Managing Brassica stem canker

Barbara Hall
South Australia Research & Development Institute (SARDI)

Project Number: VG06018

VG06018

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

The research contained in this report was funded by Horticulture Australia Ltd with the financial support of the vegetable industry.

ISBN 0 7341 2215 2

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

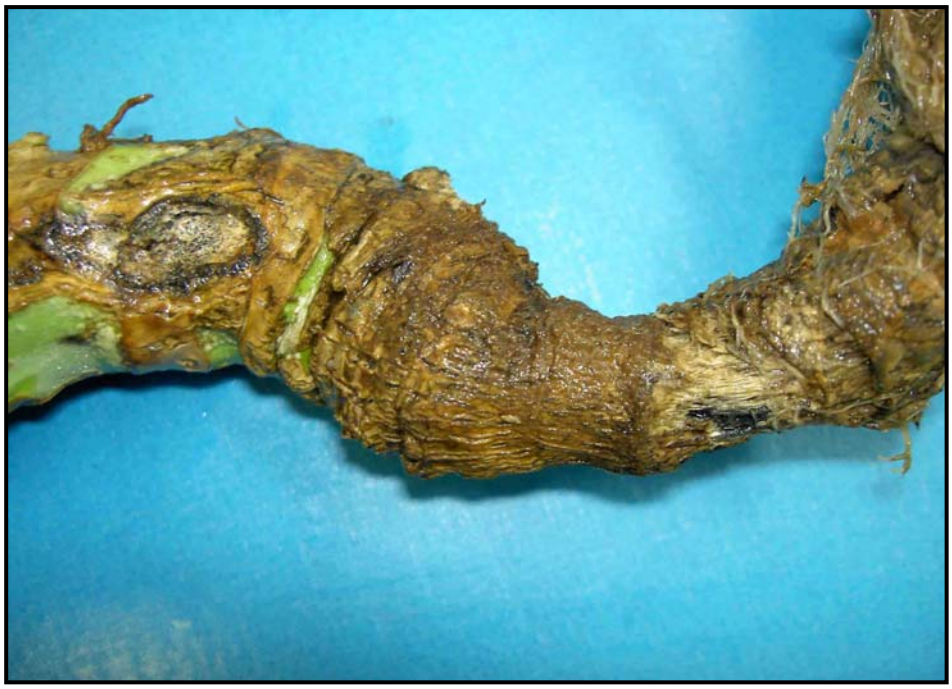
HAL

Know-how for Horticulture™

VG06018 - (December 2009)

Managing Brassica stem canker

Final Report



BH Hall *et al*
South Australian Research and Development Institute

HAL
Know-how for Horticulture™

Government of South Australia
SARDI
SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE

HAL project number: VG06018

Project leader: Barbara Hall
South Australia Research and Development Institute
GPO Box 397, Adelaide, South Australia 5001
Phone: (08) 8303 9562
Email: barbara.hall@sa.gov.au

Principal investigators: Lynette Deland
Phone: (08) 8303 9587
Email: lynette.deland@sa.gov.au

Belinda Rawnsley
Phone: (08) 8303 9409
Email: Belinda.rawnsley@sa.gov.au

Tiffany Barlow
Phone: (08) 8303 9585
Email: Tiffany.barlow@sa.gov.au

Catherine Hitch

This report presents results of experiments determining the cause and epidemiology of Brassica stem canker, and evaluations of management strategies.

This project was facilitated by HAL and funded by the National Vegetable Levy. The Australian Government provides matched funding for all HAL's R&D activities.

Table of contents

1. MEDIA SUMMARY

Stem girdling cankers have been observed on many Brassica crops in Australia with varying degrees of severity. In recent years growers in South Australia have reported losses of up to 80% as a result of stem canker. Symptoms range from a darkened superficial scurfing on the skin of the stems, variously sized irregular lesions mostly with black edges on the above ground stem, through to whole stems rotted with complete plant collapse. Often the scurfing or separate lesions will develop into complete stem rot. Disease surveys in 2005/06 showed that a complex of soil borne fungi including *Rhizoctonia* sp., *Leptosphaeria maculans*, *Pythium* sp. and *Fusarium* sp. were the main pathogens associated with the stem canker. *Verticillium albo atrum, Phoma* sp. and *Sclerotinia sclerotiorum* were also found associated with stem damage, but did not appear to be part of the canker complex in Brassica crops.

This project was undertaken to determine: (a) the relative importance of *Rhizoctonia*, *Pythium, Fusarium* and *Leptosphaeria* in the disease complex, (b) the infection pathways and environmental conditions conducive to disease development and (c) to evaluate management strategies.

Rhizoctonia solani and *L. maculans* were the dominant pathogens of the stem canker disease complex with *R. solani* anastomosis groups AG 2.1, AG 2.2 and AG 4 associated with the cankers. The relative severity of these pathogens is related to environmental conditions. *L. maculans* is more prevalent in wetter soil and *R. solani* AG 2.1 prefers cooler and AG 4 warmer temperatures. *Pythium irregulare* and *P. ultimum* var *ultimum* were isolated from plants with canker and early infection increased the severity of cankers in the presence of *Rhizoctonia*. However later infections decreased canker development, but the root infection caused significant plant stunting. The three species of *Fusarium, F. oxysporum, F. avenaceum* and *F. equiseti* recovered from stem cankers were pathogenic to cauliflower, however they were shown to be secondary invaders and did not increase canker severity.

These studies showed that infection is from soil borne fungi and begin within the first two weeks of planting, with a rapid increase in canker severity around six to eight weeks after planting. Infected plants were not found in the nursery, whereas both *R. solani* and *L. maculans* were detected in soil pre-planting. The severity of the cankers was positively correlated to the amount of fungal DNA in the plant, however the fungi could also be detected in symptomless plants.

Chemical management strategies were targeted to pre planting and planting soil drenches of fungicides, biological agents and alternative 'soft' options aimed to prevent the early infection. Greenhouse experiments indicated that the strobilurin fungicides Amistar® and Cabrio® were the most effective against both *Rhizoctonia* and *Leptosphaeria* with Maxim® and Score® also showed some efficacy. Other fungicides effective against *Rhizoctonia* (Rizolex® and Terraclor®) were not effective against *Leptosphaeria*. In addition, some fungicides were not effective against all the *Rhizoctonia* anastomosis groups. For example Chlorothalonil® was effective against AG2.2 but not against AG2.1 and AG 4.

In field experiments, none of the fungicides controlled stem canker, although canker development was suppressed with both Amistar® and Maxim® applied pre planting. The addition of a post planting drench did not significantly improve the suppression showing that more work needs to be undertaken to determine whether there would be any cost benefit from the extra application.

The use of ‘soft’ options and biological agents were effective in greenhouse evaluations, but not in the field experiments. Work needs to be undertaken to evaluate more of these products, as some may provide additional growth benefits as well as canker suppression.

None of the cultivars evaluated were resistant to stem canker, although some were less susceptible. Their use in conjunction with other control measure should reduce crop loss. Weeds were found to be hosts to *Rhizoctonia* and may provide a mechanism for carryover of the disease. However *Rhizoctonia* is an effective saprophyte and does not need a crop host to survive. Levels of the pathogen in soil were shown to increase over 18 months of fallow and incorporation of debris by rotary hoe and burial did not reduce the pathogen load. However *Leptosphaeria* was reduced by incorporation, but levels in soil were difficult to evaluate as often none could be detected in soil where the disease developed.

Rhizoctonia and *Leptosphaeria* require different management approaches, however with their combined effect in stem canker development, management strategies must address aspects of both pathogens. Using less susceptible cultivars, managing soil moisture, applying pre-planting drenches, incorporating debris and managing weeds will all assist in reducing stem canker severity to levels where a harvestable crop is achievable.

2. TECHNICAL SUMMARY

Brassica stem canker is a disease complex of several fungi causing symptoms that range from superficial scurfing, russetting and discrete lesions on stems to complete stem rot and plant collapse. Fungal pathogens involved in the disease complex have been identified as *Leptosphaeria maculans, Rhizoctonia solani AG 2.1, 2.2 and 4, Fusarium oxysporum, F. avenaceum* and *F. equiseti, Pythium irregulare* and *P. ultimum* var *ultimum.*

Between 2006 and 2009, 46 greenhouse and 16 field experiments were undertaken to determine the relative importance of these pathogens in the disease complex, the infection pathways and environmental conditions conducive to disease development and management strategies.

The main findings of this study were:

- *Rhizoctonia solani* AG 2.1, AG 4 and *L. maculans* were the dominant pathogens of the stem canker disease complex.
- Molecular tests detected *R. solani* in soil and plants whereas *L. maculans* was often not at detectable levels in soil but was found at high levels in plants grown in that soil.
- The severity of the cankers was positively correlated to the amount of pathogen DNA in the plant, however both *R. solani* and *L. maculans* were detected in symptomless plants.
- Early infection of *Pythium* increased the severity of cankers in the presence of *Rhizoctonia*. Later infections actually decreased canker development, but the root damage by *Pythium* caused significant plant stunting.
- *Fusarium,* while pathogenic to cauliflower, was a secondary invader and did not increase canker severity.
- No evidence of *R. solani* or *L. maculans* was found in nursery plants.
- Infection of both *R. solani* and *L. maculans* was from the soil and began within the first two weeks of planting, with a rapid increase in canker severity around six to eight weeks after planting.
- The relative severity of these pathogens was related to environmental conditions, with *L. maculans* more prevalent in wetter soil and *R. solani* AG 4 preferring warmer weather.
- Some cultivars were less susceptible to either *R. solani* or *L. maculans*, but none were resistant to stem canker and few were less susceptible to both pathogens.
- Weeds were found to be potential hosts to *Rhizoctonia* and may be responsible for proving a mechanism for carryover of the disease, however *Rhizoctonia* does not need a crop host to survive.
- *Leptosphaeria* was reduced by incorporation of diseased plant material into the soil but not *Rhizoctonia*.
- Fungicides drenches did not control stem canker, although they suppressed the development of cankers. The strobilurin fungicides Amistar® and Cabrio® were the most effective against both *Rhizoctonia* and *Leptosphaeria*. Maxim® and Score® also showed some efficacy, but other fungicides effective against *Rhizoctonia* (Rizolex® and Terraclor®) were not effective against *Leptosphaeria*. Some fungicides were not consistently effective against all the *Rhizoctonia* anastomosis groups.

3. INTRODUCTION

The project VG05005 "Scoping study to determine the soilborne diseases affecting Brassica crops" (Hitch *et al* 2006) showed that a new disease complex called “Brassica stem canker” was responsible for losses in Brassica crops. First observed in South Australia in 2000, where it caused losses of 70-80% in cauliflower crops. The disease rotted stems, causing plants to collapse or stems break at or before harvest The main organisms involved were identified as the soilborne fungi *Rhizoctonia*, *Pythium*, *Fusarium* and *Phoma*. Symptoms ranged from a darkened superficial scurfing on the stem surface, variously sized irregular lesions mostly with black to purple margins on the above ground stem, through to whole stems rotted with complete plant collapse. Often the scurfing or separate lesions developed into complete stem rot.

The disease has been found in Brassica crops throughout Australia, however losses have been highest in South Australian cauliflower crops planted in winter, with up to 100% loss at harvest in spring. In South Australia around 10,000 plants are sown each week. With conservative estimates of $7,000 - 8,000 loss per week in winter plantings, for the 38 plantings inspected in the Northern Adelaide Plains the potential loss was calculated to be up to $308,000 per week (Hitch *et al* 2006).

Brassica stem canker is first observed 2-4 weeks after planting and increases in incidence and severity as plants mature. The pathogens were not found in nursery speedlings, and fungicide treatments of Terraclor® and Rovral® applied in the nursery for *Rhizoctonia* had minimal effect on disease levels.

Cauliflower seedlings inoculated with either *Rhizoctonia or Phoma* developed stem canker. All *Rhizoctonia* isolates were pathogenic to cauliflower and the Anastomosis Groups (AG), determined by PCR techniques, confirmed AG 2.1, 2.2 and 4 were the main groups associated with the disease. The *Phoma* was confirmed as *Leptosphaeria maculans*, the cause of black leg in Brassica crops.

This project was undertaken to determine: (a) the relative importance of *Rhizoctonia*, *Pythium*, *Fusarium* and *Leptosphaeria* in the disease complex, (b) the infection pathways and environmental conditions conducive to disease development and (c) to evaluate management strategies.

4. TECHNICAL REPORT

4.1 General materials and methods

4.1.1 Isolates

Unless otherwise indicated, all isolates used in testing were cultures obtained from infected Brassica plants during the scoping study VG05005 (Hitch *et al* 2006).

Storage of isolates

Long term storage: Squares of agar (1 cm x 1 cm) were cut from actively growing fungal cultures on artificial media and approximately 15 squares were added to 10 ml of sterile distilled water (SDW) in sterile McCartney bottles. Bottles were stored in the dark at ~4^0C, except for *Rhizoctonia solani* AG 4 which was stored at room temperature (~22^0C) after it was found that the cultures did not survive at ~4^0C.

Short term storage: Isolates were maintained on 90 mm artificial media plates, sealed with parafilm and stored at ~4^0C or room temperature.

Maintenance of pathogenicity: After ~12 months, the *Rhizoctonia* and *Leptosphaeria* cultures used for experimentation were re-invigorated through cauliflower seedlings to ensure that pathogenicity was maintained. Six to eight week old seedlings were inoculated as described in section 4.1.2 and affected plant material isolated to recover the pathogen. The identity of all re-cultured pathogens was confirmed by PCR.

4.1.2 Inoculation techniques

Culture retrieval

When required for inoculation, plates or bottles were removed from storage and kept at room temperature for ~24 h prior to use. *Rhizoctonia* and *Fusarium* were plated onto potato dextrose agar (PDA) and *Pythium* onto corn meal agar (CMA) and grown at room temperature for up to ten days. Pieces of *Leptosphaeria* mycelia were placed onto ¼ PDA and grown at room temperature (~22^0C) under either black light or black light with white light on a 12 h light/dark cycle for up to three weeks, until pycnidia developed.

Stem inoculation

Two 7 mm plugs of mycelia from the margin of actively growing cultures were shallow buried ~2 mm in soil near the stem of each plant. Control plants were inoculated with sterile PDA. For *Leptosphaeria*, plants were wounded at the time of inoculation by scraping the base of the stem with a sterile needle.

Mycelial slurry inoculation

Actively growing cultures were macerated in a Waring blender with sterile demineralised water, mixing one plate with ~100 ml water. A specified amount of the slurry was either poured onto the soil surface of the potted plants or mixed well with soil and left covered in the greenhouse (~24^0C) for up to seven days before potting.

Spore inoculation

Spore suspensions were produced from *L. maculans* pycnidia grown on ¼ PDA as previously described. Sterile water was poured onto the plate to 2 mm depth and the surface

of the culture scraped with a sterile spreader. The suspension was diluted to $1x10^6$ spores/ml. Sterile water was used as a control.

Root inoculation: Seedlings were gently removed from trays and the roots washed then soaked for two minutes in the spore suspension before planting. After planting the remaining spore suspension was poured evenly over the inoculated pots, providing ~10 ml suspension per pot. Control plants were dipped in sterile water before planting and ~10 ml poured over the pot.

Foliar inoculation: Leaves were sprayed to run off with the spore suspension using a hand held atomiser. Plants were enclosed in moistened plastic bags for 48 hours to create near 100% humidity. Control plants were sprayed with sterile water with Tween 20.

4.1.3 Plant & soil testing

Isolation from plant material

Culturing: Diseased tissue removed from the stems and roots of affected plants was surface sterilised using 2% sodium hypochlorite solution, rinsed thoroughly, dried in a laminar flow cabinet and plated onto ¼ PDA, PDA, TWA or CMA. Plates were incubated at 22^0C for 7-21 days with a 12 hour photoperiod and then examined for the presence of fungal growth. Identity was confirmed by microscopic examination and/or PCR test.

Incubation: Diseased stems were place into trays each containing a moistened "chux" kitchen cloth covered with paper towel and enclosed in a plastic bag to provide 100% humidity. After 3-7 days incubation at room temperature, mycelia growing from the tissue was picked off with fine-point tweezers, identified by microscopic examination or plated onto TWA and incubated at room temperature for seven days.

Soil baiting

Rhizoctonia was recovered from soil using a toothpick baiting technique based on a modified method of Paulitz and Schroeder (2005). Toothpicks inserted into the soil were removed after two days, rinsed with sterile water, dried on paper towel in the laminar flow cabinet and placed horizontally, five per agar plate, onto *Rhizoctonia*-selective Ko and Hora medium (Ko and Hora 1971). After three days of incubation at room temperature, the presence of *Rhizoctonia* was confirmed by microscopic examination. If identification to Anastomosis Group was required, the culture was tested using the PCR technique.

Eucalyptus sieberi cotyledons were used to bait *Pythium* from soil (Marks and Kassaby 1974). Approximately 50g of soil collected from the root zone was placed in a small plastic tub with 200 ml of demineralised water. The cotyledons were floated on the surface for 7 days, removed, drained and placed onto CMA. After 7-10 days incubation at 22^0C the plates were examined for the presence of *Pythium*.

PCR soil and plant testing.

Molecular techniques to identify fungi in culture, soil and plant material were conducted by the Root Disease Testing Service (RDTS) of SARDI (Plant Research Centre, 2b Hartley Grove, Adelaide, South Australia). The DNA extraction technique used is commercial in confidence. The primers for *Rhizoctonia* and *Leptosphaeria* were developed and validated for research purposes though various funding sources, including HAL (PT04016 - Project 3: DNA Monitoring Tools For Soil-borne Diseases of Potato and this project), MLA (SHP005

- Molecular Tools to Study Soil Biological Constraints to Pasture Productivity) and Bayer Crop Science.

Plant material to be tested was collected, washed and stored frozen at ~-18^{0}C until required. When ready for testing, the material was freeze dried and ground for DNA extraction.

Soil was collected using the SARDI "Accucore" 10 ml sampler. Up to 40 cores were collected in a zigzag pattern over the area to be sampled. Generally soil was oven dried, ground and DNA extracted within 24 hours of sampling. If soil needed to be stored, it was either kept at 4^{0}C for up to a week, or frozen at ~-18^{0}C for longer periods. Frozen soil was freeze dried, not oven dried before DNA extraction.

The DNA extracted was tested using the specific PCR techniques developed by SARDI for *Rhizoctonia solani* (AG 2.1, 2.2, 3, 4, 5 and 8) and *Leptosphaeria maculans* (Sosnowski *et al* 2006).

4.1.4 Plant growth and maintenance

Seedlings grown from seed in the greenhouse were either planted by hand into speedling trays or provided by a nursery pre-seeded in speedling trays of commercial seedling mix. Speedlings 6-8 weeks old were also obtained from the nursery.

Two sizes of speedling trays were used, one with cells three cm square and four cm deep, the other with cells four cm square and five cm deep. MK12 pots used are ~9 cm square and hold ~0.55L of soil. MK 9 pots are ~11 cm square and hold ~0.9L of soil. MK 6 pots are ~6 cm square and hold ~0.3L of soil. Unless otherwise stated, all pots or trays were filled with steam sterilised coco peat mix (SARDI Greenhouse Services, Plant Research Centre).

Unless otherwise stated, all greenhouse and growth room plants were watered by hand or with an automatic watering system as necessary to maintain an average soil moisture of ~35% of full water holding capacity. Soil moisture was measured when required with a Measurement Engineering GT bug. Plants were fertilised every two weeks with Thrive® applied at label rates. The greenhouse was maintained at ~24^{0}C with natural lighting only, the growth rooms at the specified temperature with 12 h light/dark cycles.

Field experiments were maintained as per normal grower practices.

4.1.5 Chemical and biological product applications

Products tested.

Table 1. Fungicides evaluated for control of stem canker.

Trade name	Active ingredient
Amistar 250 SC®	250g/L azoxystrobin
Bavistin®	500g/kg carbendazim
Cabrio®	250g/L pyraclostrobin
Captan®	800 g/L captan
Chlorothalonil 720®	720g/L chlorothalonil
Dividend®	92g/L difenoconazole
Jockey Seed Fungicide ®	167g/L fluquinconazole
Maxim 100FS ®	100g/L fludioxonil
Monceren 125 DS®	125 g/L pencycuron
Octave®	462 g/kg prochloraz
Rizolex liquid ®	500g/L tolclofos-methyl
Rovral Aquaflo®	500g/L iprodione
Score®	250g/L difenoconazole
Sumisclex 500 ®	500g/L procymidone
Terraclor Soil Fungicide®	750g/kg quintozene

Table 2. Other products evaluated for control of stem canker.

Trade name	Active ingredient
Trichoshield™	*Trichoderma harzianum, T. lignorum, Gliocladium virens, Bacillus subtilis*
Seasol®	Seaweed concentrate
Nitra Mulch®	Recycled green organic material, <25mm.
Becker Underwood Experimental	BUEXP60009
Becker Underwood Experimental – combination of two products	BUEXP60009 and BUEXP1365

Application methods

Fungicide rate determination

Fungicides were diluted to a concentration either as parts per million (ppm) of the active ingredient (specified separately in each experimental method), or as per label rate. If a label rate for drenching was not specified, the amount of fungicide applied to the pots was calculated as a proportion of what would be applied in the field. The water holding capacity of the pots or speedling cells was determined by weighing with dry soil, wetting the soil to saturation and re-weighing, calculating the volume of water required. The surface area of each pot was calculated as a percent of the soil area in the field, the amount of fungicide

required for that area calculated and added to the volume of water required to saturate the pot.

Pre planting drench

Cauliflower speedlings were drenched with fungicides to simulate drench applications in the nursery. Speedling trays were immersed in a fungicide suspension for up to five minutes to optimise penetration of the fungicide into the soil and root matrix. Treated trays were drained, then planted within 24 hours of treatment. Water was used as the control treatment.

Post planting drench

Pots were treated with fungicides to mimic a field application post planting. Fungicides were poured onto to the soil surface with enough liquid to saturate the pot with minimal run off. Water was applied as a control.

Fungicides were applied to field experiments at label rates using a pressurised back pack sprayer.

4.1.6 Assessments & experiment design

Stem cankers were assessed visually while growing using a 6 level rating system (Table 3, Fig. 3 section 4.2.1). With most experiments, the cankers were rated using the percent rating, however some initial greenhouse screenings used the 0-5 rating. These were transformed to a figure between 0 and 100 using the following formula:

Severity = ((r1+(r2*2)+(r3*3)+(r4*4)+(r5*5))/n)*(100/R)

where r1 – r5 are the number of samples in that rating, n is the total number of samples and R is the highest rating level, in this case five.

Table 3. Disease rating system used to assess stem canker symptoms

Rating	Percent rating	Description
0	0%	Healthy
1	20%	staining
2	40%	½ stem canker
3	60%	full stem canker
4	80 %	severe canker (wilt)
5	100%	plant death

At the completion of the experiment, plants were removed, washed, and a final assessment undertaken to detect cankers that may have developed below soil level. Additional measurements at harvest could include:

- Fresh weight of leaves and stem. The roots were removed at soil level, the head removed and if necessary the plant material washed and dried before weighing.
- Plant size. The overall plant size was rated comparatively where 1=small, 2=medium and 3=large plants for that experiment.
- Root ball size. The size of the root ball was rated comparatively where 1=small, 2=medium and 3=large for that experiment.
- Adventitious roots. Adventitious roots were extra roots which grew out from the stem above the soil line (Fig. 1). These were rated as present or absent.

- Presence of *Pythium*. Symptoms include root tip damage and root sloughing. These were rated as present or absent.
- The precent stem area infected by the canker. Rated as 0, 25%, 50% or 100% of the circumference of the stem area affected by the canker.

Figure 1. Adventitious roots produced above a canker on cauliflower cv. Chaser.

Unless otherwise stated, all greenhouse and field experiments were laid out in a Randomised Block design. Pathogenicity testing in the greenhouse in speedling trays was set up using blocks of five plants inoculated with the same isolate and treated as five replicates. These were separated from other blocks of five plants by at least one speedling cell.

For some experiments the Relative Area Under Disease Progression (RAUDP) curve (Pscheidt and Stevenson 1986) for the canker severity was calculated. This is a way of providing a single analysable figure for the differences in disease development over time between treatments.

For the greenhouse and field experiment summaries, the data were combined to provide a comparison of results from all experiments. The incidence and severity values for the treatments in each experiment in the greenhouse and field separately, were calculated as a percent of the untreated control and averaged over the total number of experiments where that fungicide or product was evaluated. A mean canker incidence and severity was then calculated for each treatment.

Analysis was undertaken by general ANOVA and All Pairwise Comparison of Means Least Significant Difference (LSD), Linear Regression, or Two sample T test, using the Analytical Software Statistix® V8.

4.2 Factors affecting the development of Brassica stem canker.

A series of experiments were undertaken to improve the understanding of the disease complex. Previous work (Hitch *et al* 2006) evaluated the pathogenicity of the various organisms recovered from plants with symptoms of Brassica stem canker. This testing was expanded to determine if combinations of the main pathogens were more pathogenic to cauliflower than each pathogen alone. Studies examined the effect of temperature, watering, and infection timing on symptom expression in cauliflower plants.

4.2.1 Correlations between canker rating and DNA levels in plants.

Objective: *To determine the relationship between the canker severity rating and the levels of* Rhizoctonia *and* Leptosphaeria *in the stems of the cankered plants.*

Materials and methods

Cauliflower cv. Discovery collected at harvest from a field experiment were rated in the canker severity category (Fig. 3). Ten plants from each category, except for 100% where only two plants at the rating were available, were processed and PCR tested as previously described.

Results and discussion

There was a good correlation between the canker severity rating and levels of *Leptosphaeria* DNA (Fig. 2). *Rhizoctonia* AG2.1 was more variable, however there was reasonable correlation up to the 80% canker severity rating. However the two plants with 100% canker rating had no *Rhizoctonia* present. AG 4 was detected in only 3 of the plants tested (data not presented). PCR testing in other experiments in this report had not indicated any inverse relationship between the pathogens. However those tests were on bulked plant stems and the existence of any relationship needs to be confirmed by further testing on single plants.

These results indicate a good correlation between infection and development of the canker symptoms, whereby low infection was found with low canker ratings and high levels of pathogen with the high canker ratings. However this experiment should be repeated using more plants with 100% canker ratings.

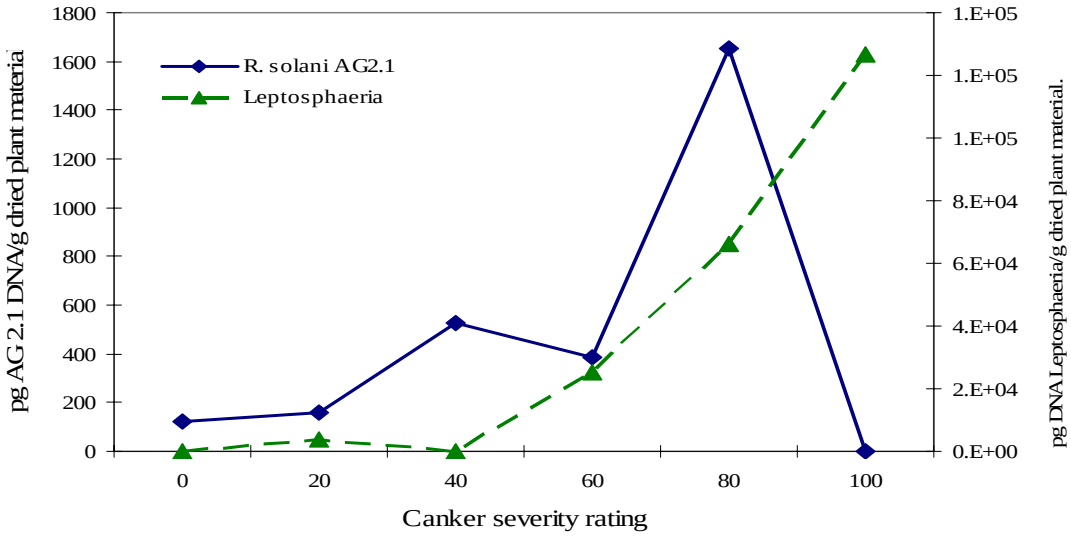


Figure 2. Levels of* Rhizoctonia *AG 2.1 and* Leptosphaeria *DNA in plants with different canker severity ratings.

(L) Canker severity 0%

(R) Canker severity 20%

(L) Canker severity 40%

(R) Canker severity 60%

(L) Canker severity 80%

(R) Canker severity 100%

Figure 3. Cauliflower with canker severity ratings used for assessment.

4.2.2 The effect of *Rhizoctonia* and *Leptosphaeria* on the development of stem canker

Rhizoctonia solani AG 2.1, 2.2 and 4 and *Leptosphaeria maculans* are pathogenic to cauliflower (Budge *et al* 2009, Pannecoucque *et al* 2008) and cause stem canker (Hitch *et al* 2006). Three greenhouse studies were undertaken; the first to confirm the effect of combined infections on disease development in cauliflower and the other two to assess the pathogenicity of *Rhizoctonia* AG 3 and AG 8. AG 3 causes the disease black scurf in potatoes and AG 8 bare patch in cereals. Both were detected at low levels in some soil and cauliflower plants during field evaluations, and cereals and potatoes are used as rotation crops with Brassica. *R. solani* AG 3 is not recognised as a pathogen of cauliflower (Budge *et al* 2009, Pannecoucque *et al* 2008). While it can penetrate the cauliflower hypocotyls and therefore be detected in the plant stems, it does not cause pectin degradation or disease symptoms and is considered weakly pathogenic or non- aggressive (Pannecoucque *et al* 2009). These studies did not include *R. solani* AG8 on cauliflower, however it was not detected in a survey in the UK where AG 2.1 and 4 where the main AG groups colonising cauliflower (Budge *et al* 2009).

Experiment 1

Objective: *To determine the influence of combined infections of* Rhizoctonia *and* Leptosphaeria *on the development of Brassica stem canker in cauliflower seedlings.*

Materials and methods

Cauliflower seedlings cv. Skywalker were germinated in speedling trays and grown in the greenhouse. Five replicate six week old seedlings were stem inoculated as previously described with 10 day old cultures of *Rhizoctonia* AG 2.1, 2.2 or 4 alone or in combination with 21 days old cultures of *Leptosphaeria* (Table 4).

Plants were maintained in the greenhouse for nine weeks and assessed for cankers at one, four and nine weeks after inoculation.

Results and discussion

After 7 days, most plants inoculated with *Rhizoctonia* developed cankers with stem thinning, whereas seedlings inoculated with *Leptosphaeria* alone appeared healthy (Table 4). The canker colour varied between the *Rhizoctonia* groups, dark cankers with AG 2.1, pale cankers from AG 2.2 and striped cankers from AG 4 (Figs. 4, 5a). This variability was not as obvious at four and nine weeks after inoculation, or with the combined inoculations. By nine weeks most inoculated plants were dead. At four weeks, only one plant inoculated with *Leptosphaeria* alone remained healthy, all others had dark cankers. Pycnidia developed on some plants inoculated with one of the *Leptosphaeria* isolates (#674) (Fig. 5b). Three of the un-inoculated control plants also died and *Rhizoctonia* was recovered from the stems. This cross contamination from the *Rhizoctonia* indicated that the separation of one speedling cell was not sufficient. The *Rhizoctonia* most likely spread through mycelial growth via the soil in the cells separating the treatments.

These results support the previous results of combined inoculations (Hitch *et al* 2005), which also showed that *Rhizoctonia* caused the early infection and plants inoculated with the combination had more severe cankers extending further up the stem, some with pycnidia.

***Table 4. Development of symptoms on cauliflower seedlings cv. Skywalker inoculated with different combinations of* Rhizoctonia *and* Leptosphaeria.**

	Percent of plants# showing symptoms after inoculation				
	1 week	**4 weeks**		**9 weeks**	
	canker	**canker**	**dead**	**canker**	**dead**
Rhizoctonia AG 2.1	100	100	0	100	60
Rhizoctonia AG 2.2	100	100	0	v	100
Rhizoctonia AG 4	100	100	0	100	100
Leptosphaeria #666	0	80	0	80	40
Leptosphaeria #674	0	40	60	100	100*
R. AG 2.1 + *Leptosphaeria* #666	80	100	0	100	60
R. AG 2.1+ *Leptosphaeria* #674	100	-	0	100	80*
R. AG 2.2+ *Leptosphaeria* #666	100	100	0	100	100
R. AG 2.2 + *Leptosphaeria* #674	100	100	0	100	100*
R. AG 4+ *Leptosphaeria* #666	100	100	0	100	100
R. AG 4 + *Leptosphaeria* #674	100	100	0	100	100
Control (not inoculated)	0	40	0	40	40

Out of 5 plants inoculated
* Pycnidia present on canker

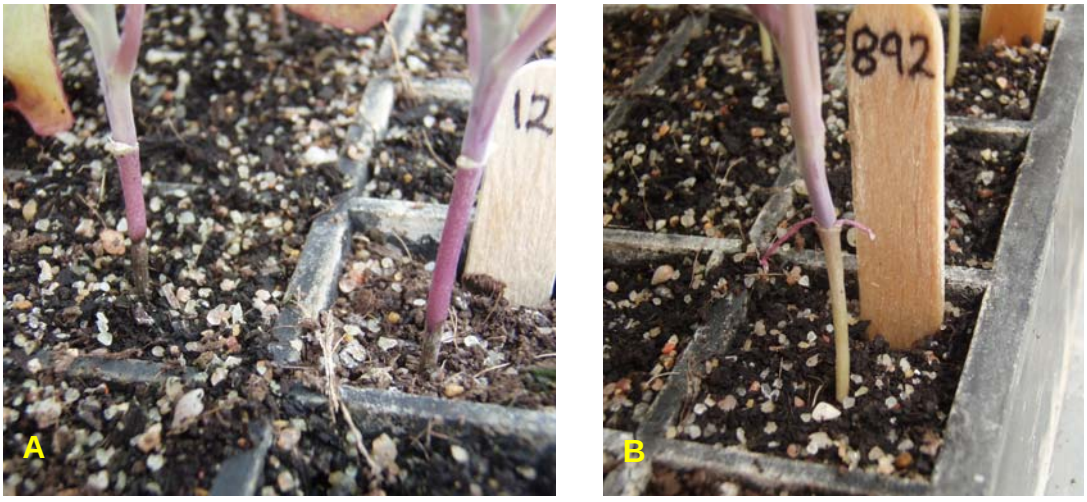


Figure 4 a,b. Stem cankers on seven week old cauliflower, seven days after inoculation with* Rhizoctonia*. AG 2.1 dark canker (A), AG 2.2 pale canker (B).

Figure 5 a,b. Stem cankers on cauliflower. (A) Striped canker on seven week old cauliflower, seven days after inoculation with* Rhizoctonia*. (B) Canker with pycnidia after inoculation with Leptosphaeria.

Experiment 2 and 3

Objective: *To determine the pathogenicity of* Rhizoctonia *AG 3 and AG 8 on cauliflower seedlings.*

Materials and methods

Cauliflower seedlings cv. Nautilus or cv. Skywalker were germinated in speedling trays and grown in the greenhouse. Five replicate six week old seedlings were stem inoculated as previously described with 10 day old cultures of 7 isolates of *Rhizoctonia* AG 3 (cv. Nautilus) obtained from potato tubers, or 2 isolates of AG 9 (cv. Skywalker) obtained from a potato tuber or from wheat roots.

Plants were maintained for eight weeks and assessed every two weeks after inoculation for stem cankers.

Results and discussion

No cankers were observed on either the inoculated or non-inoculated cauliflower plants, confirming that AG 3 and AG 8 are not pathogens of cauliflower.

4.2.3 The effect of *Pythium* on the development of stem canker

Pythium, mainly *P. ultimum* with one isolate identified as *P. irregulare,* was detected in both plant material and soil from Brassica crops with stem canker. Four greenhouse experiments were undertaken to determine pathogenicity of the *Pythium* and the effect on the development of Brassica stem canker in association with *Rhizoctonia* and/or *Leptosphaeria*.

Experiment 1

Objective: *To evaluate pathogenicity of* Pythium *isolates recovered from stem canker of cauliflower.*

Materials and methods

Isolates of *P. irregulare* and *P. ultimum* recovered from cauliflower plants with stem canker were used to inoculate up to five replicated plants per isolate of eight week old cauliflower cv. Aviron using two 7 mm mycelial plugs from ten day old cultures as previously described.

Plants were maintained in the greenhouse and assessed for stem cankers every two weeks up to ten weeks after inoculation.

Diseased plant material from the affected plants was isolated as previously described.

Results and discussion

Stem canker developed on one of the three plants inoculated with *P. irregulare* (Table 5). *P. ultimum* caused root rotting on ~36% and dark stem lesions on ~15% of the plants inoculated. Both species were recovered from the inoculated plants, while the un-inoculated control plants remained healthy.

Table 5. Effect of* Pythium *on cauliflower seedlings 10 weeks after inoculation.

	No isolates tested	**Symptom**	**No. isolates with symptoms**	**Percent of plants with symptoms**
P. irregulare	1	Canker	1	33%
P. ultimum	33	Rotted roots	12	17%
		Dark lesion	5	7%

Experiment 2

Objective: *To determine the influence of* Pythium *on Brassica stem canker of cauliflower in the presence of* Leptosphaeria.

Materials and methods

Cauliflower seedlings cv. Skywalker were germinated in speedling trays and grown in the greenhouse. Five replicate six week old seedlings were stem inoculated as previously described with 10 day old cultures of either *P. ultimum* or 21 day old cultures of *L. maculans,* alone or in combination (Table 6).

Plants were maintained in the greenhouse for nine weeks and assessed at one, four and nine weeks after inoculation for cankers.

Results and discussion

Cankers were first observed on three plants inoculated with *Leptosphaeria* one week after inoculation. After nine weeks, only one of the four combined tests had increased the incidence of stem canker on the seedlings compared to *Leptosphaeria* alone (Table 6). The un-inoculated control plants were healthy.

***Table 6. Percent of cauliflower cv. Skywalker plants developing cankers nine weeks after inoculation with* Leptosphaeria *alone or in combination with* Pythium ultimum.**

Pathogen	**% plants with canker***
Leptosphaeria #666	60
Leptosphaeria #674	40
P. ultimum #578	0
P. ultimum #934	0
Leptosphaeria #666 + *P. ultimum* #578	60
Leptosphaeria #666+ *P. ultimum* #934	60
Leptosphaeria #674+ *P. ultimum* #578	40
Leptosphaeria #674 + *P. ultimum* #934	80
Control (not inoculated)	0

* Five plants inoculated.

Experiment 3

Objective: *To determine the influence of* Pythium *on Brassica stem canker of cauliflower in the presence of* Rhizoctonia.

Materials and methods

Cauliflower seedlings cv. Skywalker were germinated in speedling trays and grown in the greenhouse. Five replicate six week old seedlings were stem inoculated as previously described with 10 day old cultures of either *P. ultimum* or *Rhizoctonia* AG 2.1, 2.2 or 4, alone or in combination (Table 7).

Plants were maintained in the greenhouse for nine weeks and assessed at one, four and nine weeks after inoculation for cankers.

Results and discussion

After 7 days, all plants inoculated with *Rhizoctonia* developed stem cankers and narrowed stems. Those seedlings inoculated with *Pythium* alone did not develop canker. The addition of *Pythium* did not increase the incidence of stem canker on the seedlings compared to symptoms caused by the *Rhizoctonia* alone (Table 7). At four weeks after inoculation, all plants had severe cankers and by nine weeks all plants except the un-inoculated control

plants were dead. *Rhizoctonia* was recovered from cankered plants inoculated only with *Pythium*, indicating cross contamination.

***Table 7. Percent of cauliflower cv. Skywalker plants developing cankers one week after inoculation with* Rhizoctonia *alone or in combination with* Pythium ultimum.**

Pathogen	**% plants with canker***
Rhizoctonia AG 2.1	100
Rhizoctonia AG 2.2	100
Rhizoctonia AG 4	100
P. ultimum #578	0
P. ultimum #934	0
Rhizoctonia AG 2.1 + *P. ultimum* #578	100
Rhizoctonia AG 2.1+ *P. ultimum* #934	80
Rhizoctonia AG 2.2+ *P. ultimum* #578	100
Rhizoctonia AG 2.2 + *P. ultimum* #934	100
Rhizoctonia AG 4+ *P. ultimum* #578	80
Rhizoctonia AG 4 + *P. ultimum* #934	100
Control (not inoculated)	0

* Five plants inoculated.

Experiment 4

Objective: *To determine if* Pythium *infection increases the susceptibility of cauliflower to* Rhizoctonia *at various plant ages.*

Materials and methods

***Pythium inoculum*:** Inoculum was prepared by soaking 200 g of white millet in a 1L Schott bottle with 200 ml sterile water for six hours. After draining, the millet was autoclaved at 120^0C for 20 mins. This process was repeated three times, allowing the bottle to cool in between cycles. An actively growing ten day old culture of *Pythium irregulare* was cut into small squares 3-5 mm wide and ~50 pieces placed into each bottle. The inoculated millet was incubated for 3 weeks at 25^0C and shaken daily to disperse the growing mycelium. The presence of *Pythium* was confirmed by placing several millet pieces onto CMA and examining microscopically after 7 days incubation at room temperature. *Pythium irregulare* was used in this test as it grows over a wider temperature range and a validated PCR test was available to detect levels in the plant.

Soil inoculation: One bottle of inoculated millet was added to 25 kg sterile coco peat in a large tub, mixed and left moist for 1 week before being potted into 200 mm diameter pots (~4.2L).

Six week old cauliflower seedlings cv. Chaser were planted into 50 pots of the *Pythium* inoculated soil and into 50 pots of non-inoculated soil. At 2, 4, 6, 8 and 10 weeks after planting, ten replicate pots each from the inoculated and un-inoculated soil were stem inoculated with a culture of *Rhizoctonia* AG 2.1.

Plants were assessed for stem cankers at 3 weeks after the first *Rhizoctonia* inoculation and then every two weeks for 16 weeks after planting. At 16 weeks plants were harvested, washed and assessed for: stem canker symptoms, plant height rated as stunted, medium or large, fresh weight of leaf and stem (excluding roots and head) and total DNA levels in the ten stems combined.

Results and discussion

Severity levels were generally low with only three plants developing cankers at 60% severity (data not presented). When results for all treatments were combined, the mean percent of plants with cankers increased at every assessment time, and apart from the first assessment time at nine weeks after planting, more plants grown in *Pythium* infected soil were infected (Fig. 6), although the differences were not statistically significant. The opposite trend was observed with canker severity (Fig. 7); at 13 and 15 weeks after planting the mean canker severity of plants that were infected was significantly higher in plants grown in soil not inoculated with *Pythium*.

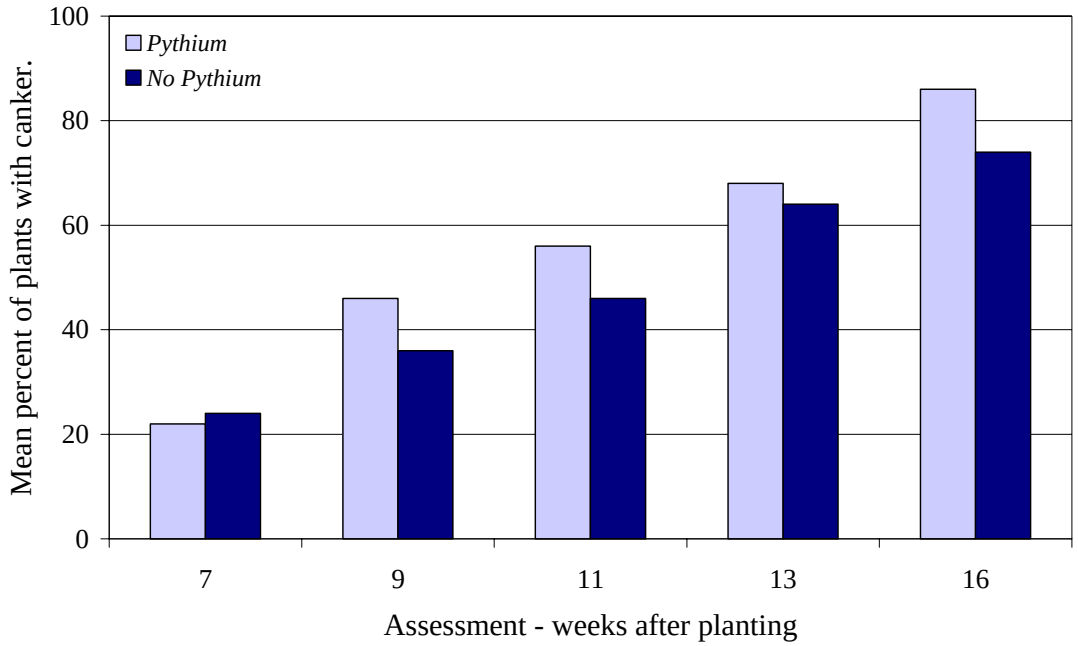


Figure 6. Mean percent of cauliflower cv. Chaser with cankers at 7, 9, 11, 13 and 16 weeks after planting, grown in soil with or without* Pythium irregulare *and inoculated with* Rhizoctonia *AG 2.1.

