h incubation at 20°C.

A number of chemicals reported to control *R. solani* elsewhere were compared for their effect on the viability of sclerotes. Chemicals and the formulations used were fenpiclonil (Beret, 40% a.i.), formaldehyde (formalin, 37% a.i.), pencycuron (Monceren, 25% a.i.), tolclofos-methyl (Rizolex, 10% a.i.), iprodione (Rovral, 25% a.i.), and sodium hypochlorite (4% available chlorine).

Organisms evaluated as potential biological treatments were (i) Tri-D25, a commercial formulation of *Trichoderma harzianum* and *T. koningii* (J. B. Biotech Inc., PO Box 7493, Ventura, CA 93006, USA), widely promoted in vegetable-growing areas of South Australia as a useful soil additive antagonistic to a wide range of soil-borne pathogens; (ii) a spore suspension of *Verticillium biguttatum* isolated from naturally infected sclerotes of *R. solani* collected from potatoes grown in sandy soils in the South East of South Australia; (iii) a spore suspension of *Gliocladium roseum* isolated from

naturally infected sclerotes as above; (iv) a spore suspension of an unknown *Bacillus* recovered from the rhizosphere of potatoes grown in the Virginia area about 30 km north of Adelaide and shown to be inhibitory to *R. solani in vitro* (Balali unpublished data 1994); and (v) Actizyme, a commercial formulation of *Bacillus subtilus* (Southern Cross Laboratories Pty Ltd, Dural, NSW) developed for use in drains and septic tanks and tested for its convenience as a commercial formulation.

Chemical treatment experiments

A series of experiments was undertaken to determine the most effective chemical concentration x dipping time combination to kill sclerotes on the surface of tubers. Tubers were dipped in either formaldehyde at 0.4, 2.0, or 4.0% a.i., or sodium hypochloride at 0.5, 1.0, or 2% available chlorine. For both chemicals, dipping times ranged from 2 to 20 min. The tubers were rinsed 3 times in water and allowed to drain and dry before sclerotes were removed from tubers and plated onto TWA. Due to the irregular availability of naturally infected tubers, different numbers of tubers were treated in the various experiments. However, at least 50 sclerotes/treatment were assessed in all experiments.

The fungicides (a.i./kg tuber) fenpiclonil (0.5 mL), iprodione (0.2 mL), and tolclofos-methyl (0.2 g) were compared for their effect on the viability of *R. solani* sclerotes. Tolclofos-methyl was applied as a dust, and the other treatments were applied as sprays. Each treatment was applied to batches of 10 tubers from which 10 replicate samples, each of at least 10 sclerotes, were picked at random and placed on TWA. Viability was assessed after 24 h incubation at 20°C.

Biological treatments

Experiment 1. Two fungi isolated from *R. solani* sclerotes, *V. biguttatum* and another subsequently identified as *G. roseum*, were evaluated as antagonists to *R. solani*. After each fungus had grown for 14 days at 20°C on potato dextrose agar (PDA), a spore suspension of 10^6 viable spores/mL was obtained by either scraping spores from the surface of the agar and adding to sterile distilled water, or macerating the fungal growth and agar in sterile distilled water using a Waring blender.

Tubers of cv. Winlock heavily infected with *R. solani* were dipped in a spore suspension for 5 min and then drained before being placed in sealed plastic bags and incubated for 49 days at 20°C. After this, at least 100 sclerotes selected at random from 10 replicates for each treatment were plated onto TWA.

Experiment 2. This experiment tested the effect of length of incubation period on the efficacy of treatments. Tubers were dipped in a spore suspension of *V. biguttatum* or *G. roseum* as described in experiment 1 and compared with pencycuron applied as a spray at 0.6 mL/kg tuber. Batches of ≥70 tubers/treatment were dipped, drained, then placed in sealed plastic containers and incubated in the dark at 20°C. Over 5 weeks, 10 tubers/treatment were removed at weekly intervals and at least 100 sclerotes from 10 replicates/treatment were picked off at random and plated onto TWA.

Experiment 3. The commercial formulation of *Trichoderma* at 1.3 g/kg tuber, the *Bacillus* isolate at $>10^6$ colony forming units/mL, the commercial formulation of *B. subtillus* at 0.06 g/mL, and the isolate of *V. biguttatum* at 10^6 spores/mL were compared for their effect on the viability of *R. solani* sclerotes. Batches of 10 tubers/treatment were either sprayed or dipped in the suspensions of the various organisms, drained, then incubated in a sealed container for 16 days at 20°C. At least 100 sclerotes from 10 replicates/treatment were removed from the tubers and plated onto TWA. Viability was assessed after 48 h incubation at 20°C.

Statistical analyses

Except for the experiments shown in Table 1, all raw data were analysed using standard chi-square tests. Where the chi-square tests showed significance, pairwise chi-square comparison of treatments was used to indicate specific differences.

Results

Chemical treatments

In most cases, ≥80% of sclerotes were viable in the control treatments. Dipping tubers in formaldehyde concentrations <2% and for ≤20 min killed some sclerotes, whereas 2% formaldehyde for 20 min and 4% formaldehyde for at least 10 min killed most sclerotes (Table 1). At lower concentrations or shorter immersion times, the viability of sclerotes was reduced compared with the control.

No sodium hypochlorite treatment killed all of the sclerotes, although at the longest immersion time (20 min), significantly ($P<0.05$) more sclerotes were killed at the highest concentration than at the other concentrations (Table 2). Similarly, at the highest concentration, significantly ($P<0.05$) more sclerotes were killed at the longest immersion time than at other immersion times.

Table 1. Viability of *R. solani* sclerotes (percentage growing) from potatoes dipped in formaldehyde at varying concentrations and immersion times

Formaldehyde (% a.i.)	Dipping time (min) 0	2	5	10	15	20
	Lenswood (cv. Atlantic)[A]					
0	80	—	—	—	—	—
0.4	—	—	70	80	30	—
2.0	—	10	10	—	—	—
	Virginia (cv. Atlantic)					
0	83[B]	—	—	—	—	—
0.4	—	—	—	—	—	22[B]
2.0	—	50[A]	30[A]	—	—	0.2[B]
0	71[C]	—	—	—	—	—
4.0	—	—	—	0[C]	—	0[C]
	Virginia (cv. Coliban)[B]					
0	83	—	—	—	—	—
0.4	—	—	—	—	—	32
2.0	—	—	—	—	—	0

[A] Assessed on a minimum of 10 sclerotes per treatment.
[B] Assessed on a minimum of 30 sclerotes per treatment.
[C] Assessed on a minimum of 100 sclerotes per treatment.

