

# **Evaluation of new seed dressing technologies for improved disease and insect control in vegetable crops**

Dr Hoong Pung  
Peracto Pty Ltd

Project Number: VG04021

## **VG04021**

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## Evaluation of new seed dressings for improved disease and insect control in vegetable crops

HAL Project VG04021

Final Report

6 July 2007

By

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## **Media Summary**

Seed treatment is the most cost-effective disease and pest control method on seed and seedlings, applied at a stage when they are most vulnerable to attack by pathogens and insect pests. Seed dressings use only a small amount of chemical, so even though the new chemicals are more expensive, their use in new seed dressings is still affordable to growers. Currently, most vegetable seed treatments rely on old broad-spectrum chemicals, such as thiram, which are indiscriminate in their target organism and could be removed from use eventually. In recent years, new chemicals that are safer and can better target pests and diseases are being developed for use in broad-acre crops like wheat and canola. This project is a two-year feasibility study conducted in 2005 and 2006 to evaluate new seed dressings in vegetable crops from the major vegetable groups of legumes (green peas and green beans), brassicas (cauliflower) and cucurbits (pumpkin).

Six new fungicide dressing formulations containing the active ingredients azoxystrobin (A), fludioxonil (F), metalaxyl-M (M) and difenconazole (D), and two new insecticide seed dressings with abamectin (Ab) or thiamethoxam (Thx), were examined at different rates to identify the appropriate concentrations for use on the different vegetable groups. These new seed dressings were compared against untreated control seeds and thiram treated seeds.

This project identified two new fungicide seed dressings, containing the active ingredients, azoxystrobin + fludioxonil + metalaxyl (AFM) and fludioxonil + metalaxyl (FM), as suitable replacements for thiram seed dressings on the major vegetable crops of green peas, green beans, pumpkin (cucurbits) and cauliflowers (brassicas).

In comparison to the standard thiram seed treatments, AFM seed treatments generally give better root rot control on the major vegetable crops. AFM was also more effective than the thiram seed treatment against *Ascochyta* infections on peas, where it prevented seed rot, and reduced collar rot. FM or seed treatment with single active seed dressings of metalaxyl-M or fludioxonil were also shown to be effective in reducing root rot severity.

Seed safety tests showed that the new fungicide and insecticide seed dressings mostly have no phytotoxic effects on garden pea, green bean, cauliflower and pumpkin seeds. Generally, the new fungicide seed dressing treatments containing azoxystrobin, fludioxonil, metalaxyl and difenconazole tend to increase seedling growth, with increased average fresh shoot weights in comparison to the untreated control and the thiram seed treatment.

There was also no obvious decline in seed viability as a result of the new fungicide seed treatments in pea, bean, pumpkin and cauliflower treated seeds after a 10-month storage period. This is important, because in practice, treated seeds are often not used immediately after treatment, and can be stored for up to one year before use.

## **Technical Summary**

Seed treatment is the most cost-effective disease and pest control method on seed and seedlings, applied at a stage when they are most vulnerable to attack by pathogens and insect pests. Seed dressings use only a small amount of chemical, so even though the new chemicals are more expensive, their use in new seed dressings is still affordable to growers. Currently, most vegetable seed treatments rely on old broad-spectrum chemicals, such as thiram, which are indiscriminate in their target organism and could be removed from use eventually. In recent years, new chemicals that are safer and can better target pests and diseases are being developed for use in broad-acre crops like wheat and canola. This project was a two-year feasibility study conducted in 2005 and 2006 to evaluate new seed dressings in vegetable crops from the major vegetable groups of legumes (green peas and green beans), brassicas (cauliflower) and cucurbits (pumpkin).

Six new fungicide dressing formulations containing the active ingredients azoxystrobin (A), fludioxonil (F), metalaxyl-M (M) and difenconazole (D), and two new insecticide seed dressings with abamectin (Ab) or thiamethoxam (Thx), were examined on green pea, green bean, cauliflower and pumpkin seeds. Various seed dressings coded according to a single or multiple actives as A, F, FM, MF, DM, AFM, Ab and Thx, were examined at three to four different rates each to identify the appropriate concentrations for use on the different vegetable groups. These new seed dressings were compared against untreated control seeds and thiram treated seeds.

### **A. Safety tests**

Seed safety tests showed that the six new fungicide seed dressings (A, F, FM, MF, DM and AFM) and their different rates of applications had no adverse effects on seedling emergence of garden pea, green bean, cauliflower and pumpkin seeds. Generally, the new seed dressing treatments containing azoxystrobin, fludioxonil, metalaxyl and difenconazole tend to increase seedling growth, with increased average fresh shoot weights in comparison to the untreated control and the thiram seed treatment.

With new insecticide seed dressings, except for the highest rate of abamectin on pumpkin, seeds treated with different rates of abamectin or thiamethoxam showed no adverse effects on seedling germination and emergence in comparison to the untreated control. Abamectin applied at the highest rate of active at 600 g ai/100 kg pumpkin seeds appeared to delay and reduce seedling emergence.

### **B. Storage effects of treated seeds**

Seed samples of the new fungicide and insecticide treatments from the initial safety test in Section A were stored for up to 10 months, and were shown to have no effects on seed viability after storage.

### **C. Efficacies of fungicide seed dressings**

The effectiveness of new fungicide seed dressings against the common damping-off pathogens, *Pythium irregulare*, *Rhizoctonia solani* and *Fusarium culmorum*, were examined on pea, bean, pumpkin and cauliflower seeds in 2005. In general, the active ingredients used in the new seed dressing combinations are highly selective against their target pathogen. Metalaxyl-M is only effective against *Pythium*, azoxystrobin and fludioxonil are effective against *Fusarium* and *Rhizoctonia*. In contrast, thiram is not selective and is effective against all of the pathogens. The high selectivity of metalaxyl-M, fludioxonil and azoxystrobin means that they are best used in seed dressing combinations in order to extend their range of pathogen control.

### **D. Evaluations of new seed dressings in field trials**

Two new seed dressing combinations, FM and AFM, were selected for further evaluation in four field trials. In a fifth trial, the effects of seed dressings that contain only one active ingredient (metalaxyl, fludioxonil or thiamethoxam only) and their combinations were also compared against the standard thiram seed treatment in a bean trial. Generally, the field trials demonstrated that the new seed dressing combinations were suitable alternatives to thiram in improving seedling establishment and reducing root rot incidence or severity. AFM seed treatments tended to give the greatest root rot control on the vegetable crops. AFM was more effective than FM or thiram seed treatment against *Ascochyta* infections on peas, where it prevented seed rot, and reduced collar rot. FM or seed treatment with single active seed dressings of metalaxyl-M or fludioxonil were also shown to be effective in reducing root rot severity.



## **Recommendations**

Research conducted in this project was meant to be a feasibility study to assist in screening potential new seed dressings. While results have been very useful in identifying effective and non-toxic rates of new active ingredients and their combinations for use on vegetables, the developments of new seed dressings for specific use in vegetable seed are still evolving to suit different industry requirements. Therefore, no recommendations for commercial use could be made at this point.

# Introduction

## Background

Seed treatment is the most cost-effective disease and pest control method on seed and seedlings, applied at a stage when they are most vulnerable to attack by pathogens and insect pests. Seed dressings use only a small amount of chemicals, so even though the new chemicals are more expensive, their use in new seed dressings is still affordable to many growers. In a scoping study (VG02105), we found that in Australia and overseas, most registered seed dressings for vegetables are based on old chemistry, while in contrast, a wide array of new chemistry has recently been registered in broad-acre crops. The use of new chemistry in seed dressing has the potential to provide more effective control of many vegetable seed, soil and air-borne diseases and insect pests, compared to old seed treatments.

Broad-spectrum chemicals, such as thiram, are indiscriminate in their target organism, have been used for the past 40 years, are increasingly seen as out-moded and could be removed from use eventually. Scientists and chemical companies have been looking at developing more sophisticated chemicals with greater accuracy at targeting the disease-causing organisms, which have been screened to ensure that they are safe and have no impact on beneficial and non-target organisms found in and around crops. The new chemicals have stringent quality control, and the development of these improved chemistries allows growers to better target pests and diseases.

Unfortunately, it has been hard to convince companies to allow new products to be tested here on vegetable seeds because they constitute a very small market in terms of seed volume compared to broad-acre crops like wheat and canola, and there isn't a great deal of financial gain to be made. Therefore, this project is the first of its kind, where the Australian vegetable industry and researchers are taking a proactive role in ensuring that new seed dressing products that have been developed for use in broad-acre crops, are also used for the benefit of the vegetable seedling industry. The participation of product manufacturers is seen as vital if we wish to get new products registered for commercial use by growers. Otherwise we will be just doing research work without the possibility of useful outcomes for the vegetable industry. This project was able to secure voluntary contributions from two companies to test and screen their new seed dressing products on vegetables for the first two years in order to identify suitable new chemistry and develop the appropriate rates of use.

Briefly, this project consists of a two-year feasibility study to evaluate and identify new chemistry for seed dressings in major vegetable crops. The study is funded by voluntary contributions from Syngenta Crop Protection Pty Ltd with matching funds from Horticulture Australia.

Major vegetable groups are considered in terms of high seed volume: legumes (green peas and green beans), cucurbits (pumpkin and squash), and brassicas (cauliflower and cabbage); minor vegetable crops are leafy vegetables (lettuces and spinach), brassica leafy vegetables (bok choy and rocket), and root vegetables (carrots and parsnips). If new seed dressings can be developed for use in major vegetable crops, applications for extended use could be made for minor vegetable crops.

## General Aims

Aims of research studies:

- Evaluate the safety of new seed dressings on seeds from three major vegetable groups;
- Determine the suitable product rates for the various types of vegetable seeds;
- Determine the storage effects of treated seeds;
- Screen the efficacies of the new seed dressings against common damping-off diseases (*Fusarium*, *Rhizoctonia* and *Pythium*);
- Investigate the effects of the new seed dressings under field conditions on major vegetable crops.

## Seed dressing product formulations

### Fungicide seed dressings

Product Code	Active Ingredient (ai)	Concentration of ai	Formulation
Thiram	thiram	800 g/kg	Wettable powder
A	azoxystrobin	100 g/L	Flowable seed dressing
MF	metalaxyl-M + fludioxonil	37.5 g/L + 25 g/L	
F	fludioxonil	100 g/L	
FM	fludioxonil + metalaxyl-M	25 g/L + 10 g/L	
DM	difenconazole + metalaxyl-M	92 g/L + 23 g/L	
AFM	azoxystrobin + fludioxonil + metalaxyl-M	75 g/L + 12.5 g/L + 37.5 g/L	

### Insecticide seed dressings

Product Code	Active Ingredient (ai)	Concentration of ai	Formulation
Ab	abamectin	500 g/L	Flowable seed dressing
Thx	thiamethoxam	600 g/L	Flowable seed dressing

## **A. Safety tests of new seed dressings**

### **Summary**

Initial safety tests of six new fungicide dressing formulations containing the active ingredients azoxystrobin (A), fludioxonil (F), metalaxyl-M (M) and difenconazole (D), and two new insecticide seed dressings with abamectin (Ab) or thiamethoxam (Thx), were conducted in 2005 on pea, bean, cauliflower and pumpkin seeds. Various seed dressings coded according to a single or multiple actives as A, F, FM, MF, DM, AFM, Ab and Thx, were examined at three to four different rates each to identify the appropriate concentrations for use on the different vegetable groups. These new seed dressings were compared against untreated control seeds and thiram treated seeds.

All the new fungicide seed dressings have no adverse effects on seedling emergence of pea, cauliflower and pumpkin seeds. Bean seed treatments generally tended to result in lower seedling emergence at the highest rate of MF, DM and AFM at 200, 260 and 200 mL/100 kg seed.

The fungicide seed treatments did not cause any obvious adverse effects on seedling growth; with no reduction in the average fresh shoot weights of pea, bean, pumpkin and cauliflower seedlings. Generally, the new seed dressing treatments containing azoxystrobin, fludioxonil, metalaxyl and difenconazole tend to increase seedling growth, with increased average fresh shoot weights in comparison to the untreated control and the thiram treated seeds. The increase in seedling growth was most obvious in peas and cauliflowers. As a result of this initial safety study, the seed dressings F, FM and AFM were selected for further study on their effects in protecting seeds and seedlings from common damping off pathogens (in Section D).

With new insecticide seed dressings, except for the highest rate of Ab on pumpkin, seeds treated with different rates of the new insecticide seed dressings with Ab or Thx showed no adverse effects on seedling germination and emergence in comparison to the untreated control. Ab applied at the highest rate of active at 600 g ai/100 kg pumpkin seeds (Ab1200) appeared to delay and reduce seedling emergence.

### **Aims**

- To determine the effects of six new fungicide dressings on pea, bean, cauliflower and pumpkin seeds on seedling emergence and growth.
- To determine the effects of two new insecticide seed dressings on pea, bean, cauliflower and pumpkin seeds on seedling emergence and growth.
- To identify the appropriate concentrations for use on the different vegetable groups.

### **Materials & Methods**

Seeds were treated with the appropriate seed dressings at different rates and compared against a standard thiram treatment and untreated seeds. Seeds were treated using a slurry treatment at 0.1 mL of seed dressing suspension per 1 g seeds, and air-dried before use.

Vegetable seeds were sown at two days after seed treatments. All seed treatments for each crop were sown in a seedling tray, with one seed per cell, as shown in the trial layout. For the fungicide seed dressing study, nine seeds of each treatment were sown in a single row on the seedling tray. For the insecticide seed dressing study, eighteen seeds of each treatment were sown in two rows on the seedling tray. There were two replicates and the results were expressed as an average of the two replicates.

At the appropriate time after sowing, when a sufficient number of seedlings had emerged, the numbers of seedlings that emerged and survived from damping-off were recorded, and the percentages of seedling emergence and survival were tabulated. For each treatment, the viable seedlings and their total fresh shoot weights were recorded at the end of the trial to determine the seedling survival rates and mean plant weight per seedling.

## A. Safety tests (Cont.)

### Seed dressings examined in the study

Product Code	Active Ingredient (ai)	Concentration of ai	Formulation
Thiram	thiram	800 g/kg	Wettable powder
A	azoxystrobin	100 g/L	Flowable seed dressing
MF	metalaxyl-M + fludioxonil	37.5 g/L + 25 g/L	Flowable seed dressing
F	fludioxonil	100 g/L	Flowable seed dressing
FM	fludioxonil + metalaxyl-M	25 g/L + 10 g/L	Flowable seed dressing
DM	difenconazole + metalaxyl-M	92 g/L + 23 g/L	Flowable seed dressing
AFM	azoxystrobin + fludioxonil + metalaxyl-M	75 g/L + 12.5 g/L + 37.5 g/L	Flowable seed dressing
Ab	abamectin	500 g/L	Flowable seed dressing
Thx	thiamethoxam	600 g/L	Flowable seed dressing

### Treatment list for fungicide seed dressings applied to green bean, green pea, cauliflower and butternut pumpkin seeds

No.	Treatment	Active ingredient	ai (g/100 kg seed)	Product Rates (per 100 kg seed)
1	UTC (Untreated control)	-	-	0
2	A50	azoxystrobin	5.0	50 mL
3	A100	azoxystrobin	10.0	100 mL
4	A150	azoxystrobin	15.0	150 mL
5	F25	fludioxonil	2.5	25 mL
6	F40	fludioxonil	4.0	40 mL
7	F50	fludioxonil	5.0	50 mL
8	FM100	fludioxonil + metalaxyl	2.50 + 1.00	100 mL
9	FM150	fludioxonil + metalaxyl	3.75 + 1.50	150 mL
10	FM200	fludioxonil + metalaxyl	5.00 + 2.00	200 mL
11	MF100	metalaxyl + fludioxonil	3.75 + 2.50	100 mL
12	MF150	metalaxyl + fludioxonil	5.63 + 3.75	150 mL
13	MF200	metalaxyl + fludioxonil	7.50 + 5.00	200 mL
14	DM130	difenconazole + metalaxyl	11.96 + 2.99	130 mL
15	DM200	difenconazole + metalaxyl	18.40 + 4.60	200 mL
16	DM260	difenconazole + metalaxyl	23.92 + 5.98	260 mL
17	AFM100	azoxystrobin + fludioxonil + metalaxyl-M	7.50 + 1.25 + 3.75	100 mL
18	AFM150	azoxystrobin + fludioxonil + metalaxyl-M	11.25 + 1.88 + 5.63	150 mL
19	AFM200	azoxystrobin + fludioxonil + metalaxyl-M	15.0 + 2.50 + 7.50	200 mL
20	Thiram	thiram	400	500 g

**A. Safety tests (Cont.)****Treatment list for insecticide seed dressings**

No.	Crop	Treatment	Active ingredient	ai (g/100 kg seed)	Product Rate* (mL/100 kg seed)
1	Peas	UTC (Untreated control)	-	-	-
2		Ab125	abamectin	62.5	125
3		Ab250	abamectin	125	250
4		Ab500	abamectin	250	500
5		Thx42	thiamethoxam	25	41.7
6		Thx83	thiamethoxam	50	83.3
7		Thx167	thiamethoxam	100	166.7
8		Thx333	thiamethoxam	200	333.0
1	Beans	UTC (Untreated control)	-	-	-
2		Ab100	abamectin	50	100
3		Ab200	abamectin	100	200
4		Ab400	abamectin	200	400
5		Thx42	thiamethoxam	25	41.7
6		Thx83	thiamethoxam	50	83.3
7		Thx167	thiamethoxam	100	166.7
8		Thx333	thiamethoxam	200	333.0
1	Pumpkin	UTC (Untreated control)	-	-	-
2		Ab300	abamectin	150	300
3		Ab600	abamectin	300	600
4		Ab1200	abamectin	600	1200
5		Thx83	thiamethoxam	50	83.3
6		Thx167	thiamethoxam	100	166.7
7		Thx333	thiamethoxam	200	333.0
8		Thx666	thiamethoxam	400	666.0
1	Cauliflower	UTC (Untreated control)	-	-	-
2		Ab667	abamectin	333	667
3		Ab1333	abamectin	667	1333
4		Ab2667	abamectin	1333	2667
5		Thx83	thiamethoxam	50	83.3
6		Thx167	thiamethoxam	100	166.7
7		Thx333	thiamethoxam	200	333.0
8		Thx666	thiamethoxam	400	666.0

\* Product rates were calculated based on the loading rate of active ingredient per seed. Product rates varied depending on the crop and its seed size.

## A. Safety tests (Cont.)

### Trial layout

#### Layout of fungicide seed treatment study

		Treatment No.																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

#### Layout of insecticide seed treatment study

		Treatment No.																			
		1	2	3	4	5	6	7	8												
X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

The trial layout of the seed safety test on a seedling tray. Butternut pumpkin seedlings at 27 days after sowing (Photograph below).



**A. Safety tests (Cont.)****Chronology of Events****Fungicide seed treatment study**

<b>DATE</b>	<b>DAYS AFTER SOWING (DAS)</b>	<b>DAYS AFTER SEED TREATMENT (DAA)</b>	<b>EVENT</b>
25/10/04	-	0	Seeds treated with fungicides.
27/10/04	0	2	Start of safety test on treated seeds shortly after treatments. Pea, bean, pumpkin and cauliflower seeds sown.
03/11/04	7	9	Check seedling for emergence.
10/11/04	14	16	Assessment for seedling emergence. The fresh shoot weights for all crops also measured except for pumpkin.
23/11/04	27	29	Assessment for seedling survival and fresh shoot weights for pumpkin.

**Insecticide seed treatment study**

<b>DATE</b>	<b>DAYS AFTER SOWING (DAS)</b>	<b>DAYS AFTER SEED TREATMENT (DAA)</b>	<b>EVENT</b>
28/11/05	-	0	Seeds treated with insecticides.
07/12/05	0	9	Pea, bean, pumpkin and cauliflower seeds sown.
04/01/06	28	37	Assessment for seedling emergence.
18/01/06	42	51	Assessment for seedling survival and fresh shoot weights for all crops.



