SOIL DISINFESTATION USING ELECTROMAGNETIC RADIATION IN THE MICROWAVE RANGE

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Project FV 272 **Project Leader: Professor Nick Christofi**

FINAL REPORT

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Report to the Horticultural Development Council

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Grower Summary

The specific objectives of the study were to determine the differential heating effects of microwaves for weed seeds (WS), fungal pathogens (FP), naturally occurring nematodes (NE) and volunteer potatoes, and, their use in destroying soil entities without incurring excessive costs. The main results are:

- Irradiation delivered from a standard domestic microwave working at a frequency of 2.45 GHz determined adequate and sufficient for soil (sandy loam) treatment.
- Microwave treatment for 40-50s with resting period of 3 min was shown to destroy all pests (Weed seeds, nematodes, sclerotia of a fungal pathogen and potato resting stages) within soil layers up to 2.5cm.
- Experiments examining kill at 5cm depth showed a requirement of 90-120s treatment with a resting period of 3 min.
- Killing dependent on water content of soil and its bulk density.
- Lifting, channeling and turning of soil using a conveyer with shielded microwave treatment from above suggested as fastest and most cost effective for the treatment of soils.

Executive Summary

The control of pathogens and pests is an important issue for growers around the world affecting the yield and quality of horticultural products. Sulfuryl fluoride and phosphine (singly and in combination with CO_2), 1.3-D and many others are alternatives to using methyl bromide in soil disinfestation. The use of these in many cases is restricted because of lack of registration and inherent problems. Phosphine, for example, causes corrosion and may not be effective because of resistance by some organisms. A number of treatments are pest specific and will not control other organisms. 1,3-D can be used for nematodes but it has limited action against fungal pathogens and requires a minimum temperature of 15^{o}C for effectiveness. Recent studies have shown that soil steaming (attaining temperatures of 50-60^{o}C) for 3 minutes resulted in 100% kill of weeds, fungal (*Sclerotinia*) spores and nematodes. Steaming has produced very poor results against root knot nematodes and the cost of treatment is approximately double the cost of methyl bromide treatment.

This preliminary work was instigated to determine the role of microwaves in destroying a range of pests and diseases in soils in a controllable way. Microwaves cause water in the soil and cells to absorb heat that results in temperatures that kill soil organisms. The experiments carried out during this study examined the effect of irradiation delivered from a standard domestic microwave working at a frequency of 2.45 GHz with an expected power output of ~1000W. Experiments to determine the optimum frequency to treat weed seeds showed that microwave absorption increased with frequencies up to 3 GHz. Between 3-5 GHz, absorption levelled off and it was decided that using a higher

frequency than that delivered by a standard microwave would not improve heating of the seeds and other constituents of the soil systems used. The study used static soil columns containing resting stages of pests and disease organisms at two depths, 2.5 and 5.0 cm, which were treated with microwave radiation to generate a predetermined temperature to affect complete kills. Originally, it was planned to treat soil to a depth of 10cm but this was modified to take into account the expected maximum depth of microwave penetration of 5cm. An energy penalty would be incurred in treating depths greater than 5cm as heating would be primarily through conduction. Our study showed that using static systems with microwave treatment of the soil surfaces ~100% weed seeds, sclerotia of the fungal pathogen *S. sclerotiorum* resting stages of the common nematode *C. elegans* and volunteer potatoes are destroyed or inactivated. This is achieved with a treatment time of 40-50s with resting period of 3 min for all pests situated in soil at a depth of 2.5 cm. Experiments examining kill at 5 cm depth showed a requirement of 90-120 s treatment with a resting period of 3 min.

Weed, nematode, fungal pathogen and potato resting stages were buried up to a maximum depth dictated by the expected optimum depth of microwave penetration. This had implications with respect to energy input. In dry soils and those with lower percentage water content (50% water holding capacity [WHC]), heating a depth of 5cm of soil was rapid and linear because of reduced water availability to absorb the microwave energy, as indicated by the experiments carried out. The heating to 70^{o}C was highly controllable and would not cause undue physicochemical and biological damage to the soil. It is obvious that soil heating is affected by water content and bulk density. Soils with a higher water content (70% water holding capacity) exhibited differential heating and there were temperature gradients with preferential heating at the surface layers. As a minimum soil temperature of 70^{o}C is required, there would be an energy penalty for heating the surface above the required temperature in soils with high water content. Calorimetry has shown that 0.045 KWh Kg^{-1} (45 KWh tonne^{-1}) soil is needed to raise the soil temperature from 10^{o}C to 70^{o}C and affect kills in the soil utilised with 50% WHC. The value is higher in 70% WHC soil, at 0.058 KWh Kg^{-1} (58 KWh tonne^{-1}).

Changes in total aerobic heterotrophic bacteria and actinomycetes populations following microwave heating showed an 1-2 log reduction but significant populations remained following treatment (>10^{5} CFU g^{-1} dry soil) that should enable recovery.

A possible methodology advocated is not to treat soil *in situ* (because of inhomogeneity of bulk soils and inherent practical, economic and other problems) but to lift and channel soil using a conveyer with shielded microwave treatment from above. The conveyer constantly mixes the soil allowing efficient and effective irradiation of the total mass of soil recovered from a field. Temperature required through the soil would be achieved quicker and more cost effectively. This would allow significantly faster soil processing and possibly using modified existing soil lifting equipment.

1. Introduction

The control of horticultural pathogens and pests is an important issue for growers around the world affecting the productivity of horticultural goods. A recent briefing from the HDC indicated that overall, there are 4 customer/retailer complaints of contaminants per 100,000 units, for the outdoor salad crop. 60% are due to insects, 20% weeds, stones etc and the remaining 20% due to discoloured leaves and other quality defects. The actual number may be 10 times more than this figure as it is estimated that only 10% of customers encountering problems actually complain. Ideas for potential engineering solutions have been sought with a number of suggestions for solving the various problems. This proposal deals with the assessment of microwaves and microwave technology in the destruction of soil-borne pathogenic (micro)organisms and pests in horticultural soils thus improving crop quality and quantity. The use of environmentally unfriendly biocides such as the widely used methyl bromide was banned by the USA in 2001 and is due to be phased out by 2005 in other industrialised countries because of potential human and environmental safety problems, such as effect on the ozone layer. Thus the need for new treatment methods is legislation led with economics also playing a major part, as it is envisaged that savings can be made in the treatment of soils by replacing current chemical methods and those including the use of steam for sterilisation.

Research, funded by the HDC at Aberdeen and Newcastle Universities, utilising low temperature-short duration steam treatment of agricultural soils, demonstrated that soil steaming (attaining temperatures of 50-60^{o}C) for 3 minutes with an eight minute resting period, resulted in 100% kill of weeds, fungal (*Sclerotinia*) spores and nematodes (Van Loenen et al., 2002; 2003). Low temperature steam sterilisation is likely to be difficult to implement and control in field situations and can be very expensive.

Microwaves constitute electromagnetic radiation that is part of the radio frequency range with frequencies above ~1 GHz. Microwaves are able to facilitate heating of non-conducting (dielectric) materials including water, fats/lipids etc. The energy of such electromagnetic radiation is absorbed by dielectric substances and converted to heat. Microwaves penetrate substances to a depth of ~ 5cm with the depth of penetration being determined by their electric properties. In the soil matrix, the heat, generated by the microwave electromagnetic radiation, is spread by conduction just as it is in conventional cooking with water content affecting heat transfer. Penetration depth is sometimes given as the depth at which approximately 63% of the energy has been absorbed. Substances, including seeds and spores, with a high sugar and fat content can heat up quicker. Little work has been done on the energy efficiency of using microwaves over thermal (steam) heating in relevant systems that do not require microwaves to penetrate soil to significant depths to destroy pests and in the process utilizing energy to heat water.

Microwave radiation with a frequency of 2.45GHz (12.25 cm) has been used as a method to sterilise soil. Microwaves cause water in the soil and cells to absorb heat that results in temperatures that kill the soil organisms. For this reason, the lethal action of microwaves

is higher in moist soils than dry soils (Alef & Nannipleri, 1995). Vela and Wu (1979) reported that bacteria, actinomycetes, fungi and bacteriophages exposed to 2.45 GHz microwaves were only destroyed when water was present. Lyophilized organisms were not destroyed by microwaves, as there was no water present to absorb adequate energy to raise the temperature and facilitate killing. Wainwright *et al.* (1980) showed that the microwave treatment of soil destroyed fungi but not heterotrophic bacteria. There is a paucity of information of the efficacy of microwaves in the destruction of resting stages of bacterial and fungal pathogens (that are not free of water in a real soil environment) that destroy horticultural plants and of weeds that interfere with their growth. Microwave treatment of soils at ~55^{o}C is sufficient to kill pathogens but work is required to demonstrate this controllable and potentially a lower cost treatment system for agricultural soils.

Important advantages of microwave heating are that there is rapid heat transfer and possible selective heating. The power is controllable as it can be switched on and off at will and the equipment can be compact. To date microwave testing has involved the delivery of electromagnetic radiation through metal waveguides at the surface of soil treating soils of various depths.

The aim of the project was to determine the efficacy of electromagnetic radiation in the microwave region in affecting the destruction of resting stages of fungal and nematode pathogens, weeds and volunteer potatoes. Ultimately, a cost-effective system may be suggested for the treatment of horticultural soils enabling the effective removal of pests, soil-borne pathogens and other problem entities affecting productivity and quality of crops. A system, if recommended, will be developed using microwave engineering companies and experts through the High Power RF Faraday Partnership and the General Physics Institute.

2. Materials and Methods

2.1. Soil

This is a freely drained sandy loam of the Darvel series obtained from the No. 3 Field at The Scottish Agricultural College (SAC) Bush Estate, Penicuik, Scotland (National Grid Reference NT243640). Batches of the soil were removed by spade from the first 30 cm of the sandy loam horizon to a depth of 20 cm to avoid contamination with the underlying loamy sand horizon. Soil collected was passed through a 10mm sieve to remove large stones and plant debris followed by sieving through a 2mm mesh screen. The soil was subsequently thoroughly mixed and its water content determined by oven drying the soil for 24h. The information on water content was used to adjust soils to 50% and 70% water holding capacity (WHC) for all killing experiments carried out.

Bulk density is a measure of the weight of the soil per unit volume. From the literature the general bulk density of a sandy loam soil is given as ~1.4 g cm^{-3} with a porosity of 48% (data was obtained from the following web site: http://www.cst.cmich.edu/users/Franc1M/esc334/lectures/physical.htm). The bulk density of the soil used in this study was determined by oven drying soil, placing in a volumetric container with tampering to loosely consolidate the soil and weighing the known volume. The bulk density is given by the weight of dry weight of oven-dried soil divided by the volume of that soil. A value of 1.26 g cm^{-3} was determined for the density of the soil used in this study suggesting that the soil was a fine sandy loam. Soil classification and characteristics are presented in Table 1.

Table 1. Soil classification and characteristics (Data from SAC, Penicuik, Scotland)

SAC No. 3 Field Soil Factors	Values
FAO classification	Eutric Cambisol
Soil texture	0 – 30cm sandy loam 30 – 95cm loamy sand >95cm sandy clay loam
pH	6.5
Organic Matter (0-30cm)	4.3%
Soil series	Macmerry
Slope	7 °
Parent material	Partially sorted glacial till
Drainage depth	7.0m
Land Use	arable stubble
Dry matter	7.7%

2.2. Effect of microwaves on soil heating

The effect of microwaves on soil heating at 2.5 and 5.0cm were examined to determine the duration of microwave heating to raise the temperature to the required level to destroy weed seeds and other soil pests and pathogens. This was done for soils with 50% and 70% WHC. 10cm x 10cm x 7.5cm (height) soil columns were prepared of soil with different WHC using microwavable plastic containers (10 x 10 x 10cm Polyethylene dessert containers from Stewart Company, Croydon CR9 4HS) and soil then subjected to irradiation for different time periods using a standard domestic touch control microwave oven with ~1000 watts output (Daewoo model KOR1A0ASL).

To ease experimentation, two polyethylene dessert containers were used nested into each other to enable a two-part soil column to be formed. The upper soil was either 2.5 or 5.0 cm deep and the material for treatment was sandwiched between the two soil layers. Holes were drilled in the plastic containers to enable temperature probes to be inserted for temperature monitoring at the 2.5cm and 5.0cm depths. Each container was filled with soil, moistened to 50% or 70% total WHC and gently tamped level separately at 25mm or 50mm marks using a flat container as the tamper. A diagrammatic representation of the irradiation system used is given in Figure 1. Experiments were carried out to determine the irradiation period required to attain a temperature between 50-80^{o}C. Temperatures of ~50-60^{o}C were found to be sufficient to kill weed seeds and pathogens by steam heating (van Loenen *et al*, 2002). 70^{o}C for 30 min is used for soil sterilisation. The time taken to heat the soil to >70^{o}C at the appropriate depth was used for all disinfestations experiments. Such a temperature was found sufficient to kill/inactivate pests in preliminary experiments using imbibed materials of seeds, spores etc. The higher temperature of ~70^{o}C was chosen for sterilisation because of the difficulty and variability in monitoring temperature in microwave soil systems where it was not possible to retain probes *in situ*. There was variability in values caused by insertion of probes and presence of voids in the heterogeneous environment. This is highlighted in Appendix 1 showing temperatures monitored at the different soil depths irradiated for different time periods ranging from 50-120s.

2.3. Direct microwave irradiation of biological material

Seeds of *E. repens* and *C. album* were distributed to 90mm polystyrene Petri dishes lined with 85mm Whatman 1 filter paper at 20 seeds per plate. Half of the seed plates were moistened with 2ml tap water and allowed to soak for 24h with the remainder immediately irradiated in triplicate with microwave energy for various time intervals in a Pyrex dish to contain any spillage of the water microwave load. Following microwave treatment, the seed plates were moistened with 2ml tap water and allowed to grow for 14 days prior to growth assessment. Seeds soaked for 24h were irradiated with microwave energy for the same times as the dry seeds before being allowed to grow for up to 14 days as above.

Imbibed weed seeds and sclerotia were prepared by placing in single layers on 2x 85mm Whatman No. 1 filter papers and moistening with tap water, covering with a third 85mm paper moistened with water and allowing to soak for 24h at room temperature.

Seed etc. pockets, comprising a monolayer of moistened material for treatment and wrapped using a single piece of PE film, were spread over a 40mm x 30mm area. Pockets were placed beyond a line 20mm from the edge of the soil in the container in a central position. The imbibed materials were irradiated in the microwave oven with the soil columns being placed in a Pyrex dish indexed so that the output of the magnetron horn waveguide was directed at the upper container soil surfaces maintained in the same position for all experiments.

Each killing experiment for weed seed and pathogens was conducted as follows:

a) The containers were irradiated for the appropriate time period determined to raise the soil temperature to ~70^{o}C with a further retention in the soil for up to 3min (resting period).

b) One package of seeds etc. was removed immediately and chilled on a cool glass surface and overlaid with an aluminium planchette filled with 10ml of cold tap water.

c) The temperature probe was inserted from the side for 50mm and temperature measurements continued for a further 3min at which time the second seed package was removed and chilled as in b) above. In some experiments, pockets of seeds etc were removed at intermediate times to determine minimum treatment times for killing.

d) The treated samples were then assessed for viability.