Table 2. Viability of *R. solani* sclerotia (percentage growing) from potato tubers dipped in sodium hypochlorite at different concentrations and immersion times

Values followed by the same letter (within rows, upper case; within columns, lower case) are not significantly different (chi-square test)

Sodium hypochlorite (% a.i.)	Dipping time (min) 0	5	10	20
0	98	—	—	—
0.5	—	96Aa	98Aa	98Aa
1.0	—	96Aa	86ABab	68Bb
2.0	—	90Aa	70Bb	46Cc

Tolclofos-methyl, fenpiclonil, and iprodione all reduced the viability of sclerotia, with iprodione the least ($P<0.001$) effective (0, 1, 27% growth of sclerotia *v.* 92% for control).

Biological treatments

Experiment 1. Verticillium biguttatum was the only treatment to reduce the viability of sclerotia. Percentages of sclerotes growing for control, *G. roseum*, and *V. biguttatum* treatments were 50, 47, and 10%, respectively.

Experiment 2. The viability of the control treatments was not measured at the first 2 samplings, but by 21 days' incubation, most of the sclerotes had grown and the viability of the controls remained >80% after 5 weeks (Table 3). After 21 days, *V. biguttatum* killed significantly ($P<0.001$) more sclerotes than either of the other treatments. After 35 days there was no significant difference between the pencycuron and *Verticillium* treatments.

Experiment 3. Most sclerotia in the control treatment grew (99%), as did those treated with *Bacillus* sp. (92%) or *Trichoderma* (99%). *Bacillus subtillus* treatment resulted in 87% viability. *Verticillium* significantly ($P<0.001$) inhibited the viability of sclerotes (6.5% viable) compared with the control and other treatments. The 2 *Bacillus* treatments were also significantly ($P<0.05$) different from the control.

Table 3. Experiment 2. Influence of incubation time on the viability (% growing) of *R. solani* sclerotia following the treatment of tubers with pencycuron or fungal antagonists

Treatment	Incubation period (days) 7	14	21	28	35
Control	—	—	96	91	84
Pencycuron (1.3 g/kg tuber)	79	42	36	21	9
V. biguttatum[A]	35	22	1.4	4	3
G. roseum[A]	77	58	47	82	42

[A] Dipped in a suspension of 10^6 spores/mL.

Discussion

The efficacy of treatments to reduce tuber-borne inoculum of *R. solani* was tested by measuring the viability of sclerotes removed from treated tubers. While this method only utilised sclerotes, there are other forms of inoculum on potato tubers, such as strands of *R. solani* mycelium, which develop on the tuber surface particularly around dormant buds. The viability of this mycelium was not tested in our experiments, as it was assumed that treatments that devitalised sclerotes would have also been effective against the exposed strands of hyphae.

Of the fungicides tested, fenpiclonil, formalin, pencycuron, and tolclofos-methyl effectively reduced the viability of *R. solani* sclerotes on the surface of seed potato tubers. Any of these chemicals should reduce or eliminate the tuber phase of *R. solani*. This is particularly important because a recent survey from seed-producing areas in Victoria found *R. solani* on 30% of potatoes (de Boer and Wicks 1994).

Although formaldehyde is widely used in the potato industry in Australia and was shown here to be highly effective in killing sclerotes, a kill of 100% was only achieved with immersion for 20 min in 2% concentration or at least 10 min in 4% concentration. This confirms other reports on the efficacy of formaldehyde (Weinhold *et al.* 1982; Carling *et al.* 1989), but those workers found 2–5 min immersion in 2% formaldehyde sufficient to kill sclerotes. Why a longer immersion was needed in our study is unclear, since the tubers were relatively free of adhering soil, which may have reduced the efficacy of the treatment. Formaldehyde is unlikely to be used because it is a dangerous chemical to handle, and large volumes of dipping solution must be disposed of after seed treatment.

Sodium hypochlorite has been used by growers as a dip treatment for potatoes, but our work confirmed the report of Weinhold *et al.* (1982) showing it to be less effective than formaldehyde.

Fungicides such as fenpiclonil, pencycuron, and tolclofos-methyl controlled *R. solani* at levels similar to formaldehyde, and applied as either dusts or sprays, they should offer practical alternatives to formaldehyde dips. However, further testing of fungicide rates and methods of application is required before recommendations can be made.

Biological control is an attractive alternative to chemicals used for the control of *R. solani*. We isolated both *V. biguttatum* and *G. roseum* from sclerotes; this was not unexpected, as both fungi have been recovered from *R. solani* sclerotes in other countries (Jager *et al.* 1979; Boogert and Gams 1988). Although the distribution of *V. biguttatum* appears to be worldwide (Boogert and Saat 1991), this is the first report of the fungus from South Australia. Our work also confirms

numerous reports from Holland showing *V. biguttatum* to be a potential biocontrol agent for *R. solani*. For example, we found that the viability of sclerotes rapidly declined by 3 weeks after inoculation with *V. biguttatum*, similar to the report of Velvis *et al.* (1989).

The fact that *V. biguttatum* can be readily cultured and easily applied to seed tubers and is capable of surviving >4 years in uncropped soil in the absence of *R. solani* (Boogert and Velvis 1992) suggests that this fungus has great potential as a biocontrol agent for *R. solani* on potatoes. Treatment of potato seed tubers to control seed-borne inoculum is becoming increasingly important in the management of potato diseases, particularly where new land is planted or where soil has been fumigated. The possibility of integrating chemical and biological treatments of seed potato tubers, utilising low dosage rates of chemicals, has shown promise overseas (Jager and Velvis 1986) and needs evaluation in Australia, particularly where the levels of infection are high.

Further work is underway to determine the presence of *V. biguttatum* in other potato-growing areas of Australia. If present, isolates can be evaluated as antagonists for use as inoculum for seed tubers.

Other antagonists to *R. solani* are required for any biological program; however, none of the other organisms we tested were as effective as *V. biguttatum*. In particular, the formulation of *Trichoderma* showed no inhibitory effect on the germination of sclerotes, which confirms the poor performance of the formulation evaluated as a seed tuber treatment in recent field experiments for the control of *R. solani* (T. J. Wicks unpublished data).

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Influence of soil fumigation and seed tuber treatment on the control of *Rhizoctonia solani* on potatoes

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Summary. Five field experiments were conducted in South Australia to determine the effect of soil fumigation (metham sodium) and chemical and biological seed tuber dressing on the severity of *Rhizoctonia solani* on potato stems and tubers.

These experiments indicated that both soil- and tuber-borne inoculum must be considered in any program aimed at controlling *R. solani*.

Tuber treatments of either a 20 min dip in 2% formaldehyde, sprays with pencycuron (0.15 mL a.i./10 kg seed), iprodione (2 mL a.i./10 kg seed) or a spore suspension of 10^6 spores/mL of *Verticillium biguttatum* or a dust with tolclofos methyl (4 g a.i./10 kg seed) were most effective if planted in soil fumigated with 500 L/ha metham sodium or soil with low levels of *R. solani*. A commercial formulation of *Trichoderma harziannum* and *T. koningii* applied as a dust at 1.3 g/10 kg seed was in most cases ineffective when treated seed was planted into either fumigated or unfumigated soil.

