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Low temperature–short duration steaming of soil kills soil-borne pathogens, nematode pests and weeds

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Abstract

Agricultural soil samples containing survival structures of the fungal crop pathogens *Verticillium dahliae*, *Sclerotinia sclerotiorum*, *Sclerotium cepivorum*, *Pythium ultimum*, potato cyst nematodes *Globodera rostochiensis* and *G. pallida* and weeds *Chenopodium album* and *Agropyron repens* [*Elymus repens*] were treated in the laboratory with aerated steam at temperatures ranging from 40 to 80 °C in a specially constructed apparatus. Steaming at 50 or 60 °C for 3 min, followed by an 8-min resting period in the steamed soil and immediate removal from the soil thereafter, resulted in 100% kill of all weeds, diseases and nematodes. When steamed at 45 °C, there was a small but significant reduction in the survival of *V. dahliae* microsclerotia but no reduction in survival of *S. cepivorum*.

Abbreviations: CFU – colony forming units; JJ2 – second stage juveniles; MS – microsclerotia; PDA – potato dextrose agar; PRD – potato root diffusate.

Introduction

Growing concern about the widespread use of pesticides has created an urgent need to develop environmentally friendly crop protection strategies. Control of soil-borne pests and diseases by soil fumigation not only destroys soil-borne pathogenic nematodes and fungi but also much of the saprophytic and beneficial soil microflora (Chen et al., 1991; Gamliel et al., 2000). Methyl bromide is a soil fumigant, which, because of its ozone depleting characteristics, is being phased out under the 1992 Montreal Protocol. Research to find replacements for this compound is currently carried out worldwide (UNEP, 1998, 2000a). Steaming has been used for more than a century as a highly effective way of soil disinfestation and is recognised as a viable alternative to methyl bromide in glasshouse production systems (Bollen, 1985; Braun and Supkoff, 1994; EPA, 1997; UNEP, 2000b). However, the high-temperature steam treatments (100–140 °C) currently used in horticultural practice have a range of negative side effects. For example, they can result in manganese and ammonium release from the soil, and to a lesser extent also nitrite and bromide and/or kill much of the soil microflora as well as the target diseases and pests (Baker, 1970; Sonneveld, 1979; Roorda van Eysinga and de Bes, 1984). Low-temperature steam treatments (60–80 °C) are thought to avoid many of these negative impacts. Exposure to steam at 60 °C for 30 min destroys most soil-borne phytopathogenic fungi and nematodes, weed seeds, insects and viruses and reduces problems of phytotoxicity and re-infestation that may persist after steaming at higher temperatures (Baker 1962; 1970; Dawson et al., 1965; Bollen, 1985). Pathogen re-infestation may be delayed because of the survival of antagonists after low-temperature treatment (Bollen, 1969; Baker, 1970).

As part of a project to develop new methods of steam disinfestations of soil for horticulture that are faster, and use less energy and a lower temperature (Pallet and Kelly, 2000) we needed to know if short duration exposure to steam at a low temperature would be sufficient to kill soil-borne diseases, pests and weeds. This paper examines the survival of the fungal pathogens *Sclerotinia sclerotiorum* (white mould), *Sclerotium cepivorum* (onion white rot), *Verticillium dahliae* (wilt disease) and *Pythium ultimum* (damping-off); the nematode pests *Globodera rostochiensis* and *G. pallida* (potato cyst nematodes) and two major weeds, annual *Chenopodium album* (fat hen) and perennial *Agropyron repens* [*Elymus repens*] (couch grass) in two different field soils which were exposed to aerated steam at a range of temperatures in a specially designed steam apparatus.

Material and methods

Soils

Soils were obtained from agricultural fields in Scotland (Dalcross, near Inverness, a well-structured loamy sand, soil D) and England (Wellesbourne, Warwick, an unstable, hard-setting sandy clay loam, soil W) and were sieved to <20 mm. Soil characteristics are listed in Table 1.