**A. Safety tests (Cont.)****Results****Table 1: Fungicide seed treatment effects on seedling emergence and growth of green beans and green peas when sown 2 days after seed treatments**

No	Treatment code	Rates (per 100 kg seed)	Green Peas (14 DAS)		Green Beans (14 DAS)	
			% Seedling emergence	Average fresh shoot weight (g/plant)	% Seedling emergence	Average fresh shoot weight (g/plant)
1	UTC (Untreated control)	0	100	0.549	100	1.33
2	A 50	50 mL	100	0.628	89	1.23
3	A 100	100 mL	94	0.529	89	1.46
4	A 150	150 mL	100	0.518	78	1.43
5	F 25	25 mL	100	0.663	100	1.38
6	F 40	40 mL	100	0.649	78	1.22
7	F 50	50 mL	100	0.623	83	1.30
8	FM 100	100 mL	100	0.651	83	1.38
9	FM 150	150 mL	100	0.661	83	1.66
10	FM 200	200 mL	94	0.611	72	1.52
11	MF 100	100 mL	100	0.592	83	1.58
12	MF 150	150 mL	100	0.676	78	1.53
13	MF 200	200 mL	100	0.656	67	1.44
14	DM 130	130 mL	94	0.627	67	1.54
15	DM 200	200 mL	100	0.589	78	1.56
16	DM 260	260 mL	100	0.592	56	1.39
17	AFM 100	100 mL	94	0.601	72	1.67
18	AFM 150	150 mL	89	0.543	83	1.33
19	AFM 200	200 mL	100	0.566	56	1.47
20	Thiram 500	500 g	100	0.517	89	1.12
p-value			0.7080	0.3545	0.1370	0.0914

## **A. Safety tests (Cont.)**

**Table 2: Fungicide seed treatment effects on seedling emergence and growth of butternut pumpkin and cauliflower when sown 2 days after seed treatments**

No	Treatment code	Rates (per 100 kg seed)	Butternut Pumpkin			Cauliflower	
			(14 DAS)	(27 DAS)	(27 DAS)	(14 DAS)	(14 DAS)
			% Seedling emergence	% Seedling emergence	Average fresh shoot weight (g/plant)	% Seedling emergence	Average fresh shoot weight (mg/plant)
1	UTC (Untreated control)	0	17 de	44	0.76	100	77 g
2	A 50	50 mL	11 e	39	0.81	100	91 cdefg
3	A 100	100 mL	28 cde	44	1.14	94	81 fg
4	A 150	150 mL	33 cde	72	0.95	94	90 defg
5	F 25	25 mL	33 cde	56	0.88	100	93 bcdefg
6	F 40	40 mL	33 cde	56	1.07	94	95 bcdef
7	F 50	50 mL	39 bcde	72	1.09	100	93 bcdefg
8	FM 100	100 mL	56 abc	61	1.27	100	89 defg
9	FM 150	150 mL	39 bcde	78	0.95	100	103 abcde
10	FM 200	200 mL	56 abc	61	1.36	89	106 abcd
11	MF 100	100 mL	50 abcd	56	0.68	89	104 abcde
12	MF 150	150 mL	72 ab	89	1.07	100	108 ab
13	MF 200	200 mL	61 abc	67	1.02	94	100 abcde
14	DM 130	130 mL	50 abcd	61	1.08	94	104 abcde
15	DM 200	200 mL	50 abcd	67	1.02	100	114 a
16	DM 260	260 mL	83 a	89	1.09	100	107 abc
17	AFM 100	100 mL	72 ab	78	1.31	94	88 efg
18	AFM 150	150 mL	72 ab	89	1.22	100	91 cdefg
19	AFM 200	200 mL	72 ab	72	1.27	94	104 abcde
20	Thiram 500	500 g	72 ab	72	1.19	100	82 fg
p-value			0.0156	0.3415	0.3657	0.6677	0.0068

## **A. Safety tests (Cont.)**

**Table 3: Insecticide seed treatment effects on seedling emergence and growth**

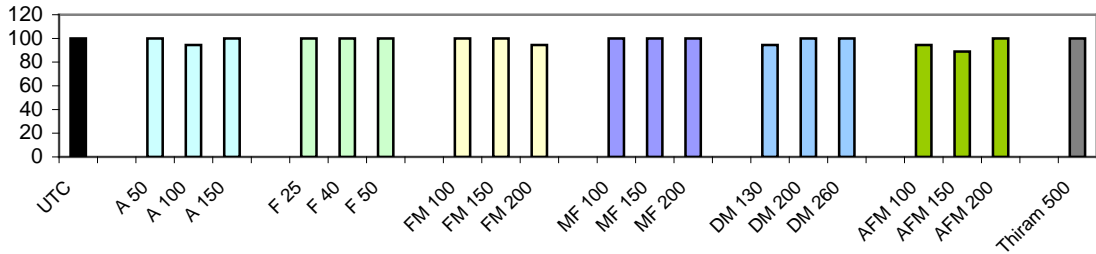
No.	Crop	Treatment	ai * (g/100 kg seed)	Product Rate* (mL/100 kg seed)	% Seedlings emerged (28 DAS)	% Surviving seedlings (42 DAS)	Average fresh shoot weight g/plant (42 DAS)
1	Peas	Untreated control	-	-	89	89	1.589
2		Ab125	62.5	125	92	89	1.475
3		Ab250	125	250	94	94	1.435
4		Ab500	250	500	94	94	1.418
5		Thx42	25	41.7	92	89	1.710
6		Thx83	50	83.3	97	97	1.469
7		Thx167	100	166.7	89	89	1.596
8		Thx333	200	333.0	89	89	1.516
p-value					0.8982	0.8542	0.5864
1	Beans	Untreated control	-	-	94	94	1.633
2		Ab100	50	100	94	92	1.722
3		Ab200	100	200	94	92	1.729
4		Ab400	200	400	92	92	1.786
5		Thx42	25	41.7	89	89	1.757
6		Thx83	50	83.3	89	83	1.798
7		Thx167	100	166.7	94	92	1.743
8		Thx333	200	333.0	89	92	1.682
p-value					0.7610	0.9449	0.9917
1	Pumpkin	Untreated control	-	-	78	89	1.741
2		Ab300	150	300	83	89	1.763
3		Ab600	300	600	69	81	1.690
4		Ab1200	600	1200	50	75	1.575
5		Thx83	50	83.3	92	92	1.896
6		Thx167	100	166.7	67	83	1.612
7		Thx333	200	333.0	94	97	1.845
8		Thx666	400	666.0	78	81	1.698
p-value					0.1175	0.4779	0.1444
1	Cauliflower	Untreated control	-	-	53	53	0.649
2		Ab667	333	667	67	67	0.524
3		Ab1333	667	1333	58	61	0.781
4		Ab2667	1333	2667	67	67	0.701
5		Thx83	50	83.3	56	56	0.538
6		Thx167	100	166.7	50	50	0.564
7		Thx333	200	333.0	53	56	0.568
8		Thx666	400	666.0	67	69	0.639
p-value					0.5530	0.5551	0.1348

\* Product rates were calculated based on the loading rate of active ingredient per seed. Product rates varied depending on the crop and its seed size.

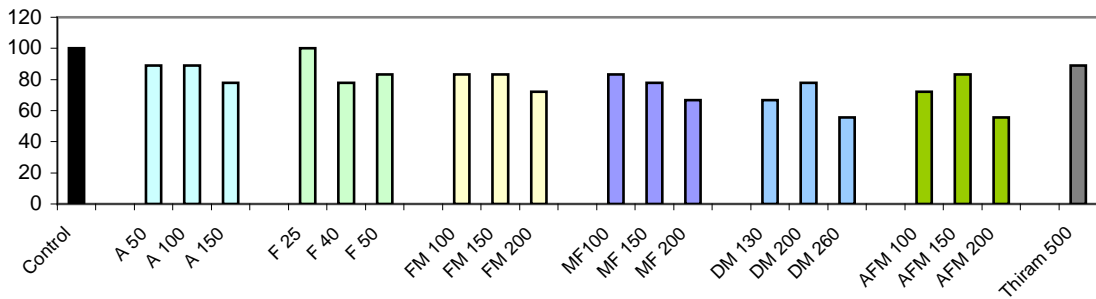
## A. Safety tests (Cont.)

**Figure 1: Effects of fungicide seed dressings on seedling emergence, when sown 2 days after seed treatment**

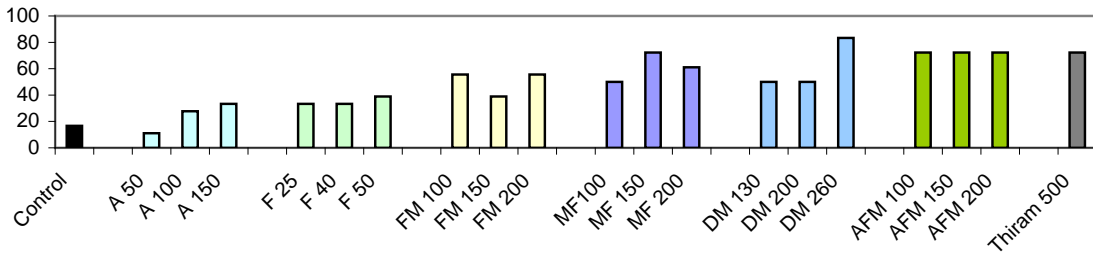
### Pea seed treatments - % seedling emergence (14 DAS)



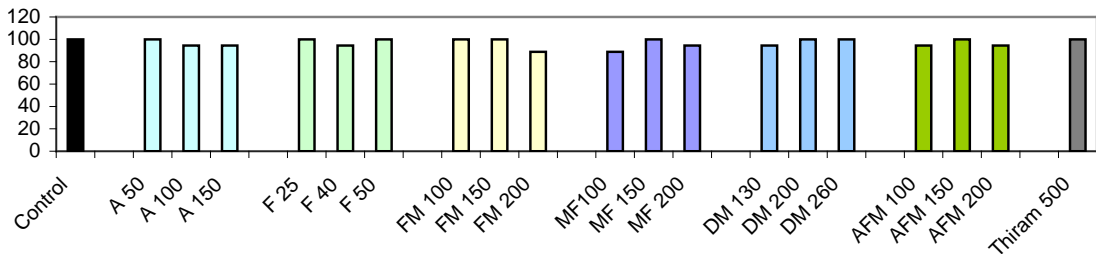
### Bean seed treatments - % seedling emergence (14 DAS)



### Pumpkin seed treatments - % seedling emergence (27 DAS)

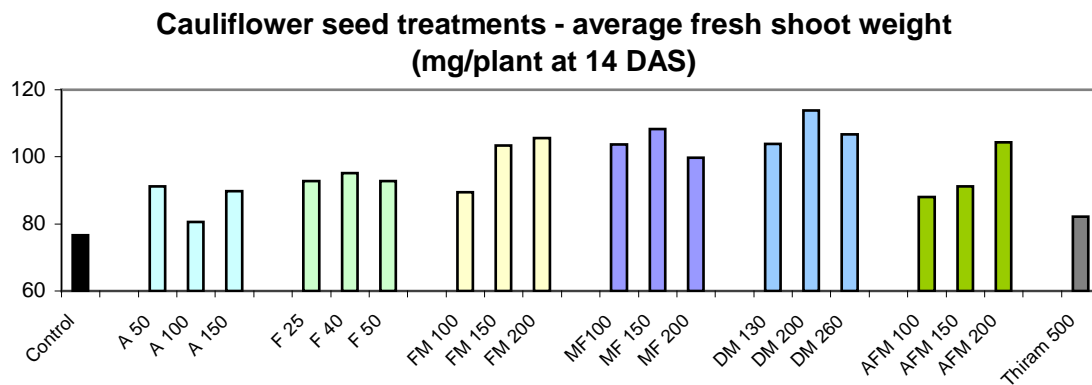
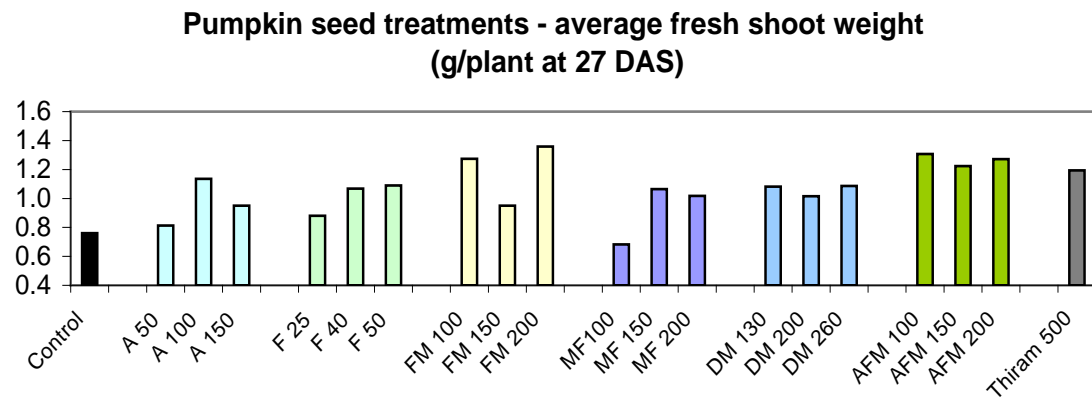
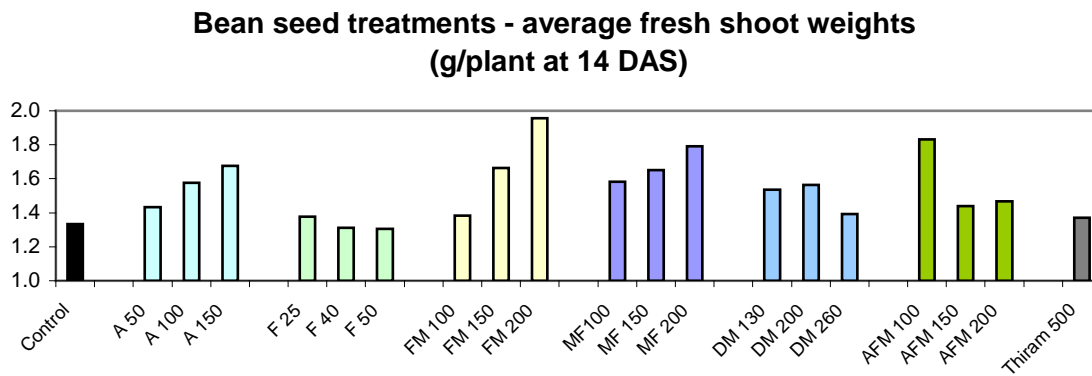
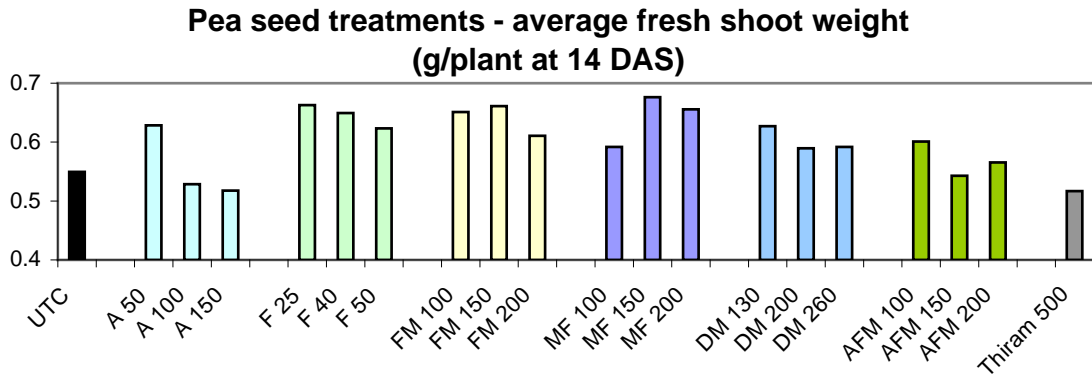


### Cauliflower seed treatments - % seedling emergence (14 DAS)



## A. Safety tests (Cont.)

**Figure 2: Effects of fungicide seed dressings on average fresh shoot weight, when sown 2 days after seed treatment**



## **A. Safety tests (Cont.)**

### **Discussion**

None of the new fungicide seed dressings or their rates of application have any adverse effects on emergence of pea, cauliflower and pumpkin seedlings (Tables 1-2). On pumpkin seeds, all new seed dressing treatments with FM, MF, DM and AFM generally improved seedling emergence (Tables 1-2, Figure 1). Although not statistically significant, bean seed treatments tended to result in lower seedling emergence at the highest rate of MF, DM and AFM at 200, 260 and 200 mL/100 kg seed (Table 1).

All new fungicide seed treatments have no adverse effects on seedling growth, causing no reduction in the average fresh shoot weight of pea, bean, pumpkin and cauliflower seedlings (Figure 2). In contrast, most of the new seed dressings tended to increase seedling growth, with increased average fresh shoot weights in comparison to untreated control and the thiram treated seeds. The increased seedling growth due to fungicide seed treatments was most obvious in peas and cauliflowers.

As a result of this initial safety study, the seed dressings F, FM and AFM were selected for further study on their effects in protecting seeds and seedlings from common damping off pathogens (refer Section D).

With the insecticide seed dressings, except for the Ab1200 treatment on pumpkin, seeds treated with different rates of the new insecticide seed dressings, with abamectin (Ab) or thiamethoxam (Thx), showed no adverse effects on seedling germination and emergence in comparison to the untreated control (Table 3). Abamectin, when applied at the highest product rate of 1200 mL/100 kg seed on pumpkin appeared to delay and reduced seedling emergence.

## B. Effects of storage on treated seeds

### Summary

Samples of fungicide treated and untreated seeds from the initial safety tests were stored for up to 10 months, and examined for any adverse effects on seed viability. Generally, there was no obvious decline in seed viability as a result of fungicide seed treatments on pea, bean, pumpkin and cauliflower treated seeds after storage.

### Aims

In practice, treated seeds are often not used immediately after treatment, and can be stored for up to one year before use. This study was, therefore, conducted to ensure that the new seed treatments have no adverse effects on seeds immediately, as well as long after, application.

### Materials & Methods

Fungicide treated seeds were stored at room temperature ranging from 15°C - 25°C, and then sown in seedling trays in seedling potting mix at 108 and 291 days after treatment applications (approximately 3.5 and 10 months). All seed treatments for each crop were sown in a seedling tray as described in the trial layout in Section A. There were two replicates and the results were expressed as an average of the two replicates. The seedling emergence after storage was compared against the emergence from seeds sown at 2 days after seed treatments (DAA).

### Chronology of Events

DATE	DAYS AFTER SEED TREATMENT	DAYS AFTER SOWING (DAS)	EVENT
			<b>2 DAA</b>
25/10/04	0	-2	All seeds treated with fungicides.
27/10/04	2	0	Start of safety test shortly after seed treatments. Pea, bean, pumpkin and cauliflower seeds sown.
03/11/04	9	7	Check for seedling emergence.
10/11/04	16	14	Assessment for seedling emergence on all crops, except for pumpkin.
23/11/04	29	27	Assessment for seedling emergence of pumpkin.
			<b>108 DAA (3.5 months)</b>
10/02/05	108	0	Start of storage effects on treated seeds at approximately 3.5 months after seed treatments. Pea, bean, pumpkin and cauliflower seeds sown.
17/02/05	115	7	Check for seedling emergence.
24/02/05	122	14	Assessment for seedling emergence on all crops, except for pumpkin.
3/03/05	129	21	Assessment for seedling emergence of pumpkin.
			<b>291 DAA (10 months)</b>
12/08/05	291	0	Start of storage effects on treated seeds at approximately 10 months after seed treatments. Pea, bean, pumpkin and cauliflower seeds sown.
26/08/05	305	14	Assessment for seedling emergence on all crops, except for pumpkin.
5/09/05	315	24	Assessment for seedling emergence of pumpkin.

**B. Effects of storage (Cont.)****Results****Table 1: Storage effects of fungicide treated seeds on seedling emergence of green beans and green peas**

No	Treatment code	Beans		Peas		
		% Seedling emergence (14 DAS)		% Seedling emergence (14 DAS)		
		2 DAA	291 DAA	2 DAA	108 DAA	291 DAA
1	Control	100	89	100	100	78
2	A 50	89	94	100	67	89
3	A 100	89	100	94	89	89
4	A 150	78	100	100	89	83
5	F 25	100	94	100	83	83
6	F 40	78	83	100	83	83
7	F 50	83	94	100	89	78
8	FM 100	83	100	100	94	78
9	FM 150	83	89	100	83	89
10	FM 200	72	94	94	94	89
11	MF 100	83	89	100	89	83
12	MF 150	78	100	100	94	94
13	MF 200	67	72	100	78	94
14	DM 130	67	100	94	94	89
15	DM 200	78	89	100	94	78
16	DM 260	56	89	100	78	72
17	AFM 100	72	100	94	100	89
18	AFM 150	83	89	89	100	83
19	AFM 200	56	72	100	94	94
20	Thiram 500	89	94	100	94	83
p-value		0.1370	0.1032	0.7080	0.2776	0.9763

DAA = days after seed treatment application at sowing.



## **B. Effects of storage (Cont.)**

**Table 2: Storage effects of fungicide treated seeds on seedling emergence of butternut pumpkin and cauliflower**

No	Treatment code	Butternut Pumpkin			Cauliflower		
		% Seedling emergence (24 DAS)			% Seedling emergence (14 DAS)		
		2 DAA	108 DAA	291 DAA	2 DAA	108 DAA	291 DAA
1	Control	17 de	78	44	100	100	100
2	A 50	11 e	56	56	100	100	89
3	A 100	28 cde	61	33	94	100	100
4	A 150	33 cde	78	22	94	94	94
5	F 25	33 cde	67	56	100	100	94
6	F 40	33 cde	56	44	94	94	100
7	F 50	39 bcde	61	39	100	100	89
8	FM 100	56 abc	89	33	100	89	100
9	FM 150	39 bcde	72	33	100	89	100
10	FM 200	56 abc	83	39	89	100	94
11	MF 100	50 abcd	67	50	89	94	94
12	MF 150	72 ab	67	39	100	94	94
13	MF 200	61 abc	72	28	94	100	100
14	DM 130	50 abcd	72	17	94	94	100
15	DM 200	50 abcd	78	22	100	100	89
16	DM 260	83 a	72	11	100	89	100
17	AFM 100	72 ab	67	33	94	89	100
18	AFM 150	72 ab	61	50	100	94	100
19	AFM 200	72 ab	78	33	94	94	100
20	Thiram 500	72 ab	72	17	100	83	94
p-value		0.0156	0.1354	0.0765	0.6677	0.8195	0.7482

DAA = days after seed treatment application at sowing.

### Discussion

Generally, there was no obvious decline as a result of fungicide seed treatments in pea, bean, pumpkin and cauliflower treated seeds after 3 and 10 months storage. The increases in the percentage seedling emergence of pumpkin seeds at 108 days after treatment application (DAA) in comparison to 2 DAA and 291 DAA appeared to be due to seasonal conditions. Seed germination and seedling emergence of pumpkin seeds are optimum under hot summer conditions at 108 DAA in February 2005 compared to the cooler conditions at 2 DAA in November 2004 and 291 DAA in August 2005.

## **C. Efficacies of fungicide seed dressings**

### **Summary**

The effectiveness of three new fungicide seed dressings against the common damping-off pathogens, *Pythium irregulare*, *Rhizoctonia solani* and *Fusarium culmorum*, were examined on pea, bean, pumpkin and cauliflower seeds in 2005 in seedling trays.

