Improved crop health and establishment using beneficial microorganisms

Horticulture LINK Project

CSA 6388/HLO 0167 LFV

Annual Report Year 1

(September 2003 – August 2004)

Project title:	Improved crop health and establishment using beneficial microorganisms
Project number:	CSA 6388/HLO 0167 LFV
Project leader:	Prof John Whipps Research Leader Warwick HRI Wellesbourne Warwick, CV35 9EF
Report:	Annual report, September 2004
Key Workers:	Dr Amanda Bennett (Warwick HRI), Rae Cook (Elsoms Seeds Ltd), Dr Richard Walker (GTG UK)
Location:	Elsoms Seeds Ltd, Spalding GTG UK, King's Lynn Warwick HRI, Wellesbourne
Project co-ordinator:	Dr Peter Halmer Research Director GTG UK Hansa Road Hardwick Industrial Estate King's Lynn Norfolk, PE30 4LG
Date commenced:	1 September 2003
Date completion due:	28 February 2007

©2004 HortLINK Consortium members

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

Whist reports issued under the auspices of the HDC are prepared from the best available information, neither the authors nor the HDC can accept any responsibility for inaccuracy or liability for loss, damage or injury from the application of any concept or procedure discussed.

The contents of this publication are strictly private to the HortLINK consortium members. No part of this publication may be copied or reproduced in any form or by any means without prior written permission of the HortLINK consortium members.

©2004 HortLINK Consortium members

Intellectual property rights are invested in the Consortium of Horticulture LINK Project CSA 6388/HLO 0167 LFV

HortLINK Consortium Members:

Department for Environment, Food and Rural Affairs

Elsoms Seeds Ltd

GTG UK

Horticultural Development Council

Warwick HRI

Government sponsor:

Department for Environment, Food and Rural Affairs

©2004 HortLINK Consortium members

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

©2004 HortLINK Consortium members

Contents

Grower Summary 1

Headline 1

Background and expected deliverables 1

Summary of the project and main conclusions 1

Application of microorganisms to seed 1

Pesticide compatibility 2

Field trials 2

Glasshouse experiment - onions 4

Glasshouse experiment -carrots 4

Shelf life studies 5

Progress against Milestones: 6

Science Section 7

Introduction 7

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. (Milestones 1.1, S1.1, S1.2) Complete 8

Introduction 8

Materials and methods 8

Bacterial inoculum preparation 8

Fungal inoculum preparation 9

Drum priming and sampling 9

Pesticide compatibility 10

Results and discussion 10

Objective 02: Assess effects of seed applied microorganisms to carrot and onion seed in field trials (Milestone 2.1) Ongoing 15

Introduction 15

©2004 HortLINK Consortium members

Materials and methods **15**
Bacterial inoculum preparation *15*
Fungal inoculum preparation *16*
Drum priming and pesticide application *16*
Reisolation of microorganisms from seed samples *16*
Seed Treatments *17*
Trial design and emergence assessments *17*
Results and discussion **19**
Advancement and germination of seed treatments *19*
Target application rates of the beneficial microorganisms *21*
Wellesbourne site, onion trial *21*
Grower site, onion trial *23*
Elsoms organic site, onion trial *24*
Wellesbourne site, carrot trial *25*
Grower site, carrot trial *27*
Elsoms organic site, carrot trial *27*
Conclusions **28**

Objective 03: Characterise growth and establishment responses to seed-applied microorganisms of carrot and onion plants grown in the glasshouse in a range of natural and pathogen infested soils. (Milestones 3.1, S3.1) Complete **29**
Introduction **29**
Materials and methods **29**
Results and discussion **30**
Onion glasshouse experiment *30*
Emergence - Soil type effects *31*
Emergence - Seed treatment effects *31*
Emergence- Pesticide effects *32*
Mean fresh weight of seedlings *33*
Carrot glasshouse experiment *35*
Emergence – Soil type effects *35*
Emergence – seed treatment effects *36*

©2004 HortLINK Consortium members

Emergence – Pesticide effects 37
Mean fresh weight of seedlings 37
Conclusions **40**

Objective 04: Determine the survival and proliferation of seed-applied microorganisms on seedlings after sowing in soil in the glasshouse and field. (Milestone 4.1) Ongoing **41**
Introduction **41**
Materials and methods **41**
Conclusions **42**

Objective 05: Characterise the long-term shelf life (up to 1 year) of seed-applied microorganisms under commercially-representative storage conditions. (Milestone S5.1) Ongoing **43**
Introduction **43**
Materials and methods **43**
Bacterial inoculum preparation 43
Fungal inoculum preparation 43
Steeping priming and microorganism reisolation 44
Results and discussion **45**
Onion 45
Carrot 48
Conclusions **50**

Technology transfer **51**
References **51**
Appendices **52**

©2004 HortLINK Consortium members

Grower Summary

Improved crop health and establishment using beneficial microorganisms

HortLINK project CSA 6388/HLO 0167 LFV

Headline

- Priming seed improves seedling emergence in carrot and onion.
- Beneficial microorganisms can be applied successfully to carrot and onion seed during the priming process.
- Compatability with pesticide seed treatments and potential biocontrol effects are being investigated.

Background and expected deliverables

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

Summary of the project and main conclusions

Application of microorganisms to seed

The beneficial bacteria *Pseudomonas fluorescens* CHA0 and *Pseudomonas chlororaphis* MA342, and fungi *Trichoderma harzianum* T22 and *Clonostachys rosea* IK726 were all applied successfully to carrot and onion seed during drum priming. Examples of microorganism survival during the different phases of the priming process are given in Figure 1. All microorganisms proliferated on carrot seed, whereas only the bacteria proliferated on onion seed. However, target

©2004 HortLINK Consortium members

application numbers of the microorganisms were achieved in all cases (5 $\log_{10}$ cfu g^{-1} dry weight seed).

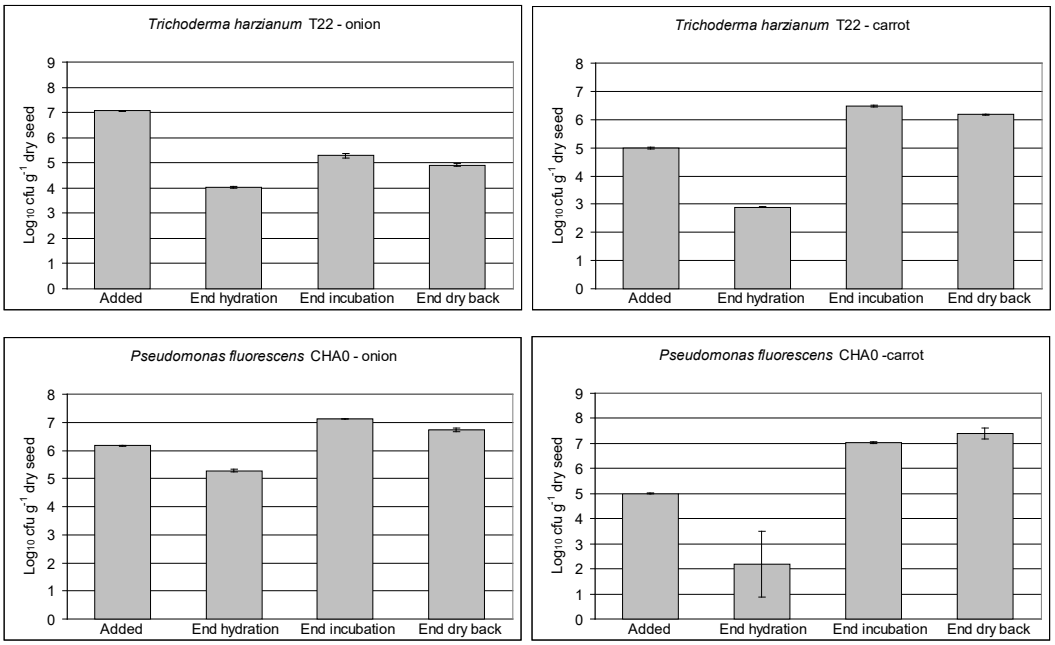


Figure 1: Examples of survival and proliferation of beneficial microorgansims on carrot and onion seed following application during priming

Pesticide compatibility

The microorganisms were found to be compatible with the following seed-applied pesticides: the fungicides Wakil XL (a.i. metalaxyl-M, cymoxanil and fludioxonil), and Apron 35 (a.i. metalaxyl-M), and the insecticide Force ST (a.i. tefluthrin). Bacteria were resistant to the fungicide HY-TL (a.i. thiram and thiabendazole) but the fungi were inhibited by the presence of this fungicide in laboratory-based tests.

Field trials

Trials have been drilled to assess the effect of the microorganism-primed treatments on crop growth and yield for both carrot and onion. Trials were drilled at Warwick HRI, Wellesbourne (conventional carrot and onion sites; sandy clay loam); T. Hammond & Sons, South Notts (conventional carrot site; sand); Elvedon Farms Ltd, Elvedon, Thetford (conventional onion site; light silt); and Elsoms (organic carrot and onion sites). Table 1 lists the treatments that were used, with all 12 treatments drilled at the conventional field sites, and treatments 1-6 drilled at the organic site.

©2004 HortLINK Consortium members

Table 1 Seed treatments produced for the Year 1 field trials

Treatment number	Description	Priming	Fungicide		Insecticide
			Carrot[a]	Onion[b]	(Force ST)
1	Primed control	✓	×	×	×
2	Primed *P. fluorescens* CHA0	✓	×	×	×
3	Primed *C. rosea* IK726	✓	×	×	×
4	Primed *P. chlororaphis* MA342	✓	×	×	×
5	Primed *T. harzianum* T22	✓	×	×	×
6	Unprimed control	×	×	×	×
7	Primed control + Pesticide	✓	Wakil XL	HY-TL & Apron 35	✓
8	Primed *P. fluorescens* CHA0 + Pesticide	✓	Wakil XL	HY-TL & Apron 35	✓
9	Primed *C. rosea* IK726 + Pesticide	✓	Wakil XL	HY-TL & Apron 35	✓
10	Primed *P. chlororaphis* MA342 + Pesticide	✓	Wakil XL	HY-TL & Apron 35	✓
11	Primed *T. harzianum* T22 + Pesticide	✓	Wakil XL	HY-TL & Apron 35	✓
12	Unprimed + Pesticide	×	Wakil XL	HY-TL & Apron 35	✓

[a] carrot seed film coated with Reichold biocide-free polymer

[b] onion seed pelleted with Propell blend

©2004 HortLINK Consortium members

Emergence has been assessed for all sites, except the organic carrot site where weed overgrowth was a problem. At the conventional field sites it was found that the application of pesticides to carrot and onion seed improved emergence. On the onion crop at the Wellesbourne site, the best emergence of the microorganism-primed treatments was found with *T. harzianum* T22 + pesticide, and *P. chlororaphis* MA342 and *C. rosea* IK726 without pesticides. However, at the other sites, *C. rosea* IK726 treatment on onion showed poor emergence. For the carrot crops, *P. chlororaphis* MA342 + pesticide or *P. fluorescens* CHA0 + pesticide treatments showed the best emergence, but *P. fluorescens* CHA0 without the pesticide treatment resulted in poor emergence. *Clonostachys rosea* IK726 without pesticide treatment resulted in poor emergence in the carrot crops.

Glasshouse experiment - onions

In glasshouse experiments, the soil type was found to have a significant effect on emergence, with light sandy loam soil resulting in a slower emergence time than sandy clay loam or peat soil. Final emergence was approximately 90% in all three soil types. The primed control seed and all seed primed with beneficial microorganisms, except *P. fluorescens* CHA0, emerged faster than unprimed seed. However, the application of pesticides did not affect emergence. The mean fresh weight of seedlings at the end of the experiment (8 weeks) was significantly higher in light sandy loam soil than the other two soil types, and higher in the peat soil than the sandy clay loam. The application of the microorganisms did not significantly increase the mean fresh weight of seedlings, although the application of pesticides did cause a small but significant decrease in the mean fresh weight compared to treatments without pesticide application.

Glasshouse experiment -carrots

Emergence of the carrot seedlings was later in the peat soil than the other soils, possibly due to slower water uptake in this soil type. For the carrot crop, the final percent emergence was significantly less in the light sandy loam and peat soils, and may have been due to the presence of a carrot pathogen in these soil types, which were collected from carrot growing areas. However, all primed seed treatments had a significantly faster emergence than the unprimed seed, and seed treated with *C. rosea* IK726 or *T. harzianum* T22 also emerged faster than the primed control.

©2004 HortLINK Consortium members

The soil type affected the mean fresh weight of seedlings, with the seedlings grown in peat soil weighing significantly more. This may have been due to the nutrient status of the soils, with peat soil providing better plant nutrition. Overall, the different seed treatments did not increase the mean fresh weight of seedlings, and the application of pesticides had no significant effect on mean fresh weight of carrot seedlings.

Shelf life studies

Shelf life studies were carried out on microorganism-primed seed, with and without the application of pesticides. On onion seed, populations of the applied microorganisms remained above the target rate (5 $\log_{10}$ cfu g^{-1} dry weight seed) for over 2 months, with the best survival seen with *T. harzianum* T22 and *P chlororaphis* MA342 for 6 months. On carrot seed, bacterial survival was better than the fungi, with *P. chlororaphis* MA342 being recovered above the target rate (5 $\log_{10}$ cfu g^{-1} dry weight seed) until approximately 9 months. Survival may be influenced by the initial numbers of cells applied to the seed, with higher doses resulting in better shelf-life.

