Ornamentals: control of pests, pathogens and weed seeds on re-used plant containers

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

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

Headline

Wet heat treatment is the most suitable and efficient method of 'sterilising' used pots, trays etc on the nursery.

Background and expected deliverables

Until recently, methyl bromide fumigation was widely used in the horticultural industry to control pathogenic organisms on plant containers that were intended for re-use. The broad spectrum of activity, low cost and relative ease of application of methyl bromide made it very suitable for sterilisation. However, methyl bromide is an ozone-depleting substance and, following international agreements, it can no longer be used for this, and most other, purposes.

This prompted a search for alternative ways of treating horticultural hardware such as pots and trays so that they could be re-used without causing a significant risk of carry-over of pests, diseases or weeds from an affected crop to a subsequent one.

The early stages of this project consisted of producing a review of potential alternatives to methyl bromide fumigation. Three categories of potential alternative treatment were identified in the review. These consisted of:

1. Alternative fumigants
2. Disinfectants
3. Heat treatments

From the list of potential alternative treatments produced during the review, the most promising were selected for practical testing. The selected treatments consisted of two fumigants Phostoxin (aluminium phosphide, generating phosphine) and Vikane (sulfuryl fluoride, approved as Profume in the UK), six disinfectants/biocides (FAM 30, Trigene Advance, citric acid, GeoSil, Virkon S and Citrox P) and three heat treatments (hot-water treatment, dry oven and microwave).

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The aim of the work was to provide growers with information that would allow them to set up a method of 'sterilising' used pots, trays etc, so that they could be re-used with a low risk of pathogen carry-over.

Summary of the project and main conclusions

Six organisms were chosen for testing in this project. All were common organisms on nurseries and each had the potential to be carried over between crops on nursery hardware. The organisms were the fungi *Rhizoctonia solani* and *Pythium intermedium*, the seeds of hairy bittercress (*Cardamine hirsuta*) and pearlwort (*Sagina subulata*), bud and leaf nematode (*Aphelenchoides* spp.), and western flower thrips (*Frankliniella occidentalis*).

The effects of two fumigants (Phostoxin and Vikane), six disinfectants (FAM 30, Trigene Advance, citric acid, GeoSil, Virkon S and Citrox P) and three methods of applying heat (hot-water treatment, dry oven and microwave) on the above organisms were examined in a series of 35 experiments conducted during 2006 and 2007.

Fumigants

- Neither of the two fumigants controlled all of the test organisms.
- Vikane, however, gave good control of the fungi and the invertebrates after 16 hours exposure and was much more effective than Phostoxin in this regard.
- The weed seeds were hardly affected by either fumigant at the concentration/time products tested.

Disinfectants

- The effects of all of the disinfectants proved to be disappointing, particularly on Rhizoctonia and the weed seeds.
- FAM 30 gave control of both Pythium and western flower thrips after 10 minutes exposure but did not control the other organisms.

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- GeoSil, Trigene Advance and Virkon-S also controlled Pythium, but only after 60 minutes exposure, and caused some mortality of western flower thrips pupae.

- Citrox P and citric acid were particularly ineffective at controlling any of the organisms tested at the concentrations used.

Heat

The application of heat proved to be the most broad-spectrum method of controlling the various test organisms.

- Wet heat (immersion in a water bath) was identified as the most effective method of application, with exposure to 60°C for 10 minutes capable of giving complete control of all of the organisms tested (with the exception of bud and leaf nematode: one nematode out of more than 4,400 tested apparently survived this treatment).

- Exposure to dry heat in an oven was not as effective, the weed seeds in particular being tolerant (e.g. all survived exposure to dry heat at 70°C for one hour).

- Microwave energy was also surprisingly ineffective on the fungi and weed seeds when these were exposed for 120 seconds. Extending the exposure period began to produce undesirable effects on the nursery hardware that was being treated.

Main conclusions

The main conclusion from the work was that use of wet heat treatment at 60°C to 'sterilise' nursery hardware was likely to provide the best solution to the problem of avoiding cross-contamination of crops via the re-used materials. This could be applied by nurserymen via a hot-water bath or by use of steam/air blends and the method is not subject to pesticide safety legislation.

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Where contamination with weed-seeds is not a problem, fumigation with Profume (the UK-approved sulfuryl fluoride product) may also be a viable option for the treatment of nursery hardware, since this seems to give good control of fungi and invertebrates at the concentration/time products tested. Profume is currently approved by the Pesticides Safety Directorate (PSD) for use on crop handling and storage structures.

Financial benefits

- Individual growers who recycle nursery hardware will reap a financial benefit that will depend on how many pots and trays they are able to recycle, and how many cycles of re-use the hardware can endure before replacement becomes necessary.

- Construction of a hot-water treatment facility or a steam/air blend treatment facility capable of treating a sufficient volume of hardware at 60°C for at least 10 minutes should not present any great difficulty. Energy costs involved will depend on factors such as the volume of water to be heated, the quantity of hardware to be 'sterilised', duration of treatment and efficiency of insulation of the treatment facility.

Action points for growers

- Consider adoption of wet heat treatment as the standard method of 'sterilising' nursery hardware that will be re-used.
- If this method is to be adopted, design a suitable system to apply hot-water treatment or treatment via steam/air blending on the nursery.
- Where weed seeds are not a problem and wet heat treatment is not considered suitable for use on the nursery, consider the use of Profume to fumigate used hardware.

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Science Section

Introduction

Until recently, methyl bromide fumigation was widely used in the horticultural industry to control pathogenic organisms on plant containers that were intended for re-use. The broad spectrum of activity, low cost and relative ease of application of methyl bromide made it very easy and cost-effective to use. However, methyl bromide is an ozone-depleting substance and, following international agreements, it can no longer be used for this purpose.

This prompted a search for alternative ways of treating horticultural hardware such as pots and trays so that they could be re-used without causing a significant risk of carry-over of pests, diseases or weeds from an affected crop to a subsequent one. The first stage of this project consisted of producing a review of potential alternatives to methyl bromide fumigation (see HNS 147 Annual report, 2006).

Three categories of potential alternative treatment were identified in the review. These consisted of:

1. Alternative fumigants
2. Disinfectants
3. Heat treatments

From the list of potential alternative treatments produced during the review, the most promising were selected for practical testing. The selected treatments consisted of two fumigants, Phostoxin (aluminium phosphide, producing phosphine) and Vikane (sulfuryl fluoride), six disinfectants/biocides (FAM 30, Trigene Advance, citric acid, GeoSil, Virkon S and Citrox P) and three heat treatments (hot-water treatment, dry oven and microwave).

Materials and Methods

Test Organisms

In order to assess the efficacy of the various treatments in controlling potential problems on re-used containers, it was necessary to select indicator organisms that could be put through practical tests. The type of organisms most likely to infect used containers, either by being attached directly to the container itself or by being

present in organic debris that was attached to, or held within, the container, were fungal pathogens, weed seeds and invertebrates. Two organisms of each type were selected to act as the indicator organisms. These were:

Fungi – *Pythium intermedium* and *Rhizoctonia solani*. Both of these are common, root-infecting fungi, found particularly on plants in the seedling stages. However, they are not closely related to each other. *Pythium* is a member of the Oomycetes, with cell walls consisting entirely of cellulose and persisting between hosts as both oospores and zoospores, whereas *Rhizoctonia* is a Basidiomycete, with chitin in the cell walls and persisting between hosts as either mycelium or sclerotia. These two fungi therefore pose a different challenge for control measures.