2.4. Weed Seeds

Seeds of *Elymus repens* and *Chenopodium album* were obtained from Herbiseed, New Farm, Mire Lane, West End, Twyford, RG10 0NJ.

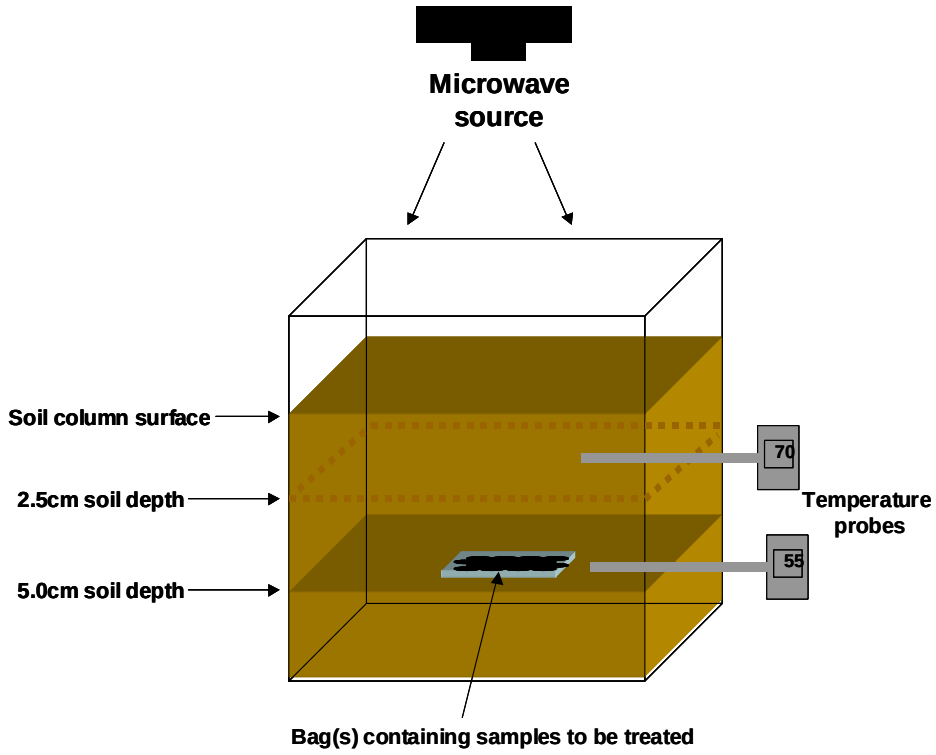
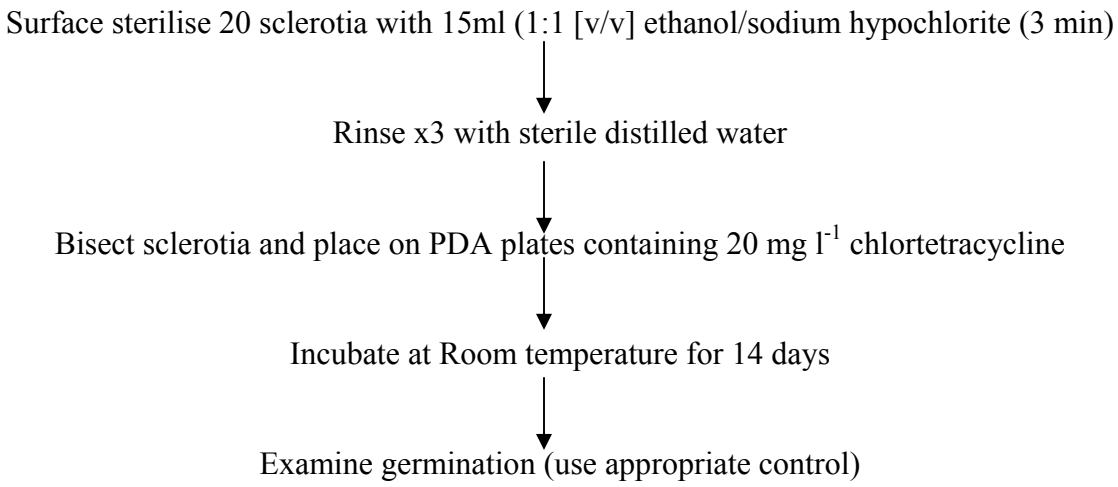


Figure 1. Diagrammatic representation of the soil irradiation experimental system

2.5. Fungal pathogen

The sclerotia (spores) of *Sclerotinia sclerotiorum* (Lib) de Bary were used in microwave killing of the fungus. The sclerotia used in these experiments were a kind gift from Dr John Clarkson, Horticultural Research Institute. Isolation of the fungus was also attempted by growing cultures on sterile wheat grain (Mylchreest and Wheeler 1987) for 3 weeks to produce sclerotia. After production, the sclerotia were separated from the wheat followed by washing, air drying and sieving to retain sclerotia of size 2-4 mm. A freeze dried culture of *S. sclerotiniorum* (IMI 101354) was obtained from CABI Biosciences, Bakeham Lane, Egham, Surrey TW20 9TY (formally International Mycological Institute- IMI). The sclerotia can be stored for up to 1 year.

Sclerotia viability was assessed as follows:



Sclerotia were moistened 24h prior to use. Moistened sclerotia were packaged in monolayers in cling film pockets and irradiated in the soil columns for the desired time periods. Non-irradiated controls and the irradiated sclerotia were separately surface sterilised for 3min in 15ml 1:1 (v/v) ethanol: sodium hypochlorite (4-15% available chlorine) followed by three rinses in sterile distilled water (Whipps and Budge, 1990). Sclerotia were then bisected using aseptic technique and each half placed carefully on a PDA+ plate at 15 bisected sclerotia per plate. Viability of sclerotia was assessed by microscopic examination and by eye. Live sclerotia produce a close white hyphal mass growing from the bisected sclerotia.

2.6. Nematode worms

Nematodes present in soil can have a beneficial or detrimental effect in horticulture. They are abundant in soils and many perform important roles in nutrient recycling living in the pore water and feeding on bacteria. A number are associated with pathogenesis including those feeding and causing diseases of plants and horticultural products, such as root-knot nematodes with more than 1000 host species, and potato cyst nematodes. A range of chemical treatments are available to control these pests embracing volatile (e.g. methyl bromide, dichloropropane) and non-volatile (e.g. sodium N-methyldithiocarbamate, 2-methyl-2-[methylthio] propionaldehyde 0-[methylcarbamoyl] oxime) nematicides.

The nematode *Caenorhabditis elegans* was chosen for experiments of this study. This free-living soil nematode is able to produce a non-feeding, almost dormant form known as the Dauer stage that is resistant to dessication and extremes of temperature and is effectively non-aging. The Dauer stage was used in killing experiment using microwaves.

A culture of the nematode *C. elegans*, maintained on a bacterial lawn of *Escherichia coli* (OP50), was obtained from Peter Geldhof, Moredun Institute, Edinburgh Technopole, Penicuik, Midlothian. *C. elegans* were cultured in sterile 90-mm disposable Petri dishes of nutrient agar inoculated with a lawn of *Escherichia coli* OP50, a uracil deficient strain. The use of a strain with a nutritional deficiency ensured that bacterial cell numbers were low enough that Dauer larvae were generated. Plates were inoculated and incubated at 37ºC overnight prior to use.

M9 buffer was used to handle worms in bulk without the osmotic stress that would be imposed by using sterile water. M9 buffer (Brenner 1974) contained Na_2HPO_4, 6.0 g; KH_2PO_4, 3.0 g; NaCl, 5.0 g; $MgSO_4.7H_2O$, 0.25 g; distilled H_2O 1000 ml.

Alkaline hypochlorite solution for use in the preparation of synchronized cultures was prepared by mixing Clorox bleach or equivalent (4-6% sodium hypochlorite; 2.0 ml) with 1 molar NaOH solution (1g in 25 ml distilled H_2O; 5.0 ml). Solutions were stored separately and mixed just before use.

Ficoll 400 is used for the preparation of Dauer larvae. This solution is diluted to 15% w/v) in 0.1 molar NaCl.

The plates of C. *elegans* were incubated at 20°C in a closed container lined with absorbent paper dampened with water to reduce evaporation. To maintain the stock culture, nematodes were transferred to a fresh lawn of *E. coli* OP50. This was done by pipetting 1-2 ml M9 buffer onto the surface of the existing stock culture, shaking gently in order to suspend the worms in the buffer and pipetting the suspension onto the new plate. A fresh culture of nematodes was used for each test. Three cultures were kept at a time to ensure that sufficient individuals were available for all the tests.

The Dauer resting larvae were used in killing experiments. Dauers were prepared from a nine-day culture. The worms were suspended in 3 ml M9 buffer from which 1 ml was removed and pipetted on top of 2 ml of Ficoll suspension in a 15 ml centrifuge tube. Care was taken not to avoid mixing of the two layers as the buffer floats on the Ficoll. The tube was left for 10 min, during which time the active adults migrated to the upper surface, while the relatively immotile Dauer larvae sank into the lower layer. The top layer was removed and the Dauer larvae washed three times with distilled water by centrifuging at 2000 rpm for 2 min and pouring off the supernatant.

0.1ml aliquots of the Dauer suspension were pipetted carefully into three Eppendorf PCR tubes for each of three experiments and 0.2 ml M9 buffer was added to each PCR tube with gentle mixing. For the irradiation experiment 0.1 ml of suspension was pipetted into each of three further Eppendorf PCR tubes with two placed at the appropriate soil depth. After assembly and irradiation of the soil column, one PCR tube was withdrawn and cooled quickly in water. The other tube was removed 3 min into the resting period.

The viability of the Dauer larvae was assessed on a Neubauer counting chamber with movement of the larvae scored as viable. This was based on the observation that if the control Dauers were stimulated physically i.e. by shear forces during manipulation the nematodes reacted by jerking/wriggling movements whereas the dead animals did not.

2.7. Volunteer Potatoes

Volunteers are potatoes remaining in soils following harvest that ultimately sprout in the subsequent year in fields used to grow other crops. Volunteers represent a problem that seems to be on the increase due to less severe weather conditions allowing overwintering of the potatoes, and, other factors. They can act as hosts harbouring pests such as late blight, potato cyst nematodes and other diseases that spread to healthy potato crops. Their growth in subsequent years can smother and reduce yield of rotation crops. Volunteer potatoes are very difficult to control. Effective strategies embrace an integrated approach. The most effective long-term volunteer control appears to be provided by the use of the herbicide glyphosate applied pre-harvest and during active growth of the volunteers. However, there is rarely total control of this pest (Turley, 2001). It has not been possible to find any work using microwave treatment to destroy volunteer potatoes and their associated problems.

In order to determine microwave effects on volunteer potatoes, seed potatoes were used. These were a kind gift from Mr Gordon Smillie, Caithness Potatoes Ltd., 26 York Place, Perth PH2 8EH, Scotland. It was suggested that we treated varieties Maris Piper and Saturna, but these were not available at the time of testing so varieties Nadine and Pentland Javelin were supplied and used in the experiments. Eyes from the seed potatoes were cut out as approx. 1cm cubes, the eye being central to one side of the cube. These were placed at the two soil depths of 2.5 and 5.0cm and irradiated for the prescribed time periods. Untreated and treated potatoes were checked for viability by attempting to grow the segments under black plastic covers on the surface of moist soil.

2.8. Microbiological media

The media used in the experiments to culture organisms are given in Appendix 2.

2.9. Calorimetric Measurements

Calorimetric measurements of the power released in the soil were performed. The equations a-e below were used to ultimately calculate the specific heat of the sandy loam. The values for the different WHC soils were utilised in the calculation of energy required to raise the temperature to that required to affect killing of weed seeds and soil-borne pathogens.

In the equations, Q_w and Q_s denote power absorbed by water and soil; m_w and m_s are the mass of water and soil; c_s and c_s the specific heat of water and soil; W is the energy in

Watts and ΔT is the change in temperature following irradiation for a given time (t). The specific heat of water is 1 calorie/gram °C = 4.186 joule/gram °C which is higher than any other common substance. The energy scattered by the chamber walls was ignored.

(a) $Q_w = c_w \cdot m_w \Delta T_w$

(b) $Q_s = Q_{w(s)} - Q_w$

(c) $W = \frac{Q}{t}$

(d) $Q_s = c_s m_s \Delta T_s$

(e) $c_s = \frac{Q_{w(s)} - Q_w}{m_s \Delta T_s}$

3. Results

3.1. Soil heating Experiments

Figure 2 shows the variations in temperature at the two depths following microwave radiation for different time periods. It is seen that soil with WHC 50% shows much more rapid and linear heating that relates to reduced absorption by the lower water content and ease of penetration of microwaves. Soils of higher water content (70% WHC) also exhibited linear heating with heating facilitated by conduction. The higher energy absorbed by the water meant longer heating periods for soil at the 5.0cm depth. The initial soil temperature obviously affects the time required to raise the temperature of the soil to that required. More energy was needed for soils with starting temperatures of 6-8^{o}C (refrigerated soil) that that at 24-26^{o}C. This data can be used to determine the duration of heating. For disinfection, it was decided to use a treatment time that would raise the temperature to between 70 and 80^{o}C in soils with different WHC.

Figure 3 shows the change in soil temperature at different depths following irradiation for 40s (soil at 50% WHC) or 110s (soil at 70% WHC). Following irradiation the *in situ* temperature of the soil was monitored for up to 300s. Irradiation of between 40-50s was found to be effective in raising the temperature of soil at 2.5cm depth and with 50% WHC to that desired. There was variability in temperatures attained caused by soil discontinuities. In some experiments, soil at 2.5cm depth did not heat up as well as that at 5.0cm and this could be due to cooling effects and other undetermined factors (Fig. 3). To heat soil with 70% WHC to the required temperature range, a minimum treatment period of 90s can be used.

