Development of methods to monitor and control Aphanomyces root rot and black root rot of beans.

Andrew Watson
NSW Department of Primary Industries

Project Number: VG08043

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NSW DPI

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This report covers the activities undertaken during the period of the project from January 2009 till July 2012 examining methods to detect and control soil borne diseases of beans.

Report Completed–July 2012.

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MEDIA SUMMARY

Aphanomyces root rot (ARR) is a soil borne fungal disease causing browning of roots and stems of green beans and in severe cases causing death of plants, often in combination with other pathogens. The fungus causing the disease is favoured by wet conditions and can build up rapidly in soil from low levels. Blocks with infected soil must remain free of beans for up to ten years to reduce the levels of the fungus. In soil surveys a number of bean farms were found to have the fungus, and therefore the potential of causing disease beans.

Using a soil bioassay before planting can give a guide to the level of the disease in blocks that are to be planted. A DNA test for the organism developed in the project will also assist in the identification of soils with the fungus. A number of rotational crops were assessed for their potential to reduce ARR and some disease reduction was identified as well as enhancing bean growth. Other management tools for reducing ARR include improving drainage by planting on beds or hills and carefully monitoring irrigation. In variety screening trials, bean varieties showed no reduction in disease levels but some were stronger plants and able to reduce the affects of the disease. The fungus affecting beans was not shown to infect peas.

Black root rot (BRR), causing blackening of stems and roots, is another soil borne fungus identified on bean farms. The fungus was found more in bean farms on Tasmania and Victoria, than in New South Wales. The fungus has a wide host range and appears to infect beans in cooler conditions. Varieties with better tolerance to the disease were identified and screening for resistance to the disease should be ongoing.

Some bacterial based soil drenches improved bean plant establishment and vigour but did not reduce ARR or BRR. In trials a soil drench of a commonly used fungicide was found to assist in the control of ARR as well as Ashy stem blight, another soil borne fungal disease of beans.

The project has identified diseases of beans, the specific symptoms of each of them, their occurrence in different growing regions, and methods to reduce disease.

TECHNICAL SUMMARY

Aphanomyces root rot (ARR) of beans is caused by *Aphanomyces euteiches* f.sp. *phaseoli*. It is a soil borne fungal disease causing browning of roots and stems of green beans (*Phaseolus vulgaris*) and in severe cases causing death of plants, often in combination with other pathogens. The fungus causing the disease is favoured by wet conditions and can build up rapidly in soil from low levels. Blocks with infected soil must remain free of beans for up to ten years to reduce the levels of the fungus. Trials conducted gave an indication on the severity of ARR and the potential that it has to wipe out whole plantings if conditions favour disease. Using a pre planting assessment is a high priority for growers, where the potential of infection from ARR is high, as it provides important information on disease levels before planting.

A DNA test was developed within the project and gives confirmation on the presence of *A. euteiches*. Growers should consider having a soil bioassay done for ARR as well as the DNA test for confirmation on observed symptoms within the bioassay. Brown hypocotyls and roots not only mean ARR they can be black root rot or types of *Pythium*. *A. euteiches* can build up in quantity rapidly given suitable conditions, from a low base level it can move throughout the crop if wet conditions continue. Zoospores can infect roots and roots can be populated by enormous numbers of oospores to infect in the current season or to survive for future seasons. Thus beans cannot be planted on the same ground regularly, less so in wetter bean growing regions.

The fungus was found to be widespread in Tasmanian bean growing soils during a survey. It had first been found in Tasmania through the project VG03002 “Managing bean root and stem diseases”.

In cross infection studies with bean isolates on peas there was no infection of the peas with the isolates of *Aphanomyces euteiches* that had been isolated from beans, indicating that the isolate from beans is *Aphanomyces euteiches* f.sp. *phaseoli*, a type specific to beans. For growers who grow beans that have ARR, there will be no cross infection with peas, providing they do not have *Aphanomyces euteiches* f.sp. *pisi*. Further work on the cross infection with other hosts should be considered and to conduct genetic comparisons between the isolates.

In trials on beans, without the influence of other pathogens, the direct yield loss due to *A. euteiches* was 70% however infected plants in the field are often overtaken by secondary fungi that can cause total plant death. Various control options were examined in the project including solarisation, soil drenches with potassium silicate, lime application, bacterial based products, fungicides and the incorporation of brassica green manures.

A number of brassica biofumigant crops were assessed as green manures for their potential to reduce ARR and some disease reduction was identified. Bean plant growth was also enhanced using brassica

crops as green manures. Other management tools for reducing ARR include improving drainage by planting on beds or hills and carefully monitoring irrigation. In variety screening trials bean varieties show no reduction in disease levels but some were stronger plants and able to reduce the affects of the disease.

Antagonism by bacteria to *A. euteiches* has been demonstrated in the greenhouse situation but transferring this to the field was not successful. Products such as Fulzyme® and Serenade Max® containing bacteria (*Bacillus* species) improved bean plant establishment in pots, but their affect on ARR disease expression was not as successful.

In trials the use of azoxystrobin (Amistar®) as a soil drench has shown some activity against ARR and also charcoal rot (ashy stem blight) caused by *Macrophomina phaseolina*. However there is no registration for this use in Australia. It was also observed in greenhouse trials that azoxystrobin can cause some phytotoxicity to seedlings, but in field trials if applied immediately at sowing as a soil drench on top of the planting row it reduces disease.

Hymexazol, the main ingredient in the product Tachigaren® marketed by Daiichi Sankyo Co. Ltd., has shown good efficacy against ARR but it is not available and unlikely to ever be in Australia.

Black root rot (BRR), causing blackening of stems and roots, is another soil borne fungus identified on bean farms. The fungus was found more on bean farms in Tasmania and Victoria, than in New South Wales. The fungus has a wide host range and appears to infect beans in cooler conditions. Varieties with better tolerance to the disease were identified and screening for resistance to the disease should be ongoing.

The project has identified diseases of beans, the specific symptoms of each of them, their occurrence in different growing regions, and methods to reduce disease. The need to continue the unravelling of the root disease “complex” is needed, further work to identify varieties with disease tolerance and the evaluation of brassica rotations to reduce disease when used as green manures.

INTRODUCTION

A survey of green bean crops in project VG03002 was carried out to identify and investigate the major root diseases that contribute to poor emergence, poor crop growth, and early senescence which caused substantial pod yield reduction. This collaborative project conducted in New South Wales (NSW), Queensland and Tasmania identified a complex of root pathogens causing bean root and hypocotyl rots, depending on each growing region. Among these pathogens, two particular bean root pathogens, root rot due to *Aphanomyces euteiches* f. sp. *phaseoli* (ARR) and black root rot due to *Thielaviopsis basicola* (BRR) were identified as the most devastating root pathogens in many traditional and major bean production areas in NSW and Tasmania.

Aphanomyces euteiches was common in NSW and was detected in several devastated bean crops in Tasmania. This was the first detection of this fungus on beans in Tasmania. *A. euteiches* is part of what was described as a root disease complex occurring in southern Queensland growing regions (Wright *et al*. 1997)). *Thielaviopsis* is a common root rot pathogen in Tasmania and Queensland.

Roots infected by these two pathogens are prone to further damage by other soilborne pathogens such as *Pythium*, *Rhizoctonia* and *Fusarium* species. Both *Aphanomyces* spp. and *Thielaviopsis* spp. can survive for many years in soil. There are currently no effective chemicals registered or cultural methods of managing ARR and BRR. There are also no practical and cost effective methods of identifying these pathogens prior to planting. Control of these pathogens currently relies on avoidance of infected fields. However, this becomes increasingly difficult as buying new land is no longer an option.

This project proposed to continue the research initiated by VG03002 by developing a DNA soil test for *A. euteiches* and to evaluate potential chemical and non-chemical strategies for managing ARR, BRR and other bean root diseases. Project VG03002 identified fungicides with potential to control ARR and these will be further investigated in VG08043.

Diseases of green beans

Green beans (*Phaseolus vulgaris* L.) consisting of French or dwarf, runner or climbing beans are a valuable crop to Australia with production approximately 30,000 tonnes worth $73M (source Australian Bureau of Statistics, 2009). Beans are grown for fresh market and for processing (i.e. canned or frozen). Queensland and Tasmania are the biggest producing states of beans in Australia. Beans are often grown on sloping sites, using diversion drains to catch water runoff. Reducing “wet feet” is a priority for healthy bean plants. Where needed irrigation is carried out using moveable aluminium pipes with overhead sprinklers, travelling irrigators or furrow irrigation.

Harvesting is carried out from 7 to 11 weeks after planting. French beans are harvested by machine or by hand. Machine harvesting only allows a single pick and harvested material is sorted in packing sheds to remove leaves etc. Like many crops, plant material not harvested remains in the paddock and is either ploughed in or eaten by stock.

Beans can be affected by a number of diseases including those listed in Table 1.

Table 1: Common diseases of beans in Australia.

Common name	**Organism**	**Symptoms**
Damping off	*Pythium, Rhizoctonia, Fusarium* sp	Damage to seedling restricting emergence before or after germination.
Ashy stem blight	*Macrophomina phaseolina*	Damage to lower stem of younger plants often lesion on one side of stem.
Sclerotium rot	*Sclerotium rolfsii*	Young plants affected causing plant death.
Fusarium root rot	*Fusarium solani, F. oxysporum*	Rotting of lower stem causing reddening and reduced plant vigour.
Aphanomyces root rot	*Aphanomyces euteiches* f.sp *phaseoli*	Watery brown colouring of roots especially tap root and hypocotyl. Whole blocks affected.
Sclerotinia rot	*Sclerotinia sclerotiorum*	Infection of stems causing weak plants or death. Pods may be affected.
Rhizoctonia stem rot	*Rhizoctonia*	Lesions on roots/hypocotyl.
Black root rot	*Thielaviopsis basicola*	Blackening of roots.
Root rot complex	*Fusarium, Pythium and Aphanomyces*	Red root. Lower hypocotyl reddish coloured. Plants survive but weak.
Bacterial brown spot	*Pseudomonas syringae pv syringae*	Leaf/pod spot
Common bacterial blight	*Xanthomonas campestris pv phaseoli*	Leaf/pod spot
Halo blight	*Pseudomonas syringae pv phaseolicola*	Leaf/pod spot
Pod twist	*Pseudomonas flectens*	Pod twist
Angular leaf spot	*Phaesiosariopsis griseola*	Leaf/pod spot
Anthracnose	*Colletotrichum lindemuthianum*	Pod spot
Ascochyta blight	*Phoma exigua (Ascochyta phaseoulorum)*	Leaf spot
Cercospora leaf spot	*Cercospora canescens*	Leaf spot
Cottony leak	*Pythium aphanidermatum*	Water soaked area on leaves and pods that may become covered in cottony growth.
Pleiochaeta brown spot	*Pleiochaeta setosa*	Leaf spot
Rust	*Uromyces appendiculatus*	Leaf spot
Viruses	Bean yellow mosaic, common mosaic, peanut mottle and bean summer death	Various symptoms from mosaic patterns on leaves to cupping and twisting or plant death.

Soil borne organisms are responsible for establishment diseases and stem rots that can be serious in certain bean growing regions. The effect of these rots can result in large areas of plantings either not germinating or causing damage at a later growth stage. Many of these organisms can survive for long periods in soils, plant material or survive on volunteer weeds or alternate crops.
Soil borne disease management is important for the bean industry to maintain a reliable supply of high quality product.

Aphanomyces root rot

In the 1980's a disease was identified in the Macksville area of NSW (North Coast). Investigations identified the disease as Aphanomyces root rot caused by *Aphanomyces euteiches* Drechs f.sp *phaseoli* Pfend & Hag (Allen *et al.* 1987). A product called Le-san® (fenuminosulph) was found to control the disease however not long after this work the permit to use Le-san® was withdrawn and since that time, when conditions are conducive to disease, large losses have resulted. *Aphanomyces euteiches* management had not been investigated thoroughly and its occurrence in other growing regions had not been fully investigated.

Aphanomyces spp. have been recorded on other crops in Australia including lucerne (Abbo and Irwin 1990), clover (Barbetti 1991), subterranean clover (Greenhalgh *et al.*1985), faba beans (Leur *et al.* 2003), peas and beetroot (Hutton and O'Brien 1986, Martin 2003). Members of this genus can also cause diseases of fish.

A thorough review of *Aphanomyces* species that affected peas and sugar beet was undertaken by Papavizas and Ayers (1974). But since then the fungus has been identified on beans and recognised as one that is specific to beans (Pfender and Hagedorn 1982). Since that time others have found it associated with bean root rot (Allen *et al.* 1987, Oyarzun and Loon 1989).

Control of this fungus currently relies on avoidance of infected fields, however this strategy is becoming difficult where land is either under development or buying new land is not possible. There is a lack of disease resistance in high yielding commercial varieties. Some resistant varieties have been identified but unfortunately the loss in other agronomic traits renders them unacceptable for production.

ARR causes browning of roots and lower stems of green beans, and in severe cases causes death of plants often in combination with other pathogens. ARR on its own reduces yield directly or by delaying flower set and making machine harvesting impossible. Structures produced by Aphanomyces include zoospores which move through water films to infect roots and oospores which are long term survival structure (Figure 1).

Fungicidal control

Control of *Aphanomyces* spp. is limited by the lack of fungicides. Those fungicides that have some efficacy against *Pythium* spp. do not control *Aphanomyces* spp. Potential fungicides for control are included in Table 2 (Erwin *et al.* 1983).

Table 2: The table below is a list of fungal organisms and the efficacy of fungicide active ingredients.

Fungicide active ingredient and control				
Soil-borne Organism	metalaxyl, fluralaxyl, benalaxyl	propamocarb	fosetyl-aluminium	hymexazol
Aphanomyces spp.	-	+/-	?	+*
Fusarium spp.	-	-	-	+
Phytophthora spp.	+	+	+	-
Pythium spp.	+	+/-	+	+

(- = no control, + = good control, +/- =variable control, ? =unknown).
* Hymexazol was found to have efficacy against *A. euteiches* examined in Project VG03002.

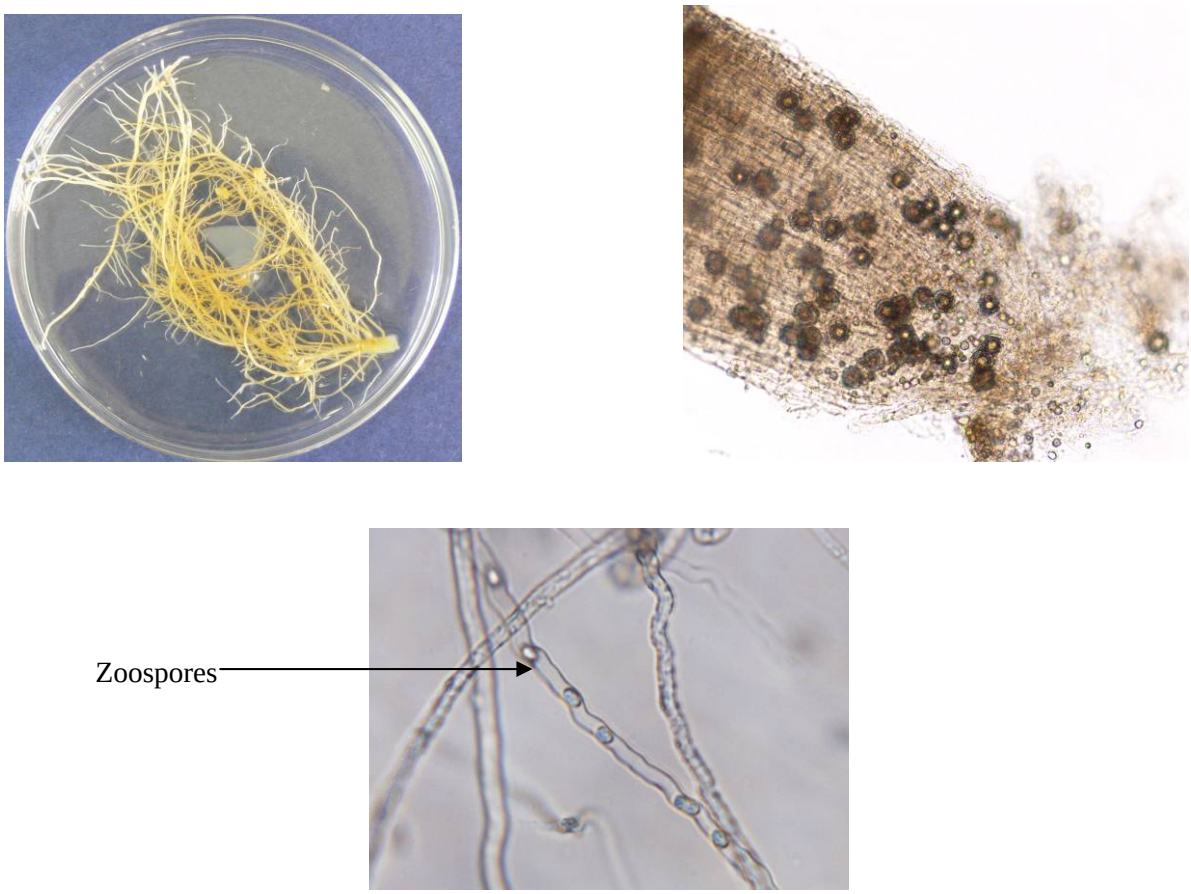

Figure 1. (From upper left) Browning of roots associated with infection from *A. euteiches* on the agar piece in the middle of the roots. Oospores in the roots on the photograph on the top right hand side. Lower photograph of zoospores ready to move out of the sporangia into water.

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NEW SOUTH WALES RESEARCH ACTVITIES

1. THE EFFECT OF SOIL SOLARISATION ON *A. EUTEICHES* SURVIVAL

Introduction

Soil borne diseases are difficult to control due to the constant supply of infective propagules and the difficulty in killing or removing these from the soil. The common methods to kill the pathogens in soil include fumigation with products such as metham sodium, methyl bromide and dazomet (Basamid®), steam sterilisation and solarisation. There are various considerations in deciding on which of these options to use. The size of the block, the type of crop, the cost of the treatment in relation to the return from the end product all need to be considered. Metham sodium is often used in larger commercial operations such as in the potato industry. Sterilisation by steam is more commonly used in nurseries with smaller quantities of soil.

Solarisation by increasing soil temperature has potential for controlling soil borne plant diseases. Although the technique is difficult to undertake in large plantings, initial studies were undertaken to examine the affects of increasing soil temperature on *A. euteiches* in infected soil. Using polyethylene films combined with the high temperatures in summer periods may offer some alternative disease control options. The use of such treatments has been shown to increase temperatures in the top 5cm of soil to 52°C (Katan 1981). These temperatures have the ability to kill fungi, nematodes and bacteria especially if these temperatures are maintained over a number of days.

However the main drawback with these examples of disease controls is that they not only kill the target pathogen but also beneficial organisms, and there is the potential that if the target pathogen survives, it has limited competition enabling it to increase its concentration in the soil.

Method

Method of disease assessment.

Disease symptoms were assessed using a 0-5 scale based on root and hypocotyl (Figure 1.1) colour and health. The 0 rating was no disease and the 5 rating was high disease. For most trials roots and hypocotyls were assessed separately, whereas with others often only the hypocotyl was assessed. The disease ratings were based on the symptoms shown in (Figure 1.2).

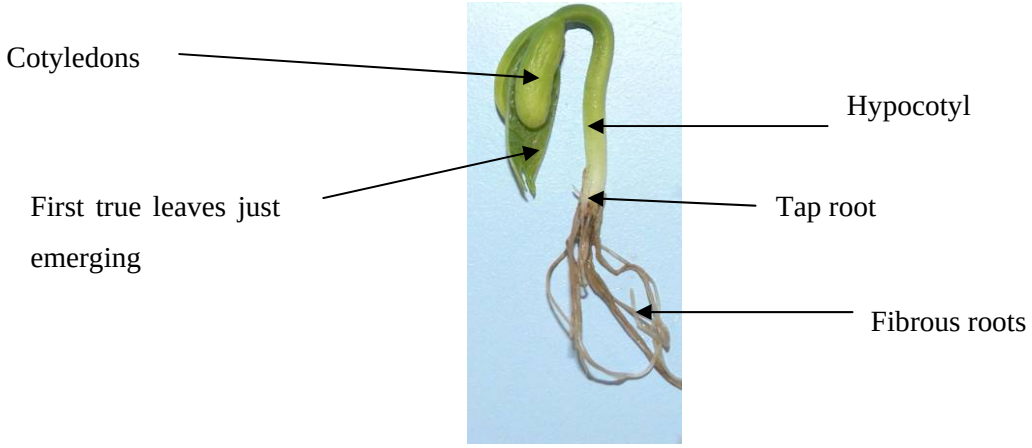

Figure 1.1. Parts of the bean plant showing the hypocotyl region which was the main area examined for disease symptoms.

Figure 1.2. Disease ratings were based around the symptoms shown in the above photograph. An examination of the hypocotyl and the finer roots was conducted allowing for browning and necrosis (root death). Often root colour was no different but showed various levels of decay.

Wetting up period

When carrying out trials in the NSW component of this report, plants were given a wet period to instigate infection by *A. euteiches*. The fungus needs free moisture to produce zoospores (swimming spores) that then use the water to move from infected tissue to non infected. These zoospores do not move across long distances possibly only as far as 10mm (Papavizas and Ayers 1974). The wetting-up period occurred at about the two leaf stage and consisted of watering the containers three times per day for three days.

For many of the greenhouse trials infected soils were used that had been collected from growers' properties. The soils were either used on their own or mixed with vermiculite. Vermiculite is a product that is commonly used in potting mixes.

Statistical analysis

All data from the trials was analysed using Genstat 11 analysis of variance.

Trial 1

The first trial consisted of placing grower soil known to contain the disease in incubators at various temperatures. Five 100mm pots were placed in incubators for one month each at temperatures of 30°C, 40°C, 50°C and 60°C. After treatment they were placed in a greenhouse at 20/27°C. Pots of soil that had not been in any heat treatment were also included. Beans were planted and assessed three week after planting after the usual wetting up treatment.

Trial 2

In trial two, known *A. euteiches* infected soil was placed into four plastic containers 220mm x 220mm x 140mm which were then placed in tubs 385mm x 290mm x 130mm. Vermiculite was then placed around the edge between the walls of plastic containers and the walls of the tubs. The tubs were then placed under black plastic on a clear section of lawn with two of the containers being removed after one week and the other two after two weeks. The trial was conducted from the 4th of February till the 11th for the one week treatment and till the 18th for the two week treatment at Yanco Agricultural Institute. Soil temperature was measured in the containers by placing loggers 100mm into the soil. After two weeks the soils were removed and placed in 100mm pots and beans (Simba) planted five per pot, there were five replications. Infected soil not under plastic was used as a control and all were placed in a greenhouse at 20/27°C. Beans were planted and assessed three week after planting after the usual wetting up treatment.

Trial 3

In a field on a trial block known to have *A. euteiches*, plastic sheets were laid on top of the soil and left for six weeks during summer. Plots were five metres long and two metres wide. Soil was collected at the end of this period and potted up in greenhouse from 5cm and 10cm below the plastic. The soil was placed in 100mm pots with 5 replicates per treatment. Beans were planted (5 per pot) and then assessed for disease symptoms after 3 weeks.

Results

Trial 1

Disease was most severe in the soil that was at ambient temperature and the soil that was maintained at 30°C (Table 1.1 and Figure 1.3). The soils maintained at 40, 50 and 60°C had minimal disease. This data provided some preliminary information on *A. euteiches* survival and also gives an indication to what temperatures need to be achieved to reduce disease levels.

Table 1.1. ARR ratings for Trial 1.

Temperature °C	**Disease rating (hypocotyl)***	**Disease rating (roots)***
60	0 a	0 a
50	0.3 a	0 a
40	0.3 a	0 a
30	4 b	2.7 b
Control	3.7 b	3.16 b
P	**<0.001**	**<0.001**
LSD 5%	**0.35**	**0.28**

*Values with the same letter not significantly different.

Figure 1.3 Soils maintained at various temperatures for one month before beans planted. From left control soil with no heat treatment, 30°C, 40°C, 50°C and 60°C.Hypocotyls clean on the 40°C, 50°C and 60°C temperatures.

Trial 2

For the one week treatment the maximum, minimum and mean temperatures were 58°C, 17°C and 35°C and for the two week treatment 45°C, 16°C and 27°C. The temperatures including maximum, minimum recorded for the period are shown in Table 1.2. Also in the table are solar radiation data and the maximum temperatures recorded in the soil under plastic. There was no ARR recorded in the heat treatments with disease rating scores of 4 in the untreated; one week was enough to reduce disease (Figure 1.4). On some days there were temperatures above 30°C measured for 20hrs. The second week was cooler with lower solar radiation levels than the first.

Figure 1.4. One week under plastic (left) and two weeks under plastic (right).

Table 1.2. Ambient temperatures for the time period of Trial 2.

February Date	Maximum	Minimum	Solar radiation MJm^{-2}	Highest temperature recorded in soil under plastic °C
4	39.7	23.5	30	47.6
5	43.3	26.6	31.5	57.9
6	43.2	25.8	31.1	58.7
7	44.8	28.1	28.9	50.4
8	42.7	27.9	29.6	52.2
9	30.7	17.2	27.3	45.0
10	27.2	12.9	27.3	42.5
11	27.4	12	28.3	43.8
12	26.5	14.8	20.5	39.9
13	24.7	13.3	19.1	30.3
14	27.4	15.2	23.6	39.0
15	28	16.2	22.3	36.0
16	30.1	15.8	25.7	39.5
17	27.2	15.8	Not available	36.8
18	28.8	18.1	Not available	39.4

Trial 3

There was no difference between the soils under plastic compared to surrounding soil when plants were assessed for ARR. Heavy rainfall had occurred in the area over the period and caused soil to be moved around the block. It also may indicate that the plot sizes were too small.

Discussion

In Trial 2 a maximum temperature of 59°C was achieved which was similar to the 52°C mentioned previously (Katan 1981), there were five consecutive days above 50°C. Beans are grown over summer and this timing may clash with this method of reducing pathogen levels in soil, however if only one week is needed then it may fit into the bean growing system. The ambient temperature at the early period of the trial was high (40°C and above) which is uncommon in most been growing regions of Australia.

This method of soil sterilisation was not adopted by growers as they could not perceive that this system would work due to large amounts of plastic needed and a method to lay it down and lift it up. Melons are often grown using plastic mulches of this type, but are left in place till the end of the crop. Therefore there is equipment available to place it over beds.

A. euteiches appears to be intolerant to heat and therefore hot periods in regions such as Queensland may reduce inoculum levels of this fungus. Beans are not grown in Queensland in the hotter part of summer. Therefore a hot fallow period may reduce inoculum. *A. euteiches* has been found to be more of an issue in northern NSW and Tasmania. Northern NSW is characterised by high rainfall (mean annual 1673mm) and mild temperatures whereas northern Tasmania has lower rainfall (mean 774mm) and lower temperatures.

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2. THE EFFECT OF *A. EUTEICHES* ON YIELD OF GREEN BEANS.

Introduction

It is difficult to demonstrate yield loss associated with *A. euteiches* infection of bean plants in the field. This can relate to the lack of disease inducing conditions required and that field observations suggest that the disease does not kill plants but instead reduces vigour resulting in less flower numbers and a poorer fruit set. In mechanically harvested blocks this means that the disease therefore stops the ability to harvest on time whereas hand picked blocks may recover enough to allow staggered picking. Based on field observations, secondary fungal organisms often invaded tissue that had already been infected by *A. euteiches*. Therefore a greenhouse trial was established to examine yield loss due to this fungus and without the influence of other pathogens.

Method

Trial 1-Preliminary trial developing application rates of *A. euteiches* inoculum

Most greenhouse studies on this disease have been undertaken using soil containing the fungus i.e. sourced from growers' properties. It was decided to examine inoculation methods to induce the disease in potting mix and therefore reduce the need for infected soil. An *A. euteiches* isolate was used that was freshly isolated from infected bean plants. The culture of the fungus once isolated, was grown on agar media (¼ strength Potato Dextrose Agar-Oxoid®) in small petri dishes (55mm diameter). After growing for one week in an incubator at 25ºC, nine petri dishes were blended with 450ml of sterile distilled water. Three different inoculation rates, chosen by volume of the fungal mixture (6.25ml, 12.5ml and 25ml) were added to five pots (100mm diameter). The same quantity of liquid was added to each pot i.e. the lower volumes were made up to 25ml with distilled water. Pots were filled with potting mix (Debco®). Pots without added *A. euteiches* were used as controls. The pots were placed in a greenhouse in a randomised complete block design.

Five Simba variety bean seeds were planted, watered with 150 ml of water and one week later the *A. euteiches* was added by placing the mixture around the roots where the potting mix had temporarily been removed. Blended agar without the *A. euteiches* was used on the controls. The pots were watered with three times a day with 150 ml of water for three days. Three weeks after sowing and after hand watering with equal quantities of water, plants were removed and rated for disease. Plants were also dried and weighed with data presented as mean weight per treatment. Data was analysed for significance between treatments.