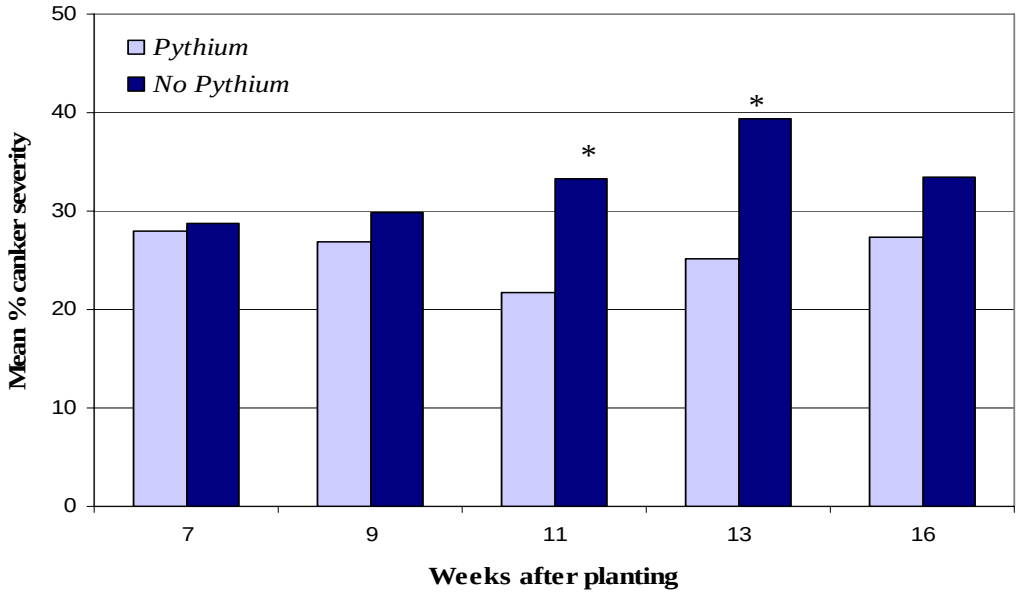


Figure 7. Mean percent canker severity of affected cauliflower cv. Chaser at 7, 9, 11, 13 and 16 weeks after planting, grown in soil with or without Pythium irregulare and inoculated with Rhizoctonia solani AG 2.1. Canker ratings of 0 are not included in means. *Values (*Pythium *vs.* non Pythium*) are significantly different (P<0.05).

At harvest (16 weeks after planting), the plants inoculated with *Rhizoctonia* 2 weeks after planting had a significantly higher level of canker severity in the plants grown in soil inoculated with *Pythium* (Table 8). The severity of canker symptoms was not significantly different in the later *Rhizoctonia* inoculations, indicating that the root damage that occurred in the presence of *Pythium* did not increase the infection by *Rhizoctonia* in older plants.

The *Rhizoctonia* DNA detected in the stems followed a similar trend. With the *Rhizoctonia* inoculation at two weeks after planting, a significantly higher level of *Rhizoctonia* DNA was detected in the stems of plants grown in *Pythium* infected soil, matching the higher canker severity (Table 8). At the last *Rhizoctonia* inoculation time (10 weeks after planting) the opposite was observed, with the mean canker severity and DNA lower in the plants grown in the *Pythium* inoculated soil.

Table 8. Mean canker severity and total DNA levels of cauliflower stems cv. Chaser at harvest (16 weeks after planting), on seedlings grown in soil with or without* Pythium irregulare *and inoculated with* Rhizoctonia solani *AG 2.1 at 2, 4, 6, 8 or 10 weeks after planting.

Time of *Rhizoctonia* inoculation	**Mean % canker severity**			**Total AG 2.1 DNA pg /g dried plant material***	
	No *Pythium*	**With *Pythium***	**P**	**No *Pythium***	**With *Pythium***
2 weeks	14	26	0.01	3	1932
4 weeks	22	20	n.s.	2837	1455
6 weeks	20	22.9	n.s.	3428	1175
8 weeks	30	32	n.s.	2914	3854
10 weeks	28	18	n.s.	1597	754

* all ten stems from each treatment were combined for the DNA test.
n.s. = means not significantly different (P=0.05)

The levels of *Rhizoctonia* DNA in the combined stems was significantly correlated with the mean percent canker severity (P=0.03), having a positive linear relationship (Fig. 8).

Analysis of canker severity on plants with the canker symptoms followed a similar trend as the total harvest data (Fig. 9). Plants inoculated with *Rhizoctonia* two weeks after planting had consistently higher levels of infection when grown in the *Pythium* infected soil. However in the older plants, plants were more severely infected when grown in clean soil. It appeared as if the *Pythium* was competing with the *Rhizoctonia* infection and reducing the ability of the fungus to colonise and cause symptoms.

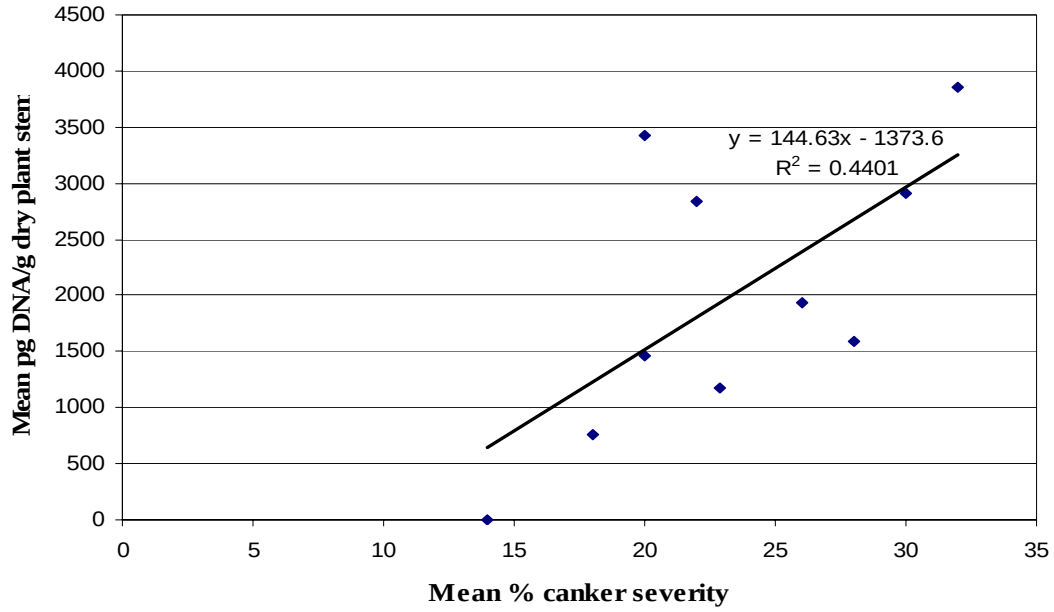


Figure 8. Linear regression of DNA and mean canker severity. Significant regression P=0.03.

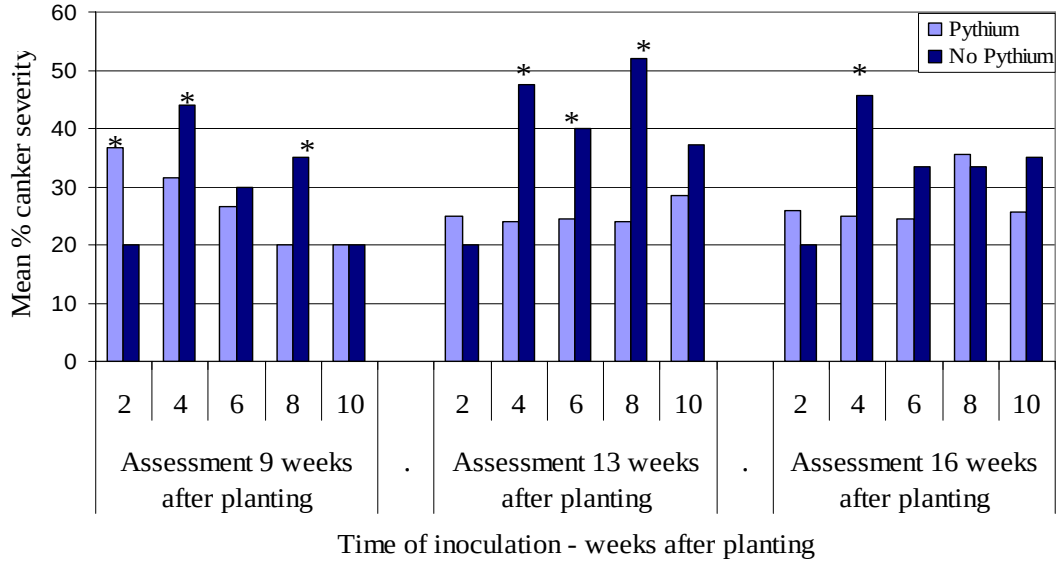


Figure 9. Mean % canker severity of cauliflower cv. Chaser with cankers at 9, 13 and 16 weeks after planting, grown in soil with or without* Pythium irregulare *and inoculated with* Rhizoctonia *AG 2.1 at 2, 4, 6, 8 or 10 weeks after planting. Canker ratings of 0 are not included in means. *Values (*Pythium *vs.* non Pythium*) are significantly different (P<0.05).

The Relative Area Under Disease Progression (RAUDP) curve for the percent canker severity (of all plants) also showed this relationship (Fig. 10). When the data for two weeks after planting was removed, there was a significant linear relationship between the time of inoculation and the canker severity of canker from four to ten weeks after planting (Fig. 10). The presence of *Pythium* appeared to make older plants less susceptible to *Rhizoctonia* and reduced canker development.

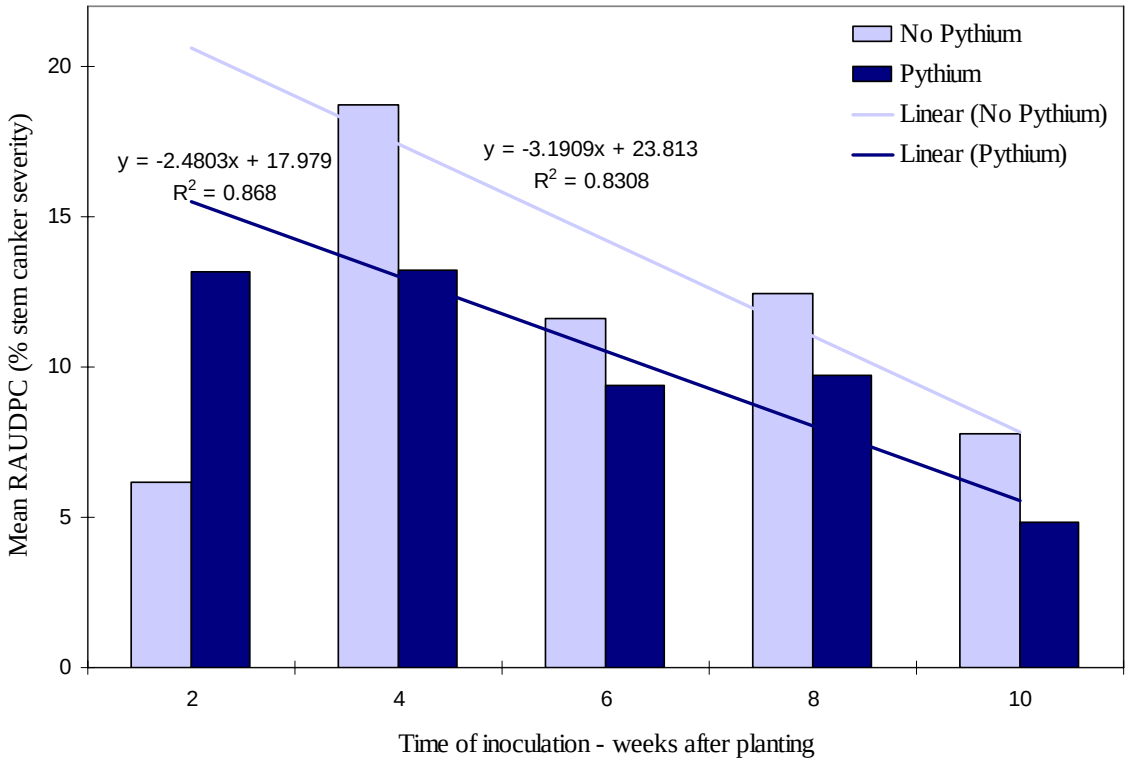


Figure 10. Relative area under disease progression curve (RAUDPC) of % canker severity of cauliflower cv. Chaser grown in soil with or without* Pythium irregulare *and inoculated with* Rhizoctonia solani *AG 2.1 at 2, 4, 6, 8 or 10 weeks after planting. Linear regressions are RAUDPC from 4 to 10 weeks only.

It was observed throughout the experiment that plants grown in the presence of *Pythium* had reduced vigour and leaf stalk thickness, irrespective of whether they were also inoculated with *Rhizoctonia* (Fig. 11). This was confirmed at harvest, where both the mean weights of stems and leaves and the head weights were consistently lower in plants grown in *Pythium* inoculated soil (Table 9).

These results show that while early infection can be increased with the presence of *Pythium* in the soil, in older plants canker severity is less in *Pythium* infected soil. However the *Pythium* infection resulted in a reduction in plant growth and yield and would cause greater economic loss than from the stem cankers.

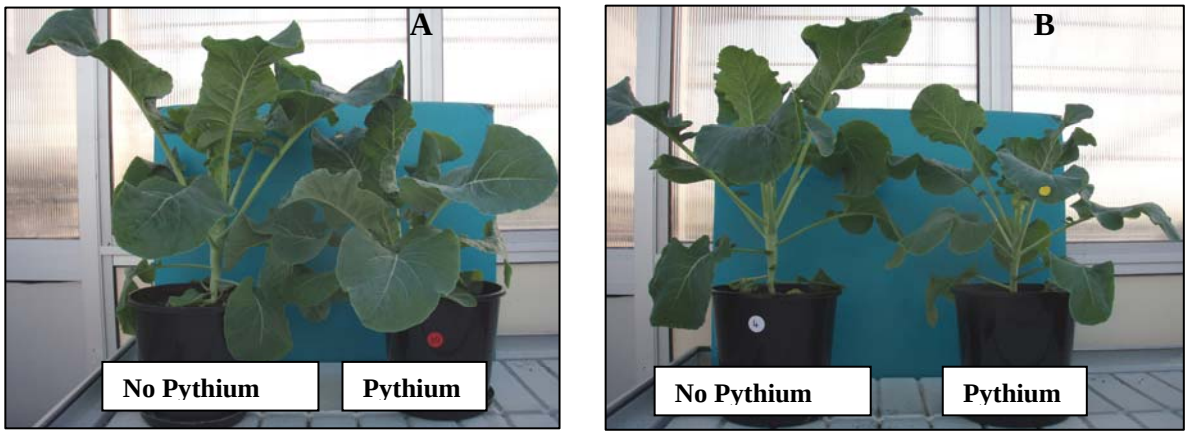


Figure 11. Vigour of cauliflower cv. Chaser seven weeks after planting into either un-inoculated soil (L) or soil inoculated with* Pythium irregulare *prior to planting (R). A: No inoculation with* Rhizoctonia*, B: Inoculated with* Rhizoctonia *2 weeks after planting.

Table 9. Mean fresh weight (stalks and leaves) and head weight of cauliflower cv. Chaser at harvest from seedlings grown for 16 weeks in soil with or without* Pythium irregulare *and inoculated with* Rhizoctonia solani *AG 2.1 at 2, 4, 6, 8 or 10 weeks after planting.

Time of *Rhizoctonia* inoculation	Mean fresh weight (g)			Mean head weight (g)		
	No *Pythium*	With *Pythium*	P	No *Pythium*	With *Pythium*	P
2 weeks	450	399	0.08	188.7	62.4	0.005
4 weeks	439	412	n.s.	210.6	123.4	0.006
6 weeks	437	316	0.00	280.1	63.1	0.00
8 weeks	483	311	0.001	244.49	119.55	0.01
10 weeks	470	406	n.s.	297.36	171.01	0.01

General discussion

Pythium irregulare causes seedling damping off (White *et al* 1984). These results confirm that *P. irregulare* causes significant root rot of cauliflower and can increase the canker severity with early infection of *Rhizoctonia*. However *P. ultimum* var *ultimum* causes stem lesions as well as root rotting on cauliflower and while it prefers warmer conditions is able to grow between 5^0C and 40^0C (Abdelzaher 2003). Therefore it would be useful to repeat experiment 4 using *P. ultimum* to determine whether that species has more impact on canker severity.

4.2.4 The effect of *Fusarium* on the development of stem canker.

Three species of *Fusarium*, *F. equiseti*, *F. oxysporum* and *F. avenaceum*, were isolated from stem cankers and were pathogenic to cauliflower (Hitch *et al* 2006). Nine greenhouse experiments were undertaken: three to confirm pathogenicity of more isolates, one to determine if plant wounding increased disease and five to investigate the interaction with *Rhizoctonia, Leptosphaeria* and *Pythium*.

Experiments 1-3

Objective: *To evaluate the pathogenicity of* Fusarium *isolates recovered from cauliflower plants.*

Materials and methods

Over a series of three greenhouse tests, isolates of *F. equiseti, F. avenaceum* and *F. oxysporum* recovered from cauliflower plants were used to inoculate up to five replicated plants per isolate of six week old cauliflower using two 7 mm mycelial plugs as previously described.

Plants were maintained in the greenhouse and assessed for stem cankers 2, 4, 6 and 8 weeks after inoculation.

Results and discussion

Three of the *F. avenaceum* isolates caused stem canker, with cv. Nautilus (Table 10) being the worst affected. As the same isolate was not tested on all three cultivars, it is not possible to determine whether this was an effect of the cultivar or the isolate. Nautilus was the only cultivar to develop stem canker with *F. oxysporum*, however *F. equiseti* produced stem cankers on both cv. Nautilus and cv. Chaser.

These results confirmed that *Fusarium* is a pathogen of cauliflower, producing symptoms ranging from root damage to stem cankers. However *Fusarium* was not as pathogenic as either *Rhizoctonia* or *Leptosphaeria* and the stem cankers were not severe.

***Table 10. Symptoms observed on cauliflower plants 8 weeks after inoculation with* Fusarium.**

	No plants tested	Cultivar	No. plants with symptoms		
			Stem staining	Root rotting	Canker
F. avenaceum	5	Chaser	3	0	0
	5	Chaser	1	1	0
	3	Chaser	0	0	0
	5	Nautilus	0	0	3
	4	Chaser	1	3	0
	4	Chaser	0	3	1
	5	Nautilus	0	0	4
F. equiseti	5	Chaser	0	0	1
	5	Nautilus	0	0	1
	5	Chaser	0	1	0
	5	Chaser	0	0	3
	5	Chaser	0	0	3
F. oxysporum	5	Skywalker	0	0	0
	5	Nautilus	0	0	1
	3	Chaser	1	0	0
	1	Chaser	0	1	0
	4	Chaser	0	3	0
	2	Chaser	0	1	0
	4	Skywalker	0	0	0

Experiment 4

Objective: *To determine the effect of wounding on the susceptibility of cauliflower seedlings to* Fusarium *infection.*

Materials and methods

Cauliflower seedlings cv. Skywalker were germinated in speedling trays and grown in the greenhouse. Ten day old cultures of either *F. equiseti*, *F. oxysporum* or *F. avenaceum* were macerated separately in ~50 ml SDW per plate. MK12 pots were half filled with coco peat and ~25 ml of the macerated fungi added in a layer and covered with more coco peat. 8 week old seedlings were wounded by either cutting the roots or scratching the stems of the seedlings at soil level, before they were planted into the inoculated pots above the layer of *Fusarium*. Seedlings without wounding or cutting were also planted into inoculated soil and plants with wounding or cut or uncut roots were planted in coco peat without inoculum as a control. 12 replicate plants were used per treatment.

Plants were maintained in the greenhouse for eight weeks and assessed every two weeks for cankers.

Results and discussion

One cauliflower seedling with cut roots planted in the *F. avenaceum* inoculated soil died around two weeks after planting. No plants died in the other treatments and stem canker did not develop in any of the treatments (data not presented).

It is not know why *Fusarium* did not affect the plants in this experiment, as both the cauliflower cultivar and the isolates had previously caused cankers and plant death. Coco peat is known to encourage *Fusarium* infection in greenhouse cereal plants, so it is unlikely to be suppressive. Previous experiments used stem inoculation rather than soil inoculation, which may have promoted the stem infection. In addition, from the previous experiments, it appeared that cv. Skywalker was less susceptible to infection by *Fusarium*.

Experiment 5

Objective: *To determine the influence of* Fusarium *on the development of Brassica stem canker on cauliflower in the presence of* Leptosphaeria.

Materials and methods

Cauliflower seedlings cv. Skywalker were germinated in speedling trays and grown in the greenhouse. Five replicate six week old seedlings were stem inoculated as previously described with 10 day old cultures of either *F. oxysporum, F. equiseti* or *F. avenaceum* or 21 day old cultures of *L. maculans* alone or in combination (Table 11).

Plants were maintained in the greenhouse for nine weeks and assessed at one, four and nine weeks after inoculation for cankers.

Results and discussion

Stem cankers were first observed on five plants inoculated with *Leptosphaeria* one week after inoculation. After nine weeks, the severity or incidence of stem canker on the seedlings did not increase in the presence of *Fusarium* compared to symptoms caused by the *Leptosphaeria* alone (Table 11). Co-inoculation with *F. oxysporum* reduced the level of stem cankers with both isolates of *Leptosphaeria*, with only one of the ten plants developing cankers compared to nine of the ten plants inoculated with *Leptosphaeria* alone. *F. avenaceum* was the only species to develop stem cankers without *Leptosphaeria*, which may have been a result of using the Skywalker cultivar.

None of the un-inoculated control plants developed cankers.

Table 11. Canker development on cauliflower cv. Skywalker inoculated nine weeks previously with* Leptosphaeria *alone or in combination with* Fusarium *sp.

Pathogen	**Percent of plants with canker***
Leptosphaeria #666	100
Leptosphaeria #674	80
F. avenaceum	80
F. oxysporum	0
F. equiseti	0
Leptosphaeria #666 + *F. avenaceum*	80
Leptosphaeria #666+ *F. oxysporum*	20
Leptosphaeria #666+ *F. equiseti*	80
Leptosphaeria #674+ *F. avenaceum*	40
Leptosphaeria #674 + *F. oxysporum*	0
Leptosphaeria #674 + *F. equiseti*	40
Control (not inoculated)	0

* five plants inoculated per pathogen

Experiment 6 & 7

Objective: *To determine the influence of* Fusarium *on the development of Brassica stem canker on cauliflower in the presence of* Rhizoctonia.

Materials and methods

Experiment 6. Cauliflower seedlings cv. Skywalker were germinated in speedling trays and grown in the greenhouse. Five replicate six week old seedlings were stem inoculated as previously described with 10 day old cultures of *Rhizoctonia solani* AG2.1, 2.2 or 4, alone or in combination with *F. equiseti*, *F. oxysporum* or *F. avenaceum.*

Plants were maintained in the greenhouse for nine weeks and inspected at one, four and nine weeks for cankers.

Experiment 7. This experiment was similar to Experiment 6 except cv. Nautilus was used as this was considered to be more susceptible to *Fusarium*. Two isolates of *Rhizoctonia* AG 2.1 were tested alone or in combination with *Fusarium*. Plants were assessed at one and four weeks after inoculation.

Results and discussion

After seven days, all plants inoculated with *Rhizoctonia* developed cankers (Table 12). Only 40% of the Nautilus seedlings inoculated with *F. avenaceum* developed cankers, but no cankers developed in Skywalker inoculated with *Fusarium* alone. The addition of *Fusarium* did not increase the incidence or severity of stem canker on the seedlings compared to symptoms caused by the *Rhizoctonia* alone.

As with previous combination inoculation experiments including *Rhizoctonia*, cross contamination occurred in Experiment 6. Most plants showed severe *Rhizoctonia* like

cankers by four weeks after inoculation and by nine weeks all plants except the un-inoculated control plants were dead. Cross contamination was not observed in Experiment 7.

These results confirm *Fusarium* as a pathogen of cauliflower. However Fusarium infection does not increase the susceptibility f the plants to canker caused by *Rhizoctonia*.

Table 12. Canker development on cauliflower cv. Skywalker or cv. Nautilus, inoculated one week previously with* Rhizoctonia *alone or in combination with* Fusarium *sp.

Pathogen	Percent plants with canker*	
	Skywalker	**Nautilus**
F. avenaceum #813	-	40
F. avenaceum #814	0	-
F. avenaceum #815	-	40
F. oxysporum	0	-
F. equiseti	0	-
***Rhizoctonia* AG 2.1 #12**	**100**	**-**
Rhizoctonia AG 2.1 #12+ *F. oxysporum*	80	-
Rhizoctonia AG 2.1 #12 + *F. equiseti*	100	-
Rhizoctonia AG 2.1 #12 + *F. avenaceum #814*	100	-
***Rhizoctonia* AG 2.1 #1002**	**-**	**100#**
Rhizoctonia AG 2.1 #1002 + *F. avenaceum #813*	-	100
Rhizoctonia AG 2.1 #1002 + *F. avenaceum #815*	-	100
Rhizoctonia AG 2.1 #1002+ *F. oxysporum*		100
Rhizoctonia AG 2.1 #1002 + *F. equiseti*	-	100
***Rhizoctonia* AG 2.1 #1010**	**-**	**100**
Rhizoctonia AG 2.1 #1010 + *F. avenaceum #813*	-	100
Rhizoctonia AG 2.1 #1010+ *F. avenaceum #815*	-	100
Rhizoctonia AG 2.1 #1010+ *F. oxysporum*		100
Rhizoctonia AG 2.1 #1010 + *F. equiseti*	-	100
***Rhizoctonia* AG 2.2**	**80**	**-**
Rhizoctonia AG 2.2+ *F. avenaceum #814*	80	-
Rhizoctonia AG 2.2 + *F. oxysporum*	100	-
Rhizoctonia AG 2.2 + *F. equiseti*	80	-
***Rhizoctonia* AG 4**	**100**	**-**
Rhizoctonia AG 4+ *F. avenaceum #814*	100	-
Rhizoctonia AG 4 + *F. oxysporum*	100	-
Rhizoctonia AG 4 + *F. equiseti*	100	-
Control (not inoculated)	0	0

* five plants inoculated per pathogen
\# two plants dead
\- not tested

Experiment 8

Objective: *To determine the influence of* Fusarium *on the development of Brassica stem canker on cauliflower in the presence of* Leptosphaeria *and* Rhizoctonia.

Materials and methods

Soil was inoculated with a slurry of *Rhizoctonia* AG 2.1, 2.2 and 4 as previously described and left moist in the greenhouse. After 7 days, two 10 day old mycelial cultures each of *F. equiseti* and *F. avenaceum* grown on PDA were macerated together with ~600 ml SDW and added to half the soil.

Six week old cauliflower seedlings cv. Chaser were root inoculated with *Leptosphaeria* as previously described, and planted into the infected soil, ten seedlings into the *Rhizoctonia* and *Fusarium* soil and ten seedlings into the *Rhizoctonia* soil.

Plants were maintained in the greenhouse for nine weeks and assessed weekly for cankers.

Results and discussion

Stem cankers developed earlier on plants inoculated with *Fusarium* compared to plants without *Fusarium*, with minor staining observed on three of the plants. However 22 days after planting plants of both treatments developed stem canker (Table 13). No differences in incidence or severity of infection developed between the non *Fusarium* inoculated plants and *Fusarium* inoculated plants.

These results confirm that while *Fusarium* was regularly isolated from stem canker symptoms and is a weak pathogen compared to *Rhizoctonia* and *Leptosphaeria*, it does not increase symptoms of stem canker.

***Table 13. Incidence and severity of stem canker in cauliflower cv. Chaser inoculated with* Leptosphaeria *and planted into* Rhizoctonia *infected soil with and without* Fusarium.**

Days from planting	Percent incidence		Severity mean*(maximum)	
	With *Fusarium*	Without *Fusarium*	With *Fusarium*	Without *Fusarium*
12 days	30	0	20 (20)	-
22 days	30	60	40 (60)	50 (60)
27 days	30	70	46.7 (60)	45.7 (60)
36 days	80	80	52.5 (60)	47.5 (60)
64 days (harvest)	100	100	60 (60)	64 (80)

*mean of infected plants only

Experiment 9

Objective: *To determine the influence of* Fusarium *and* Pythium *combined infections on cauliflower seedlings.*

Materials and methods

Cauliflower seedlings cv. Skywalker were germinated in speedling trays and grown in the greenhouse. Five replicates of six week old seedlings were stem inoculated as previously

described with 10 day old cultures of *P. ultimum* with either *F. equiseti*, *F. oxysporum* or *F. avenaceum*, alone or in combination.

Plants were maintained in the greenhouse for nine weeks and inspected at one, four and nine weeks after inoculation for symptoms of stem canker.

Results and discussion

No cankers developed on plants after nine weeks (data not presented). Roots were not inspected to determine whether *Pythium* caused root rotting.

These results show that neither *Fusarium* sp. nor *Pythium ultimum* are primary causes of stem canker on cauliflower cv. Skywalker.

General Discussion

Fusarium equiseti is associated with curd rot and plant wilt of cauliflower (Chakrabarty *et al* 1989, Saxena and Rajendra Singh 1987). *F. avenaceum* is more commonly associated with cereals and medics, but is also a cause of cauliflower damping off (Linnasalmi 1952) and has previously been isolated from cauliflower stems (APPD 1984). *F. oxysporum* is a common pathogen associated with seedling damping off and root rot and is also a common saprophyte or weak parasite, found in many soils infecting dead or dying tissue.

The results of these experiments showed that while all three species were pathogens of cauliflower, with some differences in cultivar susceptibility observed, none showed any synergistic effect on stem canker with either *Rhizoctonia* or *Leptosphaeria*.

4.2.5 The effect of temperature on infection and symptom expression

Brassica stem canker causes most crop losses in winter and spring. Two experiments were undertaken to determine the effect of temperature on the development of stem canker.

Objective: *To determine the influence of temperature on canker development following combined infections of* Rhizoctonia *and* Leptosphaeria.

Experiment 1

Materials and methods

Cauliflower seedlings cv. Skywalker were germinated in speedling trays and grown in the greenhouse. Ten replicate five week old seedlings were stem inoculated as previously described using 10 day old cultures of *Rhizoctonia* AG 2.1 or 4 alone or in combination with 21 days old cultures of *Leptosphaeria* (Table 14). One set of five replicate plants were maintained in the greenhouse at 25^0C, the other in a growth room at 15^0C.

Plants were maintained in the growth room or greenhouse for twelve weeks and stem canker assessed at eleven days and five and seven weeks after inoculation.

Results and discussion

After 11 days, most plants inoculated with *Rhizoctonia* AG 2.1 developed cankers, whereas seedlings inoculated with *Rhizoctonia* AG 4 or *Leptosphaeria* alone remained healthy (Table 14). The lack of symptoms in plants inoculated with AG 4 was unexpected, and indicates possible reduction in infectivity of the fungus with repeated re-culturing on artificial media.

This prompted the re-invigoration of some cultures before using in further greenhouse experiments (section 4.1.1).

Seven weeks after inoculation many plants were dead and pycnidia had developed on plants inoculated with *Leptosphaeria*.

Analyses of results from all isolates combined (data not shown) showed the level of canker and dead plants increased significantly with time (P=0.05), with a mean of 1.9 plants infected at 11 days with none dead and 4.5 plants infected at 7 weeks with 2.5 dead. There was also a slight but significant (P=0.05) increase in infection at 25^0C compared to 15^0C, with a mean of 3.9 and 3.1 cankered plants respectively. However there was no increase in the mean number of dead plants. At week 7, there was also significantly (P=0.04) more stems with pycnidia at 25^0C than at 15^0C, with a mean of 1.6 and 0.6 plants respectively.

The higher level of infection at 25^0C was contrary to what is observed in the field, where there are higher levels of disease in winter and spring. As the results were unexpected, the experiment was repeated using growth rooms (Expt. 2), as the larger temperature variation of the greenhouse (+/- 8^0C compared to +/- 2^0C) may have confounded the results.

Table 14. Development of canker, dead plants and pycnidia on cauliflower cv. Skywalker 11 days and seven weeks after inoculation with* Rhizoctonia *alone or in combination with* Leptosphaeria *and maintained at either 15^0C or 25^0C.

	No. plants with symptoms / 5							
	Days after inoculation							
	11		**49**					
	canker		**canker***		**dead**		**pycnidia**	
	15^0C	**25^0C**	**15^0C**	**25^0C**	**15^0C**	**25^0C**	**15^0C**	**25^0C**
Rhizoctonia AG 2.1#353	5	5	5	5	0	2	-	-
Rhizoctonia AG 2.1 #736	1	2	3	5	1	2	-	-
Rhizoctonia AG 2.1 #739	4	5	5	5	5	3	-	-
Rhizoctonia AG 4	0	0	3	5	0	0	-	-
Leptosphaeria #673	0	0	5	5	2	1	0	2
Leptosphaeria #7	0	0	4	5	4	5	4	2
R. AG 2.1 #353 + *L.* #673	3	5	5	5	1	3	1	3
R. AG 2.1 #353 + *L.* #7	4	5	5	5	5	5	0	2
R. AG 2.1 #736+ *L.* #673	1	0	2	5	1	3	0	1
R. AG 2.1 #736+ *L.* #7	0	1	5	5	1	0	2	2
R. AG 2.1 #739 + *L.* #673	5	4	5	5	4	4	0	2
R. AG 2.1 #739+ *L.* #7	0	3	5	5	4	4	0	2
R. AG 4 + *L.* #673	0	0	0	4	0	4	0	2
R. AG 4+ *L.* #7	0	0	5	5	2	4	1	4
Control (not inoculated)	0	0	1	1	0	0	0	0

* canker recorded for dead plants also

Experiment 2.

The three temperatures were chosen to represent the average temperatures in winter, summer and autumn/spring in the Northern Adelaide Plains, provided by Bureau of Meteorology historical data from the Buckland Park met station.

Materials and methods

Soil was inoculated with a slurry of *Rhizoctonia* AG 2.1, 2.2 or 4 as previously described and left moist in the greenhouse for seven days before placing into 175 mm pots (~3L) and planting with six week old cauliflower seedlings cv. Chaser. Another 30 seedlings were root inoculated with *Leptosphaeria* as previously described and planted into coco peat in 175 mm pots.

One set of ten replicate pots of each of the four pathogens were placed in each growth room, set at 14^0C, 22^0C or 30^0C.

Three weeks after inoculation, one pot for each *Rhizoctonia* AG in each temperature was baited using the toothpick technique to confirm the presence of the pathogen.

Plants were maintained in the growth rooms for 11 weeks and inspected weekly for symptoms of stem canker. Plants were harvested at 11 weeks, washed and stem canker symptoms and plant vigour assessed.

Results and discussion

Rhizoctonia was detected on all toothpicks, showing that the fungus was evenly distributed throughout the growing media.

Plants grown in soil inoculated with *Rhizoctonia* AG 2.1 developed significantly more severe stem cankers at 14^0C and 22^0C than at 30^0C (Table 15). The opposite was observed with AG 4, with stem canker significantly more severe at 30^0C and 22^0C than at 14^0C. AG 2.1 had more severe cankers at 22^0C and the number of plants with canker followed a similar trend to the severity (Fig. 12). These results confirm work by Yitbarek *et al* (1988), who found *R. solani* AG 2.1 to be more virulent to canola at lower temperatures and AG 4 at higher temperatures.

Temperature had no significant effect on stem cankers induced by *Leptosphaeria* (Table 15). However there was slightly more disease at 14^0C and 22^0C compared to 30^0C. Previous work with canola has shown greater disease with *Leptosphaeria* between 12^0C and 20^0C but this was compared to 5 to 10^0C (Naseri *et al* 2008, McGee 1977) as no evaluation was undertaken at the higher temperatures.

Table 15. Mean percent canker severity on cauliflower cv. Chaser seedlings inoculated with* Leptosphaeria *or* Rhizoctonia *AG 2.1, 2.2 or 4 at six weeks old and grown in 14, 22 or 30^0C for 16 weeks.

	Mean severity of stem canker (%)			
	14^0C	**22^0C**	**30^0C**	**P (0.05)**
Rhizoctonia AG 2.1	100	84	26	25.9
Rhizoctonia AG 2.2	0	26	4	23.3
Rhizoctonia AG 4	26	74	84	29.4
Leptosphaeria	30	33	24	n.s.
P(0.05)	22.7	22.6	26.5	

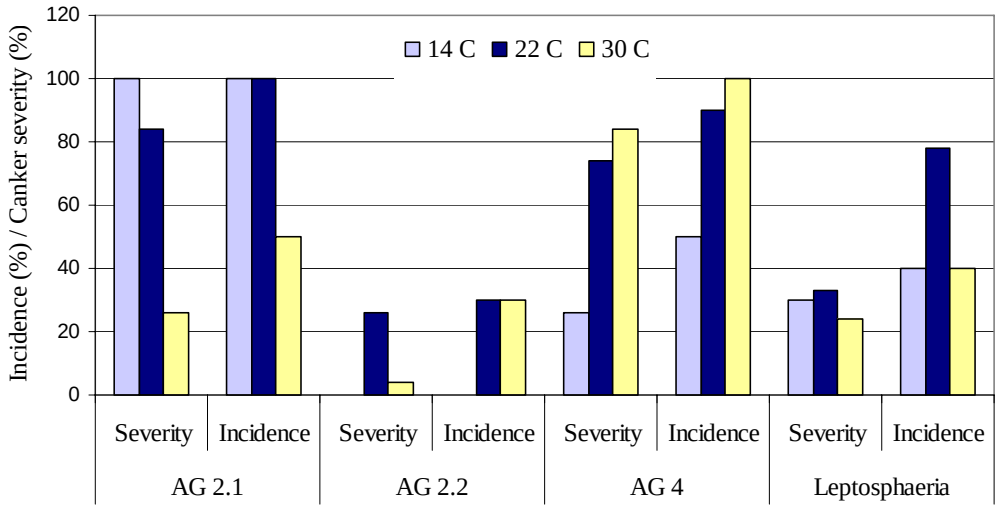


Figure 12. Mean percent canker severity and incidence on cauliflower cv. Chaser seedlings inoculated with* Leptosphaeria *or* Rhizoctonia *AG 2.1, 2.2 or 4 at six weeks old and grown in 14, 22 or 30^{0}C for 16 weeks.

Soils tested in the Northern Adelaide Plains have high levels of AG 2.1 (Section 4.2.7), which would contribute to the high canker seen in winter/spring plantings. However some also had high levels of AG 4 without a corresponding increase in canker in warmer weather. Thus the combination of *Leptosphaeria* and *AG 2.1*, both causing more canker at lower than higher temperatures, provide a synergistic effect.

4.2.6 The effect of soil moisture on infection and symptom expression

Leptosphaeria can be spread by water and rain splash, and is most destructive in wet soil. Therefore a growth room experiment was undertaken in cauliflower to determine the effect soil moisture has on the development of stem canker.

Objective: *To determine the influence of soil moisture on susceptibility of cauliflower to* Leptosphaeria *and* Rhizoctonia.

Materials and methods

Soil was inoculated with a slurry of *Rhizoctonia* AG 2.1 as previously described and left moist in the greenhouse for seven days before placing into thirty 15 cm square pots (1.7L) and planted with washed nine week old cauliflower seedlings cv. Chaser. Another 30 seedlings were root inoculated with *Leptosphaeria* as previously described and planted into coco peat in 15 cm square pots.

One set of ten replicate pots for each of the two pathogens were placed in the greenhouse and connected to an automatic watering system set to deliver one of three regimes: low watering at ~1.3 L/h, maintained on average at 10% soil moisture content (compared to full saturation); medium watering at 2.16 L/h, maintained on average at 33% soil moisture content; and high watering at 3.24 L/h, maintained on average at 45% soil moisture content. The soil moisture was measured with a Measurement Engineering GT Bug.

Three weeks after inoculation, one pot of each *Rhizoctonia* AG in each watering regime was baited using the toothpick technique to confirm the presence of the pathogen.

Plants were maintained in the greenhouse and assessed weekly for cankers from three weeks after inoculation. Plants were harvested at nine weeks after planting, washed and canker severity assessed.

Results and discussion

The mean severity of stem canker on plants inoculated with *Leptosphaeria* was higher with the high water regime at all assessment times (Table 16, Fig. 13). Conversely, the high watering regimes had a lower severity of stem canker in plants inoculated with *Rhizoctonia* AG 2.1, although only at harvest was the difference statistically significant (Fig. 14).

These results suggest that the severity of stem canker induced by *Leptosphaeria* would be greatest in wet weather or where plants are overwatered. Therefore higher levels of disease would be expected in winter in fields infected with *Leptosphaeria*. With the combination of *Leptosphaeria* and *Rhizoctonia* AG 2.1, the most severe disease would be found at medium watering. Teo *et al* (1988) found that soil moisture had no effect on *R. solani* AG 2.1 seedling infection of canola, but in mature plants increased soil moisture caused more disease.

However while soil moisture affects plant susceptibility, this needs to be balanced with the ability to produce a commercial crop. In these experiments, plants in the lowest soil moisture treatment were the least vigorous (Fig. 15). The root growth was visibly poor (Fig. 16) and heading was reduced, although these effects were not assessed. To produce a good crop, it is recommended that cauliflower should grow evenly with no moisture stress, especially immediately after transplanting and during head formation (Lancaster & Burt 2001). Curd initiation occurs 6 to 8 weeks after planting and locally water is applied at relatively high

levels during this time. This also coincides with the time of rapid canker development observed in the previous study (Hitch *et al* 2006).

Table 16. Severity of canker at harvest on cauliflower cv. Chaser inoculated with* Leptosphaeria *at nine weeks old and grown under low, medium or high water regimes.

Weeks after planting	Mean severity of stem canker (%)			
	Low	Medium	High	P (0.05)
3	6	8	26	16
4	12	18	32	n.s.
5	12	18	50	28.7
6	14	24	50	29.2
7	24	36	50	n.s.
8	42	64	68	16.9
P(0.05)	21.8	23.3	28.1	

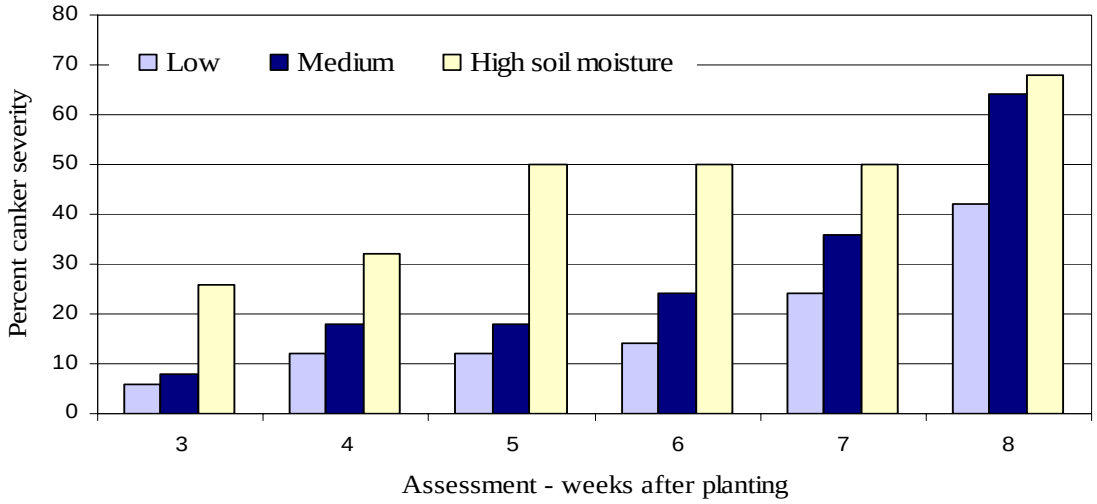


Figure 13. Incidence and severity of canker at harvest on cauliflower cv. Chaser inoculated with* Leptosphaeria *at nine weeks old and grown under low, medium or high water regimes.

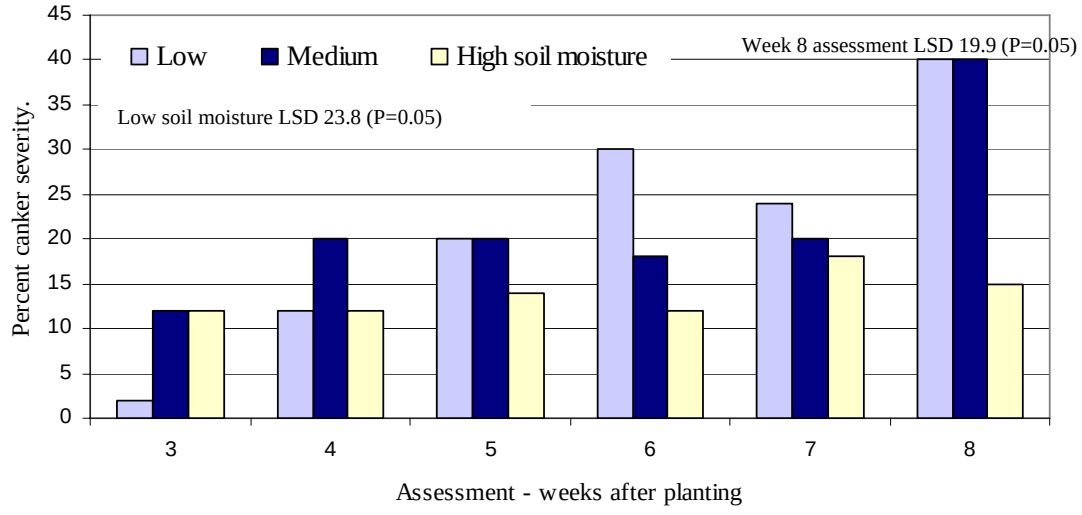


Figure 14. Incidence and severity of canker at harvest on cauliflower cv. Chaser inoculated with* Rhizoctonia *AG 2.1 at nine weeks old and grown under low, medium or high water regimes.

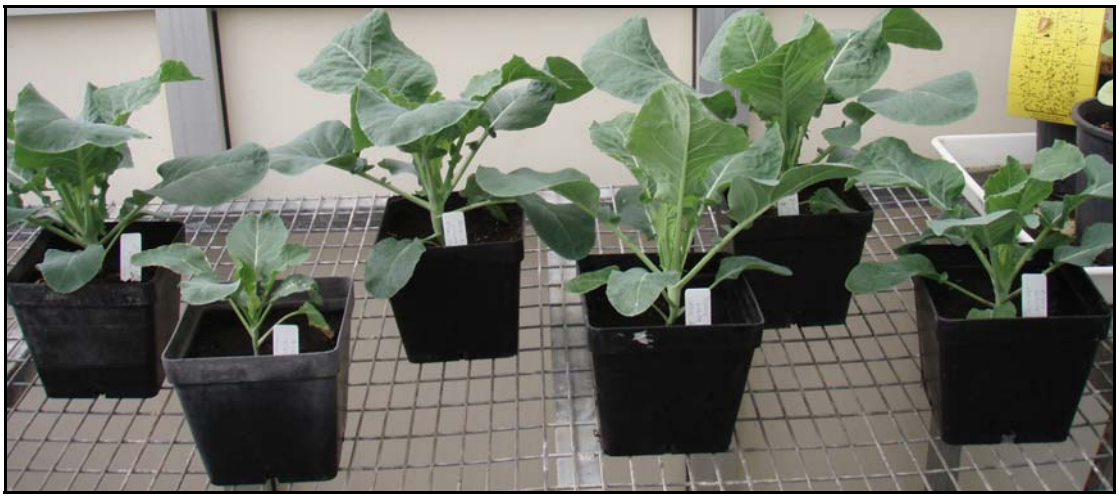


Figure 15. Plants showing variable difference in plant vigour between soil moisture regimes: Low (Left), Medium (Centre) and High (Right) in both* Leptosphaeria *inoculated plants (Front) and* Rhizoctonia *inoculated plants (Rear) 17 days after planting.