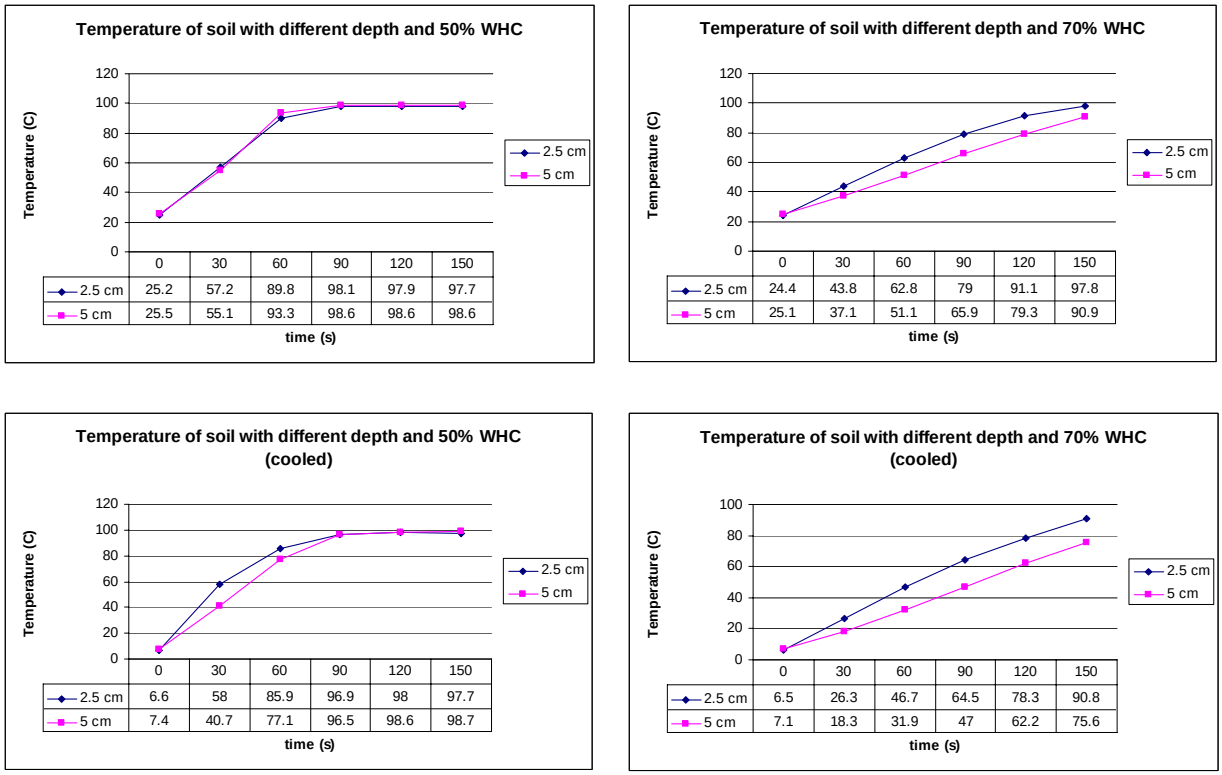


Figure 2. Changes in temperature of soil with WHC 50 and 70% at depths of 2.5 and 5.0cm after microwave irradiation for different time periods. Starting soil temperatures were 6-8^{o}C for cooled (refrigerated) soil and 24-26^{o}C for soil kept in the laboratory.

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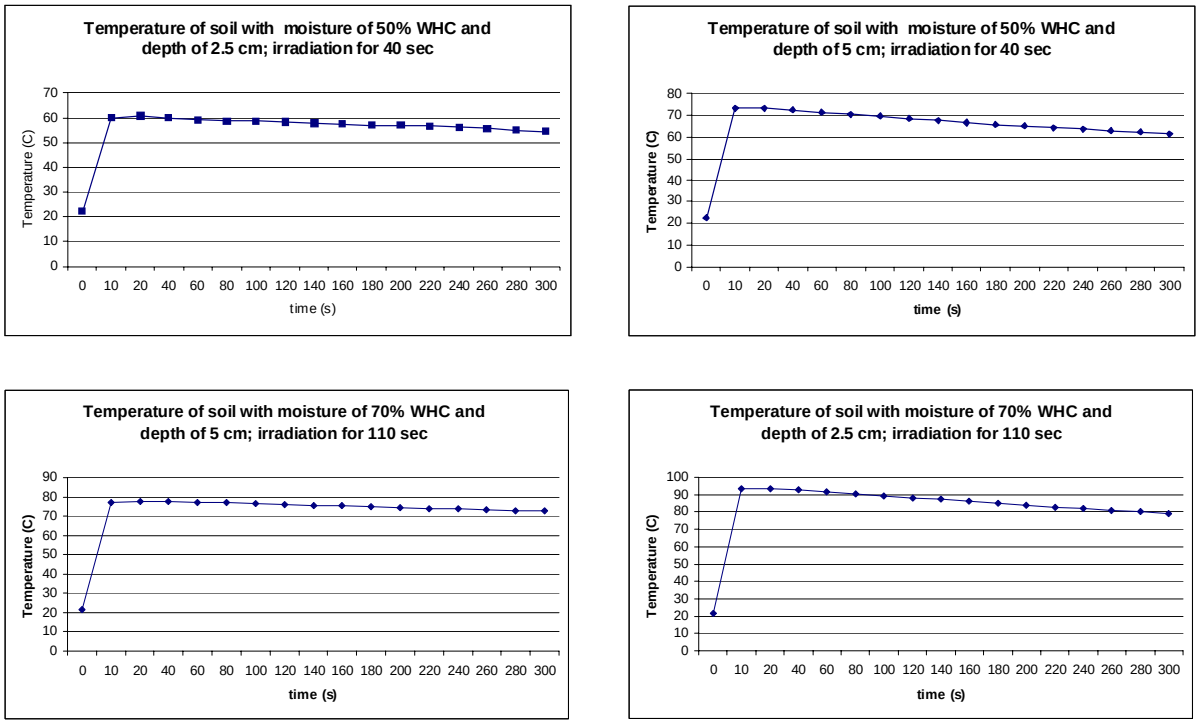


Figure 3. Temporal variation in soil temperature at depths of 2.5 and 5.0cm following microwave irradiation of soil with 50% & 70%WHC. Measurements started 10s after irradiation period. First data point in each graph represents starting temperature.

3.2. Microwave Measurements on weed seeds

The measurements made are to ascertain whether or not there is a best microwave frequency for heating each of the seed types. This information could be important in the design of commercial weed eradication system. The system used to make the loss measurements is a broadband, relatively frequency insensitive holder into which the seeds can be placed. The holder is effectively an aluminium box, which has a suspended flattened conductor running through it as shown in Fig 4.

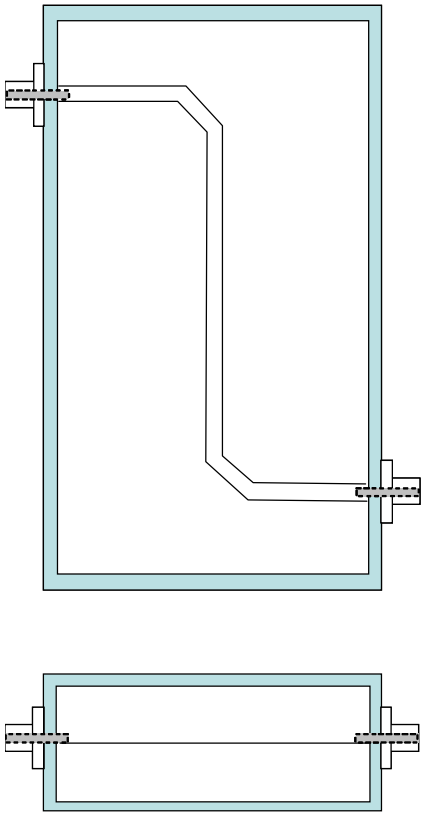


Figure 4 Sample Holder

The electric and magnetic fields associated with microwave energy propagate in the material around this centre conductor. If there are particular frequencies at which the molecules or cells of the medium are particularly lossy, that is, they absorb energy; they will show up as giving either a greater reflection loss or a greater transmission loss through the sample holder. A block diagram of the transmission system is shown in Fig. 5.

Procedurally for the transmission measurement the sample holder is first measured empty and the “normal empty transmission” is stored. This is done so that the effect of the seeds can be established.

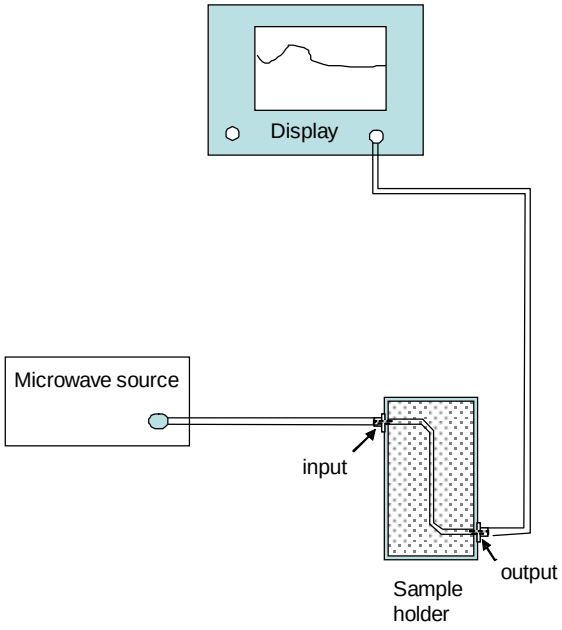


Figure 5 Transmission measurement

Similarly for a reflection, measurement can be made from the short circuited holder. Having a short circuit at the output means that the microwave signal will pass both ways through the holder before the measurement is taken. The system is illustrated in Fig 6. The reflection from an empty holder terminated in a short circuit at its output is stored as the reference level. Any loss will therefore be easier to measure.

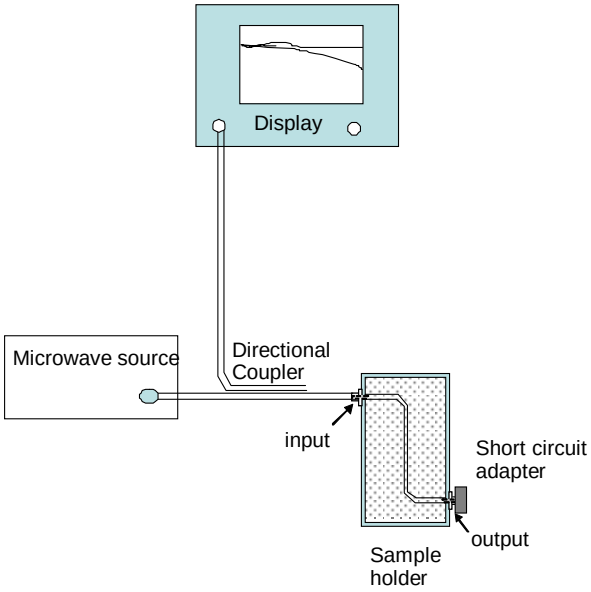


Figure 6 Reflection

The measurements were made over a frequency range of 10 MHz to 5GHz which includes the "normal microwave cooking" frequency of 2.45GHz and is marked on the graphs.

In obtaining the results the sample holder was filled with each type of seed in both ambient dry and wet conditions. The wet condition is obtained by imbibing the seeds over night.

The results in Fig 7 and 8 show that in both cases there is much more transmission (red line) and reflection (blue line) loss for wet seeds than dry ones. This is to be expected since water is a lossy material at microwave frequencies. Interestingly the dry results show that *E. repens* has very little loss over the entire frequency range. Neither seed type shows a specific characteristic loss peak either wet or dry. The peaks on the transmission and reflection characteristics are associated with multiple reflections within the sample holder when it contains the seeds, and is a function of the longest straight section of the centre conductor and the dielectric constant of the seeds.

At the frequency at which the high power experiments are done (2.45 GHz) *C. album* absorbs almost 3dB more than *E. repens*, in the transmission mode, in both wet and dry states. This equates to absorbing twice the power. This type of seed should therefore heat up more quickly than the *E. repens* and thus require less energy to eradicate. Indeed this is seen to be the case as *C. album* loses viability quicker than *E. repens*.

The reflection characteristics are slightly more difficult to interpret due to the influence of the impedance mismatch created by the seeds in the holder. The effect is to produce

multiple reflections that can interfere constructively (peaks) and destructively (nulls). For these traces it is the average that is important. Up to about 3GHz they all show the same trend as the transmission characteristic in that the absorption increases with frequency. Between 3 and 5 GHz the absorption seems to level off. This indicates that using a higher frequency would not improve heating of the seeds and that operation at 2.45 GHz is in this case almost optimal.