Target organisms

Sclerotium cepivorum Berk.
Sclerotia (isolate Kirton) were obtained from Horticulture Research International (HRI), Wellesbourne, Warwick. These sclerotia had been exposed to burial in natural soil for a period of 3 months in order to break their endogenous dormancy (Coley-Smith, 1960). Surface sterilisation was carried out using a modified version of the method described by Coley-Smith et al. (1990). Sclerotia were put in a 20-ml universal containing 15 ml sodium hypochlorite (Aldrich, available chlorine 4–20%) and shaken by hand for 90 s, followed by four rinses in sterile distilled water. Individual sclerotia were placed onto potato dextrose agar (PDA) drops. Four millilitre of agar was used to make five drops on a 9-cm petri dish. PDA contained 20 mg l^{-1} chlortetracycline (Sigma, purity 82%; Williams et al., 1998). Sclerotia were crushed with a fine forceps in the agar to stimulate germination (Coley-Smith, 1985). One replicate involved 2 mg of sclerotia (ca. 100 sclerotia) of which 30 were plated out. Plates were incubated at 20 °C and germination was established after 14 days. Only sclerotia younger than 1 year were used in trials.

Fungal pathogens

Sclerotinia sclerotiorum (Lib.) de Bary
The fungus was grown on sterile wheat grain at 20 °C for 3 weeks, followed by 4 weeks at 4 °C (Mylchreest and Wheeler, 1987), after which sclerotia were separated from the wheat, washed, air dried and sieved to give 2–4 mm sclerotia. Twenty sclerotia per replicate were used. Surface sterilisation was carried out for 3 min in 15 ml 1 : 1 (v/v) ethanol/sodium hypochlorite (Aldrich, available chlorine 4–20%), followed by three rinses in sterile distilled water (Whipps and Budge, 1990). Sclerotia were then bisected and each half placed on small disks (14 mm diameter) of PDA containing 20 mg l^{-1} chlortetracycline (Sigma, purity 82%; Williams et al., 1998). Plates were incubated at 20 °C and germination was assessed after 14 days. Sclerotia were used within 1 year.

Verticillium dahliae Kleb.
The *V. dahliae* isolate was obtained from HRI, East Malling, Kent. Microsclerotia (MS) were grown on straw cultures using a method described by Chambers

Table 1. Soil characteristics

Soil	Texture	Organic matter (C, g 100 g^{-1})	pH (H_2O)	Moisture content at FC[1] (g 100 g^{-1})	Moisture content at PWP[2] (g 100 g^{-1})
Soil D	Loamy very fine sand	6.04	6.2	28	10
Soil W	Sandy clay loam/ sandy loam	1.88	6.3	13	7

[1]FC = field capacity.
[2]PWP = permanent wilting point.

and Harris (1997). Barley straw, cut in 5 cm pieces, was soaked for 24 h in distilled water containing 0.1% KNO_3 and 0.1% sucrose in 1-l conical flasks. The straw was then drained and autoclaved (120 °C, 20 min) and a *V. dahliae* conidia suspension was added. The flasks were covered with film and incubated at 20 °C for 3–9 months. The conidia suspension was obtained by flooding a 7-day-old PDA slope culture of *V. dahliae* with 10 ml sterile distilled water and removing the conidia with a sterile loop (Chambers and Harris, 1997). After incubation the flasks were filled with tap water and MS dislodged from the straw by hand swirling and mechanical shaking (90 rpm, 16 h). The MS suspension was sieved through sieves with hole sizes of 180 and 106 μm. MS collected on the 106 μm sieve were repeatedly spun on a vortex mixer followed by repeated washing through the 106 μm sieve. Twenty millilitre of suspension of MS retained on the 106 μm sieve was then adjusted to a specific density (see below) and distributed drop wise over 200 g dry acidified sand >355 μm (Hawke and Lazarovits, 1994). The mixture was dried, stored at 4 °C and used within 1 year. MS density was established by microscopically counting MS >106 μm in a 10 μl suspension. The quantity of MS used was 1000 g^{-1} sand. After exposure to steam MS were germinated on a modified nutrient agar medium first described by Harris et al. (1993). One litre of distilled water containing 2 g $NaNO_3$, 1 g KH_2PO_4, 0.5 g $MgSO_4 7H_2O$, 0.5 g KCl, 0.01 g $FeSO_4 7H_2O$, 2 g polygalacturonic acid (sodium polypectate, Sigma) and 1 ml Tergitol NP-10 (Sigma) was adjusted with KOH to pH 6.4. The solution was microwaved to dissolve the ingredients. Fifteen gram of agar (Technical no 3, Oxoid) was added and the medium autoclaved (120 °C, 20 min). When cooled to 50 °C, 10 ml filter sterilized solution containing 0.06 g streptomycin, 0.06 g chloramphenicol, 0.06 g chlortetracycline and 0.006 g biotin was added per 100 ml agar. The medium was then poured in 9-cm petri dishes. Replicates of 1 g sand–MS were sieved through a 250 μm sieve and 30 MS placed on a petri dish. Plates were incubated at 22 °C and germination counted after 14 days.

