Application of beneficial microorganisms to seeds using priming techniques.

Horticulture LINK Project second annual report (December 2000)

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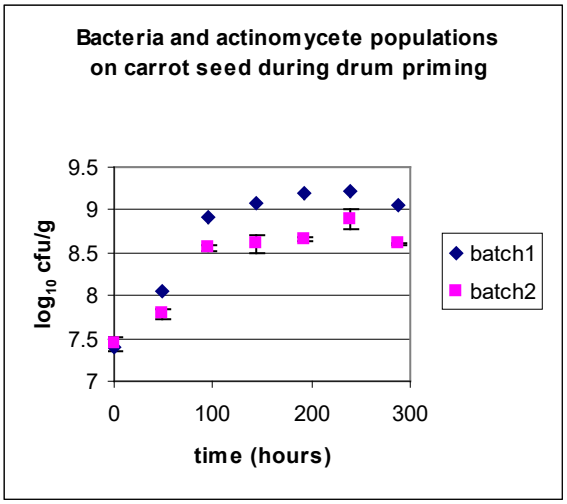
PRACTICAL SECTION FOR GROWERS

Background

Poor seedling growth and establishment continue to be problems in UK horticulture. Sometimes a high percentage of viable seeds that are sown fail to become established despite the availability of seed treatment techniques such as priming and coating. One possible approach to enhance plant growth and establishment is to apply beneficial microorganisms to seeds prior to planting. Application of specific bacteria and fungi to seeds have been shown to promote plant growth directly in a range of systems but their potential for providing beneficial effects following application during the priming systems in use in the UK have not been considered previously. Consequently, the objectives of this project are to characterise the microbial population dynamics on seeds of a range of species important to UK horticulture, during priming, and to evaluate and optimise application methods of beneficial microorganisms to seeds during priming to achieve maximum plant growth promotion.

Summary of work to December 1999

- A lab-scale drum priming system was constructed at HRI Wellesbourne to allow experiments on microbial population changes during drum priming to be evaluated. This was shown to allow priming to take place in a similar way to the large scale commercial system.
- A range of methods for isolating microorganisms from seeds was examined and it was shown that the use of grinding in water was the optimal system in general. This technique was then applied in all subsequent experiments.
- Fully replicated drum priming runs were carried out twice on carrot, leek and parsnip seeds, and populations of bacteria and fungi were found to increase during drum priming (0 to 240 hours). Bacteria but not fungi were found to decrease after drying back (288 hours). The following graph illustrates the changes in bacteria and actinomycete populations on carrot seed as an example.



- One fully replicated steeping priming run was carried out on sugar beet seeds and populations of bacteria but not fungi were found to increase during this priming process as well.
- In order to have a potential source of microorganisms that are adapted to seeds and capable of surviving the seed priming and drying back process a collection of bacterial and fungal isolates were made from drum primed carrot, leek and parsnip seeds. Samples were taken at the end of the priming process and after drying back and stored on agar slopes at 4 °C until required.
- A selection of commercially available bacterial isolates, known to promote plant growth in other systems or to have biological control properties, have been tested for compatibility with the fungicides and pesticides used commercially on carrot, leek, parsnip and sugar beet seed. None were found to be resistant to all fungicides and pesticides tested although some were resistant to most of them.

Summary of work from January 2000 to December 2000

- Two isolates of commercially available fungi, *Trichoderma harzianum* T1295-22 and *Trichoderma (Gliocladium) virens* G20, known to promote plant growth in other systems or to have biological control properties, have been tested for compatibility with the fungicides and pesticides used commercially on carrot, leek, parsnip and sugar beet seed. Both isolates were inhibited by the presence of all fungicides except but neither was inhibited by the presence of pesticides.
- One fully replicated lab-scale steeping priming run was carried out on sugar beet seeds ensuring that no Thiram was present during the process. Results suggested

that the lack of increase in the fungal populations was likely to be related to an inherent property of the sugar beet seed itself.

- One fully replicated lab-scale steeping priming run was carried out on carrot seed without the presence of Thiram and the bacterial and fungal populations increased in a similar way to drum primed seed.
- The collection of representative isolates stored on agar slopes in the first six months of the project was transferred to fresh agar slopes in May and November.
- Commercial scale drum priming runs on carrot, leek and parsnip seed have been carried out at Elsoms Seeds. Samples of seed were collected at intervals before, during and after priming and analysed for microbial populations as before at HRI. Microbial population dynamics were found to be similar to those found in the lab-scale drum priming runs done previously.
- Antibiotic resistant mutants of the commercially available isolates *Bacillus subtilis* A13 (*Bs* A13), *Bacillus subtilis* GB03 (*Bs* GB03), *Bacillus subtilis* MBI 600, (*Bs* MBI600) *Enterobacter cloacae* UW4, *Pseudomonas aureofaciens* AB254, *Pseudomonas* sp. AB842 and *Pseudomonas fluorescens* CHA0 (*Pf* CHA0), have been produced. This will enable the identification of these isolates once they have been added to, and re-extracted from, seed. Once stability was confirmed the mutants were stored in "Protect" at –80° C.
- Fully replicated lab-scale drum priming runs were carried out on carrot seed inoculated with three spore concentrations of *Trichoderma (Gliocladium) virens* G20, and one of *Trichoderma harzianum* T1295-22 (T22). There was no increase in the population of the applied fungi in any of the priming runs. The number of spores on the seed after 48 hours reflected the dosage applied, and the number of spores on the seed subsequently remained constant throughout the priming run. This indicates that neither of these fungi can proliferate on carrot seed during priming but that both can survive the drying back process.
- Fully replicated lab-scale drum priming runs were carried out on leek seed inoculated with spores of *Trichoderma (Gliocladium) virens* G20. It was not possible to recover spores of G20 when added to leek seed at a rate of 10^5 or 10^6 spores/g, suggesting that leek seed may be toxic to this fungus.
- Fully replicated lab-scale drum priming runs were carried out on parsnip seed inoculated with spore concentrations of *Trichoderma (Gliocladium) virens* G20,

and of *Trichoderma harzianum* T1295-22. Spores of T22 applied at a rate of 10^5 spores/g could be recovered from the seed during and after priming and the number of spores recovered was seen to increase from 4 to over 6 $\log_{10}$ spores/g during priming. Spores of G20, added at a rate of 10^6 spores/g were recoverable during and after priming, but did not display the same increase in numbers, remaining at approximately 3 $\log_{10}$ during priming. This suggests that there may be some seed specificity for growth and sporulation during the priming process for the two fungi examined

- Selected rifampicin (rif)-resistant *Pseudomonas* and *Bacillus* species with known growth promotion or biocontrol activity and tolerance to pesticides used in the UK were applied to carrot, leek and parsnip seed during lab scale drum priming. It was shown that *Pf* CHA0 could be added to seeds of carrot, leek and parsnip during lab-scale drum priming and that *Pf* CHA0 was recoverable from the seed during and after priming. Cells of *Bs* GB03 were added to carrot seed during lab-scale drum priming but could not be recovered after drying back the seed. Spores of *Bs* MBI600 were added to seeds of leek and parsnip during lab-scale drum priming and the spores were recoverable from the seed during and after priming.
- Further work is needed with *Bacillus* species to determine whether recovery from seed is related to the species used *per se* or the ability to produce spores during the priming process.
- Seeds of carrot inoculated with selected *Pseudomonas, Bacillus* and *Trichoderma* species during lab-scale drum priming, were assessed for germination and seedling growth parameters in soil based tests, and compared with primed and unprimed seed not treated with beneficial microorganisms. These experiments are still underway but preliminary results indicate that there are differences in germination and plant growth between different soil types used but no effect of treatments within any one soil type.

Anticipated Benefits

- Research to date has shown that populations of several species of beneficial microbial organisms on seeds (fungi or bacteria, such as "plant-growth-promoting rhizobacteria") can be enhanced when they are applied at low levels during the priming process. Currently the success of this inoculation process is seen to

depend upon both the microorganism and seed species used: it will be necessary to extend studies to each strain and species in turn. The project focus is on leek carrot and parsnip and sugar beet, crops chosen from amongst UK horticultural field crops because they are already established as commercial candidates for priming.

- The potential to increase beneficial microorganism populations on seeds through such "biopriming" technology will enable UK seed companies to use the guideline priming techniques to apply beneficial microorganisms to seeds during priming. It is expected that "biopriming" application techniques will ultimately be applicable to a range of beneficial microorganisms, with both growth-promotion and antagonist or plant-protection properties (i.e. for control of seed-borne and soil-borne fungal diseases, although these are not the subjects of the present project).
- Growers will be able to thereby take advantage of the potential of beneficial microorganisms, delivered to crops on seeds through the "biopriming" technology, to improve the reliability of establishment of uniform plant stands and increase marketable yield, to complement the improved germination and emergence performance that comes from priming. In particular, seeds with beneficial microorganisms should enhance flexibility in planting strategies and financial return.
- The technology will be particularly appropriate to organic cultivation systems. It will also be applicable to complement existing methodologies in traditional systems. (It is technically possible to produce strains of at least some beneficial microorganisms that are compatible with the fungicides and insecticides used to treat seeds of the species under study.)
- There is at present a limited selection of commercial formulated microorganisms suitable for delivery to crops on seed or in the planting medium that have been marketed in UK. Organisms with plant-protection properties need to be approved through the UK pesticide registration process.

Action Points for Growers

Growers will be invited to:

- Assist in the development of strategies for use of seed-applied beneficial microorganisms, e.g. through industry forums to discuss benefits in terms of plant

growth promotion, to help identify crops and situations where the benefits of beneficial microorganisms can be used, including discuss with fresh food produce industry, and to develop best practice for use of inoculated seed.

- Collaborate in trials work to assess beneficial responses on strains selected, using material generated by the Consortium Partners in 2002.

Milestones

The milestones given under A below are as listed in the Offer of Grant Letter. However following discussion at the last PMC, specific work to be done or initiated under these milestones by the end of 2000 was identified, (listed as an appendix to the minutes of the meeting which were circulated), and are given under B below.

A

Year 2

Primary milestones

1.3 Prime seeds of carrot, leek and parsnip using commercial-scale drum priming and steeping priming procedures and compare microbial population dynamics with those in lab-scale systems (month 24).

2.2 Prime seeds of carrot, leek and parsnip using drum and steeping priming process under commercial conditions and identify microbes that proliferate and survive well as in 2.1 (month 24).

4.3 Continue work to apply known beneficial microorganisms or novel seed-adapted microorganisms to seeds of carrot, leek and parsnip using lab-scale conditions to optimise timing and rates of application, inoculum form and culturing protocols to achieve maximum survival on seeds. Assess selected optimised combinations under commercial priming conditions (month 24).

5.1 Apply selected known beneficial microorganisms or novel seed adapted microorganisms to seeds of carrot, leek and parsnip during lab-scale priming based on information from objectives 1, 2 and 4 and assess seedling establishment and growth in the glasshouse compared with that of primed and unprimed seed not treated with beneficial microorganisms (month 24).

Secondary milestones

1.4 Prime seeds of sugar beet using lab scale steeping priming processes and monitor microbial populations on seeds with time (month 24)

2.3 Prime seeds of sugar beet in the lab using steeping priming processes and identify microbes that proliferate and survive well using microscopic observations for fungi and nutrient utilisation tests for bacteria (month 18).

3.2 Select and store representative seed adapted microorganisms from sugar beet primed in the laboratory (month 18).

3.3 Select and store representative seed adapted microorganisms from carrot, leek and parsnip primed under commercial conditions (month 24).

4.4 Apply known beneficial microorganisms or novel seed adapted microorganisms to seeds of sugar beet during lab scale priming and monitor survival of introduced beneficial microorganisms and background microflora (month 24).

5.2 Monitor survival of introduced microorganisms and changes in population of the natural endogenous microflora during seed germination and seedling growth (month 24).

