

Project title: Integrated use of Soil Disinfection and of Microbial Organic Amendments for the control of Soil Borne Diseases and Weeds in Sustainable Crop Production

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Grower Summary

HEADLINE

A new piece of machinery capable of disinfesting soil to controlled depths was developed and tested. The machine uses steam generated by a novel direct fired burner that is much more fuel and cost efficient than previous steam based methods. There are opportunities to combine the use of this machine with other non-chemical techniques for soil disinfestation.

BACKGROUND AND EXPECTED DELIVERABLES

This project aimed to improve our ability to quantify soil-borne pathogens and also to produce a cost effective adaptable method to disinfest soil. The project also aimed to investigate the potential benefits of supplementing disinfested soil with organic amendments or biological control agents.

- The pathogen detection aims were achieved by designing rapid-throughput DNA based detection systems
- The soil disinfestation aim was achieved through the development and testing of an experimental prototype demonstrating the feasibility of this method of soil disinfestation.
- The project provided the data and knowledge necessary for the post project development of commercial pathogen diagnostic kits and commercial disinfestation apparatus based on the developed technology
- The project also yielded data that suggests that the beneficial effects of compost and biological control agents may be enhanced when they are applied to disinfested soil

Much intensive production of horticultural crops in the UK, both under protection and outdoors, has come to rely on soil disinfestation from time to time to prevent the build up of soil-borne pests, weeds and diseases. As all soil disinfestation techniques tend to be harsh on the soil and costly, they should only be carried out when pathogen levels are sufficiently high to justify their use.

Methyl bromide has been the soil sterilant of choice for a number of years, but use of this compound has been phased out in the EU. Other chemical sterilants tend to be less effective and are no less damaging to the environment and consumers are demanding reductions in the levels of pesticide residues present in foods.

If the UK horticulture industry is going to respond positively to these pressures without suffering loss of crop or quality, alternative strategies need to be developed for the control of soil-borne diseases, pests and weeds. These will have to minimise pesticide residues and environmental risks. At the same time they will need to be affordable and effective.

The approach

The need for soil disinfestation can only be determined if the economic threshold for any given pathogen is known – that is the population density at which the costs of any effective treatments are less than the reduction in profit that would be caused if the disease remains untreated. Current methods tend to rely on culturing disease-causing microorganisms and are slow and labour intensive. The project tested the ability of newly emerging DNA based methods to determine thresholds using the so-called “replant problem” in strawberries cause by the fungus *Verticillium dahliae*.

Once thresholds have been established an intervention method is needed.

The current project aimed to develop a novel disinfestation method based on steam. Steam is an exceptionally efficient method of heat transfer which is why it is used routinely for sterilising surgical instruments in hospitals. It is not surprising therefore, that several manufacturers have tried to develop steam equipment to disinfest soil. Typical machines boil water in a tank until it forms steam under high pressure. This pressure is then used to force the steam from the soil surface down through the soil profile. Such machines are slow, heavy, require huge amounts of energy, and don't disinfest to depth.

Central to the project was a belief that steam is a good technology, but that it has been inappropriately applied. Hospital sterilisation requires high temperatures in excess of 100°C and these can only be achieved when steam is pressurised. Soil disinfestation can be achieved at much lower temperatures reached at atmospheric pressure therefore substantially reducing energy requirements and associated costs.

When soil is disinfested, it is likely beneficial soil microorganisms will be killed in addition to pathogens, pests and weeds. Such beneficial organisms are essential to maintain soil nutrient turnover, and maintain soil structure and physical health. In order to maintain high levels of beneficial organisms in soil we investigated the potential of introducing organic amendments into steamed soil. Composted waste material is rich in organic matter and beneficial microorganisms, and some composts are known to suppress the activity of pathogens. Thus, the project also investigated the potential benefits of introducing compost into steamed soil and tested the ability of composts made from different feedstocks to suppress plant pathogens. In addition, the use of commercial biocontrol agents and decomposing Brassica residues (biofumigation) were tested for their ability to suppress pathogens.

The project developed a novel method of generating low pressure steam and this was done at three scales using a direct fired steam generator, in which thin films of water are passed close to burning fuel. First a laboratory scale machine was developed that could be used to determine the minimum temperatures needed to control a range of soil-borne pests, diseases and weed seeds. In this machine, soil was static while steam was blown through. The outcome of this work was a temperature threshold of 70°C that should be sufficient to control all weed seeds, fungal pathogens and also potato cyst nematode.

In addition to showing our steam regime was effective at disinfesting soil, it was important to test that heating the soil to such temperatures would not damage the physical, chemical or biological condition of the soil. Thus, the effect of the steam on the stability of soil aggregates (an indication of soil structural integrity) was measured, as was the release of soil nutrients and potentially toxic elements (particularly manganese) and soil microbial activity. These investigations suggested that disinfestation would have no detrimental effects on the soil, and in fact, lead to

a short term increase in microbial activity, that enhanced nitrogen mineralisation and increased growth of crops compared to unsteamed soil.

In order to calculate maximum rates of soil transport that could be used to achieve sufficient heating a larger machine was built in which steam was passed along a conveyor while being steamed. Finally, a field-scale tractor-drawn prototype machine was constructed that could match the performance of the small-scale prototypes. This field scale prototype was field tested for its ability to control weeds at seven sites in the UK ranging from Sussex to Inverness and on a range of crops including carrot, babyleaf spinach, iceberg lettuce, onion and carrots. Trials also included treatments in which composted materials were incorporated after disinfection.

The main deliverable of the project will be an experimental prototype demonstrating the low pressure steam technology developed the knowledge necessary to take the project forward for commercial development.

SUMMARY OF THE PROJECT AND MAIN CONCLUSIONS

Soil Testing

Objective 1: *Develop a PCR based method for quantification of Verticillium wilt in soil.*

A rapid kit-based DNA extraction method and two quantitative DNA diagnostic assays were developed to detect *V. dahliae* microsclerotia in soils. One method used traditional DNA methods in which diagnostic sections of *V. dahliae* DNA are first amplified (using a technique called PCR), then visualised by running the amplified fragments on a gel. The second method used recently developed “real time” PCR techniques in which amplification and detection take place simultaneously.

Both PCR methods were tested on 13 naturally infested soil samples and compared with the current detection method in which the fungi are grown on plates of nutrient media. Our data showed how inaccurate current methods of detection are, and suggests that molecular techniques using modern “real time” PCR offer faster and more efficient identification of the pathogen in soil those currently available. Our data provides the foundation knowledge to develop routine diagnostic test for the Verticillium wilt pathogen.

Alternative soil disinfection treatments

Objective 2: *Determine the efficacy of novel steam and/or ozone disinfection treatments for killing the soil borne pathogens: Verticillium dahliae, Sclerotinia sclerotiorum, Phytophthora fragariae, Rhizoctonia, Pythium ultimum, potato cyst nematode (PCN) and Chenopodium album weed seeds.*

Agricultural soil samples containing survival structures of several crop pathogens *Verticillium dahliae* (Verticillium wilt), *Sclerotinia sclerotiorum* (white mould), *Sclerotium cepivorum* (onion white rot), *Pythium ultimum* (damping off), potato cyst nematodes *Globodera rostochiensis* (yellow potato eelworm) and *G. pallida* (white potato eelworm) and the weeds *Chenopodium album* (fat hen) and *Agropyron*

repens (couch grass) were treated in the laboratory with aerated steam at temperatures ranging from 40 to 80°C in a specially constructed apparatus. Steaming at 50 or 60°C for 3min, followed by an 8-min resting period in the steamed soil and immediate removal from the soil thereafter, and resulted in 100% kill of all weeds, diseases and nematodes, as did heating the soil to 70°C for very short holding times. When steamed at 45°C, there was a small but significant reduction in the survival of *V. dahliae* microsclerotia but no reduction in survival of *S. cepivorum*. Treatment with ozone gas did not significantly reduce viability of any test organisms and this method of control was not investigated further.

Objective 3: *Assess the effect of the most efficient novel soil disinfection method on (i) soil aggregate stability, (ii) nutrient availability, (iii) biological activity.*

No steaming treatments were found to alter the stability of soil aggregates, but if steam was applied too fast it caused entrainment (blowing away) of aggregates in some soils under conditions of very low moisture.

Soil steaming was found to increase soil respiration in the few days immediately following treatment. This is likely due to humus being broken down into sugars that can be used by microorganisms. In the same period, soil steaming caused an increase in the availability of ammonium but not nitrate, suggesting that there was increased nitrogen mineralisation, but not nitrification. Plant growth was significantly higher in steamed compared with unsteamed soils. Soil steaming at 70°C caused a slight but significant increase in available soil manganese, but this was much less than the potentially toxic levels seen when soil is steamed at 100°C.

Objective 4: *Assess the effect of soil type and soil matric potential on the disinfection efficacy of the most efficient novel soil disinfection method.*

For soils to be effectively disinfested, they need to be raised to 70°C. How much heat that is needed to achieve this will depend on numerous factors including the initial soil temperature, the soil type, soil moisture content and bulk density. Four agricultural topsoils were chosen for use in the steaming experiments to give a range of soil types and textures. The aim was to include the extreme and more typical types of soil used in horticultural production, from a very fine sandy organic soil (Dalcross) to a heavily textured (for horticulture) and structurally unstable sandy clay loam (Wellesbourne). Based on the findings of the work it was shown that under field conditions, soil that is between field capacity and slightly moister than wilting point, can be disinfested with steam. The only exception is that in sands, there may be a narrower range of suitable operating conditions and very dry sands may be unsuitable.

