Improved crop health and establishment using beneficial microorganisms

Horticulture LINK Project

CSA 6388/HL 0167 LFV

Annual report Year 3

(September 2005 – August 2006)

Project title: Improved crop health and establishment using beneficial microorganisms

Project number: CSA 6388/HL 0167 LFV

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Report: 3rd Annual report, September 2006

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Date commenced: 1 September 2003

Date completion due: 28 February 2007

Key words: Priming, carrot, onion, growth promotion, disease control, beneficial microorganisms, *Pseudomonas fluorescens* CHA0, *Pseudomonas chlororaphis* MA342, *Trichoderma harzianum* T22, *Clonostachys rosea* IK726, *Trichoderma viride* S17a, *Trichoderma viride* L4

HortLINK Consortium Members:

Department for Environment, Food and Rural Affairs

Elsoms Seeds Ltd

GTG UK

Horticultural Development Council

Warwick HRI

Government sponsor:

Department for Environment, Food and Rural Affairs

The results and conclusions in this report are based on an investigation conducted over one year. The conditions under which the experiment was carried out and the results obtained have been reported with detail and accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results especially if they are used as the basis for commercial product recommendations.

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Improved crop health and establishment using beneficial microorganisms

HortLINK project CSA 6388/HL 0167 LFV

Headline

- Beneficial microorganisms can be successfully applied to carrot and onion seed during priming.
- Improved emergence has been achieved in some instances with the application of beneficial microorganisms to seed.
- Microorganisms applied to carrot and onion seed can survive on the developing root system of the plant, and some applied microorganisms increase in number.

Background and expected deliverables

Uneven emergence and poor establishment of seedlings are problems faced by the UK horticultural industry, resulting in losses of yield and marketability of crops. This project aims to reduce the incidence of poor crop establishment and improve seedling health through the application of beneficial microorganisms to seed during priming; a technique that can be used by both conventional and organic growers. This technology may reduce pesticide usage or provide alternatives for use in integrated crop management systems. The technology developed in this project on carrot and onion seed may be applicable to other crops in the future.

Summary of the project and main conclusions

Application of microorganisms to seed

During the third project year, to extend the range of possible microorganisms that can be applied to seed, two new fungal isolates (*Trichoderma viride* S17a and *T. viride* L4) were assessed for their ability to survive and proliferate on carrot seed during the priming process. These were both applied successfully, achieving the target numbers of 5 $\log_{10}$ cfu g^{-1} dry weight seed.

Field trials

Trials have been drilled to assess the effect of applying beneficial microorganisms to primed seed on crop growth and yield for both carrot and onion. Year 2 trials have been harvested, and Year 3 trials have been assessed for emergence. Table 1 shows the locations for the field trials.

Table 1: Locations of the carrot and onion field trials for Year 2 and Year 3

Crop	Year 2	Year 3
Conventional carrot	Warwick HRI, Wellesbourne Marshall Farms, Papplewick	Warwick HRI, Wellesbourne Clumber Farms, Worksop
Organic carrot	Farcet Farms, Yaxley Fen	Hillfield, Elsoms Seeds Ltd., Spalding
Conventional onion	Warwick HRI, Wellesbourne Farcet Farms, Yaxley Fen	Warwick HRI, Wellesbourne Elveden Farms, Thetford
Organic onion	Hillfield, Elsoms Seeds Ltd., Spalding	Hillfield, Elsoms Seeds Ltd., Spalding

Table 2 lists the treatments that were used, with all 12 treatments drilled at the conventional field sites, and treatments 1-6 drilled at the organic sites. Due to poor performance over two years, *Clonostachys rosea* IK726 was not used as a seed treatment on onion for Year 3 trials, being replaced instead by *Trichoderma viride* S17a.

Table 2 Seed treatments produced for the Year 2 and Year 3 field trials

Treatment number	Description	Priming	Fungicide		Insecticide
			Carrot[a]	Onion[b]	(Force ST)
1	Primed control	✓	×	×	×
2	Primed *P. fluorescens* CHA0	✓	×	×	×
3	Primed *P. chlororaphis* MA342	✓	×	×	×
4[c]	Primed *C. rosea* IK726	✓	×	×	×
	Primed *T. viride* S17a (onion Year 3 only)	✓	-	×	×
5	Primed *T. harzianum* T22	✓	×	×	×
6	Unprimed control	×	×	×	×
7	Primed control + Pesticide	✓	Wakil XL	HY-TL	✓
8	Primed *P. fluorescens* CHA0 + Pesticide	✓	Wakil XL	HY-TL	✓
9	Primed *P. chlororaphis* MA342 + Pesticide	✓	Wakil XL	HY-TL	✓
10[c]	Primed *C. rosea* IK726 + Pesticide	✓	Wakil XL	HY-TL	✓
	Primed *T. viride* S17a + Pesticide (onion Year 3 only)	✓	-	HY-TL	✓
11	Primed *T. harzianum* T22 + Pesticide	✓	Wakil XL	HY-TL	✓
12	Unprimed + Pesticide	×	Wakil XL	HY-TL	✓

[a] carrot seed film coated

[b] onion seed pelleted

[c] Treatments 4 and 10 were *C. rosea* IK726 for both the carrot and onion seed in Year 2 trials, *C. rosea* IK726 for the carrot seed in Year 3 trials, and *T. viride* S17a for the onion seed in Year 3 trials

The harvest data for the Year 2 trials showed variable effects of microorganism treatment and pesticide application across the trial sites and crops. Positive results at the **onion** trials included:

- Pesticide increased the total weight of onion bulbs at Farcet Farms Ltd.
- *T. harzianum* T22 increased the total number of bulbs compared to the primed control (without pesticide) (Warwick HRI)
- *P. chlororaphis* MA342 + pesticide increased the mean weight of bulbs sized >60mm compared to the primed control + pesticide (Warwick HRI)

Positive results at the **carrot** trials included:

- Pesticide application increased the total number and total weight of roots (Warwick HRI)
- Pesticide decreased the percent of fanged roots, and the mean weight of roots with cavity spot or possible nematode damage (fanged) (Warwick HRI)
- *P. chlororaphis* MA342, *C. rosea* IK726 and *T. harzianum* T22 treated seed resulted in a greater weight of roots in total than the unprimed control (Warwick HRI)

There were no significant effects of microorganism treatment or pesticide at the carrot trial at Marshall Farms, and the organic carrot trial was not harvested due to weed problems. Overall, no consistent effects of microorganism treatment and pesticide application were seen across the trial sites for the onion or carrot crops in Year 1 and Year 2 for the harvest data.

The emergence data for the Year 3 trials showed pesticide application improved emergence overall at both the **onion** and **carrot** trials (Warwick HRI). Positive results at the **onion** trials included:

- All primed treatments emerged significantly faster than the unprimed control (Warwick HRI)
- *P. fluorescens* CHA0 improved emergence compared to *T.*

harzianum T22 and *P. chlororaphis* MA342 (WHRI)

- The application of pesticide improved the emergence of onion seed treated with *P. fluorescens* CHA0 and *T. harzianum* T22 (Elveden Farms)

No microorganism effects were seen at the organic onion, organic carrot or the conventional carrot grower's sites for the Year 3 emergence data. Overall, three years of emergence data showed that pesticide application improved emergence for the onion and carrot trials at Warwick HRI for all three years, and other effects of microorganism treatment were variable across the two crops and the different trial sites in all years, with significant differences being both positive and negative.

Glasshouse experiment –emergence and growth of onions

As a new seed treatment (*Trichoderma viride* S17a) was used on the onion seed for Year 3 field trials, glasshouse experiments were set up to assess the emergence and growth of seed primed with this microorganism in three soils (light sandy loam, peat and sandy clay loam). Overall, poor emergence was found with all seed treatments in the glasshouse experiment (<50% emergence). The peat soil resulted in the lowest emergence of all the soil types, and seedlings grown in light sandy loam had the greatest mean fresh weight after 8 weeks growth. There was no significant effect of microorganism treatment on emergence or fresh weight of the seedlings, but pesticide application decreased the mean fresh weight, which was consistent with previous experiments.

Glasshouse experiment –emergence and growth of carrots

Glasshouse experiments to assess the emergence and growth of all the seed treatments used in the carrot field trial were set up in three soil types, as described above. Results were similar to those found previously (2nd Annual report). Emergence was fastest in the sandy clay loam soil, but the greatest mean fresh weight of carrot seedlings was found in peat soil. One of the seed treatments, *Clonostachys rosea* IK726, improved emergence over the primed control, showing consistent results to the previous experiment (2nd Annual report).

Glasshouse experiment –onion pathogen bioassay

An experiment was set up to assess the emergence and growth of onion seed primed with different microorganisms in soil that was infested with sclerotia of *Sclerotium cepivorum* (causal agent of *Allium* white rot (AWR)). Treatments where sclerotia of *S. cepivorum* were added had a higher emergence than uninoculated treatments, possibly due to better soil aeration following the mixing of the sclerotia into the soil. The primed control treatment had a higher emergence than the unprimed control, highlighting the benefits of priming. The application of microorganisms to seed did not improve emergence compared to the primed control, and there was no reduction in the percent AWR compared to the primed control. The percent AWR may have been underestimated as only dying seedlings that had sclerotia forming on them were confirmed as having AWR. Other seedling death (no sclerotia were formed) was noted in the majority of treatments, and the total number of dead seedlings at the end of the experiment was high (including both those with AWR and those that died of other causes). The microorganism seed treatments were no worse than the primed control.

Glasshouse experiment – carrot pathogen bioassay

An experiment was set up to assess the emergence and growth of carrot seed primed with different microorganisms in soil that was infested with *Pythium ultimum* (damping-off pathogen). The pathogen inoculum was prepared in a sterile soil and chopped potato mixture. Results indicate that this carrier affected plant growth in a positive way, as there was no reduction in emergence in the treatments with the pathogen inoculum added. In fact, the addition of the chopped potato soil inoculum increased the mean fresh weight of seedling roots and shoots, possibly due to the addition of nutrients in the sterilised soil carrier. There were no effects of microorganism treatment overall. However, within the pathogen inoculated treatments some improvement in mean fresh root weight was noted with the *T. harzianum* T22 seed treatment.

Glasshouse experiment – survival of microorganisms on roots and in soil

Experiments were set up in the glasshouse to investigate the survival of

microorganisms on carrot and onion roots and in rhizosphere soil following application to seed during priming. Results from the carrot experiment showed that the bacteria generally declined in number, but *P. fluorescens* CHA0 was still recoverable from three soil types at over 3 $\log_{10}$ cfu g^{-1} after 8 weeks. *Clonostachys rosea* IK726 increased slightly in number in three soil types, both on the root and in the rhizosphere soil, and showed good survival, and *T. harzianum* T22 increased slightly in number in two soil types, and showed good survival.

For the onion experiment, only survival of the new fungal isolate *T. viride* S17a was investigated. Unfortunately, no survival data are available for this isolate on onion roots and in rhizosphere soil as it could not be recovered or identified on the *Trichoderma* selective medium. This may be due to low numbers on the seed initially.

Shelf life studies

Trichoderma viride S17a was successfully applied to carrot and onion seed for the shelf-life study. To date, survival of this microorganism is well above the target rate of 5 $\log_{10}$ cfu g^{-1} seed for both the onion seed (20 days storage), and the carrot seed (90 days storage). A repeat shelf-life study of *Trichoderma harzianum* T22 on carrot showed that a higher initial inoculum of over 7 $\log_{10}$ cfu g^{-1} seed has improved the storage time for this isolate, with numbers still over 6 $\log_{10}$ cfu g^{-1} seed at 90 days.

Financial benefits

No financial benefits are reported at this time.

Action points for growers

No action points are appropriate at this time.

Progress against Milestones:

Primary milestones: Year 3 (Sept 05 - Aug 06)

2.3 Carry out Year 3 field trials and monitor effects on seed emergence and growth (Elsoms + Warwick HRI) (Aug 2006 (month 36)) **Ongoing**

3.3 Complete Year 3 glasshouse studies and monitor effects on seed emergence and growth (Warwick HRI) (Aug 2006 (month 36)) **Complete**

4.3 Complete monitoring of microorganism survival on Year 3 field trial and glasshouse studies (GTG and Warwick HRI) (Aug 2006 (month 36)) **Complete** (The planned monitoring of survival of applied microorganisms on seedlings in field trials will not be carried out as PSD refused permission to use marked strains of the microorganisms).

6.1 Determine effect of co-inoculation of microorganisms onto carrot and onion seed (Warwick HRI) (Aug 2006 (month 36)) **Ongoing**

Secondary milestones: Year 3 (Sept 05 - Aug 06)

S2.2 Complete harvest and yield assessment of Year 2 field trials (Elsoms and Warwick HRI) (Oct 2005 (month 26)) **Complete**

S5.2 Complete second shelf life experiment (GTG) (Dec 2005 (month 28)) **Not done**

S6.1 Initiate co-inoculation experiments (Warwick HRI) (Sep 2005 (month 25)) **Complete**

S7.5 Maintain discussions with PSD concerning registration (Elsoms + GTG) **Ongoing**

S7.6 Produce a poster and attend the UK carrot and onion meeting (Warwick HRI) (Nov 2005 (month 27)) **Complete**

S7.7 Carry out grower demonstration at one Elsoms Seed field site (Elsoms) (July 2006 (month 35)) **Planned**

Science Section

Introduction

This project aims to assess the ability of selected beneficial microorganisms to survive and proliferate on onion and carrot seed during drum priming, and to subsequently improve plant growth and yield. Poor seedling establishment and plant spacing can have a negative impact on yield and marketability of crops. Priming seeds improves the time to emergence and results in a more uniform stand. It has previously been shown that microorganisms can be applied to seed during the priming process, and may proliferate (Wright et al. 2003).

Beneficial microorganisms have a range of biocontrol or growth promoting properties including the production of antibiotics or plant growth hormones, by improving plant nutrition, by being antagonistic to pathogens directly, or reducing the effects of deleterious microorganisms in the rhizosphere (Whipps 2001). Previous experimental work applying beneficial microorganisms to seed has often involved dips or slurries, and microorganisms may not remain viable for long following application in this way.

Application during priming is an alternative strategy. Priming involves the controlled application of water to seed batches to start the germination process before seed is planted, generally followed by a re-drying stage, resulting in quicker emergence and more uniform plant stands. This project aims to identify beneficial microorganisms that can survive and proliferate on seed following application during priming, and improve seedling establishment, plant growth and yield. Successful application of this technology will provide a system that can be used by both conventional and organic growers, and may reduce pesticide usage for an integrated management approach. Uniform germination of healthy seedlings will improve crop yield and marketability and will have a positive impact on revenue for growers. Crop losses from pathogens such as *Pythium* spp. (damping-off and cavity spot of carrot) and *Sclerotium cepivorum* (white rot of onion) may be reduced as well.

Objective 01: Obtain and determine ability of microorganism strains to proliferate during priming of carrot and onion seed and their compatibility with current pesticide seed treatments. (Milestone S1.5 Year 2) Complete

Introduction

Two new fungal isolates (*Trichoderma viride* S17a and *Trichoderma viride* L4) were assessed for their ability to survive and proliferate on carrot seed during drum priming, having previously been tested on onion seed (2nd Annual Report, 2005).

Materials and methods

Fungal inoculum preparation

The two selected fungi were available from the culture collection at Warwick HRI, stored in liquid nitrogen. These fungi have been shown to control *Allium* white rot in small scale field trials at Warwick HRI. Fungi were retrieved from storage, and plated onto potato dextrose agar (PDA) for routine maintenance in the laboratory at 20°C. Once the PDA plate had been covered by mycelial growth and profuse sporulation had occurred (2-3 weeks), 10 ml sterile distilled water (SDW) was added to one plate and the spores scraped into suspension. The suspension was filtered through sterile lens tissue before being serially diluted to aid haemacytometer counts, and the required amount of suspension was calculated to give an inoculum rate of 1×10^5 cfu g^{-1} dry seed.

A dilution series of the fungal spore suspension was also plated onto PDA amended with Triton X-100 (2ml l^{-1}) to verify the number of cfu present in the suspension to be added to the seed batches.

Drum priming and sampling

The drum priming process consists of three main phases: hydration (controlled addition of liquid suspension to seed batch), incubation (slow rotation of seed batch in jars for 7 days following hydration), and drying back (air drying of seed batch for 2 days following incubation). Following this, seed was transferred to long-term storage at 4^{o}C. The carrot seed had an initial moisture content of 9.28% and a target of 62% moisture content after 24 hours hydration. Seed sampling was carried out at the end of each of the three main phases stated above.

Three replicate 0.5g seed samples were ground in 10ml SDW each, using a sterile mortar and pestle. A dilution series in SDW was subsequently plated onto PDA amended with chlortetracycline (30µg ml^{-l}) and Triton X-100 (2ml l^{-l}), and colonies were counted after 7 days incubation at 20^{o}C.

Results and discussion

Both isolates followed a similar pattern of survival on the carrot seed during drum priming (Figure 1.1). Numbers dropped initially after the hydration phase, but increased after incubation to nearly 6 $\log_{10}$ cfu g^{-1} seed. A slight decrease was found on drying back the seed for storage, but the final numbers remained above the target of 5 $\log_{10}$ cfu g^{-1} seed. The general pattern of *T. viride* S17a and *T. viride* L4 survival on the carrot seed was similar to that seen with other fungal isolates previously.

Conclusions

- Both *T. viride* S17a and *T. viride* L4 can be successfully applied to carrot seed during priming.

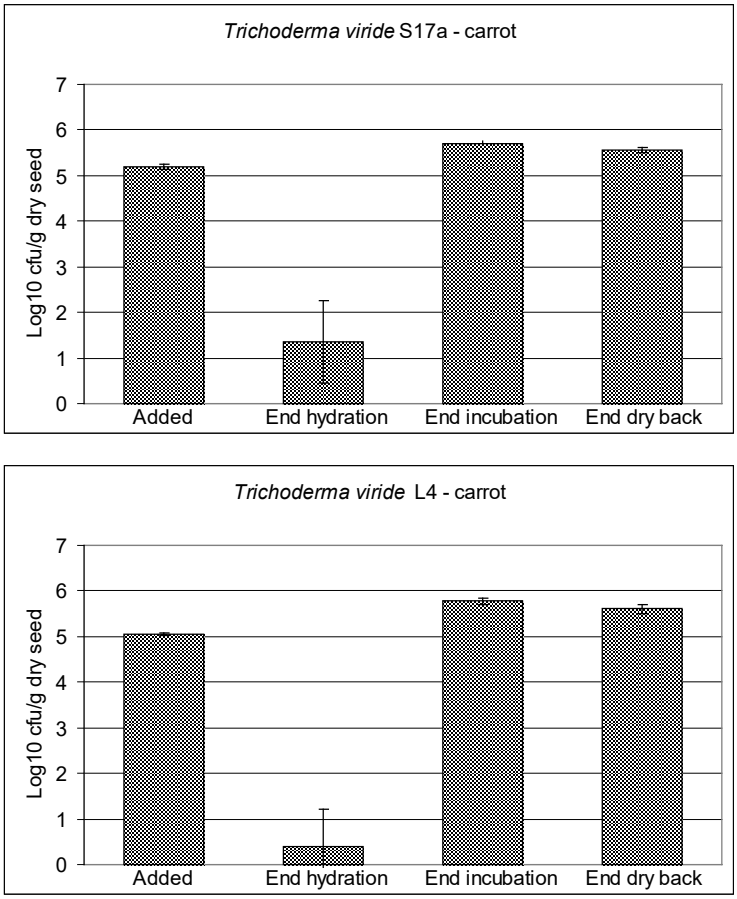


Figure 1.1: Survival and proliferation of beneficial microorganisms applied to carrot seed during drum priming. Added = initial numbers in liquid suspension applied to seed; End hydration = numbers recovered after all liquid spore suspension added to seed; End incubation = numbers recovered after seed incubated in rotating jars for 7 days; End dry back = numbers recovered when seed air-dried. Bars indicate standard error of the mean (d.f. = 2).

Objective 02: Assess effects of seed applied microorganisms to carrot and onion seed in field trials (Milestone S2.2 Complete; Milestone 2.3 Ongoing)

Introduction

Milestone S2.2 Complete harvest and yield assessment of Year 2 field trials

Year 2 field trials were set up to assess the effect of seed primed with microorganisms on emergence and yield of onion and carrot crops at two conventional sites and one organic site. Trials were drilled at Warwick HRI, Wellesbourne (conventional carrot and onion trials; sandy clay loam); Marshall Farms, Papplewick, Nottinghamshire (conventional carrot trial; sand); Farcet Farms, Yaxley Fen, Peterborough (conventional onion trial; organic carrot trial; black peat); and Hillfield, Elsoms Seeds, Spalding (organic onion trial; light silt). Emergence data was reported in the 2nd Annual Report (September 2004 – August 2005). Some harvest data from Year 2 trials were reported in the 6 month interim report (September 2005 – February 2006), except for the carrot trial at Marshall Farms. All harvest data are now presented here for completeness.

Milestone 2.3 Carry out Year 3 field trials and monitor effects on seed emergence and growth

Year 3 field trials were set up as for previous years. Trials were drilled at Warwick HRI, Wellesbourne (conventional carrot and onion trials; sandy clay loam); Clumber Farms, Worksop (conventional carrot trial; sand); Elveden Farms, Thetford (conventional onion trial; light sandy loam); and Hillfield, Elsoms Seeds, Spalding (organic carrot and onion trials; light silt). Emergence assessments have been carried out, and harvesting is scheduled for later in the year.

