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UNIVERSITÀ DEGLI STUDI DI TORINO

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Abstract The efficacy of three steam application techniques (steam injection, iron pan and sheet steaming) was evaluated against five soil-borne pathogens under controlled laboratory conditions. Injection and pan steam systems proved to be efficient and feasible alternatives to traditional sheet steaming for suppressing *Fusarium oxysporum* f.sp. *basilici* at 60% moisture field capacity in sandy-loam soil. Injecting steam was the best technique to suppress *F. oxysporum* f.sp. *basilici, F. oxysporum* f.sp. *raphani*, *F. oxysporum* f.sp. *conglutinans*, *Rhizoctonia solani* and *Phytophthora capsici*. The mycelia of *R. solani* and *P. capsici* were very sensitive to heat and were effectively killed by both injection of steam and pan steam system at 80% and 40% moisture field capacity.

Keywords soil disinfestation · *Fusarium* wilts · *Rhizoctonia solani* · *Phytophthora capsici*

Introduction

Steaming is a very effective disinfestation method for soils and substrates. Among its positive features, the broad spectrum of activity is very important: its high efficacy against soil-borne pathogens and nematodes as well as weed seeds has been known for decades (Katan 2000; Van Loenen et al. 2003; Melander and Jørgensen 2005). It was first employed in 1888 and first commercially used in the United States (Baker 1962). In general, due to its high cost, it has been applied mostly under greenhouse conditions, for high-value crops. In Italy, steam is adopted in a few greenhouses for high value

ornamental crops (rose, gerbera and potted plants) and vegetables (basil, lettuce) (Gullino et al. 2005). The main constraints are its applicability only on limited surfaces (greenhouses, raised benches, seedbeds, soilless cultivation, substrates …), high costs due to initial investments and fuel consumption.. Its high energy consumption moreover contributes to global warming due to the use of fossil fuel (Gullino et al. 2005).

Lethal temperatures for all kinds of soil-borne pathogens, pests and weed seeds have been established since the 1960's. A temperature of 70 °C for at least 30 minutes was supposed to free soil from pathogens and weeds (Bollen 1969, 1985). In many countries different steaming methods have been tested. Negative pressure steaming was assessed for efficacy by Runia (1983) for greenhouse soil disinfestation. Steam is introduced under a steaming sheet and pulled into the soil by a negative pressure, created in the soil by a fan, which sucks air out of the soil through buried perforated polypropene tubes (Runia 1983, 2000; Runia and Molendijk 2009). Steaming with the Fink system, developed for greenhouse use, is a modification of the negative pressure method: vertical suction pipes are inserted into the soil instead of horizontal ones (Ellis 1991).

Aerated steam treatment is preferable to standard steam treatment because it controls pathogenic microorganisms while allowing some of the beneficial organisms to survive. It works at 50 °C in potting media containing vermiculite infested with chlamydospores or oospores of *Phytophthora ramorum*, *Pythium irregulare*, *Thielaviopsis basicola*, and *Cylindrocladium scoparium* (Linderman and Davis 2008). Aerated steam (air-steam mixture) at 50 - 60°C for 30 min selectively controls plant pathogens such as *Fusarium* spp. and could be used for high-revenue crops (Ajwa et al. 2003).

After the phase-out of methyl bromide, the possible application of steaming has been considered for other crops, also grown in the open. New application technologies are under development which might significantly reduce the cost of soil steaming, maintaining its efficacy (Runia 2000; Runia and Greenberger 2005). A model oriented control technique to optimize treatment duration and reduce fuel consumption was presented by Dabbene et al. in 2003. Minuto et al. (2005) tried to identify the optimal soil moisture at different soil depths, in order to maximize the efficiency of sheet steaming technique on benches under greenhouse conditions.

Gay et al. (2008) investigated, by means of a small-scale plant, the effect of texture and moisture content on soil heating by supplying steam with different distribution systems, based on iron pan, buried injector and sheet steaming. Steam injection at sub-surface level proved to be more efficient

than surface application (sheet steaming and pan), obtaining quick and homogeneous heating of the soil slab above the application point. Soil heating efficiency tuned out to be strongly dependent on moisture content, in particular for sandy-loam soil as opposed to sandy soil.

The present work was carried out during the period 2007-2009 with the aim of determining, at laboratory scale, the effectiveness of different steam application techniques (sub-surface steam injection, surface steam application by means of iron pan and sheet steaming) against selected soil-borne pathogens. The role of soil moisture content was also studied, in order to determine the optimal soil conditions for efficacy of the considered techniques, also in order to implement them under practical conditions.

Materials and methods

The experiments were organized in two different phases. During the first (in 2007), a preliminary study on the effect against *Fusarium oxysporum* f.sp. *basilici* was carried out with three steam application systems (injector, pan and sheet steaming). Only one intermediate soil water content (60% of field capacity) on a sandy-loam soil was considered, corresponding with the optimal moisture value to improve sheet steaming efficiency found by Minuto et al. (2005). In this preliminary step a pilot plant equipped with a three dimensional temperature probe buried in a trial box (Gay et al. 2008) was employed.

On the basis of the results obtained, the second phase, in 2008 and 2009, focused only on steam injection and surface steam application with pan, since traditional sheet steaming proved to be the less efficient technique and not economically and technically sustainable under field condition (Runia 2000). Furthermore, the development of machines for soil steaming is required by growers as alternative to chemical soil disinfestation, therefore the employment of sheet steaming could be limited only in few cases, such as bench disinfestation.

The second set of trials, dealt with four soil-borne pathogens (*Fusarium oxysporum* f.sp. *raphani*, *F. oxysporum* f.sp. *conglutinans*, *Rhizoctonia solani* and *Phytophthora capsici*). It was carried out in a new box equipped with free temperature sensors improving experimental procedure, in particular temperature monitoring. In this case a sandy soil was used because it is considered to be one of the most difficult to steam (Runia 2000; Minuto et al. 2005). Two different water content levels, 40% and

80% of field capacity, was considered in order to evaluate the effect of moisture on treatment efficacy.