Financial benefits

No financial benefits are reported at this time.

Action points for growers

No action points are appropriate at this time.

©2004 HortLINK Consortium members

Progress against Milestones:

Work for all milestones is complete or ongoing

Primary milestones: Year 1 (Sept 03 - Aug 04)

1.1 Confirm that microorganisms can be applied to onion seed during priming (Warwick HRI)(Dec 2003 (month 4)) **Complete**

2.1 Carry out Year 1 field trials and monitor effects on seed emergence and growth (Elsoms +Warwick HRI)(Aug 2004 (month12)) **Ongoing**

3.1 Complete Year 1 glasshouse studies and monitor effects on seed emergence and growth (Warwick HRI) (Aug 2004 (month12)) **Complete**

4.1 Complete monitoring of microorganism survival on Year 1 field trial and glasshouse studies (GTG + Warwick HRI) (Aug 2004 (month 12)). The planned monitoring of survival of applied microorganisms on seedlings in field trials will not be carried out as PSD refused permission to use marked strains of the microorganisms. The glasshouse studies are underway. **Ongoing**

Secondary milestones: Year 1 (Sept 03 - Aug 04)

S1.1 Obtain microorganisms for test application to seed (Warwick HRI) **Ongoing**

S1.2 Determine pesticide compatibility of the 4 microorganisms to be applied to seed for field trials (Warwick HRI) (Feb 04 (month 6)) **Complete**

S3.1 Set up initial year 1 glasshouse trial (Warwick HRI) (March 04 (month 7)) **Complete**

S5.1 Initiate shelf life studies (GTG) (Oct 03 (month 2)) **Complete**

S7.1 Complete article for Agriculture LINK newsletter (All) (Feb 04 (month 6)) **Complete**

S7.2 Maintain discussions with PSD concerning registration (Elsoms + GTG) **Ongoing**

S7.3 Carry out grower demonstration at one Elsoms Seed field site (Elsoms) (July 04 (month 11)) **Planned for October**

©2004 HortLINK Consortium members

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.

©2004 HortLINK Consortium members

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. (Milestones 1.1, S1.1, S1.2) Complete

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 is investigating 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 four selected beneficial microorganisms on onion and carrot seed. Compatibility with commercially-applied pesticides was also tested.

Materials and methods

Four 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 fluorescens* CHA0 and *Pseudomonas chlororaphis* MA342, along with two fungal isolates, *Trichoderma harzianum* T22 and *Clonostachys rosea* IK726.

Bacterial inoculum preparation

Bacteria were available from the culture collection at Warwick HRI, and were stored at -80ºC on beads in a vial containing a cryopreservative fluid. A single bead removed from the vial was plated onto nutrient agar, an incubated at 25ºC. Single colonies resulted from this were inoculated into sterile nutrient broth (100ml) and incubated overnight at 26ºC, 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 1×10^7 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 20ºC. 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. 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 4ºC. 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 (100ug ml^{-1}) nutrient agar for bacteria, and PDA amended with chlortetracycline (30ug ml^{-1}) and Triton X-100 (2ml l^{-1}) 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 (1mg ml^{-1} metalaxyl); Wakil XL (0.38mg ml^{-1} metalaxyl, 0.22mg ml^{-1} cymoxanil, 0.11mg ml^{-1} fludioxonil); HY-TL (2.7mg ml^{-1} thiram, 2.03mg ml^{-1} thiabendazole); and Force ST (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. The onion seed had a background population of fungi that also proliferated during priming, making counts of the applied microorganisms difficult at lower dilutions. Thus, a higher initial inoculum level (1 x 10^7 cfu g^{-1} dry seed) was used for the onion seed to compensate for this. The survival and proliferation of the selected microorganisms on onion seed is shown in Figure 1.1, and on carrot seed in Figure 1.2. Generally, the bacteria increased in number on both seed types, whereas the fungi increased on carrot seed, but declined in number on onion seed. However, given a high enough inoculum dose initially, the target of

approximately 5-6 $\log_{10}$ cfu g^{-1} dry weight seed can be achieved at the end of drying back.

Table 1.1 shows the results of the pesticide compatibility testing. 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. The fungi 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. Organic growers could still use the microorganism-primed seed alone.

Conclusions

- Of the beneficial microorganisms selected for study (*P. fluorescens* CHA0, *C. rosea* IK726, *P. chlororaphis* MA342 and *T. harzianum* T22), the bacteria survived and proliferated on onion seed during drum priming, with the fungi surviving but not proliferating.
- All the beneficial microorganisms survived and proliferated following application to carrot seed during drum priming.
- All the microorganisms selected were compatible with Wakil XL, Force ST and Apron 35 in laboratory studies using agar plate tests.
- Bacterial isolates were compatible with HY-TL in laboratory studies, but the fungi did not grow on HY-TL amended agar.

©2004 HortLINK Consortium members

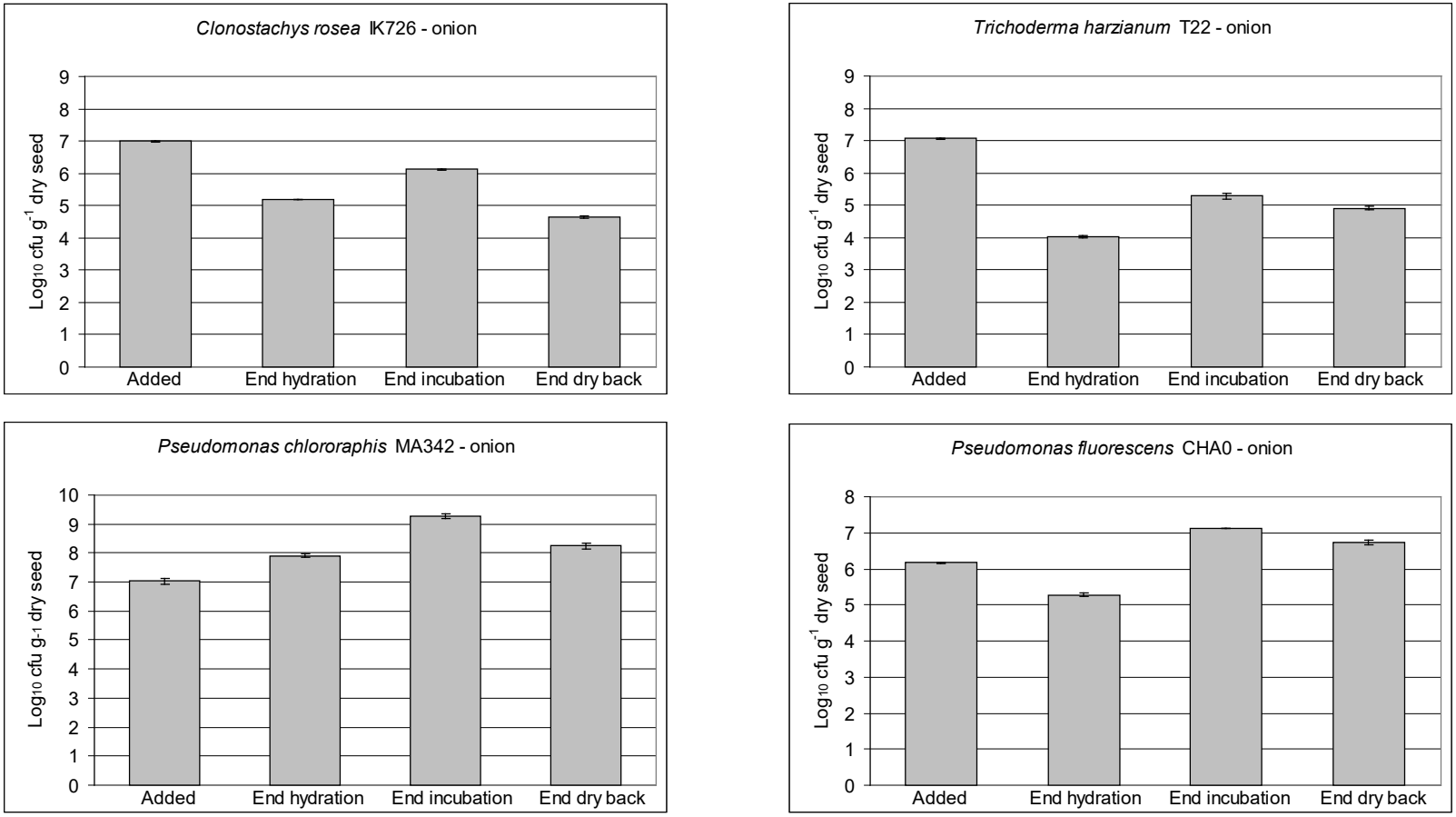


Figure 1.1 Survival and proliferation of beneficial microorganisms applied to onion seed during drum priming. Added = initial numbers in liquid suspension applied to seed; End hydration = numbers found after all liquid cell suspension added to seed; End incubation = numbers found after seed incubated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

©2004 HortLINK Consortium members

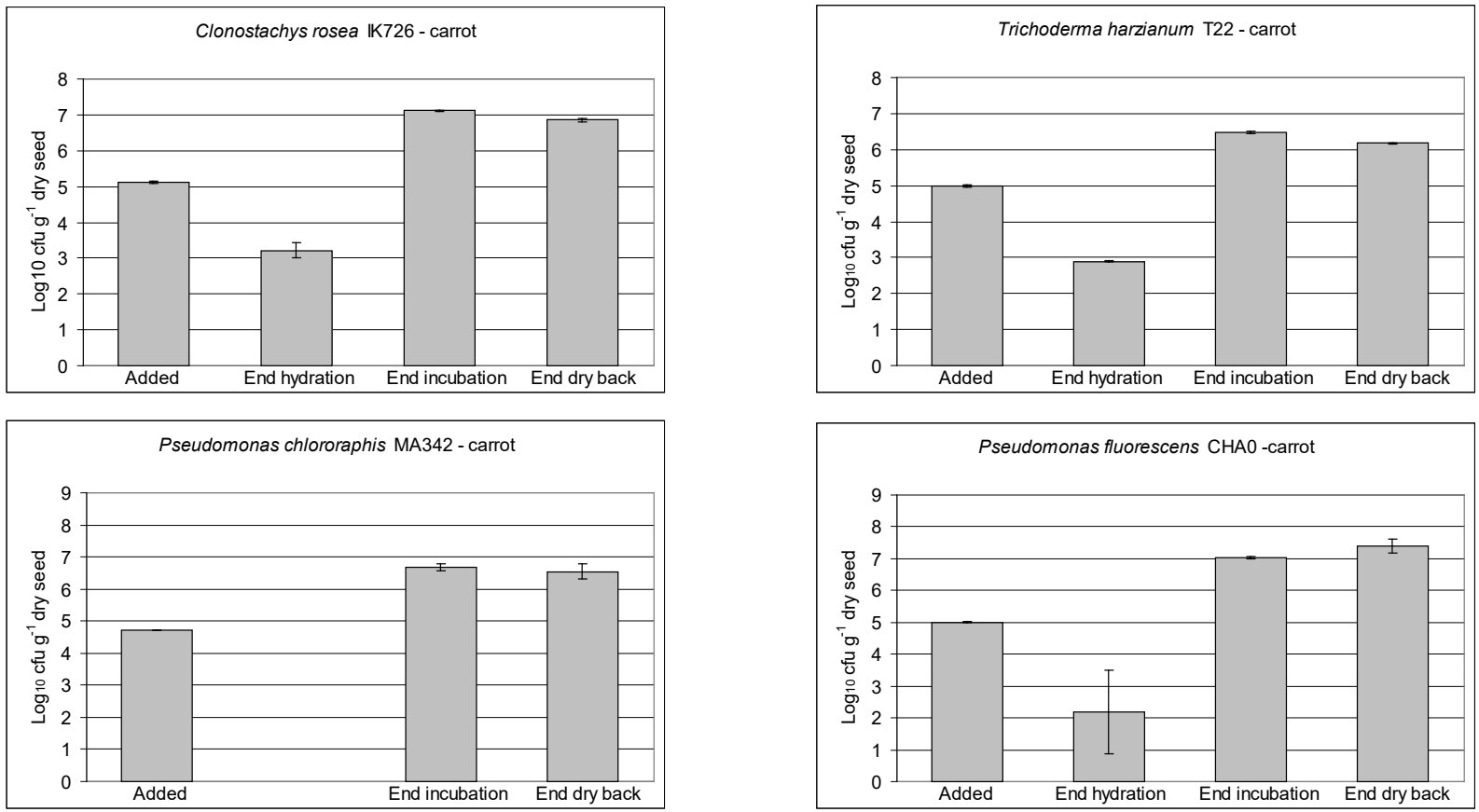


Figure 1.2 Survival and proliferation of beneficial microorganisms applied to carrot seed during drum priming. Added = initial numbers in liquid suspension applied to seed; End hydration = numbers found after all liquid cell suspension added to seed (below limit of detection for *P. chlororaphis* MA342); 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.