Objective 5: *Design and develop a field prototype of a machine for more efficient steam or ozone disinfection of soils (the target is to obtain similar efficacy and costs to methyl-bromide treatments).*

The field prototype was manufactured by Jones Engineering, Doncaster and used steam (not ozone) as the disinfesting agent. The machine was based on a potato destoning machine with a front mounted soil pickup and has an onboard tank for diesel and a tank to hold 1000 litres of water. Any tractor with a variable gearbox

can tow it. The prototype is all electric and has an on-board 15 kVA generator. The soil pick up is fixed at 1500 mm width (with 1.5 m" or 1.8 m wheeling which could go to 1.95 m") and soil is put down into new beds at the same width, but this can be varied. The soil pickup has adjustable depth settings allowing soil to be disinfested to appropriate depths depending on soil type, crop and cropping history. Once the soil has been picked up, it is passed along a conveyor and subjected to an upward flow of steam generated by a direct fired steam generator powered by an 800 kW High-Low Burner. Soil is then dropped from the rear of the conveyor and formed into new beds.

Objective 6: Compare the efficacy of the prototype in field trials against two control treatments - an existing semi-continuous whole bed steam disinfection applicator and the most appropriate existing pesticide soil treatment.

The machine was tested in series of experiments, coordinated by the Stockbridge Technology Centre (STC) during 2007 and 2008. In 2007 experiments comprised a baby leaf spinach trial at Langmead Farms, a whole head lettuce trial at Langmead Farms, a strawberry trial at Berryworld and weed control studies at Jones Engineering and STC. In 2008 trials were done on carrots at TIO, baby leaf at Langmead Farms, strawberries at Berryworld and onions at HRI Kirton. The machine performed well in all trials in terms of heating the soil. Due to low pathogen pressure, we only have good data on weed control. In the trials programme it became apparent that the initial hope that efficacy could be assured if soil temperatures merely reached 70°C was not correct and it would seem this temperature needs to be maintained for several minutes. However, even with this heating regime, significant weed control was obtained in some trials. Furthermore, in many trials, an increase in crop vigour was noticed in steamed plots, probably resulting from increased nitrogen mineralisation and in one trial this resulted in a higher crop yield.

It was not possible to use Methyl bromide in the trials, and a Regero steam hood was not available for comparisons.

Organic soil amendments

*Objective 7: Determine the effect of three compost preparations (based on (i) cattle manure and waste straw, (ii) household wastes and (iii) a mixture of green waste and cattle manure) on the soil borne pathogens *Sclerotinia sclerotiorum* and *Pythium ultimum*, potato cyst nematode (PCN) and *Chenopodium album* weed seeds.*

Composts using feedstocks (i–iii above) were prepared. All three mixtures composted successfully with a good thermophilic (disinfestation) stage. All composts showed a degree of suppressive activity towards *Pythium ultimum* but cattle manure based composts showed greatest suppressive activity. However, no composts showed suppressive activity against to *C. album* weed seeds, PCN cysts or *Sclerotinia sclerotiorum*. However, the well-established benefits of adding composts (adding beneficial microorganisms and acting as a physical conditioner that improves soil structure), suggests that compost addition after steaming will be beneficial. Our data indicate that, except when *P. ultimum* is a particular problem, the type of compost used will be of little consequence.

Objective 8: *Determine the effects of volatiles from decomposing fresh Brassica residues on a fungal pathogen (V.dahliae), free-living nematodes and a weed seed (Chenopodium album) under various combinations of soil type and conditions.*

Laboratory experiments have indicated that fresh Brassica tissue can reduce populations of *Verticillium dahliae* when incorporated in soil at 10% wt/wt. However, in field trials it was only possible to incorporate residues at less than 1% by weight and this was not effective. Our data suggest that under conditions such as in those in our trials, biofumigation is not currently feasible in the UK. The lack of effect is most likely to relate to the low amounts of plant residue incorporated, and future approaches would need to address this as well as examine modifications to the incorporation procedures

Integration of control strategies

Objective 9: *Integrate novel soil disinfection treatments with the most active (i) compost treatment, and/or (ii) three selected microbial amendments (AM-mycorrhizal fungi, Bacillus subtilis and Coniothyrium minutans) to achieve an overall efficacy similar to that of methyl bromide.*

This work focused on the survival in soil of two biocontrol agents (based on commercial availability), *Coniothyrium minutans* and *Bacillus subtilis* MBI 600, and their biocontrol activity against the pathogens *Sclerotinia sclerotiorum* and *Pythium ultimum*, respectively.

Coniothyrium minutans survived well when applied to disinfected and untreated soil for 30 days in a range of soil types, but survival was significantly enhanced in disinfested soils. *Bacillus subtilis* MBI 600 applied as a cell suspension declined in numbers in disinfested and untreated soils with spore formation being higher in disinfested than untreated soil.

Viability of *S. sclerotiorum* sclerotia was reduced equally by steam disinfestation or the application of *C. minutans*, and no further reduction in sclerotial viability was seen when the two treatments were combined. Early application of *C. minutans* to disinfested soil gave best control of the pathogen.

Pythium ultimum damping-off of lettuce was not controlled in untreated or disinfested soil by either a seed treatment or a soil amendment of *B. subtilis* MBI 600. A replacement biocontrol agent, *Pseudomonas fluorescens* CHA0, was equally unsuccessful and a different biocontrol agent may be better suited to integrated control of damping-off.

EXPLOITATION AND FUTURE APPLICATIONS

1) Soil test

For the soil diagnostics part of this project, two PCR assays for the detection and quantification of *Verticillium dahliae* in soil were successfully developed at WHRI. However, significant problems were found when correlating the PCR assays with the conventional plating assay (as is offered commercially by East Malling Research) due to variability inherent in the plating assay. Hence further development and validation was judged necessary before the PCR tests could be offered commercially. This was initially undertaken at EMR (funded by HDC) but technical problems meant this was not successful. A further HDC funded project has now started at FERA (York); this will try to circumvent the problems caused by

comparing the two types of assay by directly relating PCR detection to infectivity and disease (rather than via the conventional plating assay). If the new project is carried through successfully, an updated version of the test developed by WHRI in the HortLink project will be offered commercially by FERA (estimated time scale 3 years from 2009).

2) Soil Steamer machine

For soil disinfestation, the main output from this project has been the development of the experimental soil disinfestation machine that has been demonstrated in the field and accompanied by presentations at some HDC and HDRA grower events. The academic partners have also reported their research findings at conferences and in journals.

The consortium will continue to make use of HDC News and the trade press to disseminate information as the opportunities arise in the future.

The promising experimental results obtained within the project have lead Jones Engineering to embark on the construction of an improved prototype that will be used for further commercial trials starting during 2009. It is anticipated that the experience gained by the engineering partners in this project will be sufficient for them to design a highly improved version of the machine for commercial development offering considerable improvements in fuel, time and costs efficiencies. When trials with the new machined have been completed, results will be presented in HCD news, and it is hoped additional information on its performance will be included in Jones Engineering's sales effort.

The grower partners have had the opportunity to gain practical experience with the technology, initially with experimental equipment and the growers in the consortium are particularly keen to be involved in trials of the new machine.

3) Broadening future applications:

The broad spectrum of activity of steam disinfestation means that the technology can be applied to solve almost any soil borne pathogen, weed or pest problems. Thus, the technology has potential to be used throughout the world in many crop situations. What will limit its more widespread use is the relatively high costs. However, since all soil disinfestation treatments are expensive (and in most cases more expensive than this steam method) this should not be restrictive. Furthermore, the anticipated increases in efficiency of the next machine will lower costs considerably. It is also possible that the costs of steaming will be in part offset by increased yield caused by increased plant available nitrogen in the soil.

The academic research partners will use incite gained in the project regarding the relationships between soil biological, chemical and physical fertility in relationship to temperature to further scientific knowledge in a wide range of disciplines. In addition to crop situations, the technology has potential to aid bioremediation of contaminated land. The potential of the machine to be used to control a much broader range of fungal and nematode pests will also be considered.

4) *Exploitation plan, consortium linkages and timeframes:*

Exploitation Activity	What will be on Offer	Target (Audience)	Partner(s) Involved	Timing/ Duration
Development of quantitative commercial DNA based diagnostic kits for pathogens	A service to quantify pathogens in growers soils	Growers and their advisors	This work is now being funded by HDC at FERA, York	Commercial products estimated in 2012
Development of <u>commercial</u> prototype equipment based on the experimental prototype built during this project	Commercial prototype equipment will be available to consortium growers. Subsequently full commercial versions will be available	Growers and their advisors	Jones Engineering, Berryworld, Langmeads Farms, TIO	Work on prototype has already started
Development detailed protocols for using machine in relation to soil type, moisture etc.	Advice that could be provided by the manufacturer to optimise disinfestation performance	Growers and their advisors	All	Work on supporting agronomic information will be collected throughout trials of the commercial prototype machine.

FINANCIAL BENEFITS

It is envisaged that a test will be available, for the soil diagnostic technology, with similar, or slightly reduced costs to current methods, but results will be available more quickly (max. 5 working days from receipt of sample to result) and will be more accurate. If there is low disease pressure present in the soil, growers need not disinfest soil, representing a considerable saving (see costs below)

For the novel soil disinfestation technology an analysis based on field performance and projected capital cost suggests that the operating costs of the current machine, excluding wear and tear and depreciation, would be £3,300 per hectare. The current work rate of the machine is approximately 75 hours per hectare and fuel use is approximately 2622 litres/hectare. A guide to pricing the final developed machine would be approximately £150,000 which would have an improved work rate, and thus would be quicker and cheaper to operate.

The currently available alternative, Basamid, when applied at full rate costs approximately £5300 per hectare. Therefore the current steam machine has potential to save growers money.