Pathogens and inoculum preparations Five soil-borne pathogens were tested: *F. oxysporum* f.sp. *basilici* resistant to benomyl (FOB 009 RB), *F. oxysporum* f.sp. *raphani* resistant to benomyl (ATCC 64105 RB), *F. oxysporum* f.sp. *conglutinans* resistant to benomyl (ATCC 16600 RB), *Rhizoctonia solani* and *Phytophthora capsici*. Pathogen cultures were obtained from the American Type Culture Collection (ATCC), Manassas, Virginia, USA or from the collection of Center of Competence for the Innovation in the Agro-Environmental Sector (AGROINNOVA) of the University of Torino, located at Grugliasco (Torino), Italy. *F. oxysporum* strains (FOB 009 RB, ATCC 64105 RB and ATCC 16600 RB) were grown in 1000-ml Erlenmeyer flasks containing 250 ml of hydrolysed casein. Flasks were incubated on a platform shaker at 200 rpm, at 20-25°C. After 12 days, fungal liquid cultures were aseptically removed from the flasks and centrifuged at 8,000 g for 20 min at 20°C. The pellet was thoroughly mixed with twice the weight of dry talc powder (1:2 w/w) and kept for 10 days at 25°C as described by Locke and Colhoun (1974). The number of chlamydospores per gram of talc powder was assessed by serial plating on Komada medium (Komada 1975) containing 10 mg l^{-1} of benomyl (Benlate, 50% a.i., DuPont de Nemours, Milano, Italy).

F. oxysporum f.sp. *basilici* (FOB 009 RB) chlamydospores prepared in talc were mixed into the test soil at 2×10^4 CFU g^{-1} soil. Chlamydospores prepared in talc of *F. oxysporum* f.sp. *raphani* (ATCC 64105 RB) and *F. oxysporum* f.sp. *conglutinans* (ATCC 16600 RB) were mixed with sterile sand at 5×10^4 - 5×10^5 CFU g^{-1}soil, and placed in a fibreglass mesh bag (5cm×5cm, 6g sand per bag), which then were placed at appropriate soil depth in the steam box.

R. solani was incubated in flasks containing wheat kernel medium (300 g of wheat kernels in 320 ml of deionised water, autoclaved at 121°C for 30 min) at 25°C for seven days. *P. capsici* was propagated in flasks with wheat-hempseed medium (200 g of wheat kernels and 100 g hempseeds in 320 ml of deionised water, autoclaved at 121°C for 30 min) at 25°C in a growth chamber with a 12-h fluorescent photoperiod for two weeks. Five gram fresh biomass of wheat kernels infested with *R. solani* or *P. capsici*, respectively, were transferred into a fibreglass mesh bag as test targets.

Small-scale plant and steam application systems The equipment was made to study at laboratory scale, under controlled conditions, the temperature distribution in a defined bulk of soil, and as well as the

116 effect against the five pathogens. In particular, the injector and pan were two small scale prototypes
117 developed by Gay et al. (2008). Experiments were done in a small scale pilot plant consisted of an 8.5
118 kW electrical steam generator which supplied the distribution equipments. Steam generator was also
119 equipped with an electrical heater (1600W), set at 120°C, in order to reduce steam condensation. Steam
120 pressure and flow were set at 0.5 bar and 0.7 kg h^{-1}, respectively, during both experimental phases.
121 Steam output temperature was about 100°C. due to heat losses. Steam was applied to a box filled with
122 soil and equipped with an appropriate temperature probe connected with a data acquisition system.
123 Temperature data were recorded every 10 s for 24 hours and stored in a PC, which also automatically
124 managed the steam supply and acquisition schedules by appropriate software.
125 Two different trials boxes were used in the trials. In 2007, a Polypropylene box (a 40 cm cube)
126 equipped with a three dimensional temperature probe was used (Fig. 1). The probe consisted of three
127 square grids (15 cm side length) located at 4, 10 and 16 cm depth. On each grid were mounted 16
128 T-type thermocouples, spaced at 5 cm intervals. The thermocouples were arranged on a balsawood
129 frame coated with epoxy resin to minimize thermal coupling. As shown in Fig. 1 the probe was
130 positioned in the centre of the box, in order to avoid border effects, establishing a trial volume of about
131 15×15×12 cm. More details about the small scale plant and three dimensional temperature probe can be
132 found in Gay et al. (2008). Pathogen inoculums were directly mixed with soil one day before steam
133 application following the procedure described below.
134 In 2008 and 2009, we used a new box (44 × 44 × 36 cm high), made of epoxy resin painted wood
135 panels, equipped with a different temperature measurement system (Fig. 3). Pathogen inocula were
136 introduced into bags arranged on 4 levels (2 cm, 7 cm, 13 cm and 19 cm depth) for trials with injector
137 and on 3 levels (7 cm, 13 cm and 19 cm depth) for those performed with pan, following the schemes of
138 Fig. 4 and Fig. 5. This choice came from the results of the preliminary trials, during which complete
139 pathogen inactivation was observed in the surface layer when a pan steam distribution system was
140 employed (see Results). A thermocouple was pinned to each test bag containing the target pathogen
141 (Fig. 3, right panel), thus the temperature profile of each bag was registered. Furthermore, employing
142 free temperature sensors, bag arrangement was not bound to the grids and more layers could be
143 considered than in the previous case. Three bags with their temperature sensors were buried at each
144 level in three corresponding zones as shown in Fig. 5. During the trials carried out by using the injector,
145 bags were arranged avoiding the positions Ch 25, Ch 26 and Ch 27 at 13 cm depth because they were

close to the steam injection point. At the same time a bag for each trial was maintained at the same soil moisture level, but at room temperature as control.

In the first experiments, three steam distributions techniques (steam injection, pan and sheet steaming) were compared, while interest was focused on two of them (injector and pan) in the second phase.

Sub-surface steam was injected by a tube 21 mm in diameter and 250 mm long with a 25 mm diameter hole near the tip. Burying depth was regulated by means of a stop-collar and during trials the injector hole was at about 13 cm deep inside soil.

The pan distribution system consisted of a 20 cm square iron pan equipped with a square drilled diffusion plate (four 1 mm diameter holes), made of an iron sheet, to improve steam distribution on the soil surface. To reduce steam losses, pan edges were partially forced down into the soil.

Sheet steaming trials were made with a diffuser in order to simulate the behaviour of pipes commonly employed in this kind of treatment. It consisted of a 21mm diameter zinc-coated steel tube 70 mm long with a set of 1 mm diameter holes on the side. Treatments were carried out by resting the diffuser on the soil surface in the middle of the trial box and covering it with a plastic film.

Steam in the injection and pan treatments was supplied for 15 minutes, whereas in the sheet steaming treatments for 60 minutes.

Soil moisture In 2007, a sandy-loam soil (69.4% sand, 15.5% silt and 15.1% clay) was collected at a horticultural farm in Albenga, Italy. Moisture content was set before treatment at 9.3% (equally to 60% field capacity) during mixing, with tap water using a manual fog nozzle (Gay el al. 2008). A natural agricultural sandy soil (85.2% sand, 7.8% silt and 6.3% clay) from Moncalieri, Italy, was steamed at 12.4% and 6.2% moisture content, in the second phase of the experiments, corresponding to 80% and 40% field capacity, respectively. Both soils were sieved to eliminate stones and aggregates, and provide a more homogeneous medium, improving repeatability.