©2004 HortLINK Consortium members

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	+	-	-	+	+	+	+	+	+	+

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

©2004 HortLINK Consortium members

Objective 02: Assess effects of seed applied microorganisms to carrot and onion seed in field trials (Milestone 2.1) Ongoing

Introduction

Field trials were set up to assess the effect of microorganism-primed seed on emergence and yield of onion and carrot crops at two conventional sites and one organic site. Trials were drilled at Warwick HRI, Wellesbourne (conventional carrot and onion sites; sandy clay loam); T. Hammond & Sons, South Notts (conventional carrot site; sand); Elvedon Farms Ltd, Elvedon, Thetford (conventional onion site; light silt); and Elsoms (organic carrot and onion sites; medium to heavy silt). Initial emergence assessments have been carried out where possible and harvesting is scheduled for later in the year.

Materials and methods

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

Bacterial inoculum preparation

Wild-type 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.

©2004 HortLINK Consortium members

Fungal inoculum preparation

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

Drum priming and pesticide application

The selected microorganisms were applied to seed samples during drum priming and sub-samples of the primed seed were removed for further seed processing. Samples of carrot were film coated with Reichold biocide-free polymer, both with and without the standard rate of Wakil XL fungicide and Force ST insecticide. Samples of onion were pelleted with Propell blend both with and without standard rates of HY-TL and Apron 35 fungicides and Force ST insecticide. All treated samples of both onion and carrot were air-dried at room temperature. The pelleted seed was graded to a final size of 3.5 - 5.0 mm.

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 20ºC 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 µg ml^{-1} rifampicin for the bacteria and potato dextrose 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 analysis of variance (ANOVA).

Seed Treatments

Twelve treatments 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 (Table 2.1). Treatments 1-6 (without chemicals) of both carrot and onion were also drilled at Elsoms' own organic field trial site.

Trial design and emergence assessments

Each trial consisted of 4 replicate blocks of all the treatments (12 treatments at the conventional sites, and 6 treatments at the organic sites; Table 2.1), and a randomised design was used to allocate the treatments to plots within each replicate block. Each plot was 3m long and 4 rows were drilled across the bed. A cone drill was used, with seeding rates of 23 onion seeds/m, and 50 carrot seeds/m. At the Wellesbourne site, the carrot crop was fleeced 2 weeks after drilling to protect the crop from carrot fly. Throughout the growing season, standard commercial pesticides will be applied to each crop at the conventional sites, excluding the use of nematicides and Folicur.

Two emergence assessments were made for each trial, unless stated otherwise, and Table 2.2 shows the dates for drilling and assessment of the field trials.

©2004 HortLINK Consortium members

Table 2.1 Seed treatments produced for the Year 1 field trials

Treatment number	Description	Priming	Fungicide		Insecticide
			Carrot[a]	Onion[b]	(Force ST)
1	Primed control	✓	×	×	×
2	Primed *P. fluorescens* CHA0	✓	×	×	×
3	Primed *C. rosea* IK726	✓	×	×	×
4	Primed *P. chlororaphis* MA342	✓	×	×	×
5	Primed *T. harzianum* T22	✓	×	×	×
6	Unprimed control	×	×	×	×
7	Primed control + Pesticide	✓	Wakil XL	HY-TL & Apron 35	✓
8	Primed *P. fluorescens* CHA0 + Pesticide	✓	Wakil XL	HY-TL & Apron 35	✓
9	Primed *C. rosea* IK726 + Pesticide	✓	Wakil XL	HY-TL & Apron 35	✓
10	Primed *P. chlororaphis* MA342 + Pesticide	✓	Wakil XL	HY-TL & Apron 35	✓
11	Primed *T. harzianum* T22 + Pesticide	✓	Wakil XL	HY-TL & Apron 35	✓
12	Unprimed + Pesticide	×	Wakil XL	HY-TL & Apron 35	✓

[a] carrot seed film coated with Reichold biocide-free polymer

[b] onion seed pelleted with Propell blend

©2004 HortLINK Consortium members

Table 2.2 Dates when microorganism-primed onion and carrot field trials were drilled and assessed for emergence (1st and 2nd assessment dates)

	Wellesbourne			Grower site			Organic		
Crop	Drilled	1st	2nd	Drilled	1st	2nd	Drilled	1st	2nd
Onion	29/03	26/04	14/05	09/03	20/04	18/05	29/03[a] 18/05[b]	28/06	-
Carrot	17/05	07/06	01/07	15/05	17/06	30/06	25/06	-	-

[a] onion seed planted in modules

[b] onion seedlings planted in field

- no data available to date

Results and discussion

Advancement and germination of seed treatments

The drum priming treatments produced germination advancement responses in the pleated paper tests for both carrot and onion (Table 2.3). The *T. harzianum* T22 and *P. chlororaphis* MA342 treatments appeared to cause a slight reduction in normal germination on the onion treatments without pesticides. However, this response was not apparent on the onion treatments with pesticides or the treatments on carrot.

©2004 HortLINK Consortium members

Table 2.3 Advancement and germination data of seeds for the field trial in pleated papers.

Treatment number	Description	Carrot		Onion	
		Advancement G50 (h) [a]	Normals (%)[b]	Advancement G50 (h)	Normals (%)
1	Primed control	46.9	86.7	68.5	92.7
2	Primed *P. fluorescens* CHA0	49.5	86.7	58.6	92.0
3	Primed *C. rosea* IK726	35.1	85.7	59.5	90.0
4	Primed *P. chlororaphis* MA342	44.2	88.7	63.3	79.7
5	Primed *T. harzianum* T22	36.3	91.0	61.6	83.7
6	Unprimed control	63.6	86.7	90.0	94.7
7	Primed control + Pesticide	44.8	86.3	78.1	86.7
8	Primed *P. fluorescens* CHA0 + Pesticide	53.3	87.0	66.7	94.0
9	Primed *C. rosea* IK726 + Pesticide	34.8	83.7	76.1	93.3
10	Primed *P. chlororaphis* MA342 + Pesticide	41.4	88.0	76.9	91.7
11	Primed *T. harzianum* T22 + Pesticide	36.4	85.0	74.4	90.7
12	Unprimed control + Pesticide	67.5	85.7	97.4	91.7

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

[b] Percentage normal germination from a total of 3 x 100 seeds per treatment

©2004 HortLINK Consortium members

Target application rates of the beneficial microorganisms

All isolates were recovered in excess of the target application rates to both carrot and onion in the presence and absence of pesticides (Figure 2.1).

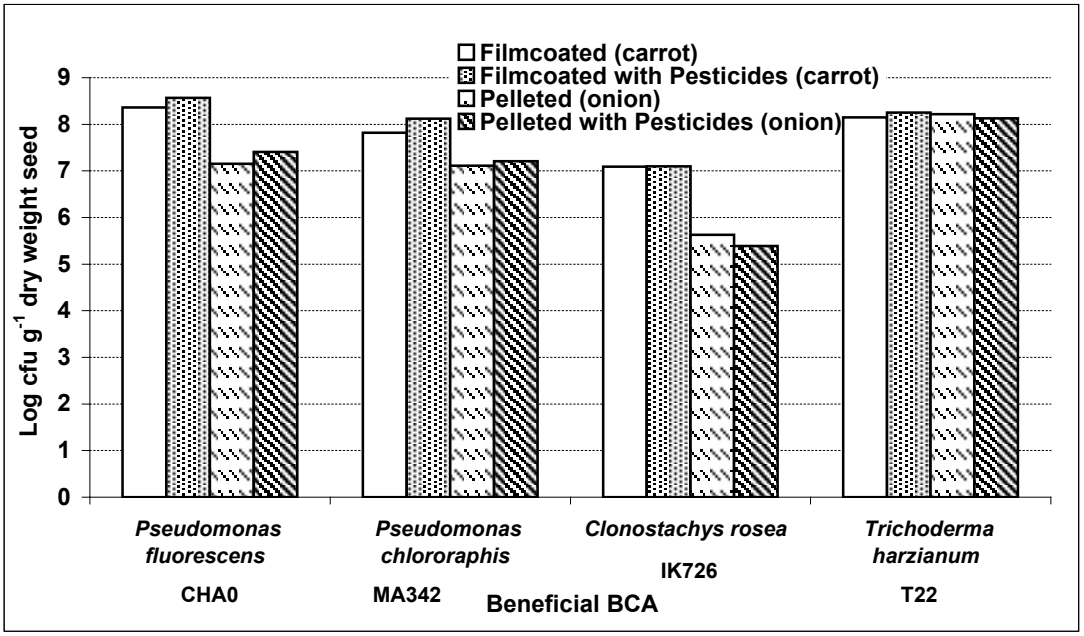


Figure 2.1: Numbers of beneficial microorganisms recovered from seed following application during priming, for seed used in Year 1 field trials (target = 5 $\log_{10}$ cfu g^{-1} dry weight seed)

Wellesbourne site, onion trial

The Wellesbourne field site for the onion trial is illustrated in Figure 2.2. Emergence counts were made for each row of each plot, excluding the first and last 25cm of the row. Figure 2.3 shows the mean emergence at 4 and 7 weeks for the 12 treatments. Emergence was very similar for all treatments at both assessment dates but some significant differences were found. For example, the addition of the pesticide to seed had a significant positive effect on emergence ($P<0.05$). At 4 weeks, seedlings treated with *T. harzianum* T22 plus pesticide had the highest mean emergence (51.4 seedlings), which was significantly better than the primed control plus pesticide treatment (49.7 seedlings). However, without the addition of the pesticide, *P. chlororaphis* MA342 treated seed had a significantly higher emergence than the primed control without pesticides. At 7 weeks, within the pesticide-amended treatments, *T. harzianum* T22 had a significantly higher mean emergence than the primed control. *Clonostachys rosea* IK726 and *P. chlororaphis* MA342 had significantly better emergence than the primed control without the addition of the

©2004 HortLINK Consortium members

pesticides.

Figure 2.2: Onion field trial at Wellesbourne (13 weeks post drilling)

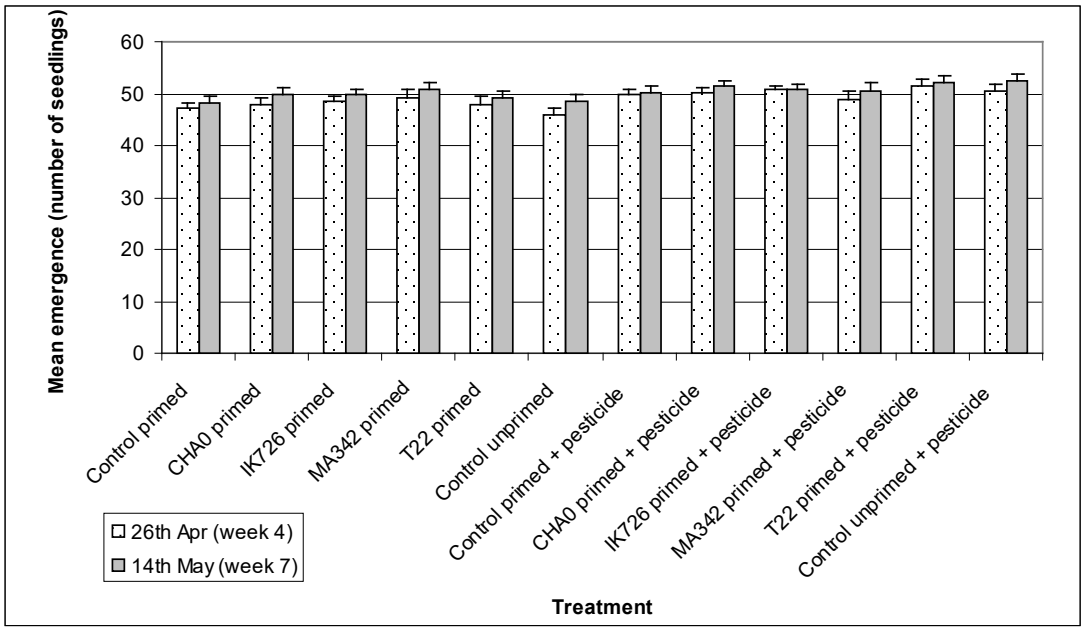


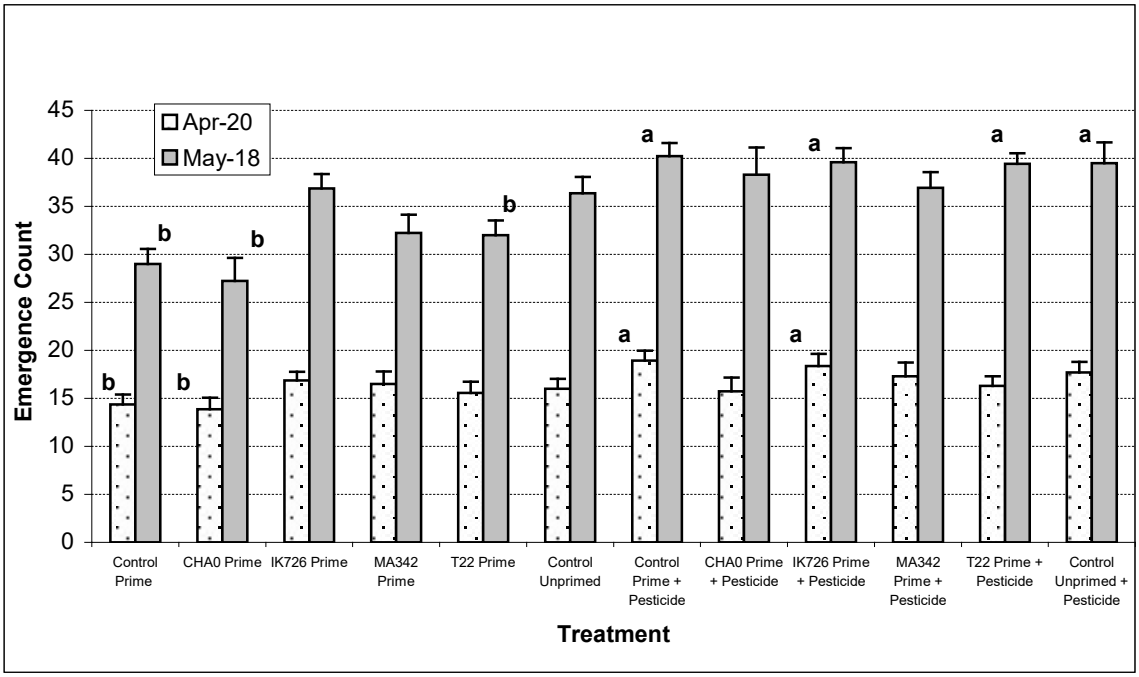
Figure 2.3: Mean emergence of treated onion seed at the Wellesbourne field site. Bars represent standard errors of the means