Weed seeds – hairy bittercress (*Cardamine hirsuta*, family Cruciferae) and heath pearlwort (*Sagina subulata*, family Caryophyllaceae). Hairy bittercress and procumbent pearlwort (*Sagina procumbens*) are two of the commonest weeds of containerised plants and both propagate very readily by seed, so they were a natural choice for this work. However, though hairy bittercress seed was available in sufficient quantity for the experimental work, supplies of viable procumbent pearlwort seed proved very difficult to come by, so *Sagina subulata* was substituted for it. *S. subulata* is very closely related to *S. procumbens*, even hybridising with it, so the substitution seems to be a reasonable compromise.

Invertebrates – bud & leaf nematodes, *Aphelenchoides* spp., and pupae of western flower thrips, *Frankliniella occidentalis.* Bud & leaf nematode is a common problem in some ornamental plant species, with a wide host range. It can persist between crops in suspended animation in fragments of dry leaf tissue and is therefore a likely candidate for transmission from crop to crop in debris attached to used containers. Western flower thrips is a pest of major importance in many glasshouse crops and pupates after dropping off the host plant to the soil. Whilst its pupation period is relatively short, probably less than a week in most cases, it could nevertheless persist for a few days in the debris clinging to a used container and could transfer to the new crop if the container was re-used fairly quickly.

Fumigation experiments

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The two fumigants selected as the most likely direct replacements for methyl bromide were phosphine (derived from the action of water on aluminium phosphide) and sulfuryl fluoride. The doses used in the experiments were based on knowledge of currently-recommended dose rates for controlling pests in other situations plus an awareness of what is practicable given the known toxicity of the gases used.

The test organisms were exposed to the fumigants in one of two 1.7 m^3 fumigation chambers in a controlled-environment room. The temperature of the room was maintained at 15°C and the relative humidity was maintained at 75%.

Concentrations of phosphine were created by dosing one of the chambers with Phostoxin pellets (Rentokil Initial UK Ltd). Each pellet contains 3g of aluminium phosphide. The concentration was monitored using gas chromatography. Two gas chromatographs (GCs) were used: a Hewlett Packard 5890 series II fitted with a 500 mm x 3.175 mm glass-lined stainless steel column packed with Poropac QS (80 to 100 mesh), and a Hewlett Packard 6890 fitted with a 30 m HP-5 wide bore column. Each GC was fitted with flame photometric detector and an automatic gas-sampling loop. KNF diaphragm pumps were used to draw gas samples, through nylon-6 gas-sampling line, to the automatic gas-sampling loops of the GCs from the fumigation chamber. The GCs were calibrated for phosphine using a 0.863g m^{-3} standard cylinder.

Concentrations of sulfuryl fluoride were created by partial evacuation of a chamber and then introduction of gas from a cylinder of Vikane® (Dow AgroSciences). The concentration was monitored using a Hewlett Packard 5890 series II GC fitted with a thermal conductivity detector, an automatic gas sampling loop and a 1 m x 3.175 mm glass-lined stainless steel column packed with Poropac QS (80 to 100 mesh). Samples were drawn from the chamber to the automatic sampling loop using a KNF diaphragm pump as before. The GC was calibrated for sulfuryl fluoride against a concentration measured using a standard thermal conductivity meter.

Exposures to fumigants are expressed as concentration-time products (CTP's), in gramme-hours per m^3 (g h m^{-3}).

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The methodology used for all of the fumigant tests was similar. A chamber was dosed with fumigant to a chosen concentration level. The chambers used each had a hatch that allowed access without permitting any leakage of gases. At time zero, the test organism as appropriate was passed into the chamber via the hatch, and it was removed again after the appropriate time had elapsed. The concentrations in the chambers were monitored over the course of the exposures by GC.

Tests with fumigants vs. fungi

Sterilised filter paper discs, 7 mm diameter, were inoculated with the test fungi by placing them on PDA-streptomycin agar plates surrounding a plug of agar containing one of the fungi. These plates were then incubated for suitable periods until the fungus had grown out of the plug, through the agar and into the paper discs. Discs were harvested for use when the appropriate stage of fungal development had been reached – the oospore stage in *Pythium* and the sclerotial stage in *Rhizoctonia.* Five plates each containing 12 discs were used to test each duration of exposure to the fumigants, and an untreated control was also included in each test. After fumigation was complete the infected paper discs were transferred onto plates containing potato dextrose agar amended with 1% streptomycin (PDA strep.). Six discs were added to each plate using heat-sterilised forceps, resulting in two plates per replicate. The plates were then incubated in the dark at 20°C. An initial assessment for growth was made after 4 days incubation and the final assessment after 8 days.

Tests with fumigants vs. weed seeds

The weed seeds were exposed to the gases on dry filter paper in a petri dish. At the completion of the exposure 100 seeds were taken at random from the fumigation sample. Twenty-five of these were transferred to each of four Petri dishes containing a damp filter paper and were incubated at 25°C and 65% R.H. in the dark. Percentage germination was recorded after 10 days.

Tests with fumigants vs. bud & leaf nematodes

For each different exposure test, four leaves infested with bud & leaf nematode were exposed to the fumigant. Four similar leaves were retained to act as an untreated control. On completion of the exposure tests the leaf pieces were immersed in aerated tap water overnight, which allowed the nematodes to leave

the leaf material. The leaf debris was then removed and the nematodes concentrated on a fine sieve before transferring to a watchglass for counting.

Tests with fumigants vs. western flower thrips

The thrips were exposed to the fumigants in 25 ml capacity glass vials with gauze lids to allow the fumigant to enter. A green bean was enclosed in each vial to stabilise the humidity whilst the test was being completed. At the end of the exposure period the thrips were visually assessed for signs of life, being gently manipulated if necessary with a fine needle.

Disinfectant experiments

The six disinfectants/biocides that were identified in the initial review (year 1 of the project) as having the greatest potential for use on re-used containers were used in tests to assess their effects on the test organisms listed above. Details of these products and their rates of use are given in Table 1. For each product, the rate chosen was the maximum recommended by the manufacturer on a product label, or the highest rate included in a Statutory Instrument where this was higher. The reasoning was that the materials should be tested at the highest permissible rate since lack of activity at this rate would preclude the product from further testing. Products that were effective at the highest rate permissible could always be re-tested at a later date to see if a lower rate of application was of satisfactory efficacy.

Table 1. List of disinfectants/biocides used in disinfectant activity experiments

Treatment	Active ingredients	Product rate & derivation
1. Untreated control	Nil (sterilised tap water only)	-
2. FAM 30	Iodine Phosphoric acid Sulphuric acid	**1:125** Diseases of Poultry Statutory Orders (England & Scotland) 2006
3. Trigene Advance	Halogenated tertiary amine. Polymeric biguanide hydrochloride < 10%. Alkyl/Didecyl Ammonium chloride < 10%.	**1:100** Feed & water bowls, foot dips (high-risk areas). For broad-spectrum activity.