Pythium ultimum Trow.

Pythium ultimum, obtained from HRI, Wellesbourne, Warwick, was grown on 3% cornmeal–sand (Martin, 1992). A 1-l Duran bottle containing 500 g of dry sand, 15 g cornmeal and 100 ml distilled water was autoclaved (120 °C, 20 min) twice, with a 24-h cooling period in between. Four plugs of a 3–5-day-old *P. ultimum* culture, grown on V8 agar, were then added. The inoculum was incubated at 20 °C and used within 4–6 weeks. Inoculum was added to the soil at 50 g l^{-1}. Viability of *P. ultimum* was assessed by a soil plating method (Stanghellini and Hancock, 1970) and with a pea bioassay. For soil plating 0.1 g soil was put in 10 ml sterile distilled water and centrifuged for 3 min. One millilitre of the suspension was distributed in 10 drops over each of four 2% water agar petri dishes. *P. ultimum* cultures were microscopically counted after incubation for 16 h at 25 °C. Peas (cv Kelvedon Wonder) were grown in 10-cm pots filled with 200 g sand/*P. ultimum* amended soil (1/1, v/v). Four pots, containing nine peas each, were used in each treatment. Germination of the pea seed was assessed after 14 days.

Potato cyst nematodes

Globodera rostochiensis and *Globodera pallida*

Cysts of *G. rostochiensis* (A97 Ro1) and *G. pallida* (E97 Pa2/3) were obtained from Scottish Crop Research Institute (SCRI), Dundee. One replicate included 10 randomly picked cysts, which were inserted in mesh bags. Hatching of cysts was carried out using procedures outlined by Shepherd (1986). Mesh containing cysts was soaked in 2–4 ml distilled water at 18 °C for 7 days. Individual cysts were then transferred to 35-mm petri dishes containing 2 ml potato root diffusate (PRD) and incubated at 15 °C for 4 weeks.

PRD was obtained from roots of potato plants (cv Désirée). Singular tubers were planted in 20-cm pots in horticultural sand in a heated greenhouse (22–25 °C). After 4 weeks the roots were removed from the young plants, washed and covered with tap water for 6 h. The diffusate was removed and filtered through 20-μm nylon mesh (1 041 M SE FAR Nitex 03-20/14, Lockertex, UK). PRD was stored either at 4 °C and used within 4 weeks or at −20 °C and used within 6 months. After incubation the contents of the dishes were transferred to a grid-marked 35-mm petri dish and second stage juveniles (JJ2) were microscopically counted.

Weeds

Chenopodium album L.

Seeds were obtained from Wageningen, the Netherlands, in 1999. They were kept in non-airtight containers at room temperature in the dark. Twenty seeds per replicate were used. Steamed seeds were put on moist filter paper at room temperature in the laboratory, and germination counted after 14 days.

Agropyron repens L. *[Elymus repens* L.*]*
Rhizomes were dug up from a local garden one day before the experiments and kept overnight in moist soil. Each replicate included two 6-node rhizomes and one 4-node rhizome. After steaming the rhizomes were placed in $20 \times 15 \times 4\,cm^3$ seed-trays on 2 cm horticultural sand, covered with 1 cm John Innes No. 1 compost (Chandrasena and Sagar, 1984). They were kept moist in a heated greenhouse at 22–25 °C. Emerged shoots were counted after 14 days.

Inserting organisms in soil samples

Soil sample holders were made of $15 \times 10\,cm^2$ steel cylinders with an open top and a 0.5 mm steel mesh base. Seeds, sclerotia, MS and cysts, were put in small, 50–450 mm^2 bags, made from 20 µm nylon mesh (1 041 M SE FAR Nitex 03-20/14, Lockertex, UK), sealed with a heat sealer. The loose rhizomes of *A. repens* [*E. repens*] and mesh bags containing the survival structures were placed in the soil at a depth of 5 cm. A total soil-depth of 10 cm was maintained in all experiments and four soil samples, each containing one replicate per organism, were used each time. For tests involving *P. ultimum* one litre of *P. ultimum* infested soil was inserted in each sample holder.