The incidence of progeny tubers with sclerotia varied between sites and ranged from 85% in an unfumigated soil planted with infected tubers to 2% in a fumigated soil planted with pencycuron-treated tubers. Except for 1 experiment where tubers were harvested early, neither seed treatments nor fumigation improved total nor marketable yield.

Introduction

Rhizoctonia solani Kühn is a widespread pathogen of potatoes that attacks stems and stolons, and forms sclerotes on the surface of tubers near harvest. Although total yield increases following *R. solani* control are rarely reported (Cother 1983) the disease is recognised by growers as a significant problem (Dillard *et al.* 1993). This is because *R. solani* prunes stolons, resulting in the production of large unmarketable tubers (Hide *et al.* 1973) and in fresh market production, the formation of sclerotia on the tubers downgrades quality.

The relative importance of seed tuber-borne inoculum and soil-borne inoculum has been studied, and it is generally considered that reducing tuber-borne inoculum is critical in controlling the disease (Frank and Leach 1980; Hide and Cayley 1982; Weinhold *et al.* 1982). However, soil-borne inoculum is also important particularly where *R. solani* survives in the soil in the absence of potato crops (Carling *et al.* 1986). Soil-borne inoculum may also be important where potatoes are planted after cereal crops as Carling and Leiner (1990) reported *R. solani* anastomosis group 8 (AG_8), a common pathogen of wheat and other grain crops, to be pathogenic to potato plants.

A wide range of chemicals and some biological agents have been evaluated as seed tuber treatments. For example, benomyl (Davis 1973), formaldehyde (Carling *et al.* 1989), iprodione (Hide and Cayley 1982; Cother 1983), pencycuron (Adam and Malcolm 1988), tolclofos-methyl (Hide and Read 1991), thiabendazole (Hide and Cayley 1982; Cother 1983), *Trichoderma viride* (Beagle-Ristaino and Papavizas 1985) and *Verticillium biguttatum* (Jager and Velvis 1986, 1988) controlled rhizoctonia when applied to seed tubers. However, in Australia few chemicals are registered for this use and apart from work by Wicks *et al.* (1995), no studies have been conducted on the use of biocontrol agents on seed tubers. In South Australia, many potato growers have not controlled *R. solani* with chemical seed treatments and some are attempting to control the disease by fumigating soil with metham sodium. While metham sodium is widely used to control soil-borne diseases of potato (Powelson and Rowe 1993) and other vegetables (Sumner and Phatak 1988) in the USA, the efficacy of its use on potatoes in Australian conditions has not been critically evaluated.

Studies were undertaken to evaluate chemical and biological treatments of seed tubers in conjunction with soil fumigation as means of controlling *R. solani*.

Material and methods

General

Three experiments evaluating seed tuber treatments with or without soil fumigation were conducted on

commercial potato-growing properties in the Virginia area, about 50 km north of Adelaide, South Australia. Another 2 experiments evaluating seed tuber treatments planted into either soil sown to potatoes the previous year or soil never planted to potatoes were conducted at the Lenswood Horticultural Centre, about 30 km east of Adelaide.

Soils were predominantly red-brown earths and loamy sands at the Virginia and Lenswood sites, respectively.

The health status of all seed lots was checked before planting by removing random samples of tubers from each seed batch and recording the incidence of *R. solani* sclerotia on the surface of at least 50 tubers from each site.

Chemical treatment of seed tubers

The chemicals and rates used per 10 kg of seed tuber were 0.6 mL Monceren (25% a.i. pencycuron), 8 mL Rovral (25% a.i. iprodione), 40 g Rizolex (10% a.i. tolclofos methyl) and formalin (37% a.i. formaldehyde). Monceren and Rovral were applied as a spray in 100 mL of water and Rizolex as a dust. Batches of 10 kg of tubers were rotated for 60–90 s using a manually operated cement mixer to ensure that the treatments evenly covered the surface of each tuber. Formaldehyde treatments were applied by dipping dormant tubers in a 2% solution for 20 min. All chemical treatments were applied at least 1 week before planting.

Except for experiment 1 where the *Verticillium* treatments were planted by hand, all other tuber seed was planted using commercial planting machines. Fertiliser applications, irrigation, and pest, foliar disease and weed control were undertaken by the grower. At the Lenswood Horticultural Centre, these operations were similar to grower practice. At Lenswood, either chlorothalonil, mancozeb or difenoconazole was applied to control *Alternaria solani* and metribuzin or fluazifop applied to control weeds. The exception to this was where weeds were manually removed from the crop 4, 11 and 14 weeks after planting.

Biological treatment of seed tubers

Biological treatments were: (i) Tri-D25, a commercial formulation of *Trichoderma harzianum* and *T. koningii* (J. B. Biotech Inc., PO Box 7493, Ventura, CA 93006, USA); (ii) a spore suspension of *Verticillium biguttatum*; (iii) a spore suspension of *Gliocladium roseum*; and (iv) a spore suspension of an unknown *Bacillus* sp.

Verticillium biguttatum and *G. roseum* were originally from naturally infected *R. solani* sclerotia on potatoes harvested isolated from a commercial property (Wicks *et al.* 1995), whereas the *Bacillus* isolate was obtained from a potato rhizosphere from potatoes in the Virginia area and shown to be antagonistic to *R. solani in vitro* (R. Balali pers. comm.).

Trichoderma was dusted onto the seed tubers at 1.3 g/10 kg of tubers, whereas the *Verticillium* and *Gliocladium* were applied separately as a spray of 100 mL containing 1 x 10^6 spores/mL. These treatments were applied on the day of planting using a manually operated cement mixer as previously described. *Bacillus* was also applied to tubers on the day of planting by dipping them in a solution of at least 1 x 10^6 colony forming units (cfu)/mL for 1.5 h and allowing to air dry.

Assessments

Between 8 and 15 weeks after planting, 5 plants per plot were selected at random and dug up to determine the incidence and severity of *R. solani* infection at each site. This was done by rating the degree of infection on the underground stems on a 0–5 scale similar to that described by Weinhold *et al.* (1982).

At harvest, plants from 8 to 10 m per plot were dug up by a single-row digger and the potatoes collected to measure total yield and tuber number. One hundred tubers selected at random from each plot were assessed for presence of sclerotia and severity of infection. The surface area of the tuber covered by sclerotia was rated on a 0–4 scale, similar to the one described by Dijst (1985). The severity of stem lesions as well as the severity of sclerotia on the tubers was converted to a 0–100 scale as described by Wicks *et al.* (1994).