Other benefits

In addition to the direct financial benefits indicated above there should be a number of other benefits which are less easy to quantify in financial terms:

- Environmental benefits resulting from reduced pesticide use
- Improved crop yield as a result of nitrogen mineralisation
- Enhanced activity of biocontrol agents
- If composts are incorporated following steam, increased soil organic matter and soil structure will be achieved

ACTION POINTS FOR GROWERS

- § In addition to efficacy of the machine at soil disinfestation, the increased nitrogen mineralisation caused by steam may increase yields and potentially reduce the need for fertilisers.
- § The machine operated reliably in a wide range of soil types and moisture conditions
- § Sandy soils close to wilting point do not get heated by steam effectively. Such soils should not be disinfested with steam until after rainfall or irrigation.
- § Soils should be maintained at 70°C for at least three minutes.
- § If compost is added and *Pythium ultimum* is present, composts based on cattle manure should be used
- § If biocontrol agents are to be added, they should be added as shortly as possible after disinfestation

SCIENCE SECTION

INTRODUCTION

The scientific approach of the project has been diverse in scope, encompassing a range of techniques from modern laboratory based molecular biology techniques, through studying the physics of soil heating, to laboratory based bioassays with a range of plant pests and full scale field experiments. The science objectives were grouped under four main themes (1) Soil testing (objective 1), (2) Develop alternative soil disinfestation treatments (objectives 2 – 6), (3) Investigate the potential benefits of adding organic amendments (compost, biological control agents) to soil (objectives 7-8), and (4) Investigating the potential to integrate the steaming and amendment techniques (objective 9).

Soil Testing

Objective 1:

Develop a PCR based method for quantification of Verticillium wilt in soil.

A rapid kit-based DNA extraction method and two quantitative PCR assays using specific primers for the quantification of *V. dahliae* microsclerotia in soils were developed. For the competitive PCR assay, a heterogeneous competitor DNA fragment was developed as an internal control and found to provide accurate quantification of *V. dahliae* DNA in the range of 2-200pg and a detection limit of approximately 60 genomes in soil extract (Figure 1). SYBR Green I PCR had sensitivity down to one genome of *V. dahliae* DNA and has detected as little as 0.1 colony forming unit per gram of soil (Figure 2). The greater sensitivity of the SYBR green real time PCR technique makes this technique more suitable for development as a commercial kit, in spite of the slightly higher costs associated with this method.

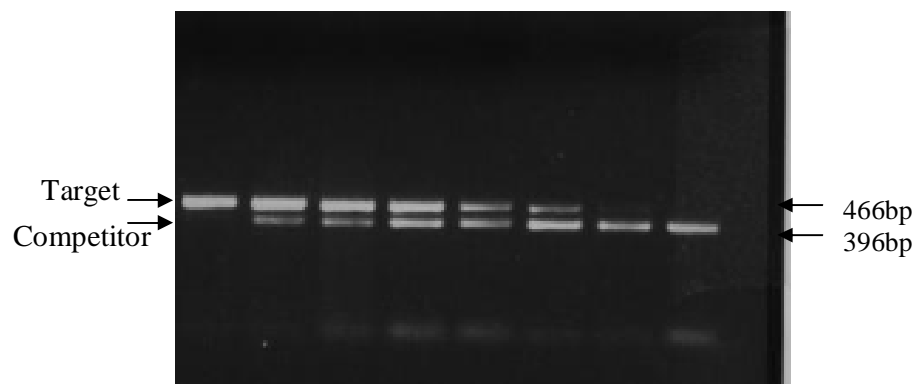


Figure 1: Competitive PCR using DB19F1 and DB22 primer and new competitor DNA. Half-log dilution of *V. dahliae* DNA co-amplified with 2fg of competitor DNA. C- competitor DNA amplified in the absence of *V. dahliae* DNA.

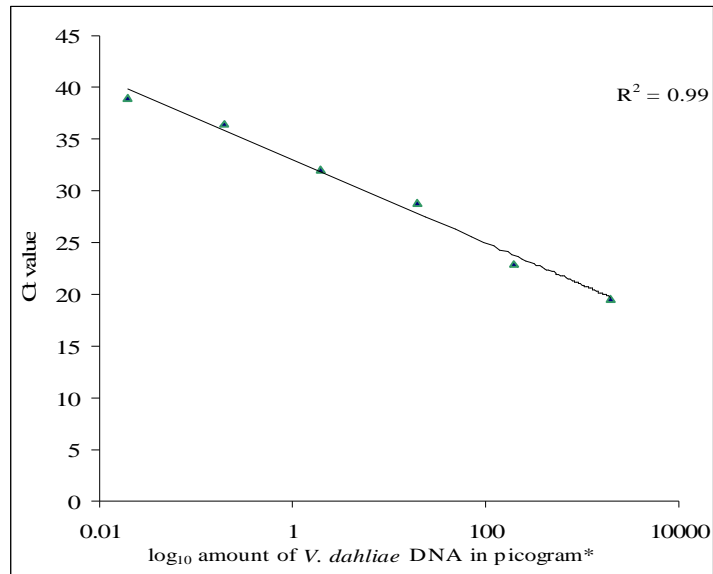
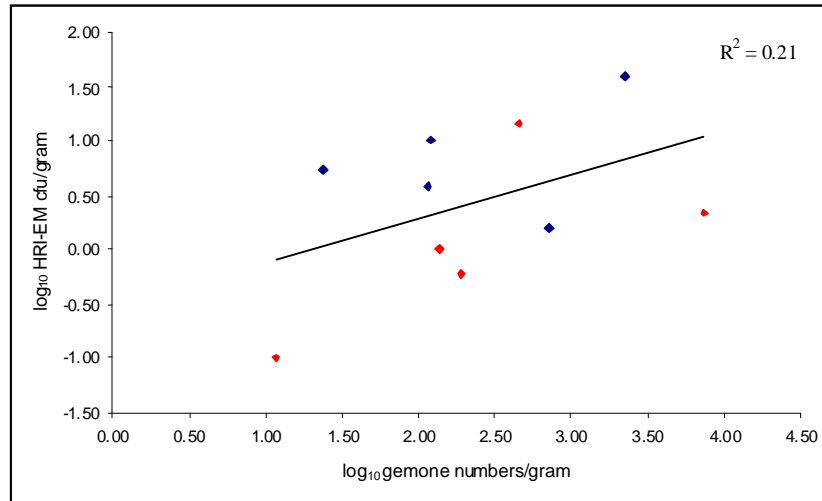


Figure 2. Plot of Ct vs starting concentration of *V. dahliae* DNA after optimisation of the SYBR Green I PCR with measurement of fluorescence at 79⁰C. * log scale 1= 1picogram= approx. 30 genomes.

Both PCR methods were tested on 13 naturally infested soil samples and compared with the current method in which the fungi are grown on plates of nutrient media. Our data showed how inaccurate current methods of detection are, with significant differences in estimates being made by HRI Wellesbourne and HRI East Malling on the same soils. However, in both cases there was strong linear relationship between quantification of *V. dahliae* by traditional plating methods and the new real time PCR method (Figure 3). Molecular techniques using modern "real time" PCR offer faster and more efficient identification of the pathogen in soil than those currently available.

A



B

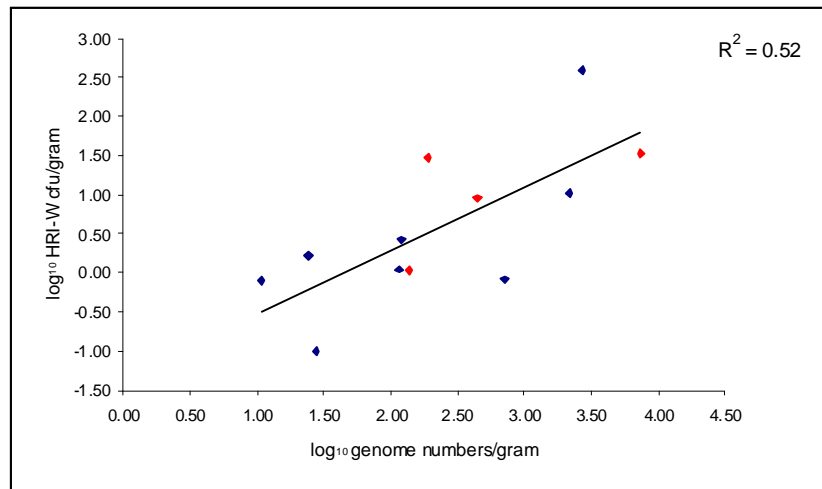


Figure 3. Plot of traditional wet plating estimates vs *V. dahliae* genomes quantified by SYBR Green I PCR. A) HRI East Malling values vs genome numbers and B) HRI Wellesbourne values vs genome numbers.

Further details of the work done under this objective can be found in Krishnamurthy, (2004).

Alternative soil disinfection treatments

Objective 2:

*Determine the efficacy of novel steam and/or ozone disinfection treatments for killing the soil borne pathogens: *Verticillium dahliae*, *Sclerotinia sclerotiorum*, *Phytophthora fragariae*, *Rhizoctonia*, *Pythium ultimum*, potato cyst nematode (PCN) and *Chenopodium album* weed seeds.*

The ability to withstand various steaming regimes was tested using a laboratory scale test rig developed specifically for the project (Figure 4). This rig could hold 4 x 1 litre soil samples. These could be spiked with the above pathogens, weeds or nematodes then steamed at a variety of soil temperatures/durations and survival could then be compared with that of unsteamed soil.



Figure 4. A) Laboratory prototype steamer, B) The four replicate cylinders used in the prototype, each of which holds 1 litre of soil. Soils were spiked with test organism prior to steaming, then survival was assessed

The test rig was used in a long term series of experiments that relate to objectives 2 – 4 investigating effects of temperature/duration on pathogen survival, investigating the effects of soil moisture on efficacy of killing pathogens, and the influence of soil type on the disinfestation process. A typical experimental output is shown in Figure 5.

The data in Figure 5 are typical in that it can be seen that temperature, soil moisture and soil type all influenced survival of the weed seed. This was similar for tests with fungal pathogens, nematodes and other weed seeds.