Materials and methods

Inoculum of the selected beneficial microorganisms (*Pseudomonas fluorescens* CHA0, *Pseudomonas chlororaphis* MA342, *Trichoderma harzianum* T22; *Trichoderma viride* S17a and *Clonostachys rosea* IK726) was prepared at GTG UK, and applied to seed during drum priming at Elsoms. Subsequent pelletting of onion seed and film coating of carrot seed took place at GTG UK.

For **carrots**, the 12 seed treatments remained the same as previous years, and are as follows:
Primed control
Primed *P. fluorescens* CHA0
Primed *P. chlororaphis* MA342
Primed *C. rosea* IK726
Primed *T. harzianum* T22
Unprimed control
Primed control + pesticide
Primed *P. fluorescens* CHA0 + pesticide
Primed *P. chlororaphis* MA342 + pesticide
Primed *C. rosea* IK726 + pesticide
Primed *T. harzianum* T22 + pesticide
Unprimed control + pesticide

For **onions**, 10 of the treatments remain the same as previously, but *C. rosea* IK726 was replaced by *T. viride* S17a. The treatments are as follows:
Primed control
Primed *P. fluorescens* CHA0
Primed *P. chlororaphis* MA342
Primed *T. viride* S17a
Primed *T. harzianum* T22
Unprimed control
Primed control + pesticide
Primed *P. fluorescens* CHA0 + pesticide
Primed *P. chlororaphis* MA342 + pesticide
Primed *T. viride* S17a + pesticide

Primed *T. harzianum* T22 + pesticide
Unprimed control + pesticide

Bacterial inoculum preparation

Wild-type strains of the bacteria supplied by Warwick HRI were cultured on nutrient agar at 25 ˚C at GTG. Single colonies were used to inoculate sterile nutrient broth incubated overnight in rotary culture (at 25 ˚C and 180 rpm). From the resulting master culture, 0.5 ml aliquots were used to inoculate fresh flasks of nutrient broth (50 ml). After incubation in rotary culture (at 25 ˚C and 180 rpm) for 4-5 h, bacterial cell numbers were determined spectrophometrically by reference to standard growth curves constructed by previous experiment. The required volume of active culture was then spun down at 12,000 g for 10 min and the resulting pellet resuspended in the volume of SDW pre-determined for seed priming. The numbers of cfu were determined by spiral plating onto nutrient agar.

Fungal inoculum preparation

Fungal isolates supplied by Warwick HRI were cultured on potato dextrose agar at 20 ˚C at GTG. Following profuse sporulation the spores were harvested by adding SDW to the solid cultures and gently scraping off the fungal growth. The resulting fungal suspension was filtered through a double layer of sterile Whatman lens tissue. Following serial dilution the spore concentration was determined by haemocytometer counts. The numbers of cfu were determined by spiral plating onto potato dextrose agar.

Drum priming and pesticide application

The selected microorganisms were applied to seed samples during drum priming and sub-samples of the primed seed were removed for further seed processing. Samples of carrot were film coated, both with and without the standard rate of Wakil XL fungicide and Force ST insecticide. Samples of onion were pelleted both with and without standard rates of HY-TL fungicide and Force ST insecticide. All treated samples of both onion and carrot were air-dried at room temperature. The pelleted seed was graded to a final size of 3.5 - 5.0 mm.

Seed treatments

Twelve treatments (Table 2.1) of both carrot and onion were produced in total for drilling at Warwick HRI and a different grower site for each crop selected on the basis of soil type and disease history. Treatments without chemicals of both carrot and onion were also drilled at the organic field trial sites.

Trial design and harvest assessments – Year 2 (Milestone S2.2)

Each trial consisted of 4 replicate blocks of all the treatments (12 treatments at the conventional sites, and 6 treatments at the organic sites; Table 2.1), and a randomised design was used to allocate the treatments to plots within each replicate block. Each plot was 6m long and 4 rows were drilled across the bed with the inner two rows allocated for the treated seed, and the outer two rows as guard rows of untreated, unprimed seed. A singulaire drill was used, with seeding rates of 20 onion seeds/m, and 50 carrot seeds/m.

Trial design and emergence assessments – Year 3 (Milestone 2.3)

Each trial consists of 4 replicate blocks of all the treatments (12 treatments at the conventional sites, and 6 treatments at the organic sites; Table 2.1), and a randomised design was used to allocate the treatments to plots within each replicate block. Each plot is 6m long and 4 rows were drilled across the bed, with the inner two rows allocated for the treated seed, and the outer two rows as guard rows of unprimed seed with the standard commercial pesticides applied as film coating (carrots) or pellets (onions). A singulaire drill was used, with seeding rates of 20 onion seeds/m, and 50 carrot seeds/m. At the Wellesbourne site, the carrot crop was fleeced 3 weeks after drilling to protect the crop from carrot fly. Throughout the growing season, standard commercial pesticides are being applied to each crop at the conventional sites, excluding the use of nematicides and Folicur.

Table 2.1 Seed treatments produced for the Year 2 and Year 3 field trials

Treatment number	Description	Priming	Fungicide		Insecticide
			Carrot[a]	Onion[b]	(Force ST)
1	Primed control	✓	×	×	×
2	Primed *P. fluorescens* CHA0	✓	×	×	×
3	Primed *P. chlororaphis* MA342	✓	×	×	×
4[c]	Primed *C. rosea* IK726	✓	×	×	×
	Primed *T. viride* S17a (onion Year 3 only)	✓	-	×	×
5	Primed *T. harzianum* T22	✓	×	×	×
6	Unprimed control	×	×	×	×
7	Primed control + Pesticide	✓	Wakil XL	HY-TL	✓
8	Primed *P. fluorescens* CHA0 + Pesticide	✓	Wakil XL	HY-TL	✓
9	Primed *P. chlororaphis* MA342 + Pesticide	✓	Wakil XL	HY-TL	✓
10[c]	Primed *C. rosea* IK726 + Pesticide	✓	Wakil XL	HY-TL	✓
	Primed *T. viride* S17a + Pesticide (onion Year 3 only)	✓	-	HY-TL	✓
11	Primed *T. harzianum* T22 + Pesticide	✓	Wakil XL	HY-TL	✓
12	Unprimed + Pesticide	×	Wakil XL	HY-TL	✓

[a] carrot seed film coated

[b] onion seed pelleted

[c] Treatments 4 and 10 were *C. rosea* IK726 for both the carrot and onion seed in Year 2 trials, *C. rosea* IK726 for the carrot seed in Year 3 trials, and *T. viride* S17a for the onion seed in Year 3 trials

Either one or two emergence assessments were made, depending on crop and site (Table 2.2). At Warwick HRI, for both the onion and carrot trials emergence counts were made for the inner two rows of each plot, excluding the first and last 50cm of the row (5m total length). At all other trial sites, the two inner rows were assessed by measuring 0.5 m in from the beginning of each plot and counting the emerged seedlings for a 4 m length of each row, with the exception of the organic onion trial where a 5m length row was assessed.

Table 2.2 Dates when onion and carrot field trials were drilled and assessed for emergence (1st and 2nd assessment dates) – Year 3

	Onion	**Carrot**
Warwick HRI		
Drilled	10th April	12th May
1st assessment	2nd May	2nd June
2nd assessment	31st May	22nd June
Grower site		
Drilled	16th March	3rd May
1st assessment	19th April	24th May (no count)
2nd assessment	10th May	13th June
Organic site		
Drilled	21st March	5th May
1st assessment	2nd May	10th July

Data analyses

All variables were subjected to analysis of variance (ANOVA), using a randomised complete block design. Percentage data were subjected to arcsine transformation prior to analyses and back-transformed data are presented. All differences noted were at the 5% significance level.

Results and discussion

Milestone S2.2 – Year 2 harvest data

Although some statistically significant differences were found with the Year 2 harvest data from all sites, many of these differences were small and are difficult to visualise on the graphs. However, significant differences are stated in the text where appropriate.

Warwick HRI onion trial

The inner two rows were harvested, excluding the first and last 50cm of each row (total length = 5m). No white rot was recorded at this site, and harvest records were made of the total number and weight of bulbs for each size category of <40mm, 40-60mm, and >60mm (Figure 2.1 – 2.4). Seed primed with *T. harzianum* T22 (without pesticide) resulted in a significantly greater total number of bulbs than the primed control (without pesticide) (Figure 2.1). However, with the application of pesticide to the seed pellet, the primed control produced a greater number of bulbs than seed primed with *P. fluorescens* CHA0 + pesticide, *C. rosea* IK726 + pesticide or *T. harzianum* T22 + pesticide.

The total weight of bulbs was significantly reduced with the treatment of *T. harzianum* T22 + pesticide compared to the primed control + pesticide. Also, *C. rosea* IK726 (without pesticide) resulted in a lower total weight than the unprimed control (without pesticide) (Figure 2.2). Although no significant effects were seen in the proportion of onion bulbs in each of the size categories (Figure 2.3), some differences were noted with the mean weight of bulbs sized >60mm (Figure 2.4). Without pesticide application, *C. rosea* IK726 was worse than both the primed and unprimed controls. *Pseudomonas chlororaphis* MA342 + pesticide had a greater mean weight of bulbs sized >60mm compared to the primed control + pesticide (Figure 2.4).

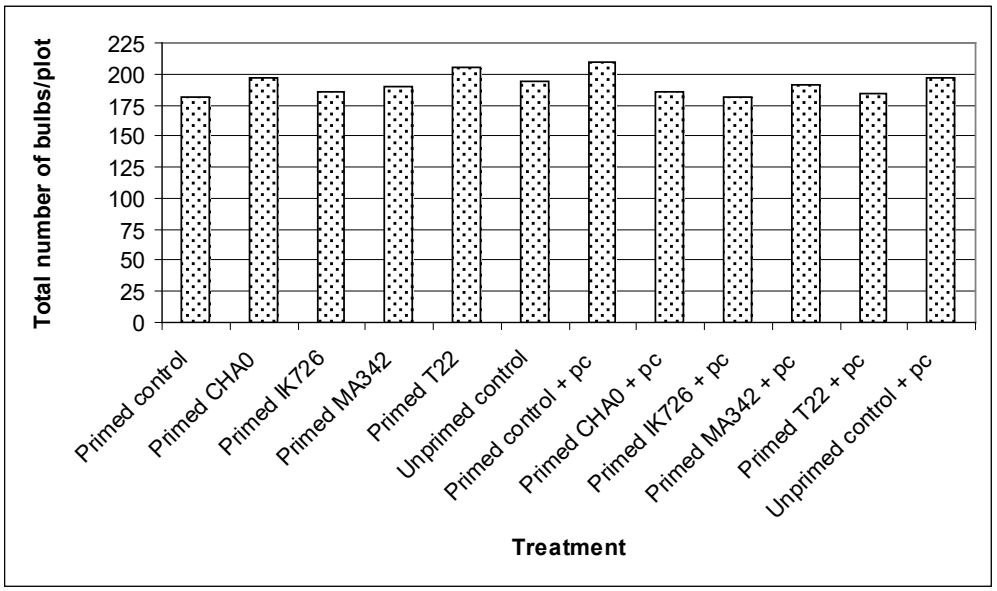


Figure 2.1: Total number of onion bulbs per plot at the Warwick HRI trial (Year 2)

LSD (5%, df = 47) comparing microorganism treatment–pesticide interaction = 19.31

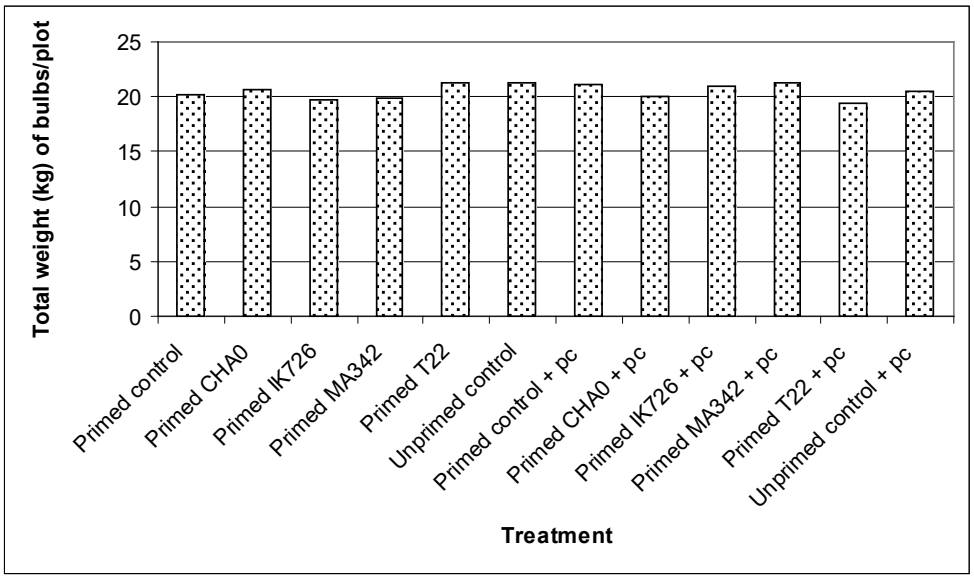


Figure 2.2: Total weight (kg) of onion bulbs at the Warwick HRI trial (Year 2)

LSD (5%, df = 47) comparing microorganism treatment–pesticide interaction = 1.493

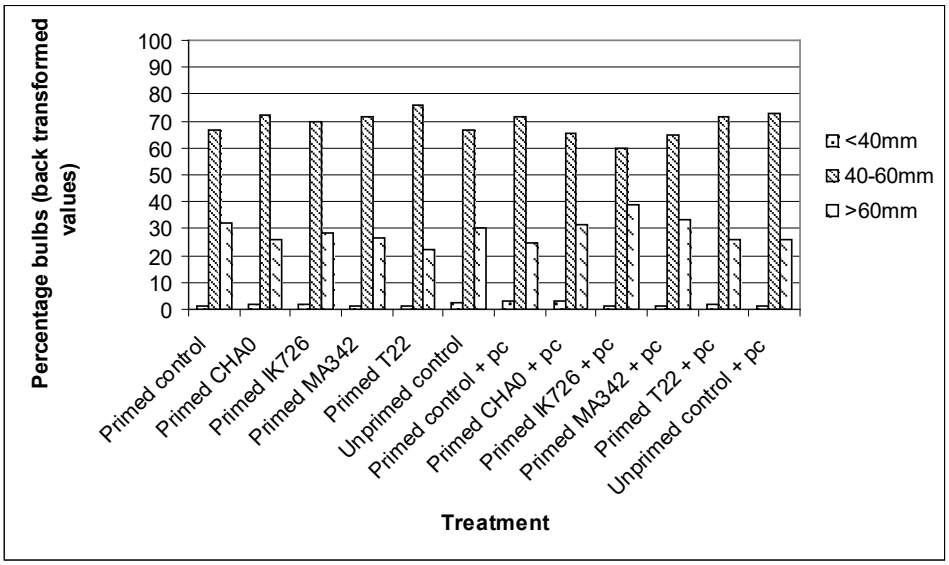


Figure 2.3: Percentage onion bulbs in different size categories at the Warwick HRI trial (Year 2)

LSD not available on back transformed data presented (analyses done on angle transformed data)

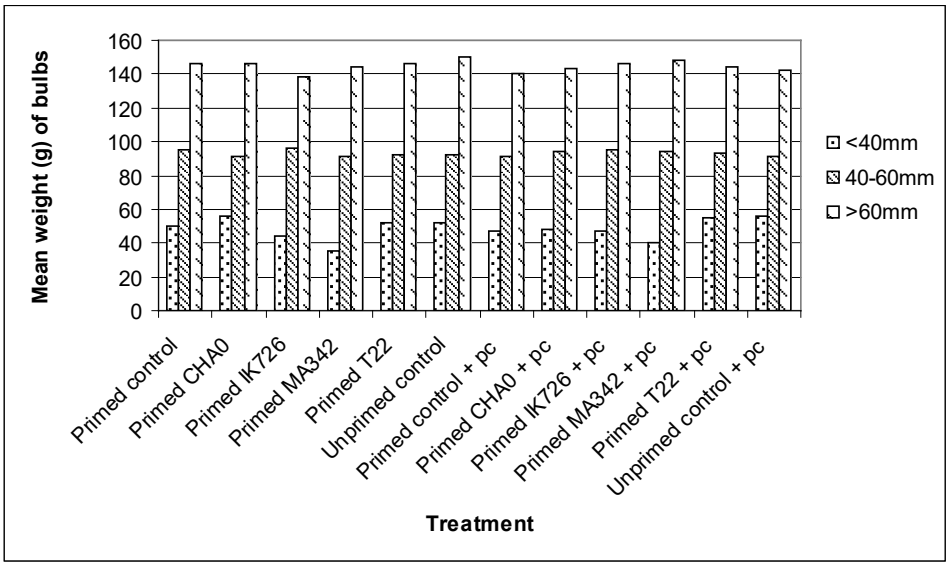


Figure 2.4: Mean weight (g) of onion bulbs in different size categories at the Warwick HRI trial (Year 2)

LSD (5%, df = 47) comparing microorganism treatment–pesticide interaction: <40mm = 18.88; 40-60mm = 6.038; >60mm = 6.856.

Farcet Farms onion trial

A 4m length of the inner two rows was harvested for each plot. No white rot was recorded at this site. A significant microorganism treatment effect was seen at the grower's site, with the total number of bulbs significantly reduced by *T. harzianum* T22 compared to the primed control (Figure 2.5). Overall, pesticide application significantly increased the total weight of bulbs, but there were no microorganism effects on the total weight (Figure 2.6).

Overall, *C. rosea* IK726 reduced the percentage bulbs sized 40-60mm compared to the unprimed control (Figure 2.7), but no significant effects were seen with the mean weight of bulbs in the size categories (Figure 2.8).

Organic onion trial

As at the grower's site, a 4m length of the inner two rows was harvested, and no white rot was recorded. No significant effects were seen regarding the total number of bulbs (Figure 2.9), total weight of bulbs (Figure 2.10), or percentage bulbs in different size categories (Figure 2.11) at the organic site. However, bulbs with the treatments *C. rosea* IK726, *P. chlororaphis* MA342 and *T. harzianum* T22 had a lower mean weight than the primed control in the size category 60-80mm (Figure 2.12). *Trichoderma harzianum* T22 was also worse than the unprimed control in this category.

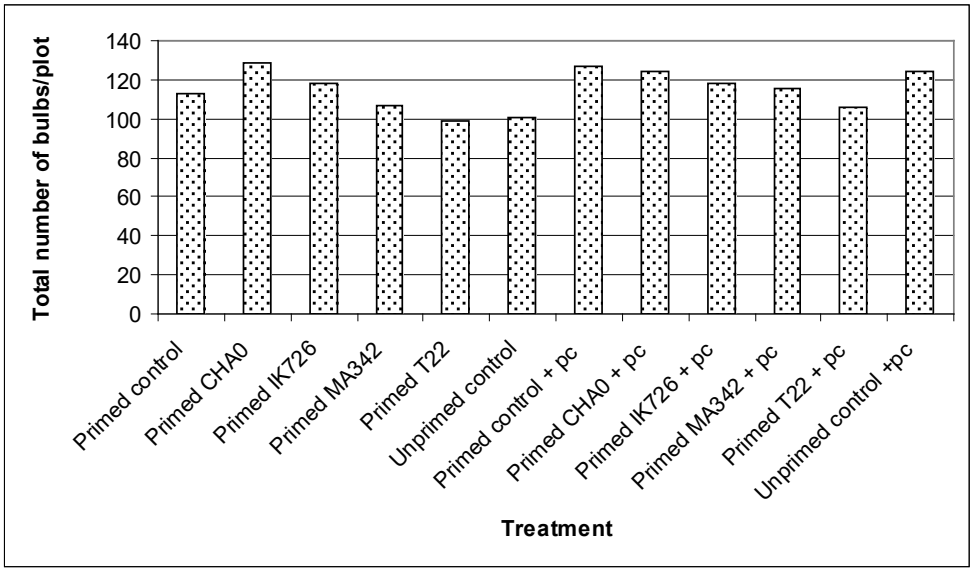


Figure 2.5: Total number of onion bulbs at the Farcet Farms trial (Year 2)

LSD (5%, df = 47) comparing microorganism treatment–pesticide interaction = 21.09.