Each of the vegetable crops was highly susceptible to damping-off by *P. irregulare*. Azoxystrobin and fludioxonil had no effect against *Pythium*, while metalaxyl and thiram were effective against the pathogen. Thiram seed dressing, and the seed dressing combinations of fludioxonil + metalaxyl-M (FM) and azoxystrobin + fludioxonil + metalaxyl-M (AFM), substantially improved seedling emergence.

Beans were highly susceptible to *F. culmorum*, with no seedling emergence, while peas and pumpkins were moderately susceptible. Even though cauliflower emergence and survival appeared to be unaffected by *Fusarium*, reduced growth was noted on seedlings produced from untreated seeds. Azoxystrobin, fludioxonil and thiram were effective against *Fusarium*. Therefore, the FM and AFM new seed dressing combinations that contain fludioxonil, as well as thiram seed dressing, substantially improved seedling emergence.

Beans were also highly susceptible to *R. solani* AG2.1, with substantially lower seedling emergence, while pumpkins were moderately susceptible. Peas and cauliflowers appeared to be tolerant to the pathogen or isolate used in this study. Azoxystrobin, fludioxonil and thiram were effective against *Rhizoctonia*. Therefore, the new seed dressings F, FM and AFM, and the old seed dressing thiram, substantially improved bean and pumpkin seedling emergence and survival.

In general, this study demonstrated the high selectivity of the active ingredients used in the new seed dressing combinations against their target pathogen groups. In contrast, thiram is not selective and is effective against all the pathogens. The broad spectrum effect of thiram could be a problem if it also inhibits other soil organisms that are beneficial to root growth. The high selectivity of the new actives means that they are best used in seed dressing combinations in order to extend their range of pathogen control.

### **Aims**

The effectiveness of new fungicide seed dressings against the common damping-off pathogens, *P. irregulare*, *R. solani* and *F. culmorum*, were examined on pea, bean, pumpkin and cauliflower seeds in 2005. After initial screening studies (refer Sections A and B to this report), the new seed dressings containing fludioxonil (F), fludioxonil + metalaxyl-M (FM) and azoxystrobin + fludioxonil + metalaxyl-M (AFM) were selected for this study.

## **C. Efficacies of fungicide seed dressings (Cont.)**

### **Materials & Methods**

The fungicide seed dressings F, FM, AFM and Thiram were applied to green bean, pea, cauliflower and butternut pumpkin seeds, as described in the treatment list. Seeds were sown into seedling trays in nursery potting mixture consisting of 70% peat and 30% composted pine bark. There were 3 replicates, with 18 seeds sown in each replicate. The fungal pathogens were inoculated at one day after sowing.

The fungal pathogens, *P. irregulare*, *R. solani* and *F. culmorum* were grown on V8 liquid medium (V8 vegetable juice) for 7 days at 20°C - 22°C, and then macerated in a blender, sieved and adjusted to concentrations of approximately  $1 \times 10^4$  colony forming units (cfu) before use. The cfu consisted mainly of hyphal fragments. Each pathogen's inoculum was applied as a water-in dose of approximately 2 mL per plant cell at one day after seed planting. *R. solani* AG2.1 is the most common sub-group of *R. solani* in soil.

### **Seed dressings examined in the study**

Product Code	Active Ingredient (ai)	Concentration of ai	Formulation
Thiram	thiram	800 g/kg	Wettable powder
F	fludioxonil	100 g/L	Flowable concentrate
FM	fludioxonil + metalaxyl-M	25 g/L + 10 g/L	Flowable concentrate
AFM	azoxystrobin + fludioxonil + metalaxyl-M	75 g/L + 12.5 g/L + 37.5 g/L	Flowable concentrate

### **Treatment list for fungicide seed dressings**

No.	Treatment Code	Active ingredient	ai (g/100 kg seed)	Product Rates (per 100 kg seed)
1	UTC (Untreated control)	-	-	0
2	F25	fludioxonil	2.5	25 mL
3	F50	"	5.0	50 mL
4	FM100	fludioxonil + metalaxyl-M	2.5 + 1.0	100 mL
5	FM150	"	3.75 + 1.5	150 mL
6	FM200	"	5.0 + 2.0	200 mL
7	FM250	"	6.25 + 2.5	250 mL
8	AFM50	azoxystrobin + fludioxonil + metalaxyl-M	3.75 + 0.625 + 1.875	50 mL
9	AFM100	"	7.5 + 1.25 + 3.75	100 mL
10	AFM150	"	11.25 + 1.875 + 5.625	150 mL
11	AFM200	"	15.0 + 2.5 + 7.5	200 mL
12	Thiram	thiram	300	500 g

## C. Efficacies of fungicide seed dressings (Cont.)

### Results

**Table 1: Seed treatment effects on peas against *P. irregulare*, *F. culmorum* and *R. solani***

Pea seed treatments				
Pathogen	Treatment Code	% Seedling emergence (18DAS)	% Seedling survival (31 DAS)	Average fresh shoot weight (g/surviving plant) (31 DAS)
<b>Nil</b>	UTC (Untreated control)	93	96	0.837 bcd
	F25	96	100	0.872 bc
	F50	96	96	0.892abc
	FM100	93	93	0.944ab
	FM150	96	96	0.950ab
	FM200	100	100	0.952ab
	FM250	93	96	1.034a
	AFM50	96	93	0.978ab
	AFM100	100	100	0.846 bcd
	AFM150	93	100	0.793 cd
	AFM200	96	96	0.715 d
Thiram	96	96	0.882 bc	
p-value		0.9298	0.6226	0.0168
<b><i>Pythium irregulare</i></b>	UTC (Untreated control)	56 b	70 b	0.566 d
	F25	33 c	44 c	0.541 d
	F50	52 b	56 bc	0.573 d
	FM100	89a	89a	0.722 bcd
	FM150	93a	93a	0.685 cd
	FM200	100a	96a	0.951a
	FM250	96a	100a	0.849abc
	AFM50	93a	93a	0.855abc
	AFM100	93a	96a	0.733 bcd
	AFM150	93a	96a	0.725 bcd
	AFM200	89a	93a	0.745abcd
Thiram	96a	96a	0.908ab	
p-value		0.0001	0.0001	0.0046
<b><i>Fusarium culmorum</i></b>	UTC (Untreated control)	63 c	67 c	0.548 d
	F25	100 a	100 a	0.993a
	F50	96 ab	100 a	0.917ab
	FM100	96 ab	93 ab	0.977ab
	FM150	96 ab	96 ab	0.943ab
	FM200	85 b	85 b	1.005a
	FM250	96 ab	96 ab	1.024a
	AFM50	93 ab	93 ab	0.907 abc
	AFM100	96 ab	96 ab	0.700 cd
	AFM150	93 ab	96 ab	0.860abc
	AFM200	89 ab	93 ab	0.776 bc
Thiram	96 ab	93 ab	1.040a	
p-value		0.0021	0.0101	0.0026
<b><i>Rhizoctonia solani</i></b>	UTC (Untreated control)	93	93	0.658 d
	F25	96	96	0.824abcd
	F50	89	96	0.869abc
	FM100	96	96	0.977a
	FM150	89	93	0.888abc
	FM200	100	100	0.942ab
	FM250	93	93	0.970a
	AFM50	89	93	0.807abcd
	AFM100	96	96	0.781 bcd
	AFM150	93	93	0.811abcd
	AFM200	89	89	0.734 cd
Thiram	89	96	0.714 cd	
p-value		0.7538	0.9043	0.0237

### **C. Efficacies of fungicide seed dressings (Cont.)**

**Table 2: Seed treatment effects on beans against *P. irregulare*, *F. culmorum* and *R. solani***

Bean seed treatments				
Pathogen	Treatment Code	% Seedling emergence (18DAS)	% Seedling survival (30 DAS)	Average fresh shoot weight (g/surviving plant) (30 DAS)
<b>Nil</b>	UTC (Untreated control)	96	32 b	1.443
	F25	89	31 b	1.430
	F50	81	31 b	1.529
	FM100	96	32 b	1.483
	FM150	96	33 b	1.569
	FM200	81	30 b	1.430
	FM250	89	30 b	1.690
	AFM50	78	30 b	1.468
	AFM100	78	81 a	1.524
	AFM150	74	93 a	1.320
	AFM200	78	93 a	1.196
	Thiram	93	96 a	1.411
p-value		0.2817	0.0001	0.2117
<b><i>Pythium irregulare</i></b>	UTC (Untreated control)	0 e	4 c	0.940 b
	F25	4 e	11 c	0.333 b
	F50	0 e	0 c	- b
	FM100	59 cd	78 ab	0.762 a
	FM150	63 bcd	81 ab	0.835 a
	FM200	85 a	85 a	0.872 a
	FM250	70 abc	70 ab	1.002 a
	AFM50	52 d	63 b	0.872 a
	AFM100	74 abc	81 ab	0.942 a
	AFM150	81 a	89 a	1.013 a
	AFM200	78 ab	81 ab	1.017 a
	Thiram	78 ab	78 ab	1.032 a
p-value		0.0001	0.0001	0.0001
<b><i>Fusarium culmorum</i></b>	UTC (Untreated control)	0 c	0 d	- d
	F25	89 ab	85 abc	1.325 abc
	F50	93 a	85 abc	1.400 ab
	FM100	85 ab	85 abc	1.283 abc
	FM150	100 a	96 a	1.388 ab
	FM200	89 ab	93 ab	1.441 a
	FM250	93 a	93 ab	1.377 abc
	AFM50	70 b	74 bc	1.308 abc
	AFM100	81 ab	78 abc	1.328 abc
	AFM150	81 ab	81 abc	1.156 bc
	AFM200	70 b	74 c	1.135 c
	Thiram	96 a	89 abc	1.249 abc
p-value		0.0001	0.0001	0.0001
<b><i>Rhizoctonia solani</i></b>	UTC (Untreated control)	19 c	19 c	1.160
	F25	85 ab	85 ab	1.390
	F50	89 ab	89 ab	2.003
	FM100	74 ab	74 ab	1.377
	FM150	85 ab	85 ab	1.491
	FM200	81 ab	81 ab	1.383
	FM250	81 ab	85 ab	1.305
	AFM50	74 ab	70 ab	1.370
	AFM100	63 b	74 ab	1.194
	AFM150	67 ab	67 b	1.287
	AFM200	85 ab	85 ab	1.269
	Thiram	93 a	93 a	1.119
p-value		0.0010	0.0006	0.3046

### **C. Efficacies of fungicide seed dressings (Cont.)**

**Table 3: Seed treatment effects on butternut pumpkins against *P. irregulare*, *F. culmorum* and *R. solani***

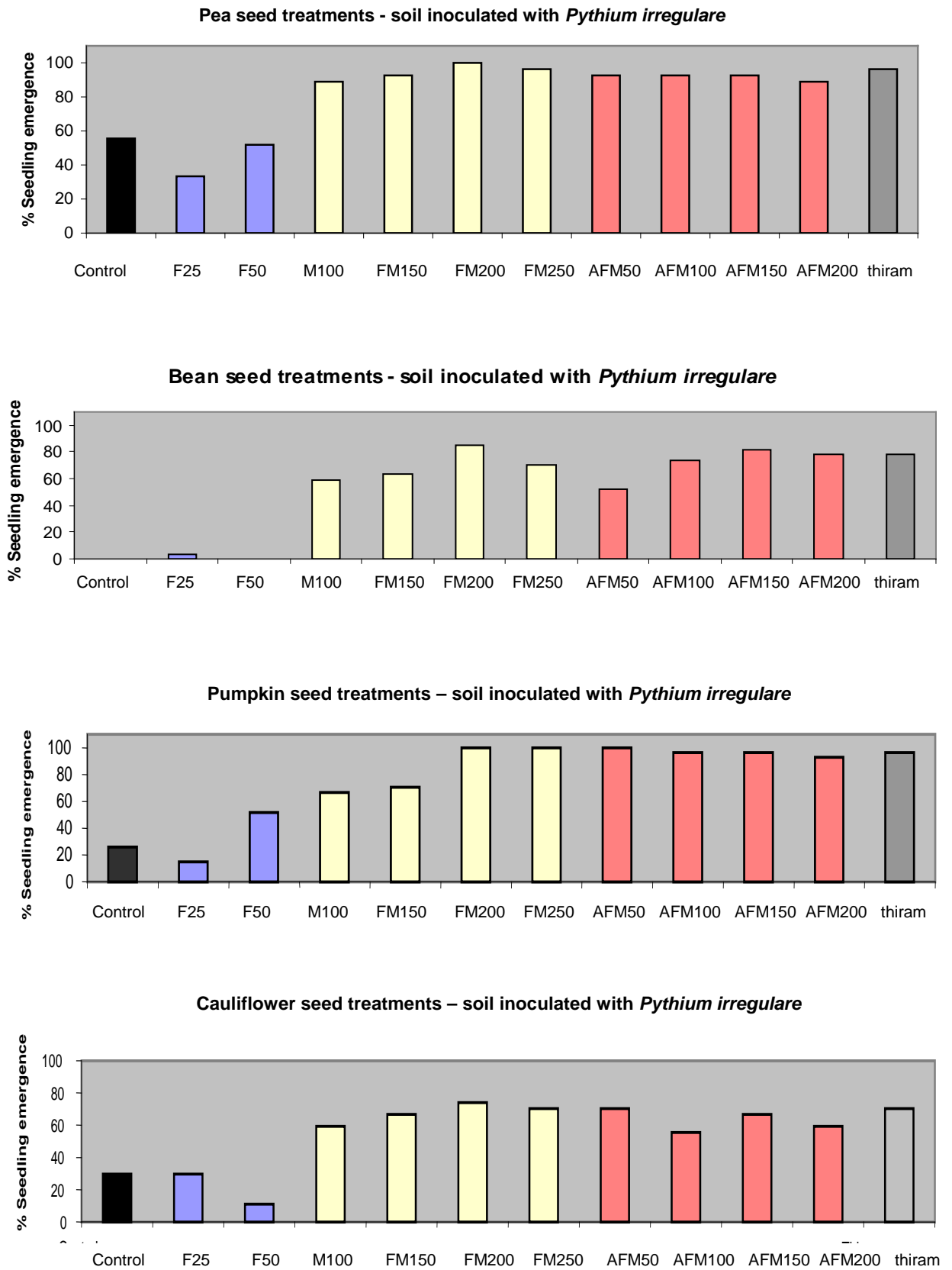
Butternut pumpkin seed treatments				
Pathogen	Treatment Code	% Seedling emergence (14 DAS)	% Seedling survival (21 DAS)	Average fresh shoot weight (g/surviving plant) (21 DAS)
<b>Nil</b>	UTC (Untreated control)	67 def	70 cde	0.869
	F25	74 bcde	74 bcde	0.836
	F50	52 f	59 e	0.870
	FM100	74 bcde	81 abcd	0.985
	FM150	59 ef	67 de	0.866
	FM200	85abcd	89abc	0.901
	FM250	89abc	89abc	0.802
	AFM50	93ab	93ab	0.771
	AFM100	100a	100a	0.836
	AFM150	89abc	89abc	0.872
	AFM200	70 cdef	78 bcde	0.894
Thiram	74 bcde	78 bcde	0.894	
p-value		0.0028	0.0336	0.4094
<b><i>Pythium irregulare</i></b>	UTC (Untreated control)	26 ef	26 c	0.707 b
	F25	15 f	11 c	0.797 b
	F50	52 de	33 c	0.952a
	FM100	67 cd	67 b	1.153a
	FM150	70 bcd	70 b	1.087a
	FM200	100a	100a	1.102a
	FM250	100a	100a	1.191a
	AFM50	100a	100a	1.194a
	AFM100	96ab	96a	1.182a
	AFM150	96ab	96a	1.111a
	AFM200	93abc	96a	1.037a
Thiram	96ab	96a	1.093a	
p-value		0.0001	0.0001	0.0002
<b><i>Fusarium culmorum</i></b>	UTC (Untreated control)	59 de	63 d	0.945
	F25	74 bcd	81 bc	0.867
	F50	48 e	59 d	1.063
	FM100	67 cde	70 cd	0.918
	FM150	48 e	56 d	0.915
	FM200	85abc	85abc	0.973
	FM250	93ab	93ab	0.919
	AFM50	100a	100a	1.009
	AFM100	93ab	93ab	1.034
	AFM150	78abcd	85abc	1.000
	AFM200	67 cde	89ab	0.869
Thiram	93ab	93ab	0.894	
p-value		0.0015	0.0001	0.3695
<b><i>Rhizoctonia solani</i></b>	UTC (Untreated control)	48 ef	59	0.918
	F25	63 cdef	74	0.893
	F50	67 bcdef	70	0.932
	FM100	44 f	63	0.906
	FM150	52 def	56	0.950
	FM200	81abc	85	0.856
	FM250	96a	96	0.841
	AFM50	96a	96	0.965
	AFM100	93ab	93	0.921
	AFM150	89abc	89	0.797
	AFM200	74abcde	85	0.945
Thiram	78abcd	81	0.869	
p-value		0.0019	0.0544	0.8328

### **C. Efficacies of fungicide seed dressings (Cont.)**

**Table 4: Seed treatment effects on cauliflowers against *P. irregulare*, *F. culmorum* and *R. solani***

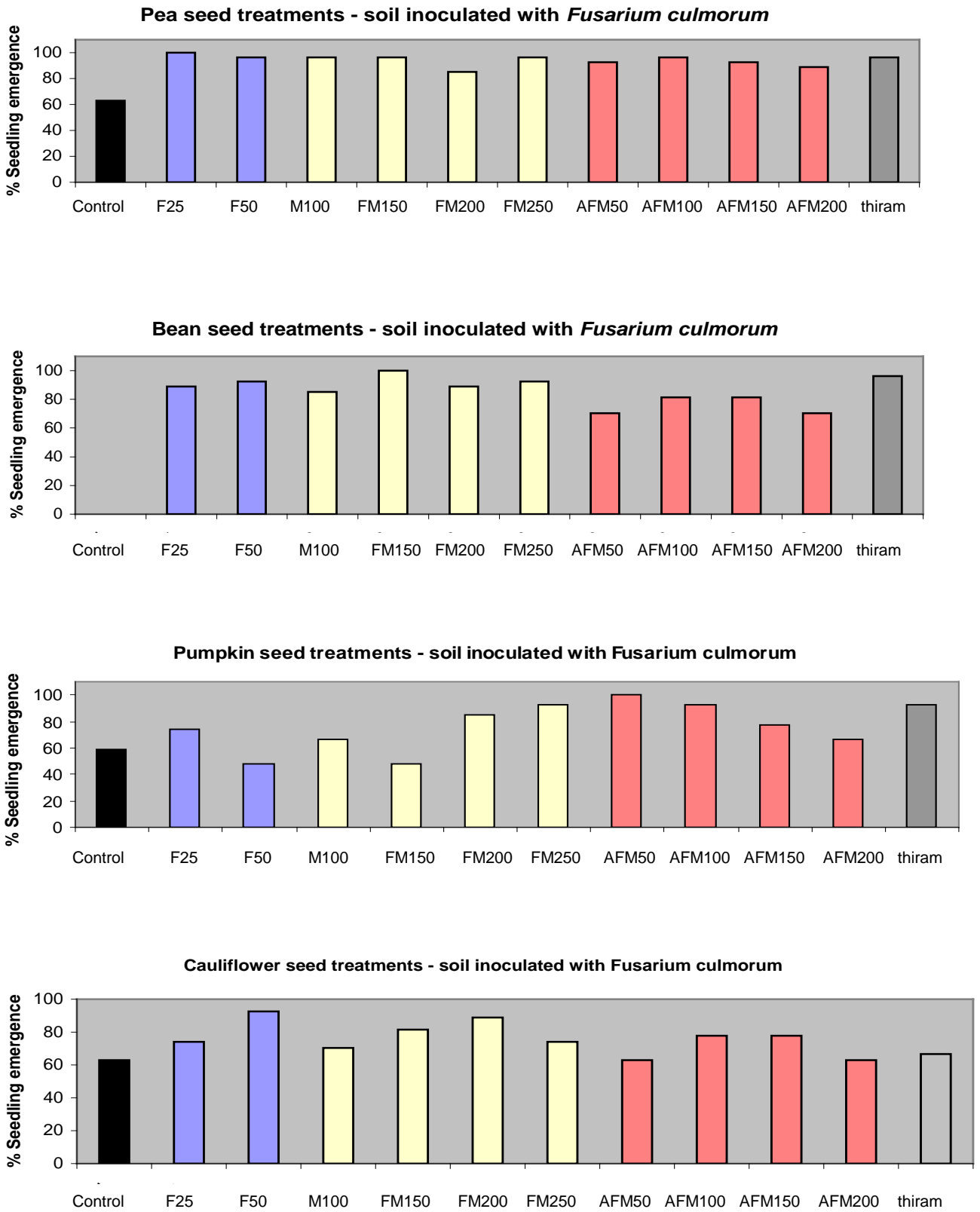
Cauliflower seed treatments				
Pathogen	Treatment Code	% Seedling emergence (8DAS)	% Seedling survival (21 DAS)	Average fresh shoot weight (g/surviving plant) (21 DAS)
<b>Nil</b>	UTC (Untreated control)	89	89	0.156 ab
	F25	89	89	0.132 bcde
	F50	85	85	0.130 cde
	FM100	70	74	0.130 cde
	FM150	81	81	0.136 bcd
	FM200	78	78	0.143 abc
	FM250	78	78	0.162 a
	AFM50	70	81	0.137 bcd
	AFM100	81	85	0.132 bcde
	AFM150	52	63	0.109 e
	AFM200	78	89	0.115 de
Thiram	85	85	0.148 abc	
p-value		0.1557	0.6231	0.0074
<b><i>Pythium irregulare</i></b>	UTC (Untreated control)	30 bc	48	0.050 bc
	F25	30 bc	44	0.043 bc
	F50	11 c	30	0.039 c
	FM100	59 ab	74	0.068 bc
	FM150	67 a	74	0.085 b
	FM200	74 a	78	0.088 ab
	FM250	70 a	78	0.083 bc
	AFM50	70 a	78	0.086 ab
	AFM100	56 ab	81	0.077 bc
	AFM150	67 a	78	0.076 bc
	AFM200	59 ab	81	0.066 bc
Thiram	70 a	63	0.165 a	
p-value		0.0122	0.1523	0.0341
<b><i>Fusarium culmorum</i></b>	UTC (Untreated control)	63 d	81	0.108
	F25	74 bcd	81	0.148
	F50	93 a	93	0.159
	FM100	70 cd	74	0.150
	FM150	81 abc	81	0.168
	FM200	89 ab	96	0.141
	FM250	74 bcd	78	0.137
	AFM50	63 d	70	0.140
	AFM100	78 abcd	89	0.124
	AFM150	78 abcd	78	0.141
	AFM200	63 d	74	0.127
Thiram	67 cd	70	0.141	
p-value		0.0183	0.1159	0.0738
<b><i>Rhizoctonia solani</i></b>	UTC (Untreated control)	93	96	0.135
	F25	78	81	0.121
	F50	74	81	0.132
	FM100	78	81	0.128
	FM150	70	78	0.132
	FM200	74	74	0.140
	FM250	74	81	0.123
	AFM50	67	78	0.125
	AFM100	63	85	0.116
	AFM150	85	93	0.129
	AFM200	78	85	0.147
Thiram	63	78	0.122	
p-value		0.7404	0.7222	0.7627