4. Citric Acid BP	Citric acid	**1:100**
5. GeoSil	Silver stabilised hydrogen peroxide	**1:33** **3%** rate recommended by supplier (2% surfaces, 5% dirty walls)
6. Virkon S	Pentapotassium bis (peroxymonosulphate) bis (sulphate) <50%. Sulphamic acid 5-10%. Sodium dodecylbenzenesulfonate 5-15%. Dipotassium peroxodisulphate <2%	**1:100** (Highest rate from 1:100 – 1:280 range given on label).
7. Citrox P	Extracts of citrus fruit. Surfactants	**1:150** label rate for containers

Tests with disinfectants vs. fungi

Sterilised filter paper discs, 7 mm diameter, were inoculated with the test fungi by placing them on PDA-streptomycin agar plates surrounding a plug of agar containing one of the test fungi. These plates were then incubated for suitable periods until the fungus had grown out of the plug, through the agar and into the paper discs. Discs were harvested for use when the appropriate stage of fungal development had been reached – the oospore stage in Pythium and the sclerotial stage in Rhizoctonia. When the inoculated paper discs were ready, solutions of the test disinfectants were made up with sterilised tap water to the concentrations listed in Table 1 and kept in beakers, two per disinfectant. Within 1 hour of the solutions being made up at least 40 inoculated paper discs were placed into each beaker. Twenty were withdrawn from the disinfectant solution after 10 minutes immersion and a further 20 remained immersed for 60 minutes before withdrawal. Immediately on withdrawal from the disinfectant solutions the paper discs were rinsed three times in sterilised tap water and were then allowed to drip dry, but not dry out.

In the earliest runs of the experiment the moist but drip-free discs were transferred to 25-well agar plates to be incubated, but problems with cross-contamination occurred and individual 9 cm diameter petri dishes were subsequently used for incubation purposes. The petri dishes were filled with PDA-streptomycin agar before they were used. The loaded dishes were transferred to an incubator set at 20°C and were incubated for a maximum of 28 days to allow growth of the fungi to occur.

The dishes were removed and examined for fungal growth after 7, 14 and 28 days. Dishes containing active fungal growth were recorded.

Tests with disinfectants vs. weed seeds

Before using the weed seeds obtained for the experiment, each was subjected to a germination test. Two simultaneous tests were done for each weed species. Each consisted of putting a sub-sample of at least 20 seeds onto a filter paper wetted with sterile tap water in a petri dish. A lid was put onto the dish, which was then transferred to a well-lit windowsill in a room with a mean temperature of approximately 20°C. The seeds were kept moist by occasional watering with sterile tap water. Following the first emergence of a radicle or shoot the dishes were left for a further 14 days. Evidence of germination was recorded on a daily basis through this period.

Both weed species used in the tests have small seeds, but those of *Sagina* are extremely small (<1 mm diameter). For the *Cardamine*, to ease handling and minimise seed losses during testing, mesh bags were used as containers, but this method was not suitable for use with the *Sagina* seed. The bags consisted of pieces of plastic mesh 100 mm x 50 mm, which were folded in half across the length and then heat sealed up the sides to make pouches 50 mm x 50 mm. Samples of at least 10 weed seeds were wrapped in a single layer of paper lens tissue that was then transferred to a mesh pouch before the latter was sealed along the fourth side.

Twenty-eight bags of *Cardamine* were prepared. Four were used with each of the six disinfectants and the remaining four were used in control treatments. Solutions of the test disinfectants were made up at the concentrations indicated in Table 1. Four bags were then immersed in each of the solutions. The remaining four bags were immersed in sterilised tap water to act as a control treatment. After 10 minutes immersion two bags were retrieved from each solution and were immediately rinsed three times in sterilised tap water to remove all traces of disinfectant. Each bag was then cut open and the contents allowed to dry partly, so that the weed seeds could easily be separated. The seeds from each bag were then transferred to a moist sterilised filter paper in a petri dish. After 60 minutes immersion the remaining bags were retrieved from the solutions and were treated similarly to those retrieved after 10 minutes. All seeds retrieved were subjected to the germination test as detailed above.

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For the *Sagina*, small batches of about 50 seeds were transferred directly into 250 ml beakers containing 200 ml of a disinfectant solution. The solutions were made up to the concentrations indicated in Table 1. One beaker was used for each disinfectant/immersion time combination. After the seed was introduced to the beakers the solutions were stirred at intervals to ensure good contact between the seed and the solution. After the appropriate time had elapsed the seeds were removed from the disinfectant by filtering through a fine mesh fitted into a funnel, and traces of disinfectant were removed by washing the seed from the filter into a beaker of sterile distilled water and re-filtering, twice. After the final rinse the seeds were washed from the filter into a 9 cm petri dish containing a filter paper disc using approximately 2 ml of sterile distilled water. The seeds were then incubated for 28 days and the germination results were recorded.

Tests with disinfectants vs. bud & leaf nematodes

The bud & leaf nematodes used in the experiments were obtained from a culture of *Aphelenchoides ritzemabosi* on the host plant Japanese anemone, maintained at ADAS Boxworth, and from naturally-infested Japanese anemone found on a commercial nursery. Ten infected leaves were used for each experiment. Each leaf was cut in half. Half was used for the experimental treatment and the other half was used as an untreated control, to allow an assessment of the level of infestation of each leaf to be made. The experimental halves of each leaf were immersed in the test solutions of the disinfectants for one hour, before removal to clean water to rinse them. The experimental and control halves of each leaf were then put separately into small (25 ml) vessels, covered in tap water and had air bubbled through using an aquarium pump for 72 hours. Sieves were then used to remove debris and concentrate the nematodes before they were transferred to a Doncaster dish for counting under a binocular microscope. It was originally intended to conduct immersion tests of i) one hour and ii) 10-minutes duration. However, the one-hour tests were done first and the results indicated that it would not be worth pursuing the 10-minute treatment option.

Tests with disinfectants vs. western flower thrips pupae

The pupae used for these tests were obtained from a culture maintained at ADAS Boxworth. The test solutions of disinfectants were made up to the concentrations indicated in table 1 using tap water. Plain tap water was used for the control

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treatment. The tests were done in 90 mm diameter plastic plant-pot saucers in the laboratory. A 90 mm diameter milk filter was placed into a saucer and a single, viable western flower thrips (WFT) pupa was placed onto the centre of the filter using a fine paintbrush. The saucer was then carefully inundated with 50 ml of one of the test solutions using a clean syringe, ensuring that the pupa was covered with the solution. The pupa was exposed to the test solution for either 10 minutes or, where this had failed to give 100% mortality in a previous test, 60 minutes. After the exposure was complete the milk filter was lifted from the solution and the pupa was transferred to a clean filter paper to shed excess solution. After 10 minutes air-drying, each pupa was assessed for mortality by gently manipulating it with a fine artists paintbrush under a binocular microscope. Pupae that produced movement were recorded as alive. Those that showed no movement were recorded as dead. Eight pupae were used as individual replicates of each treatment.

