Brassica Stem Canker: Phase 2

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Managing Brassica Stem Canker Phase 2

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South Australian Research and Development Institute

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SARDI
SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE

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This report presents results of experiments evaluating management strategies for Brassica stem canker.

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1 MEDIA SUMMARY

Stem girdling cankers have been observed on many Brassica crops in Australia since 2000 with varying degrees of severity. Previous work has shown this to be caused by a disease complex including the soil borne fungi *Rhizoctonia solani* (anastomoses groups AG2.1, AG2.2 and AG4) and *Leptosphaeria maculans.*

Soilborne diseases are often difficult to control, as the pathogens survive in soil or on alternative hosts for long periods in the absence of the crop host. While *Leptosphaeria* infects mainly Brassicas, *Rhizoctonia* has a much wider host range, infecting most vegetables.

Previous trials on commercial properties showed none of the fungicides presently registered for use on brassicas controlled the disease. However preliminary greenhouse trials showed that incidence and severity of stem canker on potted cauliflower was suppressed by the use of plant growth products and biological agents. In addition, roots were produced above the rotting and plant growth was not affected by the stem canker.

This project investigated aspects of *L. maculans* infection as part of the canker complex and evaluated commercially available plant growth products and biological agents. These were combined with fungicides shown to suppress the disease to determine whether the combination of fungicides and plant growth products improved the suppression of stem canker more than either product applied alone. Fungicides used to control *L. maculans* on canola were also included in the evaluations. Investigations were also undertaken to confirm if *L. maculans* could infected cauliflower from leaf or seed infection.

L. maculans was not found in nursery seedlings in previous work and infection was thought to develop from soil inoculum. However these studies showed seedling infection occurred in nurseries. Airborne spores of *L. maculans* were detected near cauliflower plantings indicating potential for foliar infection. Wounded leaves were shown to be susceptible to infection, the disease spreading systemically from the leaf infection down the stem. As *L. maculans* can infect both root and leaf, new infections can originate from either seed, soil or airborne inoculum.

Chemical management strategies were targeted to the use of soil drenches applied in the nursery prior to planting and in the field. The biological agents and alternative plant growth products were also applied both pre-planting and after planting as soil drenches.

None of the products evaluated provided complete control of stem canker. The fungicides Impact-In-Furrow® (flutriafol) and Jockey (fluquinconazole) in combination with Amistar ® (axozystrobin) reduced stem canker in both greenhouse and field evaluations, and registration of these products for use on vegetable Brassica crops is recommended. No cankers developed in plants grown from seed treated with fluquinconazole and this may be a useful treatment in nurseries.

Stunted plants were observed where fungicides drenches were applied prior to planting, however there was no measurable reduction in yield. These studies

confirmed previous findings that products applied after planting did not reduce canker severity and disease management was more effective with pre-planting treatments.

Overall this work has showed that the use of plant growth products and fungicides in low disease situations did not significantly reduce stem canker severity, but could improve plant growth. However the increase in yield achieved under low disease conditions may not provide economic benefit to warrant the application of the products. This needs to be further evaluated with conditions of high disease pressure, as the improved growth observed may be beneficial by providing stronger root and plant growth to achieve a marketable yield even with stem canker present. The development of pre-planting soil tests to predict disease risk would need further evaluation, as these studies showed the disease could occur where no pathogen were detected in the soil prior to planting. Currently only paddock history can be used to predict the potential severity of disease.

The use of biological and plant growth products in combination with fungicides should continue to be evaluated and the economic benefit analysed to determine whether the improvement in productivity is worth the cost of applying the product.

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. The primary fungal pathogens have been identified as *Leptosphaeria maculans and Rhizoctonia solani* AG 2.1, 2.2 and 4.

These fungi are mainly soilborne and plants were infected early after transplanting into infected soil. Applications of fungicides such as azoxystrobin and fludioxonil prior to transplanting nursery seedlings suppressed stem canker, but none provided complete control.

In 2009/10, 19 greenhouse and 9 field experiments were undertaken to improve the knowledge of stem canker and provide further management options.

The main findings of this study were:

- There was no correlation between pre-planting soil amounts of *L. maculans* and severity of stem canker at harvest.
- *L. maculans* infects through leaves, roots and seed. The fungus was detected in spore traps near cauliflower plantings, showing potential for airborne dispersal. Infection through leaves was more severe when leaves were wounded and the pathogen moved systemically into the stem. Infected plants were detected in nurseries indicating seed infection.
- Brassica weeds (white mustard, wild rocket) and other Brassica crops (radish, rocket, kale and chinese cabbage) were all susceptible to *L. maculans* when inoculated leaves were wounded. Kale and chinese cabbage were also infected without wounding.
- The fungicides fluquinconazole and flutriafol, registered to control or suppress *L. maculans* in canola, reduced or eliminated stem canker when applied as pre transplanting drenches in combination with azoxystrobin. Fluquinconazole was also affective as a seed dressing.
- Greenhouse evaluations showed suppression of canker and increased plant growth when the biological agents *Trichoderma* (Trichoshield™) and *Bacillus subtilis* (Companion®) were used with fungicides as pre-transplanting drenches. Alternative plant growth products Rootpower® and Soil-Reviva® also reduced canker severity.
- Field evaluation showed the combination of azoxystrobin and flutriafol applied prior to transplanting reduced canker severity on both cauliflower and Brussels sprouts.
- There was no benefit in canker suppression by applying azoxystrobin, biological agents or plant growth products after transplanting.
- The increase in yield with the improved plant growth and suppression of stem canker may not provide economic benefit to warrant the application of the products. This needs to be further evaluated.

3 INTRODUCTION

The project VG05005 "Scoping study to determine the soilborne diseases affecting Brassica crops" (Hitch *et al* 2006) showed that a disease complex 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 to break at or before harvest.

The project VG06018 "Managing Brassica stem canker" further defined the disease, showing the main pathogens in the fungal complex were *Rhizoctonia solani* and *Leptosphaeria maculans* (Hall *et al* 2009). In South Australia in 2007, *Rhizoctonia* was the dominant pathogen found in many of the cauliflower plantings on the Northern Adelaide Plains. However in 2008, *L. maculans* was more prevalent. *L. maculans* is the fungus that causes the disease "black leg", and has been found as part of the canker complex and also as separate lesions. *Rhizoctonia* has many sub groups known as anastomoses groups (AG) (Parmeter *et al* 1969, Carling *et al* 2002) and three sub groups of *Rhizoctonia* (AG2.1, AG2.2 and AG4) were detected in the affected Brassica plants. Both fungi are soil borne and infection occurs within 2 weeks of planting. *Rhizoctonia* is also the cause of the disease "wirestem" in Brassica, a significant issue in Western Australia where it causes early plant losses in infected paddocks (Lancaster 2006). However this symptom is rarely seen in field cauliflower in South Australia where stem canker is found.

Soilborne diseases are often difficult to control, as many pathogens survive for long periods in the absence of the crop host and often have a wide host range. While *Leptosphaeria* infects mainly Brassicas, including weeds, *Rhizoctonia* has a much wider host range, infecting most vegetables. Previous work (Hitch *et al* 2006, Hall *et al* 2009) showed *L. maculans* infection was from the soil, despite growers observing stem canker in cauliflower and Brussels sprouts planted into soil not previously planted with these. In canola, this fungus is known to be both seed borne and airborne in canola and has occurred in fumigated soil and soil not previously planted to canola (Sprague *et al* 2009).

The project VG06018 (Hall *et al* 2009) evaluated fungicides known to have efficacy against these fungi, with initial screening in greenhouse trials followed by field trials on commercial properties. The field evaluation was limited to fungicides registered for use on Brassicas, or ones that the manufacturer indicated they would support for permit use. An added complication was that some fungicides showed differences in the level of control between the different *Rhizoctonia* anastomoses groups, therefore only those which were effective against all three groups were used.

While results in the greenhouse were promising, the fungicides evaluated in commercial properties did not provide effective control, suppressing the severity but not the incidence of the disease. However preliminary greenhouse trials showed that incidence and severity of stem canker on potted cauliflower was suppressed by the use of plant growth products and biological agents. In addition, roots were produced above the rotting and plant growth was not impacted by the stem canker.

Studies have shown that nutrient availability plays a significant role in root architecture (Lopez-Bucio *et al* 2003). Improving available nutrients improves root growth, and studies have shown that plant growth promoting agents reduce the effects of disease and improve yield (Kloepper *et al* 1980, McCullagh *et al* 1996). The use of biological agents to suppress and control disease has also been extensively studied, and species of the fungus *Trichoderma* and bacteria *Bacillus* are widely used and commercially available (Papavizas 1985). Both also are effective at suppressing *Rhizoctonia* (Kwok *et al* 1987, Weindling 1934, Osaka & Shoda 1996) and are known to induce systemic resistance in plants (Hoitink *et al* 2006).

Fungicides used in the canola industry for management of black leg (flutriafol and fluquinconazole) were trialled in combination with fungicides shown to be suppressive in previous work (Hall *et al* 2009) to determine if this improves control of stem canker. Fluquinconazole is registered as a suppressant for *L. maculans*, and is applied as a seed dressing. Flutriafol can be applied coated on fertiliser and has a shorted withholding period in canola, which may have less residue issues in fresh produce brassica crops such as cauliflower and Brussels sprout.

Brussels sprout growers had reported variations in sprout size in canker affected plants, which led to multiple harvest passes and extra harvesting costs. Therefore trials were undertaken on both cauliflower and Brussels sprouts.

This project continued and expanded the work of VG06018 by further evaluating commercially available plant growth products and biological agents in greenhouse trials and on commercial properties. These were combined with fungicides to determine if the combination of products improves the suppression of stem canker. In addition the potential of *L. maculans* inoculum originating from seed and airborne spores was investigated.

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). *Leptosphaeria maculans* spore suspensions were stored at -18 degrees in 175ml Nalgene rectangular screw cap plastic bottles.

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: *Rhizoctonia* and *Leptosphaeria* cultures were inoculated onto cauliflower seedlings or kale to confirm pathogenicity of the isolates. 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 fungal isolates were required for inoculation, cultures on plates or in bottles were removed from storage and kept at room temperature for ~24 h prior to use. *Rhizoctonia* were plated onto potato dextrose agar (PDA) 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.

Frozen *Leptosphaeria maculans* inoculum was defrosted by placing the frozen bottle in a water bath maintained at 20 degrees.

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 with sterile water to $1x10^6$ spores/ml.

Root inoculation: Seedlings were gently removed from trays and the roots washed in tap water 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 water 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 24 hours to create near 100% humidity. Control plants were sprayed with sterile water with Tween 20.

Bulk soil inoculation: A specified amount of the spore suspension was mixed thoroughly with bulk soil and left covered for various times before use.

Pot soil inoculation: Adapted from a method of Hua Li *et al* (2007), three 1ml aliquots of the spore suspension were injected into the soil in a triangular arrangement (Fig. 1). A 1ml syringe was inserted 7.5ml into the soil and the spore suspension injected as the syringe was withdrawn to distribute the liquid evenly along the length of the hole. For the control pots, distilled water was applied in the same manner. Following inoculation the pots were moistened to further distribute the inoculum throughout the top of the pot.

Figure 1. *Inoculation of potted soil with Leptosphaeria maculans by injecting spore suspension (~10^6 spores/ml) into the holes indicated prior to planting.*

4.1.3 Plant & soil testing

Isolation from plant material

Diseased tissue removed from the stems and roots of affected plants was surface sterilised using 2% sodium hypochlorite solution, rinsed thoroughly in tap water, dried in a laminar flow cabinet and plated onto ¼ PDA, PDA or TWA. 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.

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.

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 ~-18^0C until required. When ready for testing, the material was freeze dried and ground prior to DNA extraction.

Soil was collected using the SARDI "Accucore" 10 ml sampler. Unless otherwise stated, 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 4^0C for up to a week, oven dried, or frozen at ~-18^0C 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 in pre-seeded speedling trays. 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. 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. Other pot sizes used are listed in table 1. Unless otherwise stated, all pots or trays were filled with steam sterilised coco peat mix (SARDI Greenhouse Services, Plant Research Centre).

Table 1*. Pot sizes and soil volumes used in greenhouse trials*

Pot	Diameter (mm)	Depth (mm)	Soil volume (L)
100mm	100	85	0.55
165mm	165	100	2.0
175mm	175	150	3.3
200mm	200	180	4.7

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 without plant growth products added were fertilised every two weeks with Thrive® applied at label rates. As the plant growth products required no additional fertiliser, other plants in these trials were maintained with the equivalent grams of nitrogen per plants as the treated plants using sulphate of ammonia.

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.

Fungicides showing suppressive effects in previous work (Hall *et al* 2009) and two registered on canola for control or suppression of *Leptosphaeria maculans* were included in greenhouse and field evaluations (Table 2). Plant growth products and biological products (Table 3) were selected that claimed to suppress diseases caused by soil borne fungi.

Application methods

Product rate determination

Unless otherwise specified, products were prepared and applied at rates shown in the label. If a label rate for drenching was not specified, the amount of product 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 product 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 products to simulate drench applications in the nursery. Speedling trays were immersed in a product suspension for up to five minutes to optimise penetration of the product into the soil and root matrix. Treated trays were drained and unless otherwise specified planted within 24 hours of treatment. Water was used as the control treatment.

Post-planting drench

Pots were treated with products applied to the soil surface to mimic a field application. Products were applied to field experiments at label rates using a pressurised back pack sprayer. Water was applied as a control.

Table 2. *Fungicides evaluated for control of stem canker.*

Trade name	**Active ingredient**
Amistar 250 SC®	250g/L azoxystrobin
Impact Endure®	500 g/L flutriafol
Jockey Seed Fungicide ®	167g/L fluquinconazole
Maxim 100FS ®	100g/L fludioxonil

Table 3. *Biological and plant growth products evaluated for control of stem canker.*

Trade name	**Ingredients**
Biological products	
Companion	*Bacillus subtilis*
Fulzyme Plus	*Bacillus subtilis*
Mycotea	*Trichoderma lignorum, Chaetomium globosum, Verticillium lacanii, Paecilomyces lilacinus, Penicillium chysogenum, Azobacter chioococcum, Bacillus polymyxa, Saccharomyces cerevisiae*
Plantmate granular	*Trichoderma atroviride/ harzianum*
Trichoshield™	*Trichoderma harzianum, T. lignorum, Gliocladium virens, Bacillus subtilis*
Plant Growth products	
Acadian	Cytokinins, seaweed extract
Bio-forge	2 % nitrogen, 2.5% potassium
Go Go Juice	Undisclosed
Manutec Zinc Sulphate	36% zinc and 17.6% sulphur
Manutec Manganese Sulphate	31% manganese 20% sulphur
Mega-Kel-P	Organic based multinutrient, cytokinins, auxins, fulvic and amino acids
Microbial	0.2 % Quaternary Ammonium compound
Profert	Multi-nutrient
Rhizotonic	Sea algae extract, polyuronic acids, iodine, trace elements, salts
Rootfeed	12% nitrogen, 8.5% calcium, 2% magnesium
Rootpower	20% zinc oxide
Seasol	Seaweed concentrate
Soil Reviva	Probiotic microbes, NPK, secondary nutrients, trace elements
TM21	Biological stimulant
X-Press	10% zinc oxide, 5% manganese oxide, 5% copper hydroxide
X-Tender	9.5% cobalt sulphate, 7.6% sodium molybdate

4.1.6 Assessments & experiment design

Stem cankers were assessed visually while growing using a 0-4 rating system (Table 4, Fig. 2).

Table 4. *Disease rating system used to assess stem canker symptoms.*

Rating	Percent rating	Description
0	0%	Healthy
1	25%	½ stem canker
2	50%	full stem canker
3	75 %	severe canker (wilt)
4	100%	plant death

Figure 2. *Disease rating system used to assess stem canker symptoms. From L-R: Rating 0, 1, 2, 2. Ratings 3 had cankers similar to 2 but with plants wilted and rating 4 the plants were dead.*

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.

Plant growth measurements included:

Fresh plant weight. The roots were removed at soil level, the head removed and if necessary the plant material washed and dried before weighing.

Stem length. The stem measured from soil level to the first expanded leaf.

Number of expanded leaves.

Presence of head.

Head size. The overall head size was rated comparatively where 1=small, 2=medium and 3=large head.

Plant size. The overall plant size was rated comparatively where 1=small, 2=medium and 3=large plants.

Root ball size. The size of the root ball was rated comparatively where 1=small, 2=medium and 3=large.

Stunting. Unless otherwise specified, stunted plants were those shorter than ¾ the height of the average plant in either the un-inoculated control (greenhouse trials) or the untreated control (field trials).

Unless otherwise stated, all greenhouse and field experiments were laid out in a Randomised Block design with 4 to 10 replicates.

4.1.7 Statistical analysis

Analysis was undertaken using either the Analytical Software Statistix® V8 or Genstat.

4.2 Soil inoculum

Previous work (Hall *et al* 2009) showed that *Leptosphaeria maculans* could be detected in high amounts in plants even where the pathogen was not detected in the soil prior to planting. Work was undertaken to determine the amount of inoculum required to cause canker symptoms in cauliflower, and the levels of infection of both *L. maculans* and *R. solani* in the plants and soil during growth.

4.2.1 Effect of Leptosphaeria maculans soil inoculum

Aim: to evaluate the effect of *L. maculans* on plant growth and canker development on cauliflower planted into infected soil.

Methods

Sterile cocopeat was inoculated with *L. maculans* as previously described. Inoculated cocopeat was then mixed thoroughly with clean sterile cocopeat 1:3 and 1:1 to provide 25% and 50% dilutions respectively.

15 replicate 200 mm pots were filled with the four rates of inoculated soil (un-inoculated, 25% dilution, 50% dilution and undiluted). The pots of soil were left for 1 week before planting with 5 week old cauliflower cv. Skywalker seedlings. Plants were irrigated with 2 L/Hr inline drippers to soil saturation daily.

Severity of stem cankers were assessed at 14 to 25 day intervals after planting and stunting assessed at 8 weeks after planting as previously described. Plants were harvested 14 weeks after planting, and plant growth assessed by measuring head weight, plant vegetative weight (not including roots or head) and length of plant stalk from soil level to first leaf. The comparative root development of each plant was rated as 1 (sparse), 2 (medium) or 3 (dense roots) (Fig. 3).

One core sample (0-10cm) was taken adjacent to the plant stem from each of the 15 replicate pots using a SARDI "Accucore" 10 ml sampler. The soil cores for each inoculation level were combined and tested for *L. maculans* by PCR as previously described.

Figure 3. *Comparative root ball size (left to right) of 3 (dense roots), 2 (medium) or 1 (sparse).*

Results and discussion

Cankers were first observed 8 weeks after planting, although there was no difference between the treatments (data not shown). At this time stunting was observed in all plants grown in the 100% inoculated soil (Figs. 4, 5, Table 5.).

Figure 4. *Comparative plant growth of cauliflower cv. Skywalker 8 weeks after planting into 100% inoculated soil (L), 50% inoculated soil (Centre) or un-inoculated soil (R).*

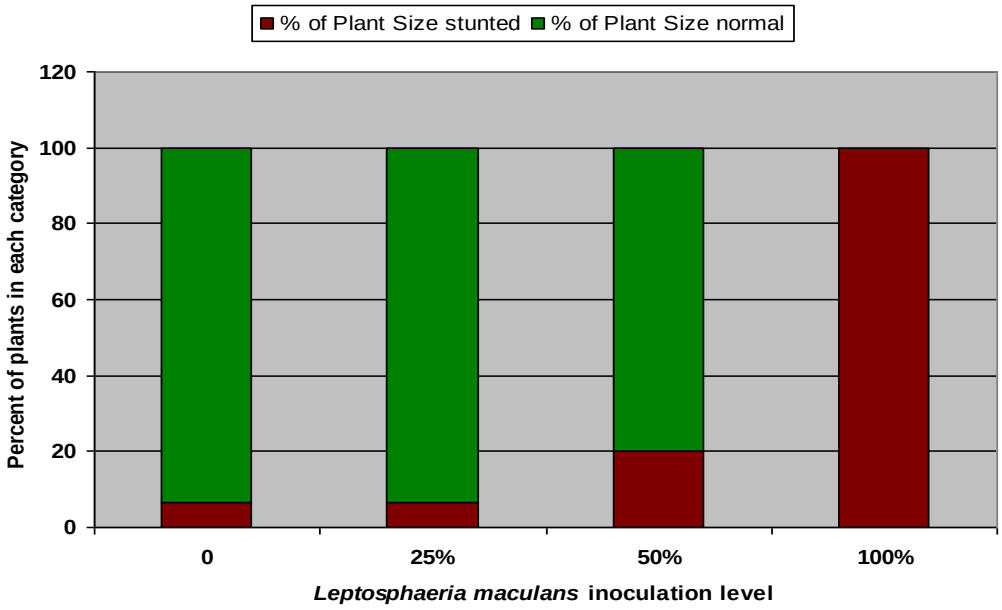


Figure 5. *Proportion of stunted cauliflower cv. Skywalker plants 8 weeks after planting into various levels of inoculated soil.*

At harvest, the greatest effect on plant growth was observed at 100% inoculum level, with significantly lower head weight and plant weight (Table 5). There was little variation in plant growth parameters in the lower inoculum rates. Severity of stem canker was low, and while there was a trend for the plants in 100% inoculated soil to

have more canker, the differences were not significantly different (Table 5). Plant fresh weight was observed to be 54% of that of cauliflowers grown in un-inoculated soil. For cauliflower production this may impact on the plants ability to produce a large head suitable for a target grade, with leaves sufficiently large enough to provide protection from curd discolouration at blanching, thereby affecting market suitability.

The PCR test detected *L. maculans* only in the soil at the 100% inoculum level. While previous work has shown this fungus to be difficult to detect in soil (Hall *et al* 2009), the low levels may explain why the canker severity was also low. *Leptosphaeria* may have caused root damage resulting in reduced plant size in the absence of physical stem cankers.

Table 5. *Amounts of DNA, plant growth and canker severity of cauliflower cv. Skywalker plants, 14 weeks after planting into various levels of Leptosphaeria maculans inoculated soil. Means with the same letter are not significantly different (P=0.05).*

Inoculation level	***L. maculans* pg DNA/g soil**	**Stem canker severity**	**Plant weight (g)**	**Head weight (g)**	**Stem length (cm)**	**Mean root ball rating**
0	0	8.3	360 a	216 a	18.7 b	2.7
25%	0	6.7	332 a	186 a	22.3 a	1.7
50%	0	8.3	329 a	217 a	19.2 ab	2.3
100%	1	13.3	193 b	88 b	16.9 b	1.7

4.2.2 Soil inoculum levels during plant growth

Aim: To monitor amounts of *L. maculans* and *R. solani* in soil and cauliflower plants over time.

Methods

Two bins, each with 54Kg cocopeat, were inoculated with either 412.5 ml of *Rhizoctonia* AG 2.1 mycelial slurry or 1.5 L of *L. maculans* spore suspension as previously described. After one week the inoculated soils were placed into Mk9 pots and 30 replicate pots of each pathogen and 30 of un-inoculated cocopeat planted with 6 wk cauliflower cv. Skywalker.

The remaining 30 replicate pots of each pathogen were left unplanted with another 30 replicate pots of un-inoculated cocopeat.

40 Kg of field soil collected from a commercial grower site in the Northern Adelaide Plains where the disease had previously been observed was steam sterilised at 120 degrees for 1 hr. Half was inoculated with 137.5 ml *Rhizoctonia solani* AG 2.1 mycelial slurry and half with 500 ml *Leptosphaeria maculans* spore suspension as previously described. After one week the inoculated soils were placed into Mk9 pots and 20 replicate pots of each pathogen planted with 6 wk cauliflower cv. Skywalker.

Pots were maintained in the greenhouse as previously described, with the unplanted pots given the same fertiliser and watering regime

Sampling

Prior to planting, four subsamples of 10 randomly chosen plants were removed from the nursery supplied trays of seedlings, root washed in sterile water, and frozen at -22 degrees before freeze drying and PCR analysis for *Rhizoctonia* and *L. maculans*.

