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Project leader:	Dr John Clarkson (Warwick HRI) and Dr Steven Roberts (Plant Health Solutions) (Joint Leaders)
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AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headlines

- Treating seed with hot water, thyme oil, Serenade ASO or an experimental product B0002 reduced *Phoma* and *Alternaria*.
- Minimising watering, incorporating composted green waste or Trianum prior to sowing reduced *Pythium* infection in transplants.

Background and objectives

The use of healthy, clean seed and planting material is an important component of effective disease management for plant propagators and is essential for organic growers who have fewer options for disease control. This was highlighted at an HDC/HDRA stakeholder day (Managing Pests, Diseases and Weeds in Organic Vegetable Production, Ryton Organic Gardens 2007) where the importance of seed quality and good disease management was identified by stakeholder discussion groups as a priority area for future research.

Diseases caused by soil and seed-borne fungal pathogens such as *Pythium* spp., *Rhizoctonia solani* (damping-off, soil-borne), *Alternaria* spp. and *Phoma* (seed-borne) are a major problem for all plant propagators, especially for high volume / high value plants such as vegetable Brassicas which have a total production value of ca. £200 million in the UK (Defra statistics, 2007). Additional disease problems may also be created for organic plant raisers through the use of companion planting (e.g. bird's foot trefoil for management of cabbage root fly) as the species employed are often fodder crops of variable seed quality which could harbour pathogens and reduce the germination and emergence of both companion and crop.

A number of products which claim to have benefits for disease management in organic transplant production are now available in the EU and are marketed as growth promoters, plant strengtheners or crop protection agents. In particular, suppressive microbial inoculants and composts have shown promise for disease control and there is increasing interest in these products in conventional production systems because of the pressure to reduce pesticide use and the potential loss of many active ingredients with the revision of EC directive 91/414.

The aim of this project was to evaluate a range of organically acceptable compost and Brassica seed treatments for their efficacy and cost effectiveness in controlling damping-off diseases caused by *Pythium* and *Rhizoctonia* and seed-borne diseases caused by *Phoma* and *Alternaria*.

During the first year of the project, compost and seed treatments were tested independently for their effects on soil-borne and seed-borne fungi respectively. In the second year the most promising treatments were tested in combination and in a more realistic simulated plant-raising set up.

Summary of results and conclusions

Compost treatments (year 1)

The microbial products Trianum, Prestop, Mycostop, Subtilex, Revive P and green waste compost inoculated with *Trichoderma* (*T. viride* S17A or *T. harzianum* from Trianum) were tested for their efficacy in controlling damping-off of cauliflower seedlings caused by *P. ultimum* and *R. solani* and compared with a fungicide treatment (thiram-treated seed) in multiple experiments. The pathogens were introduced into Bulrush Organic Modular Compost and the microbial treatments added as drenches or granules at the recommended rates. Green waste compost with or without *Trichoderma* was added at 20% v/v. The amended Bulrush compost was dispensed into modules and cauliflower seed (cv. Belot) sown. The number of healthy seedlings was assessed over time.

Damping-off disease pressure varied between experiments but overall there was no consistent or clear benefit from adding the microbial products tested for control of *P. ultimum* or *R. solani.* However, at low disease pressures there was some evidence that green waste with or without *Trichoderma* was beneficial. The thiram-treated seed consistently controlled *P. ultimum* but was less effective against *R. solani* at high disease pressures.

Seed treatments (year 1)

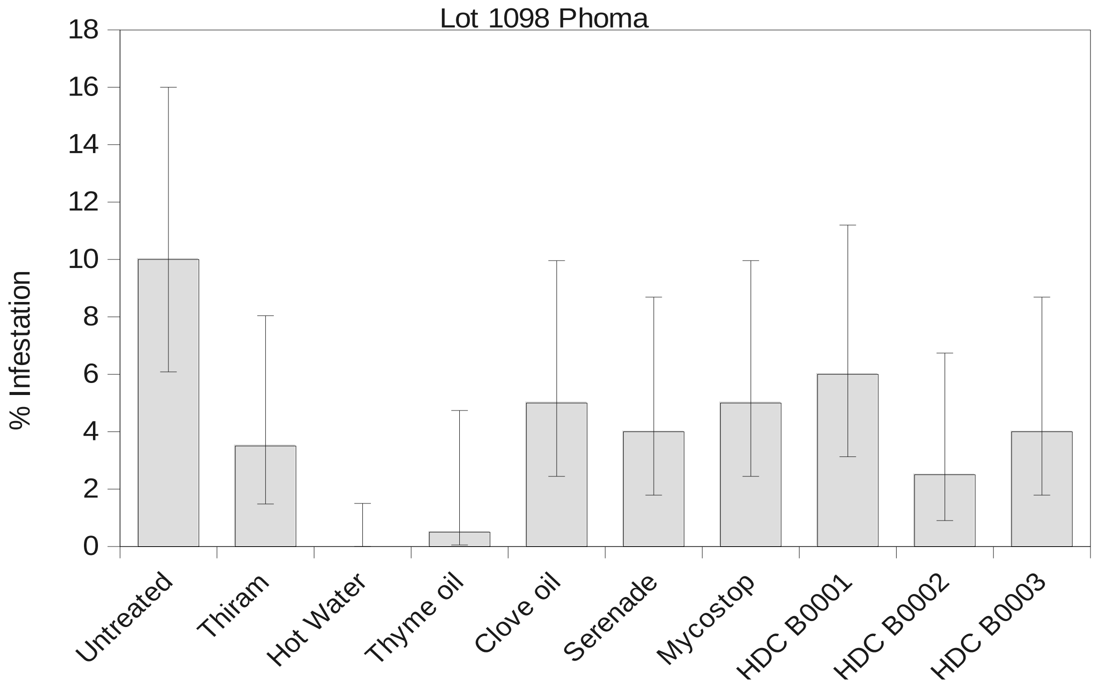
Hot water, two plant oils (thyme and clove), and five microbial products (Serenade, Mycostop and three experimental biocontrol agents) were assessed for their efficacy in the control of two seed-borne fungal pathogens of Brassicas, and improving emergence of the companion plant bird's foot trefoil. Seed was treated at recommended rates and pathogen infestation levels assessed in a standard 2,4-D blotter seed test (Brassicas) or freeze-blotter test (bird's foot trefoil). Effects on emergence and disease transmission were assessed by sowing seeds in trays of Bulrush Organic Modular Compost.