**Figure 1: Efficacies of seed treatments against *P. irregulare***

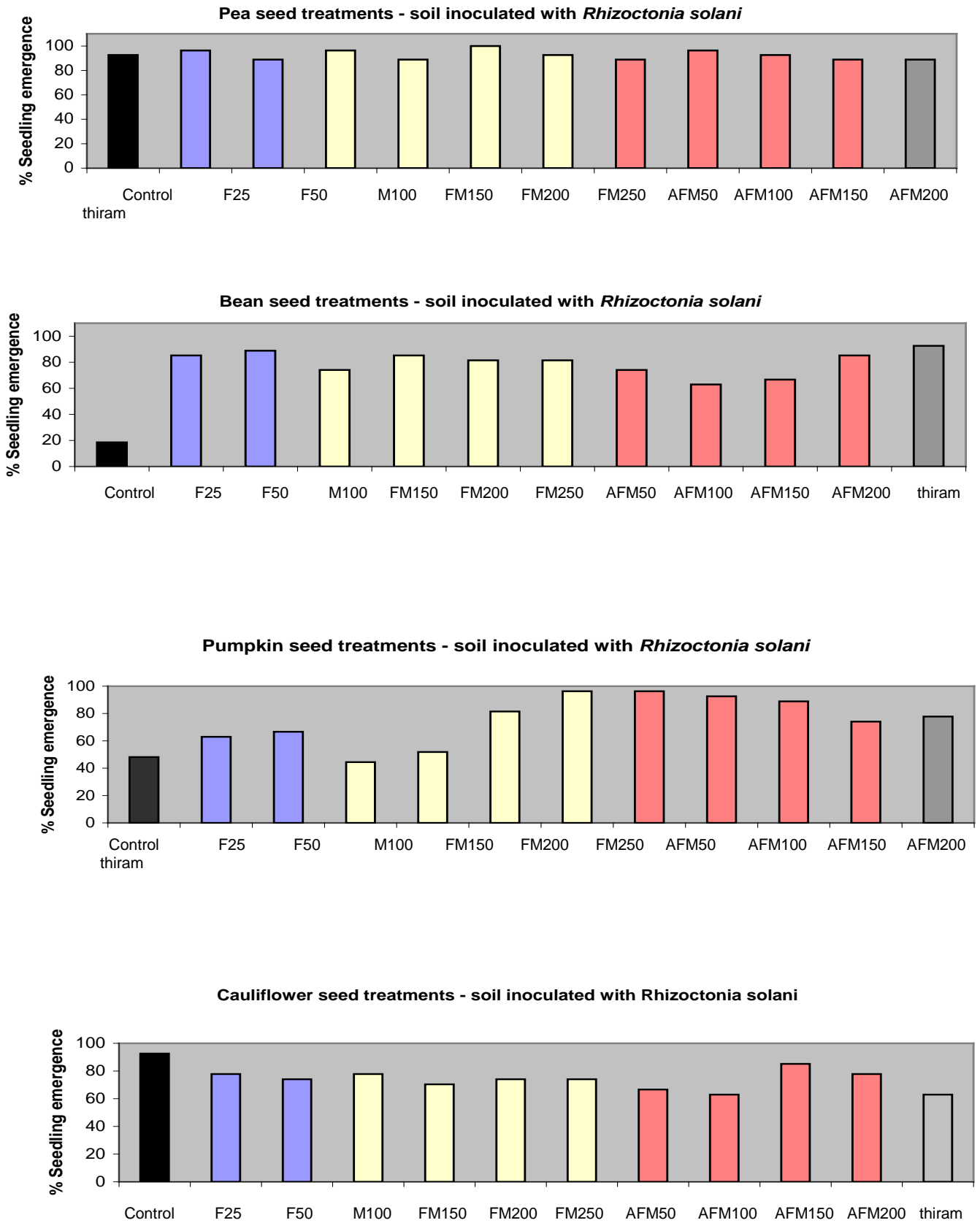




**Figure 2: Efficacies of seed treatments against *F. culmorum***



**Figure 3: Efficacies of seed treatments against *R. solani***



## **C. Efficacies of fungicide seed dressings (Cont.)**

### **Discussions**

#### ***Pythium***

All the vegetable crops were highly susceptible to damping-off by *P. irregulare* (Tables 1-4). Azoxystrobin and fludioxonil had no effect against *Pythium*, while metalaxyl and thiram were effective against the pathogen. Therefore, thiram seed dressing and the seed dressing combinations FM and AFM, which contain metalaxyl, substantially improved seedling emergence (Figure 1).

Reduced growth was recorded on almost all seedlings that survived in the *Pythium* inoculated medium, regardless of whether they were treated or not (Tables 1-4). Improved growth, however, was generally noted in seedlings produced from FM, AFM and thiram seed treatments, in comparison to the untreated control and fludioxonil only (F) seed treatments.

#### ***Fusarium***

Beans were highly susceptible to *F. culmorum*, with no seedling emergence in the untreated control, while peas and pumpkins were moderately susceptible (Figure 2). Even though cauliflower emergence and survival appeared to be unaffected by *Fusarium*, reduced growth was noted on seedlings produced from untreated seeds. Azoxystrobin, fludioxonil and thiram were effective against *Fusarium*. Therefore, the FM and AFM new seed dressing combinations, which contain fludioxonil, as well as thiram seed dressing, substantially improved seedling emergence. Reduced growth by *Fusarium* was noted on pea and cauliflower seedlings produced from untreated seeds.

#### ***Rhizoctonia***

Beans were also highly susceptible to *Rhizoctonia solani* AG2.1, with substantially lower seedling emergence, while pumpkins were moderately susceptible (Figure 3). Peas and cauliflowers appeared to be tolerant to the pathogen. Azoxystrobin, fludioxonil and thiram were effective against *Rhizoctonia*. Therefore, the new seed dressings F, FM and AFM, and the old seed dressing thiram, substantially improved bean and pumpkin seedling emergence and survival.

#### **General**

AFM seed treatments appeared to reduce pea and bean seedling growth at the high rates of 150 and 200 compared to the lower rates at 50 and 100.

## **D. Evaluations of new seed dressings in field trials**

### **General Summary**

Following initial the study (refer Section C) on the effectiveness of six new fungicide seed dressings, two seed dressing combinations fludioxonil + metalaxyl (FM) and azoxystrobin + fludioxonil + metalaxyl (AFM) were selected for further evaluation in five field trials. In a fifth trial, the effects of combining two or three seed dressing that contain only one active ingredient (metalaxyl (M), fludioxonil (F) or thiamethoxam (Thx)) were compared against the standard thiram seed treatment in a bean trial.

Three of the five field trials examined the new seed treatments on green beans within commercial bean crops because of the high susceptibility of their seedlings to many soilborne pathogens. Another two field trials examined the new fungicide seed treatments on peas, beans, cauliflowers and pumpkins.

These field trials demonstrated that the new fungicide seed dressing combinations were suitable alternatives to thiram in improving seedling establishment and reducing root rot incidence or severity. Generally, reduced seedling emergence and root rot were often caused by more than one soilborne pathogen, and therefore, new fungicide seed dressings that have combinations of two or three active ingredients were more effective than a fungicide seed dressing containing only one active ingredient.

AFM seed treatments tended to give the greatest root rot control on the vegetable crops. AFM was also shown to be most effective against *Ascochyta* infections on peas, where it prevented seed rot, and reduced collar rot. FM or combinations of the single active seed dressings of M and F were also shown to be effective in reducing root rot severity.

No conclusion could be made on the effects of Thx insecticide seed dressing due to a lack of insect damage in the bean trial.

The combinations of metalaxyl-M and fludioxonil generally demonstrated no phytotoxic effects in delaying seedling emergence or growth, while the addition of azoxystrobin in AFM may cause some reduction in shoot growth due to a delay in seedling emergence.

## **Trial 1: Screening of new seed dressings on beans, 2004/05**

### **Summary**

Following an initial pot trial study, a trial was conducted within a commercial processing bean crop, in order to screen the potential new seed dressings, azoxystrobin (A), metalaxyl + fludioxonil (MF), fludioxonil (F), fludioxonil + metalaxyl (FM), difenconazole + metalaxyl (DM), azoxystrobin + fludioxonil + metalaxyl (AFM), and thiamethoxam (Thx), under field conditions. All were new fungicide seed dressings, except for Thx, which is an insecticide seed dressing. The effects of these new seed dressings were compared against untreated control (UTC) seeds and the standard thiram seed treatment.

Due to high variability between different replicate plots, there were no statistical differences in the seedling emergence, survival, average fresh shoot weight and root rot. There was, however, a trend of increased plant shoot weight or plant size due to seed treatments with metalaxyl + fludioxonil (MF), thiram, azoxystrobin + metalaxyl + fludioxonil (AMF), and fludioxonil (F). There was also a trend of lower root rot incidence on plants produced from all fungicide treated seeds, in comparison to untreated control seeds and the insecticide thiamethoxam treated seeds.

### **Materials & Methods**

#### **Trial Summary**

<b>Treatment method</b>	Seed treated by coating with a suspension of seed dressing product at the appropriate rates, and then air-dried
<b>Grower</b>	David Chaplin
<b>Location</b>	Within a commercial bean crop at Wesley Vale, Tasmania
<b>Soil Type</b>	Grey sandy loam
<b>Variety</b>	Celtic
<b>Trial Design</b>	Randomised Complete Block
<b>Replicates</b>	3
<b>Plot Size</b>	2 m wide x 4 m long
<b>Sowing Density</b>	60 seeds sown per plot
<b>Sowing Date</b>	24/11/04

**Trial 1 (Cont.)****Seed dressing active ingredient and concentrations**

Seed Treatment Code	Active Ingredient (ai)	Concentration of ai
Thiram	thiram	800 g/kg
A	azoxystrobin	100 g/L
MF	metalaxyl + fludioxonil	37.5 g + 25 g/L
F	fludioxonil	100 g/L
FM	fludioxonil + metalaxyl	25 g/L + 10 g/L
DM	difenconazole + metalaxyl	92 g/L + 23 g/L
AFM	azoxystrobin + fludioxonil + metalaxyl-M	75 g/L + 12.5 g/L + 37.5 g/L
Thx	thiamethoxam	350 g/L

**Treatment list**

No.	Seed Treatment code	Active ingredient (ai)	Active ingredient concentration (g/100 kg seed)	Product Rate /100 kg seed
1	UTC (Untreated control)	-	N/a	N/a
2	A	azoxystrobin	5	50 mL
3	F	fludioxonil	5	50 mL
4	MF	metalaxyl + fludioxonil	5.625 + 3.75	150 mL
5	FM	fludioxonil + metalaxyl	3.75 + 1.5	150 mL
6	DM	difenconazole + metalaxyl	11.96 + 2.90	130 mL
7	AFM	azoxystrobin + fludioxonil + metalaxyl-M	7.5 + 1.25 + 3.75	100 mL
8	Thiram	thiram	400	500 g
9	Thx	thiamethoxam	140	400 mL

**Chronology of Events**

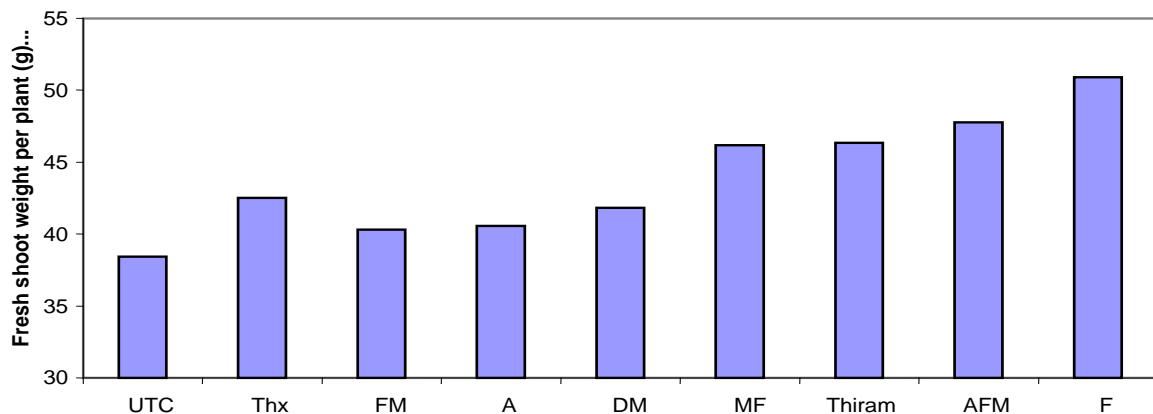
Date	Days After Application (DAA)	Days After Sowing (DAS)	Event in the trial area
25/10/04	0		Seed treatments applied.
24/11/04	30	0	Trial set up and seeds sown.
08/12/04	44	14	Seedling emergence assessment of whole plots.
11/01/05	78	48	Seedling survival assessment of whole plots.
14/01/05	81	51	Assessment for the incidence of plants with root rot, and fresh shoot weight of all surviving seedlings.

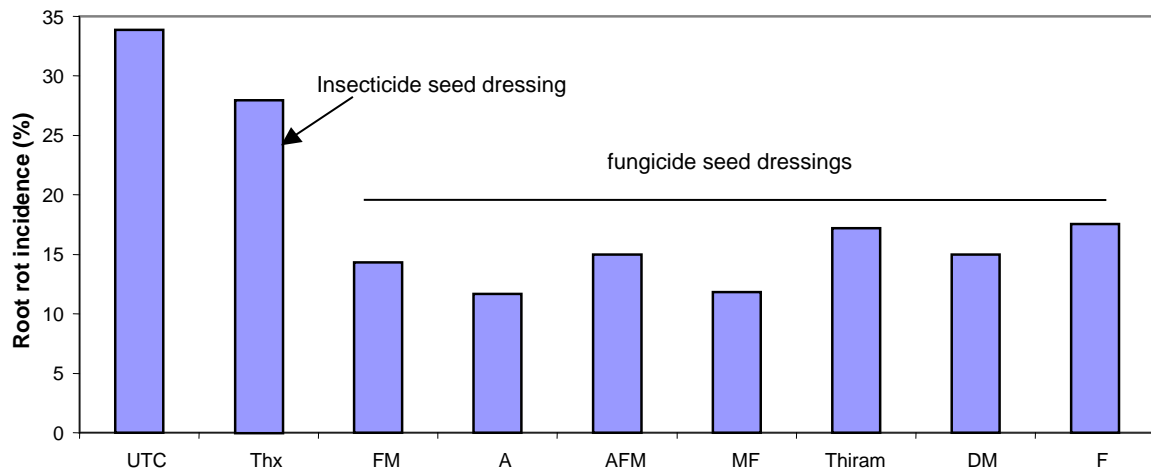
**Trial 1 (Cont.)****Results****Table 1.1: Mean seedling emergence, survival, and fresh shoot weight and root rot of surviving plants**

No.	Seed Treatment Code	Active ingredient concentration (g/100 kg seed)	% Seedling Emergence (14DAS)	% Seedling Survival (48DAS)	Fresh shoot weight (g/ plant) (51DAS)	Root Rot Incidence (%) (51DAS)
1	UTC (Untreated control)	N/a	71	84	38	34
2	A	5	55	76	41	12
3	F	5	60	79	51	18
4	MF	5.625 + 3.75	75	85	46	12
5	FM	3.75 + 1.5	48	67	40	14
6	DM	11.96 + 2.90	62	80	42	15
7	AFM	7.5 + 1.25 + 3.75	70	84	48	15
8	Thiram	400	67	86	46	17
9	Thx	140	51	72	43	28
	<b>P-value</b>		<b>0.411</b>	<b>0.183</b>	<b>0.353</b>	<b>0.469</b>

DAS = Days after sowing

Means within columns followed by the same letter are not significantly different at the 5% level according to Least Significant Difference (LSD) test.

**Figure 1.1: Seed treatment effects on the average fresh shoot weight**

**Trial 1 (Cont.)****Figure 1.2: Seed treatment effects on root rot incidence****Discussions**

The seedling emergence and survival rates were highly variable due to the poor sandy soil, the relatively dry soil conditions and the use of only three replicates. Therefore, in the analysis of variance, there were no significant differences in the seedling emergence, survival, average fresh shoot weight, or root rot incidence (Table 1.1). Poor seedling growth in the trial study was mainly due to poor root establishment in poor soil. *Rhizoctonia*, *Fusarium* and *Thielaviopsis* were found to be the causes of root rot in the trial. The root rot severity was considered to be mild to moderate. Although not significant, there was a trend of increased fresh shoot weight or plant size due to seed treatments with metalaxyl + fludioxonil (MF), thiram, azoxystrobin + metalaxyl + fludioxonil (AMF), and fludioxonil (F) (Figure 1.1). There was also a trend of lower root rot incidence on plants produced from fungicide treated seeds, in comparison to untreated control seeds and the insecticide thiamethoxam treated seeds (Figure 1.2).



## **Trial 2: Screening of new seed dressings on beans, 2005/06**

### **Summary**

A trial was conducted within a commercial bean crop to evaluate different rates of seed dressing combinations of fludioxonil + metalaxyl (FM) and azoxystrobin + fludioxonil + metalaxyl (AFM) (FM150, FM250, AFM75, AFM100 and AFM200), and compare them against untreated control seeds and the current standard thiram seed treatment. Assessments were made for seedling emergence and survival. The surviving seedlings were examined for root rot, and their average fresh shoot and root weights were recorded.

The seeds were planted under ideal field conditions, and rapid seedling emergence was noted. Although there were no significant differences in the seedling emergence and survival, there was a trend of slight improvement in seedling emergence and establishment due to the fungicide seed treatments in comparison to the untreated control. There was also a trend of slightly lower average fresh shoot weight of plants produced from seeds treated with AFM. This result was consistent with the field observation of a slight delay in seedling emergence and consequently smaller plant size with the AFM seed treatments. The delay in seedling emergence appeared to be due to azoxystrobin. However, this delay in plant emergence and growth is not expected to impact on crop yield.

*Rhizoctonia* and *Fusarium* were the main causes of root rot in the trial. AFM seed treatments resulted in significantly higher incidence of healthy roots and lower root rot severity. This finding indicates that the combination of three fungicide active ingredients, azoxystrobin, fludioxonil and metalaxyl, was more effective in protecting and reducing root rot, than the fludioxonil and metalaxyl combination and the standard thiram treatment. The rate of AFM at 200 mL/100 kg seeds appeared to be the optimum rate for root rot reduction.

### **Materials & Methods**

#### **Trial Summary**

<b>Seed Treatment Method</b>	Polymer film coating of chemical treated seeds. Seeds treated and film coated using a rotary seed treating machine.
<b>Grower</b>	D. Chaplin
<b>Location</b>	Within a commercial processing bean crop at Wesley Vale, Tasmania
<b>Soil Type</b>	Grey sandy loam
<b>Crop</b>	Garden Bush Beans
<b>Variety</b>	Strike
<b>Trial Design</b>	Randomised complete block
<b>Replicates</b>	5
<b>Sowing density</b>	40 seeds per plot
<b>Plot Size</b>	1 row x 2 m

**Trial 2 (Cont.)****Seed dressing active ingredient and concentrations**

Code	Active Ingredient (ai)	Concentration of ai
Thiram	thiram	800 g/kg
FM	fludioxonil + metalaxyl-M	25 g/L + 10 g/L
AFM	azoxystrobin + fludioxonil + metalaxyl-M	75 g/L + 12.5 g/L + 37.5 g/L

**Treatment List**

No.	Seed Treatment Code	Active ingredient	Active ingredient concentration (g per 100 kg seed)	Product Rate (per 100 kg seed)
1	UTC	-	-	-
2	FM150	fludioxonil + metalaxyl	3.75 + 1.5	150 mL
3	FM250	fludioxonil + metalaxyl	6.25 + 2.5	250 mL
4	AFM75	azoxystrobin + fludioxonil + metalaxyl	5.63 + 0.94 + 2.81	75 mL
5	AFM100	azoxystrobin + fludioxonil + metalaxyl	7.5 + 1.25 + 3.75	100 mL
6	AFM200	azoxystrobin + fludioxonil + metalaxyl	15 + 2.5 + 7.5	200 mL
7	Thiram	thiram	400	500 g

UTC = Untreated control

**Disease assessments**

At 24 and 53 days after sowing (DAS), seedling emergence and survival were recorded respectively. At 55 DAS, the surviving seedlings were also assessed for root rot incidence, and rated for severity as described below, and their average fresh root and shoot weights were recorded. Analysis of variance was conducted on the data set using Statgraphics Plus 2.0, and pairwise comparisons were made of the mean values using Least Significant Difference Test (LSD).