It was concluded that low temperature steam treatment (50oC or 60oC) for duration of only 10 minutes was enough to kill all test-organisms (which also included couch grass *Agropyron repens*). However, further studies lead us to believe that for a field operated machine for which maintenance of high temperatures for 10 minutes would not be practical, soil temperature should be raised to 70°C. If this temperature could be achieved the disinfestation would be satisfactory irrespective of soil type or moisture.

Early experiments investigated the effects of ozone gas on survival of the test organisms. However, ozone gas did not affect viability of any of the test organisms significantly and this potential method of soil disinfestation was not pursued.

Further details of the work done under this objective can be found in Van Loenen *et al.*, (2003) & Van Loenen, (2004).

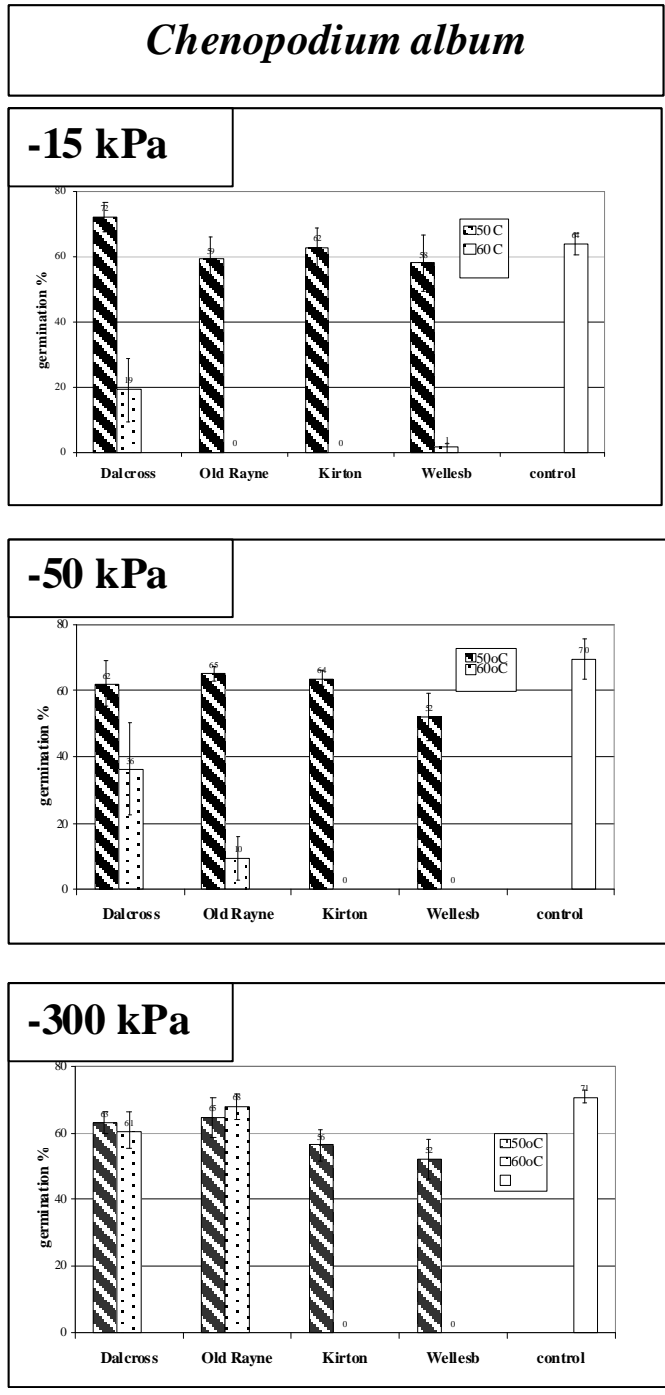


Figure 5. Typical results for experimental steaming. Germination of weed seeds of *Chenopodium album* (fat hen) when exposed to 50 or 60°C in soils of three different moisture contents (-15,-50 and -300 kPa) in four different soil types.

Objective 3:

Assess the effect of the most efficient novel soil disinfection method on (i) soil aggregate stability, (ii) nutrient availability, (iii) biological activity.

No treatments were found to alter the aggregate stability of any soils, but steaming had strong effects on both nutrient availability and biological activity.

Steaming treatments were applied using the test rig shown in Figure 4. Following a range of steaming treatments soils were sampled and analysed for available ammonium, nitrate and nitrite at three and seven days after treatment and thereafter approximately weekly until 19 weeks had elapsed. Sampling for manganese availability was conducted three days after treatment.

The most noticeable change in nutrient availability was in levels of available soil ammonium. A typical set of results for available ammonium are shown in Figure 6. It can be seen that the ammonium level in the control soil remained more or less constant throughout the sampling period. At no time did it exceed 10 mg kg^{-1} oven dried soil. In contrast, in all the steamed soils, the ammonium levels increased rapidly after steam treatment and continued to increase throughout the 19 week sampling period. As early as three days after treatment, the ammonium levels in all the treated soils were significantly greater than that of the control soil ($P < 0.05$). By the final sampling date, 19 weeks after the steam treatment, the ammonium levels in the treated soils were between 40 and 50 mg kg^{-1} OD soil.

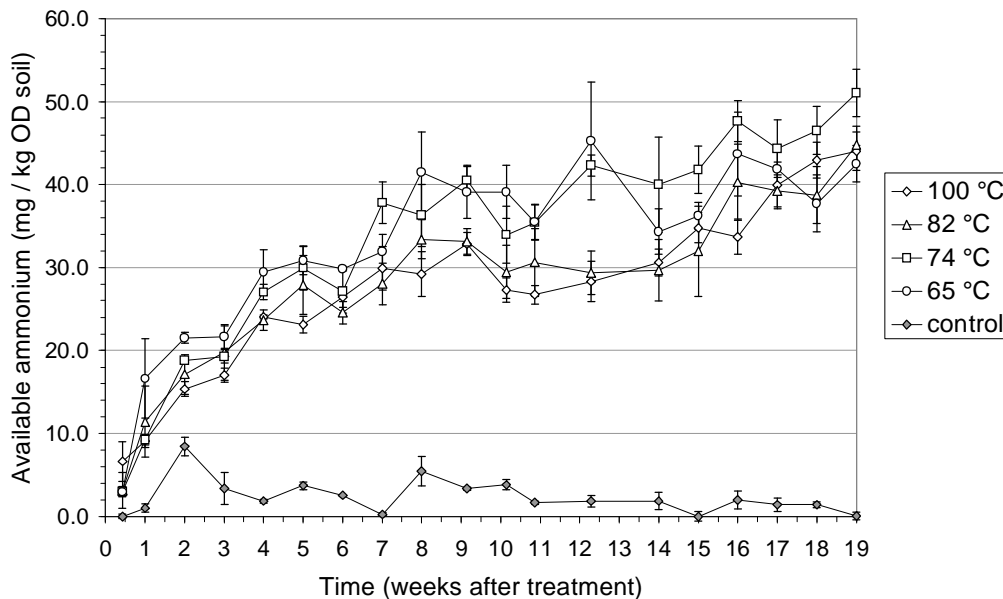


Figure 6. Changes with time in concentration of available ammonium in Old Rayne soil following steam treatment at four temperatures and in an unheated control. Error bars show ± 1 SE; $n = 3$.

One previously reported problem with steaming is increased availability of manganese that can be phytotoxic and high concentrations.

The manganese results are shown Figure 7. Treatment temperature had a clear influence on the manganese levels in the soil three days after treatment. As the treatment temperature increased, the water-soluble and exchangeable manganese rose from 3.5 mg kg⁻¹ oven dry soil in the control soil to 18.5 mg kg⁻¹ OD soil in the soil treated at 100 °C. However, the rate of increase in available manganese follows an exponential increase with increasing steam temperature. Our proposed 70°C regime is unlikely to cause phytotoxicity.

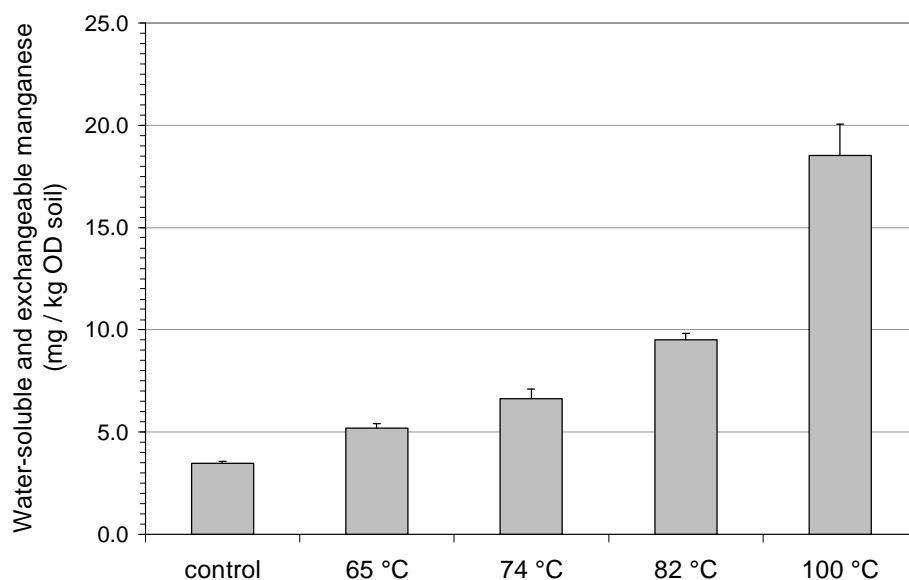


Figure 7. The concentration of water-soluble and exchangeable manganese in Old Rayne soil three days after steam treatment at four temperatures and in an unheated control. Error bars show ± 1 SE; n = 3.

In terms of biological activity, soil respiration was measured in the days following steaming by monitoring evolved CO₂ using KOH traps that were titrated against HCl. Results are shown in Figure 8. Within three days of steaming, soil respiration, an indicator of microbial activity, increased in all steamed soils compared with unsteamed controls. This phenomenon has been seen before, and is largely thought to reflect heat breaking down humic materials into sugars that can be readily be used by the microbial community. This in turn is likely to lead to increased N mineralisation, which would explain the data shown in Figure 6.