Sampling and evaluation of steam efficacy In 2007, three soil samples of 15 g were taken at 3, 9 and 15 cm depth, 24 hours after steam treatment (Fig. 1 right panel) as shown in Fig. 2. In pan and sheet steaming treatments, samples at each depth were obtained at the front of the soil section defined by the three-dimensional temperature probes, as indicated by the light gray zone in Fig. 2. Samples at each

soil depth in the injection steam treatment were taken at a distance of 9 cm from the injector with the assumption of steam quickly moving to a close distance (dark gray zone of Fig. 2). Three 5-g soil subsamples of each replicate were added individually to 50 ml sterilized deionized water, shaken for 20 minutes on a reciprocal shaker (180 rpm), and then assessed for *Fusarium* spp. by serial dilution on Komada medium (Komada 1975) containing 10 mg l^{-1} of benomyl (50% Benlate, DuPont, American) (Gamliel and Katan 1991). Initial inoculum density in soil, after inoculation of the pathogens and before steaming, was measured as control by serial dilution. In this case, soil temperature profile was assessed only for the samples taken among temperature probe grids (layers 2 and 3 of Fig 2) as average of the values measured by the eight thermocouples which enclosed each sample area.

In 2008 and 2009 bags, containing the selected pathogens, were taken from treated soil 24 hours after steam treatment (Fig. 3, 4 and 5). Survival of *F. oxysporum* f.sp. *raphani* (ATCC 64105 RB) and *F. oxysporum* f.sp. *conglutinans* (ATCC 16600 RB) was assessed as described above. Three replicates were arranged for each sample. Survival of *R. solani* and *P. capsici* with and without steam treatment, was assessed by using wheat kernels plated on PDA medium containing 25 mg l^{-1} of streptomycin sulphate or on selective Masago medium (Masago et al. 1977), respectively. One hundred wheat kernels were incubated on ten plates (10 kernels per plate) containing appropriate medium, and surviving colonies were counted after one week.

Data analysis All data were analysed by one-way ANOVA in SPSS 17.0 Windows software, and chlamydospore populations (CFU) were logarithmically ($\log_{10}$ (CFU+1)) transformed before analysis. The influence of temperature on the pathogens was examined by calculating Pearson's correlation coefficient.

Results

Efficacy of different steam distribution systems against Fusarium oxysporum *f.sp*. basilici *at 60% field capacity* The injector, pan and sheet steaming techniques were evaluated against *F. oxysporum* f.sp. *basilici* (FOB 009 RB) with 9.3% soil moisture value (corresponding with 60% field capacity) in sandy-loam soil. By pan treatment *F. oxysporum* f.sp. *basilici* FOB 009 RB was effectively suppressed at 3 cm and 9 cm soil depth, where the maximum temperature was 96.9°C (Table 2). Sheet steaming

showed similar efficacy against FOB 009 RB as pan steaming in the upper soil layers (3 cm and 9 cm depth), where a temperature above 60°C was achieved for at least 132 minutes (Table 2). At 15 cm depth, FOB 009 RB was completely killed by steam injection at soil depth of 3, 9 and 15 cm, and a maximum temperature of 99.5°C was reached, with 231 minutes above 60°C (Table 2).

Good correspondence between maximum temperature and elimination of FOB 009 RB was observed for all steam treatments, with significant negative correlation coefficient of -0.903, -0.989 and -0.908 in pan, sheet and injection steam treatments, respectively (Table 2).

Efficacy of injecting steam against soil-borne pathogens at 80% and 40% field capacity Populations of *F. oxysporum* f.sp. *conglutinans* (ATCC 16600 RB) and *F. oxysporum* f.sp. *raphani* (ATCC 64105 RB) were significantly reduced by steaming injection at 80% moisture field capacity compared with unsteamed soil, except the sample position Ch 1 and Ch 28, where the bags were located 14 cm far from the injector and at a depth of 2 and 19 cm, where lower temperatures were registered (Table 3). A survival rate of 100% of *R. solani* was obtained at the same positions with a maximum temperature of 38.2°C (Table 3). A different susceptibility to thermal treatments was observed in Ch 10 between *F. oxysporum* f.sp. *conglutinans* (ATCC 16600 RB) and *R. solani*. Particularly, the survival of *F. oxysporum* f.sp. *conglutinans* at 54.4 °C did not significantly differ from untreated samples, while surviving colonies of *R. solani* at 48 °C were 53%. *P. capsici* was significantly suppressed in all the positions tested (Table 3). At Ch 1 however some infectivity remained.

Likewise, at position Ch 1 (2 cm depth, 14 cm distance from injector) in the steaming injection treatment, *F. oxyporum* and *R. solani* populations did not significantly decrease at 40% moisture field capacity in sandy soil relative to unsteamed soil (Table 4). However, 100% efficacy against *P. capsici* was also obtained at 40% moisture field capacity by steam injection, although temperature was slightly below 60^{o}C.

A remarkable difference among the temperatures registered in different trials was observed in the sampling position Ch 10 at both moisture conditions (Table 3 and 4). This means that this position (7cm depth, 14 cm far from steam injection point) represents a border situation because in the same conditions steam flow cannot achieve it, thus the treatment efficacy is not ensured.

A significant negative correlation was observed between maximum temperature and *Fusarium* or *R. solani* survival with steaming injection at soil moisture levels 80% and 40% field capacity, respectively

(Tables 3 and 4). A negative association was found between maximum temperature and survival of *P. capsici* (correlation coefficient -0.750) in the case of 80% moisture field capacity**,** but not at 40%, due to absolute mortality for statistical analysis (Tables 3 and 4).

Efficacy of pan application system at 80% and 40% field capacity In the pan steaming experiment of soil with 80% moisture field capacity, there was significant reduction of *F. oxysporum* f.sp. *conglutinans* (ATCC 16600 RB) populations at 7 and 13 cm depth compared with unsteamed soil (Table 5). *F. oxysporum* f.sp. *raphani* (ATCC 64105 RB), inoculated in soil as chlamydospores at 80% moisture field capacity, was completely killed by pan steaming at 7 cm depth, and the sample position Ch 24 (at 13 cm soil depth) where a high steaming temperature of 87.7°C was reached and the duration of the temperature above 60°C was 22 minutes (Table 5). Recovery rate of *R. solani* was markedly decreased at 7 cm and 13 cm soil depth, but survival rate at 19 cm depth was 100% (Table 5). Moreover, at 13 cm depth, temperatures of above 60°C (maximum value 64.8°C), maintained for 10 min, could be enough to completely control *R. solani* (Table 5). At 40% soil moisture field capacity, a low *F. oxysporum* concentration was found at depth 7 cm but recovery of the population at 13 and 19 cm equalled unsteamed soil in pan steam treatment (Table 6). *R. solani* was susceptible to pan treatment only at 7 cm soil depth. Consistent reduction of *P. capsici* was obtained at 40% and 80% moisture by pan steaming at all test depths (Tables 5 and 6).