Disease symptoms were assessed using a 0-5 scale based on root and hypocotyl (Figure 1.1) colour and health. The 0 rating was no disease and the 5 rating was high disease. For most trials roots and hypocotyls were assessed separately, whereas with others often only the hypocotyl was assessed. The disease ratings were based on the symptoms shown in (Figure 1.2).

Trial 2-Yield loss trial

Five beans seeds of the variety Simba were planted into Debco® potting mix in 200mm pots. These pots were big enough to allow adequate growth and long enough to allow significant bean pod production. The pots were placed in a greenhouse at 20/25ºC. A culture of *A. euteiches* isolated from beans was applied to the pots. One week after sowing 25ml of blended culture as determined in the preliminary trial was used to inoculate the roots, by placing the mixture around the roots where the potting mix had temporarily been removed.

Different treatments were examined and there were five replicate pots per treatment that were randomly distributed around the greenhouse randomised complete block design. The treatments were designed around the "wetting up" period. *A. euteiches* with motile zoospores requires a film of water and therefore an examination of the time when this wetting period occurred and its relationship with disease incidence was undertaken. Infection appears to be possible with standard watering but the symptoms are far more extreme when there is an extended wet period. In this case therefore extra water was applied three times in an 8hr period to five pots 48hrs after the *A. euteiches* was applied (Treatment 1). Another five pots had the same treatment then a second treatment two weeks later (Treatment 2); another five pots were wet up six weeks after application of the *A. euteiches* (Treatment 3), therefore a long period between the inoculation of the fungus and the period to induce serious infection. There were five pots that had no *A. euteiches* applied (Treatment 4). Plants were watered and fertilised as required with equal quantities, so that all pots received the same amounts of water/fertiliser. 11 weeks after sowing, plants were assessed for disease, plant height and dry weights were measured, pods per pot were counted and weighed.

Results

Trial 1

The pots without *A. euteiches* added had no symptoms (Table 2.1) and the rating for disease was similar across all concentrations of inoculum but the lowest rate was significantly lower than the other two rates. The dry weight for the control plants was significantly higher than the other treatments ($P<0.05$).

Table 2.1 Hypocotyl lesion ratings and dry weights for beans grown in potting mix with different quantities of *A. euteiches* inoculum.

Treatment	**Disease rating (hypocotyl)***	**Dry weight (g)***
Control (no *A. euteiches* added)	0 a	12.39 a
6.25ml	2.68 b	10.07 b
12.5ml	3.16 c	10.08 b
25ml	3.26 c	10.61 b
P	**<0.001**	**<0.05**
LSD 5%	**0.47**	**1.16**

*Values with the same letter not significantly different.

Trial 2

Hypocotyl and root disease ratings were similar for all treatments except for the untreated control that had no symptoms (Figure 2.1). After statistical analysis of the data, pod weight was significantly different between the *A. euteiches* treated pots compared to those without *A. euteiches* (Figure. 2.2). Mean pod weight of the untreated pots was approximately 200 gm per pot compared to 60 to 80 gm per pot for the pots treated with *A. euteiches*. This was a reduction in yield of 70%. The pots that received the later wetting up period were significant for some factors compared to those that received earlier wetting up treatments including plant dry weight, height, pod number and pod weight (P<0.001). Images of the non inoculated plants compared to inoculated plants can be seen in Figures 2.3 and 2.4.

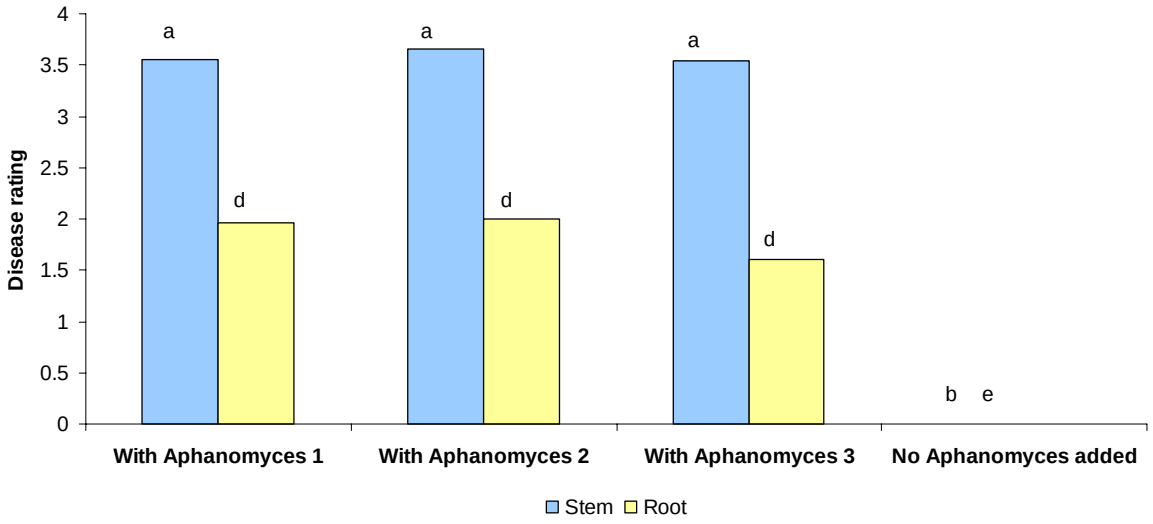

Figure 2.1. Disease ratings for hypocotyls and roots for the four different treatments. The levels were similar across all treatments except for no disease being found in the control pots (Treatment 4). There was a significant difference between those treatments that received *A. euteiches* compared to the one that did not P<0.001, LSD 5%=0.26 for hypocotyls and P<0.001, LSD 5%=0.26 for roots.

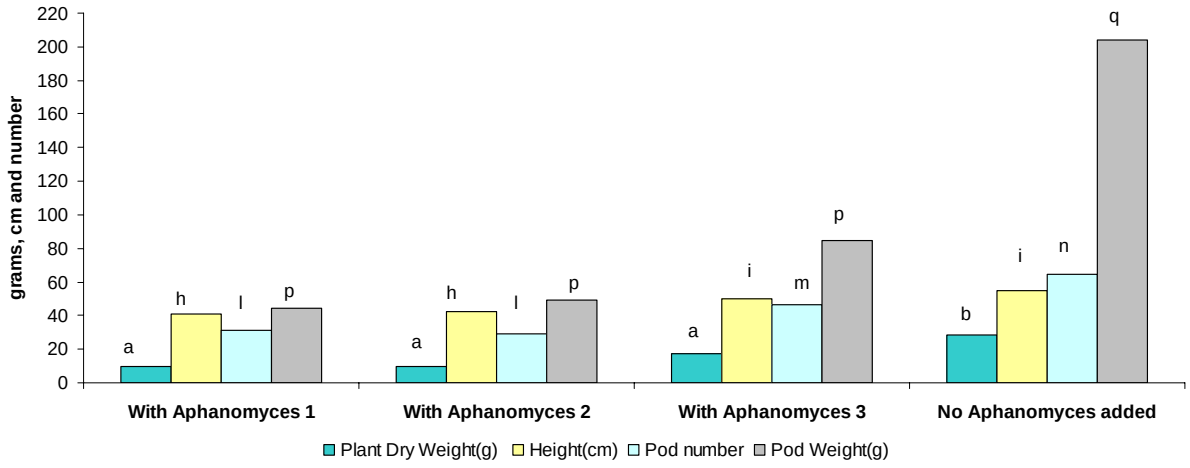

Figure 2.2 There were significant differences in all factors with P<0.001, plant dry weight (LSD 5%=7.2), height (LSD 5%=5.5), pod number (LSD 5%=10.6) and pod weight (LSD 5%=49.7) for the four treatments. Values with the same letter in each category are not significantly different.

Figure 2.3. A visual comparison of Treatment 4 (no *A. euteiches* applied) on the left and Treatment 1 (with *A. euteiches*) on the right.

Figure 2.4. The root systems of Treatment 4 (without *A. euteiches*-left) and Treatment 1 (with *A. euteiches*-right) indicating reduced root growth and lesions on the lower stem.

Discussion

This fungus did not cause bean plant death but it reduced yield considerably. Potting mix was used in this trial which is free of other potential secondary infecting fungi that could have compounded disease symptoms and may have caused death of the plants after their infection with *A. euteiches*. The bean roots infected with *A. euteiches* in the potting mix were healthier than roots on infected plants removed from the field.

The trials give an indication on the severity of ARR and the potential that it has to wipe out whole plantings if wet conditions occur that favour disease. However the disease was still able to affect those pots that had a delayed wetting up period as well, so maintenance irrigation was able to initiate infection.

3. THE INCIDENCE OF APHANOMYCES ROOT ROT IN TASMANIAN AND VICTORIAN BEAN GROWING SOILS.

Introduction

Aphanomyces euteiches is a fungus which can cause root and stem rot of beans (*Phaseolus vulgaris*) and had not been recorded in Tasmanian beans until a previous project (VG03002) found one crop to have symptoms of *A. euteiches*. A more detailed examination of Tasmanian soils was therefore the main target of this project. Forty two soils were collected by collaborators in Tasmania from different blocks on a number of farms to give an indication of how widespread this fungus was in bean growing regions. Previous pathogenicity tests had confirmed the effect of this fungus on beans (Figure 3.1). Eight soils were also included from Victorian bean growing regions.

Figure 3.1. Disease symptoms caused by *A. euteiches.* Bean plants inoculated with *A. euteiches* (left) and bean plant not inoculated with *A. euteiches* (right).

Method

After collection each of the soils was divided up into five pots (100mm). Seven Simba variety bean seeds (treated with metalaxyl) were sown into each pot and subsequently thinned to five after germination. Plants were placed in a greenhouse at 25°C in a randomised complete block design and after one week pots were watered three times per day to induce *A. euteiches* symptoms. After three weeks plants were removed and each plant assessed for hypocotyl/root lesions. Samples of roots from each soil were placed in water in a dish and in the next two days were examined microscopically for *A. euteiches* zoospore production, *A. euteiches* oospores and *Thielaviopsis* (black root rot) spores as some soils showed indications of this disease.

Plants from the Tasmania soil were also dried, weighed and soils were tested for pH (CaCl) and textured i.e. soil type was described.

Results

Both ARR and black root rot were found in a number of the soils on bean plants. *Pythium* was also found affecting some hypocotyls in some soils. In respect to the Tasmanian soils 30 were found to have ARR, 11 with black root rot, and three soils had both. Plants that were grown in the Victorian soils had fewer roots and were less vigorous; the soils were heavy and not suited to pots. There were six with severe black root rot symptoms and only one considered to have ARR. Rhizoctonia (*Rhizoctonia solani*) lesions were common on the plants grown in the Victorian soils. The results are listed in Tables 3.1, 3.2 and 3.3. Symptoms observed have been included in Figure 3.2.

In the Tasmanian soils pH ranged from 4.7 to 5.7 (Table 3.3).With those more affected by root disease having less dry weight (Figure 3.3). Images of some of the beans from four different soils are included in Figure 3.4.

Isolation of *A. euteiches* was also carried out to provide a selection of *A. euteiches* isolates for future use.

Table 3.1. Interpretation of disease on plants grown in Tasmanian soils.

Main cause of symptom observed.	**Number of soils**
Soil considered disease free.	7
Severe ARR.	4
Moderate ARR.	6
Low ARR.	20
Severe Black root rot.	2
Moderate black root rot.	1
Low black root rot.	8
Soils with both diseases observed.	3

Table 3.2. Interpretation of disease on plants grown in Victorian soils.

Main cause of symptom observed.	**Number of soils**
Soil considered disease free	0
Severe ARR.	0
Moderate ARR.	0
Low ARR.	1
Severe Black root rot.	6
Moderate black root rot.	0
Low black root rot.	0
Soils with both diseases observed.	1
Soils with *Rhizoctonia*	6

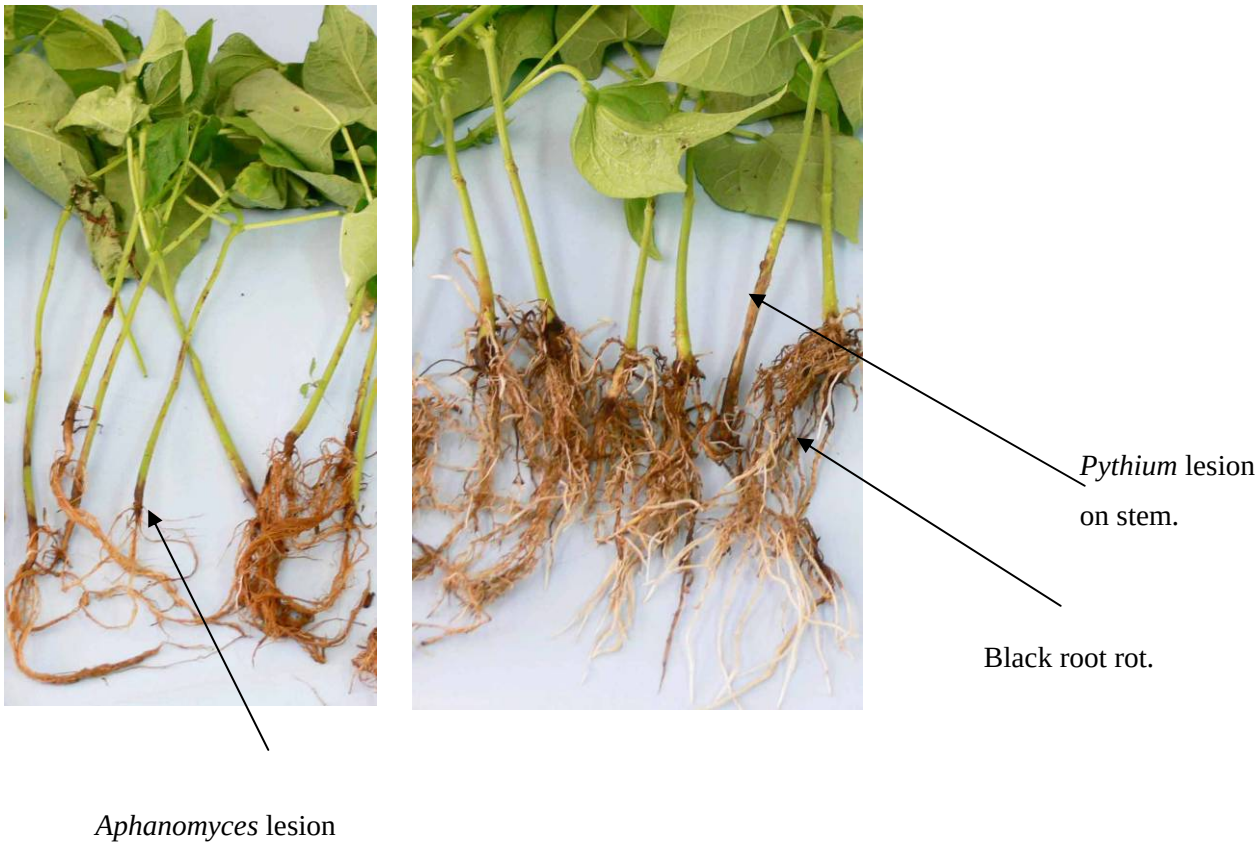

Figure 3.2. Symptoms observed of *A. euteiches* on bean plants (left) with a very restricted root mass and lesions affecting the lower hypocotyl (hypocotyl) and typical black root rot and *Pythium* symptoms (right). Note new root formation due to damage by black root rot.

Table 3.3. pH, texture and diseases observed on the Tasmanian soils. ARR=Aphanomyces root rot, BRR=black root rot, Pyt=*Pythium*. The more + signs the higher disease assessed.

Soils	**pH**(CaCl)	**Soil type**	**Disease observed**
1	5.5	Dark reddish brown loam	ARR +++
2	5.6	Very dark brown silty loam	ARR +++
3	5.3	Very dark brown silty loam	ARR +
4	5.3	Dark reddish brown loam	ARR +
5	4.9	Very dark brown fine sandy loam	ARR +
6	5.0	Very dark brown sandy loam	ARR +
7	5.3	Very dark brown silty loam	ARR +
8	5.7	Dark reddish brown clayey sand	BRR +
9	5.9	Dark reddish brown silty loam	
10	5.7	Dark reddish brown loam	BRR +
11	5.6	Dark reddish brown loam	ARR ++
12	5.5	Dark reddish brown silty loam	BRR +++
13	5.3	Very dark brown silty loam	
14	5.1	Very dark brown fine sandy loam	ARR +
15	5.2	Very dark grey silty loam	BRR +
16	5.2	Dark reddish brown silty loam	
17	5.2	Dark Brown silty loam	BRR +
18	5.5	Very dark brown silty loam	BRR +
19	5.4	Dark brown fine sandy loam	ARR +++
20	5.1	Black clay loam	ARR +++ BRR +
21	5.7	Very dark brown silty loam	
22	5.2	Very dark brown fine sandy loam	Pyt+++ BRR++
23	5.7	Black heavy clay	ARR +
24	5.6	Dark brown sandy loam	Pyt +++ BRR+++
25	5.5	Dark reddish brown fine sandy loam	
26	4.8	Dark brown clayey sand	
27	5.6	Dark reddish brown fine sandy loam	ARR + BRR +
28	5.8	Dark reddish brown sandy loam	ARR +
29	4.7	Very dark brown silty loam	ARR +
30	5.0	Dark reddish brown sandy loam	ARR +
31	5.3	Dark reddish brown sandy loam	ARR ++
32	5.3	Dark reddish brown sandy loam	ARR +
33	4.9	Dark reddish brown sandy loam	ARR +
34	5.0	Dark reddish brown loamy sand	ARR ++
35	5.4	Dark reddish brown loamy sand	ARR ++
36	5.2	Dark reddish brown loamy sand	
37	5.4	Dark reddish brown sandy loam	ARR +
38	4.9	Dark brown sandy loam	ARR ++
39	5.2	Dark reddish brown fine sandy loam	ARR +
40	4.8	Dark brown fine sandy loam	ARR ++
41	5.3	Dark brown sandy loam	ARR + BRR +
42	5.4	Dark reddish brown sandy loam	

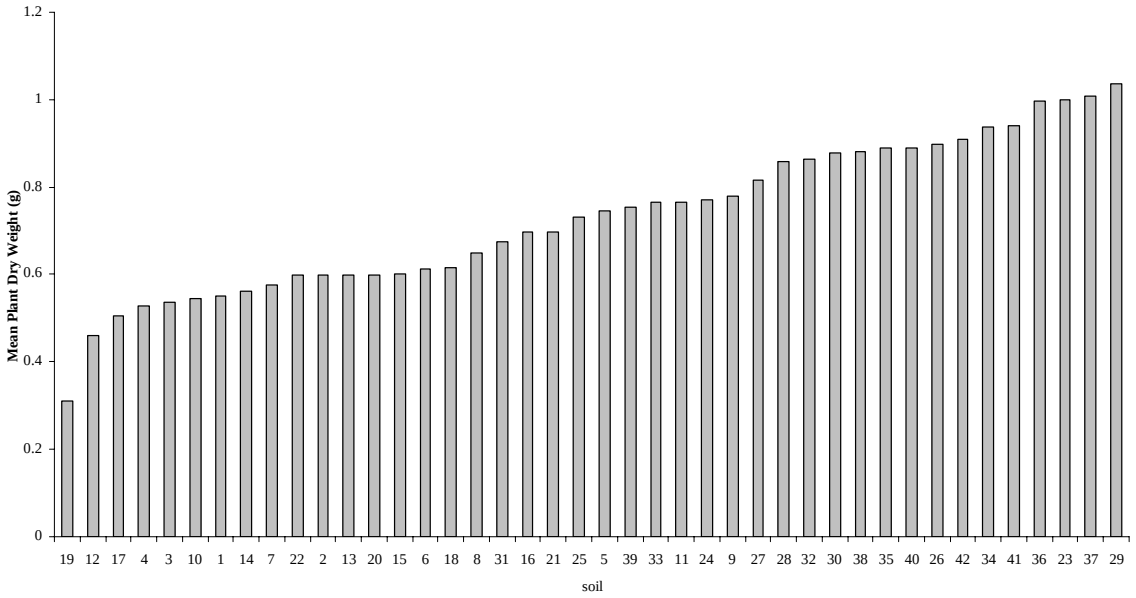

Figure 3.3. Mean plant dry weights from the various Tasmanian soils. This data was not statistically analysed. Severely infected soils included 19, 12, 1, and 2.

Figure 3.4. Beans grown in soils from Tasmania including Soil 1 with typical ARR symptoms, soil 12 with Black root rot, soil 19 with the lowest dry weight and high ARR and a disease free soil (29).

Discussion

There was a higher incidence of ARR than expected from the Tasmanian soils with 30 out of the 42 soils positive. Black root rot symptoms were high in both Tasmanian and Victorian soils. The pH and soil type did not affect the frequency of each of the diseases.

This method of detecting fungal diseases could be part of a standard test. Before planting a grower could undertake a similar bioassay method to give a pre-plant disease incidence score for a block that is being considered for planting. Both ARR and black root rot will continue to be issues for bean growing regions but awareness of this disease will assist growers in their decision making processes for bean planting.

The soils submitted were used to assess a new DNA test for *A. euteiches* detection later in the report.

4. DNA SOIL TEST FOR APHANOMYCES ROOT ROT

Introduction

As an aid for researchers and bean growers to detect *A. euteiches* in soil and improve knowledge of its potential to cause root rot, the development of a DNA soil test was proposed as part of this project. *Aphanomyces euteiches* is a fungus that is commonly difficult to isolate from plant material and observed symptoms are often confused with other pathogens or non pathogens that may be involved. The proposed soil test would provide an indication, independent of other plant pathological methods, to determine the presence or absence of the disease but also to provide some quantitative level that may be used for disease prediction. This component of the project was jointly undertaken by NSW Department of Primary Industries, and the South Australian Research and Development Institute (SARDI). The latter undertaking the development of the test and the former assisting with evaluation or comparing the test results with the actual symptoms seen in the field and or greenhouse.

Method

As part of this project SARDI setup to deliver a TaqMan® MGB (minor groove binder) real-time PCR to assist scientists assess *A. euteiches* levels in soil samples. This assay was designed (by Diana Hartley, CSIRO) but not evaluated by a prior pasture soil biology project funded by MLA, GRDC and AWI. The assay should be specific to *A. euteiches* based on the DNA sequence information in GenBank. Another assay published by Sauvage *et al*., 2007 (FEMS Microbiol Lett: 273, 64–69) was evaluated but SARDI was unable to get it to work.

Before delivering the assay, the primer and probe concentrations were optimised, standards developed and aliquoted and specificity checked (Table 4.1). Not detecting other organisms with the test is critical.

The assay is sensitive, it can detect 100 pg DNA/µL at Ct (cycle threshold) value 20 and is specific (Table 4.1). In tests with fungal cultures, the assay did not detect any of the *Pythium* species tested. *Pythium* is a common relatively closely related genus. While the assay detected *A. cochloides* 16 cycles after 4 isolates of *A. euteiches*, this does not represent a practical problem. Also the test should not detect *A. cochlioides*, as five of the last six bases of the reverse primer are mismatched, so this very low reading is probably due to a low level contamination of *A. euteiches*.

As an evaluation of the developed test, the forty two bean growing soils from Tasmania previously examined for ARR were submitted. This was the first comparison between observed symptoms (0-5 scale where 0 is no disease) and the newly developed soil test. To confirm the visual assessments, roots were physically examined for *A. euteiches* by placing in water and observing for the production of both characteristic zoospores and oospores under the microscope for the next two days.

Table 4.1. Specificity of *Aphanomyces euteiches* TaqMan® MGB real-time PCR. UD=undetected. YA1, YA4, YA7, and YA8 were isolates of *Aphanomyces euteiches*.

Species Name	Accession Number	Ct Value for 100 pg/ul DNA		Species Name	Accession Number	Ct Value for 100 pg/ul DNA
A. cochloides	YA9	36.8		*P. middletonii*	DAR 51509	UD
A. euteiches	YA 1	20.2			DAR 7328	UD
	YA4	20.2		*P. ostracoides*	DAR 66064	UD
	YA8	20.6		*P. aroecandrum*	BH40	UD
	YA7	20.3			DAR 55021	UD
Pythium debaryanum	WAC 2368	UD		*P. prolatum*	DAR 73864	UD
P. helicoides	WAC 10582	UD		*P. rostratum*	DAR 63928	UD
P. irregulare	BH 40	UD		*P. splendens*	DAR 55026a	UD
	BH 40	UD			DAR 55025	UD
	WAC 7857	UD			DAR 55026	UD
	WAC 7809	UD		*P. ultimatum*	DAR 64046	UD
	WAC 7810	UD		*P. ultimum var ultimum*	DAR 35790a	UD
	WAC 7715	UD		*P. vexans*	DAR 50450	UD
	DAR 68413	UD		*P. violae*	R .Coles	UD
	W3	UD			*P 7*	UD
	DAR 68413	UD			*P 25*	UD
	DAR 68794	UD		*P.acanthicum*	WAC2363	UD
	DAR 69783	UD		*P.debaryanum*	WAC2368	UD
	WAC 4937	UD		*P.echinocarpum*	WAC2369	UD
	D2	UD			WAC2369	UD
P. irregulare GpI	AA23323 L-NS(2)	UD		*P.echinulatum*	DAR55020	UD
	AA23346 NS-C	UD		*P.paroecandrum*	DAR55020	UD
	AA23364 L-NS	UD		*P.prolatum*	DAR3864	UD
	AA23360NS-L(2)	UD		*Pythium sp.*	BCP PAST 4	UD
P. irregulare GpII	AA23337 L-NS	UD			BCP PAST 5	UD
	AA23355S-L(1)	UD			BCP PAST 33	UD
	AA23334L-NS	UD			BCP PAST 24	UD
	AA23308(2)NS-C	UD			BCP PAST 27	UD
	AA23353NS-C(2)	UD			BCP PAST 1	UD
P. mamillatum	DAR 63997	UD				

Results

The information has been represented in Figure 4.1. As examples of the tests success samples 1, 2, 3 and 4 showed a positive detection by DNA test and visual symptoms. Soil samples 8, 9 and 10 had no detection of *A. euteiches* based on the test and visual symptoms. Others however showed some negative detection compared to visual symptoms for example soil 14; however this was a very low visual assessment score which occurred only in one rep out of four. It was confirmed as *A. euteiches*

by microscopic examination and may confirm that the soil sent for testing may not have contained *A. euteiches*.

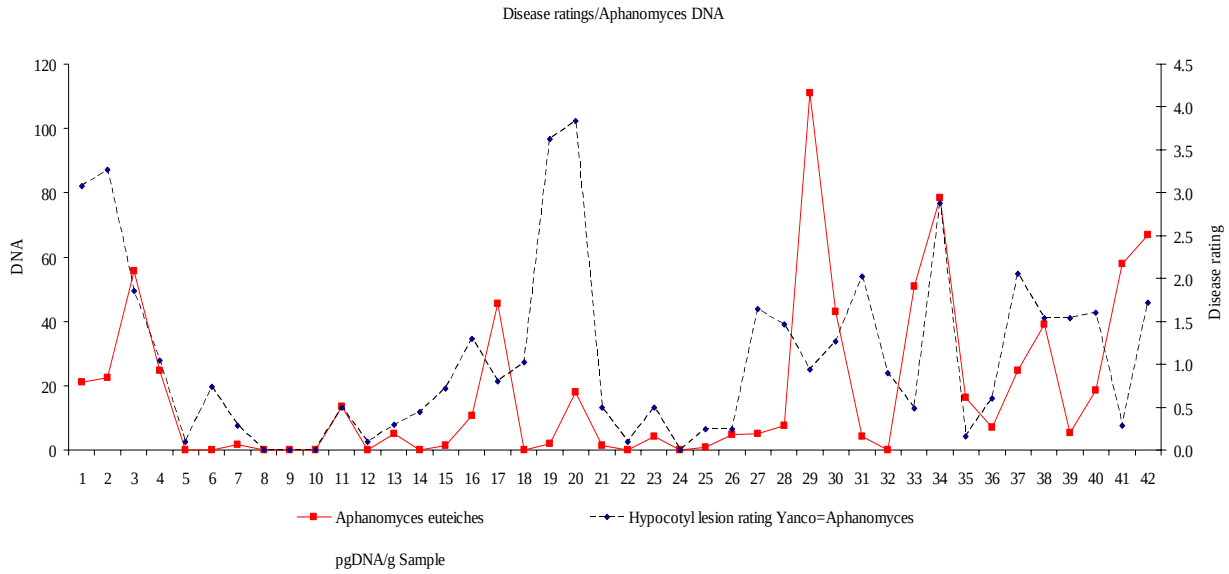

Figure 4.1. Comparison between *A. euteiches* DNA detection (primary y-axis, red line) and visual disease ratings attributed to *A. euteiches* (secondary y-axis, black line). Each of the soils is listed along the bottom x-axis. The value of the red line is not critical but greater than zero implies presence of the fungus in the soil.

Discussion

A further 50 samples of different origins were collected for *A. euteiches* DNA detection. Most of the samples were soils; however some infected bean roots were included. Soils kept for a long period register zero levels of *A. euteiches* DNA. Soils after they had been used for the bean bioassay i.e. had beans planted in pots and then sent for analysis have levels of 10-50 pgDNA/g (which is picograms of DNA per gram-Pico is 10^{-12}). Fresh roots recorded high levels of DNA in the 1000's 10-50 pgDNA/g show that the test is specific to the fungus. Soils collected from the field indicate levels of around 5 pgDNA/g.