Temperature experiments

The test organisms were exposed to elevated temperatures in three different ways. These consisted of 'wet heat', applied in a water bath, 'dry heat', applied in an oven and heat applied by molecular excitation using microwave energy.

Tests using wet heat vs. fungi

For this work, fungus-impregnated paper discs, prepared as described above ('Tests using disinfectants vs. fungi') were used. For the experiments using wet heat, mesh pouches, 50 mm x 50 mm, were used to contain the discs, always at 10 discs per bag. Two bags were used for each combination of fungal species, temperature and duration of exposure. Beakers of sterile tap water were placed in the water bath, which was then adjusted to a temperature of 20°C and allowed to stabilise. Six mesh bags of Pythium-impregnated discs were then placed into one of the beakers. After 10 minutes exposure to immersion at 20°C two bags were removed from the beaker and placed to drain. After thirty minutes exposure a further two bags were removed and again allowed to drain. Finally, after 60 minutes exposure the remaining two bags were also removed and drained. A simultaneous, similar test was done using bags containing Rhizoctonia-impregnated discs. The temperature in the water bath was then raised to 50°C and the experiment was repeated for the same combinations of fungi and duration of exposure. Further repeats were also done at 60°C and 70°C. The drained bags of fungus-impregnated paper discs were opened and the paper discs were transferred to the surface of PDA Strep. agar contained in

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9cm petri dishes, then incubated at 20°C in a dark incubator and examined at intervals for signs of active fungal growth. Un-immersed fungus-impregnated paper discs were also transferred to PDA Strep. agar in petri dishes and incubated at 20°C, to act as controls.

Tests using dry heat vs.fungi

A calibrated drying oven was used. Fungus-impregnated paper discs (produced as described above) were used for the work, as before. Polyethylene plant-pot saucers surface-sterilised with 70% ethanol were used to carry the discs, 10 discs to each saucer. Loaded saucers were transferred to the oven after it had been pre-heated to the test temperature (50°C, 60°C or 70°C) and were exposed to the heat for the required duration (10, 30 or 60 minutes). Because the oven temperature dropped temporarily when the door was opened to remove saucers the experiments were run sequentially rather than simultaneously. Saucers of discs were allowed to cool after treatment before the discs were transferred to PDA Strep. agar in 9 cm petri dishes for incubation purposes. After incubation at 20°C in a dark incubator the discs were examined at intervals for signs of active fungal growth.

Tests using microwave energy vs. fungi

A domestic 850 watt microwave oven was used. Fungus-impregnated filter paper discs, obtained as previously described, were exposed to the microwave energy in surface-sterilised polyethylene plant-pot saucers, 10 discs to each saucer. Two saucers of 10 discs were used for each exposure time. Exposure times consisted of 0, 10, 30, 60, 120 and, in the case of the Rhizoctonia only, 180 seconds at full power. Saucers were exposed to microwave heat singly. The condition of the filter-paper discs immediately after treatment was noted, and the discs were then plated out into 9cm diameter plastic petri dishes containing PDA Strep. agar and incubated for up to two months in a dark incubator kept at 20°C. The discs were regularly examined for signs of fungal growth.

Tests using heat vs. weed seeds

Before use, sub-samples of both *Cardamine* and *Sagina* seed were subjected to a germination test, as described above (see Tests with disinfectants vs. weed seeds).

Wet heat (water bath) tests: the *Cardamine* seeds were treated in 50 mm x 50 mm mesh bags, as for the tests with disinfectants. Two bags were made for each treatment. The treatments consisted of immersion in water at 20°C, 50°C, 60°C or

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70°C, for durations of 10, 30 or 60 minutes. There was also a control treatment that was not immersed in water. Treated seeds were removed from the bags and subjected to a germination test as previously described, with germination being recorded 11 and 28 days after treatment. The *Sagina* seeds were too small to be treated in mesh bags and so were treated in 2 ml glass specimen tubes filled with sterile distilled water, two tubes being used for each temperature/time combination. For the tests, the filled glass tubes, without seeds, were stoppered and placed in the water bath which had been set at the chosen temperature. The temperature of the contents of the tubes was then allowed to equalise with that of the water bath. This was checked using a thermometer in a tube set aside for this purpose. Once the temperature had equalised, sub-samples of about 50 seeds were added to each tube and these were left for the selected duration of exposure. At the completion of this period the tubes were removed from the water bath and the seeds were immediately removed by filtration, then rinsed in sterile distilled water at 20°C before being subjected to a germination test. Germination 11 and 28 days after treatment was recorded.

Dry heat tests: a scientific drying oven was used for these tests. Sub-samples of the appropriate number of weed seeds were subjected to treatment in polyethylene plant pot saucers. The saucers, which were new, were cleaned with a detergent, rinsed with distilled water and then wiped with 70% ethanol before use. Two saucers of each weed species were treated with each combination of temperature and exposure time in the oven. Treatments were carried out at 20°C, 50°C, 60°C and 70°C, for 10, 30 and 60 minutes at each temperature. On completion of the treatment the weed seeds were subjected to a germination test as previously described, the germination at 11 and 28 days after treatment being recorded.

Microwave energy test: the weed seeds were also treated in surface-sterilised polyethylene plant pot saucers. Two saucers of each species of weed seed were used for each treatment, but each was treated separately. The 850 watt microwave was used at full power for durations of 10, 30, 60, 120 and 240 seconds. At the completion of the treatment the weed seeds were subjected to a germination test as previously described.

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Tests using heat vs. bud & leaf nematodes

The bud & leaf nematodes used in the experiments were obtained from the culture of *Aphelenchoides ritzemabosi* on Japanese anemone. Pieces of leaf tissue, or whole leaves, that were infested with the nematodes were used. Half of each piece of leaf tissue was exposed to an experimental treatment and the remaining half was retained as an untreated control to confirm the viability of the nematodes.

Wet heat (water bath) tests: a water bath was used. This was calibrated against an accredited NAMAS thermometer to ensure accuracy of temperature regulation. The water bath was set to the required temperature and allowed to stabilise. Plastic pots, 60 ml capacity, were filled with tap water and the lids secured. These were then placed in the water bath to acclimatise. When the water in the pots had reached the temperature in the bath a half-piece of infested leaf tissue was introduced into each pot and the lid was re-secured. Simultaneously, the remaining half of each piece of infested leaf was placed in a similar pot filled with water at laboratory temperature, in this case 18°C. Some of the pots in the water bath were removed after 10 minutes exposure to the heat, some were removed after 30 minutes exposure and some were removed after 60 minutes. On removal each piece of leaf was extracted from its pot and placed singly into a labelled beaker containing tap water at 18°C. These beakers and the pots containing the half-pieces of leaf that had not been exposed to elevated temperature in the water bath were then left, with aeration supplied by small aquarium pumps, for any viable bud & leaf nematodes to leave the leaf tissue. After 72 hours had elapsed the contents of the first pot were passed through a 43 µm sieve, the debris and any nematodes present were backwashed into a Doncaster counting dish and the nematodes present were counted. This was repeated for each of the experimental and control pots.