At the end of each pan steaming experiment, a correlation between maximum temperature and pathogen recovery was calculated. A statistically significant negative correlation was found between temperature and pathogen survival (Tables 5 and 6).

Discussion

In 2007 the efficacy of three steam application techniques against *F. oxysporum* f.sp. *basilici* was evaluated in a sandy-loam soil at 60% of field capacity. Results showed that sheet steaming was effective at 3 and 9 cm soil depth (layer 1 and 2 in Fig. 2), because a temperature close to 70°C was reached even in the intermediate layer of the soil bulk, after 60 min of steam supply. Temperatures achieved in the deepest layer (15 cm depth) were too low (maximum temperature 47.2°C) to affect pathogen survival. In sheet steaming, steam flow involves only the surface layer, whereas the deeper

ones are heated only by heat conduction. This behavior is a consequence of a condensation front, located under the surface layer, which strongly reduces steam penetration at deeper levels (Dabbene et al. 2003; Gay et al. 2008).

Pan and injector application systems provided interesting comparisons with sheet steaming. With the pan system, steam flow also penetrates the intermediate layer as a result of the higher pressure achieved under the pan, according to Gay et al. (2008). Higher temperature can be achieved at 9 cm depth with pan treatment (96.9°C) than with sheet steaming (69.0°C), as clearly shown in Table 2.

The steam injection system was considered the most efficient in terms of pathogen inoculum reduction. Steam injected at sub-surface level (13 cm depth) naturally moved toward the soil surface, establishing a mixed liquid-vapor rising flow which involves all layers, according to Gay el al. (2008). This behavior led to homogeneous soil heating and high temperatures, so that *F. oxysporum* f.sp. *basilici* was completely suppressed at depths of 3, 9 and 15 cm (Table 2).

The duration of sheet steaming needed to control effectively the test pathogens was 60 minutes. This is four times longer than required by pan and injector systems (15 min). Furthermore, sheet steaming is very labour intensive with a low level of mechanization, thus its employment is strongly limited by high costs.

On the basis of the results obtained, injection and pan steaming were considered to be more effective than traditional sheet steaming at soil moisture of 60% field capacity. Therefore, the second part of the work was aimed to evaluate the efficacy of pan and injection on suppressing soil-borne pathogens at 80% and 40% moisture field capacity in a sandy soil.

Soil moisture did not influence pathogen suppression in the upper soil layer with pan steam treatment. However only at 80% field capacity all test pathogens were completely eradicated. This could result from the rapid heating of the soil and homogeneous diffusion of the steam in the soil profile. In the intermediate layers (13 cm depth), higher soil moisture content corresponded to higher soil heating and therefore to greater control. An increase in soil water content normally raises the thermal conductivity as well as the liquid and vapour phase diffusivity (Abu-Hamdeh 2001). No appreciable changes in soil temperature were observed in the deepest layer (19 cm) between the two moisture levels.

Steam injection strongly controlled the test fungi for both moisture contents, although moister soil improved the efficacy in particular with regard to the surface layer. We note that in bags placed furthest

292 from the injector (Ch 1, Ch 2 and Ch 28) the effect of the treatment was negligible because at these
293 distances the steam scarcely moved horizontally. This behaviour will have to be taken in account for
294 the design of equipment to implement this technique.

295 Heat can be released by pan and injection treatments at different soil depths. Injection can heat soil at
296 depth (19 cm) (Tables 3 and 4), whereas the pan distribution system can reach depths between 9 and 13
297 cm (Tables 2, 5 and 6). Thus pan steaming could be interesting for short-cycle intensive crops, such as
298 lettuce and other leafy vegetables grown for processing, which are harvested before the risk of
299 pathogen re-infestation. It would also be interesting in shallow nursery soils. Conversely, deeper steam
300 injection may be employed in open field or on long-term crops, since it allows strong pest reduction
301 even in the deepest layers as a function of injector length.

302 The minimum temperature required for pasteurization is determined by the thermal death-points of
303 pathogens. Different fungi have different temperature sensitivities, and sometimes different lethal
304 temperatures for the same genera are due to modes of survival such as chlamydospores or mycelium
305 (Mouchacca 2007; Nash et al. 1961; Schippers and Van Eck 1981). *Fusarium* spp. can be selectively
306 controlled at 50 - 60°C (Ajwa et al. 2003; Bollen 1969). Our results showed that *F. oxysporum* f.sp.
307 *conglutinans* and *F. oxysporum* f.sp. *raphani*, introduced in the soil as chlamydospores, failed to
308 survive at temperatures of 60°C or higher. Lethal temperature for *R. solani* is between 50 and 60°C
309 depending on its survival structures (Gayed et al. 1978; Pullman et al. 1981; Bollen 1969). In this study,
310 temperatures of 60°C were detrimental to *R. solani* buried in soil. *P. capsici,* as well as other species of
311 Chromista like *Pythium irregulare, P. ultimum, Phytophthora cryptogea* and *P. ramorum*, was killed at
312 50°C, according to otherstudies (Bollen 1969; Van Loenen et al. 2003; Browning et al. 2008;
313 Linderman and Davis 2008). Thus detailed information on the heat-tolerance of target microflora and
314 their survival structures is needed to implement pan or injection steam practice. Further research will be
315 needed to increase the use of steam, reducing required application times, combined with antagonists to
316 eliminate colonization of pathogens reinvading the soil and the negative effect of a "biological
317 vacuum" (Baker 1962; Katan 2000).

318 In conclusion, this study provides practical information about the possibility of using different steam
319 distribution systems to suppress different pathogens. The results show that there is not one answer for
320 all problems. In our study, steam injection was the best technique to suppress *F. oxysporum* f.sp.
321 *basilici, F. oxysporum* f.sp. *raphani*, *F. oxysporum* f.sp. *conglutinans*, *R. solani* and *P. capsici* at

different soil depths and moisture contents. The pan steam system was more effective against *R. solani* and *P. capsici*, due to their high thermal sensitivity, compared with *F. oxysporum*.

By considering the target pathogens for each crop and their survival structures, the most efficient and least costly technique can be selected for practical application. In this way, the use of steam for soil disinfestation can be implemented in a larger number of situations.

