# Improved crop health and establishment using beneficial microorganisms

Horticulture LINK Project

CSA 6388/HL 0167 LFV

Final report

February 2007

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| Project title: | Improved crop health and establishment using beneficial microorganisms |
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**HortLINK Consortium Members:**

Elsoms Seeds Ltd

GTG UK

Horticultural Development Council

Warwick HRI

**Government sponsor:**

Department for Environment, Food and Rural Affairs

**Contents**

## Grower Summary

**Improved crop health and establishment using beneficial microorganisms**
HortLINK project CSA 6388/HL 0167 LFV

**Headline**

- Priming improves emergence of onion and carrot seed.
- Beneficial microorganisms can be successfully applied to carrot and onion seed during priming. This is the first demonstration of application of microorganisms to onion seed during a commercially relevant priming procedure, as well as the first demonstration that combinations of bacteria and fungi can be applied successfully to carrot and onion seed simultaneously in this process.
- Microorganisms applied to onion and carrot seed during priming can remain viable above a target rate of 5 $\log_{10}$ cfu $g^{-1}$ seed during storage for approximately 3-6 months, depending on subsequent seed treatment or the addition of pesticides.
- Improved emergence was achieved in some instances in glasshouse experiments with the application of single beneficial microorganisms to carrot or onion seed during priming, and with a combination of two microorganisms applied simultaneously to carrot seed during priming.
- Microorganisms applied during priming to carrot and onion seed can survive on the developing root system of the plant, and fungal isolates in particular can increase in number.
- Field trials showed pesticide application consistently improved emergence of carrots and onions and increased the yield, although no consistent effects of microorganism application were seen.

**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**

*1) Application of single microorganisms to seed*

The beneficial fungi *Clonostachys rosea* IK726, *Trichoderma harzianum* T22 and *T. viride* S17a, and the beneficial bacteria *Pseudomonas fluorescens* CHA0 and *P. chlororaphis* MA342 can all be successfully applied to carrot and onion seed during drum priming, achieving a target of 5 $\log_{10}$ cfu $g^{-1}$ dry seed.

In particular, on carrot seed all the beneficial microorganisms survived and proliferated following application during drum priming, whereas on onion seed only the bacterial isolates proliferated. On onion seed, the fungal isolates survived the priming process, but did not proliferate overall and a higher initial inoculum had to be added to the seed to achieve the end target of 5 $\log_{10}$ cfu $g^{-1}$ dry seed. Another fungal isolate, *Trichoderma viride* L4, did not survive well on onion seed, and was not recovered above the target rate at the end of priming. This isolate did survive and proliferate on the carrot seed, however.

Pesticide compatibility tests on agar plates showed mixed results, with all microorganisms showing compatibility or partial compatibility with Apron 35 and Wakil XL. Although the bacterial isolates were also partially compatible with the fungicide HY-TL, none of the fungal isolates grew on HY-TL amended agar. Also, whereas *T. harzianum* T22, *C. rosea* IK726, *P. chlororaphis* MA342 and *P. fluorescens* CHA0 were compatible with Force ST, *T. viride* S17a and *T. viride* L4 did not grow on Force ST amended agar.

**Main conclusion:**

- ***Beneficial microorganisms can be successfully applied to carrot and onion seed during drum priming***
- ***First demonstration of successful application of microorganisms to onion seed using a commercially relevant procedure***

*2) Field trials - emergence and growth of onions and carrots following application of single microorganisms to seed*

Field trials were conducted over three consecutive years at various sites in the UK

(Table 1) to assess the effects on emergence and growth of onion and carrot seed primed with selected beneficial microorganisms. All beneficial microorganisms were successfully applied to carrot and onion seed during commercial drum priming and the target rate of at least 5 $\log_{10}$ cfu $g^{-1}$ seed was achieved in all years. Seed treatments are shown in Table 2, with all 12 treatments being used at the conventional sites (seed-applied pesticides allowed) and only the first 6 treatments being used at the organic sites (no pesticides). At each site, emergence counts were made at approximately 3 and 6 weeks post-planting, and growth was monitored throughout the season. At harvest, onion bulbs were counted, weighed and graded according to various size categories. Carrots were also counted and weighed, and assessed for cavity spot and possible nematode damage (fanged roots).

*Onion trials*

No consistent effects of microorganism treatment were seen for the onion trials across all three years. However, in the first two years, *Pseudomonas fluorescens* CHA0 without pesticide improved onion emergence over the primed control without pesticide at the organic sites. In Year 3 (2006) a new onion seed treatment was tried (*Trichoderma viride* S17a) and was found to increase the number and weight of onion bulbs at harvest at the grower's trial.

Across all three years, pesticide application improved emergence at the Warwick HRI onion trials and also consistently increased the weight of onion bulbs at the conventional growers' trials. In two years, pesticide application also increased the number of onion bulbs at harvest at the Warwick HRI trials.

**Table 1:** Locations of the carrot and onion field trials for Years 1-3.

| Crop | Year 1 (2004) | Year 2 (2005) | Year 3 (2006) |
|---|---|---|---|
| Conventional carrot | Warwick HRI, Wellesbourne<br>T. Hammond & Sons, South Notts | Warwick HRI, Wellesbourne<br>Marshall Farms, Papplewick | Warwick HRI, Wellesbourne<br>Clumber Farms, Worksop |
| Organic carrot | Elsoms Seeds Ltd., Spalding | Farcet Farms, Yaxley Fen | Elsoms Seeds Ltd., Spalding |
| Conventional onion | Warwick HRI, Wellesbourne<br>Elveden Farms Ltd., Thetford | Warwick HRI, Wellesbourne<br>Farcet Farms, Yaxley Fen | Warwick HRI, Wellesbourne<br>Elveden Farms Ltd., Thetford |
| Organic onion | Elsoms Seeds Ltd., Spalding | Elsoms Seeds Ltd., Spalding | Elsoms Seeds Ltd., Spalding |

**Table 2:** Seed treatments produced for the field trials

| Treatment number | Description | Priming | Fungicide | | Insecticide |
|---|---|---|---|---|---|
| | | | Carrot[a] | Onion[b] | (Force ST) |
| 1 | Primed control | ✓ | × | × | × |
| 2 | Primed *P. fluorescens* CHA0 | ✓ | × | × | × |
| 3[c] | Primed *C. rosea* IK726 | ✓ | × | × | × |
| | Primed *T. viride* S17a (onion Year 3 only) | ✓ | - | × | × |
| 4 | Primed *P. chlororaphis* MA342 | ✓ | × | × | × |
| 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[c] | Primed *C. rosea* IK726 + Pesticide | ✓ | Wakil XL | HY-TL | ✓ |
| | Primed *T. viride* S17a (onion Year 3 only) | ✓ | - | HY-TL | ✓ |
| 10 | Primed *P. chlororaphis* MA342 + Pesticide | ✓ | Wakil XL | 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. HY-TL was used in all three years. Apron 35 was also included in Year 1 only.

[c] Treatments 3 and 9 were *Clonostachys rosea* IK726 for the carrots in all three years, but on the onions in Year 1 and 2 only. *T. viride* S17a was substituted on the onion seed for Year 3 only.

*Carrot trials*

No consistent effects of microorganism treatment were seen for the carrot trials across all three years, although it was seen in the first two years that *P. fluorescens* CHA0 without pesticide and *C. rosea* IK726 without pesticide decreased the number of carrots compared to the primed control without pesticide at Warwick HRI trials.

Pesticide application consistently improved emergence at the Warwick HRI carrot trials in three years, and therefore increased the total number of carrots at harvest. Pesticide application also decreased the percentage of roots with possible nematode damage (fanged) and the mean weight of roots with cavity spot or nematode damage for two years.

**Main conclusions:**

- ***Pesticide application consistently improved emergence and yield of onions and carrots.***
- ***No consistent effects of microorganism application were seen in the field trials.***

*3) Glasshouse experiments – emergence and growth of onions and carrots following application of single microorganisms to seed*

Glasshouse experiments were conducted with the same 12 seed treatments as used in the field trials to assess the emergence and growth of onions and carrots in different soil types. Emergence was monitored until no further increase in number was seen, and after 8 weeks the seedlings were harvested and assessed for their fresh and dry weight. Three experiments with microorganism-treated onion seed showed varying results, largely due to the presence of high numbers of indigenous seed-borne fungi in the second experiment and generally poor emergence for all seed in the third experiment. In contrast, two experiments with the microorganism-treated carrot seed showed consistent results throughout.

*Onion*

In one glasshouse experiment, the application of beneficial microorganisms to seed improved the emergence compared to the primed control. Here, a population of indigenous seed-borne microorganisms was present on the seed and these increased in number during the priming process, resulting in a negative effect on emergence for the control seedlings. However, the application of beneficial

microorganisms to this seed lot during priming significantly improved emergence of the seed, resulting in better establishment of the seedlings compared to the primed and unprimed control. Although the microorganism effects were not consistent across the three experiments, it was consistently found that pesticide application decreased the mean fresh weight of onion seedlings at 8 weeks.

*Carrot*

Results from two experiments examining the emergence and growth of carrot seed primed with microorganisms were consistent and showed that all primed treatments emerged faster than the unprimed control. Also, the fungal isolate *C. rosea* IK726 consistently improved emergence over the primed control. Soil type was also found to affect the emergence of the carrot seeds, with a sandy clay loam soil resulting in the fastest emergence time. However, the greatest mean fresh weight of carrot seedlings was consistently found in peat soil.

**Main conclusions:**

- ***Beneficial microorganisms applied during priming can improve emergence of onion seed where indigenous (potentially deleterious) seed-borne microorganisms are present in high numbers***
- ***Priming consistently improves emergence of carrot seed.***
- **Clonostachys rosea *IK726 can improve the emergence time of carrot seed in glasshouse experiments.***

*4) Glasshouse experiment – onion pathogen bioassay following application of single microorganisms to seed*

A glasshouse experiment was set up to investigate the effects of onion seed primed with beneficial microorganisms on the emergence and growth of seedlings in soil infested with sclerotia of the pathogen *Sclerotium cepivorum* (causal agent of *Allium* white rot). In this experiment it was seen that the primed control had a higher emergence than the unprimed control, illustrating the benefits of priming. However, the application of microorganisms to seed did not improve emergence compared to the primed control, and there was no reduction in the percent disease compared to the primed control.

**Main conclusion:**

- ***The microorganisms tested did not reduce the percent* Allium *white rot***

***compared to the primed control***

*5) Glasshouse experiment – carrot pathogen bioassay following application of single microorganisms to seed*

A glasshouse experiment was set up to investigate the effects of carrot seed primed with beneficial microorganisms on the emergence and growth of seedlings in soil infested with *Pythium ultimum* (causal agent of damping off). Here it was found that the pathogen inoculum did not cause a reduction in carrot emergence (no damping-off recorded), and no effects of microorganism treatment were seen.

**Main conclusion:**

- ***The pathogen inoculum used in this work did not result in disease***

*6) Glasshouse experiments – survival of single microorganisms on onion and carrot roots and in rhizosphere soil following application to seed*

Glasshouse experiments were conducted to assess the survival and proliferation of seed-applied microorganisms on the roots and in the rhizosphere soil of onions and carrots. Microorganisms were applied to seed during priming, and the seed was subsequently planted in three soil types. Seedlings were harvested at 2, 4 and 8 weeks post-planting and the adhering soil was washed off and plated onto selective agar media for the re-isolation of the beneficial microorganisms (survival in rhizosphere soil). The clean roots were also ground and plated onto selective media (survival on roots). The first series of experiments showed some trends in the survival of the different microorganisms, but the volume of plant material used was small. Consequently, a larger volume of plant material was used in the second series of experiments, and this was found to give consistent results regarding the survival pattern of the seed-applied microorganisms on roots and in soil.

*Onion*

Bacterial survival on onion roots and in rhizosphere soil was variable, but numbers generally decreased with time. *Clonostachys rosea* IK726 survived well and increased in number on onion roots and in rhizosphere soil in three soil types, and *T. harzianum* T22 was recovered at all times from roots and rhizosphere soil if a high enough inoculum dose was used on the seed initially. 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.

*Carrot*

On carrot, 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, as did *T. harzianum* T22 in two soils.

**Main conclusions:**

- ***All seed-applied microorganisms could be recovered from onion and carrot roots and from rhizosphere soil in three soil types***
- ***Bacteria generally declined in number, whereas the fungi remained constant or increased in number over time***

*7) Shelf-life studies following application of single microorganisms to seed*

Shelf-life studies were conducted to assess the storage potential of onion and carrot seed primed with microorganisms, and the viability of the microorganisms over time. Beneficial microorganisms were applied to seed during steeping priming, following which sub-samples of the seed lot were pelletted (onion) or film-coated (carrot) with and without the inclusion of pesticides. Seed batches were stored for up to a year, with samples being assessed at various time points to determine the microorganisms' survival. All microorganisms were successfully applied to onion and carrot seed during steeping priming. It was found that pelletting onion seeds reduced the survival of all microorganisms in the long term, possibly due to the continued wetting and drying cycle involved in the pelletting process having a negative effect on the microorganisms. Also, the application of pesticide to the onion pellet significantly reduced the survival of the fungal isolates over time, particularly *C. rosea* IK726. However, organic growers could still use seed without the pesticide addition. On carrot, film-coating and the application of pesticides did not significantly affect the survival of microorganisms, and it was also seen with *T. harzianum* T22 that a higher initial inoculum on the seed allowed the viability of the microorganism to remain above the target of 5 $\log_{10}$ cfu $g^{-1}$ seed for a longer period. Generally speaking, the viability of microorganisms was good during storage, remaining viable over 5 $\log_{10}$ cfu $g^{-1}$ seed for up to a year in some cases.

**Main conclusions:**

- ***Beneficial microorganisms can be successfully applied to carrot and onion seed during steeping priming and can survive above the target level of 5 $log_{10}$ cfu $g^{-1}$ seed for at least 3-6 months***
- ***Pelletting onion seed reduces the long-term viability of seed-applied microorganisms***
- ***Inclusion of pesticides in the onion pellet reduces long-term viability of seed-applied fungi***
- ***Film-coating carrot seed and pesticide application do not affect the long-term viability of seed-applied microorganisms***

*8) Co-application of two microorganisms to seed*

Following on from the successful application of single microorganisms to carrot and onion seed, work was done to simultaneously co-apply two beneficial microorganisms onto the same seed lot. It was found that two microorganisms could be co-applied to the same seed, and the individual microorganisms followed a similar survival pattern on the seed during the priming process as when they had been applied singly. Combinations included: *C. rosea* IK726 and either *P. fluorescens* CHA0 or *P. chlororaphis* MA342; and *T. harzianum* T22 and either *P. fluorescens* CHA0 or *P. chlororaphis* MA342 (*ie* one bacterial and one fungal isolate were used in each combination). All microorganisms were successfully applied in combination to carrot and onion seed above the target of 5$log_{10}$ cfu $g^{-1}$ dry seed for each microorganism.

**Main conclusion:**

- ***Beneficial microorganisms can be applied in combination to carrot and onion seed during drum priming***
- ***This is the first demonstration that combinations of bacteria and fungi can be applied successfully to seed simultaneously in a commercially relevant procedure***

*9) Glasshouse experiments – emergence and growth of onions and carrots following co-application of two microorganisms to seed*

Onion and carrot seed that been treated with combinations of microorganisms

applied during priming were planted in a peat soil in a glasshouse experiment to determine the effects on emergence and growth. Emergence was monitored until no further increase in number was seen, and after 8 weeks the seedlings were harvested and assessed for their fresh and dry weight.

*Onion*

Overall, priming improved emergence of onion seed. The combination of *C. rosea* IK726 and *P. chlororaphis* MA342 significantly reduced the final percent emergence of onion seed compared to the primed control and the treatments where the microorganisms were applied on their own. This may have been due to a phytotoxic effect resulting from the high numbers of the bacterial isolate on the seed when applied in combination. At harvest, some of the combination treatments also had a negative effect on the mean fresh weight of onion seedlings. For example, the combination of *T. harzianum* T22 and *P. chlororaphis* MA342 resulted in a lower mean fresh weight per seedling than those of the primed control, unprimed control, or seeds treated with *T. harzianum* T22 on its own. In contrast, other combinations improved the mean fresh weight of seedlings. For example, the combination of *C. rosea* IK726 and *P. fluorescens* CHA0 resulted in a significantly greater mean fresh weight than when either of those microorganisms were applied singly to seed.

*Carrot*

Overall priming improved emergence of carrot seed, and all treatments with microorganisms applied during priming had improved emergence compared to the primed control. The combinations of *C. rosea* IK726 and either *P. chlororaphis* MA342 or *P. fluorescens* CHA0 showed a greater final percent emergence than all the other treatments. The microorganism treated seed also resulted in a reduced mean fresh weight per seedling at 8 weeks compared to the primed control, but this may be due to competition for space between these seedlings, as more seedlings emerged in these treatments.

**Main conclusion:**

- ***Combination microbial treatments gave variable effects on onion emergence and seedling fresh weight, but all microorganism treatments improved emergence on carrot***

*10) Glasshouse experiments – survival of two microorganisms on onion and carrot roots and in rhizosphere soil following co-application to seed*

Experiments were conducted to assess the survival and proliferation of seed-applied microorganisms on roots and in rhizosphere soil of onions and carrots. Microorganisms were applied singly and in combination to seed during priming and the seed was subsequently planted in peat soil. Seedlings were harvested at 2, 4 and 8 weeks post-planting and the adhering soil washed off and plated onto selective agar media for the reisolation of the beneficial microorganisms (survival in rhizosphere soil). The clean roots were also ground and plated onto selective agar media (survival on roots).

*Onion*

Single microorganism treatments followed a similar survival pattern on the roots and in rhizosphere soil as had been found previously. The bacteria declined in number over time, whereas the fungus *C. rosea* IK726 increased in number and *T. harzianum* T22 remained relatively constant. In combination, the microorganisms behaved similarly to when they had been applied singly to seed. The bacterium *P. chlororaphis* MA342 was recovered in higher numbers in combination with the fungi than when it had been applied singly, but this is probably due to a higher initial numbers on the seed in this case. The high bacterial inoculum may also have influenced the survival of the fungus *T. harzianum* T22, which was not recovered from onion roots when applied in combination with the bacteria, but was recovered in low numbers when applied singly.

*Carrot*

As with the onion, single microorganism treatments followed a similar survival pattern on the roots and in rhizosphere soil of carrot as had been found previously, with the bacteria generally declining and the fungi remaining constant or increasing in number. In combination, the same general survival pattern was also seen and all microorganisms were recovered at all sampling times.

**Main conclusion:**

- ***Microorganisms applied in combination to carrot and onion seed survived similarly in the rhizosphere and on the roots compared to when they were applied as individual treatments***

## Financial benefits

Priming carrot and onion seed improves emergence and establishment of the crop, resulting in greater yield. Use of seed-applied pesticides can also improve emergence and yield of carrots and onions. No clear financial benefit is to be gained from applying the microorganisms used in this project to carrot or onion seed in the field. However, should microorganisms be identified that provide reproducible increases in emergence, establishment and yield, suitable seed application procedures are now established to ensure rapid transfer to the industry.

## Action points for growers

- Using primed carrot or onion seed can improve crop establishment
- Using seed-applied pesticides can improve crop establishment and yield

## Progress against Milestones

**Primary Milestones**

| Year 1 (Sep 03 – Aug 04) | Description | Complete |
|---|---|---|
| 1.1 | Confirm that microorganisms can be applied to onion seed during priming (WHRI)(Dec 2003 (month 4)) | Yes |
| 2.1 | Carry out Year 1 field trials and monitor effects on seed emergence and growth (Elsoms +WHRI)(Aug 2004 (month12)) | Yes |
| 3.1 | Complete Year 1 glasshouse studies and monitor effects on seed emergence and growth (WHRI) (Aug 2004 (month12)) | Yes |
| 4.1 | Complete monitoring of microorganism survival on Year 1 field trial and glasshouse studies (GTG + WHRI) (Aug 2004 (month 12)) | Yes |
| **Year 2 (Sep 04 – Aug 05)** | | |
| 2.2 | Carry out Year 2 field trials and monitor effects on seed emergence and growth (Elsoms + WHRI)(Aug 2005 (month 24)) | Yes |
| 3.2 | Complete Year 2 glasshouse studies and monitor effects on seed emergence and growth (WHRI) (Aug 2005 (month 24)) | Yes |
| 4.2 | Complete monitoring of microorganism survival on Year 2 field trial and glasshouse studies (GTG + WHRI) (Aug 2005 (month 24)) | Yes |
| 5.1 | Complete first shelf life assessment (GTG)(Nov 2004 (month 14)) | Yes |

Continued

**Primary Milestones continued:**

| Year 3 (Sep 05 – Aug 06) | Description | Complete |
|---|---|---|
| 2.3 | Carry out Year 3 field trials and monitor effects on seed emergence and growth (Elsoms +WHRI) (Aug 2006 (month 36)) | Yes |
| 3.3 | Complete Year 3 glasshouse studies and monitor effects on seed emergence and growth (WHRI) (Aug 2006 (month 36)) | Yes |
| 4.3 | Complete monitoring of microorganism survival on Year 3 field trial and glasshouse studies (GTG + WHRI) (Aug 2006 (month 36)) | Yes |
| 6.1 | Determine effect of co-inoculation of microorganisms onto carrot and onion seed (WHRI) (Aug 2006 (month 36)) | Yes |
| **Year 4 (Sep 06 – Feb 07)** | | |
| 2.4 | Collate results of all field trials (WHRI) (Nov 06 (month 39)) | Yes |
| 5.3 | Complete third shelf life experiment (GTG) (Nov 06 (month 39)) | Yes |
| 7.1 | Complete drafts of all reports and submit to HDC and Horticulture LINK co-ordinator for comment (WHRI) (December 06 (month 40)) | Yes |
| 7.2 | Revise reports and submit final reports to HDC and Horticulture LINK Co-ordinator (WHRI) (Feb 07 (month 42)) | Yes |

Continued

**Secondary Milestones**

| Year 1 (Sep 03-Aug 04) | Description | Complete |
|---|---|---|
| S1.1 | Obtain microorganisms for test application to seed (WHRI) (continuous) | Yes |
| S1.2 | Determine pesticide compatibility of the 4 microorganisms to be applied to seed for field trials (WHRI) (Feb 04 (month 6)) | Yes |
| S3.1 | Set up initial year 1 glasshouse trial (WHRI)(March 04 (month 7)) | Yes |
| S5.1 | Initiate shelf life studies (GTG) (Oct 03 (month 2)) | Yes |
| S7.1 | Complete article for Agriculture LINK newsletter (All)(Feb 04 (month 6)) | Yes |
| S7.2 | Maintain discussions with PSD concerning registration (Elsoms + GTG)(continuous) | Yes |
| S7.3 | Carry out grower demonstration at one Elsoms Seed field site (Elsoms) (July 04 (month 11)) | Yes |
| **Year 2 (Sep 04-Aug 05)** | | |
| S1.3 | Obtain microorganisms for test for application on to seed during priming (WHRI) (continuous) | Yes |
| S1.4 | Determine pesticide compatibility of the microorganisms successfully applied to seed if different from year 1 (WHRI) (Feb 05 (month 18)) | Yes |
| S1.5 | Continue to assess novel microorganism colonisation on seeds during priming if required (HRI) (continuous) | Yes |
| S2.1 | Complete harvest and yield assessment of Year 1 field trials (Elsoms +WHRI) (Oct 04 (month 14)) | Yes |

Continued

**Secondary Milestones continued:**

| Year 2 (Sep 04-Aug 05) | Description | Complete |
|---|---|---|
| S7.4 | Maintain discussions with PSD concerning registration (Elsoms + GTG) (continuous) | Yes |
| S7.5 | Carry out grower demonstration at one Elsoms Seed field site (Elsoms) (July 05 (month 23)) | Yes |
| **Year 3 (Sep 05- Aug 06)** | | |
| S2.2 | Complete harvest and yield assessment of Year 2 field trials (Elsoms + WHRI) (Oct 05 (month 26)) | Yes |
| S5.2 | Complete second shelf life experiment (GTG) (Dec 05 (month 28)) | Not done |
| S6.1 | Initiate co-inoculation experiments (WHRI) (Sept 05 (month 25)) | Yes |
| S7.5 | Maintain discussions with PSD concerning registration (Elsoms + GTG) (continuous) | Yes |
| S7.6 | Produce a poster and attend the UK carrot and onion meeting (WHRI)(Nov 05 (month 27)) | Yes |
| S7.7 | Carry out grower demonstration at one Elsoms Seed field site (Elsoms) (July 06 (month 35)) | Yes |

Continued

**Secondary Milestones continued:**

| | | |
|---|---|---|
| **Year 4 (Sep 06 – Feb 07)** | | |
| S2.3 | Complete harvest and yield assessment of Year 3 field trials (Elsoms + WHRI) (Oct 06 (month 38)) | Yes |
| S3.2 | Collate all glasshouse growth experiment data (WHRI) (Oct 06 (month 38)) | Yes |
| S4.1 | Collate all microorganism monitoring data (WHRI)(Oct 06 (month 38)) | Yes |
| S5.3 | Collate all shelf life data (GTG)(Nov 06 (month 39)) | Yes |
| S7.8 | Maintain discussions with PSD concerning registration (Elsoms + GTG)(continuous) | Yes |
| S7.9 | Produce an article for HDC News (All) (Feb 07 (month 42)) | Yes |

# 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

## Introduction

It has previously been established that certain microorganisms can survive application to seeds during priming, and may proliferate during the priming process (Wright et al, 2003). This project investigated the application to seed of specific beneficial microorganisms, known to have biocontrol or plant growth promoting properties. The work carried out in Objective 01 determined the survival and proliferation of six selected beneficial microorganisms on onion and carrot seed. Compatibility with commercially-applied pesticides was also tested.

## Materials and methods

Six microorganisms were selected based on results from preliminary trials carried out in 2003, information in the literature, and availability of commercial products. Two bacterial isolates were chosen, namely *Pseudomonas chlororaphis* MA342 and *Pseudomonas fluorescens* CHA0, along with four fungal isolates, *Clonostachys rosea* IK726, *Trichoderma harzianum* T22, *Trichoderma viride* L4 and *Trichoderma viride* S17a.

### *Bacterial inoculum preparation*

Bacteria were available from the culture collection at Warwick HRI, and were stored at -80°C on beads in a vial containing a cryopreservative fluid. A single bead removed from the vial was plated onto nutrient agar, an incubated at 25°C. Single colonies resulted from this were inoculated into sterile nutrient broth (100ml) and incubated overnight at 26°C, in a rotary shaker set at 180 rpm. The following morning, 1ml of overnight culture was transferred to fresh nutrient broth and incubated for approximately 4 hours until $OD_{600}$ showed the numbers to be in the region of 1 x $10^7$ cfu $ml^{-1}$, determined from a previously prepared standard growth curve. Twenty millilitres of the bacterial broth was centrifuged (5000 rpm, 10 minutes), and the resulting pellet resuspended in 20 ml sterile distilled water (SDW). Based on the numbers calculated from the standard growth curve, the required

amount of bacterial suspension to give the desired inoculum rate (1 x $10^6$ cfu $g^{-1}$ dry seed) was combined with SDW to produce the liquid to be added to the drum priming system. However, as this inoculum dose was estimated from a growth curve and did not take into account any potential loss of cells in the centrifugation process, a dilution series of the bacterial suspension was also plated to verify the number of colony forming units (cfu) present. As such, the actual number of bacterial cells added to the seed batches ranged from just under 5 $\log_{10}$ cfu $g^{-1}$ dry seed to 7 $\log_{10}$ cfu $g^{-1}$ dry seed.