Grower site, onion trial

At the grower site (Elvedon Farm Ltd; Figure 2.4), all four rows were assessed. For the first emergence count (20th April, week 6), a 1 m length of each row was assessed. The second emergence count (18th May; week 10) was done by measuring 0.25 m in from the beginning of each plot and counting the emerged seedlings for a 2.5 m length of each row. None of the applied microorganisms had any significant effect on onion seedling emergence either in the presence or absence of the pesticides or on either assessment date (Figure 2.5). Seed treatments with the pesticide amendment showed increased emergence compared to the treatments without pesticides, and this is more apparent by the later assessment date.

Figure 2.4: Onion field trial at Elvedon Farms Ltd.

©2004 HortLINK Consortium members



Bars represent Standard Error of Means. Significant differences between samples at the same assessment date denoted by '**a**-**b**'.

Figure 2.5: Mean emergence of treated onion seed at the grower field site (Elvedon Farms Ltd). Apr 20 (week 6); May 18 (week 10)

Elsoms organic site, onion trial

Onions were grown in modules and then transplanted into the field. The two inner rows were assessed by measuring 0.5 m in from the beginning of each plot and counting the emerged seedlings for a 2 m length of each row. All the microorganisms except *C. rosea* IK726 improved seedling emergence in the organic onion trial when compared with both controls (Figure 2.6).

©2004 HortLINK Consortium members

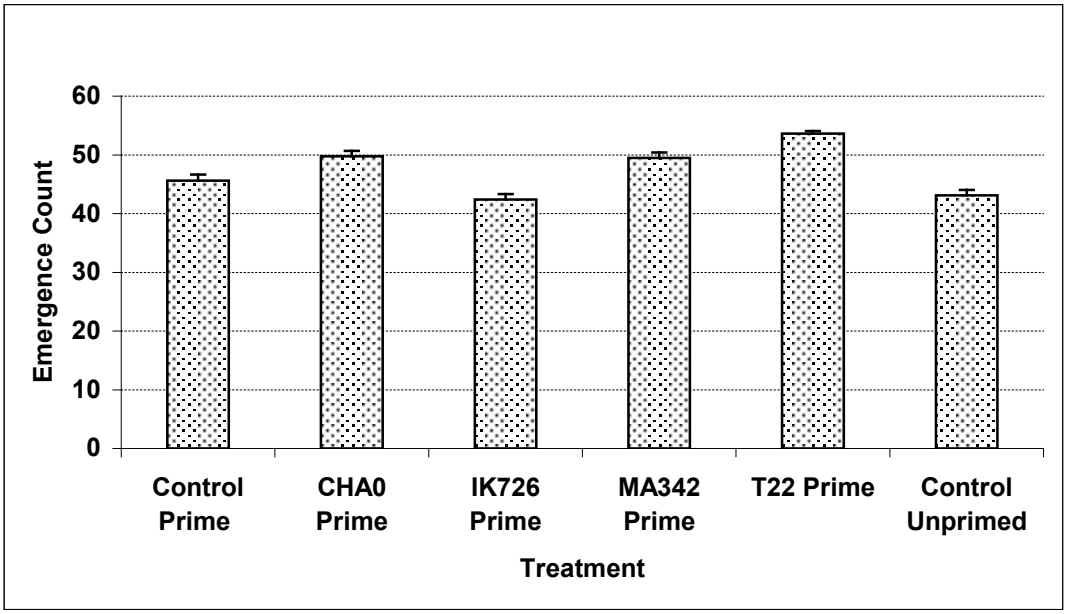


Figure 2.6: Mean emergence of treated onion seed at Elsoms organic field site (28th June, week 8). Bars represent standard errors of the means.

Wellesbourne site, carrot trial

The Wellesbourne field site for the carrot trial is illustrated in Figure 2.7. Emergence counts were made for each row of each plot, excluding the first and last 25cm of the row. Figure 2.8 shows the mean emergence at 3 and 6 weeks for the 12 treatments. Pesticide amendment had a significant positive effect on mean emergence ($P<0.05$). At 3 weeks, within pesticide-amended treatments the mean emergence was significantly higher with *P. fluorescens* CHA0 and *P. chlororaphis* MA342 treatments than the primed control. However, without the pesticide treatment, the primed control was significantly better than *P. fluorescens* CHA0 and *C. rosea* IK726. At 6 weeks, *P. chlororaphis* MA342 plus pesticides was significantly better than the primed control plus pesticides. Again, without the pesticide treatment, the primed control was significantly better than *P. fluorescens* CHA0 and *C. rosea* IK726.

©2004 HortLINK Consortium members

Figure 2.7: Carrot field trial at Wellesbourne (6 weeks post drilling)

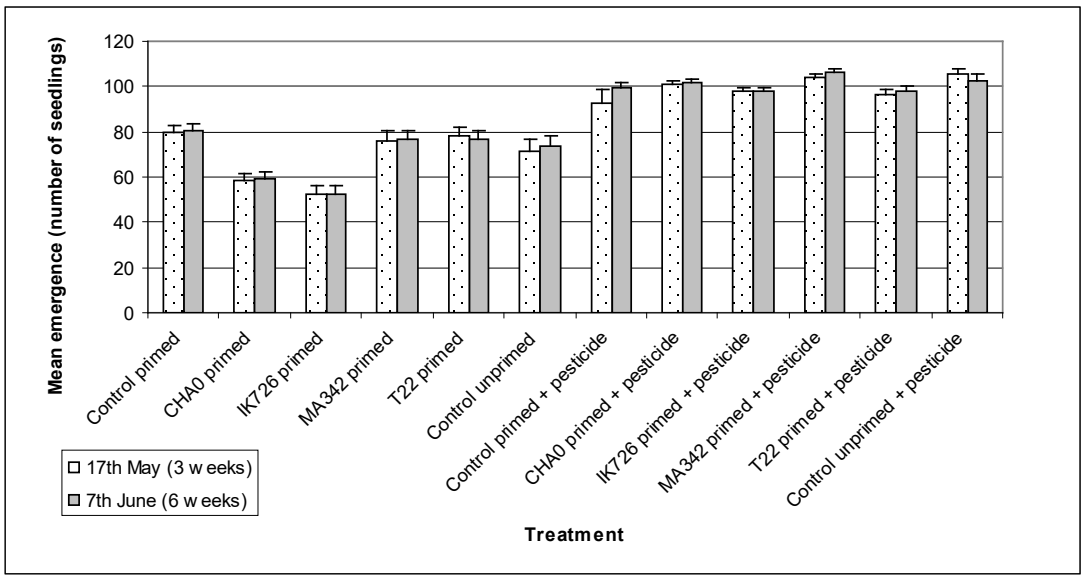
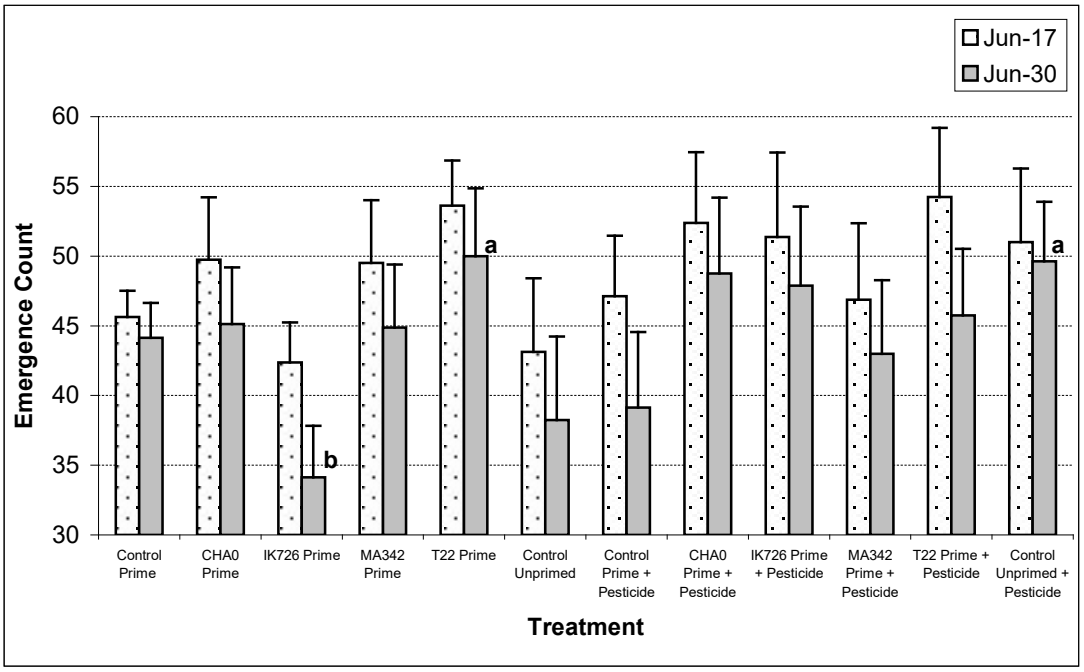


Figure 2.8: Mean emergence of treated carrot seed at the Wellesbourne field site

Grower site, carrot trial

At the grower site the two inner rows were assessed by measuring 0.5 m in from the beginning of each plot and counting the emerged seedlings for a 2 m length of each row. In the absence of pesticides, on both assessment dates all the microorganisms except *C. rosea* IK726 improved carrot seedling emergence when compared with both controls, although this was not statistically significant (Figure 2.9). In the presence of the pesticides, all four microorganisms improved emergence at the first assessment date when compared with the primed, but not the unprimed control. At the later assessment, the *T. harzianum* T22 treatment in the absence of pesticide improved emergence compared with the control treatments although not significantly.



Bars represent Standard Error of Means. Significant differences between samples at the same assessment time denoted by '**a**-**b**'.

Figure 2.9: Mean emergence of treated carrot seed at the grower field site (T. Hammond & Sons). Jun 17 (week 5); Jun 30 (week 7).

Elsoms organic site, carrot trial

The carrot trial needed to be re-drilled due to problems with weeds. Assessments will be made as soon as possible.

©2004 HortLINK Consortium members

Conclusions

- All beneficial microorganisms were successfully applied to onion and carrot seed during priming, and the target rate of 5 $\log_{10}$ cfu g^{-1} dry weight seed was achieved.
- Field trials showed that the application of pesticides to seed improved emergence, whereas the microorganisms had varied effects across the different sites, and the two crops.
- *P fluorescens* CHA0 with the addition of pesticides improved emergence at 3 weeks in the Wellesbourne carrot crop compared to the primed control with pesticides. However, this effect was not seen at later assessment times, and without the pesticide *P. fluorescens* CHA0 was worse than the primed control.
- *C. rosea* IK726 improved emergence compared to the primed control without the addition of pesticides in the Wellesbourne onion trial, but was worse than the primed control without pesticides in the organic onion trial and the Wellesboune carrot trial.
- *P. chlororaphis* MA342 improved emergence over the primed control without the addition of pesticides in the Wellesbourne onion trial, and again improved emergence over the primed control with the addition of pesticides in the Wellesbourne carrot trial
- *T. harzianum* T22 plus pesticide improved emergence compared to the primed control with the addition of pesticides in the Wellesbourne onion trial.

©2004 HortLINK Consortium members

Objective 03: Characterise growth and establishment responses to seed-applied microorganisms of carrot and onion plants grown in the glasshouse in a range of natural and pathogen infested soils. (Milestones 3.1, S3.1) Complete

Introduction

Glasshouse experiments were set up using seed from the same 12 treated batches used for the field trials (Table 2.1), to test the effects of the microorganism treatment and priming on onion and carrot emergence and growth in different soil types.

Materials and methods

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). A nutrient analysis of the soils was carried out at Warwick HRI, and is presented in Appendix 3.1. Soil moisture release curves were also determined using pressure plate apparatus at Warwick HRI, and these curves are given in Appendix 3.2.