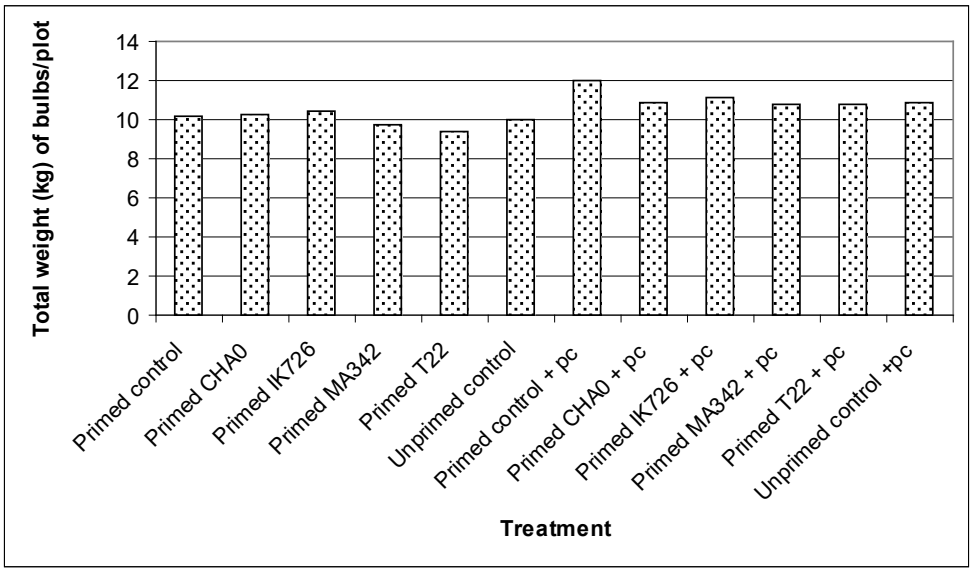


Figure 2.6: Total weight (kg) of onion bulbs at the Farcet Farms trial (Year 2)

LSD (5%, df = 47) comparing microorganism treatment–pesticide interaction = 1.777

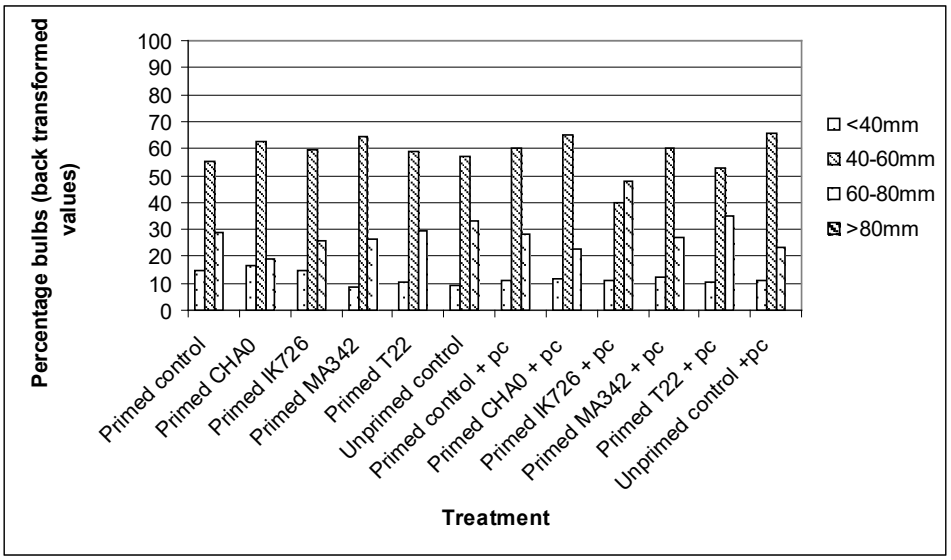


Figure 2.7: Percentage onion bulbs in different size categories at the Farcet Farms trial (Year 2)

LSD not available on back transformed data presented (analyses done on angle transformed data)

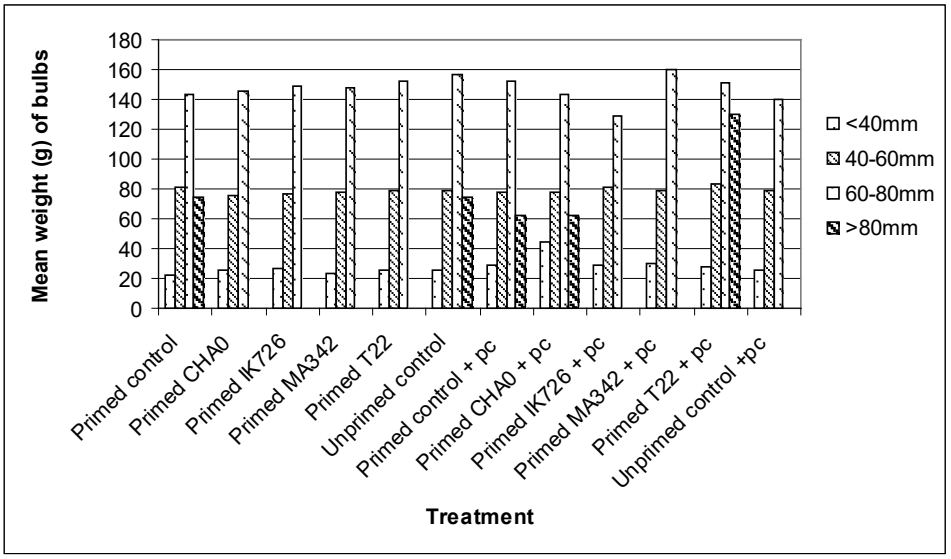


Figure 2.8: Mean weight (g) of onion bulbs in different size categories at the Farcet Farms trial (Year 2)

LSD (5%, df = 47) comparing microorganism treatment–pesticide interaction: <40mm = 16.02; 40-60mm = 7.474; 60-80mm = 20.76; >80mm = 133.6.

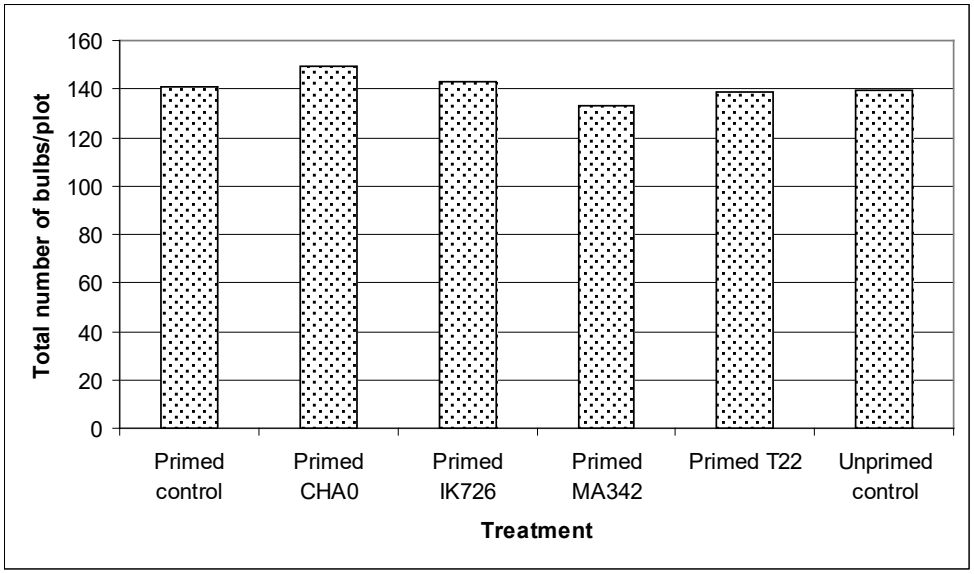


Figure 2.9: Total number of onion bulbs at the organic trial (Year 2)

LSD (5%, df = 23) comparing microorganism treatments = 14.53

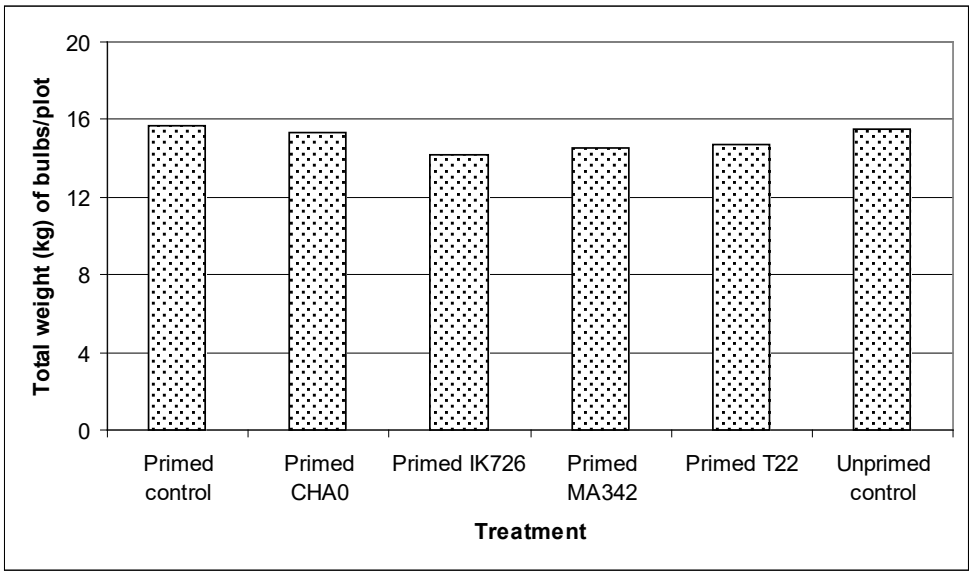


Figure 2.10: Total weight (kg) of onion bulbs at the organic trial (Year 2)

LSD (5%, df = 23) comparing microorganism treatments = 1.387

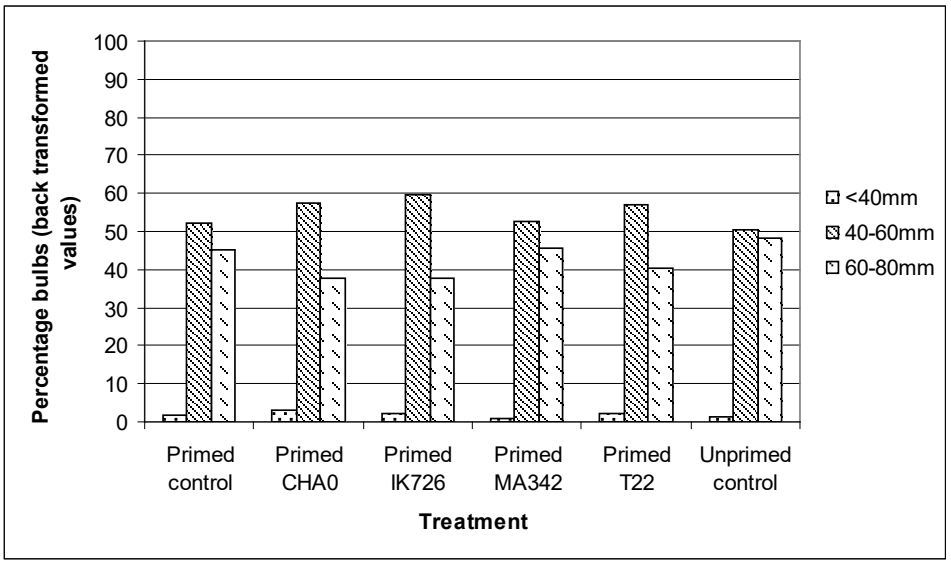


Figure 2.11: Percentage onion bulbs in different size categories at the organic trial (Year 2)

LSD not available on back transformed data presented (analyses done on angle transformed data)

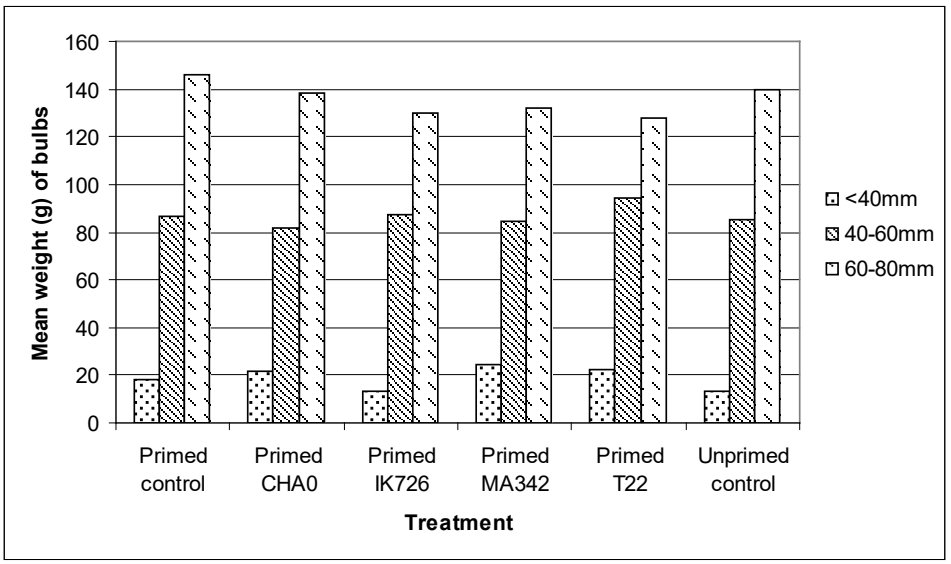


Figure 2.12: Mean weight (g) of onion bulbs in different size categories at the organic trial (Year 2)

LSD (5%, df = 23) comparing microorganism treatments: <40mm = 14.15; 40-60mm = 13.61; 60-80mm = 9.92.

Warwick HRI carrot trial

The inner two rows were harvested for each plot, excluding the first and last 50cm of each row (total length = 5m). The total number of carrots at harvest was higher with the primed control seed treatment than *C. rosea* IK726 and *T. harzianum* T22 overall (Figure 2.13). The unprimed control was also better overall than *T. harzianum* T22. Overall, the application of pesticide significantly increased the number of carrots. Without pesticide application, the primed control was better than *P. fluorescens* CHA0, *C. rosea* IK726, *T. harzianum* T22 and the unprimed control (Figure 2.13). With pesticide, *T. harzianum* T22 was also worse then the primed control + pesticide, and the unprimed control + pesticide (Figure 2.13).

Overall, the seed treated with *P. chlororaphis* MA342, *C. rosea* IK726 and *T. harzianum* T22, and the primed control resulted in a greater total weight of roots than the unprimed control (Figure 2.14). Pesticide application also resulted in a greater total weight at harvest.

The percentage of roots with cavity spot was not affected by the microorganism or pesticide treatment, although pesticide application reduced the percentage of roots with possible nematode damage (Figure 2.15).

The mean weight of roots affected by cavity spot or nematode damage (fanged) was reduced by pesticide application, but no microorganism treatment effect was seen (Figure 2.16).

Marshall Farms carrot trial

The carrots were strawed over winter (2005) and were harvested in April 2006. Several plots were missing at the edge of the blocks for each

replicate and consequently there are missing values in the analysis of this trial. A 2m length of each inner row was harvested and assessments were made as before. There was no significant difference between the treatments regarding the total number of carrots (Figure 2.17), the total weight of carrots (Figure 2.18), the percentage of roots with cavity spot or nematode damage (fanged) (Figure 2.19), or the mean weight of roots affected by cavity spot or nematode damage (fanged) (Figure 2.20).

Organic carrot trial

No harvest data are available as the organic trial suffered from problems with weeds.

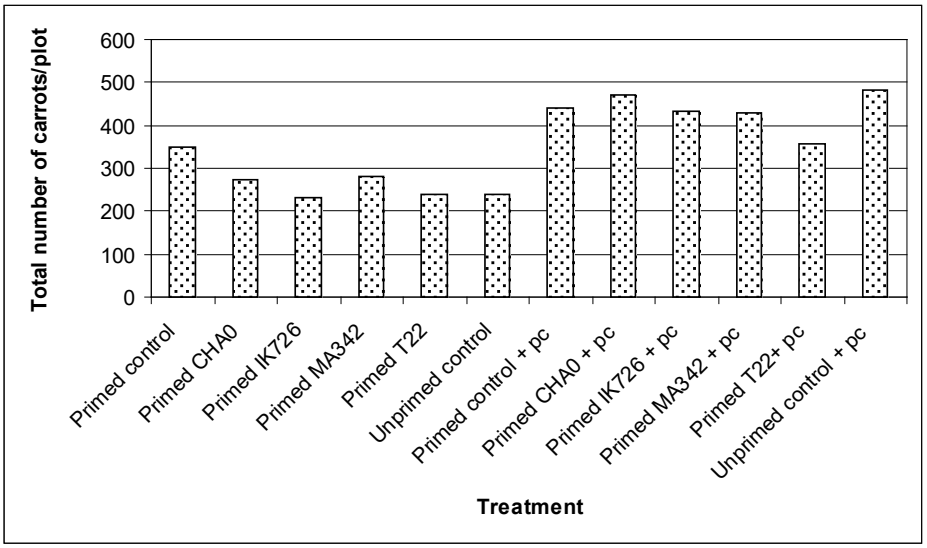


Figure 2.13: Total number of carrots per plot at the Warwick HRI trial (Year 2)

LSD (5%, df = 47) comparing microorganism treatment–pesticide interaction = 72.79

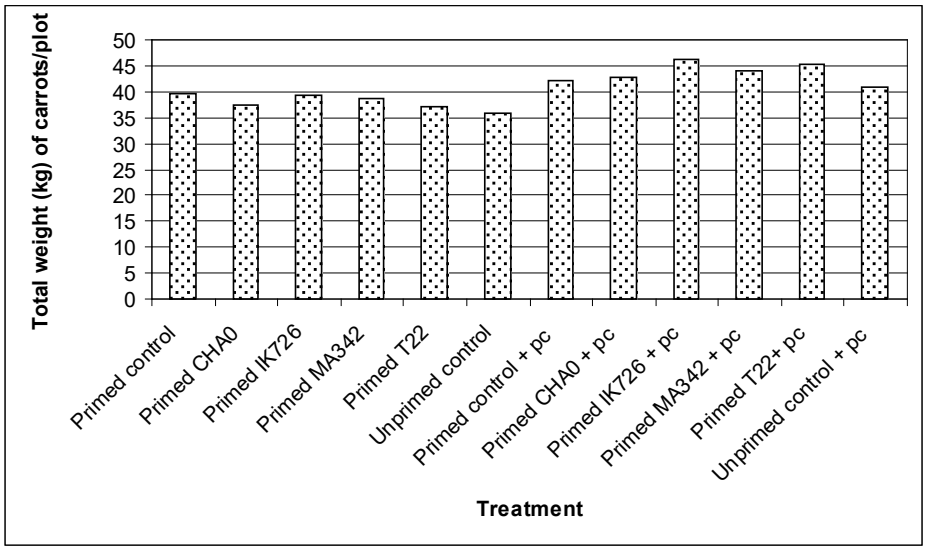


Figure 2.14: Total weight (kg) of carrots per plot at the Warwick HRI trial (Year 2)

LSD (5%, df = 47) comparing microorganism treatment–pesticide interaction = 3.453

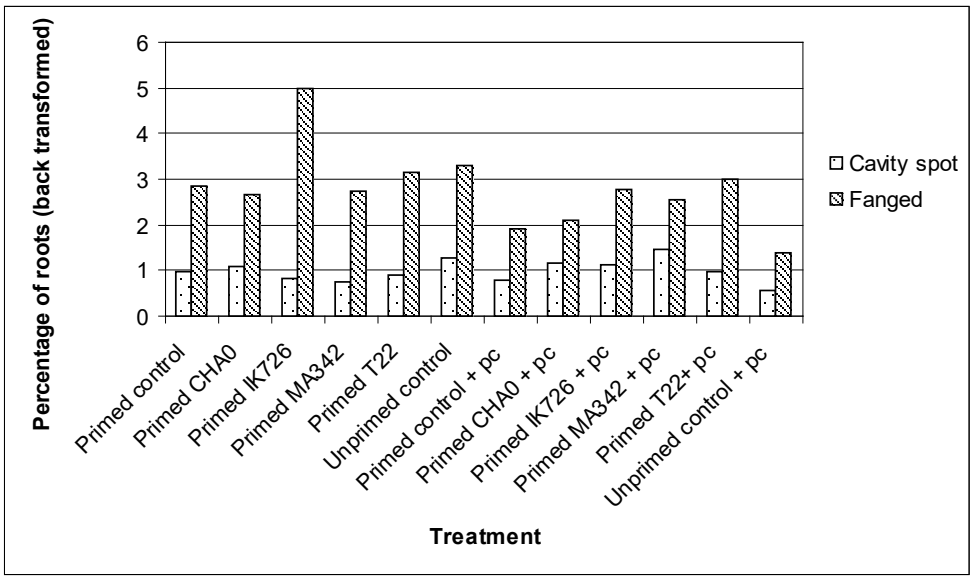


Figure 2.15: Percentage of carrot roots with cavity spot or possible nematode damage (fanged) at the Warwick HRI trial (Year 2)

LSD not available on back transformed data presented (analyses done on angle transformed data)

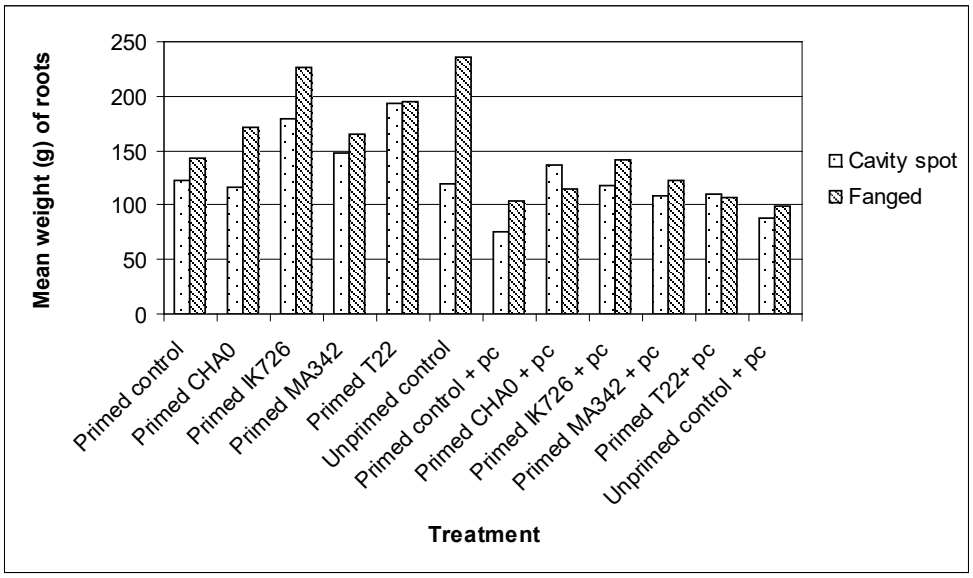


Figure 2.16: Mean weight of carrot roots with cavity spot or possible nematode damage (fanged) at the Warwick HRI trial (Year 2)

LSD (5%, df = 47) comparing microorganism treatment–pesticide interaction: Cavity spot = 84.56; Fanged = 77.36.