*Fungal inoculum preparation*

Fungi were available from the culture collection at Warwick HRI, stored in liquid nitrogen. 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 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 x $10^5$ cfu $g^{-1}$ dry seed for the carrot seed and 1 x $10^7$ cfu $g^{-1}$ dry seed for the onion seed. A higher initial inoculum was required for the onion seed as a background population of indigenous fungi was present, which prevented enumeration of the added beneficial fungi at lower levels. Preliminary priming runs also showed that the fungi decreased overall in number throughout the process and thus a higher initial inoculum was required in this case to reach the target application rate. 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°C. The initial moisture content of the carrot seed was 9.28% with a

target moisture content of 62% after 24 hours hydration. The onion seed had an initial moisture content of 8.4% and a target of 68% moisture content after 48 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 rifampicin-amended (100µg $ml^{-l}$) nutrient agar for bacteria, and PDA amended with chlortetracycline (30µg $ml^{-l}$) and Triton X-100 (2ml $l^{-l}$) for fungi. Colonies were counted after 7 days incubation at 20°C.

*Pesticide compatibility*

Pesticide dose rates were calculated so that active ingredient per litre of agar was equivalent to the active ingredient per kilogram of seed. Rates of x1, x0.1 and x10 dose were tested. Pesticides tested for compatibility with the selected microorganisms were as follows (dose rate x1): Apron 35 (fungicide) (1mg $ml^{-1}$ metalaxyl); Wakil XL (fungicide) (0.38mg $ml^{-1}$ metalaxyl, 0.22mg $ml^{-1}$ cymoxanil, 0.11mg $ml^{-1}$ fludioxonil); HY-TL (fungicide) (2.7mg $ml^{-1}$ thiram, 2.03mg $ml^{-1}$ thiabendazole); and Force ST (insecticide) (14.5mg $ml^{-1}$ tefluthrin).

The appropriate amount of pesticide was added to nutrient agar for bacterial compatibility and PDA for fungal compatibility. Control plates were also set up on unamended agar, and compatibility was assessed on the pesticide amended agar when the control plates showed sufficient growth of the microorganisms.

**Results and discussion**

All the microorganisms survived following application to carrot and onion seed during drum priming, although there were some differences between crop type and between species of microorganism. On carrot seed, all microorganisms behaved in a similar way and a representative example of the survival pattern of one bacterial and one fungal isolate is shown in Figure 1.1. All microorganisms were applied initially at approximately 5 $log_{10}$ cfu $g^{-1}$ dry seed. A decrease in numbers was found after the hydration phase, followed by an increase during the incubation phase, to reach

higher numbers than were applied initially. A small decrease in numbers for the fungal isolates was generally found after the carrot seed was dried back for storage, but the applied microorganisms still survived at over the target rate of 5 $\log_{10}$ cfu $g^{-1}$ dry seed in all cases.

On onion seed, the bacterial isolates followed a generally similar pattern to that seen on the carrot seed and a representative example is shown in Figure 1.2. However, the fungal isolates showed a different survival pattern when applied to onion seed. Here it was found that the fungi declined overall in numbers: although the isolates showed the same initial pattern of a decease in numbers following hydration, the subsequent increase during the incubation phase was not to the same extent as was seen with the carrot seed. Consequently, the numbers declined overall throughout the priming process. A representative example of the survival of a fungal isolate on onion seed is also given in Figure 1.2. To compensate for this overall decline, a higher initial inoculum of 7 $\log_{10}$ cfu $g^{-1}$ dry seed was used to achieve the target of 5 $\log_{10}$ cfu $g^{-1}$ dry seed at the end of drying back. Although this increase in initial inoculum was successful in most cases, the fungal isolate *T. viride* L4 could not be recovered at the target rate after the onion seed was dried back. Reasons for this are unclear, as another isolate, *T. viride* S17a, did reach the final target rate if the initial inoculum was high.

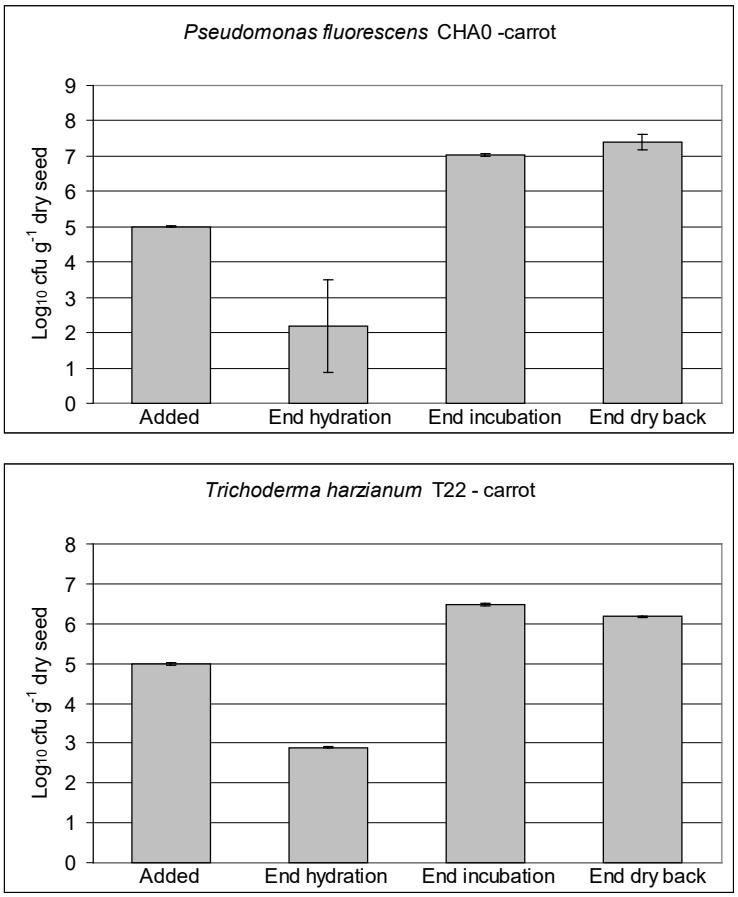


**Figure 1.1:** Examples of the survival and proliferation of selected beneficial microorganisms applied to carrot seed during drum priming. Added = initial numbers in liquid suspension applied to seed; End hydration = numbers found after all liquid cell suspension added to seed; End incubation = numbers found after seed incubated in rotating jar for 7 days; End dry back = numbers found when seed air-dried. Bars indicate standard error of the mean.

Further differences in survival of the fungal isolates on onion seed could be due to the different exudates released by this seed type compared to carrot seed during the priming process. These may have an inhibitory effect on the fungi applied during onion seed priming. Another possibility is the presence of indigenous seed-borne microorganisms on the onion seed also increasing in number during the priming process and inhibiting the applied fungi. As the bacterial isolates could be applied with equal success to both carrot and onion seed, it may be that bacteria are not affected specifically by the seed exudates of one crop type. Alternatively, it may be that the surface structure or texture of the seed varied sufficiently to provide niches better suited to survival of smaller bacterial cells than perhaps the larger sized fungi.

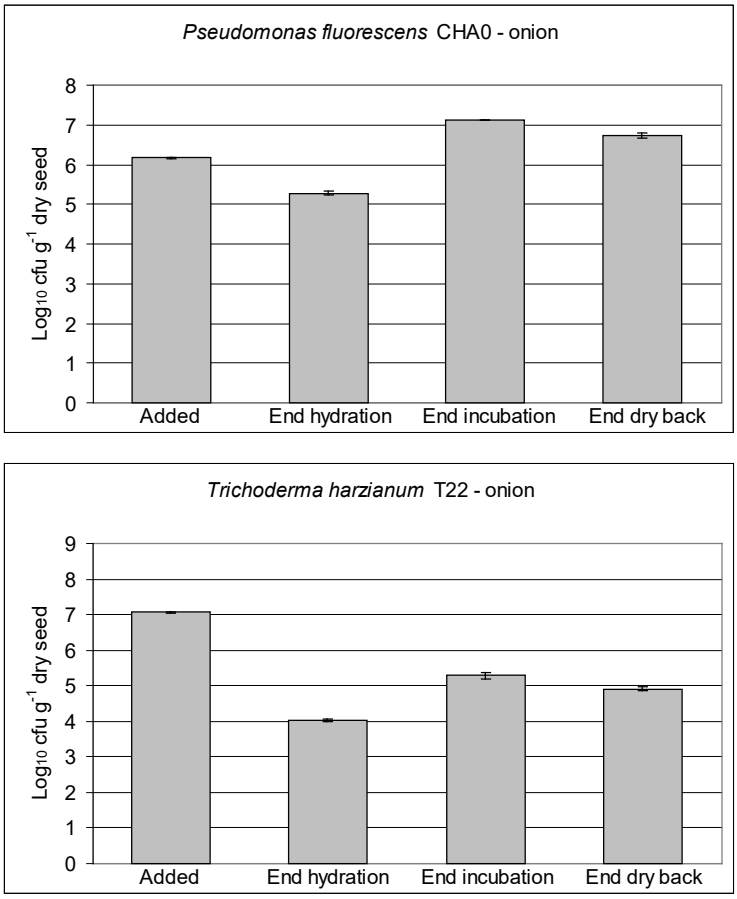


**Figure 1.2:** Examples of the survival and proliferation of selected beneficial microorganisms applied to onion seed during drum priming. Added = initial numbers in liquid suspension applied to seed; End hydration = numbers found after all liquid cell suspension added to seed; End incubation = numbers found after seed incubated in rotating jar for 7 days; End dry back = numbers found when seed air-dried. Bars indicate standard error of the mean

All the microorganisms were also assessed for their pesticide compatibility in an agar plate test (Table 1.1). The bacteria showed normal growth in a 3-way streak on all pesticide-amended agar except those amended with HY-TL, where only isolated resistant colonies developed. *Trichoderma harzianum* T22 and *C. rosea* IK726 grew normally on all pesticide-amended agar, with the exception of those amended with HY-TL, where no growth occurred. This fungicide is used for onion seeds, which are also pelleted. This may mean that if the microorganisms were applied during priming they would not come into direct contact with the fungicide in the pellet, and so such a combination treatment may still be feasible for use.

**Table 1.1:** Growth of beneficial microorganisms on pesticide-amended agar. Application rate per litre of agar equivalent to application rate per kilogram of seed (x1). Active ingredients of pesticides at x1 rate added to nutrient agar (bacteria) or potato dextrose agar (fungi) as follows: HY-TL = thiram (2.7mg $ml^{-1}$), thiabendazole (2.025mg $ml^{-1}$); Force (onion) = tefluthrin (14.5mg $ml^{-1}$); Force (carrot) = tefluthrin (18.125 mg $ml^{-1}$); Wakil = metalaxyl-M (0.3806mg $ml^{-1}$), cymoxanil (0.2175mg $ml^{-1}$), fludioxonil (0.1088mg $ml^{-1}$); Apron = metalaxyl-M (1mg $ml^{-1}$).

| **Microorganism** | **Pesticide** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | control | HY-TL x0.1 | HY-TL x1 | Force x1 (onion) | Force x1 (carrot) | Wakil x1 | Wakil x10 | Apron x0.1 | Apron x1 | Apron x10 |
| *P. fluorescens* CHA0 | + | + | ± | + | + | + | + | nt | + | + |
| *P. chlororaphis* MA342 | + | + | ± | + | + | + | + | nt | + | + |
| *T. harzianum* T22 | + | - | - | + | + | + | + | + | + | + |
| *C. rosea* IK726 | + | - | - | + | + | + | + | + | + | + |
| *T. viride* L4 | + | - | - | - | nt | ± | ± | nt | + | ± |
| *T. viride* S17a | + | - | - | - | nt | ± | ± | nt | + | ± |

\+ growth
\- no growth
± resistant colonies
nt not tested

The fungal isolates *T. viride* S17a and *T. viride* L4 were also not compatible with HY-TL or Force ST, and only partially compatible with Wakil XL. These two isolates appeared to be more sensitive to the pesticides in this agar test than the other fungal isolates, which may be a species specific phenomenon. However, organic growers could still use the microorganism-primed seed alone.

**Conclusions**

- For the first time it was shown that beneficial microorganisms can be successfully applied to onion seed during drum priming
- Beneficial microorganisms can be also be successfully applied to carrot seed during drum priming
- On carrot seed, all the beneficial microorganisms survived and proliferated following application during drum priming.
- On onion seed, bacterial isolates survived and proliferated during drum priming.
- On onion seed, the fungal isolates *T. harzianum* T22, *T. viride* S17a and *C. rosea* IK726 survived the priming process, but did not proliferate overall and a higher initial inoculum was required to achieve the target of 5 $\log_{10}$ cfu $g^{-1}$ dry seed.
- *Trichoderma viride* L4 did not survive well on onion seed, and was not recovered above the target rate at the end of priming.
- Pesticide compatibility tests on agar plates showed mixed results:
    - All isolates were compatible or partially compatible with Apron 35 and Wakil XL.
    - Bacterial isolates were partially compatible with the fungicide HY-TL.
    - None of the fungal isolates grew on HY-TL amended agar.
    - *Trichoderma harzianum* T22, *C. rosea* IK726, *P. chlororaphis* MA342 and *P. fluorescens* CHA0 were compatible with Force ST.
    - *Trichoderma viride* S17a and *T. viride* L4 did not grow on Force ST amended agar.

# Objective 02: Assess effects of seed applied microorganisms to carrot and onion seed in field trials

## Introduction

Field trials were conducted over three consecutive years at various sites in the UK (Table 2.1) to assess the effects on emergence and growth of carrot and onion seed primed with selected beneficial microorganisms. At each site, emergence counts were made at approximately 3 and 6 weeks post-planting, and growth was monitored throughout the season. At harvest, onion bulbs were counted, weighed and graded according to various size categories. Carrots were also counted and weighed, and assessed for cavity spot and possible nematode damage (fanged roots).

## Materials and Methods

*Bacterial inoculum preparation*

Wild-type strains of the bacterial isolates 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.

**Table 2.1:** Locations of the carrot and onion field trials for Years 1-3.

| Crop | Year 1 (2004) | Year 2 (2005) | Year 3 (2006) |
|---|---|---|---|
| Conventional carrot | Warwick HRI, Wellesbourne<br>T. Hammond & Sons, South Notts | Warwick HRI, Wellesbourne<br>Marshall Farms, Papplewick | Warwick HRI, Wellesbourne<br>Clumber Farms, Worksop |
| Organic carrot | Elsoms Seeds Ltd., Spalding | Farcet Farms, Yaxley Fen | Elsoms Seeds Ltd., Spalding |
| Conventional onion | Warwick HRI, Wellesbourne<br>Elveden Farms Ltd., Thetford | Warwick HRI, Wellesbourne<br>Farcet Farms, Yaxley Fen | Warwick HRI, Wellesbourne<br>Elveden Farms Ltd., Thetford |
| Organic onion | Elsoms Seeds Ltd., Spalding | Elsoms Seeds Ltd., Spalding | Elsoms Seeds Ltd., Spalding |

*Fungal inoculum preparation*

Fungal isolates supplied by Warwick HRI were cultured on potato dextrose agar (PDA) 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 PDA.

*Drum priming and pesticide application*

The selected microorganisms were applied to seed samples during drum priming at Elsoms Seeds Ltd. and sub-samples of the primed seed were removed for further seed processing at GTG. 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. In Year 1 (2004) only, the onion seed pellets also included Apron 35. 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 2.2) 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 (Treatments 1-6).

**Table 2.2** Seed treatments produced for the field trials

| Treatment number | Description | Priming | Fungicide | | Insecticide |
|---|---|---|---|---|---|
| | | | Carrot[a] | Onion[b] | (Force ST) |
| 1 | Primed control | ✓ | × | × | × |
| 2 | Primed *P. fluorescens* CHA0 | ✓ | × | × | × |
| 3[c] | Primed *C. rosea* IK726 | ✓ | × | × | × |
| | Primed *T. viride* S17a (onion Year 3 only) | ✓ | - | × | × |
| 4 | Primed *P. chlororaphis* MA342 | ✓ | × | × | × |
| 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[c] | Primed *C. rosea* IK726 + Pesticide | ✓ | Wakil XL | HY-TL | ✓ |
| | Primed *T. viride* S17a (onion Year 3 only) | ✓ | - | HY-TL | ✓ |
| 10 | Primed *P. chlororaphis* MA342 + Pesticide | ✓ | Wakil XL | 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. HY-TL was used in all three years. Apron 35 was also included in Year 1 only.

[c] Treatments 3 and 9 were *Clonostachys rosea* IK726 for the carrots in all three years, but on the onions in Year 1 and 2 only. *T. viride* S17a was substituted on the onion seed for Year 3 only.

*Reisolation of microorganisms from seed samples*

Samples were removed after drying for germination tests in pleated papers and reisolation of the applied microorganisms. For the germination tests, 3 x 100 seeds per treatment were assessed (100 seeds per pleat) under conditions of moisture and temperature consistent with ISTA standard germination testing for these crop species (35 ml water was added per pleat and the pleated papers containing the seeds were stored at 20$^{o}$C for both carrot and onion). For the reisolation of the applied microorganisms, three replicate 0.5 g seed samples were spun at high power on a vortex mixer for 3 x 1 minute in 4.5 ml of sterile distilled water (SDW). A dilution series was prepared in SDW and selected dilutions were spiral plated onto solid media (nutrient agar containing 100 $\mu$g ml$^{-1}$ rifampicin for the bacteria and potato dextrose agar containing 30 $\mu$g ml$^{-1}$ chlortetracycline for the fungi). Colonies were counted after 2 d incubation at 25 ˚C and 20 ˚C for the bacteria and fungi respectively. The numbers of colonies were expressed as cfu g$^{-1}$ dry weight seed following $\log_{10}$ transformation. Significant differences between sample means (Fisher's protected LSD ($P$ = 0.05)) were determined by analysis of variance (ANOVA).

*Design and analyses of field trials*

All trials consisted of four replicates of 12 (conventional trials) or 6 (organic trials) treatments, arranged in a randomized block design. In Year 1 (2004) plots were 3m long and had 4 rows across the bed drilled with the treated seed. A cone drill was used, with seedling rates of 23 onion seeds/m, and 50 carrot seeds/m. This method resulted in uneven seeding, and in Year 2 (2005) and Year 3 (2006) the trials were drilled with a tractor-mounted singulaire drill. Also, in the latter two years, the plot dimensions were changed to be 6m long, with the treated seed drilled only in the inner two rows, with the outer rows kept as guard rows of unprimed seed. In all years at the Warwick HRI site the carrot crop was fleeced 2 weeks after drilling to protect the crop from carrot fly. Throughout the growing season, standard commercial pesticides were applied to each crop at the conventional sites, excluding the use of nematicides and Folicur. All variables were subjected to analysis of variance (ANOVA), using a randomized complete block design. Percentage data were subjected to angular transformation prior to analysis and back-transformed data are presented. All differences noted were at the 5% significance level.

## Results and discussion

### *Advancement and germination of seed treatments*

In all years the drum priming treatments produced germination advancement responses in the pleated paper tests for both carrot and onion, and a representative example of this is shown in Table 2.3 (Year 3). Some abnormally germinated seeds were found throughout, but these were in low numbers and were not consistent across the three years.

### *Target application rates of the beneficial microorganisms*

All isolates were recovered in excess of the target application rates (5 $\log_{10}$ cfu $g^{-1}$ seed) to both carrot and onion in the presence and absence of pesticides in all three years. An example is shown in Figure 2.1 (Year 3, 2006).

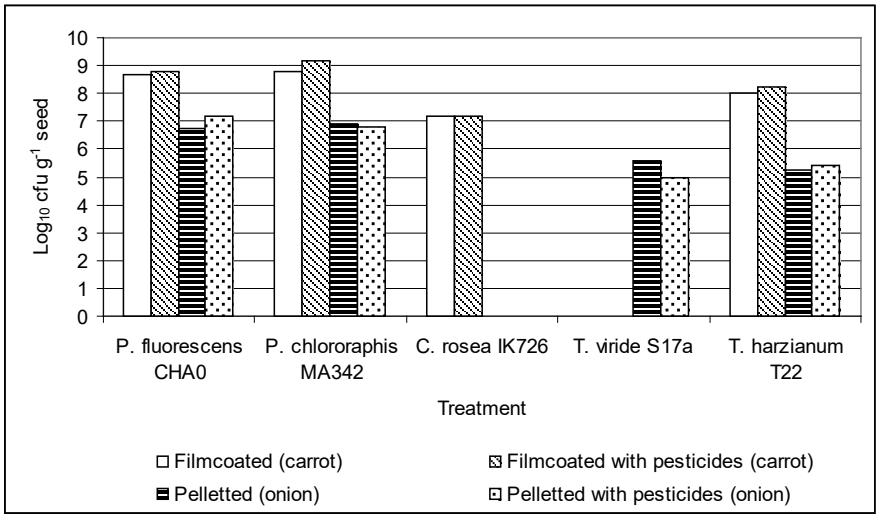


**Figure 2.1:** Target application rates of the beneficial microorganisms applied to carrot and onion seed for the Year 3 (2006) field trials. Note: *C. rosea* IK726 was used as a treatment on carrot seed only in Year 3, and was substituted by *T. viride* S17a on onion seed.

**Table 2.3** Advancement and germination data of the field trial material in pleated papers.

| Treatment Number | Description | Carrot | | Onion | |
|---|---|---|---|---|---|
| | | Normals (%)[a] | Advancement G50 (h)[b] | Normals (%) | Advancement G50 (h) |
| Treatment 1 | Primed control | 85.7 | 47.1 | 96.3 | 76.5 |
| Treatment 2 | Primed *P. fluorescens* CHA0 | 85.7 | 51.8 | 95.7 | 75.4 |
| Treatment 3[c] | Primed *C. rosea* IK726 | 80.3 | 44.3 | - | - |
| Treatment 3[c] | Primed *T. viride* S17a | - | - | 94.3 | 77.3 |
| Treatment 4 | Primed *P. chlororaphis* MA342 | 81.7 | 51.6 | 93.7 | 77.2 |
| Treatment 5 | Primed *T. harzianum* T22 | 86.0 | 49.0 | 93.3 | 77.2 |
| Treatment 6 | Unprimed control | 82.0 | 73.9 | 94.0 | 88.6 |
| Treatment 7 | Primed control + pesticide | 81.0 | 48.0 | 94.3 | 76.9 |
| Treatment 8 | Primed *P. fluorescens* CHA0 + pesticide | 85.7 | 50.7 | 94.7 | 77.2 |
| Treatment 9[c] | Primed *C. rosea* IK726 + pesticide | 81.7 | 31.8 | - | - |
| Treatment 9[c] | Primed *T. viride* S17a + pesticide | - | - | 96.3 | 78.2 |
| Treatment 10 | Primed *P. chlororaphis* MA342 + pesticide | 84.7 | 50.9 | 95.0 | 76.3 |
| Treatment 11 | Primed *T. harzianum* T22 + pesticide | 84.3 | 49.3 | 96.3 | 77.8 |
| Treatment 12 | Unprimed control + pesticide | 79.7 | 79.6 | 97.0 | 93.4 |

[a] Figures represent percentage normal germination from a total of 3 x 100 seeds per treatment

[b] G50 advancement data represents time required for 50 % of the seed in a treatment population to germinate

[c] *C. rosea* IK726 was used as a seed treatment for carrot only in Year 3 (2006), and was substituted by *T. viride* S17a on onion in the field trials

***Emergence data - onion trials***

*Warwick HRI onion trials*

In Year 1 (2004), emergence assessments were made from a 2.5m length of each of four rows per plot. In subsequent years assessments were made from a 5m length of each of the two inner rows, resulting in the same assessment area in all years (10m per plot). In all three years, it was found that **pesticide application consistently improved emergence**, although the microorganism treatments had a variable effect on onion emergence at Warwick HRI. A representative example of the onion emergence at Warwick HRI is given in Figure 2.2 (Year 3). Some positive and some negative effects were noted each year, but there were **no consistent effects of the microorganism treatment**. *Clonostachys rosea* IK726 was replaced by *Trichoderma viride* S17a as one of the seed treatments in Year 3 (2006) due to poor performance of the former in previous years.

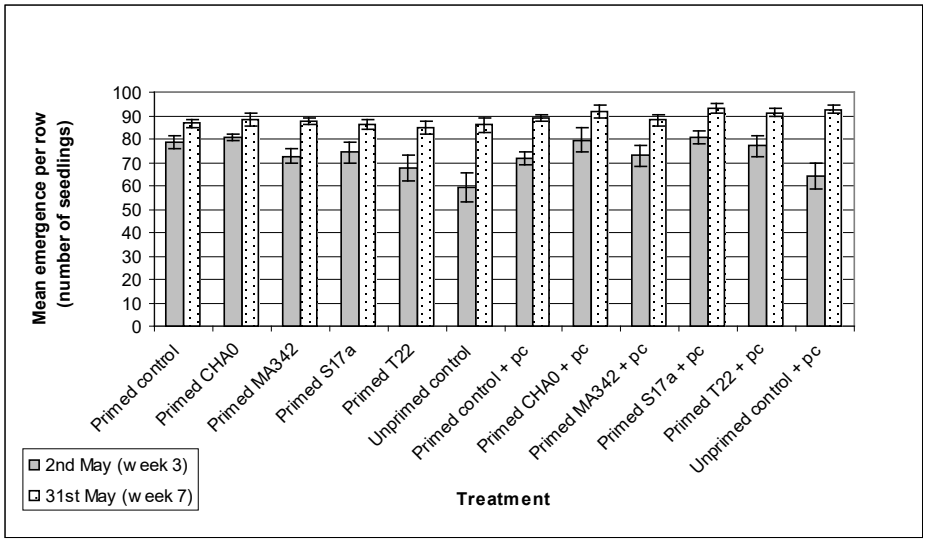


**Figure 2.2:** Mean emergence of treated onion seed at the Warwick HRI trial (Year 3). Bars represent standard errors of the mean.

*Conventional grower onion trials*

The sites for the conventional onion growers' trials were Elveden Farms in Year 1 (2004) and Year 3 (2006), and Farcet Farms in Year 2 (2005). No microorganism effects were found with emergence in Year 1, although pesticide application did improve emergence. In Year 2 (2005), the onion trial was grown with a cover crop, which made emergence assessments difficult. No microorganism treatments

resulted in improved emergence compared to the primed control. A representative example of the onion emergence at Elveden Farms (Year 3) is given in Figure 2.3. Some positive and some negative effects were noted each year, but there were **no consistent effects of the microorganism treatment**. Pesticide application improved the emergence of seed treated with *T. harzianum* T22 or *P. fluorescens* CHA0 in Year 3 (2006).