Hot water, Thyme oil, and the experimental product B0002 gave statistically significant reductions in *Phoma* seed infestation levels (shown in the Figure below), and a greater reduction than the chemical standard Thiram. Hot water in particular reduced infestation to undetectable levels (i.e. <1.5%). In transmission/emergence tests the proportion of seedlings affected by *Phoma* was significantly reduced by treatment with hot water, thiram, thyme oil and Serenade with hot water having the greatest effect.

All treatments gave a statistically significant reduction in the level of *Alternaria* infestation compared to the untreated control. The greatest reductions were achieved with hot water, Thyme oil, clove oil, the microbials Serenade and HDC B0002, and the fungicide Thiram. Again hot water reduced infestation to undetectable levels (i.e. <1.5%).

Emergence was relatively poor for the bird's foot trefoil and was not improved by any of the treatments.

Treatment with hot water led to a small but significant increase in damping off. None of the treatments gave a significant reduction in damping-off compared to the untreated controls.



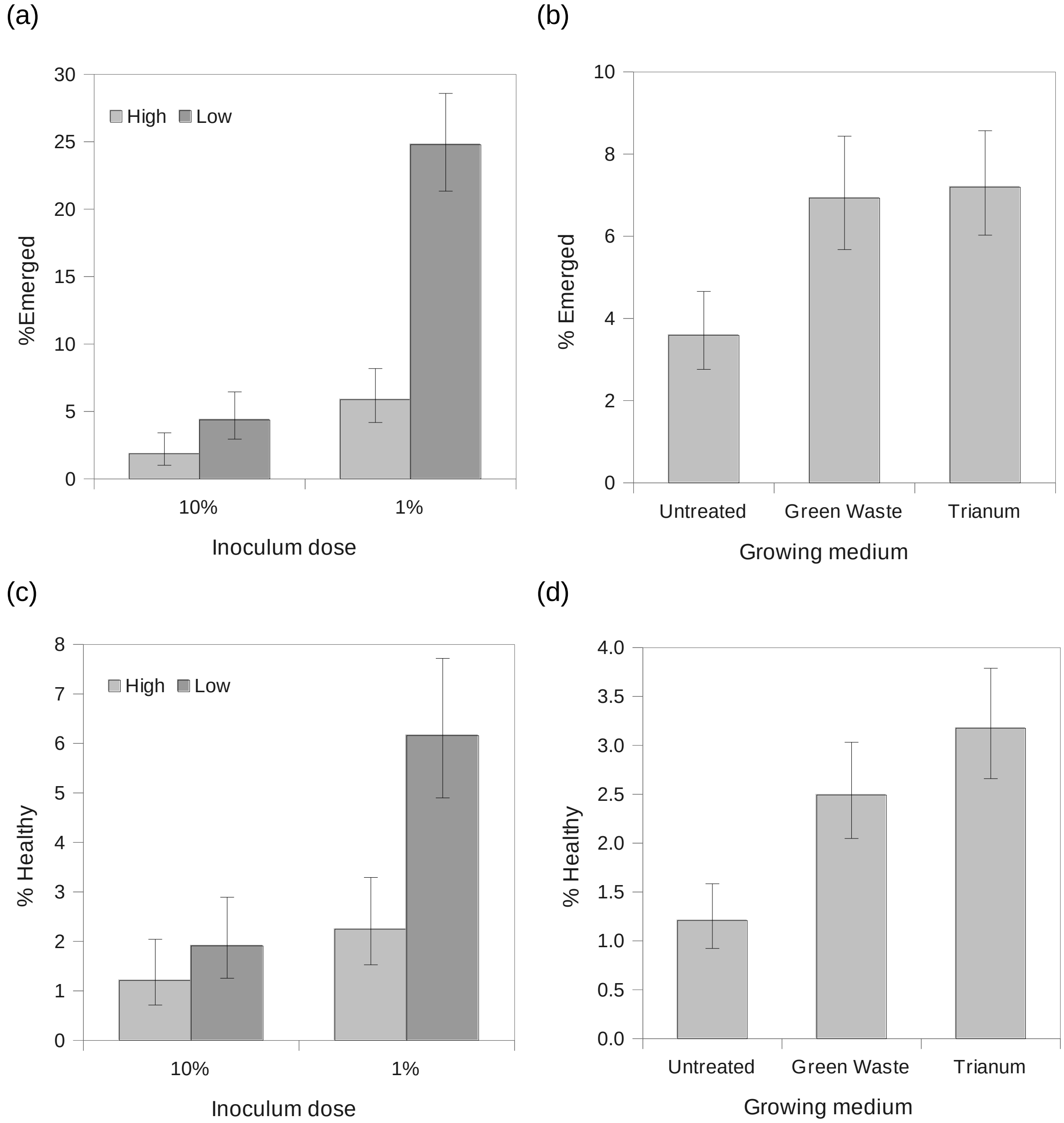
Effect of seed treatments on levels of Brassica seed infestation by *Phoma lingam* in a 2,4-D blotter seed test. Error bars represent the 95% confidence limits.