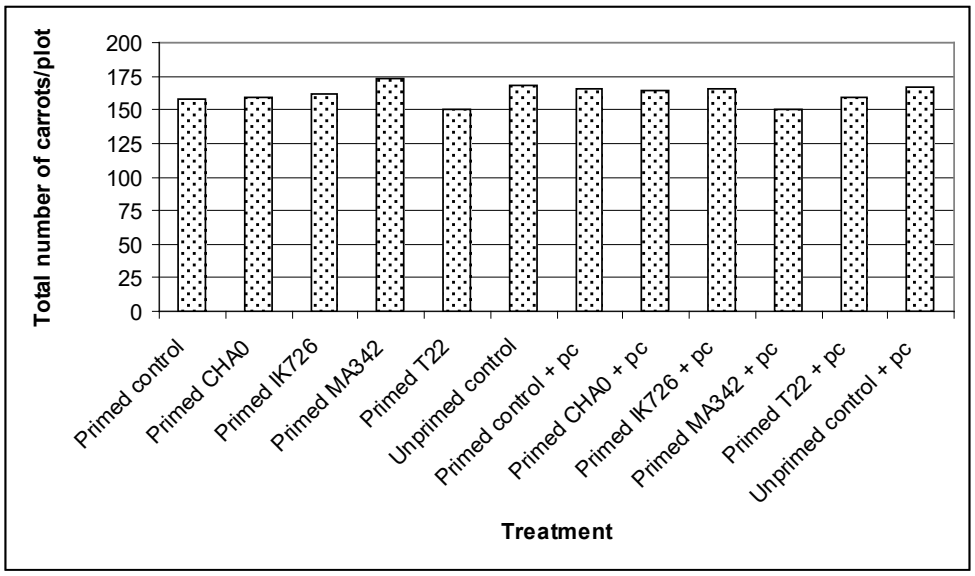


Figure 2.17: Total number of carrots per plot at the Marshall Farms trial (Year 2)

LSD (5%, df =24) comparing microorganism treatment–pesticide interaction = 42.49

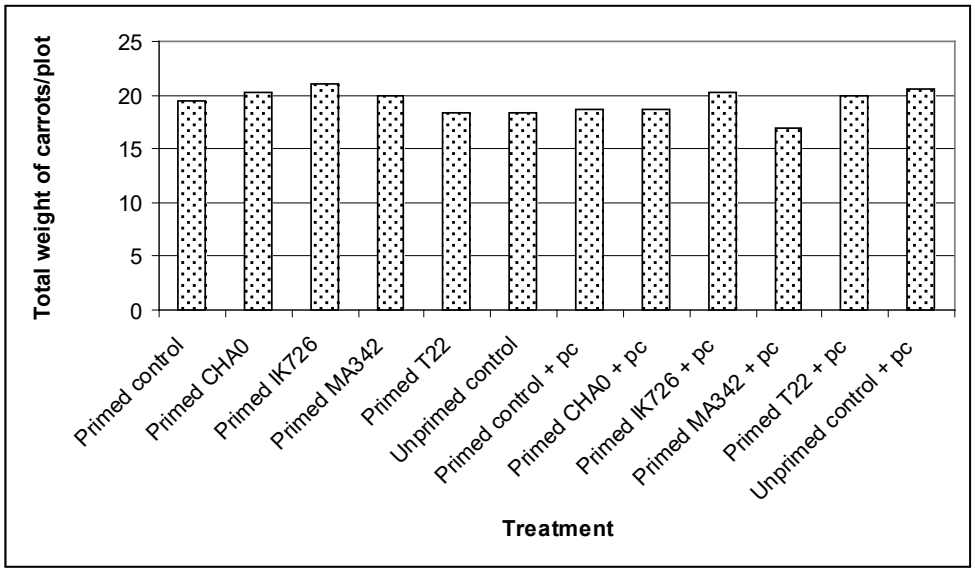


Figure 2.18: Total weight (kg) of carrots per plot at the Marshall Farms trial (Year 2)

LSD (5%, df = 24) comparing microorganism treatment–pesticide interaction = 3.18

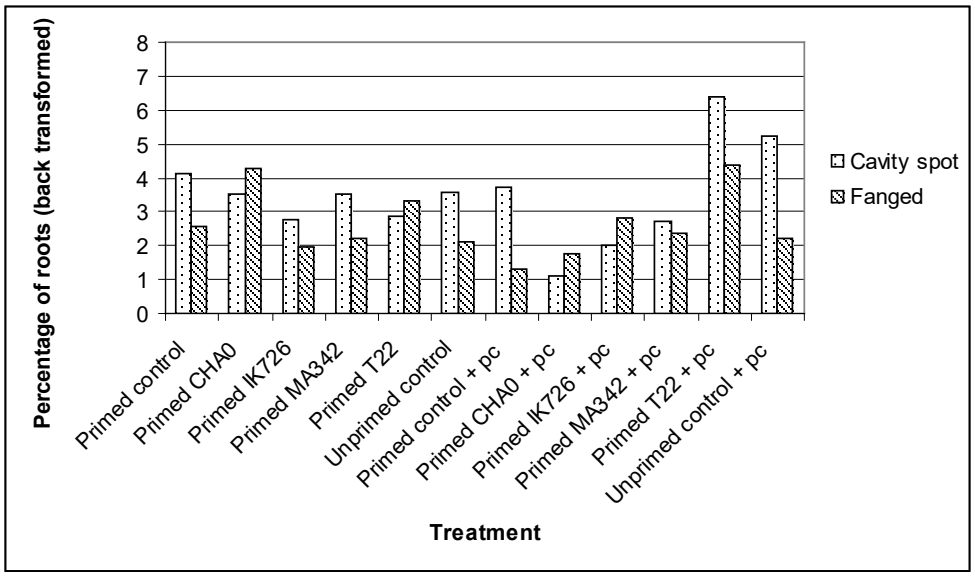


Figure 2.19: Percentage of carrot roots with cavity spot or possible nematode damage (fanged) at the Marshall Farms trial (Year 2)

LSD not available on back transformed data presented (analyses done on angle transformed data)

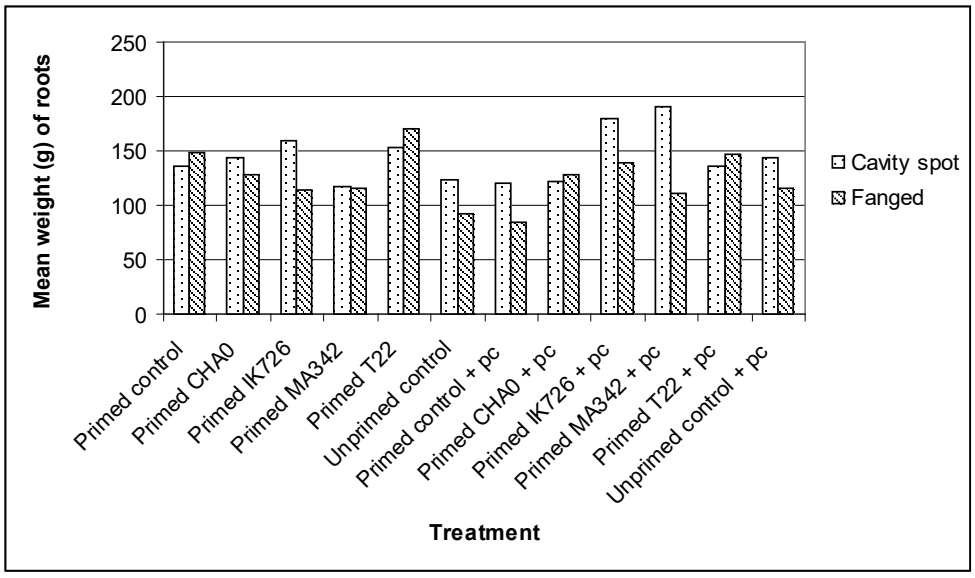


Figure 2.20: Mean weight of carrot roots with cavity spot or possible nematode damage (fanged) at the Marshall Farms trial (Year 2)

LSD (5%) comparing microorganism treatment–pesticide interaction: Cavity spot (df = 23) = 73.08; Fanged (df = 24) = 54.92

Milestone 2.3 – Year 3 emergence data

Warwick HRI onion trial

The Warwick HRI field site for the onion trial is illustrated in Figure 2.21. Emergence assessments were made by counting seedling in the inner two rows, excluding the first and last 50cm of each row (total = 5m). At 3 weeks, there was a significant difference in emergence with all the primed treatments having a higher emergence count than the unprimed control (Figure 2.22). Overall, seed primed with *P. fluorescens* CHA0 had a higher emergence that those treated with *P. chlororaphis* MA342 or *T. harzianum* T22. By 7 weeks, there was no significant effect of microorganism treatment on the emergence count, but pesticide was found to significantly improve emergence at this time (Figure 2.22).

Figure 2.21: Warwick HRI onion field trial (Year 3)

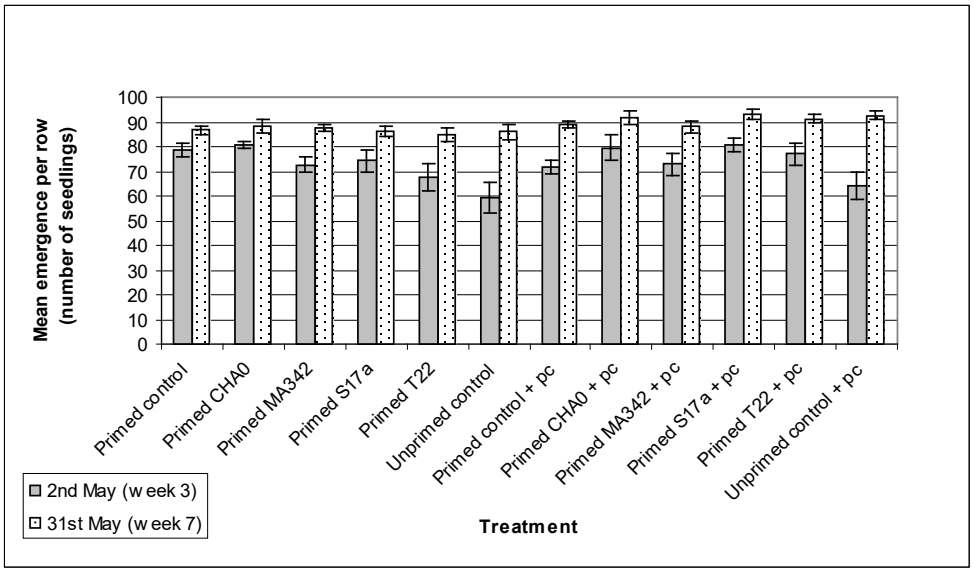


Figure 2.22: Mean emergence of treated onion seed at the Warwick HRI trial (Year 3). Bars represent standard errors of the mean.

Elveden Farms onion trial

Very poor drilling conditions were noted for the conventional onion grower's site for 2006. Emergence assessments were made at 5 and 8 weeks post planting by counting seedling in the inner two rows, for a length of 4m.

There was no overall effect of microorganism treatment or pesticide application, but at 5 weeks there was a significant treatment-pesticide interaction (P<0.01). Without pesticide application, *P. fluorescens* CHA0 was significantly worse than the primed and unprimed control, and the application of pesticide to the *P. fluorescens* CHA0 seed treatment significantly improved emergence (Figure 2.23).

By 8 weeks, further significant differences in emergence were noted. Again, without pesticide, *P. fluorescens* CHA0 was worse than the primed and unprimed control, and *T. harzianum* T22 without pesticide was also worse than the unprimed control at 8 weeks (Figure 2.23). Pesticide application significantly improved emergence for *P. fluorescens* CHA0 and *T. harzianum* T22.

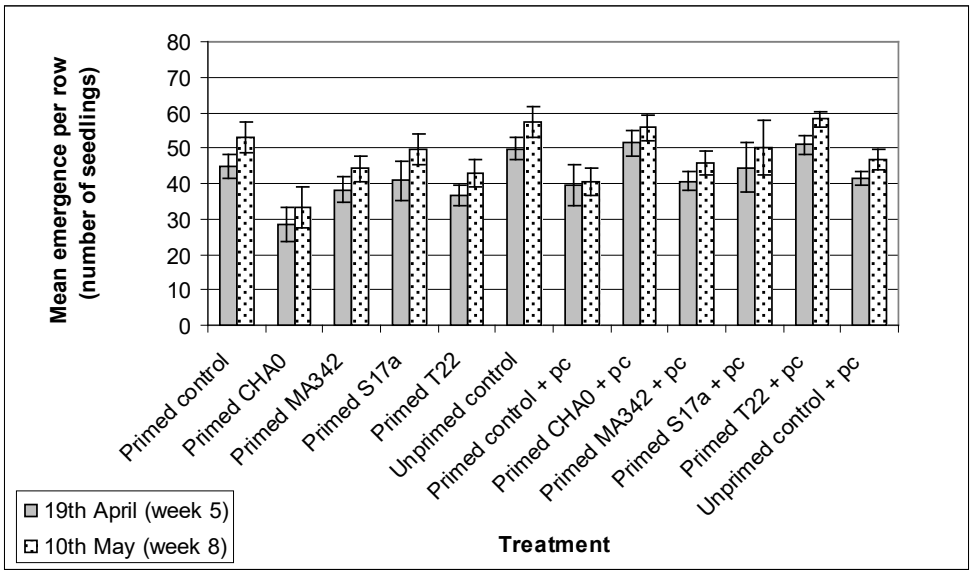


Figure 2.23: Mean emergence of treated onion seed at the Elveden Farms trial (Year 3). Bars represent standard errors of the mean.

Organic onion trial

A single emergence assessment was made at 6 weeks, counting the inner two rows of seedlings for a length of 5m per row. No significant effect of microorganism treatment was noted (Figure 2.24).

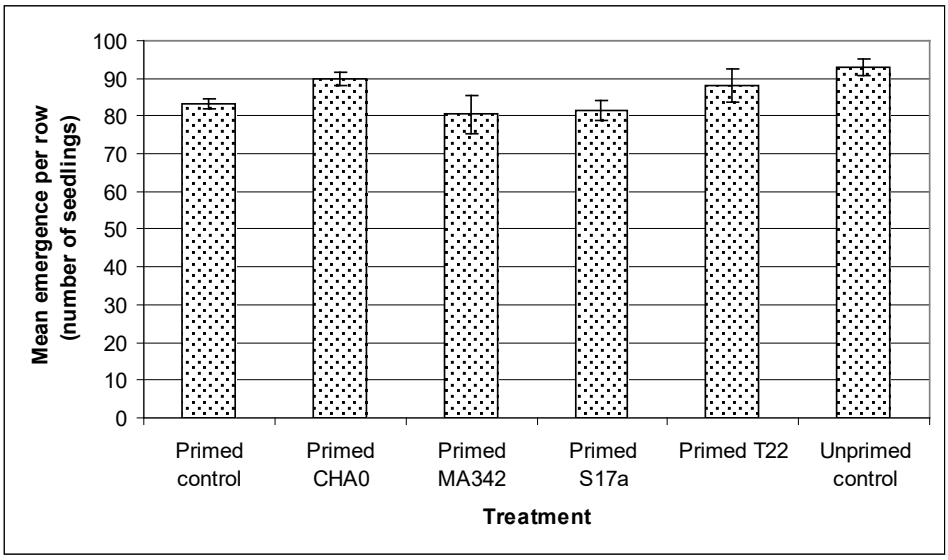


Figure 2.24: Mean emergence of treated onion seed at the organic site (Hillfield, Elsoms Seeds) (Year 3). Bars represent standard errors of the mean.

Warwick HRI carrot trial

The Wellesbourne field site for the carrot trial is shown in Figure 2.25. Emergence counts were made at 3 and 6 weeks, excluding the first and last 50cm of the inner two rows (total = 5m length per row). At 3 weeks, there was no significant effect of microorganism treatment on emergence, but emergence was significantly higher with the addition of pesticide (Figure 2.26). The same result was found with the 6 week emergence assessment, with pesticide significantly increasing emergence. At 6 weeks, the carrot field trial was weeded using a steerage hoe initially, followed by hand weeding.

Figure 2.25: Warwick HRI carrot field trial at 6 weeks (Year 3)

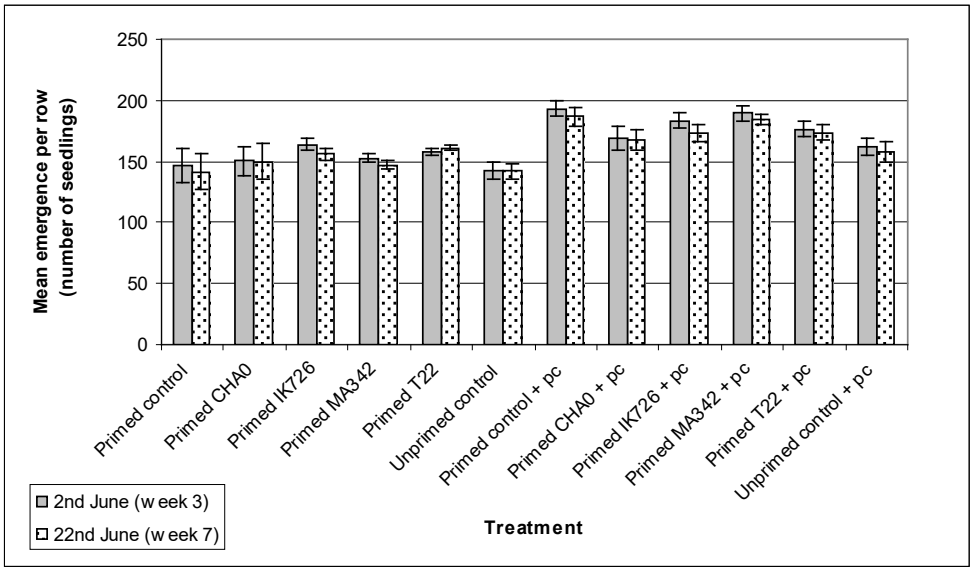


Figure 2.26: Mean emergence of treated carrot seed at the Warwick HRI trial (Year 3). Bars represent standard errors of the mean.

Clumber Farms carrot trial

On the first visit to Clumber Farms (3 weeks post-drilling) the carrots were only at the cotyledon stage and were too small to count. For the final emergence count at 6 weeks, a 4m length of row was counted for the inner

two rows. There were no significant effects of microorganism treatment or pesticide for this trial (Figure 2.27). For the primed control + pesticide treatment, some of the plots had three lines in the inner rows, instead of the usual two, whereas other plots had only one line. This resulted in higher counts for some plots than expected, but lower for others, which is why the standard error bar on the graph is large.

Organic carrot trial

A single emergence assessment was made at 10 weeks, counting the inner two rows of seedlings for a length of 4m per row. No significant effect of microorganism treatment was noted (Figure 2.28).

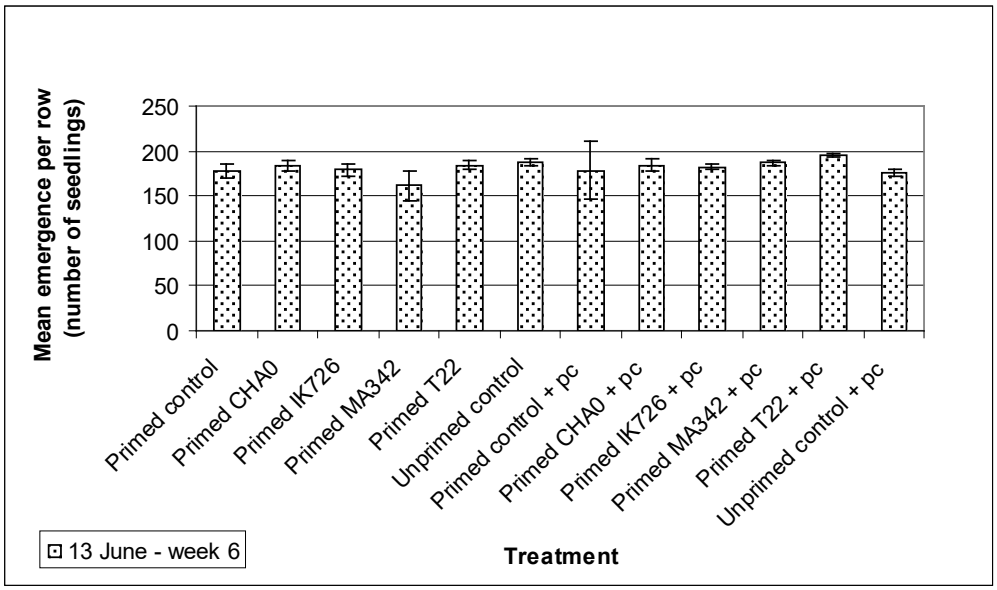


Figure 2.27: Mean emergence of treated carrot seed at the Clumber Farm trial (Year 3). Bars represent standard errors of the mean.