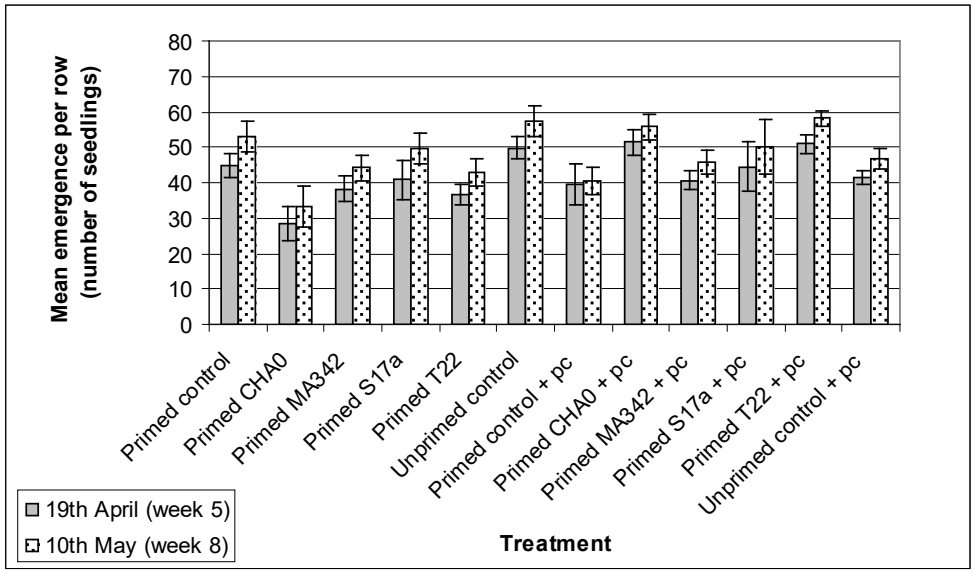


**Figure 2.3:** Mean emergence of treated onion seed at the Elveden Farms trial (Year 3). Bars represent standard errors of the mean.

*Organic site onion trials*

The organic trials were held at Hillfield, Elsoms Seeds Ltd., for all three years. In Year 1 (2004), the onion seeds were planted in modules before being transplanted as seedlings into the field. Because of this, no true "emergence" data was available, although it was found that subsequent establishment of the seedlings was improved with the seed treatments of *T. harzianum* T22, *P. fluorescens* CHA0 or *P. chlororaphis* MA342 compared to the controls. In Year 2 (2005), ***P. fluorescens* CHA0 again improved emergence over the primed control**. There were no significant effects of microorganism treatment noted in Year 3 (2006). A representative example of the onion emergence at the organic site is given in Figure 2.4 (Year 3).

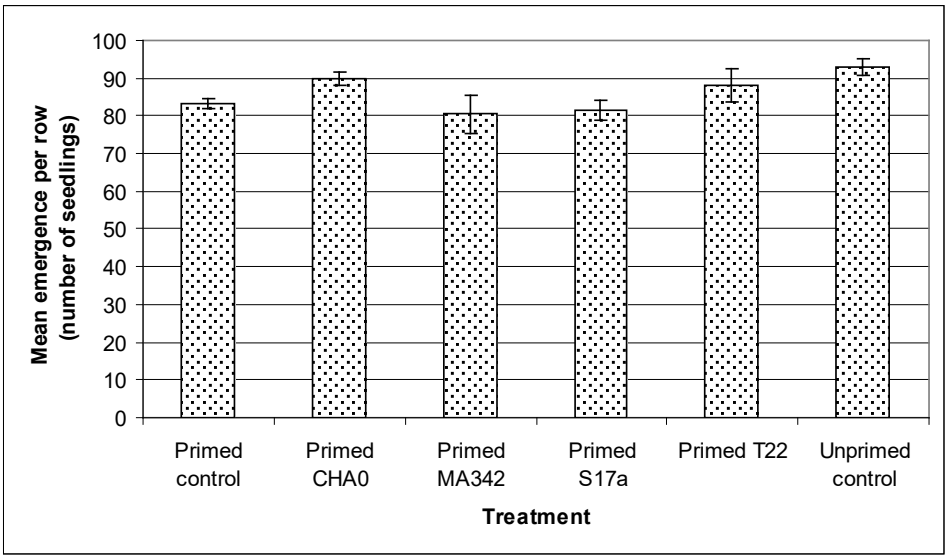


**Figure 2.4:** Mean emergence of treated onion seed at the organic site (Hillfield, Elsoms Seeds) (Year 3). Bars represent standard errors of the mean.

***Harvest data – onion trials***

*Warwick HRI onion trials*

No white rot was recorded at Warwick HRI in any of the three trial years. In Year 1 (2004) no significant effect of microorganism treatment was found. However, **pesticide application significantly increased the total number of bulbs**, and the percentage bulbs sized 40-80mm. In Year 2 (2005), various effects of microorganism treatment were noted, with some being positive and some negative. There was **no consistent effect of microorganism treatment**.

Year 3 (2006) harvest data is presented here for the first time. Although some statistically significant differences were found, many of these differences were small and are difficult to visualize on the graphs. However, significant differences are stated in the text where appropriate.

*Milestone S2.3 – Year 3 harvest data*

The two inner rows were harvested, excluding the first and last 50cm of each row (total length = 5m). No white rot was recorded, although some bulbs were found to be rotting from an undetermined disease (<1%). Pesticide application significantly reduced the percentage and the mean weight of bulbs affected by this rot (data not

 

shown). Harvest records were made of the total number and weight of bulbs, as well as the percentage and mean weight of bulbs in two size categories (Figure 2.5-2.8). **Pesticide application significantly increased the total number and weight of bulbs**; and also increased the weight of bulbs in the size range 40-80mm. **No microorganism effects were found.**

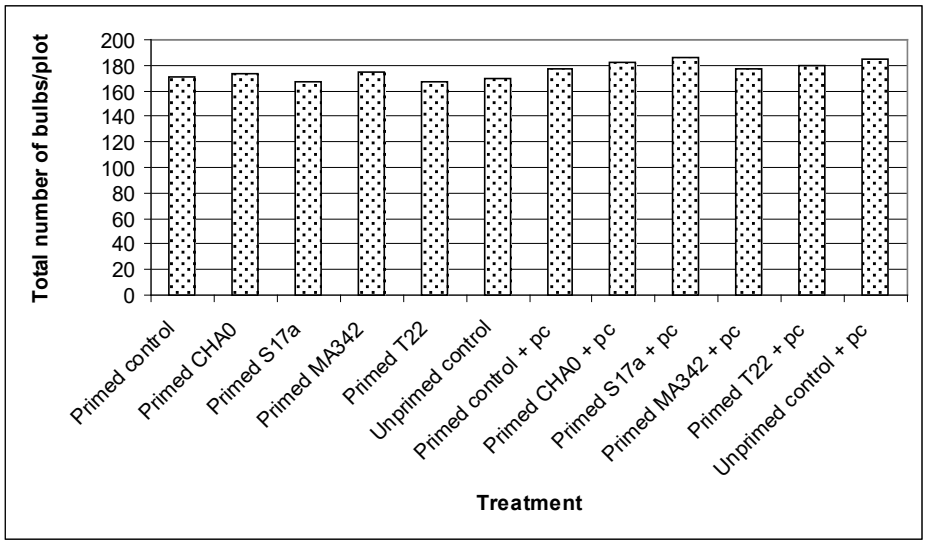


**Figure 2.5:** Total number of onion bulbs per plot at the Warwick HRI trial (Year 3). LSD (5%, df = 33) comparing microorganism treatment – pesticide interaction = 12.30

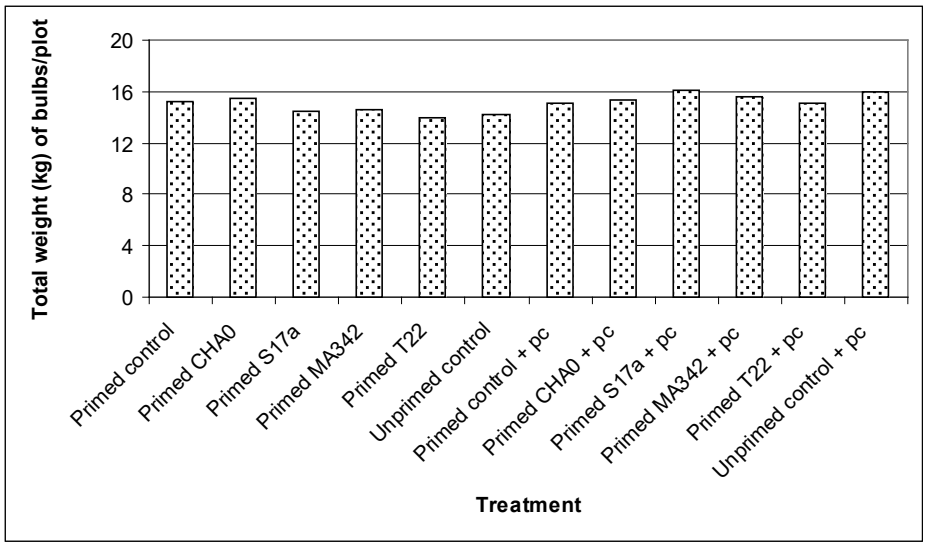


**Figure 2.6:** Total weight (kg) of onion bulbs at the Warwick HRI trial (Year 3).
LSD (5%, df = 33) comparing microorganism treatment – pesticide interaction = 1.64

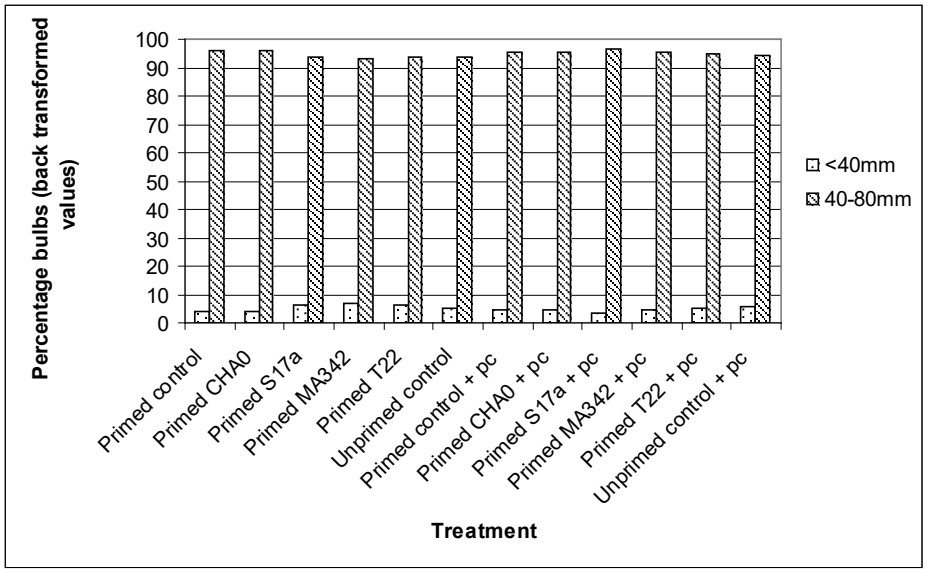


**Figure 2.7:** Percentage onion bulbs in different size categories at the Warwick HRI trial (Year 3). LSD not available on back transformed data presented (analyses done on angle transformed data)

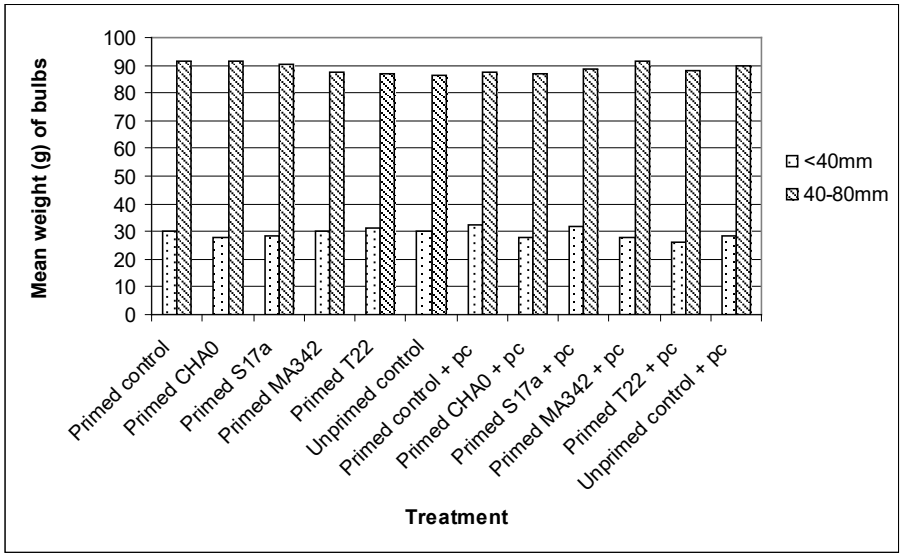


**Figure 2.8:** Mean weight (g) of onion bulbs in different size categories at the Warwick HRI trial (Year 3) LSD (5%, df = 33) comparing microorganism treatment – pesticide interaction: <40mm = 4.963; 40-80mm = 7.503.

*Conventional growers onion trial*

No white rot was recorded at any of the conventional trial sites in all three years. **Pesticide application increased the total weight of bulbs** in Year 1 (2004) and Year 2 (2005), and also increased the total number of bulbs in Year 1. In both years, some effects of microorganism treatment were also found, but these were not consistent and had both positive and negative effects.

Year 3 (2006) harvest data is presented here for the first time.

*Milestone S2.3 – Year 3 harvest data*

A 4m length of the inner two rows was harvested for each plot. No white rot was recorded at this site. Some microorganism effects were found at this trial site, and overall *T. viride* S17a increased the total number (Figure 2.9) and weight (Figure 2.10) of bulbs compared to the primed control ($P<0.05$). A microorganism-pesticide interaction was found, with the addition of pesticide improving the total number of bulbs with *P. fluorescens* CHA0 seed treatment (Figure 2.9), as well as increasing the total weight for this treatment (Figure 2.10).

The number of bulbs with *T. harzianum* T22 seed treatment was also improved by the addition of pesticide, whereas a reduction in the total number of bulbs was seen with pesticide addition in the primed and unprimed control treatments (Figure 2.9). Overall**, pesticide increased the total weight of bulbs**.

In the size category <40mm no effects of microorganism or pesticide treatment were seen. However, in the size category 40-60mm, the addition of pesticide increased the percentage of bulbs where *T. harzianum* T22 or *P. fluorescens* CHA0 had been applied as seed treatments (Figure 2.11). Pesticide application also decreased the percentage bulbs in the size 60-80mm for the *P. fluorescens* CHA0 treatment. No significant effects were seen regarding the mean weight of bulbs in the size categories (Figure 2.12).

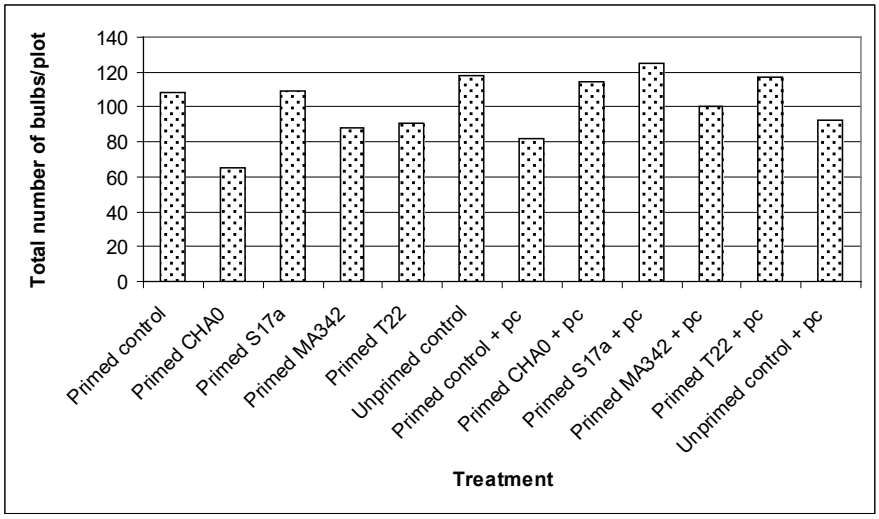


**Figure 2.9:** Total number of onion bulbs per plot at the Elveden Farms trial (Year 3). LSD (5%, df = 32) comparing microorganism treatment – pesticide interaction = 24.00

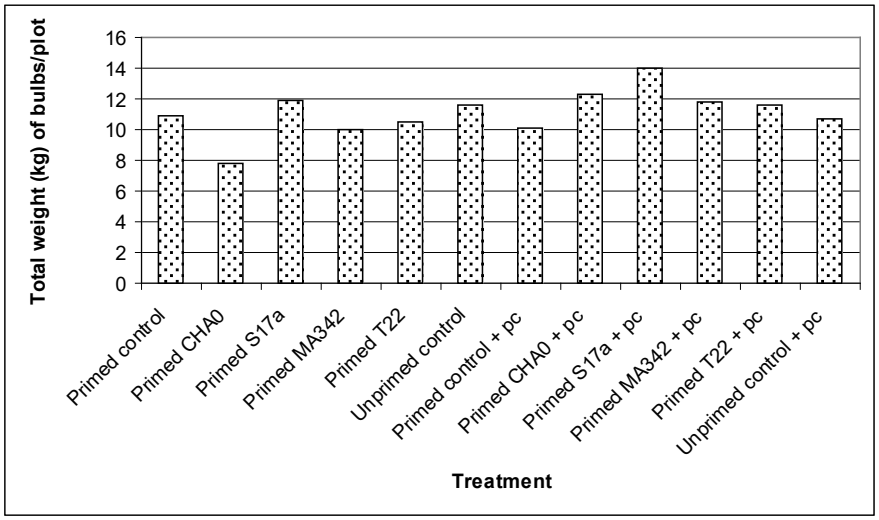


**Figure 2.10:** Total weight (kg) of onion bulbs at the Elveden Farms (Year 3).
LSD (5%, df = 32) comparing microorganism treatment – pesticide interaction = 2.497

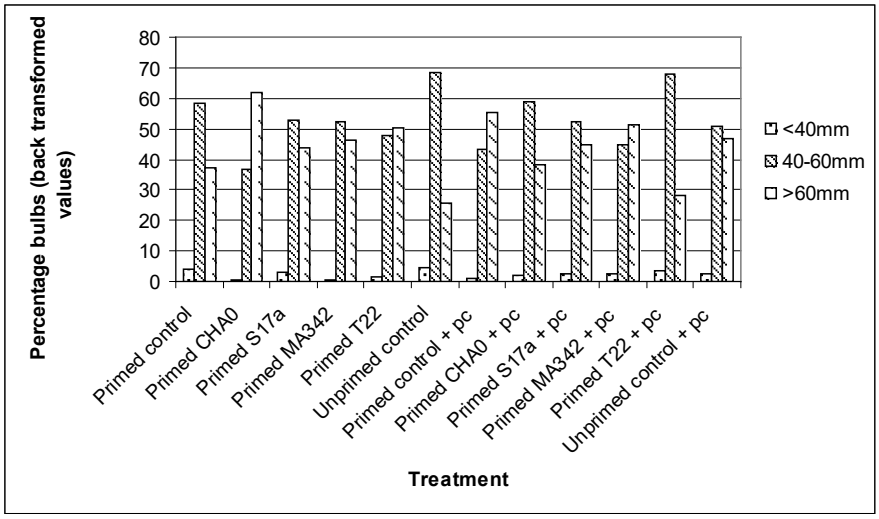


**Figure 2.11:** Percentage onion bulbs in different size categories at the Elveden Farms trial (Year 3). LSD not available on back transformed data presented (analyses done on angle transformed data)

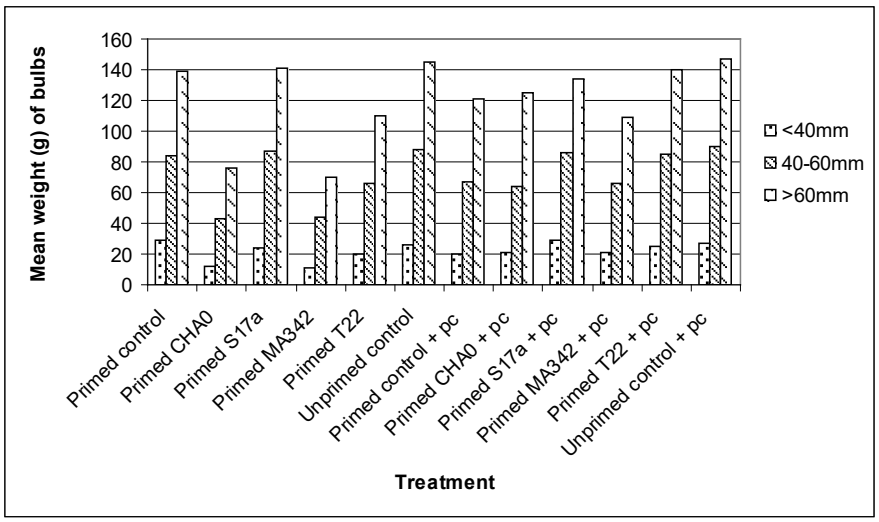


**Figure 2.12:** Mean weight (g) of onion bulbs in different size categories at the Elveden Farms trial (Year 3) LSD (5%, df = 32) comparing microorganism treatment – pesticide interaction: <40mm = 15.80; 40-60mm = 47.67; 60-80mm = 85.12.

*Organic onion trial*

No harvest data was available from the organic site in Year 1 (2004). In Year 2 (2005) no white rot was recorded. A small effect of microorganism treatment was found, with *C. rosea* IK726, *T. harzianum* T22 and *P. chlororaphis* MA342 resulting in a lower mean weight of bulbs in the size 60-80mm compared to the primed control.

Year 3 (2006) harvest data is presented here for the first time.

*Milestone S2.3 – Year 3 harvest data*

No microorganism effects were seen on the total number of bulbs (Figure 2.13); total weight of bulbs (Figure 2.14); percentage of bulbs in the size categories (Figure 2.15) or the mean weight of bulbs in the size categories (Figure 2.16).

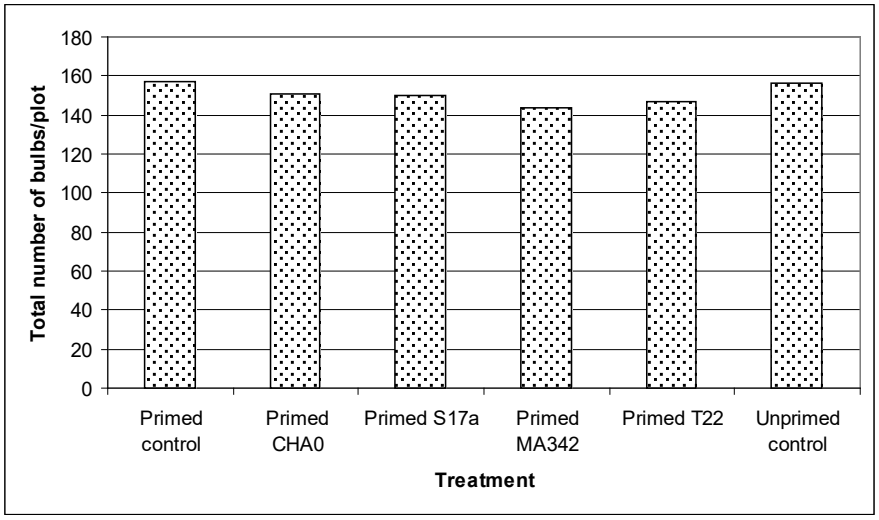


**Figure 2.13:** Total number of onion bulbs per plot at the organic trial (Year 3). LSD (5%, df = 15) comparing microorganism treatment = 22.19

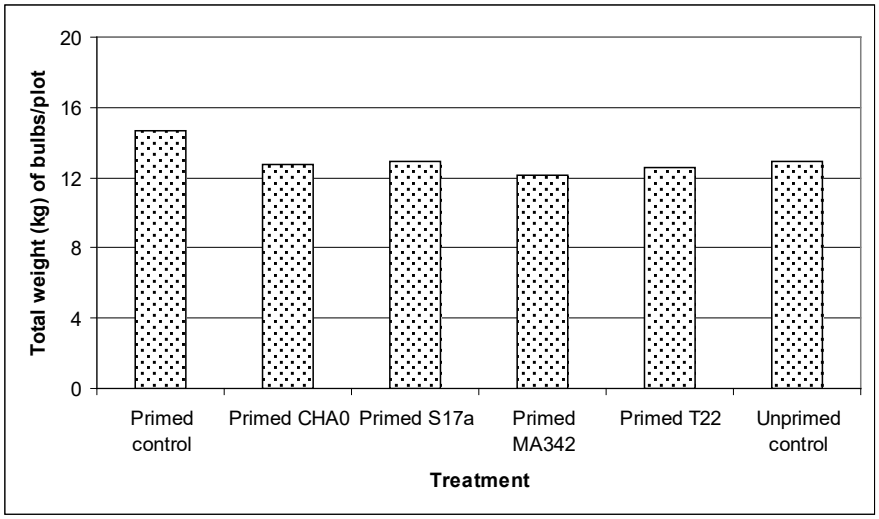


**Figure 2.14:** Total weight (kg) of onion bulbs at the organic trial (Year 3).
LSD (5%, df = 15) comparing microorganism treatment – pesticide interaction = 2.628

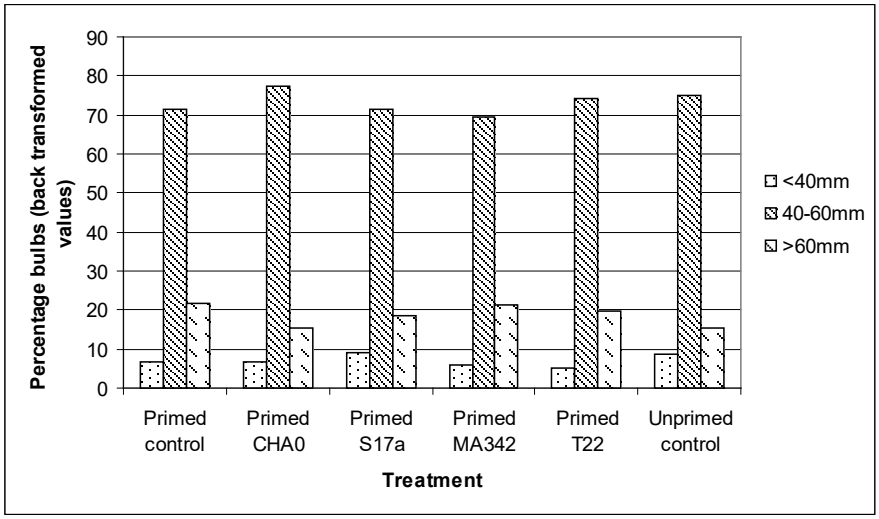


**Figure 2.15:** Percentage onion bulbs in different size categories at the organic trial (Year 3). LSD not available on back transformed data presented (analyses done on angle transformed data)

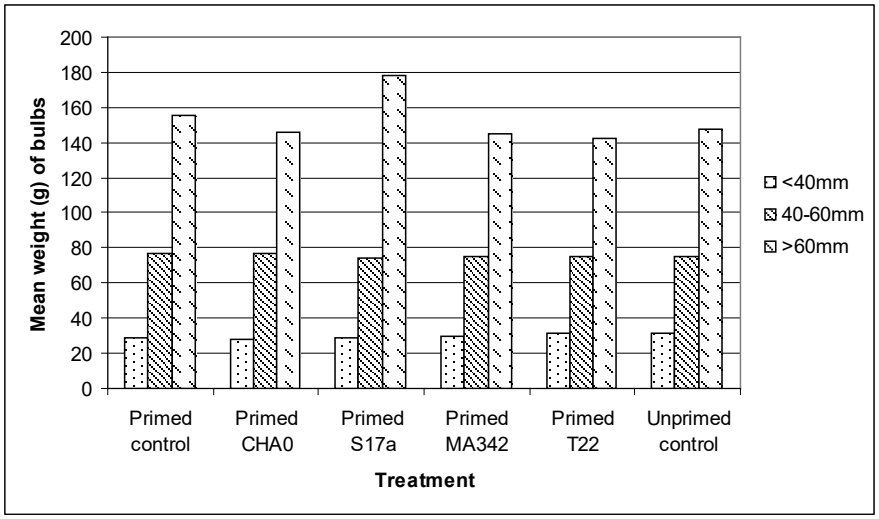


**Figure 2.16:** Mean weight (g) of onion bulbs in different size categories at the organic trial (Year 3) LSD (5%, df = 15) comparing microorganism treatments: <40mm = 4.739; 40-60mm = 11.50; >60mm = 42.49.