The DNA test gives an indication of the presence of *A. euteiches* without the need for growing the plants in soil for a few weeks and then interpreting the disease symptoms. But results indicate the ability of this fungus to increase rapidly from low levels in the presence of beans and the right conditions.

Reference

Sauvage H, Moussart A, Bois F, Tivoli B, Barray S, Laval K (2007). Development of a molecular method to detect and quantify *Aphanomyces euteiches* in soil. FEMS Microbiology Letters **273**: 64-69.

5. BIOFUMIGATION TRIALS

Introduction

Biofumigation through the incorporation of brassica green manures has been promoted as a method to reduce soil borne diseases. The means of disease control in the case of Brassica green manures is the release of isothiocyanates, a fumigant type chemical, developed from the breakdown of glucosinolates. Rangi rape and many of the Brassica group have been identified as producers of isothiocyanates (ITC's) (Matthiessen and Kirkegaard 1994). An artificial form of ITC is metham sodium which is commonly used as a soil fumigant. Research has been undertaken on this method of disease control but results are mixed and may be different for different fungal pathogens (Larkin and Griffin 2007).

Glucosinolates are common in brassicas with the early "rapeseeds" having high levels and later "canola" varieties intentionally having lower levels for human consumption. Therefore varieties high in glucosinolates are becoming more difficult to find although there is some breeding for biofumigant use still occurring. Trials were conducted in greenhouses and in the field in a rotation block to examine any effect of green manures on ARR.

Method

Trial 1

As a preliminary trial, crops that may be beneficial as green manures and biofumigants were sown into large 300mm pots containing *A. euteiches* infected soil. The crops included onion (Brown Mercedes^{TM}), canola (standard variety for oil) and B.Q. Mulch (Bioqure Mulch^{TM}) (a type of brassica specifically developed for biofumigation). The pots were placed in a greenhouse at 20/25°C till flowering for the brassicas and till bulbing for the onions. The onion plants with the bulbs removed were then incorporated into the soil, and left for three weeks. In some pots the stems and roots were incorporated and in others just the roots. The two brassica mulches were also divided up, incorporated and left for a similar period. After this time soils were and removed placed into 100mm pots. Seeds of the bean variety Simba were then planted into the soil. Beans were also planted into soil that did not have any green manures incorporated. After germination, and a wetting up period symptoms were examined on plants in the pots three weeks after sowing. At the same time some snake bean (*Vigna unguiculata* ssp. *sesquipedalis* (Fabaceae) seeds were planted in infected soil to see if they displayed disease symptoms.

Disease symptoms were assessed using a 0-5 scale based on root and hypocotyl (Figure 1.1) colour and health. The 0 rating was no disease and the 5 rating was high disease. For most trials roots and hypocotyls were assessed separately, whereas with others often only the hypocotyl was assessed. The disease ratings were based on the symptoms shown in (Figure 1.2).

Trial 2

A trial was designed to examine the effect of onion plants on ARR when incorporated at specific rates into infected soil. Onion plants (Brown Mercedes™) were grown in potting mix and at bulbing, leaf and root material were cut up and added to soil as a fresh or dried plant material. There were 5, 10, and 15g of fresh plant material and 1g of dried roots and 3g and 5g of dried stem added and incorporated each into a 100mm pot of infected soil, with five replicates of each treatment. After four weeks beans of the variety Simba were sown into the pots (five per pot). After three weeks and a wetting up period, hypocotyls and roots were assessed for disease.

Results

Trial 1

The disease assessed on bean hypocotyls in the soil with the onions incorporated was significantly different to the other treatments as seen in Figure 5.1. The incorporated B.Q. Mulch™ and canola did not reduce disease levels. The lack of disease on snake beans is shown in Figure 5.2

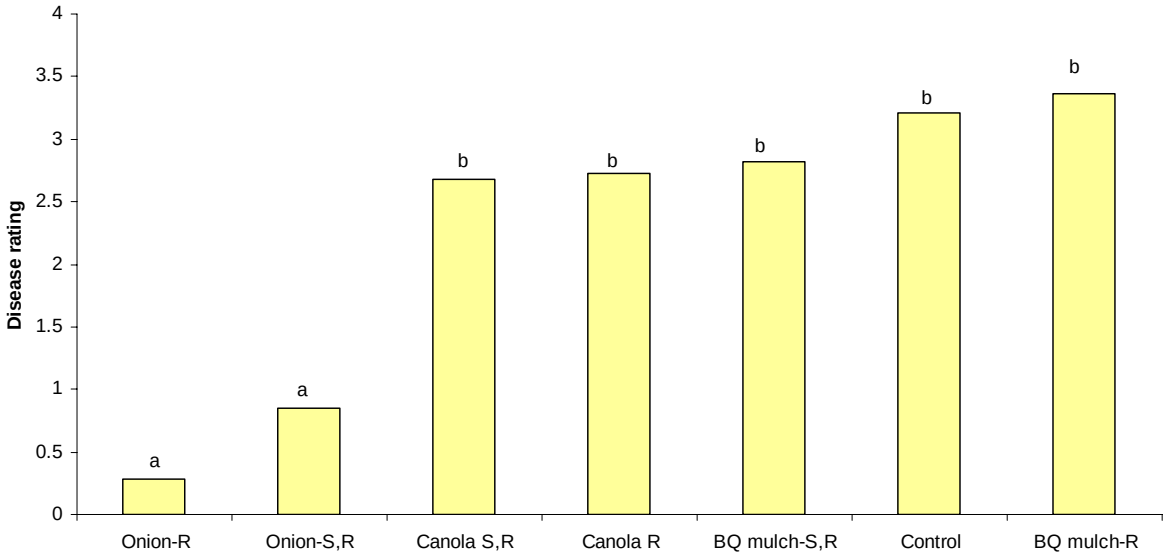

Figure 5.1. Comparison between various mulched crops, values significantly different between onions and others at P<0.001, LSD 5%=0.1442 values with the same letter not significantly different.
S=shoots incorporated, R=roots incorporated, SR=shoots and roots incorporated.

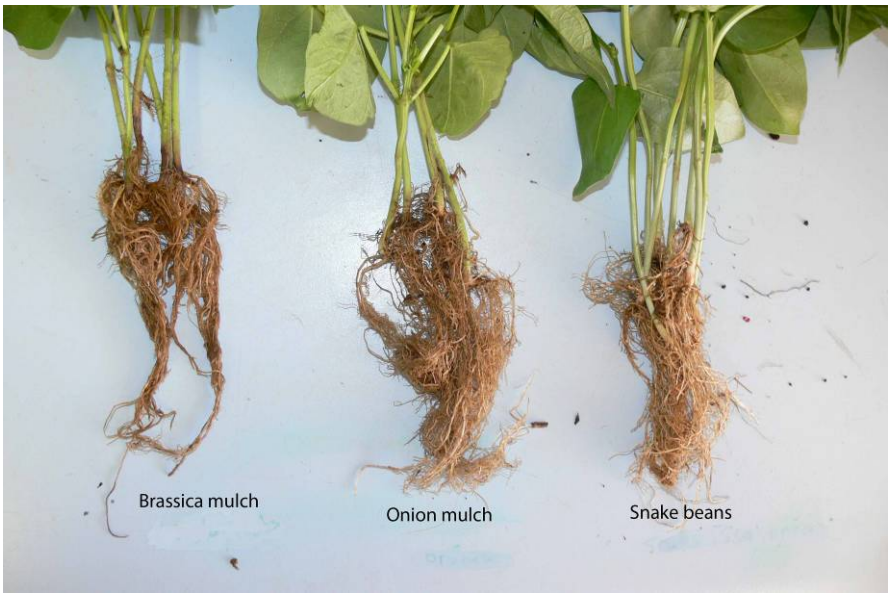

Figure 5.2. Image of disease levels of beans grown in *A. euteiches* infected soils that have had onion and B.Q. Mulch™ incorporated. Snake bean plants also grown in infected soil on the right showing zero disease.

Trial 2

The incorporation of onions in this trial had no affect on disease levels with all disease ratings similar for all treatments.

Discussion

Unfortunately the results of the first trial could not be reproduced in the second trial. This may have been due to the fact that the onions were not actually grown in the infected soil but placed into it after being grown in another pot i.e. potting mix. Another consideration when using these rotational crops is that the timing of incorporation may be critical. The lack of control from the BQ Mulch™ could be explained through this. Both the BQ Mulch™ and onion were included in a field trial reported later in the report.

An observation in the trials was that onion seedlings were found to be antagonistic to *A. euteiches* when both were placed next to each other on agar plates. This was determined to be the endemic presence of *Aspergillus niger* which is regularly associated with onions and found to be antagonistic to *A. euteiches* and also a bacteria that was isolated from the onion which also restricted growth of *A. euteiches* on agar plates.

6. ROTATION TRIAL

Introduction

This trial was established to examine the removal of some of a standard bean growing block away from the pasture/cattle/beans farming enterprise on a farm in the Valla area of NSW. It was also a block that was defined, more manageable, had a history of ARR and able to be studied more closely. A block was therefore taken out of that production system and used to sow alternative green manures in a field situation to reflect the greenhouse trials reported earlier. The block last had beans in 2007. The grower was involved in the decision making process for this trial and assisted with planting and block management. This involved a lot of work due to the rapid pasture growth and the need to maintain the block. Beans can be planted in the area from October to March and therefore rotation crops are planted in the winter/spring period to allow for breakdown and then for bean sowing. The green manure may have some biofumigant affect and thereby reducing root disease.

Method

An area of approximately 50m by 50m was separated from the rest of the block with an electric fence to exclude cattle. Pasture growth was kept to a minimum with the use of herbicides and cultivation. The block was quite sloped but typical of the local area, it was a dark soil that appears very healthy, lots of worms and very light once worked up.

2009/2010

In winter 2009 various green manure plots were planted including wheat, Rangi rape and B.Q. Mulch™ however heavy rain caused a lot of erosion and damage, mixing the soils around the plots and therefore abandoned. After the rain event a drain was placed above the trial block.

2010/2011

In winter/spring 2010 various crops were grown within the isolated block to examine any contribution they may have in reducing disease. The crops were sown in strips which were 30m long by 2m wide, but were not replicated. The crops included wheat, a canola variety known as Rangi rape which is high in glucosinolates, an indicator of a good biofumigant crop. Other strips trialled included Fulzyme® (a bacterial anti-fungal product) (label rate) and a “bed” that had been shaped i.e. lifted higher than the surrounding soil to improve drainage. Beans are currently sown on the flat which is a result of the planting equipment used and the inability to accommodate beds in the sowing process. A bedshaper was therefore used to form the bed and all plots were sown with an Earthway hand sower, two rows per bed.

Rice straw was spread over another bed. The wheat and Rangi rape were allowed to go to flower before being worked into the ground. After two months (February 2011) beans (variety Strike) were planted into the strips. At planting the Fulzyme® treatment was applied. After six weeks bean plants were assessed by scoring hypocotyl disease.

Disease symptoms were assessed using a 0-5 scale based on root and hypocotyl (Figure 1.1) colour and health. The 0 rating was no disease and the 5 rating was high disease. For most trials roots and hypocotyls were assessed separately, whereas with others often only the hypocotyl was assessed. The disease ratings were based on the symptoms shown in (Figure 1.2).

At this time, soils from the green manure plots were collected for greenhouse trials where moisture could be manipulated and therefore induce disease. Beans (five) of Simba variety were planted into pots (100mm) containing the specific soils. After germination, and one week after planting, the pots were put through the wetting up period. Three weeks after sowing, plants were removed to assess any affects of the treatments by rating visually the symptoms of the disease on the roots and lower stem or hypocotyl.

2011/2012

The block was worked up after beans in early 2011 and maintained for planting of the green manures. The same green manures were added to the beds. In a bed size gap (that did not have any crop) next to these beds the same green manure was planted. Therefore there were beds with two green manures in succession (i.e. separated by a bean crop) and a new bed with only one season of the green manure.

Therefore there was a bed with wheat-beans-wheat-beans and the block immediately adjacent was nil-nil-wheat-beans. This pattern was the same with the Rangi rape, but added for this season were onions, corn, three new brassica green manures that had become available were also included, Attack, Architect, and Doublet. Green matter was determined for the brassica rotational crops to determine how much was being incorporated back into the ground. Crops were incorporated in December, 2011 and beans planted in March, 2012. The beans were planted in five strips across the block so that there were strips with beans and strips without beans.

Plants were assessed one month after planting in April by assessing plants in each of the five planted strips in each rotation block. Soil was removed from each of the plots for disease assessments in a replicated trial in a greenhouse. Some soil was either taken within the bean strips and the strips without beans. Each soil was placed in 100mm pots with five replicates per treatment and after a wetting up period were assessed for disease symptoms two weeks after planting. A summary of the plot treatments is in Table 6.1.

Table 6.1 Treatments carried out on the 11 blocks over the three years in the field rotation trial.

Block	1	2	3	4	5	6	7	8	9	10	11
2010 (winter)	Wheat	Nil	Rangi rape	Nil	Nil	Nil	Rice straw	Nil	Nil	Nil	Nil
2011 (Feb.)	Bean	Nil	Bean	Nil	Bean	Bean	Bean	Bean	Nil	Bean (Fulzyme)	Bean
2011 (winter)	Wheat	Wheat	Rangi rape	Rangi rape	Onion	Nil	Architect Attack Doublet	Architect Attack Doublet	Nil	Onion/corn	Corn
2012	Beans	Beans	Beans	Beans	Beans	Beans	Beans	Beans	Beans	Beans	Beans

Results

2010/2011

There were not high disease scores from any of the treatments, with ratings of below two to two and a half. Disease rating scores of three or above are considered high. On assessing bean disease from the plants within the trial there were not large differences between those beans that had been grown on the different rotational crops, but there were some significantly different to the control including the incorporated rice straw and the Rangi Rape as shown in Figure 6.1. The beans planted on the bed did not show any less disease than the control plants.

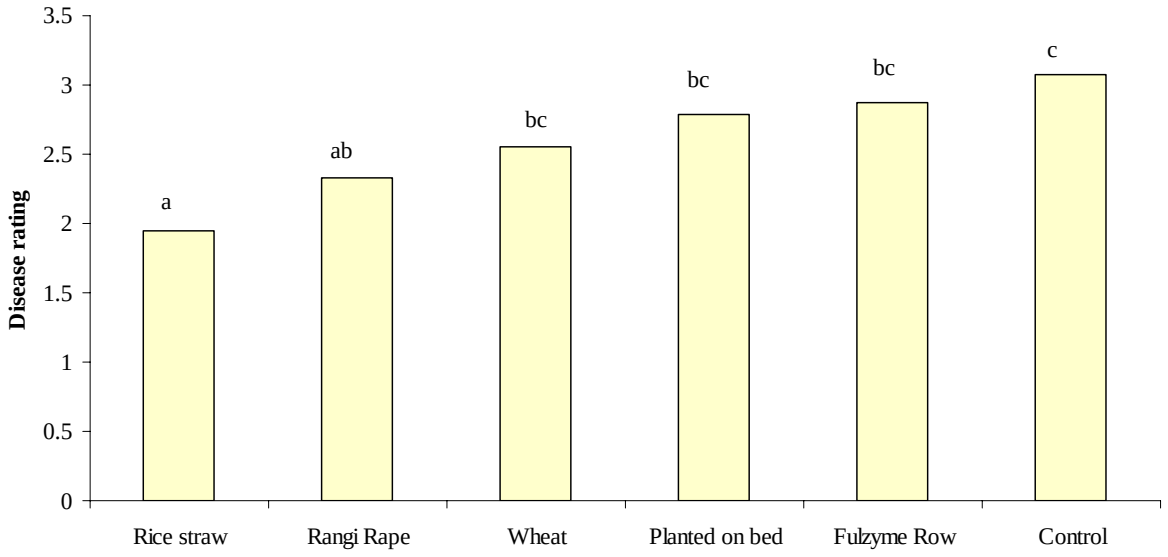

Figure 6.1. Level of disease based on a hypocotyl disease score in the field grown beans on the treatments applied to the rotation block (P<0.001 and LSD 5%= 0.6, values with the same letter not significantly different).

In the greenhouse trial, soil that came from the plots with rice straw and the Rangi rape had less root disease than soil that came from the wheat and the control soil (Figures 6.2 and 6.3). The data recorded from the potted soils was similar to the data recorded on beans in the field.

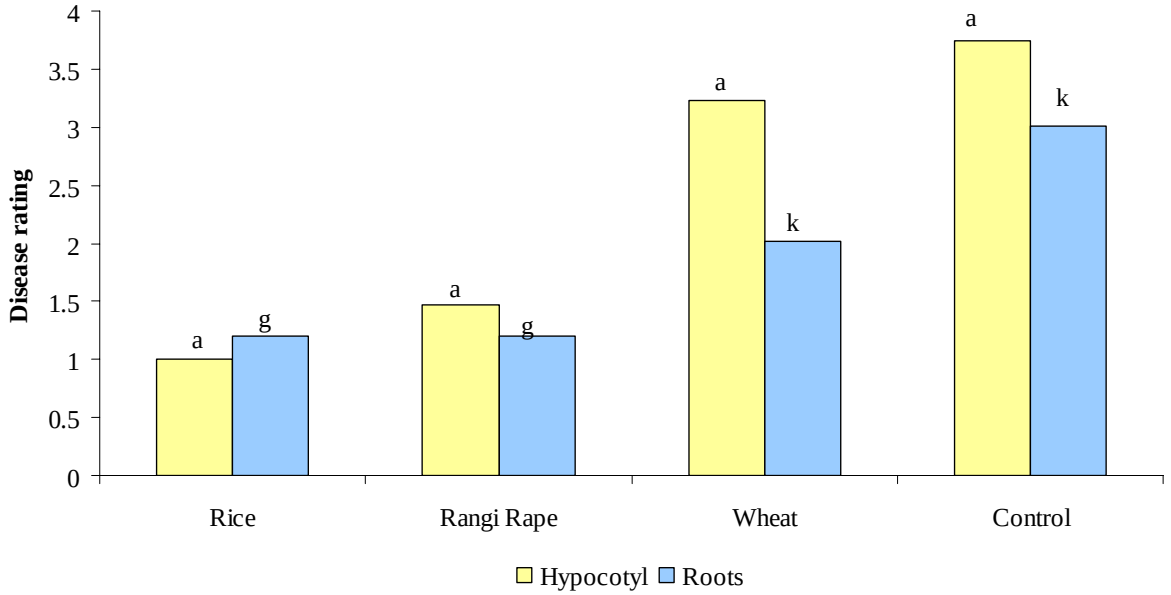

Figure 6.2. Hypocotyl and root disease ratings for ARR on beans grown in soil collected from the rotation field trial with various mulched crops based on a visual 0-5 assessment scale (P<0.001 and LSD 5%= 0.6, values with the same letter not significantly different).

Figure 6.3. Plants from the soil without the rotation crop on the left (brown lesions in the lower stem) and from the Rangi rape soil on the right (white lower stems) from the greenhouse trial.

2011/2012

In regards to the in-field assessments, disease was less on the plots that had a history of the incorporation of the brassicas and significantly better than the plot without treatment (Figure 6.4). Wheat had no effect. Disease levels were low, indicating that a wet period had not occurred on the block. It may have also been indicating that disease overall in this block was decreasing. Attack and Doublet had significantly less disease than the untreated control.

The green biomass added back to the soil was measured. Wheat was 766 g/m^2, the Rangi rape was 900 g/m^2, Attack 825 g/m^2, Doublet 3860 g/m^2, Architect 700 g/m^2, and corn 3416 g/m^2. The onion was only a very low density and not measured.

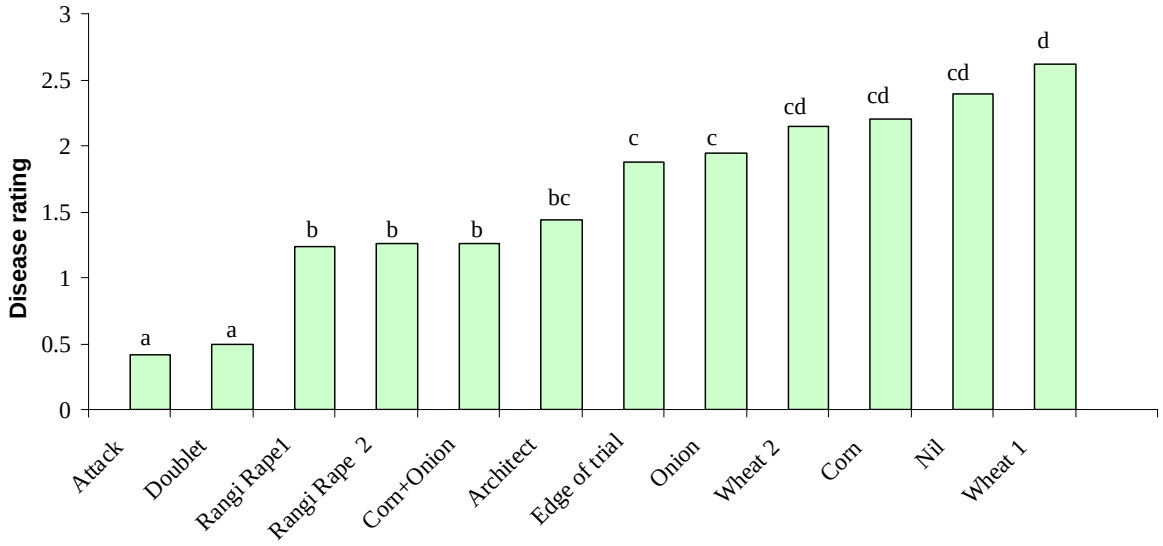

Figure 6.4. Disease rating score for the blocks that had received the various mulches (P<0.001 and LSD 5%= 0.6, values with the same letter not significantly different).

In the greenhouse trial using soils from the rotation block, the soil that came from the blocks that were currently growing beans had more disease than those that had no beans growing (Table 6.2). For example the rotation block that had no treatments (Nil) had more disease (rating of 2.9) where the soil was taken within that block that at the time of sampling had beans growing than where beans were not growing (0.08) and were significantly different (P<0.001) (Figure 6.5). The soil from the Rangi rape treatments either grown once or twice were the best of the samples from where beans were currently growing.

Overall the plots where the brassicas were incorporated had less disease than other plots.

Figure 6.5. Beans grown in the soil without any rotation crop but with beans in 2012 on the left and from the same plot without beans on the right.

Figure 6.6. Beans grown in soil taken from the onion plot with beans in 2012 on the left and without beans on the right.

Table 6.2 Mean disease ratings of the lower stem from beans grown in soils taken from the rotation trial ie greenhouse trial. NB is no beans.

Block Treatment	**Disease rating (hypocotyl)**[#]
Corn+Onion NB	0 a
Wheat * 2 NB	0 a
Attack/Doublet/Architect N.B	0.04 a
Nil NB	0.08 a
Rangi rape * 1 NB	0.38 ab
Onion NB	0.62 ab
Rangi rape * 2 NB	0.72 ab
Corn NB	0.96 bc
Edge of trial NB	0.96 bc
Rangi rape *2	1.58 cd
Rangi rape *1	1.6 cd
Wheat *2	1.72 de
Wheat *1 NB	1.88 def
Wheat *1	2.02 def
Corn	2.38 efg
Attack	2.52 fg
Architect	2.58 fg
Nil	2.92 gh
Doublet	3.12 gh
Corn+Onion	3.26 gh
Onion	3.3 h
P	**<0.001**
LSD 5%	**0.72**

[#]Values with the same letter not significantly different.

Discussion

The affect of ARR lessened over the trial period, according to growers' experience it may take ten years to go back to a block that has had beans after a serious disease epidemic. This block last had beans in 2007. In 2007 mean disease scores for this soil were 3.7, the reading for the block now (using

edge of the block) is 1 in the information from the 2012 greenhouse trial and 1.8 in the field assessment.

The disease builds up rapidly with higher disease scores in soil that was taken in the strips with the beans compared to soil in the strips without beans.

Brassicas appear to be a useful means of reducing disease as demonstrated in trials; in the field trial in 2012 the brassica plots had less root rot than other plots. The integration of these crops within the growing system will reduce disease; the mechanism for this could be biofumigation, or breaking the pasture cycle. A variety known as Caliente has been adopted by some growers for the process of biofumigation.

Further soil tests on this site are being undertaken through VG09038 “Vegetable soil health systems for overcoming limitations causing soil borne diseases”, but were not available at the time of writing.

7. LIME APPLICATION TO REDUCE APHANOMYCES ROOT ROT

Introduction

The addition of lime to soil reduces the acidity of the soil or increases the pH. Adjusting pH can reduce the effect of soil borne diseases such as clubroot of brassicas caused by *Plasmodiophora brassicae*, however this is also related to the effect of the calcium from the addition of lime (Myers *et al*, 1985). Recently it was demonstrated that the combination of lime to reduce pH with solarisation and flusulfamide was effective to control clubroot (Kowata-Dresch and May-De Mio, 2012). Although totally different organisms, there are similarities between club root and ARR, both are increased at higher soil moisture levels. An examination of the effect of lime applications to ARR infected soil was undertaken.

Method

Preliminary trials indicated on the amount of lime to add to the soil to increase the pH approximately one and two units. The two rates used were 11.4g per 1700g of air dried soil and 34.2 g per 1700g of air dried soil. The infected grower soil was divided into four 100mm pots with five Simba bean seeds per pot. Untreated soil was used as a control. Plants were maintained in a greenhouse at 20/27 °C and maintained and wet up to induce *A. euteiches* symptoms. Three weeks after planting disease was assessed and pH of the soil was measured in calcium chloride.

Disease symptoms were assessed using a 0-5 scale based on root and hypocotyl (Figure 1.1) colour and health. The 0 rating was no disease and the 5 rating was high disease. For most trials roots and hypocotyls were assessed separately, whereas with others often only the hypocotyl was assessed. The disease ratings were based on the symptoms shown in (Figure 1.2).

Results

There was no reduction in disease due to lime application with the untreated score of 2.9 and the low and high rate scores of 3.5 and 3.1 respectively. The pH of the soil without lime added was 5.0, the low lime rate was 5.8 and the higher lime rate was 6.4. The trial was repeated and similar results were achieved.

Discussion

In these trials lime application did not reduce disease levels. In the case of club root the pH required to decrease disease is around 7.2 and this pH increase was not achieved in the trial. There was however no indication that disease would be reduced by the addition of the lime with disease slightly higher in the soils with lime compared to the treatment without.

References

Kowata-Dresch LS, May-De Mio, LL (2012) Clubroot management of highly infected soils. *Crop Protection.* **35:**47-52

Myers DF, Campbell RN (1985). Lime and the control of clubroot of crucifers: effects of pH, calcium, magnesium, and their interactions. *Phytopathology* **75**: 670-673.

8. GREENHOUSE TRIALS TO CONTROL APHANOMYCES ROOT ROT

Introduction

Greenhouse trials were undertaken to examine the control of *A. euteiches* on beans by improving plant health through the use of silicon and improving plant resistance with Bion Plant Activator® (active ingredient Acibenzolar-S-Methyl 500 g/kg). Bion Plant Activator® is an inducer of host plant resistance by stimulating the natural systemic acquired resistance (SAR) response found in plants. The product does not have a direct activity against disease causing organisms. Silicon has been shown to improve the growth and disease resistance of some plants including rice (*Oryza sativa*) and other cereals, and a number of dicotyledons (Belanger *et al*. 1995, Savant *et al.* 1997, Savant *et al.* 1999). Silicon is normally applied as a foliar spray to reduce the effect of leaf diseases, but in these trials it was used as a soil drench.

The treatments used in the trials included seed dressings, soil drench and soil amendments. Products included Bion Plant Activator®, rice hulls, wheat straw and Potassium silicate (17% Silicon). The rice hulls (high in silica) were to represent an alternative silicon treatment. The products were selected because they have been recorded as having the potential to reduce the impact of soil borne diseases. These products can contribute to the control of soil borne diseases through increasing the resistance of the plant, improving soil drainage properties and possibly change the pH of the soil. These changes may directly affect the target disease organism or may also increase the antagonistic bacterial levels in the soil.

Trial 1

Bion Plant Activator® was used as a seed dressing and applied to the Simba seed. A Bion Plant Activator® soil drench was included on other pots as a separate treatment. The rice, wheat, and potassium silicate treatments were applied at two rates. The soil used on the pots was collected from northern NSW and known to be infected with *A. euteiches*. The Bion Plant Activator® was applied to seed and allowed to dry before planting, solutions of the potassium silicate were applied in 200ml of drench per pot, the wheat straw and rice hulls were dried, ground and applied to each pot. Five seeds were planted into each pot (100mm) containing the various treatments (five replicates of each treatment) and once plants were well germinated they were wet up (watered well three times a day) to induce disease symptoms. After three weeks hypocotyls and roots were rated for disease.