Root rot severity rating	Description
0	Healthy plant.
1	No hypocotyl rot and slight root discolouration.
2	Moderate hypocotyl discolouration or rot.
3	Severe hypocotyl rot or tap root rot.

**Trial 2 (Cont.)****Chronology of Events**

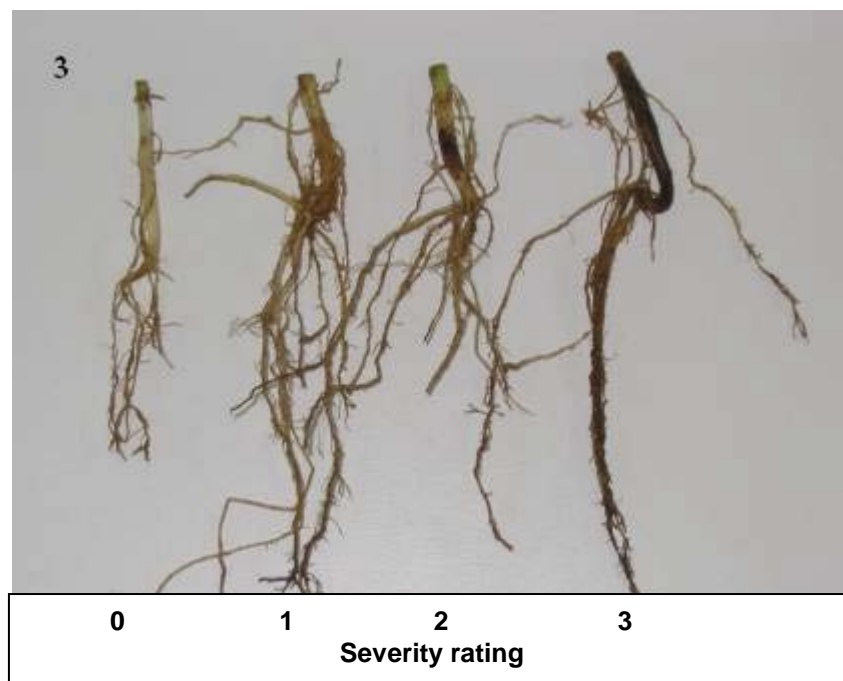
<b>Date</b>	<b>Days After Application (DAA)</b>	<b>Days After Sowing (DAS)</b>	<b>Event in the trial area</b>
05/12/05	0		Seed treatments applied.
15/12/05	10		Fertiliser drilled into the soil in the trial area in the same way as for the commercial crop outside the trial.
16/12/05	11	0	Trial set up and seeds sown.
09/01/06	35	24	Seedling emergence assessment in whole plots.
07/02/06	64	53	Seedling survival assessment in whole plots.
09/02/06	66	55	Assessment for hypocotyl and root rot, and fresh shoot weight of all surviving seedlings.

**Trial 2 (Cont.)****Photographs**

Seeds being hand sown in the trial (Photograph 1), and maturing plants in the trial within the commercial bean crop (Photograph 2)



Bean root rot severity ratings based on the proportion of roots with discolouration and constrictions (Photograph 3)

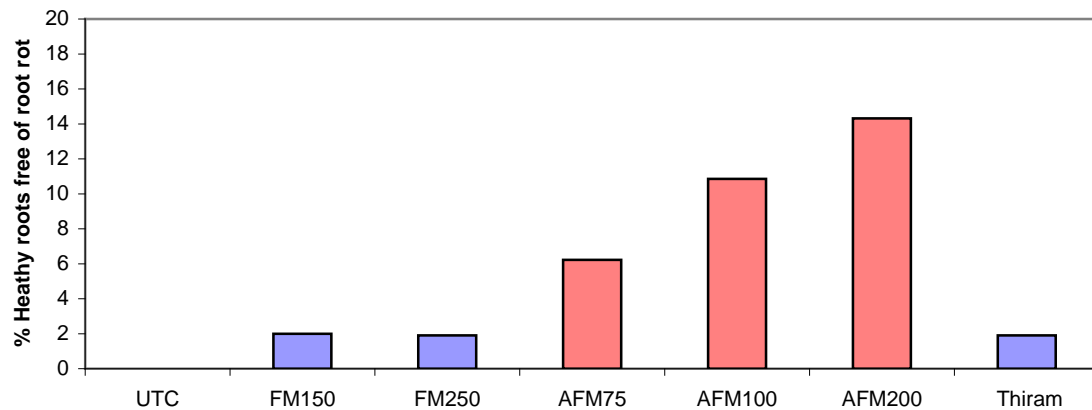


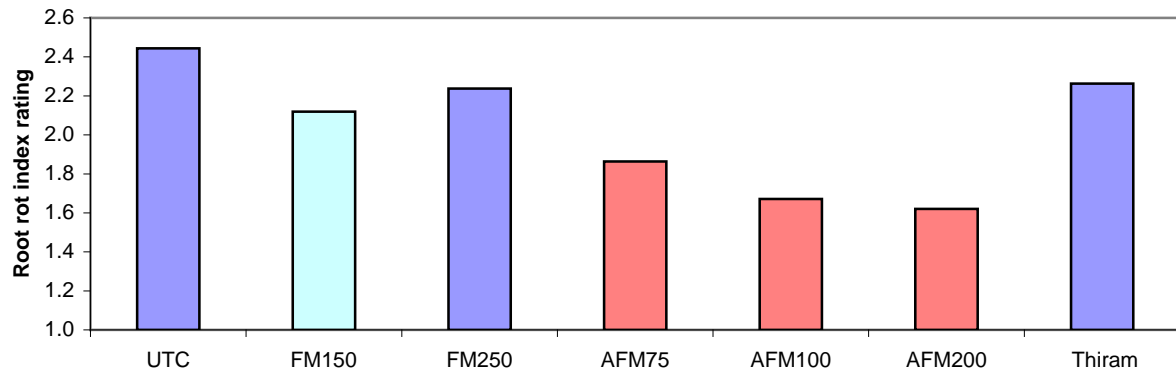
**Trial 2 (Cont.)****Results****Table 2.1: Mean seedling emergence, survival, fresh shoot weight and root rot of surviving bean plants**

No.	Seed Treatment	% Seedling emerged (24DAS)	% Seedling survival (53DAS)	Fresh shoot weight g/plant (55DAS) (assessed 20 plants/plot)	% Healthy roots free of root rot	Root rot index of surviving plants (55DAS)
1	Untreated Control	77	75	44	0 a	2.4 d
2	FM150	87	88	41	2 a	2.1 bc
3	FM250	81	80	41	2 a	2.2 cd
4	AFM75	83	81	36	6 ab	1.9ab
5	AFM100	86	85	38	11 bc	1.7a
6	AFM200	86	85	38	14 c	1.6a
7	Thiram	87	85	41	2 a	2.3 cd
	<b>P-value</b>	<b>0.434</b>	<b>0.282</b>	<b>0.243</b>	<b>0.0011</b>	<b>0.0001</b>

DAS = Days after sowing

Means within columns followed by the same letter are not significantly different at the 5% level according to Least Significant Difference (LSD) test.

**Figure 2.1: Seed treatment effects on healthy root development**

**Trial 2 (Cont.)****Figure 2.2: Seed treatment effects on root rot index****Discussions**

The seeds were planted under ideal field conditions, and rapid seedling emergence was noted. Although there were no significant differences in the seedling emergence and survival, there was a trend of slight improvements in seedling emergence and establishment due to the fungicide seed treatments in comparison to the untreated control (Table 2.1). There was also a trend of slightly lower average fresh shoot weight of plants produced from seeds treated with AFM. This result was consistent with the field observation of a slight delay in seedling emergence and consequently smaller plant size with the AFM seed treatments. The delay in seedling emergence appeared to be due to azoxystrobin. However, this delay in plant emergence and growth is not expected to impact on crop yield.

*Rhizoctonia* and *Fusarium* were the main causes of root rot in the trial. Most roots were affected by root rot, but the severity was considered to be mild to moderate. Only a small percentage of roots were healthy and completely free of rot. There were significant differences between treatments in the percentage of healthy roots and root rot index (Table 2.1). AFM seed treatments resulted in significantly higher incidence of healthy roots and lower root rot severity (Figures 2.1-2.2). This finding indicates that the combination of three fungicide active ingredients, azoxystrobin, fludioxinol and metalaxyl, was more effective in protecting the seeds and reducing root rot, in comparison to the fludioxinol and metalaxyl combination and the standard thiram treatment. Although not significantly different to AFM at 100 mL/100 kg seeds, AFM at 200 mL/100 kg seeds appeared to be the optimum rate for root rot control.

### **Trial 3: Evaluation of new seed dressing combinations on beans in a large scale study, 2005/06**

#### **Summary**

A relatively large scale trial set up to compare new alternative seed treatments against the standard thiram seed treatment on processing bean seeds within a commercial processing bean crop. The new seed treatments consisted of fludioxonil, metalaxyl + fludioxonil, metalaxyl + fludioxonil + thiamethoxam, metalaxyl + fludioxonil + thiamethoxam + zinc. The treated seeds were planted in whole bed rows and treatments were repeated eight times.

Severe root rot of all plants was noted in the trial area, as well as the whole paddock. Although the new seed treatments did not prevent root rot, they were shown to reduce the severity of the tap root rot, and hence reduced wilting of plants. All of the alternative new seed treatments with metalaxyl and fludioxonil reduced plant wilt incidence by at least 87% in comparison to the standard thiram control treatment. Similarly, the incidence of plants with rotten tap root was reduced by at least 33% by the alternative seed treatments when compared to the thiram control. A higher percentage of tap roots on plants from the thiram seed treatment were completely rotten, and there was a strong linear relationship between the tap root rot and the incidence of wilting plants.

The new seed treatments with metalaxyl and fludioxonil, with and without thiamethoxam, tended to result in lower fresh shoot weights. However, the adverse effects of the seed treatment combinations could be successfully reversed with the addition of zinc to the seed treatment combinations.

#### **Materials & Methods**

##### **Trial Summary**

<b>Treatment</b>	Seeds treated by Seed Solutions, a commercial seed treatment company. A polymer film coating was applied onto the treated seeds.
<b>Location</b>	Within a commercial processing bean crop at Sassafras.
<b>Soil Type</b>	Ferrosol
<b>Crop</b>	Processing green beans
<b>Variety</b>	Flavor Sweet
<b>Trial Design</b>	Randomised complete block
<b>Replicates</b>	8
<b>Plot size</b>	1 bed x 200 m long
<b>Trial area</b>	48 beds by approximately 200 m long
<b>Comment</b>	Seeds sown with a precision commercial seed drill.
<b>Sowing date</b>	01/12/05

##### **Seed dressing formulation**

<b>Product Code</b>	<b>Active Ingredient (ai)</b>	<b>Concentration of Active Ingredient</b>
M	metalaxyl	350 g/L
F	fludioxonil	100 g/L
Thiram	thiram	600 g/L
Thx	thiamethoxam	350 g/L

**Trial 3 (Cont.)****Treatment List**

No.	Seed Treatment	Active ingredient concentration (g per 100 kg seed)	Product Rate (per 100 kg seed)
1	Thiram		200 mL
2	M + F + Thx + Zinc	70 g + 20 g + 80.5 g	200 mL + 200 mL + 230 mL
3	M + F + Thx	70 g + 20 g + 80.5 g	200 mL + 200 mL + 230 mL
4	M + F	70 g + 20 g	200 mL + 200 mL

**Disease assessments**

At 47 days after sowing (DAS), approximately 40 plants within a 3 m plant row were assessed for wilting incidence and severity as described below. At 49 DAS, 15 consecutive plants were collected from each treatment plot – whole plants including their root system were dug from the ground using a spade, washed and then assessed for root rot severity as described below. The plants fresh root and shoot weights were also recorded. Analysis of variance was conducted on the data set using Statgraphics Plus 2.0, and pairwise comparisons were made of the mean values using Least Significant Difference Test (LSD).

Plant wilt severity rating	Description
0	No wilt.
1	Wilting of a few top leaves on one to two branches.
2	Wilting of leaves on more than two branches or up to 30% of plant.
3	Wilting on 30% to 70% of the plant.
4	Wilting on more than 70% of the plant or desiccation of the whole plant.
Root rot severity rating	Description
0	Healthy plant.
1	No hypocotyl rot and slight root discolouration.
2	Moderate hypocotyl root discolouration or root rot.
3	Severe hypocotyl rot or rotten tap root.
4	Dead or dying plant.

**Chronology of Events**

Date	Days After Application (DAA)	Days After Sowing (DAS)	Event in the trial area
08/11/05	0		Seed treatments applied.
01/12/05	23	0	Seed sown.
17/01/06	70	47	Plant wilt assessment (plants within 3 m row).
19/01/06	72	49	Tap root rot and fresh shoot weight assessments.



## **Trial 3 (Cont.)**

### **Photographs**

Different bean seed treatments sown in each plant row and repeated eight times across the paddock (Photograph 1); plants with different degree of wilting severity (Photograph 2)



Cross section of relatively healthy deep tap roots of plants grown from the new seed treatments (Photograph 3); completely rotten tap roots of plants from the standard thiram seed treatment (Photograph 4)

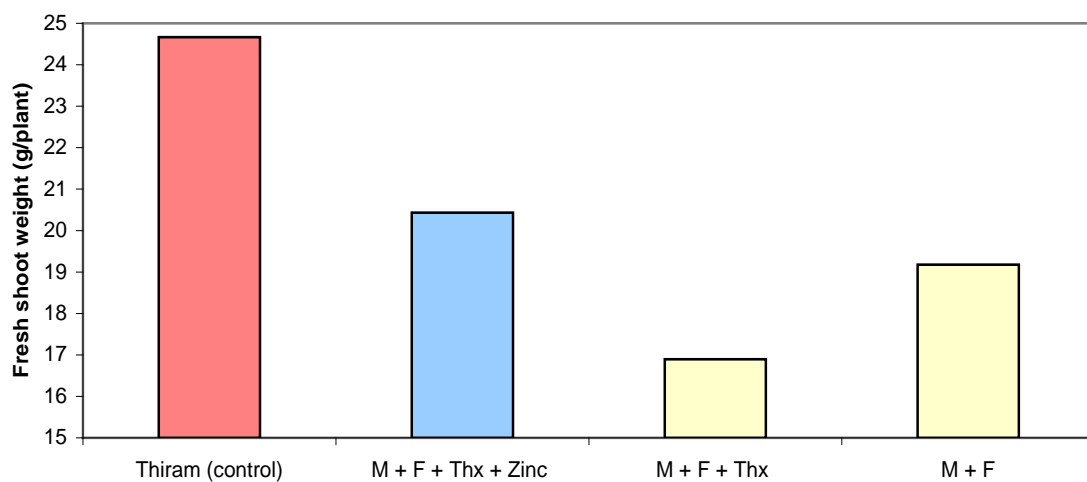
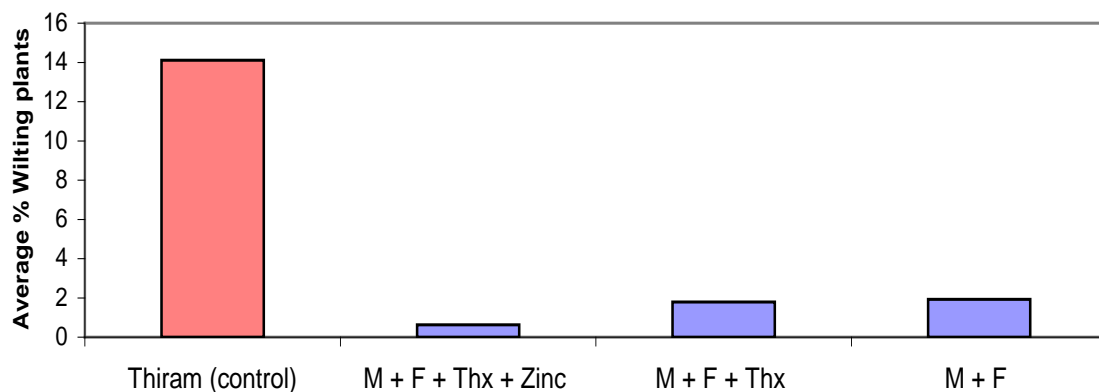


**Trial 3 (Cont.)****Results****Table 3.1: Mean fresh shoot weight, plant wilting incidence and severity, and tap root rot of beans**

No.	Seed Treatment	Plant wilt incidence (% plant affected)	Plant wilt severity index of affected plants	Average fresh shoot weight (g/plant)	Tap root rot incidence (% plants with rotten tap root)
		47DAS	47DAS	49DAS	49DAS
1	Thiram standard (control)	14.1 a	2.5	24.7 c	43 c
2	M + F + Thx + Zinc	0.6 b	2.0	20.4 b	19 a
3	M + F + Thx	1.8 b	1.3	16.9 a	21 ab
4	M + F	1.9 b	2.6	19.2 ab	29 b
	<b>P-value</b>	<b>&lt; 0.0001</b>	-	<b>0.0012</b>	<b>&lt; 0.0001</b>

DAS = Days after sowing

Means within columns followed by the same letter are not significantly different at the 5% level according to Least Significant Difference (LSD) test.

**Figure 3.1: Seed treatment effects on the average fresh shoot weight at 49DAS****Figure 3.2: Seed treatment effects on the incidence of wilting plants at 47DAS**

## Trial 3 (Cont.)

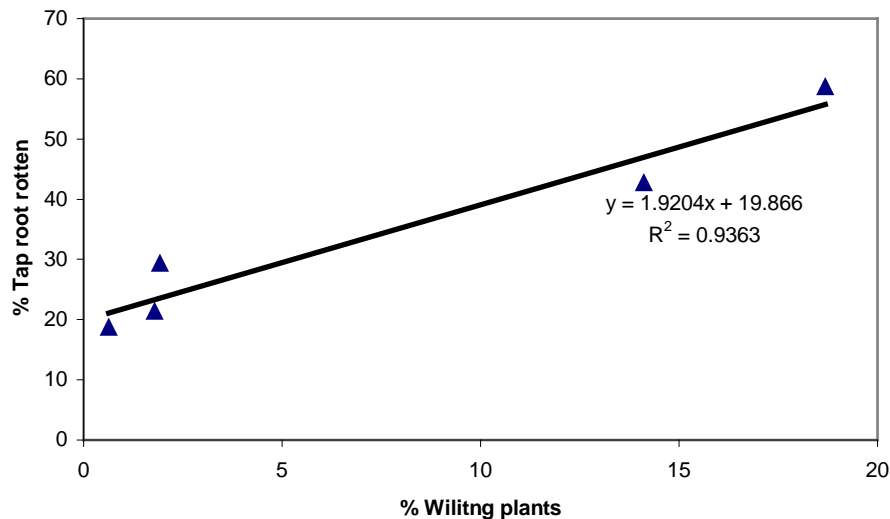
### Discussion

At the flowering period, a relatively high percentage of bean plants in the paddock, both inside and outside the trial area, were wilting. With the standard thiram seed treatment, there was an average of 14% wilt affected plants. The wilting symptoms ranged from temporary wilt to permanent wilt or complete desiccation of the plant (Photograph 2).

There were significant differences in the average fresh shoot weight between the seed treatments (Table 3.1). The new seed treatments with metalaxyl and fludioxonil, with and without thiamethoxam (Treatments 3-4) tended to result in lower fresh shoot weights (Figure 3.1). This indicates that the chemical combinations, particularly with three active ingredients in Treatment 3, could delay seed germination, and seedling emergence and growth. The addition of zinc (Treatment 2) appeared to help counter the adverse effect.

There were significant differences in the plant wilt incidence and tap root rot incidence between the seed treatments (Table 3.1). All of the alternative new seed treatments with metalaxyl and fludioxonil reduced plant wilt incidence by at least 87% in comparison to the standard thiram control treatment (Figure 3.2). Similarly, the incidence of plants with rotten tap roots were reduced by at least 33% by the alternative seed treatments when compared to the thiram control (Figure 3.2). A higher percentage of tap roots on plants from the thiram seed treatment were completely rotten (Photographs 3-4). There was a linear relationship between the tap root rot and incidence of wilting plants (Figure 3.3). This indicates that the rotten tap roots had resulted in poor water uptake, and hence wilting symptoms on the affected plants.

**Figure 3.3: The relationship between tap root rot and incidence of wilting plants**



## **Trial 4: Screening seed treatments on peas, beans, pumpkins and cauliflowers under cold conditions in Tasmania, 2006**

### **Summary**

Pea, bean, pumpkin and cauliflower seeds were treated with the seed dressing combinations fludioxonil + metalaxyl (FM) and azoxystrobin + fludioxonil + metalaxyl (AFM) at different rates (FM150, FM250, AFM75, AFM100 and AFM200), and compared with untreated control seeds and the current standard thiram seed treatment. Assessments were made for seedling emergence and survival. The surviving seedlings were examined for root rot, and their average fresh shoot and root weights were recorded.