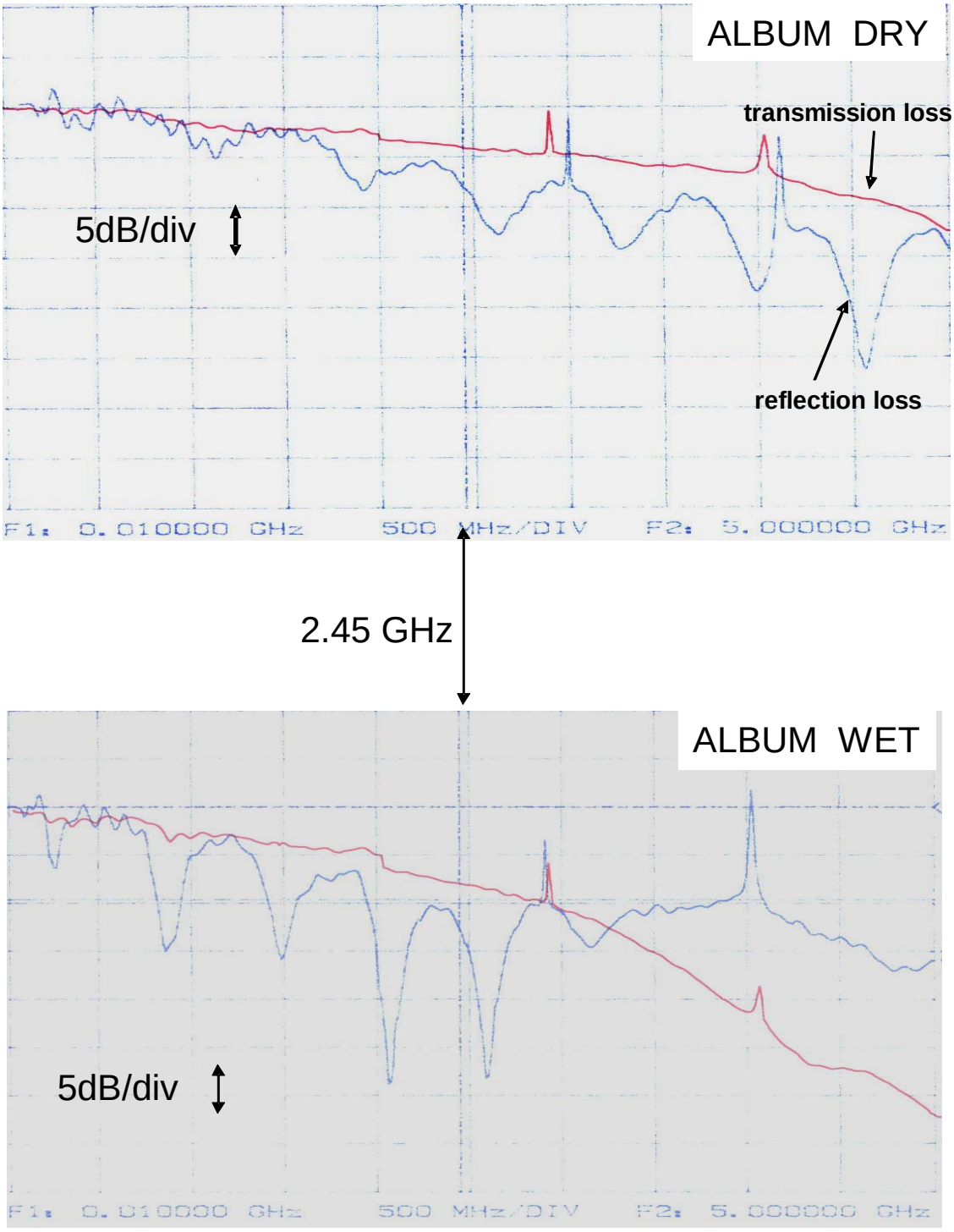


Figure 7 Transmission and return loss measurements on dry and wet Album

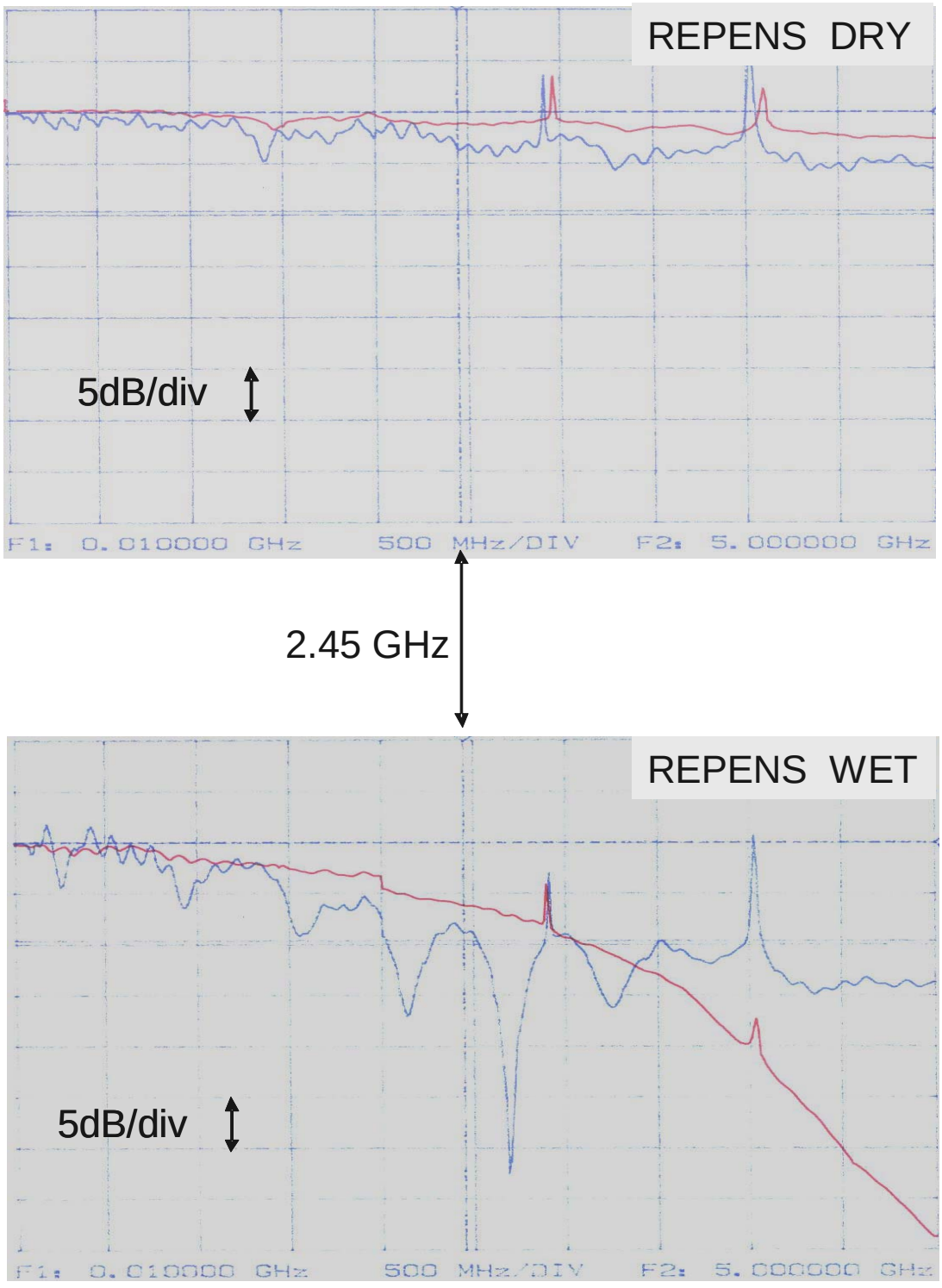


Figure 8 Transmission and return loss measurements on dry and wet Repens

3.3. Direct irradiation of dry and imbibed seeds

The results of direct irradiation of dry seeds and seeds moistened prior to treatment are presented in Tables 2-4.

E. repens	Live / Total ratio						
	Plate1	Plate2	Plate3	*Plate4*	*Plate5*	Mean	+/-SD
Irradiation Time (seconds)							
10	0.36	0.75	0.55	-	-	0.55	0.20
20	0.52	0.35	0.4	-	-	0.42	0.09
30	0.75	0.4	0.5	-	-	0.55	0.18
60	0.4	0.67	0.55	-	-	0.54	0.14
100	0.7	0.65	0.75	-	-	0.70	0.05
180	0.30	0.36	0.65	0.45	0.15	0.45	0.17
300	0.75	0.50	0.50	0.40	0.53	0.54	0.13
Control (not irradiated)	0.80	0.70	0.60	-	-	0.70	0.10

Table 2. Direct Microwave Irradiation of dry *E. repens* seeds

C. album	Live / Total ratio						
	Plate1	Plate2	Plate3	*Plate4*	*Plate5*	Mean	+/-SD
Irradiation Time (seconds)							
10	0.65	0.70	0.40	-	-	0.58	0.16
20	0.45	0.50	0.45	-	-	0.47	0.03
30	0.45	0.45	0.50	-	-	0.47	0.03
60	0.45	0.45	0.55	-	-	0.48	0.06
100	0.55	0.45	0.45	-	-	0.48	0.06
180	0.15	0.25	0.20	0.30	0.20	0.22	0.06
300	0.25	0.20	0.25	0.15	0.20	0.21	0.04
Control (not irradiated)	0.55	0.60	0.55	-	-	0.57	0.03

Table 3. Direct Microwave Irradiation of dry *C. album* seeds

C. album	Live / Total ratio				
	Plate1	Plate2	Plate3	Mean	+/-SD
Irradiation Time (seconds)					
10	0.05	0.1	0	-	-
20	0	0.2	0	-	-
30	0	0	0	-	-
60	0	0	0	-	-
100	0	0	0.1	-	-
Control (not irradiated)	0.55	0.60	0.55	0.57	0.03

Table 4. Direct Microwave Irradiation of imbibed C. *album* seeds

E. repens seeds that were moistened for 24h failed to germinate and grow following irradiation with microwave energy irrespective of exposure time from the minimum of 10s upward. With dry *E. repens*, the viability of the seeds was not significantly affected by the exposure time up to 300s (Table 2). Dry *C. album* seeds (Table 3) showed the same viability as the control non-irradiated seeds up to 100s exposure, with longer irradiation exposures reducing the seed viability by approximately 50%. The fact that the viability of *C. album* is easier to reduce is borne out by the microwave measurement experiments showing that dry (and wet) seeds of this weed absorb more microwave power. One or two of the seeds moistened prior to irradiation survived the exposure to grow (Table 4) possibly because they had failed to absorb adequate moisture prior to irradiation. There was a general viability problem with weed seeds as it was evident that a high percentage of untreated seeds were non-viable. The results confirm that water is an essential component in killing seeds by microwave irradiation but that other factors play a role possibly including sugar and lipid content.

3.4. Weed Seed destruction- Soil irradiation

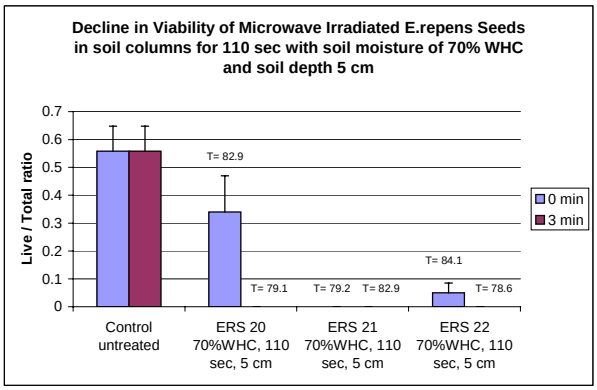
Figures 9 and 10 show the results of treating seeds of *E. repens* and *C. album* buried at two different depths and treated for different time period to attempt to achieve a temperature of >70^{o}C. The soils containing the seeds were maintained at WHC of 50% and 70% and treatment duration was chosen based on the soil heating experiments in Section 3.1 above. Following treatment, the temperature attained in the soil at the required depth was monitored within 10s and subsequently recorded after 3 min of resting period. Pockets of seeds were removed for cultivation after the initial treatment period and again after the 3 min resting period. The temperatures attained are presented above each viability histogram.

In the initial experiments with *E. repens* placed at 5cm depths in soil with 70% WHC and treated for 90-120s, the temperatures attained immediately following treatment for different systems varied widely and it was not possible to kill 100% of the seeds even after a 3 min resting period. In experiments where the temperature was maintained above 70^{o}C (Fig. 9a&b), 100% kills for the seeds were only obtained following the 3 min resting period. Reaching and maintaining temperatures of <70^{o}C in the main were insufficient to kill the seeds (Fig. 9c). *E. repens* in soils of 50% WHC and at depths of 2.5cm were inactivated following a 3 min resting period after the initial 50s irradiation (Fig. 9d).

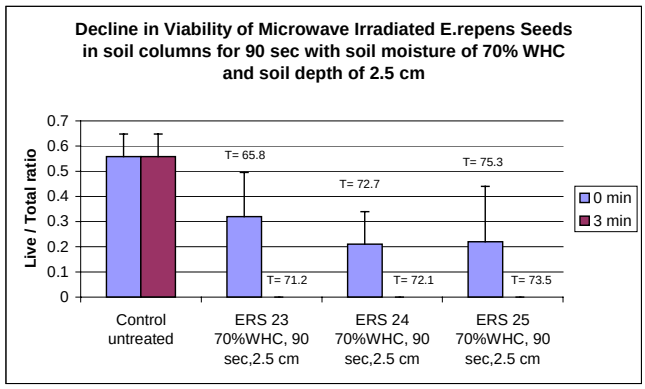
Similar results were obtained for *C. album*. The seeds were inactivated when placed at 2.5cm and 5.0cm depths in soils with 70% WHC and irradiated for 90 or 110s respectively (Figs. 10a-b). In some experiments seeds appeared to be killed at temperatures of just below 70^{o}C. Data (Fig. 10c), however, also show incomplete killing at <70^{o}C with 100% kill only achieved consistently at temperatures maintained above 70^{o}C. Seeds in soils with 50% WHC were killed following irradiation for 50s both at 2.5cm (Fig. 10d) and 5.0cm depths.

In all experiments carried out it was found that there were inconsistencies in the temperatures attained following full power microwave treatment for the set durations

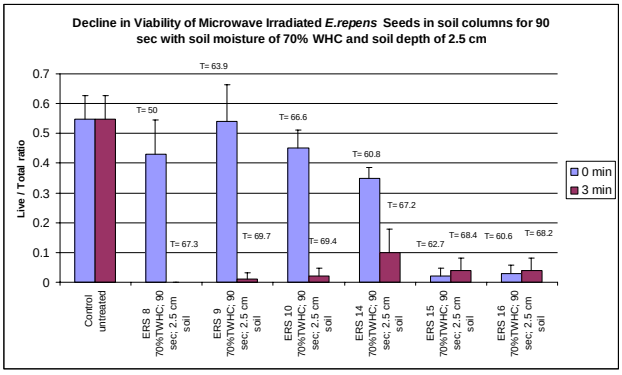
imposed at the different depths. This was caused by the inhomogeneity of the soil columns set up at the different WHC and problems in the mode of measurement of temperature with the probes.



(a)



(b)



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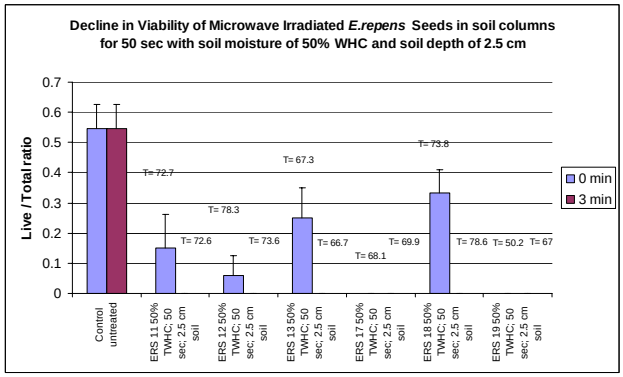
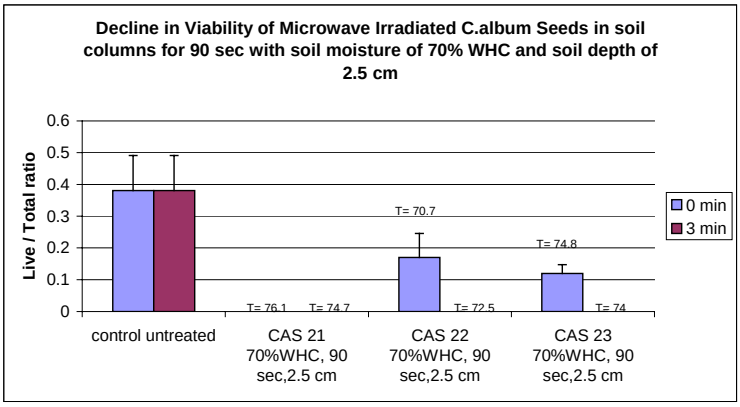
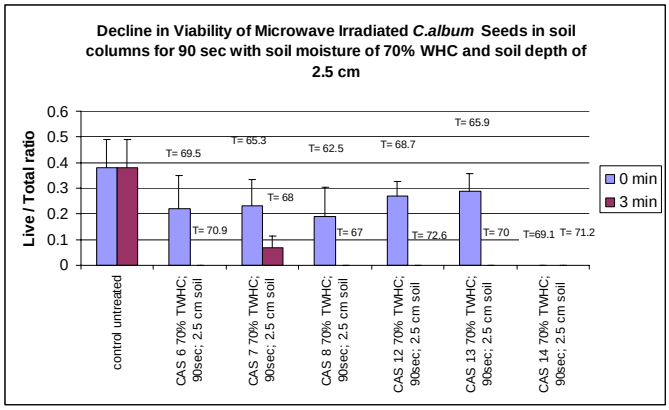


(d)

Figure 9. Effect of microwave treatment on viability of *E repens* seeds placed in soils with different water content at two depths and for various time periods



(a)



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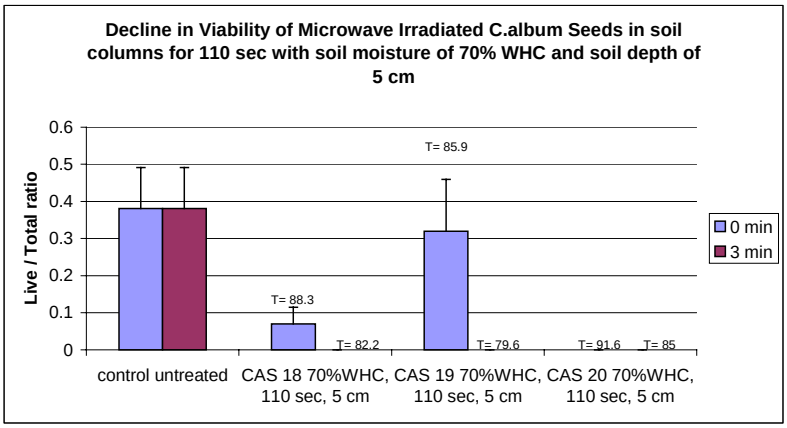

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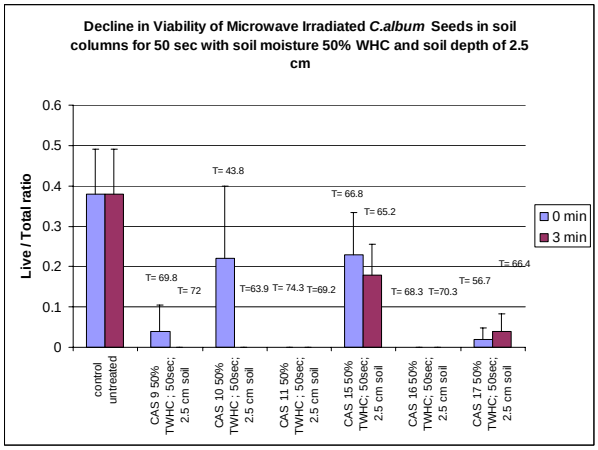

(d)

Figure 10. Effect of microwave treatment on viability of *C. album* seeds placed in soils with different water content at two depths and for various time periods

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3.5. Fungal Pathogen

Figure 11 shows the results of the killing of fungal spores (sclerotia) of the pathogen *S. sclerotiorum*. All experiments whereby sclerotia were placed at the two different depths in soils with WHC of 50 and 70% gave 100% kill following irradiation for the treatment times of 50s and 110s (for sclerotia buried at 2.5cm and 5.0cm respectively). Plate 1 shows the germination pattern of control sclerotia and lack of germination of those irradiated for 50s at a soil depth of 2.5cm.