B

Primary Milestones

1.2 Prime seeds of sugar beet, carrot, leek and parsnip using the small lab-scale steeping priming process at Germain's and monitor microbial population changes. **Actions: (1)Germain's to carry out all steeping priming;(2)HRI and Germain's to carry out microbial isolation from sugar beet at HRI; (3) Germain's to carry out microbial isolation from all other seeds with random samples checked for consistency at HRI.**

1.3 Prime seeds of carrot, leek and parsnip using commercial-scale drum priming at Elsoms and compare microbial population dynamics with those in lab based systems. In addition, samples of the same primed seed after subsequent coating treatments will also be taken. **Actions: Elsoms to prime carrot subsequently and deliver directly to HRI. Elsoms or Germain's, as appropriate, to deliver to HRI for microbial isolations.**
Prime seeds of sugar beet, carrot, leek and parsnip using the commercial-scale steeping priming process at Germain's and compare microbial population changes with those in lab-scale systems. **Actions: Germain's to initiate this work sometime in Year 2 as appropriate.**

2.2 Prime seeds of carrot, leek and parsnip using steeping priming processes under commercial conditions and identify microbes that proliferate as in 2.1. **Actions: work to be done by Germain's.**

4.3 Apply selected *Pseudomonas, Bacillus* and *Trichoderma* species with known growth promotion or biocontrol activity to seeds of carrot during lab-scale drum priming and assess survival or impact on microbial population dynamics (work after June either with other microorganisms or using other seeds to be decided in the light of the results from these initial tests). Apply selected *Pseudomonas, Bacillus* and *Trichoderma* species with known growth promotion or biocontrol activity to sugar beet during lab-scale steeping priming (Germain's) and assess survival or impact on total microbial population dynamics. **Action: HRI and Germain's.**

5.1 Apply selected *Pseudomonas, Bacillus* and *Trichoderma* species to seeds of carrot during lab-scale drum priming, using same strains and seed combinations used in milestone 4.3, and assess seedling germination, growth parameters and inoculant survival on roots, where possible, in soil based tests in comparison with primed and unprimed seed not treated with beneficial microorganisms. Other treated seed to be examined similarly as they become available. **Action: HRI to develop protocols and carry out initial experiments with option for further**

assessment tests at Elsoms. Elsoms to provide HRI with a standard sandy soil and peat soil that will be available for the duration of the project.

Interim informal discussions will take place in late September to consider progress and further identify priorities if required.

A six month report will be prepared and circulated prior to the meeting in December. **Action: HRI and Germain's.**

Secondary milestones

1.4 Prime seeds of sugar beet using lab scale steeping priming processes and monitor microbial populations on seeds with time (month 24)

3.1 The collection of microorganisms obtained from carrot, leek and parsnip during and after the small-scale drum priming process will be subcultured onto fresh agar every 6 months. **Action: HRI.**

2.3 +3.2 It was agreed that a collection of microorganisms from sugar beet during and after lab-scale steeping priming would only be made if viewed as necessary by the PMG in due course as there is a large microbial resource already available from the other species.

3.3 It was agreed that a collection of microbes from the commercial priming systems would not be done as the existing collection provided ample experimental material.

4.2 Existing known beneficial or biocontrol fungal isolates will be assessed for compatibility with Fipronil in agar plate tests in the laboratory. **Action: HRI.**

Obtain Rif resistant mutants of all existing known beneficial or biocontrol bacteria. **Action: HRI. (ADDITIONAL)**

Progress against the primary milestones from B:

Primary milestone 1.2 initiated.

Primary milestone 1.3 completed for drum priming, initiated for steeping priming.

Primary milestone 2.2 not initiated (see secondary milestones 2.3, 3.2 and 3.3 above).

Primary milestone 4.3 initiated.

Primary milestone 5.1 initiated.

Progress against the secondary milestones from B:

All secondary milestones completed.

SCIENCE SECTION

Introduction

Poor seedling growth and establishment continue to be problems in UK horticulture with sometimes a high percentage of viable seeds sown failing to become established despite the availability of seed treatment techniques such as seed priming and coating. Costs associated with the need to increase seeding rates to compensate for poor establishment and losses resulting from poor quality due to uneven spacing have been estimated from surveys and experience of UK vegetable crops to be at least £18M per annum for carrot, leek, parsnip and onion crops alone. Currently there is increasing interest in the application of beneficial microorganisms such as *Trichoderma* spp., *Bacillus* spp. and *Pseudomonas* spp. to seeds to promote seedling growth and establishment. Specific bacteria and fungi are known to promote plant growth directly, possibly through production of plant growth regulating substances or by enhancing nutrition, or indirectly, by controlling plant pathogens such as *Pythium* and *Rhizoctonia*. Importantly, some beneficial microorganisms with the capability for biocontrol can promote plant growth in the absence of pathogens.

One approach to improve seedling establishment has been to treat seeds with such beneficial microorganisms or biocontrol agents (Whipps, 1997; McQuilken, Rhodes & Halmer, 1998; Halmer, 2000). At HRI and Germain's (UK) Ltd, both bacteria and fungi have been successfully applied to seed through both thin-film coating and seed pelleting procedures (McQuilken, Whipps & Cooke, 1990; McQuilken, Budge & Whipps, 1997; Shah-Smith & Burns, 1997; McQuilken *et al.*, 1998). Several bacterial and fungal products applied to seeds are now on the market providing either plant growth promotion *per se* or control of seedling diseases (Whipps, 1997; McQuilken, Rhodes & Halmer, 1998) demonstrating a clear interest in such products, and strain isolation continues in public and private organisations worldwide. For example, SBREF-sponsored research is currently underway at IACR-Brooms Barn to isolate microbiological strains with disease-control capabilities for the sugar beet crop (M Asher, personal communication). Plant growth promotion of a range of bedding plants and lettuce has been achieved at HRI through applications of strains of *Trichoderma* to potting mixtures in the absence of known pathogens (Ousley, Lynch & Whipps, 1993, 1994a, b). These strains of *Trichoderma* as well as several bacterial isolates used commercially are available for experimental use in this

research programme. However, survival of the microorganisms in sufficient numbers during the coating or pelleting procedures and in subsequent seed storage is a common problem. An alternative approach, to be investigated in this project, is to allow the seed to come into contact with specific microorganisms during an appropriate seed priming process. In general, the objective of priming is to increase the speed and uniformity of germination, as a tool to help ensure reliably rapid, uniform and high establishment of crops, and hence improve harvest quality and yield. Currently, varying proportions of high value vegetable seeds are primed in the UK. The majority of leek, some carrot and parsnip and to some extent onion and sugar beet, are routinely primed using the patented drum priming process developed at HRI (Rowse, 1996) or a commercial steeping priming process (Elsoms Seeds Ltd and Germain's (UK) Ltd, commercial information). The latter process is now commercially applied to approx. 10% of the UK sugar beet crop. Nevertheless, there can still be poor seedling establishment in fields sown with primed seed in many cases under adverse environmental conditions.

In the USA, one patented and commercially available technique of this type, termed solid matrix priming (SMP), has been shown in experimental work to be capable of achieving high levels of several beneficial microorganisms on seeds. The patented Drum priming process developed at HRI Wellesbourne and used by Elsoms Seeds, and the steeping priming system developed and used by Germain's (UK) Ltd, share with SMP the feature of incubating moist seed for an extended period of time - conditions which are potentially highly suited to the introduction and proliferation of microorganisms. However, the application of microorganisms during the Drum priming and steeping priming systems has yet to be researched and the changes in the natural seed microflora during these processes has not been examined. A clear understanding of both the endogenous and the exogenously-applied microbial population dynamics during priming will be necessary to enable optimal establishment of beneficial microorganisms during the priming processes to be achieved. With such fundamental information for a range of plant species, specific beneficial microorganisms will be able to be applied at the optimal stage of priming and at optimal rates to give enhanced plant establishment in comparison with conventionally-treated primed or unprimed seed.

Materials and methods

The scientific protocols and methods used to analyse test material in this report are the same as those described in the First Annual Report unless stated otherwise.

Pesticide compatibility

Assessment of two fungal isolates, G20 and T22, for pesticide compatibility in agar based tests in the laboratory.

In order to establish the ability of each fungus to tolerate the different fungicides and pesticides found on commercially treated seed, an agar plate test was devised. To 500 ml quantities of molten Potato Dextrose Agar (PDA), formulations of chemical were added at rates of 0.1, 1.0 and 10.0 times the quantity used by industry on an active ingredient per kilogram of seed basis. Spores of each isolate to be tested were then spread onto three plates of each agar medium at each of the three concentrations being investigated. Plates were then incubated for 48 hours at 25 °C and examined with a light microscope for spore germination. Analysis of 10 times strength Fipronil was not possible as the agar would not set.

In cases where the opacity of the agar was too great to allow examination using a light microscope, the plates were incubated until visible colonies formed. If visible colonies had failed to form on test plates at the same time as control plates, containing only PDA, the isolate was judged to be intolerant of the pesticide.

Microbial population dynamics during priming

Determination of microbial population dynamics on sugar beet and carrot seed during steeping priming.

Sugar beet seed supplied by Germain's and carrot seed supplied from Elsoms seeds was steeping primed at Germain's. Steeping priming runs were set up such that 2, 4, 6, 8 and 10 day samples of carrot and 1, 2, 3, and 4 day samples of sugar beet could be collected on the same day for transportation in a cool box to HRI for analysis.

Determination of microbial population dynamics on carrot, leek and parsnip seed during commercial scale drum priming.

Seed primed in the commercial scale drum priming system at Elsoms seeds was sampled at intervals during priming and the seed transported on the day of sampling

to HRI for analysis. In addition, commercially drum primed carrot seed was film coated with Polycoat Bejofilm Force and then sent to HRI for analysis.

Application of fungi to seeds during priming

Addition of G20 and T22 to seeds of carrot, leek and parsnip during lab-scale drum priming.

Cultures of each isolate were grown on PDA plates until sporulation was abundant (approximately 12 days). Spores were collected by adding 10 ml of sterile distilled water to a plate and a sterile loop was then used to dislodge the spores from the culture. The resulting mixture was filtered through sterile Whatman lens cleaning tissue and the number of spores in the filtrate was calculated using a Neubauer counting chamber. The required quantity of spores were then added to water and made up to the total hydrating volume of water required for priming.

Protocols for the analysis of this seed were as previously described with the exception of the PDA+ plates, which were amended with Triton-X. This retards the growth of fungal colonies, keeping them distinct, and so allowing more accurate quantification of colony numbers.

Application of bacteria to seeds during priming

Selection of antibiotic resistant mutants of the commercially available bacteria.

In order to establish which antibiotic to use to maximise detection limits once a mutant is added to the seed, it was necessary to test the natural background population of cells on the seed for resistance to rifampicin, tetracycline, nalidixic acid and streptomycin. Carrot was chosen as a representative seed species. A sample (0.5 g) of washed, unprimed carrot seed was crushed in 9.5 ml of sterile distilled water and a one in ten dilution made. A quantity (0.1 ml) of this suspension was plated onto 0.1TSA plates containing each antibiotic individually at a rate of 100 µg/ml. After incubation, the least number of spontaneous mutants were found on agar plates amended with 100 µg/ml rifampicin. Thus it was decided to select spontaneous rif resistant mutants from the commercially available isolates *Bacillus subtilis* (*Bs*) A13*, Bacillus subtilis* (*Bs*) GB03*, Bacillus subtilis* (*Bs*) MBI600 *Enterobacter cloacae* UW4, *Pseudomonas aureofaciens* AB254, *Pseudomonas* sp. AB842 and *Pseudomonas fluorescens* (*Pf*) CHA0.

Spontaneous antibiotic resistant mutants of each isolate were collected by growing each isolate for 3 to 4 days on Nutrient agar plates until a lawn of cells was present. Cells of each isolate were then collected on a sterile swab and spread onto agar plates amended with 25, 50, 75, and 100 µg/ml rifampicin. The plates were then incubated and any colonies present were transferred onto agar amended with the next highest concentration of rifampicin. Once a mutant had been grown on agar with 100µg/ml rifampicin for five successive generations it was then grown on unamended agar for a further five generations. The stability of rifampicin resistance of the mutant was then checked by growing a further generation on agar amended with 100 µg/ml rifampicin. At this point surviving colonies, defined as rif resistant, were placed in "Protect" at –80° C until required.

Addition of bacterial cells to seed during lab-scale drum priming.

In order to add a known quantity of bacterial cells onto seed in the lab-scale drum priming system a calibration curve of optical density of the isolate in nutrient broth against cfu was prepared. From this, a known number of cells of the isolate could be collected from the broth and added to the hydrating liquid for the drum priming.

Addition of bacterial spores to seed during lab-scale drum priming.

In order to add a known quantity of bacterial spores onto seed in the lab-scale drum priming system a liquid culture of *Bacillus subtilis* MBI600 was grown for 4 days in 0.25% Brain Heart Infusion Broth. The number of spores present was then determined using a Neubauer counting chamber. The required quantity of spores were then added to water and made up to the total hydrating volume of water required for priming.

Effects of fungal and bacterial applications during priming on germination and seedling growth

Assessment of germination and seedling growth from primed and treated carrot seed in soil based tests.