Plants were removed from five of the inoculated pots at 1, 2, 3, 4, 6 and 8 weeks after planting and assessed for symptoms of stem canker. The stem was removed at soil level using a sterile scalpel from the 3, 4, 6 and 8 week plants, frozen and stored at -22 degrees prior to freeze drying and PCR analysis.

At the same times, soil from each of the five planted pots and from five unplanted pots was sampled by collecting 4 soil cores from each corner of the pots. Soil was stored at 4 degrees prior to being oven dried and PCR tested as previously described.

At 2 and 6 weeks after planting into the field soil, plants were assessed for stem canker and then separated at soil level for plant testing as previously described. For five pots, the whole pot of soil including root ball was PCR tested. For the remaining 5 pots, 4 soil cores were taken from around each plant stump for testing.

Results and discussion

Leptosphaeria maculans

L. maculans was not detected in the 40 plants tested from the nursery or at any sampling times from the un-inoculated cocopeat (data not presented). *L. maculans* was detected in all pots of inoculated cocopeat at all sampling times at amounts from 36 to 634 pg DNA/g soil (Table 6). *L. maculans* was detected in all field soil pots at 2 weeks after planting with amounts of 1 to 14 pg DNA/g soil (Table 6). However at 6 weeks after planting *L. maculans* was not detected in three of the five pots of field soil.

Severity of canker did not increase with time and of the 40 plants harvested, only six planted in cocopeat had developed cankers (Table 6). All the plants with cankers had *Leptosphaeria* DNA detected in the stems, however it was also detected in the stems of four of the remaining 34 plants, confirming previous findings (Hall *et al* 2009) that infection could be present without symptom development. There was no correlation between soil DNA and severity of stem canker (P=0.39)

The DNA amounts of *L. maculans* in the inoculated pots declined over time, even when planted with cauliflower (Fig. 6). This is contrary to previous findings (Hall *et al* 2009), where amounts at the end of a crop were generally much higher than at planting. As expected, the rate of decline was greater in unplanted soil. This indicates that pathogen carryover can be reduced by allowing ground to be fallow or rotated with non-host crops between Brassica plantings.

Table 6. *Severity of stem canker and amount of DNA detected in the soil or plant stems from 1 to 8 weeks after planting cauliflower cv. Skywalker into cocopeat or field soil inoculated with Leptosphaeria maculans. nt = not tested.*

Weeks after planting	Plant No.	Soil DNA (pg/g dried soil)	Plant DNA (pg/g dried stem)	Canker severity
Cocopeat				
1	1-5	634, 89, 84, 75, 420	nt	0
2	1-5	420, 510, 337, 565, 62	nt	0
3	1	42	0	0
	2	341	0	0
	3	380	0	0
	4	55	0	0
	5	63	1569	25
4	1	275	270	25
	2	63	0	0
	3	41	0	0
	4	69	0	0
	5	nt	320	50
6	1	nt	0	0
	2	88	0	0
	3	64	0	0
	4	97	71	0
	5	204	55	0
8	1	77	118	50
	2	116	494	50
	3	182	131	25
	4	73	0	0
	5	36	0	0
Field soil				
2	1-5	9, 1, 7, 14, 10	-	0
6	1	0	4	0
	2	0	0	0
	3	3	592	0
	4	0	0	0
	5	3	0	0

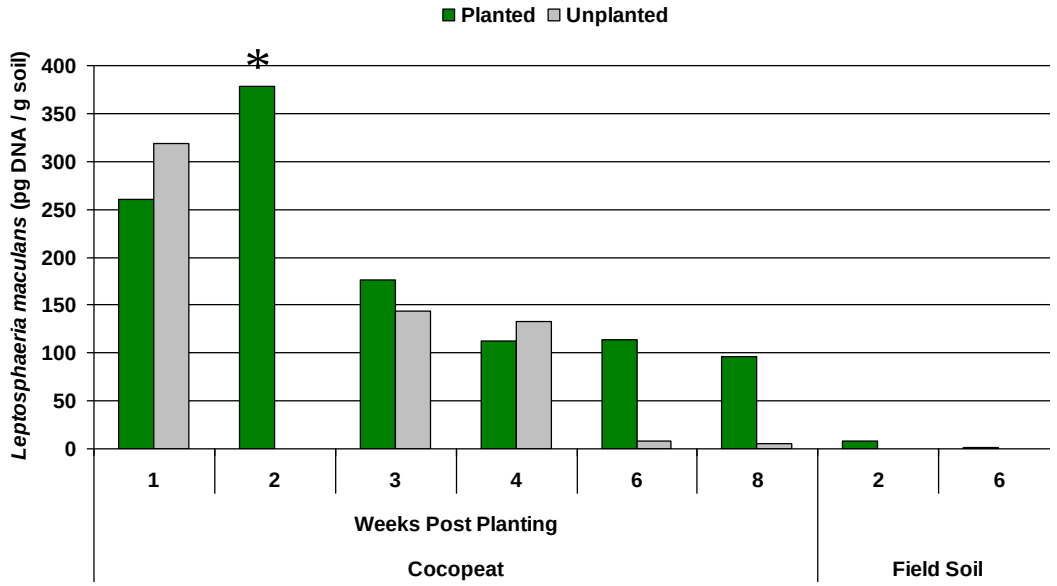


Figure 6. *Amount of Leptosphaeria maculans DNA (pg/g dried soil) in inoculated soil either not planted or planted with cauliflower cv. Skywalker.*note 2 week unplanted coco-peat not tested.*

Rhizoctonia solani

Rhizoctonia AG 2.1, 2.2 or 4 was not detected in the 40 plants tested from the nursery. *Rhizoctonia* AG 2.1 was detected in the un-inoculated pots of cocopeat 3 and 4 weeks post-planting at 0.6 and 0.2 Pg DNA/g soil respectively, indicating some cross contamination, possibly during sampling (data not presented).

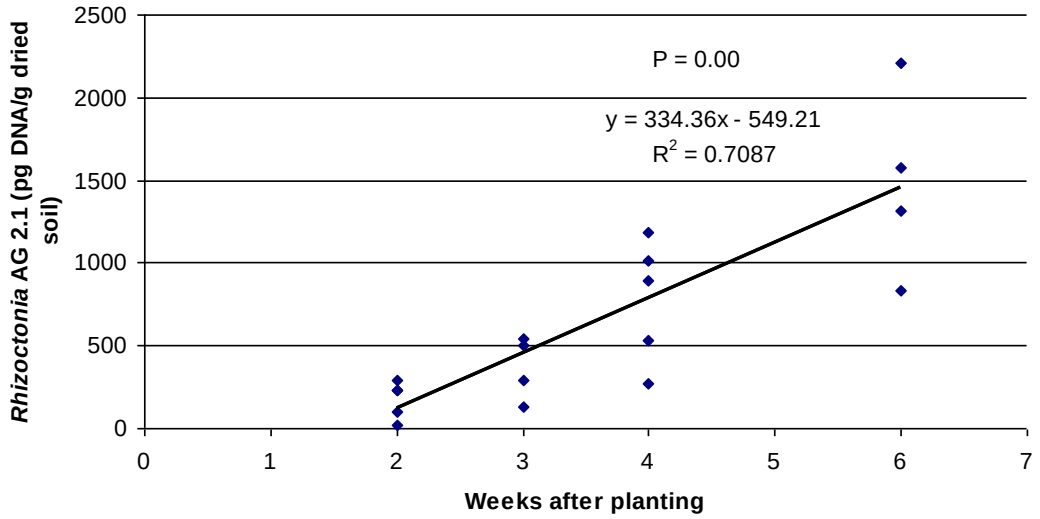
Severity of canker did not increase with time and of the 30 plants harvested from the coco-peat inoculated soil, only two had developed canker at 3 weeks after planting (Table 7). No plants grown in inoculated field soil developed canker. *Rhizoctonia* DNA was detected in the stems of both plants with canker, however it was also detected in the stems of eleven of the remaining plants, confirming previous findings (Hall *et al* 2009) that infection could be present without symptoms. There was no correlation between amounts of DNA in the plant and in the soil, or between DNA soil amounts and canker severity.

The detection of *R. solani* AG2.1 in soil was variable, both between sampled pots at each time and with time (Table 7). The amounts in cocopeat at week one were similar to those in week 6 (Table 7). However apart from one anomalous point in week three, there was a general upwards trend from 2 to 6 weeks after planting in the level of pathogen detected in planted inoculated cocopeat (Fig. 7). In field soil, two of the pots in week 6 had greater amount of DNA than in week 2, but the other three pots were lower. With the variability in the pathogen levels in both plant and soil, a much larger sample size is needed to accurately elucidate any trends.

Table 7. *Severity of stem canker and amount of DNA detected in the soil or plant stems from 1 to 8 weeks after planting cauliflower cv. Skywalker into cocopeat or field soil inoculated with Rhizoctonia solani AG2.1. nt = not tested.*

Weeks after planting	Plant No.	Soil DNA (pg/g dried soil)	Plant DNA (pg/g dried stem)	Canker severity
Cocopeat				
1	1	-	58	0
	2	-	0	0
	3	2273	3	0
	4	1312	10617	0
	5	717	0	0
2	1	19	15	0
	2	229	0	0
	3	235	0	0
	4	103	9	0
	5	294	0	0
3	1	130	1609	25
	2	3713	0	0
	3	498	809	50
	4	544	0	0
	5	293	4	0
4	1-5	897, 273, 353, 1015, 1182	0	0
6	1	-	-	0
	2	2210	34	0
	3	1578	104	0
	4	1314	0	0
	5	833	180	0
Field soil				
2	1	21	0	0
	2	13	0	0
	3	14	0	0
	4	31	2	0
	5	18	0	0
6	1	1231	0	0
	2	4	0	0
	3	8	6891	0
	4	873	0	0
	5	6	0	0

Figure 7. *Amounts of Rhizoctonia solani AG 2.1 DNA found in soil from inoculated coco-peat 2-6 weeks after being planted with cauliflower cv. Skywalker. Outlier (3713 pg/DNA) removed from week 3 data.*



4.2.3 Conclusions

These results show that not all cauliflower planted into soil infected with the pathogens *L. maculans* or *R. solani* AG2.1 developed canker. They also show that *Leptosphaeria* soil inoculum does not increase unless plants become infected, whereas *Rhizoctonia* will. They also confirm previous results that plants could be infected without showing symptoms (Hall *et al* 2009). There was no correlation between the amount of soil DNA of either *Leptosphaeria* or *Rhizoctonia* and canker severity. However other work with soil borne diseases (Heap & McKay 2004) showed inoculum in soil was rarely even and more sampling in field situations would be needed to confirm this finding.

It is possible wounding of the plant, either by other soil organisms or by damage during planting is needed to initiate infection and subsequently increase the soil inoculum.

4.3 *Leptosphaeria maculans* inoculum sources

Previous work (Hall *et al* 2009) showed that soil inoculum was the most likely source of the *Rhizoctonia* and *Leptosphaeria* causing stem canker, with none detected in nursery plants during this work and leaf inoculation techniques not successful. However *Leptosphaeria* is also known to be seed borne, and in canola, a major source of inoculum is airborne ascospores (Calderon *et al* 2002). Experiments were undertaken to confirm the possible sources of *Leptosphaeria* infection in cauliflower.

4.3.1 Seedling

Aim: To test seedlings prior to planting to detect *L. maculans.*

Method

120 plants were tested from each of four trays of seedlings, one tray from each of two planting times for two varieties cv. Skywalker and cv. Discovery. 12 subsamples of 10 plants of each cultivar and age were removed from the nursery tray. For 10 subsamples, the roots of the seedlings were washed with RO water. For the remaining two subsamples, the plant stem was cut immediately above the soil level. All equipment was cleaned in-between each subsample to prevent cross contamination.

DNA was extracted from the plants and tested for the presence of *L. maculans* by the root disease testing service at SARDI.

Results and Discussion

Low amounts of *Leptosphaeria* DNA were detected in 9 of the 48 samples of Skywalker seedlings (Table 8). In the Discovery seedlings, one of the samples had relatively high amounts of DNA, indicating at least one plant with significant infection. Seventeen of the remaining 23 subsamples had low infection ranging from 1 to 4 pg DNA/g plant material. There was no apparent decrease in disease with roots removed, indicating the infection was already in the plant stem. Previous work (Hall *et al* 2009) has shown that DNA of the pathogens could be detected in non-symptomatic plants within 2 weeks of being planted into infected field soil, with the highest amount detected here (579 pg DNA/g) being near the range where symptoms were observed 4-8 weeks after being planted in infected soil. This indicates that this seedling has been infected for some time and is most likely grown from infected seed.

From 2005, *Leptosphaeria* was not detected in nursery seedlings used for trials. The subsequent testing showing *Leptosphaeria* in nursery seedlings confirms seed borne infection. The pathogen has spread from the infected seedling to other seedlings in the same tray, resulting in infection with much lower amounts of DNA detected.

Soil infection resulting from the infected seedlings could be a source of disease in subsequent field crops, therefore it is important to ensure clean seed for planting. When grown under moist conditions with frequent rain, seed infection of 1.5% can cause significant field infection (Sherf & MacNab 1986).

***Table 8**. Amount of Leptosphaeria DNA detected in nursery seedlings.*

Cultivar	Tray	Roots included	No subsamples (total plants tested)	Number subsamples infected	mean pgDNA/g	range
Discovery	1	no	2 (20)	0	0	0
		yes	10 (100)	8	2	0-4.0
	2	no	2 (20)	2	1.3	1-1.6
		yes	9 (90)	7	1	0-2.7
		yes	1(10)	-	579*	
Skywalker	1	no	2 (20)	1	0.7	0-1.3
		yes	10 (100)	2	0.3	0-1.3
	2	no	2 (20)	1	1.8	0-3.6
		yes	10 (100)	5	1.0	0-3.1

**Single sample with high amount separated from remaining results*

4.3.2 Leaf infection

Aim: To determine if *Leptosphaeria* can cause leaf infection of cauliflower.

Methods

Experiment 1

Seeds of cauliflower cv. *Chaser* were pre-germinated in sterile coco peat in the greenhouse for 5 days before being planted into Mk 6 pots filled with sterile coco peat.

10 replicate potted seedlings were inoculated with a defrosted *L. maculans* spore suspension ~10^6 pycnidia/ml using one of 3 alternative inoculation techniques: 1) leaf misting with spore suspension until leaf saturation; 2) wounding of cotyledon followed by inoculation with a 10µl spore suspension (Fig. 8), or 3) soil inoculation at the stem base with 40 µl spore suspension.

A further 10 replicate potted seedlings were wounded and inoculated with water as controls.

Cotyledons were assessed at 11, 10 and 29 days after inoculation for lesion development at the inoculation sites using a 0-3 rating where 0 = no visible lesion, 1 = grey-green tissue collapse, 2 = collapsed tissue with a few pycnidia, 3 = collapsed tissue with multiple pycnidia.

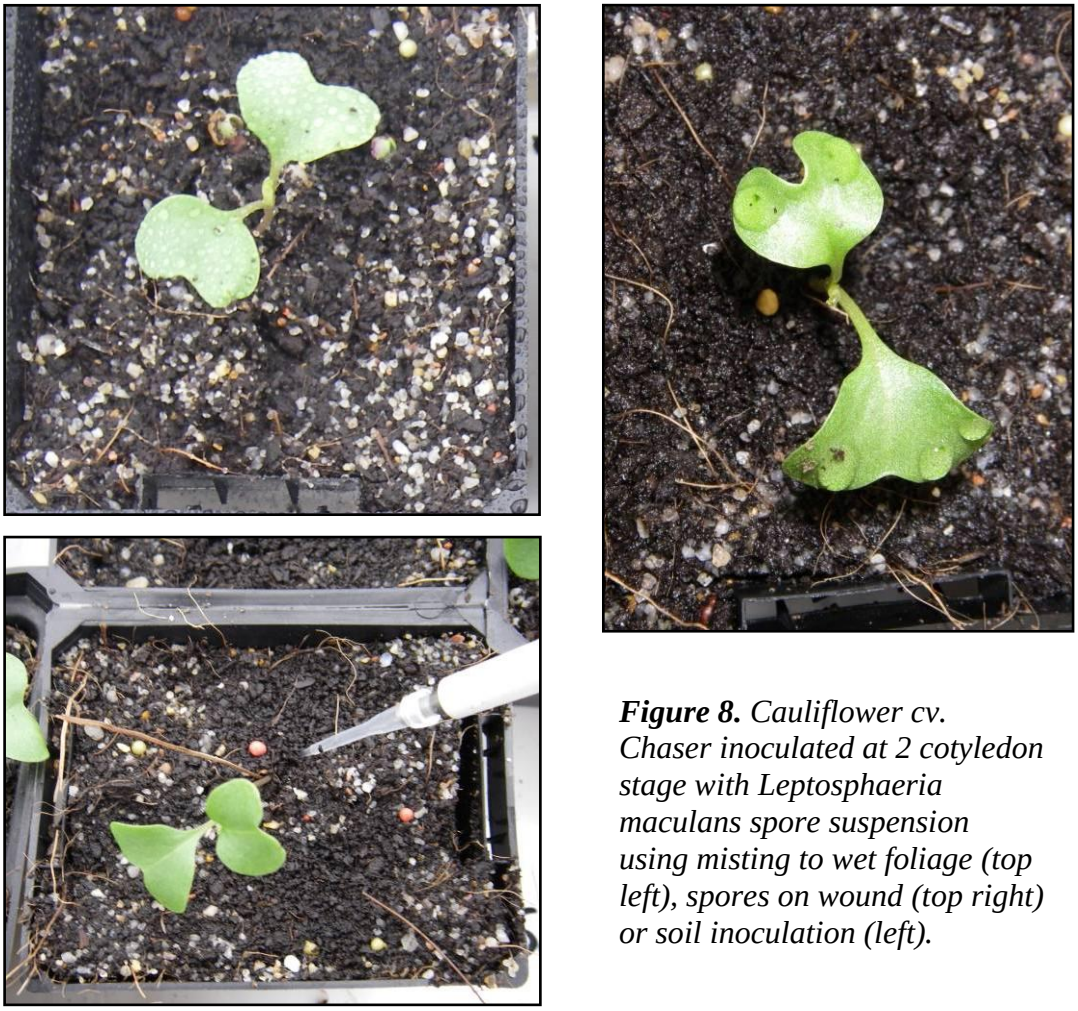


Figure 8. *Cauliflower cv. Chaser inoculated at 2 cotyledon stage with Leptosphaeria maculans spore suspension using misting to wet foliage (top left), spores on wound (top right) or soil inoculation (left).*

Experiment 2

Stem trash with symptoms of stem canker were collected from commercial Brussels sprouts paddocks in the Adelaide Hills and cauliflower paddocks in the Northern Adelaide Plains and stored in the laboratory until dry. However no fruiting bodies developed so ascospores could not be harvested. Therefore a pycnidial spore suspension of 1 x 10^6 spores/ml was prepared from culture plates as previously described.

10 replicate cauliflower cv. Skywalker were inoculated with one of the methods outlined in Table 10. The 4th leaf was cut in half and one cotyledon removed with sterile scissors to replicate the leaf cutting sometimes done in the nursery to encourage rapid plant growth. The 3rd leaf was abraded by rubbing with an eraser to remove the waxy layer (Fig. 9), replicating mechanical wounding. Plants were foliar or root inoculated immediately after wounding with either water or spore suspension as previously described.

Canker severity was assessed 8 weeks after inoculation.

Figure 9. *Wounding by cutting top of 4th leaf (L) and applying bags (R) after leaf inoculation with Leptosphaeria maculans on 5 wk cauliflower cv. Skywalker.*

Results and discussion

Experiment 1

Leaf lesions did not develop in the control plants, but were found on the wounded cotyledons (10%) 11 days after inoculation. By 29 days after inoculation some plants were infected in all the inoculation methods. Lesion incidence and severity were highest 29 days after inoculation where cotyledons had been wounded (37.5 % and 0.83) compared to the misting spray with no wounding (7.5 % and 0.2). Representative pycnidia on the infected leaves were confirmed as *L. maculans* by microscopic examination.

The plants inoculated in the soil developed leaf lesions by 29 days after inoculation, indicating systemic transferral of infection from the soil.

Table 9: *Incidence and severity of lesions on cotyledons of cauliflower cv. Chaser 11, 20 and 29 days after inoculation with Leptosphaeria maculans pycnidia by misting of spores on unwounded leaves, drops of spores on wounded leaves or soil inoculation.*

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	Mean incidence (%)			**Mean lesion severity**		
	Days after inoculation					
Inoculation method	**11**	**20**	**29**	**11**	**20**	**29**
Un-inoculated	0	0	0	0	0	0
Leaf misting no wounding	0	2.5	7.5	0	0.1	0.2
Wounding with spore drop	10	38	38	0.2	0.8	0.8
Soil inoculation	0	0	2.5	0	0	0.1

Experiment 2

Eight weeks after inoculation 100 % of the leaf inoculated plants and 90% of the root inoculated only plants were infected with blackleg compared to 10 - 60% incidence in

the un-inoculated plants (Table 10). It is likely that contamination after inoculation had infected some of the control plants.

Table 10. *Incidence and severity of stem canker following leaf wounding and inoculation through the leaves or roots with a spore suspension of Leptosphaeria maculans (✓)or water (✗). Means with the same letter are not significantly different.*

Inoculation method	Wound sites*	Leaf inoculation	Root inoculation	Mean canker severity	Incidence of plants with canker
1	3rd, 4th, cot	✗	✓	50.0a	90
2	4th, cot	✓	✗	47.5a	100
3	3rd, 4th, cot	✓	✓	52.5a	100
4	4th, cot	✗	✗	15.0ab	60
5	3rd, 4th, cot	✗	✗	2.5b	10
6	None	✗	✗	2.5b	10

** 3rd = 3rd leaf abraded to remove the waxy layer, 4th = 4th leaf cut in half, cot = one cotyledon removed with sterile scissors.*

Figure 10. *Internal stem staining in cauliflower cv. Skywalker8 wks after leaf inoculation with Leptosphaeria maculans.*

Figure 11. *The waxy cauliflower cuticle repels water resulting in leaves remaining wet up to 48 hours after irrigation.*

These results show that *Leptosphaeria* infects through the leaves and moves systemically to the stems as it does in canola (Hammond *et al*. 1985). However the internal stem staining was often absent in cauliflowers, and the degree of staining seen in cross-section (Fig. 11) did not reflect the severity of external stem canker or staining. This could be due to the multiple pathogen nature of the disease, however *L. maculans* also has an endophytic and symptomless systemic growth phase (Rouxel & Balesdent 2005), supported by the PCR detection of *L. maculans* in symptomless

cauliflower plants (Hall *et al* 2009). As cauliflower leaves mature they become harder with a waxy coating which repels water (Fig. 11). This may make mature leaves resistant to infection unless they are wounded, however the retention of water on the leaves may increase infection as both pycnidia and ascospores require free water for germination (Ash 2000).

4.3.3 Spore trapping

Aim: to detect airborne spores of *L. maculans* near cauliflower plantings in the Northern Adelaide Plains.

Methods

A Solar powered 7-day recording volumetric spore trap (Burkhardt Scientific, Rickmansworth, Hertfordshire, England) was set up alongside a 140m by 140m trial planting of Cauliflower in the Northern Adelaide Plains in 2010 (Fig. 12).

Figure 12. *Burkhardt Spore Trap (L), sited adjacent to previously infected paddock (R).*

This paddock, fallowed for the 2 years prior, was within 500m of an area planted to Rye corn that had previously experienced yield losses of 80% in the commercial grower's cauliflower planting in 2009. The trap collected airborne particles on "Tangle foot" coated Melinex tape attached to a rotating drum in the spore trap for a period of 7 days, after which time the tape was exchanged with a new one.

The spore trapping commenced in the second week of February 2010 and operated until the 25 May 2010, then continued from July until 4 January 2011. Due to motor replacement, the spore trap was not operational for the entire period of June 2010. Air was sampled at ~10 litres/min and the suction orifice was 40 cm above the ground.

Each exposed tape was cut in half lengthwise using aseptic techniques and one half further cut into 7 sections of 48mm length, placed into a single sterile falcon tube and stored at 7 degrees before PCR testing was undertaken by the SARDI Root Disease Testing Service.