3.6. Nematodes

The effects on the viability of the Dauer stage of *C.elegans* elicited by microwave irradiation in soil burial experiments is presented in Figs 12 – 15 from which it is evident that with a WHC of 50% and soil burial depths of 2.5cm and 5cm and with 2.5cm depth at 70% WHC 100% killing of the worms was achieved over a temperature range from 58 – 78 ^{o} C the preponderant temperature range achieved being in the 65 - 75 ^{o} C.

With 5cm soil depth and 70%WHC a very small minority of worms survived the irradiation trials that may have to do with the 5cm penetration depth of the 2.45 GHz radiation that would give uncertain irradiation owing to the variability of the soil in terms of chemical and air space geometry. Soil heating at this depth would therefore appear to be radiated by thermal conductivity rather than direct conversion of microwave energy into heat *in situ.*

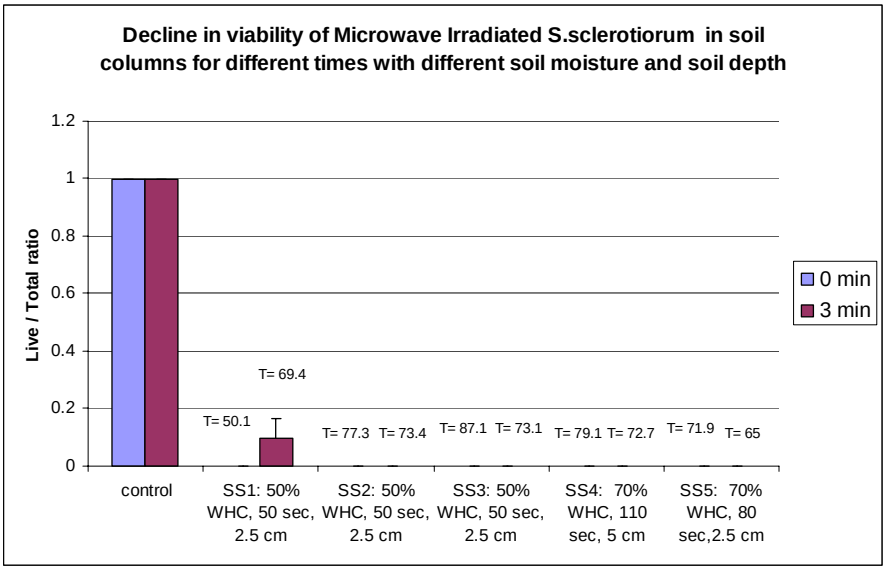

Figure 11. Decline in viability of imbibed sclerotia of *S. sclerotiorum* buried in soils with 50% and 70% WHC and irradiated for 50 or 100s.

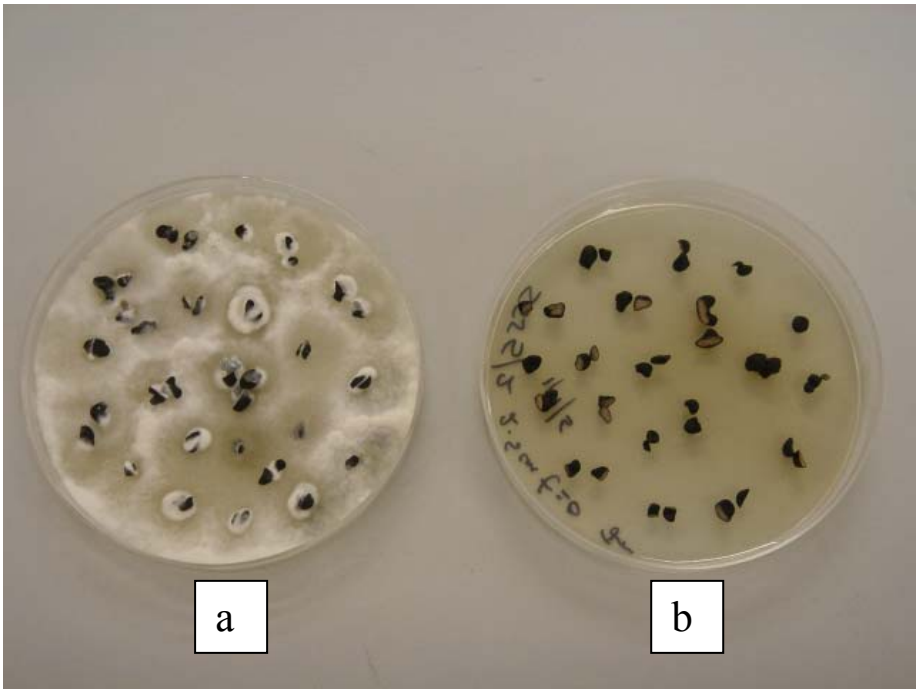

Plate 1. Germination pattern of control sclerotia (a) and lack of germination of those irradiated for 50s at a soil depth of 2.5cm (b).

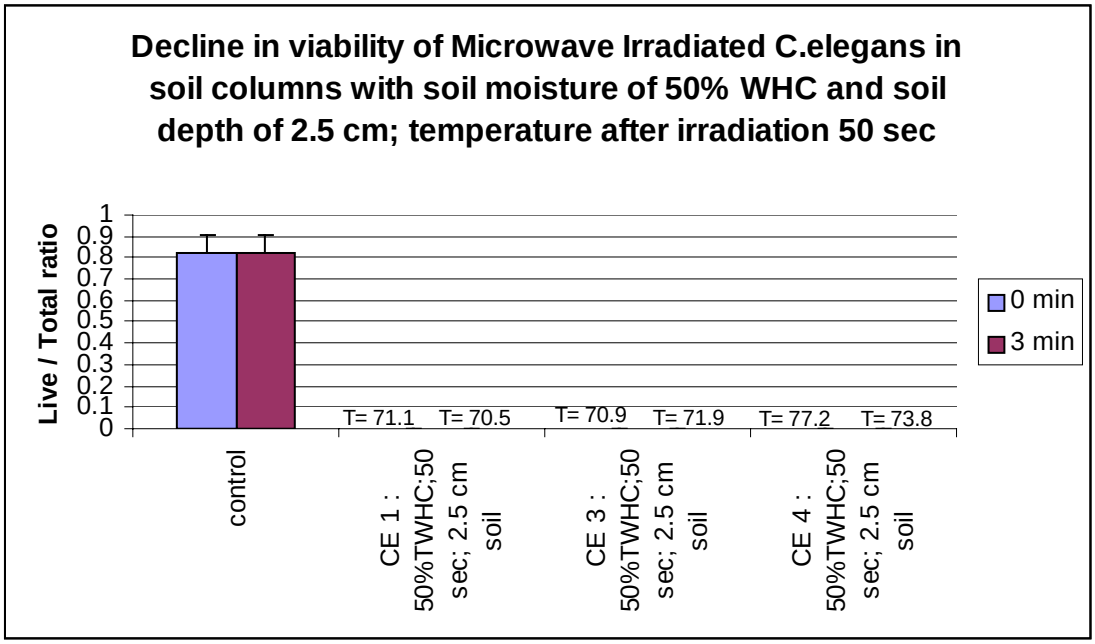

Figure 12. Effect of 50s microwave irradiation on viability of *C. elegans* placed at 2.5cm depths in soil maintained at 50% WHC.

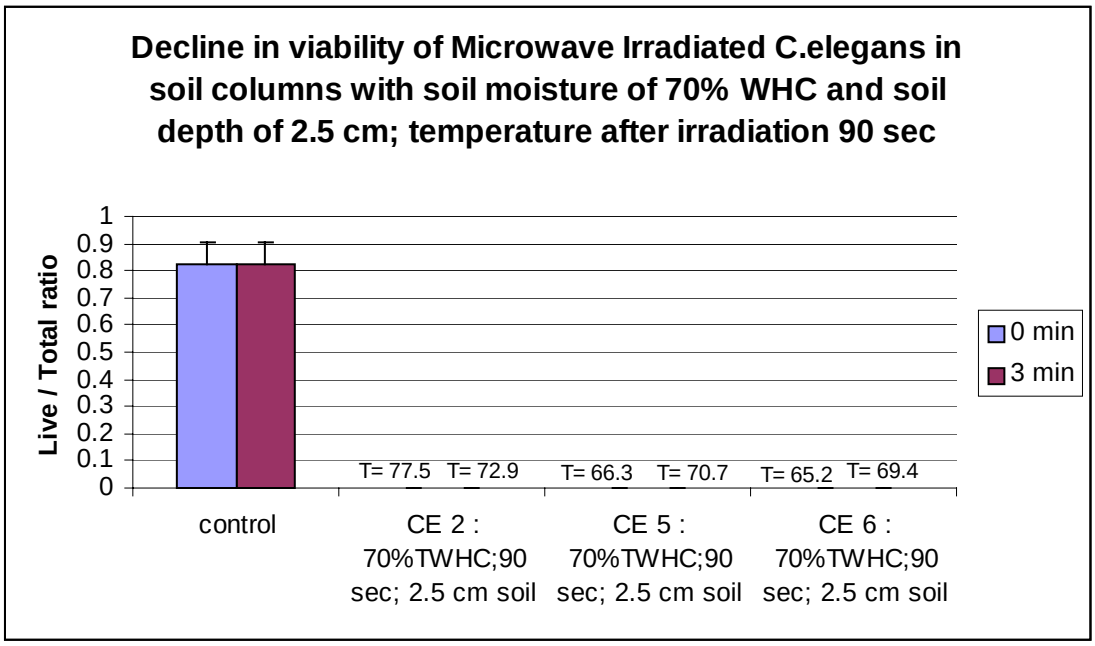

Figure 13. Effect of 90s microwave irradiation on viability of *C. elegans* placed at 2.5cm depths in soil maintained at 70% WHC.

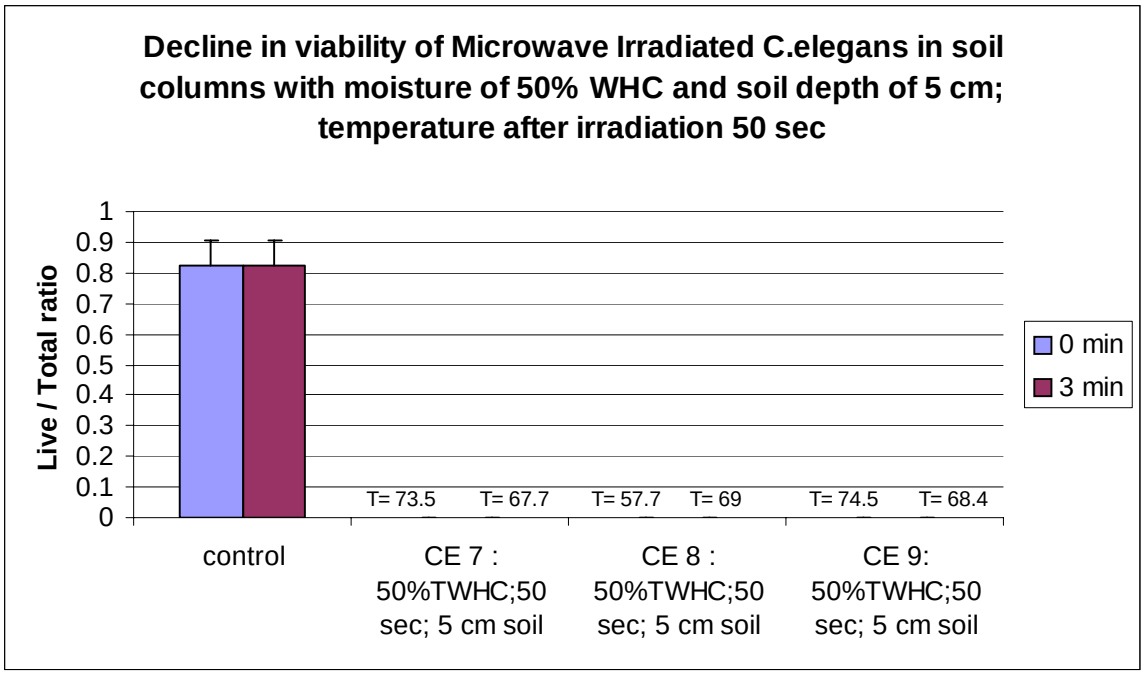

Figure 14. Effect of 50s microwave irradiation on viability of *C. elegans* placed at 5.0cm depths in soil maintained at 50% WHC.