Moisture content of soil and organisms

Soils were steamed at a moisture content close to field capacity (soil D at 23–27 and soil W at 10 g $100\,g^{-1}$ moisture content). Soil moisture content before and after steaming was determined by oven drying (105 °C, 24 h). All mesh-inserted organisms were tested in a dry and imbibed state, the latter involving soaking the organisms in distilled water for 16 h. Loose rhizomes of *A. repens* [*E. repens*] were kept in moist soil for about 16 h to allow moisture equilibration between rhizomes and soil sample. Four mesh-inserted organisms were additionally tested after soil–moisture equilibration, namely *C. album* and *S. sclerotiorum* steamed at 50 and 60 °C, and *V. dahliae* and *S. cepivorum* at 40 and 45 °C. The moisture content of *P. ultimum* infested soil was measured at the time of soil-inoculation.

Aerated steaming in test-rig

Soil samples were treated with aerated steam in a steel test-rig (Figure 1). The central body of the apparatus measured $2 \times 0.3 \times 0.3\,m^3$ (height × width × width) and

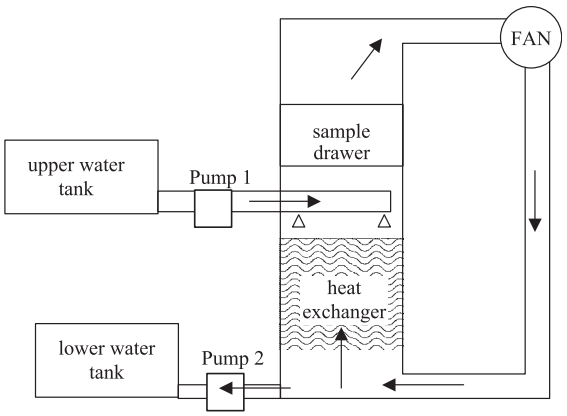

Figure 1. Water and airflow in test-rig during aerated steaming. Hot water was pumped from the upper tank and sprayed on top of a heat exchanger. A fan circulated air through the rig in the direction indicated by the arrows. Cooled water was collected at the bottom of the rig and pumped back to the lower tank.

contained a soil sample drawer and a corrugated steel heat exchanger. The drawer, containing four soil sample holders, could be moved in and out by a hydraulic unit. Hot water, produced by immersion heaters in the 50-l upper tank was sprayed from nozzles over the top of the heat exchanger. External tubing including a fan connected top and bottom of the machine. Air was driven up through the heat exchanger and recirculated as shown. Additional pipe work (not shown in Figure 1) allowed water to be pumped back to the upper tank. Thermocouples interfaced with a computer and were inserted in the soil samples at the same depth as the organisms. Temperature was recorded every 1 s. To pre-heat the test-rig water was heated 10 °C higher than the required steam temperature and aerated steam was circulated through the system for 30 s. The water was then pumped back to the upper tank and re-heated 10 °C higher than target temperature. The soil samples were inserted and steaming commenced by opening a valve in the inlet pipe and switching on the fan. The upper tank was empty after ca. 2 min and the fan was switched off after 3 min, after which the sample drawer was left inside the machine for an additional 7 min (resting period). The soil samples were then withdrawn and thermometers were immediately inserted to the same depth as the thermocouples. Temperature was recorded after a further 60 s, after which the sample holders were emptied and the organisms removed from the soil. All organisms except *A. repens* [*E. repens*] were steamed at 50, 60, 65, 70 and 80 °C. *A. repens* [*E. repens*] was steamed at 50 and 60 °C only. *V. dahliae*

and *S. cepivorum* were additionally steamed at 40 and 45 °C.

Minimum lethal steam temperatures

Temperatures were considered lethal when percent germination (seeds, sclerotia, MS), hatching (cysts) or shoot-growth (*A. repens* [*E. repens*]) was zero in all four replicates.

Statistical analysis

Two-way analysis of variance (ANOVA) was carried out to analyse data on soil-moisture increase, germination of *V. dahliae* and *S. cepivorum*, steamed at 40 and 45 °C, occurrence of *P. ultimum* (colony forming units, CFU $* 10^{-3}$) and germination of peas, using the statistical package Minitab for Windows, version 13.3 (State College, PA, USA). Significant differences between treatments were elucidated using least significance difference (LSD) values.