Compost and seed treatments (year 2)

A factorial combination of four seed treatments (untreated, thyme oil, Serenade ASO and HDC B0002) and three growing medium treatments (untreated, 20% green waste, Trianum) was examined, to give a total of 12 treatment combinations. Each treatment was applied to a whole '345' module tray, with two trays for each treatment. The growing medium was Bulrush modular organic compost and all of the growing medium was inoculated with *P. ultimum*. The trays of each treatment were set out randomly on two separate benches in a glasshouse. Watering was via an overhead sprinkler system controlled by a timer and the two glasshouse benches were subject to different watering regimes (high and low), with the high regime receiving double the amount/duration of irrigation as the low regime. Trays were initially given 20/10 minutes watering, then subsequently 8/4 min daily at 08:15 (with occasional manual cancelling, depending on weather conditions). Emergence and the presence of disease symptoms was recorded approx. 14 d after sowing. The entire experiment was repeated twice, with a reduced inoculum level in the second experiment.

Results can be summarised as follows:

- Emergence and the number of healthy Brassica seedlings was greater with the lower inoculum dose (shown below in a and c).
- Emergence and the number of healthy Brassica seedlings was greater with the low watering regime (a and c).
- Both of the growing medium treatments, green waste (20%, at sowing) and Trianum (pre-incorporated 2 weeks before sowing) increased emergence and the number of healthy Brassica seedlings (b and d).
- The high level of *Pythium* infection prevented the evaluation of the effect of treatments on seed-borne *Phoma* infection.
- None of the seed treatments had a significant positive effect on emergence or the number of healthy Brassica seedlings; Serenade ASO and thyme oil appeared to have an adverse effect.



Effects of treatments on Brassica emergence and % healthy seedlings: (a, c) watering regime (high, low) and inoculum dose; (b, d) growing medium treatment. Error bars represent the 95% confidence limits. Results based on combined data from two experiments.

Conclusions

- Keeping water applications to the minimum necessary is likely to have the biggest impact on *Pythium* infection in Brassica transplants.

- Incorporating green waste (just before sowing) or Trianum (two weeks before sowing) into the growing medium is likely to give a beneficial reduction in the levels of *Pythium* infection.
- Pre-incorporation should now be examined for other microbial products.
- Seed treatment with hot water (50°C, 30 min) gave the greatest control of *Phoma* and reduced *Alternaria* Brassica seed infestation to undetectable levels, but is not without problems and precise temperature-time conditions should be determined on a per seed lot basis.
- Seed treatment with thyme oil (1%) reduced both *Phoma* and *Alternaria* in Brassica seed, but its use is not currently approved.
- Two microbial seed treatments (Serenade ASO and an experimental product B0002) gave promising results against both *Phoma* and *Alternaria.* Their use as seed treatments is not currently approved.
- Conflicting results were obtained for the impact the Serenade ASO and thyme oil seed treatments on emergence.
- Emergence in the bird’s foot trefoil was relatively poor, but this could not be attributed to any specific fungal pathogens and none of the seed treatments gave any improvement in emergence compared to the untreated control.
- Bird's-foot trefoil appears to be less susceptible to *Pythium* infection than Brassicas.

The Table below lists the approval status of products used in this project:

Pesticide approval status of the various treatment products used in this study

Treatment/Product	A.I.	Status
Compost treatments		
Trianum	*Trichoderma harzianum*	Listed on Annexe 1 of 91/414. Currently undergoing registration.
Prestop	*Gliocladium catenulatum*	Full approval for all edible (protected) and non-edible crops (protected)
Mycostop	*Streptomyces griseoviridis*	Not approved in the UK. Approved in several EU countries.
Subtilex	*Bacillus subtilis*	Not approved.
Revive P	*Bacillus subtilis*	Not approved, but marketed as a 'Microbial Soil Treatment'
Green Waste		Approval not required.
Seed treatments		
Hot water		Approval not required.
Thiram	Thiram	Approved as a seed treatment for Brassicas.
Thyme oil		Not approved, Annexe 1 listing in progress?
Clove oil		Not approved, listed on Annexe 1 of 91/414
Serenade ASO	*Bacillus subtilis*	Not approved for application to seeds. Approved for foliar application to all crops (SOLA).
Mycostop	*Streptomyces griseoviridis*	Not approved in the UK. Approved in several EU countries.
HDC B0001		Experimental product. Not approved.
HDC B0002		Experimental product. Not approved.
HDC B0003		Experimental product. Not approved.

Financial benefits

Severe *Pythium* infection has the potential to cause complete crop loss for transplant producers. Minimisation of watering requires more attention to monitoring of the crop (and hence more staff time or equipment) but is likely to be worthwhile where *Pythium* infection has been a problem. Using growing media containing a proportion of green waste (produced according to PAS 100) should cost no more than media without, but there may be other risks and cost implications to the business, such as increased insurance costs.

Incorporating Trianum may add around £0.05 per module tray, but is probably only worthwhile after efforts have been made to accurately monitor/apply water.

Action points for growers

- Minimisation of watering should be the first consideration for the management of *Pythium* disease levels.
- Consider using growing media containing up to 20% composted green waste, but not without also considering the business, health and safety risks and insurance implications.
- Consider using growing media with Trianum pre-incorporated.
- Do not routinely use hot-water treatments without optimisation on a per-seed lot basis to avoid the potential for detrimental effects on emergence.
- Consider supporting an HDC-funded ‘commodity approval’ for thyme oil.
- Contact the manufacturers of the potential microbial seed treatments (Agraquest and Becker Underwood) or their distributors to demonstrate interest in the products.

SCIENCE SECTION

Introduction

Microbial inoculants have previously been shown to control soil and seed-borne diseases in experimental systems, but few have been marketed commercially. However, products currently available in the UK which may suppress root rots include Trianum (Koppert), Prestop and Mycostop (Fargro) which contain *Trichoderma harzianum*, *Gliocladium catenulatum* and *Streptomyces griseoviridis* respectively. These beneficial micro-organisms have been shown to reduce damping-off and root rots caused by *Pythium* and other spp. (e.g. Harman, 2000; McQuilken *et al*., 2001; Mohammadi, 1992). More recently, a HortLINK project (HL0176) demonstrated that applying the beneficial fungus *Trichoderma viride* S17A with green waste compost resulted in enhanced field suppression of the soil-borne pathogen *Sclerotium cepivorum* causing *Allium* white rot. Hence, combining microbial and compost treatments may be a promising approach for an effective disease management strategy in Brassica transplants.