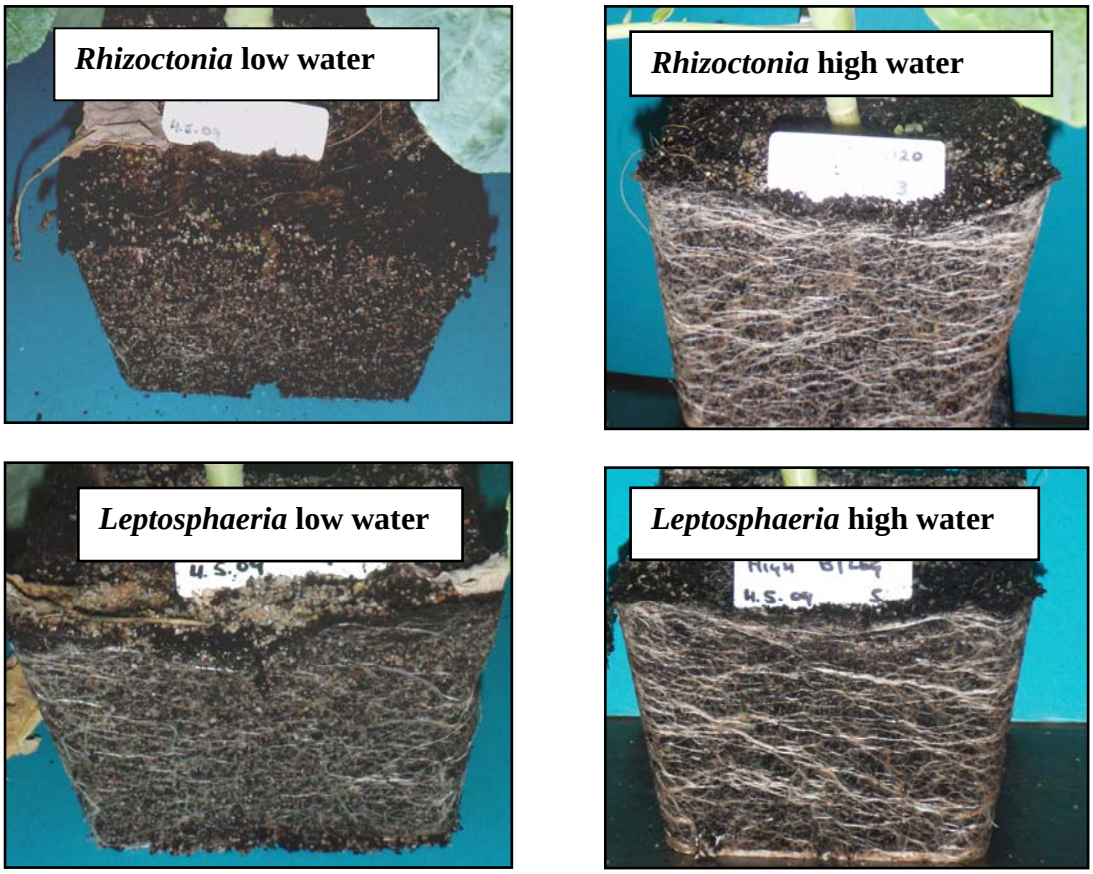


Figure 16. Root systems of potted cauliflower cv. Chaser at 7 weeks after planting with different soil watering regimes: Low (Left) and High (Right) in both* Rhizoctonia *inoculated plants (Top) and* Leptosphaeria *inoculated plants (Bottom).

4.2.7 Infection timing and source of pathogens.

To assist in determining the timing of control measures, a greenhouse experiment was undertaken to evaluate if the susceptibility of cauliflower changes as plants age. Seedlings from the two main nurseries that supply the growers at Virginia were tested for levels of *Rhizoctonia* or *Leptosphaeria* and field evaluations were used to determine when infection occurs in cauliflower seedlings after planting in infected soil and whether there is any correlation between soil levels and canker severity.

Experiment 1.

Objective: *To determine the susceptibility of cauliflower plants of increasing age to the various pathogens.*

Materials and methods

Cauliflower seedlings cv. Skywalker and Nautilus were grown in speedling trays or MK12 pots over a period of 14 weeks to provide five or ten replicate plants of various ages for stem inoculations with 14 day old cultures of one of ten pathogens. The MK12 pots were randomised, the speedling trays inoculated in two replicates each of five cells. Treatments are outlined in table 17. The pathogens were *Leptosphaeria maculans, Fusarium equiseti, F. avenaceum, F. oxysporum, Rhizoctonia* AG 2.1, AG 2.2, AG 3 and AG 4, *Pythium ultimum* and *P.* sp.

Plants were maintained in the greenhouse for a further three weeks and assessed at 13 and 21 days after inoculation for symptoms of stem canker.

Table 17. Age, cultivar, inoculation plug number and pot size of cauliflower.

Age at inoculation	Cultivar	Pot size	No. replicate plants	No. 7 mm mycelial plugs
4 weeks	Skywalker	Speedling	5	2
5 weeks	Skywalker	Speedling	10	2
6 weeks	Nautilus	Speedling	10	2
8 weeks	Nautilus	Speedling	10	2
10 weeks	Nautilus	MK12	10	10
11 weeks	Nautilus	MK12	10	10
13 weeks	Nautilus	MK12	10	10
14 weeks	Nautilus	MK12	10	10

Results and discussion

Plants up to six weeks old were highly susceptible to *R. solani, Leptosphaeria* and *Pythium* (Table 18). Plants older than 10 weeks were infected only by *R. solani* and AG 2.2 was the only pathogen to infect plants at 16 weeks. The highest level of plant infection was on six week old plants, which is when speedlings are provided to growers by the nursery. Unlike previous experiments, cankers were observed in plants inoculated with *Rhizoctonia* AG 3. However this may have been the result of cross contamination, as some controls also developed canker.

The cultivar Nautilus was used for the older 6 plantings, but a shortage of seed meant the two younger plantings were cv. Skywalker, as the nursery supplier considered these to be interchangeable varieties. Differences in susceptibility between these cultivars to the *Fusarium* sp. were observed in previous studies, however in this experiment any differences observed could equally be attributed to the age of the seedlings. Both cultivars were equally susceptible to most of the *Rhizoctonia*.

Table 18. Symptoms observed on plants of various ages three weeks after inoculation with various pathogens.

Pathogen	Percent of plants with cankers *Age of plants at inoculation (weeks)*							
	4*	***5****	***6***	***8***	***10***	***11***	***13***	***14***
Leptosphaeria	20	50	100	10	0	0	0	0
F. avenaceum	0	0	50	0	0	0	0	0
F. oxysporum	0	50	40	0	0	0	0	0
F. equiseti	0	0	0	0	0	0	0	0
R. solani AG 2.1	80	80	80	10	10	10	0	0
R. solani AG 2.2	80	100	90	10	30	60	40	80
R. solani AG 3#	0	20	0	10	0	0	0	0
R. solani AG 4	80	100	100	30	20	30	0	0
P. ultimum	20	60	70	30	0	0	0	0
Control (not inoculated)	40	10	20	0	0	0	0	0

* cv. Skywalker, all others cv. Nautilus.
# isolate from potato, all others from cauliflower.

Experiment 2

Objective: *To determine whether* Rhizoctonia *or* Leptosphaeria *could be detected in seedlings from nurseries.*

Materials and methods

Nursery 1 was sampled on two occasions. 42 six week old cauliflower seedlings cv. Elbert and 42 cv. Nautilus were collected at random in the first sampling and 100 seedlings cv. Arctic collected on the second occasion.

From Nursery 2, 84 six week old Brussels Sprout seedlings cv. Bowlan were collected at random from a batch delivered to the grower.

The roots were washed and the leaves removed before the plants were freeze dried for DNA extraction. The DNA was tested for *Rhizoctonia* AG 2.1, 2.2, 3, 4 and 8, and *Leptosphaeria*. Plants were tested individually except the Arctic from nursery one, which was bulked into 10 samples of 10 seedlings.

Results and discussion

Molecular tests did not detect any *Rhizoctonia* or *Leptosphaeria* DNA in the seedlings, indicating it was either not present or was below the level of detection.

Experiments 3-5

Objective: *To determine the natural levels of* Rhizoctonia *and* Leptosphaeria *in soil and cauliflower plants in commercial properties.*

Materials and methods

Three experiments were undertaken in cauliflower plantings located in the Northern Adelaide Plains in South Australia, approximately 50 km North of Adelaide. Experimental details are outlined in Table 19.

Soil was collected as previously described prior to or at planting and after harvest, 40 cores collected and bulked from five to ten replicated sites within the planting. For two sites the seedlings planted were from nursery stock previously tested (Expt. 2). Experiment 4 was started immediately after planting, so seedlings were not collected. Up to 100 plants were collected at intervals from two weeks after planting to harvest, either randomly over the planting, or five to ten plants selected at random from each of the replicate areas. Plants were either tested individually or bulked into samples of five or ten plants. All plants were washed and frozen on the day of collection, and stored at -18^{0}C until freeze drying, DNA extraction and PCR testing. For the earlier collection periods at two, four and six weeks, leaves were removed and the whole stem used for extraction. By eight weeks after planting, plants were too large for whole stem testing, so the stem was split vertically into three or four sections and one section used for extraction. In experiment 4 at 8, 12 and 16 weeks after planting, plants with canker were selected and tested separately from plants with no canker.

Table 19. Experimental details of disease infection experiments in commercial plantings, Northern Adelaide Plains, South Australia.

Expt.	Site (planting time)	Reps	Soil sample times	Plant sampling		Cultivar
				Weeks after planting	Total no. plants	
3	Pt Gawler (Jan 07)	9	Pre plant, post harvest	0, 2, 4	84	Elbert & Nautilus*
4	Buckland Park (June 07)	10	1 day after planting, post harvest	2, 4, 6,	100[A]	Skywalker & Savannah
				8, 12, 16	100[B]	
5	St Kilda (Aug 07)	5	Pre plant, post harvest	2, 4, 6, 8, 12	50[C]	Arctic*

A: plants bulked into 10 samples of 10 plants from each of the replicate areas.
B: plants bulked into 10 samples of 5 plants with canker, and 10 samples of 5 plants without canker, each paired sample collected from the replicate areas.
C: plants bulked into 10 samples of 5 plants, 2 samples from each of the soil replicate areas.
* seedlings from tested nursery stock.

Results and discussion

Experiment 3 – Port Gawler

Rhizoctonia AG 2.1 was found in all nine soil samples collected from each collection period, pre plant and harvest (Table 20). AG 3 was found in three pre planting soil samples (33.3%) and only one post-harvest soil sample at very low levels, whereas AG 4 was recovered from

one soil sample pre-planting and four post harvest. Neither *Leptosphaeria* nor AG 2.2 were detected in any of the soil or plant samples.

The mean levels of AG 2.1 in soil increased significantly from planting to harvest (P=0.002). At harvest, AG 4 was detected in more soil samples and the levels were higher, however the difference was not statistically significant due to the extreme variation. The level of AG 3 in soil between pre plant and harvest did not increase and DNA was detected in fewer samples, confirming that AG 3 is unlikely to be a pathogen of cauliflower.

Rhizoctonia was detected in seedlings collected from the field at 2 and 4 weeks. More plants were infected at four weeks than at two and the levels of DNA higher in the older plants. The plants detected with AG 2.1 were scattered throughout the planting, whereas the AG 4 infected plants were grouped in or near the sites of infected soil (data not presented). These results, together with the lack of infection in the nursery plants, show that stem canker originates from pathogens in the soil and *Rhizoctonia* infection starts within the first two weeks of planting.

Table 20. Levels of* Rhizoctonia *DNA found in soil pre-planting and harvest and plant material collected 2 and 4 weeks after planting and incidence of infected samples, Port Gawler, SA.

Sample	Mean DNA pg/g soil or dried plant material (*range*)*			Percent samples infected		
	AG 2.1	AG 3	AG 4	AG 2.1	AG 3	AG 4
Pre-plant soil[A]	**68** (*4-339*)	**3** (*1-4*)	**75** (*75*)	100	33.3	11.1
Post harvest soil[A]	**3453** (*879-4643*)	**4** (*4*)	**10,667** (*42-41971*)	100	11.1	44.4
Plants - 2 weeks[B]	**838** (*8-2610)*	-	**19** (*21-35*)	6.0	0	2.4
Plants - 4 weeks[B]	**958** (*1-10627*)	**345** *(10-680)*	**27,069** (*27-186,697*)	14.3	2.4	11.9

* Range and mean of infected samples only
A: 9 samples tested
B: 84 samples tested

Experiment 4 – Buckland Park

Rhizoctonia AG 2.1 was found in most of the plant and soil samples (Table 21), with the highest levels occurring just prior to harvest. There was also an increase in the level of *Rhizoctonia* AG 2.1 in soil between planting and harvest, although as the amounts detected in each sample were variable, the difference was not statistically significant. *Rhizoctonia* AG 4 was detected in 70% of the soil samples at planting and 80% post-harvest (Table 21) at levels significantly (P=0.03) higher than the AG 2.1 at both sampling times. More plant samples were infected with AG 2.1 early in the season, with DNA detected in 30% of the samples at 2 and 4 weeks after planting but in only 10% at 16 weeks after planting. AG 2.2 was not detected in any of the soil or plant samples.

Leptosphaeria maculans was detected in only 10% of the soil samples, but was found in over 70% of the plant samples (Table 21). Levels in the plants were quite low until pre harvest, where the levels in one of the plants with canker were 41,107 pg/g dried plant material (Table 21, 22). The low level of *Leptosphaeria* in soil was unexpected, considering

the level detected in the plant material. In addition, the levels in plant material were low compared to those found in other experiments, where levels in stems were up to 5.4 x 10^6 DNA pg /g dried plant material (Section 4.2.8). In this experiment, only 19% of the plant samples tested had levels over 100 DNA pg /g dried plant material (data not presented).

Although AG 2.1 was still detected in plants without cankers, the levels were significantly lower (P=0.04) than those found in plants with cankers (Table 21, Fig. 17). There was a trend for plants with canker to have higher levels of *Leptosphaeria* DNA than plants with no canker (Table 22, Fig. 17), however the variation between the samples was too great to be statistically significant.

The work undertaken to develop the PCR test on *Leptosphaeria maculans* of canola (Sosnowski *et al* 2006) showed a good correlation between disease and DNA levels in plants. They found levels in soil between 86 and 2215 pg/g soil during the year of cropping, which was higher than detected in these experiments. Therefore more work needs to be undertaken to determine correlations between soil levels and canker development in cauliflower. A more extensive survey of nursery plants may also be needed to confirm that the infection is not originating in the seedlings, although Budge *et al* (2009) showed *Rhizoctonia* was not detected in 1300 plants from six nurseries in the UK.

Table 21. Levels of* Rhizoctonia solani *AG 2.1 and AG 4 or* Leptosphaeria maculans *(L.m.) found in soil at planting and harvest and in plant material collected 2 to 12 weeks after planting and incidence of infected samples, Buckland Park, SA.

Sample and number [A]	Mean DNA pg/g soil or dried plant material (*range*) *			Percent samples infected		
	AG 2.1	**AG 4**	***L.m.***	**AG 2.1**	**AG 4**	***L.m.***
Soil at planting[A]	**61** (*9-191*)	**1,283** (*23-6,507*)	**2** (*2*)	80	70	10
Post harvest soil[A]	**137** (*6-526*)	**3,390** (*220-10,244*)	**1** (*1*)	100	80	10
Plants - 2 weeks[B]	**23.7** (*11-65*)	**108** (*31-196*)	**2.3** (*1-4*)	60	30	70
Plants - 4 weeks[B]	**7.9** (*71-21*)	**82.5** (*40-125*)	**101** (*4-646*)	80	30	100
Plants - 6 weeks[B]	**20** (*4-33)*	**13** (*13*)	**23** (*5-50*)	50	10	100
Plants - 8 weeks[C]	**417** (*3-4,096*)	**36** (*13-80*)	**62** (*8-274*)	70	15	100
Plants - 12 weeks [C]	**131** (*3-729*)	**132** (*132*)	**70** (*3-438*)	80	5	80
Plants - 16 weeks [C]	**782** (*3-7,555*)	**15** (*15*)	**2,233** (*11-41,107*)	80	10	100

* Range and mean of infected samples only
A: 10 soil samples tested
B: 10 samples each of 10 plant stems
C: 10 samples each of 5 plant stems without canker and 10 samples each of 5 plant stems with canker, results combined

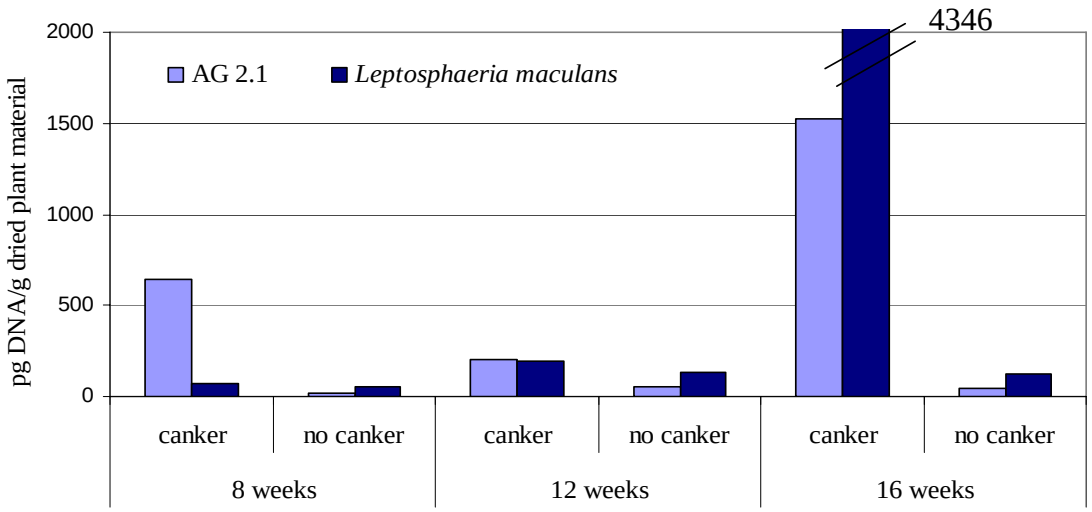


Figure 17. Rhizoctonia AG 2.1 and Leptosphaeria maculans (L.m.) detected in plant material collected 8, 10 and 12 weeks after planting with and without canker, Buckland Park, SA.

Table 22. Level of* Rhizoctonia *AG 2.1 and AG 4 or* Leptosphaeria maculans *(L.m.) in plant material collected 8, 10 and 12 weeks after planting with and without canker and incidence of infected samples, Buckland Park, SA.

Sample*	Mean pg DNA/g soil or dried plant material (*range*)#			Percent samples infected		
	AG 2.1	**AG 4**	***L.m.***	**AG 2.1**	**AG 4**	***L.m.***
Plants - 8 weeks. With canker	**641** (*3-4,096*)	**47** (*14-80*)	**69.2** (*8-274*)	90	20	100
Plants - 8 weeks. No canker	**14.2** (*5-25*)	**13** (*13*)	**54** (*13-172*)	50	10	100
Plants - 12 weeks. With canker	**207** (*3-729*)	**132** (*132*)	**19.8** (*3-47*)	80	10	100
Plants - 12 weeks. No canker	**54.5** (*4-218*)	-	**132** (*5-438*)	80	0	70
Plants - 16 weeks. With canker	**1,522** (*3-7,555*)	**15** (*15*)	**4,346** (*11-41,107*)	80	10	100
Plants - 16 weeks. No canker	**42.6** (*2-284*)	**20** (*20*)	**119** (*8-600*)	80	10	100

* 10 samples of 5 plant stems each
# Range and mean of infected samples only

Experiment 5 – St Kilda

R. solani AG 2.2 or AG 4 were not detected in soil or plant samples, whereas *Rhizoctonia* AG 2.1 was found in most soil and plant samples collected both pre plant and harvest (Table 23). The levels of AG 2.1 in soil increased between planting and harvest however the

difference was not statistically significant. In plants, AG 2.1 increased significantly over time (P=0.03) and also showed a significant linear regression with weeks after planting (Fig. 18).

Similar to the previous experiment, *Leptosphaeria* was not detected in soil collected at either planting or post harvest. However it was detected at low levels in the plant samples (Table 23).

Table 23. Number of infected plants (10 samples of 5 plants) and level of* Rhizoctonia solani *2.1 and* Leptosphaeria *(L.m) found in soil at planting and harvest and in plant material collected 2 to 12 weeks after planting, Buckland Park, SA.

Sample	Mean pg DNA/g soil or dried plant material (*range*)*		Percent samples infected	
	AG 2.1	***L.m.#***	**AG 2.1**	***L.m#***
Soil at planting	**1762** (*560-3,616)*	0	40	0
Post harvest soil	**2664** *(879–4,417*)	0	50	0
Plants - 2 weeks	**90.4** (*5-225*)	**9.4** (*3-28*)	80	90
Plants - 4 weeks	**3458** (*13-22,538*)	**32.6** (*1-199*)	80	70
Plants - 6 weeks	**412** (*2-1,419*)	**42** (*42*)	80	10
Plants - 8 weeks	**1,123** (*5-3,478*)	**338** (*7-2,479*)	100	100
Plants - 12 weeks	**36,888** (*53-196,606*)	**30.5** (*3-216*)	100	90

* Range and mean of infected samples only
# L.m. = *Leptosphaeria maculans*

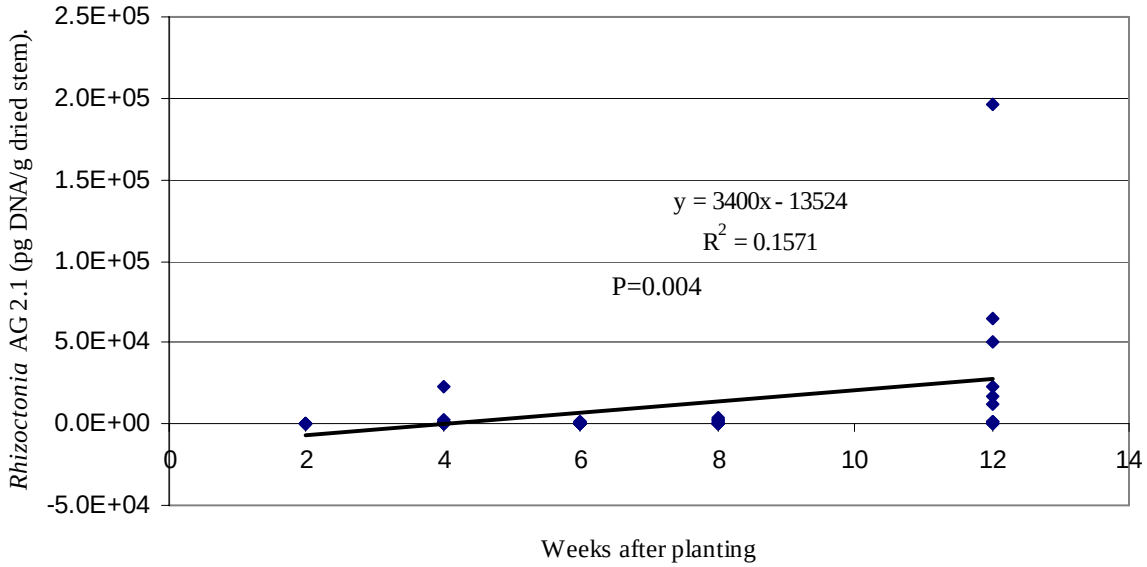


Figure 18. Amount of* Rhizoctonia solani *AG 2.1 DNA (pg/g dried stems) in cauliflower stems 2-12 weeks after planting into infected soil.

Experiment 6

Objective: *To determine if the natural infection levels of* Rhizoctonia *and* Leptosphaeria *in soil correlate with canker severity*

Materials and methods

This experiment was undertaken in a winter planting of cauliflower located in the Northern Adelaide Plains in South Australia, approximately 50 km North of Adelaide.

Soil was collected as previously described prior to planting (April 2008), at harvest and eight and ten weeks after harvest. At each sampling time 40 cores were collected and bulked for PCR testing from 16 replicated sites within the planting. Six week old seedlings of cv. Skywalker (row 1), cv. Donner (row 2) and cv. Chaser (rows 2, 3 and 4), were planted by the grower as shown in Fig. 19.

Ten plants were assessed *in situ* for canker every two weeks between planting and harvest. At harvest, from each cultivar in rows 1, 2 and 3, 10 plants that were harvested and 10 plants rejected by the grower were assessed for presence or absence of canker.

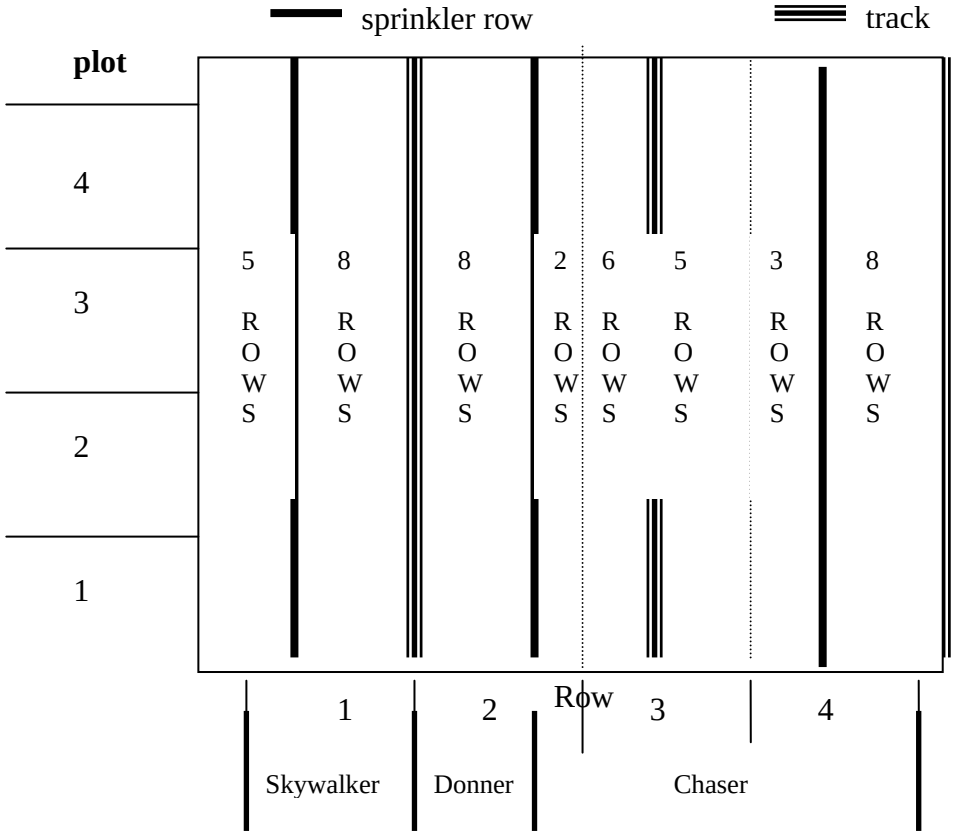


Figure 19. Trial plan, Experiment 6.

Results and discussion

Rhizoctonia AG 2.1 was widespread throughout the area, but DNA levels were highest in rows 1 and 2 (Fig. 20). *Rhizoctonia* AG 4 was more sporadic, but levels were highest in row 1 with the highest level of 1,443 DNA pg /g soil detected in plot 3. *Rhizoctonia* AG 2.2 or *Leptosphaeria* were not detected. These results show the variability of soil infection throughout a typical field planted with Brassica.

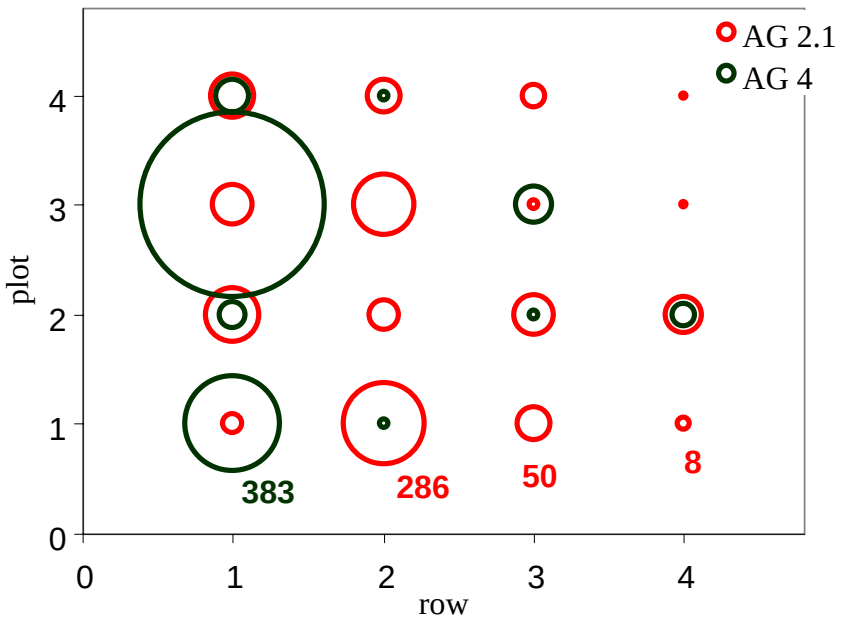


Figure 20.* Rhizoctonia solani *AG 2.1 and AG 4 in soil pre planting. The size of the circle represents the relative level of DNA detected (figures on the bottom bubbles are DNA pg/g soil).

Levels of *Rhizoctonia* AG 2.1 in soil increased significantly between pre planting and harvest and continued to increase during the 10 weeks and then 18 months of fallow after the crop residue was ploughed in (Figs. 21, 22). While the increase in each row was relatively uniform, the increase in the plot was not. Over the 19 months of fallow, the *Rhizoctonia* AG 2.1 DNA levels in plot 1 increased from ~1,600 pg /g soil at ten weeks of fallow to over 4,700 pg/g soil. AG 2.1 was detected in all soil samples whereas AG 4 was only detected in 4-5 areas each sample time and these areas were not consistent between sampling times. For example in the area with the highest level detected after 18 months fallow (2,426 pg/g soil), no DNA was detect in either the 8 or 10 weeks post harvest samples, and only 61 pg/g soil detected at harvest. However the mean levels of *Rhizoctonia* AG 4 was much higher prior to planting than after harvest (Fig. 23) and like the AG 2.1, at 18 months of fallow the levels were above those at pre planting. These results indicate that *Rhizoctonia* can proliferate in the absence of a crop host, surviving and growing on either organic matter in the soil or weeds.

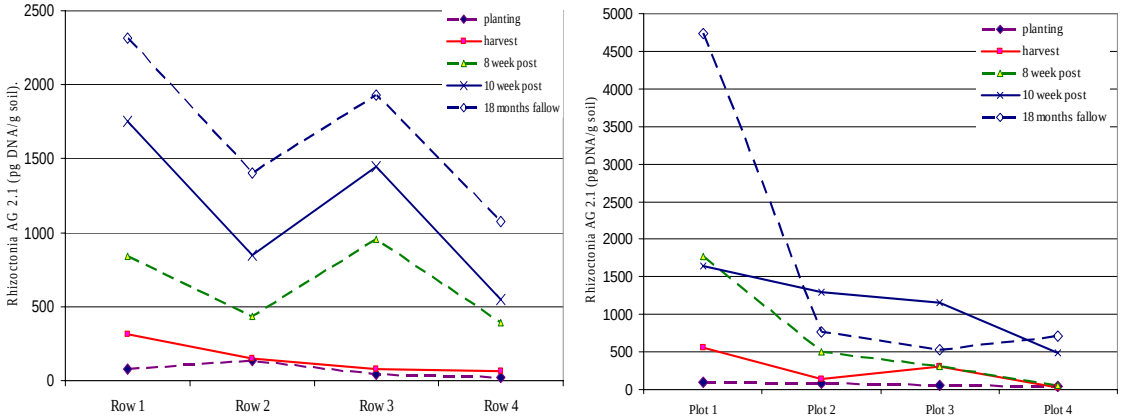


Figure 21. Rhizoctonia AG 2.1 DNA (pg/g soil) in soil at planting, harvest, eight and ten weeks after harvest and after 18 months fallow. (L) mean levels per row and (R) mean levels per plot.

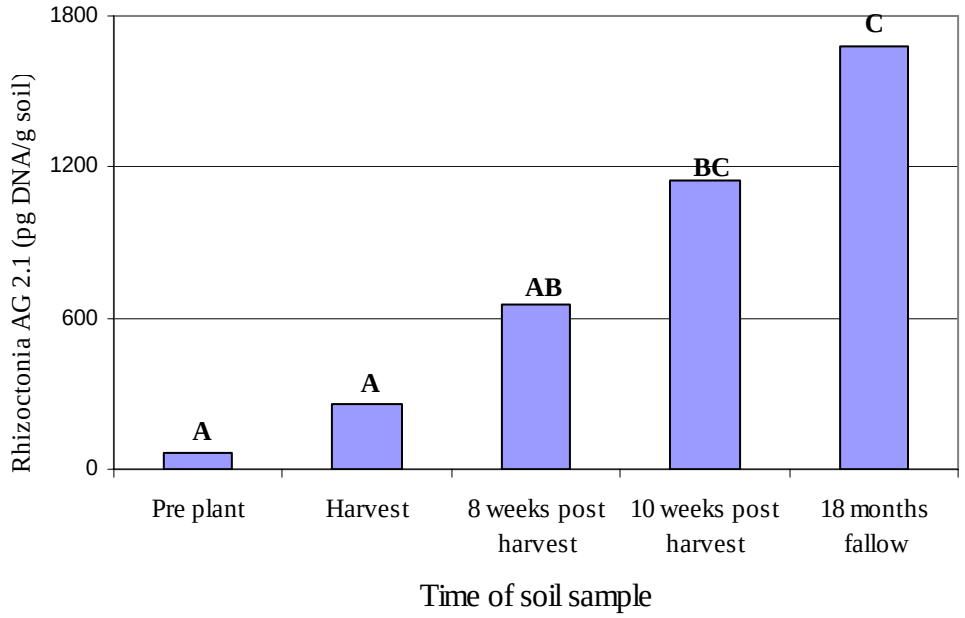


Figure 22. **Rhizoctonia** ***AG 2.1 DNA (pg/g soil) in soil at planting, harvest, eight and ten weeks after harvest. Sampling times with the same letter are not significantly different (P=0.05).***

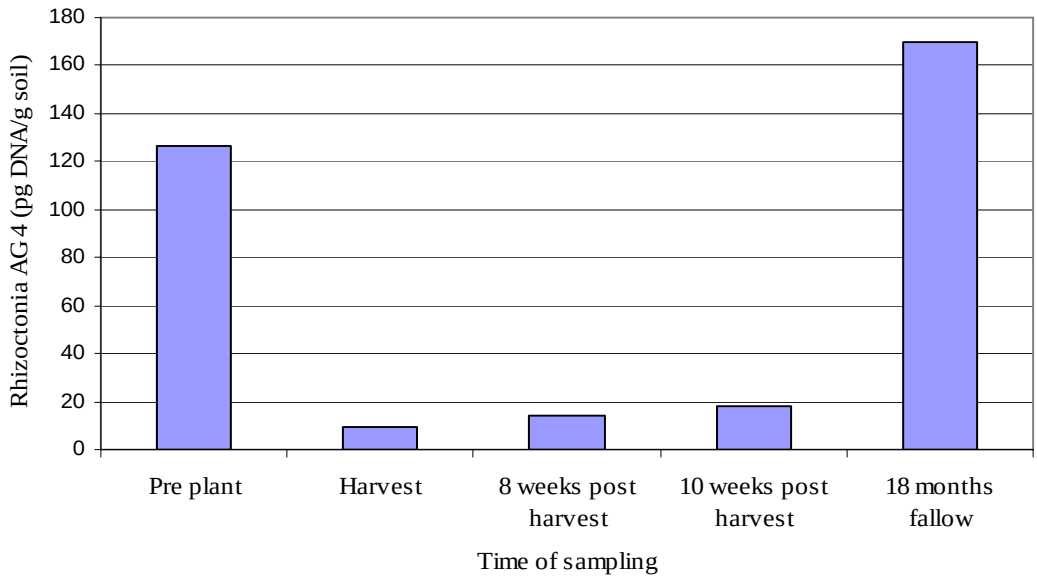


Figure 23. **Rhizoctonia** ***AG 4 DNA (pg/g soil) in soil at planting, harvest, eight and ten weeks after harvest. Means at different sampling times were not significantly different (P=0.05).***

Canker severity was low over the whole area, with only partial cankers observed (data not presented). The levels increased at 12 weeks after planting (Fig. 24), with cv. Chaser having the highest canker severity and cv. Skywalker the lowest. This concurred with results of the cultivar experiments (section 4.3.1), which showed that cv. Skywalker was the least susceptible to *Rhizoctonia*. There was also a good linear correlation between canker incidence and canker severity (Fig. 25). However there was no correlation between the canker severity and soil DNA (Fig. 26) over the rows. Plants grown in soil with high levels of *Rhizoctonia* AG 2.1 resulted in the lowest mean canker severity. This may be an effect of

the cultivar, as when the data was analysed by plot, with mixed cultivars, the relationship between canker severity and planting DNA was significant (P=0.01) (Fig. 27).

The incidence of plants with canker was not different in the harvested plants compared to those not harvested (Fig. 28). The canker severity in this crop was not severe enough to affect the marketability of the cauliflower.

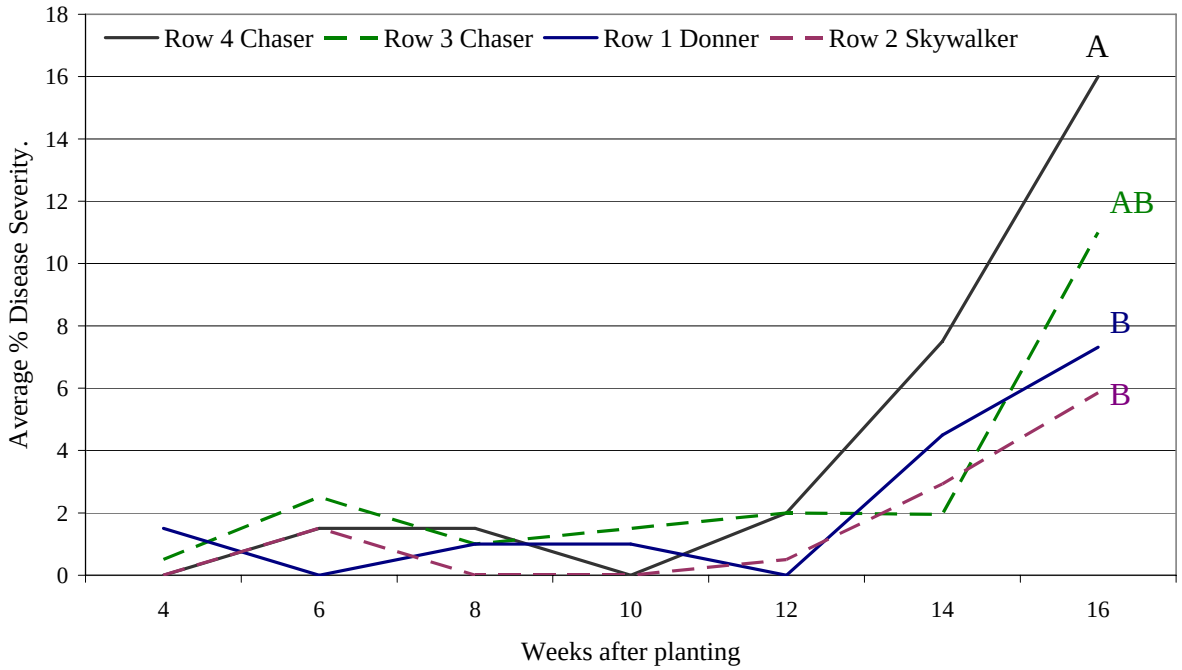


Figure 24. Canker severity in the four rows of different cultivars assessed from four to sixteen weeks after harvest. Lines with the same letter are not significantly different (P=0.05) at the 16 week assessment.

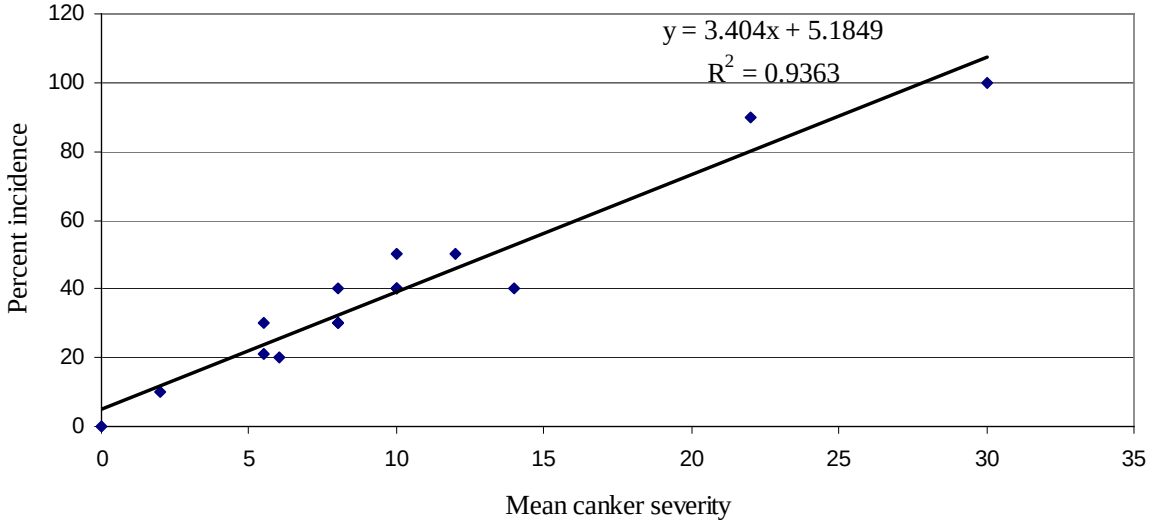


Figure 25. Correlation between canker severity and percent incidence at harvest.

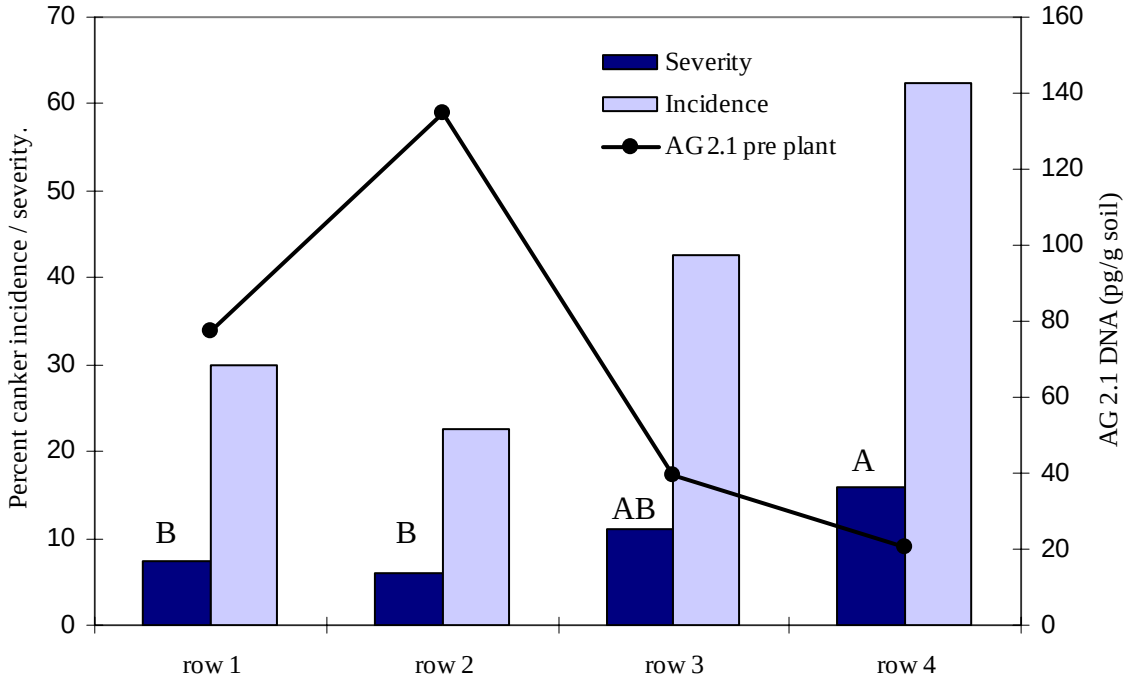


Figure 26. Canker incidence and severity at harvest in the four rows compared to levels of* Rhizoctonia solani *AG 2.1 DNA at planting. Rows with the same letter are not significantly different (P=0.05).