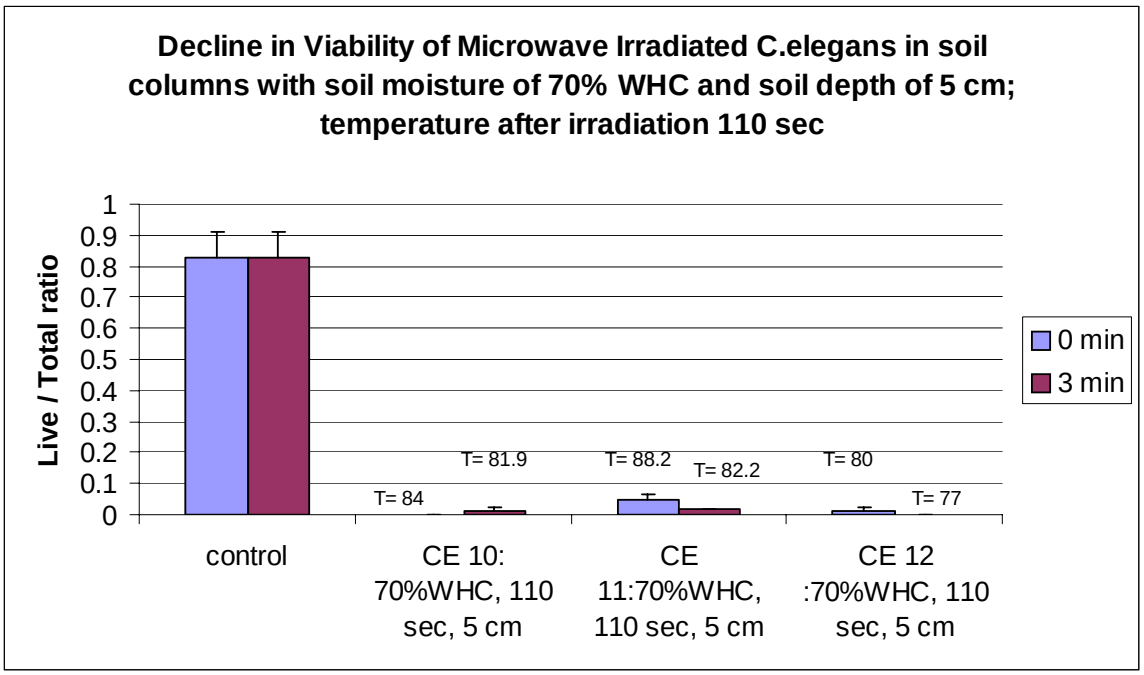

Figure 15. Effect of 110s microwave irradiation on viability of *C. elegans* placed at 5.0cm depths in soil maintained at 70% WHC.

3.7. Volunteer Potatoes

The effects on the viability of potato eyes cut from two varieties of seed potatoes, Pentland Javelin and Nadine, elicited by microwave irradiation in soil burial experiments are presented in Plates 2-5.

With potato eyes at 2.5 and 5cm soil depth and WHC set at 50% and 70% all the irradiated eyes were killed irrespective of the resting time post irradiation at which they were withdrawn from the soil mass. In treated potatoes there was rapid fungal colonisation and mycelium development on the potatoes placed on soil with ensuing degradation of the tuber material. Plant shoots develop from the eye of control potatoes planted on soil and no fungal colonisation of the potato is observed. Generally, therefore potato volunteers can be destroyed quickly with the initial microwave soil-heating period without the need for a resting period at the temperature achieved. It is believed that a much shorter irradiation regime and hence lower temperatures would be sufficient to destroy the potatoes.

Since the withdrawal of potato eyes from the irradiation system was required to be carried out separately for each post irradiation time, the highest temperatures were recorded when eyes were withdrawn at t=3min. Eye withdrawal from the soil mass at other times during the resting period caused too much soil disruption for the temperatures recorded to be meaningful. They were generally 10-15^{o}C lower than those when eyes were withdrawn at t=3min post irradiation. Very high post irradiation temperatures were recorded (over 90^{o}C in one instance), illustrating that potatoes, which have a high water content, concentrate microwave energy which would explain, in part, the higher temperatures reached in these experiments.

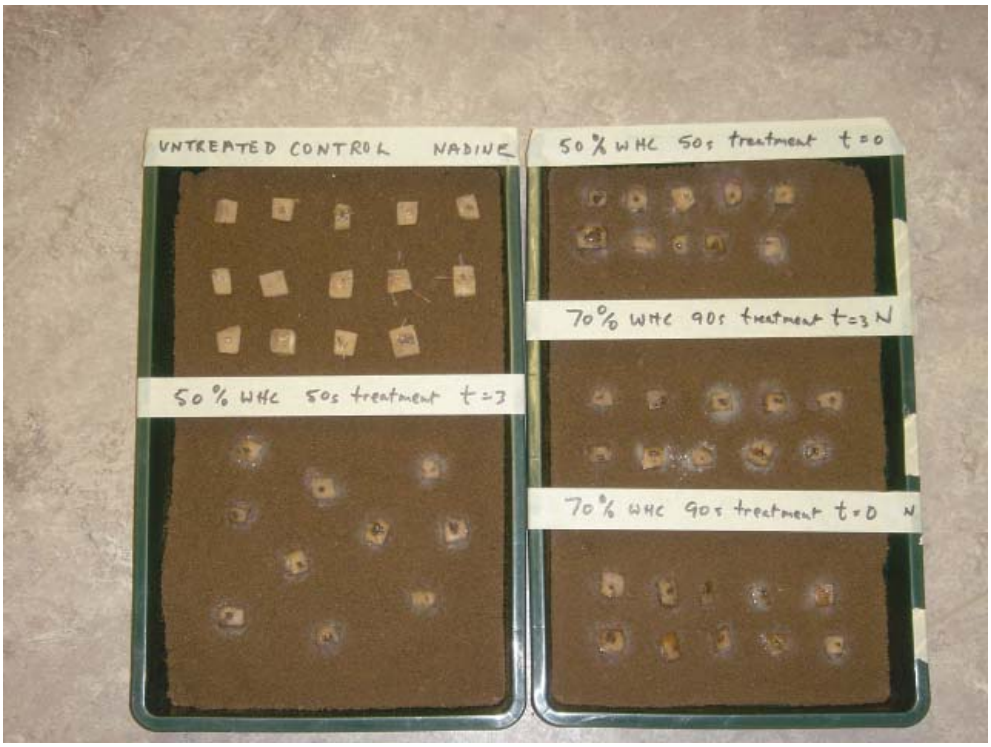

Plate 2. Effect of 50s and 90s microwave irradiation on viability of Nadine tubers with soil maintained at 50% and 70% WHC.

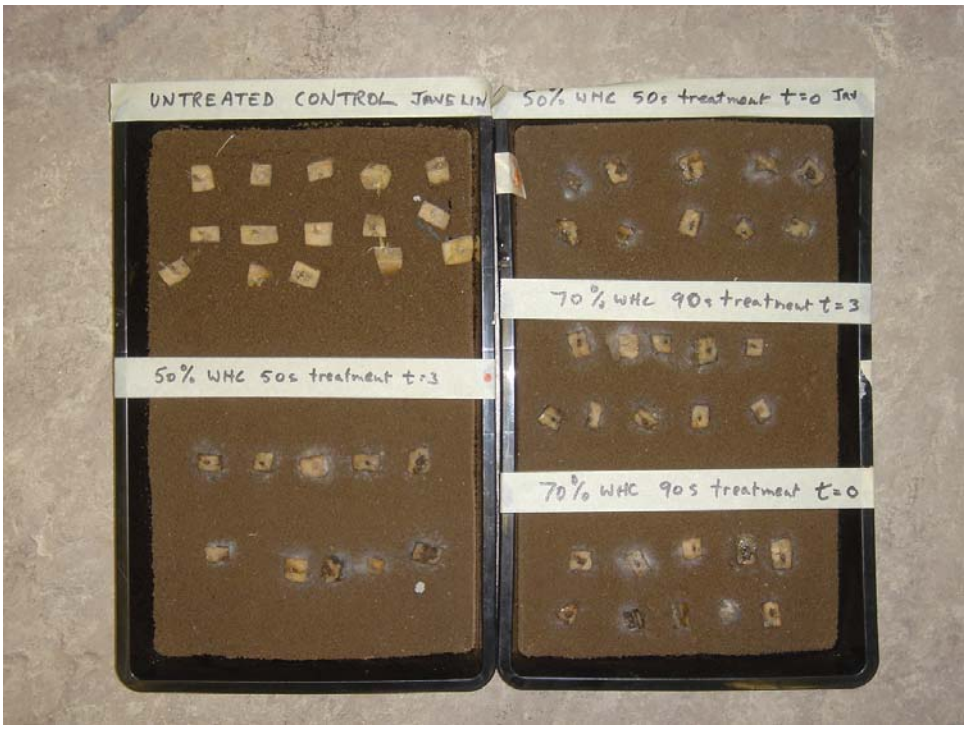

Plate 3. Effect of 50s and 90s microwave irradiation on viability of Javelin tubers with soil maintained at 50% and 70% WHC.

Plate 4. Effect of 50s and 110s microwave irradiation on viability of Javelin tubers with soil maintained at 50% and 70% WHC.

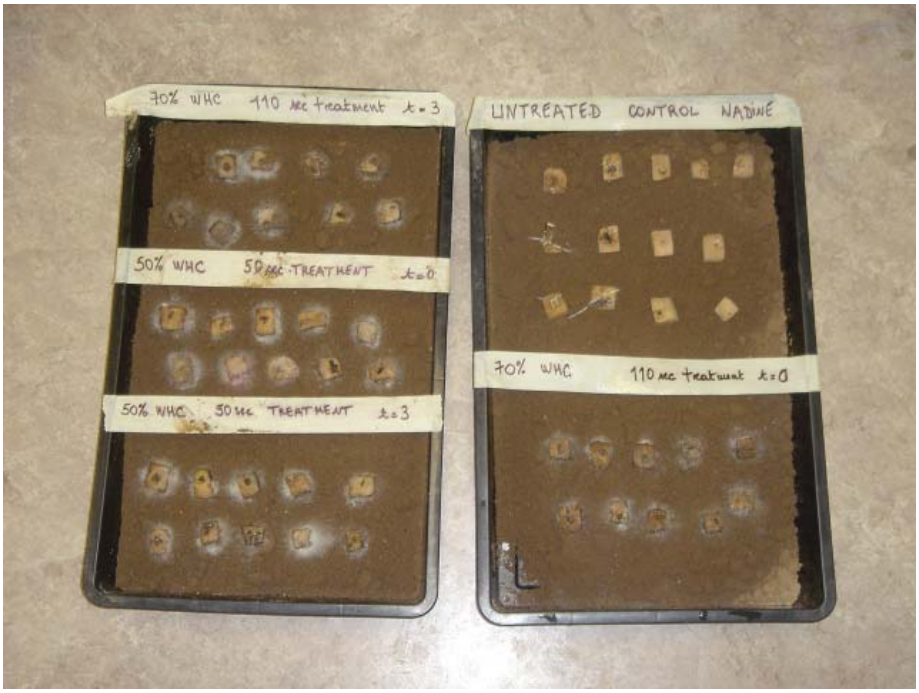

Plate 5. Effect of 50s and 110s microwave irradiation on viability of Nadine tubers with soil maintained at 50% and 70% WHC.

3.8. Changes in microbial populations in irradiated soils

In order to determine the effect of microwave irradiation on the autochthonous microbial population in the sandy loam soil total heterotrophic bacteria, fungi and actinomycetes were determined before and after heating of the soil. Standard spread plate techniques were utilised employing Soil Extract agar with actidione for total heterotrophic bacteria; Bacto-Actinomycete Isolation Agar for total actinomycetes, and Rose Bengal Streptomycin agar for fungi. The media are given in Appendix 2. No attempt was made during this study to use modern molecular techniques to determine the changes in microbial diversity or microorganisms with specific physiological function.

Soil adjusted to 70% WHC was treated with microwave irradiation for 110s to achieve the required temperature of >70^{o}C to a depth of 5cm and the system was allowed to rest at the attained temperature for a further 3 min. Thereafter the top 5cm of soil was removed and homogenised. Sub-samples were taken for microbial counting.

The results in Table 5 show that there was between 1-2 log reduction in estimated numbers with a significant population of bacteria and actinomycetes remaining. No data (ND) was obtained for fungal populations. It may be concluded therefore that following treatment the microbial populations are likely to recover quickly. The effect on pathogenic bacteria and specific physiological types that may affect biogeochemical cycling processes is not known.

	Fungi		**Bacteria**		**Actinomycetes**	
	Before	*After*	*Before*	*After*	*Before*	*After*
CFU g^{-1} dry weight soil	ND	ND	9.14 x 10^{6}	2.48 x 10^{5}	9.09 x 10^{6}	1.85 x 10^{5}

Table 5. Changes in microbial populations in sandy loam soil (70% WHC) following microwave irradiation for 110s to 70^{o}C and a three minute resting period.

3.9. Calculation of power used to heat the soil load at 50 and 70% WHC

In order to determine energy required to heat the soils at different WHC, it was decided to devise an experiment that negated the determination of parameters such as imaginary and real epsilon (ε) values required to calculate accurately absorption by the complex soil system. The experiments were done as follows to calculate the specific heat of the soils (C_s).

The same 10X10X10cm microwavable plastic containers used in the soil disinfestations experiments were utilised for the determination of power absorption (Q) for the soils. The lower plastic container was wrapped in aluminium foil as shown in Figure 16.

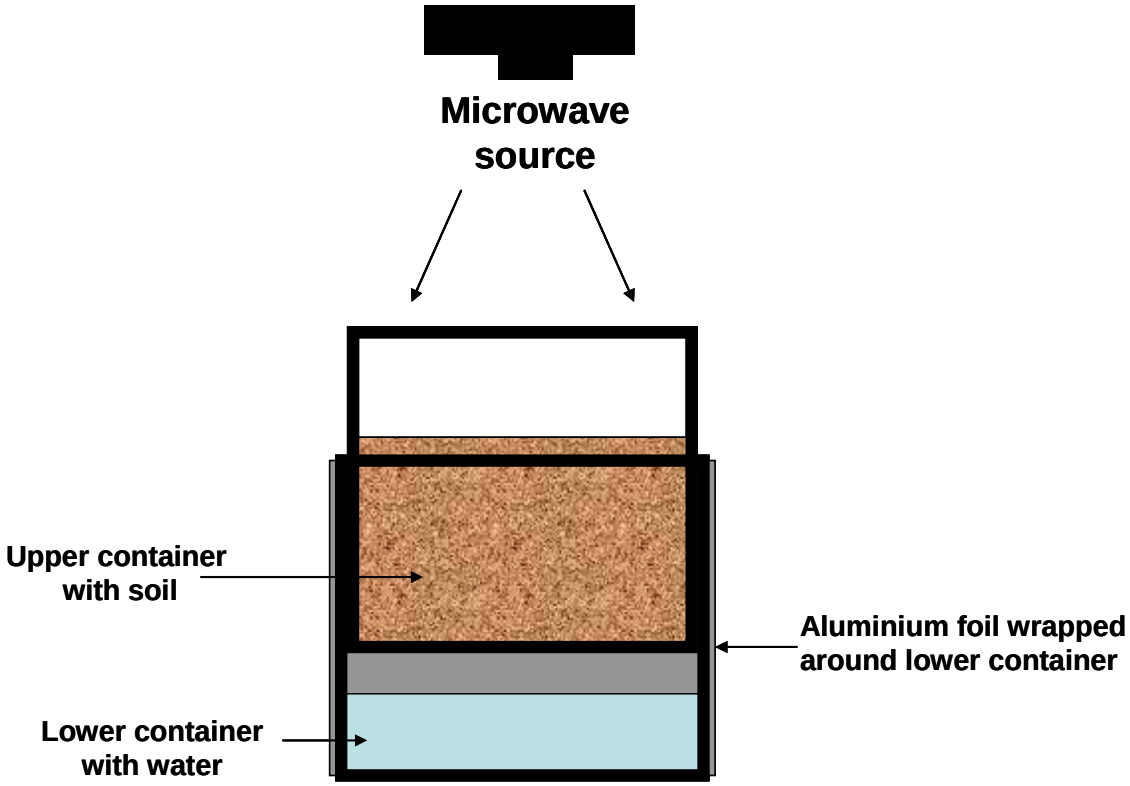

Figure. 16. Scheme for soil calorimetry experiments

The Al foil around the lower container was only wrapped to within <0.5cm of the microwave base to avoid any possible sparking and power dissipation. The wrap prevented microwave input to the water (and also soil) via side walls. The lower container had water that was irradiated from the surface for a period of time to raise the temperature by an amount avoiding evaporative losses. Generally temperature increased from around 20°C to just over 30°C. The power delivered by the microwave to the water could then be calculated using calorimetry equations in Section 2.9. In order to determine the power absorbed by soil systems, a second plastic container (upper container) with soil was utilised, inserted into the first (lower) container. Dry soil and soils at 50% and 70% WHC were treated. Various depths ranging from 0.5-5cm of soil (with corresponding weights determined) were treated. The soils received irradiation, acting as filters for the heating of the water in the lower container. The soils and water together were irradiated for a period similar to that for heating the alone. The differences in temperature generated by the different treatments of the water can be used to determine the power absorbed by the mass of soil in the upper container. This information was utilised to determine the specific heat of the soil. The energy required to facilitate killing in the soils with different bulk densities were then determined.