For experimental work, soil was sieved to a size of 5mm, and the sandy clay loam was mixed 4:1 with vermiculite to improve the soil structure. Experiments were designed for each crop, consisting of 4 replicates, with 6 pots per treatment and 4 seeds per pot (Figure 3.1). 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

©2004 HortLINK Consortium members

determined. The mean fresh and dry weight of seedlings was subsequently calculated, and $\log_{10}$ transformed before analysis. Significant differences between sample means (Fisher's protected LSD (P = 0.05)) were determined by analysis of variance (ANOVA).

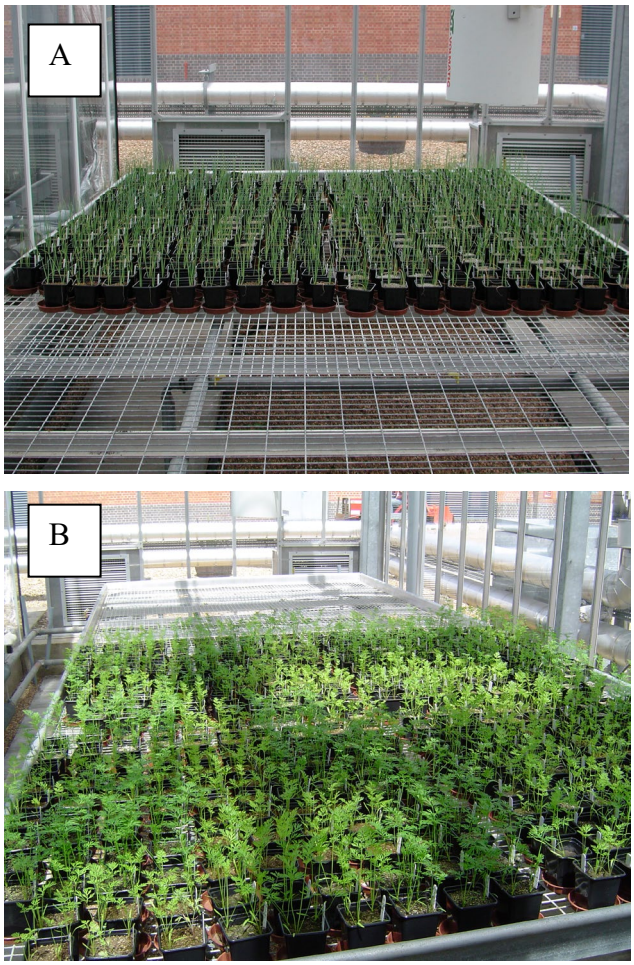


Figure 3.1: Layout of glasshouse experiments (example of two replicates) A) Onion experiment B) Carrot experiment

Results and discussion

Onion glasshouse experiment

The full analysis of all data is given in Appendix 3.3. Tables presenting the statistical analyses to illustrate the effects of soil type, seed treatment and pesticide application are given in Appendices 3.4-3.6, and these results are discussed below.

©2004 HortLINK Consortium members

Emergence - Soil type effects

Across all treatments, the soil type had a significant effect on emergence time (Figure 3.2), and the mean emergence time was significantly later in light sandy loam than the other two soil types (Appendix 3.4). Similarly, the time taken to 50% and 80% emergence of the seedlings in the light sandy loam was significantly longer than the peat or sandy clay loam soils (Appendix 3.4). The final percent emergence of onion seedlings was approximately 90% in all soil types, with no significant difference.

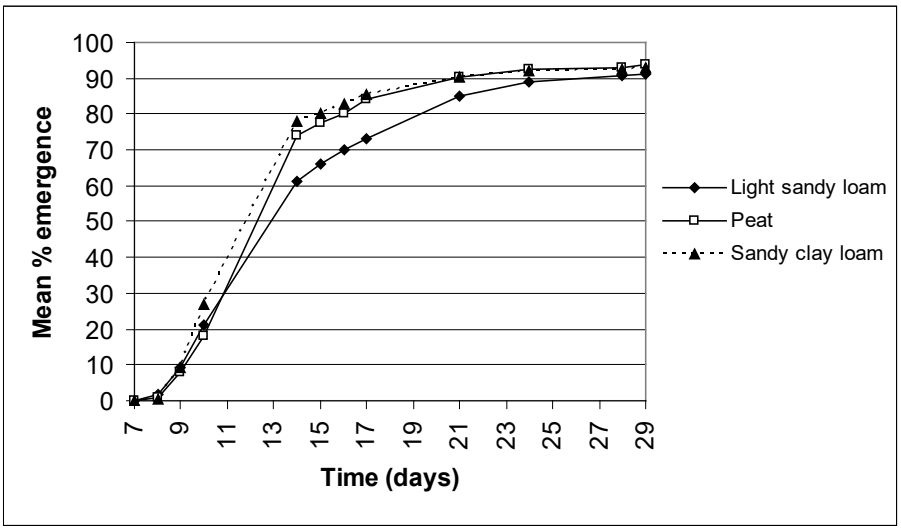


Figure 3.2: Mean percent emergence of treated onion seed over time in three soil types

Emergence - Seed treatment effects

Overall, the primed control and all microorganism treated primed seed (with the exception of *P. fluorescens* CHA0 primed seed) emerged significantly faster than the unprimed control (Figure 3.3; Appendix 3.5). Similarly, the primed control and all microorganism treated primed seed reached 50% emergence significantly faster than the unprimed control (Appendix 3.5), confirming the positive effects of seed priming. However, the addition of *P. fluorescens* CHA0 or *C. rosea* IK726 to onion seed caused a significantly slower time to 50% emergence than the primed control (Appendix 3.5). The primed control, *P. chlororaphis* MA342 and *T. harzianum* T22 primed seed reached 80% emergence significantly faster than the unprimed control,

©2004 HortLINK Consortium members

although there was no significant difference for the time taken to 80% emergence for all the primed treatments (Appendix 3.5).

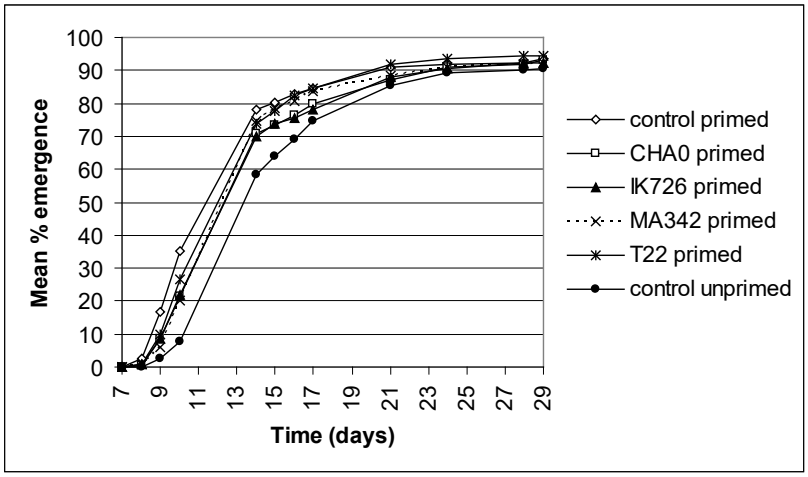


Figure 3.3: Mean percent emergence of onion seed with different microorganisms applied during priming

Emergence- Pesticide effects

Overall, the addition of pesticides had no significant effect on the emergence of the onion seed (Figure 3.4; Appendix 3.6).

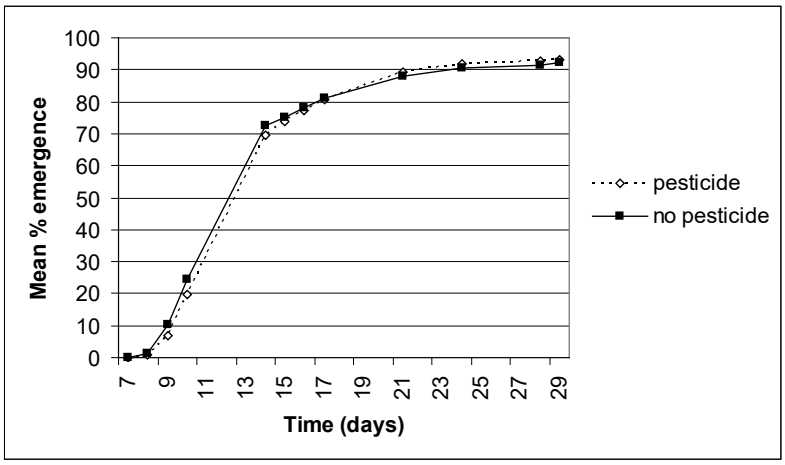


Figure 3.4: Mean percent emergence of onion seed with or without the application of pesticides

Mean fresh weight of seedlings

At harvest (8 weeks post planting), the mean fresh weight of the seedlings from the light sandy loam was significantly higher than the other two soil types, and the peat soil produced a significantly higher mean fresh weight of seedlings than the sandy clay loam (Table 3.1). These differences found with soil type may be a reflection of the nutrient status of the different soils. The application of different microorganisms to seed did not significantly increase the mean fresh weight of the seedlings (Table 3.1), although it was also found that the addition of pesticides resulted in a small but significantly lower mean fresh weight of seedlings (Table 3.2). Similar results were seen with the mean dry weights (not shown).

©2004 HortLINK Consortium members

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

Soil type	**Light sandy loam**		**Peat**		**Sandy clay loam**	
Pesticide	-	+	-	+	-	+
Primed control	961 (6.9)	827 (6.7)	796 (6.7)	781 (6.7)	424 (6.1)	451 (6.1)
Primed *P. fluorescens* CHA0	845 (6.7)	745 (6.6)	667 (6.5)	753 (6.6)	423 (6.1)	400 (6.0)
Primed *C. rosea* IK726	927 (6.8)	753 (6.6)	729 (6.6)	729 (6.6)	452 (6.1)	444 (6.1)
Primed *P. chlororaphis* MA342	852 (6.8)	748 (6.6)	830 (6.7)	700 (6.6)	459 (6.1)	437 (6.1)
Primed *T. harzianum* T22	893 (6.8)	777 (6.7)	741 (6.6)	707 (6.6)	406 (6.0)	421 (6.0)
Unprimed control	807 (6.7)	668 (6.5)	722 (6.6)	696 (6.6)	430 (6.1)	433 (6.1)
LSD_1 (0.05)	(0.25)	(0.25)	(0.25)	(0.25)	(0.25)	(0.25)
Mean	**813 (6.7)**		**743 (6.6)**		**432 (6.1)**	
LSD_2 (0.05)	(0.07)		(0.07)		(0.07)	

LSD_1 Comparing seed treatments within a soil type

LSD_2 Comparing soil types

©2004 HortLINK Consortium members

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

Treatment	Mean fresh weight (mg)
Pesticide	617 (6.4)
No pesticide	661 (6.5)
LSD (0.05)	(0.06)

Carrot glasshouse experiment

The full analysis of all data is given in Appendix 3.7. Tables presenting the statistical analyses to illustrate the effects of soil type, seed treatment and pesticide application are given in Appendices 3.8-3.10, and these results are discussed below.

Emergence – Soil type effects

Across all treatments, the soil type had a significant effect on mean emergence time, with slower emergence in the peat soil compared with the other two soil types (Figure 3.5). Similarly, the time taken to 50% and 80% emergence was significantly affected by soil type, with the peat soil showing the slowest emergence time (Appendix 3.8). This may be due to this soil taking up water slowly initially compared to the other soil types, possibly delaying the germination of the seeds. The final percent emergence in the three soil types was significantly different in the carrot trial (Appendix 3.8), reaching 86% in the sandy clay loam, 80% in the peat soil, and 70% in the light sandy loam. This may suggest the presence of a carrot pathogen in the light sandy loam, resulting in a significantly lower emergence than the other soils, and the possibility of a carrot pathogen in the peat soil as well.

©2004 HortLINK Consortium members

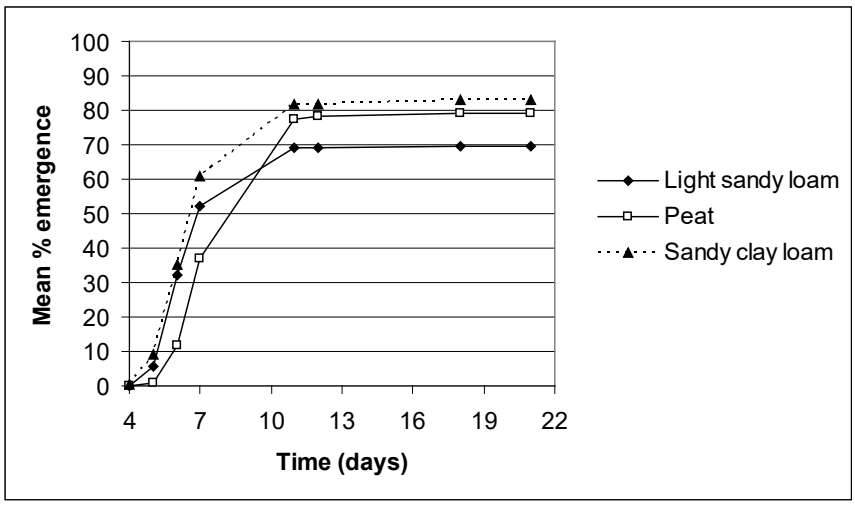


Figure 3.5: Mean percent emergence of treated carrot seed over time in three soil types

Emergence – seed treatment effects

All the primed treatments had a significantly faster mean emergence time than the unprimed seed, and seed treated with *C. rosea* IK726 or *T. harzianum* T22 emerged significantly faster than the primed control (Figure 3.6; Appendix 3.9). Similarly, all the primed treatments reached 50% and 80% emergence faster than the unprimed control, and within the primed treatments *T harzianum* T22 and *C. rosea* IK726 reached 50% and 80% emergence faster than the primed control (Appendix 3.9).