All pea seed treatments caused an obvious increase in seedling emergence and survival in comparison to the untreated control seeds, with very few seedlings in the untreated control plots. Seed rot, root rot and stem or collar rot caused by *Ascochyta* were the causes of poor seedling emergence, survival and growth from the untreated control seeds. All the new alternative seed treatments with FM and AFM at different rates generally resulted in higher seedling survival than the standard thiram seed treatment because of improved control of *Ascochyta*. AFM seed treatments at the higher rates of 100 and 200 mL product per 100 kg seeds resulted in the lowest percentage of seed nodes affected by *Ascochyta* rot.

Lower root rot severity was also recorded on bean seedlings from the AFM treated seeds in comparison to those from the other seed treatments and the untreated control. Under very cold field conditions, AFM seed treatments were shown to delay bean seedling emergence and, therefore, reduce shoot growth.

On cauliflowers, all of the fungicide seed treatments resulted in very poor seedling germination and emergence. There may have been a cross contamination with a toxic material during the cauliflower seed treatment process. No such adverse effects were detected in the initial screening trials for safety, storage and pathogenicity (refer Sections A, B and C). As a result of the severe toxic effects on all the treated seeds, no conclusions could be made on the effects of seed treatments on cauliflowers in this study.

The cold conditions in this study did not favour pumpkin germination or growth; therefore, no conclusions could be made on the effects of seed treatments on pumpkins in this study.

### **Materials and Methods**

#### **Trial Summary**

<b>Location</b>	Forthside, Tasmania
<b>Soil Type</b>	Ferrosol
<b>Crop</b>	Green beans, green peas, cauliflower, butternut pumpkin
<b>Trial Design</b>	Complete randomised block
<b>Replicates</b>	5
<b>Plot Size</b>	1 m x 1.2 m bed
<b>Comment</b>	40 seeds sown per plot in 4 rows

**Trial 4 (Cont.)****Seed dressing active ingredient and concentrations**

Code	Active Ingredient (ai)	Concentration of ai
Thiram	thiram	800 g/kg
FM	fludioxonil + metalaxyl	2.5% + 1%
AFM	azoxystrobin + fludioxonil + metalaxyl-M	7.5% + 1.25% + 3.75% w/v

**Treatment List**

No.	Crop	Treatment	Active Ingredient (ai)	ai (g/100 kg seed)	Product Rate /100 kg seed
1	Pea	UTC	-	-	-
2		FM150	fludioxonil + metalaxyl-M	3.75 + 1.5	150 mL
3		FM250	fludioxonil + metalaxyl-M	6.25 + 2.5	250 mL
4		AFM75	azoxystrobin + fludioxonil + metalaxyl-M	1.28 + 0.94 + 2.81	75 mL
5		AFM100	azoxystrobin + fludioxonil + metalaxyl-M	7.5 + 1.25 + 3.75	100 mL
6		AFM200	azoxystrobin + fludioxonil + metalaxyl-M	15 + 2.5 + 7.5	200 mL
7		Thiram	thiram	400	500 g
1	Bean	UTC	-	-	-
2		FM150	fludioxonil + metalaxyl-M	3.75 + 1.5	150 mL
3		FM250	fludioxonil + metalaxyl-M	6.25 + 2.5	250 mL
4		AFM75	azoxystrobin + fludioxonil + metalaxyl-M	1.28 + 0.94 + 2.81	75 mL
5		AFM100	azoxystrobin + fludioxonil + metalaxyl-M	7.5 + 1.25 + 3.75	100 mL
6		AFM200	azoxystrobin + fludioxonil + metalaxyl-M	15 + 2.5 + 7.5	200 mL
7		Thiram	thiram	400	500 g
1	Cauliflower	UTC	-	-	-
2		FM150	fludioxonil + metalaxyl-M	3.75 + 1.5	150 mL
3		FM250	fludioxonil + metalaxyl-M	6.25 + 2.5	250 mL
4		AFM75	azoxystrobin + fludioxonil + metalaxyl-M	1.28 + 0.94 + 2.81	75 mL
5		AFM100	azoxystrobin + fludioxonil + metalaxyl-M	7.5 + 1.25 + 3.75	100 mL
6		AFM200	azoxystrobin + fludioxonil + metalaxyl-M	15 + 2.5 + 7.5	200 mL
7		Thiram	thiram	400	500 g

UTC = Untreated control

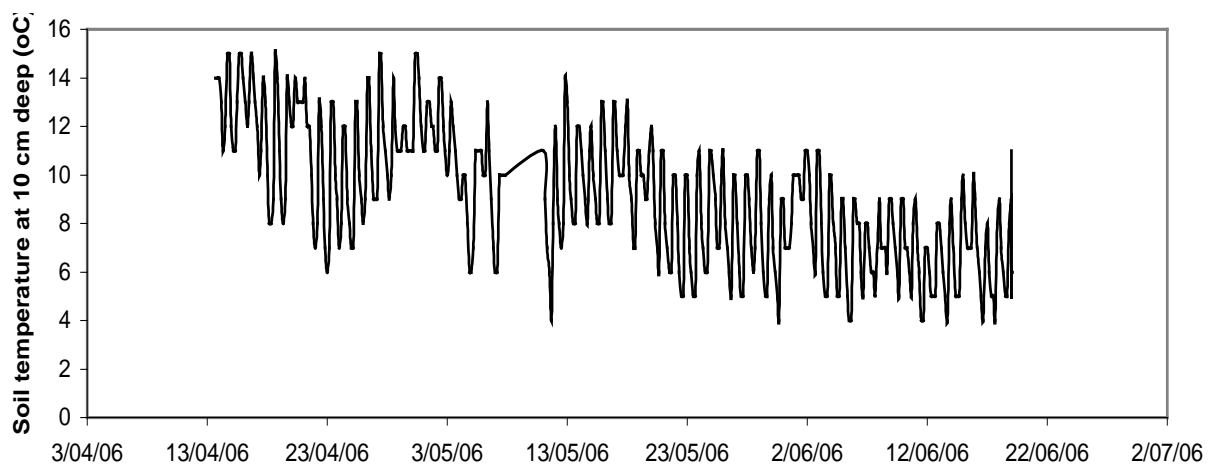
**Trial 4 (Cont.)****Disease assessments**

At the appropriate time after sowing, seedling emergence and survival were recorded. At approximately 6-7 weeks after sowing, the surviving seedlings were assessed for root rot incidence and rated for severity, as described below, and then fresh root and shoot weights were recorded. Analysis of variance was conducted on the data set using Statgraphics Plus 2.0, and pairwise comparisons were made of the mean values using Least Significant Difference Test (LSD).

<b>Root rot severity rating</b>	<b>Description</b>
	<b>Beans</b>
0	Healthy plant.
1	Root discolouration with slight to no hypocotyl rot.
2	Moderate hypocotyl discolouration or rot.
3	Severe hypocotyl rot or tap root rot.
4	Dead or dying plant.
	<b>Cauliflower</b>
0	No root rot.
1	Slight discolouration with no constriction.
2	Moderate discolouration with no constriction.
3	Constriction of the root.
4	Constriction & dead plant.
<b>Ascochyta rot Severity Rating</b>	<b>Description</b>
	<b>Peas</b>
0	Healthy plant.
1	Leaf infection only.
2	Leaf infection + traces of collar rot.
3	Leaf infection + collar rot (no constriction or partial basal stem rot).
4	Leaf infection + collar rot (around the basal stem).
5	Dead.

**Trial 4 (Cont.)****Chronology of Events**

TRIAL 4 - FORTHSIDE, TASMANIA		
DATE	DAYS AFTER SOWING (DAS)	EVENT
06/12/05		Seeds were treated by coating with the relevant seed dressings, and then sealed with a polymer film using a rotary seed treatment machine.
06/04/06	0	Seeds hand sown. Beans and peas were sown at a depth of approximately 2.0 to 2.5 cm, and cauliflowers at approximately 1 cm.
		<b>Beans</b>
06/04/06	0	Seeds sown.
04/05/06	28	Assessment for seedling emergence.
11/05/06	35	Assessment for seedling survival.
24/05/06	48	Final assessment for seedling survival, and root rots. Record fresh shoot and root weights
		<b>Peas</b>
06/04/06	0	Seeds sown.
21/04/06	15	Assessment for seedling emergence.
26/04/2006	20	Assessment for seedling survival.
01/06/06	56	Final assessment for seedling survival, and root rot. Fresh shoot and root weights recorded.
		<b>Cauliflowers</b>
06/04/06	0	Seeds sown.
11/05/06	35	Assessment for seedling emergence.
17/05/06	41	Assessment for seedling survival and root rots. Fresh shoot and root weights recorded.

**Soil temperature during the trial**

**Trial 4 (Cont.)****Photographs**

Healthy seeds and seed nodes of seedlings grown from AFM treated seeds (Photograph 4.1);  
*Ascochyta* black rot at the seed nodes of seedlings grown from untreated seeds (Photograph 4.2)



*Ascochyta* stem rot or collar rot:  
slight to moderate collar rot with ratings 1-2 (Photograph 4.3);  
severe collar rot with rating 3 (Photograph 4.4)





**Trial 4 (Cont.)**

**Bean root rot severity ratings based on the proportion of roots with discolouration and constriction (Photograph 4.5)**



**Cauliflower root rot severity ratings based on the proportion of roots with discolouration and constriction (Photograph 4.6)**

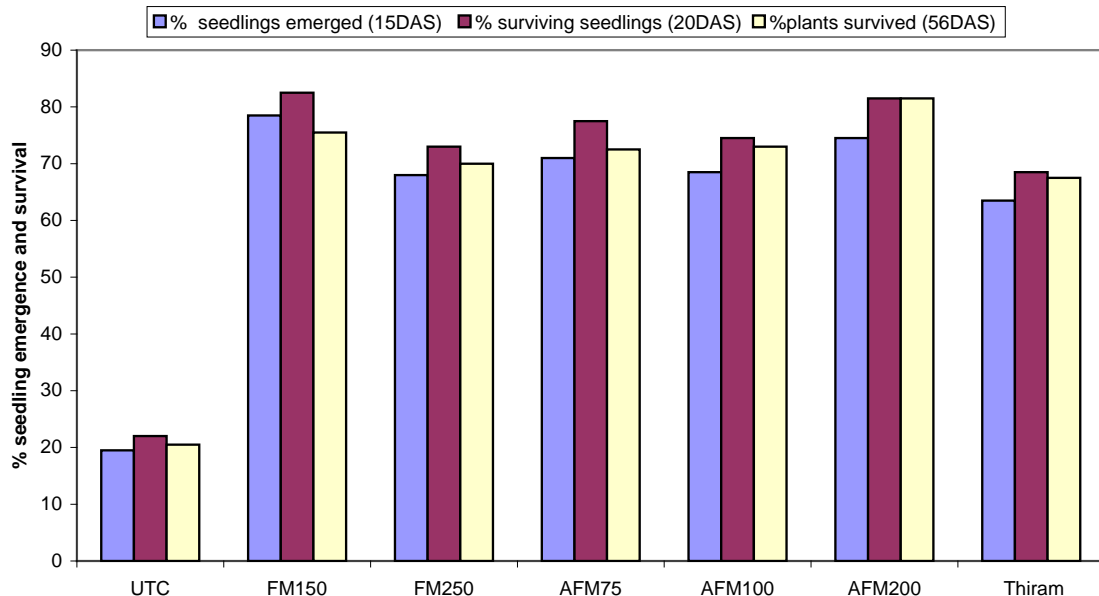


**Trial 4 (Cont.)****Results****Peas****Table 4.1: Mean pea seedling emergence and survival rates, under cold conditions in Tasmania**

No.	Treatment	% Seedlings emerged (15DAS)	% Surviving seedlings (20DAS)	% Surviving seedlings (56DAS)
1	UTC (Untreated control)	20 *	22 *	21 *
2	FM150	79 c	83 d	76ab
3	FM250	68ab	73ab	70a
4	AFM75	71abc	78 bcd	73a
5	AFM100	69ab	75abc	73a
6	AFM200	75 bc	82 cd	82 b
7	Thiram	64a	69a	68a
	<b>P-value *</b>	<b>0.046</b>	<b>0.011</b>	<b>0.041</b>

DAS = Days after sowing

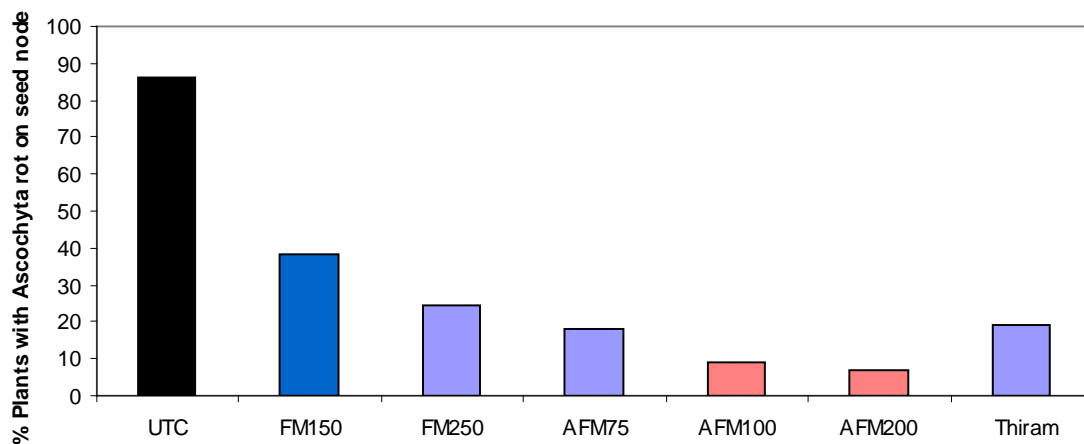
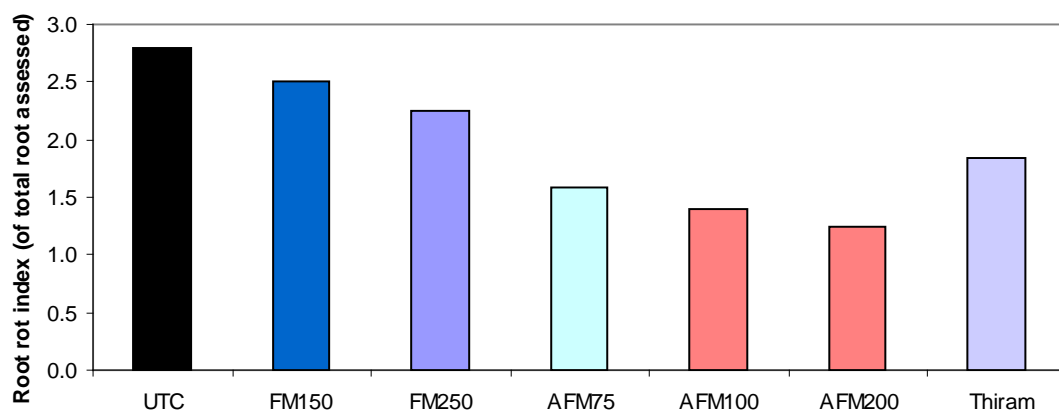
\* Untreated control data set was not included in the analysis of variance and pairwise comparative tests because it was obviously different from the chemical seed treatment. Data set was normally distributed if the untreated control data set was excluded. Means within columns followed by the same letter are not significantly different at the 5% level according to Least Significant Difference (LSD) test.

**Figure 4.1: Seed treatment effects on pea seedling emergence and survival**

**Trial 4 (Cont.)****Table 4.2: Mean *Ascochyta* seed node rot and collar rot index of surviving pea plants at 56DAS**

No.	Treatment	% Plants with <i>Ascochyta</i> seed node rot	<i>Ascochyta</i> collar rot index
1	UTC (Untreated control)	86*	2.8*
2	FM150	38 c	2.5 e
3	FM250	24 b	2.2 d
4	AFM75	18 b	1.6 b
5	AFM100	9a	1.4ab
6	AFM200	7a	1.2a
7	Thiram	19 b	1.8 c
	<b>P-value*</b>	<b>&lt;0.0001</b>	<b>&lt; 0.0001</b>

\* Untreated control data set was not included in the analysis of variance and pairwise comparative tests because it was obviously different from the chemical seed treatment. Data set was normally distributed if the untreated control data set was excluded. Means within columns followed by the same letter are not significantly different at the 5% level according to Least Significant Difference (LSD) test.

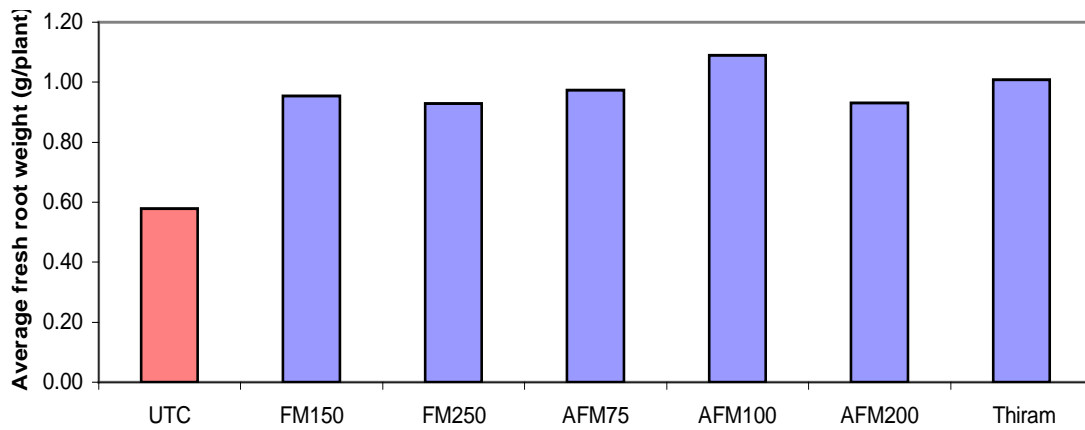
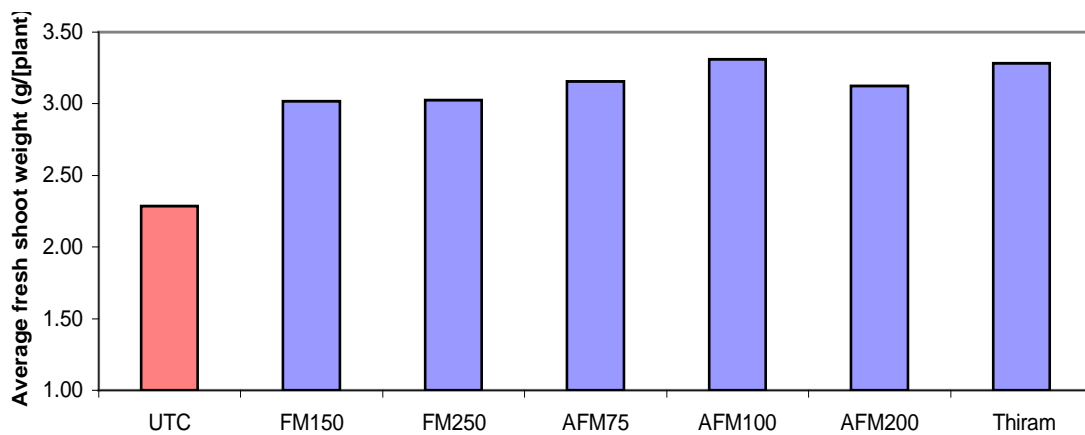
**Figure 4.2: Seed treatment effects on the incidence of *Ascochyta* rot on pea seed nodes****Figure 4.3: Seed treatment effects on the average root rot index of surviving pea plants**

***Trial 4 (Cont.)*****Table 4.3: Mean fresh root and shoot weights of surviving pea plants at 56DAS**

No.	Treatment	Average fresh root weight (g/plant)	Average fresh shoot weight (g/plant)
1	UTC (Untreated control)	0.58a	2.28a
2	FM150	0.95 b	3.02 b
3	FM250	0.93 b	3.02 b
4	AFM75	0.97 b	3.15 b
5	AFM100	1.09 b	3.31 b
6	AFM200	0.93 b	3.13 b
7	Thiram	1.01 b	3.28 b
	<b>P-value*</b>	<b>0.0001</b>	<b>0.0019</b>

DAS = Days after sowing

Means within columns followed by the same letter are not significantly different at the 5% level according to Least Significant Difference (LSD) test.