Statistical analysis

Data were analysed using analysis of variance of a randomised block design in the statistical analysis program STATISTIX (NH Analytical Software, Roseville, MN, USA).

Soil fumigation–seed treatment experiments

Experiment 1. This experiment evaluated seed tuber treatments formalin, Monceren, Rizolex, Rovral, *Trichoderma* and *Verticillium* in soil naturally infested with *R. solani*, and either with or without metham sodium fumigation.

Before the experiment was established, an indication of the incidence of *R. solani* at this site was obtained by digging up 30 volunteer potato plants from the previous crop that were selected at random and examined for stem lesions about 4 weeks after they had emerged.

On 20 December, 2 months after a previous potato crop had been harvested, metham sodium at 500 L/ha was injected under pressure behind tines set 20 cm beneath the soil surface. A roller set up immediately behind the tines compressed the soil to reduce vapour loss.

The site was planted with cv. Atlantic 6 weeks after fumigation. Plots were 15-m long and arranged in a randomised block with 6 replicates per treatment. Plots consisted of 2 rows 75 cm apart with plants 25 cm apart along the row. Harvesting commenced on 22 June 1994.

Experiment 2. Seed tuber treatments of either formalin, Monceren or *Trichoderma* were evaluated on cv. Exton at another property where the soil had been previously fumigated with 500 L/ha metham sodium 6 weeks before planting. An extra treatment included the application of *Trichoderma* at 1 g/20 L of water to 15-m of row at emergence in addition to the *Trichoderma* seed treatment.

Double row plots, 15 m long were arranged in randomised blocks with 4 replicates. Tubers were planted in September 1994 and harvested 133 days later.

Seed treatment experiments

Seed was treated to evaluate the efficacy of chemical and biological treatments applied to seed tubers before they were planted in soil, either with or without high levels of soil-borne inoculum.

Experiment 3. Efficacy of formalin, Monceren and *Trichoderma* seed tuber treatments were compared in soil known to be infested with *R. solani*. Seed of cv. Coliban were planted in March 1994 in double rows 15-m long with treatments replicated 4 times and arranged in randomised blocks. The trial was harvested prematurely in early June after the crop was severely damaged from sand blasting during a storm in late May.

Experiments 4 and 5. The experiments conducted at the Lenswood Horticultural Centre evaluated seed treatments of formalin, Monceren, *Trichoderma*, *Verticillium*, *Gliocladium* and *Bacillus* on unfumigated land. The trial design was similar to that used in experiment 1 except there were 4 replicates per treatment. Experiment 4 was planted in an area that had been sown to potatoes each year for the previous 2 years, whereas experiment 5 was planted in an area that had been under pasture for at least 5 years following the removal of trees from an apple orchard. *Gliocladium* was evaluated in experiment 4 and *Verticillium* in experiment 5.

The seed tuber source in experiments 4 and 5 was uncertified cv. Atlantic known to be infected with *R. solani*. These experiments were planted on 1 December, assessed for stem lesions 14 weeks later and harvested on 18 April (experiment 4) and 20 April (experiment 5).

Table 1. Incidence of *Rhizoctonia solani* sclerotia on seed potatoes

Expt	Cultivar	No. of tubers examined	Tubers with sclerotia (%)
1	Atlantic	50	63
2	Exton	100	0
3	Coliban	50	64
4	Atlantic	1000	100
5	Atlantic	1000	100

Results

Soil fumigation–seed treatment experiments

Experiment 1. Mycelia and lesions typical of those caused by *R. solani* were found on underground stems on 80% of the 30 volunteer potato plants examined, indicating that soil-borne inoculum was high and evenly distributed in this area. At least 63% of seed tubers used in this experiment had 1 or more viable sclerotia of *R. solani* on the tuber surface (Table 1). Untreated seed tubers planted in fumigated soil gave rise to plants with 34% of stems infected with *R. solani* (Table 2). In fumigated soil, all chemical and biological treatments reduced the incidence and severity of stem lesions caused by *R. solani*.

When the severity of stem lesions was compared between the fumigated and unfumigated plots of the same seed tuber treatment, standard *t*-tests ($P = 0.05$) showed significantly more disease developed in treatments grown in unfumigated compared with fumigated soil except for the control and formalin-treated seed. At harvest, however, similar analysis showed that there was no significant difference in the

Table 2. Experiment 1. Incidence and severity of *Rhizoctonia solani* on cv. Atlantic potatoes grown from chemically or biologically treated tubers and planted in soil either with or without metham sodium fumigation

U, untreated soil; F, soil fumigated with 500 L/ha metham sodium 6 weeks before planting

Seed treatment	Stem infection					Tuber infection				Tuber yield (t/ha)	
	Stems infected (%)		Severity		Signif. of	With sclerotia (%)		Severity			
	U	F	U	F	*t*-value	U	F	U	F	U	F
Control	63	34	33	26	n.s.	43	54	17	27	40	45
Trichoderma	67	10	40	5	*	46	52	18	24	43	45
Formaldehyde	46	9	16	6	n.s.	41	23	16	11	44	44
Rovral	56	12	30	6	*	38	21	14	10	37	44
Rizolex	50	6	22	4	*	30	22	11	9	43	41
Monceren	54	9	26	5	*	31	19	11	9	40	43
Verticillium	36	4	16	3	*	40	17	15	7	40	39
l.s.d. ($P = 0.05$)	n.s.	15.7	15.9	10.7		n.s.	19.8	n.s.	11.4	n.s.	n.s.

* $P = 0.05$, for comparison of severity values from fumigated and unfumigated areas, respectively. n.s., no significant difference.

Table 3. Experiment 2. Incidence and severity of *Rhizoctonia solani* on cv. Exton potatoes grown from chemically or biologically treated tubers and planted in soil fumigated with metham sodium

Seed treatment	Stem infection		Tuber infection		Tuber yield
	Infected (%)	Severity	With sclerotia (%)	Severity	(t/ha)
Control	22	11	23	7	26
Formaldehyde	20	11	15	5	17
Trichoderma seed + soil drench	17	10	7	3	26
Trichoderma seed only	20	11	14	4	25
Monceren	5	4	2	1	22.6
l.s.d. ($P = 0.05$)	n.s.	n.s.	11	3.7	n.s.

severity of disease between fumigated and unfumigated areas within any tuber seed treatment.

At harvest, sclerotia were detected on 43 and 54% of tubers grown from untreated seed tuber in both the fumigated and unfumigated soil respectively. In unfumigated soil, none of the tuber seed treatments reduced disease significantly compared with the control, whereas in the fumigated soil, significantly less disease developed in the chemical and *Verticillium* treatments. *Trichoderma* had no significant effect on the incidence or severity of sclerotia on the tubers. Significant increases in either total or marketable yield were not detected in any seed tuber or soil treatment.