Acknowledgements

This work was supported by grants from the Italian Ministry for Environment, Land and Sea and Piedmont Region (CIPE: Tecniche e strategie innovative e eco-compatibili per la disinfestazione dei terreni agrari in orticoltura specializzata ad alto reddito) and by INJETVAP project. The fellowship of Pingxiang Lu was co-funded by the Italian Ministry for University and Research, the University of Torino and the Italian Ministry for Environment, Land and Sea. The authors thank Dr. Robert Milne for language revision.

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Table 1. Layout of steaming trials carried out under laboratory conditions in 2007, 2008 and 2009

Pathogen	Soil texture	Soil infestation	Steam distribution (duration, minute)	% moisture field capacity	Evaluation methods on medium	Year
F. oxysporum f.sp. *basilici*	Sandy-loam	Mixing chlamydospore as talc powder	Injection (15), pan (15) and sheet steaming (60)	60	Soil dilution on Komada (10 ppm benomyl)	2007
F. oxysporum f.sp. *conglutinans*	Sandy	Mesh bags with chlamydospore as talc powder	Injector (15) and pan (15)	80 and 40	Soil dilution on Komada (10 ppm benomyl)	2008 and 2009
F. oxysporum f.sp. *raphani*	Sandy	Mesh bags with chlamydospore as talc powder	Injector (15) and pan (15)	80 and 40	Soil dilution on Komada (10 ppm benomyl)	2008 and 2009
Rhizoctonia solani	Sandy	Mesh bags of mycelium on wheat kernels	Injector (15) and pan (15)	80 and 40	Plating on PDA	2008 and 2009
Phytophthora capsici	Sandy	Mesh bags of mycelium on wheat and hemp seed	Injector (15) and pan (15)	80 and 40	Plating on Masago	2008 and 2009

Table 2. Efficacy of three steam techniques (pan, sheet and injector) against *Fusarium oxysporum* f.sp. *basilici* (FOB 009 RB) at 60% field capacity of sandy-loam soil in 2007.

Pan				Sheet			Injector			
Sampling positions (Depth, cm)	Maximum temperature (°C) and duration (min) above 60°C	CFU g^{-1} soil	Log10(CFU+1)	Maximum temperature (°C) and duration (min) above 60°C	CFU g^{-1} soil	Log10(CFU+1)	Sampling positions (Depth, cm–Distance[a], cm)	Maximum temperature (°C) and duration (min) above 60°C	CFU g^{-1} soil	Log10(CFU+1)
Unsteamed soil	-	2.0×10^{4}	4.3 e[b]	-	2.0×10^{4}	4.3 b	Not-steamed soil	-	2.0×10^{4}	4.3 e *
1A (3cm)	-	0.0	0.0 a	-	0.0	0.0 a	1A (3cm - 9cm)	-	3.7×10^{1}	1.6 cd
1B (3cm)	-	0.0	0.0 a	-	0.0	0.0 a	1B (3cm - 9cm)	-	2.0×10^{1}	1.3 b
1C (3cm)	-	0.0	0.0 a	-	0.0	0.0 a	1C (3cm - 9cm)	-	5.9×10^{1}	1.8 d
2A (9cm)	96.9 (120.7)	4.9×10^{1}	1.7 c	64.3 (132.7)	0.0	0.0 a	2A (9cm - 9cm)	99.1 (181.3)	0.0	0.0 a
2B (9cm)	94.7 (124.7)	0.0	0.0 a	65.6 (146.5)	0.0	0.0 a	2B (9cm - 9cm)	99.4 (194.8)	0.0	0.0 a
2C (9cm)	86.9 (112.5)	3.0	0.7 b	69.0 (175.7)	0.0	0.0 a	2C (9cm - 9cm)	98.6 (166.3)	2.7×10^{1}	1.4 bc
3A (15cm)	59.2	3.0×10^{4}	4.5 f	44.6	5.3×10^{4}	4.7 d	3A (15cm - 9cm)	99.5 (218.2)	0.0	0.0 a
3B (15cm)	57.1	1.1×10^{4}	4.0 d	45.6	6.0×10^{4}	4.8 d	3B (15cm - 9cm)	99.5 (231.0)	0.0	0.0 a
3C (15cm)	49.3	8.0×10^{3}	3.9 d	47.2	4.3×10^{4}	4.6 c	3C (15cm - 9cm)	99.4 (200.0)	0.0	0.0 a
Pearson's coefficient	-0.903*			-0.989*				-0.908*		

a Distance from the centre of appropriate samples to the injector (see Figure 2).

b Values of the same column, followed by the same letter, do not significantly differ according to Tukey's test ($P<0.05$).

* Significant correlation ($P\leq0.05$).

Table 3. Efficacy of steam injection treatments on *F. oxysporum* f.sp. *conglutinas*, *F. oxysporum* f.sp. *raphani*, *Rhizoctonia solani* and *Phytophthora capsici* at 80% moisture field capacity in 2008