During an EC funded project (STOVE) in which Plant Health Solutions was a partner a number of physical, microbial, resistance inducing and natural products were examined for their efficacy as seed treatments against a number of seed-borne host/pathogen combinations. The hosts included Brassicas and pathogens included *Alternaria* spp. and a *Phoma* sp. The resistance inducing products were generally unsuccessful while physical treatments were very effective and are already used/or being developed commercially by a number of seed companies. Promising results were also obtained for a number of the microbial agents, which included bacteria (*Bacillus subtilis* strains, *Streptomyces griseoviridis*, *Pseudomonas chlororaphis*) and fungi (*Clonostachys rosea* = *Gliocladium roseum*).

The aim of this project was to evaluate a range of microbial / green waste compost treatments for control of damping-off caused by *P. ultimum* and *R. solani* and organically acceptable Brassica seed treatments for control of *Phoma* and *Alternaria* diseases. During the first year of the project, compost and seed treatments were tested independently for the effects on soil-borne and seed-borne fungi respectively. The results can be summarised as follows:

- Four seed treatments showed promise for the control of *Phoma* and *Alternaria* in Brassicas: hot water, thyme oil, Serenade ASO, and an experimental microbial product.
- The companion plant bird’s foot trefoil had relatively poor emergence but this could not be improved by any of the seed treatments examined.

- Damping-off (caused by *Pythium*) was increased in hot water treated seed lots.
- The microbial inoculant Trianum appeared to decrease the emergence of cauliflower seed when mixed into the growing medium just before sowing.
- Incorporation of composted green waste with and without *Trichoderma* into the growing medium reduced damping-off at low disease pressures.

For the second (final) year of the project it was planned to test the most promising treatments from the first year, in combinations and in a more realistic simulated plant-raising set up. Based on the first year results, and following discussion with grower co-ordinator and HDC technical manager, the following treatments were selected for evaluation: the seed treatments thyme oil, Serenade ASO and experimental product B0002; and the growing medium treatments green waste and Trianum (pre-incorporated). The three seed treatments were selected on the basis that they were the best treatments in the first year. The green waste was selected as it was the most consistently effective in the first year. Trianum was selected because it is already being used commercially, and there were indications of an effect of *Trichoderma* when combined with green waste, when it was allowed to pre-colonise the substrate. It was therefore decided to add the Trianum two weeks in advance of advance of *Pythium* inoculation and sowing. This would allow 1) time for the *Trichoderma* to grow and colonise the growing medium and 2) reduce the potential for phytotoxic effects of *Trichoderma* (suggested from Year 1 results) when incorporated at sowing.

Table 1 lists the approval status of products used in this project:

Table 1. Pesticide approval status of the various treatment products used in this study

Treatment/Product	A.I.	Status
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HDC B0001		Experimental product. Not approved.
HDC B0002		Experimental product. Not approved.
HDC B0003		Experimental product. Not approved.

Materials and Methods

Overall experimental design

A factorial combination of four seed and three growing medium treatments was examined, to give a total of 12 treatment combinations. Each treatment was applied to a whole '345' module tray, with two trays for each treatment. The growing medium was Bulrush modular organic compost and all of the growing medium was inoculated with *Pythium ultimum*. The trays of each treatment were set out randomly on two separate benches in a glasshouse. The two glasshouse benches were subject to different watering regimes (high and low), with the high regime receiving double the duration of irrigation as the low regime. Glasshouse temperatures were set to a minimum 18/14°C (day/night) with venting at 20/16°C (day/night). Supplementary lighting was provided to give a minimum daylength of 12 h. Watering was via an overhead sprinkler system controlled by a timer. Trays were initially given 20/10 minutes watering, then subsequently 8/4 min daily at 08:15 (with occasional manual cancelling, depending on weather conditions). Emergence and the presence of disease symptoms was recorded approx. 14 d after sowing.

The entire experiment was repeated twice, with a reduced inoculum level in the second experiment.

Seed

One of the Brassica seed lots (S1098, cabbage) identified as infected with *Phoma* in the first year of the project was used throughout. The same seed lot of the companion plant species bird's-foot trefoil (BFT, *Lotus corniculatus*) as used in the first year was also used throughout.

Seed Treatments

Seed treatments were applied to the seed in the same way as in Year 1, at the concentrations and using the methods described in Table 2.

Table 2. Seed treatment details

Code	Treatment	Details or Concentration	mL or g per g	Application method
U	Untreated	n/a	n/a	n/a
T	Thyme oil	1% water emulsion	0.5	Added to seeds in flask or bottle, then shaken and left to stand for 30 min, before tipping out onto paper towel/filter paper and drying in air flow at RT
A	HDC B0002[1]	0.9 mL/kg	0.0036	Diluted 1+3 and added as 4x vol, then shaken.
S	Serenade ASO (*Bacillus subtilis*)	20 mg/g	0.02	Added to wall of bag/bottle and shaken

[1] Experimental biological product

Preparation of inoculum for growing medium

Pythium inoculum was prepared as in the first year of the project. Briefly, agar plugs from a culture of a known pathogenic strain of *P. ultimum* were added to a sterilised mixture of Bulrush modular organic compost (250 mL, 4 mm sieved), potato pieces (25 g, 4 mm^2) and an appropriate amount of water for an overall moisture content of 70%. After 2 weeks incubation at 18°C, the inoculum was harvested by sieving (2 mm) before use in experiments.