Dry heat tests: a scientific drying oven was used. This was set to the required temperature, which was checked against a NAMAS-approved thermometer. For each duration of heat treatment at each temperature, eight half-pieces of infested leaf tissue were exposed in the oven. The remaining half-pieces of the same infested leaf tissue were exposed to ambient temperature (approx. 18°C) for the equivalent time. At the completion of the heat treatment each leaf-piece was transferred to a beaker containing tap water at 18°C. The water was aerated using an aquarium pump. After 72 hours had elapsed the contents of the first beaker were passed

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through a 43 μm sieve, the debris and any nematodes present were backwashed into a Doncaster counting dish and the nematodes present were counted. This was repeated for each of the experimental and control treatments.

Microwave energy tests: these were done using an 850 watt domestic microwave oven. As before, half-pieces of infested leaf tissue were used as the experimental material. Each half-piece (8 per treatment in total) was treated singly in a brand-new polyethylene plant pot saucer, each of which was put separately into the microwave and treated for the selected time on full power. The control half-pieces were left in similar saucers at ambient temperature in the laboratory (approx. 18°C) for the same selected time durations. After the completion of each treatment the treated half-pieces and the control half-pieces were put into individual beakers and were inundated with tap water. The beakers were then aerated for 72 hours before the contents were put through a 47 μ sieve, the trapped nematodes and debris back-washed into a Doncaster counting dish and the live nematodes counted and recorded.

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Results and Discussion

Tables 2 to 37 contain the results of the various experiments done on the test organisms.

Fumigation experiments

Table 2. Survival of fungi after fumigation with Phostoxin (standard rate)

Exposure (hours)	Conc.-time product (g h m^{-3})	Discs with growth (%)	
		Rhizoctonia	*Pythium*
0	0	100	100
8	11	100	100
16	22	100	100
24	33	100	100
48	66	100	100

Table 3. Survival of fungi after fumigation with Phostoxin (double rate)

Exposure (hours)	Conc.-time product (g h m^{-3})	Discs with growth (%)	
		Rhizoctonia	*Pythium*
0	0	100	100
8	21	100	100
16	47	100	100
24	70	100	100
48	146	100	100

At the concentration-time product exposures given, Phostoxin failed to give any control of either *Rhizoctonia* or *Pythium* in these tests.

Table 4. Survival of fungi after fumigation with Vikane (standard rate)

Exposure (hours)	Conc.-time product (g h m^{-3})	Discs with growth (%)	
		Rhizoctonia	*Pythium*
0	0	100	100
8	330	100	100
16	660	95.0	100

24	980	73.3	25.0
48	1970	48.3	3.3

Table 5. Survival of fungi after fumigation with Vikane (double rate)

Exposure (hours)	Conc.-time product (g h m^{-3})	Discs with growth (%)	
		Rhizoctonia	*Pythium*
0	0	100	100
8	590	8.4	41.6
16	1180	0	0
24	1760	0	0
48	3530	0	0

Fumigation with Vikane gave better results than did Phostoxin, giving some mortality at the lower rate and 100% mortality of both fungi when these were exposed for 16 hours at the higher rate. *Pythium* appeared slightly more tolerant of Vikane than *Rhizoctonia*.

Table 6. Germination of weed seeds after fumigation with Phostoxin (lower rate)

Exposure (hours)	Conc.-time product (g h m^{-3})	Germination (%)	
		Cardamine	*Sagina*
0	0	53	38
8	8	44	-
16	18	55	-
24	26	38	-
48	53	48	35

Table 7. Germination of weed seeds after fumigation with Phostoxin (higher rate)

Exposure (hours)	Conc.-time product (g h m^{-3})	Germination (%)	
		Cardamine	*Sagina*
0	0	52	-
8	14	60	-
16	27	54	-

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24	43	56	-
48	88	49	-

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Table 8. Germination of weed seeds after fumigation with Vikane (lower rate)

Exposure (hours)	**Conc.-time product (g h m^{-3})**	**Germination (%)**	
		Cardamine	*Sagina*
0	0	52	-
8	350	54	-
16	700	49	-
24	1050	54	-
48	2110	47	-

Table 9. Germination of weed seeds after fumigation with Vikane (higher rate)

Exposure (hours)	**Conc.-time product (g h m^{-3})**	**Germination (%)**	
		Cardamine	*Sagina*
0	0	53	38
8	790	54	24
16	1590	52	22
24	2380	58	27
48	4760	49	25

The germination of the *Cardamine hirsuta* seed does not appear to have been affected by any of the fumigation treatments, no matter which fumigant, concentration or exposure time was used.

There was a smaller supply of *Sagina subulata* seed and so fewer tests were completed with this species. Nevertheless, fumigation with Phostoxin does not seem to have had any effect on the germination of *Sagina*. When Vikane was used as the fumigant, however, there does appear to have been an effect. The mean germination rate of the *Sagina* seed without treatment was 38%; when seed was fumigated with sulfuryl fluoride this dropped to a mean of 24.5%, which represents a reduction in germination of 34%. Strangely, there was no apparent increase in mortality of *Sagina* seed with dosage rate. A possible explanation could be that only seeds that have a damaged testa are susceptible to sulfuryl fluoride. The proportion

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of damaged seeds would be the same in all treatments, hence the similar mortality. This is, however, a speculative explanation with no corroborative observations.

Table 10. Survival of western flower thrips after fumigation with Phostoxin

Exposure (hours)	Conc.-time product (g h m^{-3})	Knockdown (%)	Control Mortality (%)
1	2.4	100	0
2	4.7	100	4
4	9.5	100	0
6	14.2	100	8
8	12.2	100	36
16	27.8	100	34
24	40.3	100	36
72	108	100	52

Table 11. Survival of western flower thrips after fumigation with Vikane

Exposure (hours)	Conc.-time product (g h m^{-3})	Knockdown (%)	Control Mortality (%)
2	165	100	3
4	330	100	7
8	660	100	7
16	1320	100	3

There was 100% knockdown of western flower thrips in each of the exposures to Phostoxin. However, some insects that had been exposed for either one or two hours began to recover after 2-3 hours. No individuals recovered after any of the other exposures. Complete mortality therefore seems to have occurred after 4 hours at the gas concentration used in the experiment. After 8 hours, significant mortality was seen in the untreated, control insects.

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Table 12. Survival of bud & leaf nematodes after exposure to Phostoxin

Exposure (hours)	**Conc.-time product (g h m^{-3})**	**Approximate nematode number in leaf samples**
0	0	5000+
8	14	5000+
16	15	5000+
24	29	5000+
48	82	100

Table 13. Survival of bud & leaf nematodes after exposure to Vikane

Exposure (hours)	**Conc.-time product (g h m^{-3})**	**Approximate nematode number in leaf samples**
0	0	5000+
8	650	0
16	1320	0
24	1960	0
48	3920	0

No mortality was observed in the nematodes treated by exposure to Phostoxin for 24 hours or less. However, after exposure to Phostoxin for 48 hours the number of surviving nematodes was drastically reduced. Vikane was more effective than Phostoxin at the exposures tested, giving complete mortality of the nematodes after only 8 hours exposure.