Disease symptoms were assessed using a 0-5 scale based on root and hypocotyl (Figure 1.1) colour and health. The 0 rating was no disease and the 5 rating was high disease. For most trials roots and hypocotyls were assessed separately, whereas with others often only the hypocotyl was assessed. The disease ratings were based on the symptoms shown in (Figure 1.2).

Trial 2

This trial was conducted to examine various rates of Bion Plant Activator® as a seed dressing and also to compare different rates of potassium silicate as a soil drench. Infected soil from a bean grower was again used. There were six rates of Bion Plant Activator® used as a seed dressing and three rates of potassium silicate. The method for planting and treatments were the same as for Trial 1. Symptoms were again assessed by rating hypocotyls and roots for disease. As the potassium silicate used was alkaline (pH 11), soil pH was measured for the three treatments and the untreated soil to rule out any pH effects.

Trial 3

In this trial molasses was the main treatment but honey was also used. The molasses was used to increase the biological activity of the soil organisms with the aim to increase bacterial antagonists. The solutions were made up in a litre of water with each pot receiving 150ml of the solution. Two types of molasses were used, one for human consumption and one that was less refined, darker and heavier called horse molasses. The same soil as the above trials was used.

Results

Trial 1

The potassium silicate treatments resulted in less disease than the control. The Bion® Plant Activator® soil drench treatment was successful at reducing disease levels but it had a negative effect on plant growth. Hypocotyl ratings are listed in Table 8.1.

Table 8.1. Disease rating of hypocotyls in Trial 1 which examined ARR control options.

Treatment	**Rate**	**Disease rating(hypocotyl)***
Potassium Silicate high	6%	0.31 a
Potassium Silicate low.	3%	0.91 ab
Bion Plant Activator® drench	0.06%	1.01 abc
Wheat high	6% w/w	1.21 abcd
Wheat low	3% w/w	1.67b cde
Rice hulls high	6% w/w	2.09 def
Control		2.42 ef
Rice hulls low	3% w/w	2.53 efg
Bion Plant Activator® seed dressing	1.2g /100kg seed	3.46 g
P		**<0.001**
LSD 5%		**0.96**

*Values with the same letter not significantly different.

Trial 2

Hypocotyls and roots were much cleaner on the plants treated with potassium silicate compared to those that had the seed treatment with Bion Plant Activator®. However the high rate of potassium silicate although had less disease did have some root distortion. The results for hypocotyl lesions are represented in Table 8.2 and images in Figure 8.1.

Table 8.2. Disease rating of hypocotyls in Trial 2 which examined ARR control options.

Treatment	**Rate**	**Disease rating (hypocotyl)***
Potassium silicate medium	9%	0.79 a
Potassium silicate low	6%	0.97 a
Potassium silicate high	12%	1.89 b
Bion Plant Activator® seed dressing rate 2	2.4g/100kg seed	2.86 c
Bion Plant Activator® seed dressing rate 3	3.6g/100kg seed	2.94 c
Bion Plant Activator® seed dressing rate 6	300g/100g seed	2.99 c
Bion Plant Activator® seed dressing rate 5	200g/100g seed	3.02 c
Control		3.37 cd
Bion Plant Activator® seed dressing rate 4	100g/100kg seed	3.62 cd
Bion Plant Activator® seed dressing rate 1	1.2g/100kg seed	3.98 d
P		**<0.001**
LSD 5%		**0.64**

*Values with the same letter not significantly different.

Figure 8.1. Bion Plant Activator® seed dressing rate 1 on the left showing brown lesions on the lower stem and white lower stems with Potassium silicate medium on the right. Roots also much whiter on the potassium silicate treated plants.

Trial 3

The higher rate of the horse molasses had significantly less disease than any of the other treatments as indicated in Table 8.3 and Figure 8.2.

Table 8.3. Hypocotyl ratings for Trial 3.

Treatment	**Rate**	**Disease rating (hypocotyl) ***
Molasses high	3% v/v	1.2 a
Honey	3% v/v	2.3 b
Refined molasses	3% v/v	3.0 c
Molasses low	1.5% v/v	3.3 c
Control		3.4 c
P		**<0.001**
LSD 5%		**0.6**

*Values with the same letter not significantly different.

Figure 8.2. Beans grown in the horse molasses treated soil on the left and the control pots on the right.

Discussion.

The potassium silicate improved bean health and may be a viable field control option. However the mechanism of disease control was not clearly identified. The pH of all the potassium silicate treatments had a mean pH of 6.1, (there was no difference in the various rates) a rise of less than one unit when compared to the untreated soil (5.3). Other trials confirmed that increasing pH with the use of lime did not reduce disease levels and therefore a fungicidal effect of potassium silicate cannot be ruled out. This has been confirmed in laboratory trials with various fungi (Hay *et al* 2009). Molasses at one rate selected reduced ARR. Under the conditions in these trials various rates of Bion Plant Activator® used as a seed dressing did not reduce disease symptoms.

References

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Savant NK, Snyder GH, and Datnoff LE (1997). Silicon management and sustainable rice production. 151-199. In: D.L. Sparks (Ed.) Adv. Agron. 58. Academic Press, San Diego, CA, USA.

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9. USING ANTAGONISTIC BACTERIA TO CONTROL APHANOMYCES

Introduction

Biological control of soil borne organisms has been investigated in many crops. There are a number of antagonistic fungi and bacteria commercially available including species of *Trichoderma* and *Bacillus* that target various pathogens. A bacterium, *Pseudomonas cepacia* strain AMMD was recognised in the United States as giving some biological control against *Pythium* and *A. euteiches.*

During routine fungal isolations a bacteria showed potential as a biocontrol for *A. euteiches*. It restricted growth of the fungus on media in petri dishes (Figure 9.1) When placed side by side the fungus would not grow near the bacteria colony compared to plates without the bacteria.

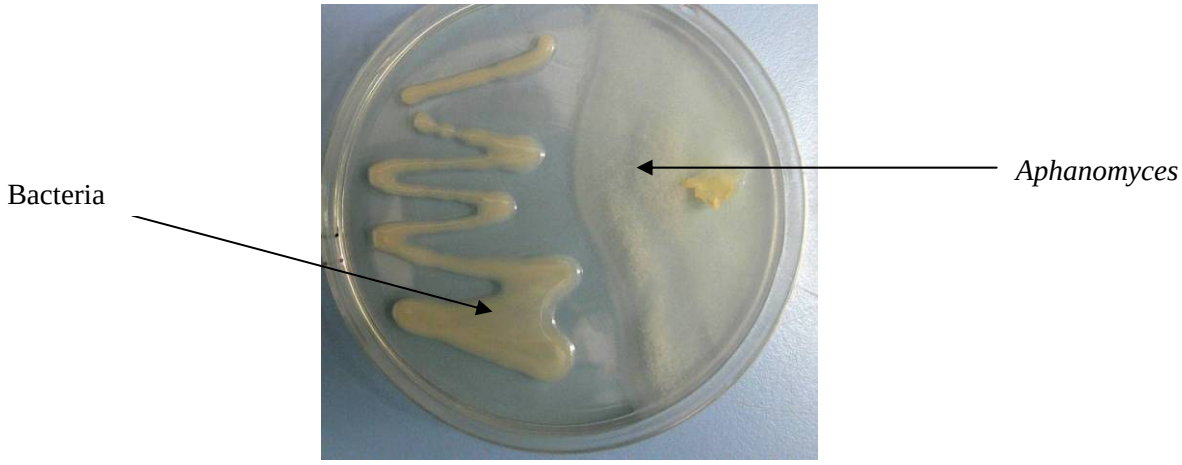

Figure 9.1. Showing restricted growth of the *A. euteiches* colony by the bacteria.

The bacterium was identified as *Burkholderia* (formerly *Pseudomonas*) *cenocepacia.* It is a part of the *B. cepacia* complex known to contain strains that are effective against a wide variety of fungi in agriculture. Some are patented in the USA however this group of bacteria has also shown to cause respiratory problems in patients with cystic fibrosis so its use as a potential biocontrol has not been pursued.

Small trials to assess the potential of assisting in the control of *A. euteiches* were carried out where bean seeds were either dipped in bacteria or bacteria/sterile water solution and then added to pots of infected soil. However no reduction in symptoms was observed as compared to beans without bacteria. Beans that had been grown in sterile vermiculite and removed after germination were transplanted to infected soil. Before transplanting, the roots of the plants were dipped in a solution of bacteria and sterile water and then transplanted. These however also developed symptoms. Some other trials were

conducted to examine whether this bacteria could be used in other ways to reduce ARR. Trials were also conducted with commercially available products for controlling diseases.

Method

Trial 1

An alternative method was adopted where the bacterium mentioned above was grown in nutrient broth to increase the concentration of the bacteria and the nutrient broth/ bacteria mixture applied to pots. Oxoid™ nutrient broth (a medium for the production of bacteria) was used (consisting of 'Lab-Lemco' powder 1g/l, Yeast extract 2g/l, Peptone 5 g/l and sodium chloride 5g/l). Nutrient broth medium was prepared and 250ml was added to six flasks and then inoculated with the bacteria (half a 90mm petri dish streaked with bacteria) after which the flasks were shaken and maintained at 30ºC in a waterbath for 48hrs. Nutrient broth without added bacteria was also made up to add to control pots. 150ml of the solution was added to each pot containing sterile vermiculite that had a layer of known *A. euteiches* infected grower soil on top into which five simba variety bean seeds were planted. The soil was then covered further with sterile vermiculite and the pots placed in a greenhouse at 17/25º C, there were five replicates of each treatment.

After six weeks, plants were removed and assessed for ARR symptoms. Disease symptoms were assessed using a 0-5 scale based on root and hypocotyl (Figure 1.1) colour and health. The 0 rating was no disease and the 5 rating was high disease. For most trials roots and hypocotyls were assessed separately, whereas with others often only the hypocotyl was assessed. The disease ratings were based on the symptoms shown in (Figure 1.2).

Trial 2

Fulzyme® is a product that contains a bacterial antagonist *Bacillus subtilis*, Superzyme® is a similar product also containing *Bacillus subtilis*, but also containing two fungal antagonistic species of *Trichoderma*.

Soil from a bean farm known to contain *A. euteiches* and observed to cause disease previously was used for the trial. Soil in 100mm pots was drenched with solutions of Fulzyme® and Superzyme® at the recommended rates i.e. and Superzyme® were applied at 50 ml of the recommended concentration (3ml/litre of Fulzyme® and 3g/litre of Superzyme®) of mixture to each pot. There were also pots without treatments indicated as "Usher" (the source of the soil) as controls. There were five replicates of each treatment. Pots were maintained in a greenhouse at 17/25º C.

After each treatment was applied, seven seeds were sown of Simba variety of green bean that had been previously treated with metalaxyl and fludioxonil, into each pot. Two weeks after sowing, a second drench of Fulzyme® and Superzyme® was applied.

Pots were observed until three weeks after sowing where the plants were removed from pots, roots and hypocotyl examined, rated for disease and subsequently dried for weighing. Plant number per pot was recorded. The roots and hypocotyl were rated using a 0-5 scale where 0 is no disease and 5 is a very brown or dead plant.

Trial 3

Trial 2 was repeated but included different rates of both Fulzyme® and Superzyme®. There were three rates of Fulzyme® 1ml, 3ml and 5ml per litre, however only 25ml of the mixture was added to each pot. Superzyme® did not show any effect previously but as to confirm this result it was used again at 1g, 3g and 5g per litre and again 25ml per pot. The products were applied to infected soil after bean seed variety Simba was planted, five seeds per pot with five replications. After 3 weeks plants were assessed for root rot, plant number and dry weight.

Results

Trial 1

The bacteria reduced disease levels in both hypocotyls and roots Figures 9.2 and 9.3. The bacteria improved growth of roots with disease affecting the roots in the control plants; however some symptoms developed on the lower stems of the bacteria treated plants. The infected soil was placed at this level but the control plants showed symptoms on all the roots. *A. euteiches* through producing motile zoospores moves readily through pots.

To investigate the possibility of reducing ARR with other biological control options, a commercial form of bacteria and bacteria/fungal mix were used in the next trial.

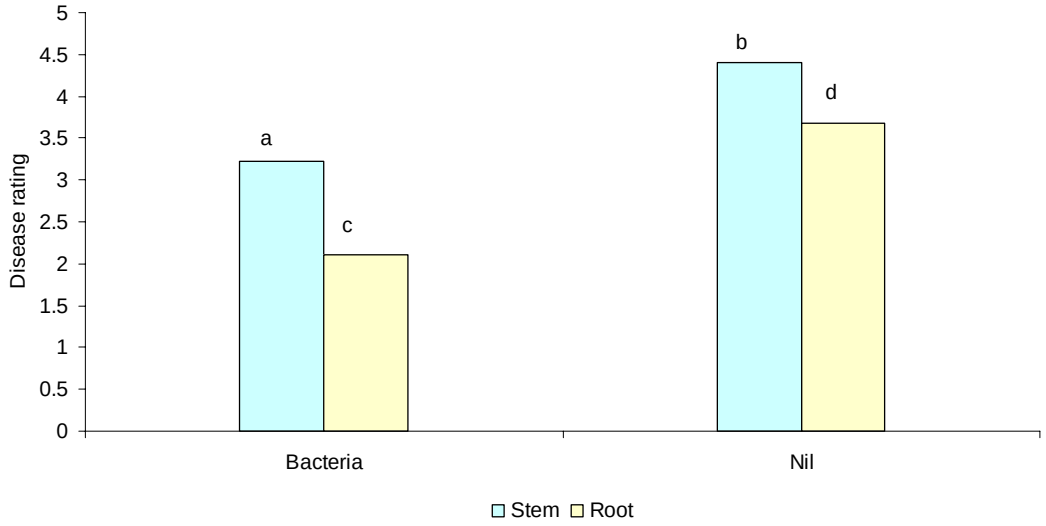

Figure 9.2. The plants that grew in the bacteria medium were not as affected as the control pots without bacteria (Hypocotyls P=0.001, LSD 5%=0.68, Roots P<0.001, LSD 5%=0.48).Values with the same letter not significantly different.

Figure 9.3. Plants grown with the bacteria medium on the left and without the bacteria medium on the right.

Trial 2

Plant number (per pot) was significantly better in pots that were treated with Fulzyme® (7) and Superzyme® (6.6) compared to a mean of three plants per pot in the untreated controls (Figure 9.4). Hypocotyl (lower stem) lesions were similar across all the treatments; however both Fulzyme® treatments were significantly less than the Usher treatment (Figure 9.4). In respect to root rot ratings, the Fulzyme® treatments were significantly better than the Usher treatment (Figure 9.4). The mean dry plant weight was also significantly different (better) between Fulzyme® and the Usher treatment (Figure 9.5). Photographs of plants from the three treatments have been included (Figures 9.6, 9.7 and 9.8).

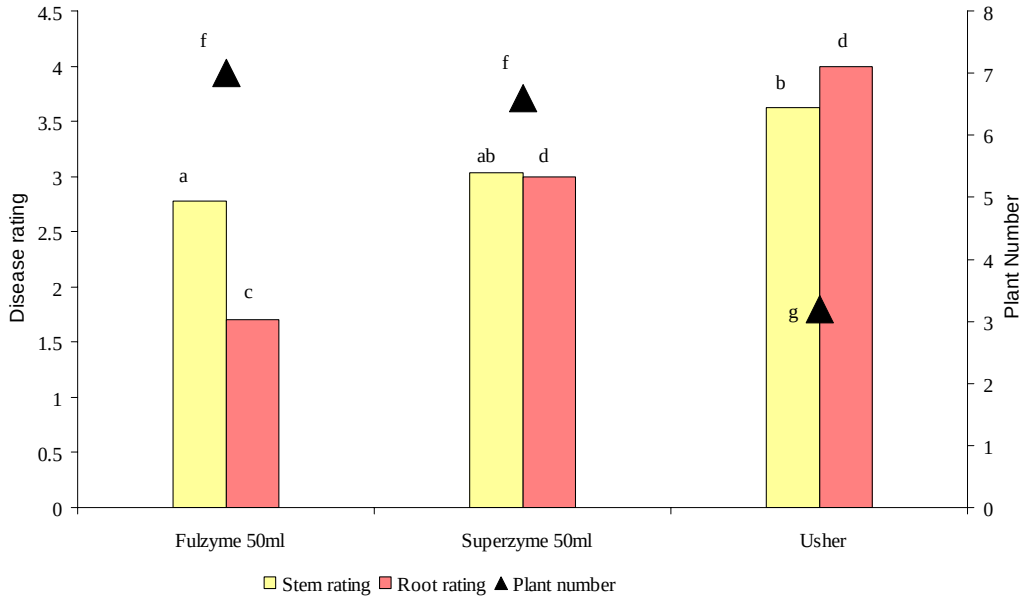

Figure 9.4. Hypocotyl, root ratings, and plant number surviving in each pot for the bacterial treatments (Hypocotyls P=0.001, LSD 5%=0.77, Roots P<0.001, LSD 5%=0.4). Values with the same letter not significantly different.

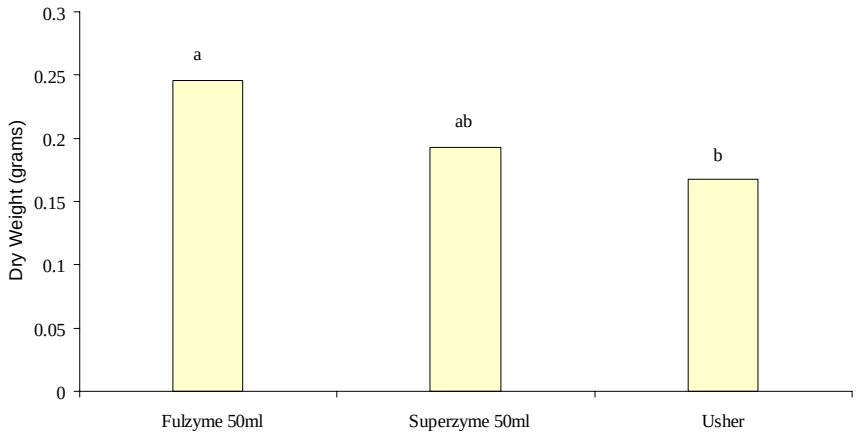

Figure 9.5. Dry weights of the treatments indicating that the Fulzyme® treatment was significantly better. (P<0.001, LSD 5%= 0.063). Values with the same letter not significantly different.

Figure 9.6. The Fulzyme® treatment showing increased root growth.

Figure 9.7. The Superzyme® treatment also showing improved root growth compared to the untreated control pots.

Figure 9.8. Usher (control) pots showing reduced root growth.

Trial 3

Disease was high across all treatments; there was less disease as per the root assessment in the Fulzyme® 5ml rate but its control was not as effective as in the earlier trial. Reducing the volume of water from 50 ml to 25 ml with the application may have reduced efficacy. There was no significant difference in plant dry weight or plant number.

Discussion

The Fulzyme®, and to a lesser extent the Superzyme® treatment, had more established plants, less disease, better root systems and higher dry weights. The Fulzyme® was superior to the Superzyme® indicating that the higher concentration of *Bacillus* in the Fulzyme® was more effective than the Superzyme® with a lower concentration of *Bacillus*. There appeared to no beneficial effect of the *Trichoderma* species; however this can be expected as they are meant to be preventative in their action rather that curative. In this case the Superzyme® may have performed better if the sowing was delayed after a reasonable period of application of the Superzyme®.

Bacteria that restrict the growth of *A. euteiches* have been identified through routine isolation. However developing these to a stage where they can be in a formulation acceptable for use in disease control is difficult. Fulzyme® already developed for this purpose is valuable.

The result in pots will however need to be evaluated in the field. In addition, the rates of Fulzyme may need to be checked to examine the quantity to be applied and also some method of application in the field.

References

King EB, Parke JL(1993) Biocontrol of Aphanomyces root rot and *Pythium* damping-off by *Pseudomonas cepacia* AMMD on four pea cultivars. *Plant Disease* **77:** 1185-1188.

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10. FIELD TRIALS TO CONTROL APHANOMYCES ROOT ROT

Introduction.

Various trials were conducted in the field based on results from greenhouse trials and to further evaluate findings of VG03002, which also examined different treatment options for ARR control. Treatments included potassium silicate, antagonistic bacteria and soil drenches with Amistar®. Field trials can be difficult to manage on growers' properties, sites chosen for trials may have variability in soil borne disease levels or the conditions to induce disease may not occur for example heavy rainfall for ARR. Sites were chosen as best possible to indicate disease, soil samples were collected for a disease bioassay before the trials were established to indicate the presence of *A. euteiches.* All the field trials were conducted on growers' properties in the Valla/Nambucca Heads area of NSW. This area has had a long history of bean growing and ARR.

Method

Trial 1

Greenhouse trials indicated that potassium silicate and molasses reduced disease in pots and therefore these treatments were to be trialled in the field. A block was selected for the trial at Valla in NSW. The farm was a newly purchased property and preliminary pot trials identified that *A. euteiches* was present in the soil.

The treatments included three rates of molasses (the horse molasses), two rates of Potassium silicate (15% potassium, 17% silicon) and two rates of Amistar® (500g/litre azoxystrobin), rates as per Table 10.1. The treatments were applied as a soil drench after Simba bean seed was sown by the grower. There were four replicates in a split block design and 2.5 litres of each solution was applied to each plot (one row by five metres) as a 200mm wide band across the row. One month after sowing 12 plants were removed from the plots (total approximately 18 plants per metre) and assessed for disease symptoms and dry weight recorded. Disease symptoms were assessed visually by rating the lower stem and tap root.

Disease symptoms were assessed using a 0-5 scale based on root and hypocotyl (Figure 1.1) colour and health. The 0 rating was no disease and the 5 rating was high disease. For most trials roots and hypocotyls were assessed separately, whereas with others often only the hypocotyl was assessed. The disease ratings were based on the symptoms shown in (Figure 1.2).

Table 10.1. Rates of the soil drenches used in field trial 1.

Drench Treatment	Solution %
Amistar® L	0.1 v/v*
Amistar® H	0.2 v/v
Molasses L	1 v/v
Molasses M	2 v/v
Molasses H	4 v/v
Potassium silicate L	6 v/v
Potassium silicate H	9 v/v

* Amistar® used in these trials was the 500g/litre azoxystrobin EC which was later replaced with Amistar SC® granule 250 g/L axoxystrobin

Trial 2

This trial included rates of Amistar® that were half and quarter the rate of the lowest rate (0.05 and 0.025%) used in the previous trial. The untreated plot was Simba and three other varieties including Bangalow, Rex and Pike. The trial was set up the same as Trial 1 with four replicates in a split block design, however the other varieties were sown by Earthway Precision Garden seeder (hand seeder). Four weeks after sowing, plant number in the five metre plots were counted, 12 plants were assessed for disease as previously described, and dried and weighed.

Trial 3

This trial was on another farm which is known to have *A. euteiches* from previous studies. The trial was established to evaluate field control options with molasses and potassium silicate. Amistar® was again included at two rates plus one applied as a foliar spray instead of application as a drench which was the method used for all the other treatments. Previcur® (propamocarb-600g/L) which has shown some control of *A. euteiches* previously was included in the trial (Table 10.2). Application was the same as in Trial 1. One month after planting (by hand seeder) and the application of treatments at planting, two metres of each row was removed and assessed for disease and dry weight. Each plot was one row by five metres with four replicates in a split block design.

There was no charcoal rot in this trial but the main infection was due to *A. euteiches*. There were not large differences however again Amistar® showed some efficacy against ARR.

Table 10.2 Control options used in Trial 3.

Treatment	% Solution
Nil	
Molasses L	1 v/v
Molasses H	2 v/v
Potassium Silicate L	6 v/v
Potassium Silicate H	9 v/v
Previcur® L	0.05 v/v
Previcur® H	0.15 v/v
Amistar ® L	0.1 w/v
Amistar® H	0.2 w/v
Amistar® Spray	1 w/v

Trial 4

A field trial was established in March as wet weather had hampered many earlier attempts to sow the trial. A site with a history of high disease incidence was available for the trial. Plants that had just germinated were used in the trial. Preliminary pot trials had shown *A. euteiches* was present in the soil but whether this would be shown in the field was not certain. At this stage the paddock already had a bean crop which had failed due to wet weather and the success of this next crop was not guaranteed due to the knowledge that *A. euteiches* was in the soil. The aim of this trial was to follow up on some success that was shown by Fulzyme® in earlier greenhouse studies.

Treatments were applied as soil drenches and included two rates of Superzyme® and Fulzyme® and one Amistar® treatment. Superzyme® is mainly a *Trichoderma* product whereas Fulzyme® is a *Bacillus* based product. The bean plants had just emerged out of the ground when each of the treatments were applied to 5 m of row and at the rate of 0.5 litres per metre in a strip of 200 mm wide along the bean plants. There were four replicates in a split block design. After two weeks plants were examined for disease symptoms and assessed for disease.

Trial 5

This trial was set up immediately after the trial above was completed and the same treatments applied in the same positions to examine if applying the products at sowing rather than on already established plants improved product efficacy. The soil drenches were applied over the top of those applied earlier. In this case however Pike variety beans were sown (with a hand seeder) and then the treatments applied. 5 m plots were used again and the same replicates (four) in a split block design. Four weeks after the trial was established, disease assessments were carried out.

Trial 6

Immediately adjacent to the trial above and at the same time a modified version of the same trial was established. The treatments were Fulzyme® (5ml/litre), Amistar®, Fulzyme® + Amistar® mixed together, Previcur® (active ingredient propamocarb), a fungicide with potential to control both *Pythium* and *A. euteiches* and Potassium silicate (shown in greenhouse trials to reduce root disease levels) and a control untreated plot i.e. water only. Plots were 5m with four replicates in a split block design with the treatments again applied as soil drenches after sowing Pike variety beans with a hand seeder.

Trial 7

After the trial above was completed, the block was cultivated for two new trials. The trial was a mixture of seed dressings and soil drenches (Table 10.3). The seed dressings included one that contained azoxystrobin, fludioxonil and metalaxyl-M (AFM) (two rates), another that contained difenoconazole and metalaxyl-M (DM), and hymexazol. The drenches included Amistar® (in these trials the Amistar SC 250 g/L a.i. was used at two rates), Previcur® (two rates), Fulzyme® (two rates), Amistar® + Fulzyme® and an untreated control. Plots again were five metres long with four replicates in a split block design. The bean variety Pike was used. The seed dressings were applied over already dressed seed (Captan®). Previous trials had shown that when Captan® is used as a seed dressing, it did not control ARR. Some of the seed with the dressings were also used in greenhouse trials. Seed was sown with a hand seeder. Disease was assessed three weeks after the trial was established.

Table 10.3 Products used in Trial 7 to control ARR, SD=seed dressing, D=drench

Treatment	**T/ment type**	**Active Ingredient**	**Concentration of ai**	**Product rate/100g seed or solution as drench.**
Hymexazol	SD	hymexazol	700g/kg	0.5kg
Amistar® + Fulzyme®	D	azoxystrobin +*Bacillus subtillus*	250g/L $2x10^{10}$ /gram	0.5% and 2.5% w/v
Amistar® L	D	azoxystrobin	250g/L	0.5% w/v
Amistar® H	D	azoxystrobin	250g/L	1% w/v
Previcur® L	D	propamocarb	600g/L	0.15% v/v
DM	SD	difenoconazole+metalaxyl-M	92g/L+23g/L	130ml
AFM L	SD	azoxystrobin+fludioxinil+ metalaxyl-M	75g/L+12.5g/L+37.5 g/L	100ml
Fulzyme® L	D	*Bacillus subtillus*	$2x10^{10}$ /gram	2.5% v/v
AFM H	SD	azoxystrobin+fludioxinil+ metalaxyl-M	75g/L+12.5g/L+37.5 g/L	150ml
Previcur® H	D	propamocarb	600g/L	0.2% v/v
Fulzyme® H	D	*Bacillus subtillus*	$2x10^{10}$ /gram	5% v/v

Trial 8

This trial was both variety trial and agronomic practice trial. It was adjacent to trial 7 so background disease levels were the same. The varieties were Simba, Rex, Bangalow and Pike. Simba is a standard variety used locally for mechanical harvesting. Rex is a newer released variety and a replacement for Simba in that role. Bangalow also is a new variety but not favoured in some regions. *A. euteiches* needs wet conditions for infection so the two other treatments consisted of examining options to improve drainage and reduce compaction. One treatment consisted of deep ripping (to 200mm) in the sowing line before sowing which was deeper than the standard rotary hoe grower practice. The other treatment was a raised bed, where the plant row was raised to 150mm above the surrounding soil.

Raised beds are considered more suitable on flatter sites and may not be as appropriate in the hilly terrain where this trial was situated but was included to examine any advantage by improving runoff. There were four replicates in a split block design, plots were three metres long and the plots were sown with a hand seeder.
After three weeks plants were assessed.

Trial 9

A field trial was established to examine nutrient effects on ARR disease levels on beans. Boron and calcium are commonly promoted as improving plant health and therefore were included as treatments. The trial site was used in previous trials and had a known disease level. Plots were five metres long with one row per plot with four replicates in a split block design. Treatments were randomly distributed in each replicate. A treatment where beds were built up higher than the surrounding soil was also included. It had been suggested that fertiliser application too close to the seed would increase disease levels, therefore fertiliser was applied either with the seed or scattered on top.