Pythium inoculum was mixed into the (pre-treated) growing medium shortly before sowing. In the first experiment inoculum was incorporated at the rate of 10% v/v, in the second a lower rate of 1% v/v was used. Care was taken to ensure that the inoculum was thoroughly and evenly mixed in the medium, by mixing appropriately sized aliquots of the total inoculum required into 10 L quantities of medium in a bucket, before combining and mixing into a single bulk.

Growing medium treatments

Trianum granules were mixed into samples of the growing medium approx. two weeks before inoculation and sowing to give a final concentration (after addition of the *Pythium* inoculum) equivalent to the standard rate of 0.75 g/L of medium. Care was taken to ensure that the treatment was thoroughly and evenly mixed into the medium, by mixing appropriately sized aliquots of the total amount of product required into 10 L quantities of medium in a bucket, before combining and mixing into a single bulk. No adjustment was made to growing medium moisture levels and following mixing the medium was stored at ambient temperature.

Green waste (4 mm sieved, Organic Recycling Ltd) was mixed into samples of the growing medium shortly before inoculation and sowing to give a final proportion of 20% by volume. Care was taken to ensure that the treatment was thoroughly and evenly mixed into the medium, by mixing 2 L aliquots of the total amount of green waste required into 8 L quantities of medium in a bucket, before combining and mixing into a single bulk.

Seed sowing

All module trays were pressure washed and disinfected with Jet 5 (peroxyacetic acid)(1%) prior to use in the experiments.

Module '345' trays (approx. 60 x 40 x 4.5 cm, 15.5 mL cell volume) were loosely filled with treated / *Pythium*-inoculated Bulrush modular organic compost. The surface of the growing medium was levelled and lightly compressed to form a depression in each cell ca. 1 cm below the rim of the tray using a proprietary 'dibber'. Seeds were hand sown into the trays, with one Brassica seed and 3-4 BFT seeds per cell.

To minimise the risks of cross-contamination all trays of each growing medium treatment were filled together. The medium was re-mixed at intervals just before and as the trays were being filled. Trays were filled in the order: untreated, green waste, Trianum. Seed was sown in the order: untreated, thyme oil, Serenade ASO, HDC B002.

After sowing, seed was covered with a light covering of sieved treated/inoculated growing medium, and transferred to glasshouse.

Statistical analysis

Data were analysed by fitting a series of generalised linear models with a binomial error distribution and logistic link function to the data for each tray (i.e. no. emerged/healthy, etc. in each tray out of the total of 345 cells). The means presented in graphs and tables were obtained as predictions after fitting model containing only significant terms. Dispersion was estimated from the data and, if greater than the theoretical minimum value of one, was used in the calculation of confidence intervals.

Results

Emergence of Brassica seedlings was generally very poor and was always less than 50%, even in the best treatments with the lower *Pythium* inoculum dose (second experiment). This compared with 90% emergence for a batch of the same seed sown in non-inoculated compost. Of those Brassica seedlings that did emerge, many were clearly stunted and diseased, and often died following emergence. For the purposes of recording/analysis, 'healthy' seedlings were considered as those which were judged as having the potential to produce a viable plant in the field (i.e. minor damage to cotyledons was ignored).

Very few *Phoma*-infected seedlings were seen: only three in the first experiment and only two in the second experiment. Therefore it was not possible to examine the effects of seed treatments on *Phoma* transmission.

A notable feature throughout was the much greater emergence of the bird's-foot trefoil companion plants in both experiments and in all treatments.

Effect of treatments on Brassica emergence and disease

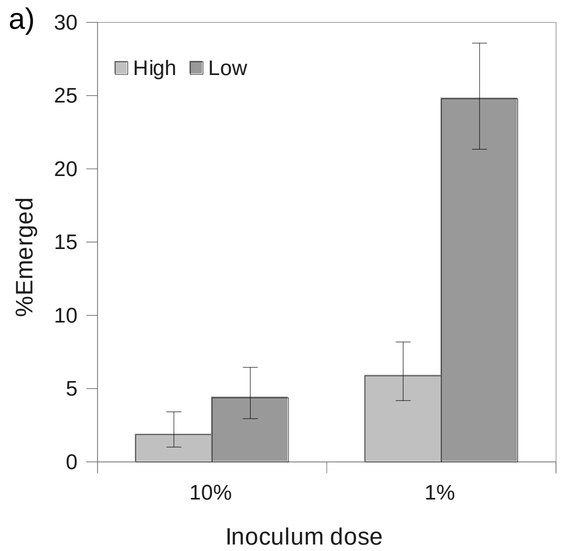
Emergence

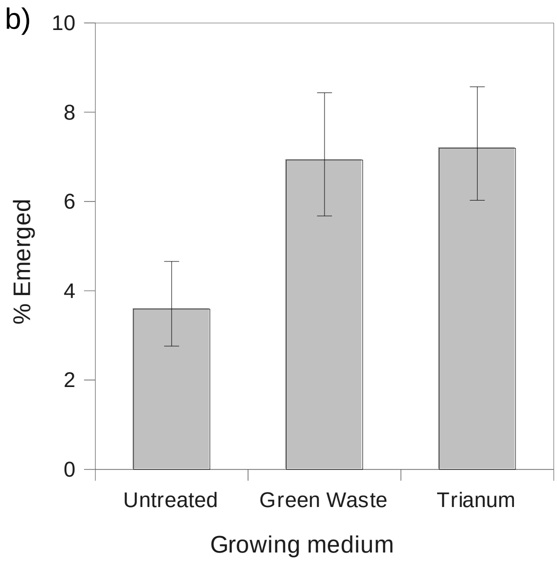
An initial analysis of deviance (Appendix 1) of the combined data for both experiments indicated significant main effects of inoculum dose (experiment), watering regime, growing medium treatment and seed treatment. There were also indications of marginal interactions between watering regime and inoculum dose (experiment) and between growing medium treatment and inoculum dose.