Sampling positions (Depth,cm–Distance[a], cm)	*F. oxysporum* f.sp. *conglutinans*			*F. oxysporum* f.sp. *raphani*			*Rhizoctonia solani*		*Phytophthora capsici*	
	Maximum temperature (°C) and duration (min) above 60°C	CFU g^{-1} soil	Log10 (CFU+1)	Maximum temperature (°C) and duration (min) above 60°C	CFU g^{-1} soil	Log10 (CFU+1)	Maximum temperature (°C) and duration (min) above 60°C	% surviving colony per pan	Maximum temperature (°C) and duration (min) above 60°C	%surviving colony per plate
Unsteamed soil	-	2.2×10^{5}	5.3 e[b]	-	8.3×10^{4}	4.9 c	-	100.0 c	-	57.0 b
Ch9 (2 - 4)	99.0 (26.2)	80.0	1.9 c	98.2 (43.6)	0.0	0.0 a	98.5 (31.5)	0.0 a	98.4 (19.3)	0.0 a
Ch5 (2 - 9)	84.4 (18.2)	27.0	1.1 abc	55.9	3×10^{3}	3.3 b	72.6 (9.2)	0.0 a	80.5 (13.3)	0.0 a
Ch1 (2 - 14)	48.3	3.2×10^{4}	4.5 de	38.8	6.8×10^{4}	4.8 c	36.1	100.0 c	42.7	3.0 a
Ch17 (7 - 4)	99.1 (76.8)	40.0	1.6 bc	98.9 (63.8)	0.0	0.0 a	98.8 (67.0)	0.0 a	98.8 (26.3)	0.0 a
Ch15 (7 - 9)	99.1 (72.7)	10.0	0.8 ab	98.9 (67.0)	0.0	0.0 a	98.8 (67.5)	0.0 a	98.7 (27.5)	0.0 a
Ch10 (7 - 14)	54.4	1.2×10^{4}	4.1 d	81.3 (20.0)	20.0	1.0 a	48.0	53.0 b	94.7 (18.5)	0.0 a
Ch24 (13 - 9)	98.4 (59.8)	0.0	0.0 a	98.0 (65.0)	0.0	0.0 a	97.9 (60.5)	0.0 a	98.0 (28.2)	0.0 a
Ch19 (13 - 14)	98.4 (54.5)	0.0	0.0 a	98.0 (40.6)	0.0	0.0 a	97.9 (34.8)	0.0 a	98.0 (27.0)	0.0 a
Ch20 (13 - 14)	97.6 (35.5)	50.0	1.7 bc	61.1 (3.5)	3.0×10^{3}	3.4 b	82.0 (11.7)	0 .0a	98.0 (24.2)	0.0 a
Ch35 (19 - 4)	99.7 (67.0)	7.0	0.7 ab	99.5 (52.8)	0.0	0.0 a	98.5 (39.5)	0.0 a	99.4 (22.5)	0.0 a
Ch33 (19 - 9)	99.6 (58.8)	0.0	0.0 a	99.4 (59.5)	7.0	0.7 a	97.7 (36.8)	0.0 a	99.4 (26.5)	0.0 a
Ch28 (19 - 14)	62.9 (8.0)	2.8×10^{4}	4.4 de	58.1	2.0×10^{4}	4.3 c	38.2	100.0 c	65.5 (10.3)	0.0 a
Pearson's coefficient	-0.902*			-0.978*			-0.944*		-0.750*	

a Distance from the centre of appropriate samples to the injector (see Figure 5).

b Values of the same column, followed by the same letter, do not significantly differ according to Tukey's test ($P<0.05$).

* Significant correlation ($P\leq0.05$).

Table 4. Efficacy of steam injection treatments on *F. oxysporum* f.sp. *conglutinas*, *F. oxysporum* f.sp. *raphani*, *Rhizoctonia solani* and *Phytophthora capsici* at 40% moisture field capacity in 2009

Sampling positions (Depth, cm–Distance[a], cm)	*F. oxysporum* f.sp. *conglutinans*			*F. oxysporum* f.sp. *raphani*			*Rhizoctonia solani*		*Phytophthora capsici*	
	Maximum temperature (°C) and duration (min) above 60°C	CFU g^{-1} soil	Log10 (CFU+1)	Maximum temperature (°C) and duration (min) above 60°C	CFU g^{-1} soil	Log10 (CFU+1)	Maximum temperature (°C) and duration (min) above 60°C	% surviving colony per plate	Maximum temperature (°C) and duration (min) above 60°C	% surviving colony per plate
Unsteamed soil	-	3.0×10^5	5.5 c[b]	-	4.2×10^5	5.6 c	-	100.0 c	-	64.0 b
Ch9 (2 - 4)	86.0 (21.0)	0.0	0.0 a	87.9 (26.0)	0.0	0.0 a	74.2 (23.0)	0.0 a	98.4 (26.0)	0.0 a
Ch5 (2 - 9)	52.6	1.7×10^4	4.2 b	53.5	6.3×10^3	3.8 b	72.4 (24.0)	0.0 a	59.3	0.0 a
Ch1 (2 - 14)	35.5	2.8×10^5	5.4 c	34.5	2.2×10^5	5.3 c	40.9	98.0 c	58.3	0.0 a
Ch17 (7 - 4)	99.0 (85.0)	0.0	0.0 a	99.0 (71.0)	3.0	0.4 a	98.9 (91.0)	0.0 a	98.6 (82.0)	0.0 a
Ch15 (7 - 9)	99.0 (85.0)	0.0	0.0 a	98.9 (74.0)	7.0	0.7 a	99.0 (88.0)	0.0 a	98.6 (92.0)	0.0 a
Ch10 (7 - 14)	74.4 (20.0)	0.0	0.4 a	45.9	2.3×10^3	3.4 b	61.7 (1.0)	0.0 a	93.5 (10.0)	0.0 a
Ch24 (13 - 9)	98.5 (81.0)	0.0	0.0 a	98.6 (66.0)	0.0	0.0 a	98.4 (65.0)	0.0 a	98.3 (87.0)	0.0 a
Ch19 (13 - 14)	97.2 (45.0)	0.0	0.0 a	98.0 (56.0)	0.0	0.0 a	98.2 (55.0)	0.0 a	98.1 (64.0)	0.0 a
Ch20 (13 - 14)	87.9 (31.0)	0.0	0.0 a	95.9 (46.0)	3.0	0.4 a	96.4 (37.0)	0.0 a	97.3 (37.0)	0.0 a
Ch35 (19 - 4)	99.6 (80.0)	0.0	0.0 a	99.5 (80.0)	0.0	0.0 a	99.5 (79.0)	0.0 a	98.1 (55.0)	0.0 a
Ch33 (19 - 9)	99.4 (75.0)	0.0	0.0 a	99.5 (86.0)	0.0	0.0 a	99.2 (54.0)	0.0 a	99.1 (71.0)	0.0 a
Ch28 (19 - 14)	56.5	1.1×10^4	4.0 b	50.1	1.2×10^3	3.1 b	53.0	37.0 b	60.0 (0.2)	0.0 a
Pearson's coefficient	-0.953*			-0.971*			-0.809*		-0.506	

a Distance from the centre of appropriate samples to the injector (see Figure 5).

b Values of the same column, followed by the same letter, do not significantly differ according to Tukey's test ($P<0.05$).

* Significant correlation ($P\leq0.05$).