There were 14 treatments in total which are included in Table 10.4. Disease levels were assessed four weeks after the trial was established. Early observations and disease assessment indicate that the fertiliser and the hilling treatments had less disease.

Table 10.4. Treatments conducted in field trial 9 to examine their effect on ARR.

Treatment	**Treatment**
Control	Nil
Foliar mix Rate 1 Consisting of Cobalt 6g, Selenium 2.5g, Copper 25g, Zinc 25g, Molybdenum 2g, Boron 10g per litre.	6.25 L /ha
Foliar mix Rate 2	12.5 L/ha
Foliar mix Rate 3	25 L/ha
Liquid calcium Rate 1.	4.5 L/ha
Liquid calcium Rate 2.	9 L/ha
Liquid calcium Rate 3.	18 L/ha
Foliar mix plus calcium	12.5 L/ha +9L /ha
Fulzyme 1 application	0.5 % v/v
Fulzyme 2 applications	0.5% * 2 v/v
Grower fertiliser incorporated with beans	25g/m
Grower fertiliser spread on the surface (allowed to water in)	25g/m
Amistar	0.2 % v/v
Beans planted on raised beds.	

Results

Trial 1

There was a high incidence of disease recorded in this block however the symptoms were not ARR but identified as Charcoal rot (also called Ashy stem blight) caused by *Macrophomina phaseolina*. The conditions had been hot and dry which is favoured by charcoal rot and not ARR. The Amistar® treatments reduced disease significantly compared to the untreated controls and other treatments (Table 10.5 and Figures 10.1 and 10.2). Plant dry weight was also significantly higher in the Amistar® treated plots.

As the Amistar® improved bean plant health, a follow up trial on the same block was established to examine lower rates of Amistar® and to observe the reaction of some new varieties.

Table 10.5. The effect of treatments including Amistar®, molasses and potassium silicate on bean health.

Treatment	**Disease rating (hypocotyl)***	**Treatment**	**Disease rating (tap root)***
Amistar® H	0.00 a	Amistar® H	0.17 a
Amistar® L	0.02 b	Amistar® L	0.54 b
Molasses M	0.58 b	Potassium silicate H	1.63 b
Potassium silicate L	0.64 b	Potassium silicate L	1.76 b
Molasses L	0.67 b	Molasses H	2.06 b
Potassium silicate H	0.69 b	Molasses M	2.33 bc
Nil	0.73 b	Nil	2.71 c
Molasses H	1.00 c	Molasses L	2.81 c
LSD 5 %	**0.23**		**0.61**
P	**<0.001**		**<0.001**

*Values with the same letter are not significantly different.

Figure 10.1. Plants treated with Amistar® (left) with clean white lower stems and untreated (right).

Trial 2

Charcoal rot was the main disease with the Amistar® treatments demonstrating significantly better disease control compared to the untreated Simba (Table 10.6). Amistar® also increased plant number and dry weight. The varieties Bangalow and Pike showed less disease symptoms as compared to Simba. This trial clearly indicated that varietal differences occur due to susceptibility to charcoal rot. Bangalow was a larger stronger bean compared to Simba and showed much less disease symptoms than the Simba variety commonly planted by the grower.

Simba drenched with Amistar® also improved the dry weight and plant number compared to the untreated Simba (Figure 10.3). There were varietal differences in dry weight and plant number but these reflected the habit of each variety and its germination ability, but also may have indicated the effect on plant survival when comparing with disease scores.

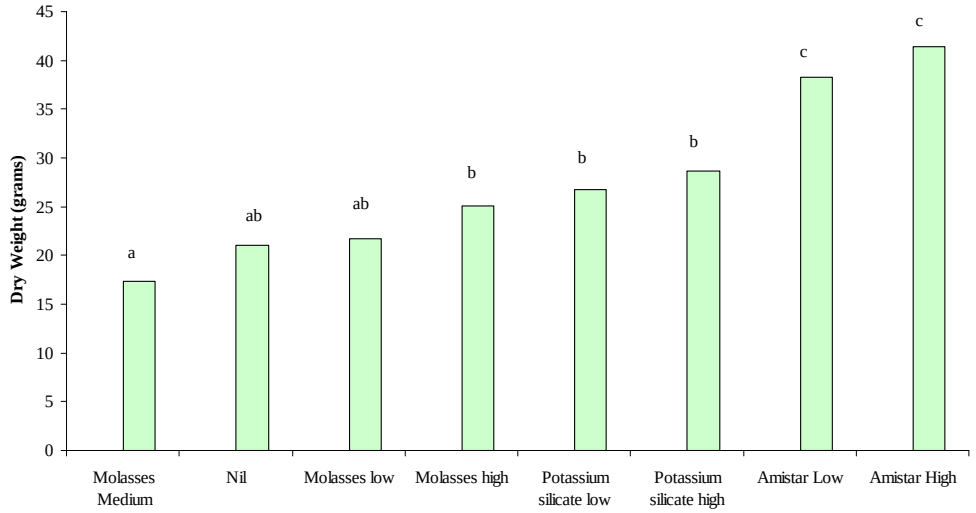

Figure 10.2. Dry weights of plants from Trial 1 indicating significantly higher dry weights of plants that had been grown in soil treated with Amistar®. Therefore charcoal rot was well controlled by the drench treatment. Values with the same letter not significantly different (P<0.001, LSD 5%=7.9).

Table 10.6. The Amistar® treatments and some varieties showed reduce disease incidence caused by charcoal rot in Trial 2.

Variety/treatment	**Disease rating (hypocotyl)***	**Variety/treatment**	**Disease rating (tap root)***
Bangalow	0.33 a	Pike	0.63 a
Simba +Amistar H	0.35 a	Bangalow	0.73 ab
Pike	0.49 ab	Simba +Amistar L	0.80 ab
Simba +Amistar L	0.97 b	Simba + Amistar H	1.13 b
Rex	1.53 c	Rex	2.33 c
Simba	1.83 c	Simba	2.59 c
P	**<0.001**		**<0.001**
LSD 5 %	**0.53**		**0.48**

*Values with the same letter not significantly different.

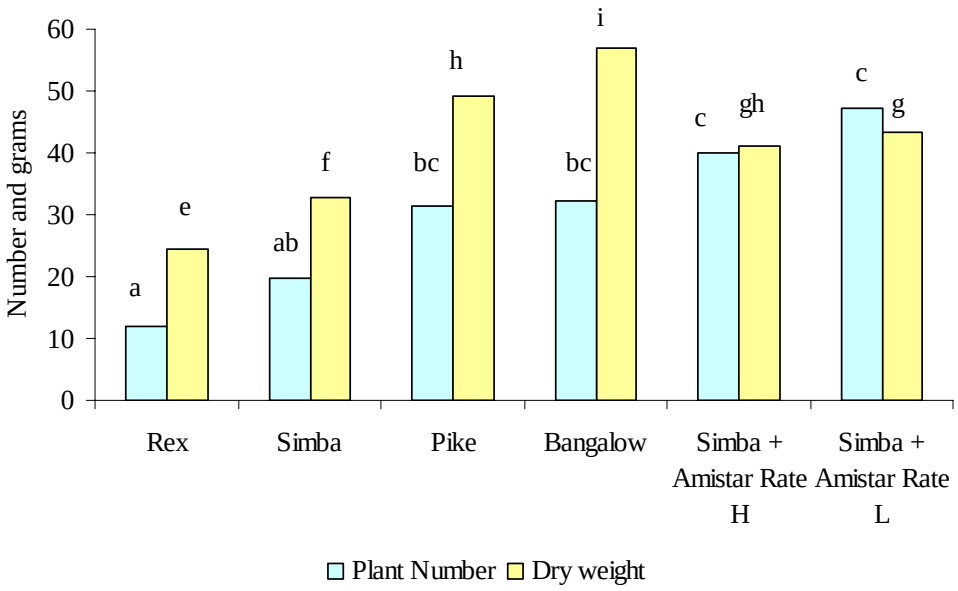

Figure 10.3. Plant number and dry weight was significantly improved by the Amistar® drench treatment in Trial 2 when compared to the untreated Simba. Values with the same letter not significantly different (Plant Number P=0.009, LSD 5% =17.9, Dry weight P=0.01, LSD 5%=16.4).

Trial 3

The main disease in this trial was ARR. Disease levels (Figure 10.4) decreased when using Amistar® as a soil drench. The overall disease level in this trial was very uniform but was not extremely high. Bangalow also exhibited less disease symptoms. There was no effect of the potassium silicate and the molasses in reducing disease levels. Previcur® which had shown some disease reduction in the past did not show the same in this trial.

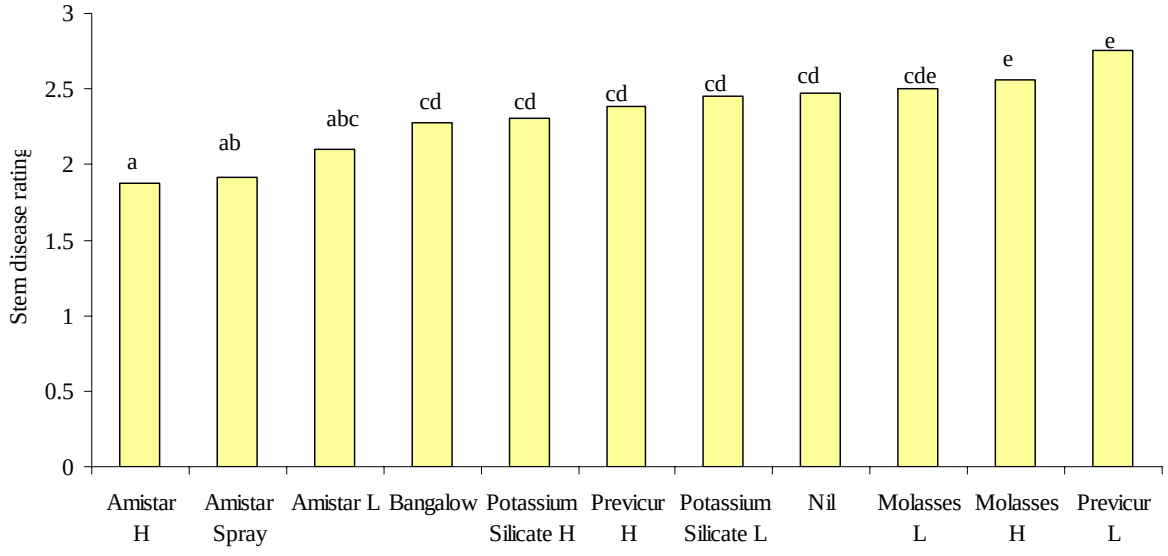

Figure 10.4. The results of the soil drenches in Trial 3. The Amistar® treatments again showed some disease reduction. Values with the same letter not significantly different (P<0.001, LSD 5%=0.18).

Trial 4.

ARR was the disease identified in this trial. There were some significant differences, for example the Amistar® treatment showed better disease ratings than other treatments, however disease was quite high across all treatments as per Table 10.6. Unfortunately the bacterial treatments did not show any improvement in disease levels. The grower had lost all his commercial plantings in the vicinity of the trial due to ARR and the prevailing wet weather conditions.

Table 10.6. Hypocotyl and root ratings for ARR on beans in Trial 4.

Treatment	**Disease rating (hypocotyl)***	**Disease rating (roots)***
Amistar®	3.0 a	2.7 a
Superzyme® (0.5%)	3.8 b	3.2 c
Fulzyme® (0.5%)	3.8 b	2.9 b
Fulzyme® (1%)	3.9 bc	3.0 b
Superzyme® (1%)	4.2 c	3.7 d
Nil	4.2 c	3.8 d
P	**P<0.001**	**P<0.001**
LSD 5%	**0.36**	**0.49**

* Values with the same letter are not significantly different.

Trial 5

Amistar showed some disease control potential and did show some significance to the other treatments and disease was slightly less than other treatments (Table 10.7).

Table 10.7. Hypocotyl and root ratings for ARR on beans in Trial 5.

Treatment	**Disease rating (hypocotyl)***	**Disease rating (roots)***
Amistar®	1.4 a	1.5 a
Fulzyme® (1%)	3.1 b	2.1 b
Fulzyme® (0.5%)	3.4 bc	2.5 cd
Nil	3.2 bc	2.4 c
Superzyme® (0.5%)	3.7 c	2.4 c
Superzyme® (1%)	3.7 c	2.7 d
P	**<0.001**	**<0.001**
LSD	**0.41**	**0.22**

*Values with the same letter are not significantly different.

Trial 6

Again four weeks after establishment disease was assessed where the Amistar® and Previcur treatments successfully reduced disease levels significantly compared to the untreated control (Table 10.8). The potassium silicate treatments and the Fulzyme® on its own treatment was not successful in reducing disease.

Table 10.8. Hypocotyl and root ratings for ARR on beans in Trial 6.

Treatment	Disease rating (hypocotyl)*	Disease rating (roots)*
Fulzyme®+Amistar®	1.2 a	1.3 a
Amistar®	2.0 b	1.5 a
Previcur®	2.7 c	2.4 b
Nil	3.8 d	3.1 d
Potassium silicate	3.9 d	2.8 c
Fulzyme® 5mls/L	4.0 d	2.9 c
P	**<0.001**	**<0.001**
LSD	**0.39**	**0.20**

*Values with the same letter are not significantly different.

Trial 7

Disease levels were lower than previously encountered on this site, but the temperature was cooler at the time and this may have been the reason. *A. euteiches* prefers warmer soil temperatures. Some treatments performed significantly better than the untreated control (Table 10.9). Hymexazol was the best overall with the hypocotyl and root rating followed by Amistar®. Hymexazol has been shown in previous trials to reduce disease levels when used as a seed dressing. It is used as seed dressing overseas in some countries including in the United States for *A. euteiches* control in the sugar beet but it is unavailable in Australia.

Table 10.9. Hypocotyl and root ratings for ARR on beans in Trial 7. L=low and H=high

Treatment	Disease rating (hypocotyl)*	Disease rating (roots)*
Hymexazol	0.0 a	0.5 a
Amistar® + Fulzyme®	0.2 ab	0.6 a
Amistar® L	0.6 b	0.6 a
Amistar® H	0.7 c	0.9 b
Previcur® L	0.5 b	1.0 b
DM	0.8 c	1.0 b
AMF L	0.4 ab	1.0 b
Fulzyme® L	0.8 c	1.1 bc
AMF H	0.3 ab	1.3 c
Previcur® H	1.3 d	1.4 c
Fulzyme® H	1.3 d	1.4 c
Nil	1.3 d	1.5 c
P	**<0.001**	**<0.001**
LSD 5%	**0.36**	**0.51**

*Values with the same letter are not significantly different.

Trial 8

Disease levels were similar to Trial 7 as they were planted at the same time (Table 10.10). Hypocotyl and root ratings were similar with the Simba and the Pike having more disease than the other varieties/treatments. Either a raised bed or in the deep ripped plots both reduced disease levels.
Disease levels were not high, the trial was later in the season and cooler than *A. euteiches* prefers. Planting on raised beds was shown to improve disease but this was at reduced disease pressure.

Table 10.10. Hypocotyl and root ratings for ARR on beans in trial 5.

Treatment	**Disease rating (hypocotyl)***	**Disease rating (roots)***
Pike(hill)	0.1 a	1.2 a
Bangalow	0.1 a	1.3 a
Rex	0.2 a	1.4 ab
Pike (deep ripped)	0.2 a	1.2 a
Simba	1.1 b	1.6 c
Nil (Pike)	1.3 b	1.5 b
P	**<0.001**	**0.002**
LSD 5%	**0.30**	**0.24**

*Values with the same letter are not significantly different.

Trial 9

All plants in this trial had disease symptoms with no treatment giving total disease control indicating high levels of disease in this trial block (Table 10.11). The treatments that had less disease were the hilling treatment and the adequately fertilised treatments. The addition of the boron and calcium had no effect on reducing disease at the application rates used. Hilling improved drainage reducing the affects of disease similarly to what was recorded in Trial 8.

Table 10.11 The hypocotyl ratings recorded for field trial 9. H=High, M=Medium. L=Low

Treatment	**Disease rating (hypocotyl)***
Hilling	2.8 a
Grower fertiliser sown with beans	2.8 ab
Grower fertiliser broadcast	3.0 abc
Fulzyme® Twice	3.1 cd
Calcium H	3.3 cd
Foliar Mix® M	3.3 cd
Amistar®	3.4 de
Foliar Mix® H	3.4 de
Fulzyme® Once	3.5 de
Foliar Mix® L	3.6 e
Calcium L	3.6 e
Fulzyme® and Superzyme®	3.6 e

Nil Treatment	3.7 e
Calcium M	3.7 e
Foliar Mix® M + Calcium M	3.7 e
P	**<0.001**
LSD 5%	**0.33**

*Values with the same letter are not significantly different.

Discussion

Many of the trials indicated that Amistar® as a drench reduced fungal diseases. Molasses and potassium silicate, although providing control in pot trials, did not have the same effect in the field. The main reasons for this could be due to quantity of product used in the field trials and the isolated nature of beans in pots in greenhouse trials.

More tolerant varieties showed the potential of reducing disease levels but the quality of bean (size, shape, etc) and plant growth habit were not acceptable for market.

Field trials indicated that Fulzyme®, which showed some response in earlier greenhouse trials, had limited affect on the disease in the field in the current field trial format. One issue that may assist in the efficacy of this product is follow-up treatments after initial application.

The success in disease control of Hymexazol (Tachigaren®) has been demonstrated in both the greenhouse and the field but its availability for this purpose in Australia is questionable. Hymexazol was registered for minor use on sugar beet in the United States to control *Aphanomyces cochlioides*. Like *Aphanomyces euteiches* on beans, *A. cochloides* once established in a field, avoidance from growing in that field is the only option to reduce disease. Sugar beet producers are also having difficulty finding new land (Harveson *et al.* 2007).

AMF showed some efficacy against ARR in the field trials but it also showed some phytotoxity which is quite likely due to the concentration of the product on the seed and the fact that one of the active ingredients is azoxystrobin. Applying adequate volumes of product to small quantities of seed is difficult. AMF is a mixture of azoxystrobin, metalaxyl-M and fludioxonil which provide good control of soil borne diseases.

Data from field and greenhouse trials indicate that Amistar® should be pursued as a soil drench. This treatment would assist in controlling a number of soil borne fungal pathogens that affect beans including ashy stem blight (*Macrophomina*), *Rhizoctonia* and ARR.
Improving drainage by planting on hills is considered a priority, it reduces moisture around the roots in periods of excess rainfall.

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11. THE INFECTION ABILTITY OF *A. EUTEICHES* ISOLATED FROM BEANS ON PEAS.

Introduction

As *Aphanomyces euteiches* causes diseases in both beans and peas, it was assumed that the situation in Australia was similar to other countries where the type causing the disease in beans is different to the one causing the disease in peas. Hence the naming of the organisms responsible as *Aphanomyces euteiches* f.sp. *phaseoli* for the type affecting beans and *Aphanomyces euteiches* f.sp. *pisi* affecting peas. The information is important because often peas are grown in similar areas to beans and often on the same ground. Anecdotal evidence was that the bean type of *Aphanomyces euteiche*s did not affect peas.

Method

Ten *Aphanomyces euteiches* isolates isolated from beans were chosen that were to be used in pathogenicity tests with peas and beans. Both beans (Simba) and peas (Oregon Giant) were grown in potting mix for one week. There were five pots of both peas and beans for each of the ten isolates. Isolates were prepared by culturing small pieces of actively growing colonies (on ¼ strength potato dextrose agar) onto small (55mm) petri dishes of ¼ strength potato dextrose agar. Four plates of each were mashed into 200ml of distilled water and 25ml of this solution was placed around the roots of the actively growing plants by removing the soil slightly and then replacing. This confirmed that the solution would be near the roots. The pots were then watered and left for two weeks and after that time the plants were removed from the pots and the plants were examined for disease symptoms.

Results

There were no symptoms of root disease on the pea plants but the bean plants had the normal amount of disease that is experienced with ARR as shown in Figure 11.1.

Figure 11.1. Bean plants (Simba) on the left showing the normal symptoms with ARR with the brown roots and lesions up the hypocotyl/stem as compared to the peas (Oregon Giant) on the right.

Discussion

There was no infection of peas with the isolates of *Aphanomyces euteiches* that had been isolated from beans. Indicating conclusively that this is *Aphanomyces euteiches* f.sp. *phaseoli,* a type specific to beans. For growers who grow beans that get ARR, there will be no cross infection with peas, providing they do not have *Aphanomyces euteiches* f.sp. *pisi*. It would be worthwhile to conduct cross infection studies with other isolates of *Aphanomyces euteiches* and conduct genetic comparisons between the isolates.

12. SOIL DEPTH AND THE PRESENCE OF *A. EUTEICHES*

Introduction

This was a preliminary investigation on the depth of *Aphanomyces euteiches* in the soil profile. As hypocotyls and roots of bean plants carry the most infection by *A. euteiches*, removal of that part of the plant or improving the breakdown of the plant material in the soil would help reduce the carry over inoculum. As with all harvested food crops the end product, in this case beans, is taken leaving a bulk of plant material left. In most bean growing areas cattle are used to eat the plant material left but the roots and lower stem are often left in the soil. The level in soil for the disease causing propagules therefore must be the level that these roots travel too. Therefore soil was collected from blocks where beans had been grown one year previously to confirm this. Zoospore movement of *Aphanomyces euteiches* is reported to be 10mm (Papavizas and Ayers 1974), therefore improved incorporation methods may keep these propagules further away from new bean growth.

Method

Soil was collected from three different depths from two sites known to have had beans and ARR. Both soils were different, with one being quite deeper than the other. Soil was collected from site A at the surface, then at 130mm below the surface then at 180mm below the surface. Soil from site B was from the surface, from 130mm below the surface and then another sample from 250mm below the surface. Site B had a deeper profile than site A. A trench was dug to carefully remove the soil so as to keep the layers separate. The soil was subsequently placed into pots and beans planted into two pots at each soil depth. The plants were allowed to grow and at one week after planting were wet up and after three weeks plants examined and rated for disease.

Disease symptoms were assessed using a 0-5 scale based on root and hypocotyl (Figure 1.1) colour and health. The 0 rating was no disease and the 5 rating was high disease. For most trials roots and hypocotyls were assessed separately, whereas with others often only the hypocotyl was assessed. The disease ratings were based on the symptoms shown in (Figure 1.2).

Results

Soil from site B taken at 250mm had lower disease incidence compared to lowest soil at Site B (180mm) as seen in Figure 12.1. Site A had similar disease levels at 130mm and 180mm.

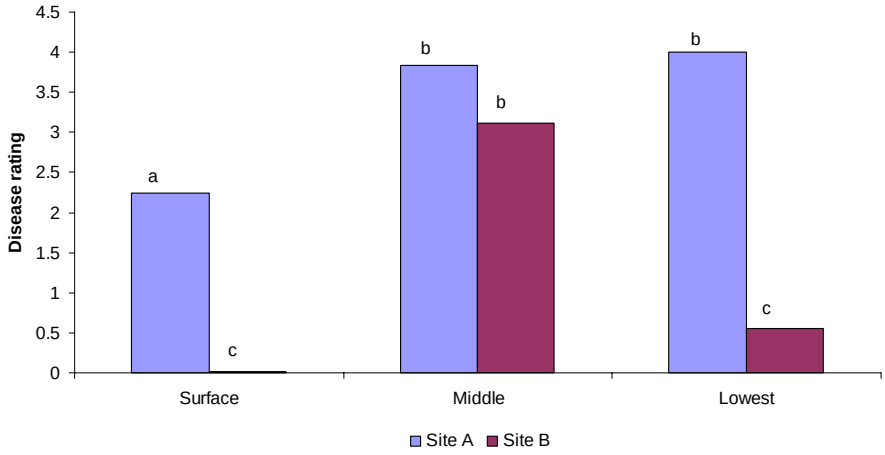

Figure 12.1 The hypocotyl ratings at each level of soil taken. There was a significant effect on the depth the soil was taken and the level of *A. euteiches*.(P<0.001, LSD 5%=0.66), values with the same letter not significantly different.

Discussion

Apart from the surface of the soil, the lowest soil at Site B had less disease. Site A however had similar levels at all three depths but more at the lower two levels. The depth that bean roots would travel down in soil would unlikely be much lower that 180mm depending on soil type, therefore taking the soil at 250mm for Soil B may have been beyond this range.

Inverting the surface layer of soil may give some control by burying infected plant material away from new crops especially in a deeper soil such as soil B. However this is not considered good management practise for maintaining soil structure, but if done only irregularly may assist in reducing the carry-over of fungal propagules.

Growers often use rotary hoes to break up plant material and work the soil before planting. This implement only reaches a certain level in the soil and can cause damage to the soil structure as well and cause a hard pan to develop. The action of the rotary hoe can also increase the amount of air in the soil which can oxidise soil humus.

A method to reduce infected plant trash would be very useful to develop to reduce soil borne disease incidence.

13. BLACK ROOT ROT-REVIEW

Black root rot is caused by a soil-borne fungus, *Thielaviopsis basicola* (Berk. & Broome) Ferraris (syn. *Chalara elegans* Nag Raj & Kendrick). The pathogen is widely distributed and causes severe root rot in ornamentals, beans, carrot, cotton, and peas. The fungus may cause diseases on its own but more often found in a complex with other soil borne pathogens.

Symptoms of black root rot include lesions on the main root (hypocotyl) and or lesions on finer roots. Lesions start on roots as purple coloured which eventually turn black. The lesions may stay minor or cause stunting or plant death. The fungus is an obligate parasite (needs a host) and does not survive saprophytically i.e. without being able to infect plants. Therefore periods without a host can reduce disease incidence in subsequent years.

There are two types of spores produced, conidia and chlamydospores (Figure 13.1). Chlamydospores are long lived, surviving for long periods in soil. The fungus can infect bean roots directly or often invade through injuries or sites of invasion of other root infecting fungi and nematodes. Fungi associated with infection by *T. basicola* include *Pythium*, *Fusarium* and Rhizoctonia.

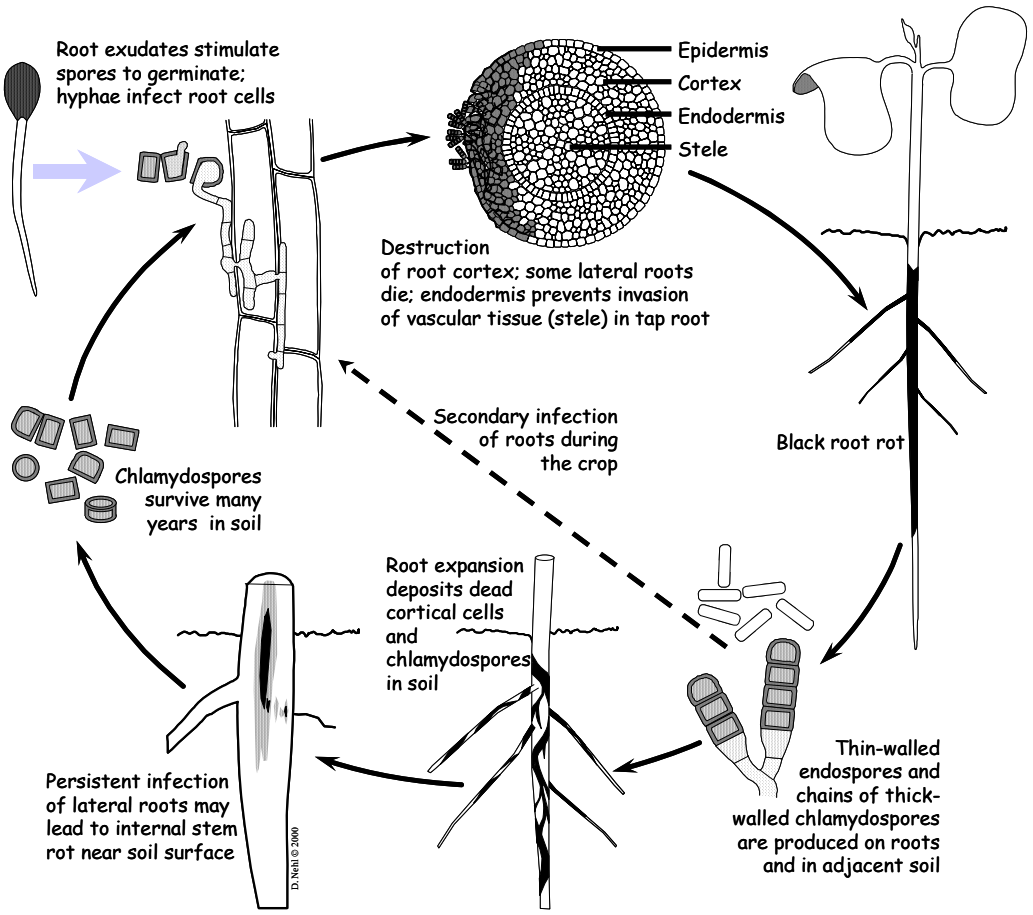

Figure 13.1. Life cycle of *T. basicola* on cotton courtesy of Dr David Nehl. The infection process would be similar for vegetable crops.

