Steam and exothermic reactions as alternative techniques to control soil-borne disease in basil

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Abstract: The search for non-chemical methods to control soil-borne pathogens has recently intensified ahead of the forthcoming phase-out of methyl bromide in 2005, and none of the previously available alternatives match this fumigant's wide spectrum control. We tested pre-planting treatments carried out with a self-propelled soil-steaming machine designed for the release of steam after incorporation in soil of a substance that causes an exothermic reaction. Experiments were conducted from 2003 to 2005 in open-field conditions by assessing the effectiveness of steam and exothermic reaction chemicals - potassium hydroxide - against *Fusarium oxysporum* f.sp. *basilici* and *Sclerotinia minor* on basil. The combination of steam and exothermic reaction chemicals reduced the incidence of *Fusarium* wilt (76.9 – 95.9%) better than using only steam (69.6 – 88.9%), and even the control of S*clerotinia minor* was better with potassium hydroxide (93.8 – 91.6%) than with steam only (83.1 – 87.0%). The effects of treatments on microbial population, yield and weed control were evaluated: the treatments caused significant reductions in the number of *Fusarium oxysporum* colonies

compared to the untreated control (cfu per gr of soil reduction of more than 99%), an increase in *Trichoderma* spp. (about 200% of increase in cfu per gr of soil) and no significant effects on total fungi and actinomycetes populations; drastic increase in yield (fresh weigh: from 6.9 – 7.5 gr in control to 23.0 – 25.5 gr in steam treatments); using steam / exothermic reaction there were significant reduction in plants m^{-2} in *Amaranthus retroflexus* (93.9 – 94.7%), *Portulaca oleracea* (96.1 – 98.9%), *Chenopodium* spp. (92,7 – 93,8%), *Hordeum vulgare* (85,6 – 90,0%), with similar results using only steam. The results show the potential for this approach to control various soil-borne pathogens and it may serve as an alternative to chemical soil disinfestation for high-value crops.

1. INTRODUCTION

Soil-borne plant pathogens cause extensive damage to many crops by affecting quality and yield. The concept of soil disinfestation consists of eradicating soil-borne pests existing in soil before planting, uniformly to the desired depth, with minimal disturbance of the biological equilibrium and without appreciably affecting chemical or physical soil properties (Katan, 2000).

For years, the most common approach to control of soil-borne pests has been soil fumigation before cropping. Methyl bromide is the most effective soil fumigant, but unfortunately it has negative attributes such as health hazards, environmental pollution, and even potential atmospheric ozone depletion. Increased environmental concern has been a major factor in triggering regulatory restrictions of the use of soil fumigants. The search for non-chemical methods to control soil-borne pathogens has recently intensified ahead of the phase-out of methyl bromide in 2005 (UNEP, 1998). The

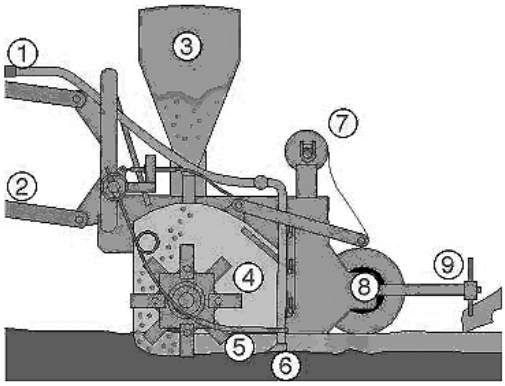
impending phasing-out of this fumigant poses new and unprecedented challenges for the agricultural research community since many major crops, especially in intensive agriculture, have become totally dependent on methyl bromide. The situation is now changing and the potential of non-chemical methods for pest control is being reassessed in an effort to include them in pest management programs.