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Disinfectant experiments

Table 14. Survival of Pythium after treatment with disinfectants (DAT = days after treatment)

Treatment	Duration of immersion (min)	% survival 27 DAT
Control (sterile tap water)	60	100
Citric acid	10	100
Citric acid	60	100
Citrox-P	10	100
Citrox-P	60	100
GeoSil	10	90
GeoSil	60	0
FAM 30	10	0
FAM 30	60	0
Trigene Advance	10	40
Trigene Advance	60	0
Virkon-S	10	70
Virkon-S	60	0

FAM 30 was the most effective disinfectant against *Pythium*, giving 100% control after 10 minutes contact. GeoSil, Trigene Advance and Virkon S all gave some control after exposure for 10 minutes and complete control after 1 hour's exposure.

Table 15. Survival of Rhizoctonia after treatment with disinfectants (DAT = days after treatment)

Treatment	**Duration of immersion (min)**	**% survival 27 DAT**
Control (sterile tap water)	60	100
Citric acid	10	100
Citric acid	60	100
Citrox-P	10	100
Citrox-P	60	90
GeoSil	10	100
GeoSil	60	90
FAM 30	10	100
FAM 30	60	90
Trigene Advance	10	70
Trigene Advance	60	70
Virkon-S	10	100
Virkon-S	60	100

Rhizoctonia proved a more difficult target for the disinfectants than did *Pythium*. Trigene Advance had the greatest effect, giving about 30% control after either 10 minutes' or 1 hour's exposure. Citrox P, Fam 30 and GeoSil gave some suppression after 1 hour's exposure, but none of the disinfectants could be described as having sufficient effect on *Rhizoctonia* to enable them to be recommended for control of this fungus.

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Table 16. Germination rate of *Cardamine* after treatment with disinfectants (DAT = days after treatment)

Treatment	Duration of immersion (min)	Mean % germination 29 DAT
Control (sterile tap water)	60	77
Citric acid	10	67
Citric acid	60	56
Citrox-P	10	62
Citrox-P	60	65
GeoSil	10	62
GeoSil	60	53
FAM 30	10	57
FAM 30	60	67
Trigene Advance	10	59
Trigene Advance	60	25
Virkon-S	10	60
Virkon-S	60	63

Seedlings produced from seeds treated with Trigene Advance (10 minutes immersion and 60 minutes immersion) and GeoSil (60 minutes immersion) were stunted, weak and had a prostrate growth habit compared to all other seedlings. Only Trigene Advance actually reduced germination however, and this only occurred after 1 hour's exposure to the product.

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Table 17. Germination rate of *Sagina* after treatment with disinfectants (DAT = days after treatment)

Treatment	**Duration of immersion (min)**	**Mean % germination 29 DAT**
Control (sterile tap water)	60	62
Citric acid	10	72
Citric acid	60	64
Citrox-P	10	64
Citrox-P	60	69
GeoSil	10	67
GeoSil	60	72
FAM 30	10	67
FAM 30	60	65
Trigene Advance	10	57
Trigene Advance	60	65
Virkon-S	10	63
Virkon-S	60	72

None of the disinfectants or biocides tested had any apparent effect on seed of *Sagina*, even after 1 hour's exposure

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Table 18. Survival of western flower thrips pupae after treatment with disinfectants

Treatment	**Duration of immersion (min)**	**Live**	**Dead**	**Survival (%)**
Control (sterile tap water)	10	56	0	100
Control (sterile tap water)	60	38	10	79
Citric acid	10	7	1	87
Citric acid	60	7	1	87
Citrox-P	10	7	1	87
Citrox-P	60	5	3	62
GeoSil	10	6	2	75
GeoSil	60	6	2	75
FAM 30	10	0	8	0
FAM 30	60	-	-	-
Trigene Advance	10	4	4	50
Trigene Advance	60	2	6	25
Virkon-S	10	4	4	50
Virkon-S	60	4	4	50

Fam 30 was by far the most effective disinfectant in controlling western flower thrips pupae. Next best were Trigene Advance and GeoSil.

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Table 19. Survival of bud & leaf nematode after treatment with disinfectants

Treatment	**Duration of immersion (min)**	**Live nematodes**		**Survival (%)**
		Treatment	**Control**	
Citric acid	60	770	762	100
Citrox-P	60	2626	2672	100
GeoSil	60	2652	2554	100
FAM 30	60	1602	2459	65
Trigene Advance	60	118	352	34
Virkon-S	60	2489	1469	100

Both FAM 30 and Trigene Advance reduced the survival of bud & leaf nematodes, with Trigene Advance proving the more effective of the two after exposure for 60 minutes.

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Heat experiments

Wet heat

Table 20. Survival of Pythium after wet heat treatment

Treatment/temperature	Duration of exposure (min)	Survival (%) after 27 days incubation
Dry heat 20°C	Constant	100
Wet heat 20°C	10	100
Wet heat 20°C	30	100
Wet heat 20°C	60	100
Wet heat 50°C	10	0
Wet heat 50°C	30	0
Wet heat 50°C	60	0
Wet heat 60°C	10	0
Wet heat 60°C	30	0
Wet heat 60°C	60	0
Wet heat 70°C	10	0
Wet heat 70°C	30	0
Wet heat 70°C	60	0

Pythium proved susceptible to wet heat treatment, 10 minutes' exposure to 50°C being sufficient to give 100% mortality.

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Table 21. Survival of Rhizoctonia after wet heat treatment

Treatment/temperature	Duration of exposure (min)	Survival (%) after 30 days incubation
Dry heat 20°C	Constant	100
Wet heat 20°C	10	100
Wet heat 20°C	30	100
Wet heat 20°C	60	100
Wet heat 50°C	10	10
Wet heat 50°C	30	30
Wet heat 50°C	60	30
Wet heat 60°C	10	0
Wet heat 60°C	30	0
Wet heat 60°C	60	0
Wet heat 70°C	10	0
Wet heat 70°C	30	0
Wet heat 70°C	60	0

Rhizoctonia was more resistant to heat than was *Pythium*. Nevertheless, 10 minutes' exposure to 60°C was sufficient to give 100% mortality.

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Table 22. Germination of *Cardamine* after wet heat treatment

Treatment/temperature	**Duration of exposure (min)**	**Germination (%) after 28 days incubation**
Dry heat 20°C	Constant	59
Wet heat 20°C	10	66
Wet heat 20°C	30	64
Wet heat 20°C	60	64
Wet heat 50°C	10	37
Wet heat 50°C	30	19
Wet heat 50°C	60	2
Wet heat 60°C	10	0
Wet heat 60°C	30	0
Wet heat 60°C	60	0
Wet heat 70°C	10	0
Wet heat 70°C	30	0
Wet heat 70°C	60	0

Though treatment at 50°C gave some useful mortality of *Cardamine* seed, with 1 hour's exposure giving a 98% reduction in germination, treating at 60°C was more effective and required only a short exposure to give complete control.