Temperature and soil moisture

T. basicola is favoured by a soil temperature of 28°C but damage to beans is most severe at lower temperatures of 15-20°C. Therefore the fungus prefers high temperatures but causes most damage on beans at cooler temperatures. Increased disease incidence and severity has been noted on cotton with reducing temperatures from 28°C down 20°C (Rothrock 1992). The same study revealed that high soil moisture also favoured disease but different soil textures had no effect.

Host range

T. basicola has been recorded infecting over 130 species of plants (Honess *et al.* 1994). Non hosts include cereals, sunflowers, brassicas and onions. Some crops such as soybeans are susceptible to black root rot but are grown in the hotter periods of the year, when conditions are not favoured for the disease. Non host crops provide alternatives for consideration in fields where black root rot is serious. Onions and garlic have also been considered as potential biofumigation crops.

Although *T. basicola* has a wide host range it is considered that the fungus has some host specificity. Therefore some strains of the fungus may favour particular hosts. For example an isolate affecting lettuce was found not to be pathogenic (i.e. infect the plant) on cotton (O'Brien and Davis 1994). In this report *T. basicola* that was isolated from lettuce was used to infect other crop plants. Disease was observed in lettuce and beans but not in cucumber, celery, tomato, capsicum, parsley, radish and watercress.

It has also been reported that when tobacco was planted into cotton soil no symptoms were recorded on the cotton (Nehl *et al.* 2004a). Nehl also suggested that continuous cotton production has selected isolates of black rot that are more virulent to cotton.

Black root rot in Australia

In Australia black root rot was first identified on ornamentals and crops such as beans (*Phaseolus vulgaris* L.), peas (*Pisum sativum* L.), tobacco (*Nicotiana tabacum* L.) lupins (*Lupinus angustifolius* L.) and various pines (Clayton 1953), (Simmonds 1966; Warcup and Talbot 1981; Allen 1990). Allen first found black root rot in cotton in Australia in Moree, NSW (1990).

Beans were found affected with black root rot in Tasmania and Victoria through a HAL funded bean project VG03002 (Watson *et al.* 2007). Symptoms seen in these plants are shown in Figure 13.2. On examining roots the chlamydospores can be seen within the root tissue and on the root itself (Figure 13.3). The density of fruiting bodies especially the chlamydospores in soil can give an indication of the severity of the disease in crops (Holtz and Weinhold 1994).

Figure 13.2. Symptoms of black root rot on beans. Notice in beans with root damage that new roots begin to form above the damaged area.

Isolating *T. basicola* from soil and plant material.

The fungus *T. basicola* is difficult to isolate from plant material. The main methods of isolation from plant material and soil include the use of selective media or carrot disks. Various media have been developed to assist with the isolation from soil and rhizosphere (Papavizas and Davey 1961; Papavisas 1964; Specht and Griffin 1985). A modification of TB-CEN agar (Specht and Griffin 1985) was used for enumeration of *T. basicola* in cotton soils (Nehl *et al.* 2004a).

O'Brien and Davis (1994) used three methods for isolation when examining the disease outbreak in lettuce. One used root pieces of lettuce which were surface sterilised, rinsed and placed onto potato-dextrose agar (PDA). The second method used root sections that were placed onto water agar for 24h after which conidia were transferred to PDA with a sterile needle. The third method consisted of placing root pieces between freshly cut carrot slices in moist Petri dishes similar to a method by Yarwood (1946). Detecting black root rot in soil can also be achieved by growing a susceptible host in the soil such as beans which can then be assessed on the presence or absence of disease symptoms and also the degree of infection.

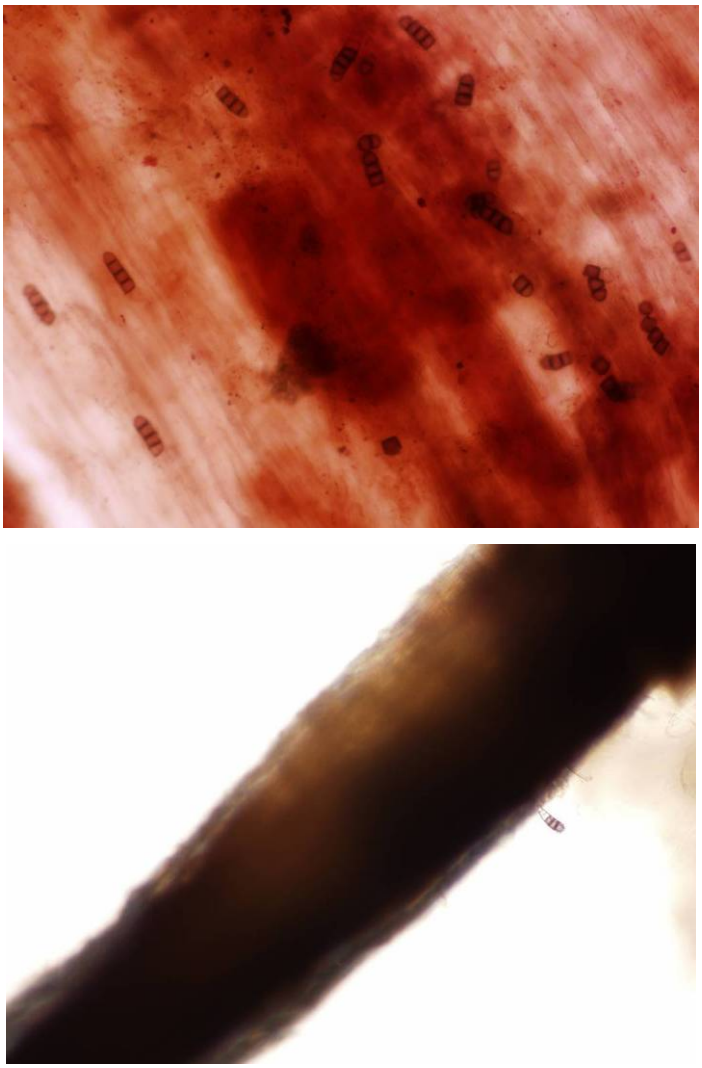

Figure 13.3. Chlamydospores in (top) and on roots (bottom) of beans.

Control options for Black root rot.

Control options for Black root rot include avoidance of infected fields, long rotations, planting at a time to reduce exposure to cool conditions, using rotation crops and biofumigant crops, use of fungicides, use of plant activators, and where available resistant or tolerant cultivars.

Resistant cultivars.

Resistant cultivars provide the best control for soil borne diseases. There have been reports of host resistance to black root rot in lettuce (O'Brien and Davis 1994; Sala *et al.* 2008) and tobacco (Haji *et al.* 2003) but there were no resistant cultivars of cotton found (Nehl 2001).

Biofumigant crops.

Biofumigant crops have been examined as a method of reducing the effects of soil-borne pathogens including black root rot on cotton. Biofumigation involves planting a crop and incorporating into the soil before flowering, the plant material then releases compounds into the soil that act similar to synthetic fumigants and are toxic to pests and diseases. The crop identified best for reducing black root rot severity in cotton was *Vicia villosa* or woolly pod vetch (Kendig and Rothrock 1991; Nehl 2001). Nehl also found a reduction in disease levels when some cultivars of mustard were incorporated into the soil as biofumigant crops. Indian mustard (Indian 651) (*B. juncea*) appeared to be the best in field trials when disease levels were assessed.

Alternative crops such as onions and garlic reduced black root rot disease levels in the USA when used in rotation with cotton. This is most likely a biofumigation response and should be considered by vegetable growers but may also depend on soil type. This method of disease management should be trialled in bean growing areas in Australia. There are onion growers in the Murrumbidgee Irrigation Area that also grow carrots but have not reported black root rot in carrots.

Fungicide options

Benlate (benomyl) was trialled as an in-furrow treatment but results were variable, it cannot be considered as a control option as the product is no longer available, however a similar product carbendazim could be considered for trials for an in-furrow application however its use is also under review. In a recent study mycobutanil as a seed treatment reduced disease levels in both artificially and naturally infested soils (Toksoz *et al.* 2009). Fungicides as seed dressings may assist in the reduction of black root rot symptoms and should be considered for further research. There are now more seed dressings available and used by field crop industries and could be available for vegetable seed.

Products that elicit systemic acquired resistance such as Bion® plant activator have been shown to reduce black root rot incidence and currently this product has registration in cotton. It was successful at reducing disease in the field when seed was soaked in the solution prior to sowing or applied as an in furrow spray (Nehl *et al.* 2004b). Bion® is only one of the plant activators, others have been trialled and shown to be successful at reducing disease levels. (Mondal *et al.* 2005).

Fumigation has been found to be successful but may not be economically viable in some situations. Fumigation is currently carried out by some growers; the main products used include metham or chloropicrin. Fumigation will also control weeds, insects and nematodes.

Farm Hygiene

Soil that has adhered to vehicles such as tractors and cars pose a potential risk of spreading black root rot from farm to farm (Nehl *et al.* 2004a). Infected plant residue can be a source of new infections.

Growers with the disease should develop a farm hygiene programme that will reduce the spread of soil and debris. It is important to restrict the movement of soil on machinery such as tractors by washing them before going from an infected block to a clean block is essential. Valuable information is available to growers for assistance with planning farm hygiene with the publication "Farm hygiene for vegetable blocks" which is a note published by Queensland DPI&F (now DAFFQ) the following web site.

http://www2.dpi.qld.gov.au/horticulture/4753.html

Other disease control options.

Alternative methods of control of black root rot have included flooding which does give control but most likely not practical for vegetable growers. Planting later in warmer conditions will reduce disease levels, but this too may bring harvesting into a hotter part of the season in some growing regions. In cotton delaying sowing in one season by 3 weeks reduced disease levels (Nehl *et al*. 2004b). This must be considered by growers that may have problems with black root rot.

Conclusion and relevance to the vegetable industry.

As black root rot has been reported more often in recent times, there is a chance that the disease will become more of an issue with vegetable industries. Industries already known to have been affected include beans, carrots and peas. But other vegetables may be affected by this soil borne pathogen with a wide host range. The importance of farm hygiene cannot be over emphasised especially when it has been diagnosed on a property. The experience of the cotton industry has demonstrated the rapid spread of the disease from farm to farm and region to region that grow cotton, and this may occur in the vegetable industry.

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14. GREENHOUSE TRIALS TO CONTROL BLACK ROOT ROT

Introduction

The control of black root rot is difficult as described in the previous review. Some preliminary trials were undertaken to examine options for controlling this disease, including soil drenches and a variety assessment to find any differences in susceptibility.

Method

Trial 1

Soil was collected from a Victorian bean farm with known bean disease problems. Preliminary tests with the soil indicated that it contained high levels of black root rot. Bion Plant Activator® was applied to Simba bean seed at three rates. There were three rates of potassium silicate (17% Silicon) as a soil drench and three rates of Bion Plant Activator® (active ingredient Acibenzolar-S-Methyl 500 g/kg) as a seed dressing. Plants were grown for six weeks and then assessed visually for disease on a similar scale to that used previously. As the disease only affected the roots only a root rating was done.

Trial 2

This trial was established to examine whether there may be differences in susceptibility of beans to black root rot caused by *Thielaviopsis basicola*. Seven bean varieties were grown in known black root rot infected soil. The soil was well mixed to increase the uniformity of fungal inoculum in the soil. Pots (100mm) were filled with a soil/sterile vermiculite mix. Five seeds were sown of each variety and then the pots were maintained at 17/23ºC as night and day temperatures.

After three weeks plants were removed from the pots and assessed for disease by rating the levels of black root rot from 0-5 with 5 being the highest level of disease. Symptoms of black root rot are typical blackened root systems.

Results

Trial 1

All of the treatments recorded high disease levels (Table 14.1) but there was an indication that potassium silicate reduced disease levels, but Bion Plant Activator® did not.

Trial 2

Although black root rot was still present in all varieties, there were some with slightly less disease. (Table 14.2, Figure 14.1).

Table 14.1. Disease scores for Trial 1 black root rot trial.

Treatment	Rate	Disease rating (hypocotyl/root)*
Potassium silicate medium	9% v/v	2.59 a
Potassium silicate low	6% v/v	2.70 ab
Bion high	3.6g/100kg seed	3.1 ab
Potassium silicate high	12% v/v	3.25 ab
Control		3.33 b
Bion medium	2.4g/100kg seed	3.59 b
Bion low	1.2g/100kg seed	3.88 b
P		**<0.001**
LSD 5%		**0.68**

*Values with the same letter are not significantly different.

Table 14.2. Black root rot disease ratings in the variety trial.

Variety	Disease rating (all roots)*
HS 697	2.17 a
Excalibur	2.46 ab
Bean 024	2.64 bc
HS 698	2.65 bc
Red Bean	2.77 bc
HS 693	2.81 bc
Valentino	2.87 c
P	**<0.001**
LSD 5%	**0.37**

*Values with the same letter are not significantly different.

Discussion

Varieties showed differences in susceptibility to black root rot and trials should be considered in areas with black root rot to assess their tolerance to the disease. Resistant cultivars provide the best control of soil borne diseases. There have been reports of host resistance to black root rot in lettuce (O'Brien and Davis 1994; Sala *et al.* 2008) and tobacco (Haji *et al.* 2003) but there were no resistant cultivars of cotton found (Nehl 2001).

Figure 14.1. Differences between black root rot disease expression with the top photograph showing less disease (i.e. black roots) than the variety underneath.

References

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Sala FC, Costa CPd, Teixeira LdD, Fabri EG, Blat SF (2008) Lettuce reaction to black root rot caused by *Thielaviospsis basicola*. *Horticultura Brasileira* **26**: 398-400.

TASMANIAN RESEARCH ACTIVITIES

15. THE EFFECT OF ROOT ROTS ON DIFFERENT BEAN VARIETIES.

Introduction

The aim of this trial was to screen 14 green bean varieties for their susceptibility to *A. euteiches* and Black root rot (*Thielaviopsis*).

Method

Two pot trials and two field trials were conducted in Tasmania to screen green bean varieties for their susceptibility to Aphanomyces and black root rots. All trials were set up as randomised block design. All bean varieties were sourced and supplied by Simplot Australia and Harvest Moon, the two largest green bean producers in Tasmania.

Pot trials

In pot trials, naturally infected field soil collected from two paddocks was used for the screening against each of the major disease (Tables 15.1-15.2). One paddock at Wesley Vale had a history of severe ARR on green beans and another paddock at Don had very severe black root rot due to *Thielaviopsis* in 2010. Ten seeds were sown on 4 Feb 11 in each pot (140 mm x 145 mm pot size) and there were two replicate pots for each variety. All surviving seedlings were assessed for survival, fresh shoot weight, root rot severity and dry root weight at 49 days after sowing for the Aphanomyces red root rot in the Wesley Vale soil and at 54 days after sowing for Thielaviopsis black root rot in the Don soil.

Field studies

In field trials, bean varieties as described in (Table 15.3) were sown by Harvest Moon in two paddocks within commercial fresh green bean crops (cv. Valentino) at Don on 25 Oct 10 and Kindred on 24 Dec 10. The varieties were sown in 6 m x 1.2 m plots with three replicates in randomised complete block designs.

Plants at the Don trial were severely affected by black root rot due to a combination of cool and wet soil conditions. Plants from the Don trial were assessed for survival and root rot severity at 50 days after sowing, and growth and pod yield at 84 days after sowing. The trial at Kindred became water-logged following frequent rainfall and all plants in the trial area as well as the rest of the paddock were severely stunted and pre-disposed to severe secondary rot by *Fusarium* species. The entire bean crop was ploughed in early. Therefore, plants from the Kindred trial were not assessed.

Table 15.1. Bean varieties screened for susceptibility to ARR in a pot trial

No.	Variety	Type	Source	Processor
1	Flavor Sweet	whole bean	Clause Pacific	Simplot Australia
2	Montano	slicing bean	Sunland	Simplot Australia
3	Goldmine	butter bean	Seminis	Simplot Australia
4	TWSC6V 0962	dwarf bean	Seminis	Simplot Australia
5	FIVC6 0998	whole bean	Seminis	Simplot Australia
6	FIVC6 0999	whole bean	Seminis	Simplot Australia
7	FIVC6 1001	whole bean	Seminis	Simplot Australia
8	FMGC6V 1006	whole bean	Seminis	Simplot Australia
9	Rex	whole bean	Farmland	Harvest Moon
10	Renegade	whole bean	-	Harvest Moon
11	Prevail	whole bean	Syngenta	Harvest Moon
12	Venice	whole bean	-	Harvest Moon
13	HMX 8122	whole bean	Osborne Seed	Harvest Moon
14	Valentino	whole bean	Seminis	Harvest Moon

Table 15.2. Bean varieties screened for susceptibility to Thielaviopsis root rot in a pot trial

No.	Variety	Type	Source	Processor
1	Flavor Sweet	whole bean	Clause Pacific	Simplot Australia
2	Montano	slicing bean	Sunland	Simplot Australia
3	Goldmine	butter bean	Seminis	Simplot Australia
4	TWSC6V 0962	dwarf bean	Seminis	Simplot Australia
5	FIVC6 0998	whole bean	Seminis	Simplot Australia
6	FIVC6 0999	whole bean	Seminis	Simplot Australia
7	FIVC6 1001	whole bean	Seminis	Simplot Australia
8	FMGC6V 1006	whole bean	Seminis	Simplot Australia
9	HMX 8122	whole bean	Osborne Seed	Harvest Moon
10	Valentino	whole bean	Seminis	Harvest Moon

Table 15.3. Bean varieties screened for susceptibility to root rot in a paddock at Don and Kindred, Tasmania

No.	Variety	Type	Source	Processor
1	Rex	whole bean	Farmland	Harvest Moon
2	Renegade	whole bean	-	Harvest Moon
3	Prevail	whole bean	Syngenta	Harvest Moon
4	Venice	whole bean	-	Harvest Moon
5	HMX 8122	whole bean	Osborne Seed	Harvest Moon
6	HMX 7111	whole bean	Osborne Seed	Harvest Moon
7	Green Valley	whole bean	-	Harvest Moon
8	Valentino	whole bean	Seminis	Harvest Moon

Root rot assessments

Roots were washed and rated for root rot severity as follows: 0 = no root rot, 1 = trace, 2 = mild, 3 = moderate, 4 = severe root rot and 5 = completely rotten root. Root rot severity index was then tabulated by the sum of number of plants in each category divided by the total number of plants assessed (Figure 15.1).

Figure 15.1 ARR severity rating (0-5) on the left and black root rot severity rating (0-5) on the right.

Results

Flavor Sweet, Montano and Goldmine varieties are the current commercial varieties that are gown in Tasmania for processing into frozen vegetables. Valentino is grown in Tasmania for fresh market green beans. All other varieties are potential new varieties.

Pot trials

Among the new varieties, Prevail, FMGC6V 1006, Renegade and TWSC6V 0962 tended to have lower root rot indices compared to all other varieties in the *A. euteiches* infected soil (Table 15.5). There was however a poor correlation between the root rot indices and the dry weights (R = 0.194) or the fresh shoot weights (R = 0.121) in a linear relationship. The shoot weight had a much better correlation to the dry root weight (R = 0.675). Therefore, the shoot weights may be a better indicator on plant vigour and their tolerance to ARR. In comparing the shoot weights, all the new varieties except for Venice appear to have greater growth than the current varieties.

Table 15.5. Effects of bean varieties on seedling growth and root rot severity in *A. euteiches* infected soil in a pot trial at 49 days after sowing

No.	Treatment	Survival count	Root rot index (0-4)	Fresh shoot weight (g/pot)	Dry root weight (g/pot)
1	Flavor Sweet	100	3.3 abc	11.13 e	0.739 e
2	Montano	95	3.1 abc	18.17 bcde	0.980 de
3	Goldmine	85	3.8 a	15.95 de	0.811 de
4	TWSC6V 0962	85	2.0 bcde	23.50 abcd	1.440abc
5	FIVC6 0998	100	3.9 a	27.87 a	1.462abc
6	FIVC6 0999	95	3.7 a	28.27 a	1.128 cde
7	FIVC6 1001	100	3.1 abc	22.74 abcd	1.152 cde
8	FMGC6V 1006	95	1.2 de	23.83 abcd	1.091 cde
9	Rex	95	3.5 ab	24.68 abc	1.603ab
10	Renegrade	70	1.7 cde	21.03 abcd	0.819 de
11	Prevail	95	0.8 e	25.52 ab	1.656 a
12	Venice	95	2.7 abcd	15.80 de	1.203 bcd
13	HMX 8122	95	3.0 abc	21.07 abcd	1.207 bcd
14	Valentino	90	2.6 abcd	16.60 cde	1.091 cde
LSD 5%		**N/A**	**1.65**	**8.420**	**0.4312**
P		**0.3567**	**0.0208**	**0.0194**	**0.0078**

Means followed by same letter do not significantly differ.

Mean comparisons performed only when AOV Treatment P(F) is significant at mean comparison OSL.

In comparing varieties in the *Thielaviopsis* infected soil, only Montano and TWSC6V 0962 tended to have lower root rot indices compared to all other varieties (Table 15.6). There was a poor correlation between the root rot indices and the dry weights (R = 0.378) or the fresh shoot weights (R = 0.156) in a linear relationship. The shoot weight was highly correlated to the dry root weight (R = 0.706). Therefore, the shoot weights also appeared to be a better indicator on plant vigour and their tolerance to black root rot. This is consistent with field observations, where the most obvious impact of black root rot is in reducing plant vigour and yield. In comparing the shoot weights, all the new varieties except for FMGC6V 1006 have greater growth than the current varieties Flavor Sweet and Goldmine.

Table 15.6. Bean varieties growth and root rot severity in *Thielaviopsis* infected soil in a pot trial at 54 days after sowing

No.	Treatment	% Survival	Black root rot index	Fresh shoot weight (g/pot)	Dry root weight (g/pot)
1	Flavor Sweet	75	3.5 abc	5.71 c	0.523
2	Montano	85	2.8 c	13.10 ab	0.840
3	Goldmine	45	3.9 ab	5.34 c	0.400
4	TWSC6V 0962	75	3.0 bc	13.45 ab	0.565
5	FIVC6 0998	80	3.5 abc	16.11 a	1.540
6	FIVC6 0999	90	3.9 ab	16.66 a	1.570
7	FIVC6 1001	70	3.8 ab	11.81 ab	1.015
8	FMGC6V 1006	70	4.3 a	8.47 bc	0.830
9	HMX8122	85	4.5 a	11.82 ab	1.715
10	Valentino	80	4.5 a	12.92 ab	1.035
	LSD	**N/A**	**1.00***	**5.93****	**N/A**
	P	**0.3053**	**0.0420**	**0.0638**	**0.4722**

Means followed by same letter do not significantly differ (P=.05, LSD)
Mean comparisons performed only when AOV Treatment P(F) is significant at mean comparison OSL.
* LSD (P = 0.05) and ** LSD (P = 0.10)

Field studies

At the Don trial, all plant roots were severely affected by black root rot because of a combination of the cool and wet soil conditions pre-disposed hypocotyls and roots to severe black rot. There were no significant differences in the black rot severity between the bean varieties (Table 15.7). Affected plants were mostly stunted with severe black root rot. There were however obvious differences in plant growth and height between the different varieties in the field. This indicates that although all varieties were susceptible to black root rot, there were differences in their tolerance to the disease. HMX 8122 appeared to be the most tolerant variety with increased shoot growth, plant height, reduced early crop senescence and yield compared to the current standard Valentino (Table 15.7, Figure 15.2). Other new varieties: Rex, Prevail and Venice also performed relatively well, producing equivalent marketable pod yields to Valentino.

Table 15.7. Bean varieties growth and root rot severity in the field trial at Don, Tasmania

No.	Treatment	Black root rot index (0-5) 52DAS	Fresh shoot weight (g/10 plants) 52DAS	Average crop height (cm) 78DAS	Crop senescence rating (0-4) 78DAS	Marketable pod 78DAS
1	Rex	3.73	279.7	26.3 ab	3.5 bc	557 ab
2	Renegade	4.00	211.0	20.3 c	3.7 b	263 bc
3	Prevail	3.77	176.3	25.0 ab	3.0 c	407 b
4	Venice	3.80	98.0	25.0 ab	2.0 d	417 b
5	HMX 8122	3.70	226.0	27.7 a	1.3 e	777 a
6	HMX 7111	3.93	153.0	20.0 c	4.3 a	97 c
7	Green Valley	3.97	199.3	24.0 abc	3.0 c	250 bc
8	Valentino	3.80	176.7	22.0 bc	3.0 c	517 ab
LSD (5%)		**-**	**-**	**4.35**	**0.637**	**307**
P		**0.2686**	**0.2197**	**0.0169**	**0.0001**	**0.0093**

Means followed by same letter do not significantly differ.
DAS: days after sowing. Black root rot index: 0 = none, 1 = trace rot, 2 = mild rot, 3 = moderate rot, 4 = severe root rot and 5 = severe root rot with rotten tap root. Crop senescence: 0 = none, 1 = 1-10%, 2 = 11-20%, 3 = 21-40% and 4 = 41-70% crop senescence.

Figure 15.2. HMX 8122 was the most tolerant (top left) to black root rot. Valentino was moderately tolerant (top right) and HMX 7111 was highly susceptible (bottom).

Discussion

All plant varieties were susceptible to ARR and black root rot. There were little or no obvious differences in the root rot severity between the different varieties. However, some varieties appeared to be more tolerant to the diseases with relatively good above-ground shoot growth and good plant vigour. In comparing varieties sown in *A. euteiches* infected soil in a pot trial, apart from poor growth by Venice, all other new varieties (TWSC6V 0962, FIVC6 0998, FIVC6 0999, FIVC6 1001, FMGC6V 1006, Rex, Renegade, Prevail and HMX 8122) appear to have greater growth than the current varieties.

In comparing varieties sown in the *Thielaviopsis* infected soil in a pot trial, except for FMGC6V 1006, all the new processing green bean varieties (FIVC6 0998, FIVC6 0999 and FIVC6 1001) have greater growth than the current varieties Flavor Sweet and Goldmine. For fresh market whole beans, the new variety HMX 8122 was equivalent to Valentino, the current standard.

In field evaluation of fresh market whole bean varieties within a commercial crop, all plant roots were severely affected by black root rot with no significant differences in the black rot severity between the bean varieties. There were however obvious differences in plant tolerance to the disease with significant differences in the above-ground plant growth and yield between the different varieties in the field. HMX 8122 appeared to be the most tolerant varieties with substantially increased shoot growth and pod yield compared to the current standard Valentino. Other new varieties: Rex, Prevail and Venice also performed relatively well, producing equivalent marketable pod yield to Valentino.

16. FUNGICIDE OPTIONS FOR APHANOMYCES ROOT ROT USING TASMANIAN SOILS

Introduction

In 2006, *Aphanomyces euteiches f. sp. phaseoli* was first recorded as causing stem and root rot in green beans in north-west Tasmania. Previously, ARR was known to occur in bean crops in Queensland and NSW, but not in Tasmania (Watson *et al* 2007). Although the pathogen is an *Oomycetes* fungus similar to *Pythium* and *Phytophthora*, the fungicide metalaxyl has little or no effect against *A. euteiches*. Therefore, currently, there are no effective fungicide treatments that can be used commercially in Australia for *A. euteiches* disease control. This study was thereby conducted to screen various fungicides as well as biocontrol and non-chemical products for *A. euteiches* control in green beans.

Methods

A pot trial was set up in 200mm pots using naturally infected soil collected from a paddock that previously had widespread and severe *A. euteiches* rot on green beans. Each pot was filled with 2 L of potting mix and then topped up with another 2 L of infected field soil. One small batch of the infected soil was sterilised by autoclaving at 121^{o}C for 15 minutes and used as a control sterilized soil.

Two varieties of green beans were used - cv. Simba and cv. Flavor Sweet. All the Simba seed have been treated commercially with Maxim XL® (fludioxonil + metalaxyl). As metalaxyl is known to have no effect on *A. euteiches*, an additional fungicide Tachigaren® (active ingredient hymexazol) was applied as a seed treatment for Treatment 2 (Table 16.1). All Flavor Sweet seed have been treated commercially with Thiram®. Flavor Sweet is a common variety sown in Tasmania to produce beans for processing into frozen vegetables.

Suspensions of fungicides or non-chemical products were prepared and then applied as a soil drench application with 200 mL water/pot. Soils in the pots were kept at full saturation throughout the trial in order to pre-dispose plants to severe *A. euteiches* infections. The experiment was conducted in a randomised complete block design with four replicates in 4 L pots.

Table 16.1 Treatment used in the trial.

No.	Variety	Product	Active Ingredient	Rate		Application Method
				AI Rate (g ai/L)	Product Rate	
1	Simba	Maxim XL®	fludioxinil + metalaxyl	-	-	Standard commercial seed treatment
2		Tachigaren	hymexaxol	-	8.3 g/kg seed	Seed treatment + commercial standard
3	Flavor Sweet (all seed treated with Thiram)	Molasses	molasses	20	20 mL/L	Soil drench
4		Parkway blend	biocontrol		20 g/L	
5		Amistar®	azoxystrobin	1.25	5 mL/L	
6		Cabrio®	pyraclostrobin	1.25	5 mL/L	
7		Previcur®	propamocarb	1.8	3 mL/L	
8		Shirlan®	fluazinam	2.5	5 mL/L	
9		Untreated control	-	-	-	Nil
10		Sterilised soil		-	-	Nil

Assessments for seedling emergence and survival were conducted at 9, 33 and 48 days after sowing. The percentage of seedlings that emerged and survived against the total number of seed sown were then tabulated. At 48 and 71 days after sowing, the fresh shoot weight of surviving plants were recorded and their roots were washed and then assessed for *A. euteiches* rot severity.