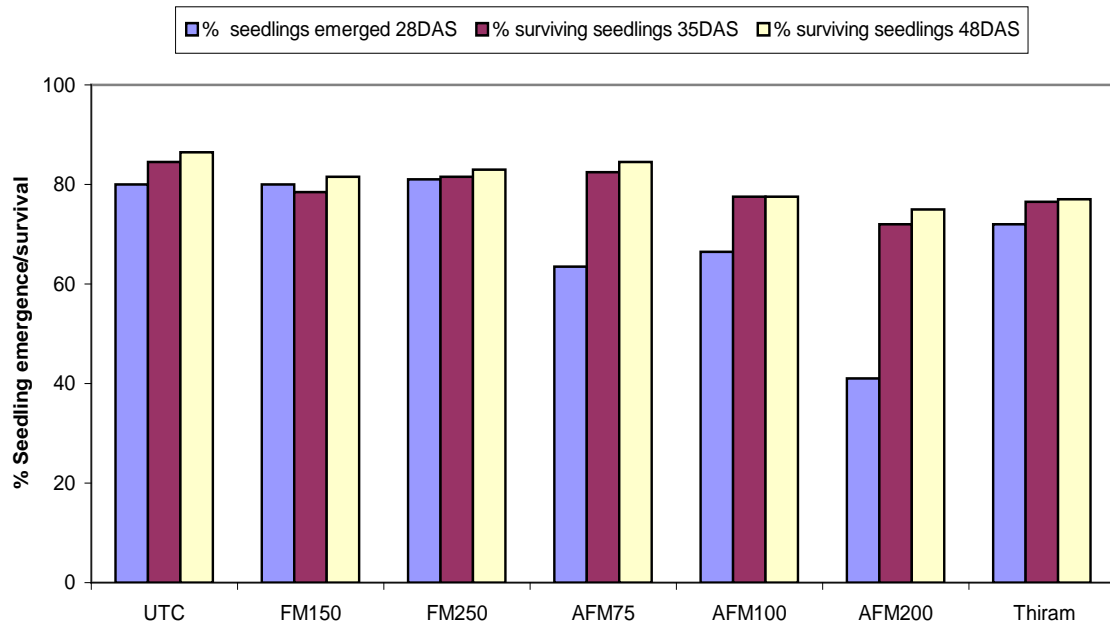
**Figure 4.4: Seed treatment effects on the average pea fresh root weight****Figure 4.5: Seed treatment effects on the average pea fresh shoot weight**

**Trial 4 (Cont.)****Beans****Table 4.4: Mean bean seedling emergence and survival under cold conditions in Tasmania**

		28DAS	35DAS	48DAS
No.	Treatment	% Seedlings emerged	% Surviving seedlings	% Surviving seedlings
1	UTC (Untreated control)	80 c	85	87
2	FM150	80 c	79	82
3	FM250	81 c	82	83
4	AFM75	64 b	83	85
5	AFM100	67 b	78	78
6	AFM200	41 a	72	75
7	Thiram	72 bc	77	77
	<b>P-value</b>	<b>&lt; 0.0001</b>	<b>0.326</b>	<b>0.370</b>

DAS = Days after sowing

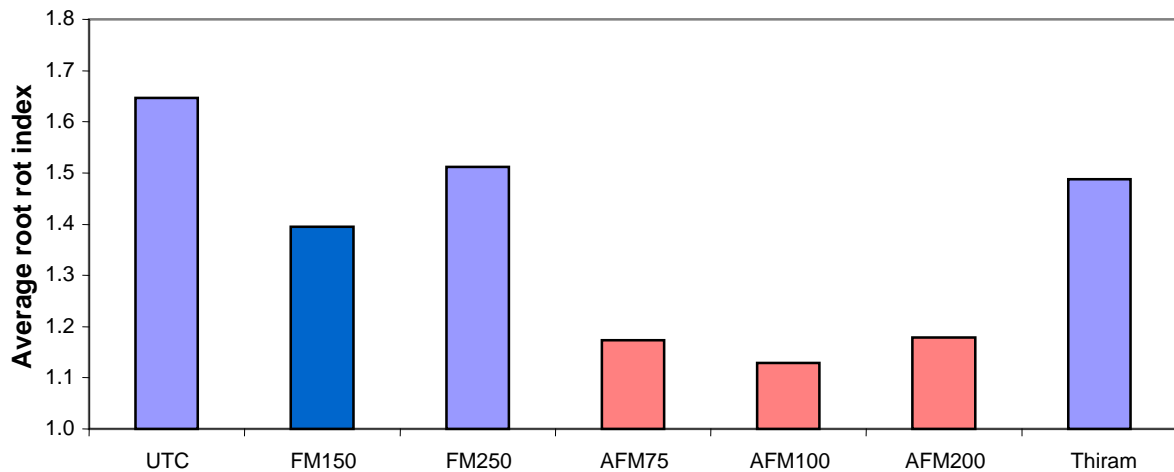
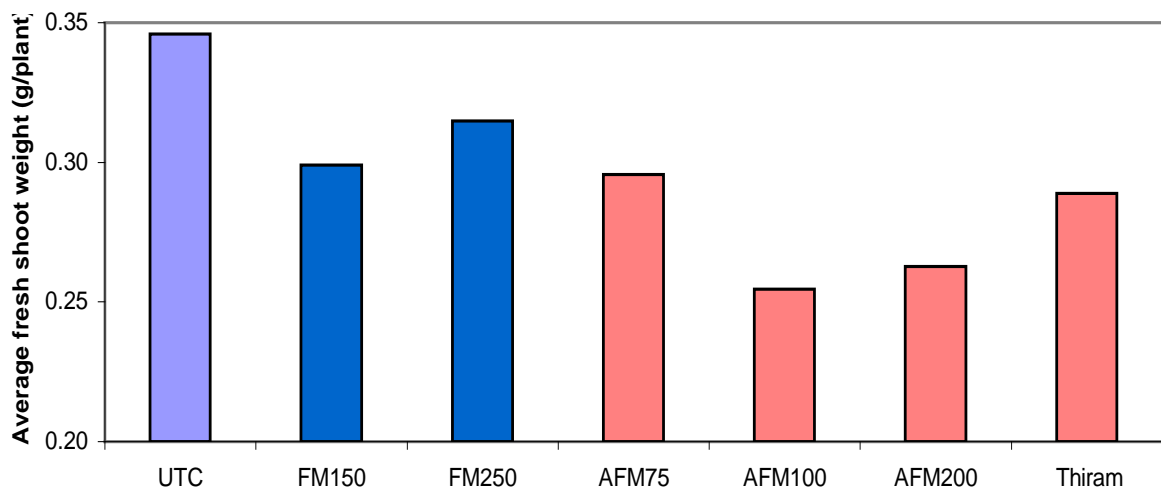
Means within columns followed by the same letter are not significantly different at the 5% level according to Least Significant Difference (LSD) test.

**Figure 4.6: Seed treatment effects bean seedling emergence and survival**

**Trial 4 (Cont.)****Table 4.5: Mean root rot index and fresh bean shoot and root weight of surviving bean plants at 48 days after sowing (DAS)**

No.	Treatment	Root rot index	Average fresh root weight g/plant	Average fresh shoot weight g/plant
1	UTC (Untreated control)	1.6 c	0.346	0.915 d
2	FM150	1.4 b	0.299	0.767 c
3	FM250	1.5 bc	0.315	0.758 c
4	AFM75	1.2a	0.296	0.738 bc
5	AFM100	1.1a	0.255	0.660ab
6	AFM200	1.2a	0.263	0.642a
7	Thiram	1.5 bc	0.289	0.665ab
	<b>P-value</b>	<b>0.0001</b>	<b>0.122</b>	<b>&lt; 0.0001</b>

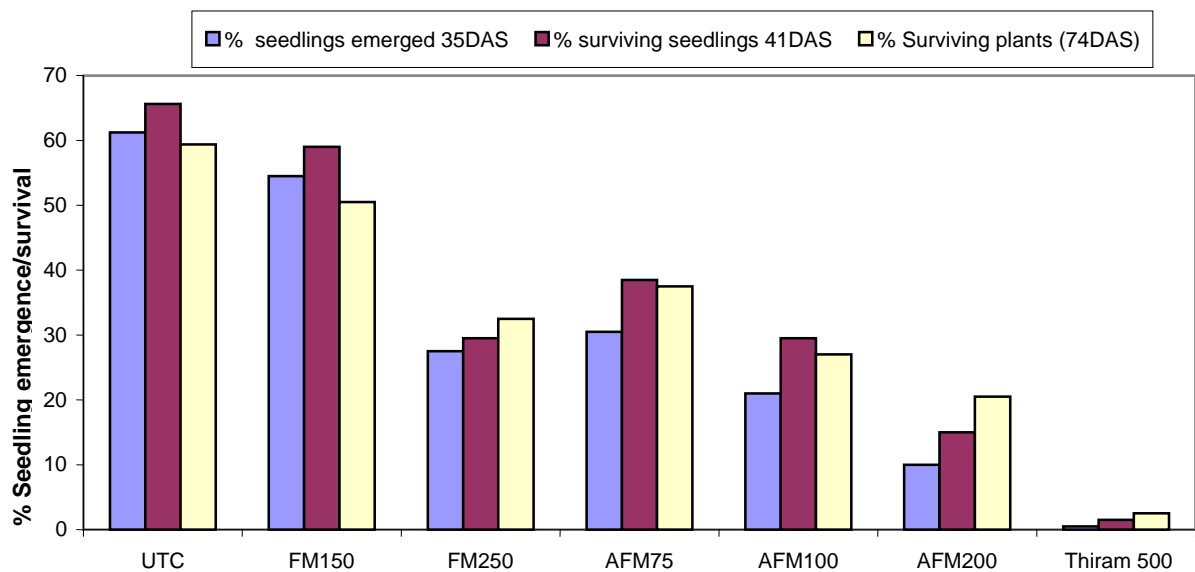
Means within columns followed by the same letter are not significantly different at the 5% level according to Least Significant Difference (LSD) test.

**Figure 4.7: Seed treatment effects on root rot index on surviving bean plants at 48DAS****Figure 4.8: Seed treatment effects on the average fresh root weight of surviving bean plants at 48DAS**

**Trial 4 (Cont.)****Cauliflowers****Table 4.6: Mean seedling emergence and survival rates under cold conditions in Tasmania**

No.	Treatment	% Seedlings emerged (35DAS)	% Surviving seedlings (41DAS)	% Surviving plants (74DAS)
1	UTC (Untreated control)	61 d	66 d	59 e
2	FM150	55 d	59 d	51 e
3	FM250	28 c	30 c	33 cd
4	AFM75	31 c	39 c	38 d
5	AFM100	21 bc	30 c	27 bc
6	AFM200	10ab	15 b	21 b
7	Thiram 500	0.5a	1.5a	2.5a
	<b>P-value</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>

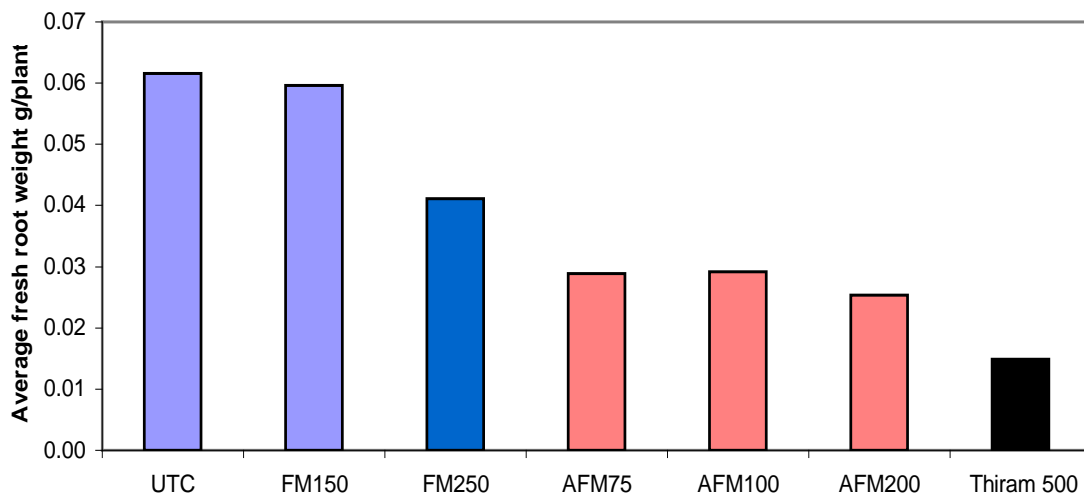
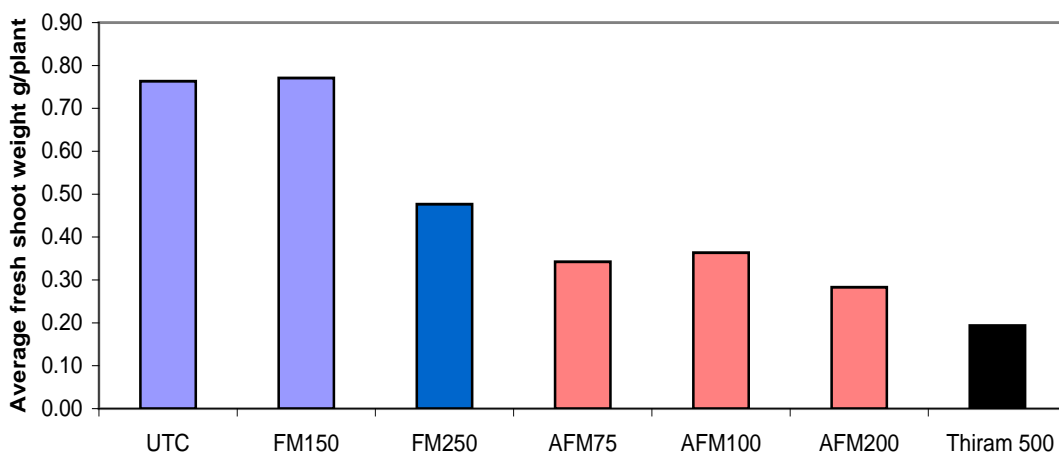
Means within columns followed by the same letter are not significantly different at the 5% level according to Least Significant Difference (LSD) test.

**Figure 4.9: Seed treatment effects on cauliflower seedling emergence and survival**

***Trial 4 (Cont.)*****Table 4.7: Mean percentage of healthy roots, root rot index, and fresh root and shoot weights of cauliflowers at 41 days after sowing (41DAS)**

No.	Treatment	% Healthy roots (no root rot)	Root rot index	Average fresh root weight (g/plant)	Average fresh shoot weight (g/plant)
1	UTC (Untreated control)	77	0.47	0.062 d	0.763 c
2	FM150	84	0.31	0.060 d	0.771 c
3	FM250	64	0.53	0.041 c	0.477 b
4	AFM75	81	0.32	0.029 b	0.342ab
5	AFM100	82	0.41	0.029 b	0.363ab
6	AFM200	79	0.43	0.025ab	0.283a
7	Thiram 500	33 *	1.00 *	0.015 *	0.193 *
	<b>P-value</b>	<b>0.517</b>	<b>0.792</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>

Means within columns followed by the same letter are not significantly different at the 5% level according to Least Significant Difference (LSD) test.

**Figure 4.10: Seed treatment effects on the average fresh root weight of cauliflowers****Figure 4.11: Seed treatment effects on the average fresh shoot weight of cauliflowers**



## **Trial 4 (Cont.)**

### **Discussion**

#### **Peas**

All fungicide seed treatments resulted in an obvious difference in seedling emergence and survival in comparison to the untreated control seeds (Table 4.1). There were very few seedlings in the untreated control plots. *Ascochyta* seed and root rot, as well as stem rot (collar rot), were the main causes of poor seedling emergence and survival from the untreated control seeds.

Generally, the new alternative seed treatments with FM and AFM resulted in higher seedling survival than the standard thiram seed treatment (Figure 4.1). AFM seed treatments at the higher rates of 100 and 200 mL product per 100 kg seeds resulted in the lowest percentage of seed nodes affected by *Ascochyta* rot (Table 4.2, Figure 4.2), reducing *Ascochyta* rot at the seed node by 90% in comparison to the untreated control. This indicates that high rates of azoxystrobin in the AFM seed dressing were especially effective in reducing *Ascochyta* rot at the seed nodes (Photographs 4.1-4.2). Similarly, the levels of *Ascochyta* collar rot or stem rot severity in the AFM seed treatments were less, with significantly lower root rot index in comparison to the untreated control and other seed treatments (Figure 4.3).

All of the seed treatments resulted in bigger seedlings, with higher average fresh root and shoot weights of surviving plants in comparison to the untreated control (Table 4.3, Figures 4.4-4.5).

#### **Beans**

Initially, at 28 days after sowing (DAS), seed treatments with azoxystrobin, fludioxonil and metalaxyl (AFM) delayed seedling emergence (Table 4.4, Figure 4.6). Later, at 35 and 48 DAS, there were no significant differences in the percentage of surviving seedlings, even though the AFM seed treatments appeared to reduce the surviving seedling density by approximately 10%. It should be noted that the beans were grown under field conditions that were considered to be too cold for beans. Therefore, the apparent reduction in seedling density due to AFM seed treatments may not be typical under warmer conditions.

The roots of surviving plants were considered to have mild to moderate levels of root rot. Roots of seedlings from the AFM treated seeds generally resulted in lower root rot index (Figure 4.7). This indicates that these seed treatments could help reduce root rot severity.

The average fresh shoot weights of the AFM seed treatments were generally slightly lower due to the initial delay in seedling emergence (Figure 4.8).

#### **Cauliflowers**

Except for FM150 seed treatment, there was poor seedling emergence and survival due to the chemical seed treatments (Table 4.6). This phytotoxic effect was surprising, as no phytotoxicity was observed at the same rates in the initial screening study in pot trials. In this trial, the cauliflower seeds were treated using a rotary seed treatment machine. It is possible that toxic chemical residue may be present in the rotary seed machine. Further investigations must be carried out to determine if different seed batches or contaminant may have contributed to the poor seedling emergence. Generally, most of the surviving plants were free of root rot (Table 4.7).

## **Trial 5: Screening seed treatments on peas, beans, pumpkins and cauliflowers under hot conditions in Queensland, 2006**

### **Summary**

Pea, bean, pumpkin and cauliflower seeds were treated with the seed dressing combinations fludioxonil + metalaxyl (FM) and azoxystrobin + fludioxonil + metalaxyl (AFM) at different rates (FM150, FM250, AFM75, AFM100 and AFM200) and compared against untreated control seeds and the current standard thiram seed treatment. Assessments were made for seedling emergence and survival. The surviving seedlings were examined for root rot, and their average fresh shoot and root weights were recorded.

All pea seed treatments caused an obvious increase in seedling emergence and survival in comparison to the untreated control seeds, with very few seedlings in the untreated control plots. Of the two new seed dressings, AFM seed treatments gave significantly higher seedling emergence and survival than FM seed treatments.

Bean seed treatments with FM150, FM250, AFM75 and thiram increased seedling emergence and survival compared to the untreated control and AFM100 and AFM200. The high rates of AFM at 100 and 200 appeared to cause a slight adverse effect and slightly reduced plant density. Under hot field conditions that were more favourable to bean crops in this study, AFM seed treatments did not cause any delay in seedling emergence or growth. The AFM bean seed treatments at increasing rates increased the average fresh root weights when compared to the untreated control and the thiram seed treatment. There was a trend of lower root rot index with all of the AFM seed treatments and the FM250 treatment, when compared to the untreated control and the thiram seed treatment.

Pumpkin seeds germinated and emerged very quickly under hot and moist conditions, and there were no significant differences in the seedling emergence and survival, or average fresh root and shoot weights, between treatments. There were, however, trends of slight increases in seedling emergence and survival and lower root rot levels with all of the fungicide seed treatments, compared to the untreated control.

### **Materials and Methods**

#### **Trial Site Details**

<b>Location</b>	Bowen, Queensland
<b>Soil Type</b>	Sandy loam
<b>Crop</b>	Green beans, green peas, cauliflowers, butternut pumpkins
<b>Trial Design</b>	Complete randomised block
<b>Replicates</b>	4
<b>Plot Size</b>	5 m x 1 bed (2 plant rows per bed)
<b>Comments</b>	Green peas and beans - 82 seeds sown per plot Butternut pumpkin - 30 seeds sown per plot Cauliflower – 52 seeds sown per plot

**Trial 5 (Cont.)****Seed dressing active ingredient and concentrations**

Product Code	Active Ingredient (ai)	Concentration of ai
Thiram	thiram	800 g/kg
FM	fludioxonil + metalaxyl	25 g/L + 10 g/L
AFM	azoxystrobin + fludioxonil + metalaxyl-M	75 g/L + 12.5 g/L + 37.5 g/L

**Treatment List**

No.	Crop	Treatment	Active Ingredient (ai)	ai (g/100 kg seed)	Product Rate /100 kg seed
1	Pea	UTC	-	-	-
2		FM150	fludioxonil + metalaxyl-M	3.75 + 1.5	150 mL
3		FM250	fludioxonil + metalaxyl-M	6.25 + 2.5	250 mL
4		AFM75	azoxystrobin + fludioxonil + metalaxyl-M	1.28 + 0.94 + 2.81	75 mL
5		AFM100	azoxystrobin + fludioxonil + metalaxyl-M	7.5 + 1.25 + 3.75	100 mL
6		AFM200	azoxystrobin + fludioxonil + metalaxyl-M	15 + 2.5 + 7.5	200 mL
7		Thiram	thiram	400	500 g
1	Bean	UTC	-	-	-
2		FM150	fludioxonil + metalaxyl-M	3.75 + 1.5	150 mL
3		FM250	fludioxonil + metalaxyl-M	6.25 + 2.5	250 mL
4		AFM75	azoxystrobin + fludioxonil + metalaxyl-M	1.28 + 0.94 + 2.81	75 mL
5		AFM100	azoxystrobin + fludioxonil + metalaxyl-M	7.5 + 1.25 + 3.75	100 mL
6		AFM200	azoxystrobin + fludioxonil + metalaxyl-M	15 + 2.5 + 7.5	200 mL
7		Thiram	thiram	400	500 g
1	Cauliflower	UTC	-	-	-
2		FM150	fludioxonil + metalaxyl-M	3.75 + 1.5	150 mL
3		FM250	fludioxonil + metalaxyl-M	6.25 + 2.5	250 mL
4		AFM75	azoxystrobin + fludioxonil + metalaxyl-M	1.28 + 0.94 + 2.81	75 mL
5		AFM100	azoxystrobin + fludioxonil + metalaxyl-M	7.5 + 1.25 + 3.75	100 mL
6		AFM200	azoxystrobin + fludioxonil + metalaxyl-M	15 + 2.5 + 7.5	200 mL
7		Thiram	thiram	400	500 g

UTC = Untreated control

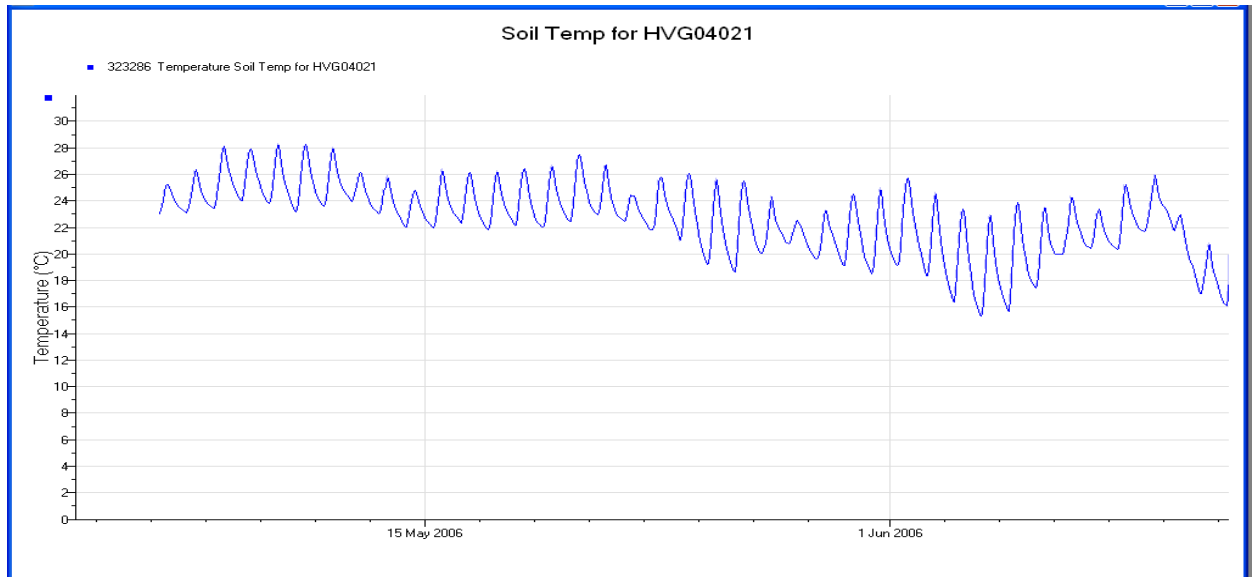
**Trial 5 (Cont.)****Disease assessments**

At 14 and 28 days after sowing, whole plots were assessed for seedling emergence and survival. At approximately 6 weeks after sowing, the surviving seedlings in each plot were assessed for root rot incidence and severity ratings as described below, and their fresh root and shoot weights were then recorded. Analysis of variance was conducted on the data set using Statgraphics Plus 2.0, and pairwise comparisons were made of the mean values using Least Significant Difference Test (LSD).