A selection of full length tape halves remaining after the initial splitting of the tape which had collected air spore during these conditions, one for each month, were scanned using a compound microscope at 200 x magnification.

Weather conditions were tabulated from the nearby Edinburgh weather station for the entire period of trapping, with temperature and rainfall records used to determine times most conducive to *L. maculans* ascospore release.

Results and discussion

Spores of *Alternaria* spp. were evident on a number of tapes, particularly during October. *L. maculans* ascospores and pycnidiospores were not detected on the tapes by microscopic examination, possibly due to the level of other spores and dirt present.

The PCR results showed three main spore loads in April, August/Sept and December (Fig. 13). These corresponded to the rainfall peaks in 2010 (Fig. 14). The spore number is estimated based on results of a dilution series of pycnidiospores undertaken prior to this test (data not presented). However the DNA amounts of the spore tapes from the field do not distinguish between DNA of ascospore or pycnidiospores. Correlations between spore number and the spore type producing the positive DNA result requires further trial work. Ascospores germinate in the presence of free water in temperatures from 4-28^{o}C, whereas pycnidia require 16 hours of continual wetness at the optimal range of 20-25^{o}C (Ash 2000). Therefore ascospore germination could occur at all three times, whereas only the December weather conditions would favour pycnidial infection.

No cauliflower was planted near the spore trap during this time. The late November planting adjacent to the spore trap had very little disease observed, indicating low levels of infection in the December peak spore load.

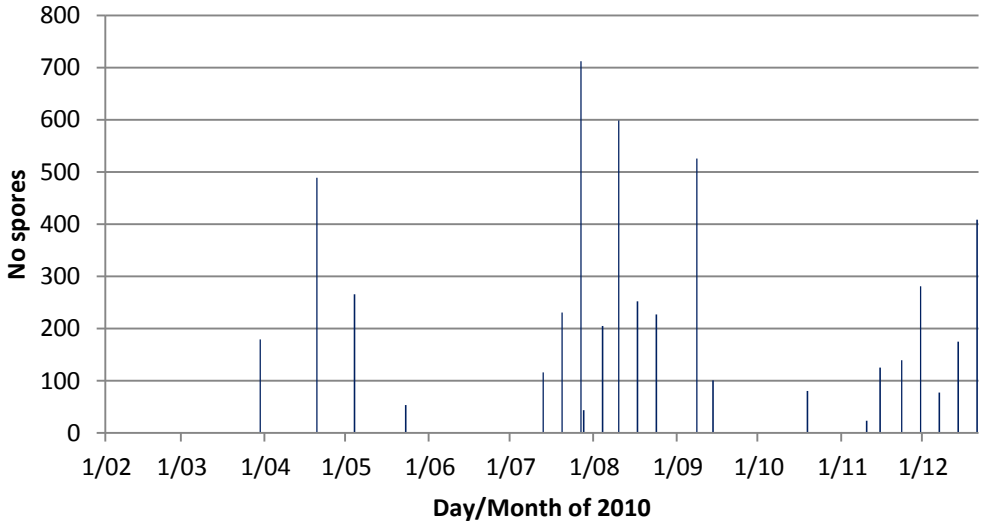


Figure 13. *Number of spores detected on the tape for each week in 2010. (No tapes were collected in June).*

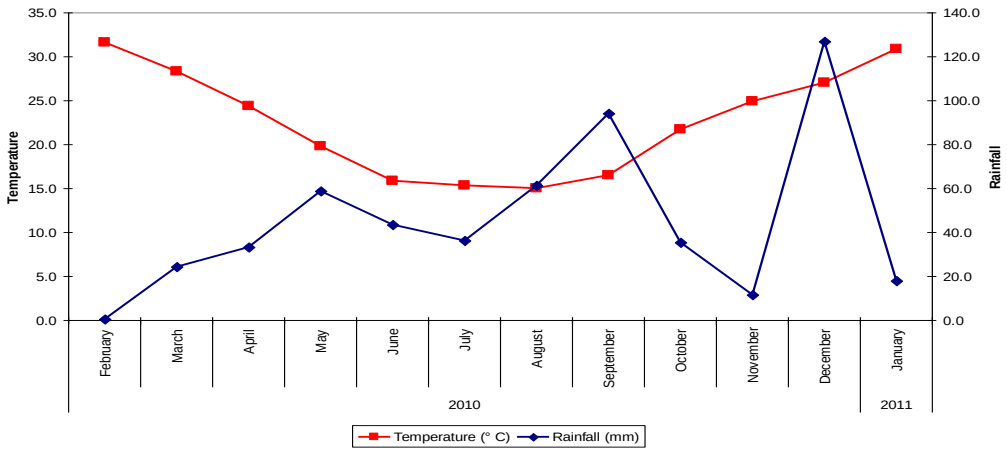


Figure 14. *Temperature (monthly mean maximum) and rainfall (monthly total) measured at the BOM weather station closest to the spore trapping site in 2010.*

4.3.4 Conclusions

Infection by *L. maculans* can occur in both root and leaf, originating from seed, soil and airborne inoculum. Growers surveyed in the 2005 scoping study VG05005 (Hitch *et al* 2005) observed stem canker in cauliflower and Brussels sprouts planted into soil not previously planted with these crops and that the disease was most prevalent in late winter.

Research into *L. maculans* infection in canola has shown that with soil borne infection, pycnidia will cause more plant death than ascospores (Hua Li *et al* 2007). However ascospore infection of leaves has the potential to severely increase the severity of canker in canola compared to conidial infection alone (Somda *et al* 1998). Sprague *et al* (2009) also found that cankers and root rots caused by *L. maculans* could be found on canola planted into both fumigated soil and soil not previously planted to canola, with infection from airborne spores and infected seed.

Infection of Brassicas through leaves was more effective with wounding the leaves and reducing wounding may be an effective way of minimising potential infection. The practice of cutting the leaves to encourage growth will increase the potential of foliar infection.

Therefore the *L. maculans* infection in cauliflower is most likely from low levels of soil borne and seed infection and is supplemented by ascospore infection in the late winter causing the increase in disease observed by growers. Early infection of canola caused a more severe stem canker, particularly at lower temperatures of 11 -18^{o}C (Hua *et al* 2006). These temperatures were common in winter (Fig. 14) and ascospore infection during this time with wounded plants would result in the higher disease levels observed.

The low level of stem canker in the commercial cauliflower crop adjacent to the sporetrap in summer may be due to low ascospore release. Khanghura *et al* (2007) found with canola in Western Australia, different patterns of ascospore discharge

occurred in different environments and different years. Therefore to provide a clear picture of the effect of air borne spores in the disease cycle with Brassica vegetable crops, the spore trapping would need to be repeated over a number of seasons and should be correlated with crop damage.

It would also be useful to be able to differentiate between the two spore types on the tapes and to determine whether the spores detected are pathogenic to cauliflower, as *L. maculans* is a highly variable fungus, with many strain variations detected (Rouxel *et al* 1995, Balesdent *et al* 2005).

4.4 Relative susceptibility of Brussels sprouts cultivars

Aim: To compare the susceptibility of six varieties of Brussels sprouts to *Leptosphaeria maculans*.

Methods

Sandy loam soil was collected from a commercial paddock in Virginia which had been fallow for one season following a cauliflower planting. The field soil was steam sterilised at 60 degrees for 120 minutes. PCR testing of the soil was undertaken following sterilisation for the presence of AG 2.1, 2.2 or 4 and *Leptosphaeria maculans* before the soil was transferred into 80, 200mm sterile pots.

Each pot was pot soil inoculated as previously described.

Pre-germinated seeds of BS137, Abacus, BS141, BS144, Romulus and Cumulus were planted in a standard commercial multicell speedling tray and maintained in a greenhouse with approximate day/night temperatures of 30/20 degrees.

Five weeks post-seeding 10 replicate plants of each cultivar were planted into the previously inoculated pots of field soil.

Planted pots were maintained in a controlled environment room with a day night temperature of 20 degrees and a 12 hr day/night light cycle. Plants were watered to field capacity and were fertilised fortnightly with Thrive up to 8 weeks then Maxfeed until 20 weeks.

Canker severity was assessed at 5, 8 and 12 wks after planting. The plant height from soil level to first expanded leaf was measured 5 weeks after planting and plant weights measured 17 weeks after planting included weight of the plant minus the rootball and the weight of the stalk including immature sprouts.

Results and discussion

L. maculans, R. solani AG 2.1, 2.2 or 4 was not detected in the steam sterilised soil.

No canker developed in any of the treatments, indicating the inoculation technique was not successful, and this trial should be repeated.

While differences in plant growth were observed, in the absence of any canker symptoms it is unknown whether these can be attributed to varietal differences or whether the *Leptosphaeria* was affecting the root growth. However the similarity in growth of cv. Abacus in the inoculated soil and the not inoculated soil suggests that the *Leptosphaeria* was not influencing growth.

Cultivar BS 137 was the most stunted of all cultivars two weeks after transplanting into the inoculated soil. The cultivar is classed as an early season cultivar suited to the higher temperatures of the February and March, and may have been less suited to the 20 degree growing conditions.

At harvest cultivar BS144 was the most consistent in plant size with a mean size of 12 cm at harvest and the largest mean plant weight of 162.52g and stalk weight of 11.71g (Table 11).

Table 11. *Growth of Brussels sprouts cultivars in soil inoculated with Leptosphaeria maculans. Means with the same letter are not significantly different from one another (P=0.05).*

Cultivar	**Average height (cm) 5 wks**	**Average plant weight (g) 17 wks**	**Average stalk weight (g) 17 wks**
BS137	9.2 a	94.5 a	4.7 a
Abacus F1 (not inoculated)	10.2 ab	90.7 a	6.3 a
Abacus F1	10.4 abc	108.5 ab	7.1 a
BS141	11.6 bcd	137.2 bc	11.7 b
BS144	12.0 cd	162.5 c	11.7 b
Romulus F1	14.2 e	144.9 c	11.1 b
Cumulus F1	16.3 f	159.8 c	13.3 b

Conclusion

This experiment needs to be repeated, as the use of cultivars with reduced susceptibility to disease is required as part of a management strategy.

4.5 Biological and fungicide compatibility

Many of the biological products were to be applied mixed with fungicides as a pre-planting drench. Therefore it was necessary to determine whether the fungicides were detrimental to the biological agents.

Aim: To evaluate *in vitro* the affect of fungicides on growth of biological products.

Methods

Potato dextrose agar (PDA) was autoclaved and cooled to 70˚C, before adding commercial formulations of fungicides (Table 12) to a final concentration of 1, 10 and 100ppm active ingredient (a.i.). Agar amended with azoxystrobin also had 100 µg/ml salicylhydroxamic acid (SHAM) dissolved in methanol added to inhibit the induction of alternative oxidase respiration (Olaya & Koller 1999). The biological products Mycotea, Companion and Trichoshield were mixed at recommended rates and 20µL of the solution spread over the surface of 5 replicate plates of the amended agar with a sterile spreader. Unamended agar and agar amended with SHAM were used as the controls. Plates were assessed for presence of fungal or bacterial growth after 9 days incubation in the laboratory (~22^0C)

Results and Discussion

Growth of the components in Trichoshield™ was completely inhibited by all fungicides at 100 ppm a.i. (Table 12) and fluquinconazole, fludioxonil and pyraclostrobin inhibited growth at all concentrations. Flutriafol had the least effect with no inhibition at 1 and 10ppm. The growth of Companion (*Bacillus subtilis*) was reduced at 10 and 100ppm with iprodione, fludioxonil and pyraclostrobin (Table 12, Fig. 15), but there was no inhibition of growth from azoxystrobin, fluquinconazole or flutriafol. Inhibition of Mycotea occurred with iprodione and pyraclostrobin (Table 12). This product contains a range of eight biological agents and while the total product was not reduced by the other fungicides tested (azoxystrobin, fluquinconazole, fludioxonil) the relative growth of the constituents changed with increasing fungicide concentrations (data not presented), which may impact on the efficacy.

While the rates of the fungicides used in this test cannot be directly related to the concentration of product applied to the plants, the results show some products are not compatible with fungicides. For example while Mycotea and Companion could be tank mixed with fungicides, Trichoderma products may need to be applied separately to prevent reduction in efficacy of the product. This is reflected in the product label, which notes potential reduction in efficacy when used with fungicides.

Table 12. *Percent of amended agar plates at various concentrations with no growth of biological agent after 9 days incubation.*

Fungicide	BIOLOGICAL PRODUCT											
	Mycotea				Trichoshield				Companion			
	FUNGICIDE CONCENTRATION (ppm)											
	0	**1**	**10**	**100**	**0**	**1**	**10**	**100**	**0**	**1**	**10**	**100**
Amistar 250SC (250g/L azoxystrobin)	0	0	0	0	0	0	100	100	0	0	0	0
Jockey Seed (167g/L fluquinconazole)	0	0	0	0	0	40	80	100	0	0	0	0
Maxim 100FS (100g/L fludioxonil)	0	0	0	0	0	60	100	100	0	0	40	100
Rovral Aquaflo (500g/L iprodione)	0	0	100	80	0	0	100	100	0	0	100	100
Impact Endure (500g/L flutriafol)	-	-	-	-	0	0	0	100	0	0	0	0
Cabrio (250g/L pyraclostrobin)	0	20	80	40	0	20	100	100	0	0	100	100

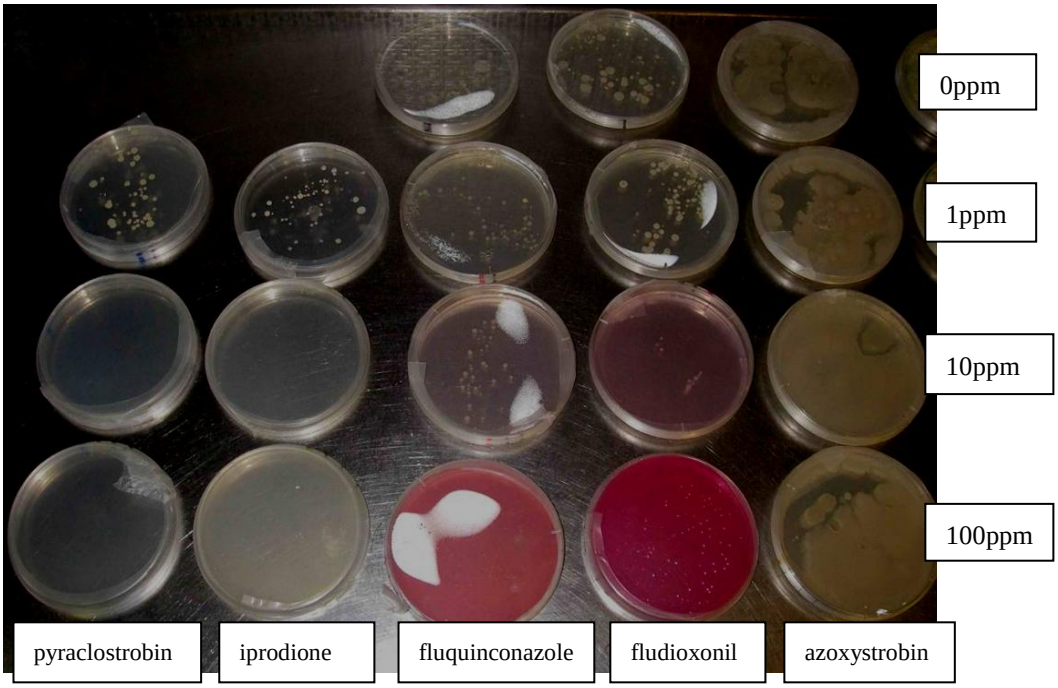


Figure 15. *Growth of Companion (Bacillus subtilis) after nine days incubation on PDA amended with 0ppm (top) 1, 10 and 100ppm (bottom) of pyraclostrobin iprodione, fluquinconazole, fludioxonil and azoxystrobin (left to right).*

4.6 Greenhouse efficacy trials

Previous work (Hall *et al* 2009) showed that fungicide drenches suppressed the development of stem canker. A series of greenhouse trials were undertaken to determine whether alternative products improve control of the disease. The efficacy of various fungicides and commercially available biological and plant growth products were evaluated in their ability to control stem canker symptoms when applied in various combinations, application times and methods. Azoxystrobin (Amistar®), iprodione (Rovral®) and fludioxonil (Maxim®) were used as these had previously showed to suppress stem canker (Hall *et al* 2009). Flutriafol (Impact Endure®) and fluquinconazole (Jockey Seed®) were also evaluated as they are registered to use in canola for *Leptosphaeria* (black leg).

4.6.1 Experiment 1. Azoxystrobin and biological products

Aim: To evaluate the efficacy of biological products applied as drenches with and without azoxystrobin.

Methods

Sterile cocopeat (106 L) was inoculated with 400ml of a mycelial slurry of *Rhizoctonia* AG2.1 and 110 ml of a spore suspension of *Leptosphaeria* as previously described. After 1 week, 175 mm pots were filled with the inoculated soil. Non-inoculated sterile cocopeat was used as a control.

The presence of *Rhizoctonia* was confirmed in the bulk soil by toothpick baiting as previously described.

Four biological products Trichoshield, Companion, Mycotea and Rhizoctonic and the fungicide azoxystrobin (Amistar ®) were applied as previously described alone and in various combinations of pre-plant and post-plant drenches (Table 13) to cauliflower seedlings cv. Donner planted into the inoculated soil. Each treatment was applied to 10 replicate plants, with another 10 replicate plants of water treated control seedlings.

Plants were assessed for symptoms of stem canker at 2, 4, 6, 8 and 12 weeks after planting as previously described. The stem weight, size of head and size of the root ball were assessed 12 weeks after planting as previously described.

Results

Stem cankers were first observed 6 weeks after planting (data not presented) and by harvest 12 weeks after planting canker had developed in all treatments (Table 14). Disease severity was low with the inoculated controls having a mean incidence of 60 % and severity of 17.5 % at harvest. Plants treated with Mycotea had significantly higher incidence (100%) and severity (30%) of canker than the untreated control. Treatments 4, 5, 7 and 8 (Companion alone and azoxystrobin combined with Trichoshield or Rhizotonic) significantly reduced canker incidence and severity compared to the untreated control.

Table 13. *Treatments applied in Experiment 1. - = not applied. * Amistar post-planting applied once at 2 weeks only.*

Treatment	Product	Pre-plant drench rate /100L		Drench after planting – product rate per plant	
		Amistar (20ml/100L)	Product	24 hours	Every 2 weeks
1	Amistar	20ml	-	-	
2	Amistar	20ml	-	-	.002ml*
3	Companion	20ml	125ml	-	0.0125ml
4	Trichoshield	20ml	-	2g	.005g
5	Trichoshield	20ml	500g	-	.005g
6	Mycotea	20ml	50g	-	.005ml
7	Rhizotonic	20ml	400ml	-	1ml
8	Companion	-	125ml	-	0.0125ml
9	Mycotea	-	50g	-	.005ml
10	Rhizotonic	-	400ml	-	1ml
11	Trichoshield	-	500g	-	.005g

Table 14. *Stem canker incidence and severity at harvest on cauliflower cv. Donner treated with various products and planted into soil inoculated with Leptosphaeria maculans and Rhizoctonia solani AG 2.1. Treatments with the same letter are not significantly different from one another (P=0.05).*

Treatment	Pre-planting	Post-planting	Canker incidence %	Canker severity %
1	Amistar	Water	70 bcd	20.0 bc
2	Amistar	Amistar	60 cd	15.0 cd
3	Amistar + Companion	Companion	50 de	15.0 cd
4	Amistar / 24h Trichoshield	Trichoshield	25 efg	6.3 e
5	Amistar + Trichoshield	Trichoshield	10 fg	2.5 e
6	Amistar + Mycotea	Mycotea	70 bcd	17.5 bc
7	Amistar + Rhizotonic	Rhizotonic	30 ef	7.5 de
8	Companion	Companion	20 fg	7.5 de
9	Mycotea	Mycotea	100 a	30.0 a
10	Rhizotonic	Rhizotonic	90 ab	25.0 ab
11	Trichoshield	Trichoshield	80 abc	22.5 abc
12	Water	Water	60 cd	17.5 bc
	LSD		28	7.5

The addition of azoxystrobin to the products as a pre-planting drench reduced stem canker severity significantly for all products except Companion, where the addition of azoxystrobin increased disease compared to Companion alone. There was no negative effect on Companion by azoxystrobin *in vitro*, so this result was unexpected and warrants further investigation. No improvement in canker control was observed by delaying the planting application of Trichoshield until 24 hours after the azoxystrobin application, indicating that the negative effect of azoxystrobin with Trichoshield detected *in vitro* did not cause loss of field efficacy in this trial.

Of the growth parameters measured, only mean stalk weight was significantly different between the treatments (Table 15). However this was not correlated with canker development, with the greatest mean stalk weight seen in the Rhizotonic treatment (Treat. 10), which had one of the higher levels of canker symptoms. However larger stems may improve plant strength and maintain yield in the presence of the disease.

The treatments including Rhizotonic, Mycotea or Trichoshield had a higher proportion of larger heads than the Companion treatments, indicating a possible growth advantage (Fig. 16). However this trend was not seen in the root ball sizes (data not presented) with none of the biological treatments having as many plants with large root balls as the non-inoculated control plant (90%). While there was variation between the treatments with both head size and root ball size, no correlations to canker severity were observed.

Table 15*. Mean stalk weight at harvest of cauliflower cv. Donner planted into soil inoculated with Leptosphaeria maculans and Rhizoctonia solani AG 2.1 with pre-and post-planting treatments of various fungicide and biological products. Means with the same letter are not significantly different from one another (P=0.05).*

Treat.	Pre-planting	Post-planting	Mean stalk weight (g)
1	Amistar	Water	8.7 abcde
2	Amistar	Amistar	8.3 bcde
3	Amistar + Companion	Companion	6.5 e
4	Amistar / 24h Trichoshield	Trichoshield	8.1 cde
5	Amistar + Trichoshield	Trichoshield	7.6 cde
6	Amistar + Mycotea	Mycotea	9.7 abc
7	Amistar + Rhizotonic	Rhizotonic	9.8 abc
8	Companion	Companion	7.5 cde
9	Mycotea	Mycotea	7.1 de
10	Rhizotonic	Rhizotonic	10.8 a
11	Trichoshield	Trichoshield	9.4 abcd
12	Water	Water	7.1 de
	LSD		10.5 ab

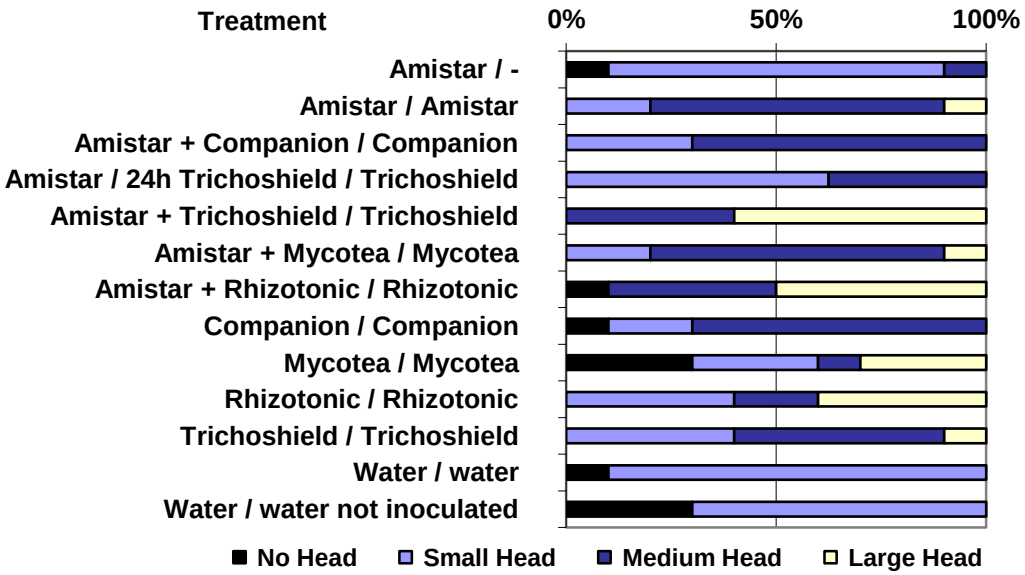


Figure 16. *Relative incidence of mean head size of cauliflower cv. Donner 12 weeks after planting into soil inoculated with Leptosphaeria maculans and Rhizoctonia solani AG 2.1 .following various pre- and post-planting treatments.*

This trial indicates that apart from Companion, the biological products trialled are unlikely to provide adequate economical disease suppression of stem canker when used alone, although this would need to be proven in field conditions, where the different soil or pathogen levels may impact efficacy.