***Emergence data – carrot trials***

*Warwick HRI carrot trials*

**Pesticide significantly improved emergence in carrot trials at Warwick HRI for three years**. In Year 2 (2005), the primed control showed improved emergence over the fungal seed treatments. *Pseudomonas fluorescens* CHA0 without pesticide and *C. rosea* IK726 without pesticide were worse than the primed control without pesticide in two years. No other consistent effects of microorganism were seen over three years. A representative example of carrot emergence at Warwick HRI is shown in Figure 2.17 (Year 3).

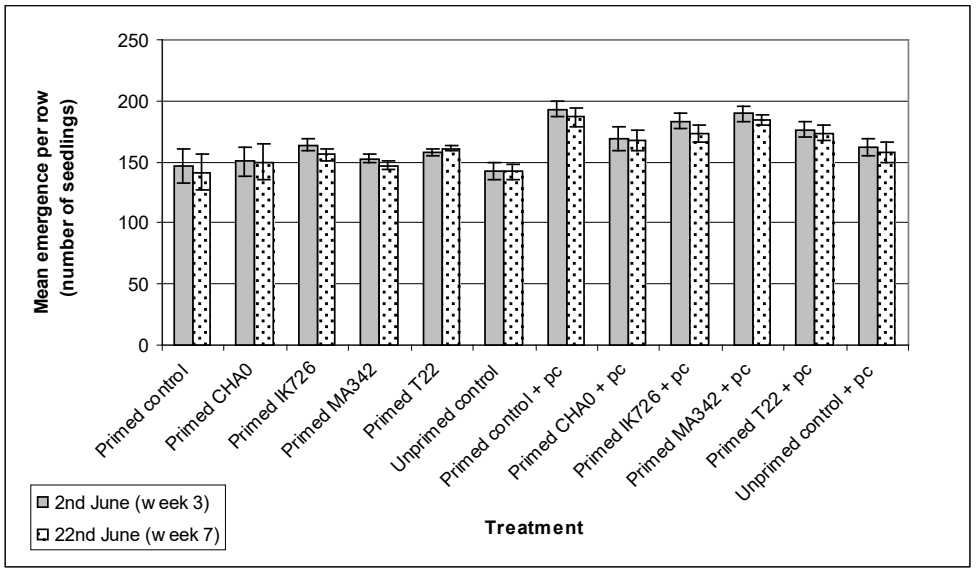


**Figure 2.17:** Mean emergence of treated carrot seed at the Warwick HRI trial (Year 3). Bars represent standard errors of the mean.

*Conventional grower carrot trials*

Some small microorganism effects were noted in Year 1 (2004), but these were not statistically significant. No microorganism or pesticide effects were found in Year 2 (2005) or Year 3 (2006). A representative example of the carrot emergence at the conventional grower's site (Clumber Farms, Year 3) is given in Figure 2.18.

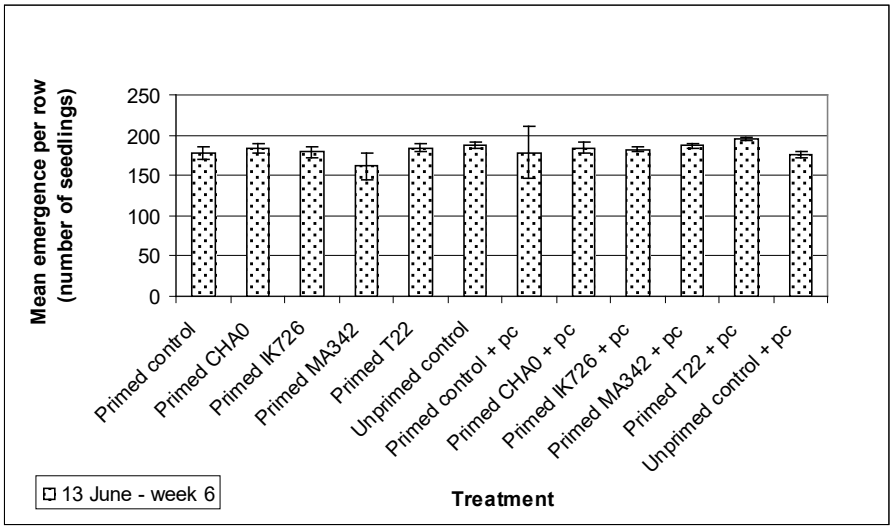


**Figure 2.18:** Mean emergence of treated carrot seed at the Clumber Farm trial (Year 3). Bars represent standard errors of the mean.

*Organic carrot trials*

No data were available for Year 1 (2004) because of problems with weeds, and there were no significant effects of microorganism treatment in either Year 2 (2005) or Year 3 (2006). A representative example or the carrot emergence at the organic site is shown in Figure 2.19 (Year 3).

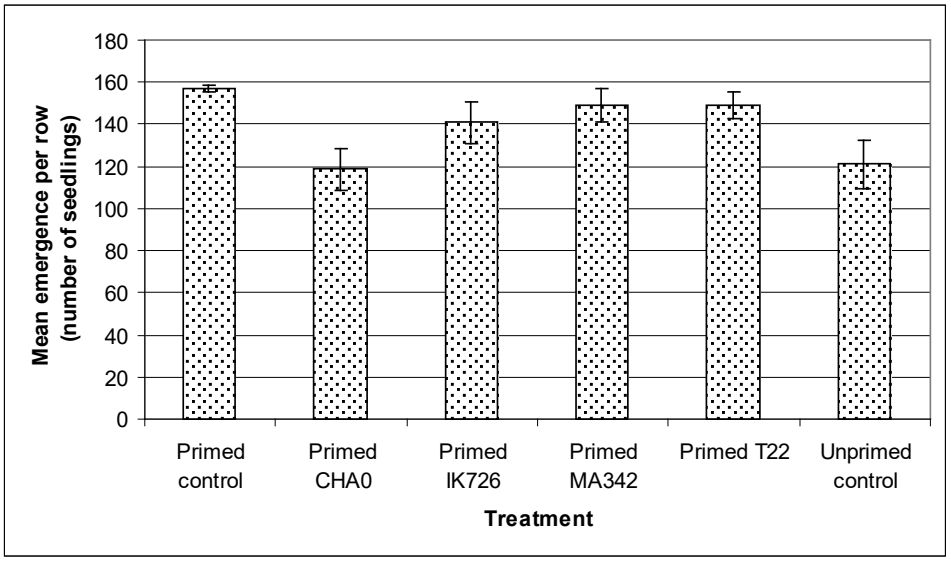


**Figure 2.19:** Mean emergence of treated carrot seed at the organic site (Hillfield, Elsoms Seeds) (Year 3). Bars represent standard errors of the mean.

***Harvest data – carrot trials***

*Warwick HRI, carrot trials*

**Pesticide application significantly increased the number of carrots** at harvest in Years 1 and 2 and increased the weight of carrots in Year 2. **Pesticide also decreased the percentage of fanged roots**, and decreased the mean weight of roots with cavity spot or possible nematode damage (fanged) in Years 1 and 2. There was no significant effect of microorganism treatment in Year 1 (2004), but in Year 2 (2005) the fungal seed treatments had a lower number of carrots than the primed control.

Year 3 (2006) harvest data is presented here for the first time

*Milestone S2.3 – Year 3 harvest data*

**Pesticide significantly increased the total numbe**r (Figure 2.20) **and total weight** (Figure 2.21) **of carrots**. **Overall, no microorganism effects were seen**. A single microorganism treatment-pesticide interaction was seen with *P. chlororaphis* MA342, where the addition of pesticide in combination with this treatment increased the percentage of roots affected with cavity spot, compared to the microorganism alone (Figure 2.22). No other significant effects were seen with the percentage of roots with cavity spot or nematode damage, or the mean weight of roots with cavity spot or nematode damage (Figure 2.23).

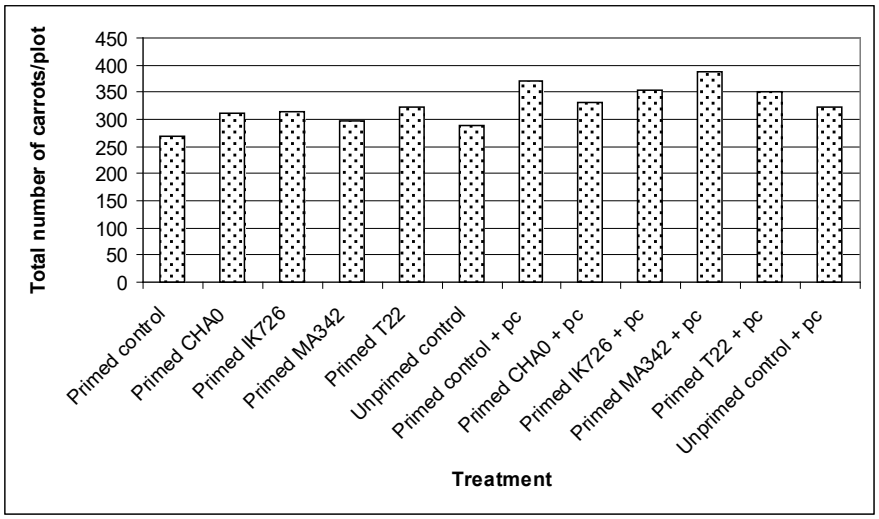


**Figure 2.20:** Total number of carrots per plot at the Warwick HRI trial (Year 3)
LSD (5%, df = 29) comparing microorganism treatment-pesticide interaction = 61.05

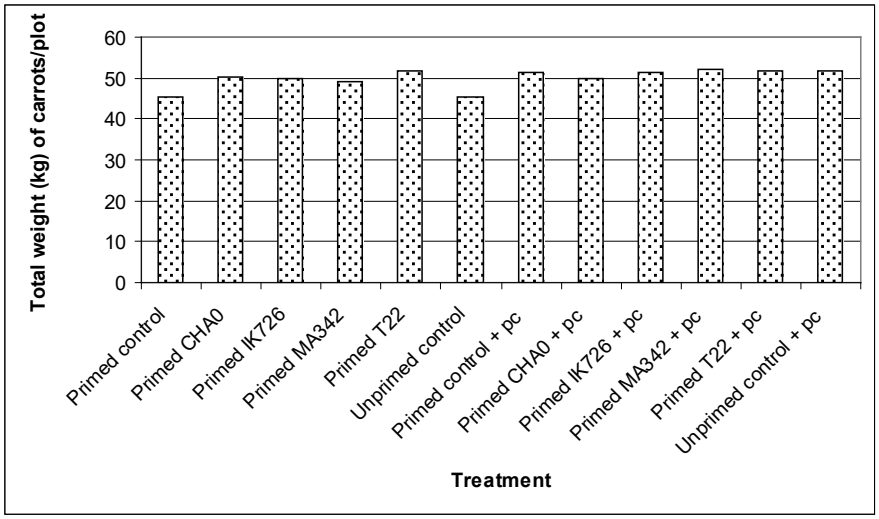


**Figure 2.21:** Total weight (kg) of carrots per plot at the Warwick HRI trial (Year 3) LSD (5%, df =29 ) comparing microorganism treatment-pesticide interaction = 5.329

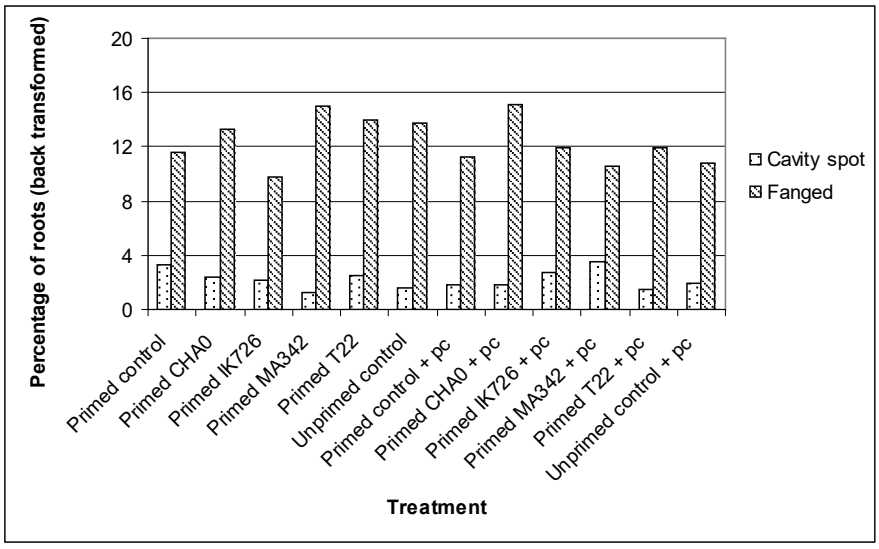


**Figure 2.22:** Percentage of carrot roots with cavity spot or possible nematode damage (fanged) at the Warwick HRI trial (Year 3) LSD not available on back transformed data presented (analyses done on angle transformed data)

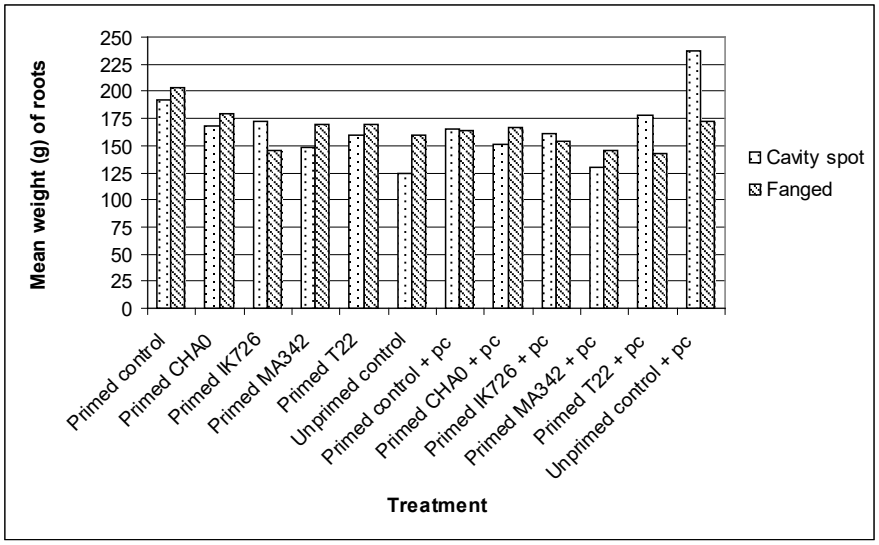


**Figure 2.23:** Mean weight of carrot roots with cavity spot or possible nematode damage (fanged) at the Warwick HRI trial (Year 3) LSD (5%, df = 29) comparing microorganism treatment-pesticide interaction: Cavity spot = 105.7; Fanged = 47.78

*Conventional growers carrot trials*

No significant effects of pesticide or microorganism treatment were found in Year 1 or Year 2. Similar results were found in the third trial, and Year 3 harvest data is presented here for the first time.

*Milestone S2.3 – Year 3 harvest data*

The inner two rows per plot were harvested. No significant effects of microorganism or pesticide treatment were found with the total number of carrots (Figure 2.24). Similarly, there was no effect of pesticide or microorganism treatment on the total weight of carrots (Figure 2.25). The percentage of roots affected by cavity spot or nematode damage (fanged) was low overall (Figure 2.26) and not affected by pesticide or microorganisms treatment. The mean weight of roots affected by cavity spot was significantly higher with the primed control than all other treatments (primed with microorganisms or the unprimed control) (Figure 2.27). However, no effect was seen with the mean weight of roots with nematode damage (Figure 2.27).

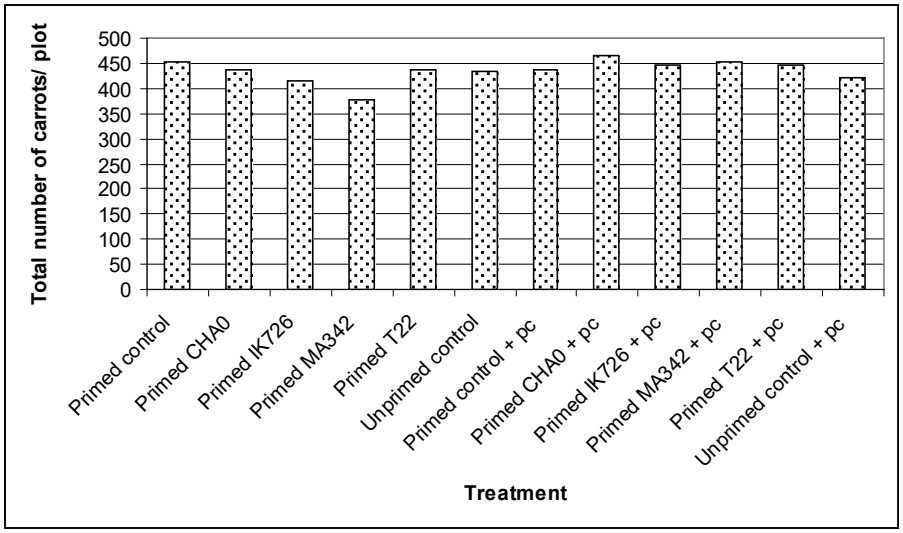


**Figure 2.24:** Total number of carrots per plot at the Clumber Farms trial (Year 3)
LSD (5%, df = 33) comparing microorganism treatment-pesticide interaction = 106.2

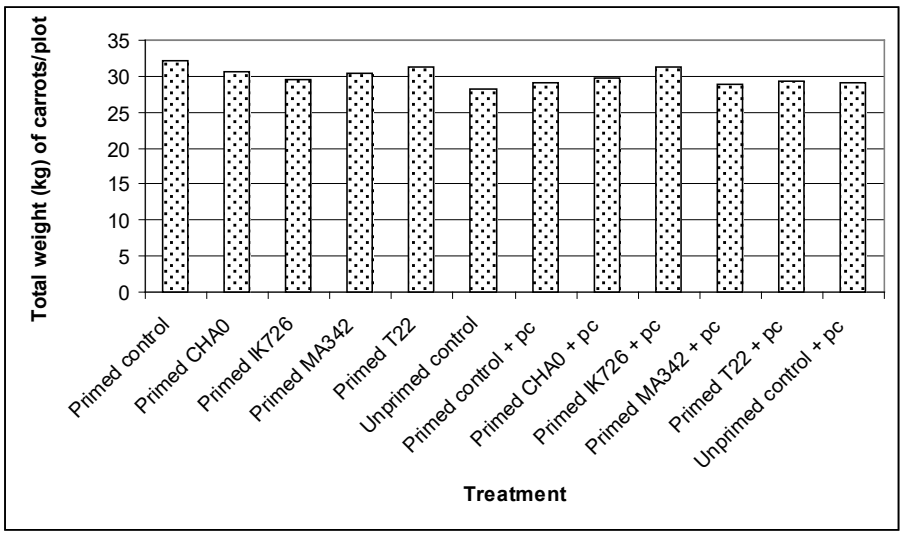


**Figure 2.25:** Total weight (kg) of carrots per plot at the Clumber Farms trial (Year 3)
LSD (5%, df =33) comparing microorganism treatment-pesticide interaction = 3.58

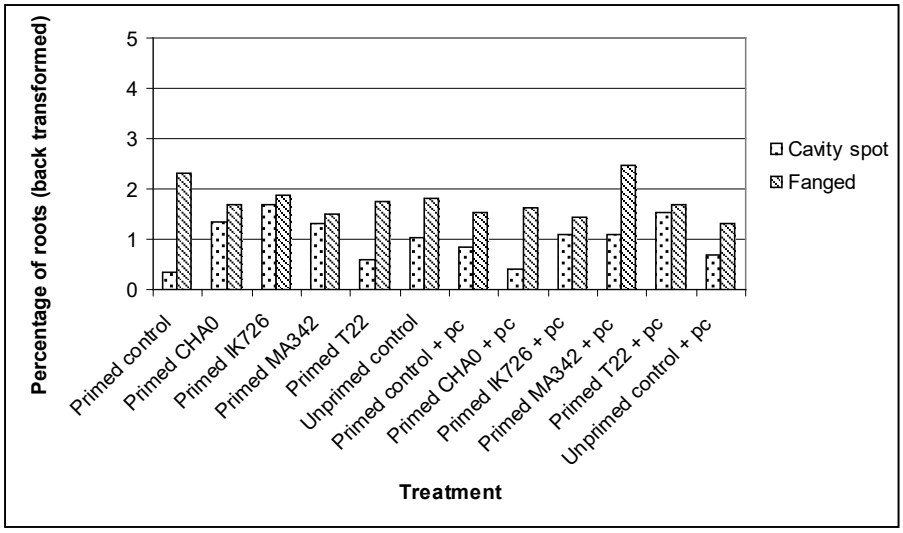


**Figure 2.26:** Percentage of carrot roots with cavity spot or possible nematode damage (fanged) at the Clumber Farms trial (Year 3) LSD not available on back transformed data presented (analyses done on angle transformed data)

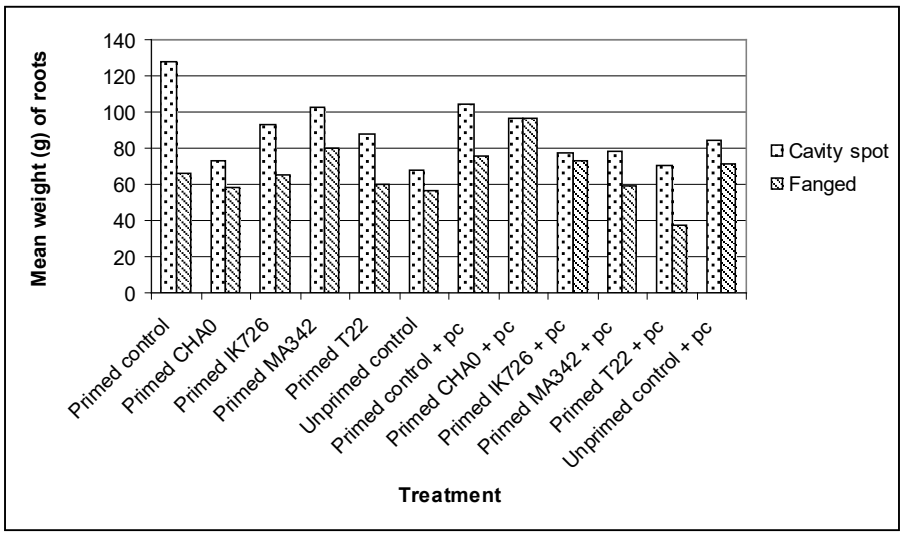


**Figure 2.27:** Mean weight of carrot roots with cavity spot or possible nematode damage (fanged) at the Clumber Farms trial (Year 3) LSD (5%) comparing microorganism treatment-pesticide interaction: Cavity spot (df = 28) = 34.1; Fanged (df = 33) = 44.9

*Organic carrot trials*

The organic trials for the carrot crop were badly affected by weeds in both Year 1 and Year 2 and as such no data are available. Year 3 harvest data is presented here for the first time.

*Milestone S2.3 – Year 3 harvest data*

Regarding the total number of carrots at harvest (Figure 2.28), all primed treatments apart from seed primed with *P. fluorescens* CHA0 had a significantly higher number of carrots than the unprimed control. *Pseudomonas fluorescens* CHA0 had a significantly lower number of carrots than the primed control (Figure 2.28). All primed treatments, with the exception of *P. fluorescens* CHA0, had a significantly greater total weight at harvest than the unprimed control (Figure 2.29). Microorganism treatment did not affect the percentage of roots with cavity spot or nematode damage (Figure 2.30), or the mean weight of carrots with cavity spot or nematode damage (Figure 2.31).