Experiment 2. Sclerotia of *Rhizoctonia* were not found in tubers used for seed in this experiment (Table 1) although microscopic examination detected hyphae of *Rhizoctonia* on the surface of 4% of the tubers. The assessment 8 weeks after emergence, showed that the incidence and severity of *Rhizoctonia* stem lesions was not significantly different between treatments, although it was lowest in plants grown from tubers treated with Monceren. By harvest, however, the incidence of tubers with sclerotia and severity of sclerotia on the tubers was greatest in the plots of untreated tubers but only the *Trichoderma* seed tuber treatment plus soil drench and the Monceren treatments were significantly less than that of the controls (Table 3). None of the treatments improved tuber yield.

Seed treatment experiments

Experiment 3. In this experiment, 64% of the tuber seed carried sclerotia of *Rhizoctonia* (Table 1). Untreated seed tuber planted in infested soil resulted in 51% of plants with stem lesions and sclerotia on 85% of the progeny tubers (Table 4). None of the treatments reduced the severity of stem lesions compared with the untreated control whereas all treatments significantly reduced the severity of sclerotia on the tubers at harvest. The severity of tuber infection was lowest in the Monceren treatment

Table 4. Experiment 3. Incidence and severity of *Rhizoctonia solani* on cv. Colfban potatoes grown from seed tubers treated with either chemicals or *Trichoderma*

Seed treatment	Severity of stem infection (%)	Tuber infection with sclerotia (%)	Severity	Tuber yield (t/ha)
Control	51	85	31	9.9
Trichoderma	47	65	21	14.2
Formaldehyde	39	46	18	15.4
Monceren	39	49	15	14.4
l.s.d. ($P = 0.05$)	n.s.	11.7	3	3.7

Table 5. Experiments 4 and 5. Influence of seed treatment and cropping history on the incidence and severity of *Rhizoctonia solani* on cv. Atlantic potatoes

O, old area planted to potatoes the previous two years; N, new area never planted to potatoes

Seed treatment	Stem infection				Tuber infection				Mean tuber weight (g)	
	Infected (%)		Severity		With sclerotia (%)		Severity			
	O	N	O	N	O	N	O	N	O	N
Control	41	47	21	28	69	51	23	21	136	143
Formaldehyde	45	13	14	6	60	22	18	7	133	139
Monceren	43	7	19	1	28	26	8	8	150	130
Trichoderma	55	32	39	17	52	43	15	15	144	136
Verticillium	—	22	—	9	—	9	—	3	—	134
Gliocladium	30	—	10	—	41	—	13	—	154	—
Bacillus	43	8	20	3	53	37	18	13	152	133
l.s.d. ($P = 0.05$)	n.s.	22.9	n.s.	18.5	21.1	16.9	9.2	8.9	n.s.	n.s.

although it was not significantly ($P>0.05$) different from formalin. All treatments significantly increased yield compared with the control, but there were no significant differences between treatments.

Experiments 4 and 5. All tubers used in these experiments had 1 or more sclerotia of *R. solani* on the tuber surface (Table 1). The incidence and severity of stem lesions were similar in the control plots of both the old area previously planted to potatoes and the new area (Table 5). In the old area, none of the seed tuber treatments significantly reduced the severity of the stem infection; however, the severity was significantly reduced by Monceren, formalin and *Bacillus* in the new area.

At harvest, all treatments in the new area except *Trichoderma* had fewer tubers with sclerotia compared with the control. In the old area, only Monceren and *Gliocladium* treatments significantly reduced the sclerotia severity; however, in the new area formalin, Monceren and *Verticillium* significantly reduced the severity of sclerotia on the tubers. None of the treatments improved yield in either the new or old areas.

Discussion

Limited sampling of potato tuber seed used in these experiments and more extensive sampling of seed potatoes in Victoria (de Boer and Wicks 1994) shows that potato seed tubers used in Australia are frequently infected with *R. solani*. High levels of *R. solani* infection of seed tubers have been reported from England and Wales (Hide 1981). In addition, we found hyphae of *R. solani* on tubers free of visible sclerotia, which was also reported by Hide *et al.* (1973) and confirms the conclusion of Frank and Leach (1980) that seed piece treatments should not be confined to tubers with only visible sclerotia. Treatments to eliminate or at least reduce the level of *R. solani* on seed tubers are warranted particularly when new land is brought into potato production or fumigated soil is planted to potatoes. The chemicals formalin, Rovral, Rizolex and Monceren and the mycoparasite *Verticillium biguttatum* were the most effective of the tuber seed treatments tested.

Verticillium performed as well as chemical treatments in experiment 1, suggesting that it has potential to be developed as an alternative to chemical control of *Rhizoctonia*. Our field results with *Verticillium* confirm both reports from other countries and previous laboratory studies on the efficacy of this fungus in inactivating sclerotia of *R. solani* (Jager and Velvis 1986, 1988; Wicks *et al.* 1995). However, as with the report of Jager and Velvis (1986), we found *Verticillium* less effective in soils with high levels of *R. solani* inoculum. The control achieved with *Trichoderma* applied as a soil drench after emergence suggests that this method of application needs further evaluation. Applying a spore suspension to the soil surface either just before rain or irrigation should ensure that the antagonist is leached into and dispersed throughout the soil profile and likely to be more effective than a seed coating. As *Verticillium* is capable of surviving in uncropped soil in the absence of *R. solani* (van den Boogert and Velvis 1992) it may be possible to induce suppressiveness to *R. solani* by applying a spore suspension of the antagonist to fumigated soil.

Although our experiments showed other biological treatments were, in most cases, less effective than chemicals in reducing the development of sclerotia on tubers at harvest, further work with these and other biocontrol agents are warranted as *Gliocladium*. *Trichoderma* and binucleate *Rhizoctonia* are reported as useful biocontrol agents (Papavizas 1985; Escande and Echandi 1991). In particular, Beagle-Ristaino and Papavizas (1985) showed that seed tuber treatments with certain strains of *Gliocladium* and *Trichoderma* significantly reduced disease incidence and severity as well as the viability of tuber-borne sclerotia of *R. solani*.

In soils previously planted to potatoes and where levels of soil inoculum were high, tuber seed treatments were less effective or, in most cases, did not inhibit the development of *R. solani* sclerotia on progeny tubers. The development of sclerotia on 30% or more of tubers grown from treated seed tubers emphasises the importance of reducing the level of soil inoculum before planting. In some of our experiments this was achieved by fumigating the soil with metham sodium, which is effective against *R. solani* (Sumner and Phatak 1988). However, no direct measurements of *R. solani* inoculum levels were made in soil before and after fumigation which would have enabled the efficacy of the fumigation treatment to be measured. The high incidence of stem lesions on volunteer plants examined before fumigation at site 1 suggested that high levels of *R. solani* were evenly distributed in the soil. At this site, the low incidence of stem lesions on plants in the fumigated compared with the unfumigated area showed that the fumigation treatment was effective. Comparison of these levels with the incidence of stem lesions on plants growing from treated tubers also indicated that metham sodium fumigation reduced the level of soil *R. solani* inoculum early in the season.