<b>Root Rot Severity Rating</b>	<b>Description</b>
0	Hypocotyl and roots white and firm; no root pruning.
1	Slightly brown or discoloured hypocotyl and roots; hypocotyl firm under pressure from thumb and forefinger; slight root pruning.
2	Brown or discoloured hypocotyl and roots; hypocotyl and root spongy under pressure from thumb and forefinger; moderate root pruning.
3	Darkly discoloured hypocotyl and roots; hypocotyl and roots collapse easily under pressure from thumb and forefinger; extensive root pruning.
4	Very darkly discoloured hypocotyl and roots; hypocotyl completely collapsed or would collapse easily under pressure from thumb and forefinger; severe root pruning.

**Trial 5 (Cont.)****Chronology of Events**

DATE	DAYS AFTER SOWING (DAS)	EVENT
06/12/05		Seeds were treated, by coating with the relevant seed dressings; they were then sealed with a polymer film using a rotary seed treatment machine.
		<b>Peas and Beans</b>
04/05/06	0	Seeds sown.
18/05/06	14	Emergence count.
01/06/206	28	Survival count.
15/06/06	42	Survival and growth assessment.
		<b>Pumpkins and Cauliflowers</b>
4/05/06	0	Seeds sown.
18/05/06	14	Emergence count.
01/06/06	28	Survival count.
13/06/06	40	Survival and growth assessment.

**Soil temperature during the trial**

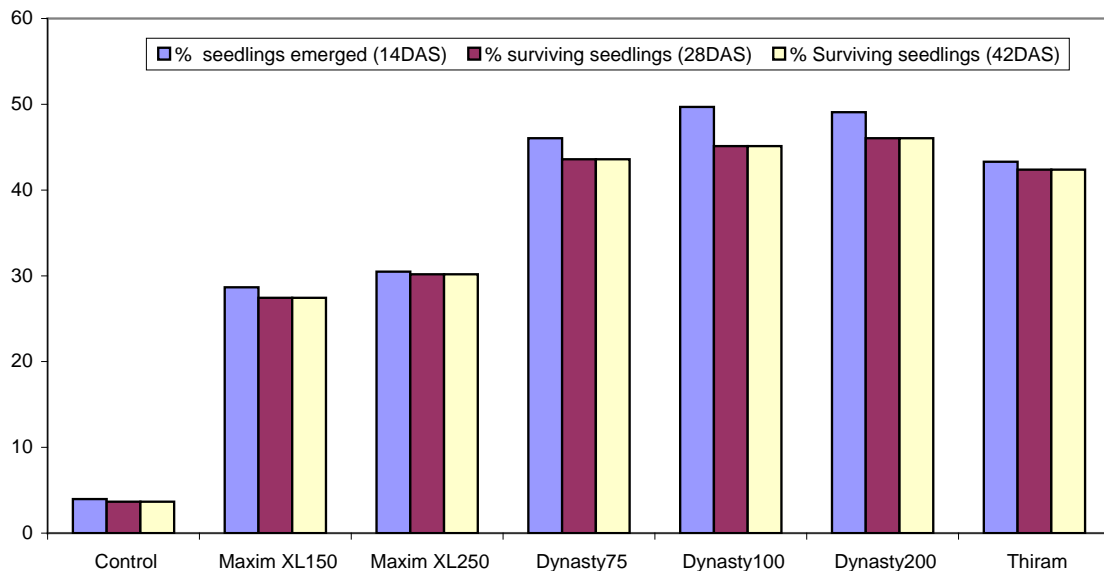
**Trial 5 (Cont.)****Photographs****Pea root rot severity ratings 0-4 (Photograph 5.1)****Bean root rot severity ratings 0-4 (Photograph 5.2)**

**Trial 5 (Cont.)****Results****Peas****Table 5.1: Mean pea seedling emergence and survival**

		14DAS	28DAS	42DAS
No.	Treatment	% Seedlings emerged	% Surviving seedlings	% Surviving seedlings
1	UTC (Untreated control)	4*	4*	4*
2	FM150	29a	27a	27a
3	FM250	30a	30a	30a
4	AFM75	46 b	44 b	44 b
5	AFM100	50 b	45 b	45 b
6	AFM200	49 b	46 b	46 b
7	Thiram	43 b	42 b	42 b
	<b>P-value *</b>	<b>0.0001</b>	<b>0.0001</b>	<b>0.0001</b>

DAS = Days after sowing

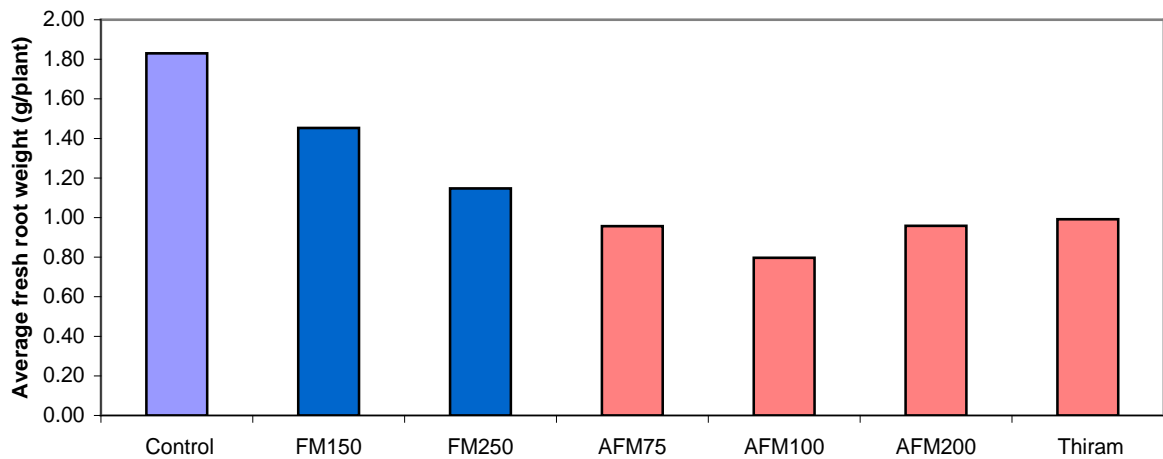
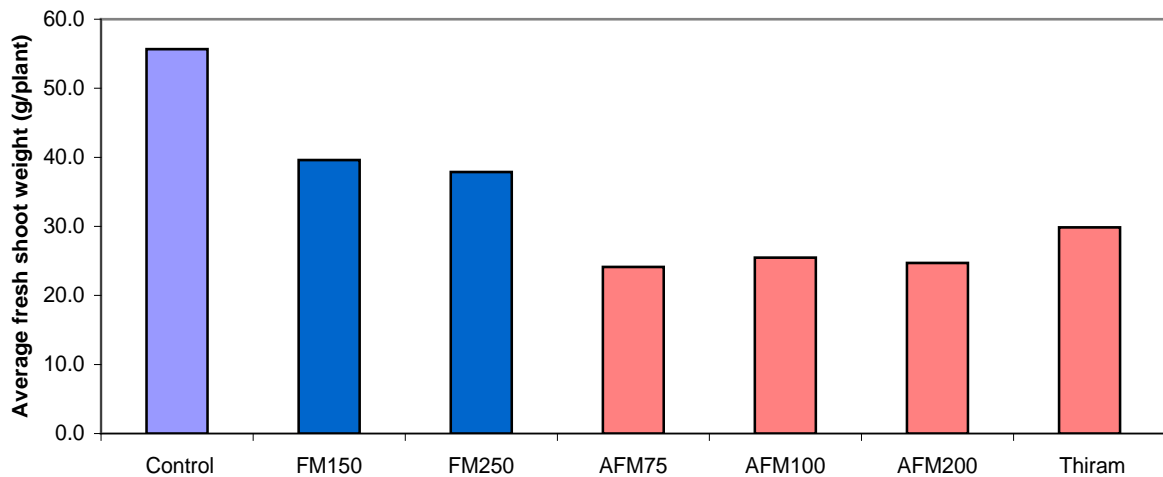
\* Untreated control data set was not included in the analysis of variance and pairwise comparative tests because it was obviously different from the chemical seed treatment. Data set was normally distributed if the untreated control data set was excluded. Means within columns followed by the same letter are not significantly different at the 5% level according to Least Significant Difference (LSD) test.

**Figure 5.1: Seed treatment effects on pea seedling emergence and survival**

***Trial 5 (Cont.)*****Table 5.2: Mean root rot index and fresh shoot and root weight of surviving pea plants at 48 days after sowing (DAS)**

No.	Treatment	Root rot index	Average fresh root weight g/plant	Average fresh shoot weight g/plant
1	UTC (Untreated control)	0.25	1.83 d	55.7 c
2	FM150	0.08	1.45 c	39.6 b
3	FM250	0.10	1.15 bc	37.9 b
4	AFM75	0.03	0.96ab	24.1 a
5	AFM100	0.08	0.80a	25.5a
6	AFM200	0.00	0.96ab	24.7a
7	Thiram	0.03	0.99ab	29.9ab
	<b>P-value</b>	-	<b>0.0001</b>	<b>&lt; 0.00001</b>

Means within columns followed by the same letter are not significantly different at the 5% level according to Least Significant Difference (LSD) test.

**Figure 5.2: Seed treatment effects on the average pea fresh root weight****Figure 5.3: Seed treatment effects on the average pea fresh shoot weight**

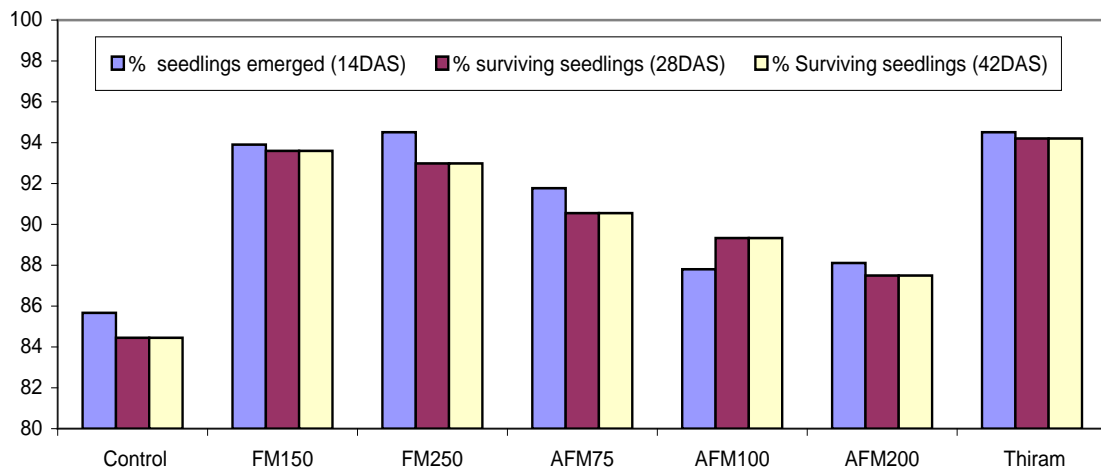


**Trial 5 (Cont.)****Beans****Table 5.2: Mean percentage bean seedling emergence and survival under hot conditions in Bowen, Queensland**

		14DAS	28DAS	42DAS
No.	Treatment	% Seedlings emerged	% Surviving seedlings	% Surviving seedlings
1	UTC (Untreated control)	86	84a	84a
2	FM150	94	94 c	94 c
3	FM250	95	93 bc	93 bc
4	AFM75	92	91 bc	91 bc
5	AFM100	88	89abc	89abc
6	AFM200	88	88ab	88ab
7	Thiram	95	94 c	94 c
	<b>P-value</b>	<b>0.080</b>	<b>0.018</b>	<b>0.018</b>

DAS = Days after sowing

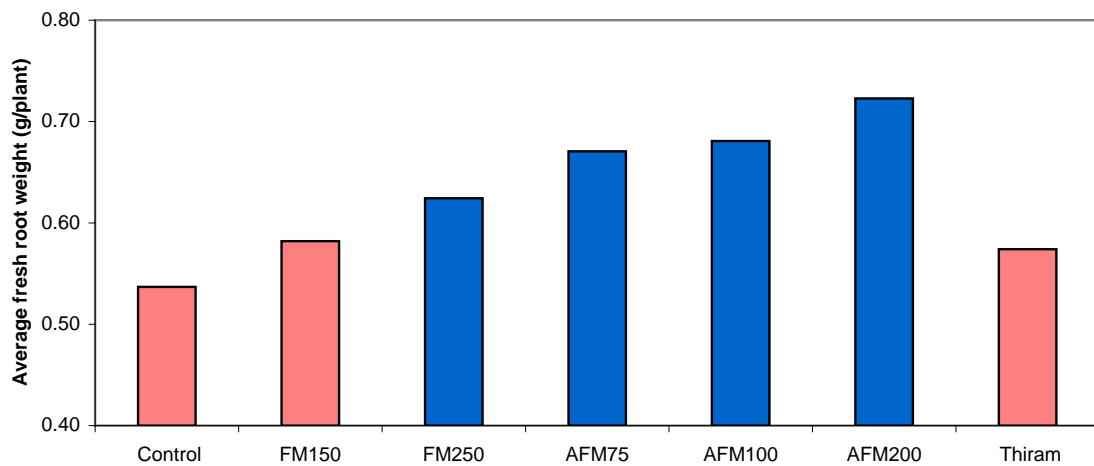
Means within columns followed by the same letter are not significantly different at the 5% level according to Least Significant Difference (LSD) test.

**Figure 5.4: Seed treatment effects on bean seedling emergence and survival**

**Trial 5 (Cont.)****Table 5.4: Mean root rot index and fresh shoot and root weights of surviving bean plants at 48 days after sowing (DAS)**

No.	Treatment	Root rot index <sup>1</sup>	Average fresh root weight g/plant	Average fresh shoot weight g/plant
1	UTC (Untreated control)	1.71	0.54a	18.6
2	FM150	0.28	0.58ab	19.1
3	FM250	0.15	0.62 bc	22.7
4	AFM75	0.08	0.67 cd	21.1
5	AFM100	0.08	0.68 cd	20.6
6	AFM200	0.03	0.72 d	21.4
7	Thiram	0.23	0.57ab	19.8
	<b>P-value</b>	-	<b>0.0023</b>	<b>0.511</b>

Means within columns followed by the same letter are not significantly different at the 5% level according to Least Significant Difference (LSD) test.

**Figure 5.5: Seed treatment effects on the average bean fresh root weight**

**Trial 5 (Cont.)****Pumpkins****Table 5.5: Mean pumpkin seedling emergence and survival under hot conditions in Bowen, Queensland**

		14DAS	28DAS	42DAS
No.	Treatment	% Seedlings emerged	% Surviving seedlings	% Surviving seedlings
1	UTC (Untreated control)	83	83	81
2	FM150	89	90	90
3	FM250	88	89	89
4	AFM75	89	88	88
5	AFM100	95	95	95
6	AFM200	94	92	92
7	Thiram	89	87	87
	<b>P-value</b>	<b>0.352</b>	<b>0.498</b>	<b>0.360</b>

DAS = Days after sowing

**Table 5.6: Mean root rot index and fresh shoot and root weight of surviving pumpkin plants at 48 days after sowing (DAS)**

No.	Treatment	Root rot index <sup>1</sup>	Average fresh root weight g/plant	Average fresh shoot weight g/plant
1	UTC (Untreated control)	0.78	4.45	193.4
2	FM150	0.15	4.27	179.5
3	FM250	0.18	4.28	203.4
4	AFM75	0.13	4.21	202.5
5	AFM100	0.13	4.17	189.3
6	AFM200	0.00	4.85	199.5
7	Thiram	0.00	4.85	213.7
	<b>P-value</b>	<b>-</b>	<b>0.604</b>	<b>0.727</b>

**Trial 5 (Cont.)****Cauliflowers****Table 5.7: Mean cauliflower seedling emergence and survival under hot conditions in Bowen, Queensland**

		14DAS	28DAS	40DAS
No.	Treatment	% Seedlings emerged	% Surviving seedlings	% Surviving seedlings
1	UTC (Untreated control)	63	56	56
2	FM150	25	23	23
3	FM250	12	9	9
4	AFM75	4	3	3
5	AFM100	4	3	3
6	AFM200	1	1	1
7	Thiram	0	0	0

DAS = Days after sowing

**Table 5.8: Mean root rot index and fresh shoot and root weight of surviving cauliflower plants at 48 days after sowing (DAS)**

No.	Treatment	Root rot index	Average fresh root weight g/plant	Average fresh shoot weight g/plant
1	UTC (Untreated control)	0	1.55	31.2
2	FM150	0	0.57	10.2
3	FM250	0	0.45	7.8
4	AFM75	0	0.41	9.2
5	AFM100	0	0.24	4.2
6	AFM200	0	3.11	6.9
7	Thiram	0	*	*

\* No surviving seedlings

## **Trial 5 (Cont.)**

### **Discussion**

#### **Peas**

All pea seed treatments caused an obvious increase in seedling emergence and survival in comparison to the untreated control seeds. There were very few seedlings in the untreated control plots (Table 5.1, Figure 5.1). Of the two new seed dressings, AFM seed treatments gave significantly higher seedling emergence and survival than FM seed treatments (Table 5.1). AFM seed treatments gave similar performance in improving seedling establishment as the thiram seed treatment.

AFM and thiram seed treatments generally resulted in significantly lower average fresh root and shoot weights when compared to FM or untreated control treatments at 48 days after sowing (DAS) (Table 5.2, Figures 5.2-5.3). These lower weights, however, may be a consequence of increased seedling emergence and survival and hence greater plant density and smaller plants due to increased competition between plants. There were negligible levels of root rot on the surviving seedlings. Plant roots from the untreated control tended to have slightly higher levels of root discoloration when compared to roots from fungicide treated seeds (Table 5.2).

#### **Beans**

The seed treatments FM150, FM250, AFM75 and thiram increased seedling emergence and survival compared to the untreated control and AFM100 and AFM200 treatments. The high rates of AFM at 100 and 200 appeared to cause a slight adverse effect and slightly reduced plant density.

In contrast to Trial 4, which was conducted under cold conditions, there was no delay in seedling emergence by AFM under hot field conditions that were more favourable to bean crops in Trial 5 (Table 5.2). AFM seed treatments at increasing rates increased the average fresh root weights (Figure 5.5) when compared to the untreated control and thiram seed treatment. Although not significant, there was also a trend of higher average fresh shoot weights with the AFM seed treatments. FM250 also resulted in a similar increase in the average fresh root and shoot weights as the AFM treatments.

Although the levels of root rot on the surviving bean plants were considered to be very low and mild in severity, there was a trend of lower root rot index with all the AFM seed treatments and FM250 treatment (Table 5.4).

#### **Pumpkins**

Pumpkin seeds germinated and emerged very quickly under hot and moist conditions, and there were no significant differences detected in the seedling emergence and survival, or average fresh root and shoot weights between treatments (Tables 5.5-5.6). It is noteworthy, however, that although not significant, all of the fungicide seed treatments appeared to increase seedling emergence and survival.

Although the levels of root rot on the pumpkin seedlings were considered to be low and mild in severity, there was a trend of lower root rot index with all the fungicide seed treatments (Table 5.6).

#### **Cauliflowers**

As in Trial 4, all of the fungicide seed treatments resulted in very poor seedling germination and emergence. At the initial screening trials for safety, storage and pathogenicity (refer Sections A, B and C), no such adverse effects were detected. The cauliflower seeds used in both, Trial 4 and Trial 5, came from the same batches of treated seeds, and it is possible that there may have been a cross contamination with a toxic material in the seed treatment machine during the cauliflower seed treatments.