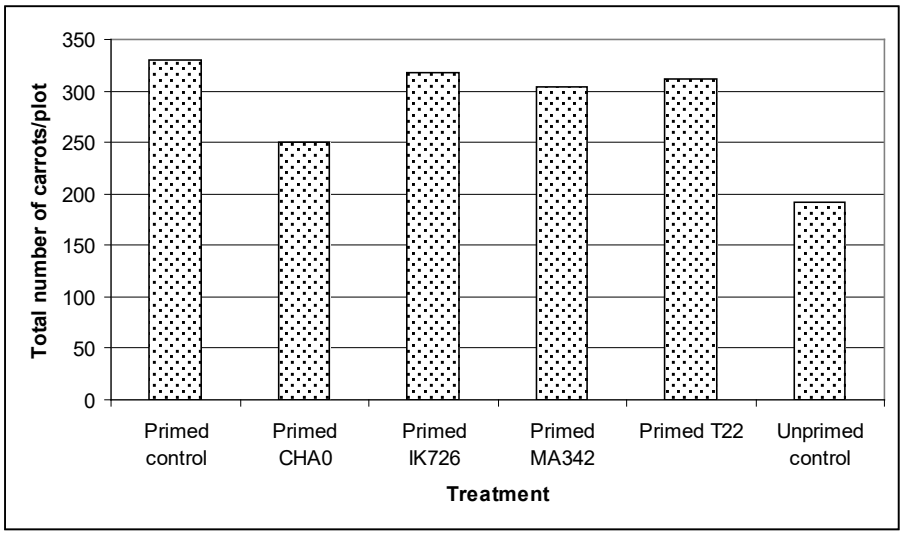


**Figure 2.28:** Total number of carrots per plot at the organic trial (Elsoms Seeds, Ltd) (Year 3). LSD (5%, df = 15) comparing microorganism treatment = 71.65

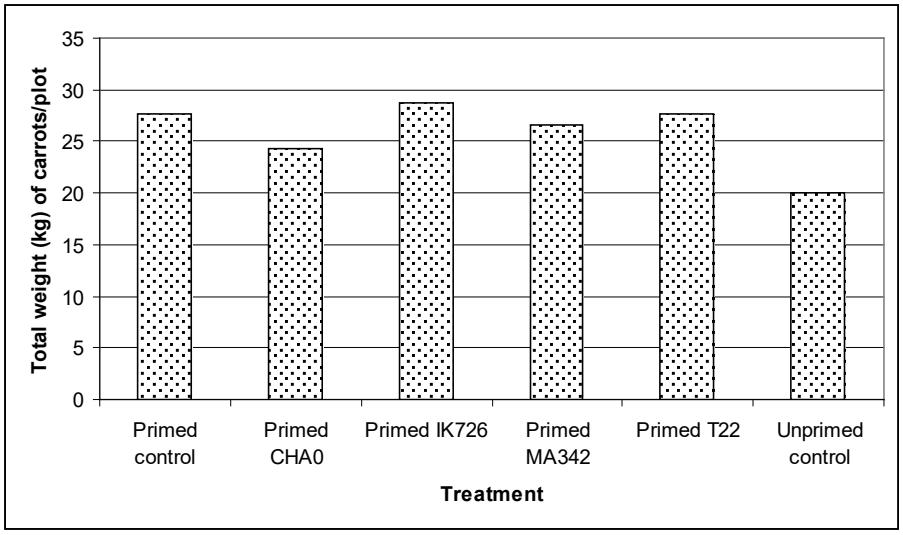


**Figure 2.29:** Total weight (kg) of carrots per plot at the organic trial (Elsoms Seeds, Ltd) (Year 3). LSD (5%, df =15) comparing microorganism treatment = 5.37

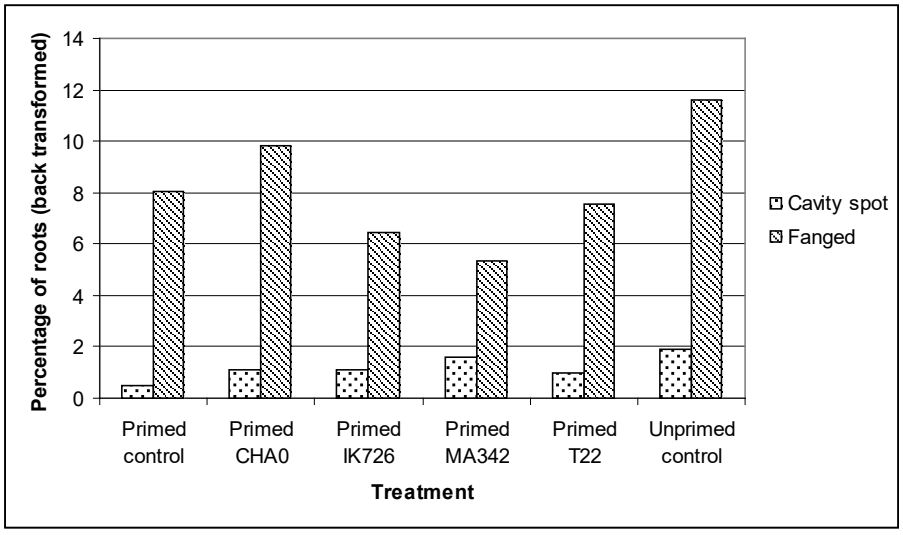


**Figure 2.30:** Percentage of carrot roots with cavity spot or possible nematode damage (fanged) at the organic trial (Elsoms Seeds, Ltd.) (Year 3). LSD not available on back transformed data presented (analyses done on angle transformed data)

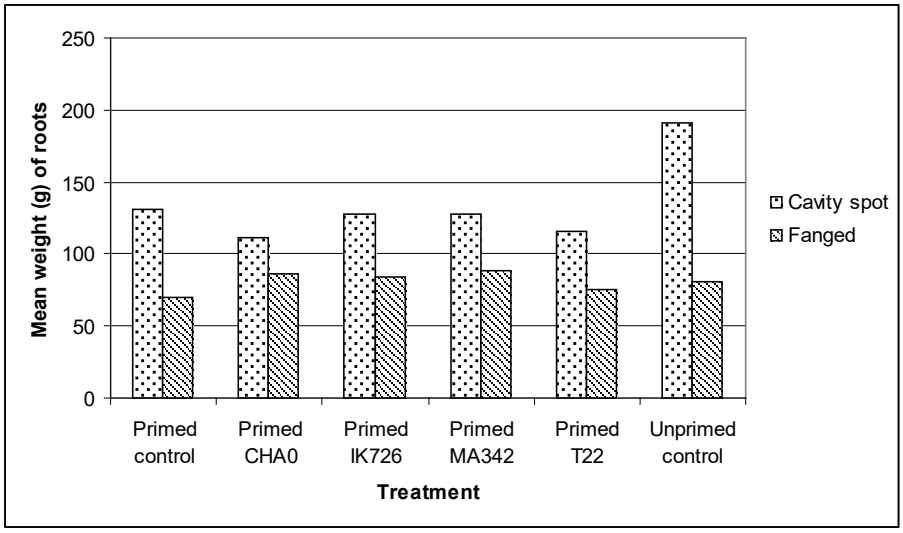


**Figure 2.31:** Mean weight of carrot roots with cavity spot or possible nematode damage (fanged) at the organic trial (Elsoms Seeds, Ltd.) (Year 3). LSD (5%) comparing microorganism treatment: Cavity spot (df = 12) = 57.7; Fanged (df = 15) = 28.7

**Conclusions:**

- All beneficial microorganisms were successfully applied to carrot and onion seed during commercial drum priming and the target rate of at least 5 $\log_{10}$ cfu $g^{-1}$ seed was achieved in all years.

**Onion trials:**

- Pesticide application improved emergence at the Warwick HRI onion trials in three years.
- Pesticide application increased the number of onion bulbs at harvest at the Warwick HRI trials in two years.
- Pesticide application increased the weight of onion bulbs at the conventional growers' trials in three years.
- *Pseudomonas fluorescens* CHA0 without pesticide improved onion emergence over the primed control without pesticide at the organic sites in Year 1 and Year 2.
- The new seed treatment for Year 3 (*Trichoderma viride* S17a) increased the number and weight of onion bulbs at harvest at the grower's trial.
- No consistent effects of microorganism treatment were seen for the onion trials for three years.

**Carrot trials:**

- Pesticide application improved emergence at the Warwick HRI carrot trials in three years.
- Pesticide application increased the number of carrots at harvest at the Warwick HRI trials in three years.
- Pesticide application decreased the percentage of roots with possible nematode damage (fanged) and the mean weight of roots with cavity spot or nematode damage for two years.
- *Pseudomonas fluorescens* CHA0 without pesticide and *C. rosea* IK726 without pesticide decreased the number of carrots compared to the primed control without pesticide at Warwick HRI trials in two years.
- No consistent effects of microorganism treatment were seen for the carrot trials for three years.

# 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

## Introduction

Glasshouse experiments were set up using seed from the same 12 treated batches used for the field trials, to test the effects of the microorganism treatment and priming on onion and carrot emergence and growth in different soil types. Experiments were also conducted in pathogen-infested soils, to determine the potential for seed-applied microorganisms to reduce disease.

## Materials and methods

### *Emergence and growth bioassays*

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. Two experiments were designed for each crop, consisting of 4 replicates of 12 treatments, with 6 pots per treatment and 4 seeds per pot. A third experiment for onion only had 8 treatments to assess the emergence and growth of the new microorganism in Year 3 (2006). In all experiments, emergence was recorded until no further increase in seedling number was noted. Some seedlings died after emergence, but were included in the calculations for the emergence data, which comprised the mean percent emergence, the mean time to emergence, as well as the time taken to 50% and 80% emergence. The final number of surviving seedlings was also determined to give a final percent emergence.

After 8 weeks, the experiment ended and the surviving seedlings were harvested. Seedlings from all six 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 per seedling was subsequently calculated, and $\log_{10}$ transformed before analysis. Significant differences between sample means ($P$ = 0.05) were determined by analysis of variance (ANOVA).

***Pathogen bioassays***

Sclerotium cepivorum *pathogen bioassay on onions*

*Sclerotium cepivorum* sclerotia (causing *Allium* white rot) 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.

Pythium ultimum *pathogen bioassay on carrots*

An isolate of *Pythium ultimum* was provided by Dr. 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. 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 x $10^3$ pathogen cfu $g^{-1}$ soil). 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 ANOVA.

**Results and discussion**

***Emergence and growth bioassays***

*Onion glasshouse experiments*

Three experiments were conducted in total, using the same seed lots used in the field trials for Years 1-3. The first two experiments used all 12 seed treatments, but the third experiment used 8 selected seed treatments. In the first experiment, **all the primed treatments** (including treatments primed with microorganisms) **emerged faster than the unprimed control**, illustrating the benefits of priming. Microorganism treatments did not significantly improve emergence over the primed control in this experiment, and nor did the addition of pesticide to the seed pellet. However, pesticide application did reduce the mean fresh weight of onion seedlings at harvest (8 weeks). Soil type affected the emergence rate of the seedlings, with emergence being significantly slower in the light sandy loam soil than the other two soil types. However, seedlings grown in the light sandy loam soil had the greatest mean fresh weight at harvest.

In the second onion experiment, the seed batch was contaminated with a deleterious microorganism that increased in number during priming and resulted in the primed control having the worst emergence of all the treatments. However, this experiment showed the beneficial effects of applying microorganisms to seed, as ***P. fluorescens*** **CHA0,** ***T. harzianum*** **T22 and** ***P. chlororaphis*** **MA342 all improved emergence significantly compared to the primed and unprimed controls** (Figure 3.1). **Pesticide application also improved emergence** (Figure 3.2), but as in the first experiment it also reduced the mean fresh weight of the seedlings. Interestingly, pesticide application also improved the emergence of seed treated with *C. rosea* IK726. It may have been that the inoculum of this microorganism was too high on the seed, as laboratory germination studies at Elsoms seeds indicated this seed treatment showed signs of “white mould” (the colour of the sporulating fungus). If so, the pesticide may have reduced the numbers of *C. rosea* IK726 on the seed, possibly reducing any phytotoxic effects. The peat soil resulted in the fastest emergence and the highest mean fresh weight of the seedlings in the second experiment (Figure 3.3). In the first two experiments it was also seen that *P. chlororaphis* MA342 and *T. harzianum* T22 treatments reached 50% and 80 % emergence faster than the unprimed control.

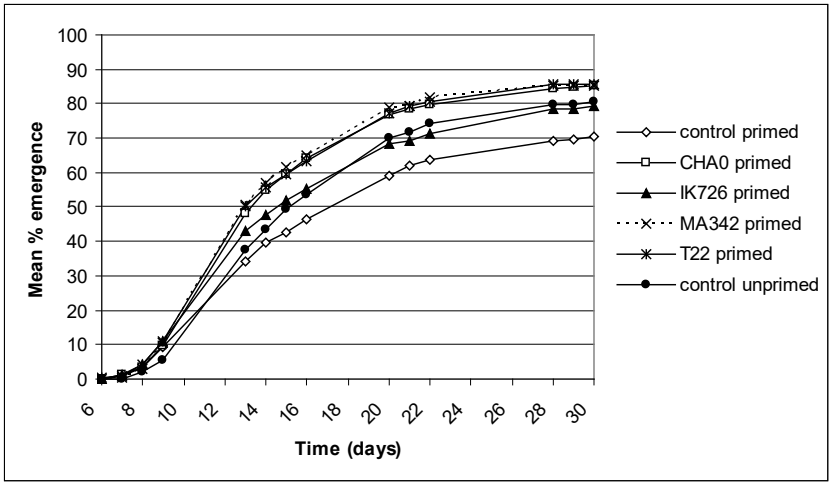


**Figure 3.1:** Mean percent emergence of onion seed primed with different microorganisms (experiment 2)

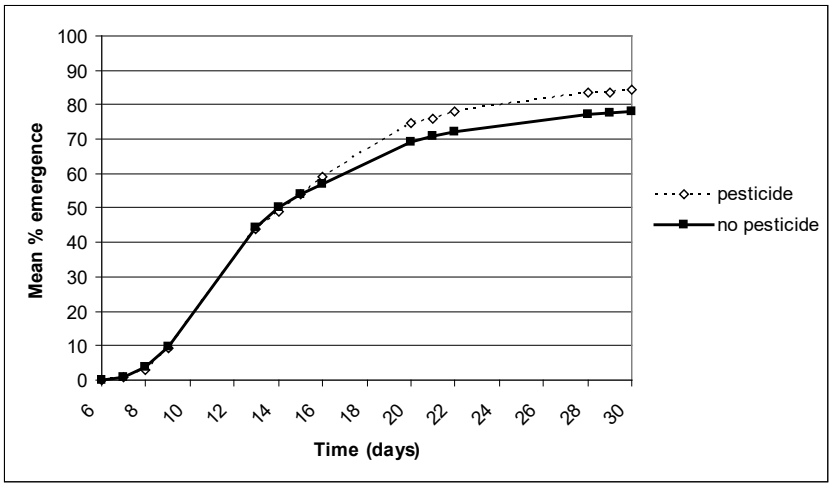


**Figure 3.2:** Mean percent emergence of onion seed with or without the application of pesticides (experiment 2)

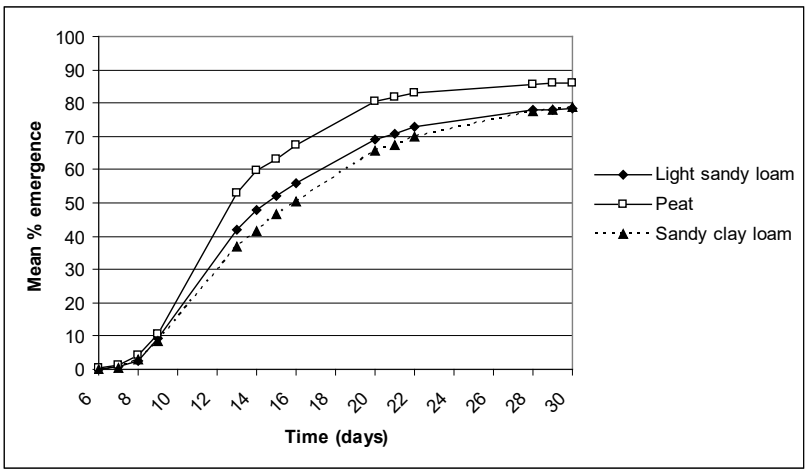


**Figure 3.3:** Mean percent emergence of treated onion seed over time in three soil types (experiment 2)

In the third experiment, the new fungal isolate being used in the field trials (*T. viride* S17a) was being tested, with the primed control, unprimed control and *T. harzianum* T22 as a comparative control to relate back to the earlier experiments. Unfortunately, emergence was poor throughout the experiment, reaching only 50% by the end of 8 weeks, and direct comparisons with the first two experiments could not be made. **No microorganism effect was seen** regarding emergence or mean fresh weight. Pesticide application increased the mean emergence time, but did not affect the final percent emergence at 8 weeks. The peat soil resulted in the lowest final percent emergence in this third experiment.

As there was no consistency with the three experiments, it is difficult to compare the results of the onion glasshouse work over three years. **Importantly, however, results from the second experiment did show that applying microorganisms to seed can improve emergence if the seed batch is contaminated with a deleterious or possibly pathogenic microorganism.**

*Carrot glasshouse experiments*

Unlike the onion experiments, the carrot experiments showed consistent results for two consecutive years. As there was no change in the carrot seed treatments throughout this work, the carrot glasshouse experiment was not repeated for the third year. Representative examples of the results are given in Figure 3.4 – 3.6 (experiment 2). **All the primed treatments emerged significantly faster than the unprimed control** (Figure 3.4). **Seed treated with *C. rosea* IK726 emerged faster than the primed control in both experiments**, although there was no significant difference in the final percent emergence. **Pesticide application also improved emergence** (Figure 3.5). Soil type influenced emergence time, with the sandy clay loam soil resulting in the fastest emergence in both experiments (Figure 3.6). Emergence was also faster in the light sandy loam than the peat soil.

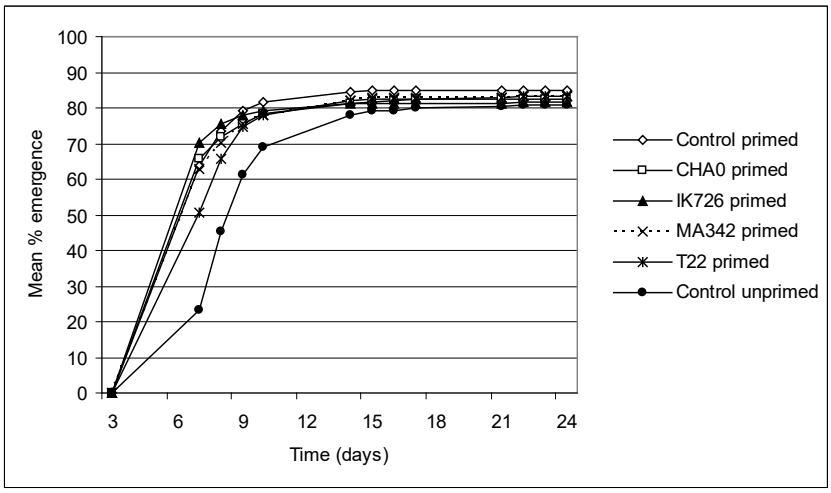


**Figure 3.4:** Mean percent emergence of carrot seed primed with different microorganisms (experiment 2)

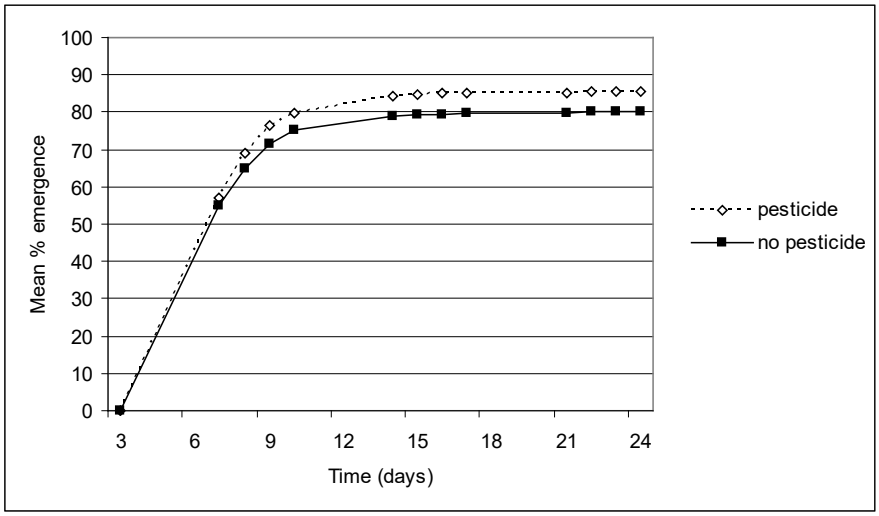


**Figure 3.5:** Mean percent emergence of carrot seed with or without pesticide application (experiment 2)

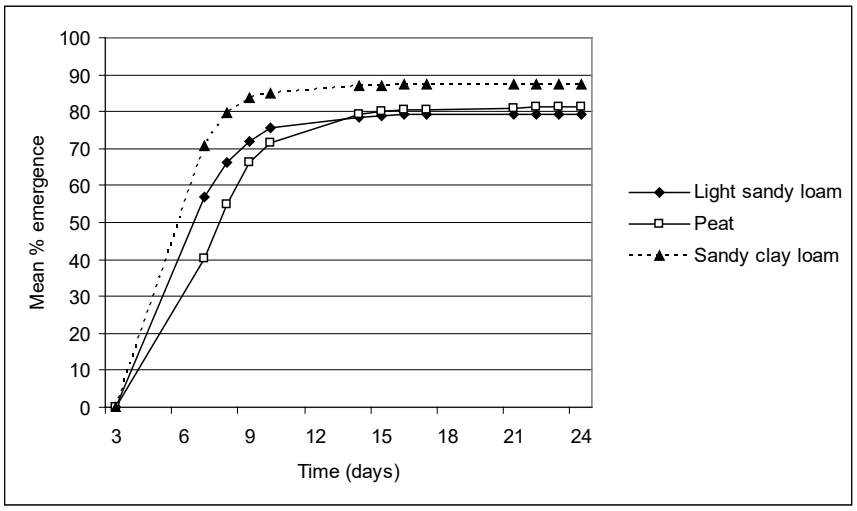


**Figure 3.6** Mean percent emergence of treated carrot seed over time in three soils types (experiment 2)

***Pathogen bioassays***

Sclerotium cepivorum *pathogen bioassay on onions*

The overall emergence count was determined from all seedlings that emerged, including those that subsequently died (Figure 3.7). 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 3.7).

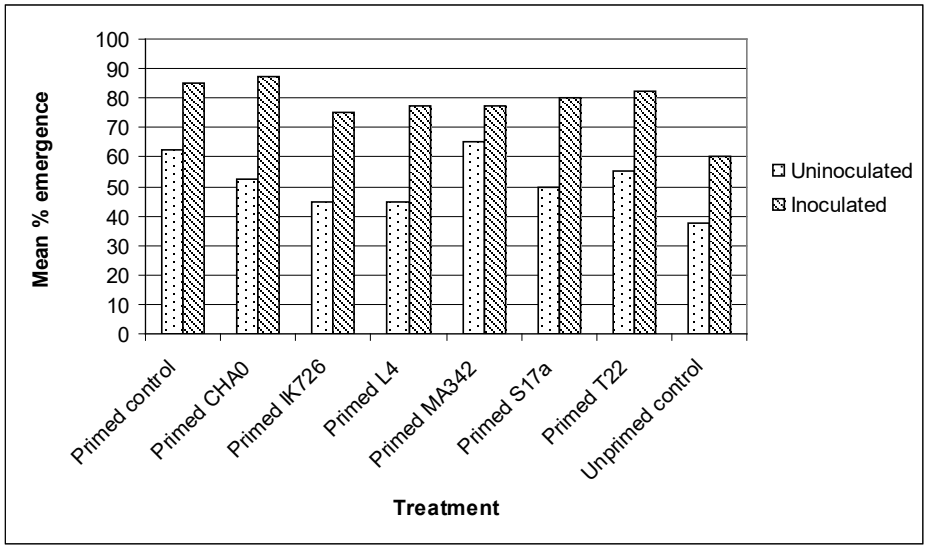


**Figure 3.7:** Mean percent emergence of treated onion seed in soil inoculated or not with *Sclerotium cepivorum* sclerotia (5 sclerotia/g soil)

Of the emerged seedlings, the proportion of those affected by *Allium* white rot (AWR) was calculated (Figure 3.8). Statistically, there was no significant difference in the percent AWR between the primed control and those treatments with microorganisms applied to the seed.

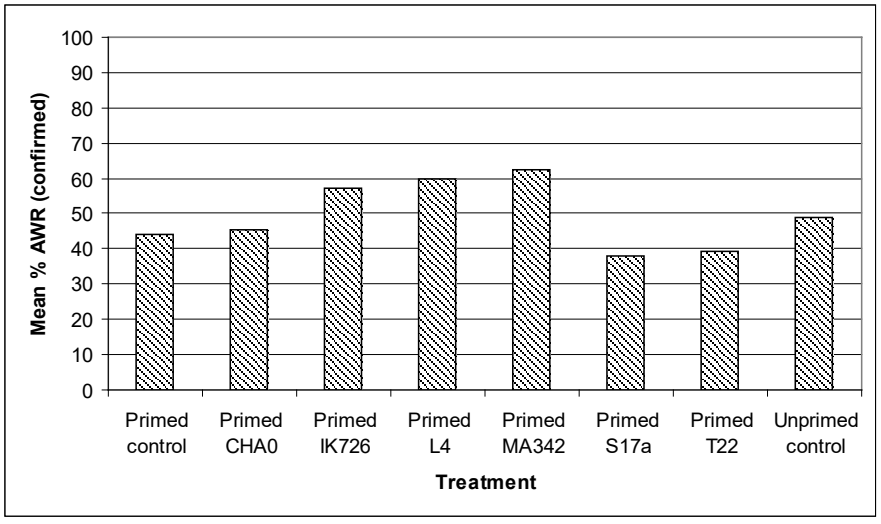


**Figure 3.8:** Mean percent of onion seedlings with confirmed *Allium* white rot (sclerotia present), following growth in soil amended with 5 sclerotia/g soil.

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

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 3.9). There was no significant effect of microorganism treatment on the overall emergence.

Fresh root weight per seedling was significantly higher when the pathogen inoculum was added (Figure 3.10). 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 3.10).

The addition of the CPS inoculum also caused a significant increase in the fresh shoot weight per seedling (Figure 3.11). 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.

The pathogen inoculum in this experiment affected the results, and consequently the

use of a different inoculum source will be required to reduce the effect of additional nutrition from the sterilised soil carrier in the future.

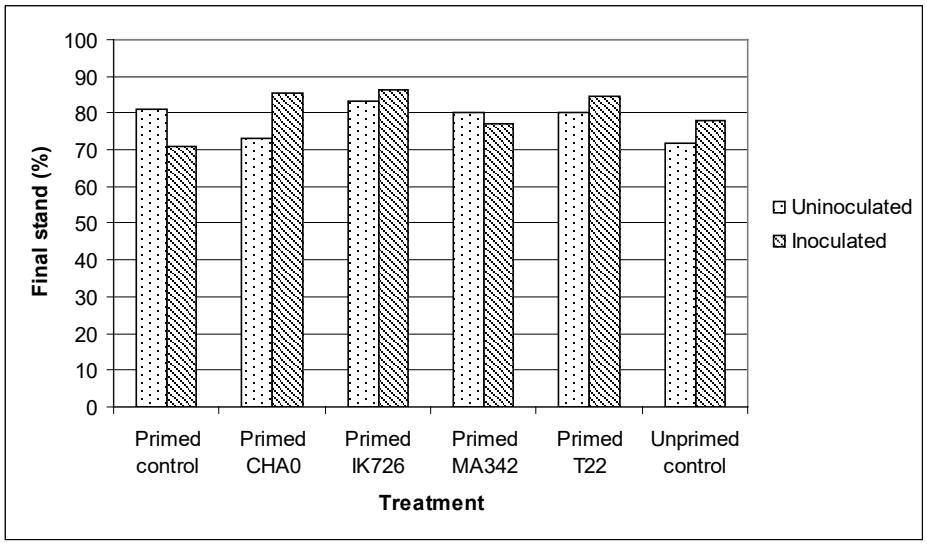


**Figure 3.9:** Overall emergence of carrot seed primed with different microorganisms, in soil either inoculated with *Pythium ultimum* (1 x $10^3$ cfu $g^{-1}$ soil) or left uninoculated.