Results

Temperature of steamed soil

It took 100–120 s after commencement of steaming before the soil surrounding the organisms reached the required temperature (Figure 2). During fan operation temperature recording was erratic, due to electrical noise from the fan, but stabilised after the fan was switched off. The temperature stayed very close to target temperature during the resting period. Thermometer readings taken immediately after the tests showed a maximum deviation of 2 °C in comparison to thermocouple readings.

Soil moisture increase

The increases in soil moisture content were low in both soils, ranging from 1.1 to 3.9 g 100 g^{-1} with an average of 1.9 g 100 g^{-1}. There was a trend of soils becoming progressively wetter when steamed at higher temperatures, but this was not statistically significant.

Minimum lethal steam temperatures

All organisms in a moist state were killed at 50 or 60 °C (Table 2). Average germination/hatching/shoot-growth of untreated controls were: *C. album*, 74 ± 3%; *S. sclerotiorum*, 100%; *S. cepivorum*, 71 ± 5%; *V. dahliae*, 85 ± 5%; peas, 72 ± 7%; *G. pallida*, 57 ± 8 JJ2; *G. rostochiensis*, 159 ± 26 JJ2; *A. repens* [*E. repens*] 6.2±0.2 shoots. Dry and imbibed *V. dahlia*, *S. cepivorum* and *G. pallida* were killed in both soils after steaming at 50 °C (Table 2). *G. rostochiensis* was also eliminated at 50 °C in both soils, except for a very small number of JJ2 that hatched from imbibed cysts in soil D (Table 3). Imbibed *C. album* and *S. sclerotiorum* were killed at 60 °C in both soils, but showed reduced sensitivity to steam treatment in their dry state, with *S. sclerotiorum* needing 70 or 80 °C to be completely killed. However, when these organisms were allowed to equilibrate with soil moisture the minimum lethal temperature was the same as those for the imbibed organisms (Table 2). The survival rate at 50 and 60 °C steam treatment of those organisms that were not, or only partly, killed after 50 °C steaming is given in Table 3.

Table 2. Minimum lethal temperatures (±2 °C) for dry, imbibed (Imb) and soil moisture equilibrated (SME) target organisms, steamed in agricultural soil at about 70% field capacity. Steaming lasted 3 min and was followed by a 8-min resting period before removal of the organisms from the soil

Target organisms	Soil D			Soil W		
	Dry	Imb	SME	Dry	Imb	SME
S. cepivorum	50	50	—	50	50	—
V. dahliae	50	50	—	50	50	—
G. pallida	50	50	—	50	50	—
G. rostochiensis	50	60	—	50	50	—
C. album	65	60	60	65	60	60
S. sclerotiorum	80	60	60	70	50	50
A. repens	—	—	60	—	—	60
P. ultimum	—	—	60	—	—	60

Figure 3 shows the effect of steaming of *P. ultimum* infested soil on pea germination and occurrence of *P. ultimum* CFU. Peas were killed pre-emergence in non-steamed, *P. ultimum* infested soil. *P. ultimum* was considered to be eliminated when there was no significant difference in germination of peas in inoculated, steamed soil and non-inoculated, non-steamed soil. *P. ultimum* could only be recovered from infested, non-steamed soil, apart from a very small number in soil D after 50 °C treatment (see also Table 2). The unexpected high germination of peas grown in infested soil D after 60 °C treatment was thought to be related to bio-stimulation of the seed batch used in soil D trials, which showed reduced vigour compared with seeds used in soil W trials.