The level of sclerotial infection that occurred by harvest in the fumigated areas suggests that the level of *R. solani* inoculum increased over the growing season. This could have initiated from: (i) seed-borne inoculum not killed by the seed treatments; (ii) soil-borne inoculum not eradicated from treated soil; (iii) recontamination of the fumigated area from wind-

blown inoculum; or (iv) from *R. solani* growing up from beneath the fumigated zone. Nevertheless, comparison of disease incidence and severity of stem lesions on plants in unfumigated and fumigated areas suggested that most infections in the fumigated area arose from tuber-borne inoculum. These results emphasise the importance of eliminating or reducing tuber-borne inoculum when planting fumigated areas.

A significant yield increase following treatment of tubers was detected in only 1 experiment and this was where the tubers were harvested early. The failure to detect significant difference in either total or fresh marketable yields between treatments was surprising, particularly where large differences in the incidence and severity of *Rhizoctonia* occurred between treatments. Single plant assessment as described by Weinhold *et al.* (1982) or larger plot sizes may have detected significant field increases.

Potato growers in South Australia are continuing the use of soil fumigation as they believe it has other benefits such as control of weeds, volunteer potato plants and soil-borne diseases such as powdery scab (*Spongospora subterranea*) and common scab (*Streptomyces scabies*).

Conclusions

These experiments have shown that both soil- and tuber-borne inoculum must be considered in any program aimed at controlling *R. solani*. For example, seed tuber treatments that control the tuber-borne phase of *R. solani* are only effective if treated tubers are planted in fumigated soil or soil with nil to low levels of the fungus. Also the benefits of soil fumigation are lost unless the treated area is planted with tubers treated to eliminate or reduce the level of tuber-borne inoculum.

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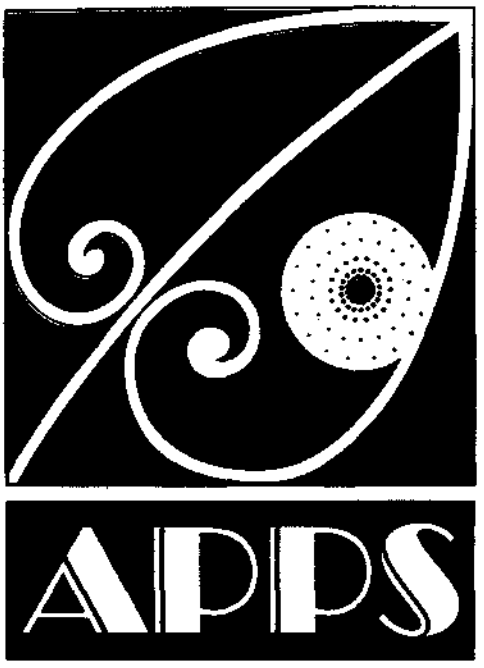

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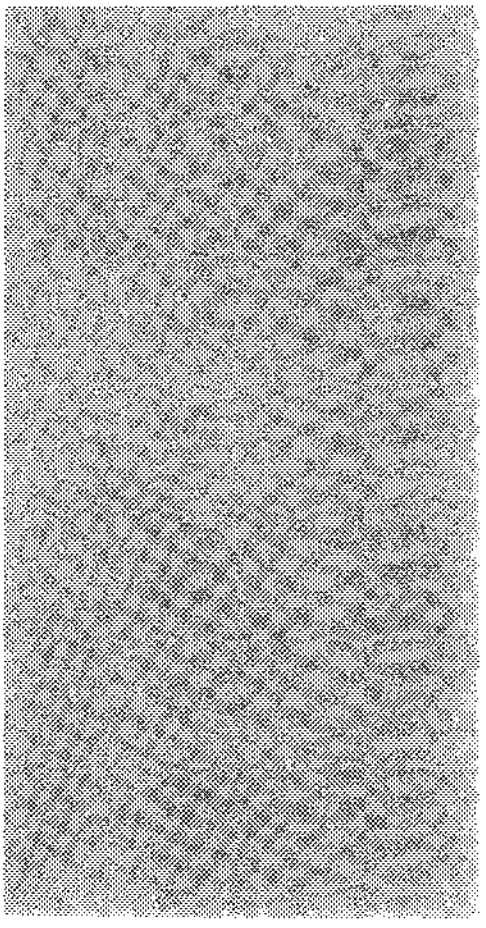

10th Biennial Australasian Plant Pathology Society Conference

Scientific Programme and Abstracts

Lincoln University
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28 - 30 August 1995
(Workshops 23 - 26 Aug & 31 Aug - 2 Sep)

48. An investigation of the epidemiology and control of *Ascochyta* diseases of peas in Tasmania

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A field survey combined with disease monitoring was conducted in several pea paddocks in the North West of Tasmania in the 1994 season. The collar rot incidence tended to increase between four weeks after sowing and the end of the growing season at which time most plants had become infected. Due to drier weather conditions this year the disease did not cause any major yield losses. All pathogen cultures isolated from collar rot affected pea plants appear to resemble *Phoma medicaginis* var. *pinodella*. *Ascochyta pinodes* was isolated from leaves and seed, but there was no evidence of *A. pisi*. Evidence from pot trials with various soil samples indicated that collar rot pathogens are universal in Tasmanian soil and may survive in soil for several years. The protection provided by the current seed treatment with Apron (Metalaxyl) / P-Pickel T (Thiram + Thiabendazole) is very effective in eliminating disease from seed but is not durable enough to protect pea plants from soil-borne infection. Some alternative fungicides were found to inhibit fungal growth in agar and may offer improvements to the duration of disease control. Results from a field trial indicated that the use of the herbicide metribuzin led to a 10 % increase in the incidence of collar rot.

49. An investigation of potato black dot disease in Tasmania and its control

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Field surveys conducted in the early 1990s revealed widespread presence of the disease in all potato growing areas in Tasmania. Only some paddocks with no previous history of potatoes were free of the disease. The disease is caused by *Colletotrichum coccodes* and seems to perpetuate itself through infected seed, haulms, and infested soil. Weeds like *Solanum nigrum* and volunteer potato plants may serve as a reservoir for the pathogen. Soil and / or seed-borne infection appears to be necessary for early dying symptoms, quality defects, and yield decreases to be produced.
In *vitro* and in *vivo* investigations have shown promising results with Octave (Prochloraz), Shirlan (Fluazinam), and Saphire (Fludioxonil). Prochloraz, in addition to suppressing disease, showed growth promotion and yield increases in the field. *Gliocladium roseum* as a biological control agent was trialed in pots and resulted in better growth and protection from early dying symptoms without adversely affecting tuber quality. The two methods provide the potato industry in Tasmania and elsewhere with a potential integrated approach not only to control black dot, but also other soil-borne pathogens.