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Table 23. Germination of *Sagina* after wet heat treatment

Treatment/temperature	Duration of exposure (min)	Germination (%) after 28 days incubation
Dry heat 20°C	Constant	61
Wet heat 20°C	10	66
Wet heat 20°C	30	65
Wet heat 20°C	60	67
Wet heat 50°C	10	58
Wet heat 50°C	30	20
Wet heat 50°C	60	3
Wet heat 60°C	10	0
Wet heat 60°C	30	0
Wet heat 60°C	60	0
Wet heat 70°C	10	0
Wet heat 70°C	30	0
Wet heat 70°C	60	0

The *Sagina* seed behaved in a very similar way to the *Cardamine* when exposed to wet heat. Treatment at 50°C gave some useful mortality, with 1 hour's exposure giving a 97% reduction in germination, but treating at 60°C was more effective and required only a short exposure to give complete control.

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Table 24. Survival of western flower thrips pupae after wet heat treatment

Treatment/temperature	Duration of exposure (min)	Live pupae	Dead pupae	Survival (%)
Control 20°C	5	8	0	100
Wet heat 50°C	5	0	8	0
Control 20°C	10	8	0	100
Wet heat 50°C	10	0	8	0

Western flower thrips pupae proved to be extremely susceptible to wet heat, with 5 minutes' exposure to 50°C being enough to give complete control.

Table 25. Survival of bud & leaf nematodes after wet heat treatment

Treatment/temperature	Duration of exposure (min)	Live nematodes in treated tissue	Live nematodes in control tissue	Treated as a % of control
Wet heat 50°C	10	3	1830	0.17
Wet heat 50°C	30	3	3503	0.09
Wet heat 50°C	60	0	2088	0.00
Wet heat 60°C	10	1	4442	0.02
Wet heat 60°C	30	3	4987	0.06
Wet heat 60°C	60	-	-	-

Most individual bud & leaf nematodes succumbed quite quickly to exposure to wet heat, but a few survived for quite long periods. Longer exposure to heat seems to be the key to controlling the nematode, perhaps because it requires a longer exposure to raise the temperature of the bulky leaf material in which the nematode lives. One hour's exposure to 50°C was effective in eradicating all bud & leaf nematodes, whereas some even survived 30 minutes' exposure to 60°C.

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Dry Heat

Table 26. Survival of Pythium after dry heat treatment

Treatment/temperature	Duration of exposure (min)	Survival (%) after 6 days incubation
Dry heat 20°C	Constant	100
Dry heat 50°C	10	100
Dry heat 50°C	30	100
Dry heat 50°C	60	100
Dry heat 60°C	10	100
Dry heat 60°C	30	95
Dry heat 60°C	60	90
Dry heat 70°C	10	100
Dry heat 70°C	30	90
Dry heat 70°C	60	45

Pythium proved tolerant of dry heat, even 60 minutes' exposure to 70°C failing to give more than 55% control.

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Table 27. Survival of Rhizoctonia after dry heat treatment

Treatment/temperature	**Duration of exposure (min)**	**Survival (%) after 6 days incubation**
Dry heat 20°C	Constant	89
Dry heat 50°C	10	65
Dry heat 50°C	30	40
Dry heat 50°C	60	45
Dry heat 60°C	10	45
Dry heat 60°C	30	40
Dry heat 60°C	60	45
Dry heat 70°C	10	60
Dry heat 70°C	30	15
Dry heat 70°C	60	10

Rhizoctonia was slightly more susceptible than Pythium to the effects of dry heat. However, long exposures to 50°C or 60°C failed to give more than 60% control. The most effective treatment was exposure to 70°C for one hour, which gave 90% mortality.

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Table 28. Germination of *Cardamine* after dry heat treatment

Treatment/temperature	Duration of exposure (min)	Germination (%) after 10 days incubation
Dry heat 20°C	Constant	59
Dry heat 50°C	10	62
Dry heat 50°C	30	59
Dry heat 50°C	60	53
Dry heat 60°C	10	64
Dry heat 60°C	30	56
Dry heat 60°C	60	53
Dry heat 70°C	10	55
Dry heat 70°C	30	56
Dry heat 70°C	60	51

Table 29. Germination of *Sagina* after dry heat treatment

Treatment/temperature	Duration of exposure (min)	Germination (%) after 10 days incubation
Dry heat 20°C	Constant	45
Dry heat 50°C	10	64
Dry heat 50°C	30	56
Dry heat 50°C	60	58
Dry heat 60°C	10	48
Dry heat 60°C	30	56
Dry heat 60°C	60	44
Dry heat 70°C	10	33
Dry heat 70°C	30	29
Dry heat 70°C	60	41

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None of the treatments applied appeared to affect the germination of either the *Cardamine* or the *Sagina* seed

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Table 30. Survival of western flower thrips pupae after dry heat treatment

Treatment/temperature	**Duration of exposure (min)**	**Survival (%) after treatment**
Dry heat 20°C	Constant	100
Dry heat 50°C	10	0

Western flower thrips pupae did not survive the minimum dry heat treatment applied, namely 10 minutes' exposure to 50°C.

Table 31. Survival of bud & leaf nematode after dry heat treatment

Treatment/temperature	**Duration of exposure (min)**	**Live nematodes in treated tissue**	**Live nematodes in control tissue**	**Treated as a % of control**
Dry heat 50°C	10	8387	11687	71.7
Dry heat 50°C	30	1829	12869	14.2
Dry heat 50°C	60	629	9200	6.8
Dry heat 60°C	10	170	11744	1.4
Dry heat 60°C	30	8	5233	0.2
Dry heat 70°C	10	0	11903	0.0

Most of the dry heat treatments listed had some effect on the survival of bud & leaf nematode. Ten minutes' exposure to 70°C gave complete control.

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Table 32. Survival of Pythium after microwave treatment (850 watt microwave)

Treatment	**Duration of exposure (sec)**	**Survival (%) after 26 days incubation**
Control	0	100
Experimental	10	100
Experimental	30	100
Experimental	60	60
Experimental	120	20

Increasing exposure to microwave energy gave increasing mortality of *Pythium*, but even 120 seconds' exposure failed to give complete control.

Table 33. Survival of Rhizoctonia after microwave treatment (850 watt microwave)

Treatment	**Duration of exposure (sec)**	**Survival (%) after 26 days incubation**
Control	0	100
Experimental	10	100
Experimental	30	100
Experimental	60	100
Experimental	120	100
Experimental	180	100

None of the microwave treatments were effective in controlling *Rhizoctonia*, including the extreme of 180 seconds' exposure.

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Table 34. Germination of *Cardamine* after microwave treatment (850 watt microwave)

Treatment	Duration of exposure (sec)	Germination (%) after 13 days incubation
Control	0	58
Experimental 1	10	62
Experimental 2	30	55
Experimental 3	60	53
Experimental 4	120	42
Experimental 5	240	38

There was a tendency for the germination of *Cardamine* seed to decrease with increasing exposure to microwaves. However, the maximum exposure given, 240 seconds, only reduced germination by 34%.

Table 35. Germination of *Sagina* after microwave treatment_(850 watt microwave)

Treatment	Duration of exposure (sec)	Germination (%) after 13 days incubation
Control	0	68
Experimental 1	10	52
Experimental 2	30	53
Experimental 3	60	57
Experimental 4	120	58
Experimental 5	240	32

The germination of *Sagina* seed did not appear to be affected by exposure to microwaves for up to 120 seconds. Exposure for 240 seconds did however reduce subsequent germination by 52% compared to the control.