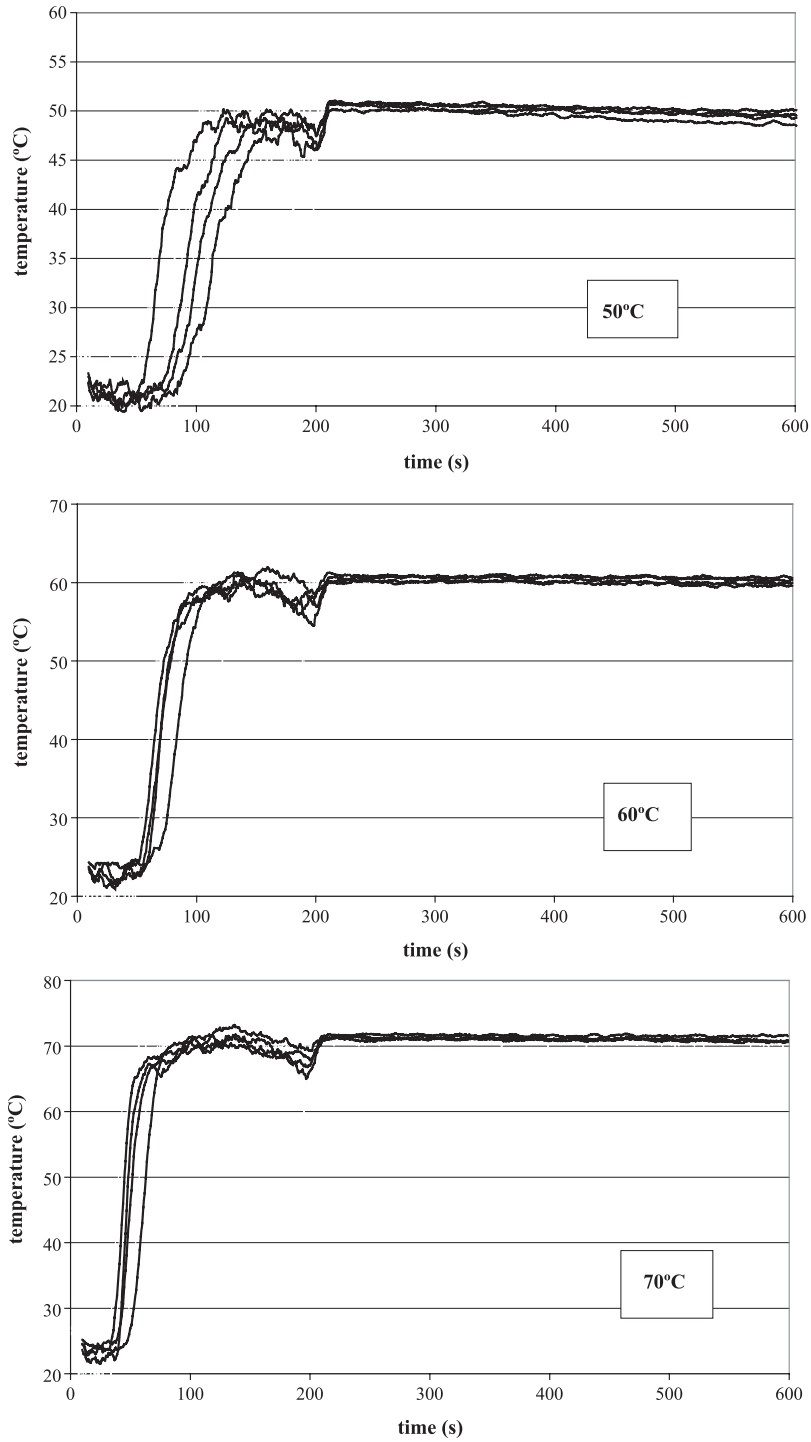

Figure 2. Temperature–time curves of thermocouples inserted at 5 cm depth in four soil samples and steam-treated at target temperatures of 50, 60 and 70 °C. The gas flow was stopped after 3 min, causing a transient drop in temperature.

Table 3. Survival rates as a percentage of the control after 50 and 60 °C steaming of dry, imbibed (Imb) and soil moisture equilibrated (SME) target organisms, not or only partly eliminated after 50 °C treatment

Target organisms	Soil D						Soil W					
	Dry		Imb		SME		Dry		Imb		SME	
	50	60	50	60	50	60	50	60	50	60	50	60
G. rostochiensis	0	0	3	0	—	—	0	0	0	0	—	—
C. album	100	62	100	0	70	0	87	13	104	0	106	0
S. sclerotiorum	100	98	42	0	40	0	100	100	0	0	0	0
A. repens	—	—	—	—	63	0	—	—	—	—	4	0
P. ultimum (CFU)	—	—	—	—	3	0	—	—	—	—	0	0