Table 6 shows the estimated power and energy required to heat the sandy loam soil, with different WHC, to the required temperature to affect killing of all biological pests and pathogens tested. The specific heat (C_s) of the 50% and 70% soils were 2.698 and 3.455 respectively. Dry soil tested gave a value of 1.47-2.02. This value is higher than that given in the literature for a sandy loam of ~0.77-.85. A value of 1.38 is given for sandy clay and this approaches the lower value obtained in this study. It is possible that the soil used was not a sandy loam but a mixture of soil from the top 1 m soil horizon of the Darvel series (Table 1) with higher clay content. This is borne out by the bulk density determined. The structure of the soil was not determined during the study but assumed to be that given for the top 30cm of the SRC No. 3 field. (Table 1).

ΔT (°C)	**50% WHC soil (C_s= 2.698)**		**70% WHC soil (C_s= 3.455)**	
	KJ Kg^{-1}	**KWh Kg^{-1}**	**KJ Kg^{-1}**	**KWh Kg^{-1}**
40	107.9	0.030	138.1	0.038
50	134.9	0.037	172.7	0.048
60	161.9	0.045	207.3	0.058

Table 6. Calculated values of energy and power required to raise the temperature of sandy loam soils, with 50% and 70% WHC, by 40-60°C.

Figures 17 and 18 show the power absorbed by the different soils with increasing soil mass (representing different depths of soil within the 100cm^2 area). There are errors involved in the determination of the specific heat of the soils that rely on accurate temperature measurements. It is evident that it is not easy to record temperatures following microwave treatment of soil and water accurately because of time delays. The actual specific heat values obtained are therefore higher than expected and energy values calculated for heating soils are expected to be worst case. In our killing experiments irradiation facilitated heating of soil from ~20^{o}C to ~70^{o}C. This represents a ΔT of 50^{o}C. In Table 6, it is seen that 0.037 KWh Kg^{-1} (37 KWh tonne^{-1}) soil is needed to affect kills in soil with 50% WHC. The value is higher in 70% WHC soil, at 0.048 KWh Kg^{-1} (48 KWh tonne^{-1}). It has not been possible to obtain data on steam heating to compare costs. More realistic energy requirements should use a ΔT of 60^{o}C to represent a temperature increase from ~10^{o}C (mean soil temperature in the UK) to 70^{o}C. The energy requirement for this is higher as given in Table 6.

That the data overestimate power and energy required to affect kills is clear, as there are problems in carrying out calorimetric experiments using conventional microwave ovens. In heating soils there is also power loss within the system through the walls of the microwave containers used to house the soil columns and within the microwave chamber. The energies calculated are based on a specific heat value that is high and if it is accepted that the value is twice that expected, then all the values calculated should be divided by a factor of ~2.

Microwave can be an effective treatment system that can be applied to field situations. In the microwave treatment system envisaged, soil would be lifted and channelled through the microwave radiation in a tumbling manner so that all soil constituents receive disinfecting radiation near the source. The microwaves would not need to penetrate to any great depth of soil and time taken to achieve the required temperature (>70^{o}C) for disinfestations would be much reduced. In the experimental systems used for this study, microwave radiation was used to attain a temperature of >70^{o}C at soil depths of 2.5-5cm where pests were situated. Most energy and heating to destroy pests is delivered at the surface of soil with a gradient in temperature toward the required depths (heating is considered here rather than any microwave effects). A soil tumbling treatment system would negate excessive waste in microwave energy in heating soil.

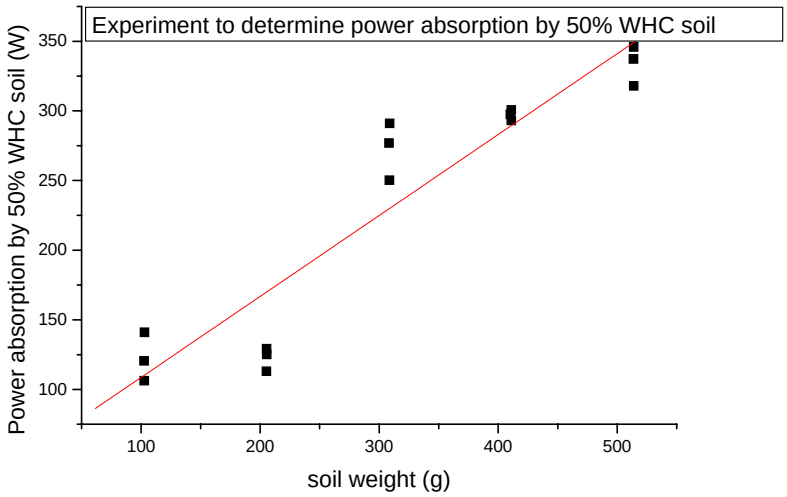

Figure 17. Effect of 50% WHC bulk soil mass on microwave power absorption

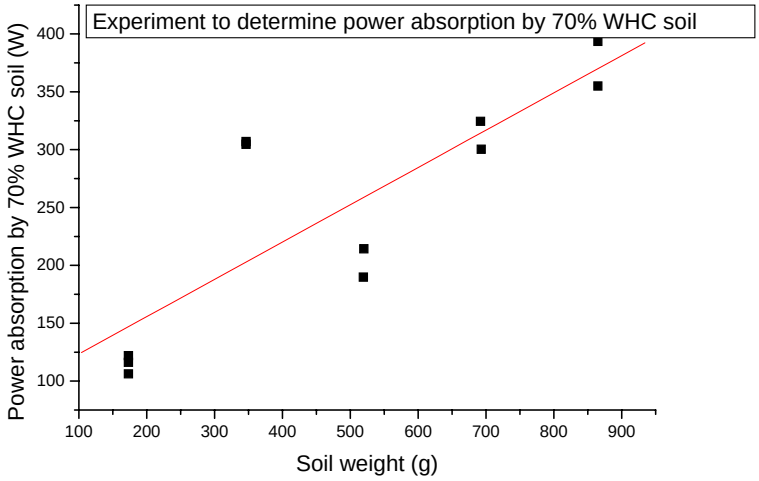

Figure 18. Effect of 70% WHC bulk soil mass on microwave power absorption

4. Discussion

Sulfuryl fluoride and phosphine (singly and in combination with CO_2), 1.3-D and many others are alternatives to using methyl bromide in soil disinfestation. The use of these in many cases is restricted because of lack of registration and inherent problems. Phosphine, for example, causes corrosion and may not be effective because of resistance by some organisms. A number of treatments are pest specific and will not control other organisms. 1,3-D can be used for nematodes but it has limited action against fungal pathogens and requires a minimum temperature of 15^{o}C for effectiveness.

The experiments carried out during this study examined the effect of irradiation delivered from a standard domestic microwave working at a frequency of 2.45 GHz with a power output of ~1000W. Weed, nematode, fungal pathogen and potato resting stages were buried in soil columns at two depths, 2.5 and 5.0cm with the maximum dictated by the expected optimum depth of microwave penetration. It was initially conceived that microwaves, through dielectric heating of constituents, could preferentially affect target pests. The dielectric constant of soil is found to be strongly dependent on moisture content. Materials with a high fat and sugar content heat up to higher temperatures in a shorter time. The microwaves generated are attracted and absorbed by water, fats, sugar and other dielectric substances in soils. It has been shown that a number of factors affect the response of seeds of different weeds, for example, to microwaves, and, because of tolerance differences, irradiation can reduce the viability of one seed type while not affecting other plant seeds in a mixture (Diprose *et al*, 1978). Our, and other studies demonstrated the difficulty in destroying dry weed seeds and the need for a moistened state. There is a need to irradiate open structured soils or break soil structure up mechanically to produce air gaps which act as "inefficient waveguides" ensuring the presence of enough water to moisten the weed seeds, sclerotia etc. an essential factor in killing by microwave.

It is likely that the damage to weed seeds is due to heating in the absence of evidence of a microwave effect. Microwave penetration to the required depth may not be effective and temperatures generated may be due to conductive processes, at least for soils with high water content. This is shown in our systems, containing soil at 70% WHC, where a longer irradiation period was required at a depth 5.0cm to attain the necessary temperature for treatment, than at 2.5cm.

Sclerotia are the resting survival spore stage of fungal pathogens such as *S. sclerotiorum*. These remain dormant in soil until stimulated by the presence of the host plant. Control of soil sclerotia can be achieved by destroying or inactivating the spore. It is evident that this can easily be achieved using microwave treatment of soil to a desired temperature. This has also been achieved using steam sterilisation techniques, pulsed electric fields (PEF) etc.

The Dauer resting stages of *C. elegans* were easily killed by the microwave treatment regime. The treatment would obviously destroy natural populations of native non-pathogenic nematodes but these should recover quickly in soils. It was not possible to carry out studies using cysts of pathogenic nematodes during this short study.

Microwave irradiation destroys volunteers under the conditions used and these volunteers can be left in the field to be degraded by the natural microflora. The conditions utilised may also be successful in destroying pests and diseases associated with the potatoes. Microwave treatment would negate the need for landfill disposal of harvested volunteers where green waste is increasingly being rejected. Herbicides used to remove volunteer viability may not be effective if applied at the wrong time in the growing season. The high cost of herbicides would also not remove pests and diseases that are soil-borne or that can survive without a host, something achievable with microwave treatment.

It was necessary to ensure that irradiation created temperatures over 70^{o}C and to allow at least 3min residence time post irradiation for killing of all soil pests, weeds and pathogens. Technically it is feasible to heat soil to a minimum temperature and to maintain this target temperature for a sufficient time to eradicate pests. Usually soils have to be heated to temperatures exceeding the target temperature in order to reach that desired. This can be achieved by mixing air with steam at specific proportions. Higher temperature treatment may affect soil physicochemical and biological characteristics in a detrimental way. Costs involved for heating are very high. It has been determined that soil steaming is approximately double the cost of methyl bromide treatment. Steaming has produced very poor results against root knot nematodes. An important consideration is that steaming soil at ~60°C (for 30 min) would eliminate most soil-borne pests and disease organisms but leave numerous saprophytes that would be antagonistic to the pathogens. In addition, such temperatures may alleviate the problems of phytotoxicity where higher temperatures cause toxic manganese and/or ammonium to be released (Dawson et al, 1965). This study used microwave power to heat soils to ~70^{o}C, measured using thermistor probes at the appropriate soil depths requiring disinfestations, as this was necessary to eradicate the various resting stages of pests and disease-causing organisms. This temperature is not considered excessive as 70^{o}C for a period of 30 min is used to disinfest glasshouse soils. Attaining a lower temperature gave inconsistent results with microwaves and this is probably due to uneven soil heating of soil blocks used in the study. This would not be a problem in the proposed microwave treatment system envisaged (see below). Microwave penetrates up to 5cm soil unreliably as evidenced by the recorded temperatures, however it should be borne in mind that contact thermistor measurements inside microwave irradiated soil masses require excellent contact for accuracy. Care needs to be exercised, therefore, when interpreting the data. Irrespective of this, however, microwave irradiation of soils up to 5cm deep and 70% WHC containing volunteer potatoes, Dauer stage nematode larvae, and fungal sclerotia kills 100% of these pests and disease-causing organisms. This study has used appropriate soils at higher water content (70 & 50% WHC) to mimic worst-case scenarios. Obviously the use of soils with lower moisture content would require less energy to heat the constituents to the required temperature, affecting kill.

5. Technology Transfer: Can microwaves be used in soil disinfestation?

Energy requirement for microwave treatment has to be considered in concert with the use of other control methods and the costs involved in utilising a range of treatment methods. Work by van Loenen et al (2002) examined the disinfestation of resting structures of fungal pathogens, a nematode and weed seeds in soils using aerated steam in a laboratory model system. They found that temperatures maintained at between 50-60^{o}C for 3 min with a resting period of 8 min were sufficient to achieve 100% kill for all structures of weeds, fungi and nematode. The authors used two types of soils (loamy sand and sandy clay loam) with moisture levels of 70% field capacity similar to this study. Our study showed that in static systems with microwave treatment of the soil surfaces using 2.45 GHz microwave delivering ~1 KW of power, ~100% weed seeds, sclerotia of the fungal pathogen *S. sclerotiorum* resting stages of the common nematode *C. elegans* and volunteer potatoes are destroyed or inactivated. This is achieved with a treatment time of 40-50s with resting period of 3 min for all pests situated in soil at a depth of 2.5cm. Experiments examining kill at 5cm depth showed a requirement of 90-120s treatment with a resting period of 3 min.

Irradiation has been used in the US to treat soils. Menges and Wayland (1974) used microwave treatment of weed seeds placed at 2 cm depths in irrigated and non-irrigated soils. Wayland *et al* (1975) used microwave generators over field plots to non-selectively kill seeds of different plants at energy levels above 70 J cm^{-2}. Using a frequency of 2.45 GHz and energies of 45 to 730 J cm^{-2} have been found effective in killing seeds of a number of species without any effect on the crop subsequently planted. 180 J cm^{-2} was sufficient to achieve 96-100% kill for seeds of all but one species of weed (81% for common purslane). The soil temperature had reached 80^{o}C after 1 min treatment. The work showed that all seeds were destroyed following 360 J cm^{-2} treatment of dry soils containing non-imbibed seeds. To inhibit germination of the weed seeds a minimum energy input of 210 J cm^{-2} was necessary for moist soils. Obviously the authors found that moisture content and initial soil temperature are important factors in the economics of using microwaves to destroy soil pests. This relates to the time taken to heat soils to temperature affecting kill. Safety for operators and other people and animals of exposure to microwaves is of paramount importance in the development of field microwave treatment systems.