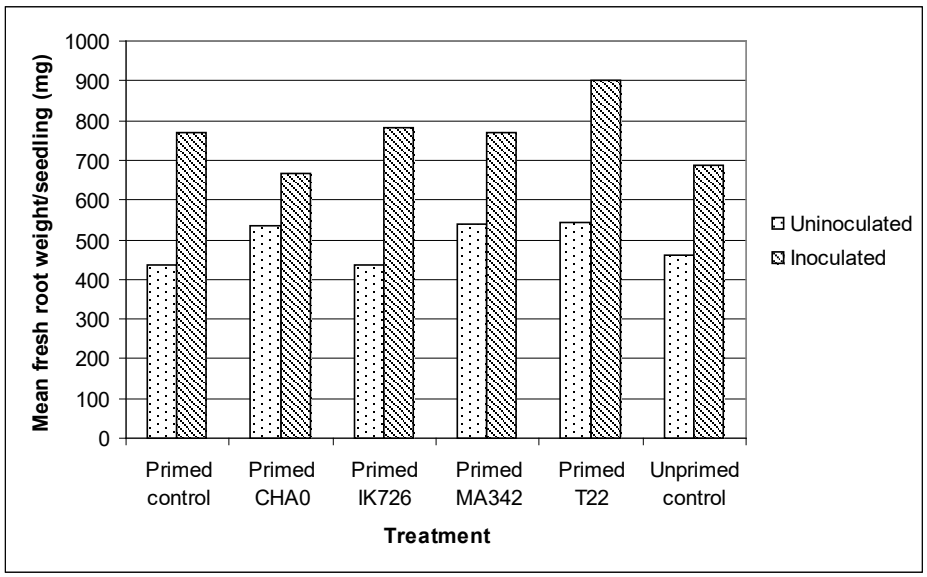


**Figure 3.10:** Mean fresh root weight per carrot seedling at harvest, following growth for 10 weeks in soil inoculated with *Pythium ultimum* (1 x $10^3$ cfu $g^{-1}$ soil) or left uninoculated

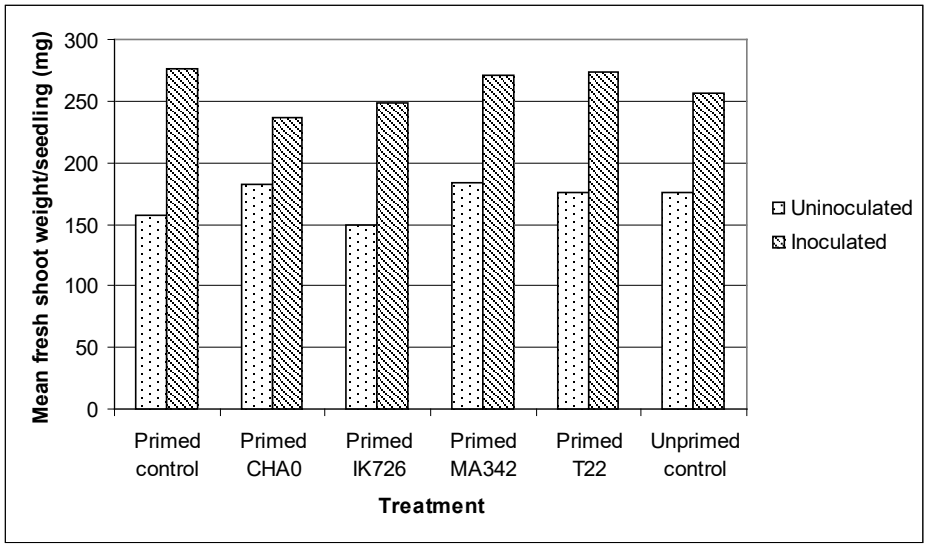


**Figure 3.11:** Mean fresh shoot weight per carrot seedling at harvest, following growth for 10 weeks in soil inoculated with *Pythium ultimum* (1 x $10^3$ cfu $g^{-1}$ soil) or left uninoculated

**Conclusions: Emergence and growth bioassays**

*Onion*

- Application of beneficial microorganisms to seed improved the emergence of seed contaminated with a deleterious microorganism
- Pesticide application decreased the mean fresh weight of onion seedlings at 8 weeks, which was consistent in three experiments.
- No consistent microorganism effects were seen over the course of three experiments, although a direct comparison is difficult due to variable emergence of the treated seed in experiments 2 and 3.

*Carrot*

- Results from two experiments examining the emergence and growth of carrot seed primed with microorganisms were consistent.
- All primed treatments emerged faster than the unprimed control.
- *Clonostachys rosea* IK726 improved emergence over the primed control.
- Emergence was fastest in the sandy clay loam soil, but the greatest mean fresh weight of carrot seedlings was found in peat soil.

**Conclusions: Pathogen bioassays**

*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.

*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.

# Objective 04: Determine the survival and proliferation of seed-applied microorganisms on seedlings after sowing in soil in the glasshouse and field

## Introduction

Experiments were run in the glasshouse to monitor the survival of marked strains of microorganisms on onion and carrot roots and in rhizosphere soil, following application to seed during drum priming. 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 or *T. viride* S17a, 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.

The planned monitoring of survival of applied microorganisms on seedlings in field trials was not carried out as PSD refused permission for use of the marked strains of microorganisms.

## Materials and methods

*Seed preparation and initial cfu counts*

Seed samples were drum primed at Warwick HRI, as described in Objective 01. Enumeration of applied microorganisms was carried out as described in Objective 01, followed by plating onto appropriate media, *ie*. rifampicin amended (100µg $ml^{-1}$) agar for the bacteria; Triton X-100 (2ml $l^{-1}$) and hygromycin B amended (150µg $ml^{-1}$) agar for *C. rosea* IK726; and *Trichoderma* semi-selective medium for *T. harzianum* T22 and *T. viride* S17a (Appendix 4.1).

*Glasshouse design and set up*

For the first experiment for each crop type (carrot and onion), four replicates were set up in the glasshouse at different times. There were five different seed treatments including a primed control, primed *P. fluorescens* CHA0, *P. chlororaphis* MA342, *C. rosea* IK726 and *T. harzianum* T22. Each treatment was set up in three soil types (light sandy loam, peat soil and sandy clay loam), with one pot per seed-treatment/soil-type combination and 4 seeds planted per pot. For the second experiment for each crop type, the experiment remained the same, except that the number of pots per treatment was increased to 4 pots (increasing the maximum up to 16 seedlings). This was done so that more root material was available, particularly for the 2 week sample, and also to get a better representation of the microorganism survival on a larger number of roots.

Enough pots were set up to allow for sampling at 2, 4 and 8 weeks post-planting. Emergence and growth of seedlings for all treatments was monitored, and survival of the microorganisms on roots and in rhizosphere soil was assessed at the different sample times. At each time interval, the seedlings were harvested and rhizosphere soil was washed off in 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 SDW water using a sterile mortar and pestle, and a dilution series was similarly plated onto selective media (root count).

A smaller third experiment was set up to determine the survival of *T. viride* S17a on onion roots and in rhizosphere soil, as this was a new seed treatment in Year 3 (2006). Treatments for this experiment included a primed control, primed *P. fluorescens* CHA0, primed *T. harzianum* T22 and primed *T. viride* S17a. As for the second experiment, four pots per treatment per sampling time were set up.

**Results and discussion**

The first set of experiments showed some trends in the survival of the microorganisms on the onion and carrot roots and in rhizosphere soil, but the larger amount of material in the second set of experiments allowed for a more rigorous analysis. Consequently, representative examples of the survival of the seed-applied microorganisms from the second set of experiments are given in Figure 4.1-4.4. Results from one soil type only (light sandy loam) are shown, as the survival patterns were largely similar in all three soils.

*Onion glasshouse experiments*

On onion, *Pseudomonas fluorescens* CHA0 declined in number overall from what was applied to the seed initially (Figure 4.1). At 8 weeks less than 1 $\log_{10}$ cfu per seedling was recovered for root and soil samples. However, there was some increase in number on the roots from 4 to 8 weeks in the peat and sandy clay loam soil, and these were above 2 $\log_{10}$ cfu per seedling at the end of the experiment (not shown). *Pseudomonas chlororaphis* MA342 survived poorly on onion roots and in rhizosphere soil (Figure 4.1). There was a decline in numbers over time to approximately 1 $\log_{10}$ cfu per seedling or less by the end of 8 weeks in all soils.

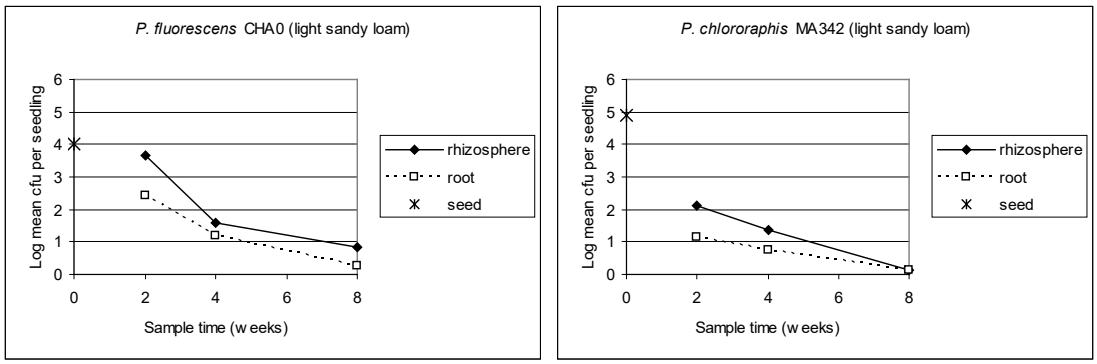


**Figure 4.1:** Survival of bacterial isolates *Pseudomonas fluorescens* CHA0 and *Pseudomonas chlororaphis* MA342 on onion roots and in the rhizosphere in light sandy loam soil (Experiment 2). LSD (0.05) comparing same microorganism at different times in the same soil: rhizosphere (df = 72) = 1.09; root (df = 70) = 1.00

Overall, *C. rosea* IK726 showed very good survival on onion roots and in rhizosphere soil (Figure 4.2). Although the numbers recovered at 2 weeks post planting were lower than had been applied to the seed initially, the numbers increased significantly over time to achieve 4 $\log_{10}$ cfu per seedling in the rhizosphere soil, and 3 $\log_{10}$ cfu per seedling on roots. *Trichoderma harzianum* T22 did survive on the onion roots and in rhizosphere soil, although not in high numbers on the roots (Figure 4.2). A drop in numbers was found at the 2 week sampling time from what was found on the seed initially. However, in the light sandy loam soil, an increase was seen over time, with numbers greater than 3 $\log_{10}$ cfu per seedling in the rhizosphere soil at 8 weeks. No data are available for *T. viride* S17a survival on onion roots and in rhizosphere soil as this isolate could not be recovered or identified on the agar plates. This may be due to low numbers of this isolate on the seed initially.

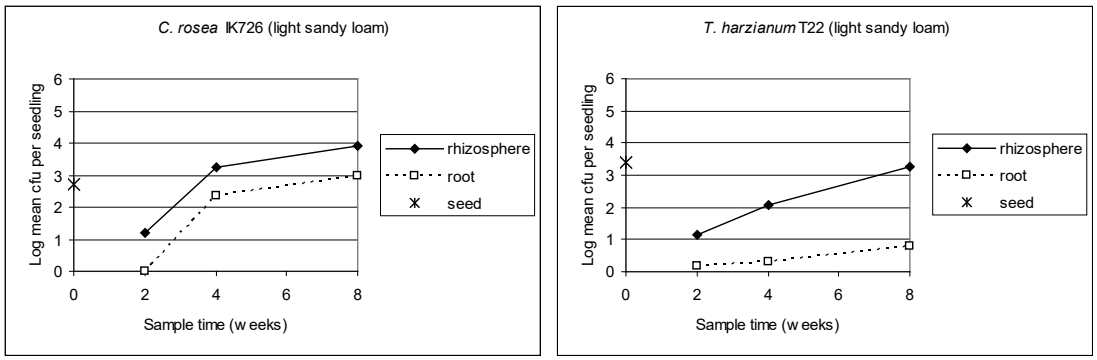


**Figure 4.2:** Survival of fungal isolates *Clonostachys rosea* IK726 and *Trichoderma harzianum* T22 on onion roots and in the rhizosphere in light sandy loam soil (Experiment 2). LSD (0.05) comparing same microorganism at different times in the same soil: rhizosphere (df = 72) = 1.09; root (df = 70) = 1.00

*Carrot glasshouse experiments*

*Pseudomonas fluorescens* CHA0 was recoverable from the carrot roots and rhizosphere soil at all sampling time in all three soils (Figure 4.3). In the light sandy loam the numbers decreased in the rhizosphere from 2 to 4 weeks, but increased again by 8 weeks post planting. There was an increase in numbers recovered from the roots at all times in this soil type. However, numbers declined overall from what was found on the seed initially. *Pseudomonas chlororaphis* MA342 did not survive well on the carrot roots or in the rhizosphere soil, with numbers recovered below 2 $\log_{10}$ cfu per seedling at 4 and 8 weeks (Figure 4.3).

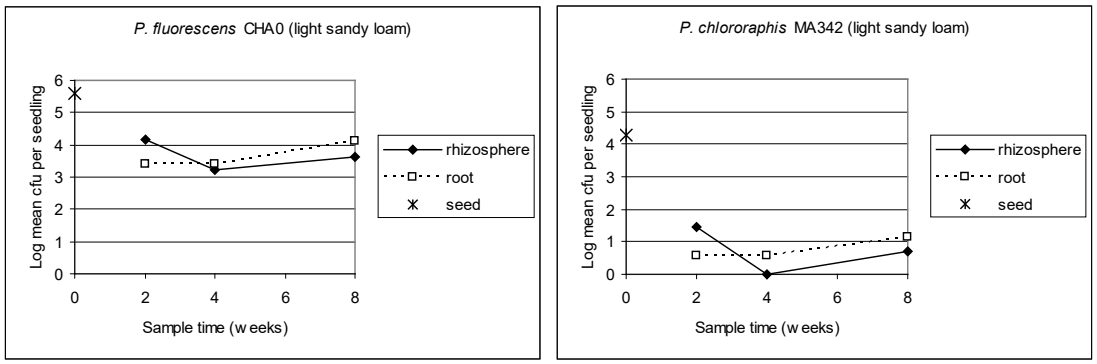


**Figure 4.3:** Survival of bacterial isolates *Pseudomonas fluorescens* CHA0 and *Pseudomonas chlororaphis* MA342 on carrot roots and in the rhizosphere in light

sandy loam (Experiment 2). LSD (0.05) comparing same microorganism at different times in the same soil: rhizosphere (df = 71) = 0.88; root (df = 72) = 1.11

*Clonostachys rosea* IK726 increased in number over time in all the soil types (Figure 4.4). From the initial numbers found on the seed, a drop in numbers was found at 2 weeks on the roots and rhizosphere soil. However, this was followed by an increase in number on the carrot roots in all soil types at all sampling times. In the rhizosphere soil, *C. rosea* IK726 increased in all soil from 2 to 4 weeks, but declined slightly in number from 4 to 8 weeks in the light sandy loam soil. Overall *C. rosea* IK726 survived well and was still recovered at over 3 $\log_{10}$ cfu per seedling at 8 weeks in all soils. Like *C. rosea* IK726, *T. harzianum* T22 decreased in number from what was initially on the seed by the 2 week sampling time (Figure 4.4). However, a continued increase in number was seen on the roots and in rhizosphere soil for the light sandy loam at all times, and by 8 weeks the numbers were recovered at approximately 4 $\log_{10}$ cfu per seedling.

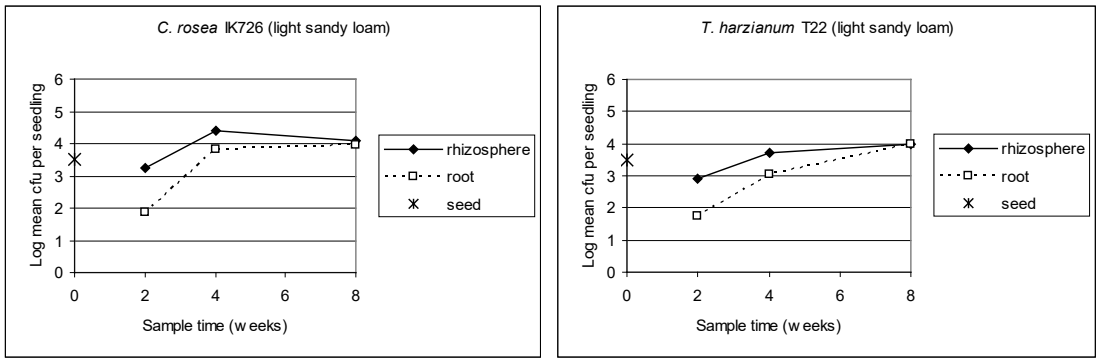


**Figure 4.4:** Survival of fungal isolates *Clonostachys rosea* IK726 and *Trichoderma harzianum* T22 on carrot roots and in the rhizosphere in light sandy loam soil (Experiment 2). LSD (0.05) comparing same microorganism at different times in the same soil: rhizosphere (df = 71) = 0.88; root (df = 72) = 1.11

**Conclusions:**

- A larger volume of plant material allowed for improved recovery of the microorganisms from roots and rhizosphere soil.

*Onion*

- Bacterial survival on onion roots and in rhizosphere soil was variable, but generally decreased with time.
- *Clonostachys rosea* IK726 survived well and increased in number on onion roots and in rhizosphere soil in three soil types.
- *Trichoderma harzianum* T22 was recovered at all times from roots and rhizosphere soil, if a high enough inoculum dose was used on the seed initially.
- 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.

*Carrot*

- 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

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

## Introduction

Work was undertaken in Objective 05 to monitor the long-term survival of the selected beneficial BCAs on stored seed following priming inoculation. The effects of subsequent film coating of carrot and pelleting of onion, with and without pesticides applied at the standard rate, on survival of the beneficials was also determined.

## Materials and methods

All the selected beneficial microoganisms used in the field trials and glasshouse experiments (*P. fluorescens* CHA0, *P. chlororaphis* MA342, *T. harzianum* T22, *C. rosea* IK726, *T. viride* S17a) were applied to carrot and onion seed during the steeping priming process (GTG UK). The minimum target of the initial populations was 5 $\log_{10}$ cfu $g^{-1}$ dry seed.

### *Bacterial inoculum preparation*

*Rif* $^{+}$ - marked strains of the bacteria supplied by Warwick HRI were cultured on nutrient agar at 25 ˚C. 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 PDA at 20 ˚C. Following profuse sporulation (7-10 d for *T. harzianum* T22 and 12-15 d for *C. rosea* IK726) 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 PDA.

*Steeping priming and microorganism reisolation*

In these experiments, microorganisms were applied to seed during steeping priming rather than drum priming. 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 microorganisms was carried out at the end of each of these phases as well as during subsequent storage at 15 ˚C. After drying back, sub-samples of the primed seed were removed for further seed processing.

Samples of carrot seed were film coated both with and without the standard rate of Wakil XL. Samples of onion seed were pelleted both with and without standard rates of HY-TL (with the exception of *T. viride* S17a, which was tested on raw seed only). All treated samples of both onion and carrot seed were air-dried at room temperature. The pelleted seed was graded to a final size of 3.5 - 5.0 mm. Initial seed samples were removed for analysis after drying and again at 3, 7, 14 and 21 d storage with the dried back sample constituting the T = 0 sample. Subsequent samples were analysed after 1, 2, 3, 6 and 12 months, with the exception of *T. viride* S17a on carrot and onion, which were sampled until 3 months only.

Three replicate 0.5 g samples of seed 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 solid media (nutrient agar containing 100 μg $ml^{-1}$ rifampicin for the bacteria and potato dextrose agar agar containing 30 μg $ml^{-1}$ chlortetracycline for the fungi). Colonies were counted after 2 d incubation at 25 ˚C and 20 ˚C for the bacteria and fungi respectively. The numbers of colonies were expressed as cfu $g^{-1}$ dry weight seed following $log_{10}$ transformation. Significant differences between sample means (Fisher's protected LSD ($P$ = 0.05)) were determined by ANOVA.

**Results and discussion**

*Onion seed shelf-life studies*

The long-term survival of a representative fungal and bacterial isolate are represented in Figures 5.1-5.2. Pelleting had a significant negative impact on all beneficials. Application of the pesticides had no effect on survival of the bacteria when compared with pelleting alone but significantly reduced the survival of both fungal isolates, with the effect on *C. rosea* IK726 being particularly profound (Figure 5.1). Results from the pesticide compatibility studies (Objective 01) suggest that this effect was due to the HY-TL fungicide.

The population of *C. rosea* IK726 on the pelleted treatment remained within the target application range up to the 180 d storage interval although the population had declined below this level after 85 d storage after application of the pesticides (Figure 5.1). *Trichoderma harzianum* T22 maintained populations of all treatment above the target application range up to the 180 d storage interval (data not shown).

*Pseudomonas chlororaphis* MA342 maintained populations of all treatment above the target application range up to the 6 month storage interval, whereas pelleted populations of *P. fluorescens* CHA0 had declined below this level after approximately 120 d and 85 d storage in the presence and absence of the pesticides respectively (Figure 5.2).

The new isolate for the Year 3 (2006) onion field trials, *T. viride* S17a showed good survival on raw onion seeds for up to 90 days, remaining above the target of 5 $\log_{10}$ cfu $g^{-1}$ seed (Figure 5.3).

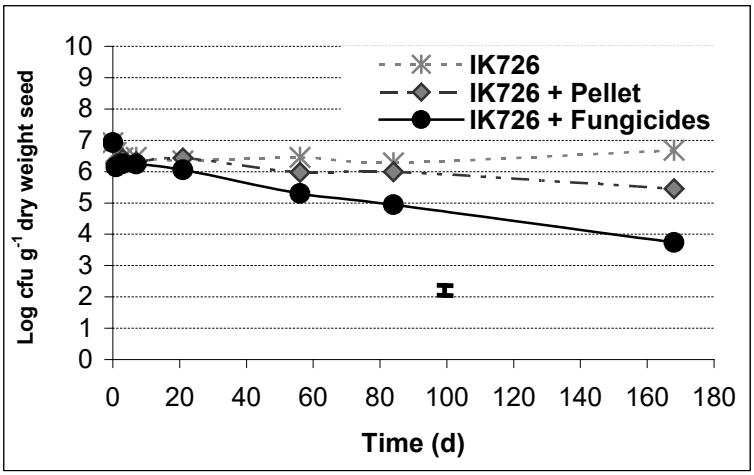


**Figure 5.1:** Shelf life study illustrating survival of *Clonostachys rosea* IK726 on onion seed following steeping priming. Bar indicates least significant difference.

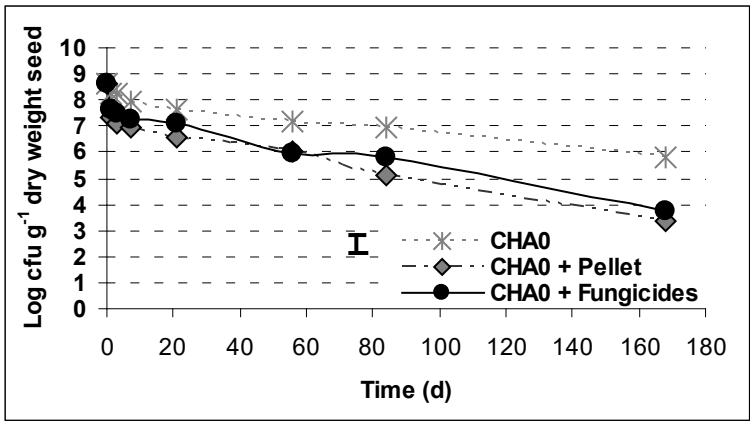


**Figure 5.2:** Shelf life study illustrating survival of *Pseudomonas fluorescens* CHA0 on onion seed following steeping priming. Bar indicates least significant difference.

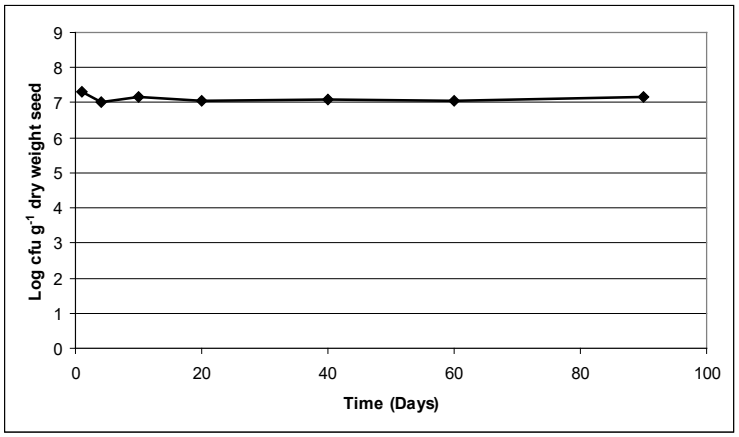


**Figure 5.3:** Shelf life study illustrating survival of *Trichoderma viride* S17a on onion seed following steeping priming.

*Carrot seed shelf-life studies*

The long-term survival of a representative fungal and bacterial isolate on carrot seed is given in Figures 5.4-5.5. Film coating with and without the fungicide had a marginal negative impact on the microorganisms which was more pronounced on the fungal isolate, *T. harzianum* T22. Despite this, neither film coating nor addition of the fungicide significantly affected survival of any of the fungal isolates (*eg.* Figure 5.4). From the original application rates achieved in this experiment, film coated treatments of *T. harzianum* T22 maintained populations within the target application range for approximately 60 d and 100 d in the presence and absence of the fungicide respectively in the first shelf-life study. A later study showed that *T. harzianum* could survive above the target rate on carrot seed for up to 90 days if applied at a higher rate initially (data not shown). Film coated treatments of *P. chlororaphis* MA342 and *P. fluorescens* CHA0 (*eg.* Figure 5.5) maintained populations within the target application range for approximately 275 d and 150 d respectively.

The isolate *T. viride* S17a was also used in a shelf-life study on raw carrot seed (not fimcoated, nor filmcoated with pesticides). Results show that this fungus could survive up to at least 90 days (length of the test) above the target rate (data not shown).

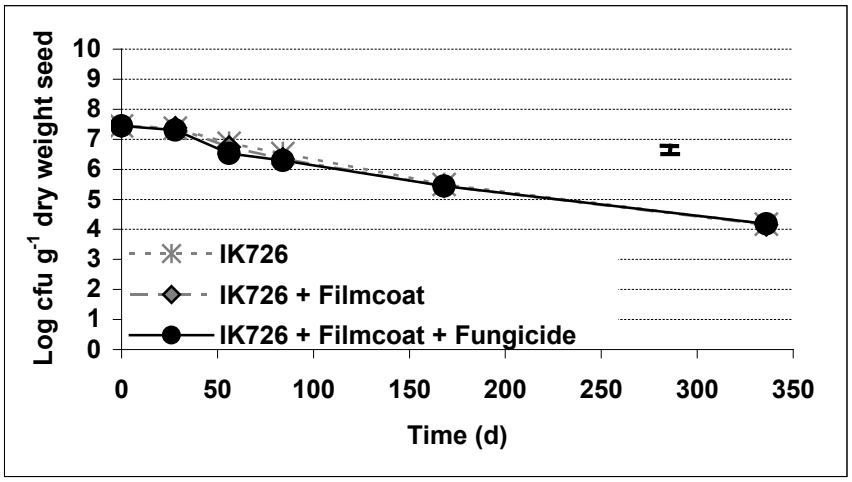


**Figure 5.4:** Shelf life study illustrating survival of *C. rosea* IK726 on carrot seed following steeping priming. Bar indicates least significant difference.