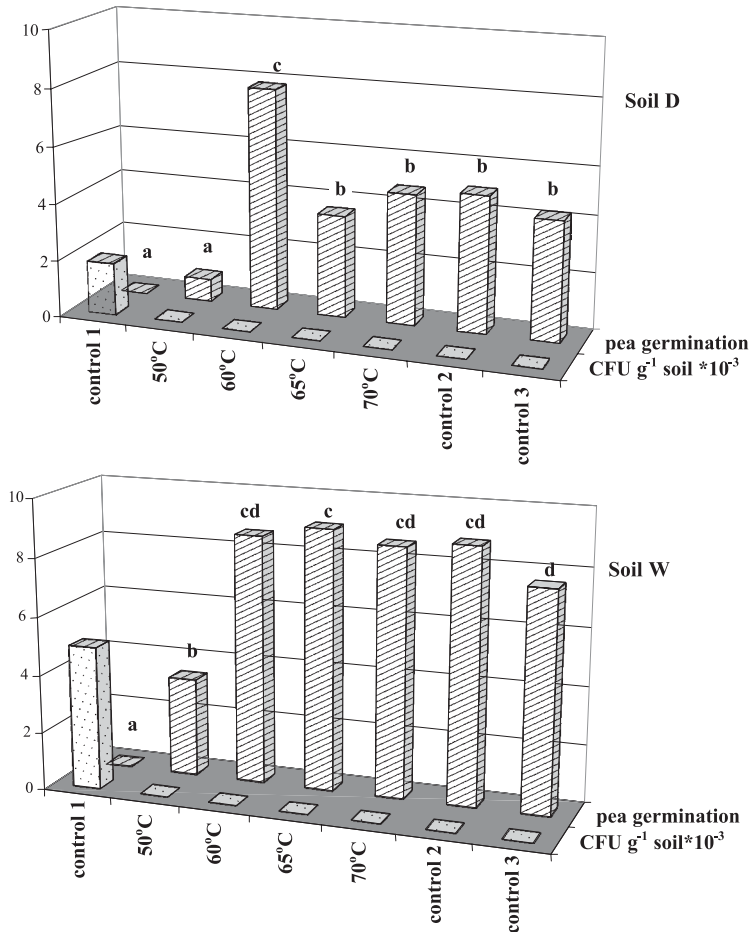

Figure 3. Effect of steaming soil infested with *P. ultimum* at a range of temperatures on germination of pea seeds (cv Kelvedon Wonder), out of a total of 9 per pot (background) and on occurrence of *P. ultimum* (foreground). Control 1 was non-steamed, infested soil, resulting in zero pea germination. (*P. ultimum* was only found in control 1 soils, except for a trace after 50 °C treatment in soil D). Control 2 was non-steamed, non-infested soil and control 3 non-steamed soil infested with sterilized *P. ultimum.* Treatments that do not share the same letter differ significantly ($P \leq 0.05$, Fisher's LSD test).

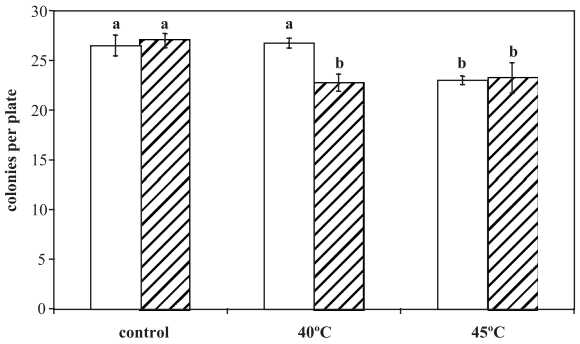

Figure 4. Germination of *V. dahliae* MS after steam treatment at 40 and 45 °C in two soils (open bars = soil D, hatched bars = soil W). Thirty MS were plated out on agar ($n = 4$). Treatments that do not share the same letter differ significantly ($P \leq 0.05$, Fisher's LSD test).

Steam treatment of *V. dahliae* and *S. cepivorum* at 40 and 45 °C was not lethal, although there was a small but significant reduction of *V. dahliae* survival at 45 °C in both soils and at 40 °C in one soil (Figure 4). There was no decrease in survival of *S. cepivorum* after 40 or 45 °C steaming.