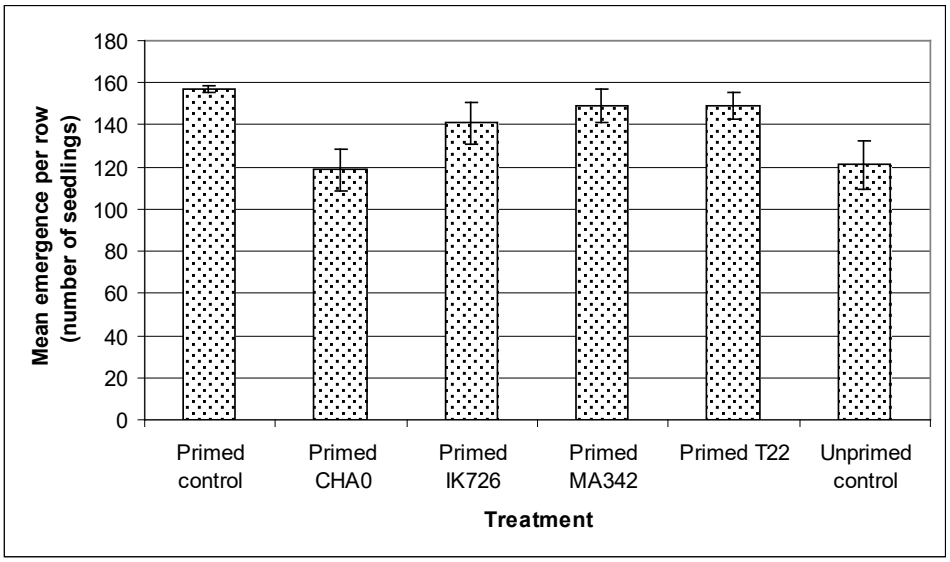


Figure 2.28: Mean emergence of treated carrot seed at the organic site (Hillfield, Elsoms Seeds) (Year 3). Bars represent standard errors of the mean.

Conclusions Milestone S2.2 – Year 2 harvest data:

- Variable effects of microorganism treatment and pesticide application were seen across the trial sites in Year 2.
- Pesticide increased the total weight of **onion** bulbs at Farcet Farms Ltd.
- Positive results at the **onion** trials included:
    - *T. harzianum* T22 increased the total number of bulbs compared to the primed control (without pesticide) (WHRI)
    - *P. chlororaphis* MA342 + pesticide increased the mean weight of bulbs >60mm compared to the primed control + pesticide (WHRI)
- Negative results at the **onion** trials included:
    - *P. fluorescens* CHA0 + pesticide, *C. rosea* IK726 + pesticide and *T. harzianum* T22 + pesticide resulted in fewer bulbs in total than the primed control + pesticide (WHRI)

  - *T. harzianum* T22 + pesticide resulted in a lower weight in total than the primed control + pesticide (WHRI)
  - *C. rosea* IK726 resulted in a lower weight in total than the unprimed control (without pesticide) (WHRI)
  - *C. rosea* IK726 resulted in a lower mean weight of bulbs sized >60mm compared to both the primed and unprimed controls
  - *C. rosea* IK726 reduced the percent bulbs sized 40-60mm compared to the unprimed control (Farcet Farms Ltd.)
  - *T. harzianum* T22 resulted in fewer bulbs overall compared to the primed control (Farcet Farms Ltd.)
  - The mean weight of bulbs sized 60-80mm was higher for the primed control than those treated with either *C. rosea* IK726, *P. chlororaphis* MA342 or *T. harzianum* T22 (Organic site) and was also higher for the unprimed control than those treated with *T. harzianum* T22 (Organic site)
- Positive results at the **carrot** trial at Warwick HRI included:
  - Pesticide application increased the total number and total weight of roots
  - Pesticide decreased the percent of fanged roots, and the mean weight of roots with cavity spot or possible nematode damage (fanged)
  - *P. chlororaphis* MA342, *C. rosea* IK726 and *T. harzianum* T22 treated seed resulted in a greater weight of roots in total than the unprimed control
- Negative results at the **carrot** trial at Warwick HRI included:
  - *C. rosea* IK726 and *T. harzianum* T22 resulted in fewer carrots overall compared to the primed control
  - *T. harzianum* T22 resulted in fewer carrots overall compared to the unprimed control
  - Without pesticide, *P. fluorescens* CHA0, *C. rosea* IK726, *T. harzianum* T22 and the unprimed control resulted in fewer roots overall than the primed control without pesticide
- There were no significant effects of microorganism treatment or

pesticide at the **carrot** trial at Marshall Farms and the organic carrot trial was not harvested due to weed problems.

Conclusions Milestone 2.3 – Year 3 emergence data:

- Due to poor performance over two years, *Clonostachys rosea* IK726 was not used as a seed treatment on onion for Year 3 trials, being replaced instead by *Trichoderma viride* S17a.
- Pesticide application improved emergence overall at both the **onion** and **carrot** trials (WHRI)
- Positive results at the **onion** trials included:
    - All primed treatments emerged significantly faster than the unprimed control (WHRI)
    - *P. fluorescens* CHA0 improved emergence compared to *T. harzianum* T22 and *P. chlororaphis* MA342 (WHRI)
    - The application of pesticide improved the emergence of onion seed treated with *P. fluorescens* CHA0 and *T. harzianum* T22 (Elveden Farms)
- Negative results at the **onion** trials included:
    - Without pesticide application, *P. fluorescens* CHA0 resulted in worse emergence than the primed and unprimed controls (Elveden Farms).
    - Without pesticide application, *T. harzianum* T22 resulted in worse emergence than the unprimed control at the 8 week seedling count (Elveden Farms).
- No microorganism effects were seen at the organic onion, organic carrot or the conventional carrot grower's sites.

General conclusions – Year 1 and 2 harvest data

- No consistent effects of microorganism treatment and pesticide application were seen across the trial sites for the onion or carrot crops in Year 1 and Year 2.

General conclusions – Year 1, 2 and 3 emergence data

- Pesticide application improved emergence for the onion and carrot trials for all three years (WHRI)
- *P. fluorescens* CHA0 (without pesticide) was worse than the primed control (without pesticide) for the carrot crop in Years 1 and 2 only (WHRI).
- *C. rosea* IK726 (without pesticide) was worse than the primed control (without pesticide) for the carrot crop in Years 1 and 2 only (WHRI)
- Other effects of microorganism treatment were variable across the two crops and the different trial sites in all years, with significant differences being both positive and negative.

Objective 03: Characterise growth and establishment responses to seed-applied microorganisms of carrot and onion plants grown in the glasshouse in a range of natural and pathogen infested soils. (Milestones 3.2 and 3.3) Complete

Introduction

The glasshouse experiment assessing emergence and growth of carrot seed primed with different microorganisms, using the same seed as used in the 2005 field trials (Milestone 3.2) is complete. As these results were consistent with those found in the first experiment (2004, 1st Annual report), no further glasshouse experiments were carried out using the carrot seed for the 2006 field trials.

For the onion seed, an experiment was conducted to assess emergence and growth of seed primed with *T. viride* S17a as this was a new seed treatment for this years' field trials. *Trichoderma harzianum* T22 was used as a comparative seed treatment control, as this isolate had been used in previous years as well (Milestone 3.3). The bacterial isolates were not used in this experiment.

Experiments on emergence and growth of carrot and onion seed in pathogen-infested soil have also been carried out to see whether the microorganisms applied during priming could improve the establishment of the seedlings when challenged with a soil-borne pathogen. Two different experiments were conducted: a carrot bioassay using *Pythium ultimum* infested soil, and an onion bioassay using soil containing sclerotia of *Sclerotium cepivorum*, the causal agent of *Allium* white rot.

Materials and methods

Emergence and growth bioassays (carrot (Milestone 3.2) and onion (Milestone 3.3))

Three soil types were used: sandy clay loam (Wellesbourne, Warwickshire); light sandy loam (West Winch, Nr Kings Lynn, Norfolk); and peat (Isleham, Nr Soham, Cambridgeshire). For experimental work, soil was sieved to a size of 5mm, and the sandy clay loam was mixed 4:1 with vermiculite to improve the soil structure. Experiments were designed for each crop, consisting of four replicates, with 6 pots per treatment and 4 seeds planted per pot. Emergence was recorded for up to 30 days, based on previous work that showed that no further increase in seedling number usually occurred following this time. Some seedlings died after emergence, but these were included in the calculations for the emergence data, which comprised the mean emergence time, as well as the time taken to 50% and 80% emergence. The final number of surviving seedlings at harvest (8 weeks) was also determined to give a final percent emergence.

After 8 weeks, the experiments ended and the surviving seedlings were harvested. Seedlings from all 6 pots per treatment were grouped together, comprising a single replicate. All soil was washed off the roots, before they were blotted dry with tissue paper and the fresh weight of the final seedling stand was recorded. The seedlings were then dried to a constant weight and the dry weight per treatment was determined. The mean fresh and dry weight of seedlings was subsequently calculated and $\log_{10}$ transformed before analysis. Significant differences between sample means were determined by analysis of variance (ANOVA).

Pythium ultimum *pathogen bioassay on carrots*

An isolate of *Pythium ultimum* was provided by Mark McQuilken (SAC, Auchincruive) and stored at 4°C on cornmeal agar slopes. Inoculum for the bioassay was prepared using the chopped potato soil (CPS) method. Briefly, this consisted of mixing 500ml of sieved sandy clay loam soil with 50g chopped potato in 1l Duran bottles and autoclaving this twice, on consecutive days. This sterile potato-soil mix was then inoculated with cubes of *P. ultimum* grown on cornmeal agar. The CPS inoculum was incubated at 25°C for 4-6 weeks to allow for colonisation by *P. ultimum* before being sieved and used in the bioassay. Numbers of colony forming units (cfu) were determined by dilution plating a suspension of the CPS.

The bioassay was set up in one soil type only: Wellesbourne soil, (sandy clay loam) mixed 4:1 with vermiculite. Four replicates were set up, each consisting of 6 seed treatments: primed control, unprimed control, primed with *P. fluorescens* CHA0, primed with *P. chlororaphis* MA342, primed with *T. harzianum* T22 or primed with *C. rosea* IK726. These seed treatments were planted in soil either with or without the addition of the CPS inoculum (achieving a rate of 1×10^3 cfu g^{-1} soil), and there were 6 pots per treatment, with 4 seeds planted per pot.

Pots were watered from below as necessary and emergence was recorded weekly for four weeks. After 10 weeks, the experiment ended, and the seedlings were harvested. Seedlings from all 6 pots per treatment were grouped together, comprising a single replicate. All soil was washed off the roots, before they were blotted dry with tissue paper and the fresh root and shoot weight were recorded separately. The roots and shoots were then dried to a constant weight and the dry weight per treatment was determined. The mean fresh and dry weight was subsequently calculated and $\log_{10}$ transformed before analysis. Significant differences between sample means were determined by analysis of variance (ANOVA).

Sclerotium cepivorum *pathogen bioassay on onions*

Sclerotium cepivorum sclerotia were available for use from stocks at

Warwick HRI (Kirton isolate). An initial dose response assay indicated that approximately 60% onion seedling death could be achieved with a dose rate of 5 sclerotia per gram of soil. The bioassay was set up in one soil type: Wellesbourne soil, (sandy clay loam), mixed 4:1 with vermiculite. In this bioassay there were four replicates, each consisting of 8 seed treatments: primed control, unprimed control, primed with *P. fluorescens* CHA0, primed with *P. chlororaphis* MA342, primed with *T. harzianum* T22, primed with *C. rosea* IK726, primed with *T. viride* S17a or primed with *T. viride* L4. These seed treatments were planted in soil with or without the addition of sclerotia of *S. cepivorum* (5 sclerotia per gram), and there were 10 pots per treatment with a single seed planted in each.

Pots were watered from below as necessary. Emergence was recorded and any dying seedlings were removed to the laboratory to determine if *S. cepivorum* was the causal agent. This test consisted of placing the seedling in a Petri dish of water and leaving it at room temperature. If white mycelium and sclerotia formed on the seedling, *Allium* white rot (AWR) was confirmed. Some seedlings did not form sclerotia in this way, and were plated onto PDA amended with chlortetracycline (30µg ml^{-1}) and Triton X-100 (2ml l^{-1}). If sclerotia subsequently formed on the agar plates, AWR was confirmed. Dead or dying seedlings which did not form sclerotia in either of these tests were classified as having died from “other” causes.

The number of emerged seedlings expressed as a proportion of the number sown (10), and the number of seedlings confirmed dead due to AWR, number of seedlings dead not due to AWR, and the total number of dead seedlings, all expressed as proportions of the number of emerged seedlings, were analysed within a generalised linear model (GLM) assuming a binomial distribution and logit link function. Overall differences between treatments (microorganisms), the effect of sclerotial inoculation, and the interaction between treatments and the sclerotial inoculation effect were assessed via an analysis of deviance using a chi-square test.

Results and discusssion

Emergence and growth bioassay – carrot (Milestone 3.2, 2005)

The full analysis of all data is given in Appendix 3.1. Tables presenting the statistical analyses to illustrate effects of soil type, seed treatment and pesticide application are given in Appendices 3.2-3.4 and these are discussed below.

Emergence - Soil type effects

Soil type had a significant effect on emergence of carrot seedlings (*P* <0.05). Mean emergence time and time to 50% and 80% emergence were all significantly faster in the sandy clay loam soil than the other soil types, and also significantly faster in the light sandy loam soil than the peat soil (Figure 3.1). Final percent emergence was also significantly greater in the sandy clay loam (88%) than the peat (80%) or light sandy loam (77%). Compared to the first experiment on emergence and growth of carrot seed primed with microorganisms (1st Annual Report, Sep 2004), the soil type effects were similar with the peat soil having the slowest emergence time. This may be due to slower uptake of water in the peat soil compared to the other soil types. Also, the final percent emergence was similar in the sandy clay loam and peat soils, although emergence in the light sandy loam was better in the second experiment (this report).

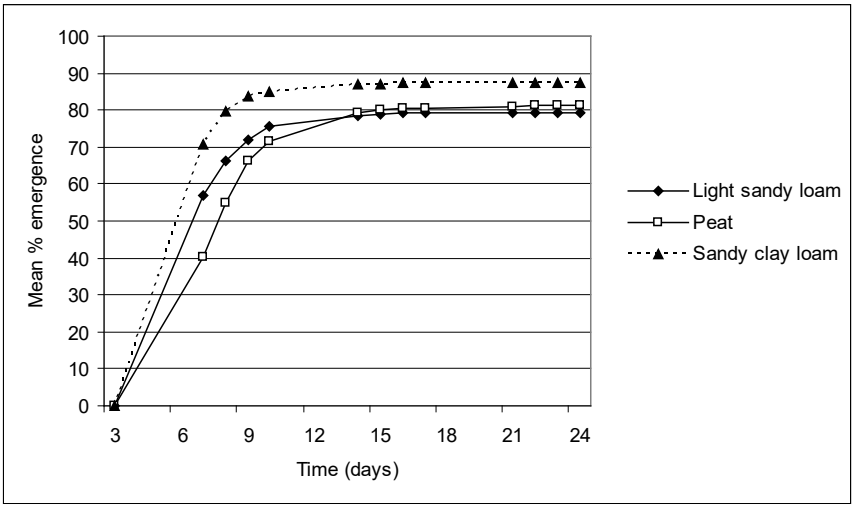


Figure 3.1: Mean percent emergence of treated carrot seed over time in three soils types

Emergence - Seed treatment effects

All primed seed treatments emerged faster than the unprimed control treatment (Figure 3.2). The mean emergence time, and time to 50% and 80% emergence, of seed treated with *C. rosea* IK726 were faster than the primed control, but that of *T. harzianum* T22 was slower than the primed control. However, overall there was no significant difference between treatments with the final percent emergence (Figure 3.2).

Compared to the first experiment (1st Annual report, 2004), the results were similar, with the primed treatments emerging faster than the unprimed control. Also, *C. rosea* IK726 was consistent in that it emerged faster than the primed control in both experiments. A discrepancy was noted with *T. harzianum* T22, which resulted in faster emergence than the primed control in the first experiment, but was worse that the primed control in the second experiment. This may be due to the initial inoculum of *T. harzianum* T22 on the carrot seed. In the first experiment, the inoculum was approximately 8 $\log_{10}$ cfu g^{-1} seed, whereas in the second experiment it was 7 $\log_{10}$ cfu g^{-1} seed.

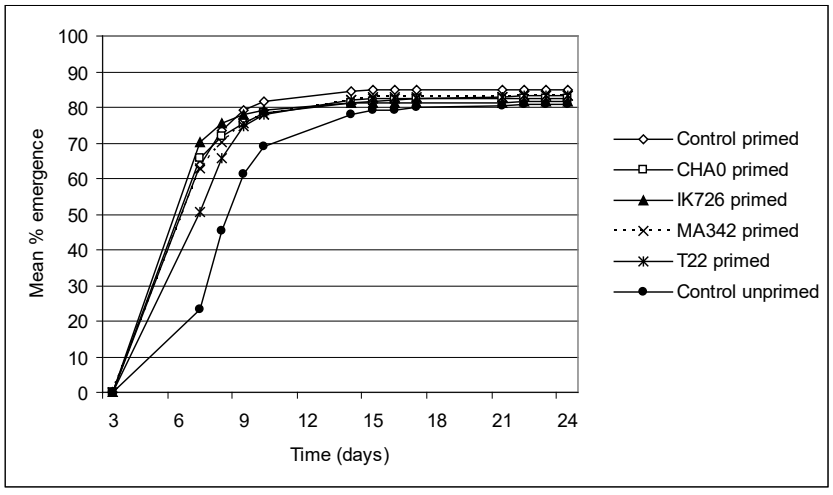


Figure 3.2: Mean percent emergence of carrot seed with different microorganisms applied during priming

Emergence - Pesticide effects

Pesticide application did not affect the emergence time of the carrot seed, but the final percent emergence of seed treated with pesticide was significantly higher than seed without pesticide (Figure 3.3). These results are consistent with those found in the first experiment (1st Annual Report, 2004).

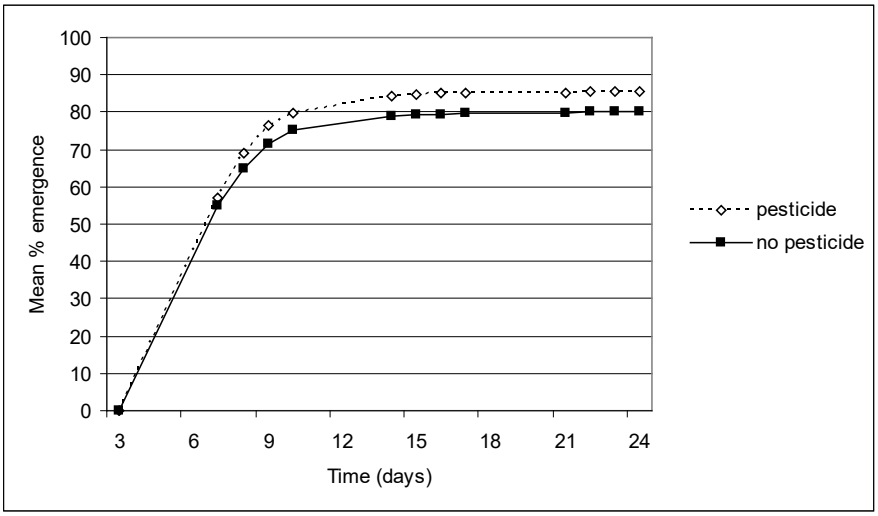


Figure 3.3: Mean percent emergence of carrot seed with or without pesticide application

Mean fresh weight of seedlings

At harvest (8 weeks post planting) the mean fresh weight of seedlings from peat soil was significantly greater than seedlings from the other two soil types (Table 3.1). Also, seedlings from the light sandy loam soil had a greater mean fresh weight than those in the sandy clay loam soil. No effects of microorganism treatment or pesticide were seen (Table 3.1 and 3.2). These results are consistent with those from the first experiment (1st Annual Report, 2004)

Table 3.1: Mean fresh weights (mg) of carrot seedlings primed with different microorganisms. Values have been log transformed and the analysis carried out on the transformed data. Transformed data are in parentheses.

Soil type	Light sandy loam		Peat		Sandy clay loam	
Pesticide	-	+	-	+	-	+
Primed control	954 (6.9)	1472 (7.3)	1997 (7.6)	2052 (7.6)	1051 (7.0)	1314 (7.2)
Primed *P. fluorescens* CHA0	1643 (7.4)	1344 (7.2)	1915 (7.6)	2126 (7.7)	1166 (7.1)	1329 (7.2)
Primed *C. rosea* IK726	1465 (7.3)	1509 (7.3)	2116 (7.7)	2129 (7.7)	1226 (7.1)	1371 (7.2)
Primed *P. chlororaphis* MA342	1259 (7.1)	1683 (7.4)	2047 (7.6)	1892 (7.6)	1068 (7.0)	1299 (7.2)
Primed *T. harzianum* T22	1365 (7.2)	1623 (7.4)	2102 (7.7)	1997 (7.6)	1113 (7.0)	1145 (7.0)
Unprimed control	1417 (7.3)	1566 (7.4)	1762 (7.5)	1813 (7.5)	1078 (7.0)	1288 (7.2)
Mean	1427 (7.3)		1992 (7.6)		1199 (7.1)	

LSD_1 (0.05, d.f. = 105) Comparing seed treatments within a soil type = 0.43

LSD_2 (0.05, d.f. = 105) Comparing soil types = 0.12

Table 3.2: Overall effect of pesticides on the mean fresh weight (mg) of microorganism-primed carrot seedlings. Values have been log transformed and the analysis carried out on the transformed data. Transformed data are in parentheses.