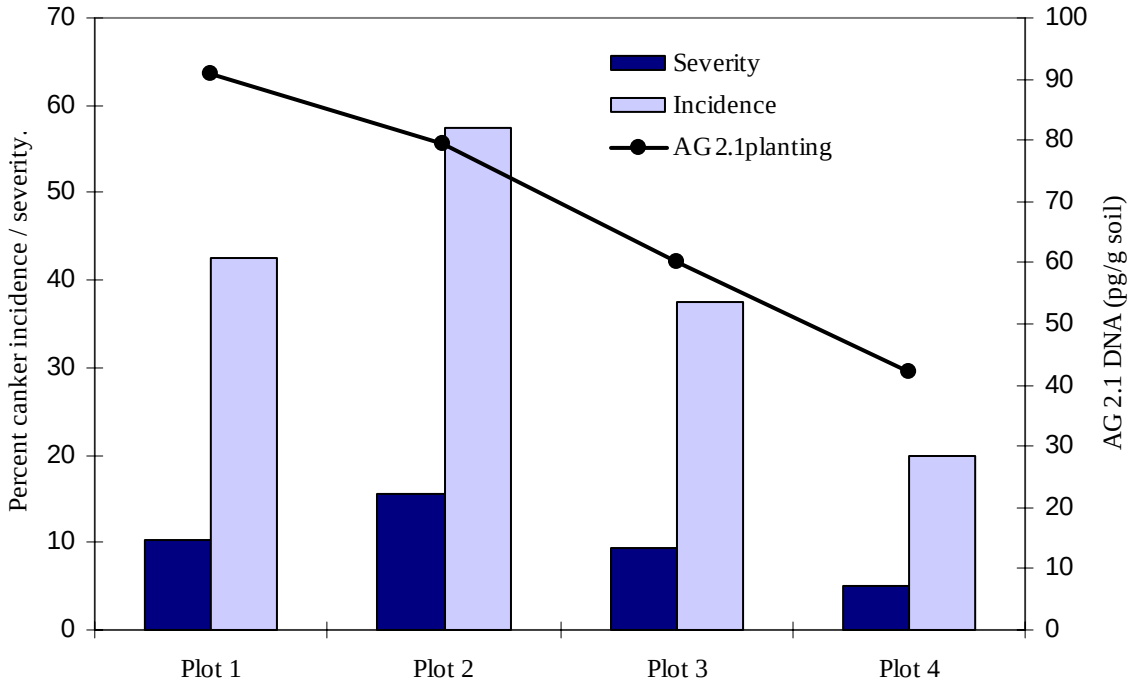


Figure 27. Canker incidence and severity at harvest in the four plots compared to levels of* Rhizoctonia solani *AG 2.1 DNA at planting.

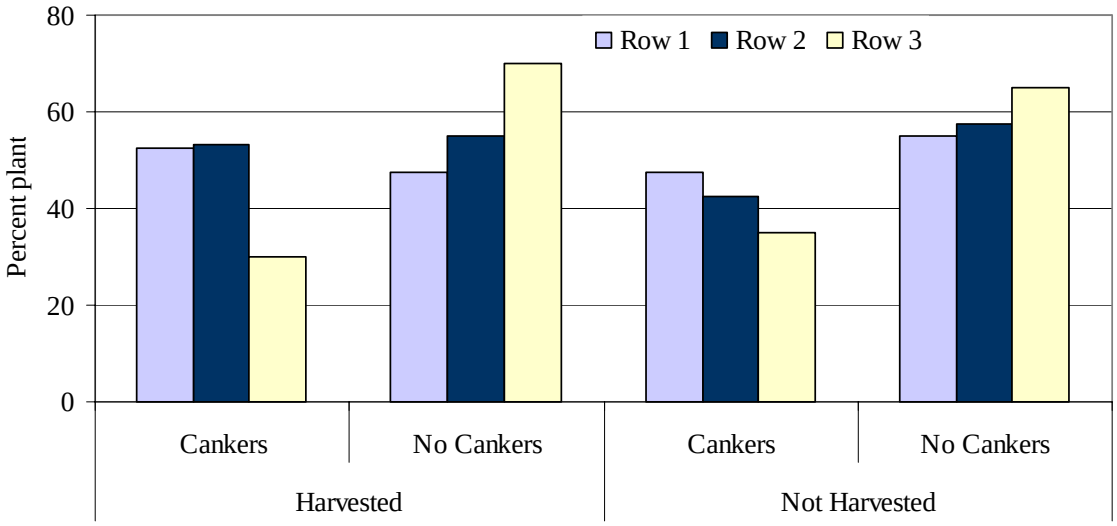


Figure 28. Incidence of cankers on plants harvested or not harvested by grower.

General discussion

These results show that the levels of pathogen in stems usually increased over time, however the relationship was not always linear. From previous results (Hitch *et al* 2006), the canker became more obvious at 6-8 weeks after planting and it was expected that there would be a significant increase in DNA in the plant near this time. The plants with canker generally had higher levels of DNA than those without canker and it may be that the levels in plants chosen at random were too variable to obtain meaningful correlations. However it is not always possible to choose plants with canker, or know which plants will become infected in an uncontrolled field situation. In addition, *Rhizoctonia* and *Leptosphaeria* were detected in plants with no canker. More work needs to be done to determine whether there is a threshold level of DNA in plants above which cankers are observed. Disease severity from *Rhizoctonia* on cauliflower is related to infection rate, pectin degradation and lack of host response (Pannecoucque and Hofte 2009). Budge *et al* (2009), who also found a high level of non-symptomatic plants infected with *Rhizoctonia*, suggested that while plant defences could not prevent infection by the pathogen, they may prevent damage to the host. The symptom expression could therefore be influenced by the host and the environmental conditions and not directly to the levels of DNA detected.

These results highlight the variability of inoculum in the field. More inoculum was found in the soil after a crop, and the levels continued to increase after harvest. However there was no clear correlation between amount of inoculum in soil and disease levels as found in other studies with *Rhizoctonia* solani AG 4 on cabbage (Keinath 1995).

4.2.8 Alternate weed hosts

Rhizoctonia was previously recovered from the roots of weeds, including fat hen, knotweed, thistles, rye grass, sour sob and paddy melon, however no obvious stem canker were found. Weeds in and around cauliflower plantings in the Northern Adelaide Plains were tested in a series of greenhouse experiments to determine whether stem canker developed on plants infected with either *Rhizoctonia* or *Leptosphaeria.*

Experiments 1-7

Objective: *To determine if weeds infected with* Rhizoctonia *AG 2.1, 2.2 and 4 and* Leptosphaeria maculans develop *stem canker.*

Materials and methods

Seeds of fat hen (Expt. 1 - *Chenopodium peutablum* and *C. album*) were collected and germinated in the greenhouse. At 2-4 leaf stage, seedlings were planted into 6-celled punnet trays of coco-peat. Other weeds (Expt. 2-7 - Table 24) were collected from the field as seedlings, returned to the laboratory where they were washed and planted into MK16 pots. After 3 days 6 (Expt. 1 only) or 8 replicate plants were inoculated with 2 (Expt. 1 only) or 4 plugs of actively growing *Rhizoctonia* AG 2.1, 2.2, 4 or *L. maculans* isolates as previously described, using clean PDA as the control. Plants were maintained in the greenhouse at ~22-25^{0}C for up to 3 months.

Plants were inspected at 7-14 day intervals after inoculation to assess symptom expression, using the rating system of 0= healthy, 1= minor stem staining, 2=stem canker, 3=full stem canker, 4=wilt, 5=plant death. Where wilt and plant death occurred, weeds were deemed to be susceptible only where the number of inoculated plants affected were significantly greater that the un-inoculated plants.

Results

Fat hen and stinging nettle were the only plants that developed cankers after inoculation with *Rhizoctonia* (Table 24) and none developed canker when inoculated with *Leptosphaeria*.

Table 24. Common weeds in Brassica crops and susceptibility to stem canker caused by* Rhizoctonia solani *AG 2.1, AG 2.2, AG 4 or* Leptosphaeria maculans (L.m.)*. Symbol (√) indicates susceptibility.

Common weed	Scientific name	AG 2.1	AG 2.2	AG 4	L.m.
Broadleaf	*Fumaria parviflora*				
Fat hen	*Chenopodium album*		√		
Fat hen	*Chenopodium peutablum*	√	√	√	
Fennel	*Foeniculum vulgare*				
Marshmallow	*Malva parviflora*				
Hoary cress	*Cardaria draba*				
Rye grass	*Lolium multiflorum*				
Soursob	*Oxalis pes-caprae*				
Stinging nettle	*Urtica urens*		√	√	
Sow thistle	*Sonchus oleraceus*				
Sweet clover (King Island Melilot)	*Melilotus indicus*				
Wireweed (knotweed)	*Polygonum aviculare*				
Wild mustard	*Sinapis arvensis*				

Experiment 8.

Objective: *To evaluate the susceptibility of various weeds to Rhizoctonia AG 2.1, 2.2 and 4.*

Materials and Methods

Weeds (Table 25) germinated in soil collected from a cauliflower paddock at Virginia, SA and used in greenhouse experiments were washed and potted into coco peat in MK 12 pots. After 3 days 3 replicate plants were inoculated with 2 plugs of actively growing isolates of *Rhizoctonia* AG 2.1, 2.2 or 4 as previously described, using clean PDA as the control. Plants were maintained in the greenhouse at ~22-25^{O}C.

After 10 weeks, plants were removed, washed, any disease symptoms noted and roots plated from each treatment onto TWA. Stem sections from diseased plants were also plated onto TWA. The presence of *Rhizoctonia* on the plates was assessed after 4 days incubation at ~24^{O}C.

Results

Dark lesions developed on the stems of common purslane inoculated with *Rhizoctonia* AG 2.1 and 2.2 and white growth was observed on the wireweed stems inoculated with *Rhizoctonia* AG 2.1 (Table 25). *Rhizoctonia* was re-isolated from both weeds (Table 26).

Rhizoctonia was isolated from diseased roots of fat hen (AG 2.2) and paddymelon (AG 2.1) (Fig. 29) and from roots of fat hen (AG 2.1) and paddymelon (AG 4) with no observed rotting (Table 26).

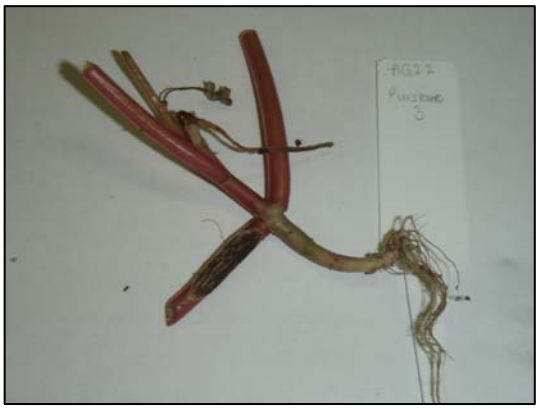
***Table 25. Symptoms developing on weeds artificially inoculated with* Rhizoctonia solani.**

	AG 2.1	AG 2.2	AG 4
Fat hen	nil	Main root slightly dark and degraded	-
Wireweed	Some white growth on stem	nil	-
Common purslane	(1) stem v. dark section almost rotted through. Roots damp & sloughing (2) lesion on one part of main root	Dark lesion on stem (stem resting on soil?)	-
Paddymelon	Root split & degraded	nil	nil
Grass	-	Very pink roots	-
Fat hen (other sp.)	-	-	Stems very red

Table 26.* Rhizoctonia *isolated from roots and stems of artificially inoculated weeds. √= Rhizoctonia *recovered, X = no* Rhizoctonia *recovered, - = plant material not isolated.

	AG 2.1		AG 2.2		AG 4	
	Stem	**Roots**	**Stem**	**Roots**	**Stem**	**roots**
Fat hen	-	√	-	√	-	-
Wireweed	√	X	-	X	-	-
Common purslane	√	X	√	X	-	-
Paddymelon	-	√	-	X	-	√
Grass	-	-	-	X	-	-
Fat hen (other sp.)	-	-	-	-	-	X

Paddymelon AG2.1: root split and degraded.



Purslane AG2.2: dark lesion on stem.

Figure 29. Symptomatic plants from which* Rhizoctonia *was isolated:

Discussion

These results show that several common weeds growing in land used for cauliflower production are susceptible to the fungus causing stem canker and can be non-symptomatic hosts. This highlights the importance of weed control in soil borne disease management. As alternative hosts of *Rhizoctonia,* the presence of weeds provides potential for carry over of the pathogen between crops and could contribute to the build up of inoculum.

4.2.9 Survival of pathogens in crop residues

After harvest, many growers incorporate crop residues by rotary hoe, cultivating once to depths of 200 mm and sometimes ripping to 600 mm prior to leaving fallow. As plant debris can be a source of inoculum, three experiments were set up to evaluate the survival of the pathogen on buried plant material.

Experiment 1-3

Objective: *To determine the effect of depth and time of burial on the survival of pathogens in crop residues.*

Materials and methods

Details for each of the three experiments are outlined in Table 27. Diseased cauliflower stems of at least level 3 (60%) canker severity were collected from the field, washed, the roots cut off and stems either left whole or cut into 6 or 8 sections to mimic chopping by rotary hoeing. Each stem was put into a small hessian bag 10 cm x 10 cm, labelled and the bag sealed. Replicate bags were either placed on the surface or buried at a depth of 10 or 30 cm. After 0 (initial levels) 1, 3, 6 or 12 months, ten replicate bags from each depth were dug up, washed and frozen. Once all bags had been collected, samples were freeze dried and analysed for levels of *Rhizoctonia* AG 2.1, 2.2, 4 or *Leptosphaeria maculans* by PCR analysis.

Table 27. Details of debris burial experiments.

Experiment	Location	Stem type		Reps	Sample time				
		Whole	Cut		0	1	3	6	12
1 – April 2007	Pt. Gawler		✓	10	✓	✓	*	-	-
2 – Oct. 2007	Pt. Gawler	✓	✓	5	✓	✓	✓	✓	✓
3 – Sept. 2007	St. Kilda	✓	✓	5	✓	✓	✓	✓	#

* The debris experiment was ploughed in by grower and bags destroyed.
# The property was sold and experiment abandoned.

Results

Experiment 1

This experiment was ploughed in at 3 months by the grower, allowing only the one month burial samples to be collected. No significant differences were observed in the levels in stems before or after burial for one month (Table 28). No *Rhizoctonia* AG2.2 was detected in the plant material before or after burial. The levels of *Rhizoctonia* and *Leptosphaeria* in the stems were extremely variable. For example one stem had *R. solani* AG 2.1 DNA of 5 pg/g dried plant material, whereas another had *R. solani* AG 2.1 DNA of 88,117 pg and AG 4 DNA of 34,433 pg/g dried plant material. Only 10 of the 30 stems assessed had all three pathogens (*R. solani* AG 2.1, 2.2 and *Leptosphaeria*).

Table 28. DNA in plant material after 1 month burial at 10 cm or 30 cm.

Burial details	**Mean DNA pg/g dried plant material (***range*)			
	Leptosphaeria maculans	***Rhizoctonia solani***		
		AG 2.1	**AG 2.2**	**AG4.**
Pre burial sample	**49,373** (*0-444,236*)	**24,886** (*0-142,306*)	0	**77,058** (*0-169,998*)
1 month burial at 10 cm	**10.3** (*0-46*)	**10,462** (*0-103,574*)	0	**8,449** (*0-40,469*)
1 month burial at 30 cm	**7.6** (*0-61*)	**13,887** (*0-88,117*)	0	**51,210** (*0-34,433)*

Experiment 2

Results of DNA in stem were variable, with no AG2.2 observed (Table 29). When all data were analysed together, significant differences ($P<0.05$) were observed with the levels of *Leptosphaeria* and *Rhizoctonia* AG4 but not with AG2.1 (Table 30). *Leptosphaeria* was significantly lower in stems after burial, with the highest DNA level being 2.5 $x10^6$ pg/g dried plant material in surface stems at three months, down to 44 pg/g dried plant material in stems buried at 10 cm for six months (Table 29). Combining the parameters of cut/whole stems showed that any burial of stems at or below 10 cm reduced the levels of *Leptosphaeria* DNA at 1 month to 12.7% compared to stems left on the surface and at six months this was reduced to 0.04% (Table 31).

Rhizoctonia levels decreased over time (Table 32), but not by depth of burial or cutting. However there was a trend overall for cut stems to have lower levels of DNA, with full stems having a mean level of DNA per gram of dried plant material of 24,596 pg AG 2.1 and 8,410 pg AG 4 compared to cut stems with 14,091 pg AG 2.1 and 4,528 pg AG 4. There was also a trend for stems left on the surface of the ground to have lower levels of *Rhizoctonia* AG 4 than buried stems, with 2,158 pg AG4 in unburied stems compared to 9,704 pg AG4 at 10 cm.

Table 29. DNA in cut or whole cauliflower stems after 1-12 months either on the surface of the soil or buried at 10 cm or 30 cm.

Burial details	Mean DNA pg/g dried plant material			
	Leptosphaeria maculans	*Rhizoctonia solani*		
		AG 2.1	AG 2.2	AG 4
1 month on surface – cut stem	1,755,810	35,395	0	0
1 month burial at 10 cm – cut stem	31,764	7	0	50,490
1 month burial at 30 cm – cut stem	385,154	34,453	0	117
1 month on surface – full stem	1,423,970	348	0	77,382
1 month burial at 10 cm – full stem	46,273	39,413	0	3,166
1 month burial at 30 cm – full stem	347,797	64,996	0	0
3 month on surface – cut stem	1,765,987	4,267	0	10
3 month burial at 10 cm – cut stem	2,720	88,283	0	34
3 month burial at 30 cm – cut stem	362	14	0	13,173
3 month on surface – full stem	2,497,111	16,874	0	0
3 month burial at 10 cm – full stem	914	2,376	0	4,999
3 month burial at 30 cm – full stem	380	16,201	0	3,495
6 month on surface – cut stem	239,380	245	0	17
6 month burial at 10 cm – cut stem	399	277	0	34
6 month burial at 30 cm – cut stem	69	2	0	60
6 month on surface – full stem	846,246	108,533	0	200
6 month burial at 10 cm – full stem	44	37,609	0	1,324
6 month burial at 30 cm – full stem	311	7,138	0	0
12 month on surface – cut stem	1,173,504	5,704	0	20
12 month burial at 10 cm – cut stem	134	389	0	229
12 month burial at 30 cm – cut stem	433	51	0	420
12 month on surface – full stem	1,396,071	14	0	0
12 month burial at 10 cm – full stem	641	1,193	0	82
12 month burial at 30 cm – full stem	408	457	0	0

Table 30. Significance of interactions between parameters of burial depth, time and whether stems were cut or whole.

Parameters	P value (ANOVA)		
	Leptosphaeria maculans	*Rhizoctonia solani*	
		AG 2.1	AG 4
Cut (cut stems or full stems)	0.396	0.435	0.403
Depth (0, 10 or 30 cm)	0.000	0.918	0.394
Time (1, 3, 6 or 12 months)	0.028	0.455	0.008
Cut * depth	0.468	0.786	0.036
Cut * time	0.797	0.306	0.834
Depth * time	0.025	0.557	0.105
Cut * depth * time	0.923	0.369	0.001

Table 31. Amount of* Leptosphaeria maculans *DNA in cauliflower stems after 1-12 months, and the percent variation on levels between stems from the surface of the soil or buried. Means with the same letter are not significantly different (P=0.05).

Time	Mean pg DNA *Leptosphaeria maculans* /g dried plant material			
	All stems	Surface	Buried	%variation by burial
1 month	665,128 A	1,589,890	202,747 A	12.8
3 months	711,246 A	2,131,549	1,094 B	0.05
6 months	181,075 B	542,813	206 B	0.04
12 months	392,265 AB	1,284,788	404 B	0.03

Table 32. Amount of* Rhizoctonia *DNA in cauliflower stems after 1-12 months. Means with the same letter are not significantly different (P=0.05).

Time	Mean pg *Rhizoctonia solani* AG 4 DNA/g dried plant material
1 month	21,859 A
3 months	3,036 B
6 months	855 B
12 months	125 B

Experiment 3

This experiment was only collected for six months due to the property being sold. *Leptosphaeria* was found in all plant stems and *Rhizoctonia* AG 2.1 in 75% (Table 33). *Rhizoctonia* AG2.2 was not present and AG 4 was found in only seven samples. The levels of DNA in the plant material were very again variable, with Rhizoctonia AG 2.1 ranging from 0 - 3,369,238 pg/g dried plant material and Leptosphaeria from 82 - 11,360,716 pg/g dried plant material. DNA levels of *Leptosphaeria* were significantly reduced with burial at either 10 cm or 30 cm (Table 34), but there was no significant effect over time or between whole or cut stems. While there were no significant differences in levels of *Rhizoctonia* DNA between any of the parameters, there was a trend for levels of AG 2.1 DNA to decrease over time, with 560 pg /g dried plant material at six months compared to 140,770 pg/g dried plant material at one month.

Table 33. DNA in cut or whole cauliflower stems after 1-6 months either on the surface of the soil or buried at 10 cm or 30 cm.

	Mean DNA pg/g dried plant material			
	Leptosphaeria maculans	***Rhizoctonia solani***		
Burial details		**AG 2.1**	**AG 2.2**	**AG 4**
Control plants pre burial - cut stem	337,180	280	0	0
Control plants pre burial - full stem	709,819	1603	0	0
1 month on surface – cut stem	573,889	46	0	0
1 month burial at 10 cm – cut stem	702,026	10	0	0
1 month burial at 30 cm – cut stem	956,636	740,770	0	0
1 month on surface – full stem	770,554	1,346	0	0
1 month burial at 10 cm – full stem	491,244	6,601	0	0
1 month burial at 30 cm – full stem	832,280	95,850	0	0
3 month on surface – cut stem	4,218,189	182,831	0	190,543
3 month burial at 10 cm – cut stem	66,272	57	0	219
3 month burial at 30 cm – cut stem	7,248	45,021	0	0
3 month on surface – full stem	3,644,489	3,086	0	14
3 month burial at 10 cm – full stem	3,116	4,279	0	0
3 month burial at 30 cm – full stem	2,272	3,090	0	0
6 month on surface – cut stem	3,378,504	3,298	0	0
6 month burial at 10 cm – cut stem	5,920	10	0	0
6 month burial at 30 cm – cut stem	266	1	0	0
6 month on surface – full stem	1,535,566	42	0	0
6 month burial at 10 cm – full stem	552	9	0	2,540
6 month burial at 30 cm – full stem	464	2	0	0

Table 34. Amount of* Leptosphaeria maculans *DNA in cauliflower stems after 1-6 months, and the percent variation on levels between stems from the surface of the soil or buried. Means with the same letter are not significantly different (P=0.05).

Time	Mean *Leptosphaeria maculans* DNA pg/g dried plant material			
	All stems	Surface	Buried	%variation by burial
1 month	721,105 A	672,221	745,547 A	110.9
3 months	1,323,598 A	3,931,339	19,727 B	0.50
6 months	820,212 A	2,457,035	1,800 B	0.07

Discussion

These results show that incorporation and burial of infected plant debris for at least 6 months reduced inoculum in plant material, particularly *Leptosphaeria*. However, unlike previous work with sugar beet (Herr 1976), burial did not increase the reduction in *Rhizoctonia* inoculum. Cutting the stems may aid in stem breakdown, however it did not increase the inoculum reduction over the 12 months of burial. It would be useful to evaluate whether additional treatments to increase break down the stems would also reduce the inoculum and what effect exposing the undecomposed plant residue by tilling soil would have on inoculum levels.

4.3 Management of Brassica stem canker

Management options evaluated included an understanding of the relative susceptibility of cauliflower cultivars and the efficacy of fungicides or alternative products at various application timings.

4.3.1 Cultivar susceptibility

Four greenhouse experiments were undertaken to evaluate susceptibility of the main commercial cultivars of cauliflower. Three different inoculation techniques were used to test susceptibility of the cultivars to *Leptosphaeria maculans* through stem, root or leaf infection, whereas only stem inoculation was used for *Rhizoctonia*.

Experiment 1

Objective: *To determine the susceptibility of eleven cauliflower cultivars to* Rhizoctonia solani *AG 2.1, 2.2 or 4.*

Materials and methods

Six week old seedlings of eleven cauliflower cultivars Appia, Atlantis, Chaser, Discovery, Donner, Elbert, Moby, Nautilus, Nova, Skywalker and Whistler were planted into MK 12 pots. One week later 12 replicate pots of each cultivar were stem inoculated as previously described with seven day old cultures of *R. solani* AG 2.1, 2.2 or 4. Plugs of PDA with no fungal growth were used as a control on another 12 replicated pots of each cultivar.

Plants were maintained in the greenhouse for eight weeks and assessed weekly for stem canker.

At eight weeks, soil was tested for presence of *R. solani* using the toothpick bait method as previously described.

Results and discussion

All eleven cultivars were susceptible to *Rhizoctonia,* the canker severity varying with different AG groups and cultivar (Fig. 30). Overall cv. Chaser and cv. Nova were the most susceptible and cv. Skywalker and cv. Atlantis the least susceptible to *Rhizoctonia* (Fig. 31).

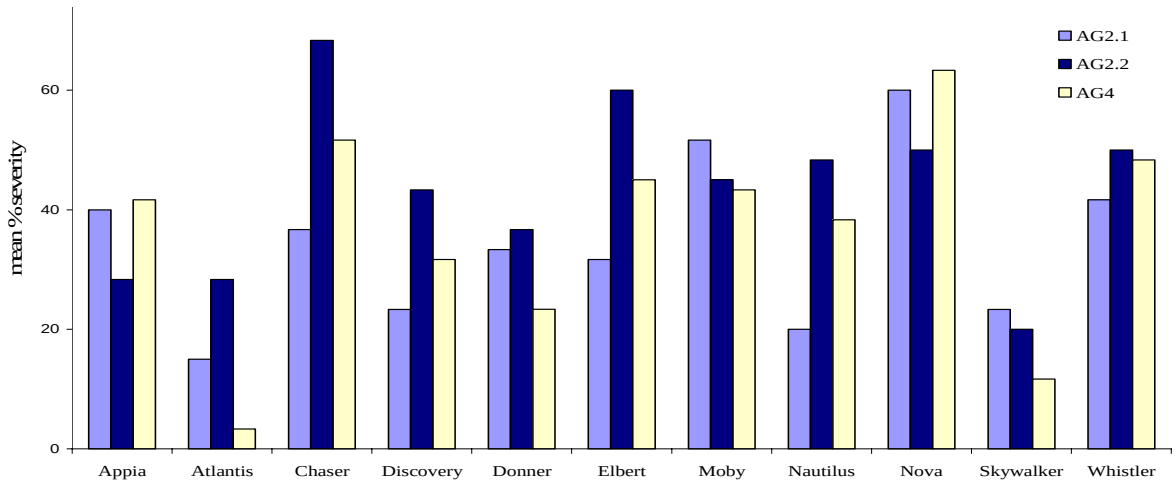


Figure 30. Severity of stem canker on 11 cultivars of potted cauliflower eight weeks after inoculation with* Rhizoctonia solani *AG 2.1, 2.2 or 4.

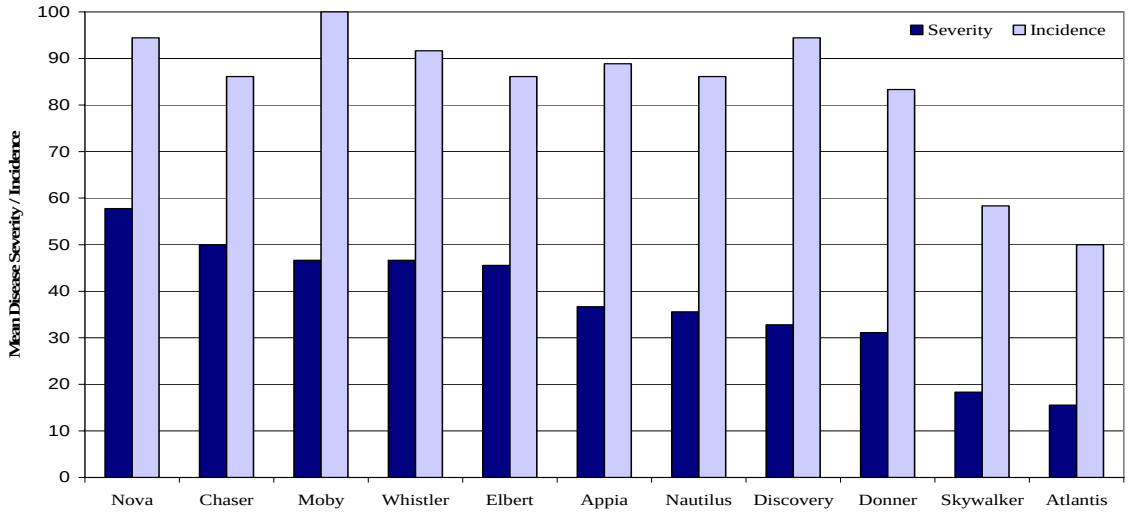


Figure 31. Incidence and severity of stem canker on cauliflower cultivars 8 weeks after inoculation. Data is the mean of results from inoculations with* Rhizoctonia solani *AG 2.1, 2.2, 4.

The toothpick baiting confirmed *Rhizoctonia* in soil of 100%, 85% and 90% of the pots inoculated with AG 2.1, 2.2 and 4 respectively. In the tests that showed cv. Skywalker and cv. Atlantis to be the least susceptible cultivars, soil in all pots were infected with *Rhizoctonia*. *Rhizoctonia* was not detected in soil in the control pots and no control plants developed symptoms.

Experiments 2-4

Objective: *To determine the relative susceptibility of cauliflower cultivars to* Leptosphaeria maculans.

Materials and methods

Experiment 2 – stem inoculation

Seven week old seedlings of ten cauliflower cultivars Appia, Atlantis, Chaser, Donner, Elbert, Moby, Nautilus, Nova, Skywalker and Whistler were planted into MK 12 pots. One week later ten replicate pots of each cultivar were stem inoculated with wounding as previously described with ten day old cultures of *L. maculans*. Plugs of PDA with no fungal growth were used as a control on another 10 replicated pots of each cultivar. Plants were maintained in the greenhouse for eight weeks and assessed weekly for stem canker.

Experiment 3 – foliar inoculation

Ten replicate pots of two week old seedlings of eleven cauliflower cultivars Appia, Atlantis, Chaser, Discovery, Donner, Elbert, Moby, Nautilus, Nova, Skywalker and Whistler were foliar inoculated as previously described. Plants were maintained in the greenhouse for three weeks and assessed weekly for stem canker.

Experiment 4 – root inoculation

Ten replicate pots of two week old seedlings of eleven cauliflower cultivars Appia, Atlantis, Chaser, Discovery, Donner, Elbert, Moby, Nautilus, Nova, Skywalker and Whistler were root inoculated as previously described and potted into MK9 pots, with ten replicate inoculated pots and ten control pots per cultivar. Plants were maintained in the greenhouse for eight weeks and assessed weekly for stem canker.

Results and discussion (Expt. 2-4)

Plants developed cankers in both the stem and root inoculation experiments (2 and 4), however only three plants in the foliar inoculation were affected (Expt. 3). One seedling cv. Discovery and one cv. Skywalker had severe narrowing of the stem, while stem rotting occurred on one seedling cv. Whistler. Although this technique has been used successfully on canola, the failure in these tests may be due to cauliflower leaves being much thicker than canola and having a waxy coating. Cauliflowers are therefore less likely to be infected through the leaf surface.

Therefore experiment 3 results were discarded and not included in the analysis.

All cultivars developed cankers, however the cankers were least severe on cultivars Elbert and Nautilus (Figs. 32, 33a, Table 35). Many growers consider cv. Chaser to be resistant to black leg, the disease caused by *L. maculans*, however this was not supported in these tests.

Disease symptoms were first observed 12 days after plants were stem inoculated (Expt. 2) and 18 days after root inoculation (data not presented). Disease levels were generally higher and more severe when plants were root inoculated (Table 35). There were some variations in results, with the root inoculation technique causing significantly more disease in some cultivars. For example cv. Moby had a mean canker severity of 26% when stem inoculated, but 96% with root inoculation. However the overall susceptibility of the cultivars was comparable with both techniques.

While 15% or less of the root and stem inoculated control plants developed superficial stem damage, there was no evidence of *L. maculans* infection by either isolation of infested stem tissue or microscopic examination for the presence of pycnidia.

Plants inoculated by the root dip technique often developed lesions at the leaf scars well above soil level and black vascular staining was observed. *L. maculans* was isolated from both these areas of affected tissue, indicating a systemic spread of the infection from the roots (Fig. 33b).

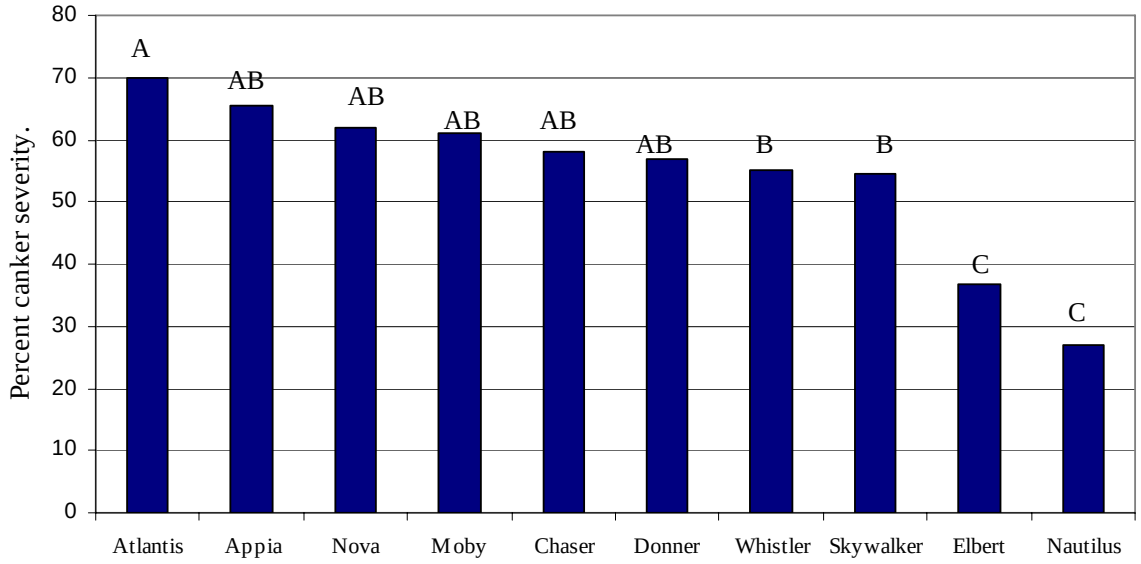


Figure 32. Severity of stem canker on cauliflower seedlings eight weeks after inoculation with* L maculans*. Combined results of stem and root inoculations. Varieties with the same letter are not significantly different (P=0.05).

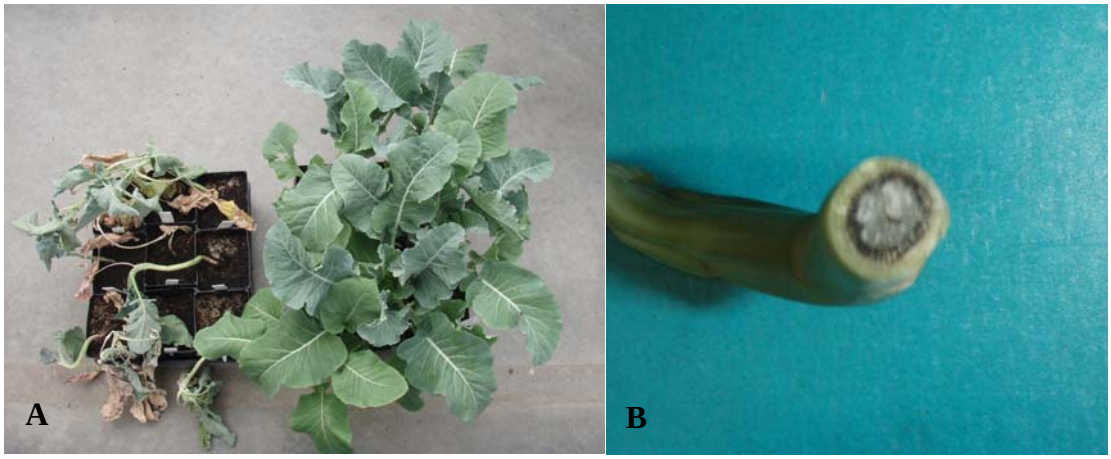


Figure 33 a, b. (A) cv. Appia (left) and cv. Nautilus (right) eight weeks after root inoculation with* L. maculans*. (B) Vascular staining from systemic infection by* L. maculans *eight weeks after root inoculation, cv. Nova.

Table 35. Severity of stem canker on cauliflower seedlings eight weeks after inoculation with* L maculans *by either stem or root inoculation technique. *Varieties with the same letter are not significantly different (P=0.05).

Cultivar	Stem inoculation		Root inoculation	
	Disease incidence	Mean percent canker severity*	Disease incidence	Mean percent canker severity*
Appia	78	33 ABCD	100	98 A
Atlantis	100	50 A	100	90 AB
Chaser	90	34 ABC	100	82 AB
Discovery	-	-	90	82 AB
Donner	60	38 ABC	100	76 B
Elbert	56	16 D	90	56 C
Moby	90	27 BCD	100	96 A
Nautilus	70	22 CD	80	32 D
Nova	80	44 AB	100	80 AB
Skywalker	80	28 BCD	100	90 AB
Whistler	90	30 BCD	100	80 AB

General discussion

These results confirm the variability in susceptibility to both *Rhizoctonia* and *Leptosphaeria* between the different cauliflower cultivars. This information is useful to growers in selecting cultivars to plant in areas of known infection or with a history of high disease.

4.3.2 Screening fungicides for *Rhizoctonia* – greenhouse experiments

Experiments were undertaken on potted greenhouse cauliflower seedlings to evaluate the efficacy of fungicides as potential controls for stem canker caused by *Rhizoctonia*. Many of the early screening experiments used cv. Skywalker, however this cultivar was found to be less susceptible (Section 4.3.1) so some experiments were repeated with a more susceptible cultivar.

Experiment 1-5

Objective: *To evaluate fungicide drenches applied after planting for the control of Rhizoctonia AG 2.1, 2.2 or 4 in cauliflower.*

Materials and methods

Cauliflower seedlings cv. Skywalker were germinated in nursery speedling trays and transferred into punnet trays (six cells per tray) after four weeks. Seedlings were stem inoculated at five weeks of age as previously described with 10-14 day old cultures of *Rhizoctonia* AG 2.1, AG 2.2 or AG 4. The same cultures were used for each experiment. After four days 100 ml of water or fungicide solution were applied to each seedling in two replicate punnet trays. Treatments are outlined in table 36.

In experiment 2, soil was inoculated by mixing ten mycelial plugs into the soil in each of 12 replicate MK 12 pots seven days prior to planting the five week old seedlings cv. Skywalker. Seedlings were drenched with 120 ml of water or fungicide solution on the day of planting.

Table 36. Fungicides and rates used in the five experiments.

Fungicide	Rate of active ingredient of fungicide used (ppm a.i.)				
	Expt. 1	Expt. 2	Expt. 3	Expt. 4	Expt. 5
Water	✓	✓	✓	✓	✓
Amistar 250 SC®	250	250	-	-	250
Cabrio®	-	-	-	500	250
Captan®	-	-	1000	-	-
Chlorothalonil 720®	1000	1000	-	-	-
Dividend®	500	500	-	-	-
Jockey Seed®	-	-	500	-	250
Maxim 100FS®	500	500	250	100	100
Monceren 125 DS®	-	-	-	500	250
Rizolex liquid®	-	-	-	500	250
Rovral Aquaflo®	500	500	-	-	-
Score®	-	-	-	500	250
Sumisclex 500®	-	-	-	500	250
Terraclor®	-	-	2000	-	750

All experiments included an untreated un-inoculated control treatment of 12 seedlings.

Plants were maintained in the greenhouse for six weeks and assessed weekly for canker using the 0-5 severity rating. Plant height from soil level was also measured in some experiments.

In experiments 3 and 4 the presence of *Rhizoctonia* in four pots selected at random from each treatment was assessed by baiting with toothpicks as previously described.

Results and discussion

Experiment 1

Cankers were first observed seven days after inoculation, with over 83% of the control plants infected within six weeks (Table 37). Maxim® and Amistar® were the most effective fungicides, showing no canker development on plants inoculated with AG 4 and AG 2.2 and only 17% on the AG 2.1 plants. The fungicides were not equally effective on the AG groups, for example Dividend® was effective on AG 4 but not AG 2.1 or 2.2 (Table 37, Fig. 34).

Table 37. Incidence and severity of cankers on cauliflower plants cv. Skywalker 6 weeks after inoculation with* Rhizoctonia solani *AG 4, AG 2.1 or AG 2.2 and drenched with various fungicides. Treatment severity means with the same letter are not significantly different (P=0.05).

Treatment	AG 4		AG 2.1		AG 2.2	
	Incidence	Severity	Incidence	Severity	Incidence	Severity
Untreated	92	82 a	100	97 a	83	78 a
Chlorothalonil®	58	50 b	92	77 b	8	8 cd
Rovral Aquaflo®	25	20 c	75	47 b	58	53 b
Dividend®	8	2 c	83	70 c	25	23 c
Maxim®	0	0 c	17	10 d	0	0 d
Amistar®	0	0 c	17	10 d	0	0 d

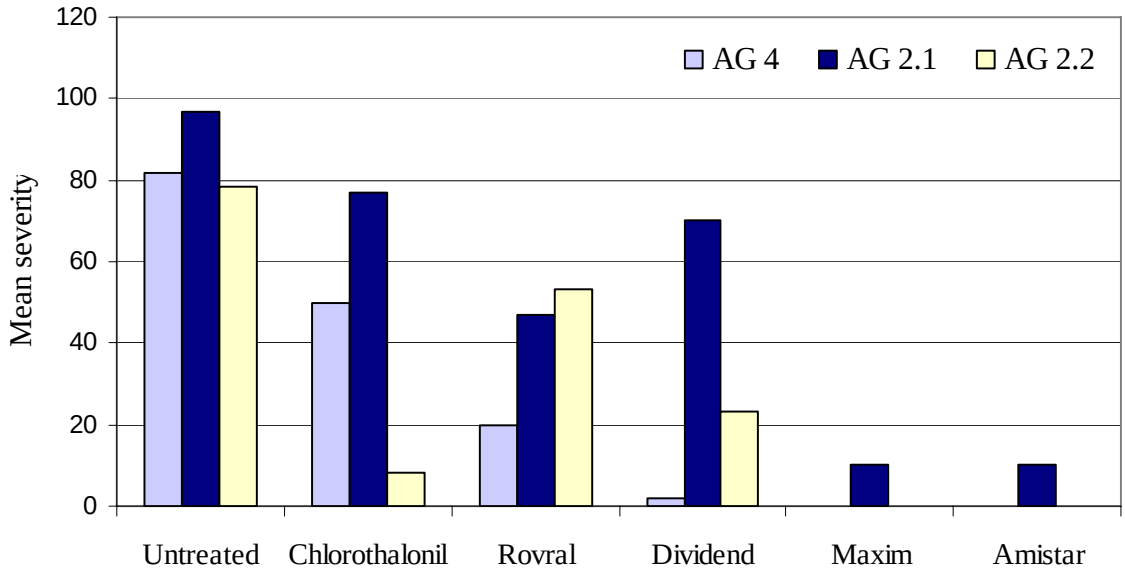


Figure 34. Mean severity of cankers on cauliflower plants cv. Skywalker 6 weeks after inoculation with* Rhizoctonia solani *AG 4, AG 2.1 or AG 2.2 and drenched with various fungicides.

Experiment 2

Cankers developed on the untreated plants inoculated with AG 2.1 and AG 4 within seven days of inoculation (data not presented) and by six weeks after treatment 92 % of the AG 2.1 and 50% of the AG 4 untreated plants were infected (Table 38). However no seedlings developed canker planted in soil inoculated with AG 2.2. While no testing of soil was undertaken to confirm the presence of *Rhizoctonia*, these results indicated that inoculating soil with mycelial plugs before planting was not as effective as inoculation on the soil surface. In this experiment plants treated with Dividend® were stunted with some leaf distortion, which was not observed in the previous experiment one where the same rate of application was used. Maxim® and Amistar® were again the most effective fungicides, preventing canker development on inoculated plants.

Table 38. Incidence and severity of cankers on cauliflower plants cv. Skywalker 6 weeks after inoculation with* Rhizoctonia solani *AG 4, AG 2.1 or AG 2.2 and drenched with various fungicides. Treatment severity means with the same letter are not significantly different (P=0.05).

Treatment	AG 4		AG 2.1		AG 2.2	
	Incidence	Severity	Incidence	Severity	Incidence	Severity
Untreated	50	50 a	92	83 a	0	0
Chlorothalonil®	0	0 b	33	20 c	0	0
Rovral Aquaflo®	0	0 b	50	50 b	0	0
Dividend®	0	0 b	0	0 d	0	0
Maxim®	0	0 b	0	0 d	0	0
Amistar®	0	0 b	0	0 d	0	0

Experiment 3

Cankers developed on the untreated plants within seven days of inoculation, but not on the Captan® treated plants until the following week (data not presented). Cankers developed on over 75% of the control plants by six weeks after inoculation (Table 39). Maxim® was the most effective fungicides, as no cankers developed on any plants. No cankers developed on AG 4 and AG 2.2 inoculated plants treated with Terraclor® and Jockey®, but these fungicides were less effective against AG 2.1, with cankers developing on 8.3% of the plants.

Table 39. Incidence and severity of cankers on cauliflower plants cv. Skywalker 6 weeks after inoculation with* Rhizoctonia solani *AG 4, AG 2.1 or AG 2.2 and drenched with various fungicides. Treatment severity means with the same letter are not significantly different (P=0.05).

Treatment	AG 4		AG 2.1		AG 2.2	
	Incidence	Severity	Incidence	Severity	Incidence	Severity
Untreated	83*	83 a	92*	63 a	75*	73 a
Captan®	8.3*	8.3 b	83*	47 a	8.3*	6.7 b
Jockey®	0*	0 b	8.3	1.7 b	0	0 b
Maxim®	0	0 b	0	0 b	0	0 b
Terraclor®	0	0 b	8.3	8.3 b	0	0 b

* treatments with positive toothpick bait (*Rhizoctonia* detected)

The toothpick baits confirmed *Rhizoctonia* in soil of untreated pots, pots treated with Captan® for all AG groups and pots inoculated with AG 4 and treated with Jockey (Table 39). These results indicate the other fungicides prevented infection from spreading from the mycelial plugs to the plants and soil.

Captan® was not effective as a soil drench for *Rhizoctonia*, as cankers developed on plants and *Rhizoctonia* was detected by toothpick bait in all AG groups.

Experiment 4

Cankers developed on the untreated plants inoculated with *Rhizoctonia* AG 2.1 within seven days of inoculation, but not on untreated plants inoculated with AG 4 until the third week (data not presented). Incidence of canker on the control plants by six weeks after inoculation with AG 2.1 was 92 % (Table 40). No plants inoculated with AG 2.2 developed cankers. Cankers developed on 8.3% of plants inoculated with AG 4 and treated with Sumisclex®.