Table 5. Efficacy of steam pan treatments on *F. oxysporum* f.sp. *conglutinas*, *F. oxysporum* f.sp. *raphani*, *Rhizoctonia solani* and *Phytophthora capsici* at 80% moisture field capacity in 2008

Sampling positions (Depth, cm)	*F. oxysporum* f.sp. *conglutinans*			*F. oxysporum* f.sp. *raphani*			*Rhizoctonia solani*		*Phytophthora capsici*	
	Maximum temperature (°C) and duration (min) above 60°C	CFU g^{-1} soil	Log10 (CFU+1)	Maximum temperature (°C) and duration (min) above 60°C	CFU g^{-1} soil	Log10 (CFU+1)	Maximum temperature (°C) and duration (min) above 60°C	% surviving colony per plate	Maximum temperature (°C) and duration (min) above 60°C	% surviving colony per plate
Unsteamed soil	-	6.1×10^4	4.8 d[a]	-	1.5×10^5	5.2 e	-	100.0 c	-	50.0 d
Ch10 (7cm)	98.9 (51.3)	0.0	0.0 a	99.0 (51.0)	0.0	0.0 a	97.9 (48.0)	0.0 a	98.4 (48.2)	0.0 a
Ch14 (7cm)	98.9 (59.8)	0.0	0.0 a	98.4 (54.0)	0.0	0.0 a	98.5 (53.0)	0.0 a	99.1 (58.7)	0.0 a
Ch18 (7cm)	98.9 (54.3)	0.0	0.0 a	98.9 (54.0)	0.0	0.0 a	98.4 (55.2)	0.0 a	99.0 (58.2)	0.0 a
Ch20 (13cm)	80.5 (22.0)	0.0	0.0 a	55.2	5×10^4	4.7 b	53.6	10.0 b	84.7 (1.0)	0.0 a
Ch24 (13cm)	71.9 (17.8)	2×10^3	3.3 c	87.7 (22.7)	0.0	0.0 a	55.5	10.0 b	85.3 (37.5)	0.0 a
Ch25 (13cm)	78.4 (17.5)	33.0	1.5 b	62.8 (10.8)	7.9×10^4	4.9 c	64.8 (10.0)	0.0 a	57.0	0.0 a
Ch28 (19cm)	27.9	5.6×10^4	4.7 d	30.0	1.2×10^5	5.2 e	27.7	100.0 c	30.5	19.0 c
Ch32 (19cm)	31.9	4.3×10^4	4.6 d	31.1	5.7×10^4	4.8 b	27.3	100.0 c	32.2	10.0 b
Ch36 (19cm)	30.1	5.4×10^4	4.7 d	28.6	9.5×10^4	5.0 cd	31.6	100.0 c	28.2	4.0 ab
Person's coefficient	-0.951*			-0.932*			-0.947*		-0.742*	

a Values of the same column, followed by the same letter, do not significantly differ according to Tukey's test ($P<0.05$).

* Significant correlation ($P\leq0.05$).

Table 6. Efficacy of steam pan treatments on *F. oxysporum* f.sp. *conglutinas*, *F. oxysporum* f.sp. *raphani*, *Rhizoctonia solani* and *Phytophthora capsici* at 40% moisture field capacity in 2009

Sampling positions (Depth, cm)	*F. oxysporum* f.sp. *conglutinans*			*F. oxysporum* f.sp. *raphani*			*Rhizoctonia solani*		*Phytophthora capsici*	
	Maximum temperature (°C) and duration (min) above 60°C	CFU g^{-1} soil	Log10 (CFU+1)	Maximum temperature (°C) and duration (min) above 60°C	CFU g^{-1} soil	Log10 (CFU+1)	Maximum temperature (°C) and duration (min) above 60°C	% surviving colony per plate	Maximum temperature (°C) and duration (min) above 60°C	% surviving colony per plate
Unsteamed soil	-	5.7×10^4	4.8 d[a]	-	1.6×10^5	5.2 b	-	100.0 b	-	70.0 f
Ch10 (7cm)	98.5 (52.0)	13.0	1.1 a	98.1 (52.0)	3.0	0.4 a	98.5 (50.0)	0.0 a	98.3 (52.0)	0.0 a
Ch14 (7cm)	98.5 (59.0)	10.0	0.8 a	98.1 (54.0)	17.0	0.9 a	98.8 (60.0)	0.0 a	98.1 (57.0)	0.0 a
Ch18 (7cm)	98.5 (47.0)	17.0	1.2 a	98.5 (53.0)	17.0	0.9 a	98.5 (54.0)	0.0 a	98.4 (45.0)	0.0 a
Ch20 (13cm)	45.2	1.6×10^4	4.2 c	42.2	1.2×10^5	5.1 b	49.4	100.0 b	49.2	7.0 ab
Ch24 (13cm)	43.3	7.0×10^3	3.8 b	45.1	1.4×10^5	5.2 b	48.1	100.0 b	42.5	19.0 cd
Ch25 (13cm)	43.4	1.8×10^4	4.2 cd	40.9	7.9×10^4	4.9 b	37.6	100.0 b	37.8	12.0 bc
Ch28 (19cm)	25.8	1.7×10^4	4.2 cd	27.4	1.2×10^5	5.1 b	25.4	100.0 b	26.2	33.0 e
Ch32 (19cm)	27.4	5.1×10^4	4.7 d	26.6	4.3×10^4	4.6 b	27.3	100.0 b	27.2	26.0 de
Ch36 (19cm)	27.2	5.6×10^4	4.8 d	27.2	1.5×10^5	5.2 b	27.1	100.0 b	26.5	27.0 de
Pearson's coefficient	-0.984*			-0.959*			-0.964*		-0.905*	

a Values of the same column, followed by the same letter, do not significantly differ according to Tukey's test ($P<0.05$).

* Significant correlation ($P\leq0.05$).

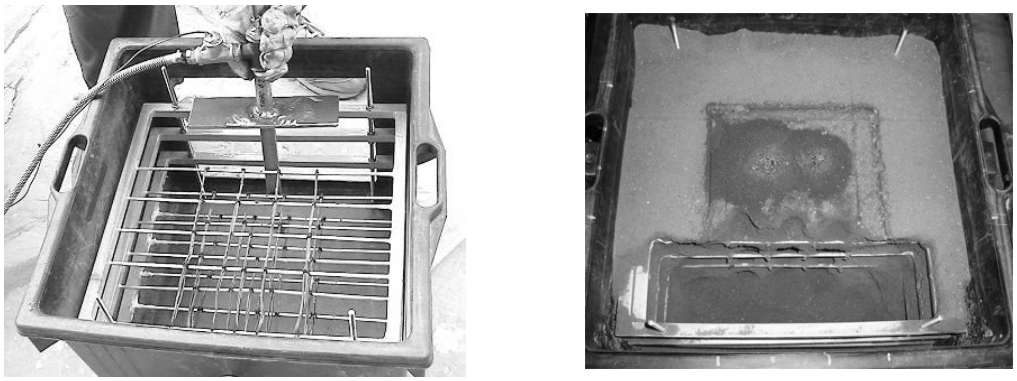

Fig. 1. Trial box with three-dimensional temperature probe (on left), situation after soil sampling at the end of a treatment with pan steaming (on right) in 2007.