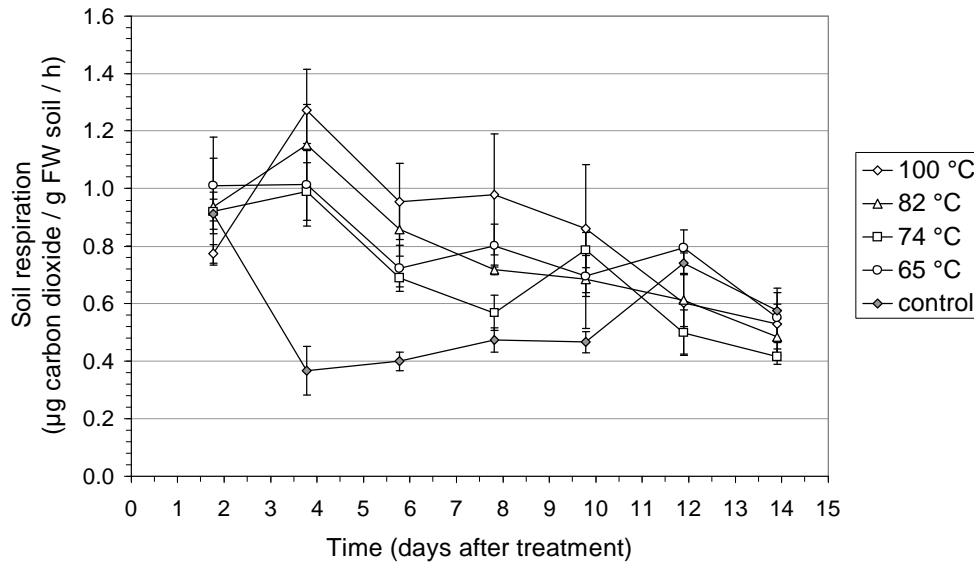


Figure 8. Changes with time in the respiration rate of Old Rayne soil following steam treatment at four temperatures and in an unheated control. Error bars show ± 1 SE; $n = 3$.

Further biological tests done on steamed soil included testing the ability of crops to grow in steamed soil.

The day after the steam treatment (at 65, 74, 82 or 100°C), soil was packed into four 6.3 cm flowerpots per treatment. Four further pots were filled with untreated soil to act as controls. Four lettuce seeds (cv. Little Gem, Unwin's Seeds, Histon, Cambridge, UK) were then sown in each pot at a depth of approximately 5 mm and the soil was gently pressed over them. The pots were placed in a lidded propagator, arranged in a randomised block and moved to a phytotron for 48 h (high humidity; light 16 h, 18 °C; dark 8 h, 10 °C) until the seedlings had emerged. After emergence, the propagator was moved to the greenhouse and its lid removed. Nine days after the steam treatment the lettuces were thinned to leave one seedling per pot. The growing seedlings were kept moist by frequent watering with deionised water. The lettuces were harvested 43 d after sowing using the following procedure.

Fig 9 shows the oven-dry weight of the lettuce shoots and roots at harvest. Steam treatment using either method (i.e. the new method or the conventional treatment at 100 °C) and at all temperatures significantly increased the weight of both the lettuce shoots ($P < 0.001$) and roots ($P < 0.001$) compared to the control. The only significant differences between the steam treatments were found in the shoots; those that grew in the soil steamed at 82 and 100 °C were heavier than those that grew in the 65 °C soil ($P < 0.05$). The increase in above ground weight caused by steaming was proportionately greater than for roots, and this resulted in visibly larger plants in steamed soils, irrespective of the steaming temperature (Figure 10).

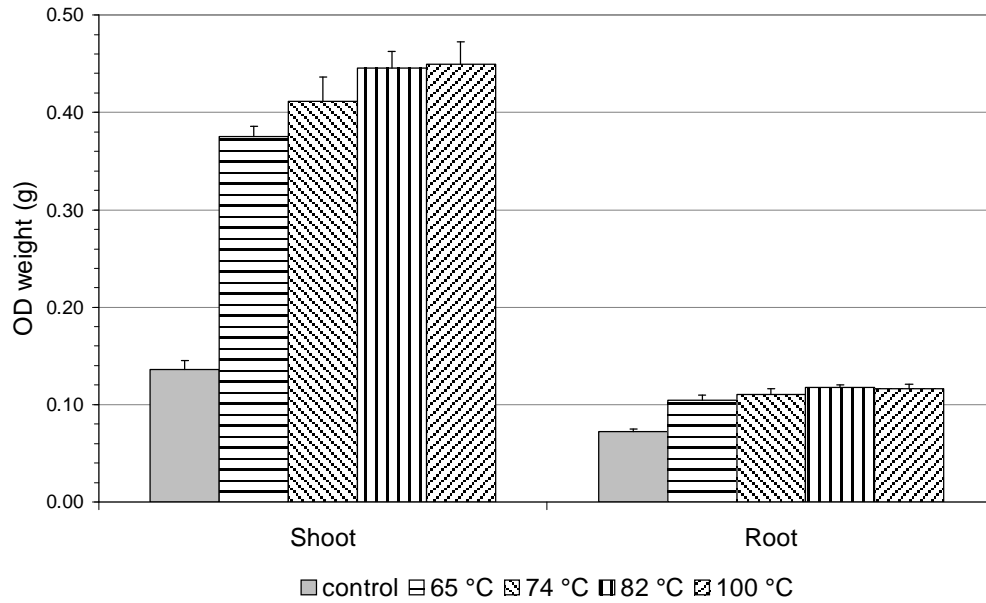


Figure 9. The oven-dry weight of lettuce (cv. Little Gem) shoots and roots at harvest following 43 d of growth in freshly steamed (at 65, 74, 82 or 100 °C) or unheated (control) Old Rayne soil. Error bars show ± 1 SE; n = 4.



Figure 10. Lettuce (cv. Little Gem) plants shortly before harvest following 43 d of growth in freshly steamed (at 65, 74, 82 or 100 °C) or unheated (control) Old Rayne soil.

In conclusion, low temperature soil steaming, in addition to the beneficial effects discovered under objective 2 (reduced numbers of weeds, pathogens & nematode

pests) also increases availability of ammonium and increases plant growth. A more detailed account of work done under this objective can be found in Turbett (2004).

Objective 4:

Assess the effect of soil type and soil matric potential on the disinfection efficacy of the most efficient novel soil disinfection method.

Four different soils were tested using the laboratory steamer described under Objective 2, and it was found that the finer textured soils (Dalcross and Old Rayne) were more difficult to steam with increasing soil dryness. In the driest conditions (-300 kPa) Dalcross and Old Rayne steam did not penetrate at all, due to blocked pores. In contrast, steam-treatment of the other two soils (Wellesbourne and Kirton) was adequate at all three matric potentials that were tested: -15kPa (wet), -50kPa (medium) and -300kPa (dry). Wellesbourne and Kirton soil were better aggregated, when dry than Dalcross and Old Rayne, and soil type thus, matric potential must be considered important factors, when trying to achieve satisfactory steam-results. Difficulties with steam-treatment of certain sand and loam soils in greenhouses have been reported by others (Runia, 1983; IKT-AC, 1992).

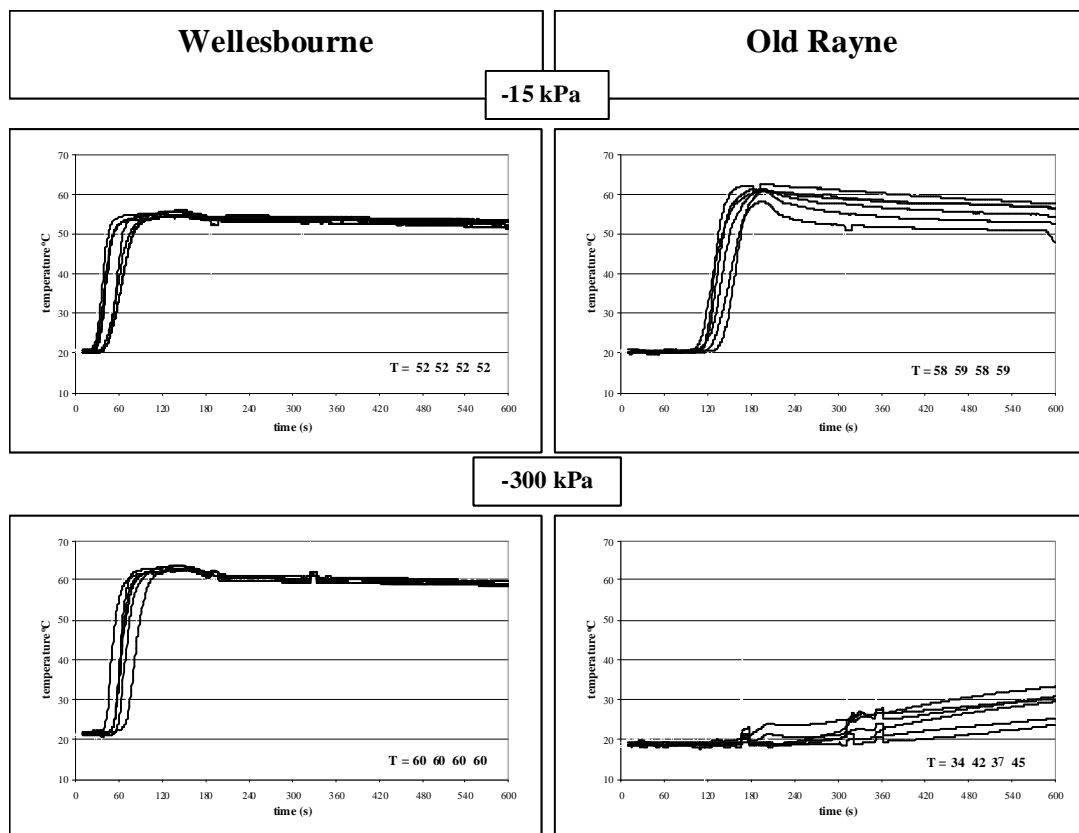


Fig. 11. Temperature-time profiles of steam-treated Old Rayne and Wellesbourne soils. Steam target temperatures were 50oC (shown on charts at lhs) and 60oC (shown on charts at rhs). Soils were steamed at matric potentials of -15, and -300 kPa. In drier conditions steam penetration was poor in Old Rayne, but adequate penetration for Wellesbourne was achieved at both matric potentials.