The toothpick baits confirmed the presence of *Rhizoctonia* in soil in untreated pots, including AG 2.2 where no cankers developed (Table 40). It is possible that the cultures being used had lost some pathogenicity in storage.

The mean height of Sumisclex® treated plants was lowest in all AG groups and Rizolex® treated plants highest in AG 2.1 and AG 4 plants (data not presented). When the height data for all AG groups was combined, Sumisclex® treated plants were significantly shorter than all other treatments and Rizolex® tallest (Fig. 35). Moceren® treated plants were also significantly shorted than the untreated control.

Table 40. Incidence and severity of cankers on cauliflower plants cv. Skywalker 6 weeks after inoculation with* Rhizoctonia solani *AG 4, AG 2.1 or AG 2.2 and drenched with various fungicides.

Treatment	AG 4		AG 2.1		AG 2.2	
	Incidence	Severity	Incidence	Severity	Incidence	Severity
Untreated	17*	5	92*	92	0*	0
Cabrio®	0	0	0	0	0	0
Maxim®	0	0	0	0	0	0
Monceren®	0*	0	0	0	0	0
Rizolex®	0	0	0	0	0	0
Score®	0*	0	0*	0	0	0
Sumisclex®	8.3*	1.7	0	0	0	0

* treatments with positive toothpick bait (*Rhizoctonia* detected)

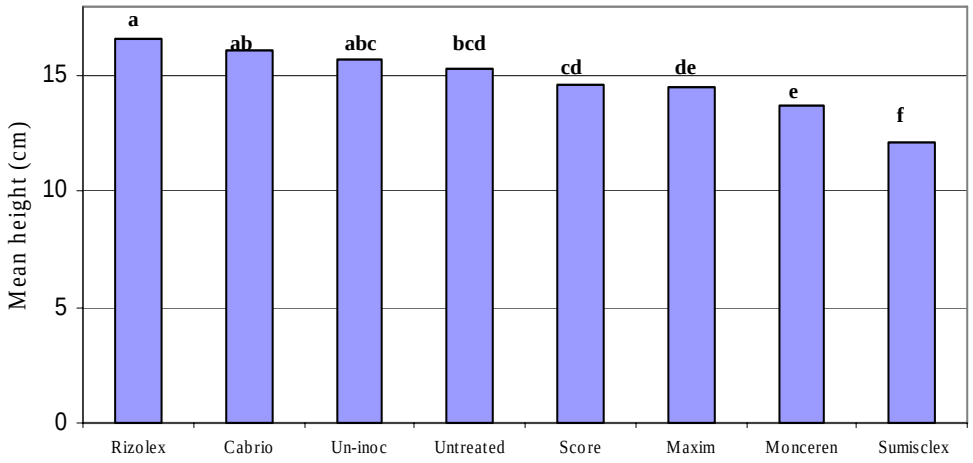


Figure 35. Mean height of cauliflower plants cv. Skywalker at six weeks after inoculation with* Rhizoctonia *and drenching with various fungicides. Treatments with the same letter are not significantly different (P=0.05).

Experiment 5

The untreated plants inoculated with AG 2.1 and AG 4 developed cankers within seven days of inoculation, but cankers were not observed on the AG 2.2 plants until the third assessment (data not presented). By six weeks after inoculation, 75% of untreated plants inoculated with *R. solani* AG 2.1 developed canker (Table 41), with 17% and 42% on AG 4 and AG 2.2 respectively. Plant treated with Maxim®, Rhizolex®, Sumisclex® and Cabrio® did not develop cankers.

Table 41. Incidence and severity of cankers on cauliflower plants cv. Skywalker 6 weeks after inoculation with* Rhizoctonia solani *AG 4, AG 2.1 or AG 2.2 and drenched with various fungicides. Treatment severity means with the same letter are not significantly different (P=0.05).

Treatment	AG 4		AG 2.1		AG 2.2	
	Incidence	Severity	Incidence	Severity	Incidence	Severity
Untreated	17	13 a	75	55 a	42	27 a
Jockey®	0	0 b	33	32 b	8.3	1.7 b
Terraclor®	0	0 b	8.3	8.3 c	0	0 b
Monceren®	0	0 b	8.3	8.3 c	0	0 b
Amistar®	0	0 b	8.3	1.7 c	8.3	5 b
Rizolex®	0	0 b	0	0 c	0	0 b
Cabrio®	0	0 b	0	0 c	0	0 b
Maxim®	0	0 b	0	0 c	0	0 b
Sumisclex®	0	0 b	0	0 c	0	0 b
Score®	0	0 b	8.3	1.7 c	0	0 b

Experiments 6, 7

Objective: *To evaluate fungicide drenches applied before and after planting for the control of Rhizoctonia AG 2.1, 2.2 or 4 in cauliflower.*

Materials and methods

Coco peat was inoculated with macerated *Rhizoctonia* plates as previously described and potted into six celled punnet trays after 18 days. Cauliflower seedlings cv. Skywalker (Expt. 6) or cv. Chaser (Expt. 7) were germinated in nursery speedling trays. Twelve replicate plants per treatment were pre-plant drenched as previously described. Another 12 replicate plants per treatment were planted into the infected soil and drenched with 60 ml of fungicide solution per plant. Treatments are outlined in table 42. All experiments also had an untreated un-inoculated control treatment of 12 seedlings.

Plants were maintained in the greenhouse and assessed every 2 weeks for canker.

Table 42. Fungicides and rates used in experiments 6 and 7.

Fungicide	**Rate of fungicide used (ppm a.i.)**	
	Expt. 6	**Expt. 7**
Water	✓	✓
Amistar 250 SC®	-	250
Cabrio®	100	-
Jockey Seed®	-	500
Maxim 100FS®	50	-
Monceren 125 DS®	250	-
Rizolex liquid®	100	-
Rovral Aquaflo®	-	1000
Score®	-	250
Sumisclex 500®	100	-
Terraclor®	500	-

- = no treatment

Results and discussion

Infection with *Rhizoctonia* AG 2.1 was not as severe in these experiments as the previous ones, with cankers developing on only 42% of plants in both experiments by six weeks after inoculation (Tables 43, 44). This may be due to the inoculation technique, with the macerated agar mixed with soil providing less inoculum than surface mycelial plugs. However, while AG 2.1 had consistently high canker incidence in experiments 1-5, incidence of infection for AG 2.2 and AG 4 was more variable. In experiment 6 and 7, the infection was more consistent and generally higher, with canker incidence of 42% and 67% in AG 2.2 and AG 4 respectively in experiment 7 (Table 44).

Generally the fungicides applied pre planting was not as effective as those applied post planting, although the difference was not significant. No cankers developed on plants treated with Jockey®, Score®, Cabrio® or Terraclor® after planting. None of the fungicides applied to speedlings prior to planting in infected soil prevented canker development, but

apart from Maxim® and Rovral Aquaflo®, all significantly reduced canker development compared to the untreated control.

Table 43. Incidence and severity of cankers on cauliflower plants cv. Skywalker 6 weeks after inoculation with* Rhizoctonia solani *AG 4, AG 2.1 or AG 2.2 and drenched with various fungicides prior to or after planting. Treatment severity means with the same letter are not significantly different (P=0.05). (Experiment 6).

Treatment		AG 4		AG 2.1		AG 2.2	
		Incidence	Severity	Incidence	Severity	Incidence	Severity
Untreated	pre*	25	5 ab	42	15 abc	17	15 a
	post#	25	10 a	42	22 a	17	13 a
Maxim®	pre	0	0 b	33	17 ab	0	0 b
	post	0	0 b	8.3	8.3 bcd	0	0 b
Rizolex®	pre	0	0 b	50	17 ab	0	0 b
	post	0	0 b	0	0 d	0	0 b
Cabrio®	pre	0	0 b	8.3	1.7 d	0	0 b
	post	0	0 b	0	0 d	0	0 b
Sumisclex®	pre	0	0 b	25	5 d	17	13 a
	post	8.3	1.7 b	8.3	5 d	0	0 b
Terraclor®	pre	0	0 b	17	3.3 cd	0	0 b
	post	0	0 b	0	0 d	0	0 b

* fungicide application by speedling drench pre planting into infected soil
# fungicide application by post planting soil drench

Table 44. Incidence and severity of cankers on cauliflower plants cv. Chaser 6 weeks after inoculation with* Rhizoctonia solani *AG 4, AG 2.1 or AG 2.2 and drenched with various fungicides prior to or after planting. Treatment severity means with the same letter are not significantly different (P=0.05). (Experiment 7).

Treatment		AG 4		AG 2.1		AG 2.2	
		Incidence	Severity	Incidence	Severity	Incidence	Severity
Untreated	pre*	50	43 a	42	23 ab	42	28 b
	post#	58	48 a	33	28 a	67	60 a
Amistar®	pre	8.3	5 b	0	0 c	17	10 cd
	post	0	0 b	0	0 c	0	0 d
Jockey®	pre	0	0 b	8.3	5 c	0	0 d
	post	0	0 b	0	0 c	0	0 d
Rovral Aquaflo®	pre	0	0 b	17	13 bc	33	27 bc
	post	0	0 b	17	10 bc	33	23 bc
Score®	pre	8.3	8.3 b	8.3	5 c	17	10 cd
	post	0	0 b	0	0 c	0	0 d

* fungicide application by speedling drench pre planting into infected soil
# fungicide application by post planting soil drench

Experiments 8, 9

Two experiments were undertaken using the most susceptible cultivar cv. Chaser. In the first experiment *Rhizoctonia* infection in the pots was variable and poor infection occurred in the untreated controls. Therefore the *Rhizoctonia* infection in the soil was tested in the second experiment before commencing fungicide treatments.

Objective: *To evaluate fungicide drenches applied after planting for the control of Rhizoctonia AG 2.1, 2.2 or 4 in cauliflower seedlings using a known susceptible cultivar.*

Experiment 8

Materials and methods

Cauliflower seedlings cv. Chaser were germinated in nursery speedling trays and transferred into MK 6 pots. Seedlings were stem inoculated at six weeks of age as previously described with 10 - 14 day old cultures of *Rhizoctonia* AG 2.1 or AG 2.2. After 4 days 40 ml of water or fungicide solution were applied to six replicated seedlings. Treatments and rates are shown in table 45 and included an untreated un-inoculated control treatment of 12 seedlings.

Plants were maintained in the greenhouse for seven weeks and assessed weekly for canker using the percent severity rating. The presence of *Rhizoctonia* was assessed at ten days after inoculation and again at seven weeks by baiting with toothpicks as previously described.

Results and discussion

Inoculation with *Rhizoctonia* AG 2.1 and 2.2 was inconsistent, with the severity of the untreated controls lower than those of some treatments (Table 45). The un-inoculated untreated control had no canker or *Rhizoctonia* detected (data not presented).

None of the fungicides prevented canker formation in AG 2.1 and only Rovral Aquaflo® at 100 ml/100L prevented cankers forming on plants infected with AG 2.2. The severity of canker development with AG 2.1 was lowest in plants treated with Amistar®, Cabrio®, Maxim®, or Rovral Aquaflo® at 50 ml/100L (Table 45).

Rhizoctonia was detected in soil from 100% of the untreated pots inoculated with AG 2.1 and 83% with AG 2.2 ten days after inoculation (Table 46), indicating the inoculation technique was not as effective with AG 2.2. No *Rhizoctonia* was detected in the soil from plants treated with Maxim® or Terraclor®, however cankers developed in plants of both treatments, with 100% infection with AG 2.1 occurring in the Terraclor® treatment. Either the *Rhizoctonia* had infected prior to the fungus being eradicated, or the toothpick bait was not effective. *Rhizoctonia* was detected at ten days after inoculation in soil from some of the pots treated with Amistar® or Sumisclex®, but not at seven weeks after inoculation. Cankers developed on plants in both treatments. There was no correlation between detection of *Rhizoctonia* in soil and the severity of canker.

Table 45. Incidence and severity of cankers on cauliflower plants cv. Chaser 6 weeks after inoculation with* Rhizoctonia solani *AG 2.1 or AG 2.2 and drenched with various fungicides. Treatment severity means with the same letter are not significantly different (P=0.05).

Treatment	Rate of product / 100L	AG 2.1		AG 2.2	
		Incidence	Severity	Incidence	Severity
Untreated	-	100	23 cde	17	6.7
Amistar 250 SC®	50 ml	50	10 e	50	10
Amistar 250 SC®	100 ml	33	10 e	33	6.7
Cabrio®	40 ml	50	20 de	67	20
Jockey Seed®	100 ml	83	30 bcde	50	10
Maxim 100FS®	40 ml	33	20 de	17	17
Rizolex liquid®	20 ml	83	50 abcd	67	27
Rizolex liquid®	40 ml	67	40 abcde	67	17
Rovral Aquaflo®	50 ml	67	13 e	33	13
Rovral Aquaflo®	100 ml	100	53 abc	0	0
Sumisclex®	75 ml	100	57 ab	100	30
Terraclor®	200 g	100	70 a	33	6.7

Table 46. Percent of pots with* Rhizoctonia *detected by toothpick bait ten days and seven weeks after inoculation with* Rhizoctonia solani *AG 2.1 or AG 2.2 and drenching with various fungicides.

Treatment	Rate of product / 100L	AG 2.1		AG 2.2	
		% toothpick infection			
		10 days	7 weeks	10 days	7 weeks
Untreated	-	100	83	83	17
Amistar 250 SC®	50 ml	100	0	50	0
Amistar 250 SC®	100 ml	33	0	33	0
Cabrio®	40 ml	83	17	67	0
Jockey Seed®	100 ml	83	100	0	0
Maxim 100FS®	40 ml	0	0	0	0
Rizolex liquid®	20 ml	100	100	33	33
Rizolex liquid®	40 ml	50	67	0	17
Rovral Aquaflo®	50 ml	100	100	17	50
Rovral Aquaflo®	100 ml	100	83	17	17
Sumisclex®	75 ml	83	0	17	0
Terraclor®	200 g	0	0	0	0

Experiment 9

Materials and methods

Coco peat in MK 12 pots was stem inoculated with 10 - 14 day old cultures of *Rhizoctonia* AG 2.1, AG 2.2 or AG 4 and left covered for one week in the greenhouse. Each pot was tested for the presence of *Rhizoctonia* using the toothpick baiting method as previously described.

As only low levels of infection were detected, the soil was removed from the pots, re-inoculated using mycelial slurry as previously described, and replaced into the same pots.

Cauliflower seedlings cv. Chaser were germinated in nursery speedling trays and at six weeks 30 replicate seedlings were pre-plant drenched with water or a fungicide solution. Treatments are outlined in table 47 and included an untreated un-inoculated control treatment of 10 seedlings. The treated seedlings were planted into the inoculated soil, ten replicate treated seedlings per AG inoculation.

Plants were maintained in the greenhouse for nine weeks and assessed weekly for canker using the percent severity rating.

The presence of *Rhizoctonia* was assessed at four, five and six weeks after drenching for the AG 2.1, 2.2 and 4 inoculated pots respectively, by baiting with toothpicks as previously described.

Results and discussion

Canker developed on all the AG 2.1 untreated controls (Table 47), however lower levels developed on both AG 2.2 (70%) and AG 4 (90%). None of the fungicides inhibited canker development, however all fungicides but Rizolex® reduced the severity of cankers caused by AG 2.1 compared to the untreated control. In AG 4 inoculated soil, all fungicides except Rizolex® and Rovral® reduced the severity of cankers compared to the untreated control. The AG groups were not equally suppressed by the fungicides, for example Rizolex ® applied at 40 ml/100L effectively reduced the incidence and severity of cankers that developed on plants grown in AG 2.2 inoculated soil, but not in the other two AG groups (Fig. 36).

The un-inoculated untreated control had no canker or *Rhizoctonia* detected (data not presented).

None of the fungicides eradicated *Rhizoctonia* as the toothpick bait detected the fungus in soil of at least 80% of AG 2.1 pots and 90% of AG 4 (Table 48). *Rhizoctonia* AG 2.2 was detected in 10% of soil from the untreated pots and between 10% and 40% in those treated with fungicides. These results show that the bulk soil inoculation technique is more effective that the stem inoculation in obtaining an even infection of *Rhizoctonia* in all pots.

Table 47. Incidence and severity of cankers on cauliflower plants cv. Chaser 6 weeks after inoculation with* Rhizoctonia solani *AG 2.1 or AG 2.2 and drenched with various fungicides. Treatment severity means with the same letter are not significantly different (P=0.05).

Treatment	Rate of product / 100L	AG 2.1		AG 2.2		AG 4	
		Incidence	Severity	Incidence	Severity	Incidence	Severity
Untreated	-	100	74 ab	70	24 a	90	46 b
Amistar 250 SC®	50 ml	90	20 ef	20	4 bc	50	10 e
Amistar 250 SC®	100 ml	80	16 ef	50	10 bc	70	14 e
Cabrio®	40 ml	90	16 ef	60	12 abc	80	22 de
Jockey Seed®	100 ml	40	10 f	30	6 bc	100	26 cde
Maxim 100FS®	40 ml	80	16 ef	30	6 bc	50	10 e
Rizolex liquid®	20 ml	70	78 a	50	14 abc	100	42 bc
Rizolex liquid®	40 ml	100	62 bc	10	2 c	100	82 a
Rovral Aquaflo®	50 ml	100	36 d	30	6 bc	90	36 bcd
Rovral Aquaflo®	100 ml	70	30 de	30	6 bc	100	34 bcd
Sumisclex®	75 ml	80	18 ef	70	12 abc	70	18 de
Terraclor®	200 g	100	52 c	60	16 abc	70	24 cde

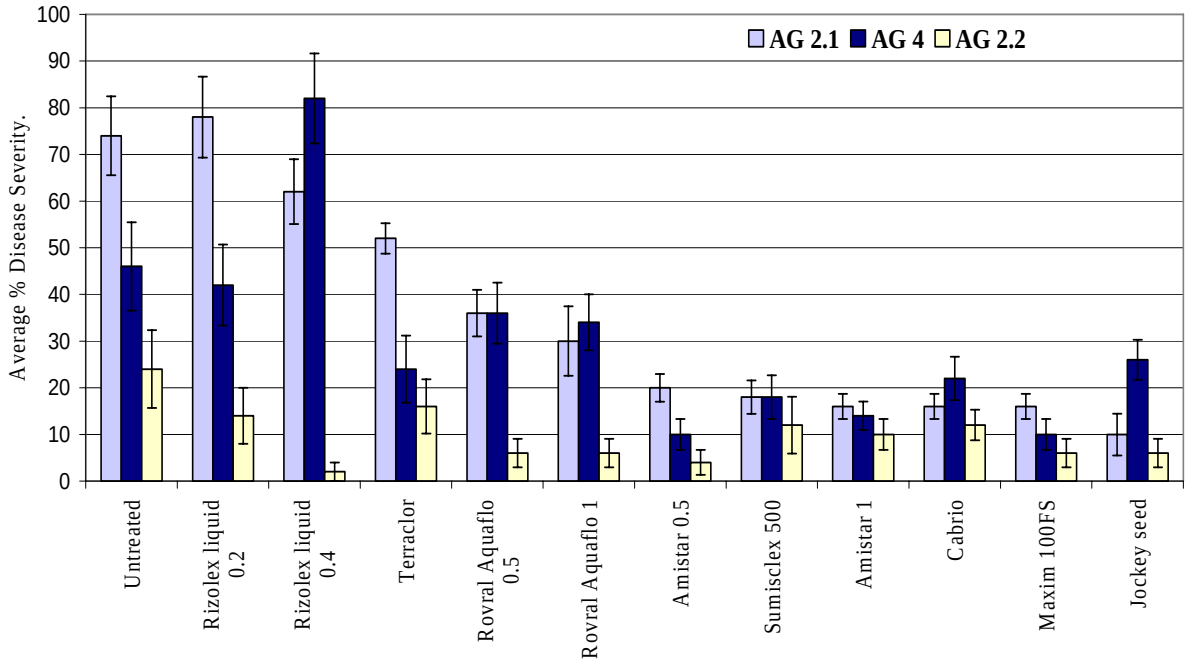


Figure 36. Severity of cankers on cauliflower cv. Chaser drenched with various fungicides before being planted in soil inoculated with* Rhizoctonia solani *AG 2.1, 2.2 or 4.

Table 48. Percent of pots with Rhizoctonia detected by toothpick bait after inoculation with Rhizoctonia solani AG 2.1, AG 2.2 or AG 4 and drenching with various fungicides. AG 2.1 was tested four weeks, AG 2.2 five weeks and AG 4 six weeks after inoculation.

Treatment	Rate of product / 100L	% toothpick infection		
		AG 2.1	AG 2.2	AG 4
Untreated	-	100	10	100
Amistar 250 SC®	50 ml	80	40	90
Amistar 250 SC®	100 ml	70	10	100
Cabrio®	40 ml	90	20	100
Jockey Seed®	100 ml	90	20	100
Maxim 100FS®	40 ml	100	10	100
Rizolex liquid®	20 ml	100	10	100
Rizolex liquid®	40 ml	100	40	100
Rovral Aquaflo®	50 ml	100	20	100
Rovral Aquaflo®	100 ml	100	20	100
Sumisclex®	75 ml	100	20	90
Terraclor®	200 g	100	20	100

Experiment 10, 11

A fungicide screening experiment was undertaken in the greenhouse using field soil. Initial tests were undertaken to determine whether plants would be infected in field soil in a greenhouse

Objective: *To evaluate fungicide drenches applied after planting for the control of stem canker in cauliflower planted in field soil.*

Experiment 10

Materials and methods

Soil (clay-loam) was collected from three properties in the Northern Adelaide plains, ~50 km North of Adelaide, from areas known to have crops with stem canker. Site 1 was a fallow area 3 months after the remains of an infected crop was rotary hoed. Site 2 was a fallow area 1 month after the remains of an infected crop was rotary hoed. Site 3 was a potato crop planted nearby, paddock history unknown.

Half of all soil was steam pasteurised at the Plant Research Centre and allowed to cool. Soil from each site was tested for *Rhizoctonia* using the toothpick bait method as previously described. Soil was placed into MK12 pots and planted with four week old seedlings cv. Skywalker. Plants were maintained in the greenhouse for eight weeks and assessed twice for canker.

Results and discussion

Toothpick baits detected *Rhizoctonia* in soil from site 1 and 2, but not from site 3 (potato field). *Rhizoctonia* was not detected in any of the pasteurised soil.

All plants grown in the pasteurised soil remained healthy. Plant growing in the non pasteurised soil from site 2 and 3 were stunted and showed wirestem symptoms typical of *Rhizoctonia* infection. Plants growing in the non-pasteurised soil from site 1 remained healthy.

This indicated that some field soil could be successfully used in greenhouse trials.

Experiment 11

Materials and methods

Soil was collected from an area known to be infected with stem canker near site 2 (Expt. 10) and a 200g sub-sample tested for *Leptosphaeria* and *R. solani* by PCR.

Soil was placed into five MK 12 pots and five 150 cm pots (~1.9L). Five replicate four week old seedlings cv. Nautilus and five replicate cauliflower seedlings (cultivar unknown and age ~six weeks) were drenched with fungicide solutions as previously described at the rates listed in Table 49. Cauliflower seedlings cv. Nautilus were planted in the MK12 pots and the unknown cultivar in the six inch pots, maintained in the greenhouse for twelve weeks and assessed at 2, 3 and 4 weeks after planting for canker.

At 12 weeks after planting, three pots selected at random from each treatment were tested for the presence of *Rhizoctonia* by toothpick bait assay, the plants harvested and washed and fresh weights of stem and leaf measured.

Results and discussion

Rhizoctonia AG 4 DNA of 146 pg/g and *Leptosphaeria* DNA of 3 pg/g were detected in the field soil. No *Rhizoctonia* AG 2.1 or 2.2 was detected.

Rhizoctonia was detected by toothpick bait in soil from three untreated pots, three pots treated with Rizolex® and one pot in each of the Maxim® and Rovral Aquaflo® treatments (Table 49). No *Rhizoctonia* was detected in the soil of pots planted with seedlings drenched with Amistar®.

Only 40% of the seedlings developed cankers (Table 49). While *Rhizoctonia* was detected in soil of the untreated plants, the level in the original field soil was not high. There was no correlation between detection of *Rhizoctonia* in the soil and canker development. For example, no cankers developed in seedlings treated with either Amistar® or Rizolex®, however *Rhizoctonia* was detected in soil from the Rizolex® treatment but not the Amistar® treatment.

The fresh weight of untreated plants was significantly lower than those treated with all fungicides except Rizolex® (Table 49). This may be a result of the fungicides controlling root infection of the *Rhizoctonia*.

Table 49. Incidence of canker at 4 weeks after planting, presence of* Rhizoctonia *and mean fresh weight of cauliflower leaves and stems at 12 weeks after planting cauliflower seedlings drenched with various fungicides in field soil. Means with the same letter are not significantly different (P=0.05).

Treatment	**Rate of product applied***	**No of pots out of three with *Rhizoctonia* detected.**	**Incidence of plants with canker at 4 weeks after planting**	**Mean fresh weight of stems and leaves**
Control	Water	3	40%	29.53 b
Amistar 250 SC®	1.25L/100L	0	0	67.83 a
Maxim 100FS®	93.6 ml/100L	1	10%	51.83 a
Rizolex liquid®	150 ml/100L	3	0	47.13 ab
Rovral Aquaflo®	250 ml/100L	1	10%	58.23 a

* calculated from the label rate to a per plant rate. Each speedling took up 40 ml of water, so the fungicide was added to provide the in field per plant amount in 40 ml.

4.3.3 Screening fungicides for *Leptosphaeria* – greenhouse experiments

Experiment 1

Objective: *To evaluate fungicide drenches applied before and after planting for the control of* Leptosphaeria maculans *in cauliflower seedlings.*

Materials and methods

Cauliflower seedlings cv. Chaser were germinated in nursery speedling trays and transferred into MK 12 pots. Seedlings were stem inoculated with stem wounding at four weeks of age as previously described with 21 day old cultures of *Leptosphaeria maculans*.

Two days before inoculation, six replicate pots were drenched with 50 ml of water or each fungicide solution as a pre-infection treatment. Two days after inoculation, another six replicate pots were drenched with 50 ml of water or each fungicide solution as a post-infection treatment. Treatments and rates are shown in table 50 and included an untreated un-inoculated control treatment of 12 seedlings.

Plants were maintained in the greenhouse for twelve weeks and assessed weekly for canker using the percent severity rating.

Results and discussion

The first cankers were observed on untreated plants within three weeks after planting, however cankers were not observed on many of the treated plants until six to eight weeks after inoculation (Fig. 37) compared to 1-2 weeks with *Rhizoctonia*. Cankers developed earlier in the treatments where fungicides were applied after inoculation.

All pre-inoculation fungicides drenches significantly reduced the canker severity compared to the control (Table 50, Fig. 37). The most effective treatment was Amistar® applied at 100 ml/100L before inoculation, with no cankers detected. Both rates of Amistar®, Cabrio ® and the higher rate of Rovral Aquaflo® when applied before inoculation provided acceptable control, reduced the cankers to less than 10% severity. The control of the post inoculation treatments did not produce a high canker severity (27%) even with 100% of the seedlings infected, therefore none of the treatments improved the canker severity compared to the control. Sumisclex® was the least effective fungicide, as plants drenched after inoculation had a significantly higher canker severity (67%) than the control and plants drenched prior to inoculation had the highest canker severity (40%) of all the treatments.

When the severity data for all treatments was combined, the mean canker severity of the pre-inoculation treatments at 16.4 was significantly (P=0.001) lower than the mean canker severity of the post-inoculation treatments at 30.0.

The un-inoculated, untreated plants all developed cankers, with a mean severity rating of 33% (Fig. 38). The cankers were first observed at eight weeks, similar to other treatments but late than the untreated controls, indicating it was more likely to occur from disease spread by water splash or soil movement than by accidental inoculation.

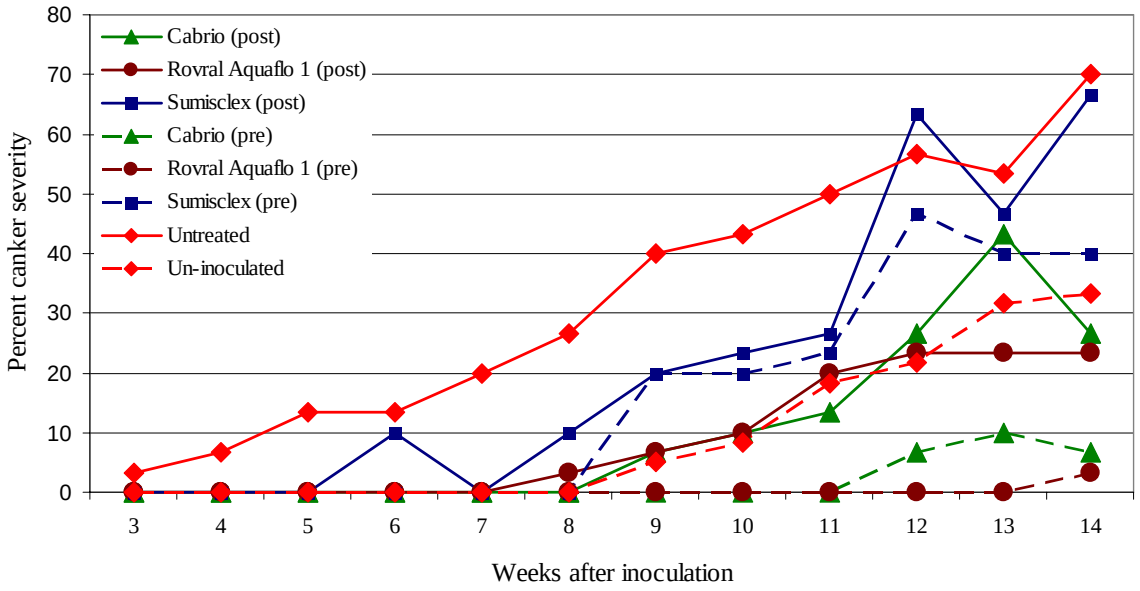


Figure 37. Progression of canker severity over time on cauliflower plants cv. Chaser after inoculation with* Leptosphaeria maculans *and drenched with fungicides either 2 days before inoculation (pre) or two days after (post). Treatment severity means with the same letter are not significantly different (P=0.05).

Table 50 Incidence and severity of cankers on cauliflower plants cv. Chaser 14 weeks after planting with* Leptosphaeria maculans *and drenched with various fungicides. Treatment severity means with the same letter are not significantly different (P=0.05).

Treatment	Rate of product / 100L	Pre inoculation drench*		Post inoculation drench*	
		Incidence	Severity	Incidence	Severity
Untreated	-	100	70 a	100	27 bc
Amistar 250 SC®	50 ml	50	13 cde	67	23 c
Amistar 250 SC®	100 ml	0	0 e	67	20 c
Cabrio®	40 ml	33	6.7 de	100	27 bc
Jockey Seed®	100 ml	83	27 bc	100	23 c
Maxim 100FS®	40 ml	33	6.7 de	100	20 c
Rizolex liquid®	20 ml	67	17 cde	83	27 bc
Rizolex liquid®	40 ml	83	30 bc	100	27 bc
Rovral Aquaflo®	50 ml	83	17 cde	100	43 b
Rovral Aquaflo®	100 ml	17	3.3 de	100	23 c
Sumisclex®	75 ml	100	40 b	100	67 a
Terraclor®	200 g	100	20 cd	100	30 bc

* Pre inoculation drench applied two days before inoculation and post inoculation drench applied two days after inoculation

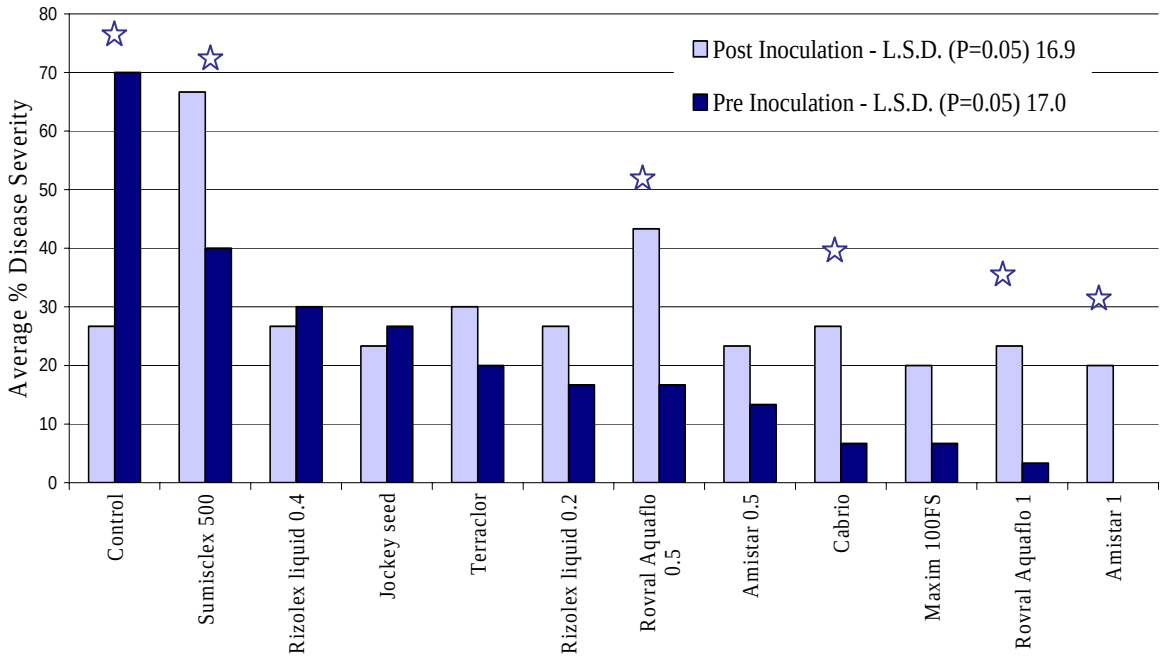


Figure 38. Incidence and severity of cankers on cauliflower plants cv. Chaser 14 weeks after planting with* Leptosphaeria maculans *and drenched with various fungicides. Fungicide treatments with a star indicate the mean severities of the post inoculation and pre inoculation treatments for that fungicide are significantly different (P<=0.05).

4.3.4 Summary – greenhouse fungicide screening

Overall these results show that none of the fungicides evaluated were effective in controlling both *Rhizoctonia* and *Leptosphaeria* (Fig. 39). However the strobilurin fungicides Cabrio® and Amistar® were the most inhibitory when all data were combined, suppressing the canker severity to below 20% of the untreated control. This may be enough suppression to allow the plants to mature to a harvestable crop.

The combined data showed that fungicides applied as post planting drenches were more effective against *Rhizoctonia* than as pre-planting drenches. None of the pre-plant drenches effectively eradicated cankers caused by *Rhizoctonia*, however Amistar®, Cabrio® and Score® reduced the severity of cankers to under 50% of the untreated controls (Fig. 40). A wider range of fungicides were effective as post planting drenches against *Rhizoctonia*, with all but Captan® and Rovral® reducing the canker severity to less than 50% of the untreated controls (Fig. 41). Eight of the eleven in this group reduced canker severity to less than 10% of the untreated control.

The application of pre-planting drenches to speedlings in the nursery is widely used in the industry, with the usual choice being Rovral®. Alternatives for that purpose need to be evaluated, as while this application method was less effective for *Rhizoctonia* it was more effective for *Leptosphaeria* (Section 4.3.3).

The results of these experiments also show the benefit of using a less susceptible cultivar, as all the experiments using cv. Skywalker had lower levels of canker severity than the later experiments with cv. Chaser. Using less susceptible cultivars may also provide higher levels of suppression by the fungicides.

This summary of combined results provides an overview of fungicides suitable for further screening in field experiments. However the choice of fungicides available for use on Brassica is restricted and for field evaluations preference would be for fungicides already registered or permitted for use, or fungicides that the manufacturers would be willing to allow a permit for this use to be developed.

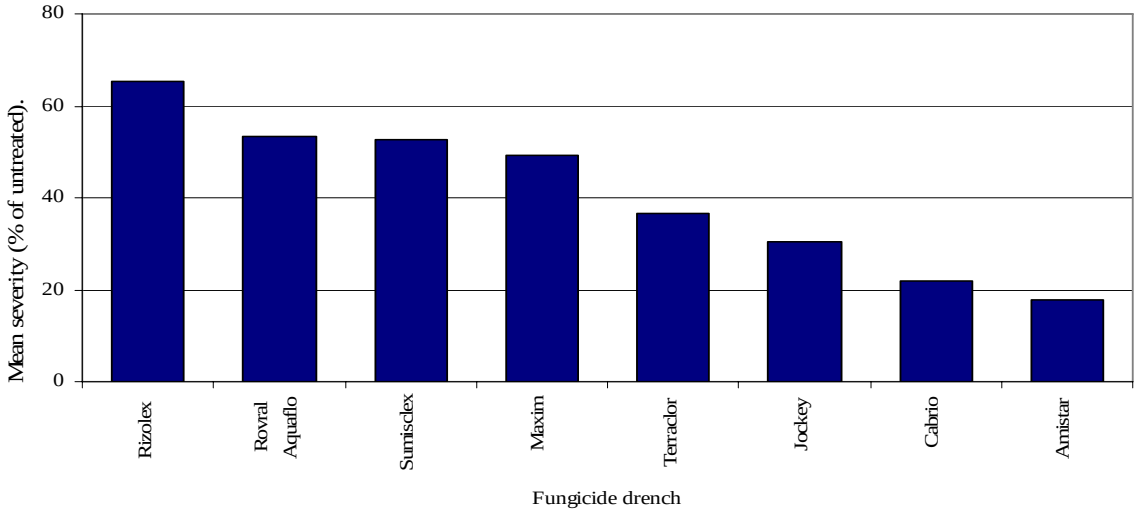


Figure 39. The combined mean severity of canker on cauliflower planting into soil inoculated with* Rhizoctonia solani *AG 2.1 or* Leptosphaeria maculans*, with fungicides applied as a pre-infection or post-infection drench. The results of each trial were calculated as a percent of the untreated control and averaged.

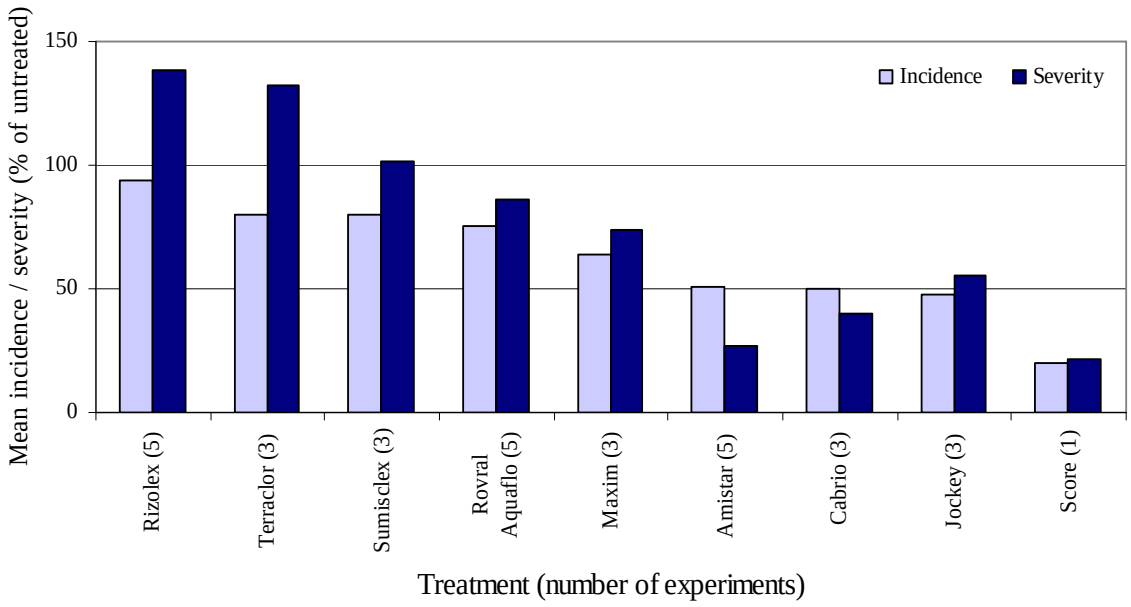


Figure 40. The mean incidence and severity of canker on cauliflower with fungicides applied as a pre-planting drench before planting into soil inoculated with* Rhizoctonia solani *AG 2.1. The results of each trial were calculated as a percent of the untreated control and averaged over the number of experiments, listed in brackets after the treatment name.

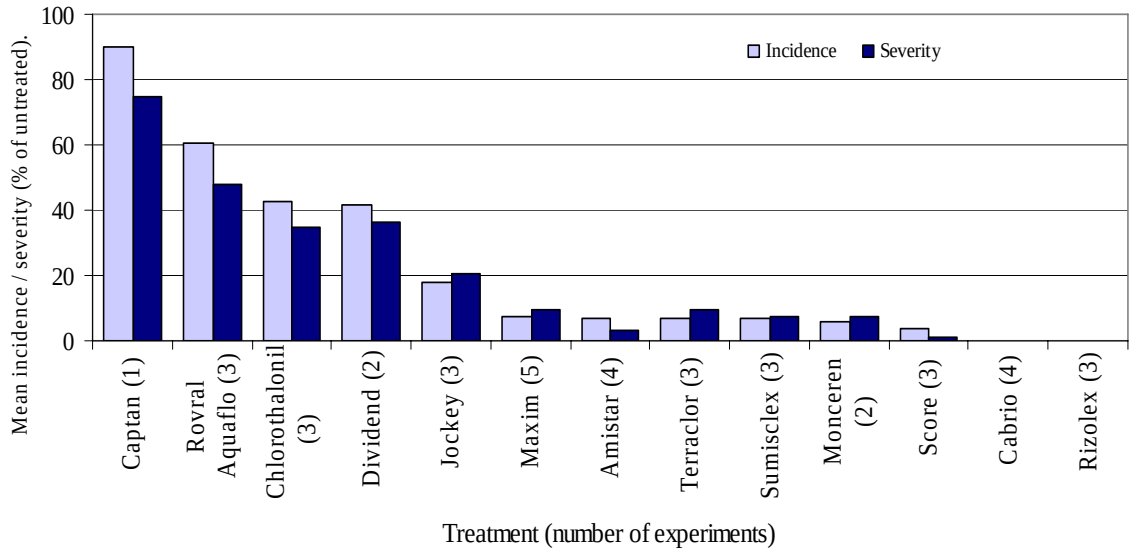


Figure 41. The mean incidence and severity of canker on cauliflower planted into soil inoculated with* Rhizoctonia solani *AG 2.1 and drenched with fungicides after planting. The results of each trial were calculated as a percent of the untreated control and averaged over the number of experiments, listed in brackets after the treatment name.

4.3.5 Screening alternative products – greenhouse experiments

Objective: *To evaluate alternative products applied before and after planting for the control of stem canker in cauliflower.*

Materials and methods

Sterilised coco-peat was inoculated with macerated mycelial suspensions of *Rhizoctonia* (AG 2.1, 2.2 and 4 combined) or *Leptosphaeria maculans* as previously described and after twelve or seven days respectively transferred to 175 mm round pots (~2L volume).

Cauliflower seedlings cv. Chaser were germinated in nursery speedling trays. Ten replicate nine week old seedlings were placed for one to five minutes in either water or one of three biological treatment solutions until the soil was saturated and planted into each of the inoculated soils. Another ten replicate seedlings were planted into each of the inoculated soils and the pot drenched with 500 ml of water or biological treatment solution. Trichoshield™ and Seasol® were applied as per label recommendations after planting. A 5 cm layer of Nitra Mulch® was placed on the inoculated soil prior to planting. Treatment details are outlined in Table 51.

Table 51. Products used, application methods and rates.

Treatment	**Rate and application**	
	Pre plant treatment	**Post plant treatment**
Water	✓	✓
Becker Underwood Experimental	5ml in 5L water, soak speedlings for 5 minutes	40ml in 20L, 500ml solution per pot
Becker Underwood Experimental – combination of two products	5ml of each product combined in 5 L water, soak speedlings for 5 minutes	40ml of each product combined in 20L water, 500ml solution per pot
Trichoshield™	Pre water pots, soak speedlings for 1 minute pre-planting in 5g product/L. At 4 weeks after planting apply 500ml per pot of 0.1g/10 L.	
Seasol®	500ml of 5ml/L per pot at planting and every 2 weeks.	
Nitra Mulch®	5cm layer on pots prior to planting. Watered in with 500 ml water.	

Two pots from each treatment and four pots from the untreated controls were tested for the presence of *Rhizoctonia* three weeks after planting using the toothpick bait method as previously described.

Plants were maintained in the greenhouse for ten weeks and assessed weekly for canker using the percent severity rating. Plants were harvested at 10 weeks and a final assessment on washed plants included canker severity and the presence of adventitious roots above the canker.

A high population of fungus gnats occurred in pots with Nitra Mulch®, which were treated with a combination of insecticide (Confidor®) and commercially available biological predators (*Aphidius* and *Hypoaspis*)

Results and discussion

Rhizoctonia was detected in all the pots inoculated with *Rhizoctonia*, confirming that the inoculation was successful and that none of the treatments eradicated the pathogen from soil. There was no evidence of cross contamination between pots as no *Rhizoctonia* was detected in the soil inoculated with *Leptosphaeria*.

Cankers were first detected on plants one week after planting in *Rhizoctonia* inoculated soil (data not presented). Several of the seedlings had died within two weeks after planting and *Rhizoctonia* was confirmed by isolation from the dead tissue (Table 52).

Table 52. Death of cauliflower cv. Chaser planted in Rhizoctonia inoculated soil (combination of AG 2.1, 2.2 and 4) with various biological treatments.