Among physical methods, steaming is still the major tool for soil disinfestation. The basic principle is to heat the soil to a temperature that will effectively control all existing soil-borne pests, namely pests, weeds and arthropods. The lethal temperature of all kinds of soil-borne pathogens and weed seeds was established in the 1960's. On the basis of these studies suggestions for soil steaming were drawn up: a temperature of 70°C should be maintained for at least 30 min to free the soil from the greater part of plant diseases and weeds. But it has been noted that temperatures above 70°C, and particularly approaching 100°C, become detrimental to most soil biota (Bollen, 1969; 1985), and undesirable effects include a shift in the soil's microbial equilibrium resulting in diminished suppressiveness, i.e. a biological vacuum, increased population of certain pathogens, a detrimental effect on beneficial organisms such as rhizobia and mycorrhizae and microbial antagonists (Dawson and Johnson, 1965; Katan, 1984).

The thermal values achieved in soil with steam treatment change if energy-releasing exothermic reaction chemicals are spread onto soil prior to treatment, thereby generating a temperature peak ("thermal flash"), and the energy released in an exothermic reaction could maintain a useful temperature for controlling pests in the soil during treatment with a mobile steam generator. Moreover, the combination steam / exothermic reaction could change soil thermal levels through the distribution of temperatures in the soil profile as well as to avoid biological vacuum, with minimal concerns with regard to environmental impact or worker safety.

In this article, we describe pre-planting treatments carried out with a self-propelled soil-steaming machine (Figure 1) designed to release steam after incorporation in soil of a substance that causes an exothermic reaction (Peruzzi et al., 2003), for control of two soilborne pathogens in basil, *Fusarium oxysporum* f.sp. *basilici* and *Sclerotinia minor*, recently described in United States as cause of basil stem rot (Koike and O'Brien, 1995). Moreover, the effects of treatments on microbial population, yield and weed control were evaluated, compared to the use of only steam.

Figure 1. Scheme of the system to perform soil disinfection.



(1) steam feed-pipe; (2) connection arms; (3) hopper of the exothermic reaction product; (4) incorporation in the soil by means of a blade rotor; (5) adjustment of working depth; (6) steam injection; (7) plastic mulch spool; (8) roller; (9) ridging-mulching machine.

2. MATERIALS AND METHODS

Experimental design and cultural practices

Experimental plots (120 x 300 cm) were established in open-field conditions at University of Pisa, Italy in 2003 – 2005 (thirty-six in 2003 – 2004 and eighteen in

2005). A split-plot layouts with three replicates were used, preparing different plots in adjacent pieces of land each year. The land was characterized by a sandy soil, with homogeneous properties without significant difference among the plots before the treatments (62.5% sand; 21.0% silt; 16.5% clay; 1.0% organic matter; pH 7.4; moisture holding capacity 14.8%). We used a uncultivated land from 1999, and a soil disinfestation with metham sodium was performed in 2002. The artificial soil inoculations with pathogens were performed in spring each year (April 14, 2003; April 12, 2004; April 11, 2005), and the experimental fields were treated with steam, steam / exothermic reaction chemical and without soil sterilization as control, about a month after inoculation (May 15, 2003; May 14, 2004; May 12, 2005). Non-inoculated plots were used for yield and weed assessments. A drip irrigation system was established the day after treatments; seedling of a common Italian variety of basil (*Ocimum basilicum* L. cv. Genovese gigante), highly susceptible to *F. oxysporum* f.sp. *basilici* and *S. minor*, were transplanted with 20 cm spacing between the plants two days after soil treatments, 30 plants m^{-2}. No chemical fertilizers, herbicides and insecticides were applied throughout the growing season.

Inoculation with the pathogens

Monosporic isolate of *F. oxysporum* f.sp. *basilici* was obtained from wilted basil plants; each year, infected wheat kernels were produced adding 100 ml of tap water to 100 gr of wheat kernels in a 500 ml flask and autoclaved on two successive days for 20 min at 120°C and 1.06 kg cm^{-2} steam pressure, according to Imolehin et al. (1980) with minor modification. The autoclaved kernels were inoculated with mycelial disks from

3-day-old cultures of monosporic isolate of *F. oxysporum* f.sp. *basilici*: cultures were aged in the flasks at room temperature (about 20°C) for 6 weeks before use.