4.6.2 Experiments 2-7. Azoxystrobin, fludioxonil and plant health products

Five small experiments were set up to evaluate different plant health products with and without fungicide.

Aim: To evaluate azoxystrobin (Amistar®) and fludioxonil (Maxim®) in combination with various plant health products to control stem canker.

Materials and methods:

A suspension of *Leptosphaeria maculans* was prepared by blending 20 plant reinvigorated cultures on ¼ PDA plates in a Waring blender with one Litre of sterile water. The suspension was filtered with Mira cloth before placing the filtrate on a stirrer for 5 minutes, a spore concentration was determined using a haemocytometer and the filtrate diluted to a 1 x 10^6 concentration using a further one and a half litres of RO water. A *Rhizoctonia solani* AG 2.1 slurry was prepared by blending two and a half plates of actively growing cultures on full PDA with 250 ml RO water.

144 Kg of coco-peat was inoculated with 2.5L of *L. maculans* spore suspension and 250ml of *R. solani* AG2.1 slurry as previously described, mixed thoroughly and 160 MK9 pots filled with the inoculated soil. 20 MK9 pots were filled with un-inoculated soil for the control. Pots were watered briefly to moisten the soil and stored in the

greenhouse for 24 hours before being planted with six week old cauliflower cv. Skywalker pre-treated with either water or product as outlined in Table 16, 10 replicate plants per treatment.

Fungicides and plant health products applied as pre-plant drenches were mixed together and the speedlings soaked in the mixture for 5 mins before planting. Soil Reviva was applied immediately prior to planting, stirred thoroughly into the coco-peat and watered in with 100mls immediately prior to planting.

Post-planting applications of Companion, Rootpower and Acadian were applied at recommended rates in 100 ml per Mk9 pot at planting and 200 ml at two, four, six and eight weeks after planting, sufficient volume to saturate the soil matrix.

Plants were maintained with a fortnightly application of Maxfeed applied to the soil at label rates and soil moisture kept to field capacity by keeping the pot submerged in water to 2cm depth or about 1/5th of the pots depth, simulating typically saturated commercial field conditions. Plants were overhead watered at planting and once a week until 8 weeks post-planting.

Table 16. *Treatments applied for each experiment as drench to speedlings pre-planting. *Expt. 2 Soil-Reviva applied to pot as a soil drench immediately before planting.*

Expt.	**Treatment**	**Pre-planting drench (rate product/plant)**	**Product applied after planting**	
			rate ml/plant	**timing**
All	Water	Water	-	-
1	Profert	Profert (0.04ml)	-	-
1	M-Profert	Maxim (0.006ml) + Profert (0.04ml)	-	-
2*	Soil Reviva	Soil Reviva (16g/pot)	-	-
2*	A-Soil Reviva	Amistar (0.01ml) + Soil Reviva(16g/pot)	-	-
3	Rootpower	Rootpower (0.035ml)	0.035	2 wks
3	A-Rootpower	Amistar (0.01ml) + Rootpower (0.035ml)	0.035	2 wks
4	Acadian	Acadian (0.1ml)	0.1	2, 4, 6, 8 wks
4	A-Acadian	Amistar (0.01ml) + Acadian (0.1ml)	0.1	2, 4, 6, 8 wks
5	Maxim	Maxim (0.006ml)	-	-
5	Companion	Companion (0.0125ml)	0.125	2, 4, 6, 8 wks
5	M-Companion	Maxim (0.006ml) Companion(0.0125ml)	0.125	2, 4, 6, 8 wks

A visual assessment of plant health and stunting was undertaken four weeks after planting. At 11 weeks after planting the plants were harvested by cutting the stem at the soil line. Symptoms of stem canker were rated as previously described and internal stem staining of the stem cross section noted. Plant growth parameters included head weight and plant weight (without the head). The rootballs were given a comparative rating of small, medium or large.

Results and discussion

The variation in canker incidence and severity between the inoculated controls in the various experiments was not significant (Table 17). However it illustrated the difficulty in achieving consistent infection with this disease, as all these controls were the same cultivar planted into the same batch of inoculated soil. There was also a low level of infection detected in the un-inoculated treatment, indicting possible pathogen spread through contamination or water splash.

Significant differences were observed between the controls in both head weight and plant weight (Table 17), with the mean head weight negatively correlated to the canker severity (Fig. 17). The mean head weight and plant weights were lowest (7.6g and 82g respectively) in the control from Expt. 2 which had the highest canker severity (35%) and highest (18.4 g and 136g respectively) in the un-inoculated control with the lowest canker severity (1%).

Table 17*. Incidence and severity of stem canker and plant growth of cauliflower cv. Skywalker treated with water only and planted into un-inoculated soil or soil inoculated with Leptosphaeria maculans and Rhizoctonia solani AG 2.1. Treatments with the same letter are not significantly different from one another.*

Expt.	Soil treatment	Canker incidence %	Canker severity %	Head weight (g)	Plant weight(g)
all	Un-inoculated	5 a	1 a	18.4 b	136 b
1	Inoculated	50 b	20 b	18.1 b	125 ab
2		70 b	35 b	7.6 a	82 a
3		40 b	18 b	14.9 b	150 b
4		40 b	30 b	8.6 ab	104 ab
5		30 b	20 b	16.8 ab	94 ab

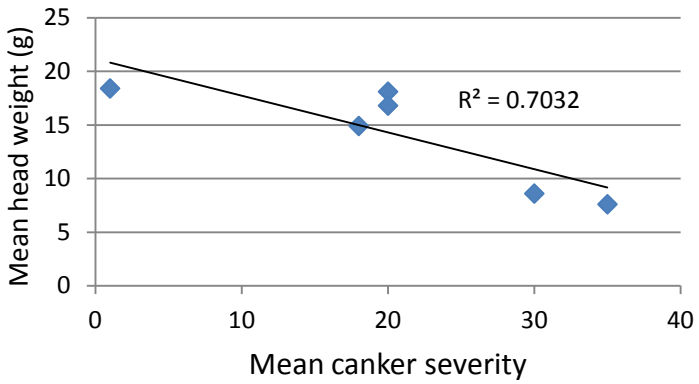


Figure 17. *Correlation between mean head weight and mean canker severity in cauliflower cv. Skywalker treated with water only and planted into un-inoculated soil or soil inoculated with Leptosphaeria maculans and Rhizoctonia solani AG 2.1.*

Figure 18. *Symptoms of canker on untreated dead cauliflower cv Skywalker 10 weeks after planting into soil inoculated with Leptosphaeria maculans and Rhizoctonia Ag2.1. L. maculans was isolated from the canker.*

Three untreated inoculated plants died before harvest, and were excluded from mean plant weight harvest data. *L. maculans* was isolated from the stem canker (Fig. 18). Internal stem staining developed in the inoculated controls (Fig. 19). *L. maculans* was recovered from the stem staining, indicating systemic infection from the roots.

Figure 19. *Internal stem staining observed in untreated plants with stem canker. Leptosphaeria maculans was isolated from the staining.*

General brown discoloration of the lower stem was observed on many plants by 8 weeks after planting, darkening more towards harvest (Fig. 20). As it was observed on 80% of the un-inoculated controls (data not presented) and was not considered typical of the usual stem canker early symptoms, it was attributed to a factor other than stem canker and not included in the disease ratings. One possible cause was the higher soil moisture maintained by under pot watering compared to other experiment using drip irrigation.

Figure 20*. Brown discoloration observed at harvest on cauliflower grown in un-inoculated soil and attributed to maintenance of high soil moisture (L) compared to stem canker (C) and unstained stems (R).*

The severity of stem canker showed significant differences in some experiments and canker did not develop in plants treated with Profert + Maxim or Soil Reviva (Table 18). Except for Expt. 5 with Companion, the severity of stem canker in the treated plants was lower or equivalent to the untreated control. The application of Maxim or Companion alone numerically increased stem canker severity compared to the untreated control. Except for Soil Reviva, adding the fungicide gave better or equivalent control to the plant health product alone.

The head weights were variable with no significant differences between treatments except Soil Reviva (Table 18), where the treated plants had smaller heads and some plant did not develop heads by harvest. The mean head weight of plants treated with Companion, Profert and Amistar + Arcadian were numerically greater than the un-inoculated controls. There was no correlation between mean canker severity and head weight (data not presented), indicating the treatments were affecting the head weight irrespective of the canker development.

Plant weight (without head) showed significant differences in some experiments (Table 18). The products Soil Reviva, Arcadian and Companion all increased plant weight compared to the inoculated control, whereas Profert and Rootpower reduced plant weight. Plants treated with Soil Reviva and Maxim were noticeably stunted at 4 weeks after planting (Fig. 21), however both improved with time to have higher plant weight than the controls. Plants treated with Soil Reviva were also chlorotic at 2 weeks after planting (Fig. 21), indicating the soil amendment was either applied at too high a rate or may have needed more time to incorporate before planting. This product should be field tested to confirm the positive growth effects and reduction in stem canker.

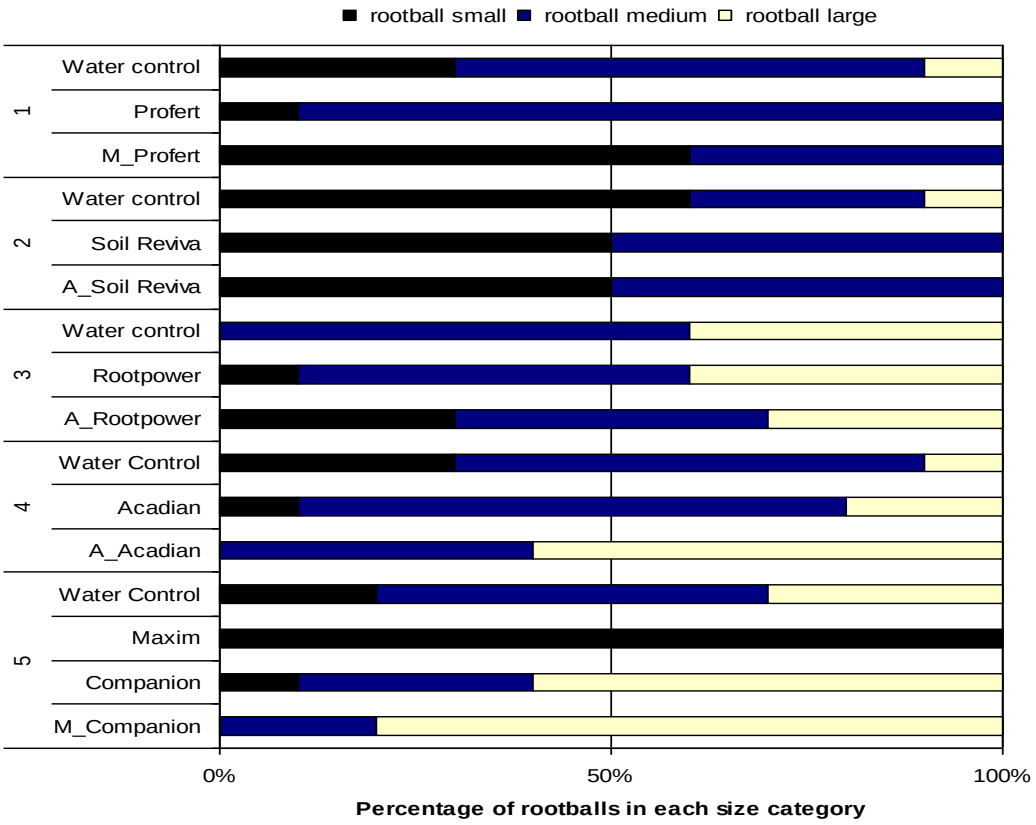
Table 18*. Incidence and severity of stem canker and plant growth of cauliflower cv. Skywalker treated with various fungicides and planted into soil inoculated with Leptosphaeria maculans and Rhizoctonia solani AG 2.1. Treatments with the same letter in the same experiment are not significantly different from one another (P=0.05).*

Expt.	Treatment	Canker incidence %	Canker severity %	Head weight (g)	Plant weight (g)
1	Water control	50	20 b	18.1	124 a
	Profert	40	20 b	21.1	87 b
	M-Profert	0	0 a	8.8	80 b
2	Water control	70	35 b	7.6a	82 a
	Soil Reviva	0	0 a	0.5b	104 a
	A-Soil Reviva	30	15 a	1.6b	115 a
3	Water control	40	18 a	14.9	150 a
	Rootpower	20	5 a	7.2	131 a
	A-Rootpower	10	5 a	13.5	136 a
4	Water control	40	30 b	9.7	104 a
	Acadian	20	10 ab	6.8	122 a
	A-Acadian	10	5 a	14.5	141 a
5	Water control	30	20 ab	15.1	104 b
	Maxim	60	25 ab	10.9	132 ab
	Companion	70	33 b	24.6	134 ab
	M-Companion	20	10 a	14.5	150 a

Figure 21. *Cauliflower plants cv. Skywalker planted into soil treated Soil Reviva, showing chlorotic leaves at 2 weeks after planting (L) and stunting with no chlorosis at 4 weeks after planting (C) compared to the inoculated control (R).*

Comparison of rootball size showed differences between the inoculated controls in each experiment, as well as between the treatments (Fig. 22). All the controls had some large rootballs, however no large rootballs developed in plants treated with Maxim alone or Soil Reviva or Profert alone or with fungicide. This indicates these products either did not encourage root growth, or the lack of root growth contributed to the early stunting or lower plant weight at harvest.

Figure 22. *Incidence of rootball size of cauliflower cv. Skywalker 11 weeks after planting into soil inoculated with Leptosphaeria maculans and Rhizoctonia solani AG 2.1 following pre- and post-planting treatments (*Experiments *1 to 5).*



While the addition of Maxim to Profert increased the proportion of small rootballs, the addition of Companion to Maxim improved root growth and plants treated with Companion + Maxim or Companion alone had rootballs larger than or similar to those of the un-inoculated controls (Fig. 23). This may indicate Maxim used as a pre-planting root drench may have a negative impact on root growth in the confined pots, which could impact on the plants ability to adequately supply nutrients.

Figure 23. *Rootballs of cauliflower cv. Skywalker treated with Maxim (L) or Maxim and Companion (R) 11 weeks after planting into soil inoculated with Leptosphaeria maculans and Rhizoctonia AG2.1.*

4.6.3 Experiments 8, 9. Azoxystrobin, flutriafol and plant health products

Aim: To evaluate the efficacy of azoxystrobin combined with Companion and Rootfeed, alone or in combination with low rates of flutriafol, based on the seed application rates used in canola calculated as a per plant dose.

Methods

Sterile cocopeat was inoculated with a mycelial slurry of *Rhizoctonia* AG2.1 as previously described and after one week placed into either 175mm pots (Expt. 8) or MK9 pots (Expt. 9). Each pot was inoculated with 10ml or 4.5ml *Leptosphaeria* spore suspension on the soil surface and watered with ~200ml or ~100ml of tap water in Expts. 8 and 9 respectively. Un-inoculated cocopeat was used as the control. Pots were kept moist in the greenhouse for three weeks before planting.

Experiment 8: Five week old cauliflower seedlings cv. Chaser were treated with a tank mix of azoxystrobin (Amistar 1ml/L) and flutriafol (Impact Endure 0.1ul/L), alone or with either Companion (1.25ml/L) or Rootfeed (20ml/L) as a pre-plant drench using 17 replicate plants per treatment. Another 17 replicate plants were treated with water as the inoculated control Either Rootfeed (0.8 ml/L) or Companion (0.05ml/L) were applied in 250 ml water to the treated pots at 2, 4, 6 and 8 weeks after planting. An equivalent amount of nitrogen using the product Thrive was applied in place of Rootfeed to the un-inoculated and inoculated control pots.

Experiment 9: Five week old cauliflower seedlings cv. Appia were treated with a mix of azoxystrobin (Amistar 1ml/L), Companion (1.25ml/L) and Rootfeed (20ml/L) with and without flutriafol (Impact Endure 0.1ul/L) as a pre-plant drench with 10 replicate plants per treatment. Another 10 replicate plants were treated with water as the inoculated control Rootfeed (0.8ml/L) was applied in 250 ml water to all treated pots at 2, 4, 6 and 8 weeks after planting. An equivalent amount of nitrogen using the product Thrive was applied in place of Rootfeed to the un-inoculated and inoculated control pots.

Cankers were assessed as previously described 2, 4, 6, 8, 12 and 17 weeks after planting. At six weeks after planting, stunting was rated as the number of plants with plant height less that 75% of the height of the plants in the un-inoculated controls. Plant growth parameters assessed at harvest 17 weeks after planting included: head weight, stalk weight; plant weight, and root-ball size (Fig. 24).

Figure 24. *Root ball size of cauliflower cv. Appia assessed at harvest as large (L), medium (C) and small (R).*

Results and Discussion

Experiment 8: Only five plants developed cankers at harvest and there was no significant difference between the treatments (data not presented). Plant growth was similar between treatments (data not presented) with no indication of stunting.

Experiment 9: Cankers were first observed six weeks after planting in 8% of the azoxystrobin, Companion and Rootfeed treated plants and 4% of the azoxystrobin, Companion, flutriafol and Rootfeed treated plants. By harvest 17 weeks after planting both treatments had numerically less canker than the inoculated control, and the addition of flutriafol reduced canker (P=0.06) compared to the azoxystrobin + companion + Rootfeed alone (Table 19).

The un-inoculated controls were the largest plants, with significantly higher mean plant weight and stalk weight (Table 20). However these plants also had no large rootballs, indicating that the size of the rootball was not a limiting factor for growth and was more than adequate to provide sufficient nutrients for plant growth. None of the plants in the inoculated controls were stunted, however there was some stunting in the treated plants (data not presented) indicating a possible effect from the treatment.

The stunting was reduced from 16% to 3% where flutriafol was included in the treatment, but this difference was not significant.

Table 19. *Incidence and severity of stem canker in potted cauliflower cv. Appia 17 weeks after application of various products and planting into soil inoculated with Rhizoctonia AG2.1 and Leptosphaeria maculans.*

Pre-planting treatment	**Mean canker incidence (%)**	**Mean canker severity**
Inoculated control	40	10
azoxystrobin + Companion + Rootfeed	24	6
azoxystrobin + Companion + Rootfeed + flutriafol	8	2

Table 20. *Growth of cauliflower cv. Appia 17 weeks after application of various products and planted into soil inoculated with Rhizoctonia and Leptosphaeria maculans. Means with the same letter are not significantly different from one another.*

Treatment	**Mean stalk weight (g)**	**Mean plant weight (g)**	**Mean head weight (g)**	**% incidence rootball size**		
				Small	**Medium**	**Large**
azoxystrobin + Companion + Rootfeed + flutriafol	17.5 a	58.3 a	10.5	20	40	40
azoxystrobin + Companion + Rootfeed	17.5 a	54.9 a	6.5	12	72	16
Inoculated control	17.2 a	54.0 a	9.1	17	58	25
Uninoculated control	26.6 b	78.3 b	12.9	20	80	0

Due to the low disease severity little can be interpreted on the relative efficacy of the treatments. However even with the minimal canker detected, the plant growth was obviously impacted by the presence of the disease and not improved by either treatment. The addition of flutriafol may provide some benefit in canker suppression but needs to be further evaluated.

4.6.4 Experiment 10. Flutriafol

Aim: To evaluate the efficacy of higher rates of flutriafol applied as a preplanting drench in Brussels sprout cv. Cumulus grown in field soil inoculated with *L. maculans*. Rates of flutriafol were increased from those used in experiment 8 and 9 following advice from overseas work using rates for foliar and soil applications.

Methods

Sandy loam soil was collected from a commercial paddock in Virginia which had been fallow for one season following a cauliflower planting. The field soil was steam sterilised at 60 degrees for 120 minutes then PCR tested for the presence of AG 2.1, 2.2 or 4 and *L. maculans.*

The soil was transferred into 80, 200mm sterile pots and each pot was soil inoculated as previously described.

Pre-germinated seeds cv. Cumulus were planted in a standard commercial multicell seedling tray and maintained in a greenhouse with approximate day/night temperatures of 30/20 degrees. Five weeks post-seeding 10 replicate plants were treated by drenching with flutriafol (8ml/100L Impact Endure) for 5 minutes before being planted into the inoculated pots of field soil. 10 replicate plants were planted into inoculated pots without fungicide treatment.

Planted pots were maintained in a controlled environment room with a day night temperature of 20 degrees and a 12 hr day/night light cycle. Plants were watered to field capacity and were fertilised fortnightly with Thrive up to 8 weeks then Maxfeed until 20 weeks.

Stem canker was assessed 5, 8 and 12 wks after planting and growth parameters included plant height from soil level to first expanded leaf at 5 weeks post-planting, and weight of the plant minus the rootball and the weight of the stalk including immature sprouts was undertaken at 17 weeks post-transplanting.

Results and discussion

No *Leptosphaeria maculans* or *Rhizoctonia solani* AG 2.1, 2.2 or 4 were detected in the sterilised soil.

No cankers were observed on any plants, indicating that the inoculation was not successful. While there was no significant difference in plant growth, numerically the untreated plants were larger than the treated plants (Table 21), indicating a possible phytotoxic effect of the fungicide that would need further investigation.

Table 21. *Growth at 5 and 17 weeks after planting Brussels sprouts cv. Cumulus drenched with flutriafol in field soil inoculated with Leptosphaeria maculans. No significant difference between treatments (P=0.05).*

Treatment	**Mean height (am) at 5 wks**	**Mean plant weight (g) at 17 wks**	**Mean stalk weight (g) at 17 wk**
Cumulus F1 Treated	11	145.24	8.38
Cumulus F1 Untreated	16.3	159.80	13.28

4.6.5 Experiment 11. Azoxystrobin, flutriafol and plant health products

Aim: To evaluate the efficacy of various biological and plant growth products in comparison with azoxystrobin and flutriafol.

Methods

100 L of sandy loam soil was collected from a commercial cauliflower paddock, steam-pasteurised, combined thoroughly with 100 L of sterile cocopeat and placed into 175mm pots. Each pot was inoculated with a 3ml mycelial slurry of *Rhizoctonia* AG2.1 on the soil surface and injected with 3ml of a spore suspension of *Leptosphaeria* as previously described and the soil mixed thoroughly in each pot.

A combined 200g soil subsample was taken randomly from both the inoculated and uninoculated pots and PCR tested. Pots were left to stand in the greenhouse for 7 weeks and kept moist. The pots had *Hypoaspis* applied to control fungus gnats.

Cauliflower seedlings cv. Chaser were collected at 4 weeks of age from a commercial nursery and two subsamples of 20 plants each removed, roots washed and the entire plant stored at -22 degrees until PCR tested. The remaining seedlings were maintained in the greenhouse for two weeks before treatment and planting. Two pots of each treatment were also toothpick baited as previously described prior to planting.

Ten replicate plants were treated prior to or at planting with a range of products and treatment methods outlined in Table 22. Treatment methods included pre-plant drenching plants as previously described or one of two pre-plant soil treatments. Flutriafol was applied by applying 10ml of diluted product into the planting hole to simulate in furrow application. Other treatments were applied in trench 4cm deep and 1cm wide, either as a granular product or as 250 ml liquid product, covered with a 2mm layer of soil and drenched with 250 ml water before planting seedlings in the trench, simulating commercial field planting into a pretreated trench. Post-plant treatments were applied at 2, 4 and 6 weeks after planting in 250 ml water, sufficient to saturate the entire pot.

Ammonium sulphate was applied fortnightly at 3 g/L with each pot receiving 100ml.

Stem canker and stunting was assessed at 3, 5, 8, 12 and 15 weeks after planting as previously described. A plant was considered stunted if it was less than half the height of the plants in the un-inoculated controls. At harvest, 15 weeks after planting, comparative rootball size, stalk length and dry weight of combined stems were measured.