Treatment	Pre plant speedling drench		Post plant pot drench	
	No. plants dead	**No. confirmed *Rhizoctonia***	**No. plants dead**	**No. confirmed *Rhizoctonia***
Water	2	2	0	0
Becker Underwood experimental	1	1	5	5
Becker Underwood experimental combination	2	2	3	3
Trichoshield™	-	-	1	1
Seasol®	-	-	0	0
Nitra Mulch®	-	-	6	4

Cankers developed rapidly in the seedlings planted into *Rhizoctonia* inoculated soil (Fig. 43), with all treatments having a level of canker detected by 12 days after planting. The highest level of canker was observed in plants treated with Nitra Mulch®, developing 95% severity on all plants by 57 days after planting (Fig. 43, Table 53). Both experimental products applied post-planting were also worse than the untreated control, however unlike the Nitra Mulch® the differences were not statistically significant. The experimental products applied pre-planting were the most effective.

Cankers developed more slowly in seedlings planted into the soil inoculated with *Leptosphaeria* (Fig. 44) and the severity lower (<27%) than the severity in the *Rhizoctonia* infected plants. The plants grown in the Nitra Mulch® treated soil had variable canker severity, as there was significant staining on the lower stems of the plant which did not develop and were possibly a phytotoxic reaction to the treatment. Seasol had the lowest canker severity, but the differences in the means were not statistically significant.

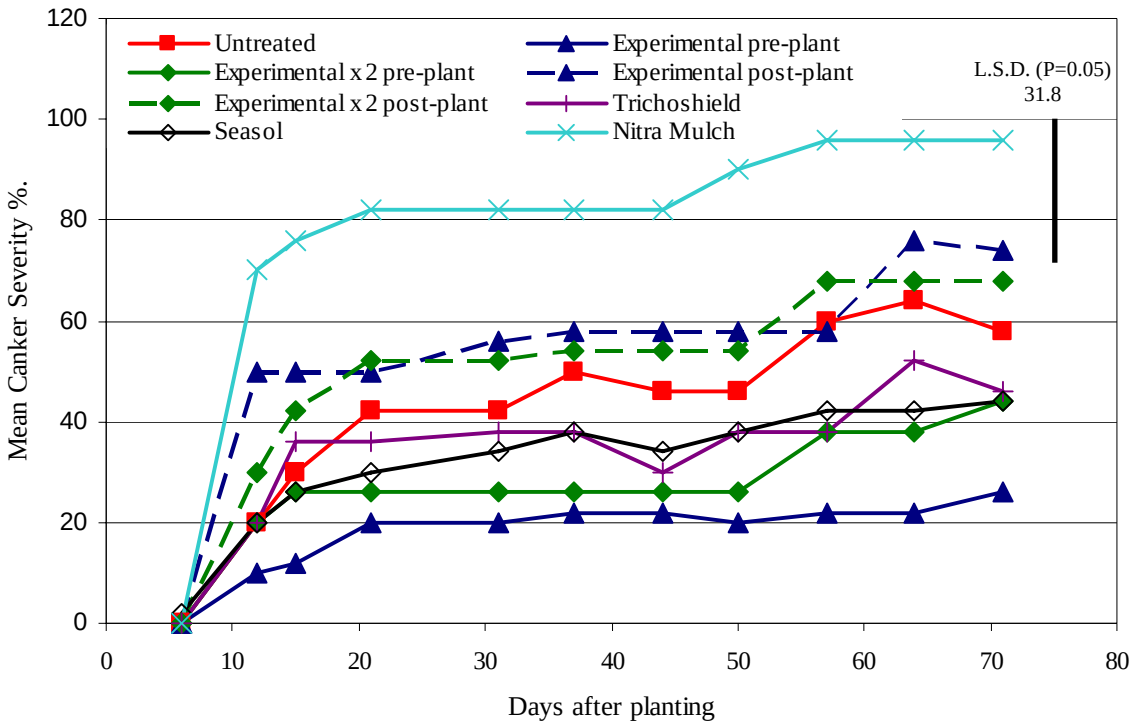


Figure 43. Canker development on cauliflower cv. Chaser up to ten weeks after planting into soil inoculated with* Rhizoctonia *and treated with various biological treatments applied pre or post planting.

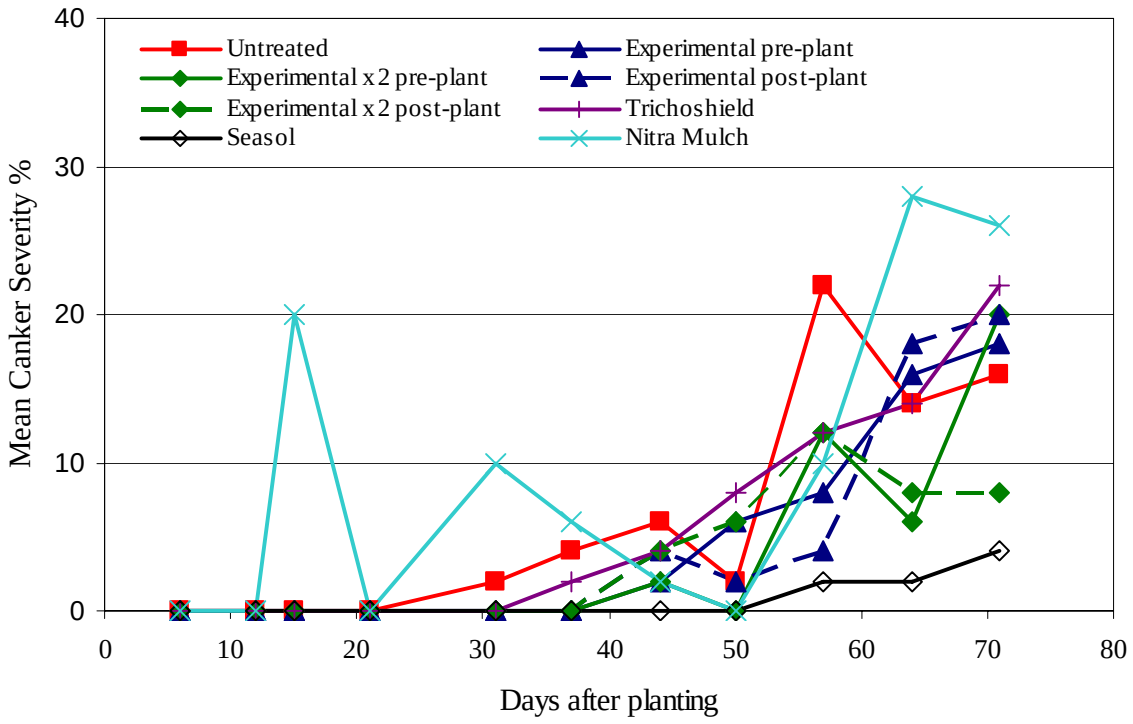


Figure 44. Canker development on cauliflower cv. Chaser up to ten weeks after planting into soil inoculated with* Leptosphaeria *and treated with various biological treatments applied pre or post planting.

When grown in soil inoculated with *Leptosphaeria*, the experimental products gave similar disease control when applied either pre-planting or post-planting (Table 53). Plants treated with Seasol® had significantly less disease than the untreated control and plants grown in Nitra Mulch® had the most disease.

Up to 80% of the untreated plants grown in soil inoculated with *Rhizoctonia* developed adventitious roots. While there were significant differences between the incidence of plants with adventitious roots, there was no correlation with canker severity (Table 53). For example 100% of plants treated with Nitra Mulch® using soil inoculated with *Leptosphaeria* developed adventitious roots, whereas none were produced on plants in *Rhizoctonia* soil with the same treatment. It was anticipated that this growth would be encouraged by some of the biological treatments, however this was not confirmed. Some plants produce these adventitious roots above the canker and allowed the plant to keep growing.

Table 53. Incidence and severity of canker and incidence of adventitious roots on cauliflower cv. Chaser at harvest, ten weeks after planting into soil inoculated with* Rhizoctonia *and treated with various biological treatments applied pre or post planting. Treatment means with the same letter are not significantly different (P=0.05).

Treatment	*Rhizoctonia*			*Leptosphaeria*		
	Incidence	Severity	% Adv roots	Incidence	Severity	% Adv roots
Untreated	100	44 bc	80 a	100	20	0 c
Becker Underwood experimental: pre-plant	100	24 c	30 bc	100	10	40 b
Becker Underwood experimental: post-plant	100	74 ab	10 c	100	26	10 c
Becker Underwood experimental combination pre-plant	100	44 bc	10 c	100	26	10 c
Becker Underwood experimental combination post-plant	100	50 bc	10 c	100	18	0 c
Trichoshield™	100	48 bc	50 ab	100	16	20 bc
Seasol®	100	44 bc	10 c	100	20	0 c
Nitra Mulch®	100	95 a	0 c	100	20	100 a

The relative area under disease progression curve showed Nitra Mulch® and the two experimental products applied after planting had more disease that the untreated controls in *Rhizoctonia* inoculated soil (Fig. 45). None of the treatments significantly improved the RAUDPC compared to the untreated control, however both experimentals applied pre planting had significantly less disease than the plants grown in Nitra Mulch®.

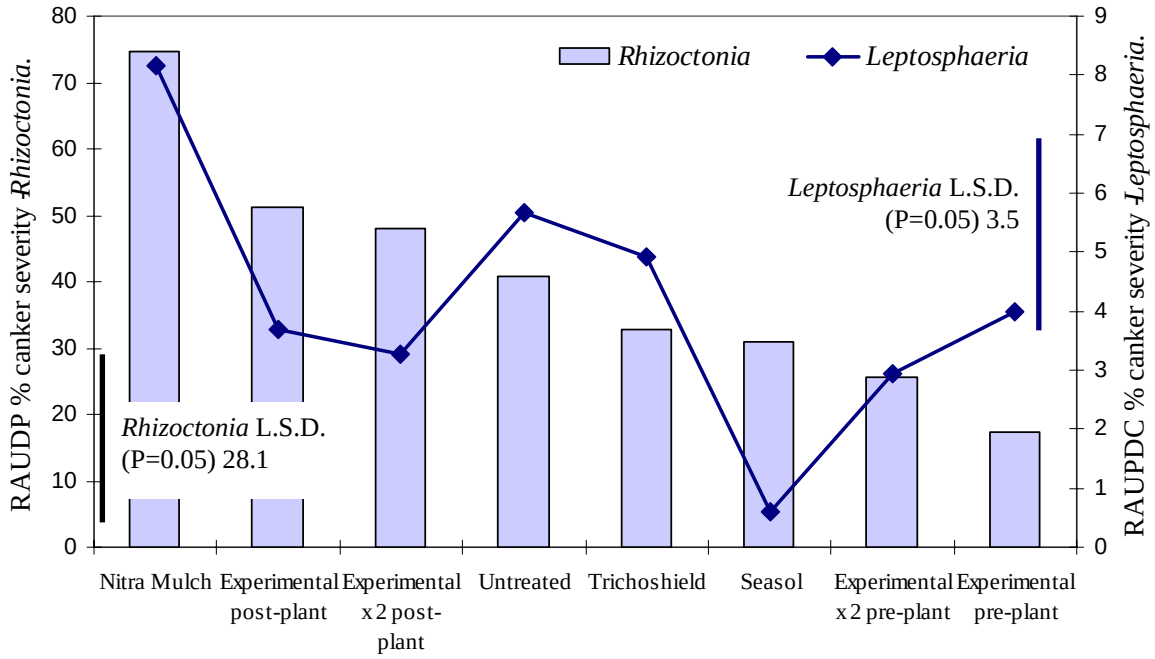


Figure 45. Relative area under disease pressure (RAUDPC) of mean percent canker severity in cauliflower cv. Chaser planted into soil inoculated with* Rhizoctonia *or* Leptosphaeria *and treated with biologicals as either pre or post-planting drenches.

4.3.6 Evaluating fungicides and alternatives – field experiments

Fungicide treatments were evaluated in experiments undertaken on commercial properties in the Northern Adelaide Plains (NAP) ~50 km north of Adelaide, or on commercial properties and at the Research Station in the Adelaide Hills (AH) ~40 km west of Adelaide. While initial experiments used several fungicides, the later experiments concentrated on products available to the growers, either registered or with a permit for use. Maxim® has a permit for use on broccoli for *Rhizoctonia* damping off, Amistar® a permit for cauliflower and broccoli for white blister rust and *Sclerotinia* and Rovral® a permit for *Rhizoctonia* on broccoli. Treatment application methods were focussed on pre-planting drenches simulating nursery applications before delivery to growers and post-planting drenches could be applied through irrigation or a boom spray.

Objective: *To determine the efficacy of various fungicides and methods of application for the control of stem canker on cauliflower.*

Experiment 1 – NAP October 2006

Materials and methods

Cauliflowers cv. Skywalker were planted into the field on 10th October 2006 at plant spacings of 50 cm and row spacing of 60 cm. Treatment plots consisted of four rows by seven plants with the spray zone including 10 cm either side of the plant and foliage, with four replicate plots per treatment arranged in a randomised block design. Amistar was applied at 300 or 600 ml/Ha at 3 days and 2 weeks after planting, using a back pack sprayer to apply three litres of fungicide solution to each plot.

Plants were maintained as per normal grower practices and up to eight plants in the centre of each plot were assessed at seven weeks and ten weeks after planting.

Results and discussion

Infection was low in the planting, with only 11% of the plants with canker at ten weeks after planting (Fig. 46). None of the fungicide treatments eradicated the canker, however all provided some suppression. The higher rate of Amistar (600 ml/ha) applied two weeks after planting was the only treatment to have significantly lower canker incidence than the untreated control.

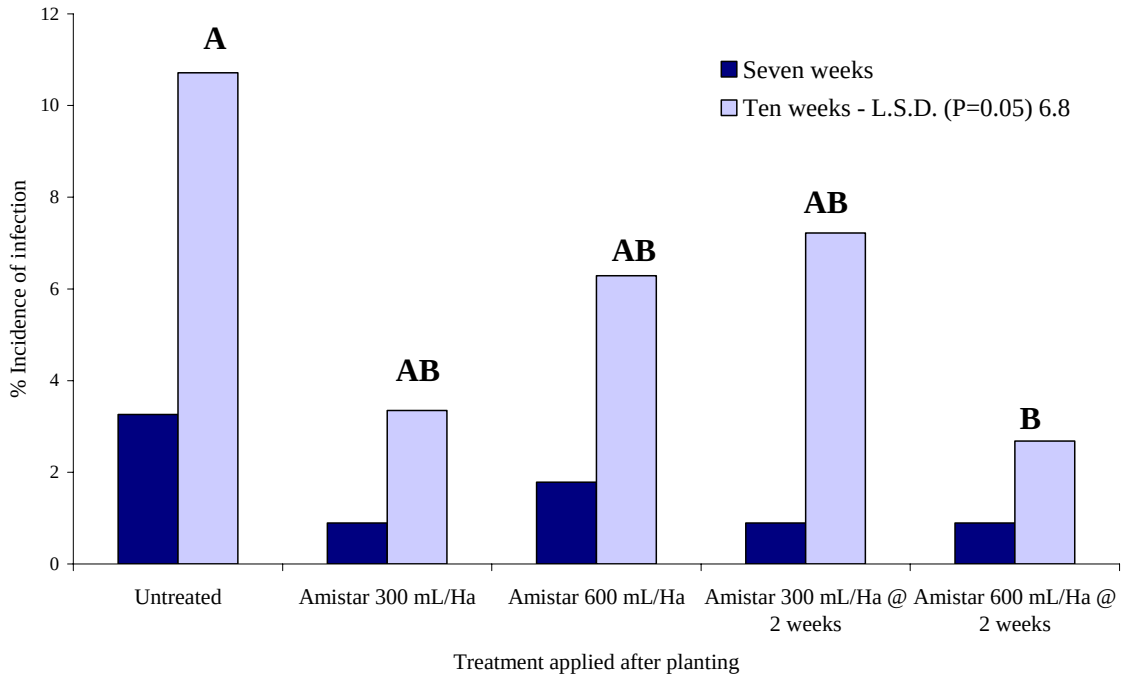


Figure 46. Progression of canker incidence on cauliflower cv. Skywalker between seven and ten weeks after planting on plants treated with fungicide drenches at planting or at two weeks after planting. Ten week means with the same letter are not significantly different (P=0.05)

Experiment 2 – NAP February 2007

Materials and methods

Cauliflowers cv. Savannah were planted into the field on 8th February 2007 at plant spacings of 50 cm and row spacing of 60 cm. Treatment plots consisted of four rows by three meters (approximately six plants per row) with four replicate plots per treatment arranged in a randomised block design. Soil and plants were treated with fungicides applied 10 cm either side of the plant including the foliage. Fungicides including Amistar (1 ml/L), Rovral (1 ml/L), Bavistin (1 ml/L) or Octave (2 g/L) were applied 1 day after planting using 3 L of fungicide solution to each plot.

Plants were maintained as per normal grower practices and up to eight plants in the centre of each plot were assessed for canker every 2 weeks.

Results and discussion

By 12 weeks after planting, cankers were found on 17% of the untreated controls (Fig. 47). Octave® and Amistar® initially reduced disease development with significantly less plants infected at 8 weeks after treatment. However by twelve weeks the incidence of canker, while less than those in the untreated control, the difference was not statistically significant.

At 12 weeks after planting, plants treated with Octave® had significantly less canker that the untreated control (Fig. 47). The Relative Area Under Disease Progression (RAUDP) of canker severity was significantly higher with the untreated control than with all treatments, with the least disease developing in the Octave® treated plants (Fig. 48). Before analysis of both these data sets (final severity and RAUDP), one outlier in the data was removed. One replicate of the Bavistin treatment had a high number of dead plants, which skewed the data. The cause of the death was not determined.

These results indicate that fungicides applied at planting slowed the development of disease, however a subsequent application may improve control.

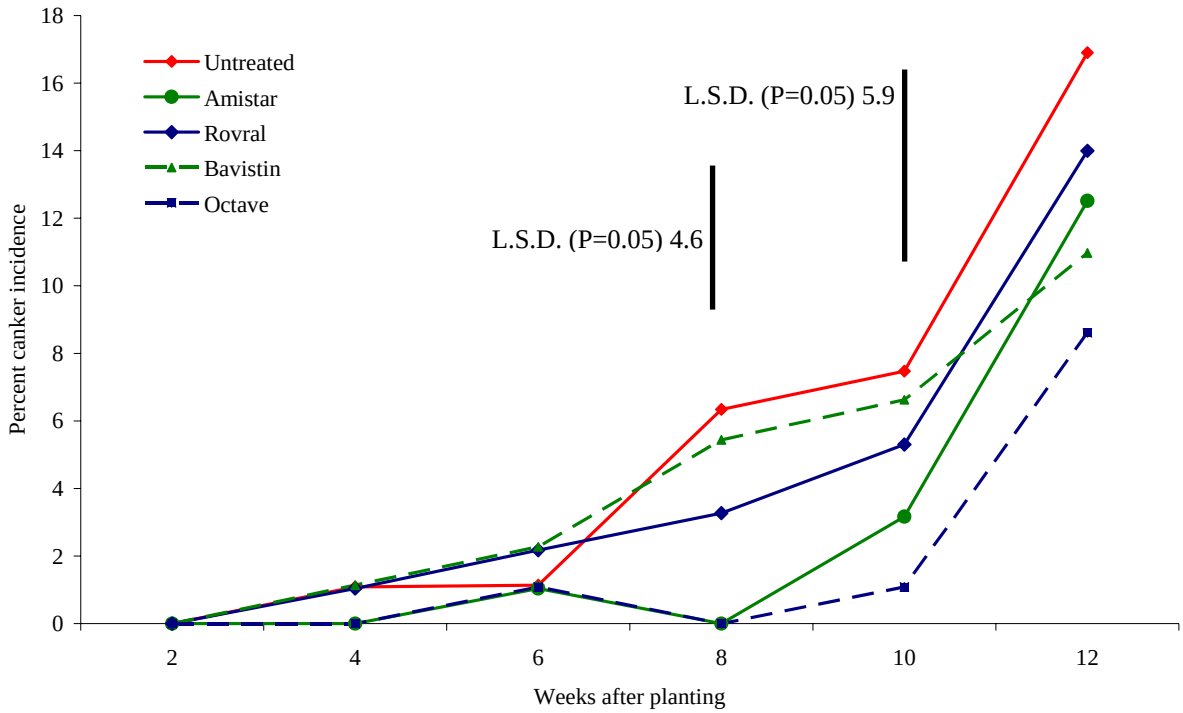


Figure 47. Progression of canker incidence on cauliflower cv. Savannah over 12 weeks on plants treated with fungicide drenches one day after planting. Significant differences (P=0.05) observed between treatment means at 8 weeks after planting(L.S.D 4.6) and 10 weeks after planting (L.S.D. 5.9)

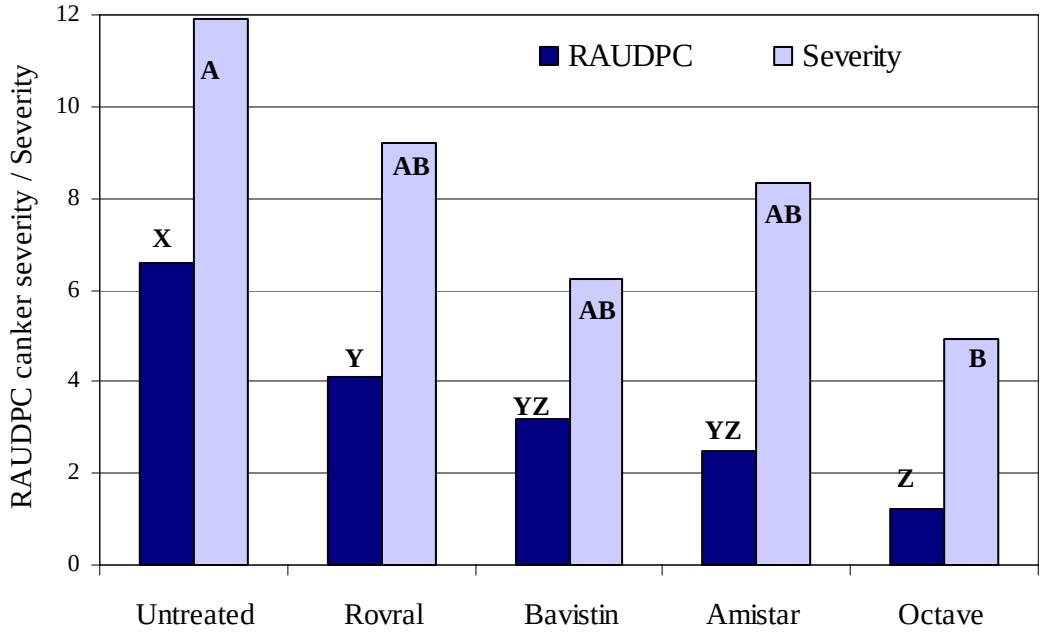


Figure 48. Severity of canker at 12 weeks after planting and the Relative Area Under Disease Progression (RAUDP) of canker severity on cauliflower cv. Savannah over 12 weeks on plants treated with fungicide drenches one day after planting. Bars with the same letter are not significantly different (P=0.05).

Experiment 3 – NAP June 2008

Materials and methods

Soil was collected as previously described prior to planting and again at harvest and eight weeks after harvest, with 40 cores collected and bulked for testing from nine replicated sites within the planting. Cauliflowers cv. Arctic were treated with fungicides at the nursery 24 hours before planting. Two seedlings trays of 196 plants were each drenched for ~1 minute in water or a solution of 0.5 ml/L Amistar® or 40 ml/L Maxim®.

The treated plants were planted into the field on 6th June 2008 at plant spacings of 50 cm and row spacing of 60 cm using the commercial planter. The two trays of each fungicide treatment were planted in one block of four rows wide until the trays were empty (~98 plants per row), with the water treated control planted between the two fungicide treated areas.

Plants were maintained as per normal grower practices. 100 plants per treatment, ten groups of ten plants selected at intervals along the four rows, were assessed for incidence and severity of canker every two weeks until harvest. Plants were harvested by the grower 20 weeks after planting and 100 harvested and 100 non harvested plants assessed for canker.

Results and discussion

Rhizoctonia AG 2.1 and 4 were detected in all soil samples collected pre planting (Fig. 49), with a mean of 366 pg/g AG 2.1 DNA and 185 pg/g AG 4 DNA. Between 1 and 8 pg/g *Leptosphaeria* DNA was detected in 5 of the soil samples.

By eight weeks after harvest (Fig. 50), the levels of AG 2.1 in the soil, while still present in every sample, had reduced to a mean of 139 pg/g AG 2.1 DNA. The *Leptosphaeria* had also reduced, with only one sample of soil infected with 9 pg/g. However the AG 4 levels had increased, with a mean of 840 pg/g.

After eighteen months fallow (Fig. 51), the levels of AG 2.1 in the soil had increased slightly, with a mean of 191 pg/g. However the levels of DNA were variable between the sampling times for each sample area. For example plot 2 row 2 had 836 pg/g soil pre planting, 32 pg/g eight weeks after harvest and had risen to 886 pg/g after 18 months fallow. The AG 4 levels had fallen after fallow to a mean of 243 pg/g soil, with similar variations in magnitude as AG 2.1 between the sampling times. These results showed that regardless of the variability in the levels detected, *Rhizoctonia* survived in soil even after 18 months without a host crop and posed a serious threat of disease to new plantings.

Canker severity was significantly reduced by Maxim® compared to the untreated control at both 15 and 17 weeks after planting (Figs. 52, 53). At 15 weeks, Amistar® treated plants also had lower canker severity. The mean severity of canker increased significantly ($P=0.001$) after 13 weeks post-planting (Fig. 52), with the mean severity of all treatments combined being 0.3% at 13 weeks increasing to 10.7% at 17 weeks.

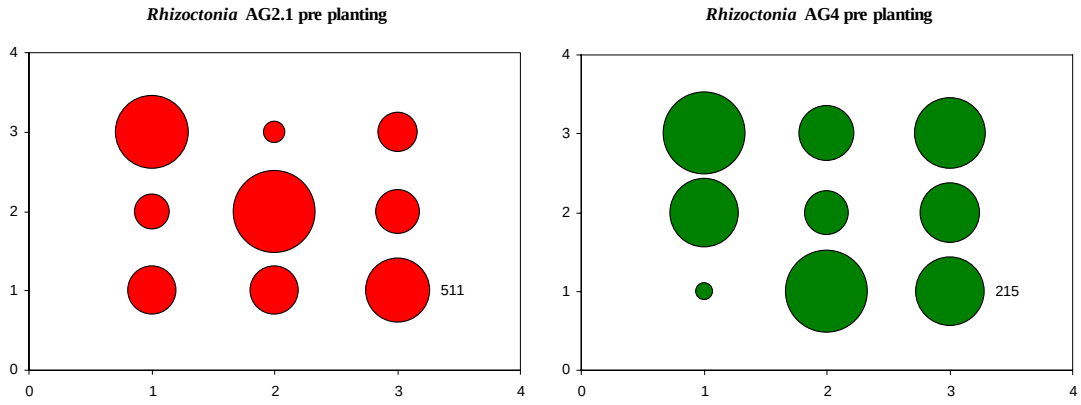


Figure 49. Relative distribution of* Rhizoctonia *AG2.1 and AG4 in soil pre planting. The figure is the value of the adjacent bubble (pg/g soil) to provide relative size.

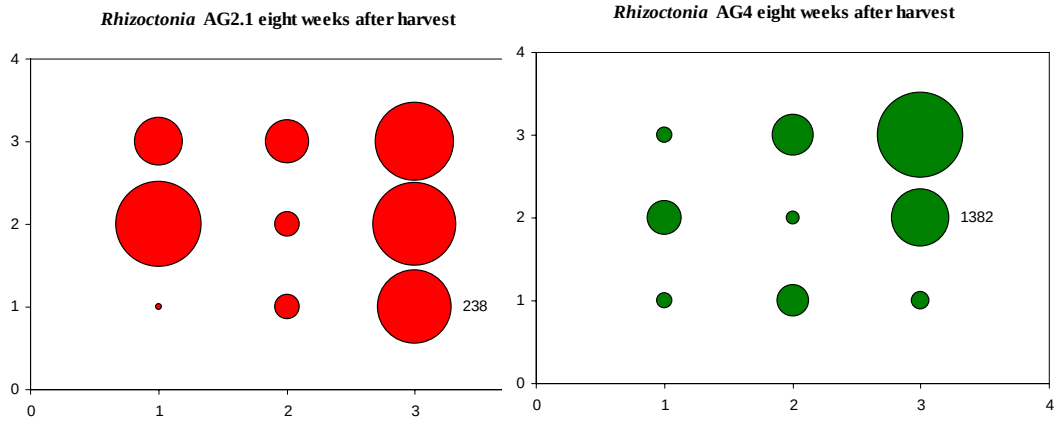


Figure 50. Relative distribution of* Rhizoctonia *AG2.1 and AG4 in soil eight week after planting. The figure is the value of the adjacent bubble(pg/g soil) to provide relative size.

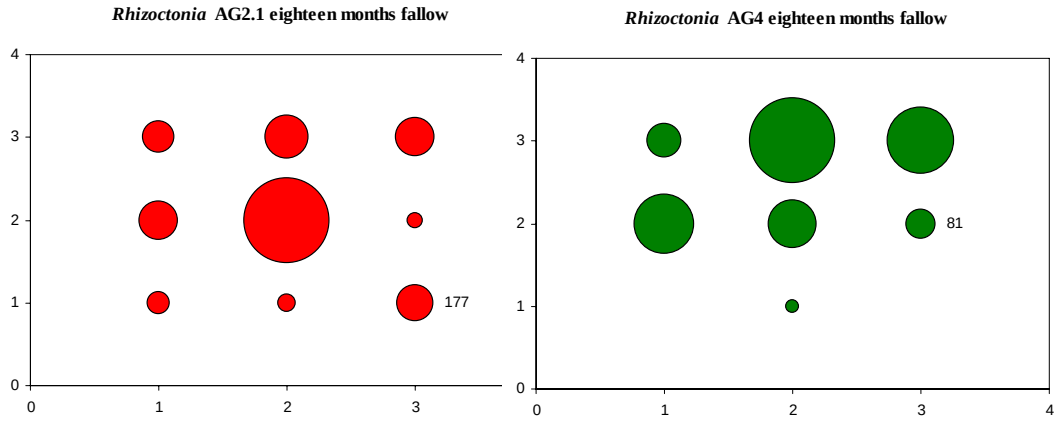


Figure 51. Relative distribution of* Rhizoctonia *AG2.1 and AG4 in soil after eighteen months fallow. The figure is the value of the adjacent bubble(pg/g soil) to provide relative size.

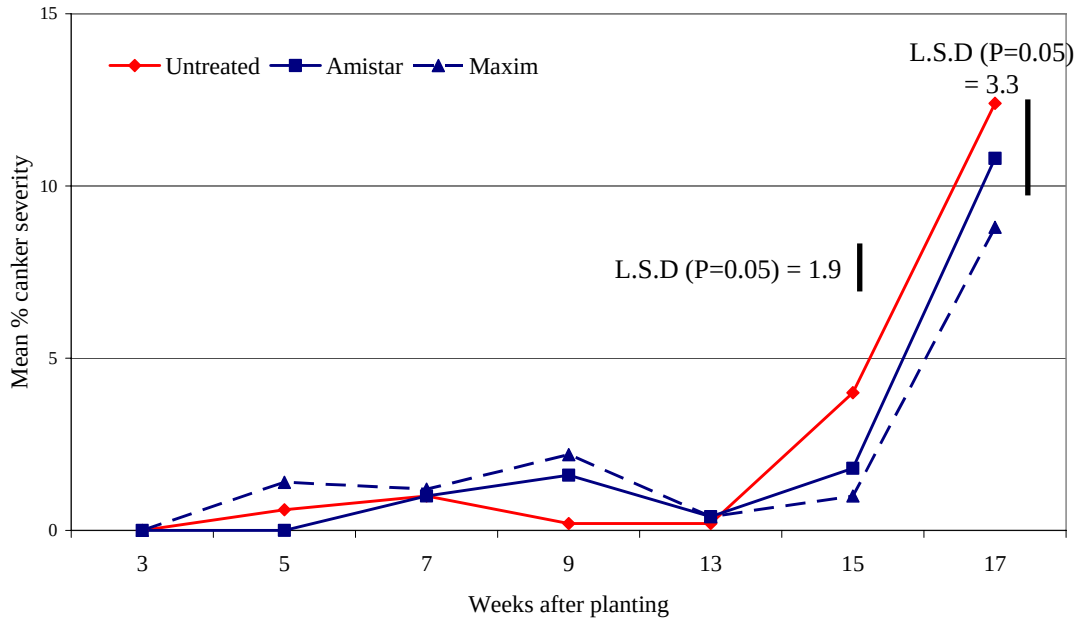


Figure 52. Mean canker severity from 3 to 17 weeks after planting on cauliflower seedlings cv. Arctic drenched pre-planting with Maxim®, Amistar® or water. Significant differences (P=0.05) observed between treatment means at 15 weeks after planting (L.S.D 1.9) and 17 weeks after planting (L.S.D. 3.3)

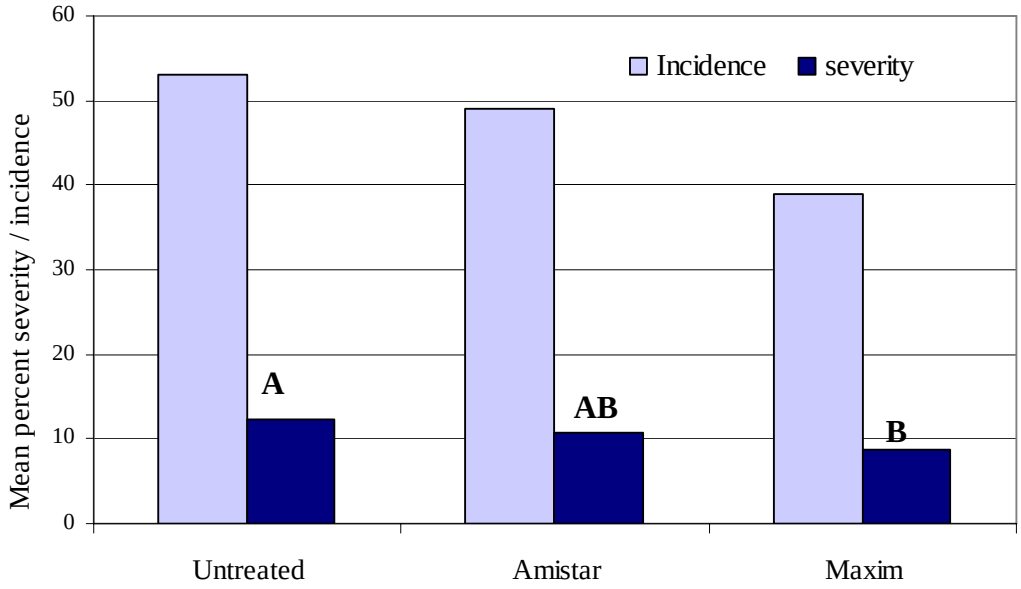


Figure 53. Mean incidence and severity of canker at harvest from cauliflower cv. Arctic drenched pre-planting with Maxim®, Amistar® or water. Severity means with the same letter are not significantly different (P=0.05).

The canker incidence and severity in the fungicide treated areas was lower than in the untreated areas among the harvested plants (Fig. 54). There were no significant differences observed between treatments in the non harvested plants, although there was a trend for fewer cankers to develop. For both fungicide treatments there was no significant difference between the canker incidence and severity in harvested and non harvested plants. However in the untreated area, the incidence and severity in the harvested plants was significantly

(P<0.05) higher than in the non harvested plants. These results indicate that while the cankers had some influence on the marketability of the crop, they were not the main reason for not harvesting the plant. It would also have been useful to obtain a percent loss from the crop in each treatment area, to determine whether the suppression of canker achieved by the application of the fungicides was of economic benefit.

From grower observation, there was less canker in the fungicide treated areas than the adjacent non trial area. This was not confirmed by sampling and may also have been due to other factors such as soil inoculum levels.

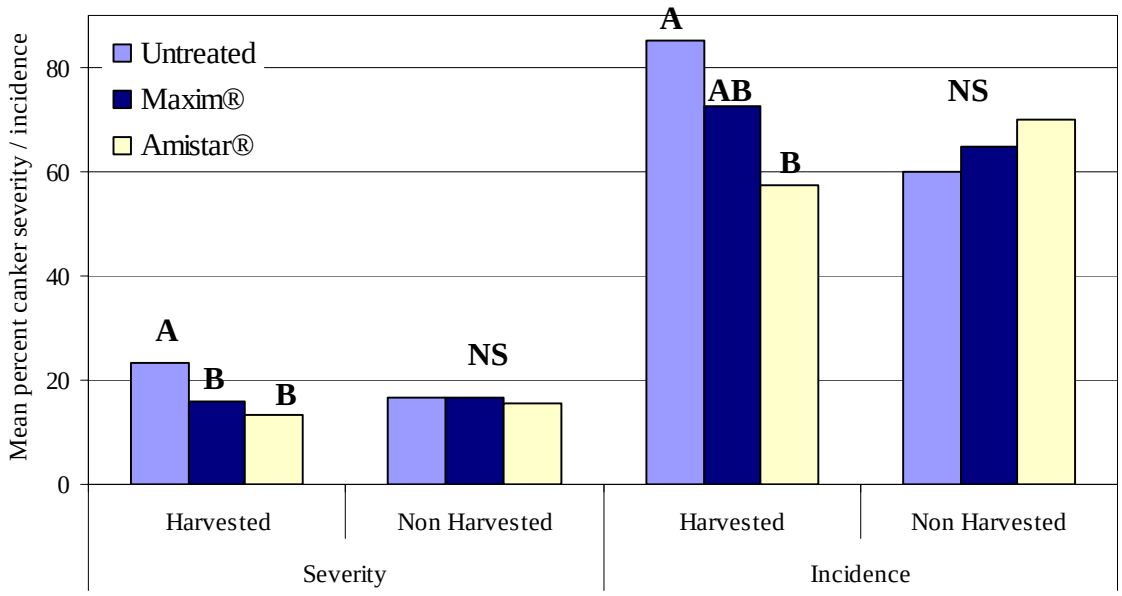


Figure 54. Mean incidence and severity of canker on harvested and not harvested cauliflower cv. Arctic drenched pre-planting with Maxim®, Amistar® or water. Means with the same letter in each group are not significantly different (P=0.05). NS indicates there is no significant difference (P=0.05) in the means within that group.

Experiment 4 – AH December 2008

Materials and methods

Two adjacent plots at the Lenswood Research Centre were inoculated with *Rhizoctonia* and *Leptosphaeria*. Ten culture plates each of *Rhizoctonia* AG 2.1, 2.2 and 4 were macerated in 3L of water and spread evenly over the two plots. Inoculated soil and infected plants from greenhouse experiments were also spread over the area and incorporated with a rotary hoe. Approximately 390 six week old cauliflower seedlings cv. Discovery were root inoculated with *Leptosphaeria* and planted evenly over the trial area. After three weeks these were also rotary hoed in, and the ground left for one week.

Six week old cauliflower cv. Moby were drenched by immersing speedling trays in either Maxim® at 40 ml/100L, Amistar® at 100 ml/100L or water for five minutes and allowed to drain. After 24 hours, treated seedlings were planted by hand on 19th December at plant spacings of 50 cm and row spacing of 80 cm, two rows of five plants per replicate for each treatment. 18 days after planting, drenches of Rovral Aqauflo® at 1L/ha or Amistar® at 600 ml/ha were applied with a back pack sprayer using 1L of water per row of five plants. The sprays were applied in a 10 cm band along the full row until soil saturation. There were six

replicated areas with treatments arranged in a randomised block. Treatment details are outlined in Table 54.

Table 54. Treatments – fungicides and rates applied as pre-planting and post-planting drenches.

Treatment	Pre-plant drench	Post-plant drench
Maxim/-	Maxim® 40 ml/100L	Water
Maxim/Amistar	Maxim® 40 ml/100L	Amistar® 600 ml/ha
Maxim/Rovral	Maxim® 40 ml/100L	Rovral® 1 L/ha
Amistar/-	Amistar® 100 ml/100L	Water
Amistar/Rovral	Amistar® 100 ml/100L	Rovral® L/ha
Untreated	Water	Water

Soil was collected as previously described 4 weeks after planting from the mid row area to avoid the treated soil and plants, bulking soil to obtain an overall level for each of the two plots. Five cores of soil were collected from beneath the plants in each replicate. The cores were bulked for each treatment from replicates 2, 3, 5 and 6 and the lower disease replicates of 1 and 4 were bulked for each treatment and tested separately.

Plants were watered by overhead irrigation to the equivalent of ~20 mm rainfall every 2 days from planting, with the exception of withholding irrigation for four days after the post planting drench was applied. On 24th February, following a significant heat wave of 40+^{0}C late January and poor plant growth in some areas of the plot, three T Bug SM200 probes from Measurement Engineering Australia were installed to measure percent soil moisture. Weeds were controlled by hand hoeing and Confidor® and Dipel® each applied once to control aphids, LBAM and cabbage white butterfly.

Plants were assessed for cankers at 3, 4, 7, 8, 9, and 11 weeks after planting. Plants were harvested by hand 12 weeks after planting, roots washed and the plants returned to the laboratory for a final assessment of: canker, presence of *Pythium* on roots by root damage symptoms, the comparative size of the root ball (1=small, 2=medium and 3=large), the size of the plant (1=small, 2=medium and 3=large) and the presence of adventitious roots.

Results and discussion

The watering of the block was not even, and it was observed that the plants in replicate four at the top of one plot were stunted and replicate six plants and the lower end of the other plot were much larger that plants in the other replicates (Fig. 55). When the moisture probes were installed, the soil moisture was 12% in replicate four and 30% in replicate six whereas the other plots were between 20 and 25% (data not presented).

Figure 55. Cauliflower plants in plot at Lenswood, February 2009. Replicate four under watered plants in foreground and replicate six overwatered plants at far end of plot.

All replicate data

Analysis of canker for all replicates irrespective of treatment showed that the replicate effect was significant, with the under-watered plot four having a lower canker severity and incidence and the overwatered plot six having the highest (Table 55). A similar pattern was observed in the other plot: while the plant height difference was not as obvious there was more disease in the "wetter" replicate three. The treatment differences for any of the three disease measurements, canker severity, incidence or RAUDPC were not significantly different (Table 56).

Table 55. Incidence and severity of cankers on cauliflower plants cv. Moby 11 weeks after planting. Replicate means with the same letter are not significantly different (P=0.05).

Replicate	Water	Percent incidence of plants with canker	Percent canker severity	RAUDPC
1	Medium	50.0 bc	22.0 cd	9.1 bc
2	Medium	41.7 cd	15.3 d	5.9 bc
3	High	68.3 b	35.7 b	15.2 a
4	Low	25.0 d	14.3 d	4.8 c
5	Medium	61.7 b	27.8 bc	10.0 b
6	High	90.0 a	48.7 a	16.2 a

Table 56. Incidence and severity of cankers on cauliflower plants cv. Moby 11 weeks after planting, treated with various fungicides pre planting and three weeks after planting.

Treatment	Percent incidence of plants with canker	Percent canker severity	RAUDPC
Amistar / Rovral	55.0	23.7	8.4
Amistar / -	58.3	30.3	10.8
Maxim / Amistar	56.7	27.7	9.1
Maxim / Rovral	58.3	30.8	11.4
Maxim / -	55.0	20.7	9.8
Untreated	53.3	30.7	11.6

The disease levels were more related to the watering levels than the treatments, so the two most extreme replicates were removed from the data analysis and only those with equivalent moisture levels were retained.

Data from replicates 1, 2, 3 and 5 only

Plants treated with Amistar® at planting had lower canker incidence and severity than the plants treated with Maxim® (Figs. 56, 57). The RAUPDC was significantly lower with the plants treated with Amistar® at planting followed by a Rovral® drench at three weeks than either the untreated plants or those treated with Maxim® then Rovral®.

The application of the fungicide drench at three weeks after planting slowed the development of canker, at four weeks after planting the untreated and Maxim/Water treatments had less disease than the other treatments (Fig. 56).

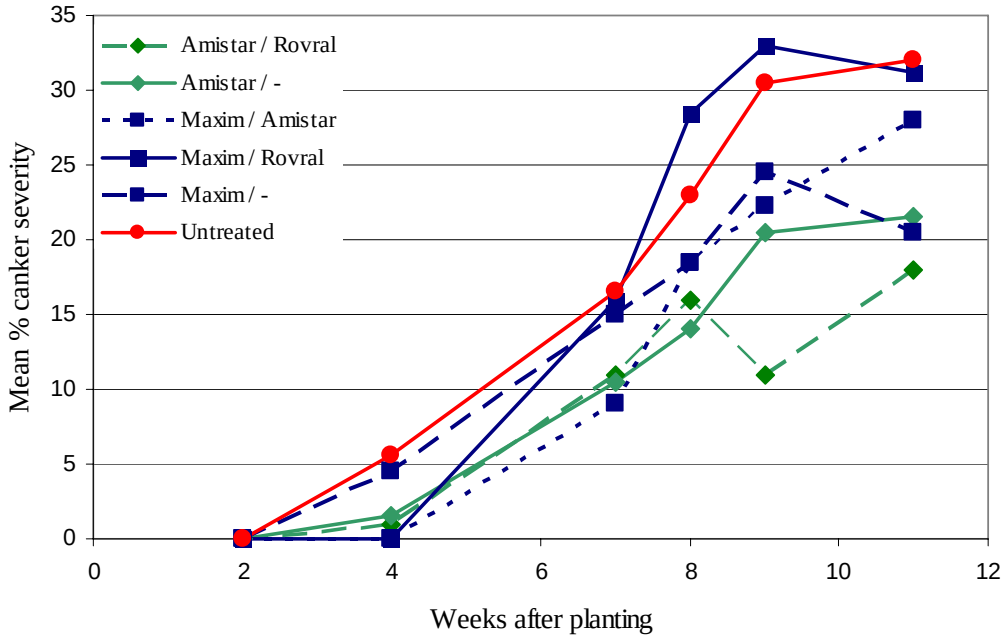


Figure 56. Mean canker severity from 2 to 11 weeks after planting on cauliflower seedlings cv. Moby drenched pre-planting and post planting with fungicides. Data from replicates 1, 2, 3 and 5 only.