In order to determine whether any of the priming treatments given to the carrot seed provided a growth promotion effect, the following experiment was designed. Twenty four, six inch pots with saucers were filled with sandy soil, black soil or Levington F1

compost, sieved to pass a 4 mm mesh. Pots were filled to overflowing with the soil to be used and given three sharp taps on the work bench. The soil was then levelled to the brim of the pot using a flat edge. Ten small, evenly spaced, indentations were then made into the surface of the soil. One seed was then placed into each indentation and covered with 2-3 mm of soil. The pots were then placed in saucers in a Gallenkamp Fi-totron 600H growth cabinet, running at 20 °C with 85 % RH, 16 hours light / 8 hours dark and watered daily. Initially watering was carried out from above but this caused the sandy soil structure to deteriorate and cap. Subsequently watering was carried out from below. This capping could have been responsible for the poor germination of some seeds.

Each day a count of germination was made and after ten days, shoot length and root length (length of primary root) of each individual plant were measured. Total shoot and root fresh weights for each pot were also recorded. The fresh material was then oven dried (80 °C overnight) and weighed to obtain dry weight value.

As all treatments could not be run within the growth cabinet simultaneously, a specific experimental protocol was designed with the aid of the Biometrics Department. The design of the experiment involved the use of three soil types, six seed treatments (unprimed, primed and four primed treatments) in two growth cabinets with 4 replicate pots per treatment. All plots within a cabinet on a particular occasion contained pots of the same soil (i.e. only one soil type per cabinet at any one time) and two different soil types are assessed on each occasion (i.e. one in each cabinet). Each soil type appears twice in each cabinet over the whole trial.

Results

Pesticide compatibility

Assessment of two fungal isolates, G20 and T22, for pesticide compatibility in agar based tests in the laboratory.

Table 1 shows the mycelial growth from spores of G20 and T22 on formulations of fungicides and pesticides used commercially on the seeds of interest. Although low levels of Metalaxyl and Iprodione did not appear to inhibit initial germination of the fungal spores and production of a germ tube, they did prevent further growth and production of mycelia. Thiram, Apron, Thiabendazole and Hymexazol all prevented spore germination in both T22 and G20. Tefluthrin, Fipronil and Imidacloprid did not effect the growth of spores of G20 or T22 at any of the concentrations tested. This is in contrast with most of the bacteria tested previously which could all grow on the fungicides Apron, Metalaxyl, Thiabendazole and the two pesticides at least (Table 4 First Annual Report).

Microbial population dynamics during priming

Determination of microbial population dynamics on sugar beet seed during steeping priming.

Figs. 1 and 3 show that as with drum priming for carrot, leek and parsnip, bacteria and pseudomonad populations increase sharply in the initial stages of priming and then level out at a maximum between days 2 and 4. Bacterial populations increase from approximately 5.5 $\log_{10}$ cfu/g in unprimed seed to over 9 $\log_{10}$ cfu/g by day 2, with pseudomonad populations representing around one tenth of this level. The drying back process causes a slight decrease in numbers of bacteria and pseudomonads. Fig. 2 shows that the numbers of fungi and yeasts on sugar beet during steeping priming do not appear to increase in contrast with drum primed carrot, leek and parsnip. An initial increase from around 3.5 $\log_{10}$ cfu/g to just under 5 $\log_{10}$ cfu/g on day two was followed by a decrease in filamentous fungi and yeast populations back to around the level of the unprimed seed. No data were available for the day 4 treatment as the population in the extraction was below detectable limits in the dilution series used. Overall, these results suggest that it was not just the Thiram present in the previous experiment on steeping primed sugar beet (Figs. 14 to 17, First Annual Report) which

inhibited the population growth of fungi and yeast from increasing. It would seem that the sugar beet seed itself has an inherent inhibitory effect on fungal growth.

Determination of microbial population dynamics on carrot seed during steeping priming.

The aim of this experiment was to determine whether steeping priming itself might have been the cause of inhibition of fungi and yeast population growth seen in previous experiment. Figs. 5 and 7 show that bacteria and pseudomonad populations on steeping primed carrot seed followed the same trend as those on drum primed carrot seed (see Figs. 2 and 4, First Annual Report). Bacteria increased from under 7 $\log_{10}$ cfu/g at time zero to over 9 $\log_{10}$ cfu/g at the end of priming. Pseudomonad populations increased from under 5.5 $\log_{10}$ cfu/g to over 8.5 $\log_{10}$ cfu/g by the end of priming. Fig. 6 shows that between 48 and 144 hours the population of fungi and yeasts on steeping primed carrot increased by one log unit in a similar fashion to drum primed seed. However, by 196 hours, contamination with a Penicillium-like fungus was observed to have spread into the system and was the dominant fungus recovered at this point. The results clearly indicate that the steeping priming system does not inhibit the increase of fungal populations on carrot seed. Fig. 8 shows that spore forming bacteria on steeping primed carrot seed increased during the priming process from 2 $\log_{10}$ cfu/g after 48 hours to 4.5 $\log_{10}$ cfu/g after 10 days.

Determination of microbial population dynamics on carrot, leek and parsnip seed during commercial scale drum priming.

Results of this analysis for carrot can be seen in Figs. 9 to 12, for leek Figs.13 to 16 and for parsnip Figs. 17 to 20. These results show that the populations of microorganisms on commercially drum primed seed of carrot, leek and parsnip are very similar in number to those on lab-scale drum primed seed. In the analysis of commercially primed carrot seed, time zero represents the washed but unprimed seed. At 12 hours all the hydrating fluid has been added to the seed. The point at 240 hours represents the end of the incubation phase of priming, after which the seed is dried back represented by the point at 288 hours. The final point at 336 hours represents primed, dried seed coated with Polycote Bejofilm Force. Populations of bacteria and actinomycetes (Fig. 9) on commercially drum primed seed increase during priming to over 8.5 $\log_{10}$ cfu/g and remain at this level after drying back. This pattern and

number of microorganisms is the same as that found earlier on lab-scale, drum primed, carrot seed.

Fluorescent pseudomonad populations on commercially primed carrot seed (Fig. 11) increase in the same pattern as those seen on lab-scale drum primed carrot seed reaching a level of 7.5 $\log_{10}$ cfu/g after drying back. As previously determined in lab-scale drum priming, the population of fluorescent pseudomonads represents approximately one tenth of the overall bacterial population.

Fungi and yeast populations on commercially drum primed carrot seed (Fig. 10) increase during priming to just under 6.5 $\log_{10}$ cfu/g. This is a similar level to that seen on lab-scale drum primed carrot seed (6.5 and 7 $\log_{10}$ cfu/g at 240 hours, see Fig. 3, First Annual Report) although the level of fungi and yeasts appears to increase more quickly in the commercial scale system.

Spore forming bacteria populations on commercially primed carrot seed (Fig. 11) increase to over 4.5 $\log_{10}$ cfu/g at 240 hours. This represents 0.5 to 1 log unit greater than spore forming bacteria seen on lab-scale drum primed carrot seed (Fig. 5 First Annual Report).

The populations of microorganisms on the Polycote treated carrot seed (336 hours) are very similar to the populations on the seed after priming and drying back (240 hours). The Polycote treatment does not appear to have an effect on the population of microorganisms on the seed.

Bacteria and actinomycete populations on leek seed were over 9 $\log_{10}$ cfu/g after 240 hours (Fig. 13) and represents 0.5 $\log_{10}$ units/g greater than that reached by seed in the lab-scale system at this time. Fungi and yeast populations, Fig. 14, were similar at more than 6 $\log_{10}$ cfu/g in both the lab-scale and commercial scale runs at 240 hours. Pseudomonad populations on commercially drum primed leek seed represent around one tenth of the overall bacterial population on the seed (Fig. 15). This is also the same as on seed primed in the lab-scale system. Spore forming bacteria (Fig. 16) increase in a uniform fashion to 4.5 $\log_{10}$ cfu/g at 240 hours.

Bacteria and actinomycete populations on commercially drum primed parsnip seed (Fig. 17) were just under 9.5 $\log_{10}$ cfu/g after 240 hours, which was slightly higher than the equivalent lab-scale primed seed. Fungi and yeast populations on commercially primed parsnip seed (Fig. 18) are similar to those on seed primed in the lab-scale system. Fluorescent pseudomonads (Fig. 19) represent around one tenth of the overall bacterial population. Levels of spore forming bacteria on commercially

drum primed leek and parsnip seed (Figs. 16 and 20 respectively) are similar to those on seed in the lab-scale system. It is interesting to note that the increase in spore forming bacteria in the commercial scale system appears to be smoother and more uniform than was observed in the lab-scale drum priming system.

Examination of the PDA+ plates showed that generally the same fungal species were isolated from both commercially primed carrot, leek and parsnip seed and those primed in the lab-scale system. These included *Cladosporium*, *Penicillium* like species, *Alternaria*, *Aureobasidium*, white filamentous fungi and various pink and white yeasts.

Application of fungi to seeds during priming

Addition of G20 at three different rates to carrot seed during lab-scale drum priming.

The aim of these experiments was to determine whether a beneficial fungus could increase in number after application to seed and to examine the survival of the fungus on seed during the priming process. Three rates of application were used: $8.5x10^7$ spores/g seed (10^5 spores/seed), 10^5 spores/g seed ($1.18x10^2$ spores/seed) and 10^4 spores/g seed (12 spores/seed). Figs. 21 and 22 show the populations of bacteria and G20 recovered from seed inoculated during drum priming with spores added at a level equal to $8.5x10^7$ spores/g seed (10^5 spores/seed). This experiment was designed to determine the maximum number of spores that could be added to the seed. The pattern of increase in the bacterial population on the carrot seed was similar to that observed in previous experiments (Fig. 21), with approximately 8.5 $\log_{10}$ cfu/g in this experiment and 9 $\log_{10}$ cfu/g previously at 240 hours. After 48 hours, when all the spores and hydrating media were added, the level of recoverable spores was $6.4x10^6$ cfu/g seed. This level is maintained for the full duration of priming and drying back of the seed. No other fungi could be detected. Consequently at 240 hours approximately $6x10^6$ cfu/g seed ($7.06x10^3$ cfu/seed) were recoverable. This equates to 7% of the number of spores added to the system. It is likely that some spores attached to the walls of the fluid reservoir and the apparatus between the reservoir and the priming drum, as well as attaching to the drum itself. Other spores may simply have lost their viability or been non-viable in the first place. It is known from previous experiments (Fig. 3, First Annual Report) that the maximum number of fungi and yeasts recovered

on carrot seed after 240 hours is approximately 7 $\log_{10}$ cfu/g (10^6 cfu/g) for Batch 1 seed and 6.4 $\log_{10}$ cfu/g (3.5×10^6 cfu/g) for Batch 2 seed. This suggests that in Fig. 22 the carrying capacity of the seed for fungi and yeasts had been reached.

Figs. 23, 24 and 25 show the bacterial, G20 and fungi and yeast populations on carrot seed respectively when 10^5 spores/g seed (1.18×10^2 spores per seed) are added. The bacterial populations increase similarly to the previous runs to reach 10^8 to 10^9 cfu/g seed (Fig. 23), and this time other fungi and yeasts were detected to increase to 5 $\log_{10}$ cfu/g seed (2×10^5 cfu/g seed, Fig. 25). This is at least 1 log unit lower than the number of fungi and yeasts present on Batch 1 and 2 carrot seed drum primed in the absence of G20 (see Fig. 3, First Annual Report). G20 is known to produce a range of inhibitory secondary metabolites and this may be involved in the inhibition of fungal growth.

From Figs. 21 and 22 we know that the seed can support G20 at a level much higher than 10^5 spores per gram so we would expect all of the added spores to be recoverable after 240 hours if all were viable and present on the seed. Fig. 24 shows that after 240 hours 4.6 $\log_{10}$ cfu/g were recoverable from the seed. This represents 4×10^4 spores per gram of seed, or 40% of the added spores. This means that some spores must have been lost in the priming apparatus, were not viable or were out competed by the microorganisms already on the seed.

In addition, spores were also added at a rate of 10^4 spores per gram of seed (12 spores per seed, data not shown), G20 spores were not recoverable from the priming run.

Addition of T22 to carrot seed during lab-scale drum priming.

Figs. 26 and 27 show the populations of bacteria and T22 recovered from drum primed carrot seed to which T22, at a rate equivalent to 8.5×10^7 cfu/g seed (10^5 cfu/seed) has been added. The results follow the same pattern as those for G20. The bacterial population increased to between 10^8 to 10^9 cfu/g seed and the population of T22 remaining constant at approximately 6.75 $\log_{10}$ cfu/g seed (6.6×10^3 spores/seed), or 7% of the total number of spores added, after the hydration phase of priming.

Samples of both G20 treated seed and T22 treated seed were sent to Germain's for germination analysis. Neither G20 nor T22 appear to have an adverse effect on carrot seed germination.

Addition of G20 to leek seed during lab-scale drum priming.