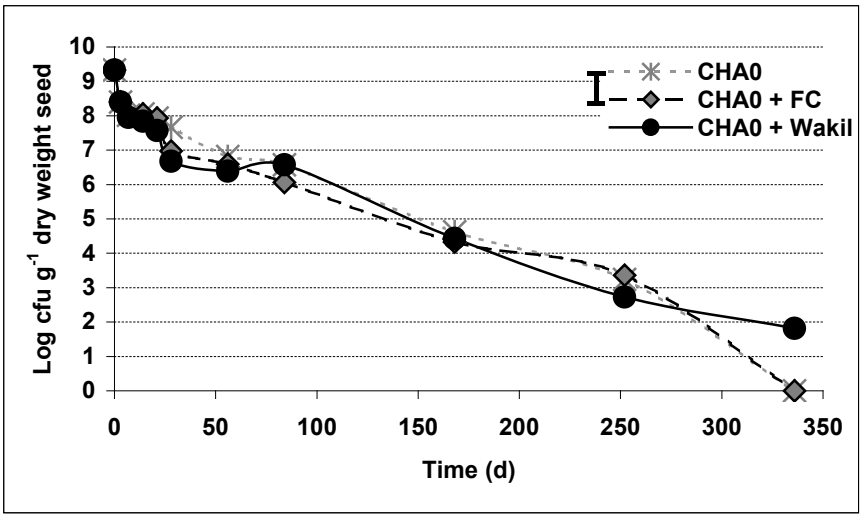


**Figure 5.5:** Shelf life study illustrating survival of *Pseudomonas fluorescens* CHA0 on carrot seed following steeping priming. Bar indicates least significant difference.

**Conclusions**

- All microorganisms were successfully applied to onion and carrot seed during steeping priming.
- A higher initial inoculum on the seed allowed the microorganisms to survive at 15°C for a longer storage time above the target of 5 $\log_{10}$ cfu $g^{-1}$ seed.
- Pelletting onion seeds reduced the survival of all microorganisms in the long term possibly due to the continued wetting and drying cycle involved in the pelletting process.
- The application of pesticide to the onion pellet significantly reduced the survival of the fungal isolates, particularly *C. rosea* IK726.
- Filmcoating, and the application of pesticides during filmcoating, did not significantly affect the survival of microorganisms on carrot seed.

# Objective 06: Examine the ability to co-apply selected microorganism combinations

## Introduction

Following on from the successful application of microorganisms applied singly to carrot and onion seed, further work involved assessing the ability to co-apply beneficial microorganisms to seed. Experiments in the glasshouse were then set up to look at the emergence and growth of seeds primed with a combination of microorganisms, as well as the survival of the co-applied microorganisms on roots and in rhizosphere soil.

## Materials and methods

*Microorganism preparation and application to seed*

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 x $10^5$ cfu $g^{-1}$ dry seed for each fungal isolate for carrot, and 1 x $10^7$ cfu $g^{-1}$ dry seed for each fungal isolate for onion. 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.

Bacteria were retrieved from a cryopreservative bead storage system and plated onto nutrient agar. Single colonies resulted from this were inoculated into sterile nutrient broth (100ml) and incubated overnight at 26°C, in a rotary shaker set at 180 rpm. The following morning, 1ml of overnight culture was transferred to fresh nutrient broth and incubated for approximately 4 hours until spectrophotometry ($OD_{600}$) showed the numbers to be in the region of 1 x $10^7$ cfu $ml^{-1}$, determined from a previously prepared standard growth curve. Twenty millilitres of the bacterial broth was centrifuged (5000 rpm, 10 minutes), and the resulting pellet resuspended in 20 ml sterile distilled water (SDW). Based on the numbers calculated from the standard

growth curve, the required amount of bacterial suspension to give the desired inoculum rate was added to the water to be used in the priming process. A rate of 1 x $10^5$ cfu $g^{-1}$ dry seed for each bacterial isolate was used for the carrot combination priming, and 1 x $10^6$ cfu $g^{-1}$ dry seed for each bacterial isolate for the onion combination priming. A dilution series of the bacterial suspension was also plated to verify the number of colony forming units (cfu) present.

Once prepared, the fungal and bacterial suspensions were mixed together and topped up with SDW as required to achieve the correct volume of liquid for the priming process. The combined suspension was added to seed batches during the hydration phase.

*Seed sampling during priming*

Seed sampling was carried out at the end of each of the three main phases of the drum priming process: hydration, incubation and drying back (see Objective 01). At each sampling time, 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 $l^{-l}$) for the fungi, and 0.1 tryptone soya agar amended with rifampicin (150µg $ml^{-1}$) for the bacteria. Colonies were counted after 7 days incubation at 20°C. Three replicate 0.5g seed samples were also dried to a constant weight to determine the cfu $g^{-1}$ dry seed.

*Glasshouse experiment – emergence and growth*

Glasshouse experiments were set up in peat soil only for each of the carrot and onion crops. For each experiment there were four replicates, each with 10 seed treatments: primed control, unprimed control, primed with *P. fluorescens* CHA0, primed with *P. chlororaphis* MA342, primed with *C. rosea* IK726, primed with *T. harzianum* T22, primed with a combination of *P. fluorescens* CHA0 and *T. harzianum* T22, primed with a combination of *P. fluorescens* CHA0 and *C. rosea* IK726, primed with a combination of *P. chlororaphis* MA342 and *T. harzianum* T22, and primed with a combination of *P. chlororaphis* MA342 and *C. rosea* IK726. Each treatment consisted of 6 pots, with four seeds planted in each.

Pots were watered from below and emergence was assessed until no further increase in numbers was seen. After 8 weeks the experiment was 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 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).

*Glasshouse experiment – survival of microorganisms*

Experiments were conducted in peat soil only to investigate the survival of microorganisms applied in combination to carrot and onion seed. For each experiment there were four replicates, each with 9 seed treatments: primed control, primed with *P. fluorescens* CHA0, primed with *P. chlororaphis* MA342, primed with *C. rosea* IK726, primed with *T. harzianum* T22, primed with a combination of *P. fluorescens* CHA0 and *T. harzianum* T22, primed with a combination of *P. fluorescens* CHA0 and *C. rosea* IK726, primed with a combination of *P. chlororaphis* MA342 and *T. harzianum* T22, and primed with a combination of *P. chlororaphis* MA342 and *C. rosea* IK726. Each treatment consisted of 12 pots, with four seeds planted in each. At each sampling time of 2, 4 and 8 weeks post-planting, four pots were removed from each treatment. The seedlings from these four pots were grouped together and made up the treatment sample for that assessment time. Rhizosphere soil was washed off the roots and the clean roots were crushed in a mortar as described in Objective 04. Dilution series were prepared and plated onto selective agar media, and the numbers of seed-applied microorganisms surviving on the roots and in the rhizosphere soil were enumerated as described previously.

An estimate of the numbers on the seed initially was also made by grinding a single sample (0.5g) of seed and preparing a dilution series on selective agar.

**Results and discussion**

*Survival during priming*

The survival patterns for the combinations were found to be similar to the patterns seen when the microorganisms were applied separately to carrot and onion seed during priming (see Objective 01). A representative example of one fungal/bacterial combination is given for each of the onion and carrot seed in Figure 6.1 and 6.2. On onion seed, the fungi decreased in numbers overall, as they were found to do when applied singly (Figure 6.1; see also Objective 01). The bacteria were applied at a slightly higher rate to onion seed initially, based on results of previous work where they had been applied singly. Previous work also showed that fungal isolates tended to decrease in number on onion seed following application during priming, so consequently they were applied at a much higher rate initially to compensate for this.

The survival patterns of the microorganisms applied in combination were similar to those seen when applied individually (Figure 6.1). The bacteria increased in number to the end of the incubation phase before decreasing slightly after drying back. The final numbers were higher than the initial inoculum level for all of the treatments, apart from *P. fluorescens* CHA0 in combination with *C. rosea* IK726, which dropped in number overall but remained above the target after drying back the seed. The fungal isolates all decreased in number from the initial application rate by the end of the drying back process, but were still recovered above the target application of 5 $\log_{10}$ cfu $g^{-1}$ dry seed.

A similar situation was found with the carrot seed, where microorganisms applied in combination followed the same survival trends as when they were applied singly. Generally the bacteria dropped in number after the hydration phase, but recovered to high numbers after incubation, with a slight decrease again after drying back (Figure 6.2). The fungi were largely unrecoverable after the hydration phase, but increased in numbers after incubation, achieving a final rate higher than the initial application rate on carrot (Figure 6.2).

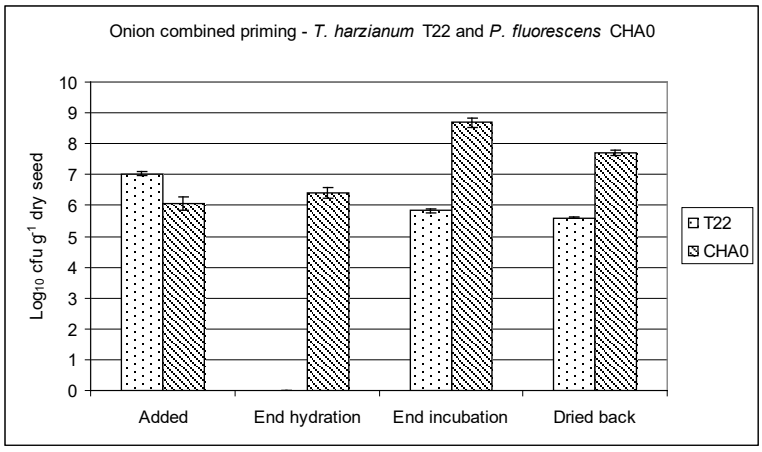


**Figure 6.1:** Survival and proliferation of beneficial microorganisms applied in combination to onion seed during drum priming. Added = initial numbers in liquid suspension applied to seed; End hydration = numbers recovered after all liquid spore suspension added to seed; End incubation = numbers recovered after seed incubated in rotating jars for 7 days; Dried back = numbers recovered when seed air-dried. Bars indicate standard error of the mean (d.f. = 2).

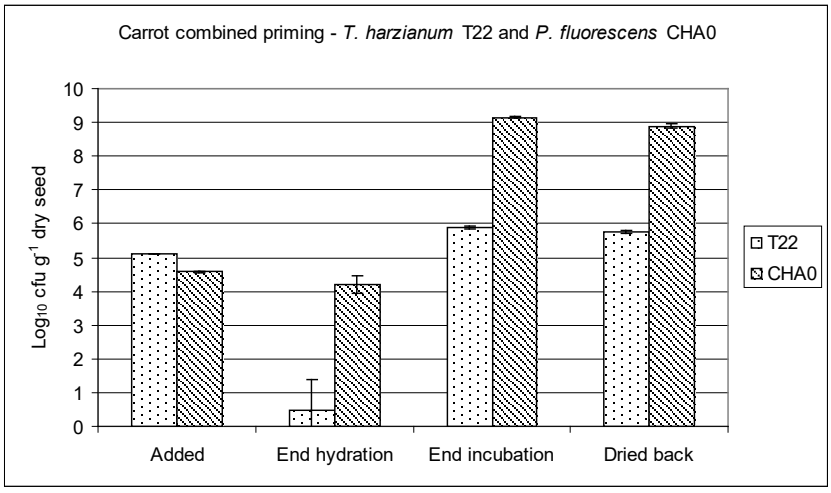


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**Figure 6.2:** Survival and proliferation of beneficial microorganisms applied in combination to carrot seed during drum priming. Added = initial numbers in liquid suspension applied to seed; End hydration = numbers recovered after all liquid spore suspension added to seed; End incubation = numbers recovered after seed incubated in rotating jars for 7 days; Dried back = numbers recovered when seed air-dried. Bars indicate standard error of the mean (d.f. = 2).

*Glasshouse experiment – onion emergence and growth*

Data for the onion glasshouse experiment are presented here for the first time. The mean emergence time, time to 50% and time to 80% emergence are based on the percent emergence achieved with any one particular seed treatment by the end of the assessment time at approximately 4 weeks. Consequently the difference in the percent emergence may have influenced the emergence time factors (Figure 6.3; Table 6.1). The final percent emergence as shown in Table 6.1 is based on the final stand at harvest at 8 weeks post planting.

Overall, priming improved emergence compared to the unprimed control. The final percent emergence of onion seed (as determined at harvest at 8 weeks) was significantly worse with co-application of *C. rosea* IK726 and *P. chlororaphis* MA342 compared to either of the microorganism treatments applied singly (Table 6.1). Similarly, the final percent emergence of seed treated with a combination of *T. harzianum* T22 and *P. chlororaphis* MA342 was significantly lower that *P. chlororaphis* MA342 applied alone. However, the initial number of *P. chlororaphis* MA342 on the seed in combination was at a much higher rate than the single treatment (ranging from 5.2-5.5 $\log_{10}$ cfu per seed in combination, compared to 3.4 $\log_{10}$ cfu per seed applied singly), and it may have had a phytotoxic effect at this high dose. The treatment with the highest final percent emergence was *P. chlororaphis* MA342 applied singly (Table 6.1).

A separate analysis showed that combination priming as a whole significantly reduced the final percent emergence of onion seedlings (Table 6.2). However, as mentioned above, it may be that the high dose of the bacterial isolates in the combination treatments with *C. rosea* IK726 had a phytotoxic effect, which may have influenced the results.

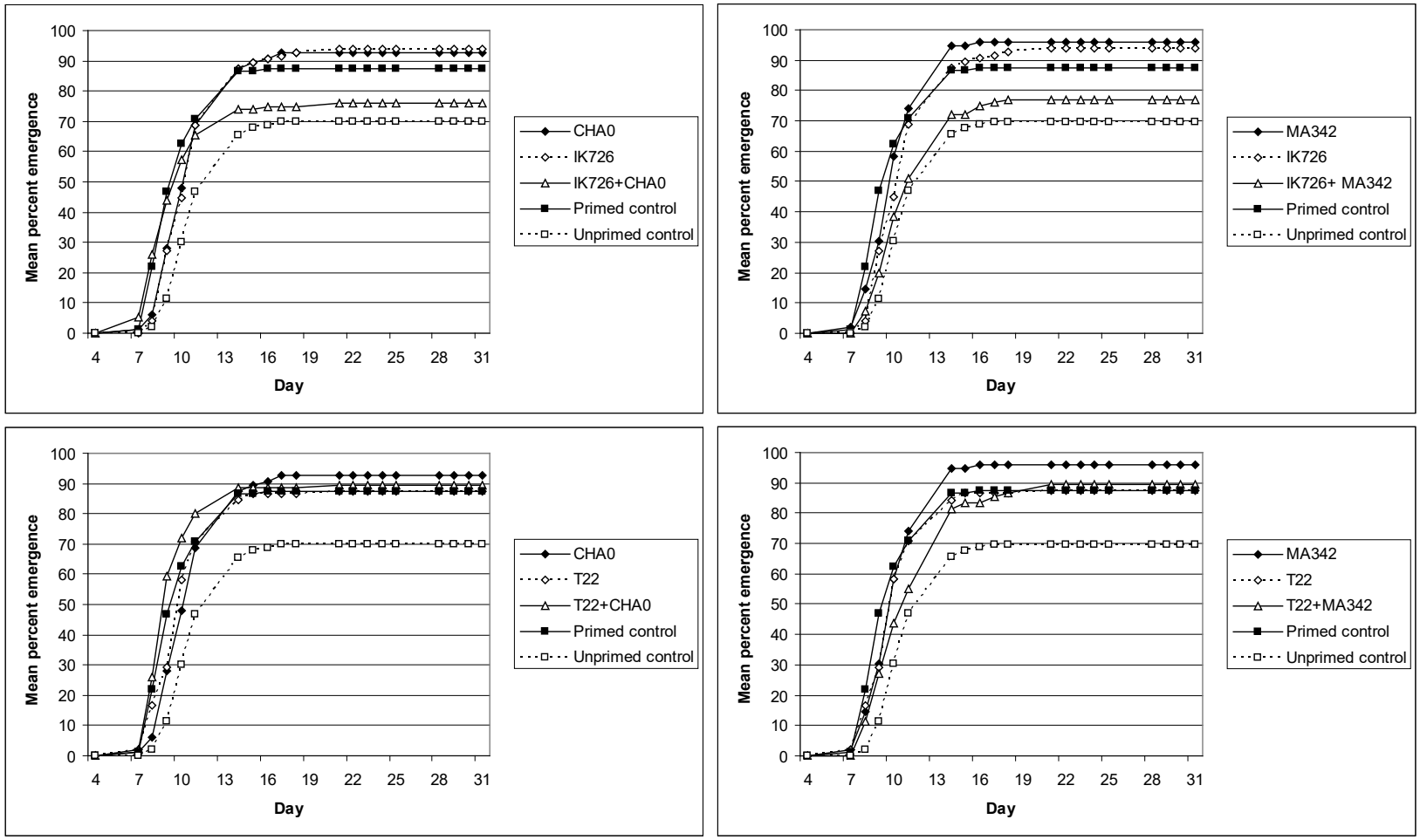


**Figure 6.3:** Emergence of onion seed primed with a combination of microorganisms, in peat soil.

**Table 6.1:** Emergence of onion seed primed with combinations of microorganisms, grown in peat soil. Data for the final percent emergence are back-transformed, and values in parentheses are means after arcsine transformation of data. Analyses were carried out on transformed data.

| Treatment | Mean emergence time (days)[a] | Time to 50% emergence (days)[a] | Time to 80% emergence (days)[a] | Final % emergence[b] |
|---|---|---|---|---|
| Unprimed control | 10.01 | 9.69 | 11.47 | 66.70 (54.76) |
| Primed control | 9.76 | 9.31 | 11.37 | 83.55 (66.07) |
| Primed CHA0 | 10.05 | 9.52 | 11.86 | 91.10 (72.64) |
| Primed MA342 | 9.91 | 9.64 | 11.13 | 94.81 (76.84) |
| Primed IK726 | 10.19 | 9.79 | 11.95 | 91.92 (73.49) |
| Primed T22 | 10.31 | 9.98 | 11.75 | 87.23 (69.07) |
| Primed IK726 + CHA0 | 9.36 | 9.14 | 10.26 | 80.28 (63.63) |
| Primed IK726 + MA342 | 10.16 | 9.55 | 11.88 | 73.05 (58.72) |
| Primed T22 + CHA0 | 10.19 | 9.81 | 11.63 | 86.76 (68.66) |
| Primed T22 + MA342 | 10.06 | 9.65 | 11.36 | 77.06 (61.38) |
| LSD (0.05; df = 27) | 1.170 | 1.184 | 1.769 | (12.126) |

[a] Emergence time factors are related to the percent emergence recorded at approximately 4 weeks post-planting

[b] Final percent emergence is based on the number of seedlings at harvest, 8 weeks post-planting

**Table 6.2:** Emergence of onion seed primed with a single microorganism, or a combination of two microorganisms, grown in peat soil. Data for the final percent emergence are back-transformed, and values in parentheses are means after arcsine transformation of data. Analyses were carried out on transformed data.

| Treatment | Mean emergence time (days)[a] | Time to 50% emergence (days)[a] | Time to 80% emergence (days)[a] | Final % emergence[b] |
|---|---|---|---|---|
| Single microorganism | 10.12 | 9.73 | 11.67 | 91.46 (73.01) |
| Combination of microorganisms | 9.94 | 9.54 | 11.28 | 79.53 (63.10) |
| LSD (0.05, df = 27) | 0.585 | 0.593 | 0.884 | (6.053) |

[a] Emergence time factors are related to the percent emergence recorded at approximately 4 weeks post-planting

[b] Final percent emergence is based on the number of seedlings at harvest, 8 weeks post-planting

The mean fresh weight per seedling after 8 weeks growth was significantly affected by the microorganism treatments (Table 6.3). The combination of *T. harzianum* T22 and *P. chlororaphis* MA342 resulted in a significantly lower mean fresh weight per seedling than either the primed or unprimed controls. In addition, seedlings treated with *T. harzianum* T22 in combination with *P. chlororaphis* MA342 had a lower mean fresh weight than those where *T. harzianum* T22 was applied on its own. In contrast, seedlings treated with *T. harzianum* T22 in combination with *P. fluorescens* CHA0 had a significantly greater mean fresh weight per seedling than those treated with *T. harzianum* T22 alone. The combination of *C. rosea* IK726 and *P. fluorescens* CHA0 resulted in a significantly greater mean fresh weight than either of the microorganism treatments applied singly (Table 6.3). The different combinations of microorganisms may have different interactions with each other and with the plant, possibly indicating that various combinations would need to be tested on each crop to achieve the best selection for that species.

**Table 6.3:** Mean fresh weight per seedling (mg) of onions primed with a combination of microorganisms and grown in peat soil. Data presented are back-transformed, and values in parentheses are means after $log_{10}$ transformation of data. Analyses were carried out on transformed data.

| Treatment | Mean fresh weight per seedling (mg) |
|---|---|
| Unprimed control | 250.6 (5.52) |
| Primed control | 243.5 (5.50) |
| Primed CHA0 | 232.3 (5.45) |
| Primed MA342 | 237.2 (5.47) |
| Primed IK726 | 231.5 (5.45) |
| Primed T22 | 221.6 (5.40) |
| Primed IK726 + CHA0 | 286.4 (5.66) |
| Primed IK726 + MA342 | 225.5 (5.42) |
| Primed T22 + CHA0 | 262.0 (5.57) |
| Primed T22 + MA342 | 181.5 (5.20) |
| LSD (0.05; df = 27) | (0.129) |

*Glasshouse experiment – carrot emergence and growth*

Emergence of the carrot seed was variable, with as much as a 20% difference in final percent emergence between treatments. A representative example of the emergence of seed treated with *C. rosea* IK726 and *P. fluorescens* CHA0 is given in Figure 6.4.

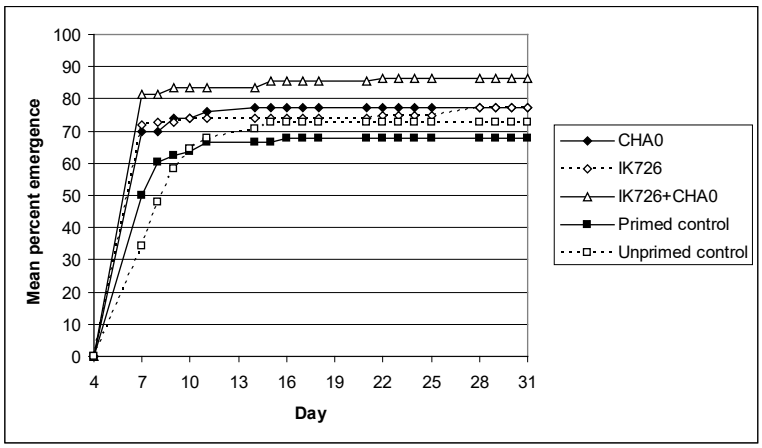


**Figure 6.4**: Emergence of carrot seed primed with a combination of microorganisms, in peat soil.

Primed treatments as a whole had a significantly greater final percent emergence than the unprimed control, and **all treatments with microorganism application** (single or combined) **were significantly better than the primed control** Although the combination primed treatments achieved the highest final percent emergence, the increase was neither additive nor synergistic and was not statistically different to the single treatments. A separate analysis showed that combination priming as a whole did not significantly improve emergence compared to single microorganism priming statistically speaking, although the increase in emergence may be important biologically for increased yield.

The mean fresh weight per seedling after 8 weeks growth was significantly affected by the microorganism treatments. All microorganism treatments had a lower mean fresh weight compared to the primed control, and all but the treatment with *P. chlororaphis* MA342 applied individually and *T. harzianum* T22 and *P. fluorescens* CHA0 applied in combination had a lower mean fresh weight than the unprimed control. However, the control treatments also had the lowest emergence, and it may be that the other treatments had a lower mean fresh weight because there were more seedlings in the pot competing for space and nutrients. There was no significant difference in the total fresh weight per treatment (all seedlings from 6 pots combined) (data not shown).

*Glasshouse experiment – survival of microorganisms on onion*

Survival data for the microorganisms applied in combination to onion seed are presented here for the first time. At all sample times, the primed control seedlings were sampled and plated onto selective media to assess the background populations of the microorganisms that had specifically been applied to the seed. *Clonostachys rosea* was not recovered from the primed control, nor were any rifampicin resistant bacterial isolates. Indigenous *Trichoderma* spp. were recovered from rhizosphere soil at 2 and 4 weeks only and this was less than 0.5 $\log_{10}$ cfu per seedling.

Single microorganism seed treatments followed a generally similar survival pattern on the roots and in rhizosphere soil as was found previously with onion (Figure 6.5). *Pseudomonas fluorescens* CHA0 decreased significantly in number in the rhizosphere soil over time, and despite a recovery in number between 4 and 8 weeks it was found below 2 $\log_{10}$ cfu per seedling at 8 weeks (Figure 6.5). *Pseudomonas*

*chlororaphis* MA342 also decreased in number over time in the rhizosphere soil and on the root.

Both fungal isolates declined in number from what was on the seed initially (Figure 6.5). *Clonostachys rosea* IK726 was below the level of detection at 2 weeks post planting, but subsequently increased significantly in numbers at 4 and 8 weeks, reaching over 3 $\log_{10}$ cfu per seedling in the rhizosphere soil and over 2 $\log_{10}$ cfu per seedling on the root. *Trichoderma harzianum* T22 showed the highest recovery at 4 weeks sampling, but generally remained at about 1 $\log_{10}$ cfu per seedling throughout the experiment in the rhizosphere soil. Recovery of this isolate from roots was poor, below 1 $\log_{10}$ cfu per seedling (Figure 6.5). The findings here were similar to those from previous work (Objective 04).