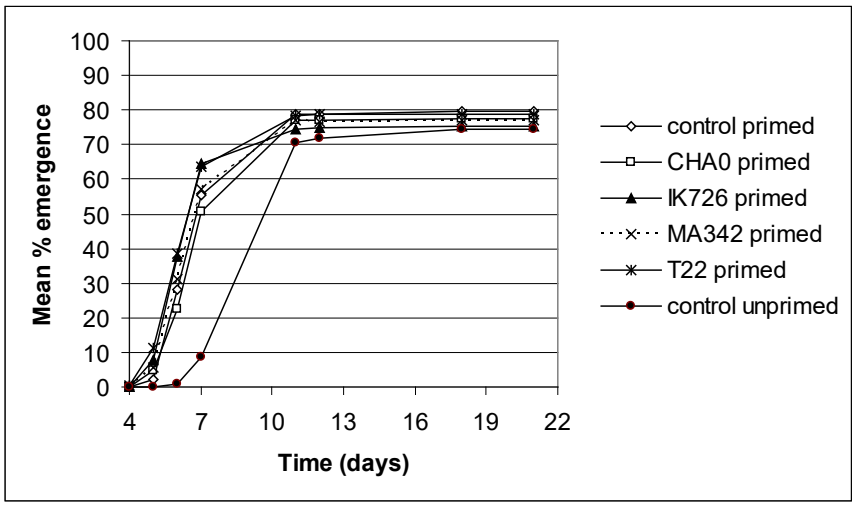


Figure 3.6: Mean percent emergence of carrot seed with different microorganisms applied during priming

©2004 HortLINK Consortium members

Emergence – Pesticide effects

There was no significant difference in mean emergence time between seeds treated with pesticides and those without (Figure 3.7, Appendix 3.10). Seeds without pesticides reached 50% emergence slower than those with pesticide, but by 80% emergence there was no significant difference. Overall, the final percent emergence was significantly higher with pesticide treated seed, again suggesting the presence of a carrot pathogen in one of the soils (Appendix 3.10).

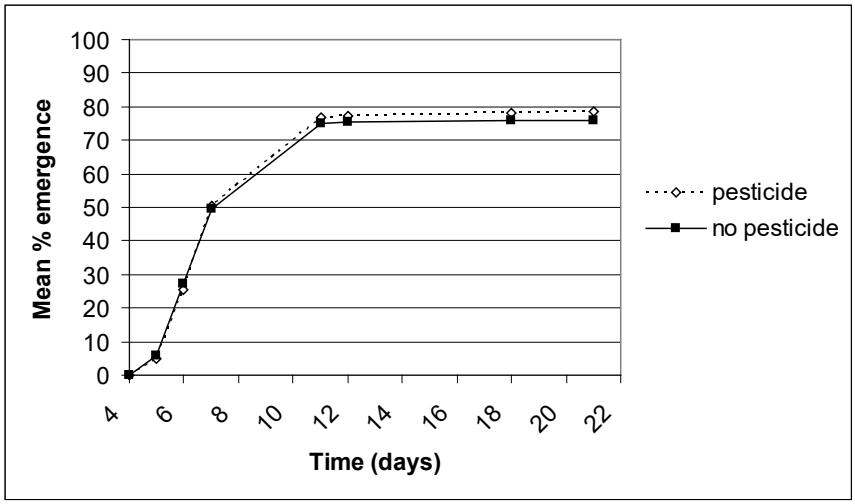


Figure 3.7: Mean percent emergence of carrot seed with or without the application of pesticides

Mean fresh weight of seedlings

There was a clear difference in the seedling growth in the three soil types (Figure 3.8). The mean fresh weight of seedlings was significantly greater in peat soil than in the other two soil types, and seedlings in the light sandy loam had a significantly greater mean fresh weight than those in the sandy clay loam (Table 3.3). This may be due to the nutrient status of the soils, with the peat soil providing the best plant nutrition. There was no significant effect of the different seed treatments (Table 3.3) or addition of pesticides on mean fresh weight of seedlings (Table 3.4). Similar results were found with the mean dry weight (not shown).

©2004 HortLINK Consortium members

Figure 3.8: Comparison of growth of primed (control) carrot seedlings without pesticide in three soil types. Left to right: Peat, light sandy loam and sandy clay loam soils

©2004 HortLINK Consortium members

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

Soil type	**Light sandy loam**		**Peat**		**Sandy clay loam**	
Pesticide	-	+	-	+	-	+
Primed control	770 (6.7)	735 (6.6)	1355 (7.2)	1361 (7.2)	603 (6.4)	667 (6.5)
Primed *P. fluorescens* CHA0	574 (6.4)	859 (6.8)	1492 (7.3)	1429 (7.3)	632 (6.5)	617 (6.4)
Primed *C. rosea* IK726	536 (6.3)	915 (6.8)	1424 (7.3)	1430 (7.3)	625 (6.4)	676 (6.5)
Primed *P. chlororaphis* MA342	646 (6.5)	788 (6.7)	1306 (7.2)	1419 (7.3)	612 (6.4)	661 (6.5)
Primed *T. harzianum* T22	727 (6.6)	671 (6.5)	1626 (7.4)	1674 (7.4)	574 (6.4)	603 (6.4)
Unprimed control	791 (6.7)	730 (6.6)	1421 (7.3)	1598 (7.4)	543 (6.3)	555 (6.3)
LSD_1 (0.05)	(0.50)	(0.50)	(0.50)	(0.50)	(0.50)	(0.50)
Mean	**721 (6.6)**		**1457 (7.3)**		**613 (6.4)**	
LSD_2 (0.05)	(0.14)		(0.14)		(0.14)	

LSD_1 Comparing seed treatments within a soil type

LSD_2 Comparing soil types

©2004 HortLINK Consortium members

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

Treatment	Mean fresh weight (mg)
Pesticide	898 (6.8)
No pesticide	830 (6.7)
LSD (0.05)	(0.12)

Conclusions

- Primed seed emerged faster than unprimed seed.
- The soil type had a significant effect on emergence, with the slowest emergence occurring in the light sandy loam for the onions, and the peat soil for the carrots.
- The light sandy loam and peat soils may contain a carrot pathogen, as there was a significant reduction in carrot emergence in these soils compared to the sandy clay loam.
- Onion seed treated with *P. fluorescens* CHA0 or *C. rosea* IK726 was slower to reach 50% emergence than the primed control.
- However, carrot seed treated with *C. rosea* IK726 or *T. harzianum* T22 improved emergence over the primed control.
- The application of pesticides to seed did not affect emergence.
- Soil type had a significant effect on the mean fresh weight of seedlings, possible due to the nutrient status of the soils. Seedlings in peat soil had a greater mean fresh weight than those grown in light sandy loam or sandy clay loam.
- The application of microorganisms did not affect the mean fresh weight of seedlings after 8 weeks growth for either the carrot or onion.
- Pesticide application caused a small but significant reduction in the mean fresh weight of onion seedlings, but did not affect the carrots.

©2004 HortLINK Consortium members

Objective 04: Determine the survival and proliferation of seed-applied microorganisms on seedlings after sowing in soil in the glasshouse and field. (Milestone 4.1) Ongoing

Introduction

An experiment has been set up in the glasshouse to monitor the survival of marked strains of microorganisms on onion seedlings, following application to onion seed during priming. Rifampicin-resistant isolates of the bacteria and a hygromycin B-resistant isolate of *C. rosea* are being used to aid recovery of the microorganisms from roots. There is no marked strain of *T. harzianum* T22, but a *Trichoderma* selective medium is being used to monitor numbers of this fungus. In order to ensure the activity of the marked strains is similar to the wild-type strains, a comparison of the marked and wild-type isolates has been incorporated into the design of this experiment, with assessments being made on emergence and growth. The planned monitoring of survival of applied microorganisms on seedlings in field trials will not be carried out as PSD refused permission to use the marked strains of the microorganisms in the field.

Materials and methods

Seed samples were drum primed at Warwick HRI, as described in Objective 01. Table 4.1 shows the treatments used in this experiment, and, where appropriate, the initial numbers of applied microorganism surviving on the seed after drum priming. It was not possible to determine the numbers of wild-type bacteria due to the inability to distinguish them from other bacteria associated with the seed. 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; hygromycin B amended (150µg ml^{-1}) agar for *C. rosea* IK726; and *Trichoderma* selective medium for *T. harzianum* T22).

Emergence and growth of seedlings in all treatments will be assessed, and survival of the marked strains on roots will be assessed at 2, 4 and 8 weeks. A similar experiment is planned to monitor the survival of marked strains on carrot seedlings, and will be set up later in the year.

©2004 HortLINK Consortium members

Table 4.1: Onion seed treatments used in the glasshouse trial investigating the survival of microorganisms on roots following application to seed during drum priming

Treatment	**Number of cfu g^{-1} dry seed**	**Log_{10} cfu g^{-1} dry seed**
Unprimed control	NA	NA
Primed control	NA	NA
Wild-type *P. fluorescens* CHA0	NA	NA
Rifampicin- resistant *P. fluorescens* CHA0	4.1×10^5	5.6
Wild-type *P. chlororaphis* MA342	NA	NA
Rifampicin- resistant *P. chlororaphis* MA342	3.5×10^7	7.5
Wild-type *C. rosea* IK726	2.1×10^5	5.3
Hygromycin B-resistant *C. rosea* IK726	1.2×10^5	5.1
T. harzianum T22	7.9×10^4	4.9

NA = not applicable

Conclusions

- The beneficial microorganisms were all applied to onion seed targeted to reach a rate of 5 log_{10} cfu g^{-1} dry seed. *Trichoderma harzianum* T22 was recovered from the seed batch in numbers slightly below the target, but the other microorganisms were recovered at or above the target rate.

©2004 HortLINK Consortium members

Objective 05: Characterise the long-term shelf life (up to 1 year) of seed-applied microorganisms under commercially-representative storage conditions. (Milestone S5.1) Ongoing

Introduction

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

Four beneficial microorganisms, selected on criteria described previously, 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 potato dextrose agar 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 potato dextrose agar.

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 with Reichold biocide-free polymer, both with and without the standard rate of Wakil XL fungicide. Samples of onion seed were pelleted with Propell blend both with and without standard rates of HY-TL and Apron 35 fungicides and Force ST insecticide. 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.

Three replicate 0.5 g samples were spun at high power on a vortex mixer for 3 x 1 minute in 4.5 ml of SDW. A dilution series was prepared in SDW and selected dilutions were spiral plated onto 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.

©2004 HortLINK Consortium members

Results and discussion

There were clear differences between the isolates with respect to their survival during long-term storage. Although each isolate produced an individual survival profile, the overall trends followed a similar general pattern, especially in response to the effects of the film coating of carrot, the pelleting of onion and the application of pesticides.

Onion

The long-term survival of the microorganisms on onion seed is represented in Figures 5.1-5.4. 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. 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. *Trichoderma harzianum* T22 maintained populations of all treatment above the target application range up to the 180 d storage interval, possibly in response to the higher initial application rate compared with the studies on carrot.

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.

©2004 HortLINK Consortium members

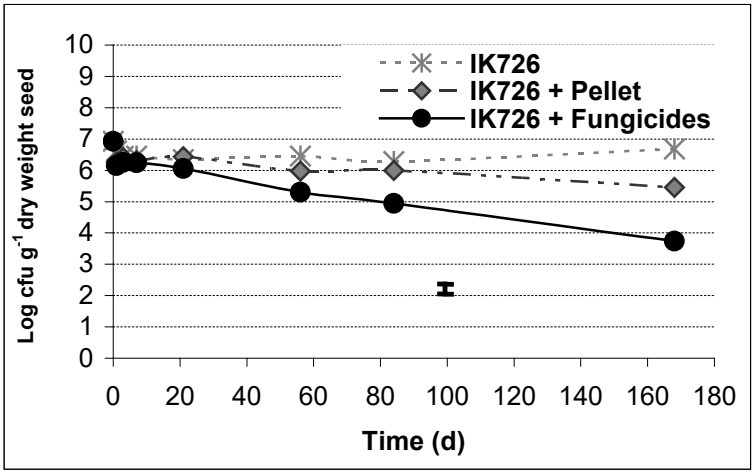


Figure 5.1: Shelf life study illustrating survival of *Clonostachys rosea* IK726 on onion seed following steeping priming. Bar indicates least significant difference.