Treatment	Mean fresh weight (mg)
Pesticide	1579 (7.36)
No pesticide	1434 (7.27)
LSD (0.05, d.f. = 105)	(0.101)

Emergence and growth bioassay - onion (Milestone 3.3, 2006)

As a new fungal isolate, *Trichoderma viride* S17a was used for the onion field trials in 2006, the emergence and growth of onion seed primed with this microorganism was investigated in three soil types. As a comparative control, *T. harzianum* T22 was also used. Seed primed with the bacterial isolates (*P. fluorescens* CHA0 and *P. chlororaphis* MA342) was not used in this experiment. The full analysis of all data is given in Appendix 3.5. Tables presenting the statistical analyses to illustrate the effects of soil type, seed treatment and pesticide application are given in Appendices 3.6-3.8, and these results are discussed below.

Emergence – Soil type effects

Overall, emergence was poor (Figure 3.4). By 30 days, less than 50% of the seed had emerged and assessments were stopped at this time. Soil type had no significant effect on the emergence time, but it did affect the final percent emergence ($P<0.01$). The final stand was significantly lower in the peat than the other soil types (Appendix 3.6).

Compared to previous experiments (1st and 2nd Annual reports), the emergence of onion seedlings was low in all three soil types. Also, there was no consistency with regard to the soil type with the highest percent emergence, as the peat soil produced the largest final stand in 2005 (2nd

Annual report), but the lowest in 2006 (this report).

Emergence – Seed treatment effects

Although seed treated with *T. viride* S17a did seem to show improved emergence over the other seed treatments in the 30 day emergence assessment, there were no statistically significant effects of microorganism treatment (Figure 3.5). Some seedling emergence continued after the assessments had stopped at 30 days, although the increase in numbers after this time was not high. At 8 weeks when the experiment was harvested, there was no significant difference in the final stand, with all treatments having less than 50% emergence (Appendix 3.7).

A comparison of this experiment with those from previous years is difficult as the treatments in common between the experiments (unprimed control, primed control and *T. harzianum* T22) did not perform consistently over the three experiments (1st, 2nd and current Annual reports). However, it may be that *T. viride* S17a has potential to improve emergence of onion seeds, even though the difference is not significant statistically.

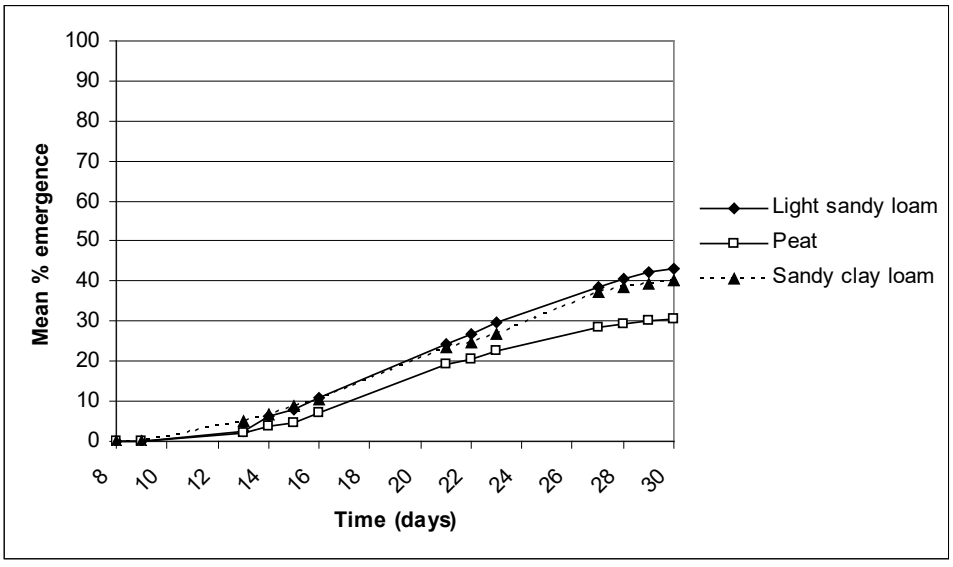


Figure 3.4: Mean percent emergence of treated onion seed over time in three soil types.

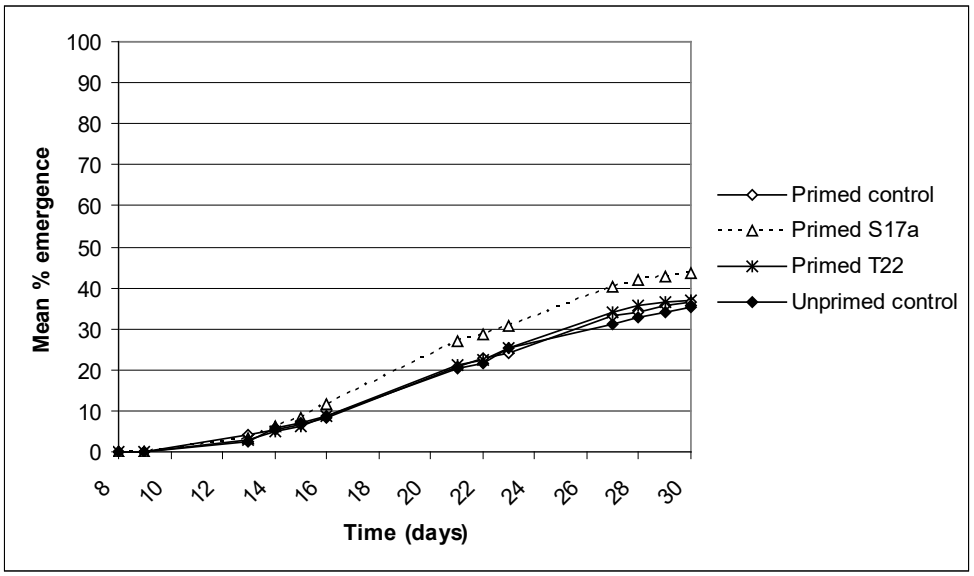


Figure 3.5: Mean percent emergence of onion seed with different microorganisms applied during priming

Emergence – Pesticide effects

Pesticide application to the pellet significantly increased the mean emergence time, time to 50% emergence and time to 80% emergence (Figure 3.6). The final percent emergence after 8 weeks was not significantly different, however (Appendix 3.8). This was different to the previous experiment on onion (2nd Annual report) where the application of pesticide improved emergence. However, that seed batch was affected by a deleterious microorganism that increased during priming, so the pesticide application may have had a positive effect there. The current seed batch did not appear to be affected by a deleterious microorganism, hence the pesticide had no effect.

The poor emergence of seed in this glasshouse experiment cannot be explained, as the same seed used for the field trials in 2006 (Objective 02) did not suffer from poor emergence.

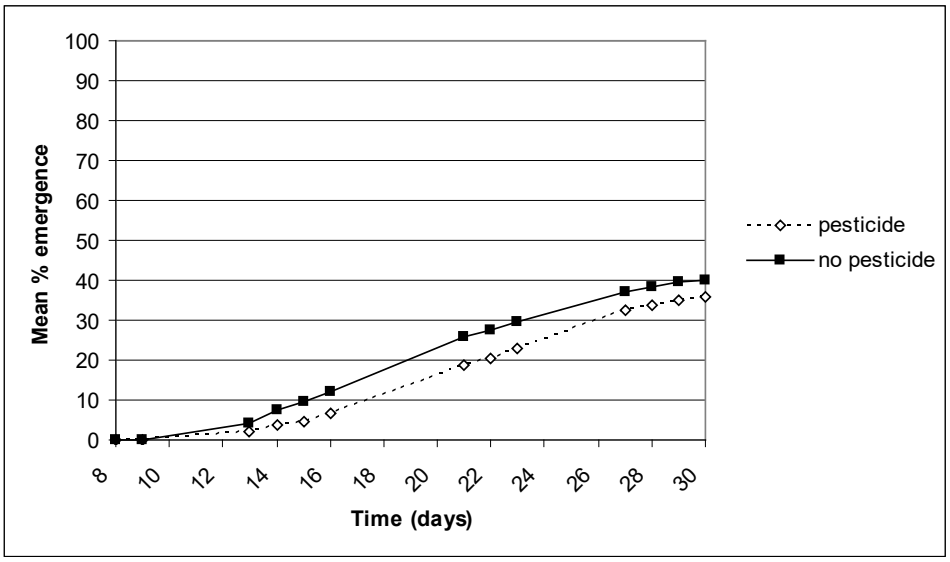


Figure 3.6: Mean percent emergence of onion seed with or without the application of pesticides.

Mean fresh weight of seedlings

At harvest (8 weeks post planting) the mean fresh weight of seedlings from the light sandy loam soil was significantly higher than the other soils (Table 3.3). This is consistent with the results found in the first experiment (1st Annual report), but different to the second experiment where the peat soil resulted in the greatest mean fresh weight (2nd Annual report). As in all previous experiments, there was no microorganism treatment effect on the mean fresh weight, and the addition of pesticides resulted in a small but significantly lower mean fresh weight of seedlings (Table 3.4).

Table 3.3 Mean fresh weights (mg) of onion seedlings primed with different microorganisms. Values have been log transformed and the analysis carried out on the transformed data. Transformed data are in parentheses.

Soil type	Light sandy loam		Peat		Sandy clay loam	
Pesticide	-	+	-	+	-	+
Primed control	448 (6.10)	314 (5.75)	247 (5.51)	227 (5.42)	265 (5.58)	252 (5.53)
Primed *T. viride* S17a	373 (5.92)	436 (6.08)	294 (5.68)	218 (5.38)	315 (5.75)	276 (5.62)
Primed *T. harzianum* T22	543 (6.30)	361 (5.89)	304 (5.72)	251 (5.53)	253 (5.53)	265 (5.58)
Unprimed control	470 (6.15)	361 (5.89)	288 (5.66)	183 (5.21)	262 (5.57)	225 (5.42)
Mean	408 (6.01)		248 (5.51)		263 (5.57)	

LSD_1 (0.05, d.f. = 69) Comparing seed treatments within a soil type = 0.467

LSD_2 (0.05, d.f. = 69) Comparing soil types = 0.165

Table 3.4: Overall effect of pesticides on the mean fresh weight (mg) of microorganism-primed onion seedlings. Values have been log transformed and the analysis carried out on the transformed data. Transformed data are in parentheses.

Treatment	Mean fresh weight (mg)
Pesticide	273 (5.61)
No pesticide	327 (5.79)
LSD (0.05, d.f. = 69)	(0.135)

Pythium ultimum *pathogen bioassay on carrots*

Few seedlings (both uninoculated and inoculated with *P. ultimum*) died post-emergence in this experiment. Consequently, the final seedling stand at 10 weeks was used to assess the overall emergence. The addition of the pathogen inoculum did not significantly reduce emergence, and the final stand was over 70% for all treatments (Figure 3.7). There was no significant effect of microorganism treatment on the overall emergence.

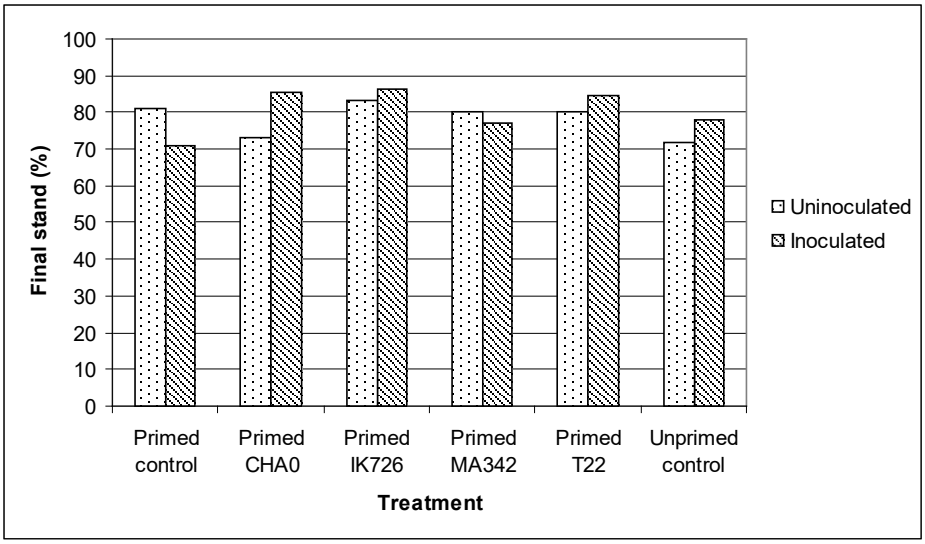


Figure 3.7: Overall emergence of carrot seed primed with different microorganisms, in soil either inoculated with *Pythium ultimum* (1 x 10^3 cfu g^{-1} soil) or left uninoculated.

Fresh root weight per seedling was significantly higher when the pathogen inoculum was added (Figure 3.8). This was contrary to expectations, but a possible explanation could be that the addition of the CPS inoculum provided the plants with further nutrients. A relatively high amount of CPS was added to each pot to achieve the pathogen inoculum of 1 x 10^3 cfu g^{-1} soil (36g CPS +180g soil per pot), which meant that a large amount of sterilised soil was added to the inoculated treatments. Sterilising soil can cause a release of nutrients that would otherwise be unavailable for plant uptake, and these nutrients could have allowed for improved growth of the plants in inoculated treatments. Overall, there were no significant effects of the microorganism treatments on the fresh root weight of seedlings, although within the pathogen inoculated treatments, *T. harzianum* T22 resulted in a higher fresh root weight compared to *P. fluorescens* CHA0 and the unprimed control (Figure 3.8).

The addition of the CPS inoculum also caused a significant increase in the fresh shoot weight per seedling (Figure 3.9). Again there were no significant effects of microorganism treatment on the fresh shoot weight, but within the pathogen inoculated treatments *Pseudomonas fluorescens* CHA0 primed seed resulted in a significantly lower mean fresh weight than the primed control, primed *P. chlororaphis* MA342 and primed *T. harzianum* T22.

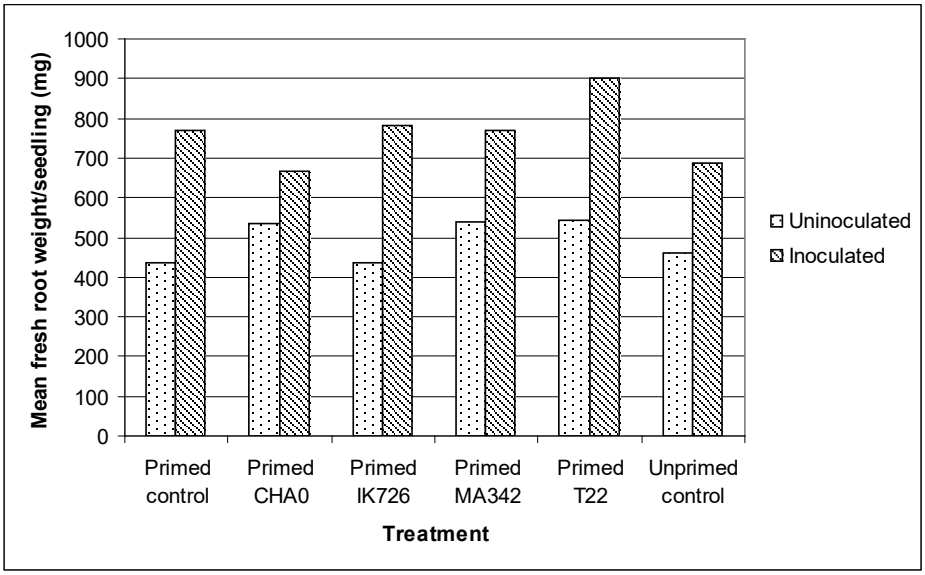


Figure 3.8: Mean fresh root weight per carrot seedling at harvest, following growth for 10 weeks in soil inoculated with *Pythium ultimum* (1 x 10^3 cfu g^{-1} soil) or left uninoculated

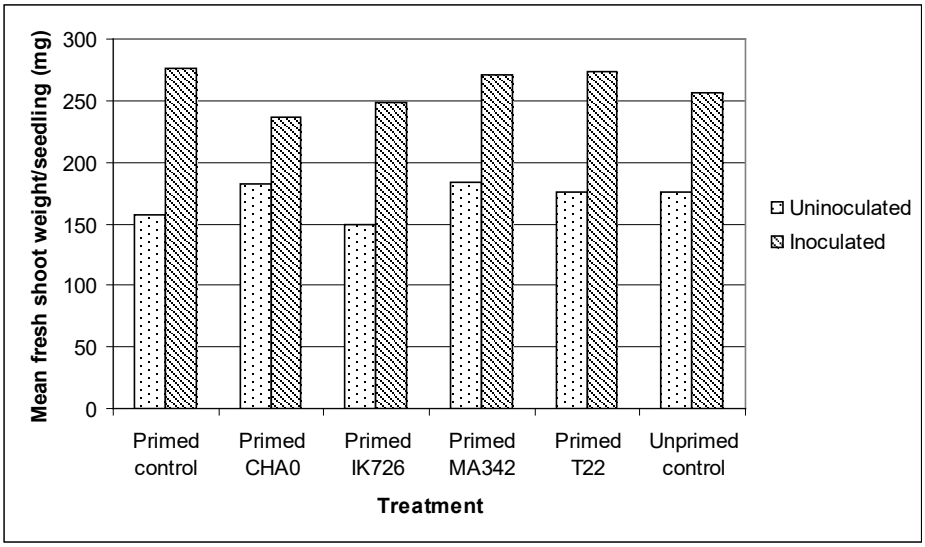


Figure 3.9: Mean fresh shoot weight per carrot seedling at harvest, following growth for 10 weeks in soil inoculated with *Pythium ultimum* (1 x 10^3 cfu g^{-1} soil) or left uninoculated

The choice of pathogen inoculum in this experiment may have affected the results and it may be that a different inoculum source (for example cornmeal sand) may be better to reduce the effect of additional nutrition from the sterilised soil carrier.

Sclerotium cepivorum *pathogen bioassay on onions*

The overall emergence count was determined from all seedlings that emerged, including those that subsequently died (Figure 3.10). Higher emergence was noted for treatments where sclerotia had been mixed into the soil (inoculated), and this was a significant difference for all the treatments apart from the seed primed with *P. chlororaphis* MA342 (P<0.05). This may be due to the inoculated treatments having more aerated soil than the uninoculated treatments, following the manual mixing of soil and sclerotia in a bag before being transferred to the pots. Uninoculated soil was placed directly in the pots without any mixing.

In both the uninoculated and inoculated treatments, the primed control had a higher emergence than the unprimed control (P<0.05), confirming the positive effects of priming. Compared to the primed control, the application of microorganisms to seed had no significant effect on seedling emergence (Figure 3.10).

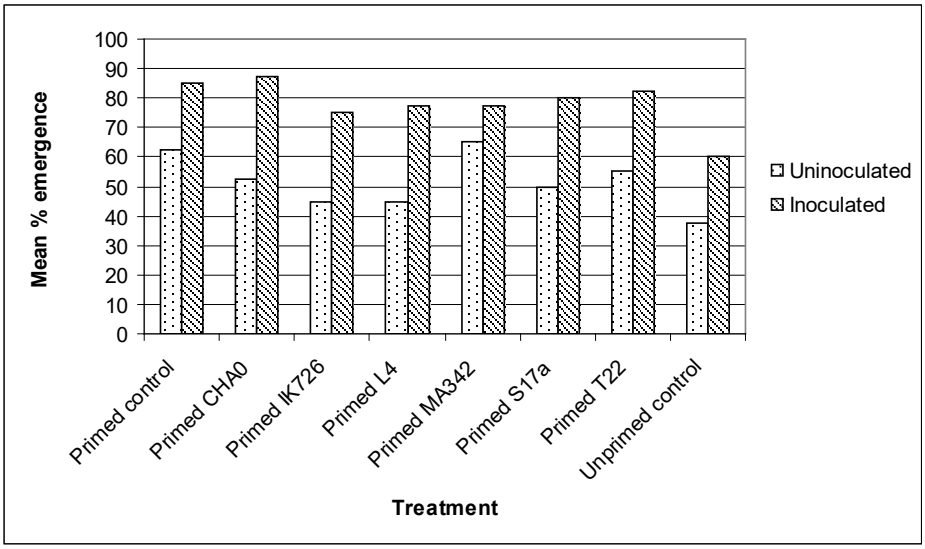


Figure 3.10: Mean percent emergence of treated onion seed in soil inoculated or not with *Sclerotium cepivorum* sclerotia (5 sclerotia/g soil)

Of the emerged seedlings, the proportion of those affected by *Allium* white rot (AWR) was calculated (Figure 3.11). Statistically, there was no significant difference in the percent AWR between the primed control and those treatments with microorganisms applied to the seed.