In conclusion it appears that on light textured soils steam is capable of raising the soil temperature effectively, irrespective of soil moisture content. However, fine textured soils should not be steamed under very dry conditions.

Further details of the work done under this objective can be found in Turbett, (2004).

Objective 5:

Design and develop a field prototype of a machine for more efficient steam or ozone disinfection of soils (the target is to obtain similar efficacy and costs to methyl-bromide treatments).

Specifications of the machine were based on knowledge of heat transfer, soil temperature heating characteristics and the lethal temperatures of weeds, pests and pathogens gained in objectives 2-4. The field prototype was manufactured by Jones Engineering, Doncaster. The machine was based on a potato destoning machine with a front mounted soil pickup. The soil is then passed along a conveyor during which steam is passed through the soil. The steam is passed through the soil twice. Soil closest to the burner is steamed at high temperature, then the cooled steam/exhaust gas mix is passed through soil immediately behind the soil pickup as a preheating treatment. A schematic drawing of the machine is shown in Figure 12, as is a photograph of the machine in operation on land owned by Jones Engineering.

The prototype is all electric and has an on-board 15 kVA generator. The soil pickup is fixed at 1500 mm width (with 1.5 m or 1.8 m wheeling which could go to 1.95 m) and soil is put down into new beds at the same width, but this can be varied. The soil pickup has adjustable depth settings allowing soil to be disinfested to appropriate depths depending on soil type, crop and cropping history. Once the soil has been picked up, it is passed along a conveyor and subjected to an upward flow of steam generated by a direct fired steam generator powered by an 800 kW High-Low Burner. Soil is then dropped from the rear of the conveyor and formed into new beds.

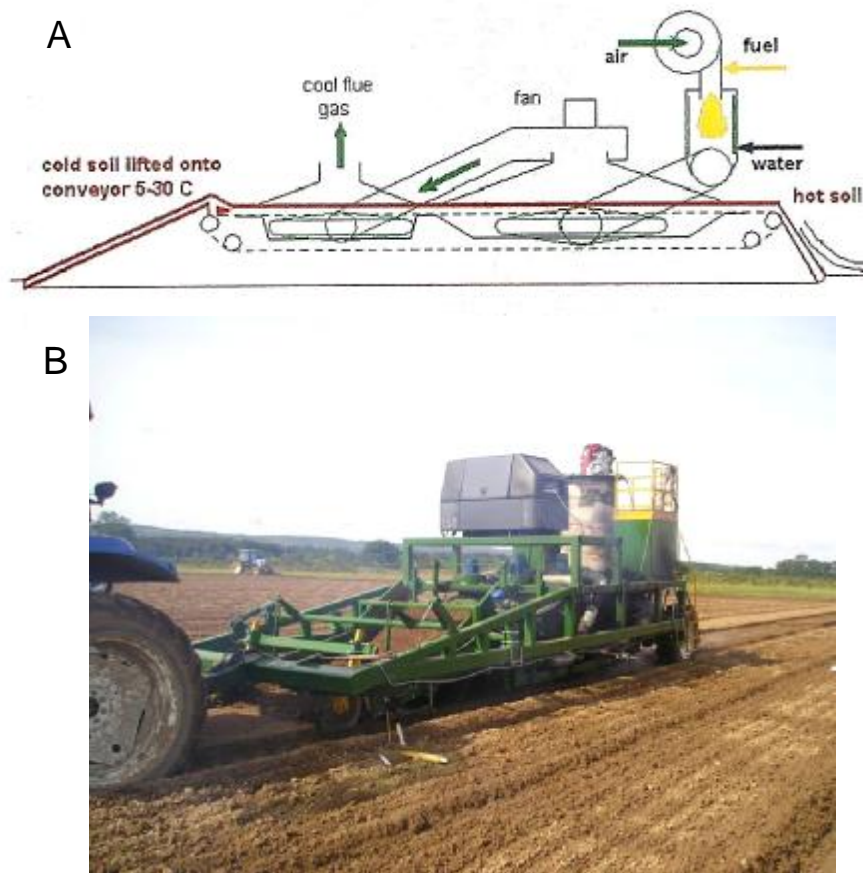


Figure 12. Schematic diagram (A) and photograph (B) of the prototype soil disinfection machine being tested by Jones Engineering staff.

Objective 6

Compare the efficacy of the prototype in field trials against two control treatments: an existing semi-continuous whole bed steam disinfection applicator and the most appropriate existing pesticide soil treatment.

The machine described in Objective 5 was tested in series of experiments, coordinated by the Stockbridge Technology Centre (STC) during 2007 and 2008. In 2007 experiments comprised a baby leaf spinach trial at Langmead Farms, a whole head lettuce trial at Langmead Farms, a strawberry trial at Berryworld and weed control studies at Jones Engineering and STC. In 2008 trials were done on carrots at TIO, baby leaf at Langmead Farms, strawberries at Berryworld and onions at HRI Kirton.

The machine performed well in all trials in terms of soil heating.

However, in most trials weed control was not as high as hoped and differences in weed numbers were rarely significant. An exception was an experiment done on carrots at TIO in 2008.

This experiment investigated steaming to three different depths (8-10 cm, 15 cm and 20-25 cm) and also looked at the ability of compost at two levels (50t/ha & 100t/ha) either alone or in combination to disinfest the soil. The trial was started on 2nd June and assessments of weed cover were made on 26 June, 14th July, 4th August and 3rd November. Data for weed cover are shown in Table 1.

Table 1. Weed cover (%) on 26 June, 14 July, 4 August and 3 November 2008 (%) – the range is given in brackets.

Treatments	Weed cover (%)			
	26 June	14 July	4 August	3 November
Untreated control	1.6	42.5 (20-50)	80.0 (70-90)	52.5 (50-55)
Compost @ 50t/ha	1.0	22.5 (10-30)	73.8 (50-100)	11.0 (4-15)
Compost @ 100t/ha	1.0	25.0 (25-25)	45.0 (15-75)	32.0 (4-25)
Steamed 8-10cm	0.5	7.8 (1-15)	30.0 (15-70)	9.5 (3-20)
Steamed 15cm	0.3	1.4 (0.5-3)	13.8 (5-25)	7.0 (0-20)
Steamed 20-25cm	0.4	3.1 (0.5-10)	21.5 (1-70)	7.5 (0-20)
Steamed 20-25cm + compost @ 50t/ha	0.3	1.3 (0-3)	5.0 (2-10)	3.5 (0-10)
Steamed 20-25cm + compost @ 100t/ha	0	1.0 (0-3)	10.8 (1-20)	11.8 (0-30)
LSD for comparing treatments	0.53 (***)	11.2 (***)	27.6 (***)	12.7 (***)

Weed cover was very low in late June but within 3 weeks had increased significantly on the non-steamed plots. On 14 July there was significantly lower weed cover on the steamed plots but with some variability between the plots of the same treatment. The non-steamed plots were hand hoed on 14 July to remove the very small weeds. There was some evidence that when the soil had been steamed at 8-10cm and a soil temperature of only 65-70°C had been achieved then poorer weed control resulted.

By 4 August weed cover had increased but results were still encouraging for the 15cm and 20-25cm depth treatments. The crop canopy was developing well and the steaming treatments had provided effective weed control during the critical stage of establishment and early growth. Although the weed cover appears quite high on the steamed plots the delay in weed emergence allowed the crop to out-compete the weeds. There was still some variation in the level of weed control between plots of the same treatment. The non-steamed plots were to be steered hoed by tractor in mid August but this was not carried out.

By 3 November the main reason for the higher weed cover on the steamed plots was due to a small number of large weeds (mainly *Chenopodium alba* - Fat hen) that had not been controlled or removed. There was significantly more weed cover on the untreated control. Highly significant differences ($P < 0.001$) were found on all assessment days with highest weed coverage always being in the untreated control plots. All steam treatments caused significant reductions in weed cover on all assessments. Compost treatments also reduced weed coverage but to a lesser extent than steaming. The difference in weed coverage was easily distinguishable between steamed and control plots (Figure 13).



Figure 13. Carrot trial at TIO, Inverness 9 weeks after treatment. Steamed plot left with very low weed cover, and untreated control plot on right with high weed infestation

Another observation was that in many trials, crops on steamed soil appeared to show increased growth, and in some instances this translated into increased yield (probably resulting from increased Nitrogen mineralisation associated with steaming). For example, in one Iceberg lettuce trial done in Selsy, West Sussex, the final trimmed head weights for lettuce were significantly higher for all steamed treatments compared with control plots, although the weights of non-trimmed heads were similar for all treatments except for the steaming plus compost treatment where they were higher (Table 2).

Table 2. Mean head weights for trimmed and untrimmed heads at harvest (g) – excluding plots in bed 4.

Treatments	Mean head weights (g)	
	Trimmed heads *	Untrimmed heads #
Untreated control	275	670
Conventional	275	642
Compost @ 25t/ha	283	639
Compost @ 50t/ha	303	679
Prototype machine 10cm	340	649
Prototype machine 20cm	341	661
Prototype machine 20cm + compost @ 25t/ha	376	722
Prototype machine 20cm + compost @ 50t/ha	384	737

* mean of 30 heads

mean of 10 heads

Due to low pathogen pressure, we only have good data on weed control. In the trials programme it became apparent that the initial hope that efficacy could be

assured if soil temperatures merely reached 70°C was not correct. In the successful trial at TIO described above soil temperatures varied from 70°C – 90°C with a mean temperature of 81°C, significantly higher than that reached in other trials. It would appear from these trial results that either 70°C has to be maintained for a longer period (>2 minutes) or the target temperature needs to be raised.