Table 22.*Treatments and rates of products applied either as 1) pre-plant root drench of the seedling, 2) drenching the soil prior to planting,3) applying product in a trench in the soil and watering in before planting into the trench or 4) drenching the soil after planting.*

Treatment	**Rate/L of product and application method**			
	1) Pre-plant root	**2) Pre-plant soil**	**3) Pre-plant trench**	**4) Post-plant**
Untreated				
Impact Endure (flutriafol)		1.35ml		
Impact Endure + Amistar (azoxystrobin)	 0.2ml	1.35ml		
Manutec Zinc Sulphate + Manganese Sulphate				1.28mg 1.04mg
Trichoshield	5g			0.02g
Go Go Juice	50ml			2ml
Plantmate granular			19 g	
Companion			1.25ml	0.05ml
Microbial			1 ml	0.5ml

Results and discussion

At two weeks after planting, root and stem damage was observed on seedlings (Fig. 25) and two of the Zinc/Manganese treated plants had died. *Rhizoctonia* was recovered from the stems of the dead plants. Zinc has been shown to suppress development of black leg symptoms caused by *L. maculans* in canola (Rouxel *et al* 1990), however in this experiment it did not suppress *Rhizoctonia* infection. The plants in this treatment also appeared pale and smaller than other plants, which may have increased their susceptibility to disease.

Further seedling deaths with wire-stem symptoms attributed to *Rhizoctonia* occurred at three weeks after planting, with two plants from the Microbiol treated plants and one in each of the untreated, Companion, Plantmate, GoGo Juice and flutriafol treated plants. Toothpick baiting had detected *Rhizoctonia* in the inoculated pots prior to planting.

Figure 25. *Root and stem damage caused by Rhizoctonia on cauliflower cv. Chaser 2 weeks after planting into soil inoculated with Rhizoctonia AG 2.1 and Leptosphaeria maculans.*

The canker severity of Microbial 15 weeks after planting was highest at 42.5% while no canker was observed in plants treated with Trichoshield or the Impact + Amistar combined treatment (Table 23).

At 15 weeks after planting, all plants treated with flutriafol (Impact) were stunted, however no stunting was observed where this product had been combined with azoxystrobin (Table 23).

There were no significant differences in stem weight between the treatments, with the highest stem weights observed in the plants treated with Companion and the untreated control. Plants treated with Plantmate had the lowest stem weight (Fig. 26)

Table 23. *Severity and incidence of stem canker and stunting and stem dry weight of cauliflower plants cv. Chaser 15 weeks after planting and treatment with various fungicides and biological products.*

Treatment	**Mean % Severity**	**Mean % Incidence**	**Mean % Stunting**	**Stem dry weight (g)**
Untreated	15 b	20	10	5.3
Impact	12.5 ab	20	100	3.9
Impact & Azoxystrobin	0 a	0	0	4.4
Companion	15 b	30	0	5.3
Trichoshield	0 a	0	0	4.5
Plantmate	10 ab	10	60	3.1
Go Go Juice	10 ab	10	10	3.9
Microbial	42.5 c	50	0	4.5
Manutec	20 bc	20	0	3.8

Figure 26. *Stems of cauliflower seedlings cv. Chaser 15 weeks after treated with Plantmate granular (top) or Companion (bottom) and planted into soil inoculated with Rhizoctonia AG2.1 and Leptosphaeria maculans.*

4.6.6 Experiment 12. Azoxystrobin and fluquinconazole

Aim: To evaluate the efficacy of fluquinconazole (Jockey ®) for suppression of stem canker when applied as a seed treatment to cauliflower seed.

Methods

Untreated cauliflower seeds cv. Snowball were coated with either 58 µl sterile water or 8µl Jockey® mixed with 8µl sterile water. Seed was coated by placing in a Petri dish with the treatment and gently agitating until the seed was coated. Treated seed was dried and sown into 6 punnet seedling trays filled with sterile cocopeat and watered from underneath.

There was only 17% emergence of the 222 seeds, 17 fluquinconazole treated seedlings and 14 sterile water treated seedlings, with no difference in initial emergence noted between the treatments. At six weeks after germination, half the seedlings from the water treatment were root drenched as previously described in water and the other half in azoxystrobin. Seedlings treated with fluquinconazole (Jockey®) were root drenched in either azoxystrobin or a mixture of fluquinconazole and azoxystrobin.

Treated seedlings were planted into 165mm pots of steam pasteurised field soil previously inoculated with 3 plugs of *Rhizoctonia* AG2.1 mycelia and 6ml of *L. maculans* spore suspension per pot. 500g of inoculated soil was collected as 50g samples from ten randomly selected pots, mixed together and PCR tested for amounts of *Rhizoctonia* and *Leptosphaeria*. Two toothpicks were also placed in each pot to confirm the presence of *Rhizoctonia*.

Plant stunting (calculated as the percent of plants with height less than 1/2 the height of the tallest controls) and canker severity were assessed 7 weeks after transplanting.

Results and discussion

L. maculans was detected in the inoculated soil at 4 pg DNA/g soil and *Rhizoctonia* AG 2.1 at 100 Pg DNA/g soil. The toothpick bait confirmed the presence of *Rhizoctonia* in all pots. None of the plants grown from seed treated with fluquinconazole and drenched with azoxystrobin with or without fluquinconazole developed canker (Table 24), whereas the canker developed on 21.4% of plants seed treated with water and drenches with azoxystrobin.

Untreated plants were variable in height, with over 57% being classified as stunted (Table 24). Seedlings grown from seed treated with fluquinconazole had more even growth, with less stunted plants than other treatments. The addition of azoxystrobin as a pre-plant drench increased stunting except when mixed with fluquinconazole.

Table 24. *Incidence and severity of canker on cauliflower cv. Snowball 7 weeks after planting into inoculated soil, using seedlings grown from treated seed and pre-plant drenched with various fungicides.*

Seed treatment	Pre-plant drench	Mean canker incidence	Mean canker severity	Mean % stunting
fluquinconazole	azoxystrobin	0	0	33.3
	fluquinconazole and azoxystrobin	0	0	12.5
Water	azoxystrobin	21.4	22.9	85.7
	Water	7.1	8.6	57.1

4.6.7 Conclusions

The fungicides flutriafol and fluquinconazole are used to manage black leg caused by *L. maculans* in canola. Fluquinconazole is only registered as a suppressant for *L. maculans* on canola and flutriafol has been found to be more effective (Sprague *et al* 2010). However neither have registration or permits for fresh vegetable production and as both provided suppression of canker in the greenhouse trial it would be beneficial to field evaluate both products and to develop efficacy data for potential registration. Using the combination of products to control both *L. maculans* and *Rhizoctonia* should provide better control of stem canker.

Many of the fungicides evaluated in greenhouse stunted plants, which needs further field evaluation. The use of alternative products that promote plant growth may be of benefit in providing stronger root and plant growth to achieve a marketable yield even with stem canker present.

4.7 Alternative hosts

Aim: to determine if Brassica weeds and other cruciferous crops are alternate hosts of *Leptosphaeria maculans*.

Materials and methods

9 trays (20 cm x 30 cm x 15cm deep with 6 25mm holes in the base) were lined with chux cloths, filled with sterile coco peat and placed in a greenhouse with approximate night temperatures of 20 degrees and day temperature of 30 degrees.

Each tray was seeded with one of eight Brassica plants (Table 25). At 5 days after seeding, individual seedlings were transferred into 100mm pots and supported with sterile plastic rods to prevent the plants touching the soil. Thrive was applied fortnightly to the soil of each pot at 0.02 g/plant.

A white mustard weed was collected from near Brassica crops in the Adelaide Hills (Fig. 27) and air dried for 1 week. Seeds were removed from seedpods and placed on moistened cotton wool in a petri dish, sealed with parafilm and incubated at 25°C. 2 days after incubation the germinated seeds were transferred into the 10cm pots.

Table 25. *Brassica species trialled for susceptibility to Leptosphaeria maculans.*

Common name	**Genus**	**Cultivar / type**
White mustard	*Brassica alba (Sinapsis alba)*	
Radish	*Raphanus sativus*	Scarlet Globe
Rocket	*Eruca sativa*	
Kale	*Brassica oleraceae* ssp. *acephala*	
Chinese cabbage	*Brassica rapa* ssp. *chinensis*	Buk choy -
Chinese cabbage	*Brassica rapa* ssp. *chinensis*	Wombok -
Wild rocket	*Diplotaxis tenuifolia*	
Radish	*Raphanus sativus*	Salad Crunch

The cotyledons of ten 7 day old seedlings of each crop were wounded with a sterile needle, twice on each cotyledon. The wound sites were inoculated with a 10µl conidial suspension of defrosted *L. maculans* (~1 x 10^6 spores/ml) (Fig. 28) or sterile water as a control.

The cotyledons of a further 5 seedlings of each alternate crop had a 10µl droplet of the same spore suspension placed on each lobe without wounding.

Figure 27. Mustard weed in inter-row of cauliflower plantings Adelaide Hills.

Figure 28. *Inoculation of wounded white mustard cotyledons with 10 µl Leptosphaeria maculans spore suspension.*

Eight 28 day old plants for each alternate crop were wounded on the 4th and 5th expanded leaf and inoculated with a 10µl conidial suspension of defrosted *L. maculans* (~1 x 10^{6} spores/ml).

Stem staining and leaf lesions at the point of inoculation were assessed 11, 20 and 29 days after the cotyledon inoculation and 14 days after the mature leaf inoculation. Leaf lesions were identified and distinguished from other pathogens using the description of Laing (1996). Lesions at each inoculation site were rated using a 0-3 rating, where 0 = no visible lesion, 1 = small area of grey-green tissue collapse, 2 = lesion with tissue collapse and a few pycnidia, 3 = collapsed tissue with masses of pycnidia (Fig. 29). A mean incidence and severity rating for all lobes of each inoculation technique was determined for each plant type.

Figure 29. *Leptosphaeria maculans infection. Lesion ratings 1 (L), 2 (Centre) and 3 (R).*

Results and discussion

No control plants developed lesions whereas 11 days after inoculation lesions were present on the inoculated cotyledons except the white mustard and the organic rocket (Table 26). The pycnidia in the lesions were confirmed *L. maculans* by microscopic examination and isolation onto PDA. The rocket had a low incidence of 2.5% at 20 and 29 days after inoculation. The kale, wombok and buk choy were the most susceptible of the Brassica plants, with 90, 67.5 and 55% incidence respectively at 29 days after inoculation. They were also the only plants to develop lesions without wounding, with lesion incidence of 5, 10 and 12.5% respectively.

Cotyledons were also more susceptible to infection than the mature leaves, with only kale, wombok, buk choy and radish (cv. salad crunch) developing lesions on the mature leaves at 14 days after inoculation. It is possible that lesions on the mature leaves may take longer to develop, however it is more likely that increased plant maturity may impact on leaf susceptibility to *L. maculans* infection.

Kale was also the only Brassica to develop stem staining on 4 of the 10 leaf wounded and inoculated plants (Fig. 30), indicating significant systemic activity of the fungus.

Two of the white mustard weeds developed lesions on the wounded and inoculated cotyledons 6 weeks after inoculation (data not presented). No lesions were present on the control plants, the non-wounded cotyledons or the mature leaf inoculated plants.

These results show that Brassica plants, particularly Kale and Chinese cabbage (wombok and buk choy), as well as Brassica weeds could act as alternative hosts and inoculum source for *L. maculans*. Reducing volunteer Brassica weed species during fallow could limit pathogen carryover during fallow and rotation cropping.

Figure 30. *Stem lesion (L) and canker (R) on Kale caused by* Leptosphaeria maculans *28 days after cotyledon wounding and inoculation.*

Table 26: *Incidence and severity of lesions on wounded and unwounded Brassica seedlings (n=15 wounded, 5 unwounded) 11 to 29 days after inoculation with* Leptosphaeria maculans *on cotyledons or 4th and 5th expanded leaves.*

Brassica crop	Cotyledon infection						4th and 5th exp. leaf	
	Mean % lesion incidence			Mean severity			sev	% inc.
	Days after inoculation							
	11	20	29	11	20	29	14	
Wounded								
Buk choy	45	55	55	0.9	1.5	1.5	0.7	34
Kale	53	88	90	1.1	2.6	2.6	0.7	34
Radish (salad crunch)	20	38	45	0.4	0.9	1.1	0.3	13
Radish (scarlet globe)	18	40	40	0.4	0.8	0.8	0	0
Rocket	2.5	2.5	2.5	0.1	0.1	0.1	0	0
Rocket (wild)	10	10	10	0.2	0.2	0.2	0	0
White mustard	0	0	10	0	0	0.2	0	0
Wombok	48	68	68	1.0	1.5	1.5	0.7	28
Unwounded								
Buk choy	0	13	13	0	0.3	0.3	Not tested	
Kale	10	5	5	0.2	0.1	0.1		
Radish (salad crunch)	0	0	0	0	0	0		
Radish (scarlet globe)	0	0	0	0	0	0		
Rocket	0	0	0	0	0	0		
Rocket (wild)	0	0	0	0	0	0		
White mustard	0	0	0	0	0	0		
Wombok	5	5	10	0.1	0.1	0.2		

4.8 Field efficacy trials – South Australia

Products showing efficacy in greenhouse experiments were further evaluated in field trials in soil previously shown to be infected with both pathogens.

4.8.1 Trial 1. Adelaide Hills

Aim: To evaluate preplant treatments with azoxystrobin and flutriafol combined with plant growth products or Companion followed by post-plant treatments on control of stem canker in cauliflower planted into infected soil.

Materials and methods

Five week old cauliflower cv. Skywalker were drenched as previously described with fungicides and the alternative products outlined in Table 27 and planted 24 hrs later into soil known to be infected with *R. solani* and *L. maculans*. The trial area had been previously inoculated with infected soil from greenhouse trials. Each plot consisted of 6 rows of three plants, spaced at 50 cm intervals in rows spaced 50 cm apart, with each treatment replicated 6 times.

Companion and Rootfeed were also applied 2, 4 and 6 weeks after planting to Treatments 2 and 3 respectively using a Hortex Insecticide and Fertiliser Hose on sprayer. Thrive® was applied to treatment 2 and the untreated control to provide the equivalent g per plant of Nitrogen applied in the form of Rootfeed® to treatment 3. Maxfeed was used fortnightly on all plants to provide adequate Phosphorous and Nitrogen requirements for normal growth.

Canker severity was assessed on all plants three, five, eight and eleven weeks after planting. 20 random plants from each of the three treatments were cut at the soil line, weighed and assessed for staining in the stem cross section.

Table 27. *Products and rates applied as pre-planting and post-planting drenches.*

	Rate/L	**Treatment**		
		1	**2**	**3**
Water		pre-plant		
Azoxystrobin	1ml		pre-plant	pre-plant
Flutriafol	0.1ml		pre-plant	pre-plant
Companion	1.25ml		pre-plant + 2, 4, 6 weeks after planting	
Bioforge	0.6ml			pre-plant
X-tender	0.5ml			pre-plant
Rootfeed	0.6ml			pre-plant + 2, 4, 6 weeks after planting

Results and discussion

Incidence of canker in the untreated control increased from 10% 5 weeks after planting to 27% 11 weeks after planting (Table 28). Both treatments significantly reduced the level of canker at all assessments, with canker not observed in treatment 3 until 8 weeks after planting. While there was no significant difference between the two fungicide treatments, less disease was observed initially in the treatment 3, where the plant growth products were applied with the fungicides before planting. The post-planting applications of Companion numerically reduced the canker severity at week 11 compared to the plant growth products, with 1.2% and 3.2% respectively.

Maximum temperatures during the trial period were almost 2 degrees below average and rainfall in excess of 70mm occurred within a 4 day period, causing waterlogged conditions when seedlings were 8 weeks of age. While *Rhizoctonia* is favoured by moderately wet conditions rather than dry or saturated soils, *Leptosphaeria* is known to be most destructive in wet conditions with persistent dews (Sherf & MacNab 1986)**.**

Table 28. *Incidence and severity of stem canker 5, 8 and 11 weeks after treatments and planting cauliflower cv. Skywalker into soil infected with* Rhizoctonia solani *and* Leptosphaeria maculans. *Means with the same letter are not significantly different (P=0.05).*

Treatment	**Incidence**			**Severity**		
	5wks	**8wks**	**11wks**	**5wks**	**8wks**	**11wks**
1 (untreated)	10.2 a	13.9 a	26.9 a	5.6 a	7.2 a	13.7 a
2 (+companion)	3.7 b	0.9 b	4.6 b	1.4 b	0.5 b	1.2 c
3 (+ plant growth)	0.0 b	2.8 b	9.3 b	0.0 b	0.9 b	3.2 b

Only one control plant had staining present in the stem cross section, indicating internal stem staining was not correlated to external canker symptoms in this trial. The mean plant weight was significantly higher in the treated plants than the controls. Mean plant weight in the controls was the lowest at 15.26 g while there was no statistical difference between the two treatments with a mean plant weight of 29.11 g in treatment 3 (azoxystrobin, flutriafol and plant growth products) and 27.75 g in treatment 2 (azoxystrobin, flutriafol and Companion).

4.8.2 Trial 2. Adelaide Hills

Aim: To evaluate preplant treatment with azoxystrobin and flutriafol combined with plant growth products or Companion followed by post-plant treatments on control of stem canker in cauliflower planted into infected soil.

Materials and methods

Five week old cauliflower cv. Skywalker were drenched as previously described with fungicides and the alternative products outlined in Table 29 and planted 24 hrs later

into soil known to be infected with *R. solani* and *L. maculans*. The trial area had been previously inoculated with infected soil from greenhouse trials and left for three weeks before planting. Each plot consisted of 24 rows of five plants, spaced at 50 cm intervals in rows spaced 50 cm apart. The treatments were planted in a large unreplicated block in order to minimise treatment interactions from water and post-planting product application runoff due to the sloping land.

Post-planting applications of Rootfeed and Companion were undertaken 2, 4 and 6 weeks after planting using a Hortex Insecticide and Fertiliser Hose on sprayer until soil saturation of the entire treatment area. Thrive® was applied to provide the equivalent g per plant of Nitrogen as that applied in the form of Rootfeed® to the other treatments. Maxfeed was used fortnightly on all plants to provide adequate Phosphorous and Nitrogen requirements for normal growth.

Seasol was applied twice two weeks apart (10 and 12 weeks after planting) in the same manner as the Rootfeed to ½ each area of the two treatments.

Table 29. *Products and rates applied as pre-planting and post-planting drenches.*

	Rate/L	**Application**	**Treatment**				
			1	**2**	**3**	**4**	**5**
Water		pre-plant	✓				
Azoxystrobin	1ml	pre-plant		✓	✓	✓	✓
Flutriafol	0.1ml	pre-plant		✓	✓	✓	✓
Companion	1.25ml	2, 4, 6 weeks after planting		✓	✓		
Bioforge	0.6ml	pre-plant				✓	✓
X-tender	0.5ml	pre-plant				✓	✓
Rootfeed	0.6ml	pre-plant				✓	✓
Seasol	3.25ml	10, 12 weeks after planting			✓		✓

At 3 and 5 weeks after planting, canker severity was assessed on 6 plants at 6 areas within the treatment, a total of 36 plants per treatment. At 8, 11 and 13 weeks after planting, canker severity was assessed on 10 plants at 6 areas within the treatment, a total of 60 plants per treatment.

Results and discussion

Canker developed in 38% of plants in the untreated controls compared to 8 to 23% in the treated plants (Table 30). All treatments reduced canker incidence and severity, however there were no significant differences between treatments. While the differences were not significant, in both treatments the addition of seasol at 10 and 12 weeks numerically increased the incidence and severity of canker.

Table 30. *Incidence and severity of stem canker on cauliflower cv. Skywalker 13 weeks after planting into soil infected with Leptosphaeria maculans and Rhizoctonia solani, treated with azoxystrobin and flutriafol with various pre-plant and post-plant products. Means with the same letter are not significantly different from one another (P=0.05).*

Treatment	Incidence	Severity
1 Untreated	38.3 a	18.4 a
2 (+ companion)	15.0 b	5.00 b
3 (+ companion, seasol)	23.3 b	7.08 b
4 (+ plant growth products)	8.3 b	2.50 b
5 (+plant growth products, seasol)	10.0 b	3.33 b

Plants were slow growing, very stunted and did not form heads by 13 weeks in all treatments and consequently were not assessed for plant weights or yield. A significant frost occurred at 10 weeks after planting and may have reduced growth.

4.8.3 Trial 3. Northern Adelaide Plains

Aim: To evaluate the efficacy of fluquinconazole (Jockey) as a preplant seedling drench for control of stem canker, applied alone and in combination with the fungicides azoxystrobin and fludioxonil in a commercial planting of cauliflower.

Materials and methods

Speedling trays containing 196 seedlings of 6 wk old Skywalker seedlings were drenched prior to planting as previously described using the treatments outline in Table 30, one tray per treatment. One tray was drenched in water as the untreated control, and another PCR tested as previously described for presence of *R. solani* or *L. maculans*.

The trays of treated seedlings were planted by the grower using a commercial planting machine, interplanting each treated row with cauliflower cv. Brittany provided by the grower and planted in the surrounding area.

Eight days after planting, azoxystrobin was applied using a motorised knapsack as a soil drench in a banded application 10cm wide along the rows (Fig. 31).

Watering and fertilisers were applied by the grower as per normal practice.

3, 8 and 15 weeks after planting, the crop was assessed for canker symptoms on 20 random plants in each treatment, 5 consecutive plants from 4 areas within the row.

Table 30. *Timing and rates of fungicide drenches applied to cauliflower cv. Skywalker.*

Tmt No.	Pre-plant treatment	Rate/plant	Post-plant treatment (8 days)
1	Jockey (fluquinconazole)	0.04ml	-
2	Amistar (azoxystrobin)	0.02ml	-
3	Amistar + Jockey	0.02 +0.04ml	-
4	Maxim (fludioxonil)	0.016ml	-
5	Jockey	0.04ml	Amistar (0.96ml/plant)
6	Maxim	0.016ml	Amistar (0.96ml/plant)
7	Water	-	Water

Figure 31. *Applying post-planting azoxystrobin as a 10cm banded fungicide drench 8 days after planting on cauliflower cv. Skywalker.*

Results and Discussion

Three weeks after planting stunting was observed in seedlings pre-plant drenched with fluquinconazole, however by harvest all plants had achieved similar height. The product label notes Jockey® may shorten the hypocotyl length in canola.

Cankers were observed on some plants at 8 weeks after planting, and by 15 weeks (harvest) the untreated control had significantly more infection at 15% severity than any of the treatments (0-8.75%) (Fig. 32, Table 31). There was no significant difference in mean canker severity between any of the fungicide treated plants, however no disease was observed in plants treated before planting with the combination of fluquinconazole and azoxystrobin.

Marcroft *et al* (2004) found during fungicide screening trials for control of blackleg in canola, suppression of canker due to *L. maculans* was only significant in seasons of

high disease pressure. Therefore repetition of this trial under a higher disease pressure would be an advantage.