50. Effects of tuber and in-furrow chemical treatments on powdery scab control on potatoes

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Powdery scab, caused by *Spongospora subterranea*, is still considered the most important problem facing the New Zealand Potato Industry. Field trials over a three year period evaluated tuber and in-furrow treatments for powdery scab control. Fluazinam and mancozeb have consistently provided good disease control when applied as either tuber treatments against tuber-borne inoculum or in-furrow treatments against soil-borne inoculum. Dichlorophen-Na, dichlofluanid and formaldehyde solution have also provided effective control of tuber-borne powdery scab when applied as a tuber treatment. During the 1994/95 season, two field trials were conducted to test a more extensive range of chemical rates and additional chemicals for control of powdery scab. In trial 1, 28 tuber treatments were applied to infected tubers of the cultivar Agria. In trial 2, 15 in-furrow treatments were applied at the time of planting uninfected Agria seed tubers into plots inoculated with powdery scab. The trials were irrigated six times to provide conditions favourable for disease development. Plant emergence was assessed and the trials were harvested at crop maturity when all tubers were assessed for powdery scab infection. Yield parameters were also determined. Powdery scab infected seed tubers (Trial 1), treated with dichlorophen-Na, fluazinam, flusulfamide and mancozeb gave progeny with significantly less powdery scab incidence and severity compared to untreated controls. None of the treatments affected total yield. Compared to untreated controls, progeny from tubers planted into soil inoculated with powdery scab (Trial 2) showed significantly less powdery scab incidence and severity from plots treated with cyprodinil, dichlorophen-Na, fluazinam, flusulfamide, mancozeb and sulfur. Cyprodinil, dichlorophen-Na, flusulfamide, mancozeb and sulfur also increased total yield of tubers.

51. Control of *Rhizoctonia solani* on potatoes with tuber seed treatments and soil fumigation

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Rhizoctonia solani (AG3) is a widespread pathogen of potatoes that attacks the stems and stolons and forms sclerotes on the surface of tubers near harvest. Fumigation with metham sodium is widely used in South Australia to reduce soil inoculum and control weeds and volunteer plants. In 3 different field experiments, potato seed tubers treated with chemicals and biological control agents were planted in soil with or without fumigation using 500 L/ha metham sodium. Tubers were naturally infected with more than 63% viable sclerotia of *R. solani*. In fumigated soil, seed treatments of formaldehyde, the fungal antagonist *Verticillium biguttatum*, pencycuron, iprodione or tolclofos methyl significantly reduced stem infection and the incidence and severity of sclerotia on tubers at harvest. Trichoderma reduced stem infection only. No treatment reduced the severity of sclerotial infection at harvest in unfumigated soil.
The experiments showed that both soil and tuber borne inoculum must be considered in any program to control *R. solani*, as tuber seed treatments are only effective when planted in fumigated soil or soil with nil to low levels of fungi. Also soil fumigation is most effective when planted with tubers treated to eliminate seed borne inoculum.

Poster Session 7 : Vegetables

192. Chemical and biological control of *Rhizoctonia solani* on potato seed tubers

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Black scurf caused by *Rhizoctonia solani* is a serious disease of potatoes and the development of sclerotes on tubers grown for fresh market reduces marketability and results in economic loss. Reduction of tuber-borne inoculum is considered crucial in the control of this disease.
Tubers cv. Atlantic naturally infected with *R. solani* (AG3) and with 5 or more visible sclerotes were used to test chemicals and potential antagonists. After treatment tubers were incubated at 30°C in high humidity for 14 days before sclerotes were tested for viability. Sclerotes were considered viable if growth was detected on tapwater agar after 48 hours at 20°C. Formaldehyde, toclofos methyl, fenpiclonil and pencycuron all reduced sclerotial viability. Formaldehyde reduced viability to nil when used at 2% a.i. for 20 minutes. At the same rate, sodium hypochlorite was not as effective, reducing viability from 98% to 46%. Of the fungal antagonists tested, only *Verticillium biguttatum* significantly reduced sclerotial viability from 99% on untreated tubers to 6.5% on treated tubers. The longer the incubation period after treatment with *V. biguttatum*, the greater the reduction in sclerotial viability. Neither *Bacillus sp.*, *Bacillus subtilus* (Actizyme®) , *Trichoderma sp.* (Tri D 25®), nor *Gliocladium roseum* significantly reduced viability.

193. *In vitro* evaluation of Indian mustard as a control for *Rhizoctonia solani* on potatoes

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The Indian mustard plant (*Brassica juncea*) contains glucosinolates, which when broken down produce methyl isothiocyanate. This is the active ingredient of the fumigant metham, commonly used to control soil fungi.
Experiments were initiated to test the efficiency of Indian mustard against *R. solani* (AG3) from potatoes. Indian mustard plants were grown to 50% flowering and freeze dried after washing and drying the roots. The tops and roots were ground in a blender to powder and chaff and tested separately and combined. The growth of *R. solani* (AG3) on artificial media was totally inhibited when exposed to vapours from 0.01g or greater of seed meal. At least 0.25g of freeze dried tops or 1g of roots were required for total inhibition. Another test combining freeze dried roots and tops was less effective, with 2 g of plant material inhibiting growth of *R. solani* by only 58%.
Further evaluation of Indian mustard as a green manure crop and seed meal as a soil additive are underway.

194. Phosphonic acid fails to control powdery scab and black scurf of potatoes

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Phosphate based fungicides used to control diseases such as downy mildew and Phytophthora root rots are highly systemic and move rapidly within plants from sprayed leaves to roots. Because of this property, and reports of foliar sprays inhibiting formation of pink rot in potato tubers, some growers have used these materials in the hope of controlling other diseases such as black scurf and powdery scab. Fourteen post emergent sprays of 10 or 20 ml/L phosphonic acid were applied to pot grown potatoes (cv. Kennebec) planted in soil artificially inoculated with *Rhizoctonia solani*. Three sprays of 15 or 20 ml/L phosphonic acid were applied to field potatoes (cv. Atlantic) grown in naturally infected soil. Potatoes (cv. Atlantic) grown in pots or field artificially inoculated with powdery scab spores were sprayed on 3 occasions with either 15 or 20 ml/L phosphonic acid.

Phosphonic acid treatments did not reduce the number of tubers infected with sclerotes or powdery scab lesions, nor the severity of infection at harvest.