It was not possible to use Methyl bromide in the trials, and a Regero steam hood was not available for comparisons.

Investigate the potential benefits of adding organic amendments (compost, biological control agents) to soil

Objective 7

*Determine the effect of three compost preparations (based on (i) cattle manure and waste straw, (ii) household wastes and (iii) a mixture of green waste and cattle manure) on the soil borne pathogens *Sclerotinia sclerotiorum* and *Pythium ultimum*, potato cyst nematode (PCN) and *Chenopodium album* weed seeds.*

Composts were prepared from all the three potential feedstocks. All composted well with a strong thermophilic (disinfestation) phase (See Fig 14). Weed seeds and nematode cysts are both destroyed during composting, and compost has previously been shown to suppress weed emergence when used as a surface mulch.

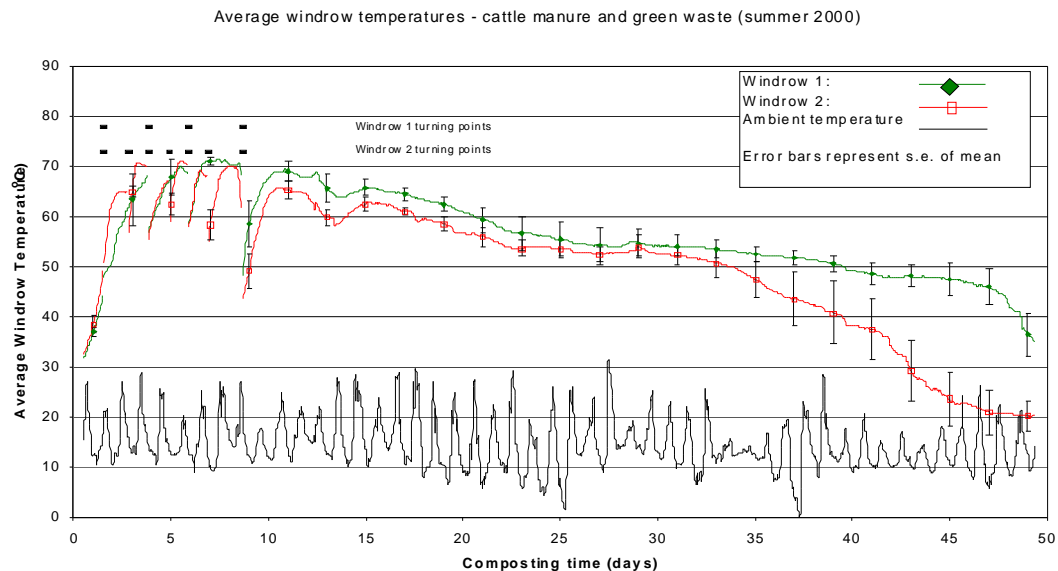


Fig. 14. Heat profile over time in two composting windrows Using a cattle manure and green waste feedstock

Of the three composts prepared during this project, those based on farm yard manure were found to be more effective than compost based on green waste for suppressing *Pythium* damping off disease (Fig 15). The composts needed to be allowed to mature to increase suppressiveness. None of the composts significantly affected the infectivity of white mould (*Sclerotinia sclerotiorum*).

While the composts produced in this project killed weed seeds and cyst nematodes present in the feedstocks, they did not have any significant effects on the viability of

weed seeds or potato cyst nematode cysts if they were already present in the soil to which compost was added. Thus compost should not be relied on as the sole method of disease prevention, but is expected to be important in extending the lifetime of the beneficial effects of soil disinfestation.

The compost based on cattle manure and green waste is recommended. This has shown good suppressiveness against *Pythium* damping off disease, and maximises the benefits of a limited resource (cattle manure). It will also confer higher chemical fertility benefits than the green waste compost, and has a better structure than the cattle manure/straw mix, which makes it easier to handle.

Objective 8

Determine the effects of volatiles from decomposing fresh Brassica residues on a fungal pathogen (V.dahliae), free-living nematodes and a weed seed (Chenopodium album) under various combinations of soil type and conditions.

Initial laboratory studies in which chopped white cabbage (1cm² pieces), was added to jars containing 200g infested soil (moisture content 9.6%). Three replicates were set up with 0%, 0.5%, 1%, 2%, 4.7%, 9% incorporation. Jars were incubated for 28 days at 20°C, before analysis of *V. dahliae* by standard wet sieving and plate counting techniques. After 28 days, the degradation of the cabbage was estimated at 90-95% of the mass originally added, regardless of incorporation rate. Results are shown in Fig. 15

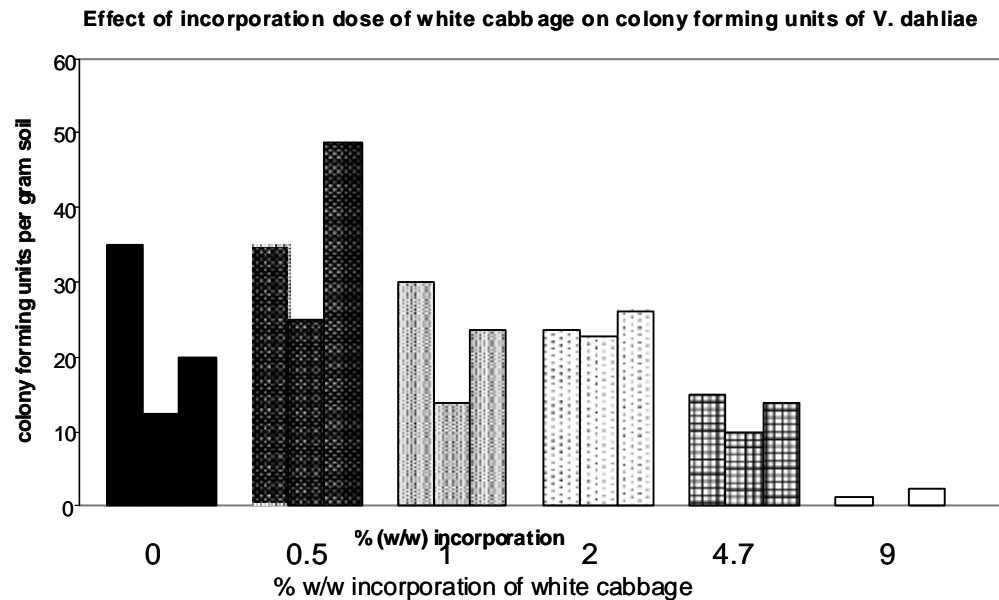


Figure 15 Colony forming units of *V. dahliae* after white cabbage incorporation into soil at different rates. Three replicates per dose are shown

Figure 15 indicates that there is a decline in colony forming units with increasing cabbage dose. However, this is only significant at $P < 0.05$ when comparing controls with 9% incorporation or 0.5% incorporation with 4.7% and 9%. Hence, it appears that greater than 4.7% incorporation rate is needed to significantly reduce *V. dahliae*

levels. The data appears to suggest that low incorporation levels (0-1%) may lead to an increase in colony forming units of the fungus, something that has been suggested in previous experiments. However, this was not statistically significant.

Following the successful laboratory experiment, a field trial was conducted in 2002. The experiment aimed to assess the ability of incorporation of two different Brassica crops on *Verticillium dahliae* infection in strawberry. A field site infested with *V. dahliae* was selected, and split into three replicated blocks of five different treatments.

1. Basamid
2. Incorporated white mustard
3. Incorporated rape kale
4. Rape kale grown, but not incorporated after removal
5. No amendments (control)

After Brassicas were incorporated, *Verticillium dahliae* presence was analysed using a variation on the plating technique and *Pythium ultimum* numbers were estimated using the most probably number technique. Data for survival of these pathogens are shown in Tables 3 & 4 respectively.

Table 3. Counts of *V. dahliae* (cfu /g soil) after biofumigation trial treatments were performed. Bracketed figures indicate increase or decrease in *V. dahliae* relative to change in control plots.

Treatment	<i>V. dahliae</i> counts (cfu /g) for blocks		
	I	II	III
None (control)	45	89	109
Winter Brassica, incorporated	63 (+17)	100 (-45)	144 (+18)
Winter Brassica, unincorporated	94 (+51)	77 (-1)	97 (-20)
Spring Brassica, incorporated	65 (+33)	77 (-30)	111 (-1)
Basamid	0.1 (-44)	6 (-140)	6 (-114)

Table 4. Estimates of *Pythium* most probable numbers (mpn) before and following biofumigation trial treatments

Treatment	<i>Pythium</i> (mpn counts/g soil)					
	Block I		Block II		Block III	
	Pre-treat	Post-treat	Pre-treat	Post-treat	Pre-treat	Post-treat
Control	622	354	521	1092	417	743
Rape kale, incorporated	670	316	932	559	539	536
Rape kale, unincorporated	539	316	659	559	659	417
White mustard, incorporated	406	308	342	362	1009	539
Basamid	346	12	1569	38	1242	243

Only the application of Basamid resulted in a significant decrease ($P < 0.05$) in counts of microsclerotia compared to other treatments

Combined analysis of the three blocks indicated that significant reduction in *Pythium* numbers had been achieved using Basamid compared to all other treatments ($P < 0.05$), but that also, all Brassica treatments had reduced numbers compared to the control.

However, the decrease in *Pythium* caused did not translate into increased yield for the Brassica treatments, whereas Basamid did increase yield (Table 5)

Table 5 Yield of strawberry fruits from field trial.