Inoculum dose (experiment) and watering regime had the biggest effects on emergence: with greater numbers at the lower inoculum dose and low watering regime (Figure 1a). The slight interaction effect suggested that watering regime may have a greater impact at the lower inoculum dose.

Both of the growing medium treatments (green waste, Trianum) significantly increased emergence compared to the untreated control (Figure 1b). The slight interaction effect suggested that the green waste may have a greater impact at the lower inoculum dose.

For the seed treatments it appeared that both Serenade and thyme oil gave a significant reduction in emergence (Figure 1c).





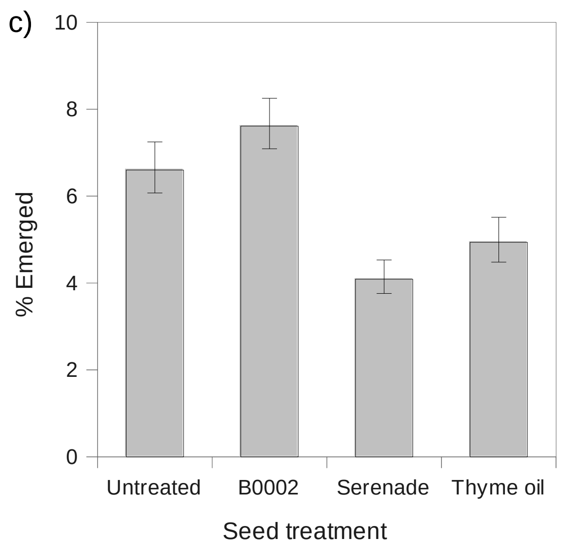


Figure 1. Effects of treatments on Brassica emergence: (a) watering regime (high, low) and inoculum dose (experiment); (b) growing medium treatment; (c) seed treatment. Error bars represent the 95% confidence limits. Results based on combined data from two experiments.

Healthy seedlings

An initial analysis of deviance (Appendix 1) of the combined data for both experiments revealed a similar pattern to that for emergence and indicated significant main effects of experiment, watering regime, growing-medium treatment, and seed treatment, but no interactions between these effects.

Inoculum dose (experiment) and watering regime had the biggest effects on the number of healthy seedlings: with greater numbers at the lower inoculum dose and low watering regime (Figure 2a). Both of the growing-medium treatments (green waste, Trianum) significantly increased the number of healthy seedlings compared to the untreated control (Figure 2b).

For the seed treatments it appears that Serenade gave a significant reduction in the number of healthy seedlings (Figure 2c).

Bird's-foot trefoil

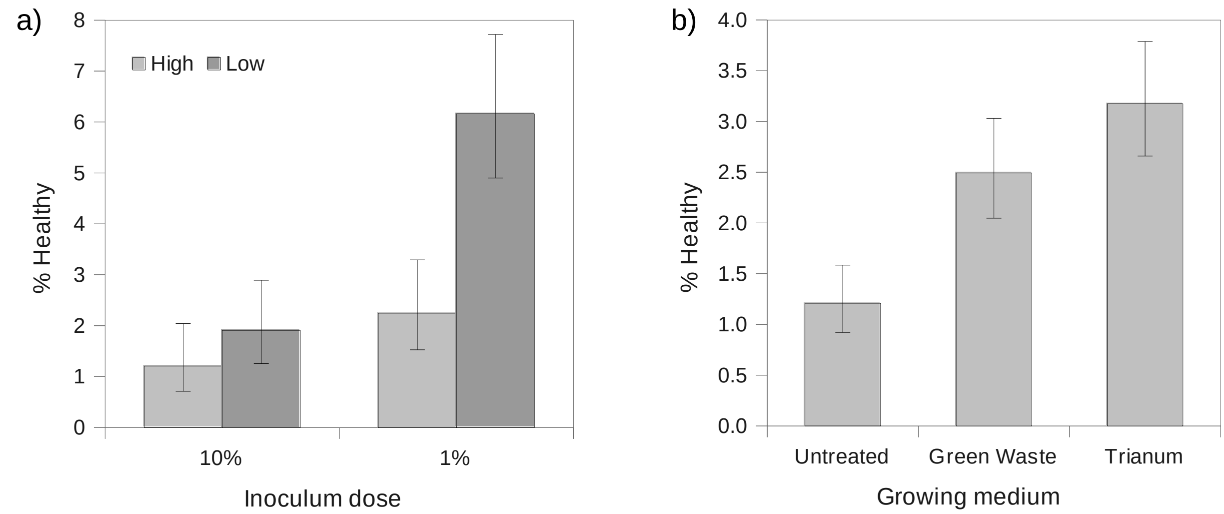
The initial analysis of deviance (Appendix 1) of the combined data for both experiments indicated significant main effects for inoculum dose (experiment) and growing medium, smaller effects for watering-regime and a marginal watering-regime x experiment interaction.

The BFT appeared to be less susceptible to *Pythium* than the Brassicas. Nevertheless the biggest effect on BFT emergence was inoculum dose, with much greater emergence at the lower inoculum dose (Figure 3a). At the lower dose there was also significant improvement in low watering regime (Figure 3a). Of the growing-medium treatments, only green waste gave a significant improvement in emergence (Figure 3b).

Discussion

Watering-regime

These results clearly indicate that watering-regime has the biggest impact on *Pythium* infection in Brassica seedlings. Thus, if *Pythium* is present or suspected in the growing medium, reducing the amount of applied water to the absolute minimum should be the first action to be considered. It is also important that water is applied uniformly to avoid 'wet' spots.