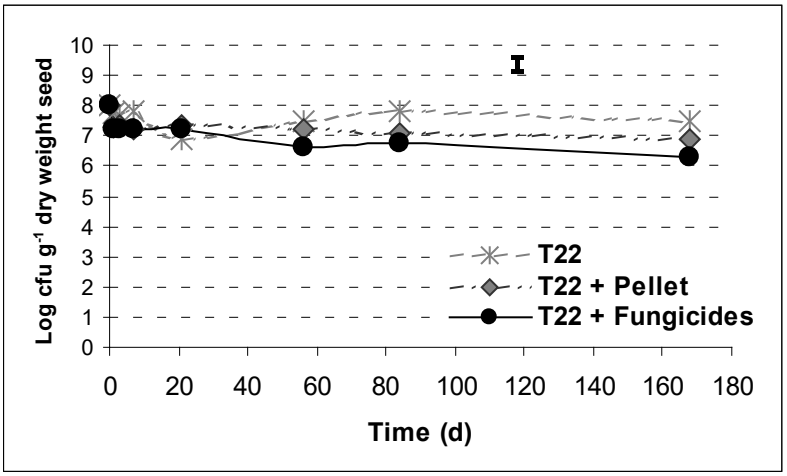


Figure 5.2: Shelf life study illustrating survival of *Trichoderma harzianum* T22 on onion seed following steeping priming. Bar indicates least significant difference.

©2004 HortLINK Consortium members

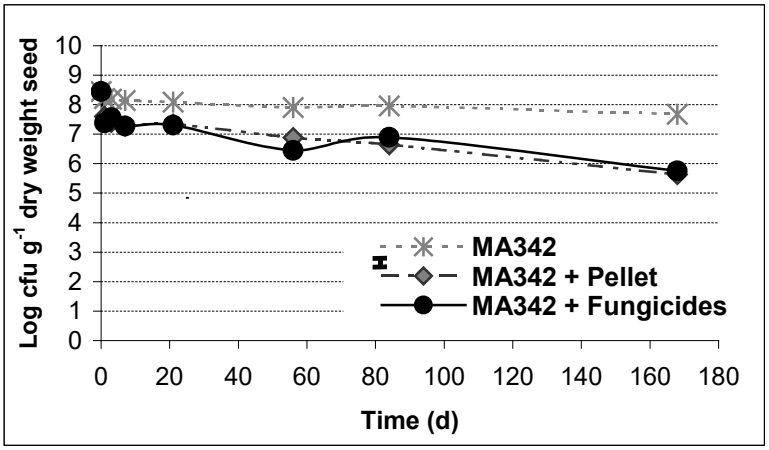


Figure 5.3: Shelf life study illustrating survival of *Pseudomonas chlororaphis* MA342 on onion seed following steeping priming. Bar indicates least significant difference.

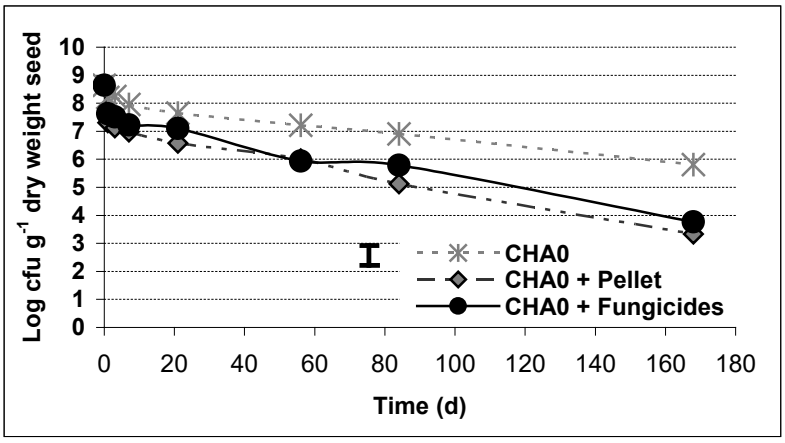


Figure 5.4: Shelf life study illustrating survival of *Pseudomonas fluorescens* CHA0 on onion seed following steeping priming. Bar indicates least significant difference.

Carrot

The long-term survival of the microorganisms on carrot seed is represented in Figures 5.5-5.7 (with the shelf-life of *C. rosea* IK726 on carrot currently in progress). 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 isolates. 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. Film coated treatments of *Ps. chlororaphis* MA342 and *Ps. fluorescens* CHA0 maintained populations within the target application range for approximately 275 d and 150 d respectively.

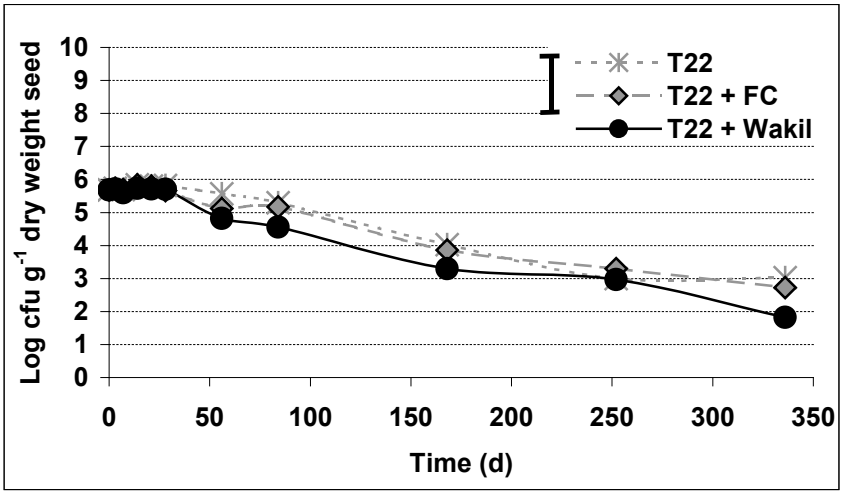


Figure 5.5: Shelf life study illustrating survival of *Trichoderma harzianum* T22 on carrot seed following steeping priming. Bar indicates least significant difference.

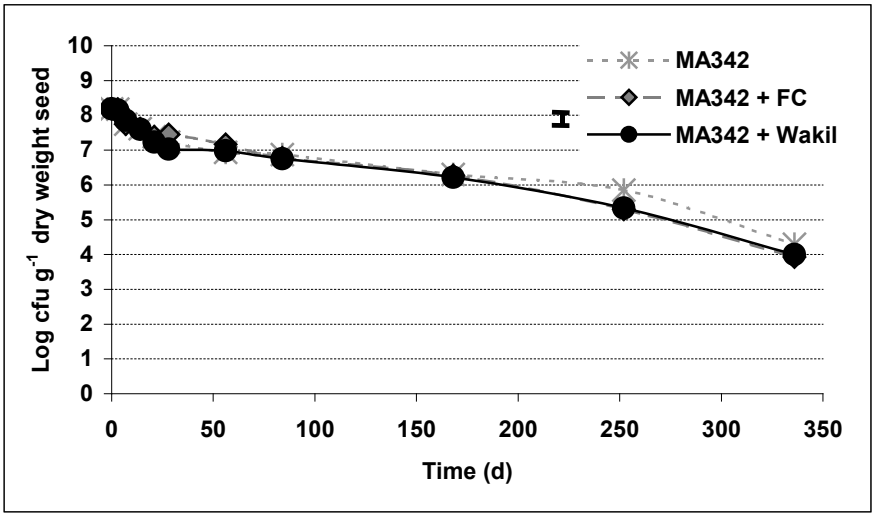


Figure 5.6: Shelf life study illustrating survival of *Pseudomonas chlororaphis* MA342 on carrot seed following steeping priming. Bar indicates least significant difference.

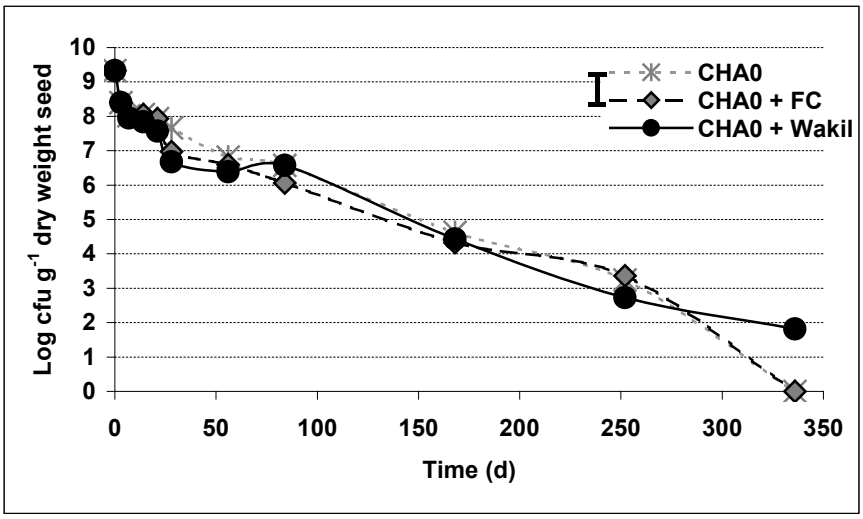


Figure 5.7: Shelf life study illustrating survival of *Pseudomonas fluorescens* CHA0 on carrot seed following steeping priming. Bar indicates least significant difference.

Conclusions

- *C. rosea* IK726 was affected most noticeably by the application of fungicides to the onion pellet in shelf-life studies.
- With the comparative data available so far (up to the 180 d storage interval) there appears to be little apparent difference in survival of the two bacterial isolates as film coated treatments on carrot compared with pelleted treatments on onion. These isolates so far appear to survive more readily as primed only treatments on onion than on carrot. All treatments of *T. harzianum* T22 survived better on onion than on carrot where the 100-fold higher initial application rate may have played a role in stabilising these populations.

©2004 HortLINK Consortium members

Technology transfer

Objective 07: Technology transfer and exploitation planning (Milestones S7.1, S7.2, S7.3)

- An Agriculture LINK newsletter article has been published.
- Discussions have taken place with PSD regarding the use of different isolates and marked strains.
- Presentations on the project have been made to Horticulture LINK 2004 and the Vegetable Consultants group.
- The Wellesbourne field trials were on display to the public at the Warwick HRI Open Day, held on the 10th July 2004.
- A poster of the work is planned to be exhibited at the British Carrot Growers Association site in October 2004.
- A demonstration of the organic field trial of this project is planned for the Elsoms organic day in October 2004.

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*.

©2004 HortLINK Consortium members

Appendices

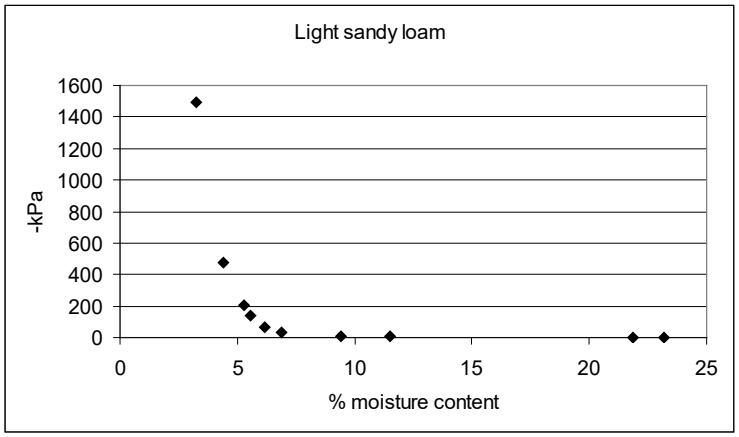
Appendix 3.1: Nutrient analysis of soil types used in glasshouse experiments

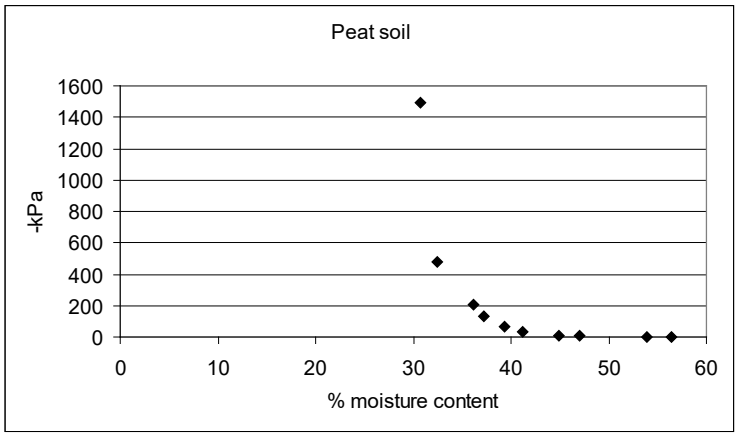
	Light sandy loam	Peat	Sandy clay loam
Fresh soil			
% water	14.14	38.38	6.65
NO_3-N ppm	0	114	15
NH_4-N ppm	6.8	9.2	5.9
Total N%	0.056	1.688	0.053
Total C%	1.224	21.840	0.994
Air dried soil			
pH	6.8	7.5	7.0
e.c.µS*	2043	2726	2138
P mg/L	102	98	73
K mg/L	177	275	164
Mg mg/L	88	77	61
NO_3-N mg/L	0	106	20