Spores of G20 were added to leek seed during lab-scale drum priming at 10^5 and 10^6 spores/g. It was not possible to recover any spores of G20 from the seed during priming. It is possible that the survival of spores on the coat of leek seed could be prevented by the exudation of fungicidal substances from the seed.

Addition of G20 and T22 to parsnip seed during lab-scale drum priming.

Spores of G20 were added to parsnip seed during lab-scale drum priming at 10^5 and 10^6 spores per gram. When 10^5 spores/g of G20 were added to parsnip seed, no spores were recoverable from the seed during priming.

When 10^6 spores of G20/g parsnip seed were added during drum priming, spores of G20 were recoverable from the seed at approximately 3 $\log_{10}$ units/g ($1.5x10^3$ spores/g) after all the spores were added (Fig. 29). This was maintained throughout priming and increased to 4 $\log_{10}$ units/g after drying back. The population of general bacteria and actinomycetes (Fig. 28) increased in the pattern seen previously to approximately 9 $\log_{10}$ units/g after 240 hours of drum priming.

Fig. 31 shows the results of adding 10^5 spores of T22 to parsnip seed during drum priming. No spores were recoverable until 144 hours when approximately 4 $\log_{10}$ units/g ($1.8x10^3$ spores/g) were recovered. This level increased to over 6 $\log_{10}$ units/g by 240 hours. Again the general population of bacteria and actinomycetes increased to approximately 9 $\log_{10}$ units/g at 240 hours (Fig 30).

In each case above, only a small quantity of the applied spores (less than 1 % for G20 and 18% for T22) survives the initial application onto the seed.

It was shown previously that when spores of G20 and T22 were added to carrot seed no increase in number occurred during priming. It is possible that the shape of parsnip seed causes it to move in a different way in the incubation vessel to seed of leek and carrot and allows growth and reproduction of these fungi.

Application of bacteria to seeds during priming

Addition of a rif resistant Pf *CHA0 to carrot seed during lab-scale drum priming.*

Pf CHA0 was added to the system at a rate of 10^5 cells/g seed during hydration. The level of *Pf* CHA0 recovered on carrot seed after the addition of the hydrating mix of water and cells (48 hours, Fig. 33) was 5 $\log_{10}$ cfu/g seed (10^5 cfu/g seed). Between 48 and 240 hours this increased to 7.9 $\log_{10}$ cfu/g seed (8.5×10^7 cfu/g seed). The overall population of fluorescent pseudomonads on the seed at 240 hours (Fig. 32) was 8.2 $\log_{10}$ cfu/g seed (1.8×10^8 cfu/g seed). The *Pf* CHA0 therefore represents a high proportion (47%) of the total fluorescent pseudomonad population on the seed at 240 hours and is clearly seed adapted and may inhibit other pseudomonads. It was not possible to ascertain the overall population of bacteria and actinomycetes on the seed as the rapid development of *Pf* CHA0 on the TSA plates made colony counting impossible.

In order to determine the reproducibility of these results, the experiment was repeated on a different batch of carrot seed. The results from the repeat experiment (not shown) are very similar to the first experiment, showing that the result is reproducible.

Addition of a rif resistant Pf *CHA0 to leek seed during lab-scale drum priming.*

In an initial experiment, leek seed was inoculated during priming with 10^5 cfu/g of *Pf* CHA0. It was not possible during, or after priming, to recover cells of *Pf* CHA0 from the leek seed. The experiment was then repeated with an inoculum level of 10^6 cfu/g seed at which level cells of *Pf* CHA0 were recoverable during and after priming. The general population of bacteria and actinomycetes showed an increase from 7 $\log_{10}$ cfu/g seed at 48 hours to over 8 $\log_{10}$ cfu/g seed at the end of priming (Fig. 34).

After 48 hours, the level of *Pf* CHA0 on the seed (3 $\log_{10}$ cfu/g) (Fig. 36), represented less than 1% of the population of fluorescent pseudomonads (approx. 6.9 $\log_{10}$ cfu/g). The number of surviving cells of *Pf* CHA0 after 48 hours represented less than 1% of the added inoculum. By 240 hours, the population of fluorescent pseudomonads on the seed (Fig. 35) consisted solely of *Pf* CHA0 and these formed 33% of the overall bacterial population. This appears to show that once applied to leek seed in high enough numbers, *Pf* CHA0 can compete with the population of other

bacteria on the seed and increase in numbers during the priming process. It is also evident that the applied *Pf* CHA0 can withstand the drying back process.

Addition of a rif resistant Pf *CHA0 to parsnip seed during lab-scale drum priming.*

In an initial experiment, parsnip seed was inoculated during priming with 10^5 cfu/g of *Pf* CHA0. It was not possible, during or after priming, to recover cells of *Pf* CHA0 from the parsnip seed. The experiment was then repeated with an inoculum level of 10^6 cfu/g seed at which level cells of *Pf* CHA0 were recoverable during and after priming. The general population of bacteria and actinomycetes (Fig. 37) showed an increase from 6 $\log_{10}$ cfu/g seed to over 9 $\log_{10}$ cfu/g seed during priming. Analysis of Figs. 38 and 39 showed that after only 48 hours the total population of fluorescent pseudomonads (5 $\log_{10}$ cfu/g) on the seed was composed entirely of *Pf* CHA0. This trend continued with 88 % of the total population of bacteria on the seed (9 $\log_{10}$ cfu/g, Fig.37) being composed of *Pf* CHA0 by 240 hours. After 48 hours, 7% of the added inocula was recoverable from the seed, greater numbers than seen previously on carrot and leek. This suggests that *Pf* CHA0 is very well adapted to parsnip seed and can compete effectively with the population of endogenous bacteria on the seed provided it is applied at 10^6 cfu/g seed.

Addition of cells of a rif resistant Bs *GB03 to seeds of carrot during lab-scale drum priming.*

Figs. 40, 41 and 42 show the populations of general bacteria and actinomycetes, rif resistant *Bs* GB03 and spore forming bacteria recovered from lab-scale drum primed carrot seed inoculated with rif resistant cells of *Bs* GB03 at a rate of 10^6 cfu/g seed. There was an increase in the overall bacterial population increasing from 7 to 8.5 $\log_{10}$ cfu/g (Fig. 40) following the pattern already seen for carrot seed during drum priming.

The population of spore forming bacteria on the seed (Fig. 42) increased in the pattern seen before for carrot seed and to a similar level around 4 $\log_{10}$ units/g at 240 hours. The level of *Bs* GB03 on the seed (Fig. 41) also increased from 4 $\log_{10}$ units at 144 hours to 5 $\log_{10}$ units/g at 240 hours. However, after the seed was dried back, no cells of *Bs* GB03 were detected on rifampicin plates. Spore forming bacteria were still detected at a level of over 3 $\log_{10}$ units/g. This suggests that the added *Bs* GB03 has not formed spores on the seed and is destroyed by the drying back process.

Addition of spores of* Bs *MBI600 to seeds of leek and parsnip during lab-scale drum priming.

In order to overcome the problem of the cells of *Bacillus* not surviving the drying back process it was decided to add spores instead. The results of these experiments can be seen in Figs. 43 to 50. For both leek and parsnip the general population of bacteria and actinomycetes follows the pattern of increase seen in previous experiments (Figs. 43 and 47). For leek, bacteria and actinomycete populations increased from 6 to 8.5 $\log_{10}$ cfu/g and for parsnip from under 8 to over 9 $\log_{10}$ cfu/g.

When 10^5 spores of *Bs* MBI600 were added to both leek and parsnip (Figs. 44 and 48) the population of spore formers recoverable from the seed (3 to 4 $\log_{10}$ cfu/g) is similar after 48 hours to that seen previously on the seed in the absence of *Bs* MBI600. The level of spore forming bacteria on leek then remained constant throughout the priming period but on parsnip increased from 3.5 to 5 $\log_{10}$ cfu/g.

To determine whether increasing application rate affected colonisation and survival, 10^7 spores of *Bs* MBI600 were added to leek and parsnip seed during priming. With this treatment the general population of bacteria and actinomycetes followed the same pattern of increase seen for both leek and parsnip in previous experiments (Figs. 45 and 49). For leek, bacteria and actinomycete populations increased from 6 to 8 $\log_{10}$ cfu/g and for parsnip from 7.5 to 9.5 $\log_{10}$ cfu/g. Figs. 46 and 50 show the behaviour of *Bs* MBI600 on leek and parsnip seed, respectively. After 48 hours of priming, the level of spore formers recoverable from the seed was approximately 2 $\log_{10}$ units/g higher than that seen on the equivalent seed primed only with water (First Annual Report, Figs. 9 and 13). For leek seed (Fig. 46) this level of approximately 6.5 $\log_{10}$ units/g was maintained throughout priming and drying back. However on parsnip seed, spore forming bacteria continued to increase from 6 $\log_{10}$ units/g at 48 hours to 7.5 $\log_{10}$ units/g at 240 hours (Fig 50). After 48 hours 30% and 10% of the added inocula were recoverable on leek and parsnip seed, respectively. The continued increase in numbers of spore forming bacteria on parsnip seed could be a consequence of the shape of parsnip seed and the way that it moves in the incubation vessel providing a more amenable environment for microbial development. Increasing spore application of *Bs* MBI600 from 10^5 to 10^7 cfu/g seed increased recovery at the end of the drying back process by at least 2 $\log_{10}$ units.

Effects of fungal and bacterial applications during priming on germination and seedling growth

This experiment is only part way through and so a full analysis of the results is not possible. However, some tentative comparisons are made based on the mean values from each experimental run.

Germination of carrot seeds grown in compost, black soil and sandy soil.

Figs. 51 to 55 show the pattern of germination of carrot seed with six different treatments in three soil types. In compost (Figs. 51 and 52), black soil (Figs. 53 and 54) and sandy soil (Fig. 55), primed seed and that primed and treated with *Pf* CHA0 showed the greatest total germination. In most cases primed seed treated with *Pf* CHA0 germinated in a shorter time than seed of other treatments.

Average shoot and root length in 10 day old carrot seedlings.

Figs. 56 to 60 show the average shoot and root length of 10 day old carrot seedlings grown in compost (Figs. 56 and 57), black soil (Figs. 58 and 59) and sandy soil (Fig. 60). No trend or pattern in the data was immediately obvious.

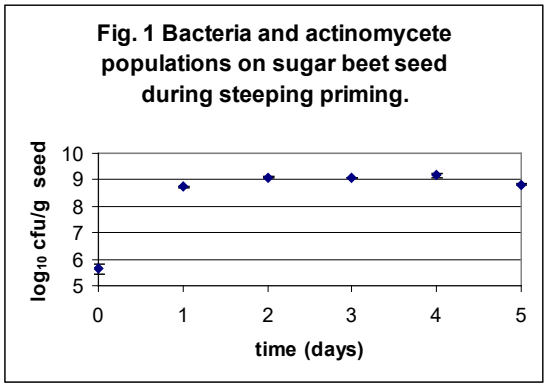
Shoot to root ratio for carrot seedlings in compost, black soil and sandy soil.

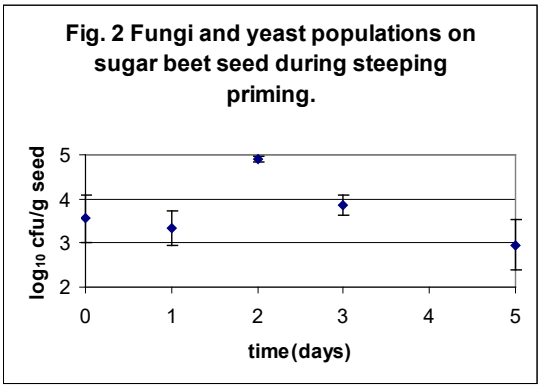
Figs. 61 to 65 show the average dry weight shoot to root ratios for 10 day old carrot seedlings in compost (Figs. 61 and 62), black soil (Figs. 63 and 64) and sandy soil (Fig. 65). Differences between soil types were evident. For example, the shoot to root ratios of seedlings in compost were around 1.5 to 2, in sandy soil were around 0.3 and in black soil most were around 0.8. Any differences between treatments within a soil type were not evident in this analysis.

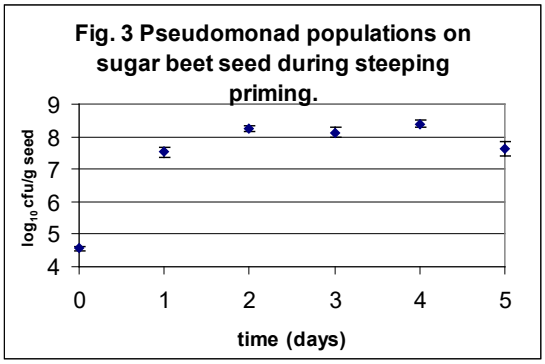
Table 1. Mycelial growth from spores of *Trichoderma harzianum* T1295-22 and *Trichoderma (Gliocladium) virens* G20 on formulations of fungicides and pesticides used commercially on the seeds of interest.