The *A. euteiches* stem and root rot severity rating were assessed according to the following ratings:

0 = no stem or root rot

1 = no stem/hypocotyl rot, slight root discolouration

2 = < 10% stem/hypocotyl rot, some root discolouration

3 = 11-30% stem/hypocotyl rot, root discolouration

4 = 31-60% stem/hypocotyl rot, root discolouration

5 = >60% stem/hypocotyl rot, root discolouration

The disease severity index was calculated from the disease severity ratings as the sum of the total number of diseased plants, multiplied by their rating values, and divided by the total number of infected plants.

Results

A. euteiches rot on stem bases and roots was the only disease observed on plants grown in the field infected soil. In the heat sterilised field soil, there was little or no root rot by *A. euteiches*. Generally, the pathogen appeared to have little or no effects on seedling emergence and seedling survival (Table 16.2). Plants affected by *A. euteiches* were however, weak and stunted in their growth with severe

basal stem and root rot, therefore resulting in lower fresh shoot weight and high root disease severity (Table 16.3).

Fungicide soil drench applications with the strobilurins fungicides azoxystrobin (Amistar) and pyraclostrobin (Cabrio) were phytotoxic to the Flavor Sweet bean seeds resulting in a delay as well as reduced seedling emergence (Table 16.2). Compared to the untreated control, there was a reduction of 18% to 23% in seedling establishment at 33 days after sowing by azoxystrobin and pyraclostrobin, respectively. Pyraclostrobin appeared to be slightly more phytotoxic than azoxystrobin.
Among the soil drench treatments, pyraclostrobin followed by azoxystrobin were found to be the most effective fungicides in reducing the severity of *A. euteiches* infections and improving plant growth (Table 16.3). Propamocarb and fluazinam also have some activity against *A. euteiches* and help reduced root disease severity and increased shoot growth. The two non-chemical products, molasses and Parkway blend containing a mixture of beneficial bacteria and fungi have no effects on the pathogen.

As for fungicide seed treatments, the two standard commercial treatments used with Thiram and Maxim XL® (fludioxonil + metalaxyl) have no obvious effects in controlling *A. euteiches* rot. A significant reduction in the fresh shoot weights was recorded as a result of severe *A. euteiches* rot by the two seed treatments in Treatment 1 with Simba seed variety and Treatment 9 with Flavor Sweet variety. The additional seed treatment with hymexazol (Tachigaren®) onto Maxim XL® treated Simba seed did not prevent *A. euteiches* infections, but did reduce the stem and root disease severity and improves plant growth (Table 16.3). This indicates that Tachigaren has effects against *A. euteiches*, but the small quantity used in the seed treatment was insufficient to provide long-term protection under high disease pressure and highly disease favourable conditions.

Table 16.2. Treatment effects on seedling emergence and survival

No.	Treatment	Seed Variety	Product Rate	% Seedling emergence (9DAS)	% Seedling survival (33DAS)	% Seedling survival (48DAS)
1	Simba-Maxim XL®	Simba	std	93 a	83 bcd	71 b
2	Simba-Tachigaren®	Simba	8.3 g/kg seed	90 a	94 ab	85 a
3	Molasses	Flavor Sweet	20 ml/L	93a	93 ab	93 a
4	Parkway blend	Flavor Sweet	20 g/L	89a	89 abc	86 a
5	Amistar®	Flavor Sweet	5 ml/L	15 b	79 cd	74 b
6	Cabrio®	Flavor Sweet	5 ml/L	4 c	74 d	69 b
7	Previcur®	Flavor Sweet	3 ml/L	92a	93 ab	93 a
8	Shirlan®	Flavor Sweet	5 ml/L	90a	95 a	95 a
9	Untreated control	Flavor Sweet	-	94a	96 a	95 a
10	Sterilized soil	Flavor Sweet	-	91a	95 a	94 a
	P			0.0001	0.0030	0.0001
	LSD 5%			9.7	11.5	10.6

Means within columns followed by the same letter are not significantly different at the 5% level according to Least Significant Difference (LSD) test. DAS = Days after sowing, std= simba seed with standard seed dressing.

Table 16.3. Treatment effects on plant weights and root rot severity

Treatment	Active Ingredient	Active Ingredient rate	Seed Variety	Product Rate	Number of surviving plants/pot	Fresh shoot weight of surviving plants (g/pot) **	Root rot severity index
Simba-Maxim XL®	Fludioxonil+ metalaxyl-m	25g/L+10g/L	Simba	std	14	40 de	4.7 a
Simba-Tachigaren®	hymexazol	700g/L	Simba	8.3 g/kg seed	17	87 ab	3.6 b
Molasses			Flavor Sweet	20 mL/L	19	22 f	3.8 b
Parkway blend	Beneficial soil bacteria and fungi		Flavor Sweet	20 g/L	17	36 e	4.0ab
Amistar®	azoxystrobin	250g/L	Flavor Sweet	5 mL/L	15	67 bcd	2.5 c
Cabrio®	pyraclostrobin	250g/L	Flavor Sweet	5 mL/L	14	86 abc	2.0 c
Previcur®	propamocarb	600g/L	Flavor Sweet	3 mL/L	19	70 cd	2.7 c
Shirlan®	fluazinam	500g/L	Flavor Sweet	5 mL/L	19	69 abc	3.4 b
Untreated control			Flavor Sweet	-	19	34 a	3.9 d
Sterilised soil			Flavor Sweet	-	19	96 e	1.2 b
P						0.0001	0.0001
LSD 5%						*	0.69

Means within columns followed by the same letter are not significantly different at the 5% level according to Least Significant Difference (LSD) test.
* Data analysis was conducted on transformed data using log(x+0.5)
** Untransformed mean values are presented in the table.

Discussion

This study demonstrates the impact of *A. euteiches* f. sp. *phaseoli* in causing basal stem and root rot, resulting in stunted and weak plants. These symptoms were consistent with those observed in commercial crops that were affected by the disease in previous years. Even though the last affected bean crops were sown in the paddock in 2005, the pathogen was still present at high and damaging level when the soil was collected in 2008. This demonstrates the persistence of the pathogen in the soil. Therefore, crop hygiene and restrictions of soil movements from infected paddocks are vital in preventing the spread of this devastating disease.

The study examined two applications methods with soil drench applications and seed treatments. The fungicides, azoxystrobin, hymexazol, propamocarb, pyraclostrobin and fluazinam were shown to have activity against *A. euteiches*. Seed treatment with hymexazol delayed and reduced the disease severity, but the small quantity coated onto seed was insufficient to provide long term control under high disease pressure. With the soil drench treatments, pyraclostrobin followed by azoxystrobin were the most effective fungicides. However, these two fungicides can also be phytotoxic if applied at high rates. Relatively high fungicide rates were used in this study in order to screen the fungicides efficacies against *A. euteiches*. If they are to be developed for commercial use, much lower affordable rates should be further investigated. At the lower rates, less phytotoxic effects will be expected. Azoxystrobin has been developed for use in the Dynasty seed dressing combination (contains azoxystrobin + fludioxonil + metalaxyl) by Syngenta Crop Protection. However, it is not available for use in Australia. Recently some batches of commercial bean seed had been treated with Dynasty overseas in the U.S.A. and imported to Tasmania. Improved crop vigour and productivity has been reported in crops produced from the Dynasty treated seed. Even though azoxystrobin may slightly delay seedling emergence, no long term adverse effects were noted in the commercial crops.

The two non-chemical soil treatments with Parkway Blend and molasses were not effective against *A. euteiches*. These were both applied in the expectation that the high rates of beneficial bacteria and fungi in Parkway Blend and increased microbial activity stimulated by molasses may be able to help suppress the pathogen. It is possible that these products may work better if applied several weeks prior to sowing.

References

Watson, A., Pung, H. and Duff, J.Managing bean root and stem diseases. HAL Project VG 03002 Final Report 2007.

17. EVALUATION OF POTENTIAL TREATMENTS FOR THE CONTROL OF ARR IN A POT TRIAL

Introduction.

To determine the effectiveness of fungicides and biocontrol agents in controlling ARR on green bean.

Methods

Two pot trials were conducted in Devonport, Tasmania: the first trial was set up on 30 Dec 11 to evaluate 15 treatments for root rot control (Table 17.1) and the second trial was set up on 15 Feb 12 to determine the optimum rates of the most effective product in the first trial (Table 17.2). Naturally infected field soil collected from a paddock at Wesley Vale that had a history of severe ARR on green beans was used. Ten seeds were sown in each pot (140 mm x 145 mm pot size) and there were three replicates in the first trial and four replicates in the second trial. Pots were set up in a randomised complete block design. Plants were assessed for emergence, survival, fresh shoot weight and dry root weight.

Table 17.1. Seed and drench treatments evaluated in the first pot trial for *A. euteiches* control

No.	Product	Active ingredient	Application method	Product Rate
1	Untreated control	Nil	Nil	Nil
2	Dynasty CST®	azoxystrobin + fludioxonil	Seed treatment	2 ml/kg seed
3	Exp 042	fungicide*	Seed treatment	2 ml/kg seed
4	Previcur®	propamocarb	Seed treatment	2 ml/kg seed
5	Switch®	cyprodinil + fludioxonil	Seed treatment	2 g/kg seed
6	Fulzyme®	*Bacillus subtilis*	Seed treatment	undiluted
7	BL Bacillus	*Bacillus subtilis*	Seed treatment	undiluted
8	Fulzyme®	*Bacillus subtilis*	Drench application	50 ml/L (5% v/v)
9	BL Bacillus®	*Bacillus subtilis*	Drench application	50 ml/L (5% v/v)
10	Bio-shot®	Growth promotant	Drench application	50 g/L (5% w/v)
11	Serenade Max®	*Bacillus subtilis*	Drench application	50 g/L (5% w/v)
12	Amistar SC®	azoxystrobin	Drench application	5 ml/L (0.5% v/v)
13	BAS 651	initium + dimethomorph	Drench application	5 ml/L (0.5% v/v)
14	NC 224	amisulbron	Drench application	5 ml/L (0.5% v/v)
15	Exp 042	fungicide*	Drench application	5 ml/L (0.5% v/v)
16	sterilised soil	Nil	Nil	Nil

* Experimental fungicide

Table 17.2. Rates of Serenade Max applied in drench applications in the second pot trial for *Aphanomyces* control

No.	Product	Active ingredient	Application method	Product Rate
1	Untreated control	Nil	Nil	Nil
2	Serenade Max®	*Bacillus subtilis*	Drench application	6 g/L (0.6% w/v)
3	Serenade Max®	*Bacillus subtilis*	Drench application	3 g/L (0.3% v/v)
4	Serenade Max®	*Bacillus subtilis*	Drench application	0.3 g/L (0.03% v/v)

Results

Currently, there are no effective strategies for ARR control on beans. This study compares various potential products as well as two methods of applications (seed treatment and soil drench application) depending on the product. Seed treatment is the most cost effective and convenient method for soilborne disease control. Soil drench however distributes the product to a larger surface area and may provide longer disease control than seed treatment.

Among the seed treatments, only Dynasty CST showed potential in improving plant survival and vigour at 20 days after sowing (Table 17.3).

Among the soil drench treatments, only Serenade Max showed potential in improving plant survival and vigour at 20 days after sowing (Table 17.3). Serenade Max is a bacterial biocontrol product based on *Bacillus subtilis* and is specially formulated for use in soil applications. Serenade Max at 0.5% w/v is a very high rate, completely covering the entire surface of the soil with its carrier. In a second trial conducted to investigate its potential under lower rates, Serenade Max was only effective at the highest rate of 6% w/v. There was a rate response with increased survival and vigour at increasing rates. Other methods of application such as in-furrow application with the seed or soil incorporation should be considered to improve its efficacy under lower rates.

Fulzyme® and BL Bacillus® are also based on *B. subtilis* and they were less effective than Serenade Max in drench application. BL Bacillus had an adverse effect on seedling emergence and survival when applied as a seed treatment. Fulzyme however appeared to be more effective when applied as a seed treatment.

Amistar SC® was highly toxic in a drench application, substantially reducing seedling emergence and survival. Previous trial had shown that Amistar was moderately toxic and highly effective in reducing ARR. This trial was carried out under cooler conditions, which may have pre-disposed the seeds and seedlings to severe toxicity by the fungicide.

Table 17.3 Effects of seed and drench treatments on plants grown in *A. euteiches* infected soil**.**

No.	Product	Application method	Product Rate	% Emergence 14DAS	% Survival 20DAS	% Plant biomass 20DAS
1	Untreated control	Nil	Nil	57 bcd	37 def	35 cd
2	Dynasty CST®	Seed treatment	2 ml/kg seed	63 abc	57 abcd	52abc
3	Exp 042		2 ml/kg seed	70 abc	50 bcde	39 bcd
4	Previcur®		2 ml/kg seed	67 abc	40 cde	31 de
5	Switch®		2 g/kg seed	57 bcd	40 cde	42 abcd
6	Fulzyme®		undiluted	73 ab	53 bcd	37 bcd
7	BL Bacillus®		undiluted	33 de	13 fg	15 ef
8	Fulzyme®	Drench application	50 ml/L (5% v/v)	47 cde	27 ef	47 abcd
9	BL Bacillus®		50 ml/L (5% v/v)	70 abc	47 bcde	42 abcd
10	Bio-shot®		50 g/L (5% w/v)	63 abc	43 bcde	46 abcd
11	Serenade Max®		50 g/L (5% w/v)	80 ab	80a	58 a
12	Amistar SC®		5 ml/L (0.5% v/v)	27 e	0 g	3 f
13	BAS 651		5 ml/L (0.5% v/v)	83 a	67 ab	37 bcd

14	NC 224		5 ml/L (0.5% v/v)	73 ab	53 bcd	36 bcd
15	Exp 042		5 ml/L (0.5% v/v)	80 ab	63 abc	49 abc
16	sterilised soil	Nil	Nil	77 ab	67 ab	54 ab
LSD (P=5%)				**26.5**	**23.5**	**18.1**
P				**0.0033**	**0.0001**	**0.0001**

Means followed by same letter do not significantly differ.
Mean comparisons performed only when AOV Treatment P(F) is significant at mean comparison OSL.

Table 17.4. Effects of Serenade Max applied at three rates on plants grown in *A. euteiches* infected soil

No.	Product	Application method	Product Rate	% Emerge 12DAS	% Survive 19DAS	% Biomass 19DAS
1	Untreated control	Nil		30.0a	30.0a	18 b
2	Serenade Max®	Drench application	0.3 g/L (0.03% w/v)	16.7a	16.7a	8 b
3			3 g/L (0.3% v/v)	33.3a	40.0a	33 b
4			6 g/L (0.6% v/v)	66.7a	56.7a	87a
LSD (P=5%)				**-**	**-**	**47**
P				**0.2024**	**0.2925**	**0.0253**

Means followed by same letter do not significantly differ (P=.05, LSD)
Mean comparisons performed only when AOV Treatment P(F) is significant at mean comparison OSL.

Discussion

Among the seed treatments, only Dynasty CST® showed potential in improving plant survival and vigour in beans grown in *A. euteiches* infected soil. Among the soil drench treatments, only Serenade Max® showed potential in substantially improving plant survival and vigour. Amistar SC® was highly toxic in a drench application, substantially reducing seedling emergence and survival.

18. INVESTIGATIONS IN SELECTING AND EVALUATING THE POTENTIAL OF BIOFUMIGANT GREEN MANURE CROPS FOR GREEN BEAN DISEASE CONTROL

Introduction

This study aimed to evaluate brassica green manure varieties that may be suitable for use as a break crop under the cold climatic conditions in Tasmania. The potential of these varieties as part of an integrated pre-plant management strategy for soilborne disease control in the subsequent green beans were examined. The varieties evaluated were Indian mustard (*B. juncea*), white mustard (*Sinapis alba*), forage rape (*B. napus*), oilseed radish (*Raphanus sativus*) and Ethiopian mustard (*B. carinata*). If shown to be suitable in Tasmania, these new varieties will provide growers with a greater choice in the types of brassica green manure crops, which can be used in crop rotations to replenish organic matter as well as for soilborne disease control.

Methods

New biofumigant varieties were evaluated in Tasmania in two sites, Merseylea and Sassafras (Table 18.1). The paddock at Merseylea was known to have very high *Sclerotinia* disease pressure and low to moderate levels of black root rot. The varieties were sown in May 2009, to determine their suitability as winter break crop in paddocks that were later sown with green beans. The varieties were sown in large non-replicated blocks, each measuring 7 m x 100 m with a commercial seed drill. The rest of the paddock was sown with ryegrass at Merseylea and oats at Sassafras. At Merseylea, two by one metre quadrats in the middle of each of the biofumigant crops were assess for plant density, biomass and height. The crops at Sassafras were not assessed for plant biomass and height as they were slashed by the grower before they could be assessed, because of great concern of cross pollinations by bees with a brassica seed crop in the vicinity. The green manure crops were not irrigated and relied on rainfall, while the green bean crop was irrigated. The sequences of events are described in Table 18.2. An analysis of variance was conducted on the results using ARM and Statgraphic Plus 2.0, and pairwise comparisons were made of the mean values using Least Significant Difference Test (LSD).

Table 18.1. Biofumigant plant varieties

Variety Name	Scientific Name	Common Name
Architect	*Sinapus alba*	white mustard
Abraham	*Sinapus alba*	white mustard
Attack	*Sinapus alba*	white mustard
Mustclean	*Brassica juncea*	Indian mustard
B. carinata	*Brassica carinata*	Ethiopian mustard
BQ Mulch	*Brassica napus + B.campestris*	rape + turnip
Greenland	*Brassica napus*	rape
Adios	*Raphanus sativus*	oilseed radish
Arena	*Raphanus sativus*	oilseed radish
Doublet	*Raphanus sativus*	oilseed radish

Table 18.2. Chronology of events

Date	Chronology of Events
Merseylea, Tasmania	
22/05/09	Biofumigant varieties sown in large single non-replicated plots.
05/08/09	Plant density recorded.
16/10/09	Assessed plant biomass.
01/11/09	All biofumigant varieties slashed and incorporated into soil.
14/12/09	Green beans cv. Flavor Sweet sown.
01/02/10	1st application of Filan, followed by another two applications at 7 day intervals.
18/02/10	White mould disease assessment.
Sassafras, Tasmania	
18/05/09	Biofumigant varieties sown in large single non-replicated plots.
11/10/09	All biofumigant varieties slashed early to prevent cross contamination of a brassica seed crop nearby.
08/12/09	Green beans cv. Orlando sown.
02/02/10	Obvious differences noted in the growth of green beans.
17/02/10	Assessed for plant biomass and yield of green beans prior to commercial harvest.

Results

Evaluations of biofumigant crops before planting green beans

The biofumigant varieties showed difference in their tolerance to frost conditions (Table 18.3). The white mustard, Architect and Abraham were highly susceptible to frost damage, whereas Attack was tolerant to it. Mustclean, an Indian mustard, was moderately tolerant. All the oilseed radish varieties, Adios, Arena and Doublet, and BQ Mulch were highly tolerant.

Table 18.3. Observations on the growth of the biofumigant varieties sown in winter and spring in 2009

Variety name	Common name	Frost tolerance (May – August)	Crop maturity		Growth habit
			Sown in May, flowering in October	Sown in October, flowering in December	
Architect	white mustard	Very low	Mid	Early	Tall single stem
Abraham	white mustard	Low	Mid	Early	
Attack	white mustard	High	Early	Early	
Mustclean	Indian mustard	Medium	Late	Mid	
B. carinata	Ethiopian mustard	-	-	Late	Short rosette
BQ Mulch	rape + turnip	High	Late	Mid	Medium multi stem
Greenland	rape	-	-	Late	
Adios	oilseed radish	High	Early	Mid to late	Short rosette
Arena	oilseed radish	High	Early	Mid to late	
Doublet	oilseed radish	High	Early	Mid to late	

All the brassica green manure crop growth was relatively slow during winter, but plant biomass increased rapidly at the onset of warmer spring conditions in September. Oilseed radish varieties, rape and rape/turnip appeared to be better suited for sowing in the autumn and winter, when frost conditions may occur in Tasmania. Early sowing in March and April, prior to the onset of the very cold conditions may be more conducive to growing oilseed radish, rape and turnip varieties for greater plant biomass. The mustard varieties were more suitable for sowing in spring, where they can establish and grow rapidly. With green manure crops sown in winter, the white mustards (Architect, Abraham and Attack) and oilseed radishes (Adios, Arena and Doublet) matured about the same time. The Indian mustard (Mustclean) and rape/turnip (BQ-Mulch) matured about two weeks later.

Currently, on many farms, ryegrass is the most commonly used break crop in autumn and winter, between vegetable crops in spring and summer, in Tasmania. The brassica green manure crops produced vastly different root systems compared to the ryegrass root systems. Ryegrass produces fibrous root systems, while the brassica crops produced a main tap root, with or without lateral root

branching as well as fine fibrous roots. Oilseed radish and *B. carinata* have enlarged tap roots and are recommended for use to reduce soil compaction. The soil benefits from each type of root systems are expected to be different. Therefore, the use of different types of break crops is preferable to using only one type of green manure crop. In Europe, the use of combinations of two or three brassica green manure varieties are recommended to growers in order to obtain the multiple benefits of their different root structures.

At Merseylea and Sassafras, plant density and growth were also affected by the soil type and water logging under wet winter conditions. At Sassafras, the biofumigant crops had high plant densities and produced relatively large plants in the well-drained deep red ferrosol soil in the northern part of the site compared to the sparse and stunted plants in the poorly drained duplex soil in the southern part of the site. At Merseylea, the plant density of Attack was reduced by approximately 50% when it was assessed for plant biomass at flowering (Table 18.4), because of foot rot due to saturated soil conditions after frequent rainfall in September and October.

Plant biomass was not available for the brassica green manure crops at Sassafras, because the crops were slashed by the grower on a Sunday, one day before the crops were scheduled to be assessed for plant density and biomass, because of concerns of cross pollination and contamination by bees of a cauliflower seed crop that was nearby. This highlights the precaution that growers need to consider, when growing biofumigant crops to ensure that there is no brassica seed crop nearby.

At Merseylea and Sassafras, the green manure crops were incorporated in October 2009, and green beans were sown in December 2009. At 3 months after the green manure crops had been slashed and rotary hoed into the soil, there was still a lot of undecomposed crop residue at Merseylea in late January 2010. But there was little or no crop residue at Sassafras. This was not surprising as the crop residue fragments were much larger at Merseylea compared to those at Sassafras. At Merseylea, many of the tap roots of oilseed radish remained intact and contained sclerotia of *S. sclerotiorum*. No sclerotia were found in the mustard or rape crop residues. Before incorporation, only very low levels of infected plants of less than 0.1% were noted in the biofumigant crops at Merseylea. No *Sclerotinia* was noted at Sassafras in the biofumigant crops, before or after incorporation. Chopping the plants into fragments is not sufficient to extract the full benefits of the biofumigation process. Brassica plant tissue must be broken down at cellular level with a mulching implement that is equipped with hammers to pulverize the above plant material to generate the biofumigant compounds (Matthiessen & Kirkegaard 2006). Large pieces of undecomposed brassica crop residue may re-grow or become colonized by the *Sclerotinia* pathogens.

Evaluations of the subsequent green bean crops

The bean crop at Merseylea was irrigated with a centre pivot irrigator, and there was no visual difference in the green bean plant growth between inside and outside the biofumigant crop site. When the green beans were ready for harvest, the white mould incidence at this site was very high, ranging from 63% to 88% plants infected (Table 18.4). The disease incidence outside the trial site was similar. However, we noted that the white mould severity in the whole biofumigant site was much higher with many wilting plants compared to fewer wilting plants outside the trial area. Green beans in the paddock, including the trial site were treated with three commercial applications of Filan at 1 kg/ha. The biofumigant crops did not reduce the disease incidence, and instead, appeared to cause the disease to spread more rapidly on the infected plants. This is believed to be due to the higher crop residue and greater water retention in the green manure crop area, hence creating a more ideal environment for the rapid spread of the disease in infected plants at close to harvest. There was little or no root rot noted on the bean roots. Therefore, no root rot assessment was carried out in the trial at this site.

At Sassafras, the green bean crop was irrigated with travelling gun irrigation. The growth of green bean plants in the biofumigant area was more vigorous with larger plants compared to the areas previously sown with oats in the same paddock. The differences in green bean plant growth were recorded in the plant biomass assessment (Table 18.5). In field observations, the soil beneath the bean plant canopy was moist in the biofumigant area. In the oat area, soil surface was dry and compacted. The surface soil temperature between the rows was measured with an infra-red heat sensor was considerably different: 20-21^{o}C in between the large plant rows in the biofumigant crop area versus 35-40^{o}C in between the small plants in the oat area. Changes in soil and crop conditions due to the biofumigant crops appeared to promote growth of the bean crop. Unfortunately, white mould caused by *Sclerotinia sclerotiorum* disease is also favoured by the moist soil conditions. In the biofumigant area, there was a small hot spot area with many *Sclerotinia* infected plants. Elsewhere, there was no white mould disease. This indicates that changes in crop management strategies, particularly with irrigation intervals and fertiliser application, may have to be considered following biofumigant green manure crops. All roots were affected by black root rot and the black rot severity was moderate. The root severity of bean roots planted in the oat area was similar to the biofumigant crops. This indicates that the biofumigant crops did not reduce black root rot but may have increased plant tolerance to the disease instead through changes in soil conditions and crop vigour.

Table 18.4. Biofumigant crop growth over winter and spring in 2009 at Merseylea

	Biofumigant crops						Green bean crop
Variety Name	**Sowing Rate kg/ha**	**Plant density/m2**	**Average crop height (m)**	**Biomass plant density/m2**	**Biomass fresh weight (kg/m2)**	**Biomass dry weight (kg/m2)**	**White mould % Plants infected**
		05/08/09	16/10/09	16/10/09	16/10/09	16/10/09	18/02/10
Architect[1]	10	37	1.00	27	0.55	0.14	63
Abraham	10	44	1.50	30	2.18	0.46	85
Attack	10	116	1.80	56	3.18	0.73	74
Mustclean	10	123	1.40	59	3.04	0.50	83
BQ Mulch	10	142	1.50	123	6.18	0.86	88
Adios	15	56	1.60	45	8.32	1.52	82
Arena	15	96	1.50	55	n/a	n/a	88
Doublet	15	97	1.40	75	6.48	0.99	75

[1] Sparse and stunted plants due to frost

Table18.5. Effects of biofumigant crops sown in winter on the subsequent green bean crop at Sassafras

Variety	**Sowing Rate kg/ha**	**Bean plant biomass (09/02/10)**		**Black root rot severity Root rot index (0-5)**
		Fresh weight (kg/20 plants)	**Dry weight (kg/20 plants)**	
Oat - control		1.83	0.48	2.5
Architect	10	2.14	0.57	2.8
Abraham	10	2.36	0.66	2.8
Attack	10	2.02	0.50	2.8
Mustclean	10	2.48	0.57	2.9
Adios	15	2.53	0.77	2.6
Arena	15	2.02	0.52	2.8
Doublet	15	2.03	0.52	2.8

Discussion

The white mustards, Architect and Abraham were highly susceptible to frost damage, whereas Attack was tolerant to it. Mustclean, an Indian mustard, was moderately tolerant. All the oilseed radish varieties, Adios, Arena, Doublet, and BQ Mulch were highly tolerant. Plant stems and root growth were affected by plant density, soil type and drainage. Increases in the biomass of green bean plants were recorded at one of the two sites because of changes in soil conditions due to the biofumigant crops. Increases in the crop vigour of the subsequent green bean plants however would also pre-dispose them to white mould. The biofumigant crops did not reduce black root rot but appeared to increase green bean plant tolerance to the disease as a result of increased crop vigour.

Reference

Matthiessen, J. & Kirkegaard, J.A., 2006. Biofumigation and enhanced biodegradation: opportunity and challenge in soilborne pest and disease management. Critical Reviews in Plant Sciences, 25: 235-265.

GENERAL DISCUSSION

Aphanomyces root rot (ARR) causes browning of roots and stems of green beans, and in severe cases causes death of plants often in combination with other pathogens. ARR on its own reduces yield directly or by delaying flower set and making machine harvesting impossible. Trials conducted gave an indication on the severity of ARR and the potential that it has to wipe out whole plantings if conditions favour disease. Using a pre planting assessment is a high priority for growers where the potential of infection from ARR is high, as it provides important information on disease levels before planting.

One of the important findings from this project was the number of farms in Tasmania that had ARR with 30 out of 42 soils examined showing to be positive for the organism. It had first been found in Tasmania through the project VG03002 “Managing bean root and stem diseases”. The case that bean root rot has been considered previously as a complex makes bean root rot control difficult. And to define the pathogens contributing to the root disease complex and their specific roles is needed. The role of *A. euteiches* in the bean root rot complex has therefore been clarified.