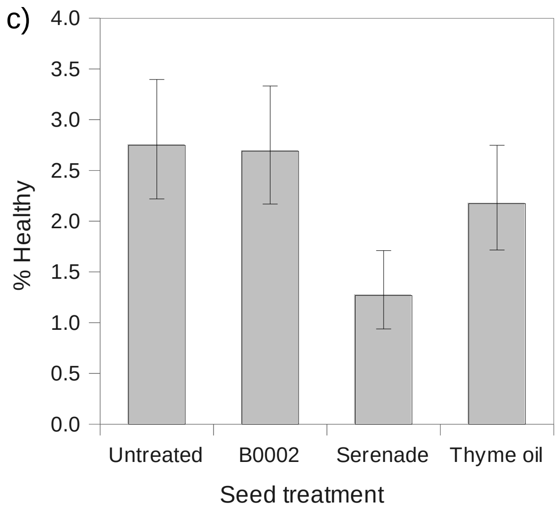


Figure 2. Effects of treatments on the percentage of healthy Brassica transplants:

(a) watering regime (high, low) and inoculum dose (experiment);

(b) growing medium treatment;

(c) seed treatment. Error bars represent the 95% confidence limits. Results based on combined data from two experiments.

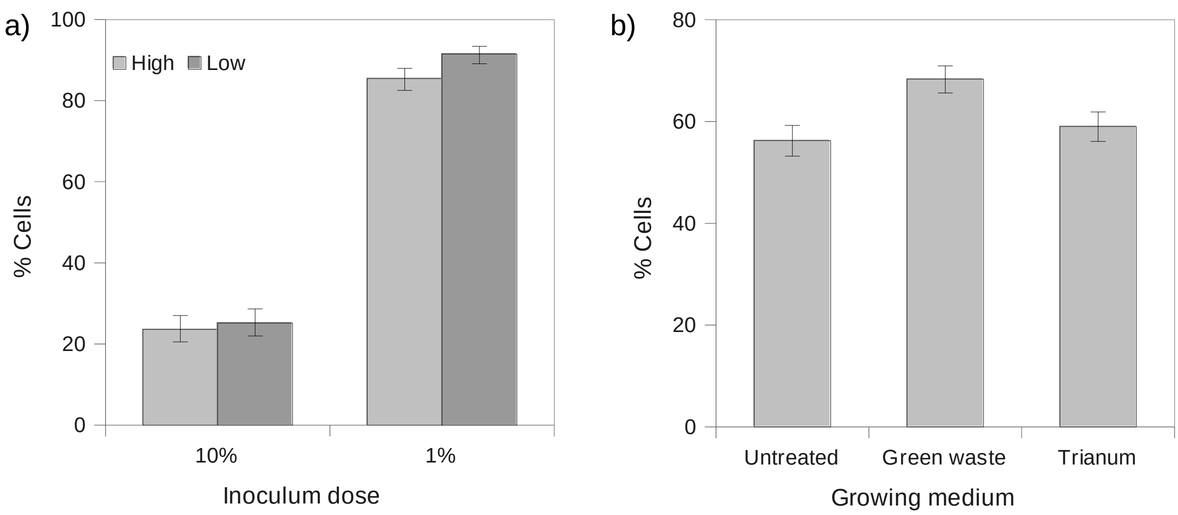


Figure 3. Effects of treatments on the percentage of module cells with at least one bird's foot trefoil seedling: (a) watering regime (high, low) and inoculum dose (experiment); (b) growing medium treatment. Error bars represent the 95% confidence limits. Results based on combined data from two experiments.

Growing medium treatments

Both growing medium treatments, green waste (incorporated just before sowing) and Trianum (incorporated two weeks before sowing) gave significant improvements in both emergence and the number of healthy Brassica seedlings. The green waste also gave an improvement in the emergence of BFT. The results for green waste are consistent with the results obtained in the first year. However, the commercial use of media containing composted green waste is not without issues, and plant-raisers also need to consider other (perceived) risks associated with green waste both for themselves and down the production chain, particularly in relation to insurance cover.

The results for Trianum were in contrast with those from the small scale trials in the first year where there appeared to be either no effect or a detrimental effect. However, a critical difference was that in the small scale trials in Year 1, all growing medium treatments were incorporated just before sowing and at the same time as inoculation with *Pythium*, whereas in these Year 2 experiments the Trianum was incorporated two weeks before sowing and inoculation. It is likely therefore that pre-incorporation gives the *Trichoderma* an opportunity to multiply and colonise the growing medium, and possibly the same effect could also be obtained by increasing the dose of the BCA. Also in-contrast to the results in the first year, pre-incorporation of Trianum appeared to remove any potential detrimental effect on the seedlings. The results for Trianum also suggest that it may be worthwhile re-examining the other microbial treatments examined in Year 1 using this pre-incorporation/colonisation approach.

At a cost of about £0.05 per module tray growers/plant-raisers may consider that the inclusion of Trianum in the growing medium may be beneficial if *Pythium* is present or suspected in the growing medium, and particularly during colder months when the risks from *Pythium* may be greater.

The effects of the growing-medium treatments did not appear to be consistent between the Brassica and BFT: only GW gave a significant improvement in BFT. BFT appeared to be less susceptible to *Pythium* than the Brassica, thus if the effect of Trianum on emergence in the Brassica is primarily a result of direct effects on *Pythium*, then less impact would be expected in the BFT.

The green waste used in this project all came from a single source, green waste from other sources may have different impacts on disease.

Seed treatments

The major impact of *Pythium* inoculation on Brassica emergence effectively masked infection by Phoma. This made it impossible to determine the effects of any of the seed treatments on Phoma.

In the first year experiments, seed treatment with thyme oil significantly reduced *Phoma* infection when evaluated in both direct seed health test and emergence/transmission experiments. The microbial seed treatments with Serenade ASO and the experimental BCA B0002 also reduced *Phoma* infection: Serenade gave a significant reduction in the direct seed health test, and also gave a slight reduction in the seedling transmission experiments (although not statistically significant). B0002 gave a significant reduction in the seedling transmission experiment and also gave a reduction in the seed health test (but not statistically significant).