Steam treatment of soils requires that water be boiled and passed through soil for a period of time that is longer than that required to microwave heat soil. The energy required in microwave heating of soils is more controllable and would be significantly less than that required to boil water that will ultimately be injected to soils or mixed with air in cold steaming methods for soil disinfection.

This study used a microwave oven for the rapid heating of soils to affect destruction of soil-borne diseases and pests. The premise that microwave radiation generated within a microwave oven will lead to even heating throughout a load placed within it is erroneous. The size and shape of the load and its physical properties will determine the power absorption. Diprose (2001) highlighted the fact that equal volumes of water

in different shaped containers reach different final temperatures. This is shown in the present experiments utilising a complex load of mineral particles, water and organic materials exhibiting differential and variable heating patterns for specific soil 'formulations'. There was extreme variability in temperature generated at different soil depths and more control would be required, as disinfection must maintain specific heating conditions. The use of microwave guides delivering high frequency directly into a soil surfaces may not provide the required control for effective treatment, although waveguides delivering lower frequencies may provide the necessary control. The microwave horn geometry with respect to soil irradiation and killing needs to be optimised for maximum efficiency for a given energy input rate.

This work was instigated to determine the role of microwaves in destroying a range of pests and diseases in soils in a controllable way. The study used static systems whereby soil at a specific depth, containing resting stages of pests and disease organisms, were heated to a set temperature to affect complete kills. This has implications with respect to energy input. In dry soils with low percentage water content, heating a depth of 5cm of soil is rapid and linear because of reduced water availability to absorb the microwave energy, as indicated by the experiments carried out. The heating to 70^{o}C is highly controllable and would not cause undue physicochemical and biological damage to the soil. It is obvious that soil heating is affected by water content and bulk density. Soils with a high water content exhibited differential heating and there were temperature gradients with preferential heating at the surface layers. As a minimum soil temperature of 70^{o}C is required, there would be an energy penalty for heating the surface above the required temperature in soils with high water content.

A possible methodology advocated is not to treat soil *in situ* (because of inhomogeneity of bulk soils and inherent practical, economic and other problems) but to lift and channel soil using a conveyer with shielded microwave treatment from above (see Figure 19). The conveyer constantly mixes the soil allowing efficient and effective irradiation of the total mass of soil recovered from a field. Temperature required through the soil would be achieved quicker and more cost effectively. This would allow significantly faster soil processing. The system would enable the treatment of deeper soil harbouring pests and pathogens that may interfere with horticultural productivity.

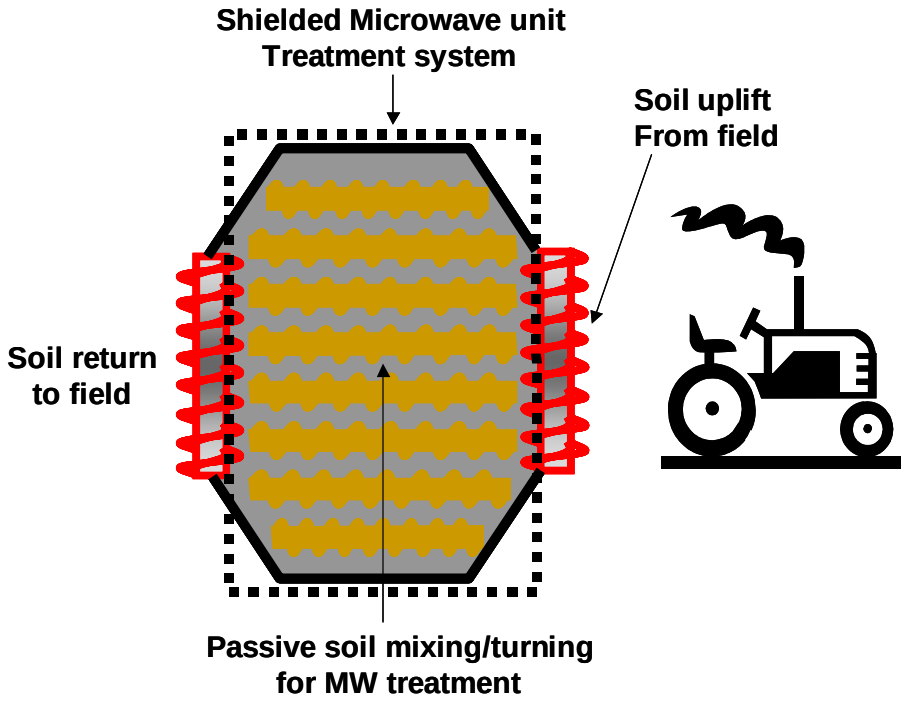

Figure 19. Diagrammatic representation of a field microwave treatment system.

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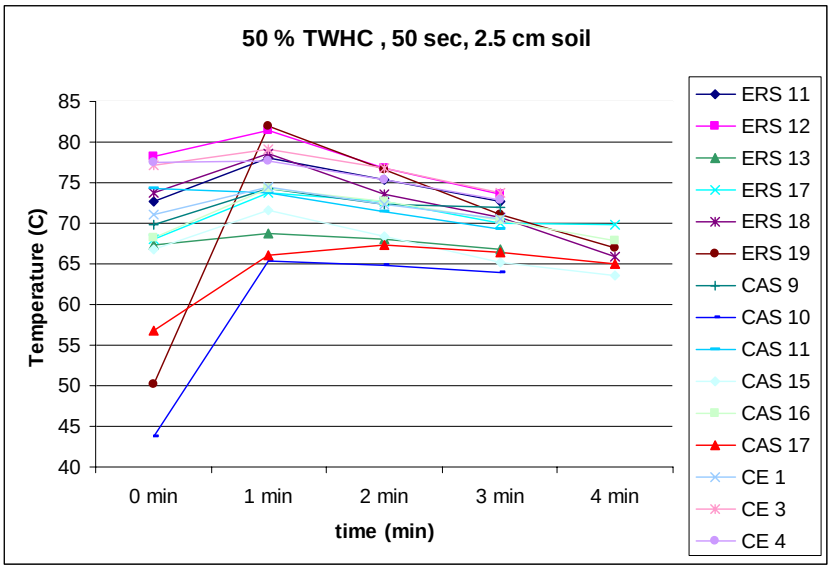
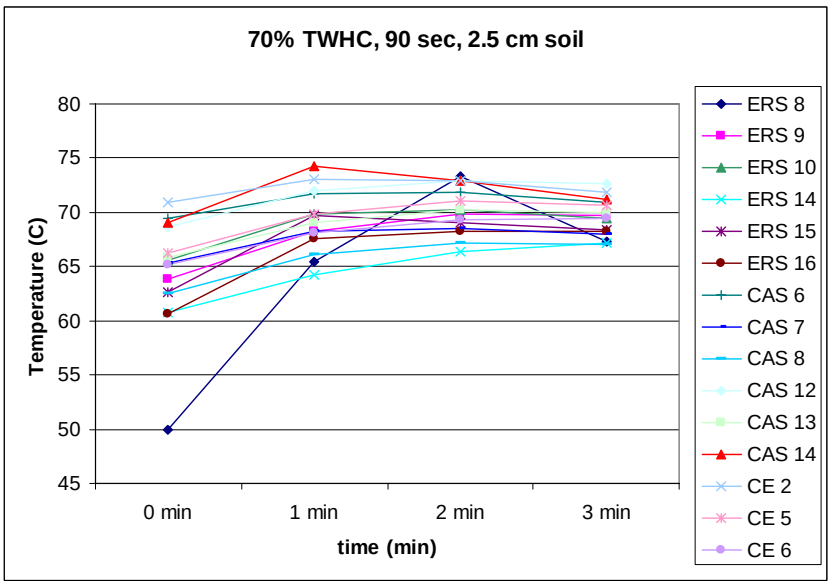
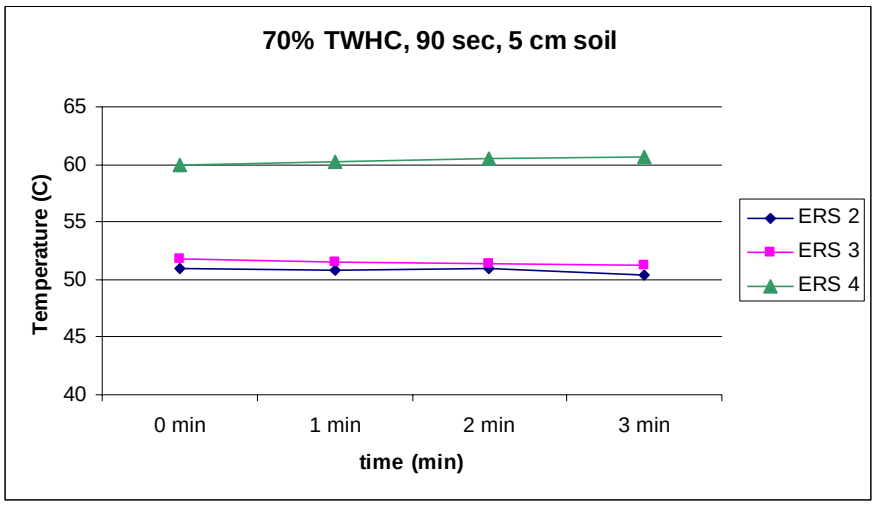
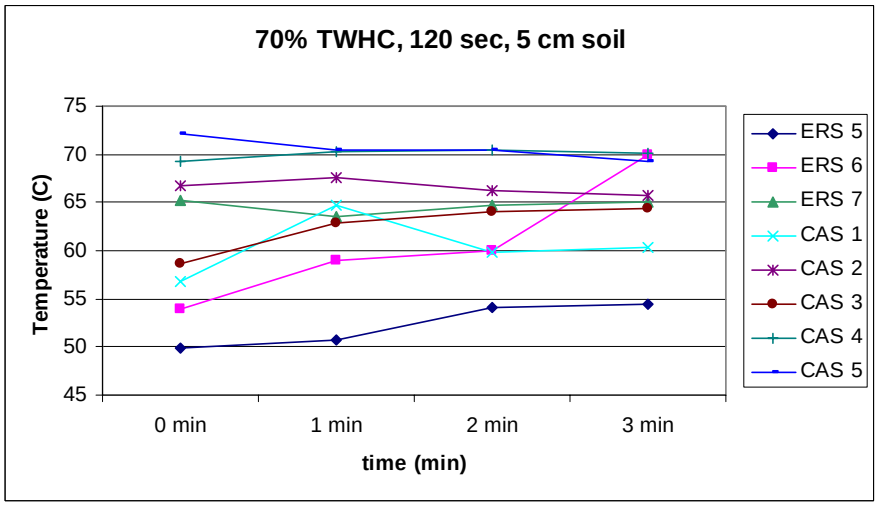
Appendix 1- Soil Temperature Changes after irradiation

Changes in temperature of soil at depth irradiated for a set time and subsequent temperature retention for a period of 3 min. T_0 min represents the start of the resting period and temperature following the initial microwave irradiation time.






70% TWHC, 90 sec, 5 cm soil
Temperature (C)
65
60
55
50
45
40
0 min
1 min
2 min
3 min
time (min)
ERS 2
ERS 3
ERS 4


70% TWHC, 120 sec, 5 cm soil
Temperature (C)
75
70
65
60
55
50
45
0 min
1 min
2 min
3 min
time (min)
ERS 5
ERS 6
ERS 7
CAS 1
CAS 2
CAS 3
CAS 4
CAS 5

Appendix 2. Microbiological culture media

NGM Agar contained NaCl, 3g; Agar, 17g; Peptone, 2.5g; Cholesterol (5mg ml^{-1} in ethyl alcohol), 1ml; Deionised water, 975ml. Sterilise by autoclaving at 121 ^{o} C for15min and then add using sterile technique, the following sterile solutions: $CaCl_2$ 1M, 1ml; $MgSO_4$ 1M, 1ml; KH_2PO_4 1M pH 6, 25ml.

Potato Dextrose/chlortetracycline Agar (PDA+).
PDA was prepared with 20mgl^{-1} chlortetracycline autoclaved (15min x 121^{o} C) cooled to 45 ^{o} C and distributed into sterile 9cm diameter polystyrene Petri dishes.

Oatmeal Agar (OA)
This was prepared by boiling 30g powdered oatmeal in approx 800mL distilled water for 1h with stirring in a boiling water bath followed by straining through a muslin bag. After straining, the volume was made up to 1l, 20g agar added and reboiled to dissolve the agar. OA was sterilised (20min x 121^{o} C) in 15ml (glass universal bottles for slopes) and 500ml aliquots (sterile 9cm Petri dishes).

Wheat grain culture medium
Sclerotia from *Sclerotinia sclerotiorum* were prepared from cultures grown on wheat grains. A 2cm layer of moistened organically grown wheat grains was sterilised (15min x 121 ^{o} C) in a 2l Erlenmeyer flask prior to inoculation with sclerotia of *S. sclerotiorum.*

Soil Extract Agar Plus Actidione for Total Bacteria

This contained Agar, 15.0 g; Glucose, 1.0 g; K_3HPO_4, 0.5 g; Soil Extract, 100.0 ml; Tap Water, 900.0 ml. All ingredients except 6 are placed in a sterilising bottle and autoclaved for 15 minutes at 121^{o}C. The medium is cooled to 50^{o}C followed by addition of 1 ml of the actidione solution (650 mg actidione in 100ml water), mixed gently and poured into Petri dishes for spread plates.
One litre of tap water is added to 1 kg of garden soil and autoclaved (15 lbs pressure, 121^{o}C) for 30 minutes. Next 0.5 g of calcium carbonate is added and the supernatant filtered through Whatman filters until clear. The clear extract is bottled, sterilised in 100 ml amounts, and stored at 2^{o}C to 5^{o}C until used.

Bacto Actinomycete Isolation Agar for Total Actinomycetes

This was Sodium caseinate; 2.0 g; Asparagine, 0.1 g; Sodium propionate, 4.0 g; Dipotassium phosphate, 0.5 g, Magnesium sulphate, 0.1 g; Agar, 15.0 g, Ferrous sulphate, 1.0 mg, H_2O, 1000ml. The ingredients are dissolved by boiling and 5 g 1^{-1} of Glycerol is added prior to sterilisation. The final pH of the medium is 8.1 ± 0.2.

Rose Bengal Streptomycin Agar for Total Fungi

This consists of Dextrose, 10.0 mg; Peptone, 5.0 g; KH_2PO_4, 1.0 g; $MgSO_4.7H_2O$, 0.5 g; Agar, 20.0 g; Tap Water, 1000ml, Rose Bengal, 33.0 mg. All ingredients except Rose Bengal are placed in a sterilising bottle and brought to boiling. Rose Bengal is added and mixed followed by autoclaving 15 minutes at 121°C. After the medium cools and before pouring plates, 30 mg per litre of streptomycin is added. Final pH is 6.8 ± 0.2.