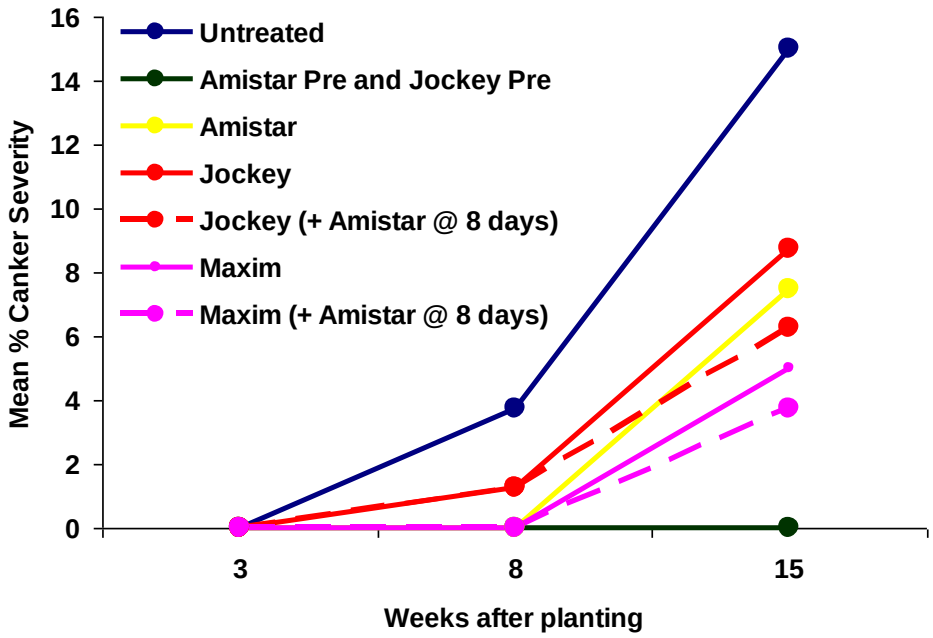


Figure 32. *Severity of stem canker at 3, 8, and 15 weeks after planting cauliflower cv Skywalker treated with various fungicides pre-planting or 8 days after planting.*

Table 31*. Canker incidence and severity at harvest on cauliflower cv. Skywalker treated with various fungicides and planted into a field previously infected with stem canker (Northern Adelaide Plains). Severity of untreated control greater than all treatments (P=0.05).*

Treatments		**Canker incidence (%)**	**Canker severity (%)**
Pre-planting	**After planting**		
Untreated control (water)	-	35	15
Jockey		20	8.8
Amistar		25	7.5
Jockey	Amistar	15	6.3
Maxim		10	5.0
Maxim	Amistar	5	3.8
Amistar and Jockey		0	0

All plants at harvest were assessed as having a head: the grower rated them of equal harvestable quality and consequently no yield losses were recorded due to stem canker in this planting.

4.8.4 Trial 4. Northern Adelaide Plains grower trial

Aim: To evaluate applications of azoxystrobin applied in the nursery before planting combined with commercial formulations of *Bacillus subtilis* or a plant growth promotant Mega-Kel-P applied both in the nursery before planting and in field after planting by the grower.

Materials and methods

12 Speedling trays each containing 196 plants of 5 week old cauliflower cv. Skywalker were preplant drenched at commercial rates at the nursery with a combination of azoxystrobin (Amistar) and *Bacillus subtilis* (Companion) 24 hours prior to collection by the grower for planting (Table 32). Seedlings were machine planted in paired rows with an interplant spacing of 50 cm and row spacing of 75cm adjacent to the growers planting of seedlings of the same age and cultivar but predrenched in the nursery with Mega-Kel-P. All seedlings were maintained as per normal commercial grower practice.

Two weeks after planting the grower applied a drench of Mega-Kel-P to the plants previously treated with this product and planted adjacent to the trial site. Four weeks later (six weeks after planting) the grower applied an alternative *Bacillus subtilis* product (Fulzyme) through the overhead irrigation system, leaving an equivalent area untreated in both the trial site and the adjacent planting of 4 rows by 24 plants of seedlings.

Table 32. *Products, timing and rates applied as either pre-plant or post-planting soil drenches for each treatment.*

Product	**Timing**	**Rate**	**Treatment**			
			1	**2**	**3**	**4**
Amistar	pre-plant	100 ml/100L	✓	✓		
Companion	pre-plant	120ml/100L	✓	✓		
Mega-Kel-P	pre-plant	1 L/100L			✓	✓
Fulzyme	post-plant @ 6 weeks	1 L/Ha		✓		✓
Mega-Kel-P	post-plant @ 2 weeks	5 L/Ha			✓	✓

Four replicate areas of 20 randomly chosen plants from each treatment were assessed for stem canker severity and stunting as previously described 11 days, 7, 12 and 15 weeks post-planting.

Results and discussion

The incidence and severity of stem canker increased between 12 and 15 weeks after planting, with 48-54% of plants infected at 15 weeks compared to 5-12.5% at 12 weeks (Table 33). There were no significant differences in canker between any of the treatments. Stunting appeared in 20% of plants 7 weeks after planting (data not presented) however this had reduced to between 7.5 and 12.5% at 15 weeks after planting, although the differences between treatments was not statistically significant.

Table 33. *Incidence and severity of stem canker twelve and fifteen weeks and stunting at 15 weeks after planting cauliflower cv. Skywalker treated with various products before and after planting. No significant differences between treatments were detected.*

Treatment	Stem canker (%)				Stunting (%) 15 weeks
	12 weeks		15 weeks		
	Incidence	Severity	Incidence	Severity	
Azoxystrobin + Companion	6.3	1.9	48.1	14.2	8.8
Azoxystrobin + Companion + Fulzyme	8.8	2.8	50.0	15.9	7.5
Mega-Kel-P	12.5	3.1	53.8	15.9	8.8
Mega-Kel-P + Fulzyme	5.0	1.6	56.2	17.2	12.5

4.8.5 Trial 5. Adelaide Hills

Aim: To evaluate the efficacy of preplant seedling drenches of flutriafol and azoxystrobin alone and in combination on Brussels sprouts in a commercial field known to be infected with *L. maculans* and *R. solani*.

Materials and methods

The soil from three areas on a property in the Adelaide Hills was collected using a combined 40 cores sample and PCR tested for the presence of *R. solani* and *L. maculans* as previously described to determine a suitable site for a trial. The sites were either adjacent to an infected crop or planted with a crop showing symptoms (Fig. 33). The site where both *R. solani* AG 2.1 and *L. maculans* were detected was chosen for the trial area.

Five week old Brussels sprouts seedlings cv. Romulus were treated pre-planting with fungicide as previously described with azoxystrobin, azoxystrobin + flutriafol at two rates, or water as the untreated control (Table 34).

Fig 33. *Commercial Brussels sprout crop with diseased patches indicated on the area chosen as the trial site.*

Table 34. *Fungicides and rates applied pre-planting.*

Treatment	Active	Rate product per L in drench
Untreated	water	
Amistar/Impact 8	500g/Kg azoxystrobin	1 ml
	500g/L flutriafol	0.08ml
Amistar/Impact 4	500g/Kg azoxystrobin	1 ml
	500g/L flutriafol	0.04 ml
Amistar	500g/Kg azoxystrobin	1ml

Seedlings were stored for 12 hours at 25 degrees in the greenhouse after drenching before delivery to the grower, then maintained in coolstore at 2 degrees for 10 days by the grower due to delayed planting, receiving one light water prior to planting. It was decided not to re-treat the seedlings as advice from the manufacturer was that the fungicide should still be active in the soil. The seedlings were planted by the grower in two rows 70cm apart, with each treatment in one block and a control at both top and bottom of the rows. The trial rows were planted into freshly prepared soil following harvest, adjacent to crop about to be harvested and later planted to rye corn (Fig. 34). The trial area was not irrigated and relied on natural rainfall. Fertiliser was applied by hand 18 weeks after planting.

Soil samples consisting of 40 core samples taken using an accucore sampler were collected from each treatment area midway between the cauliflowers immediately after planting. The combined samples from each area were PCR tested for *L. maculans* and *R. solani* as previously described.

At 2 weeks after planting 10 random plants in each treatment were assessed for stem canker as previously described. Six, eight and twelve weeks post-planting, 5 plants were randomly chosen from each of four replicate areas for each treatment (total of 20 plants per treatment) and assessed for stem canker.

Figure 34. *Brussels sprouts cv. Romulus planted adjacent to old crop (L), with old crop area planted to rye corn after harvest, leaving two trial rows (R).*

At harvest (22 wks after planting) 15 plants per replicate area were chosen at random (60 plants per treatment) and assessed for stem canker. The height of the plant was measured and rated as normal (greater than 50 cm), small (between 40cm and 50 cm) or stunted (less than 40 cm) (Fig. 35). The size of the majority of sprouts on the stem was classed as small (less than 3 cm) or large (greater than 3 cm).

Figure 35. *Indicative stunted (left), small (centre) and large (right) Brussels sprout cv. Romulus 22 weeks after planting.*

At harvest soil was removed from the rootball of each of six randomly selected untreated 'normal' plants, six treated 'normal' plants and six treated 'stunted' plants. 100g of the soil from each plant was toothpick baited for *Rhizoctonia* as previously described. The remainder was pooled to provide 2 replicate 250g subsamples of soil removed from stunted plants and 2 replicated 250g samples from non-stunted plants, which was PCR tested for *L. maculans*.

Results and discussion

R. solani AG2.1 was detected in all treatment areas at planting, whereas AG4 was detected in two areas and *L. maculans* in one (Table 35). Given the level of disease in the previous crop, it was expected that *L. maculans* would be at much higher amounts in the soil, however previous work has also shown this pathogen to be difficult to detect in soil.

Table 35. *Amounts of Rhizoctonia solani and Leptosphaeria maculans DNA (pg/g soil) detected in soil collected at planting.*

Treatment	**DNA pg/g soil**			
	AG2.1	**AG2.2**	**AG4**	***L. maculans***
Untreated top control	95	0	0	0
Amistar/Impact 8	274	0	0	0
Amistar/Impact 4	60	0	19	2
Amistar	53	0	0	0
Untreated bottom control	195	0	4	0

At 2 weeks after planting, cankers typical of *L. maculans* infection were found in the untreated controls (Fig. 36) with 30% of the plants with cankers in the untreated top control and 60% in the bottom control with severity of 6 and 18% respectively. The higher incidence and severity in the controls located downhill may be attributed to the higher amount of AG2.1 detected and the positive detection of AG4, known to produce the more severe cankers (Hall *et al* 2009). One plant in the Amistar/Impact 8 treatment was also infected.

Figure 36. *Leaf staining from Leptosphaeria maculans infection in Brussels sprout cv. Romulus control 2 weeks after planting into infected soil.*

Two weeks after planting more than 50% of plants in the bottom control treatment had been destroyed by rabbits and these plants were excluded from the future assessments. Treatment replicates were pooled for statistical analysis where plant numbers were reduced due to rabbit damage.

At 8 weeks after planting, symptoms observed in the fungicide treated plants were less severe than the untreated controls except for the Amistar treatment (Fig. 37). However by 12 weeks after planting, symptoms in the Amistar treatments had become less obvious (Fig. 38), whereas those in the untreated control had a higher severity compared to all treatments (Fig 37).

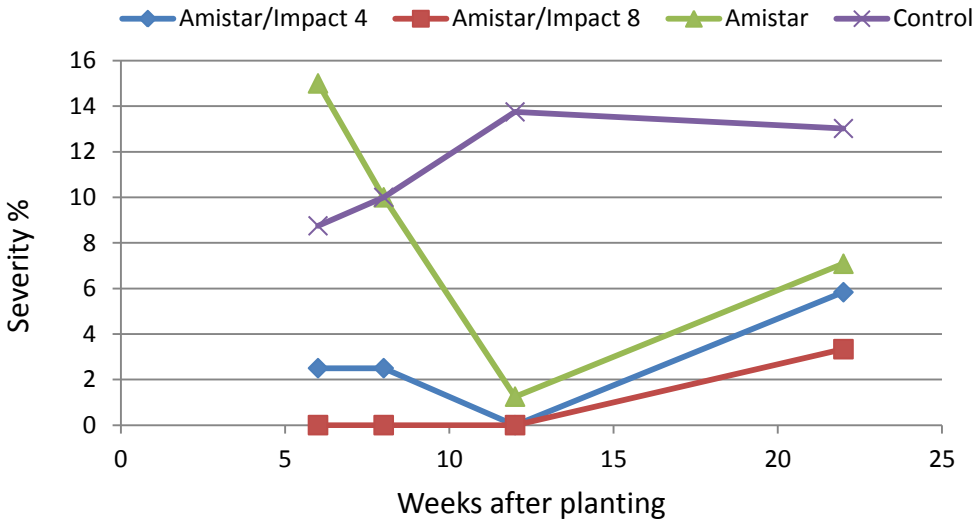


Figure 37. *Canker severity at 6, 8, 12 and 22 weeks after planting Brussels sprout cv. Romulus treated prior to planting with various fungicides.*

Figure 38. *Canker development on Brussels sprout cv. Romulus treated prior to planting with azoxystrobin at 6 (left), 8 (centre) and 12 (right) weeks after planting. Note the symptom is less obvious at 12 weeks.*

It was noted at 12 weeks that the soil moisture in the commercial trial plot was at a much lower field capacity than previous assessments, as the irrigation had been removed following harvest of the surrounding commercial crop and there had been limited natural winter rainfall to irrigate the remaining trial. Soil moisture increased significantly as winter rainfalls commenced prior to harvest.

Assessment of stem canker severity at 22 weeks after planting showed the higher rate of flutriafol with azoxystrobin (Azoxystrobin/Impact 8) provided the best reduction in canker severity (Table 36).

Table 36. *Incidence and severity of canker in Brussels sprout cv. Romulus seedlings 22 weeks after planting into soil infected with Rhizoctonia and Leptosphaeria maculans following treatment with various products. Means with the same letter are not significantly different (P=0.05).*

Treatment	Incidence	Severity
Control	38.5 a	12.9 a
Amistar/Impact 8	10.0 b	3.1 b
Amistar/Impact 4	20.0 ab	5.8 ab
Amistar	16.7 ab	7.1 ab

A comparison between canker severity of untreated plants and plants treated with the highest dose rate of flutriafol in combination with azoxystrobin was significant (LSD = 7.55). Canker severity of plants treated with the lower dose of flutriafol combined with azoxystrobin appeared to be intermediate between these two, possibly indicating a dose effect of flutriafol, however this was not statistically significant in this trial.

All treatments reduced the incidence of larger plants and larger sprouts (Fig. 39). Stunting of plants was greatest in plants treated with the azoxystrobin + flutriafol combination at the higher dose rate, but the difference was not significant.

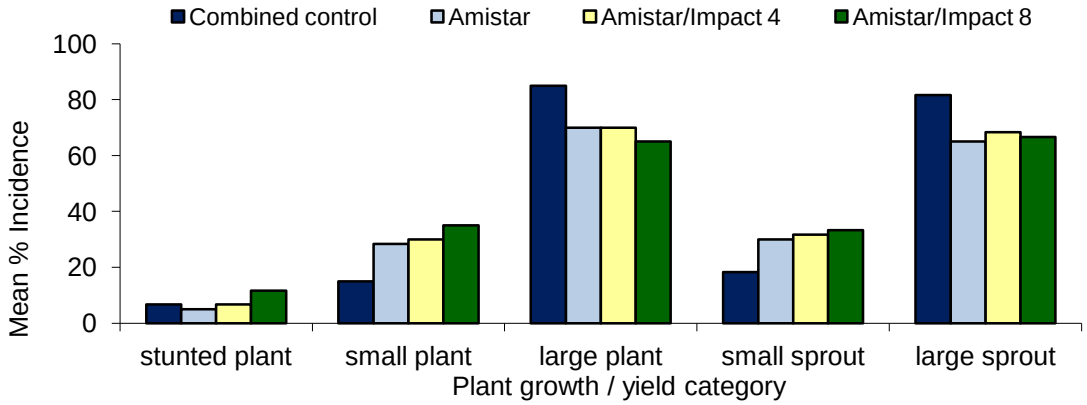


Figure 39. *Incidence of relative plant and sprout size of Brussels sprout cv. Romulus plants 18 weeks after various treatment applications and planting into a paddock infected with Leptosphaeria maculans and Rhizoctonia solani.*

Conclusions

While there was low disease severity in these field trials, with no plant collapse, these results show there is potential to reduce severity of stem canker by applying prior to planting combined products with active ingredients that suppress both pathogens, such as azoxystrobin and flutriafol or fluquinconazole. However the use of these products in situations of low disease pressure may not provide sufficient economic benefit to warrant their application. Further repeated testing under higher disease pressure and in a range of soil conditions is warranted. In seasons of low disease pressure, a reduction in longer term field spore load may be the only ongoing return on the cost of chemicals and their application, rather than an immediate, once off return in yield or reduced number of harvesting passes from the immediate planting. However plant stunting occurred with pre-planting fungicides drenches, particularly in the first few weeks of growth. In some trials this stunting was no longer visible by harvest, however further works needs to be undertaken to determine whether the stunting has yield implications.

There was no additional benefit in reducing canker severity from the use of biological or plant growth products in canker severity. However there were variations in plant growth observed which may provide benefit in yield from growth effects in plants infected with canker as well as uninfected plants.

Additional testing of biological products is needed in large scale experiments. Early applications can establish the bacteria in the soil and enable sufficient time for the successful colonization of the organism, helping to prevent invasion and establishment of the pathogens in the transplant (Ramarathnam *et al* 2011). It was intended in one of the grower trials to apply *Bacillus subtilis* to soil prior to planting to confirm whether this would reduced stem canker, however the infrastructure required to apply the product was not installed in time.

It may be beneficial to repeat this trial with the additional and correctly timed applications and to include an untreated area for comparison.

4.9 Field efficacy trial - Western Australia

In Western Australia, *Rhizoctonia* is a significant issue in the Brassica production area. A trial was set up to evaluate various fungicides and plant growth products on a research station known to be infected with *R. solani.*

Aim: To determine the effectiveness of biological agents and fungicides used alone and in combination for controlling *R. solani* in cauliflower.

Materials and methods

A 40 core sample of soil was collected from each research bay and combined for PCR testing for *R. solani* as previously described. The results were used to determine the optimum site for the trial.

Four replicates of the 15 treatments outlined in Table 37 were arranged in a randomised complete block design, each plot 5.1m wide by 5 m long with 322 plants cauliflower cv. Boris planted per plot with a commercial finger planter.

Table 37. *Products, rates and timings applied for each treatment*

Tmt. no.	Product	Rate	Application and timing
1	Amistar 250SC	100mL/100L	Preplant drench
2	Rovral Aquaflo	100mL/100L	At transplanting
3	Maxim	0.016mL/plant	Preplant drench
4	Rhizotonic	400mL/100L (1mL/plant)	Preplant drench + applied again at 2 and 4 weeks
5	Amistar 250SC	100mL/100L	Preplant drench
	Rhizotonic	400mL/100L (1mL/plant)	Preplant drench and applied at 2 and 4 weeks post-transplant
6	Bio-forge	0.006mL/plant	Preplant drench and applied again at 2 and 4 weeks
7	Amistar 250SC	100mL/100L	Preplant drench
	Bio-forge	0.006mL/plant	Applied at 2 and 4 weeks post-transplant
8	Amistar 250SC	100mL/100L	Preplant drench
	Bio-forge Extender	0.006mL/plant 0.005mL/plant	Applied at 2 and 4 weeks post-transplant
9	Root-feed	0.02mL/plant	Post-transplant drench and applied again at 2 and 4 weeks
10	Amistar 250SC	100mL/100L	Preplant drench
	Root-feed	0.02mL/plant	Applied at 2 and 4 weeks post-transplant
11	Bio-forge Extender	0.006mL/plant 0.005mL/plant	Preplant drench
	Root-feed	0.02mL/plant	Applied at 2 and 4 weeks post-transplant
12	TM21	250mL/ha	Boomspray at transplanting + 2 and 4 weeks
13	Amistar 250SC	100mL/100L	Preplant drench
	TM21	250mL/ha	Applied at 2 and 4 weeks post-transplant
14	TM21	250mL/ha	Boom spray application 2 weeks prior to transplant and applied again at 2 and 4 weeks post-transplant
15	Control, no products applied		

Pre-plant drenches were applied to the seedling trays using watering cans. For post-planting applications Bioforge, Extender, Rootfeed and TM21 products were applied in a knapsack sprayer directed at the base of plants.

From 3 to 8 weeks after planting, 11 plants from the inner four rows of each plot were assessed weekly for symptoms of canker as previously described. A small hand shovel was used to collect soil from an area within 5 cm of the stem of five plants showing symptoms of infection by *R. solani* and tested to confirm the presence of the pathogen in the soil by toothpick baiting previously described.

10 soil cores were collected taken from all plots of treatments 1-5 and 15 at harvest, the soil from each treatment combined and tested for the presence of *R. solani* by PCR as previously described.

At harvest 10 plants per plot were assessed for symptoms of stem canker and curd measurements were taken from 44 plants (the inner 11 plants of the inner 4 rows) from each plot. The crop was harvested over 5 picks from 80 to 93 days after planting, with individual curds picked when ready for harvest. Curd measurements included weight, quality and density. Quality assessments included curd size, colour, shape (lumpy/flat/misshapen), leaves in curd and damage (insect, rodent, rot, slug or snail, splits). The quality score depended on the number of issues the curd had and their severity, with a rating of 1-7 (Table 38).

Table 38. *Quality rating scores. Quality issues include curd size, colour, shape (lumpy/flat/misshapen), leaves in curd and damage (insect, rodent, rot, slug or snail, splits). Minor issue affected <5% of the curd, major >5%.*

Rating	**Marketable**	**Description**
1	No	Inedible or with rot
2	No	Two or more major quality issues
3	No	One major quality issue
4	No	Not in marketable size, or three minor quality issues
5	Yes	Two minor quality issues
6	Yes	One minor quality issue
7	Yes	No quality issues

The density was measured on a 1-3 scale, where 1 = open curd with large gaps between florets, 2 = more open but marketable, 3 = a dense closed curd without any gaps between florets and is marketable. Off-types and non-hybrid plants were excluded from all calculations of average weight, yield, quality, density and % rejected as well as % picked at each harvest. The number of picks to compete harvest were assessed, with only commercial size picks included (5% or greater of the crop harvested).

Results and Discussion

Stem canker

The 'wirestem' symptom caused by *R. solani*, was first observed in week 3, with more developing in weeks 4 and 5. The number of plants per treatment affected by *R. solani* ranged between zero and three (Table 39). Due to the minimal incidence of *R. solani* infection in the trial no statistical analysis has been done on the results. However no plant loss occurred in pre-plant drench treatments containing Amistar (azoxystrobin) or Maxim (fludioxonil), indicating early control of *Rhizoctonia*.

No other stem canker symptoms were observed when plants were assessed in weeks 3-8 or in the final week of harvest.

Table 39: *Incidence and timing of appearance of 'wirestem', a symptom of Rhizoctonia solani infection.*

Tmt. no.	**Product**	**No. of plants showing symptoms (rep in which they occurred)**			
		Week 3	**Week 4**	**Week 5**	**Total**
1	Amistar	0	0	0	0
2	Rovral Aquaflo	1 (4)	0	0	1
3	Maxim	0	0	0	0
4	Rhizotonic	1 (4)	0	0	1
5	Amistar + Rhizotonic	0	0	0	0
6	Bio-forge	2 (3)	0	1 (3)	3
7	Amistar + Bio-forge	0	0	0	0
8	Amistar + Bio-forge + Extender	0	0	0	0
9	Root-feed	3 (1)	0	0	3
10	Amistar + Root-feed	0	0	0	0
11	Bio-forge + Extender + Root-feed	0	0	1 (3)	1
12	TM21	2 (3, 4)	0	0	2
13	Amistar + TM21	0	0	0	0
14	TM21	1 (2, 3)	0	1 (2)	3
15	Untreated control	1 (3)	1 (3)	0	2

Yield

There were no significant differences between the treatments for any of the curd measurements, including average curd weight, average marketable curd weight, total yield, marketable yield, the number of picks required to remove the crop and average curd quality and density scores (Table 40). The variability of results in crop growth and harvest uniformity between treatments using the same product would suggest that for most of the products tested there is no direct link between a particular product and increased uniformity of harvest. Any improvement in crop growth uniformity may be due to complex interactions between products.