195. Integrated management of early and late potato blights

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Automatic weather recorders have been established in a number of potato crops in Tasmania and South Australia. Data from these loggers is being used to evaluate both early blight (target spot - *Alternaria solani*) and Irish blight (*Phytophthora infestans*) forecasting systems. Regular monitoring of crop disease symptoms and weather records are being undertaken to compare the efficiency of current chemical management strategies.
Several trials in both states have been set up to test various fungicide sprays and timing of application. Significant reduction in the severity of early blight infection with 2 less spray applications was achieved using 500ml/Ha Score on an eradicant spray schedule. In Tasmanian trials, correct timing of the initial spray resulted in marketable yield increases of up to 11% . Where Dithane was applied on a regular spray schedule for Irish blight protection, good control of early blight was achieved by replacing 5 sprays of Dithane with 3 sprays of Score in the middle of the spray schedule after the appearance of the first lesions.
After evaluation of all data is finalised, development of a forecasting system for potato leaf blights, suited to conditions in Australia, will be undertaken.

196. *Verticillium biguttatum* in South Australia

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Verticillium biguttatum occurs naturally on sclerotes of *Rhizoctonia solani* on potato tubers, and when used as a pre-plant dip shows promise as a biocontrol agent for *R. solani*. Investigations into the geographical spread of *V. biguttatum* in South Australia and the amount of natural sclerotial infection are underway. So far the fungus has been found in most of the main potato growing areas of South Australia, with 40-100% of the sclerotes infected. Some tubers from the southern areas of South Australia, although heavily infected with *R. solani* sclerotes, were not naturally infected with *V. biguttatum*.
In vitro tests have shown that *V. biguttatum* mycelia grows on artificial media between 15 and 30°C, with the best growth at 20°C. *R. solani* (AG3) grew in the same temperature range, however it was most active at 25°C. Further evaluation of the different geographical isolates is underway to ascertain if they differ in their efficacy against *R. solani* (AG3).

197. *Rhizoctonia* disease of potato in South Australia

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Isolates of *Rhizoctonia* collected from the stems, roots, tuber sclerotia and soil of potato crops in Virginia and Lenswood, South Australia, were identified based on number of nuclei per cell and anastomosis group (AG). Of the 305 isolates of *R. solani* tested, 89% were AG-3, 7% were AG-4, 2% were AG-5 and 1% were AG2-1; 12 isolates were binucleate *Rhizoctonia* spp. All AG-3, AG-4 and AG-5 isolates tested caused rhizoctonia disease symptoms on the potato cultivar Coliban in pathogenicity trials conducted in glasshouse conditions. Both AG-3 and AG-5 isolates caused black scurf and stem cankers, although symptoms of black scurf were more severe with AG-3. AG-4 isolates produced severe stem and stolon cankers. The pathogenicity of tuber-borne inoculum was confirmed by growing plants from sclerotia-infested tubers. This is the first report of isolates of AG-4 and AG-5 causing disease in potato crops in South Australia. AG-8 isolates from diseased barley and wheat produced severe root cankers and caused loss of feeder roots on inoculated potato plants. AG2-1 isolates, recovered only from tuber-borne sclerotia, were not used in pathogenicity tests. Rhizoctonia disease in potato crops in South Australia appears to be caused by a combination of different anastomosis groups and this has important implications for crop rotations.

198. Detection of benzimidazole resistance in isolates of *Monilochaetes infuscans* (Scurf) from Kumara in Northland

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In New Zealand three fungicides are currently registered for use on kumara (sweet potato, *Ipomoea batatas*). Benomyl (Benlate) and thiophanate-methyl (Topsin M-4A) treatments are recommended for use prior to planting out for control of scurf (*Monilochaetes infuscans*); and dicloran (Botran 75 WP) has recently been re-introduced, for use as a post-harvest treatment for control of *Rhizopus* rot. The majority of New Zealands kumara crop is produced in Northland. In a written survey completed by 33 kumara growers in the Northland region in June 1994, scurf was indicated (by 22 growers) as their biggest disease problem. In November 1994, cultures of *M. infuscans* were isolated from scurf lesions on kumara storage roots that had been grown commercially in the Ruawai district of Northland (where the benzimidazole fungicides benomyl and thiophanate-methyl are regularly used during commercial production); and also from experimental kumara plantings grown in the Pukekohe district of South Auckland (where fungicides are not used during production). Cultures of *M. infuscans* isolated from South Auckland kumara grew on un-amended media *in vitro*, but failed to grow on media containing 5 mg/l benomyl; cultures isolated from Northland kumara, however, grew on media containing 0, 5, 10, and 50 mg/l benomyl. The overuse of benzimidazole fungicides by Northland kumara growers in the past is likely to have been responsible for the development of the benomyl-resistant population of *M. infuscans* in the Ruawai district.

199. Spatial dynamics of dark leaf spot of chinese cabbage

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The spatial dynamics of epidemics of dark leaf spot disease of Chinese cabbage caused by *Alternaria brassicicola* were monitored over two months in three inoculated plots, each with 1000 plants. Runs analysis of the data indicated that disease was aggregated in all plots with higher aggregation within than across rows. Spatial Lag Autocorrelation Analysis (LCOR) revealed that diseased plants were almost always clustered during the epidemics. Proximity patterns were simple when disease incidence was less than 20%, and complex between 20% - 80%. Two-dimensional distance class (2DCLASS) analysis was revealed only one cluster of diseased plants in each plot and spread of disease between adjacent plants. The size of the core cluster increased with the increase of disease incidence over time. Minimum core cluster size increased from 216 to 310, 173 to 323 and 99 to 326 plants over time in field 1, 2 and 3, respectively.

200. Detection of Powdery scab (*Spongospora subterranea*) from potato tubers with PCR based detection systems

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Powdery scab has become a major concern for New Zealand potato growers and in particular they are concerned at the risk of spreading this disease through the planting of infected seed tubers.
There is a need to be able to detect this disease at low level on tubers.
Initially DNA was extracted from a number of sources:

1 infected potato tissue (including both potato skin and scab lesions)
2 individual *Spongospora* spores (collected under a dissecting microscope)
3 infected potato roots
4 pure potato tissue (sterile tissue culture)

This DNA was amplified with a standard ITS set of primers (Chambers et al. 1986).
The *Spongospora* specific band was deduced and sequenced. From this sequence *Spongospora* specific set of primers (Spongo 1&2) were designed.
Amplification conditions were optimised and specificity for *Spongospora* was verified against a range of common soil pathogens. Crude DNA extracts of infected potato skin and associated soil debris were prepared and amplified with Spongo 1 & 2 primers. The results confirmed that the primers were capable of detecting *Spongospora* against a high background of conflicting DNA sources.
A range of geographically distant *Spongospora* isolates were successfully amplified with this primer set.
Work is underway to determine the lowest detectable level of infection on a seed tuber and improved rapid isolation of PCR competent DNA.