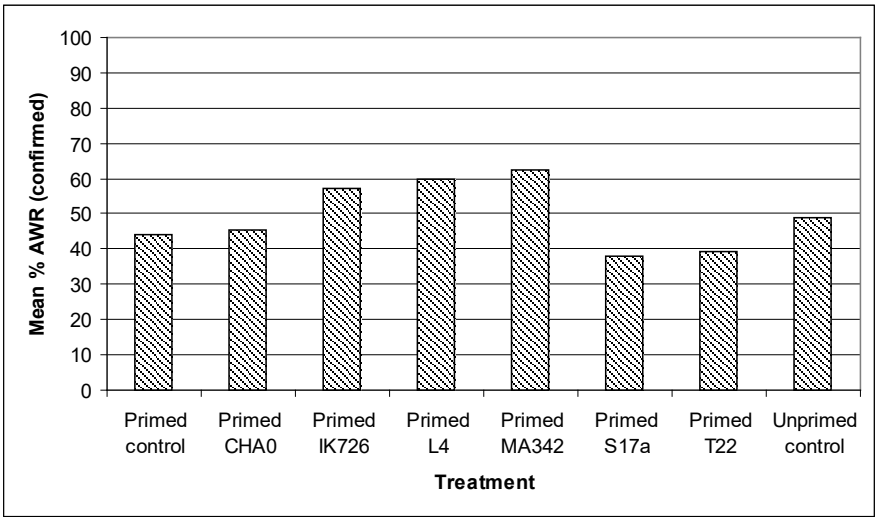


Figure 3.11: Mean percent of onion seedlings with confirmed *Allium* white rot (sclerotia present), following growth in soil amended with 5 sclerotia/g soil.

During the experiment a proportion of seedlings died where the death could not be confirmed as AWR as no sclerotia were produced on the seedlings in the Petri dishes or on agar plates. However, some of these seedling deaths may have been due to *S. cepivorum* in the inoculated treatments, and the overall numbers of AWR may be underestimated in this case. For example, in the primed control treatment only, the effect of sclerotial inoculation on the percent "other" seedling death showed that the addition of sclerotia to the soil resulted in more seedling death than where no sclerotia had been added. This suggests that some of these "other" seedling deaths could be attributed to AWR even though no sclerotia were formed on the dead seedlings. However, as there was a relatively high proportion (up to 16%) of "other" seedling deaths occurring in uninoculated treatments, these deaths were kept separate in the analyses.

Figure 3.12 shows the total percent dead seedlings at the end of the experiment, including those that died from AWR and those that died of other causes. The total number of dead seedlings was high in the inoculated treatments (63-87%). However no significant difference was found with the total percent seedling death comparing the microorganism treatments to the

primed control for both the uninoculated or inoculated treatments.

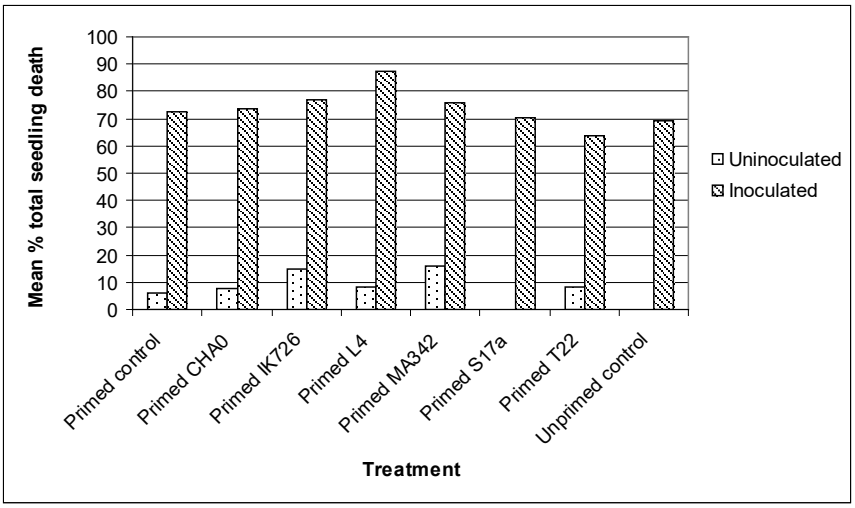


Figure 3.12: Mean percent of seedlings that died overall (due to AWR or other causes), following growth of treated onion seed in soil amended with sclerotia of *S. cepivorum* (5 sclerotia/g) or left uninoculated.

Conclusions: Emergence and growth bioassays

Carrot (Milestone 3.2)

- Results from two experiments examining the emergence and growth of carrot seed primed with microorganisms were similar.
- Emergence was fastest in the sandy clay loam soil, but the greatest mean fresh weight of carrot seedlings was found in peat soil.
- *Clonostachys rosea* IK726 improved emergence over the primed control in two experiments.
- *Trichoderma harzianum* T22 did not perform consistently over two experiments, but this may have been due to the initial inoculum on the seed.

Onion (Milestone 3.3)

- Poor emergence was found with all seed treatments in the glasshouse experiment (<50% emergence).
- The peat soil resulted in the lowest emergence of all the soil types, and seedlings grown in light sandy loam had the greatest mean fresh

weight.

- There was no significant effect of microorganism treatment on emergence or fresh weight of the seedlings.
- Pesticide application decreased the mean fresh weight, which was consistent with previous experiements.
- No other consistent effects were seen with experiments from previous years.

Conclusions: Pathogen bioassays

Pythium ultimum *on carrots*

- The chopped potato soil (CPS) pathogen inoculum did not cause a reduction in emergence.
- The addition of the CPS increased the mean fresh weight of seedling roots and shoots, possibly due to the addition of nutrients in the sterilised soil carrier.
- Overall there were no effects of microorganism treatment, although within the pathogen inoculated treatments some improvement in mean fresh root weight was noted with the *T. harzianum* T22 seed treatment.
- It would be better to use a different inoculum source in future experiments.

Sclerotium cepivorum *on onions*

- Treatments where sclerotia of *S. cepivorum* were added had a higher emergence than uninoculated treatments, possibly due to better soil aeration.
- The primed control had a higher emergence than the unprimed control.
- The application of microorganisms to seed did not improve emergence compared to the primed control, and there was no reduction in the percent AWR compared to the primed control.
- The percent AWR may have been underestimated as only dying

seedlings that had sclerotia forming on them were confirmed as having AWR.

- The total number of dead seedlings at the end of the experiment was high, but there was no significant difference comparing the primed control with the microorganism seed treatments.

Objective 04: Determine the survival and proliferation of seed-applied microorganisms on seedlings after sowing in soil in the glasshouse and field. (Milestone 4.2 and 4.3) Complete

The planned monitoring of survival of applied microorganisms on seedlings in field trials will not be carried out as PSD refused permission to use marked strains of the microorganisms

Introduction

An experiment was set up in the glasshouse to monitor the survival of marked strains of microorganisms on carrot roots and in rhizosphere soil, following application to seed during drum priming. Results from this experiment for Milestone 4.2 were not available for the 2nd Annual report (2005) and are presented here. To facilitate recovery of the applied microorganisms, rifampicin-resistant strains of the bacteria were used, and a hygromycin-B resistant strain of *C. rosea* IK726 was used. There was no marked strain of *T. harzianum* T22, but a *Trichoderma* selective medium was used for isolation and colonies were compared to a reference plate of the same fungus at each sampling time.

A similar experiment was also set up to monitor the survival of the new isolate *T. viride* S17a on onion roots and in rhizosphere soil, as this isolate was being used for the first time in field and glasshouse trials in 2006. There was no marked strain of this isolate, but the *Trichoderma* selective medium mentioned above was used for re-isolation of the applied fungus.

Materials and methods

Seed preparation and initial cfu counts

Seed samples were drum primed at Warwick HRI and Table 4.1 shows the treatments used in the experiments with the initial numbers of applied microorganism surviving on the seed at the time when the experiments were

set up.

Table 4.1: Seed treatments used in the glasshouse experiments investigating the survival of microorganisms on roots and in rhizosphere soil following application to seed during drum priming. Values are the mean number of cfu found on the seed before the experiments were set up in the glasshouse.

Treatment	**Log_{10} cfu g^{-1} dry seed**	
	Carrot	**Onion**
Primed control	NA[a]	NA
P. fluorescens CHA0	8.3	NU[b]
P. chlororaphis MA342	7.0	NU
C. rosea IK726	6.2	NU
T. harzianum T22	6.3	5.6
T. viride S17a	NU	4.2

[a] NA = not applicable

[b] NU = not used

Experimental set up

For each experiment, four replicates were set up in the glasshouse. The carrot experiment consisted of five seed treatments, whereas the onion experiment had three seed treatments (Table 4.1). Each seed treatment was planted in three soil types (light sandy loam, peat and sandy clay loam), with four pots per seed treatment-soil-type combination and four seeds per pot. Enough pots were set up initially to allow for sampling at 2, 4 and 8 weeks post-planting.

At each time interval, the seedlings from four pots (up to 16 seedlings) for each seed treatment-soil-type combination were harvested. Rhizosphere soil was washed off in 10ml sterile distilled water (SDW) and plated in a dilution

series onto agar selective for the various microorganisms (soil count). Roots were then blotted dry and ground in 10ml SDW water using a sterile mortar and pestle, and a dilution series was similarly plated onto selective media (root count). At the 4 and 8 week sampling time for the carrot experiment and the 8 week sampling time for the onions, the rhizosphere soil was washed off in 50ml SDW due to the size of the root mass, and ground roots were also suspended in 50ml SDW. Values are expressed as $\log_{10}$ cfu g^{-1} fresh weight of soil or root for comparison with previous experiments (2nd Annual reports), but Appendix 4.1 also shows the graphs for the carrot experiment expressed as $\log_{10}$ cfu per seedling.

Results and discussion

Carrot experiment (Milestone 4.2)

The bacterial isolates on roots and in rhizosphere soil generally declined in numbers over the 8 week period, with *P. chlororaphis* MA342 being recovered in much lower numbers than *P. fluorescens* CHA0 (Figure 4.1). At the final sampling time, *P. fluorescens* CHA0 was recovered at over 2 $\log_{10}$ cfu g^{-1} soil or root in all soil types, whereas *P. chlororaphis* MA342 declined to below 1 $\log_{10}$ cfu g^{-1} soil or root.

Compared to the first experiment looking at survival of the microorganisms on carrot roots and in rhizosphere soil, numbers of bacteria recovered were significantly higher. Previously, bacteria were recovered in low numbers from peat soil only, whereas both isolates were consistently recovered from all three soils in the second experiment. However, the improved recovery in the second experiment may be due to the larger amount of material used.

Survival of the fungal isolates is shown in Figure 4.2. *Clonostachys rosea* IK726 showed a slight increase in numbers in all soil types, both on the root and in rhizosphere soil, with final numbers over 3 $\log_{10}$ cfu g^{-1} at 8 weeks. *Trichoderma harzianum* T22 increased slightly in light sandy loam and peat soil, being recovered at over 3 $\log_{10}$ cfu g^{-1} at 8 weeks, but declined slightly in

sandy clay loam soil. However, at 8 weeks, *T. harzianum* T22 was still recovered at over 2 log_{10} cfu g^{-1} (Figure 4.2). The fungal survival patterns were less variable in the second experiment compared to the first (2nd Annual report), again reflecting the larger sample size.

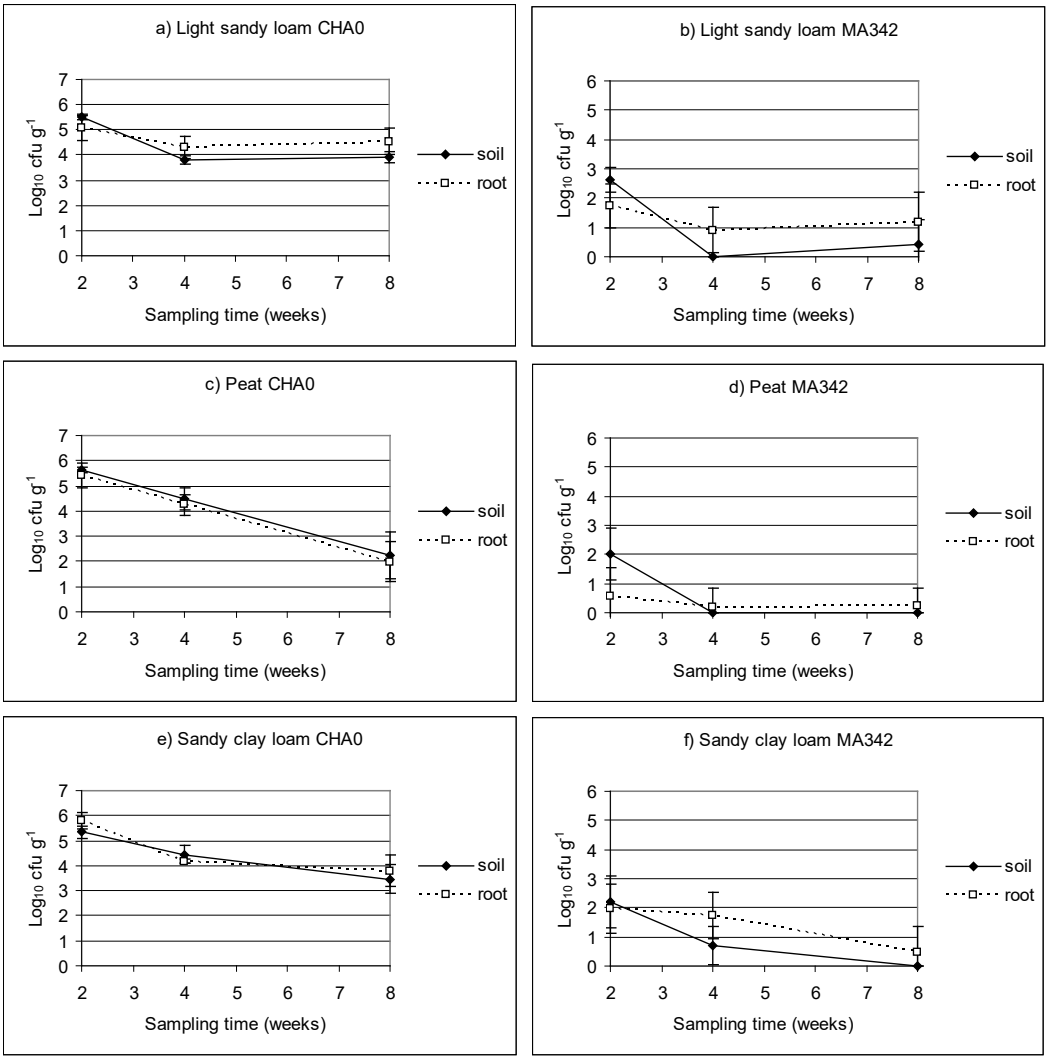


Figure 4.1: Survival of bacterial isolates *Pseudomonas fluorescens* CHA0 and *Pseudomonas chlororaphis* MA342 on carrot roots and in the rhizosphere in three soil types

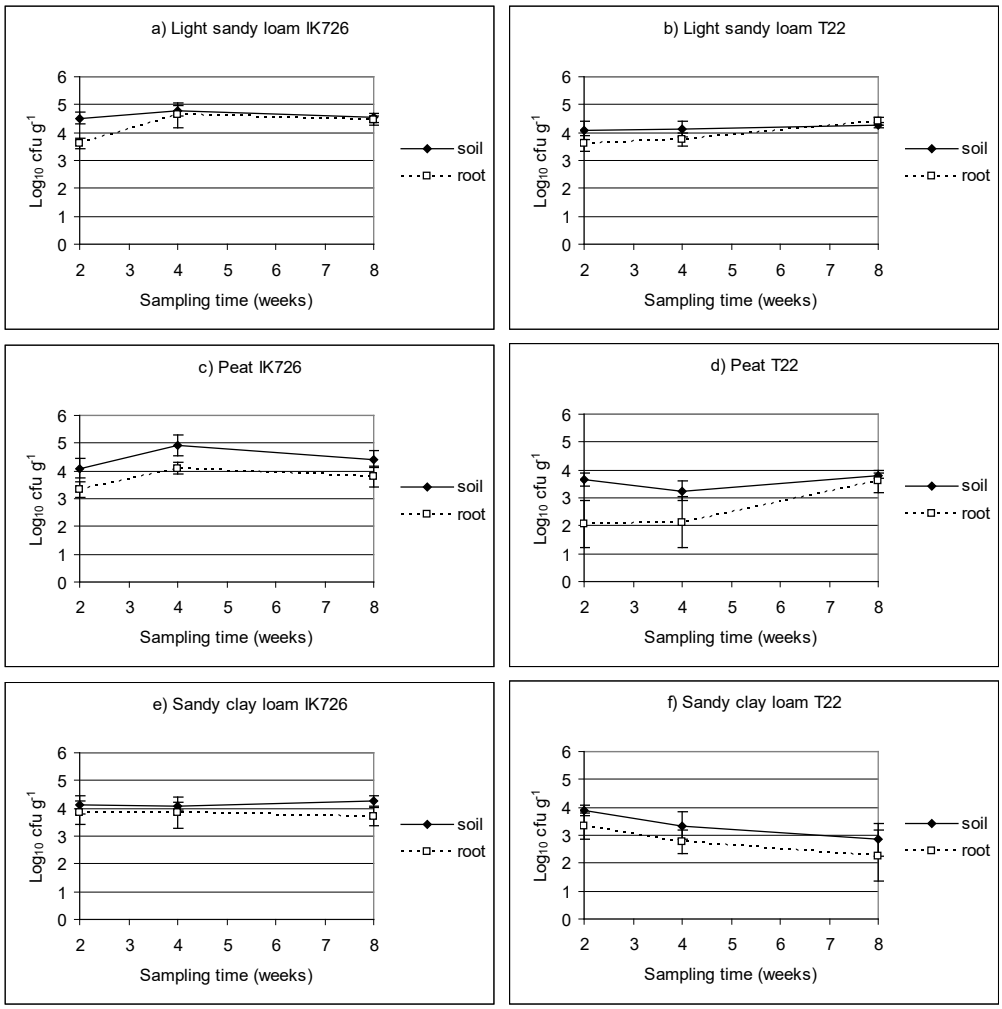


Figure 4.2: Survival of fungal isolates *Clonostachys rosea* IK726 and *Trichoderma harzianum* T22 on carrot roots and in the rhizosphere in three soil types.

Onion experiment (Milestone 4.3)

As a new fungal isolate, *Trichoderma viride* S17a was used for the onion field trials, the survival of this microorganism on onion roots and in rhizosphere soil was investigated in this experiment. Unfortunately, *T. viride* S17a could not be recovered and identified on the plates used for assessment. This may be due to a large background population of other fungi being present on the plates, or possibly because the numbers on the seed initially were too low. Consequently, no data on the survival of this fungal isolate on onion roots and in rhizosphere soil are available.

Conclusions:

Carrot

- The larger volume of material used in this experiment allowed for improved recovery of the microorganisms from roots and rhizosphere soil (compared to the first experiment described in the 2nd Annual report).
- Bacteria generally declined in number, but *P. fluorescens* CHA0 was recoverable from three soil types at over 3 $\log_{10}$ cfu g^{-1} after 8 weeks
- *Clonostachys rosea* IK726 increased slightly in number in three soil types, both on the root and in the rhizosphere soil, and showed good survival
- *Trichoderma harzianum* T22 increased slightly in number in two soil types, and showed good survival

Onion

- No survival data are available for *T. viride* S17a on onion roots and in rhizosphere soil as this fungus could not be recovered or identified on the *Trichoderma* selective medium. This may be due to low numbers on the seed initially.

Objective 05: Characterise the long-term shelf life (up to 1 year) of seed-applied microorganisms under commercially-representative storage conditions. (Milestone 5.2) Ongoing

Introduction

Although it has been demonstrated previously that beneficial microorganisms can be applied successfully to horticultural seed during priming (Wright et al, 2003), no long-term survival studies have been undertaken to determine subsequent shelf-life of the BCAs under typical commercial storage conditions. Work was undertaken in Objective 05 to monitor the long-term survival of the selected beneficial BCAs on stored seed following priming inoculation. This work is ongoing.

Materials and methods

The new fungal isolate *Trichoderma viride* S17a was applied to both onion and carrot seed during the steeping priming process (Germain's Technology Group), and *T. harzianum* T22 was applied to carrot seed only, at a higher rate than was used in previous studies (2nd Annual report).

Fungal inoculum preparation

Fungal isolates supplied by Warwick HRI were cultured on potato dextrose agar at 20 ˚C. Following profuse sporulation the spores were harvested by adding SDW to the solid cultures and gently scraping off the fungal growth. The resulting fungal suspension was filtered through a double layer of sterile Whatman lens tissue. Following serial dilution the spore concentration was determined by haemocytometer counts. The numbers of cfu were determined by spiral plating onto potato dextrose agar.

Steeping priming and BCA reisolation

The process of steeping priming is similar to drum priming in that it also consists of the three main phases of hydration, incubation and drying back.

Reisolation of the BCAs was carried out after drying and again during subsequent storage at 15°C, at 4, 10, 20, 40, 60 and 90 days.

Three replicate 0.5 g samples were spun at high power on a vortex mixer for 3 x 1 minute in 4.5 ml of SDW. A dilution series was prepared in SDW and selected dilutions were spiral plated onto potato dextrose agar containing 30 μg ml^{-1} chlortetracycline. The numbers of colonies were expressed as cfu g^{-1} dry weight seed following logarithmic transformation.

Results and discussion

Trichoderma viride S17a was successfully applied to onion seed during priming, at a rate of over 7 $\log_{10}$ cfu g^{-1} seed (Figure 5.1). Samples had been taken up to 20 days storage at the time of writing this report, and at this time *T. viride* S17a was surviving well, with numbers still being recovered at over 7 $\log_{10}$ cfu g^{-1} seed. This isolate was also successfully applied to carrot seed. Here numbers decreased by less than 1 $\log_{10}$ cfu g^{-1} seed from the initial dose, remaining above 7 $\log_{10}$ cfu g^{-1} seed after 90 days of storage (Figure 5.2).