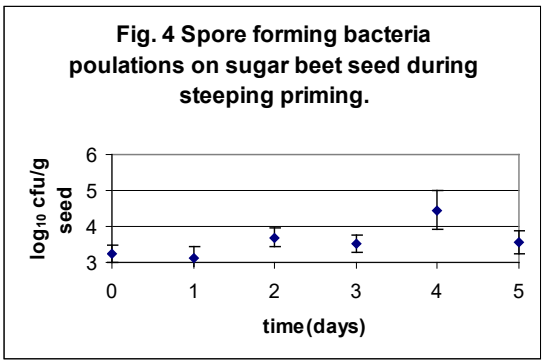
	Formulation																									
	Thiram			Apron			Metalaxyl			Thiabendazole			Iprodione			Hymexazol			Tefluthrin			Imidacloprid			Fipronil	
Concentration	0.1a	1	10	0.1	1	10	0.1	1	10	0.1	1	10	0.1	1	10	0.1	1	10	0	1	10	0.1	1	10	0.1	1
Isolate																										
Trichoderma harzianum T1295-22	0c	0	0	0	0	0	b	b	0	0	0	0	b	0	0	0	0	0	1	1	1	1	1	1	1	1
Trichoderma(Gliocladium)virens G20	0	0	0	0	0	0	b	b	0	0	0	0	b	0	0	0	0	0	1	1	1	1	1	1	1	1

a = concentration is 0.1, 1.0 and 10 times the rate of active ingredient applied to seed on a per kilogram of seed basis.
b = spores germinate but then are inhibited.
c 0 = no growth, 1 = growth.

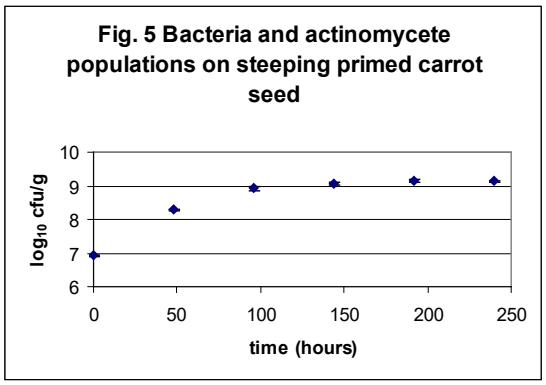


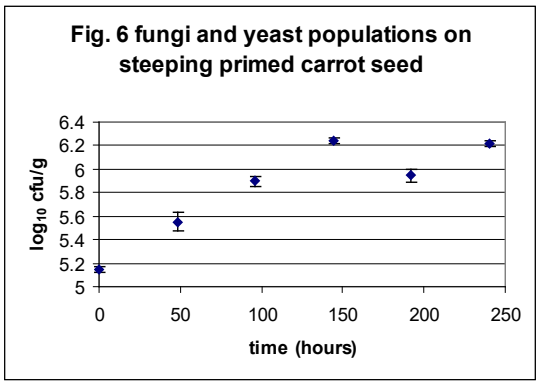


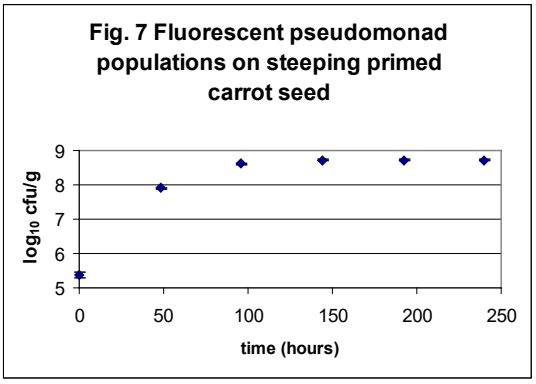


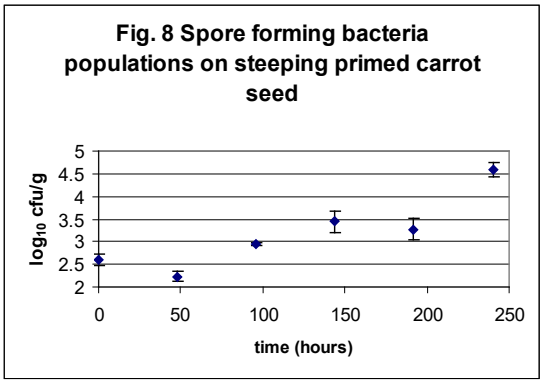


Time 0 represents washed, unprimed sugar beet seed. Times 1, 2, 3 and 4 represent days of priming and time 5 represents the dried back seed.
Note: No Thiram was added to seed in this run.









Time 0 represents washed but unprimed carrot seed.

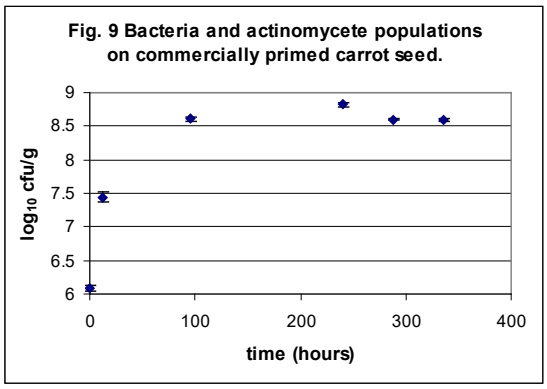


Fig. 9 Bacteria and actinomycete populations on commercially primed carrot seed.
log10 cfu/g
9
8.5
8
7.5
7
6.5
6
0
100
200
300
400
time (hours)

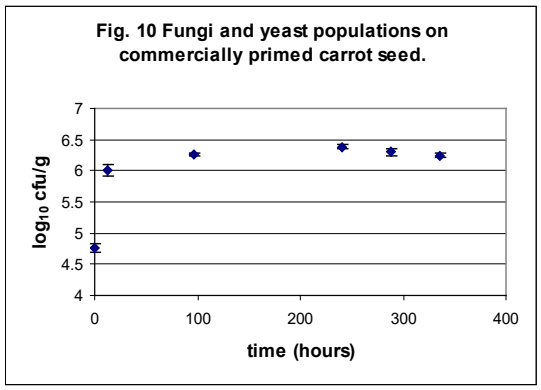


Fig. 10 Fungi and yeast populations on commercially primed carrot seed.
log10 cfu/g
7
6.5
6
5.5
5
4.5
4
0
100
200
300
400
time (hours)

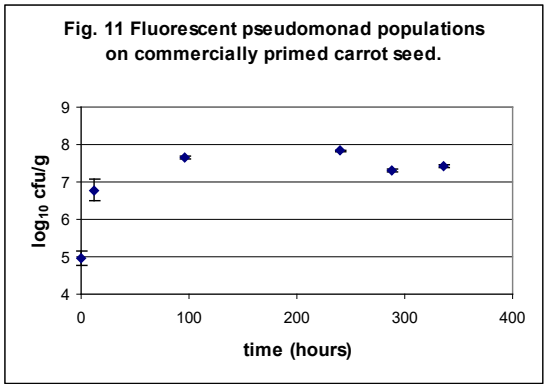


Fig. 11 Fluorescent pseudomonad populations on commercially primed carrot seed.
log10 cfu/g
9
8
7
6
5
4
0
100
200
300
400
time (hours)

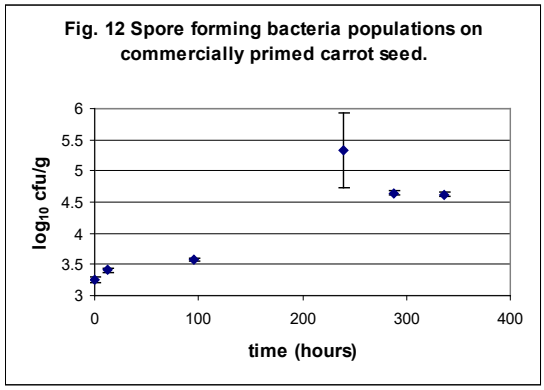


Fig. 12 Spore forming bacteria populations on commercially primed carrot seed.
log10 cfu/g
6
5.5
5
4.5
4
3.5
3
0
100
200
300
400
time (hours)

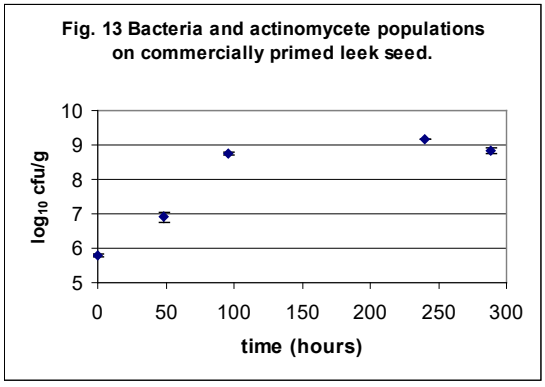


Fig. 13 Bacteria and actinomycete populations on commercially primed leek seed.
log10 cfu/g
10
9
8
7
6
5
0
50
100
150
200
250
300
time (hours)

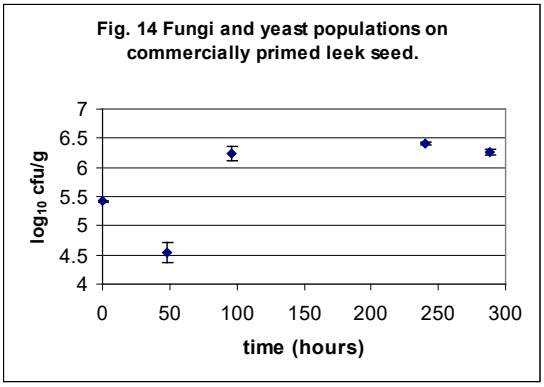


Fig. 14 Fungi and yeast populations on commercially primed leek seed.
log10 cfu/g
7
6.5
6
5.5
5
4.5
4
0
50
100
150
200
250
300
time (hours)

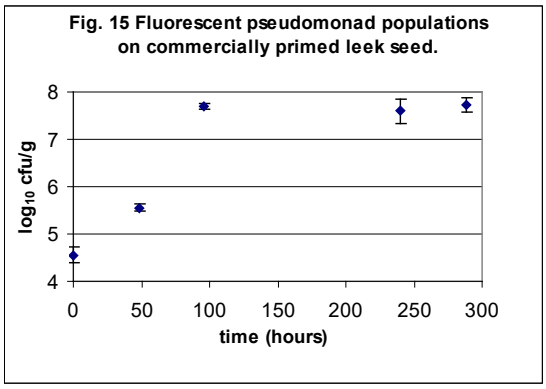


Fig. 15 Fluorescent pseudomonad populations on commercially primed leek seed.
log10 cfu/g
8
7
6
5
4
0
50
100
150
200
250
300
time (hours)