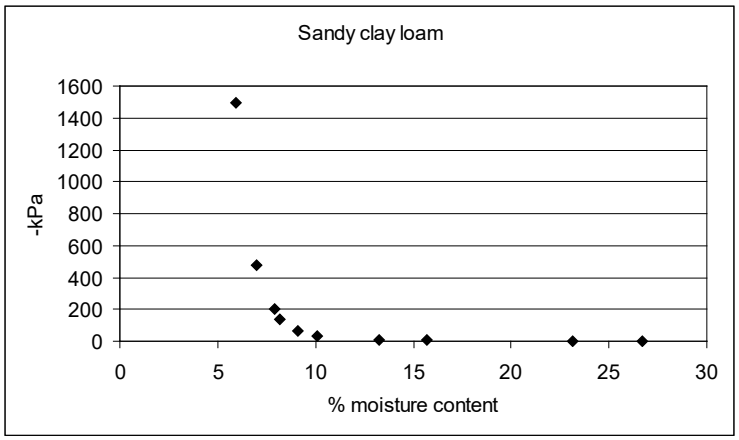
* electrical conductivity in microSiemens

©2004 HortLINK Consortium members

Appendix 3.2: Soil moisture release curves for the three soil types used in glasshouse experiments







©2004 HortLINK Consortium members

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

Soil type	Treatment	Pesticide	Mean emergence time (days)	Time to 50% emergence (days)	Time to 80% emergence (days)	Final percent emergence
Light sandy loam	Primed control	+	12.0	11.2	14.4	88.8 (70.4)
Light sandy loam	Primed CHA0	+	14.6	14.3	14.7	83.4 (66.0)
Light sandy loam	Primed IK726	+	13.8	13.3	16.2	90.7 (72.2)
Light sandy loam	Primed MA342	+	13.3	12.4	15.5	89.9 (71.4)
Light sandy loam	Primed T22	+	14.0	12.8	17.8	98.3 (82.5)
Light sandy loam	Unprimed control	+	14.7	14.6	17.5	89.6 (71.2)
Light sandy loam	Primed control	-	12.2	11.6	14.4	85.2 (67.4)
Light sandy loam	Primed CHA0	-	14.4	13.7	18.0	89.8 (71.4)
Light sandy loam	Primed IK726	-	14.3	14.0	17.3	80.8 (64.0)
Light sandy loam	Primed MA342	-	12.0	11.2	13.9	93.1 (74.7)
Light sandy loam	Primed T22	-	12.5	12.2	14.7	94.8 (76.8)
Light sandy loam	Unprimed control	-	14.2	13.4	17.8	82.7 (65.4)
LSD (0.05)			**2.42**	**2.59**	**3.90**	**(11.54)**

©2004 HortLINK Consortium members

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

Soil type	Treatment	Pesticide	Mean emergence time (days)	Time to 50% emergence (days)	Time to 80% emergence (days)	Final percent emergence
Peat	Primed control	+	11.9	11.5	12.9	95.5 (77.7)
Peat	Primed CHA0	+	12.0	11.4	14.0	89.2 (70.8)
Peat	Primed IK726	+	12.5	11.7	14.6	97.4 (80.6)
Peat	Primed MA342	+	13.4	12.5	15.9	90.0 (71.5)
Peat	Primed T22	+	12.0	11.5	13.8	90.3 (71.9)
Peat	Unprimed control	+	14.5	13.4	17.8	93.9 (75.7)
Peat	Primed control	-	12.1	11.1	14.4	89.3 (70.9)
Peat	Primed CHA0	-	12.6	11.9	14.9	94.1 (76.0)
Peat	Primed IK726	-	13.3	13.0	15.5	91.4 (72.9)
Peat	Primed MA342	-	12.2	11.7	13.3	89.6 (71.2)
Peat	Primed T22	-	12.3	12.0	14.2	89.9 (71.4)
Peat	Unprimed control	-	13.4	13.3	15.3	91.6 (73.1)
LSD (0.05)			**2.42**	**2.59**	**3.90**	**(11.54)**

©2004 HortLINK Consortium members

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

Soil type	Treatment	Pesticide	Mean emergence time (days)	Time to 50% emergence (days)	Time to 80% emergence (days)	Final percent emergence
Sandy clay loam	Primed control	+	11.1	10.3	12.4	91.1 (72.6)
Sandy clay loam	Primed CHA0	+	12.7	11.5	14.1	93.1 (74.8)
Sandy clay loam	Primed IK726	+	12.6	12.1	14.5	91.9 (73.4)
Sandy clay loam	Primed MA342	+	13.0	12.1	15.5	90.8 (72.4)
Sandy clay loam	Primed T22	+	12.3	11.9	14.5	89.3 (70.9)
Sandy clay loam	Unprimed control	+	13.7	13.3	15.9	91.4 (73.0)
Sandy clay loam	Primed control	-	12.0	11.0	14.2	99.0 (84.1)
Sandy clay loam	Primed CHA0	-	12.2	11.4	13.9	89.6 (71.2)
Sandy clay loam	Primed IK726	-	11.7	10.5	13.3	95.3 (77.4)
Sandy clay loam	Primed MA342	-	11.4	10.7	12.9	89.8 (71.4)
Sandy clay loam	Primed T22	-	11.4	10.6	13.3	95.5 (77.7)
Sandy clay loam	Unprimed control	-	13.6	12.9	15.9	87.7 (69.4)
LSD (0.05)			**2.42**	**2.59**	**3.90**	**(11.54)**

©2004 HortLINK Consortium members

Appendix 3.4: Overall effect of soil type on emergence of microorganism-primed onion seedlings

Soil type	Mean emergence time (days)	Time to 50% emergence (days)	Time to 80% emergence (days)	Final percent emergence
Sandy clay loam	12.3	11.5	14.2	92.4 (74.0)
Peat	12.7	12.1	14.7	92.1 (73.7)
Light sandy loam	13.5	12.9	16.3	89.5 (71.1)
LSD (0.05)	**0.70**	**0.75**	**1.12**	**(3.33)**

Appendix 3.5: Overall effect of seed treatment on emergence of microorganism-primed onion seedlings

Treatment	Mean emergence time (days)	Time to 50% emergence (days)	Time to 80% emergence (days)	Final percent emergence
Primed control	11.9	11.1	13.8	92.3 (73.9)
Primed *P. fluorescens* CHA0	13.1	12.4	15.4	90.1 (71.7)
Primed *C. rosea* IK726	13.0	12.4	15.2	91.9 (73.4)
Primed *P. chlororaphis* MA342	12.5	11.8	14.5	90.6 (72.1)
Primed *T. harzianum* T22	12.4	11.8	14.7	93.5 (75.2)
Unprimed control	14.0	13.5	16.7	89.7 (71.3)
LSD (0.05)	**0.99**	**1.06**	**1.59**	**(4.71)**

©2004 HortLINK Consortium members

Appendix 3.6: Overall effect of pesticide application on emergence of microorganism-primed onion seedlings

Treatment	Mean emergence time (days)	Time to 50% emergence (days)	Time to 80% emergence (days)	Final percent emergence
Pesticide	13.0	12.3	15.3	91.7 (73.3)
No pesticide	12.7	12.0	14.8	91.0 (72.6)
LSD (0.05)	**0.57**	**0.61**	**0.92**	**(2.72)**

©2004 HortLINK Consortium members

Appendix 3.7: Effects of soil type, seed treatment and pesticide application on emergence of carrot seedlings (continued overleaf)

Soil type	Treatment	Pesticide	Mean emergence time (days)	Time to 50% emergence (days)	Time to 80% emergence (days)	Final percent emergence
Light sandy loam	Primed control	+	6.3	6.3	7.0	72.2 (58.2)
Light sandy loam	Primed CHA0	+	7.0	6.4	7.3	63.8 (53.0)
Light sandy loam	Primed IK726	+	6.3	6.0	6.7	67.3 (55.1)
Light sandy loam	Primed MA342	+	6.3	6.1	7.0	73.1 (58.7)
Light sandy loam	Primed T22	+	6.3	6.0	7.1	84.5 (66.8)
Light sandy loam	Unprimed control	+	8.6	8.4	9.7	64.6 (53.5)
Light sandy loam	Primed control	-	6.1	6.0	6.7	62.3 (52.1)
Light sandy loam	Primed CHA0	-	6.7	6.3	7.2	71.5 (57.7)
Light sandy loam	Primed IK726	-	5.9	5.8	6.5	71.2 (57.6)
Light sandy loam	Primed MA342	-	6.2	5.9	6.8	71.9 (58.0)
Light sandy loam	Primed T22	-	6.0	5.6	6.5	70.6 (57.1)
Light sandy loam	Unprimed control	-	8.3	7.8	9.3	61.6 (51.7)
LSD (0.05)			**0.60**	**0.57**	**0.73**	**(8.95)**

©2004 HortLINK Consortium members

Appendix 3.7 continued: Effects of soil type, seed treatment and pesticide application on emergence of carrot seedlings (continued overleaf)

Soil type	Treatment	Pesticide	Mean emergence time (days)	Time to 50% emergence (days)	Time to 80% emergence (days)	Final percent emergence
Peat	Primed control	+	7.4	7.1	8.4	88.1 (69.8)
Peat	Primed CHA0	+	7.6	7.3	8.8	82.0 (64.9)
Peat	Primed IK726	+	6.9	6.5	7.7	83.6 (66.1)
Peat	Primed MA342	+	7.7	7.3	8.4	86.8 (68.7)
Peat	Primed T22	+	7.0	6.8	7.7	76.2 (60.8)
Peat	Unprimed control	+	9.6	9.5	10.8	73.1 (58.7)
Peat	Primed control	-	7.7	7.5	8.7	83.4 (66.0)
Peat	Primed CHA0	-	7.6	7.3	8.8	79.4 (63.0)
Peat	Primed IK726	-	6.7	6.4	7.1	74.5 (59.7)
Peat	Primed MA342	-	7.5	7.2	8.6	72.2 (58.2)
Peat	Primed T22	-	7.0	6.7	8.1	76.2 (60.8)
Peat	Unprimed control	-	9.1	9.0	10.1	85.1 (67.3)
LSD (0.05)			**0.60**	**0.57**	**0.73**	**(8.95)**

©2004 HortLINK Consortium members

Appendix 3.7 continued: Effects of soil type, seed treatment and pesticide application on emergence of carrot seedlings

Soil type	Treatment	Pesticide	Mean emergence time (days)	Time to 50% emergence (days)	Time to 80% emergence (days)	Final percent emergence
Sandy clay loam	Primed control	+	6.3	6.0	6.7	91.4 (72.9)
Sandy clay loam	Primed CHA0	+	6.7	6.3	7.4	88.1 (69.8)
Sandy clay loam	Primed IK726	+	5.9	5.6	6.4	83.2 (65.8)
Sandy clay loam	Primed MA342	+	6.1	6.0	6.9	85.5 (67.6)
Sandy clay loam	Primed T22	+	5.9	5.8	6.7	85.9 (68.0)
Sandy clay loam	Unprimed control	+	8.3	8.1	9.3	91.1 (72.6)
Sandy clay loam	Primed control	-	6.6	6.2	7.3	88.8 (70.4)
Sandy clay loam	Primed CHA0	-	6.5	6.4	7.4	81.5 (64.5)
Sandy clay loam	Primed IK726	-	6.1	5.8	6.7	78.4 (62.3)
Sandy clay loam	Primed MA342	-	6.2	5.9	6.8	85.6 (67.7)
Sandy clay loam	Primed T22	-	6.1	5.8	6.3	88.4 (70.1)
Sandy clay loam	Unprimed control	-	8.5	7.9	9.6	82.4 (65.2)
LSD (0.05)			**0.60**	**0.57**	**0.73**	**(8.95)**

©2004 HortLINK Consortium members

Appendix 3.8: Overall effect of soil type on emergence of microorganism-primed carrot seedlings

Soil type	Mean emergence time (days)	Time to 50% emergence (days)	Time to 80% emergence (days)	Final percent emergence
Sandy clay loam	6.6	6.3	7.3	86.1 (68.1)
Peat	7.7	7.4	8.6	80.4 (63.7)
Light sandy loam	6.7	6.4	7.3	69.7 (56.6)
LSD (0.05)	**0.17**	**0.16**	**0.21**	**(2.58)**

Appendix 3.9: Overall effect of seed treatment of emergence of microorganism-primed carrot seedlings

Treatment	Mean emergence time (days)	Time to 50% emergence (days)	Time to 80% emergence (days)	Final percent emergence
Primed control	6.8	6.5	7.5	82.0 (64.9)
Primed *P. fluorescens* CHA0	7.0	6.7	7.8	78.2 (62.2)
Primed *C. rosea* IK726	6.3	6.0	6.9	76.6 (61.1)
Primed *P. chlororaphis* MA342	6.7	6.4	7.4	79.6 (63.2)
Primed *T. harzianum* T22	6.4	6.1	7.1	80.9 (64.1)
Unprimed control	8.7	8.4	9.8	77.3 (61.5)
LSD (0.05)	0.25	0.23	0.30	**(8.95)**

©2004 HortLINK Consortium members

Appendix 3.10: Overall effect of pesticide application on emergence of microorganism-primed carrot seedlings

Treatment	Mean emergence time (days)	Time to 50% emergence (days)	Time to 80% emergence (days)	Final percent emergence
Pesticide	7.0	6.8	7.8	80.8 (64.0)
No pesticide	6.9	6.6	7.7	77.4 (61.6)
LSD (0.05)	**0.14**	**0.13**	**0.17**	**(2.11)**

©2004 HortLINK Consortium members