Table 40. *Yield and curd parameters measured at harvest. No significant difference between treatments.*

Tmt no.	Avg curd wt(g)	Total yield (t/ha)	Avg marketable curd wt(g)	Marketable yield (t/ha)	No picks	Avg quality score	Avg density score
1	756	23.7	823	20.1	3.00	5.07	2.83
2	768	24.2	802	17.0	3.00	4.86	2.81
3	746	24.1	812	19.1	3.00	4.94	2.75
4	799	24.5	832	20.5	2.75	5.14	2.91
5	717	21.2	783	17.4	2.75	4.97	2.82
6	780	22.6	857	17.2	3.25	4.89	2.85
7	719	22.7	797	19.5	2.50	5.06	2.89
8	791	25.0	827	22.1	3.00	5.20	2.85
9	810	26.5	853	21.1	3.00	4.90	2.73
10	763	23.4	837	19.1	3.00	5.04	2.90
11	797	25.0	839	18.0	3.50	4.83	2.74
12	798	24.7	824	20.4	3.25	5.13	2.89
13	731	22.7	801	17.6	3.00	4.99	3.30
14	793	23.8	835	18.9	2.75	4.92	2.81
15	839	26.0	872	20.1	3.25	4.97	2.84
P. value	0.388	0.368	0.746	0.313	0.949	0.121	0.287

There were statistical differences between the percentages of crop harvested in the largest pick for various treatments ($P<0.05$) (Table 41). A higher percentage indicates a higher uniformity of growth and harvest. Treatment 5 (Amistar plus Rhizotonic pre-plant and Rhizotonic twice post-plant) had the highest percentage of crop harvested in one pick with 71.1%.

Table 41: *Percentage of crop harvested in the largest pick for each treatment. Means with the same letter are not significantly different from one another.*

Treatment No.	**Treatment applied (timing of application)**	**% harvested in largest pick***
11	Bio-forge (preplant drench) Extender (preplant drench) Root-feed (2 and 4 weeks post-transplant)	41.8 a
3	Maxim (preplant drench)	47.1 ab
12	TM21 (boomspray at transplanting; 2 and 4 weeks post-transplant)	47.1 ab
4	Rhiztonic (preplant drench; 2 and 4 weeks post-transplant)	48.7 ab
1	Amistar (preplant drench)	49.2 ab
6	Bio-forge (preplant drench; 2 and 4 weeks post-transplant)	50.2 ab
2	Rovral Aquaflo (at transplanting)	50.4 ab
14	TM21 (boomspray 2 weeks prior to transplanting; 2 and 4 weeks post-transplant)	51.8 ab
8	Amistar (preplant drench) Bio-forge (2 and 4 weeks post-transplant) Extender (2 and 4 weeks post-transplant)	53.5 abc
15	Control	54.1 abc
9	Root-feed (post-transplant drench; 2 and 4 weeks post-transplant)	54.3 abc
10	Amistar (preplant drench) Root-feed (2 and 4 weeks post-transplant)	56.0 bc
13	Amistar (preplant drench) TM21 (2 and 4 weeks post-transplant)	58.7 bcd
7	Amistar (preplant drench) Bio-forge (2 and 4 weeks post-transplant)	66.3 cd
5	Amistar (preplant drench) Rhizotonic (preplant drench; 2 and 4 weeks post-transplant)	71.1 d
	P. value	0.010
	l.s.d.	13.46

Most products tested were used in a number of treatments. No analysis was done comparing products due to the complexity of combinations and possible interactions. However, it should be noted that four of the six products that were used in multiple

treatments had results that were significantly different from each other. For example, Rhizotonic was used in treatments 4 and 5 which had largest pick results of 48.7% and 71.1%, which are statistically different from each other. Only the two treatments using Extender and the three treatments using TM21 produced results that were not statistically different from themselves.

There was no correlation between the percent harvested in the largest pick and the yield, with treatment 5 having the largest pick (71%) but the lowest yield (21.2 t/ha).

Soil testing

R. solani AG2.1 and 2.2 were detected in the trial area both prior to planting and at harvest (Table 42). *R. solani* AG 4 was found prior to planting but not at harvest. The amounts of *R. solani* in the soil at harvest were lower at harvest than at planting, and also very low compared to those found in other trials (Hall *et al* 2009). This indicated that the conditions during the trial were not conducive to infection and build up of the pathogen in the soil. No correlations could be made between the fungicides applied and the change in amounts of *Rhizoctonia* from pre-planting to harvest.

Table 42: *DNA amounts of Rhizoctonia solani AG2.1, 2.2 and 4 (pg DNA/g soil) at harvest for selected treatments.*

	R. solani		
	AG2.1	***AG2.2***	***AG4***
Pre-plant amounts	19-24	40-291	0-28
Control	2	5	0
Amistar + Rhizotonic	0	2	0
Rhizotonic	10	2	0
Maxim	1	115	0
Rovral	2	0	0

Conclusions

Due to the low disease incidence and low levels of *Rhizoctonia* in the soil at harvest, no conclusions can be drawn on the comparative effectiveness of any of the products or treatments used in controlling stem canker. However plants treated pre-planting with azoxystrobin had no wirestem symptoms from *Rhizoctonia*.

While there were variations in plant growth and harvest uniformity, these could not be correlated with any particular treatment. This trial, or similar, would have to be repeated before any recommendations could be made in the use of biological products to economically improve crop growth.

4.10 Field trials – MT09045.

Three trials was undertaken as part of a related project (MT09045 "Overcoming Onion Stunting and Brassica stem canker by the use of liquid fertilisers") and were reported in Milestone 105. However they have been included in this report to ensure all available data is presented to provide management options for stem canker.

4.10.1 Field trial 1. Brussels sprout

Aim: To evaluate combinations of fungicides and plant growth products for control of Brassica stem canker in cauliflower planted in commercial properties previously infected with Rhizoctonia and Leptosphaeria.

Materials and methods

Brussels sprout seedlings cv. Helemus were drenched with various treatments outlined in Table 43 for 3 mins as previously described 12 hours prior to planting in a property known to be infected with *Rhizoctonia* and *Leptosphaeria*. Seedlings drenched in water were used as the untreated control.

Table 43. *Products and rates of application used for each treatment*

Product	Preplanting rate ml /plant	Treatment						
		1	2	3	4	5	6	7
Amistar	0.01	✓	✓					
Cabrio	0.018			✓	✓			
Maxim	0.016					✓	✓	
Bio-Forge	0.04		✓		✓		✓	✓
Rootpower	0.036							✓

Two replicates of ~100 plants per treatment in paired rows, ~50 plants per row were planted by the grower with a commercial planter, 35cm between plants and 50cm between rows.

Five plants selected at random were assessed in each row (20 plants per treatment) 6, 8, 13 and 22 weeks after planting using the rating scale previously described. Assessments of plant size and yield were undertaken prior to harvest at 22 weeks after planting. The healthy plants with good crop load were found to be over 80cm in height, so to assess the effect of treatments on vegetative size plants were rated as having a stalk size less than or greater than 80cm measured from soil line to growing tip. As a measure of potential yield, each plant was given a relative rating between 0 (no sprouts) and 3 (maximum potential yield calculated from the healthy plants with good crop load). The ratings 1 and 2 were less than 50% and greater than 50 %

respectively of the crop load of plants with rating 3. Disease and growth assessments on 20 plants were also made of the grower planting adjacent to the trial.

The plants were managed by the grower as per normal practice.

Results and discussion

Severity of stem canker symptoms increased from 6 to 13 weeks after planting in all treatments except Maxim Bio-Forge (Fig. 40). The addition of Bio-Forge did not reduce disease progression except when applied with Maxim, however due to the variability none of the differences at each sampling time were statistically significant.

At harvest, none of the treatments gave significantly better control of stem canker than the untreated control or grower treatment, however the Cabrio and Cabrio/Bio-Forge treatment had the least disease observed and gave significantly better control than the Amistar/Bio-Forge treatment (Fig. 41). The effect of the combination of fungicides with Bio-Forge was variable, improving the suppressive effect of Maxim and Cabrio but reducing the suppressive effect of Amistar.

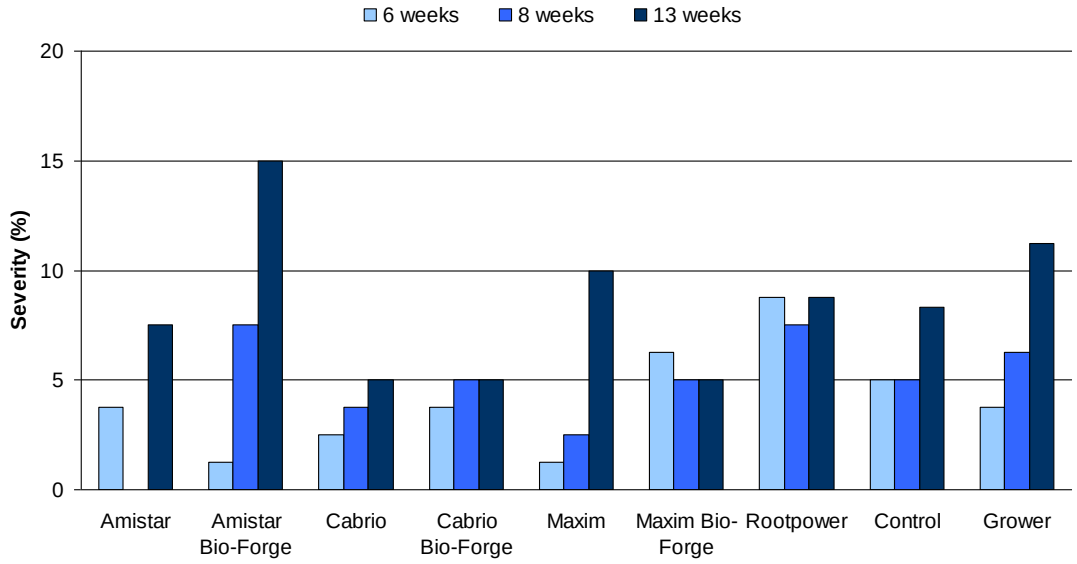


Figure 40. *The effect of various treatments on severity of stem canker on Brussels sprouts cv. Helemus from 6 to 13 weeks after planting into commercial soil known to be infected with Rhizoctonia and Leptosphaeria. No significant differences observed between treatments.*

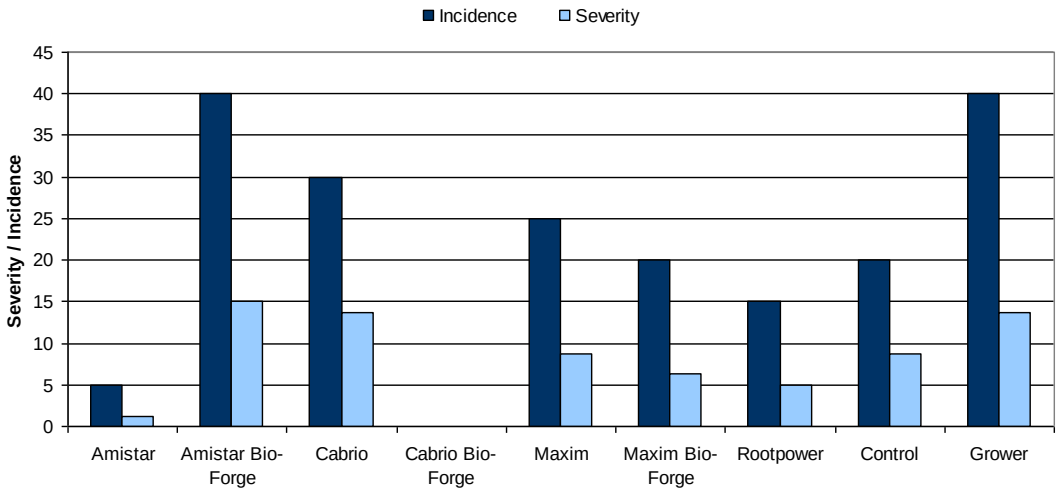


Figure 41. *The effect of various treatments on incidence and severity of stem canker on Brussels sprouts cv. Helemus 22 weeks after planting into commercial soil known to be infected with Rhizoctonia and Leptosphaeria.*

The severity of stem canker was lower at the 22 week assessment in many of the treatments. This could be due to a combination of factors. The plants were assessed in situ, and excessive amounts of rain and associated mud made assessing at ground level difficult. Also there was a large amount of new root growth at soil level, which may have overgrown the earlier canker sites.

Plants bearing no sprouts occurred only in 5% of the untreated control plants and those treated with the Maxim and Bio-Forge combination (Fig. 42). These plants did not have visual staining or cankers typical of Stem Canker, hence may have been impacted by some other factor. At least 35% of untreated plants and those treated with Maxim and Bio-Forge were of a lower yield compared to all other treatments having less than 25% of plants with the same yield. For all other treatments more than 80 % of the crop was assessed as having an equally high yield rating. Plants treated with Rootpower or Azoxystrobin and Bioforge had the greatest number of plants with full crop load equivalent to the remainder of the growers planting.

The grower rated all plants as having sprouts of equal maturity suitable for harvesting as one machine pass.

The plant size did not correspond with crop load or disease severity. A greater proportion (75 and 70%) of plants were observed in the > 80cm category in the untreated plants and those treated with the combined Azoxystrobin and Bio-Forge products, however these treatments also had the highest stem canker severity (Fig. 43). Plants treated with Rootpower or Azoxystrobin and Bioforge had the highest proportion of plants with full crop load, but Rootpower had one of the lowest proportions of tall plants while Azoxystrobin and Bioforge had one of the highest. This indicates that the height of the plant was not a good indicator of crop load or plant health.

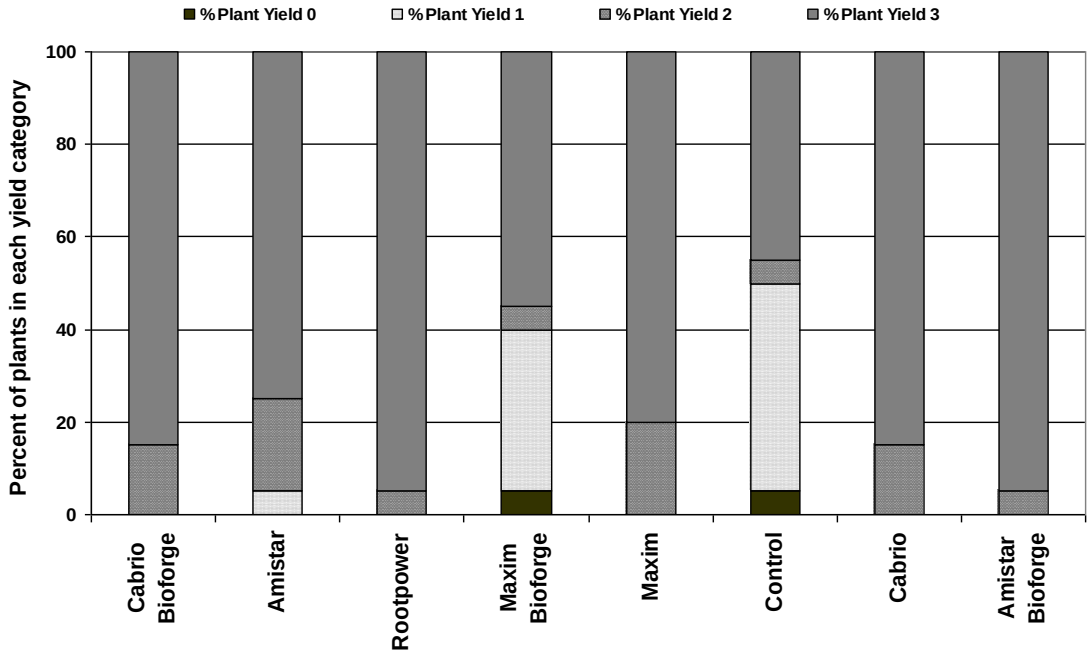


Figure 42. *The effect of various treatments on yield of Brussels sprouts cv. Helemus 22 weeks after planting into commercial soil known to be infected with Rhizoctonia and Leptosphaeria. Yield 0 = no sprouts, 1 = <50%, 2=>50 % and 3=full plant load of sprouts.*

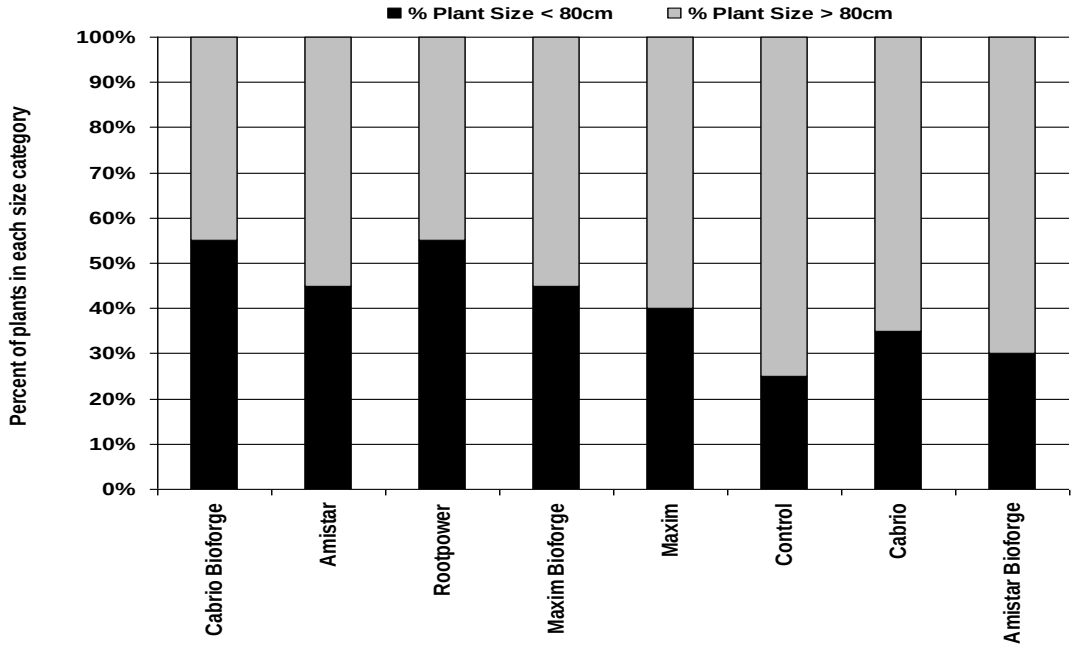


Figure 43. *The effect of various treatments on plant size of Brussels sprouts cv. Helemus 22 weeks after planting into commercial soil known to be infected with Rhizoctonia and Leptosphaeria.*

The use of the plant growth promoting product Bio-Forge in conjunction with fungicides as a pre-planting drench gave variable results depending on the fungicide used. However the levels of disease observed in this trial were not severe enough to significantly impact on plant yield, and more work would be needed to determine whether there was an economic benefit gained from application of these products. It is possible that the benefit from these products would be in preventing the early seedling death from *Rhizoctonia* observed in the adjacent grower paddock (Figs. 44, 45), however no seedling death was observed in the trial area.

Figure 44. *Patches of Rhizoctonia affected Brussels sprouts in planting adjacent to trial site*

Figure 45. *Stunted Brussels sprouts seedlings cv. Helemus 4wks after planting, infected with Rhizoctonia.*

4.10.2 Field Trial 2. Cauliflower.

Materials and Methods

Eight week old cauliflower cv. Skywalker seedlings were drenched 36 hrs prior to planting with the treatments outlined in Table 44. Product rates were applied to each plant equivalent to that recommended for per plant field application. For pre-planting drenches, trays of seedlings were immersed for 5 minutes in the treatment solution to within 5 mm of the seedlings base. Each seedling absorbed ~10ml of mixed product. Untreated control plants were immersed in sterile water.

Table 44. *Products and rates of application used for each treatment*

Product	Pre-planting rate ml /plant	Post-planting rate ml/plant	Treatment			
			1	2	3	4
Azoxystrobin	0.01		✓	✓		
Maxim	0.016				✓	
Bio-Forge	0.006		✓	✓	✓	✓
X-Press	0.0006		✓			
X-Tender	0.005			✓	✓	✓
Rootfeed	0.2		✓	✓	✓	✓
Rootfeed		0.2	✓	✓	✓	✓

Seedlings were planted as per commercial practice by the grower, plants 50 cm apart on mounds spaced 70 cm apart. The trial was laid out in a randomised block with four replicate plots of 24 plants (2 rows of 12 plants) for each treatment.

Rootfeed was applied every two weeks after planting in 2,300 L/Ha of water (until soil saturation) using a knapsack sprayer as a 10 cm banded application offset to the stem of the cauliflower. This was followed by a foliar application of water, ~ 20 ml/plant also using a knapsack sprayer. The dual application was to simulate a fertigation application of the product, which is followed by short irrigations to wash the nutrients off the leaves into the soil and to prevent burning. Ammonium sulphate was applied 2 weekly to controls at 3.3 Kg/Ha to provide an equivalent nitrogen dose as the Rootfeed. Plants were watered by overhead irrigation and maintained as per standard grower practices.

Assessment

Symptoms of stem canker were assessed as previously described at 2 weeks, 8 weeks and 12 weeks (harvest). At 8 weeks the soil was removed from the stem area before assessing, as the grower has banked the soil and covered the stems up to 10cm.

The yield of plants was assessed by head size and weight. Head size of each plant was rated at harvest as small (<10cm diameter), medium (10cm-12cm diameter) or large (>12cm diameter) (Fig. 46). The heads of 12 plants from each row of the four

replicates for each treatment were combined and weighed and a mean weight of harvested heads determined.

Figure 46*. Cauliflower head size cv. Skywalker, R to L small (<10cm diameter), medium (10cm-12cm diameter) and large (>12cm diameter).*

Soil sampling

A 40 core soil sample was taken pre-planting from each of the replicates and PCR tested for *Rhizoctonia* AG2.1, 2.2, 4 and *Leptosphaeria maculans*. Post-harvest a 40 core soil sample was taken from each of replicates 1 and 2 combined and replicates 3 and 4 combined as the grower had already ploughed the marked trial plot before individual soil samples could be taken from each treatment area.

Results and discussion

At 8 weeks after planting, Maxim/Bio-Forge/X-Tender/Rootfeed had the highest stem canker severity (14.84 %) and was not significantly different from the controls (10.7%). Azoxystrobin/Bio-Forge/ X-tender/Rootfeed had the lowest stem canker severity (8.1%) but was only statistically significantly lower than Maxim/Bio-Forge/X-Tender/Rootfeed treated plants (Table 45). At 16 weeks after planting, the plants treated with Azoxystrobin/Bio-Forge/X-Tender/Rootfeed pre-planting has the lowest level of stem canker severity (16.9%), significantly lower than all other treatments except the Maxim/Bio-Forge/X-Tender/Rootfeed (21.6%). However the variation in severity was not large and the treatments would unlikely to have been economically viable. While the addition of X-tender to the fungicide treatments numerically reduced the canker severity and incidence, it did not influence the leaf infection.

Table 45. *The effect of various treatments on the incidence and severity of stem canker symptoms on cauliflower cv. Skywalker 8 and 16 weeks after planting into paddocks known to be infected with Rhizoctonia and Leptosphaeria. Treatments with the same letter are not significantly different from one another (P=0.05).*

Treatments			8 weeks after planting		16 weeks after planting		
	Pre-plant	**Post-plant**	**% canker severity**	**% canker incidence**	**% canker severity**	**% canker incidence**	**% leaf infection**
1	Azoxystrobin Bio-Forge X-Press Rootfeed	Rootfeed	10.7 c	11.5c	26.2 ab	57.3	25.0
2	Azoxystrobin Bio-Forge X-Tender Rootfeed	Rootfeed	8.1 bc	20.8 bc	16.9 c	65.6	45.8
3	Maxim Bio-Forge X-Tender Rootfeed	Rootfeed	14.8 a	35.4 a	21.6 bc	57.3	32.3
4	Bio-Forge X-Tender Rootfeed	Rootfeed	10.4 abc	20.8 bc	26.8 ab	66.7	42.7
	Water Only		10.7 ab	32.3 ab	29.69 a	55.2	40.6

Head size and weight

Head weight was not significantly different between treatments (Fig. 47) with mean head weights between 296g and 545g. The head weights from the untreated control, averaging 578 g were only significantly larger than the Azoxystrobin/Bioforge/X-Press/Rootfeed treated plants. The control treatments also had the lowest proportion of large heads (45%), with the largest proportion of large heads (55%) in the plants treated with Azoxystrobin/Bio-Forge/X-Press/Rootfeed (Fig. 48). The product X-Press contains Zinc oxide, which may improve growth.