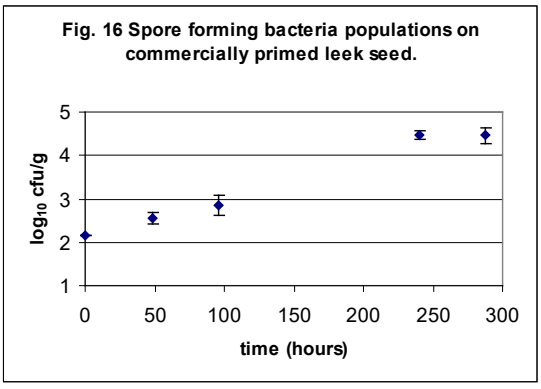
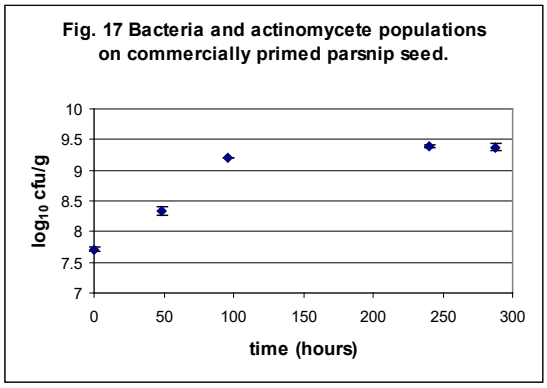
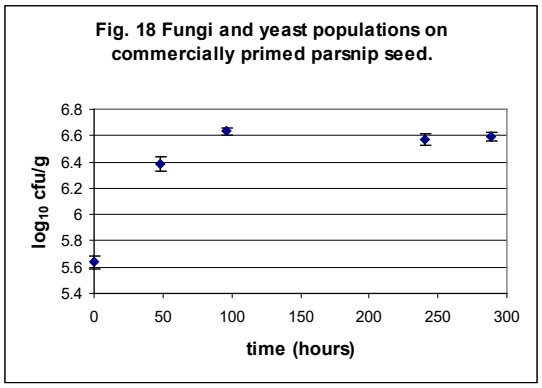
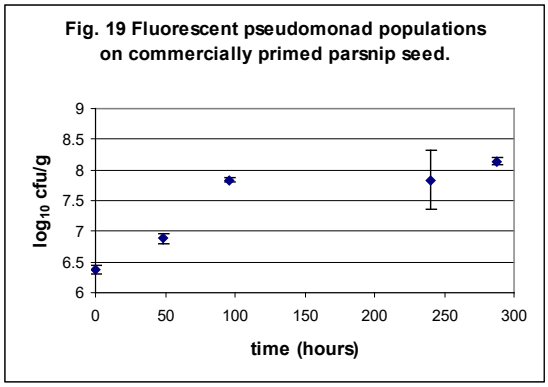
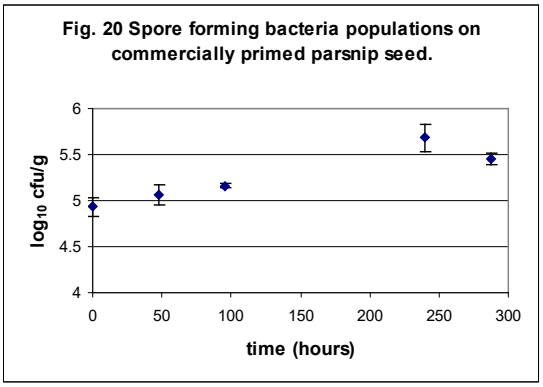


Fig. 16 Spore forming bacteria populations on commercially primed leek seed.
log10 cfu/g
5
4
3
2
1
0
50
100
150
200
250
300
time (hours)









Zero hours represents the unwashed leek seed (Figs. 13 to 16) or washed parsnip seed (Figs. 17 to 20). The point at 48 hours represents seed after hydration (Figs. 13 to 20). Between 48 and 240 hours the seed is incubating and 288 hours the seed has been dried back.

Fig. 21 Bacteria and actinomycete populations on G20 inoculated carrot seed 8.5x10^7 spores/g seed) during drum priming

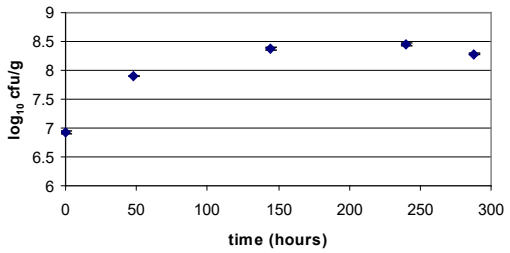
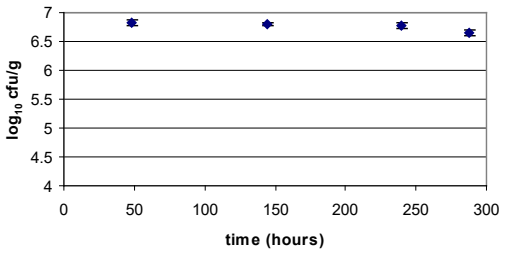


Fig. 22 Population of G20 recovered from carrot seed inoculated with 8.5x10^7 spores/g seed during drum priming



.

Fig. 23 Bacteria and actinomycete populations on carrot seed inoculated with 10^5 spores of G20 per gram seed.

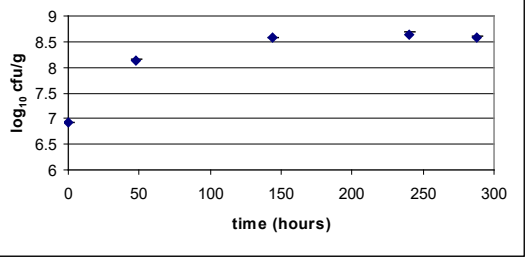


Fig. 24 Population of G20 recovered from carrot seed inoculated with 10^5 spores of G20 per gram seed during drum priming.

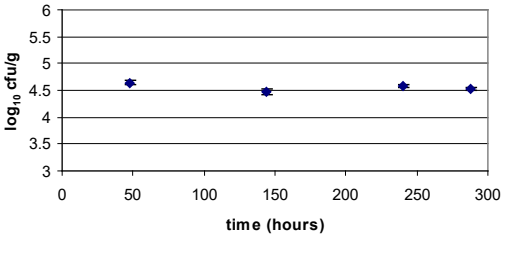


Fig. 25 Fungi and yeast populations on carrot seed inoculated with 10^5 spores of G20 per gram seed during drum priming.

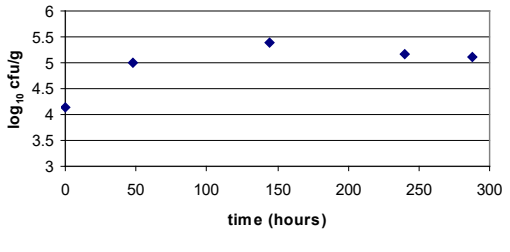


Fig. 26 Bacteria and actinomycete populations on T22 inoculated carrot seed (8.5x10^7 spores/g seed) during drum priming.

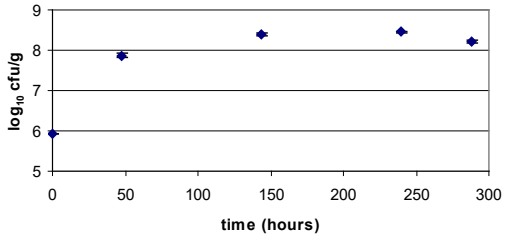
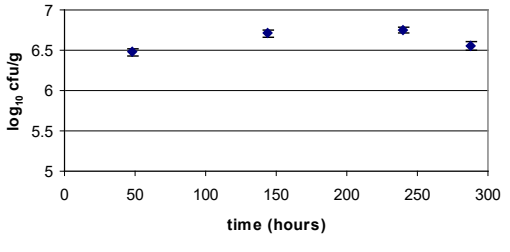
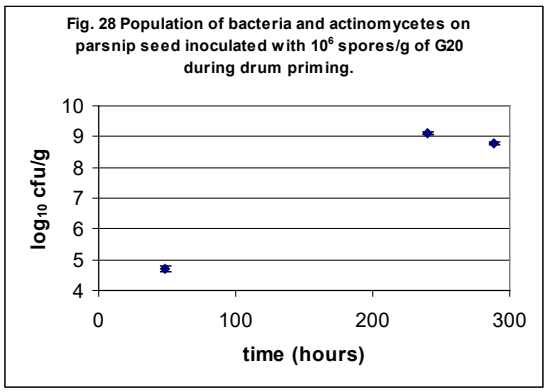
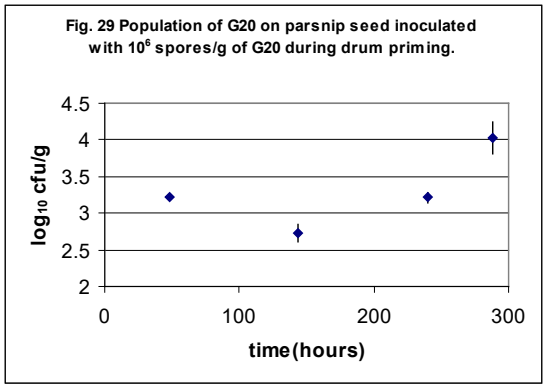
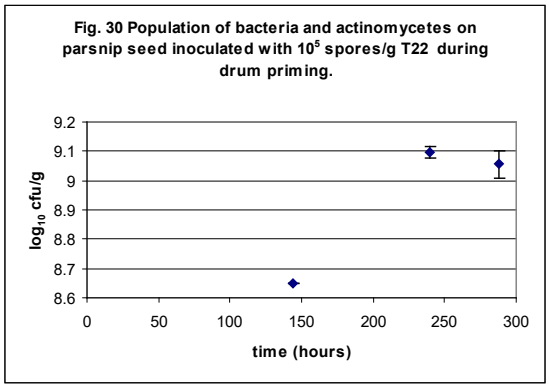


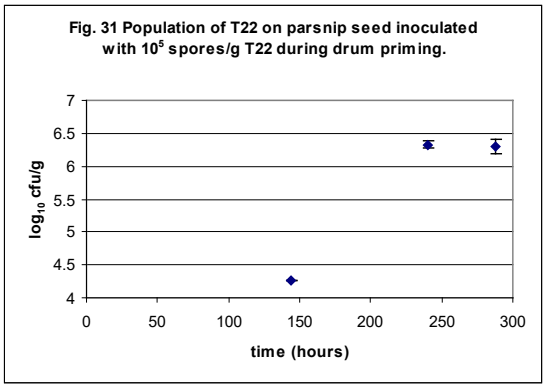
Fig. 27 Population of T22 recovered from carrot seed inoculated with 8.5x10^7 spores of T22 per gram seed during drum priming.



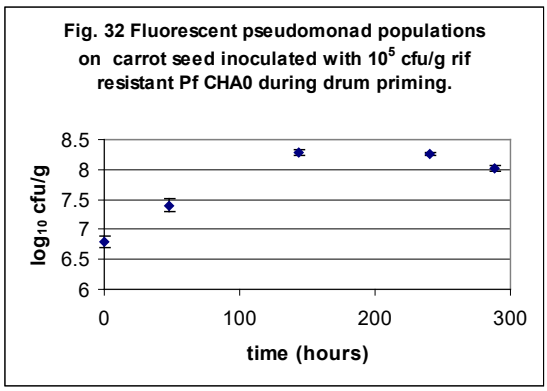








Figs. 21 to 31. The point at 48 hours is taken from seed after the addition of the hydrating mix of water and spores. Between 48 and 240 hours the seed is incubating and at 288 hours has been dried back



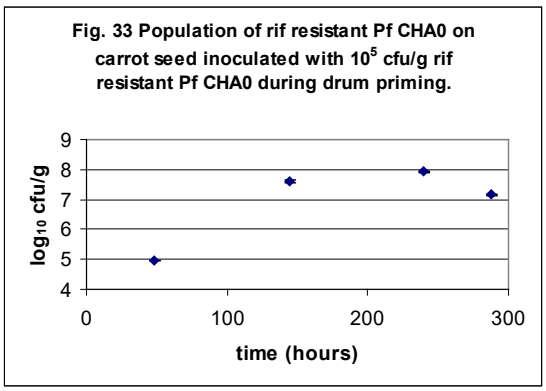


Fig. 34 Population of general bacteria and actinomycetes on leek seed inoculated with 10^6 cfu/g Pf CHA0 during drum priming.

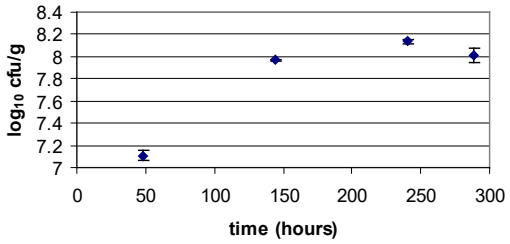


Fig. 35 Fluorescent pseudomonad populations on leek seed inoculated with 10^6 cfu/g Pf CHA0 during drum priming.

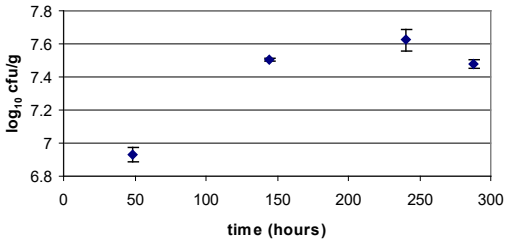


Fig. 36 Population of rif resistant Pf CHA0 on leek seed inoculated with 10^6 cfu/g Pf CHA0 during drum priming.

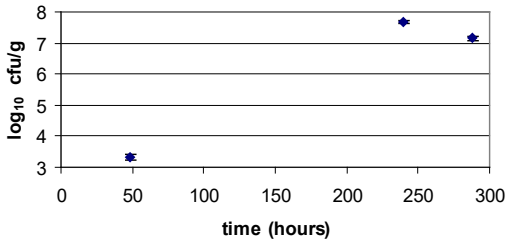


Fig. 37 Population of general bacteria and actinomycetes on parsnip seed inoculated with 10^6 cfu/g Pf CHA0 during drum priming.