Treatment	Yield of strawberries (kg)		
	Block I	II	III
None (control)	4.8	5.2	2.1
Winter Brassica, incorporated	3.2	3.9	3.6
Winter Brassica, unincorporated	3.9	3.4	2.4
Spring Brassica, incorporated	4.2	5.3	3.5
Basamid	7.1	4.7	5.2

Table 5 shows that in blocks I and III, the highest yields were obtained following application of Basamid. In block II, despite the reduction in *V. dahliae* being as great following Basamid application, yield was no better than other treatments.

The results to date suggest that under conditions such as in this trial, biofumigation is not currently feasible in the UK. The lack of effect is most likely to relate to the low amounts incorporated, and future approaches would need to address this as well as examine modifications to incorporation procedures.

Investigating the potential to integrate the steaming and amendment techniques

Objective 9

Integrate novel soil disinfection treatments with the most active (i) compost treatment, and/or (ii) three selected microbial amendments (AM-mycorrhizal fungi, Bacillus subtilis and Coniothyrium minutans) to achieve an overall efficacy similar to that of methyl bromide.

This work focused on the survival in soil of two biocontrol agents (based on commercial availability), *Coniothyrium minutans* and *Bacillus subtilis* MBI 600, and their biocontrol activity against the pathogens *Sclerotinia sclerotiorum* and *Pythium ultimum*, respectively.

An experiment was done to determine if *P. ultimum* could be controlled by *B. subtilis* incorporated into soil. This experiment comprised six treatments (uninoculated control; sterile talc amendment; *B. subtilis* MBI 600 talc amendment; *P. ultimum* amendment; sterile talc and *P. ultimum* amendment; and *B. subtilis* MBI 600 talc

and *P. ultimum* amendment) in each of two soil states (non-sterile and pasteurised). There were ten replicate pots (400g soil each) per treatment per soil state, and five lettuce seeds were planted per pot. The *B. subtilis* MBI 600 talc formulation was added to the pasteurised soil in the bags in which it had been pasteurised, with each bag containing enough soil to set up the 10 replicate pots for that treatment.

Results are shown in Table 6. It can be seen that *Pythium ultimum* damping-off of lettuce was not controlled in untreated or disinfested soil by neither a seed treatment nor a soil amendment of *B. subtilis* MBI 600. A replacement biocontrol agent, *Pseudomonas fluorescens* CHA0, was equally unsuccessful and a different biocontrol agent may be better suited to integrated control of damping-off (Table 7).

Table 6. Effect of *Bacillus subtilis* MBI 600 soil amendment on emergence and mean fresh weight of lettuce seedlings in non-sterile and pasteurised soil amended with *Pythium ultimum*. Percentage emergences are back-transformed data and values in parentheses are means after arcsine transformation of data. Analyses were carried out on transformed data.

Soil	<i>P. ultimum</i>	<i>B. subtilis</i> MBI 600 only	Sterile talc only	% emergence	mean fresh weight (mg)
Non-sterile	no	no	yes	99.9 (87.8)	211
Non-sterile	no	no	no	94.3 (76.2)	219
Non-sterile	no	yes	no	98.0 (82.0)	201
Non-sterile	yes	no	yes	39.9 (39.2)	206
Non-sterile	yes	no	no	67.0 (54.9)	199
Non-sterile	yes	yes	no	52.0 (46.2)	190
Pasteurised	no	no	yes	99.9 (87.8)	211
Pasteurised	no	no	no	99.0 (84.2)	184
Pasteurised	no	yes	no	99.8 (87.3)	213
Pasteurised	yes	no	yes	31.0 (33.9)	182
Pasteurised	yes	no	no	29.0 (32.6)	166
Pasteurised	yes	yes	no	0.2 (2.4)	201
LSD (5%)				(12.81)	33.3

Significant differences between any treatment mean are calculated from the least significant difference (LSD),

Table 7. Effect of *Pseudomonas fluorescens* CHA0 soil amendment on emergence and mean fresh weight of lettuce seedlings in non-sterile and pasteurised soil amended with *Pythium ultimum*. Percentage emergence is back-transformed data and values in parentheses are means after arcsine transformation of data. Analysis carried out on transformed data.

Soil	<i>P. ultimum</i>	<i>P. fluorescens</i> CHA0	% emergence	mean fresh weight (mg)
Non-sterile	no	no	98.7 (83.4)	130
Non-sterile	no	yes	93.9 (75.7)	161
Non-sterile	yes	no	50.1 (45.0)	129
Non-sterile	yes	yes	49.9 (44.9)	122
Pasteurised	no	no	93.9 (75.6)	123
Pasteurised	no	yes	93.9 (75.7)	132
Pasteurised	yes	no	48.3 (44.0)	161
Pasteurised	yes	yes	36.2 (37.0)	149
LSD (5%)			(14.80)	37.6

Because neither of these biocontrol agents was effective, the possibility of combining them with steam disinfestation was not pursued.

The work investigating the potential of *Coniothyrium minitans* to control *Sclerotinia sclerotiorum* was much more successful and experiments investigated the possibility of integrating *C. minitans* with steam disinfestation. Soil was pasteurised in an autoclave, using a temperature of 80 °C for 3 min to simulate the possible temperatures reached by soil steaming for field use. *Coniothyrium minitans* was subsequently applied to the pasteurised soil to assess the effects of the combination of control measures in reducing sclerotial viability of *S. sclerotiorum*.

C. minitans typically survived better in soils that had been pasteurised than in untreated soil (Figure 16).

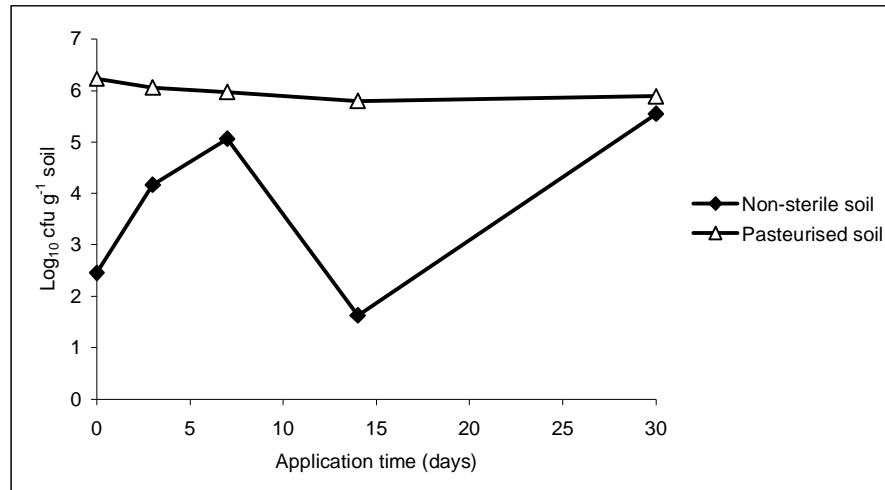


Figure 16 Numbers of *Coniothyrium minitans* in non-sterile and pasteurised Dalcross soil, where sclerotia of *Sclerotinia sclerotiorum* isolate SB have been incubated. C LSD (5%) = 1.868

Both methods used individually were effective in decreasing the number of viable sclerotia, but no further reduction in sclerotial viability was seen when the two methods were combined. However, this was because in the laboratory, pasteurisation was completely effective (Figure 17) so the benefits of combining the two methods could not be proven. However, our data suggest that in the field, efficacy may be greater if the two methods were combined. *Coniothyrium minitans* was found to colonise pasteurised sclerotia significantly quicker than untreated sclerotia, and it was seen that there was an increase in number of *C. minitans* in pasteurised soil in the presence of sclerotia. Experiments were also conducted to investigate the effect of application timing of the biocontrol agent to soil following pasteurisation, in relation to sclerotial infection. Here, two different isolates of *S. sclerotiorum* were used, with similar results. Application of *C. minitans* to soil immediately following pasteurisation resulted in sclerotial infection by the mycoparasite, but application 7 days or more after soil pasteurisation resulted in low recovery of the biocontrol agent from sclerotia, possibly due to the mycoparasite being masked by the presence of other fungi which colonised the sclerotia first.

This suggests that early application of *C. minitans* is advantageous.

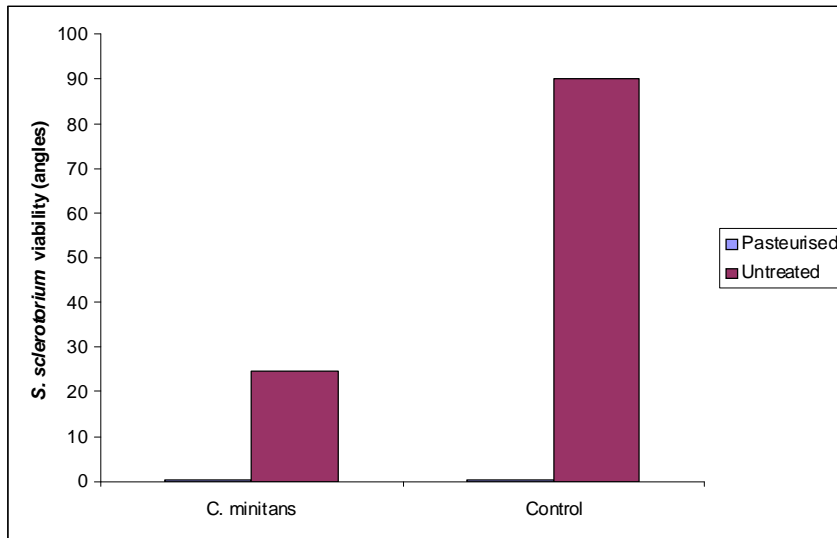


Fig 16. Viability of *S. sclerotiorum* sclerotia when grown on Pasteurised or untreated soil, in the prescens or absences of *C. minitans* (recorded 14 days after treatment)

Further details of the work done under this objective can be found in Bennett 2004 and Bennett et al. (2003; 2005; 2006).

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