Discussion

In earlier work in which small soil samples were treated with aerated steam, treatment times almost invariably lasted 30 min (Dawson et al., 1965; Bollen, 1969; 1985; Baker, 1970; Sylvia and Schenck, 1984). This excluded time for 'warming-up' (time needed to reach target temperature of the soil samples) and 'cooling-down' (time between treatment and further assessment). Bollen (1969) and Dawson et al. (1965) reported warming-up periods of 3–7 and 10 min respectively, while Sylvia and Schenck (1984) needed 14–35 min to heat their soils to 50–80 °C. Cooling-down periods are mostly not mentioned by these authors, except by Dawson et al. (1965), who reported 24 h. In our experiments the warming-up period was included in the treatment time, while the cooling-down period ('resting' period) lasted 8 min after which temperature of soil samples was quickly returned to room temperature. Soil samples were actively treated for a period of 3 min only, which is less then a tenth of treatment times reported in those earlier works.

In the field (e.g. after hood steaming where soil is typically steamed for 8 min (Pinel et al., 2000)) soil will cool more slowly but cooling may be faster near to the soil surface and to the base and sides of the treated zone. However, when we filled 15 cm deep pits with soil at 70 °C (both at wilting point and at field capacity) in an outside plot in December ($T_{air} = 10$ °C), even heated soil within 3.5 cm of the base or sides of the pit took >4 min to cool by 10 °C and most of the soil took >7 min to cool by 5 °C (full data not shown). Thus our procedure is a realistic representation of the high temperature exposure of organisms in most of a field plot after short steam heating to 5 °C above target temperature.

Although soil pasteurisation (70 °C, 30 min) is the recommended method for steam disinfestation of glasshouse soils (Bollen, 1985; Runia, 2000) commonly used steam practices such as sheet steaming tend to last much longer (6–7 h) and result in temperatures of 100 °C in the top 10-cm of soil (Runia, 1983; IKC-AT, 1992). Also, because of the high costs involved, steam treatment is currently limited to a few high value horticultural and floricultural crops. Our findings that major soil pests, pathogens and weeds are killed in steamed soil after only 11 min at 50–60 °C suggest that a smaller amount of energy may be needed to achieve soil disinfestation with steam and costs of steaming field soils could be reduced. Many soil-borne pests, diseases and weeds have been controlled in Southern European and Mediterranean countries with low-temperature (45–60 °C) 'steam' soil treatment, based on soil solarization (Davis, 1991; Elmore, 1991; Stapleton and Heald, 1991), apparently without negative effect on chemical, physical or microbial soil properties (Chen et al., 1991). This relatively new disinfestation technique has been recognised as a non-chemical alternative to methyl bromide (UNEP, 2000b; 2001), but is only suited to warm dry climates. Low temperature–short duration steam treatment of soil could become a comparable disinfestation method in cooler climates. Further investigations into soil properties such as matric potential, nutrient content and microbial life in relation to soil steaming are in progress.

The fungal structures used in the experiments were produced in the laboratory without influences that natural inoculum would normally experience, such as the presence of other soil microbes and climatic fluctuations. Reports on vitality of natural *versus* cultured sclerotia are inconclusive or contradicting (Coley-Smith, 1959; Merrimen, 1976; Leggett et al., 1983; Littley and Rahe 1992). In our case a number of procedures were undertaken to produce inoculum that was as natural as possible. Sclerotia of *S. cepivorum* were buried in non-sterile soil for 3 months; sclerotia of *S. sclerotiorum* were exposed to a period of 4 weeks at 4 °C, to mimic winter conditions, and MS of *V. dahlia* were allowed to grow on straw

for at least 3 months, before collection. Follow-up field trials with natural infested soil using similar steaming techniques as described here are planned and should bring to light any over- or underestimation of the steam results that may have occurred by the use of laboratory produced inoculum.

It is quite possible that a shorter period of exposure to the target temperature than 11 min may achieve the same results as those shown here. However, even if a target temperature exposure time of 11 min were necessary, this could still be achieved using a shorter period of steam heating but with a slightly higher treatment temperature. For example, nearly all of a soil heated to 70 °C would take more than 11 min to cool to a target disinfestation temperature of 60 °C. A further practical consideration is that soil heterogeneity requires that current steaming techniques incorporate a margin of error (e.g. because steam penetration under a hood or sheet cannot be entirely uniform).

Finally, it should be remembered that the work presented in this paper is an early part of a commercial enterprise, aimed at improving current soil steaming techniques towards more modern and environmentally acceptable standards.

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