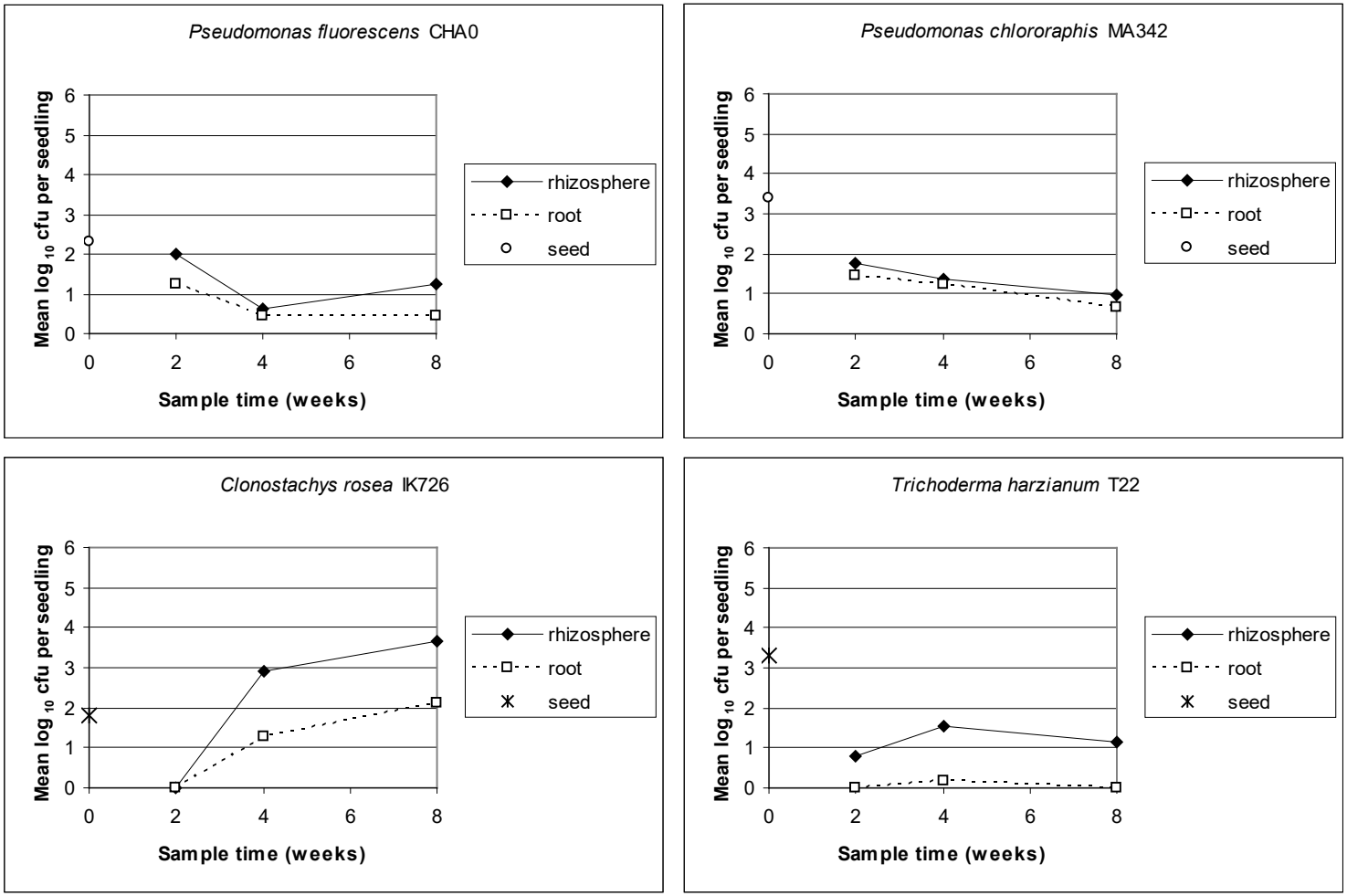


**Figure 6.5:** Survival on roots and in rhizosphere soil of beneficial microorganisms applied singly to onion seeds during priming and planted in peat soil. LSD (0.05; df = 72) comparing the same microorganism at different times: rhizosphere = 1.02; root = 0.91

In combination with *C. rosea* IK726, *P. fluorescens* CHA0 fluctuated in its survival in the rhizosphere, although none of the changes were significant (Figure 6.6). *Clonostachys rosea* IK726 behaved similarly in combination as to when it was applied singly to onion seed, with a significant overall increase in numbers over time in the rhizosphere soil and on the roots. Although numbers dropped from what was on the seed initially, by the end of 8 weeks they had increased again to 4 $\log_{10}$ cfu per seedling in the rhizosphere, and nearly 3 $\log_{10}$ cfu per seedling on the roots (Figure 6.6).

A similar situation was found when *C. rosea* IK726 was in combination with *P. chlororaphis* MA342, with the fungus again increasing significantly in number between 2 and 8 weeks (Figure 6.6). In this combination, *P. chlororaphis* MA342 was recovered in higher numbers throughout the experiment than when it was applied singly to onion seed. However, this may be due to the higher initial inoculum of this isolate on seed when applied in combination (Figure 6.6). On the roots, *P. chlororaphis* MA342 showed a significant increase in numbers at 4 weeks sampling when in combination with *C. rosea* IK726, and by 8 weeks it was recovered at approximately 3 $\log_{10}$ cfu per seedling (Figure 6.6).

In combination with *T. harzianum* T22, both bacterial isolates followed similar patterns, decreasing in number overall, although not significantly (Figure 6.6). Although the numbers recovered from the combination primed seed were higher (Figure 6.5 and 6.6), this is probably due to the high initial inoculum of the bacterial isolates when applied in combination. This high inoculum may have influenced the survival of *T. harzianum* T22 on the onion roots, as the fungus was not recovered from roots at any time when applied in combination with the bacteria to onion seed (Figure 6.6). When applied singly, recovery of *T. harzianum* T22 was poor from onion roots, and in combination with the high numbers of applied bacteria, this may have reduced the populations of the fungus to below the level of detection. However, *T. harzianum* T22 was recovered from rhizosphere soil and showed a significant increase in numbers when in combination with *P. fluorescens* CHA0 (Figure 6.6). Overall, a similar pattern of survival was found in the rhizosphere soil for *T.* harzianum T22 as when it was applied singly to seed.

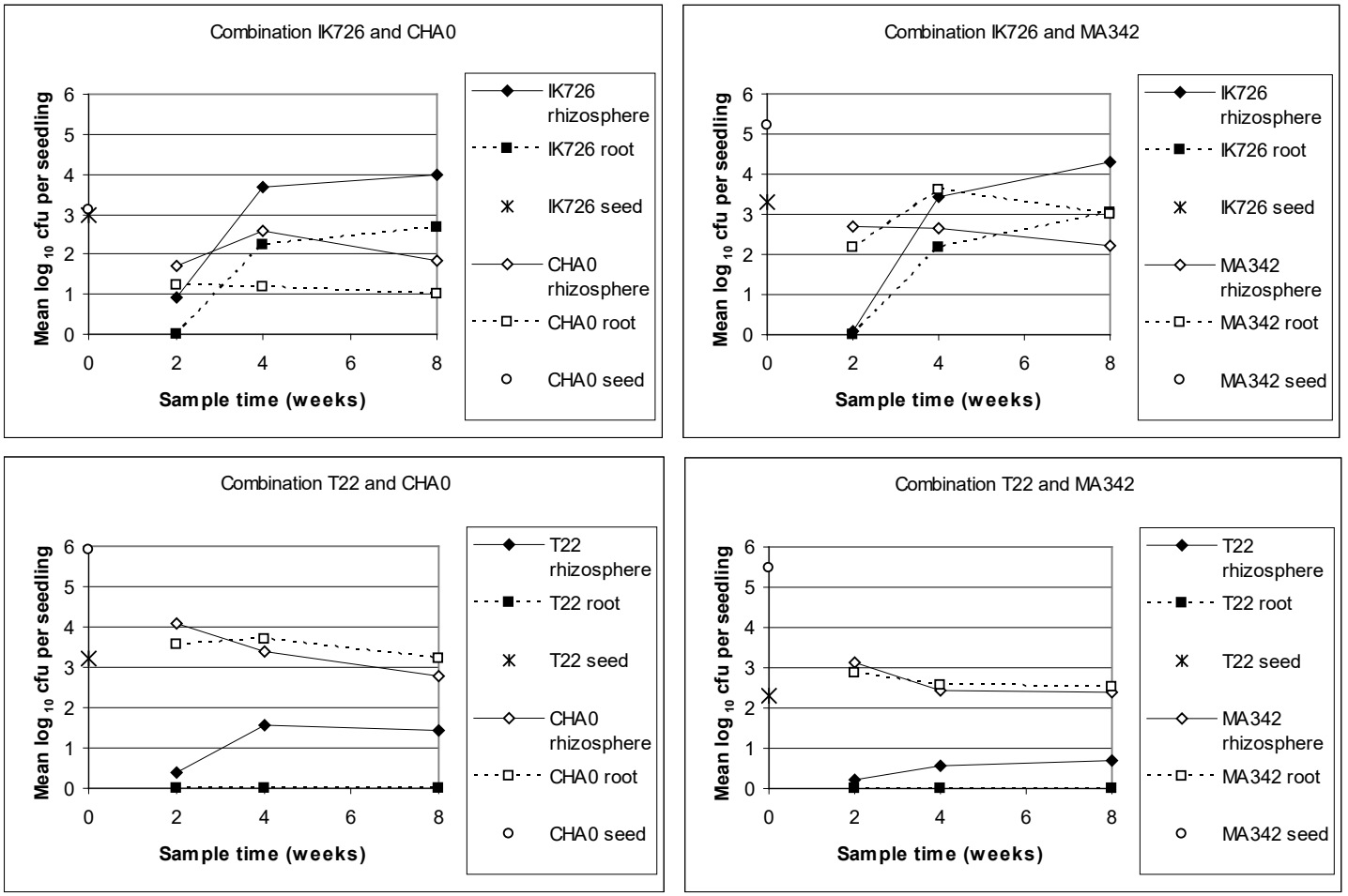


**Figure 6.6:** Survival on roots and in rhizosphere soil of microorganisms applied in combination to onion seeds during priming and planted in peat soil. LSD (0.05; df = 72) comparing the same microorganism at different times: rhizosphere = 1.02; root = 0.91

*Glasshouse experiment – survival of microorganisms on carrot*

Survival data for the microorganisms applied in combination are presented here for the first time. At all sample times, the primed control seedlings were sampled and plated onto selective media to assess the background populations of the microorganisms that had also been applied to the seed. *Clonostachys rosea* was not recovered from the primed control, and indigenous *Trichoderma* spp. were recovered only once from the root sample at 2 week (<0.5 $\log_{10}$ cfu per seedling). Rifampicin-resistant bacteria were recovered from the primed control samples at 8 weeks, but this background population was low (approx 1 $\log_{10}$ cfu per seedling).

Single microorganism seed treatments followed a generally similar survival pattern on the roots and in rhizosphere soil as was found previously with carrot (Figure 6.7). *Pseudomonas fluorescens* CHA0 declined significantly in number over the 8 weeks in the rhizosphere soil, dropping below 1 $\log_{10}$ cfu per seedling at the end of the experiment (Figure 6.7). The other bacterial isolate, *P. chlororaphis* MA342 survived poorly and was not recovered above 1 $\log_{10}$ cfu per seedling throughout the 8 weeks. From the initial numbers found on the seed, both fungal isolates decreased in number in the first 2 weeks, but subsequently showed an increase in numbers over time (Figure 6.7). *Clonostachys rosea* IK726 increase to over 3 $\log_{10}$ cfu per seedling by the end of 8 weeks, and *T. harzianum* T22 was recovered at over 2 $\log_{10}$ cfu per seedling.

Following application in combination to carrot seed, all the microorganisms were recoverable from roots and rhizosphere soil for 8 weeks (Figure 6.8). In combination with *C. rosea* IK726, *P. fluorescens* CHA0 was found to follow a similar survival pattern over 8 weeks as when it was applied singly to the seed, with recovery below 1 $\log_{10}$ cfu per seedling at 8 weeks. In this combination, *C. rosea* IK726 declined initially from what was on the seed, then increased significantly in number again by the 4 week sample in the rhizosphere soil and on the roots (Figure 6.8). This was similar overall to the pattern found with single application. Despite a reduction in numbers at 8 weeks in combination with *P. fluorescens* CHA0, possibly indicating a negative interaction between the microorganisms, this decrease was not significant.

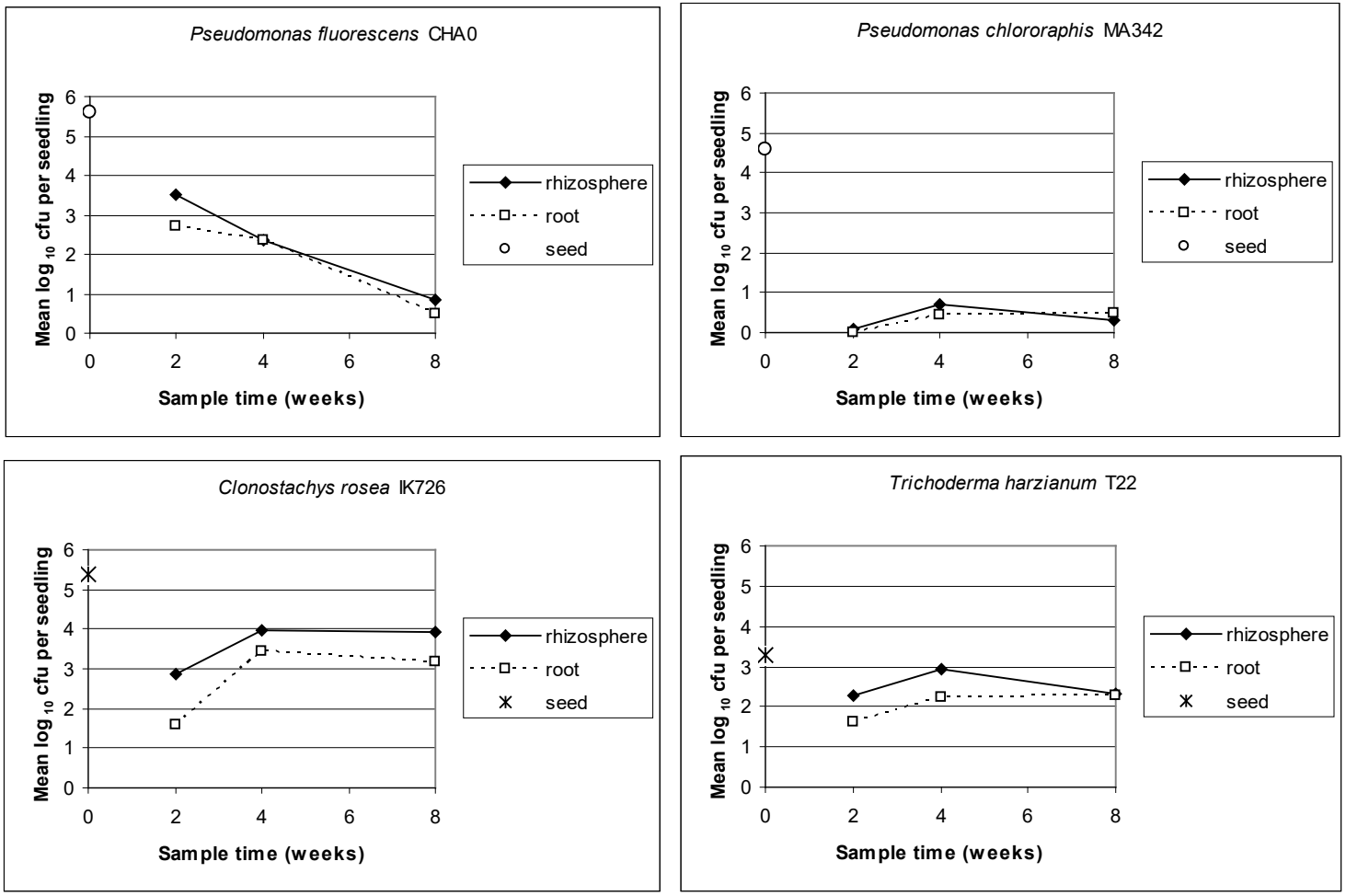


**Figure 6.7:** Survival on roots and in rhizosphere soil of beneficial microorganisms applied singly to carrot seeds during priming and planted in peat soil. LSD (0.05, df = 72) comparing the same microorganism at different sample times: rhizosphere = 1.38; root = 1.19.

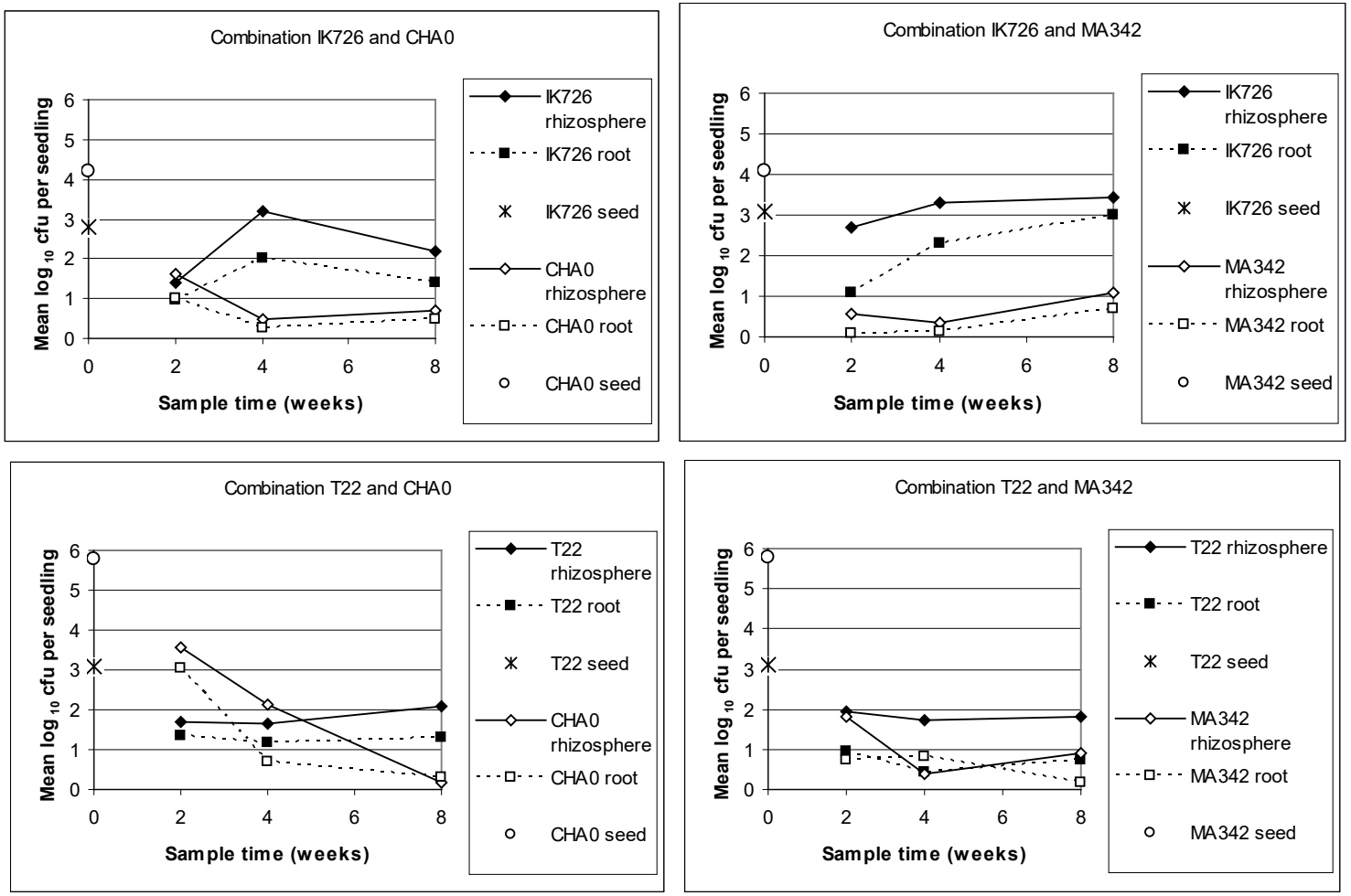


**Figure 6.8:** Survival on roots and in rhizosphere soil of microorganisms applied in combination to carrot seeds during priming and planted in peat soil. LSD (0.05; df = 72) comparing the same microorganism at different times: rhizosphere = 1.02; root = 0.91

In combination with *C. rosea* IK726, *P. chlororaphis* MA342 was recovered in low numbers, as it had been when applied singly to the carrot seed (Figure 6.8). Although it appears that the numbers of the bacterial isolate are increasing in the rhizosphere after 8 weeks when in combination with *C. rosea* IK726, this may be the variability of the experiment and is not significant. Further experiments would need to be carried out to assess the longer-term effect of the interaction between these two microbial species on the carrot roots. In this combination, *C. rosea* IK726 behaves in a similar manner to when it was applied singly to carrot seed, *ie* with an overall increase in numbers from 2 to 8 weeks (Figure 6.8).

In combination with *T. harzianum* T22, *P. fluorescens* CHA0 again behaved in a similar manner as when it was applied singly, declining significantly in number throughout the 8 weeks (Figure 6.8). *Trichoderma harzianum* T22 also followed a similar pattern to previously, although the recovery of this fungus seemed lower on the root samples when in combination with *P. fluorescens* CHA0. However, this may be due to initial numbers on the seed being slightly different.

In combination with *T. harzianum* T22, *P. chlororaphis* MA342 was recovered from roots in low numbers, as when it was applied singly (<1 $\log_{10}$ cfu per seedling) (Figure 6.8). In this combination, *T. harzianum* T22 was recovered from rhizosphere soil and roots in generally lower numbers than when applied singly. It may be that the presence of *P. chlororaphis* MA342 had an inhibitory effect in this case.

**Conclusions:**

- Beneficial microorganisms can be applied in combination to carrot and onion seed during drum priming, achieving the target of 5 $\log_{10}$ cfu $g^{-1}$ dry seed for each microorganism.
- This is the first demonstration that combinations of bacteria and fungi can be applied successfully to seed simultaneously, using commercially relevant procedures.
- On onion seed, the combination of *C. rosea* IK726 and either bacterial isolate resulted in significantly worse emergence compared to the microorganisms applied singly to the seed.
- *Trichoderma harzianum* T22 in combination with either bacterial isolate on onion seed did not improve the emergence compared to the microorganisms applied singly.

- The combination of *C. rosea* IK726 and either bacterial isolate improved the emergence of carrot seedlings, but the effect was neither additive nor synergistic and was not statistically different to the single treatments.
- Microorganisms co-applied to carrot and onion seed could be recovered from roots and rhizosphere soil samples for up to 8 weeks, following generally the same survival pattern as when they were applied singly to the seed.
- The initial dose of the microorganisms on the seed may influence the subsequent level of their survival in the rhizosphere and possibly influence any interactions between the species.

# Objective 07: Technology transfer and exploitation planning

**Poster displays**

- British Carrot Growers Association event (5th October 2004)
- Elsoms Organic open day (14th October 2004)
- British Carrot Growers Association event (6th October 2005)
- UK Carrot and Onion Conference (23rd-24th November 2005)
- Horticulture LINK 2006 (23rd February 2006)
- British Carrot Growers Association event (October 2006)

**Field trial open days**

- Warwick HRI Open Day (10th July 2004)
- Organic field trial open day at Elsoms Seeds Ltd. (14th October 2004)
- Warwick HRI Growers and Gardeners Event (17th August 2005)
- Warwick HRI Growers and Gardeners Event (June 2006)

**Articles**

- An Agriculture LINK newsletter article was published.
- An AgLINK publicity leaflet was prepared and distributed
- HDC News article – Giving seeds a head start - March 2007, pp. 18-19.

**Presentations**

- Horticulture LINK 2004 (John Whipps, June 2004)
- Vegetable Consultants group 2004 (John Whipps, December 2004)
- Association of Applied Biologists meeting in Oxford (John Whipps, December 2004)
- IOBC conference in Spa, Belgium (Amanda Bennett, 6th - 10th September 2006)

**Non-refereed papers**

- Bennett, A. J. and Whipps, J. M. (2006) Application of beneficial microorganisms to seed during priming to improve crop health and

establishment IOBC/wprs Bulletin *in press*

**Refereed papers**

- Bennett, A. J. and Whipps, J. M. (2007) Beneficial microorganism survival on roots and in rhizosphere soil following application to seed during priming. *Soil Biology & Biochemistry* (*in preparation*)
- Bennett, A. J. and Whipps, J. M. (2007) Combinations of beneficial microorganisms can be applied to seed during drum priming. (*in preparation*)

**Other**

- Discussions have taken place with PSD regarding the use of different isolates and marked strains.

## Future research and development resulting from this project

In the view of GTG, Elsoms and the HDC, there remains a clear need for research to deepen the understanding of the interaction between microorganisms as constituents of a consortium and between microbial consortia and the host plant, with particular reference to plant growth promotion and on reduction of inputs by the grower.

The outputs of the present project are supporting GTG's development work in the following areas;

1) Disinfection of seed prior to application of beneficial microorganisms.

2) Demonstrating means by which microorganisms can be applied to seed.

3) To test the efficacy of beneficial microorganisms on plant species other than those used in the present project.

4) Benefits derived from the use of combinations of co-applied microorganisms.

As an example of this further development, as of mid-February 2007, GTG will have commenced testing in collaboration with Washington State University. The test will look at the potential for a seed-applied microorganism from the present project to provide protection against soil-borne fungal pathogens when applied to spinach (*Spinacia oleracea*) in glasshouse experiments.

Additionally, GTG recognises a need to obtain unequivocal data on the ability of seed-applied beneficial microorganisms to improve plant health (meaning growth rate and yield against input requirements, as opposed to providing protection against pathogens) under commercial growing conditions. Hence, GTG is building on the results of the present project by establishing a project during 2007, with a commercial collaborator, to determine the influence of other beneficial microorganisms on the health and productivity of field crops.

The HDC is not currently planning any further research, having established the technique of successfully applying micro-organisms to seed during priming. The next step needs to be either enhancing their effect and reliability or identifying alternative microorganisms and this work will now be progressed by GTG.

## Industrial relevance and plans for future commercial exploitation

GTG and Elsoms continue to focus on enhancing plant health and protection. Strategies for which involve a diverse range of technologies such as priming and seed-applied treatments as well as coating and pelleting. Moreover, with the diminishing range of conventional seed protection products there is an imperative need to develop suitable replacements.

However, the piecemeal adoption of disparate seed technologies, limited to a narrow range of target species, is unlikely to lead to satisfactory new product candidates. Hence GTG and Elsoms are seeking to integrate seed technologies. To this end the present project has started to draw these technologies together.

The project has yielded data which is highly relevant to GTG and Elsoms commercial aims. At present however there are no outputs for immediate commercialisation.

The outputs of the project are to be fed directly into further development studies as detailed above.

The HDC recognise that seed is the most significant input in crop production and support measures and research to improve crop establishment and crop health. Whilst no direct outputs for growers have arisen from this work, the HDC hope that ultimately the techniques established in this project, will yield outputs (seed treatments) in the future after further commercial development.

## 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* **13 (6)** 599-614.

## Acknowledgements

We would like to thank Andrew Mead for assistance with all the statistical analyses, and the staff at Horticultural Services at Warwick HRI for helping to run and manage the field trials at Wellesbourne.

# Appendices

## Appendix 4.1: *Trichoderma* semi-selective medium

**Basal medium ingredients (for 1 litre):** Autoclave

0.20g $MgSO_4.7H_2O$
0.90g $K_2HPO_4$
0.15g KCl
1.00g $NH_4NO_3$
3.00g D (+) glucose
0.15g rose bengal
20.0g agar
950ml sterile distilled water (SDW)

**Biocidal ingredients:** mix in a mortar and pestle and suspend in 50ml SDW before adding to the autoclaved basal medium

0.25g chloramphenicol
0.15g quintozene
0.60ml propamocarb
0.025g captan