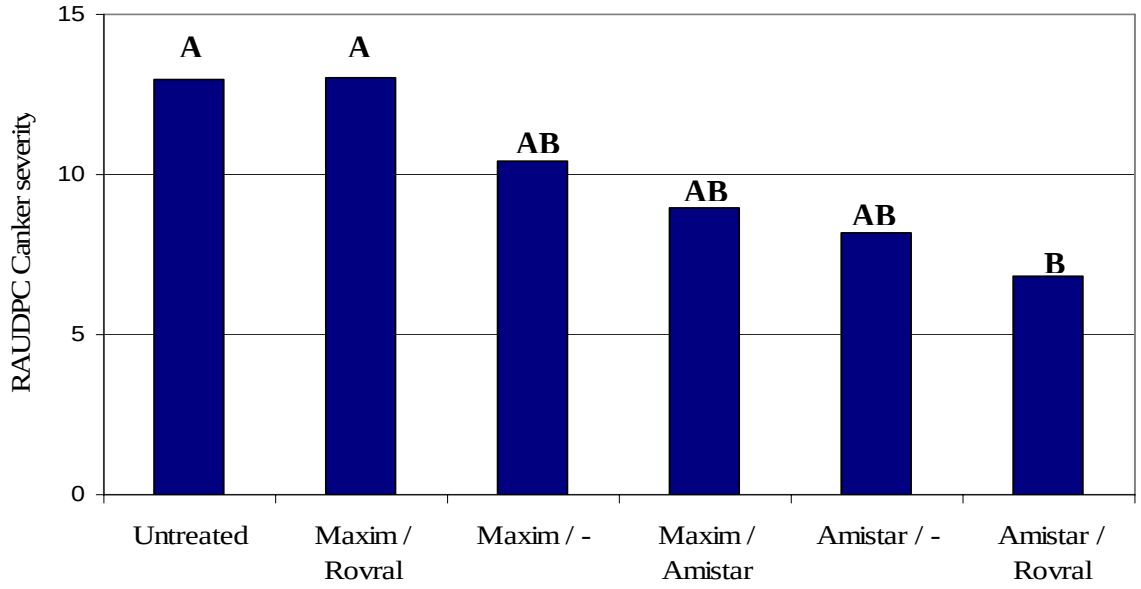


Figure 57. Relative area under disease pressure curve (RAUDPC) of canker severity from 2 to 11 weeks after planting on cauliflower seedlings cv. Moby drenched pre-planting and post planting with fungicides. Data from replicates 1, 2, 3 and 5 only. Bars with the same letter are not significantly different (P=0.05).

There were no significant differences between the treatments for incidence of plants with symptoms of *Pythium* or the size of plants (data not presented). There were also no differences between treatments in the size of the root ball or the presence of adventitious roots (Fig. 58), however there was an inverse relationship observed between the two measurements. For example the treatment with the highest incidence of plants with adventitious roots, Amistar® at pre-planting, also had the lowest mean root ball size. From these results, the development of adventitious roots was in response to the reduced root area and not from any treatment effect.

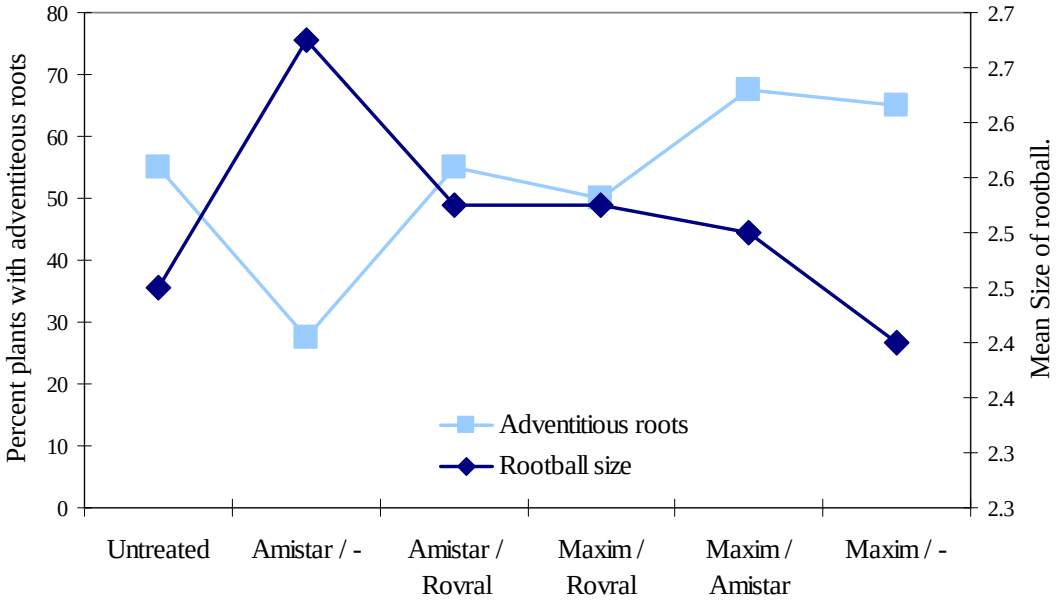


Figure 58. The percent of cauliflower cv. Moby plants with adventitious roots at harvest 12 weeks after planting on drenched pre-planting and post planting with fungicides and the mean comparative root ball size, where 1=small, 2= medium and 3=large root ball. Data from replicates 1, 2, 3 and 5 only.

Soil data

Higher levels of *Rhizoctonia* AG 2.1, AG 4 and *Pythium* clade F (which includes *P. irregulare*) were detected in the initial soil sample in Plot 2 (Table 56). No *Leptosphaeria* was detected in either plot.

There was no effect of treatments of detection of *Rhizoctonia* post harvest, with a slight overall increase in levels of *Rhizoctonia* post harvest compared to the initial sample (Table 56). *Rhizoctonia* AG 2.2 was detected in two of the treatments, but not in the initial sampling. There was a significantly (P=0.006) lower level of *Pythium* in the low disease replicates of 1 and 4 compared to the other replicates. This corresponds to the replicates with the higher soil moisture levels.

Table 56. DNA levels (pg/g) pre-plant and post-harvest in soil planted with cauliflower cv. Moby drenched pre-planting and post planting with fungicides.

Sample	AG 2.1		AG 2.2		AG 4		Pythium clade f	
	Reps 1&4*	**Reps 2,3,5,6**	**Reps 1&4**	**Reps 2,3,5,6**	**Reps 1&4**	**Reps 2,3,5,6**	**Reps 1&4**	**Reps 2,3,5,6**
Plot 1 initial sample	3		0		11		54	
Plot 2 initial sample	7		0		412		189	
Mean initial sample	**5**		**0**		**211.5**		**121.5**	
Untreated	0	5	0	0	495	14	164	187
Amistar® / -	0	0	0	0	83	99	59	173
Amistar® / Rovral®	0	1	0	1	229	155	73	192
Maxim® / -	0	0	0	0	375	64	131	130
Maxim® / Rovral®	60	7	32	0	284	51	57	166
Maxim® / Amistar®	3	0	0	17	14	1,250	72	176
Mean	**10.8**	**2.2**	**5.3**	**3.0**	**247**	**272**	**93#**	**171#**

*Reps 1 and 4 had lower disease levels overall than the other replicates.
Significant difference between the *Pythium* levels in rep 1&4 and reps 2,3,5,6 by Two-sample T test, P=0.006.

Experiment 5 – AH June 2009

This experiment utilised the two adjacent plots at the Lenswood Research Centre inoculated and planted in experiment 4. The compatibility of Trichoshield™ with Maxim® and Amistar® was tested *in vitro* by spreading Trichoshield™ onto fungicide amended plants. Trichoshield™ grew equally well on both PDA and PDA amended with either fungicide.

Materials and methods

Soil was collected prior to planting as previously described, sampling each of the six replicate areas separately.

Five week old cauliflower cv. Discovery were drenched by immersing speedling trays in Maxim® at 40 ml/100L, Trichoshield™ at 5g/1L or water for five minutes and allowed to drain. After 24 hours, treated seedlings were planted by hand on 23 June 2009 at plant spacings of 50 cm and row spacing of 80 cm, two rows of five plants per replicate for each treatment. 14 days after planting, drenches of Seasol® at 15 ml/m^2 or Trichoshield™ at 2 kg/ha were applied with a back pack sprayer using 1L of water per row of five plants was sprayed in a 10 cm band along the full row until soil saturation. There were six replicated areas with treatments arranged in a randomised block. Treatment details are outlined in Table 56.

On 30th June, more than 30 mm of rain flooded the area. The plants were waterlogged, and the constant rain prevented effective insect control. On 15th July it was decided to abandon the experiment. Ten plants from each of replicate areas 3, 5 and 6 were collected and bulked for PCR testing and the area with the remaining plants was rotary hoed for replanting.

Table 56. Treatments – products and rates applied as pre-planting and post-planting drenches.

Treatment	**Pre-plant drench**	**Post-plant drench @ 14 days**
Maxim	Maxim® 40 ml/100L	Water
Maxim / Seasol	Maxim® 40 ml/100L	Seasol 15 ml/m^2
Maxim / Trichoshield	Maxim® 40 ml/100L	Trichoshield 2 kg/ha
Seasol	Water	Seasol 15 ml/m^2
Trichoshield	Trichoshield™ 5g/1L	Trichoshield 2 kg/ha
Untreated	Water	Water

Results and discussion

Rhizoctonia AG 2.1 and 4 were detected in all replicates areas (Table 57). AG 2.2 was detected at 73 pg/g soil in replicate area four only in the pre planting soil test (data not presented). *Pythium* clade f was also detected, but no *Leptosphaeria*.

Leptosphaeria was detected in high levels in the young plant material. *Rhizoctonia* AG 2.1 was also detected, but no AG 4. While AG 4 was proven to be pathogenic in the greenhouse, it appears that plants are not as easily colonised in the field by this group compared to AG 2.1. These results confirm that detection levels in soil of *Leptosphaeria* are not sensitive enough for cauliflower and infection will occur even with no soil detection.

The levels of *Pythium* were higher than those detected at the end of the previous trial (Experiment 4, Table 56) approximately two months earlier. There replicate 1 and 4 had significantly lower levels than the other replicates (mean 93 pg/g soil), whereas in these results replicate 1 had the second highest level at 1,869 pg/g soil. It is possible the populations increased with the advent of wet and cooler weather.

Table 57. Levels of* Rhizoctonia solani *and* Pythium *clade f (pg/g soil) in replicates in plot 1 and 2 at Lenswood prior to planting and from plants collected ~4 weeks after planting.

	DNA pg/g soil or plant material: total or mean (*range*)					
	***R. solani* AG 2.1**		***R. solani* AG 4**		***Pythium****	***Leptosphaeria#***
Rep. area	**Pre-planting soil**	**Plants**	**Pre-planting soil**	**Plants**	**Pre-planting soil**	**Plants***
1	20	-	146	-	1,869	-
2	11	-	294	-	675	-
3	4	**7** (*0-22*)	36	0	1,042	**1,191** (*0-3,338*)
4	16	-	278	-	184	-
5	8	**45** (*0-85*)	133	0	616	**3,507** (*1,127-7,793*)
6	942	**2,996** (*703-7,224*)	372	0	2,276	**5,170** (*325-14,460*)

* *Pythium* tested in soil but not in plants.
\# *Leptosphaeria* not detected in soil.

Experiment 6 – AH June 2009

This experiment utilised another plot at the Lenswood Research Centre.

Materials and methods

This plot was inoculated with macerated plates and soil and infected plants from inoculated greenhouse experiments as described in Experiment 4, however due to time constraints, no additional *Leptosphaeria* inoculated plants were planted and incorporated.

Soil was collected prior to planting as previously described, sampling each of the five replicate areas separately.

Five week old cauliflower cv. Discovery were drenched by immersing speedling trays in Maxim® at 4 0ml/100L, Amistar® at 100 ml/100L or water for five minutes and allowed to drain. After 24 hours, treated seedlings were planted by hand on 23 June 2009 at plant spacings of 50 cm and row spacing of 80 cm, one row of 20 plants per replicate for each treatment. Seven and 14 days after planting, drenches of Rovral® at 1 L/ha or Amistar® at 600 ml/ha were applied with a back pack sprayer using ~4L of water per row of twenty plants, sprayed in a 10 cm band along the full row until soil saturation. There were five replicated areas with treatments arranged in a randomised block. Treatment details are outlined in Table 58.

Plants were watered when needed by overhead irrigation to the equivalent of ~20 mm rainfall every 2 days from planting, the soil moisture monitors indicating average soil moisture of ~30%. Weeds were controlled by hand hoeing and Confidor® and Dipel® applied to control aphids, LBAM and cabbage white butterfly.

Table 58. Treatments – products and rates applied as pre-planting and post-planting drenches.

Treatment	Pre-plant drench	Post-plant drench @ 7 days	Post-plant drench @ 14 days
Maxim	Maxim® 40 ml/100L	Water	Water
Maxim / Rovral	Maxim® 40 ml/100L	Rovral® 1L/ha	Rovral® 1L/ha
Maxim / Amistar / Rovral	Maxim® 40 ml/100L	Amistar® 100 ml/100L	Rovral® 1L/ha
Amistar	Amistar® 100 ml/100L	Water	Water
Amistar / Rovral / Rovral	Amistar® 100 ml/100L	Rovral® 1L/ha	Rovral® 1L/ha
Untreated	Water	Water	Water

On 30th June, more than 30 mm of rain flooded the area. The plants were waterlogged as in Experiment 4, however effective insect control was maintained and the experiment was continued. However the wet weather and associated low temperatures retarded plant growth.

Plants were assessed every two weeks for presence of canker. Ten plants from each replicate were harvested on 23rd September, ~13 weeks after planting roots washed and the plants returned to the laboratory. A final assessment was undertaken of canker, using the percent rating system and also the area of stem affected (0, 25%, 50% or 100%) and the size of the plant (1=small, 2=medium and 3=large). Plants from replicates 1, 2 and 3 for each of the treatments were bulked and tested for levels of pathogens by PCR.

The remaining plants were harvested 13th October ~16 weeks after planting, roots washed and the plants returned to the laboratory for a final assessment of the canker, stem area infected, presence of *Pythium* on roots by root damage symptoms, the comparative size of the root ball (1=small, 2=medium and 3=large), the presence of adventitious roots and the fresh weight of the stems and leaves.

Soil was collected from replicates 1, 2 and 3 of each treatment post harvest and tested for levels of pathogens by PCR.

Results and discussion

Canker incidence and severity

Canker development increased rapidly after 8 weeks after planting (Fig. 59). At 10 weeks after planting plants treated with Amistar® and Maxim® pre planting only had the highest incidence of canker with 26% and 18% of plants infected respectively. The incidence of plants with canker increased in all treatments between 10 and 12 weeks after planting. The greatest increase was in the untreated control, from 10 to 42%, and the smallest increase was the Amistar® pre planting followed by two post-planting drenches of Rovral®, increasing from 8 to 16% of plants with canker. By the second harvest 16 weeks after planting, the incidence of canker had increased to 100% in the untreated controls, 96% in all the Maxim® pre-plant drench treatments and Amistar® as a pre-plant drench. The lowest incidence was the Amistar® pre planting followed by two post-planting drenches of Rovral®, with 14% of plants with canker.

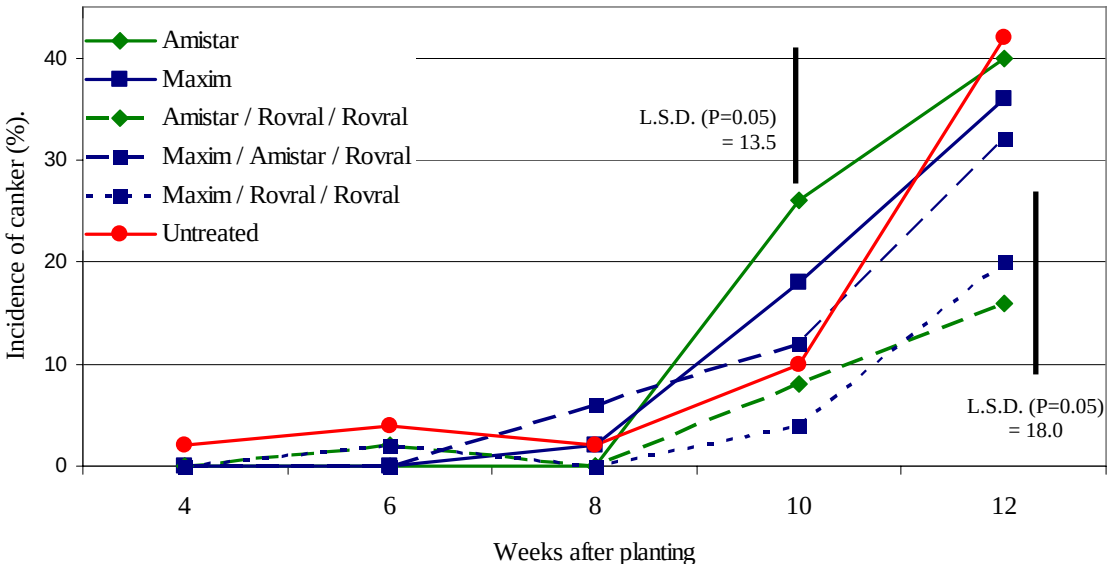


Figure 59. Mean percent canker incidence from 4 to 12 weeks after planting on cauliflower cv. Discovery drenched pre-planting and post planting with various fungicides. Significant differences (P=0.05) observed between treatment means at 10 weeks after planting(L.S.D 13.5) and 12 weeks after planting (L.S.D. 18.0)

There was an increase in mean canker severity between the plants harvested at 13 weeks and 16 weeks for all treatments (Fig. 60). At 13 weeks after planting, all treatments except Amistar® pre planting followed by two post-planting drenches of Rovral® had significantly lower canker severity than the untreated control. At 16 weeks after planting, only the plants treated with Maxim® pre-planting followed by two Rovral® drenches and the Amistar® pre planting had lower canker severity than the untreated control. There was no correlation between the incidence at 12 weeks and the severity at 13 and 14 weeks for the different fungicide treatments. However from 12 weeks after planting, the untreated control had the highest level of canker.

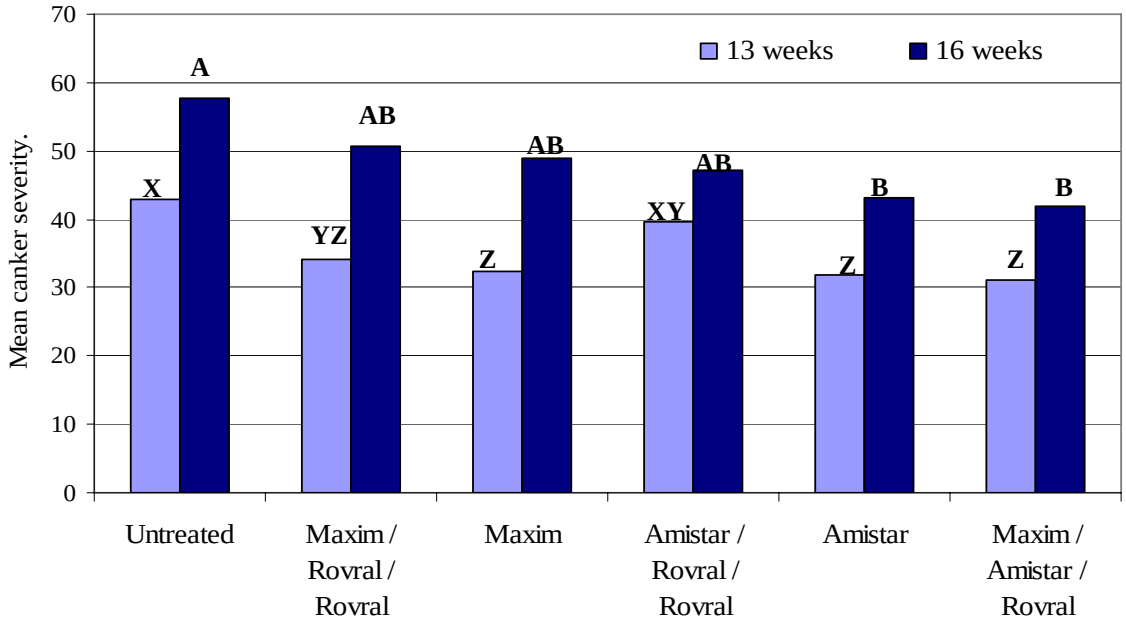


Figure 60. Mean canker severity at 13 and 16 weeks after planting on cauliflower cv. Discovery drenched pre-planting and post planting with various fungicides. Treatment means with the same letter are not significantly different.

All treatments significantly reduced the mean percent stem area infected at 13 weeks after harvest compared to the untreated control (Fig. 61). By 16 weeks only the two treatments with Rovral® applied twice post planting were significantly better than the untreated control. Plants in the pre-planting Maxim® treatment had more stem infected than the untreated, although the difference was not statistically significant.

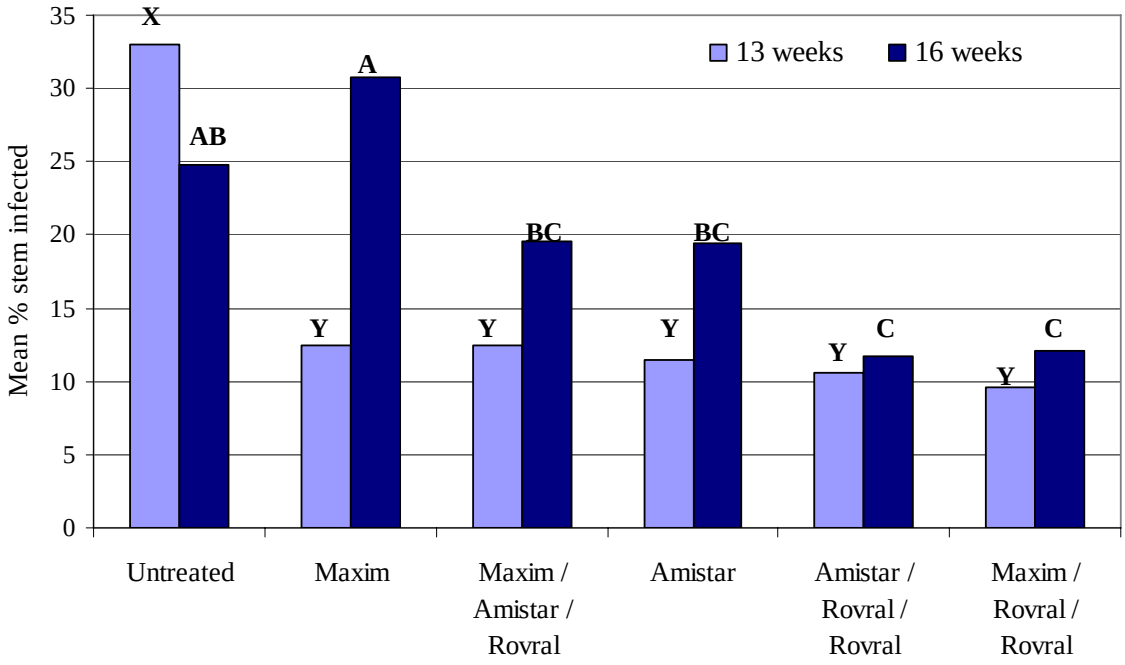


Figure 61. Mean percent area of stem infected with canker at 13 and 16 weeks after planting on cauliflower cv. Discovery drenched pre-planting and post planting with various fungicides. Treatment means with the same letter are not significantly different.

Plant size

All fungicide treatments produced a significantly (P=0.05) smaller proportion of small plants at 13 weeks after planting than the untreated control (Fig. 62). There was no significant difference between the proportions of medium sized plants, however the untreated control and Maxim® pre-planting followed by two Rovral® drenches had the lowest number of large plants.

At 16 weeks after planting, plants of all treatments had increased in size, with a higher proportion of large plants. The Amistar® pre-planting followed by two Rovral® drenches had the highest number of large plants and the lowset number of small plants (Fig. 63).

There were no significant differences between treatments in the fresh weight of tops (Fig. 64), although the untreated control had the lowest weight. The size of the root ball was significantly lower in the untreated plants than in both treatments with Amistar® applied as a pre plant drench. The opposite was observed in the percentage of plants with *Pythium*, with significantly higher number of plants in the untreated control compared to both treatments with Amistar® applied as a pre plant drench. As *Pythium* infects the feeder roots, this inverse relationship is not unexpected, as infection with *Pythium* will reduce the root size.

The root rotting from *Pythium* infection was also partially correlated with the proportion of small plants (Fig. 65). Increased *Pythium* and smaller root mass would cause smaller plants. However there was no relationship observed between the *Pythium* and the canker severity as found with the greenhouse trial, possibly because the infection was not as severe and did not significantly impact the size of all plants.

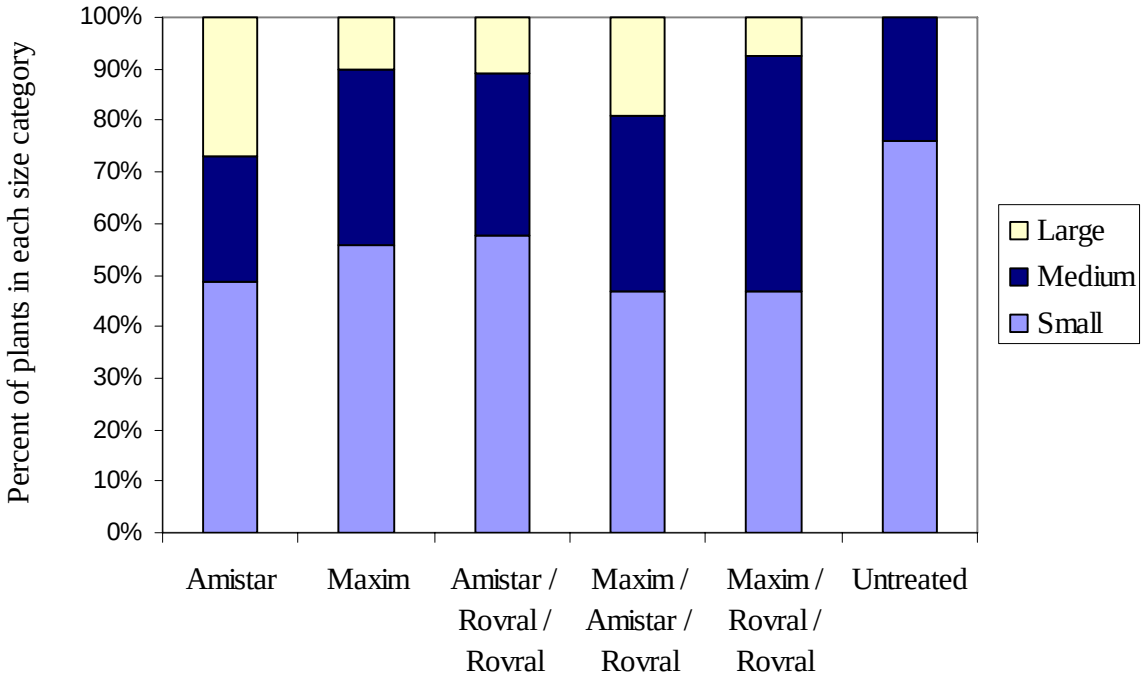


Figure 62. Proportion of comparative plant sizes of cauliflower cv. Discovery plants at 13 weeks after planting, drenched pre-planting and post planting with various fungicides.

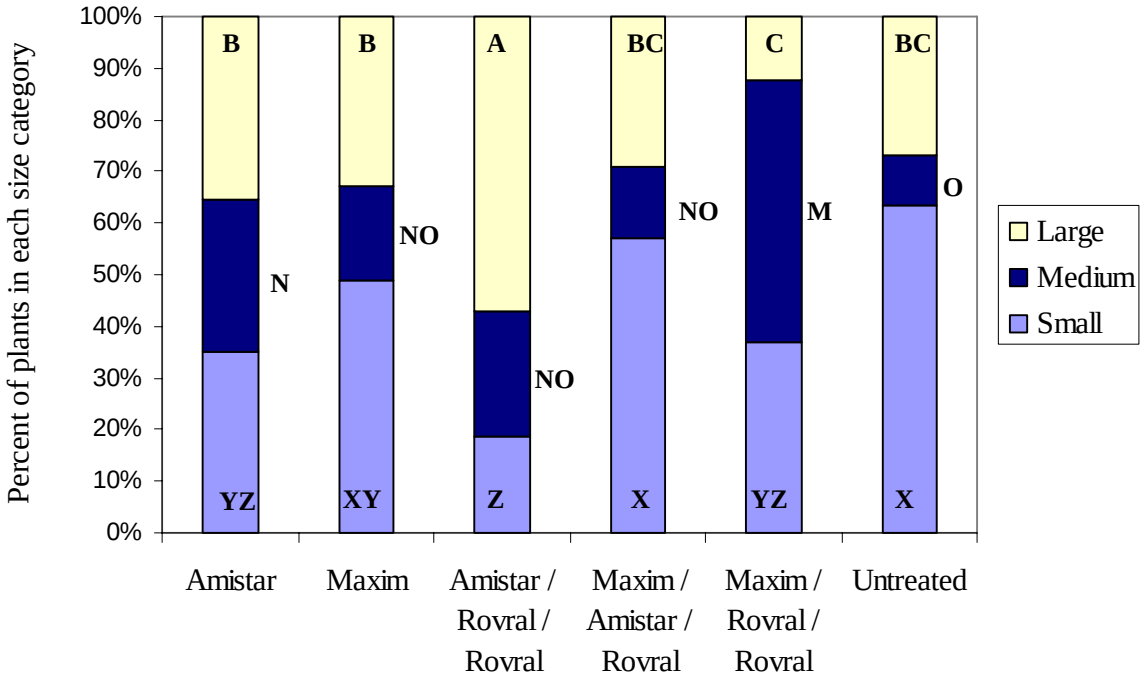


Figure 63. Proportion of comparative plant sizes of cauliflower cv. Discovery plants at 13 weeks after planting, drenched pre-planting and post planting with various fungicides. Treatments with same letter are not significantly different (P=0.05).

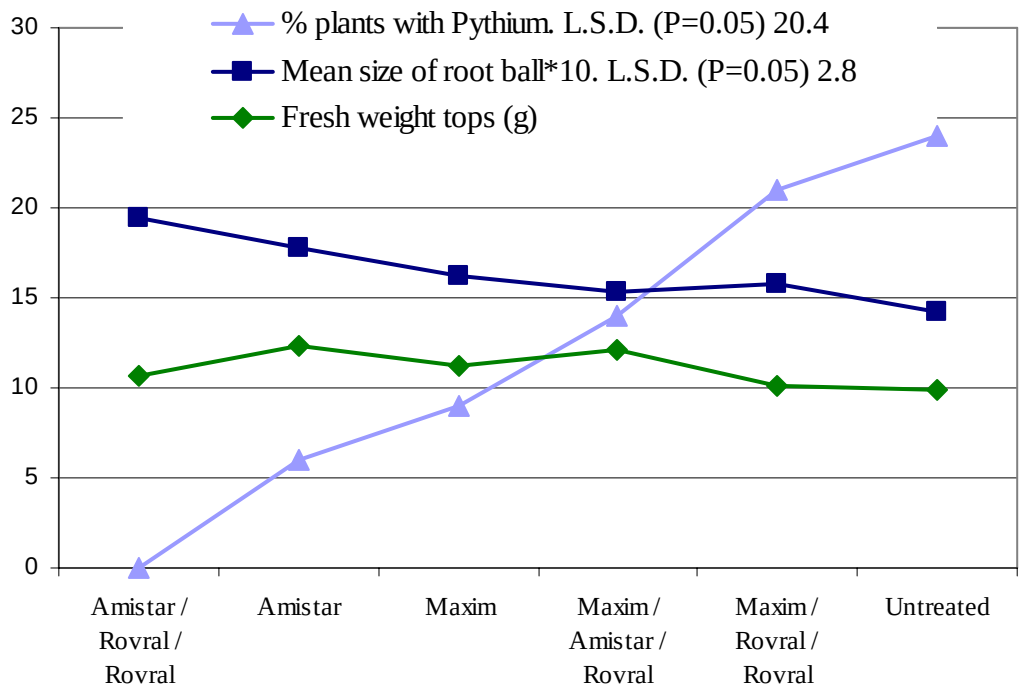


Figure 64. Percent of plants with symptoms of* Pythium*, mean comparative size of root ball (1=small, 2= medium and 3=large) and fresh weight of tops of cauliflower cv. Discovery plants harvested at 16 weeks after planting, drenched pre-planting and post planting with various fungicides. The values of the mean size of the root ball have been multiplied by ten to allow better presentation on the graph.

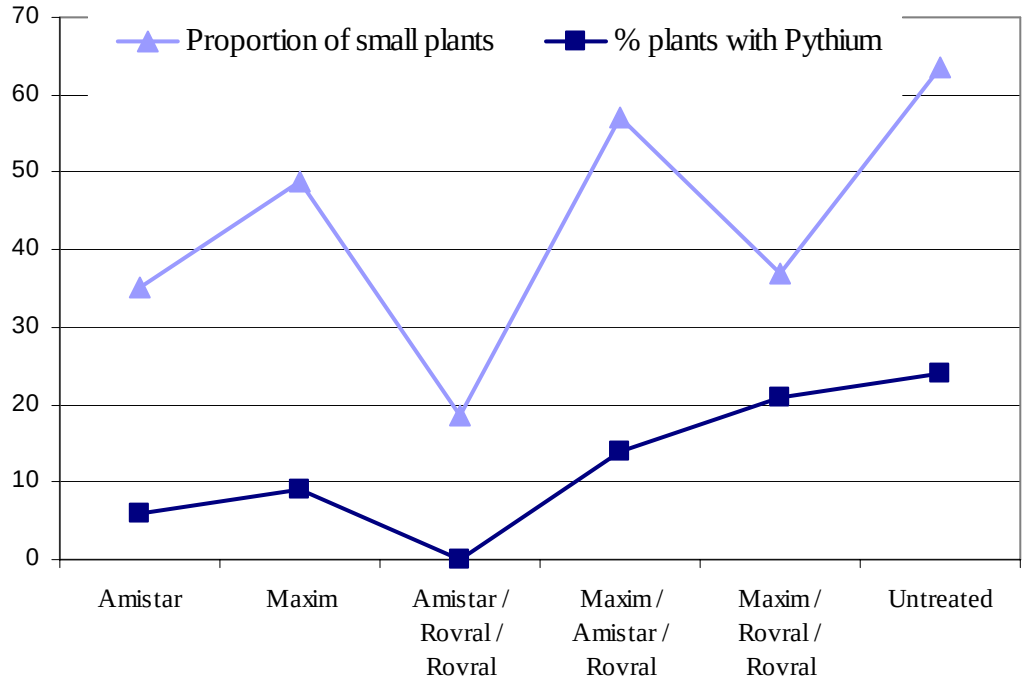


Figure 65. Percent of plants with symptoms of* Pythium *and proportion of small plants of cauliflower cv. Discovery plants harvested at 16 weeks after planting, drenched pre-planting and post planting with various fungicides.

Soil and plant infection.

The pre-plant soil levels were low and neither *Rhizoctonia* nor *Leptosphaeria* were found in all replicates (Table 59). *Rhizoctonia* AG 2.1 was detected in soil from all three replicates tested post-harvest, with levels similar to those pre-planting. No AG 2.2 or 4 were detected post-harvest. *Leptosphaeria* was also detected in soil from all three replicates post-harvest where none had been detected pre-planting.

Table 59. Mean and range of pre-plant and post-harvest soil levels of* Rhizoctonia *and* Leptosphaeria *DNA (pg/g soil) in each replicate area. Post-plant sampling was not undertaken in replicates 4 and 5.

	DNA pg/g soil: total or mean (range**)**							
	Rep 1		**Rep 2**		**Rep 3**		**Rep 4**	**Rep 5**
	Pre-plant*	Post-harvest	Pre-plant	Post-harvest	Pre-plant	Post-harvest	Pre-plant	Pre plant
AG 2.1	0	**138** (0-801)	**21**	**21** (0-98)	**504**	**31** (3-42)	0	**26**
AG 2.2	0	0	**9.0**	0	0	0	0	0
AG 4	0	0	**131**	0	0	0	0	0
L. maculans	0	**44** (2-218)	0	**2.6** (0-6.3)	0	**10** (3-26)	**0.04**	0

* pre-plant testing undertaken on one sample, post-harvest five samples per replicate.

At harvest, *Rhizoctonia* AG 2.1 and *Leptosphaeria* were found in all treatments in both soil and plants (Table 60). No AG 2.2 or 4 were detected in soil or plants from any of the treatments. High levels of *Leptosphaeria* DNA were detected in the plants, indicating that the levels in soil that can infect plants are often below the detectable limits of this test. All fungicide treatments had significantly lower levels of *Leptosphaeria* in plant material post harvest than the untreated control.

There were no correlations between soil levels post-harvest and plant levels in either *Rhizoctonia* or *Leptosphaeria*. There was extreme variability between the results; therefore the sampling strategy may need to be amended to obtain correlations. As Rhizoctonia is an effective saprophyte and does not need a host plant to multiply, the levels in soil can be extremely variable and further work on sampling techniques is underway in other crops (McKay *pers. comm*.) in *Rhizoctonia* has shown that this pathogen can often be highly variable in soil distribution.

No correlations were found between the DNA levels of either pathogen in soil or plant and the severity of canker.

Table 60. Pre plant and post-harvest soil levels of* Rhizoctonia *and* Leptosphaeria *DNA (pg/g soil or pg/g dried pant material) in each replicate area (rep 1, 2 and 3 only).

Treatment	DNA pg/g soil or dried plant material: mean (range)			
	AG 2.1		*Leptosphaeria*	
	Soil	Plant	Soil	Plant*
Maxim	**60** (16-142)	**2,389** (155-4,250)	**11.8** (1-23)	**13,247** (6,516 – 18,176)
Maxim / Rovral / Rovral	**35** (3-98)	**4,352** (981 – 10,282)	**7.7** (6-9)	**51,824** (32,856 – 64,030)
Maxim / Amistar / Rovral	**3.1** (0-9)	**498** (0-867)	**74.2** (5-218)	**44,593** (21,505 – 79,344)
Amistar	**2.5** (0-4)	**2,676** (13-7,429)	**3.3** (0-5)	**31,348** (1,627 – 64,058)
Amistar / Rovral / Rovral	**268** (0-801)	**510** (46-1,296)	**4.6** (2-8)	**46,065** (37,031 – 59,871)
Untreated	**12.4** (3-25)	**516** (372 – 802)	**11.0** (2-26)	**92,857** (75,261 – 116,523)

* Untreated mean of *Leptosphaeria* DNA in plant material significantly higher (P=0.05) than in all fungicide treatments.

Experiment 7 – AH September 2009

This experiment was the replanted Experiment 4 in the two adjacent plots at the Lenswood Research Centre.

Materials and methods

Treatments were applied as outlined in Experiment 5 (Table 61), using five week old cauliflower cv. Discovery. The soil was not retested prior to planting.

Table 61. Treatments – fungicides and rates applied as pre-planting and post-planting drenches.

Treatment	Pre-plant drench	Post-plant drench every 14 days
Maxim	Maxim® 40 ml/100L	-
Maxim / Seasol	Maxim® 40 ml/100L	Seasol® 15 ml/m^2
Maxim / Trichoshield	Maxim® 40 ml/100L	Trichoshield™ 2 kg/ha
Seasol	Seasol® 330 ml/100L	Seasol® 15 ml/m^2
Trichoshield	Trichoshield™ 5g/1L	Trichoshield™ 2 kg/ha
Untreated	Water	-

Plants were watered by overhead irrigation to the equivalent of ~20 mm rainfall every 2 days from planting. Extra sprinklers were installed to alleviate the watering irregularities found in Experiment 3. Weeds were controlled by hand hoeing and Maverik®, Confidor® and Dipel® applied to control aphids, LBAM and cabbage white butterfly.

Plants were assessed every two weeks for presence of canker. Plants were harvested by hand 14 weeks after planting, roots washed and the plants returned to the laboratory for a final assessment of canker. For replicates 1,2 and 3, assessments were also made of: presence of *Pythium* on roots by root damage symptoms, the comparative size of the root ball (1=small, 2=medium and 3=large), the size of the plant (1=small, 2=medium and 3=large) and the presence of adventitious roots.

Results and discussion

Cankers were observed four weeks after planting, with more developing between six and eight weeks after planting (Fig. 66). This concurs with the timing of canker development observed in previous work (Hitch *et al* 2006). Many of the cankers were lesions or only part of the stem affected, as the incidence of plants with cankers completely girdling the stem was much lower (Fig. 67). By 12 weeks after planting, the untreated plants had the highest incidence of canker and Trichoshield™ the lowest, however the differences were not statistically significant.

Trichoshield™ had the highest canker severity at harvest (Table 62), significantly higher than the untreated and treatments with a pre plant drench of Maxim®. Therefore while the incidence was lower, each canker was more severe.

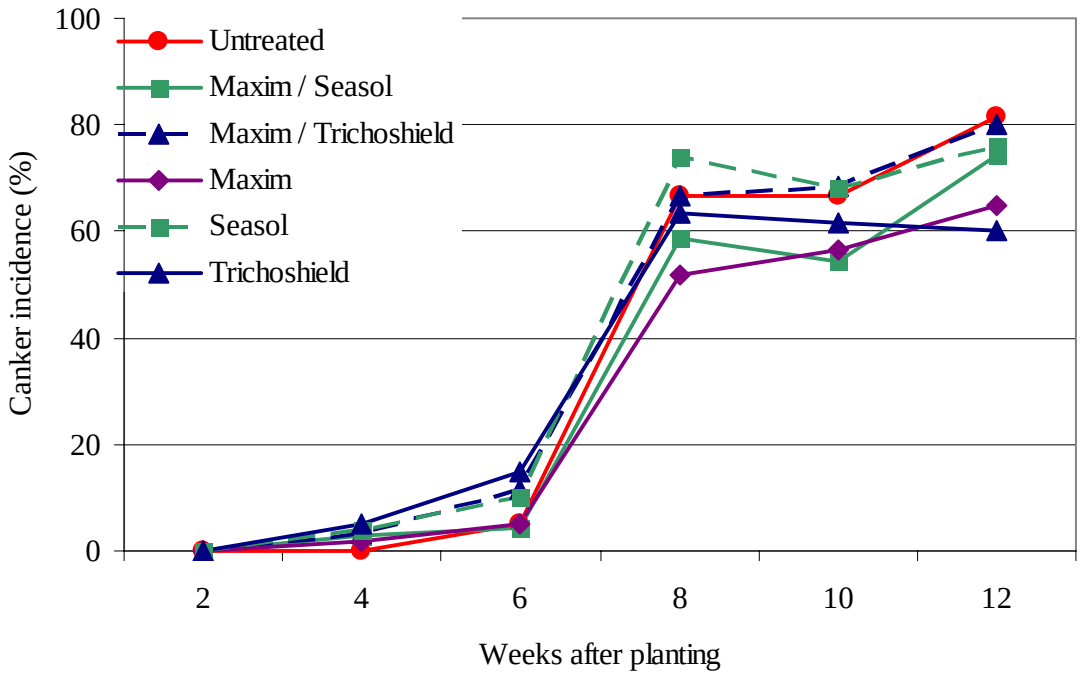


Figure 66. Mean canker incidence from 0 to 12 weeks after planting on cauliflower cv. Discovery drenched pre-planting and post planting with fungicides and biologicals.

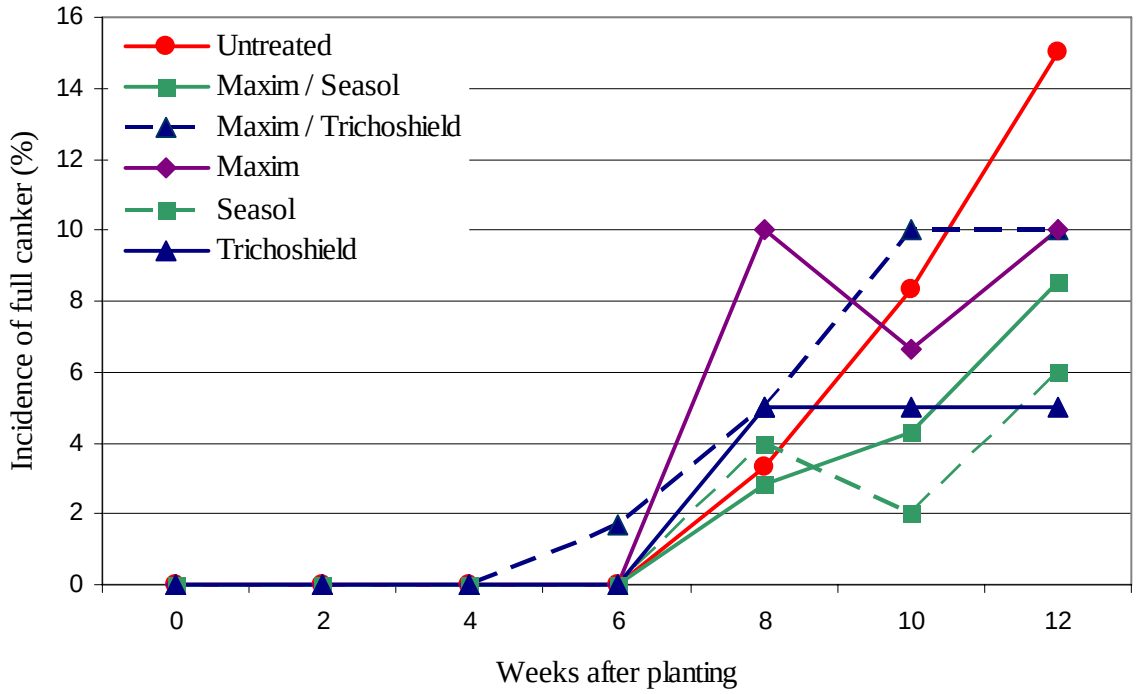


Figure 67. Mean incidence of cauliflower plants cv. Discovery with cankers girdling the stem from 0 to 12 weeks after planting, drenched pre-planting and post planting with fungicides and biologicals.

There were no significant differences in the other harvest data collected (data not presented). Plants treated with Trichoshield™ had the lowest levels of adventitious roots (9% of plants) and Maxim® the highest (28% of plants), but unlike Experiment 3 these did not correlate to the root ball size. There was also no correlation between the plants with *Pythium* symptoms and the *Pythium* levels in the soil pre planting (Table 62). Replicate 3, which was the downhill wetter end of the plot, had the lowest levels of plants with *Pythium*, 27% compared to 43% in the other two replicates.