A repeat study of the shelf-life of *T. harzianum* T22 on carrot was undertaken, as the earlier study showed the numbers of this isolate decreased over the storage time (2nd Annual report). However, in this earlier work, the initial numbers on the seed were less than 6 $\log_{10}$ cfu g^{-1} seed, which may have contributed to the poor long-term survival. In the current study, the initial numbers were over 7 $\log_{10}$ cfu g^{-1} seed, and after 90 days storage the numbers were still over 6 $\log_{10}$ cfu g^{-1} seed (Figure 5.3). This is a much better survival time than the previous study, where numbers had dropped below the target of 5 $\log_{10}$ cfu g^{-1} seed by 90 days (2nd Annual report). For this isolate on carrot, a higher initial inoculum improved the shelf-life.

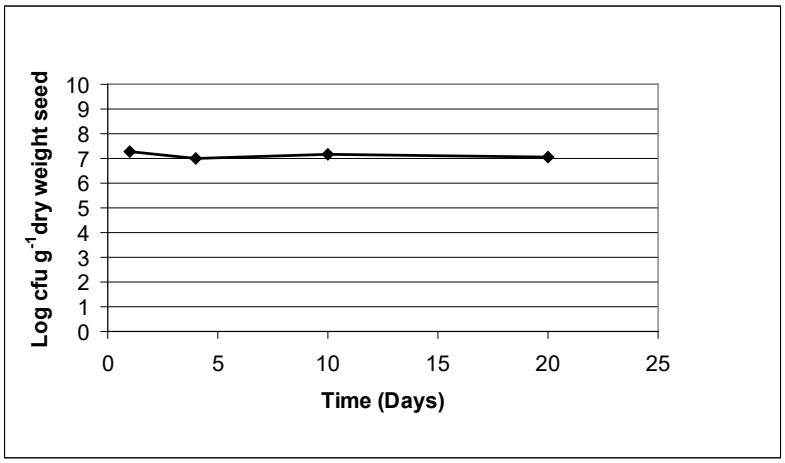


Figure 5.1: Shelf-life study of *Trichoderma viride* S17a on onion seed.

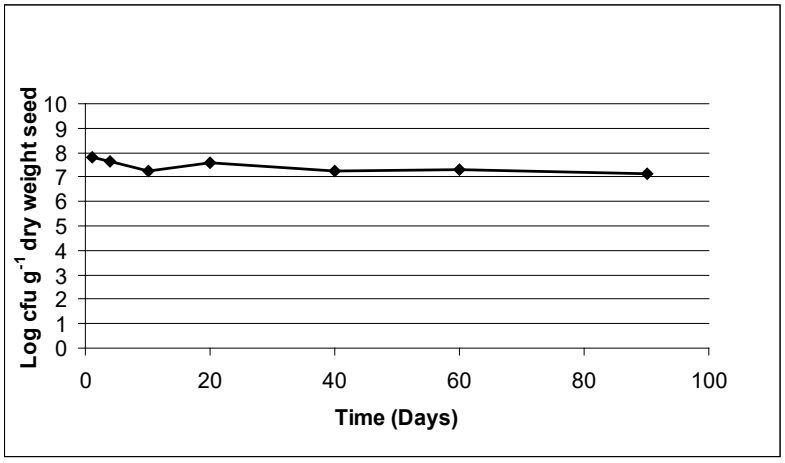


Figure 5.2: Shelf-life study of *Trichoderma viride* S17a on carrot seed.

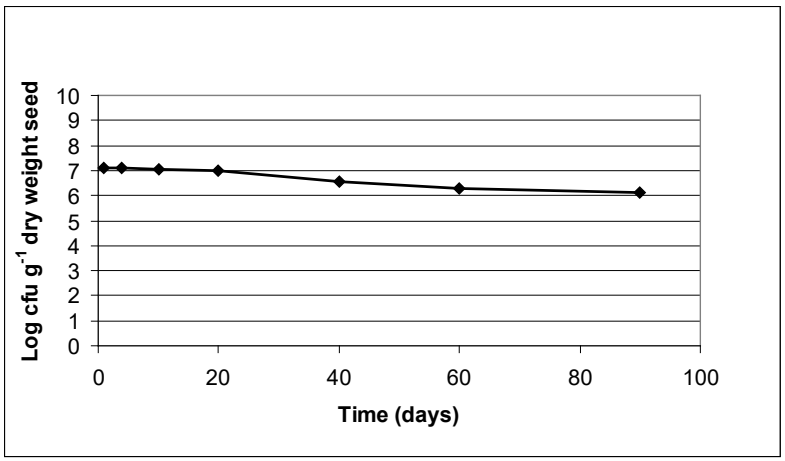


Figure 5.3: Shelf-life study of *Trichoderma harzianum* T22 on carrot seed.

Conclusions:

- *Trichoderma viride* S17a was successfully applied to carrot and onion seed during steeping priming for the first time.
- *Trichoderma viride* S17a survived above 7 $\log_{10}$ cfu g^{-1} seed for 20 days on onion seed and 90 days on carrot seed.
- A higher initial application (7 $\log_{10}$ cfu g^{-1} seed) of *Trichoderma harzianum* T22 to carrot seed resulted in an extended shelf-life compared to earlier work, with recovery above 6 $\log_{10}$ cfu g^{-1} seed by 90 days.

Objective 06: Examine the ability to co-apply selected microorganism combinations (Milestone 6.1; S6.1) Ongoing

Work for this objective is confidential (see Annex 1).

Objective 07: Technology transfer and exploitation planning (Milestones S7.5, S7.6, S7.7)

Poster display

- BCGA event, 6th October 2005
- UK Carrot and Onion Conference, 23rd-24th November 2005

Field trials

- Highlighted at Growers and Gardeners event at Warwick HRI, June 2006
- Grower demonstration planned for Elsoms Seeds field site

Presentation

- Oral presentation given at IOBC conference, Spa, Belgium 6th - 10th September 2006. “Application of beneficial microorganisms to seed during priming to improve crop health and establishment”. Paper to be published in meeting proceedings.

References

Whipps, J. M. (2001) Microbial interactions and biocontrol in the rhizosphere*, Journal of Experimental Botany,* **52,** 487 - 511.

Wright, B., Rowse, H. R. and Whipps, J. M. (2003) Application of beneficial microorganisms to seeds during drum priming*, Biocontrol Science and Technology*.

Appendices

Appendix 3.1: Effects of soil type, seed treatment and pesticide application on emergence of carrot seedlings (Milestone 3.2) (continued)

Soil type	Treatment	Pesticide	Mean emergence time (days)	Time to 50% emergence (days)	Time to 80% emergence (days)	Final percent emergence
Light sandy loam	Primed control	+	6.2	5.9	7.4	82.6 (65.4)
Light sandy loam	Primed CHA0	+	5.5	5.3	6.8	81.9 (64.8)
Light sandy loam	Primed IK726	+	5.7	5.4	6.8	79.5 (63.1)
Light sandy loam	Primed MA342	+	6.3	5.6	8.1	77.6 (61.7)
Light sandy loam	Primed T22	+	6.8	6.7	8.1	81.8 (64.7)
Light sandy loam	Unprimed control	+	7.9	7.6	8.9	77.4 (61.6)
Light sandy loam	Primed control	-	5.8	5.5	7.0	80.9 (64.1)
Light sandy loam	Primed CHA0	-	5.8	5.3	6.8	72.5 (58.4)
Light sandy loam	Primed IK726	-	5.4	5.2	6.2	74.1 (59.4)
Light sandy loam	Primed MA342	-	5.9	5.8	7.0	74.1 (59.4)

Light sandy loam	Primed T22	-	6.9	6.6	8.4	79.5 (63.1)
Light sandy loam	Unprimed control	-	7.8	7.4	8.9	59.5 (50.5)
LSD (0.05, d.f. = 105)			0.90	0.80	1.23	(9.36)

Appendix 3.1 continued: Effects of soil type, seed treatment and pesticide application on emergence of carrot seedlings (Milestone 3.2) (continued)

Soil type	Treatment	Pesticide	Mean emergence time (days)	Time to 50% emergence (days)	Time to 80% emergence (days)	Final percent emergence
Peat	Primed control	+	7.0	6.7	8.1	80.0 (63.4)
Peat	Primed CHA0	+	7.6	7.3	9.7	79.3 (62.9)
Peat	Primed IK726	+	6.0	5.7	7.5	83.9 (66.3)
Peat	Primed MA342	+	7.0	6.7	8.5	83.7 (66.2)
Peat	Primed T22	+	7.4	7.1	8.7	86.3 (68.3)
Peat	Unprimed control	+	9.5	8.9	10.4	89.2 (70.8)
Peat	Primed control	-	7.1	7.0	8.5	80.8 (64.0)

Peat	Primed CHA0	-	6.8	6.4	8.3	78.9 (63.3)
Peat	Primed IK726	-	6.4	6.0	7.5	72.4 (58.3)
Peat	Primed MA342	-	7.0	6.8	8.2	75.3 (60.2)
Peat	Primed T22	-	8.1	7.5	9.6	70.4 (57.0)
Peat	Unprimed control	-	9.0	8.6	10.2	77.5 (61.7)
LSD (0.05, d.f. = 105)			0.90	0.80	1.23	(9.36)

Appendix 3.1 continued: Effects of soil type, seed treatment and pesticide application on emergence of carrot seedlings (Milestone 3.2)

Soil type	Treatment	Pesticide	Mean emergence time (days)	Time to 50% emergence (days)	Time to 80% emergence (days)	Final percent emergence
Sandy clay loam	Primed control	+	5.5	5.3	6.4	93.1 (74.7)
Sandy clay loam	Primed CHA0	+	5.4	5.3	6.4	86.1 (68.1)
Sandy clay loam	Primed IK726	+	5.3	5.2	6.2	85.6 (67.7)
Sandy clay loam	Primed MA342	+	5.6	5.3	6.4	83.7 (66.2)
Sandy clay loam	Primed T22	+	6.0	6.2	7.1	93.0 (74.6)

Sandy clay loam	Unprimed control	+	7.5	7.3	8.6	83.4 (66.0)
Sandy clay loam	Primed control	-	5.9	5.8	6.9	89.2 (70.8)
Sandy clay loam	Primed CHA0	-	5.5	5.8	6.5	85.7 (67.8)
Sandy clay loam	Primed IK726	-	5.4	5.1	6.1	81.5 (64.5)
Sandy clay loam	Primed MA342	-	5.7	5.4	6.6	94.9 (77.0)
Sandy clay loam	Primed T22	-	6.1	6.0	7.0	81.7 (64.7)
Sandy clay loam	Unprimed control	-	7.2	7.0	8.2	90.2 (71.7)
LSD (0.05, d.f. = 105)			0.90	0.80	1.23	(9.36)

Appendix 3.2: Overall effect of soil type on emergence of microorganism-primed carrot seedlings (Milestone 3.2)

Soil type	Mean emergence time (days)	Time to 50% emergence (days)	Time to 80% emergence (days)	Final percent emergence
Light sandy loam	6.3	6.0	7.5	77.0 (61.4)
Peat	7.4	7.1	8.8	80.2 (63.6)
Sandy clay loam	5.9	5.8	6.9	87.7 (69.5)
LSD (0.05, d.f. = 105)	0.26	0.23	0.36	(2.70)

Appendix 3.3: Overall effect of seed treatment on emergence of microorganism-primed carrot seedlings (Milestone 3.2)

Treatment	Mean emergence time (days)	Time to 50% emergence (days)	Time to 80% emergence (days)	Final percent emergence
Primed control	6.2	6.0	7.4	84.8 (67.1)
Primed *P. fluorescens* CHA0	6.1	5.9	7.4	81.1 (64.2)
Primed *C. rosea* IK726	5.7	5.4	6.7	79.7 (63.2)
Primed *P. chlororaphis* MA342	6.2	5.9	7.5	82.3 (65.1)
Primed *T. harzianum* T22	6.9	6.7	8.2	82.7 (65.4)
Unprimed control	8.2	7.8	9.2	80.4 (63.7)
LSD (0.05, d.f. = 105)	0.37	0.33	0.50	(3.82)

Appendix 3.4: Overall effect of pesticide application on emergence of microorganism-primed carrot seedlings (Milestone 3.2)

Treatment	Mean emergence time (days)	Time to 50% emergence (days)	Time to 80% emergence (days)	Final percent emergence
Pesticide	6.6	6.3	7.8	84.1 (66.5)
No pesticide	6.5	6.3	7.7	79.5 (63.1)
LSD (0.05, d.f. = 105)	0.21	0.19	0.29	(2.21)

Appendix 3.5: Effects of soil type, seed treatment and pesticide application on emergence of onion seedlings (Milestone 3.3) (continued)

Soil type	Treatment	Pesticide	Mean emergence time (days)	Time to 50% emergence (days)	Time to 80% emergence (days)	Final percent emergence
Light sandy loam	Primed control	+	21.3	20.0	26.1	67.9 (55.5)
Light sandy loam	Primed S17a	+	20.6	19.7	26.1	64.9 (53.7)

Light sandy loam	Primed T22	+	20.3	20.2	24.4	58.7 (50.0
Light sandy loam	Unprimed control	+	22.1	22.3	26.6	55.4 (48.1)
Light sandy loam	Primed control	-	18.8	18.9	22.8	43.5 (41.3)
Light sandy loam	Primed S17a	-	20.3	20.0	25.7	51.0 (45.6)
Light sandy loam	Primed T22	-	19.0	18.4	23.9	41.5 (40.1)
Light sandy loam	Unprimed control	-	19.8	20.0	26.7	43.5 (41.3)
LSD (0.05, d.f. = 69)			2.77	3.47	3.77	(10.48)

Appendix 3.5 continued: Effects of soil type, seed treatment and pesticide application on emergence of onion seedlings (Milestone 3.3) (continued)

Soil type	Treatment	Pesticide	Mean emergence time (days)	Time to 50% emergence (days)	Time to 80% emergence (days)	Final percent emergence
Peat	Primed control	+	21.4	21.2	25.5	23.7 (29.1)
Peat	Primed S17a	+	19.5	20.3	22.3	23.1 (28.8)
Peat	Primed T22	+	20.9	20.5	26.0	33.2 (35.2)
Peat	Unprimed control	+	19.9	19.9	23.5	22.7 (28.4)
Peat	Primed control	-	19.8	19.7	23.6	38.3 (38.3)
Peat	Primed S17a	-	20.1	19.4	23.9	42.6 (40.8)
Peat	Primed T22	-	18.3	19.0	21.5	26.8 (31.2)
Peat	Unprimed control	-	18.2	18.1	21.6	38.4 (38.3)
LSD (0.05, d.f. = 69)			2.77	3.47	3.77	(10.48)

Appendix 3.5 continued: Effects of soil type, seed treatment and pesticide application on emergence of onion seedlings (Milestone 3.3)

Soil type	Treatment	Pesticide	Mean emergence time (days)	Time to 50% emergence (days)	Time to 80% emergence (days)	Final percent emergence
Sandy clay loam	Primed control	+	20.6	21.0	24.2	33.3 (35.3)
Sandy clay loam	Primed S17a	+	20.0	20.1	24.8	54.3 (47.5)
Sandy clay loam	Primed T22	+	20.6	21.3	24.9	56.4 (48.7)
Sandy clay loam	Unprimed control	+	22.1	21.8	26.1	47.9 (43.8)
Sandy clay loam	Primed control	-	19.8	19.1	24.7	56.3 (48.6)
Sandy clay loam	Primed S17a	-	17.7	16.2	22.8	50.1 (45.1)
Sandy clay loam	Primed T22	-	21.5	22.1	24.8	45.8 (42.6)
Sandy clay loam	Unprimed control	-	20.4	19.7	24.9	46.9 (43.2)
LSD (0.05, d.f. = 69)			2.77	3.47	3.77	(10.48)

Appendix 3.6: Overall effect of soil type on emergence of microorganism-primed onion seedlings (Milestone 3.3)

Soil type	Mean emergence time (days)	Time to 50% emergence (days)	Time to 80% emergence (days)	Final percent emergence
Light sandy loam	20.3	19.9	24.9	53.4 (46.9)
Peat	19.8	19.8	23.5	30.9 (33.7)
Sandy clay loam	20.3	20.2	24.6	48.9 (44.3)
LSD (0.05, d.f. = 69)	0.98	1.23	1.34	(3.70)

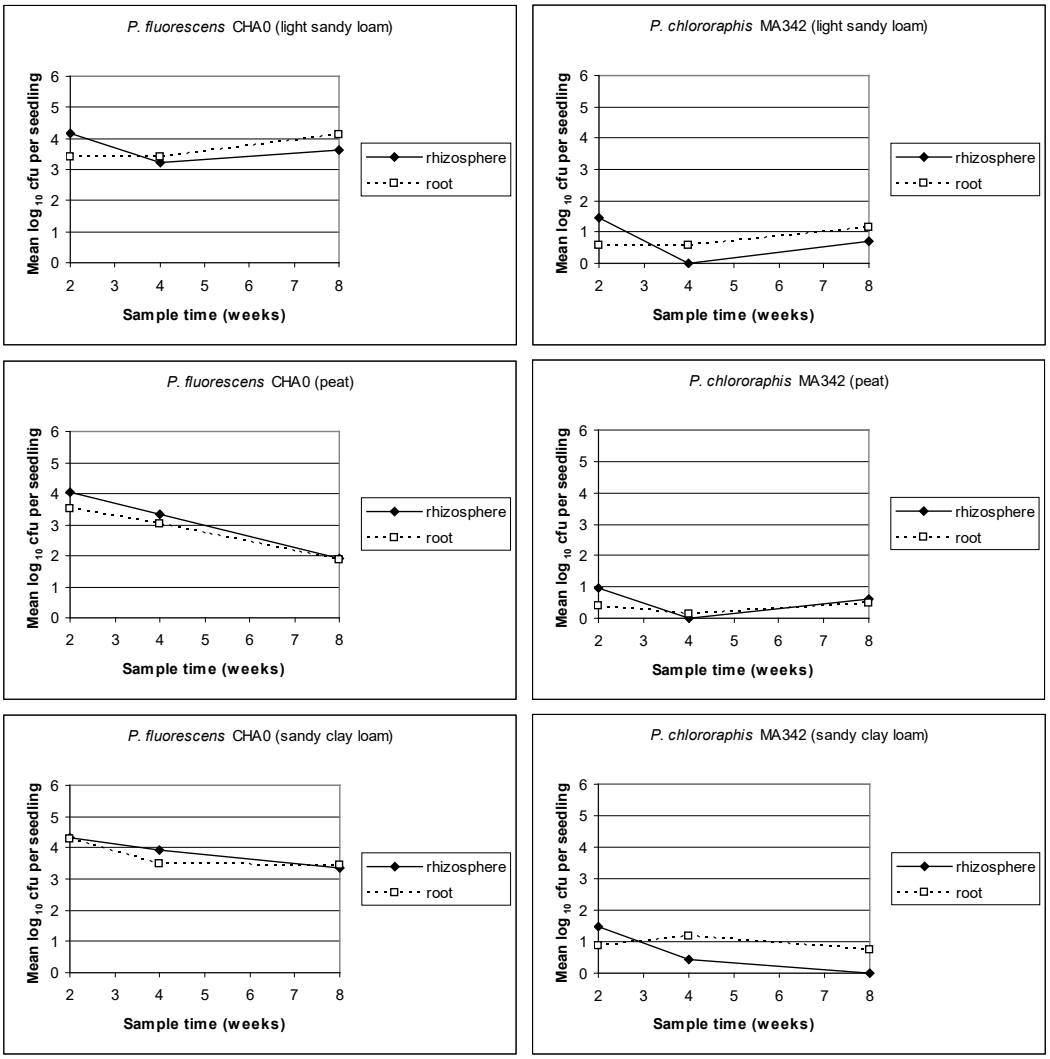
Appendix 3.7: Overall effect of seed treatment on emergence of microorganism-primed onion seedlings (Milestone 3.3)

Treatment	Mean emergence time (days)	Time to 50% emergence (days)	Time to 80% emergence (days)	Final percent emergence
Primed control	20.4	20.0	24.5	43.6 (41.3)
Primed *T. viride* S17a	19.7	19.3	24.3	48.5 (43.6)
Primed *T. harzianum* T22	20.1	20.2	24.2	43.5 (41.3)
Unprimed control	20.4	20.3	24.4	42.2 (40.5)
LSD (0.05, d.f. = 69)	1.13	1.41	1.54	(4.28)

Appendix 3.8: Overall effect of pesticide application on emergence of microorganism-primed onion seedlings (Milestone 3.3)

Treatment	Mean emergence time (days)	Time to 50% emergence (days)	Time to 80% emergence (days)	Final percent emergence
Pesticide	20.8	20.7	25.0	44.8 (42.0)
No pesticide	19.5	19.2	23.6	43.6 (41.4)
LSD (0.05, d.f. = 69)	0.80	1.00	1.09	(3.02)

Appendix 4.1: Survival of microorganisms on carrot roots and in rhizosphere soil following application to seed during primed (expressed per seedling) (continued overleaf) (Milestone 4.2).



Appendix 4.1 continued: Survival of microorganisms on carrot roots and in rhizosphere soil following application to seed during primed (expressed per seedling) (Milestone 4.2)