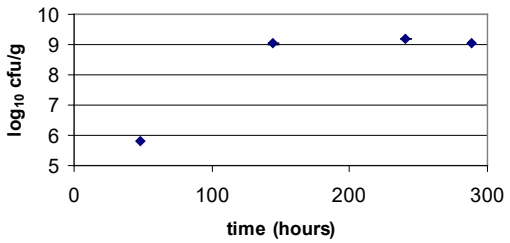


Fig. 38 Population of fluorescent pseudomonads on parsnip seed inoculated with 10^6 cfu/g Pf CHA0 during drum priming.

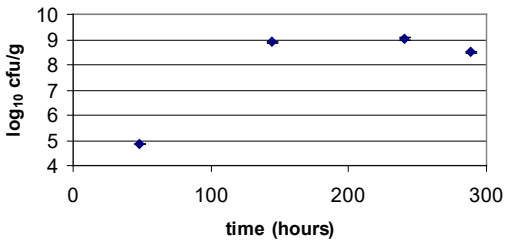


Fig. 39 Population of rif resistant Pf CHA0 on parsnip seed inoculated with 10^6 cfu/g Pf CHA0 during drum priming .

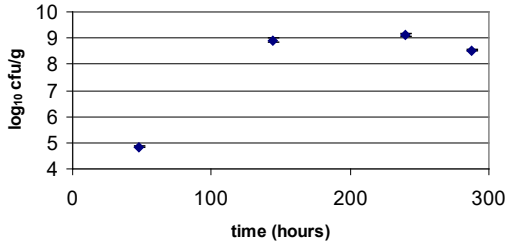


Fig. 40 Bacteria and actinomycete populations on carrot seed inoculated with 10^6 cfu/g GB03 during drum priming.

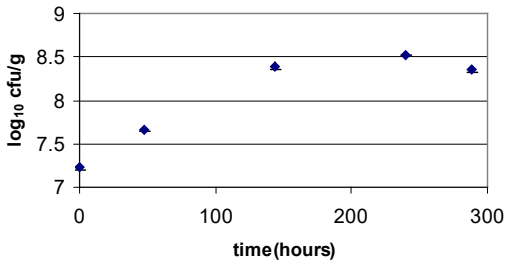


Fig. 41 Population of GB03 on carrot seed inoculated with 10^6 cfu/g GB03 during drum priming.

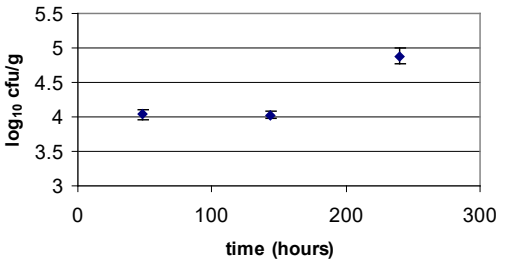


Fig. 42 Population of spore forming bacteria on carrot seed inoculated with 10^6 cfu/g GB03 during drum priming.

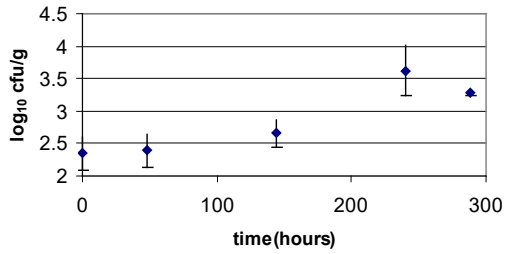


Fig. 43 Population of bacteria and actinomycetes on leek seed inoculated with 10^5 MBI600 spores per gram during priming.

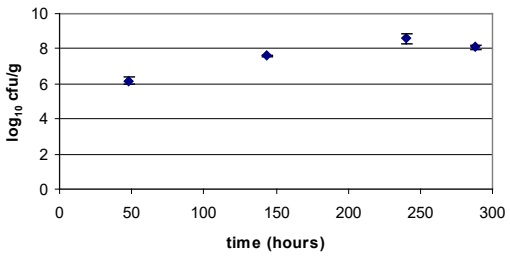


Fig.44 Population of spore forming bacteria on leek seed inoculated with 10^5 MBI600 spores per gram during priming.

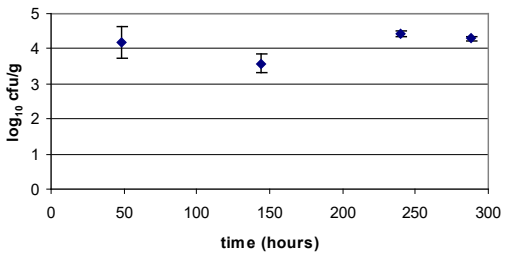


Fig. 45 General Bacteria and actinomycete population on leek seed inoculated with 10^7 MBI600 spores per gram.

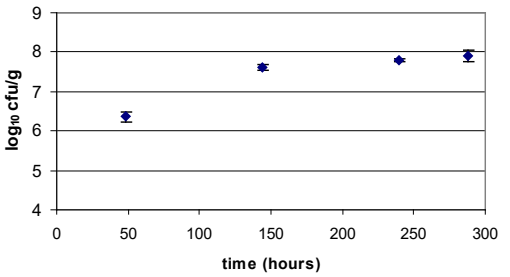


Fig. 46 Population of spore forming bacteria on leek seed inoculated with 10^7 spores MBI600 per gram.

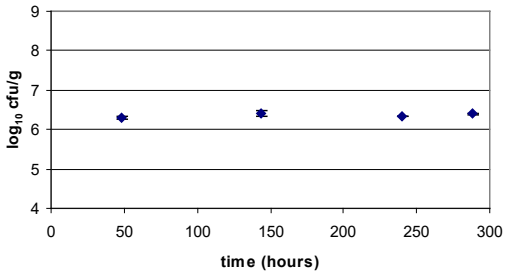


Fig. 47 Population of general bacteria and actinomycetes on Parsnip seed inoculated with 10^5 spores MBI600 per gram during priming.

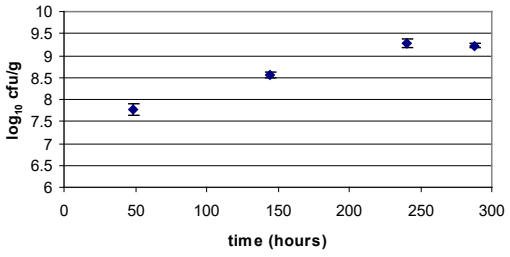


Fig. 48 Population of spore forming bacteria on parsnip seed inoculated with 10^5 spores MBI600 per gram during priming.

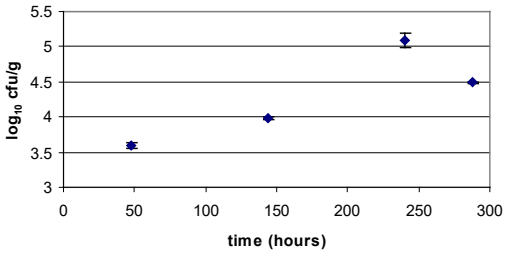


Fig. 49 Population of general bacteria and actinomycetes on parsnip seed inoculated with 10^7 spores MBI600 per gram during priming.

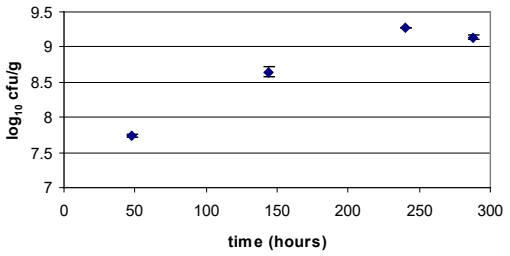
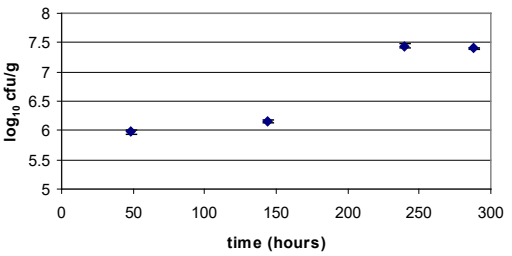
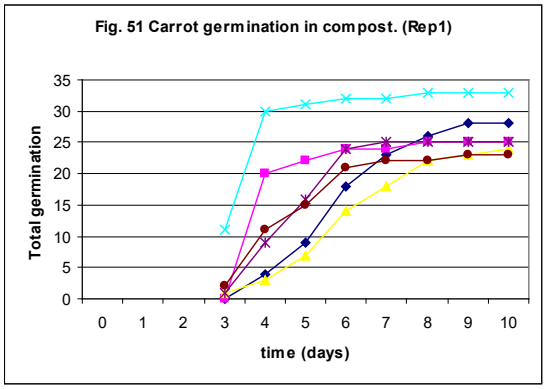
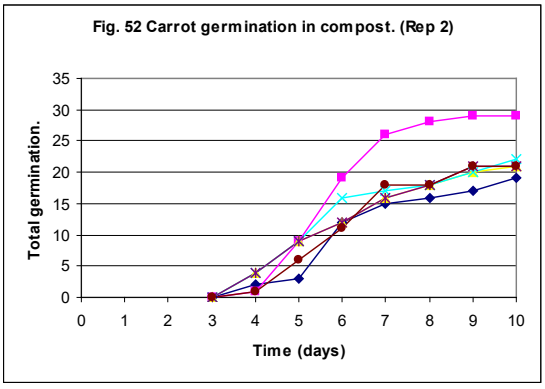


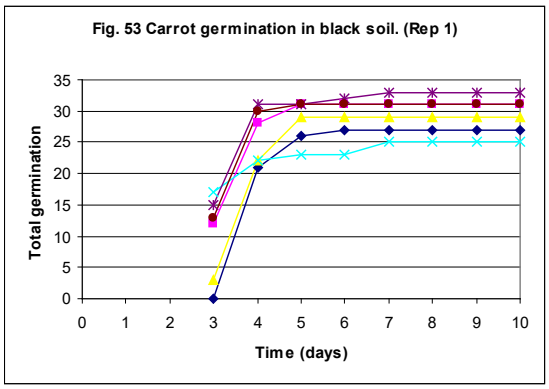
Fig. 50 Population of spore forming bacteria on parsnip seed inoculated with 10^7 spores MBI600 per gram during priming.

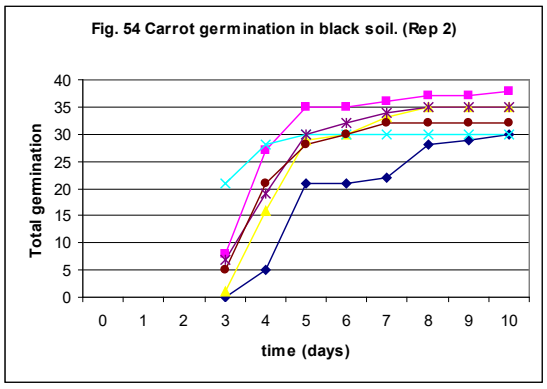


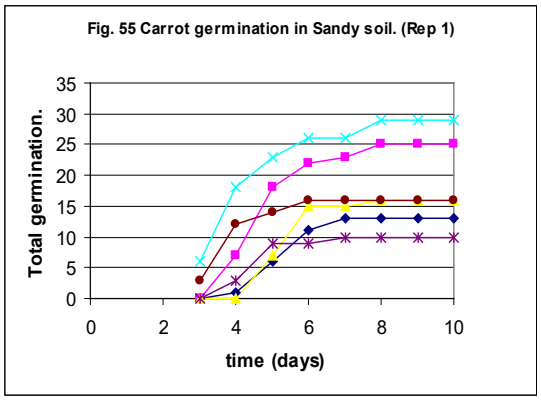
Effect of microbial treatment during priming on germination in soil for carrot seed.

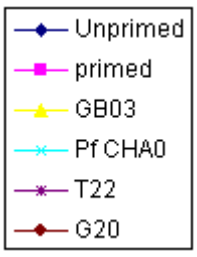












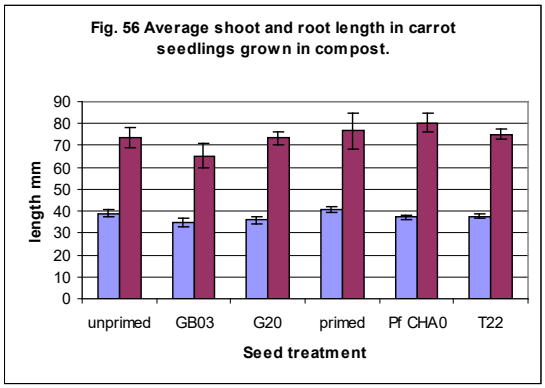


Fig. 56 Average shoot and root length in carrot seedlings grown in compost.
length mm
90
80
70
60
50
40
30
20
10
0
unprimed
GB03
G20
primed
Pf CHA0
T22
Seed treatment

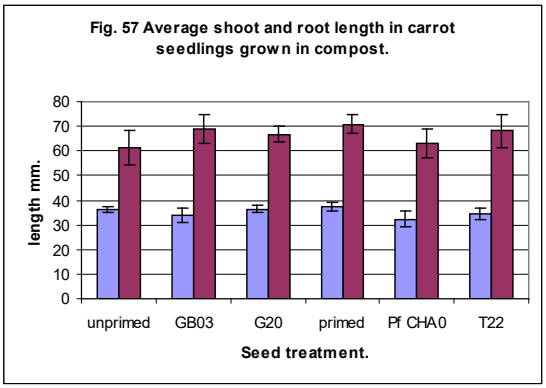


Fig. 57 Average shoot and root length in carrot seedlings grown in compost.
length mm.
80
70
60
50
40
30
20
10
0
unprimed
GB03
G20
primed
Pf CHA0
T22
Seed treatment.