In these second year experiments, both the Serenade ASO and thyme oil seed treatments appeared to have a detrimental effect on emergence, and the Serenade ASO also reduced the number of healthy seedlings. These results are in marked contrast with the results from the first year, where there was no indication of an adverse effect of either of these treatments on emergence, and both increased the number of healthy seedlings. It is possible that these treatments increased the susceptibility of the seed to the high level of *Pythium* present, but it is difficult to envisage a mechanism for this, and in previous work such effects have not been encountered. The experimental BCA B0002 on the other hand did not have any adverse effects.

Taken together, the results from the two years highlight some of the classic problems of biological controls/bio-pesticides: that of demonstrating consistent effects in a cost-effective manner with relatively limited resources. It is also clear from these results that the precise details of the experimentation, such as the timing of incorporation of compost treatments may also play a role in outcomes, highlighting the care needed when evaluating reports of both successful and unsuccessful trials with BCAs.

Conclusions

- Keeping water applications to the minimum necessary is likely to have the biggest impact on *Pythium* infection in Brassica transplants
- Incorporating green waste (just before sowing) or Trianum (two weeks before sowing) into the growing medium is likely to give a beneficial reduction in the levels of *Pythium* infection.
- Pre-incorporation should now be examined for other microbial products.

- Hot water (50°C, 30 min) gave the greatest control of *Phoma* and reduced *Alternaria* Brassica seed infestation to undetectable levels, but is not without problems and precise temperature-time conditions should be determined on a per seed lot basis.
- Thyme oil (1%) reduced both *Phoma* and *Alternaria* in Brassica seed, but its use is not currently approved.
- Two microbial treatments (Serenade ASO and an experimental product B0002) gave promising results against both *Phoma* and *Alternaria.* Their use as seed treatments is not currently approved.
- Conflicting results were obtained for the impact the Serenade ASO and Thyme oil seed treatments on emergence.
- Emergence in the bird's foot trefoil was relatively poor, but this could not be attributed to any specific fungal pathogens and none of the seed treatments gave any improvement in emergence compared to the untreated control.
- Bird's-foot trefoil appears to be less susceptible to *Pythium* infection than Brassicas.

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Appendix 1

This appendix gives extracts of the output from the statistical analyses, showing the summary analyses of deviance tables. Treatments are abbreviated as follows: Exp = inoculum dose (experiment); Bench = watering regime; Seed = seed treatment; Med = growing medium treatment; Effects considered to be significant are indicated by an * symbol. Effects of marginal significance are indicated by a ? symbol. Note that because these are generalised linear models, significance tests (F-probabilities) are not exact and should only be considered as a guide.

Accumulated analysis of deviance for Brassica emergence

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.	
+ Exp	1	797.692	797.692	105.11	<.001	*
+ Bench	1	628.460	628.460	82.81	<.001	*
+ Exp.Bench	1	23.367	23.367	3.08	0.086	
Residual	44	333.936	7.589			
+ Seed	3	79.185	26.395	9.39	0.002	*
+ Med.Seed	6	27.528	4.588	1.63	0.221	
+ Exp.Med	2	19.991	9.996	3.56	0.061	
+ Exp.Seed	3	1.982	0.661	0.24	0.870	
+ Bench.Med	2	5.753	2.876	1.02	0.389	
+ Bench.Seed	3	5.436	1.812	0.64	0.601	
+ Exp.Med.Seed	6	13.312	2.219	0.79	0.595	
+ Exp.Bench.Seed	3	17.631	5.877	2.09	0.155	
+ Exp.Bench.Med	2	4.065	2.032	0.72	0.505	
Residual	12	33.724	2.810			
Total	47	1783.455	37.946			

Accumulated analysis of deviance for healthy Brassicas

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.	
+ Exp	1	107.398	107.398	29.37	<.001	*
+ Bench	1	81.433	81.433	22.27	<.001	*
+ Exp.Bench	1	6.960	6.960	1.90	0.175	
Residual	44	160.878	3.656			
+ Med	2	65.510	32.755	17.68	<.001	*
+ Seed	3	37.473	12.491	6.74	0.006	*
+ Med.Seed	6	10.218	1.703	0.92	0.514	
+ Exp.Med	2	1.969	0.984	0.53	0.601	
+ Exp.Seed	3	3.163	1.054	0.57	0.646	
+ Bench.Med	2	1.381	0.691	0.37	0.697	
+ Bench.Seed	3	6.138	2.046	1.10	0.385	
+ Exp.Med.Seed	6	5.132	0.855	0.46	0.824	
+ Exp.Bench.Seed	3	4.340	1.447	0.78	0.527	
+ Exp.Bench.Med	2	3.324	1.662	0.90	0.433	
Residual	12	22.232	1.853			
Total	47	356.669	7.589			

Accumulated analysis of deviance for bird's-foot trefoil

Change	d.f.	deviance	mean deviance	deviance ratio	approx F pr.	
+ Exp	1	7564.337	7564.337	1195.32	<.001	*
+ Bench	1	41.644	41.644	6.58	0.014	?
+ Exp.Bench	1	35.856	35.856	5.67	0.022	?
Residual	44	278.445	6.328			
+ Med	2	152.204	76.102	25.12	<.001	*
+ Seed	3	5.042	1.681	0.55	0.655	
+ Med.Seed	6	13.226	2.204	0.73	0.636	
+ Exp.Med	2	18.885	9.442	3.12	0.081	
+ Exp.Seed	3	5.267	1.756	0.58	0.640	
+ Bench.Med	2	8.026	4.013	1.32	0.302	
+ Bench.Seed	3	10.463	3.488	1.15	0.368	
+ Exp.Med.Seed	6	10.684	1.781	0.59	0.734	
+ Exp.Bench.Seed	3	15.241	5.080	1.68	0.225	
+ Exp.Bench.Med	2	3.052	1.526	0.50	0.616	
Residual	12	36.356	3.030			
Total	47	7920.282	168.517			