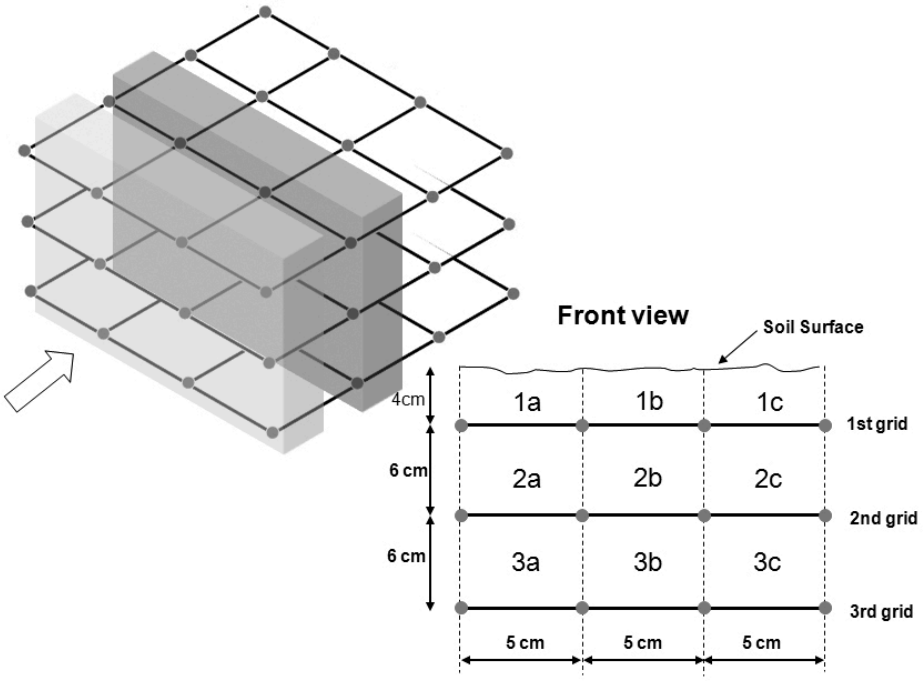

Fig. 2. Sampling scheme adopted during the preliminary trials carried out in 2007: in dark gray the region considered for injector and in light gray that for pan and sheet steaming. Points indicate the positions of the thermocouples.

Fig. 3. Second trial box equipped with free thermocouples (on left) adopted in 2008 and 2009; Every test bag attached to one thermocouple arranged on soil layer (on right).

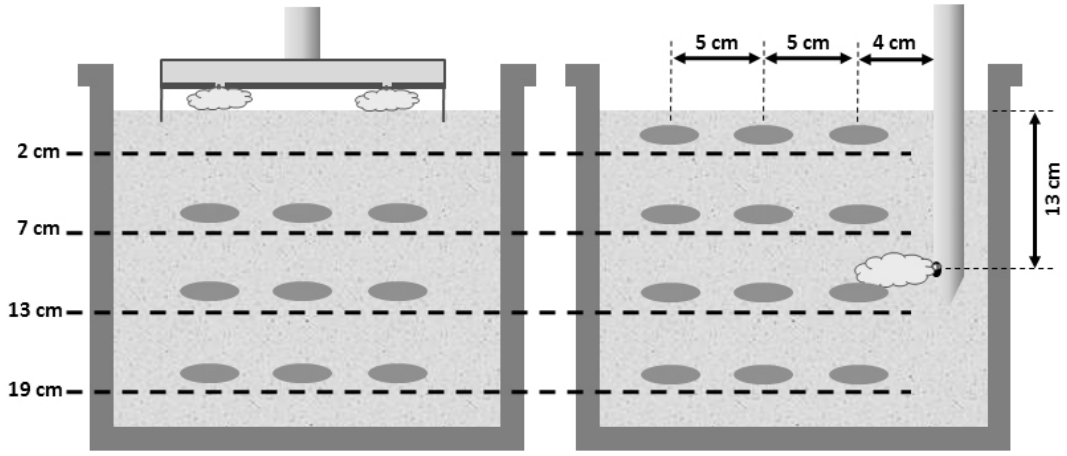

Fig. 4. Scheme of samples (in light blue) vertical arrangement during trials with the box equipped with free temperature sensors: pan distribution system (on left) and injector (on right) in 2008 and 2009.

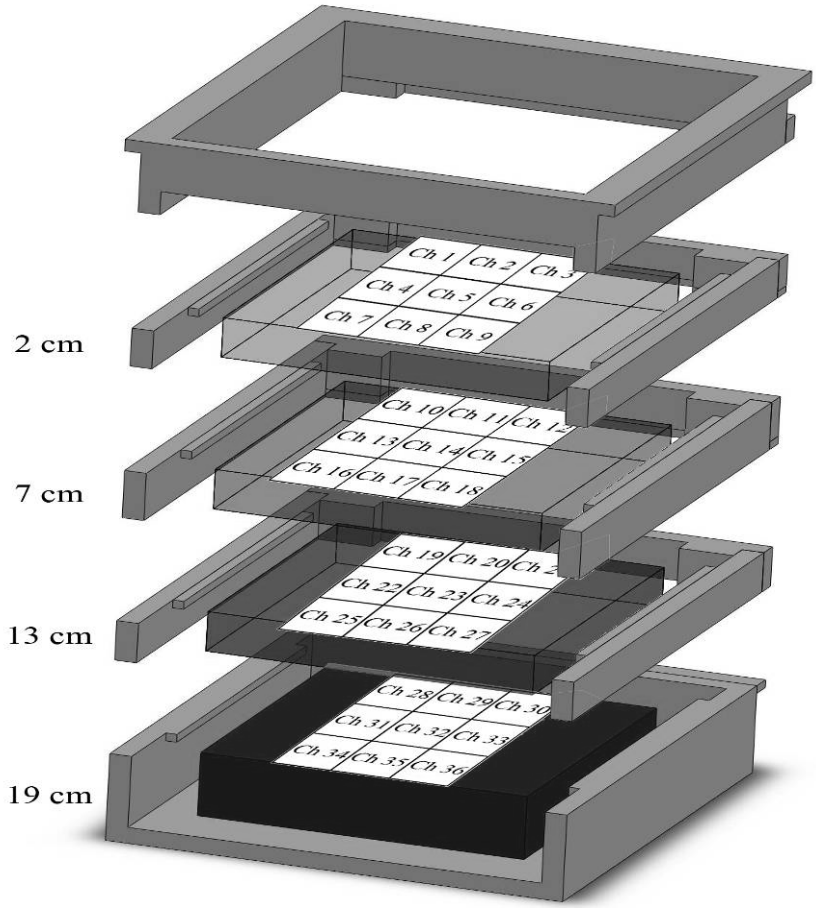

Fig. 5 Layout of test bags adopted for trials in 2008 and 2009.