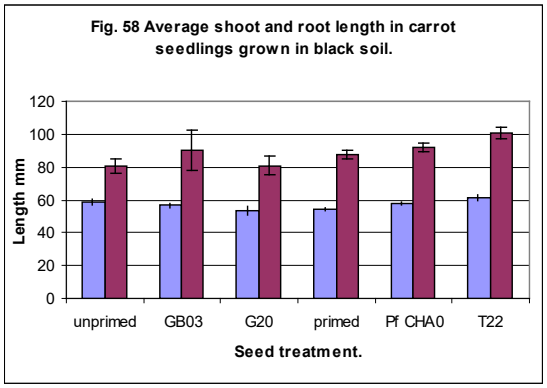


Fig. 58 Average shoot and root length in carrot seedlings grown in black soil.
Length mm
120
100
80
60
40
20
0
unprimed
GB03
G20
primed
Pf CHA0
T22
Seed treatment.

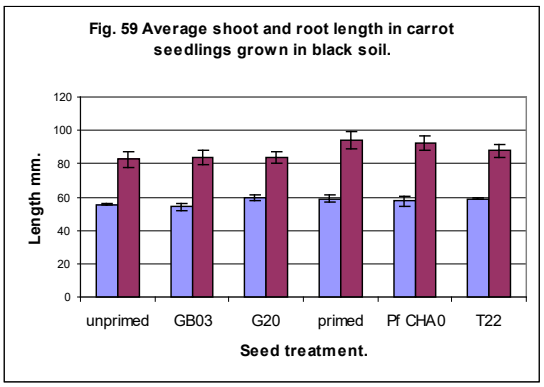


Fig. 59 Average shoot and root length in carrot seedlings grown in black soil.
Length mm.
120
100
80
60
40
20
0
unprimed
GB03
G20
primed
Pf CHA0
T22
Seed treatment.

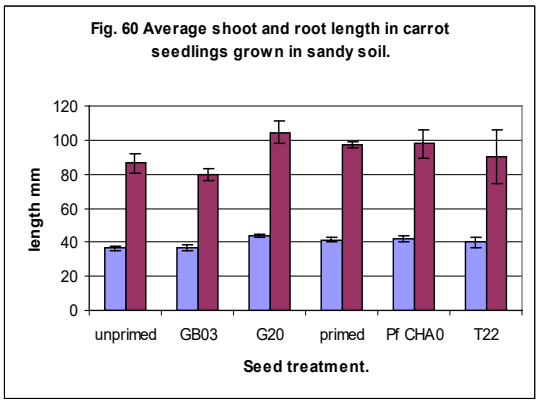
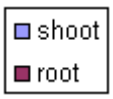


Fig. 60 Average shoot and root length in carrot seedlings grown in sandy soil.
length mm
120
100
80
60
40
20
0
unprimed
GB03
G20
primed
Pf CHA0
T22
Seed treatment.


shoot
root

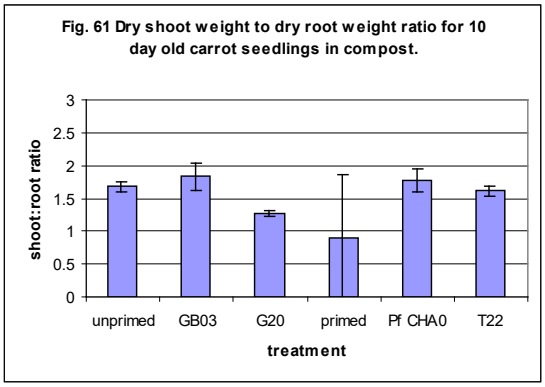


Fig. 61 Dry shoot weight to dry root weight ratio for 10 day old carrot seedlings in compost.
shoot:root ratio
3
2.5
2
1.5
1
0.5
0
unprimed
GB03
G20
primed
Pf CHA0
T22
treatment

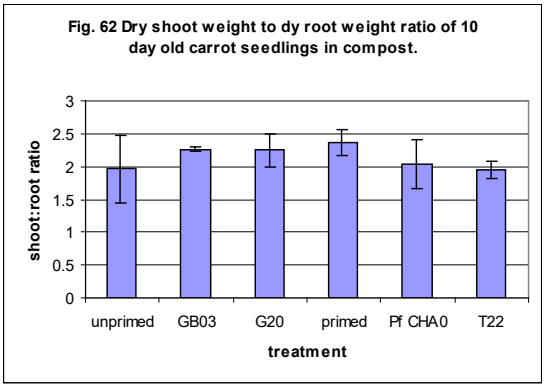


Fig. 62 Dry shoot weight to dy root weight ratio of 10 day old carrot seedlings in compost.
shoot:root ratio
3
2.5
2
1.5
1
0.5
0
unprimed
GB03
G20
primed
Pf CHA0
T22
treatment

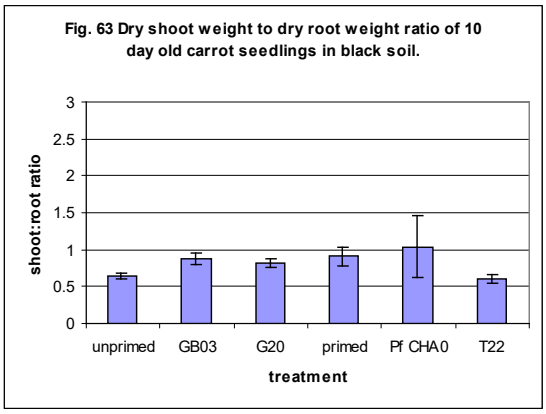


Fig. 63 Dry shoot weight to dry root weight ratio of 10 day old carrot seedlings in black soil.
shoot:root ratio
3
2.5
2
1.5
1
0.5
0
unprimed
GB03
G20
primed
Pf CHA0
T22
treatment

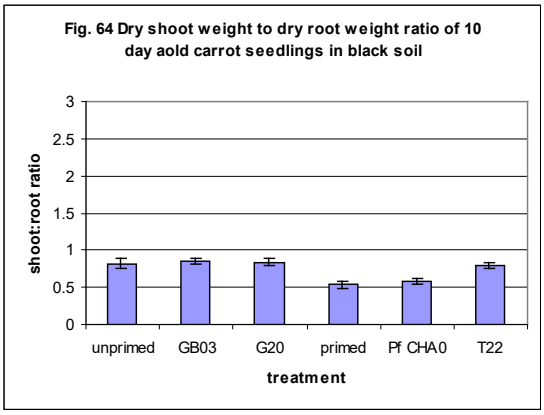


Fig. 64 Dry shoot weight to dry root weight ratio of 10 day aold carrot seedlings in black soil
shoot:root ratio
3
2.5
2
1.5
1
0.5
0
unprimed
GB03
G20
primed
Pf CHA0
T22
treatment

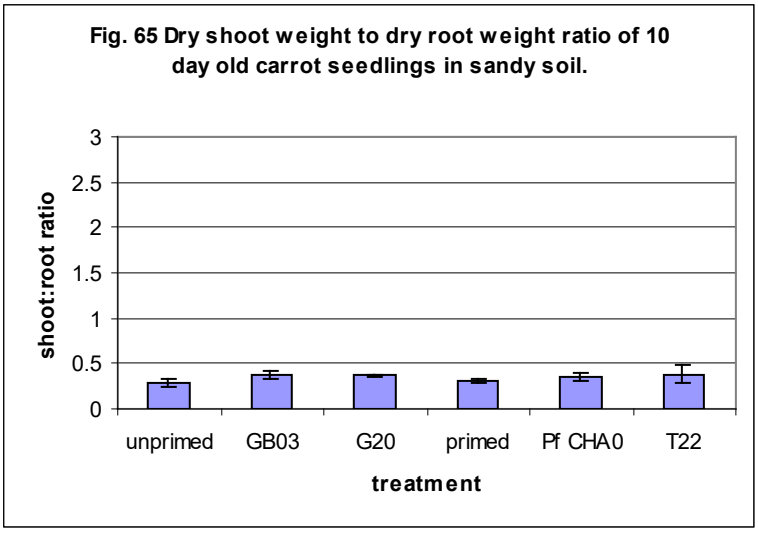


Fig. 65 Dry shoot weight to dry root weight ratio of 10 day old carrot seedlings in sandy soil.
shoot:root ratio
3
2.5
2
1.5
1
0.5
0
unprimed
GB03
G20
primed
Pf CHA0
T22
treatment

CONCLUSIONS

- Two isolates of commercially available fungi, *Trichoderma harzianum* T1295-22 (T22) and *Trichoderma (Gliocladium) virens* G20, known to promote plant growth in other systems or to have biological control properties, have been tested for compatibility with the fungicides and pesticides used commercially on carrot, leek, parsnip and sugar beet seed. Both isolates were inhibited by the presence of all fungicides except Fipronil but neither was inhibited by the presence of pesticides.
- Lab-scale steeping priming of sugar beet seed increased the population of bacteria on the seed in a similar pattern to that seen on drum primed carrot, leek and parsnip seed. Fungi and yeast populations did not increase during the priming run. Further investigation suggested that this was a property of the sugar beet seed itself.
- Fully replicated commercial drum priming runs carried out on carrot, leek and parsnip seed showed the populations of microorganisms changed in a similar way to those on lab-scale drum primed seed. It was also shown that coating with Polycote Bejofilm Force did not affect microbial numbers on carrot seed.
- It was not possible to recover spores of G20 when added to leek seed, during lab-scale drum priming, at a rate of 10^6 spores/g suggesting that leek seed may be toxic to this fungus.
- Both G20 and T22 could be recovered when applied to seed of carrot and parsnip during priming but there was no proliferation of G20 on either seed whereas T22 did increase in population on parsnip. This suggests that there may be some seed specificity for growth and sporulation during priming for these two fungi.
- It was shown that when 10^5 cfu/g seed of *Pf* CHA0 was added to seeds of carrot during lab-scale drum priming, *Pf* CHA0 was recoverable from the seed during and after priming with 8.5×10^7 cfu/g seed recovered at 240 hours, representing 47% of total pseudomonad population.
- In order to recover cells of added *Pf* CHA0 from leek seed an inoculum of 10^6 cfu/g seed rather than 10^5 cfu/g seed had to be added to the priming run. *Pf* CHA0 numbers were shown to increase during priming on leek seed, dominating the pseudomonad population, to form 33% of the overall bacterial population by 240 hours.

- In order to recover cells of added *Pf* CHA0 from parsnip seed an inoculum of 10^6 cfu/g seed had to be added to the priming run. *Pf* CHA0 numbers were shown to increase during priming on parsnip seed, dominating the pseudomonad population, to form 88% of the overall bacterial population by 240 hours.
- *Pf* CHAO is clearly seed adapted and, providing the correct application rate is used, can dominate the microbial population on primed seed. It can also survive the drying back process.
- When 10^6 cells/g of *Bacillus subtilis* GB03 were added to carrot seed during lab-scale drum priming, numbers of GB03 were 5 $\log_{10}$ units at 240 hours but no cells of GB03 could be recovered after drying back the seed.
- Spores of *Bacillus subtilis* MBI600 added at 10^5 and 10^7 cfu/g to seeds of leek and parsnip during lab-scale drum priming could be recovered from the seed during and after priming. Approximately 2 $\log_{10}$ cfu/g higher number of spores were detected on seed inoculated with the higher dose rate.
- Further work is needed with *Bacillus* species to determine whether recovery from seed is related to the species used *per se* or the ability to produce spores during the priming process.
- Seeds of carrot inoculated with selected *Pseudomonas, Bacillus* and *Trichoderma* species during lab-scale drum priming, were assessed for germination and seedling growth parameters in soil based tests in comparison with primed and unprimed seed not treated with beneficial microorganisms. These experiments are still underway but preliminary results indicate that there are differences in germination and plant growth between different soil types used but no effect of treatments within any one soil type.

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