Table 62. Incidence and severity of cankers on cauliflower plants cv. Discovery at harvest 14 weeks after planting, treated with various fungicides and biologicals pre planting and post-planting. Means with the same letter are not significantly different (P=0.05).

Treatment	Percent incidence of plants with canker	Percent canker severity
Trichoshield	100	57.3 a
Seasol	100	49.6 ab
Maxim / Seasol	100	43.4 bc
Maxim / Trichoshield	96.7	39.3 c
Maxim / -	93.3	37.7 c
Untreated	100	43.7 bc

Trichoshield™ and Seasol® treated plants had the largest proportion of large plants (Fig. 68), with Seasol® treated plants also having the greatest number of small plants and few in the mid size range.

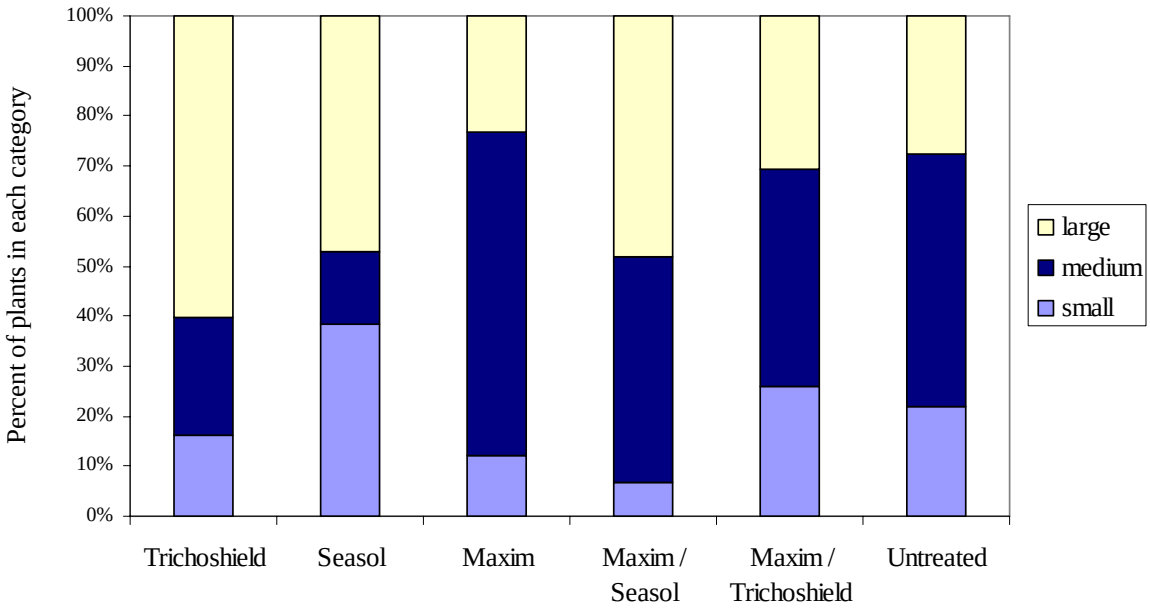


Figure 68. Mean proportion of cauliflower cv. Discovery plants in each size category at harvest, 14 weeks after planting and drenched pre-planting and post planting with fungicides and biologicals.

Seasol and Trichoshield did not reduce canker development to the same extent as the greenhouse trials. However there was a growth benefit and this may translate to improved harvest levels even with higher canker levels. Further experiments should be undertaken to determine the economic benefits of using these products in comparison to fungicides.

Experiment 8 – AH September 2009

This experiment was planted on a grower's property where high levels of black leg (caused by *Leptosphaeria)* had been detected in a Brussels sprouts crop in summer 2008/9. Soil tests showed high levels of *Leptosphaeria* and *Rhizoctonia* and the grower agreed to plant cauliflower in the affected area after a short fallow. The area was rotary hoed to 150 mm on 9th February to incorporate the old crop, followed by a ripping pass, and planed to oats. The oats were sprayed with Fusilade (12 ml/15L) on the experimental area only.

Materials and methods

Soil was collected one week after planting from each replicate area in the trial site. Cores were taken mid row to avoid the treated plants.

Six week old cauliflower cv. Discovery were drenched by immersing speedling trays in Amistar® at 100 ml/100L, Seasol® at 300 ml/100L, Trichoshield™ at 550g/100L or water for five minutes and allowed to drain. After 24 hours, treated seedlings were planted by hand on 25 June 2009 at plant spacings of 50 cm and row spacing of 80 cm, one row of 20 plants per replicate for each treatment. As required after planting, drenches of Seasol® or Trichoshield™ at label rates were applied with a back pack sprayer using ~1L of water per row of twenty plants, sprayed in a 10 cm band along the full row until soil saturation. There were five replicated areas with treatments arranged in a randomised block. Treatment details are outlined in Table 63.

Plants were watered by hand once to provide the equivalent of ~5 mm rainfall as there was sufficient rainfall to not require irrigation. The crop was managed as per normal grower

practice. There was significant insect attack despite the insecticides applied, and the cool, wet and windy conditions slowed plant growth. Therefore 10 plants of similar size were marked and assessed every two weeks for 12 weeks after planting for presence of stem canker.

Table 63. Treatments – products and rates applied as pre-planting and post-planting drenches.

Treatment	Pre-plant drench	Post-plant drench
Amistar	Amistar® 100 ml/100L	-
Amistar	Amistar® 100 ml/100L	Trichoshield™ once at 2 weeks
Amistar	Amistar® 100 ml/100L	Seasol once at 2 weeks
Seasol	Seasol® 330 ml/100L	Seasol every 2 weeks 15 ml/m^2
Trichoshield	Trichoshield™ 500 g/100L	Trichoshield™ every 2 weeks 2 kg/ha
Untreated	Water	-

All plants were harvested on 23rd September ~13 weeks after planting, the roots washed and the plants returned to the laboratory. A final assessment was undertaken of canker, using the percent rating system, the size of the plant (1=small, 2=medium and 3=large) and the presence of adventitious roots.

Ten plant stems were bulked from each replicate of the untreated, Amistar® pre-planting, Seasol® and Trichoshield™ treatments and tested for levels of pathogens by PCR. Soil was collected from the same treatments post harvest and also tested for levels of pathogens by PCR.

Results and discussion

Analysis of the parameters measured detected no significant differences between the harvest data from the continually assessed ten plants and the others from the same treatments and replicates, therefore all data was combined for analysis.

Canker incidence and severity

The canker incidence increased between 8 and 10 weeks after planting, from below 10% to between 38 and 58% (Fig. 69). There were no significant differences in incidence of canker, however plants treated with Amistar® as a pre-plant drench only had the lowest incidence.

All treatments with an Amistar® pre-plant drench significantly reduced the level of canker compared to the untreated control (Fig. 70).

There was no correlation between plant size and canker severity. The three treatments with the lowest canker severity (Amistar® pre-planting) had both the lowest number of large plants (Amistar® pre-planting and Amistar® pre-planting with Trichoshield™ post-planting) and the highest number of large plants (Amistar® pre-planting with Seasol® post-planting) (Fig. 71).

All treatments had between 28 and 40% of plants with adventitious roots (data not presented) and there were no significant differences between the treatments.

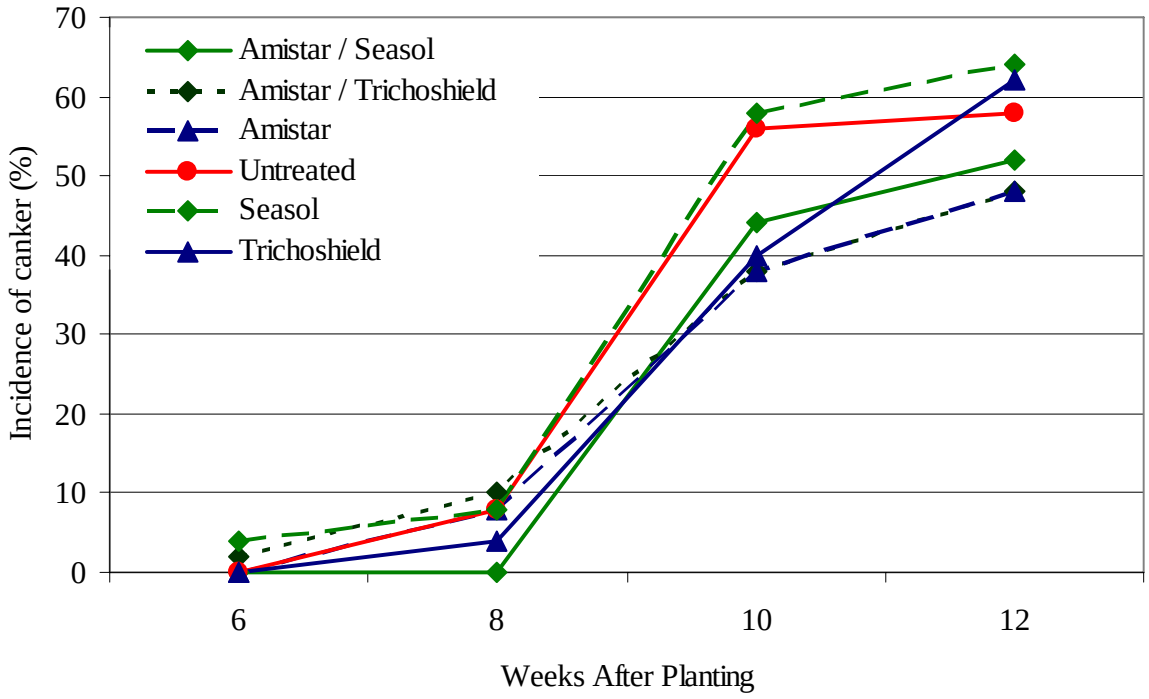


Figure 69 Mean canker incidence from 6 to 12 weeks after planting on cauliflower cv. Discovery drenched pre-planting and post planting with fungicides and biologicals.

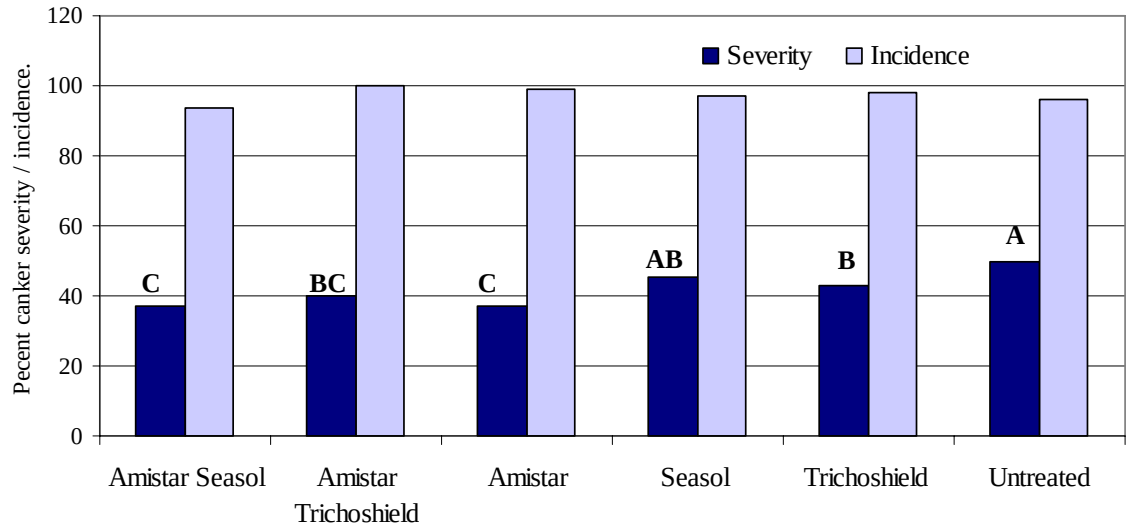


Figure 70. Incidence and severity of cankers on cauliflower plants cv. Discovery at harvest 14 weeks after planting, treated with various fungicides and biologicals pre planting and post-planting. Treatment means of severity with the same letter are not significantly different (P=0.05).

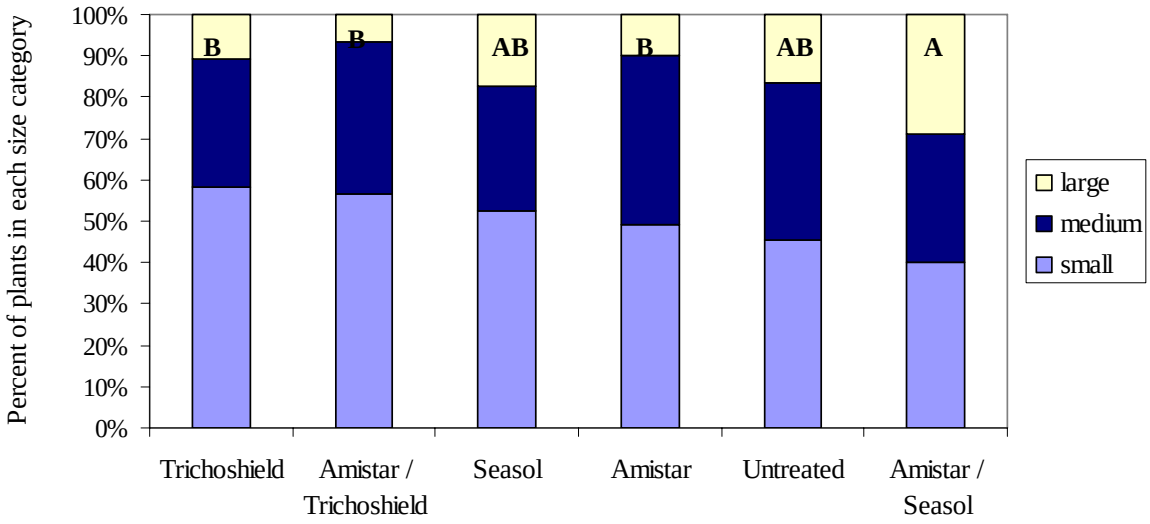


Figure 71. Percent of large, medium or small cauliflower plants cv. Discovery at harvest 14 weeks after planting, treated with various fungicides and biologicals pre planting and post-planting. Means of large plants with the same letter are not significantly different (P=0.05).

Soil and plant infection

Rhizoctonia AG 2.1 was found in all soil and plant material tested (Tables 64, 65). While *Rhizoctonia* AG 4 was detected in the initial soil and in three of the replicates sampled at planting, it was detected in only one sample of soil post-harvest and it was not detected in any of the plant material. Rhizoctonia AG 2.2 was not detected in any of the samples. The soil levels of *Rhizoctonia* 2.1 were lower in replicate one and increased up to replicate five. The opposite was found in both the *Rhizoctonia* AG 4, with higher levels detected in replicate one and reducing to replicate five. This graduation was also observed with the *Leptosphaeria*, while it was detected in all plant material, it was not detected in the soil at planting in replicates 4 and 5. This may be a result of the sloping block where the experiment was set up (Fig. 67), with replicate one at the top of the hill. The original disease in the Brussels sprouts was observed to be worse at the top of the hill. This was identified as *Leptosphaeria*, with no *Rhizoctonia* recovered from the plants, potentially the source of the higher levels of *Leptosphaeria* detected in the planting soil.

The levels of *Rhizoctonia* and *Leptosphaeria* DNA in the soil were lower after harvest than when sampled both initially and at planting (Table 64). The first sample was taken three weeks after incorporation of the Brussels sprouts crop, which may have increased the initial levels. However while there was significant plant infection with *Leptosphaeria*, the soil levels after harvest were low compared to pre-harvest levels. This was not a result of the fungicide treatments, as even the untreated soil samples post-planting had lower levels of *Rhizoctonia* and *Leptosphaeria* than the pre-planting samples. If the plants did not become infected with AG 4, the soil levels after harvest would not expect to be elevated.

Plants treated before planting with Amistar® had the lowest levels of *Leptosphaeria* and the lowest canker severity, while Seasol® treated plants with significantly higher canker severity had the highest level of *Leptosphaeria* and *Rhizoctonia* AG 2.1 detected (Table 66). As in other experiments, the DNA levels were too variable to obtain consistent statistically significant correlations between DNA and canker severity.

Table 64. Mean and range of pre-plant and post-harvest soil levels of* Rhizoctonia *and* Leptosphaeria *DNA (pg/g soil) over the trial area

	DNA pg/g soil: mean (range**)**		
	Initial*	**Planting**	**Post-harvest**
***Rhizoctonia* AG 2.1**	921	1,564 (623-2,808)	672 (121-2,898)
***Rhizoctonia* AG 2.2**	0	0	0
***Rhizoctonia* AG 4**	229,801	650 (225-2,320)	355 (0-1,419)#
Leptosphaeria	122	67 (11-261)	18 (3-60)

* Initial testing undertaken on one sample, planting and post-harvest mean of 12 samples.
# AG 4 detected in only one sample

Table 65. Mean and range of pre-plant and post-harvest soil levels of* Rhizoctonia *and* Leptosphaeria *DNA (pg/g soil) in each replicate area.

	DNA pg/g soil: mean (range**)**					
	***Rhizoctonia* AG 2.1**		***Rhizoctonia* AG 4**		***Leptosphaeria***	
Rep	**Planting***	**Post-harvest**	**Planting**	**Post-harvest**	**Planting**	**Post-harvest**
1	769	-	2,320	-	261	-
2	623	-	705	-	22	-
3	1,574	**512** (283-756)	225	0	31	**24** (10-60)
4	2,049	**1,159** (122-2,899)	0	**355** (0-1,419)#	13	**18** (6-39)
5	2,808	**347** (129-737)	0		11	**12** (3-20)

* planting testing undertaken on one sample, post-harvest four samples per replicate 3, 4 and 5 only.
# only one sample with AG4 detected post harvest.

Table 66. Pre plant and post-harvest soil levels of* Rhizoctonia *and* Leptosphaeria *DNA (pg/g soil or pg/g dried pant material), growing cauliflower cv. Discovery treated pre-planting with fungicides or biological agents.* Leptosphaeria *DNA means from plant material with the same letter are not significantly different (P=0.05).

Treatment	**pg DNA / g soil or dried plant material: mean (**range**)**			
	***Rhizoctonia* AG 2.1**		***Leptosphaeria***	
	Soil	Plant	Soil	Plant*
Seasol	**1,379** (501-2,899)	**80,813** (3,165-180,993)	**12** (6-20)	**308,380a** (172,465–436,791)
Trichoshield	**269** (122-509)	**14,133** (6,315-27,995)	**30** (11-60)	**192,443ab** (173,822-229,456)
Amistar	**409** (129-816)	**9,196** (3,861-17,333)	**19** (3-39)	**55,833b** (50,456-64,262)
Untreated	**632** (343-798)	**9,023** (7,731-10,289)	**11** (9-12)	**184,016ab** (101,247-261,708)

Figure 67. Experimental site in Adelaide Hills, Experiment 8.

4.3.7 Summary – field fungicide evaluations

The combined results of all products screened against stem canker in the field showed that none were effective (Fig. 68), but some provided suppression. All experiments used susceptible varieties and future work should compare cultivars in the same experiment with differing sensitivities to the pathogens, as this may enhance the suppressive effect. Maxim® and Amistar® applied as pre plant drenches suppressed the development of canker without the addition of post planting drenches. The combination of Maxim® pre planting and Amistar® post planting was the most effective treatment, although it was evaluated in only one experiment and further evaluation is warranted.

The two biological products tested, Seasol® and Trichoshield™, were not effective. However they showed some activity in greenhouse studies and should also be included in future evaluations as well as other similar products.

Future evaluations need to include an assessment of economic benefit, to determine whether the suppressive effect observed is of benefit considering the chemical and application costs. It addition, there needs to be a better understanding of the soil inoculum effect and environmental effects, as manipulation of some of these variables may be a management option.

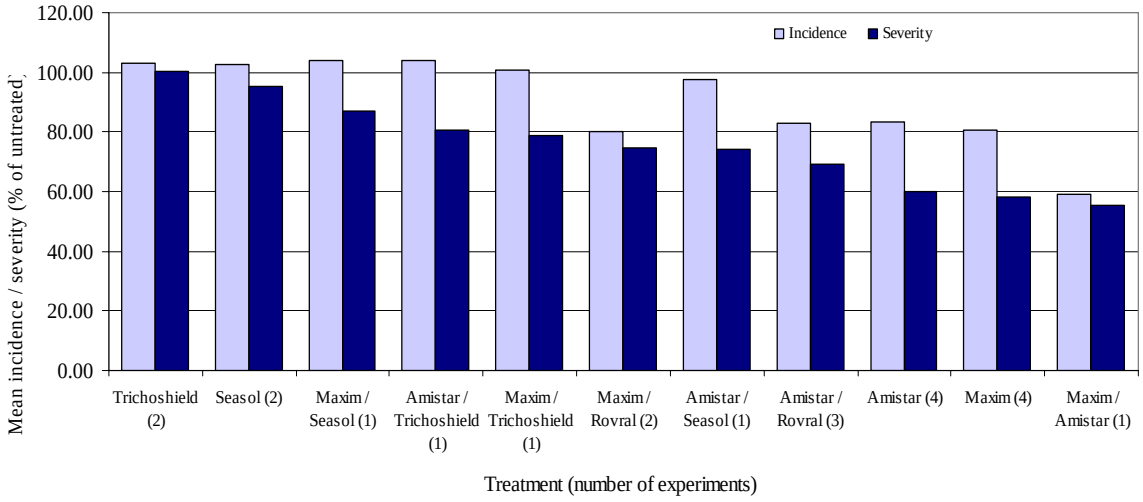


Figure 68. The mean incidence and severity of canker on cauliflower planted into infected soil drenched with fungicides before and after planting. The results of each trial were calculated as a percent of the untreated control and averaged over the number of experiments, listed in brackets after the treatment name.

5. REFERENCES

Abdelzaher, HMA (2003) Biological control of root rot of cauliflower (caused by *Pythium ultimum* var. *ultimum*) using selected antagonistic rhizospheric strains of *Bacillus subtilise New Zealand Journal of Crop and Horticultural Science 31*: 209–220.

Budge GE, Shaw MW, Lambourne C, Jennings P, Clayborn R, Boonham N and McPherson M (2009). Characterisation and origin of infection of *Rhizoctonia solani* associated with *Brassica oleracea* crops in the UK. *Plant pathology* 58:1059-1070.

Chakrabarty PK, Shyam KR, Bhardwaj SS (1989) Some new records of micro-organisms associated with curd rot of cauliflower. *Indian Journal of Mycology and Plant Pathology* 19:118-120.

Herr LJ (1976) In field survival of *Rhizoctonia solani* in soil and in diseased sugarbeets. *Canadian Journal of Microbiology* 22:983-8.

Hitch CJ, Hall BH and Wicks TJ (2006) Scoping Study to Determine the Soilborne Diseases Affecting Brassica Crops. Final report to HAL VG05005, 30 July 2006.

Keinath AP (1995) Relationships between inoculum density of *Rhizoctonia solani*, wirestem incidence and severity, and growth of cabbage. *Phytopathology* 85: 1487-1492.

Ko W and Hora FK (1971) A selective medium for the quantitative determination of *Rhizoctonia solani* in soil. Phytopathology **61**: 707-710.

Lancaster R and Burt J (2001) Cauliflower production in Western Australia. Bulletin 4521, WA Department of Agriculture.

Marks GC and Kassaby FY (1974) Detection of *Phytophthora cinnamomi* in soils. *Australian Forestry* **36**: 198-203.

McGee DC (1977) Black leg (*Leptosphaeria maculans* (Desrn.) Ces. et de Not.) of rapeseed in Victoria: sources of infection and relationships between inoculum, environmental factors and disease severity. *Australian Journal of Agricultural Research* **28:** 53-62.

Naseri B, Davidson JA and Scott ES (2008) Effect of temperature, cultivar and plant tissue on the germination of, and hyphal growth from, ascospores of *Leptosphaeria maculans*. *Australasian Plant Pathology* **37**:365–372

Pannecoucque J, Van Beneden S and Höfte M (2008) Characterization and pathogenicity of *Rhizoctonia* isolates associated with cauliflower in Belgium *Plant Pathology* 57: 737–746

Pannecoucque J and Höfte M (2009) Interactions between cauliflower and *Rhizoctonia* anastomosis groups with different levels of aggressiveness *BMC Plant Biology* 9:95

Paulitz, TC and Schroeder KL (2005) A new method for the quantification of *Rhizoctonia solani* and *Rhizoctonia oryzae* from soil. *Plant Disease* **89**: 767-772.

Pscheidt JW and Stevenson WR (1986) Comparison of forecasting methods for control of potato early blight in Wisconsin. *Plant Disease* **70**:915-920

Sosnowski MR, Scott ES and Ramsey MD (2006) Survival of *Leptosphaeria maculans* in soil on residues of *Brassica napus* in South Australia *Plant Pathology* **55**: 200–206

White JG, Crute IR, Wynn C (1984) A seed treatment for the control of *Pythium* damping-off diseases and *Peronospora parasitica* on brassicas *Annals of Applied Biology* 104:**241-247**

Saxena VC and Rajendra Singh (1987) A new wilt of cauliflower caused by *Fusarium equiseti* in India. *Indian Journal of Plant Pathology* 5:211-212.

Yitbarek SM, Verma PR, Gugel RK and Morrall RAA (1988) Influence of soil moisture, seeding date, and *Rhizoctonia solani* isolates (AG 2-1 and AG 4) on disease incidence and yield in canola. *Canadian Journal of Plant Pathology* 10:151-158

6. TECHNOLOGY TRANSFER

Research findings contained in this report have been presented to Industry by one-to-one contact, at grower meetings, through newsletters and magazine articles. Seminars and poster presentations have been presented to the scientific community in Australia and overseas.

Newsletters:

- Newsletter: Brassica Stem Canker. Issue 1, February 2007.
- Newsletter: Brassica Stem Canker. Issue 2, June 2008.

Industry magazines:

- Article: SARDI Communicator March 2007.
- The South Australian Grower, "National study puts canker under microscope", July 2007.
- PIRSA Primetime, "Canker Conundrum", Autumn 2007.
- Australian Vegetable Review "Brassica Disease study", September 2007.
- Article in HAL Vegetable Annual Industry Report, 2008.
- Article in Vegetables Australia magazine, 2009.
- Article in HAL Vegetable Annual Industry Report, 2009.

Conference proceedings/posters:

- Oral presentation at Australasian Plant Pathology Society (APPS) 16th biennial conference, Adelaide September 2007.

  C.J. Hitch, B.H. Hall and T.J. Wicks (2007). Brassica stem canker – the role of *Rhizoctonia*. In "Proceedings of the 16th Biennial APPS Conference, Adelaide" p34.

- Presentation of poster at the International Congress of Plant Pathology, Italy, August 2008.

  C. Hitch, B. Hall and T. Wicks (2008). Brassica Stem Canker - a disease complex of Brassicas. In "Proceedings of the 9th International Congress of Plant Pathology (ICPP) in Turin, Italy, 24 – 30 August 2008." Journal of Plant Pathology 90(2):S2.409

- Oral presentation at the International Rhizoctonia symposium, Germany in August 2008.

  Barbara H Hall, Catherine J Hitch, Belinda Rawnsley, Alan McKay, Trevor J Wicks, Sue Pederick and Kathy Ophel-Keller (2008). Using molecular diagnostic techniques to study Rhizoctonia solani infections in Brassica and onion crops. In "Proceedings of the 4th International Symposium on Rhizoctonia, Berlin, Germany, 20-23rd August 2008".

- Oral and poster presentation at the Australasian Soilborne Diseases Symposium, February 2009

  Hall B, Barlow T, Rawnsley B, Hitch C, Deland L (2009). Varietal resistance of cauliflower cultivars to soil borne diseases: *Rhizoctonia solani* and *Leptosphaeria maculans*. 5th Australian Soil borne diseases symposium, Thredbo NSW, p61-62

- Poster at Vegetable Conference, Melbourne, May 2009

- Oral presentation at Australasian Plant Pathology Society (APPS) 17th biennial conference, NSW September 2009.
  Hall BH, Deland L, Rawnsley B, Barlow, T, Hitch C, Wicks TJ (2009) Evaluation of fungicides to manage Brassica stem canker. In "Proceedings of 17th Australasian Plant Pathology Conference, Newcastle NSW" p 88.

Scientific seminar:

- Seminar: Brassica Stem Canker. Part of the SARDI Horticulture Seminar Series, Plant Research Centre, 23rd November 2006.

Industry/grower updates:

- Brassica Grower meeting, SA April 2007.
- Brassica Grower meeting, SA, July 2007.
- Brassica growers meeting, Victoria March 2008.
- National Vegetable expo, Werribee, May 2009.

Presentations at HAL / Industry meetings:

- Brassica think tank August 2008
- Rhizoctonia technical workshop, Melbourne, October 16-17 2007

7. MAIN OUTCOMES

7.1 Recommendations – scientific and industry

No single treatment will control stem canker. However there are several management strategies that will suppress the disease, including:

- Pre plant seedling drenches of 40 ml/100L Maxim® or 100 ml/100L Amistar®.
- Planting less susceptible cultivar such as Skywalker.
- Avoiding overwatering.
- Controlling weeds such as stinging nettle, fat hen, wireweed, common purslane and paddymelon that host *Rhizoctonia*.
- Incorporating of plant residue after harvest and leaving for at least 12 months to reduce inoculum load.

7.2 Recommended further work

Studies need to continue to evaluate more effective management strategies, including:

- Determining the economic threshold of stem canker.
- Evaluating biological products and soft options such as plant growth promotants to determine whether they will suppress the disease.
- Evaluating additional fungicides and application timings.
- Evaluation of rotation crops and bio-fumigation crops that may reduce inoculum load.
- Additional testing to confirm disease free status of nursery plants.
- Determining the soil inoculum levels of *Leptosphaeria* that infect plants and sources of inoculum other than soil borne.
- Evaluating cultivars of other Brassica crops such as Brussels sprouts and cabbage, and new cauliflower cultivars.
- Testing single stems to determine whether there is any relationship between *Leptosphaeria* and *Rhizoctonia* in plants.

8. ACKNOWLEDGEMENTS

We wish to thank and acknowledge Brassica growers of South Australia for their co-operation in allowing field experiments to be conducted on their properties: Frank Musolino, George Pannucio and Scott Samwell. A special thank you to Frank Musolino and son Steven for allowing continual access to their properties and providing their valuable time to transfer information and answer questions, and Gino Guidotto from Gino's Nursery for providing large numbers of seed and seedlings.

We gratefully acknowledge funding from the Vegetable Industry levy and the Commonwealth of Australia through Horticulture Australia Limited

Many people have been involved in undertaking this project we wish to thank for their valuable help: to the grower committee of Frank Mussolino, Gino Guidotto and Domenic Cavallaro for their input; to Mana, Ian, Shona, Matt, David for technical assistance; to the farm staff at Lenswood Research Centre and staff of the Plant Research Centre for their help; to the Root Disease Testing Service of SARDI and to Chris Dyson, SARDI statistician.

9. APPENDIX – NEWSLETTERS & POSTER

Newsletter 1

Brassica Stem Canker

Issue 1 February 2007

What is Brassica Stem Canker?

Brassica stem canker is a disease complex caused by several fungi. The symptoms range from superficial scurfing/russetting and discrete lesions on the stem to complete stem rot and plant collapse (Photos of symptoms overleaf).

Brassica stem canker was first observed on the Northern Adelaide Plains in South Australia in 2000. In recent years growers have reported losses of up to 80% due to stem canker.

A 12 month project funded by the Australian Vegetable Industry and Commonwealth Government through Horticulture Australia Limited started in July 2005.

The aims of the project were to:

- determine the extent of the problem in Australia
- investigate where the problem originates
- identify the cause of plant death
- determine the economic importance

A 3 year project started in July 2006 to investigate management strategies for brassica stem canker.

Survey Results - 2005/06

- 113 brassica crops were surveyed throughout Australia.
- Stem canker was found in all mainland states.
- Brassica stem canker was most severe in cauliflower but also affected Brussels sprout, red and green cabbage and broccoli.
- The disease was observed 4-6 weeks after planting and increased in incidence and severity as plants matured.
- The incidence of stem canker was highest in South Australia where 100% of some plantings were affected.
- On the Northern Adelaide Plains in South Australia the potential loss was calculated to be as much as $309,000 per week when disease levels were high.
- Brassica stem canker was found in ground continuously cropped, used in rotations and not previously planted to brassicas.
- The fungi *Rhizoctonia* (Wirestem), *Phoma* (*Leptosphaeria maculans* the cause of Black leg), *Fusarium* and *Pythium* were frequently recovered from diseased plants. No bacteria were detected.
- Brassica stem canker is a disease complex involving several fungi. Managing only one of these fungi will not effectively control the disease.
- Management strategies will need to be a mixture of cultural and chemical control.

Table 1. Brassica stem canker survey results 2005/06.

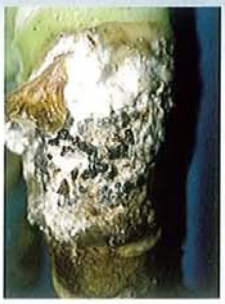
State	Crop	# plantings assessed	% plants affected
SA	Cauliflower	52	0-100
	Brussels sprout	6	64-100
	Red cabbage	3	49-83
	Broccoli	3	0-7
NSW	Cauliflower	13	4-74
	Brussels sprout	2	2-6
	Red cabbage	5	0-51
	Green cabbage	1	15
	Broccoli	1	1
Vic	Cauliflower	12	0-38
WA	Cauliflower	11	0-28
Qld	Cauliflower	4	1-25

For further information please contact

Catherine Hitch
SARDI Horticulture Pathology
GPO Box 397, Adelaide, SA, 5001

Tel: (08) 8303 9587 Fax: (08) 8303 9393
Email: hitch.catherine@saugov.sa.gov.au
www.sardi.gov.au

Stem Canker Symptoms



White mycelial growth

Most common fungus: *Sclerotinia.*

Other fungi: *Phoma, Rhizoctonia* and *Fusarium.*

Symptoms: White fluffy growth with black fruiting bodies (sclerotes).

Occurs: Common in mature plantings, random throughout.

Leads to: Plant collapse.

Phoma like lesions

Most common fungus: *Phoma.*

Other fungi: *Pythium, Rhizoctonia, Sclerotinia* and *Fusarium.*

Symptoms: Brown to purple lesions usually high on stem. Lesion contain small pinhead black dots (pycnidia).

Leads to: Stem canker and plant death.

Soft stem russetting

Most common fungus: *Phoma*

Other fungi: *Pythium, Rhizoctonia* and *Fusarium.*

Symptoms: Soft brown sunken lesions just above root zone.

Leads to: Stem canker and plant death.

Severe stem canker

Most common fungi: *Phoma* and *Fusarium.*

Other fungi: *Pythium, Rhizoctonia* and *Sclerotinia.*

Symptoms: Dry rot and stem death.

Leads to: Plant collapse and death.

Leaf lesions

Most common fungus: *Phoma.*

Other fungi: *Rhizoctonia* and *Fusarium.*

Symptoms: Black lesions on lower leaves close to stem usually contain pycnidia.

Leads to: Stem lesions and plant collapse.

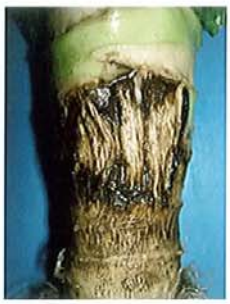
Lesion where leaf removed

Most common fungi: *Fusarium* and *Rhizoctonia.*

Other fungi: *Phoma, Pythium* and *Sclerotinia.*

Symptoms: Tan lesions with black margins located where older leaves have fallen off.

Leads to: Lesion increase in size and can cause stem canker.



Black cracked russetting

Most common fungi: *Pythium, Rhizoctonia* and *Fusarium.*

Other fungus: *Phoma.*

Symptoms: Superficial russetting of outer layer of stem. Black and usually pitted.

Leads to: Extensive pitting of stem can cause plant collapse.

Water soaked lesions

Most common fungus: *Pythium.*

Other fungi: *Phoma* and *Fusarium.*

Symptoms: Grey watery lesions high on stem.

Leads to: Lesion increase in size and can cause stem canker.

Lesion:	Area of diseased tissue.
Sclerotes:	Hard black structures, 1-5 mm. Produced to enable the survival of the fungus on debris and in the soil.
Pycnidia:	Small black structures (like poppy seeds) filled with spores of the fungus.

We would like to acknowledge funding from the Vegetable Industry and the Commonwealth Government through Horticulture Australia Limited.

Newsletter 2

Brassica Stem Canker

Issue 2 June 2008

In this issue - the role of *Rhizoctonia* in Brassica stem canker

Brassica stem canker.....

- **is a disease complex caused by several fungi**
- **can cause superficial symptoms through to plant collapse**
- **research is continuing at SARDI!**

Brassica stem canker was first observed on the Northern Adelaide Plains in South Australia in 2000. In recent years growers have reported losses of up to 80% due to stem canker. A 3 year project started in July 2006 to investigate management strategies for brassica stem canker.

As part of the SARDI research program, the incidence of stem canker was surveyed throughout Australia and the main symptoms of stem canker have been described. Stem canker was found in all mainland states and is most severe in cauliflowers. Results of the survey were presented in Issue 1 which is still available by contacting Tiffany Barlow.

For further information please contact:

Tiffany Barlow
Tel: (08) 8303 9587
Email: barlow.tiffany@saugov.sa.gov.au

or
Barbara Hall
Ph: (08) 8303 9562
Email: hall.barbara@saugov.sa.gov.au

SARDI Horticulture Pathology
GPO Box 397, Adelaide, SA 5001
Fax: (08) 8303 9393
www.sardi.sa.gov.au

Disease progression of *Rhizoctonia*

Brassica stem canker is caused by several fungi including *Rhizoctonia* sp., *Leptosphaeria maculans*, *Pythium* sp., and *Fusarium* sp.. *Rhizoctonia* has been the dominant fungus found in cauliflowers on the Northern Adelaide Plains. There are many different groups of *Rhizoctonia* fungi, and three groups are involved in Brassica stem canker, AG 2.1, AG 2.2 and AG 4.

To determine when *Rhizoctonia* infects the cauliflowers, soil and plants were tested using DNA analysis. No infection was found in the seedlings before planting, but the field soil was infected with *Rhizoctonia*. Two weeks after planting, *Rhizoctonia* was identified in seedlings, indicating that infection from the field soil occurred within 2 weeks of planting. However symptoms of stem canker did not appear until 8 weeks after planting (see table below - bold figures show when symptoms were observed). The levels of DNA in the plant material were highest just before symptoms were observed.

Levels of *Rhizoctonia* detected by DNA testing of stems and soil (pg/g)

weeks after planting - stems	AG 2.1	AG 4
0	0	0
2	15	34
4	2,200	965
6	59,080	918
8	**2,254**	**19**
12	**96**	**7**
16	**626**	**2**
Soil before planting	48	769
Soil after harvest	137	2,712

These tests showed that the amount of *Rhizoctonia* in the soil increased during the life of the crop, and that control of *Rhizoctonia* needs to occur early.

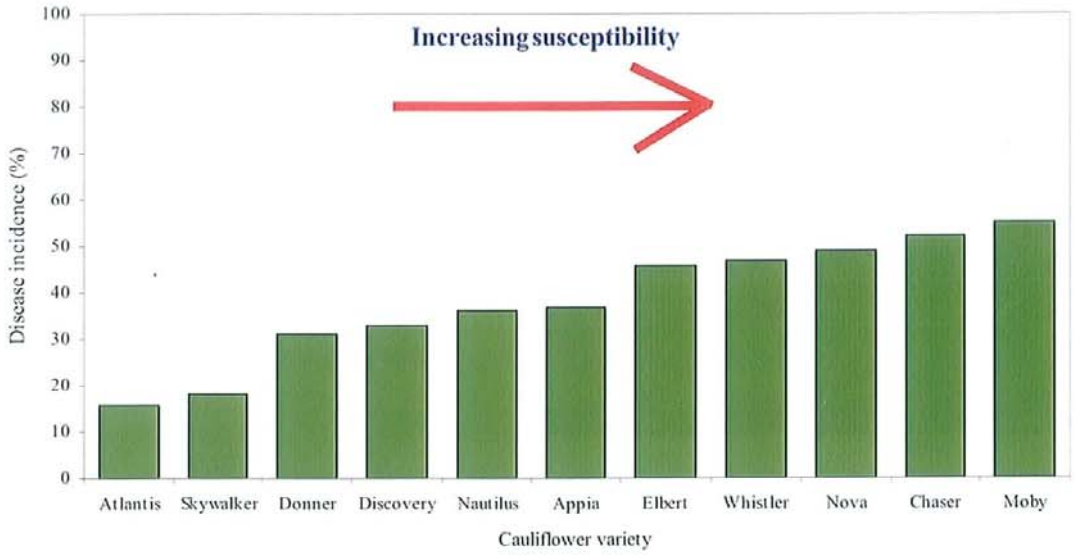
Brassica Stem Canker

Are some cauliflowers varieties more susceptible to *Rhizoctonia* than others?

In a greenhouse experiment at SARDI, eleven cauliflower varieties were treated with *Rhizoctonia*, to see which varieties were the most susceptible.

The caulifower varieties Nova, Chaser and Moby were the most susceptible to *Rhizoctonia* and a higher incidence of disease symptoms were seen on these plants. Disease was less obvious on Skywalker and Atlantis plants. At this stage it appears that variety can play a role in susceptibility to at least one component of stem canker, *Rhizoctonia*. Research in this area is ongoing to determine if these varieties are also less suceptible to the other fungi involved in the stem canker complex.

Susceptibility of cauliflower varieties to *Rhizoctonia*



What's next for stem canker research?

- A greenhouse trial is underway to test the susceptibility of cauliflower varieties to other stem canker fungi including *Leptospaeria maculans* (black leg). Eleven common varieties are being assessed for stem canker symptoms on a weekly basis. This will add valuable information on variety selection for fields with a history of stem canker.
- Fungicides are being tested on cauliflower seedlings being grown in infected soil - so far the results are promising and field trials are planned to support the greenhouse experiments.
- We don't yet know how long stem canker pathogens can persist in the field. Experiments are underway to test the level of pathogens in crop debris over time, these trials have been established at Virginia and will be monitored over a 12 month period.

This project was facilitated by HAL in partnership with AUSVEG and was funded by the National Vegetable Levy. The Australian Government provides matched funding for all HAL's R&D activities.

Poster presentation, ASDS 2009.

SARDI

South Australian Research and Development Institute

Varietal resistance of cauliflower cultivars to soilborne diseases: *Rhizoctonia solani* and *Leptosphaeria maculans*

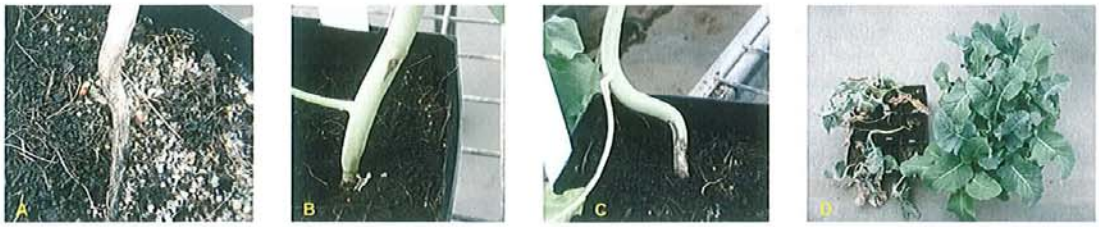
B.H. Hall, T. Barlow, B. Rawnsley, C.J. Hitch & L. Deland

Plant Research Centre, GPO Box 397, Adelaide SA 5001, Australia

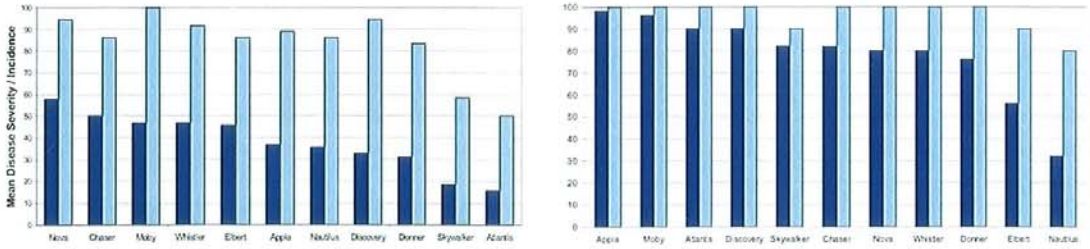
Government of South Australia

Brassica stem canker is a disease complex of several soilborne fungi, the dominant pathogens being *Rhizoctonia solani* (Anastomosis groups AG 2.1, AG 2.2, AG 4) and *Leptosphaeria maculans*. Symptoms vary from superficial scurfing to complete rotting of the stem and plant death. While all Brassica are affected, cauliflower are the most susceptible. The use of resistant varieties is an effective management strategy for soilborne diseases, therefore tests were undertaken to evaluate the susceptibility of cauliflower to *R. solani* and *L. maculans* using cultivars commonly planted in South Australia.

Stem inoculation with mycelial plugs (A) caused cankers with *R. solani* (B) and *L. maculans* (C). **Foliar inoculation** (D) with *L. maculans* was not successful.



***L. maculans* root dip inoculation.** Symptoms after 8 weeks: complete stem death (A), localised lesions at leaf scars (B), lower stem lesions at ground level (C). Appia was the most susceptible cultivar (D - left) and Nautilus the least susceptible cultivar (D - right).



Incidence and severity of stem canker on cauliflower cultivars 8 weeks after soil inoculation with *R. solani* (L) or root dip inoculation with *L. maculans* (R). *R. solani* graph shows the means of results from inoculations with AG2.1, 2.2 and 4.

None of the cultivars tested were resistant to both *R. solani* (AG 2.1, 2.2 and 4) and *L. maculans*, although some had reduced susceptibility. Skywalker and Atlantis were least infected by *R. solani*, and Nautilis and Elbert least infected by *L. maculans*. Investigations into effective management strategies for Brassica stem canker are continuing.

AUSVEG

This work was facilitated by Horticulture Australia Ltd (HAL) in partnership with AUSVEG and funded by the National Vegetable Levy. The Australian Government provides matched funding for all HAL's R&D activities.

HAL