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Table 36. Survival of western flower thrips pupae after microwave treatment_(850 watt microwave)

Treatment	Duration of exposure (sec)	Survival 10 min. after treatment (%)	Comment
Control	0	100	No physical symptom
Experimental 1	10	100	No physical symptom
Experimental 2	30	100	No physical symptom
Experimental 3	60	25	No physical symptom
Experimental 4	120	0	Shrivelling visible

Western flower thrips pupae survived exposure to microwaves for up to 30 seconds with no apparent ill-effect. However, exposure for 60 seconds reduced survival by 75% and exposure for 120 seconds caused 100% mortality.

Table 37. Survival of bud & leaf nematode after microwave treatment_(850 watt microwave)

Treatment	Duration of exposure (sec)	Live nematodes 72 hours post-treatment
Control 1	0	3440
Experimental 1	10	3
Control 2	0	7908
Experimental 2	30	0
Control 3	0	4484
Experimental 3	60	0

Bud & leaf nematode proved to be very susceptible to exposure to microwave treatment when compared to the other organisms tested. Ten seconds' exposure

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caused 99.9% mortality, and none survived when exposed to microwave energy for 30 seconds.

The data in Table 38 are abstracted from tables 2 – 37 above and provide a summary of the effects of the various significant treatments on the test organisms.

Table 38. Summary of effects of treatments on test organisms

Control method	**Exposure criteria**	Pythium	Rhizoctonia	*Cardamine*	*Sagina*	Western Flower thrips	Bud & leaf nematode
Phostoxin (High rate)	8 hours	-	-	-	n.d.	+++	-
	16 hours	-	-	-	n.d.	+++	-
	48 hours	-	-	-	- (low rate)	+++	++
Vikane (High rate)	8 hours	++	++	-	+	+++	+++
	16 hours	+++	+++	-	+	n.d.	+++
	48 hours	+++	+++	-	+	n.d.	+++
Citric Acid	10 min	-	-	-	-	-	-
	60 min	-	-	-	-	-	-
Citrox P	10 min	-	-	-	-	-	n.d.
	60 min	-	+	-	-	+	-
GeoSil	10 min	+	-	-	-	-	n.d.
	60 min	+++	+	-	-	-	-
FAM 30	10 min	+++	-	-	-	+++	n.d.
	60 min	+++	+	-	-	+++	+
Trigene Advance	10 min	++	+	-	-	+	n.d.
	60 min	+++	+	++	-	++	++
Virkon-S	10 min	+	-	-	-	+	n.d.
	60 min	+++	-	-	-	+	-
Wet heat	10 min, 50°C	+++	++	+	-	+++	++
	60 min, 50°C	+++	++	++	++	n.d.	+++
	10 min, 60°C	+++	+++	+++	+++	n.d.	++
	30 min, 60°C	+++	+++	+++	+++	n.d.	++
Dry heat	10 min, 50°C	-	+	-	-	+++	+
	10 min, 70°C	-	+	-	-	n.d.	++
	60 min, 70°C	++	++	-	-	n.d.	+++
Microwave	60 sec	+	-	-	+	++	+++
	120 sec	++	-	+	+	+++	n.d.
	240 sec	n.d.	n.d.	+	++	n.d.	n.d.

Key:
+++ = Complete control.
++ = >50% control.
+ = <50% control.
- = no control.

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n. d. = not done

Conclusions

Table 38 was compiled from the results of more than 150 individual tests and is inevitably a simplification of the results, but it does allow for some direct comparisons of the effectiveness of the various methods of controlling the test organisms.

The organisms.

The most durable organisms in the test programme proved to be the weed seeds, which were only effectively controlled by exposure to hot water at 60°C for 10 minutes. Longer exposures at lower temperatures (e.g. 60 minutes at 50°C) were not as effective, and none of the fumigants, disinfectants, dry heat nor microwave energy gave adequate control at the temperature/exposure parameters used in the experiments. There was little to choose between the two weeds in their susceptibility to the treatments used in the work.

The two fungi used in the test programme, whilst not as tolerant of the treatments as the weed seeds, were more durable than either of the invertebrates. Wet heat was again the most effective method of control. *Pythium* was less tolerant of wet heat than *Rhizoctonia*, being controlled by 10 minutes' exposure to 50°C whereas it took 10 minutes' exposure to 60°C to control *Rhizoctonia*. The pattern of *Rhizoctonia* being slightly less susceptible to the treatments than *Pythium* was true for most methods of control. Apart from wet heat, Vikane (sulfuryl fluoride) was also reasonably effective against both organisms, as were some of the disinfectants.

The invertebrates were more susceptible to the control methods used the either the weed seeds or the fungi. Western flower thrips (WFT) was the least resilient organism of all, being susceptible to wet heat, dry heat, microwave energy, both fumigants and at least one disinfectant. Bud & leaf nematode proved more tolerant of treatments than WFT, with the exception of microwave energy to which it appears to be very vulnerable.

The treatment methods

Wet heat was overall the most effective control method. 10 minutes' exposure to 60°C was sufficient to give complete control all of the test organisms with the exception of the bud & leaf nematode, of which a very small number of individuals (0.06%) apparently survived 30 minutes' exposure to this temperature.

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Dry heat was nothing like as effective as wet heat, with a proportion of both fungi and all the weed seeds surviving exposure to 70°C for one hour. Microwave energy controlled both invertebrates well but was much less effective against both of the weed seeds and both fungi.

Of the fumigants, Phostoxin (generating phosphine) was only really effective in controlling the WFT and gave no control at all of either the weeds or the fungi. Vikane however, after 16 hours exposure at the concentrations tested, gave complete control of the fungi and the invertebrates and even reduced the germination of the pearlwort seed by about 25%.

The disinfectants were in general disappointing. GeoSil, FAM 30, Trigene and Virkon S all controlled Pythium after 60 minutes' exposure, and FAM 30 also controlled WFT, but the other organisms were only partially controlled, if at all, by the disinfectants tested. Citric acid and Citrox P were particularly ineffective in these trials.

Overall conclusion

The clear conclusion from this work is that wet heat treatment is likely to prove the most effective method of treating nursery hardware that might be contaminated by one or more of a range of unwanted plant pathogens and weeds. Treatment at 60°C for ten minutes should be sufficient to destroy most pathogens and weeds that might be commonly found on a nursery. Wet heat treatment does not require any approval from the authorities. Wet heat could be applied in a water bath or as an air-steam mixture that supplies the same exposure criteria. If a water bath is used, disposal of the used dip presents no problem.

If weed seed transmission is not a problem on the nursery, then fumigation with sulfuryl fluoride (approved as Profume in the UK) offers an alternative to wet heat treatment as this material seems to be efficient at controlling both fungi and invertebrates. Profume is currently approved for use on structures used for crop handling and storage, but could not, of course, be handled by a grower.

Technology transfer

The results of this work have not been presented to HDC members to date.

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A presentation will be made at a suitable meeting at the request of the HDC.