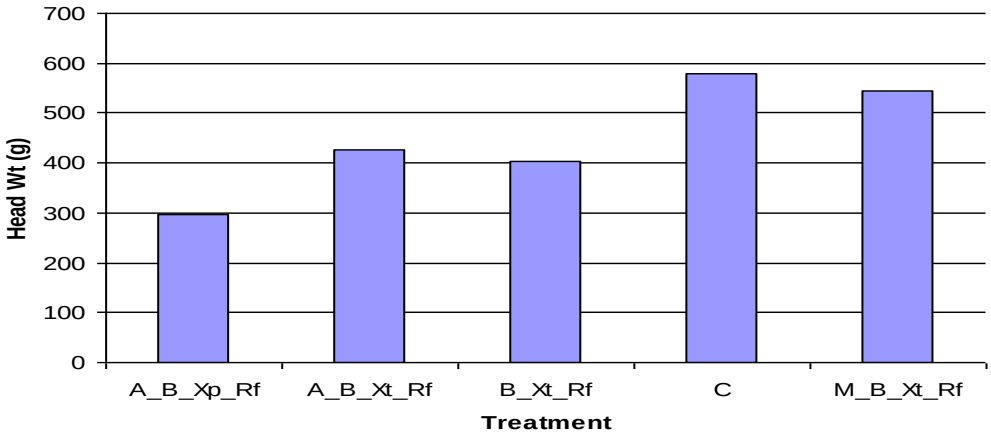


Figure 47. *The effect of various treatments on the mean headweight of cauliflower cv. Skywalker at harvest after planting into paddocks known to be infected with Rhizoctonia and Leptosphaeria. A= Azoxystrobin, M=Maxim, B=Bio-Forge, Xp=X-Press, Xt=X-Tender, Rf = Rootfeed, C=untreated*

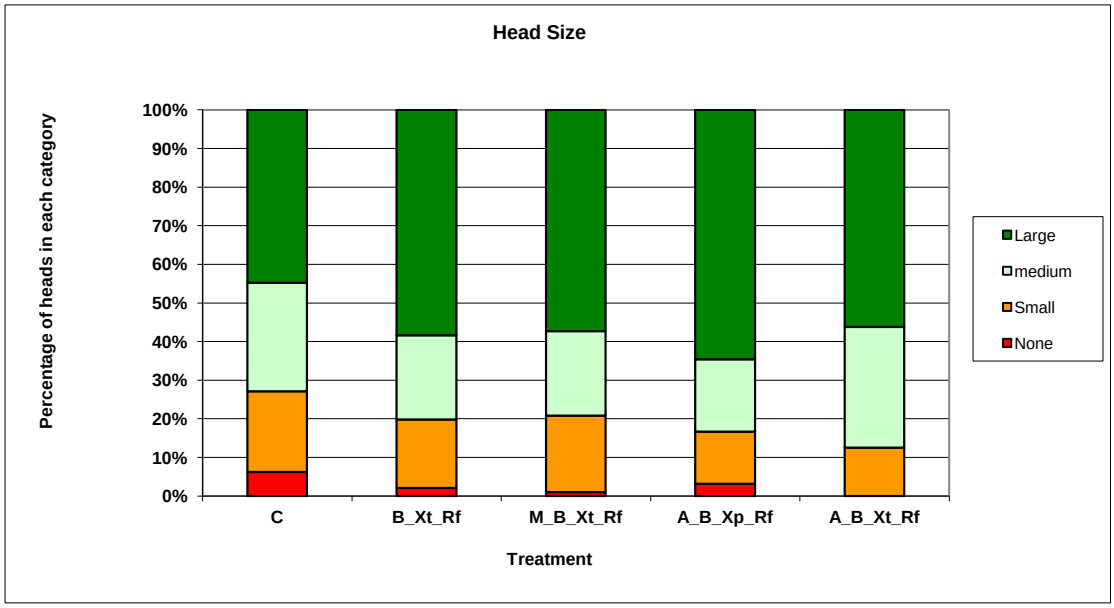


Figure 48. *The effect of various treatments on the comparative head size of cauliflower cv. Skywalker at harvest after planting into paddocks known to be infected with Rhizoctonia and Leptosphaeria. A= Azoxystrobin, M=Maxim, B=Bio-Forge, Xp=X-Press, Xt=X-Tender, Rf = Rootfeed, C=untreated. Small = <10cm diameter, medium =10cm-12cm diameter, large >12cm diameter.*

Payment for cauliflower is on price per head for domestic markets not weight. Differentiation in head size needs to be obvious, enabling personnel to select heads for different market grades at harvest, increasing payment received per head to have an impact on farm gate revenue. The increase in head size and weight in the treated plants may have more to do with the growth promoting products used, as there was no obvious correlation with the level of canker suppression.

Soil sampling

Rhizoctonia solani AG2.1 was detected in soil at low levels both pre-and post-planting, and AG4 at pre-planting only (Table 46). No AG2.2 or *Leptosphaeria* was detected at either time.

The mean maximum temperature for the trial duration between the 16th September 2010 and the 7th December 2010 was 22 degrees. During the growing period the nearby weather station at Edinburgh recorded 138 days with a mean temperature between 14 and 20 degrees, 67 between 20 degrees and 28 degrees and 24 above 28 degrees. Previous work in (Hall *et al* 2009) showed the severity of disease in cauliflowers inoculated with AG 2.1 was greater at temperatures of 14 and 22 degrees than either AG2.2 or 4. The higher severity of canker recorded in replicate three (data not shown) may be attributed to the higher amount of AG 2.1.

While no *L. maculans* was detected in the soil, there was evidence of the disease in the plants, confirmed with PCR testing of affected stems (Table 47, Fig. 49). No *Rhizoctonia* AG2.2 or 4 was detected, and the higher amounts of AG2.1 and Blackleg

generally correlated with the more severely rated stalks. However both pathogens were also detected in symptomless plants.

Table 46. *Detection of Rhizoctonia and Leptosphaeria in soil pre-planting and post-harvest.*

Area	***R. solani* AG 2.1**	***R. solani* AG 2.2**	***R. solani* AG 4**	***L. maculans***
Preplanting				
Replicate 1	0	0	3	0
Replicate 2	1	0	3	0
Replicate 3	112	0	0	0
Replicate 4	3	0	0	0
Post-Harvest				
Rep1 and 2	6	0	0	0
Rep 3 and 4	4	0	0	0

Table 47*. Leptosphaeria and Rhizoctonia AG2.1 DNA recovered from plants grouped into the severity ratings.*

Canker severity at harvest	**No stems bulked per test**	***Rhizoctonia* AG2.1 pg DNA/g**	***L. maculans* pg DNA/g**
0	16	263	60
25	10	9	1
50	10	49	157
75	10	5942	12

Figure 49. *Stem canker on cauliflower cv. Skywalker, with Leptosphaeria recovered from affected tissue.*

4.10.3 Field trial 3. Cauliflower

This trial area had previously been infected with both pathogens though addition of infected soil from greenhouse trials and cauliflower inoculated with *Leptosphaeria* planted and incorporated after 4 weeks with a rotary hoe.

Material and methods

Six week old cauliflower cv. Discovery were drenched in various treatments (Table 48) for 5 minutes as previously described, before being planted into the pre-infected soil. Water was used for the untreated control. Six replicated plots of 10 plants per treatment were planted in September at 70 cm intervals into rows spaced 50 cm apart.

Soil was collected from the whole trial area as a bulked 40 core sample, and again at 16 weeks after planting from each treatment as a bulked sample of 7 soil cores per replicate for each treatment. Samples were tested for *Rhizoctonia* AG 2.1, 2.2 and 4 and *L. maculans*.

Canker severity was assessed as previously described on all plants at 4, 8 and16 weeks after planting. A relative assessment of plant size was undertaken at 16 weeks after planting, with plants rated as small, medium or large, relative to the size of all other plants in the trial area.

Table 48. *Products and rates of application used for each treatment.*

Product	**Preplanting rate ml /plant**	**Treatment**				
		1	**2**	**3**	**4**	**5**
Azoxystrobin	0.01	✓			✓	
Maxim	0.016		✓			✓
Jockey	0.04	✓				
Bio-Forge	0.006	✓		✓	✓	✓
X-Tender	0.005	✓		✓	✓	✓
Rootpower	0.006	✓		✓	✓	✓

Results and discussion

Soil sampling

Soil sampling showed a moderate amount of *Rhizoctonia* AG 2.1 pre-planting, but no evidence of *Leptosphaeria*, although this area was known to have been infected in the previous year (Table 49). Flooding from 6 to 10 days has been reported to effectively eliminate *L. maculans* from residues. The trial area had received heavy winter rains after the incorporation of infected material and prior to planting, which may in part explain why none was detected in the soil.

Rhizoctonia AG2.1 was found at harvest in all treatment areas, and was lowest in the Maxim treated area. *Leptosphaeria* was not detected at harvest in any of the areas treated with fungicide, however detection of *Leptosphaeria* is variable in soils.

Table 49. *Pathogen amounts in soil collected pre-planting from the trial area and post-harvest from the different treatment areas. A= Azoxystrobin, M=Maxim, J= Jockey, B=Bio-Forge, Xt=X-Tender, Rp = Rootpower.*

Treatment	*R. solani AG 2.1* **DNA pg/g soil**	*L. maculans* **DNA pg/g soil**
Preplanting		
Whole Plot	120	0
Postplanting		
Control	279	1
Maxim	151	0
M_B_Xt_Rp	475	0
A_B_Xt_Rp	639	0
B_Xt_Rp	197	1
A_J_B_Xt_Rp	405	0

Stem canker assessment

At 2 weeks after planting 4% of plants were dead from severe stem constriction due to *Rhizoctonia* (Fig. 50). 25% of these were in the controls with no significant difference between the remaining treatments. At 8 weeks 5% of control plants were dead compared to fewer than 2 % for all other treatments (data not presented separately).

Figure 50. *Stunted cauliflower cv. Discovery with stem narrowing and stripped roots caused by Rhizoctonia.*

Plants treated with Maxim alone and Maxim or Amistar combined with the plant growth promoting products had significantly less canker than the other treatments and the untreated control (Table 50). Adding Jockey to the Amistar mix reduced disease

control, indicating a potential antagonistic effect of combining the two products which needs further investigation.

The addition of either fungicide to the plant growth products improved the control of stem canker compared to the products alone (Table 50). No significant decrease in disease severity was achieved by the addition of biological products to Maxim. At 8 weeks the products tended to increase disease levels. At 16 weeks where either Amistar or Maxim had been combined with Bio-Forge /X-Tender / Rootpower the incidence of infection was reduced. This may be a result of the Maxim alone providing better initial suppression of the *Rhizoctonia*, either through reducing the soil levels or by protecting the plant.

Table 50. *The effect of various treatments on the incidence and severity of stem canker symptoms on cauliflower cv. Discovery 8 and 16 weeks after planting into an area known to be infected with Rhizoctonia and Leptosphaeria. Treatments with the same letter are not significantly different from one another (P=0.05).*

Treatment	8 weeks after planting		16 weeks after planting	
	% severity	% incidence	% severity	% incidence
Maxim	1.7 cd	5.0 cd	17.9 b	60.0
Maxim Bio-Forge X-Tender Rootpower	4.6 bcd	6.7 bcd	18.8 b	60.0
Azoxystrobin Bio-Forge X-Tender Rootpower	0.4 cd	1.7 cd	16.3 b	51.7
Azoxystrobin Jockey Bio-Forge X-Tender Rootpower	11.3 ab	30.0 a	35.8 a	76.7
Bio-Forge X-Tender Rootpower	11.3 ab	18.3 ab	37.1 a	70.0
Untreated	15.0 a	26.7 a	37.9 a	65.0

Plant size

Visual assessment of plant vigour 8 weeks after planting indicated plants treated with Azoxystrobin, Bio-Forge, X-Tender and Rootpower appeared to be larger. This was confirmed at 16 weeks after planting where assessment showed a higher proportion of large plants (25%) and smaller proportion of small plants (10%) than the other treatments (Fig. 51). The addition of Jockey to the treatment reversed this trend, with only 3.3% of large plants and 41.7% of small.

At 8 weeks after planting, plants treated with Maxim alone appeared to have more consistent plant size than all other treatments with no plant deaths. At 16 weeks the addition of the plant growth products to Maxim did not improve the plant size, with similar numbers of small plants (18.3%), although Maxim alone had slightly more large plants (20% compared to 13.3%).

High soil moisture and cool weather retarded plant growth across all treatments, preventing effective head formation by the end of the trial period, with small head forming on only 7% of plants in the trial. The high incidence of plant stunting is also possibly due to the levels of *Rhizoctonia*, which is known to cause plants to bolt rather than produce a commercial head.

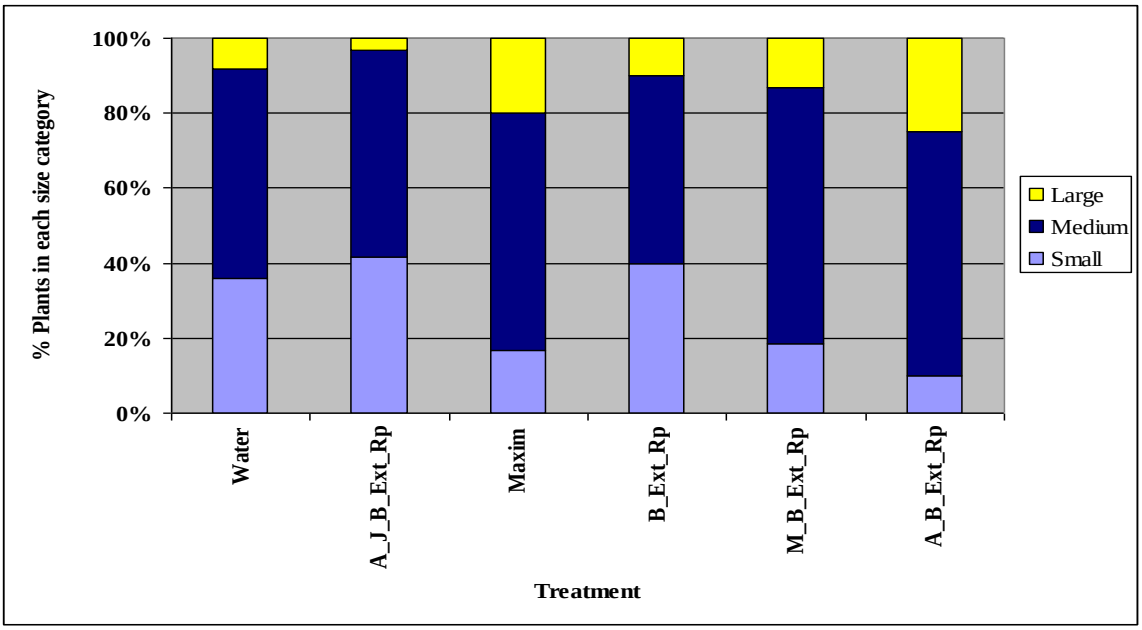


Figure 51. *The effect of various treatments on the comparative plant size of cauliflower cv. Skywalker 16 weeks after planting into an area known to be infected with Rhizoctonia and Leptosphaeria. A= Azoxystrobin, M=Maxim, J= Jockey, B=Bio-Forge, Ext=X-Tender, Rp = Rootpower.*

Conclusion

Overall this work has showed no significant additional benefit in suppression of stem canker by the addition of plant growth products to fungicides. However these trials were in low disease situations, and the plant growth benefits from these products might be more beneficial with high disease pressure.

Canker symptoms appeared to be predominately caused by the *Rhizoctonia*, and including fluquinconazole in the fungicide mix was not as effective as in other trials. Fluquinconazole is used for *Leptosphaeria* control and if this fungus was not dominant in the complex the benefits may not occur. It is possible that too many products were included in the treatment mix, so whilst no physical incompatibility occurred such as visible flocculation or heating when combining the products, there may have been a detrimental effect to the efficacy of one or more of the products.

Trials were planted in areas identified as high disease risk by the grower, however often the pre-planting soil results indicated only low disease pressure. While there was some benefit in disease suppression and head size from the treatments, it is unlikely to be economically viable in seasons of low to moderate disease pressure.

Recuperation of product and application costs would be by the actual number of heads harvested due to reduced plants collapse, or uniformity of heads enabling a greater percentage of harvest to occur in each harvesting pass. Potentially higher yield impacts may occur under conditions of greater disease pressure.

The use of such products in combination with fungicides should continue to be evaluated, and the economic benefit analysed to determine whether the improvement in productivity is worth the cost of applying the product.

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5 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.

Conference proceedings/posters:

Poster presentation at the 4th Asian Conference on Plant Pathology concurrent with the 18th Biennial Australasian Plant Pathology Society Conference, April 2011.

Grower meetings:

- Brassica Vegetable Growers Researchers Update, "Brassica Stem Canker Phase 2" Lenswood S.A, August 2010

Industry magazines:

- Article in HAL Vegetable Annual Industry Report, 2009/10
- Article in HAL Vegetable Annual Industry Report, 2011
- Article in Stock Journal Smartfarmer, November 2011

Scientific Journals:

Three journal articles are in preparation from this work and previous research (VG06018)

6 MAIN OUTCOMES

6.1 Recommendations – scientific and industry

Suppression of stem canker was achieved with pre-planting drenches of fungicides (azoxystrobin or fludioxonil plus fluquinconazole or flutriafol), however some stunting was observed. The addition of *Bacillus subtilis* or *Trichoderma* products either with the fungicide or as post-plant drenches improved disease suppression. The use of these products may not be economical in situations of low disease.

None of these fungicides have a permit or are registered for this use. Azoxystrobin (Amistar 250SC Fungicide) has a permit for use on cauliflower for white blister, The other three products (fludioxonil, fluquinconazole and flutriafol) are registered for black leg on canola.

Infection from *Rhizoctonia* is soil borne, however *Leptosphaeria* has the potential to infect from soil, seed and foliar infection from airborne spores.

Management should be a combination of strategies including:

- Use healthy disease free seed
- Plant less susceptible varieties
- Avoid plant wounding
- Treat nursery plants with fungicides prior to planting
- Control weeds and alternate hosts
- Minimise soil inoculum by incorporating plant residue after harvest and use fallow or non-host crop for at least 12 months.

6.2 Recommended further work

- Assess the economic threshold of canker and the cost benefit of fungicide, biological and plant growth product applications in both high and low disease pressure situations.

- Further investigate the role of foliar infection from airborne spores of *Leptosphaeria*. Repeat spore trapping over a number of seasons and correlated with crop damage.

- Determine flutriafol and fluquinconazole residues on treated crops to assist with registration or minor permit for use in vegetable Brassica crops.

- Continue to evaluate new fungicides for stem canker management

- Continue screening of Brassica vegetable varieties for susceptibly to *Rhizoctonia* and *Leptosphaeria*.

- Undertake pathogen race studies of *L. maculans* populations and determine the resistance genes needed to minimise disease. This will enable cultivar choice to be suited to the race of *L. maculans* present.

7 ACKNOWLEDGEMENTS

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8 APPENDICES

8.1 APPS/ACPP 2011 Conference abstract

PLANT HEALTH PRODUCTS AND FUNGICIDES INCREASE BRASSICA STEM CANKER SUPPRESSION.

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Brassica Stem Canker is a disease complex caused by several pathogens including *Leptosphaeria maculans* (Blackleg) and 3 anastomosis groups of *Rhizoctonia solani* (wirestem); AG 2.1, 2.2 and 4.

Most severe in winter plantings for spring harvest, the complex occurs in Cauliflower, Broccoli, Cabbage and Brussel sprout. Crop losses up to 80 % from complete stalk collapse in cauliflowers in the Northern Adelaide Plains of South Australia, were first noted in 2000. More than 15 different fungicides trialled as root drenches prior or post transplanting of seedlings, have only suppressed the disease, the degree depending on the anastomosis present. Alternatives to traditional fungicides for suppression of Brassica Stem Canker were trialled.

Products Trichoshield®, Bioforge®, Mycotea® and Companion® which claim to alleviate plant stress, or improve plant; fungal defence, strength, nutrition or root mass, were applied as a seedling root drench to 6 week old cauliflower cultivar Donner alone, and in combination with the fungicide Amistar®. Mean severity ratings of staining and cankers every 14 days until harvest on 10 replicate artificially soil inoculated plants of each treatment were used to calculate the Relative area under a disease progression curve for each treatment, enabling comparison between products of Brassica Stem Canker suppression.

Greenhouse trials demonstrated some plant health products in combination with Amistar provided additional benefit in reducing the staining and canker symptoms of Brassica Stem Canker than when Amistar was used alone. Such products may assist plants to resist disease sufficiently to allow a harvestable age and quality to be achieved.

8.2 APPS/ACPP 2011 Conference poster

Brassica stem canker:
Improved suppression in the greenhouse by combining biological products with fungicides

SARDI
SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE

Lynette Deland, Barbara Hall and Catherine Hitch, South Australian Research and Development Institute, GPO Box 397, Adelaide, S.A, 5001, Australia

What is brassica stem canker ?

- Disease causing canker of plant stalk resulting in plant collapse
- Complex involving the pathogens: *Leptosphaeria maculans*, *Rhizoctonia solani; AG 2.1, 2.2 and 4*
- Affects cauliflower, brussels sprouts, broccoli and cabbage

2 min preplant drench cauliflower *cv.* Donner until saturation

Planted into coco peat inoculated with AG 2.1 + *L.maculans*

Post plant drench (where applied) to soil only, no leaf contact

Canker severity assessed every 2 weeks for 12 weeks. A Relative Area Under Disease Progression Curve (RAUDPC) of canker severity is then calculated

Plant health products with fungicides improved disease suppression and root growth

Comparative RAUDPC of stem canker severity. SE=2.14

Amistar alone RAUDPC=12.5	Plant Health Product			
	Companion®	Trichoshield™	BioForge® + X-Tender + Rootfeed™	Myco-Tea™
Product only	14.33	13.5	11	19
Amistar® + product	10.33	5	5.33	12.33

Improved rootball structure

Control Trichoshield™

Amistar® + Trichoshield™ Amistar®

- Brassica stem canker cannot be controlled with fungicides currently registered or permitted. The fungicide Amistar suppresses *Rhizoctonia.*
- Plant health products improve plant growth and can provide some disease suppression
- Combination of fungicide with plant health products improves suppression of disease
- Growth of biological products on fungicide amended agar indicate Trichoshield™ (Trichoderma) growth was reduced when used in combination with some fungicides
- Improvement in root structure combined with improved disease suppression will mean growers can harvest plants even with disease present
- Further work is underway to confirm improved suppression under field conditions and evaluate combinations with products registered to control *Leptosphaeria maculans* in canola

Improving suppression and root growth will reduce crop loss

Government of South Australia

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

HAL Horticulture Australia

8.3 Stock Journal Smartfarmer, November 2011

SARDI research reducing Brassica collapse:

Funded by the vegetable industry through Horticulture Australia SARDI has been investigating the cause and management of Brassica stem canker, a disease causing losses up to 80% in cauliflowers, Brussells sprouts and cabbage. The disease first appears below the first leaf as a coffee coloured stain on the stalk and develops into a cracked region with a dark surrounding area. The damage may lead to plant collapse, mostly in late winter plantings.

The cause of Brassica stem canker is a complex of the soil borne fungi Leptosphaeria maculans (the cause of black leg) and several strains of Rhizoctonia solani (AG 2.1, 2.2 and 4). Plants are infected within the first few weeks of planting into infested soil.

To reduce Brassica stem canker:

- Plant less susceptible varieties. Of the eleven cauliflower varieties tested all were susceptible to both pathogens. Nautilus had relatively low susceptibility to both pathogens. Skywalker and Atlantis had least disease when infected with Rhizoctonia and Elbert and Nautilus were best with Leptosphaeria.
- Bury old crop residues below 10cm for at least 6 months to reduce carryover of Leptosphaeria.
- Remove weed hosts such as; fat hen, wireweed, common purslane, paddymelon and stinging nettle which are hosts of Rhizoctonia.

Fungicide and biological treatments:
None of the 15 fungicides tested alone or in combination controlled the disease. Some suppressed disease symptoms when applied as a root drench at transplanting and may assist in minimising damage to provide a harvestable crop, but may not be of economic benefit. Bacillus subtilis GB03 applied both at planting and as post planting drenches also suppressed symptom expression.

Need to know more? Findings from the research projects VG05005, VG06018 and soon VG09129 can be found on the HAL website www.horticulture.com.au. Research was undertaken at SARDI Horticulture Pathology, 83039585.