Hymexazol, the main ingredient in the product Tachigaren® marketed by Sankyo Co. Ltd, Tokyo, Japan (now Daiichi Sankyo Co. Ltd.), shows good efficacy against ARR but it is not available and unlikely to ever be in Australia. This product was made available for the sugar beet industry in the USA (Harveson *et al*. 2007). Sugar beet is affected by *Aphanomyces cochlioides* in many growing regions of the world and the industry successfully gained access to Tachigaren® as a seed dressing to aid in its control. Tachigaren® has registration on crops in Hungary including, cucumbers, green peppers, melons, onions, peas, rice, soybeans, sugar beet and tomatoes.

The fungal group that are responsible for the inability to establish a good stand of beans are *Pythium* species, which cause damping off and root rot and the use of seed dressings to target these fungi needs to be maintained. These seed dressings are generally based on metalaxyl products that have good efficacy against *Pythium* species but not *A. euteiches*, but having an alternative such as Tachigaren® which may control *Pythium* as well as *A. euteiches* is needed.

Soil borne diseases are difficult to control. General control options include avoidance of infected fields, growing alternate crops that are not affected by the disease, fumigation and growing resistant varieties. Bean varieties are developed overseas for the Australian market. There have been attempts to develop some resistance to ARR without success or with success but the end product is not up to market expectations. Varieties have been identified in the course of this project that are much stronger

than other varieties and able to resist disease better but they often do not produce the type of bean for the market.

There are some control options that can reduce ARR but they may be difficult to consider financially or mechanically. Fumigation is possible but often not economic to consider for beans. Some soil types may be too heavy for fumigation. Planting on beds or hills should be considered to provide better water runoff, reducing the conditions conducive to infection. Careful monitoring of irrigation is critical so as not to over water.

Brassica biofumigants were shown within the project to reduce disease levels. ARR was reduced in NSW trials, but in Tasmania plants were shown to be stronger but no clear reduction in disease levels. Results from Victorian trials (Anonymous, 2010) suggested control of soil borne diseases of beans was improved through the use of brassica biofumigants high in glucosinolates. Caliente, which is an Indian Mustard, has been adopted as a rotation for diseases suppression by some growers.

The DNA test developed gives some confirmation on the presence of *A. euteiches*. Growers should consider having a soil bioassay done for ARR as well as the DNA test for confirmation on observed symptoms within the bioassay. Brown hypocotyls and roots not only mean ARR they can be black root rot or types of *Pythium* as well. *A. euteiches* can build up in quantity rapidly given suitable conditions, from a low base level it can move throughout the crop if wet conditions continue. Zoospores can infect roots and roots can be populated by enormous numbers of oospores to infect in the current season or to survive for future seasons. Thus beans cannot be planted on the same ground regularly, less so in wetter bean growing regions.

There was no infection of peas with the isolates of *Aphanomyces euteiches* that had been isolated from beans, indicating conclusively that this is *Aphanomyces euteiches* f.sp. *phaseoli,* a type specific to beans. For growers who grow beans that are infected by ARR, there will be no cross infection with peas, providing they do not have *Aphanomyces euteiches* f.sp. *pisi*. It would be useful to conduct cross infection studies with other isolates of *Aphanomyces euteiches* and conduct genetic comparisons between the isolates.

The region where these trials were conducted in both NSW and Tasmania have a long history of growing beans. The NSW region has high rainfall, especially during the period when beans are grown; rainfall of over 100mm in one day is not uncommon. The normal cropping system in that region is based around cattle/pasture/beans. The system is a good one, pasture is plentiful for cattle due to regular rainfall, and bean plants remaining after harvest can be cleaned up by the cattle. However the

part of the bean that contains most of the fungal survival structures are in the roots, which remain behind.

A management system that would improve the breakdown of this plant material is needed. One that could be considered is a thorough working up of any remaining plant material, planting a brassica biofumigant immediately, allowing it to flower then working in and then converting back to pasture, and left for as long a period as possible. On bringing out of pasture, prepare the ground well in advance, planting another brassica biofumigant, working this in and prepare for planting. The brassicas also provide some weed control. The optimum time to reincorporate into the soil is at flowering, this is the time period of highest biofumigant effect and also stops seed set. The frost consequence of the biofumigants as recorded in Tasmania must also be considered.

Planting on beds or hills should be also examined as this achieves better drainage for the bean plants. Some growers have looked at controlled traffic options and this is a good idea. Compaction of soil does not allow adequate root penetration and can lead to water logging.

The use of azoxystrobin (Amistar®) as a soil drench has shown some activity against ARR. It has been demonstrated in the trials that it can cause some phytotoxicity to seedlings, but in field trials if applied immediately at sowing as a soil drench on top of the planting row it reduces disease. The fungicide has a broad spectrum of activity, against other fungi such as *Pythium, Rhizoctonia* and *Sclerotinia* and many leaf affecting fungi such as the powdery and downy mildews.

The soils where work has been done on ARR (NSW) were considered to be healthy soils, all other factors when considering healthy soils were there, good structure, adequate pH, high levels of biological activity, earthworms present, but the presence of *A. euteiches* refutes the definition that it is a healthy soil for beans. Growers were able to grow alternative crops for example corn, zucchinis and peas without any disease issues, the soils were “healthy” for these crops.

Antagonism by bacteria to *A. euteiches* has been demonstrated in the greenhouse situation but transferring this to the field was not successful. Products such as Fulzyme® and Serenade Max® containing bacteria (*Bacillus* species) improved bean plant establishment in pots, but their affect on ARR disease expression was not as successful. The use of bacteria to control soil borne diseases is a viable alternative and more environmentally friendly than fungicides. The soils that ARR occurs is quite likely full of antagonistic bacteria but pushing the balance from the pathogenic fungi to antagonistic bacteria may well be the key to reducing the attack from soil borne diseases.

Black root rot (*Thielaviopsis basicola*) is a troublesome disease in many crops and has been found in Victorian and Tasmanian bean growing regions. It has been observed in NSW but it is normally associated with crops later in the season (cooler) and is not of concern. This disease is of ongoing concern for the cotton industry. For southern states with cooler conditions during the growing season the crops will be more at risk to the disease. Controlling this disease would be best with resistant varieties and some varieties tested in trials showed not so much reduced disease levels but better tolerance to black root rot through improved vigour. However for the same reasons as mentioned for ARR these varieties may be not up to the market standard. The work undertaken in this project has given a better picture of the spread of this disease and its impact on the bean industry. A DNA test for black root rot would be a valuable consideration; it affects many vegetables and cotton.

During trials azoxystrobin (Amistar®) was shown to reduce the effect of *Macrophomina phaseolina* - Charcoal rot (ashy stem blight) on stem infection. The results were very good with a clear reduction of disease recorded.

A permit/registration for azoxystrobin as a soil drench when planting beans should be considered to reduce the impact of soil borne diseases.

RECOMMENDATIONS

Further research is needed on the effects of brassica biofumigants on soil borne disease. This would include screening varieties in different growing regions and soil types on soils known to have soil borne diseases.

A DNA test for black root rot would be a valuable consideration; it affects many crops and a collaborative project to develop one between the cotton industry and vegetable industry would benefit both parties.

It would be useful to conduct cross infection studies with other isolates of *Aphanomyces euteiches* that are sourced from other hosts and conduct genetic comparisons between the isolates.

Examine the effect of azoxystrobin as a soil drench on black root rot.

Fungicide options for Sclerotium stem rot (*Sclerotium rolfsii*) need to be investigated.

The depth of planting and various planting dates could be examined if there is an affect on disease.

TECHNOLOGY TRANSFER

Vegetable Pathology Program Workshop.

Presentation at Vegetable Pathology Program Workshop, Best Western Airport Motel and Convention Centre, 33 Ardlie Street, Attwood, Vic., November 27th and 28th, 2008.

The project and current disease information was presented to attendees at the meeting. Those present included plant pathologists, extension staff and agricultural consultants.

NSW IDO Newsletter.

A contribution was made to the NSW Industry Development Officer (IDO) newsletter indicating the beginning of the project. This was also distributed to other states IDO newsletters.

Ausveg Conference Booklet

Booklet page prepared for Ausveg Conference in Melbourne

Field officer/industry meeting in Devonport Tasmania, 6th August 2010.

Report was presented on the results of the soil assessments with the Tasmanian soils, presented handout with soil borne disease information of beans.

Poster at the Australian soil borne diseases symposium, Queensland, 9th -11th 2010

Watson A, Browne L and Snudden M (2010) Root rot of green beans caused by *Aphanomyces euteiches*: symptoms detection and management implications. Soil borne diseases symposium.

Report at the grower meeting in Gympie, Queensland, 11th August, 2010.

Handout prepared and distributed. Appendix 3

Report at the grower meeting in Gatton, Queensland, 12th August, 2010.

Handout prepared and distributed. Appendix 3

Queensland Visit

Andrew Watson visited two industry agronomists/growers in the Gatton area of Queensland to discuss bean disease issues mainly regarding Sclerotium stem rot (*Sclerotium rolfsii*) on beans and carrots. Terraclor®, which gave good control of *Sclerotium rolfsii*, has been withdrawn. There are no alternative control options for this disease however there is a permit for Amistar® use in peanuts for *Sclerotium rolfsii*, this should be investigated for the same disease on beans. There was also discussion on other disease issues in vegetables.

Plant Pathology Conference

Poster was presented at the Australian Plant Pathology Conference

Watson A, Pung H, Browne SL, Snudden MG, Cross S (2011) Soilborne diseases of green beans in Australia, significance and management. Poster presentation. Australasian Plant Pathology Conference, Darwin, April.

Australasian Plant Pathology Society-Pathogen of the month

A short article on ARR was written for the 'Pathogen of the Month' (December 2011). http://www.appsnet.org/Publications/potm/index.html

Scientific paper.

Watson A, Browne SL, Snudden MG, and Mudford EM Aphanomyces root rot of beans and control options. (2012) (Journal to be advised).

Victorian Grower Visit

IDO, vegetable growers and resellers were visited in Lindenow, Victoria on the 28th-30th May 2012, powdery mildew was discussed as were best management options.

Primefact 1237 Detection and management of root diseases of beans.

Appendix 2

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APPENDIX I

Fungicides used in trials and their efficacy on certain fungi

Fungicide	Group	Company	Active ingredient	Soil borne organisms affected
DM	C+D	N/A	difenconazole + metalaxyl-M	*Rhizoctonia* and *Pythium*
AFM	D+K+L	N/A	azoxystrobin + fludioxonil + metalaxyl-M	*Pythium* and *Rhizoctonia*
Hymexazol	Heteroaromatic	Daiichi Sankyo Co. Ltd.	hymexazol	*Fusarium, Aphanomyces* and *Pythium*
Amistar	K	Syngenta	azoxystrobin	*Pythium* and *Rhizoctonia*
Previcur	Y	Bayer	propamocarb	*Pythium, Phytophthora* and *Aphanomyces*

Source: Tomlin, CDS (2003). The Pesticide Manual Thirteenth Edition

Terms used in the report.

"wetting up" application of water three times a day.

"two leaf stage" after the cotyledon stage when two true leaves have emerged.

"cotyledons" first two leaves that emerge from the seed.

"hypocotyl" region of the stem between the seed and the cotyledons.

APPENDIX 2

Primefact 1237

Detection and management of root diseases of beans.

Andrew Watson, Yanco Agricultural Institute

Introduction

- French Beans, *Phaseolus vulgaris,* are an important fresh market and processing crop for Australia.

- Beans are susceptible to an array of soil-borne fungal pathogens including *Aphanomyces euteiches, Thielaviopsis basicola*, *Macrophomina phaseolina*, *Rhizoctonia solani*, and species of *Pythium* and *Fusarium*. They can be associated with bean root disease singly or in combinations, often referred to as a “complex”.

- These are all fungal organisms and beans appear highly susceptible to these pathogens but often this is dependant on conditions that favour each pathogen.

- Beans planted regularly in the same ground increase the levels of these diseases.

- Root rots caused by either *Thielaviopsis basicola* (black root rot) and/or *Aphanomyces euteiches* (Aphanomyces root rot) are common to many bean growing regions.

- This brochure has some information on Aphanomyces root rot, black root rot and charcoal rot caused by *Macrophomina phaseolina*.

- The control of these diseases is difficult and apart from fumigation there is no “silver bullet” approach to their control.

- An integrated approach is needed to manage these diseases and this guide proposes to give information on developing this management plan.

- Resistant varieties are the ultimate control option but loss of favourable agronomic traits commonly occurs with the more resistant selections.

- Although both diseases cannot be eliminated, reducing the infection by promoting healthy plants, making correct decisions on where to plant and adequate rotations will help reduce the diseases.

Horticulture Australia

July, 2012
Primefact 1237 first edition
Yanco Agricultural Institute

Symptoms

Aphanomyces root rot (Arr) causes browning of roots and lower stem. It may not kill the plant but will reduce yield and cause uneven bean maturity making machine harvesting impossible. Other fungi can affect the plants as they are weakened by Arr.

The disease needs excess moisture through over irrigation or heavy rainfall therefore good drainage is critical.

Aphanomyces infected plants are shown in Figure 1.

Figure 1. Aphanomyces root rot (ARR) on the left and disease free on the right with healthy roots (vermiculite still present on roots).

Black root rot (Brr) also affects the roots and lower stem but the lesions appear much darker than ARR (Figure 2). Brr does not need excess water and beans are more seriously affected at cooler temperatures.

Figure 2. Black root rot (BRR) showing black lesion on the lower stem and root.

Charcoal rot (also called ashy stem blight) can seriously affect beans causing plant death and loss of vigour.

Figure 3. Charcoal rot lesion up the stem.

The obvious symtpoms is a brown lesion often only on one side of the stem (Figure 3). Disease is worse in hot conditions especially when the surface of the soil is hot, 27ºC in the top 2.5cm.

How these fungi cause disease

Aphanomyces has two types of spores. One type (zoospores) requires water to move through and infect roots. The other type (oospores) are round, tough and survive in roots and soil for long periods (Figure 4).

Oospores can germinate to produce zoospores to start infections. Zoospores can also be produced from the hyphae (fibrous structure of the fungus).

There is no varietal resistance to this disease; however more vigorous varieties resist the disease better.

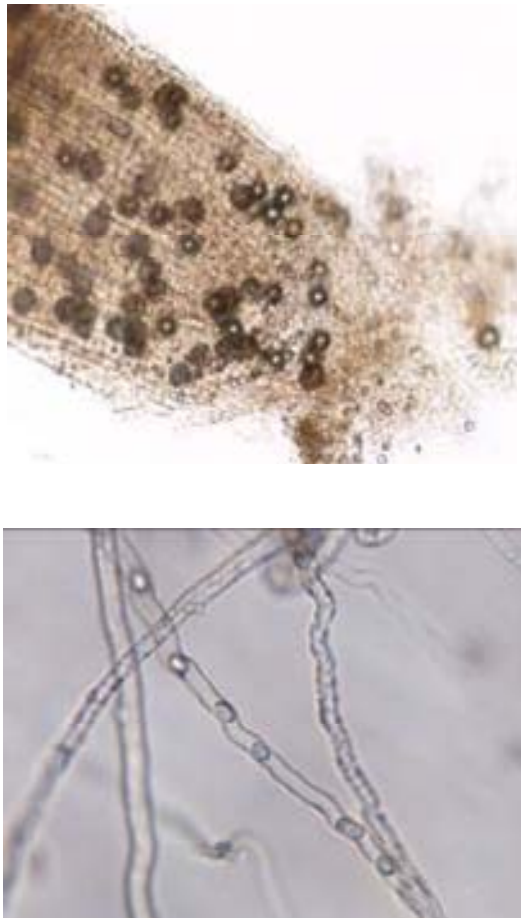

Figure 4. Aphanomyces oospores (top) and zoospores (bottom).

Black root rot does not have swimming spores although it does have two spore types, one for infection within the crop and the other a long lived spore, to carry it from season to season (Figure 5). Some varieties are more susceptible to others.

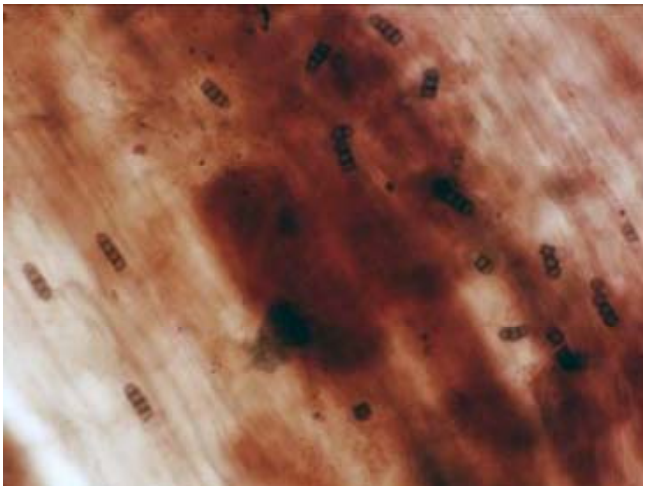

Figure 5. Black root rot spores within plant material.

Charcoal rot is noticeable by the black structures on the stem which can be either sclerotia (hard long lived structures) or pycnidia which produce spores (Figure 6). It infects many hosts but can also be seed borne. Some varieties are more susceptible than others.

Figure 6. Charcoal rot pycnidia on the stem.

How to control these diseases

Diagnose problems when they are occur to find what diseases are present. This is critical for future decision making**.** To reduce all fungal diseases allow adequate time for previous crop residues to break down thoroughly.

Aphanomyces root rot

If Aphanomyces has been diagnosed, it lasts for many years in soil therefore the land has to be kept free of beans.

- it has a narrow host range, the bean *Aphanomyces* only affects beans
- peas are not susceptible to the bean type

- have soil tested for disease levels with a soil bioassay and DNA test
- improve drainage, plant on hills or beds if practical
- reduce compaction
- increase where possible the breakdown of old crop residue especially the roots and parts of beans left in the ground
- mustards or oilseed radishes are examples of brassica biofumigants can reduce disease, so consider planting these as a rotation crop.
- give seedlings the best conditions for establishment possible
- do not over irrigate
- heavy rain causes more infection
- bacteria common in soils are very antagonistic to *Aphanomyces*. Bacteria populations are depressed by dry conditions, acidity, salinity, soil compaction and lack of organic matter
- liming has not shown a reduction in disease
- No seed dressings currently available controls ARR however it has been demonstrated that hymexazol seed dressing controls this disease but it is not available in Australia

Black root rot

- wide host range, more than 130 species, other vegetables that can be infected include carrots, peas, tomato and corn
- non hosts include cereals, sunflowers, brassicas and onions therefore use these in rotation
- improve drainage, plant on beds or hills if practical, although moisture is not required by the fungus, stressed plants can increase infection
- rotate with non hosts for up to three years avoiding legumes and controlling weeds
- biofumigant brassicas, woolly pod vetch (*Vicia villosa*) and onions can reduce disease when used as rotation crops
- avoid infected blocks
- plant so that beans do not grow in the cooler part of the growing season

Charcoal Rot (ashy stem blight)

- has a wide host range however infection occurs in hot dry weather
- allow adequate irrigation
- allow adequate breakdown of the previous crop residue before planting beans
- seed can carry the disease
- in research trials, drenching the soil with azoxystrobin controlled the disease however there is no registration for this purpose.

This project has been partly funded by HAL using the vegetable industry levy, and matched funds from the Australian Government.

Contacts

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APPENDIX 3

Handout for growers at meetings.

Root diseases of green beans.

Andrew Watson, Industry and Investment NSW, Yanco Agricultural Institute, Yanco, 2703.

With the assistance of Lee Browne, Meryl Snudden, Hoong Pung, Susan Cross, John Duff, Alan Mckay, Rob Dimsey, Rob O'Brien, Dominie Wright, Heidi Martin and many growers who have assisted in each state.

This handout is a brief update of research on soil-borne diseases of beans past and present.

French Beans, *Phaseolus vulgaris* L., are susceptible to an array of soil-borne fungal pathogens including *Aphanomyces euteiches, Thielaviopsis basicola*, *Macrophomina phaseolina*, *Rhizoctonia solani*, and species of *Pythium* and *Fusarium*. They can be associated with bean root disease singly or in combinations, often referred to as a "complex".

Aphanomyces root rot.
Bean plants on the left displaying symptoms after inoculation with *Aphanomyces*. Showing brown roots and stems and reduced root growth compared to the non-inoculated beans on the right.

VG024-BEAN ROOT ROT AETIOLOGY AND CONTROL

Rob O'Brien and Dominie Wright (QDPI) undertook work into bean root diseases concentrating on the Gympie district (VG 024-1999) on a disease known as "red root". A disease that occurred in the cooler parts of the year.

They considered that a complex of fungi were involved in the "red" root issue and found fungi including *Rhizoctonia*, *Fusarium solani*, *Fusarium oxysporum*, *Aphanomyces euteiches* and *Pythium*. They considered that *Pythium* was the most likely main cause of "red" root especially in earlier plantings.

They identified that shallow planting reduced disease and also that the soil type from Gympie (red Kraznozem) was far more prone to disease than a soil collected from Cooroy (red earth).

Black Root Rot (*Thielaviopsis basicola*).

Charcoal rot (*Macrophomina phaseolina*) (black lesion) in combination with *Tobacco yellow dwarf* virus (bean summer death).

VG 03002-MANAGING BEAN ROOT AND STEM DISEASES.

Project VG 03002 was to carry on from VG024, but to also cover New South Wales and Tasmania.

Disease surveys of beans were undertaken in the three regions in 2004.

QLD (Gympie)

Isolates identified included, *Fusarium solani*, *Fusarium oxysporum, Fusarium compactum, F. culmorum, Pythium sp.*, *Rhizoctonia solani*, *Sclerotium rolfsii* (Sclerotium stem rot) and *Macrophomina phaseolina* (charcoal rot).

Tasmania (Devonport)

The fungi included *Fusarium solani, Fusarium oxysporum, Fusarium compactum, F. culmorum, Thielaviopsis basicola* (black root rot), *Pleiochaeta setosa* (brown spot), *Pythium irregulare*, *P. acanthicum*, *Rhizoctonia solani* and *Aphanomyces euteiches* (first record on beans).

NSW (Mid- north coast)

Fungi isolated included *Aphanomyces euteiches Rhizoctonia solani, Fusarium oxysporum, Pythium irregulare* and *P. acanthicum.*

Further investigations within the project included control options for Aphanomyces root rot (ARR) trialling seed dressings and soil drenches. *Aphanomyces*, although similar to *Pythium* fungi, are not controlled by products such metalaxyl. A seed dressing, with the active ingredient hymexazol was found to reduce ARR and increased plant dry weight, however this product is not available in Australia.

In a pot trial, bean seeds were treated with various seed dressings and planted in known *Aphanomyces* infected soil. Bean roots and stems were rated for disease and plant weight measured (Fig.1-lower rating =lower disease).

In pot and field trials where soil drenches were applied a similar result was achieved with some commonly available fungicides (Fig.2-pot trial). In this case Azoxystrobin showed potential as a soil drench to reduce ARR.

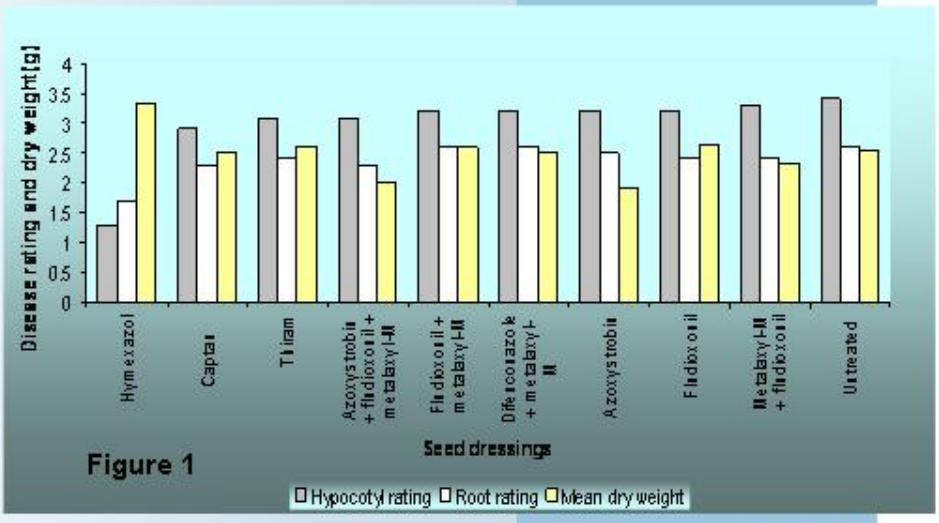

Figure 1

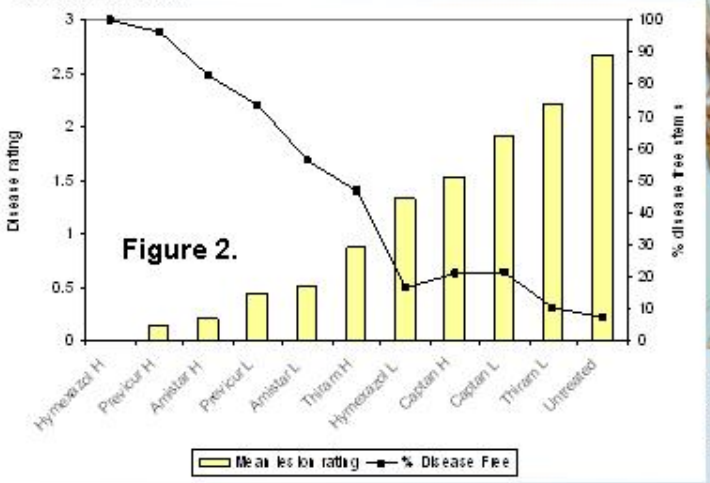

Figure 2.

Figure 3. Bean plants grown from hymexazol treated seed (r) and untreated beans(l). Note larger root mass and increased nodulation.

VG 08044-DEVELOPMENT OF METHODS TO MONITOR AND CONTROL APHANOMYCES ROOT ROT (ARR) AND BLACK ROOT ROT OF BEANS (BRR).

Main priorities

- **This project was established to examine the incidence of ARR on bean farms in Tasmania and Victoria. ARR was common on Tasmanian bean growing soils but not so in Victorian soils. Black root rot caused by *Thielaviopsis basicola* was common to both regions.**
- **Development of a DNA soil test with SARDI to detect presence of *Aphanomyces* in soil. This has required development of the test and validation. Soils are also being examined for Rhizoctonia solani groups. There are DNA soil tests for the groups of *Rhizoctonia solani* available, what type is in the soil may assist in disease management.**
- **Control options for soil borne disease of beans continued.**

Rhizoctonia lesion on a bean stem.

Black root rot caused by *Thielaviopsis basicola*

•*T. basicola* is favoured by a soil temperature of 28°C but damage to beans most severely occurs at lower temperatures of 15-20°C.

•*T. basicola* has been recorded infecting over 130 species of plants. Non hosts include cereals, sunflowers, brassicas and onions.

•Control options for Black root rot include avoidance of infected fields, long rotations, planting at a time to reduce exposure to cool conditions, using rotation crops and biofumigant crops, use of fungicides (although these are very limited), use of plant activators, and where available resistant or tolerant cultivars (Figure 4).

Figure 4. Black root rot has affected the variety on the right more than that on the left.

VG 08044-continued

Charcoal rot (*Macrophomina phaseolina*) control.

- In a soil drench trial examining the effect of molasses(3 rates), potassium silicate and Amistar® both at two rates, root disease due to charcoal rot was reduced (Figures 5 and 6). Disease levels also varied between varieties.

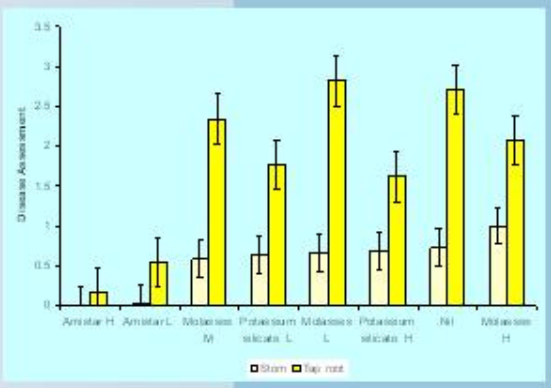

Figure 5.Left. Clean bean stems on the left from the Amistar® treated plots and untreated on the right. The main contributor to disease was charcoal rot.

Figure 6. Right. Disease assessments for each of the treatments.

Conclusions

•Beans are affected by many soil borne fungi and any work on any one fungus should include others in control and assessments etc.

•A soil drench with the fungicide Amistar® has been shown to reduce the effects of some of these fungi, however there is no registration for this use.

•Bacteria are antagonistic to these fungi and improving bacterial levels in soil may reduce fungal populations.

•Make sure you are aware of what fungi are associated with beans in your region. This can be achieved through greenhouse tests in pots and/or soil tests for some of the pathogens.

•Beans appear more susceptible to root disease in soils that had beans previously therefore rotation and forward planning essential.

•Varieties have shown some resistance to these diseases especially charcoal rot and black root rot.