An isolate of *S. minor* was obtained from diseased lettuce plants and sclerotia were produced each year on autoclaved oat seeds using the previous method. The autoclaved oat seeds were inoculated with mycelial disks from 3-day-old cultures of the isolate of *S. minor*: cultures were aged in the flasks at room temperature (about 20°C) for 12 weeks before use. Sclerotia were recovered by using 1.98 mm (to retain coarse oat debris) and 0.25 mm (to retain sclerotia) sieve sizes. Finally sclerotia were dryed at room temperature for 24 h and stored at 4 °C until needed.

Artificial soil infestation was carried out about a month prior to application of soil treatment, to simulate a realistic field condition when basil sown was performed. Infected wheat kernels were applied to soil at 20 g m^{-2} in three rows of 10 x 300 cm at 8-10 cm depth in 2003-2005, and sclerotia, 15 g m^{-2}, were applied to soil in three rows of 10 x 300 cm at 4-6 cm depth in 2004-2005. Propagule counts were performed each years before inoculating the soil for producing similar disease incidences in control.

Soil treatments

The equipment used was a self-propelling machine able to inject steam deep down through special feeding devices as it moves (Celli S.p.A., Forlì - Italy).

The machine is able to spread on the soil the exothermic reaction chemical from an hopper, and then it is incorporated in the soil by means of a blade rotor. Behind the rotor, a steam injection system is collocated and connected to the steam generator via feed – pipe: this steam dispenser can be filled at various depth (in ours trials was set at 15 cm depth), by a system for the adjustment of rotor working depth. After the release

of steam or steam / exothermic reaction chemical, a plastic film cover the soil by the action of a roller and a ridging – mulching machine. The steam discharge is 600 kg h^{-1}, with a pressure of 1.18 MPa. The injection system is 160 cm large with 70 holes for steam dispensing: from 2004 an improved steam dispenser, characterized by different holes profiles, was designed by Settore Meccanica Agraria of the Dipartimento di Agronomia e Gestione dell'Agroecosistema - University of Pisa (Pisa, Italy) and used.

The treatments performed by the self-propelling machine were steam and steam / potassium hydroxide (KOH) treatments, using 1.000 kg ha^{-1} of KOH and with a feeding speed of the machine was 60 m h^{-1}.

After each treatment the soil was covered with a 40-μm-thick black polyethylene film for 24 hr, then it was removed. The control plots were uncovered.

Temperature, disease, microbial and growth assessment, and weed control

Temperatures in the soil profile were monitored in each trial using PT100 sensors that send a voltage signal to data loggers from which data are acquired and recorded on a personal computer using specially designed software. Temperatures were recorded at 0 – 10 and 11 – 20 cm depth for 3 hr after treatments, reporting the duration of 4 thermal periods (<40°C, 40-60°C, 60-80°C, >80°C) according to depth and treatments (data from Settore Meccanica Agraria of the Dipartimento di Agronomia e Gestione dell'Agroecosistema, University of Pisa, Pisa, Italy).

The plant monitoring was performed daily, and disease incidence was evaluated at the end of experiments by counting the number of healthy and diseased plants (without considering the stage of disease) in the plots, considering all the plants in each plots, for one month after transplant. Disease evaluation was performed considering all

symptomatic plants and re – isolating pathogens from diseased plants, using Komada medium for *F. oxysporum* f.sp. *basilici* and potato dextrose agar (PDA) for *S. minor*. A microscopical identification of pathogens was performed, and 30% of non – symptomatic plants per plot were processed for pathogens isolation. Effectiveness of treatments on basil was expressed as disease reduction (%) compared to untreated inoculated plots.

The effects of treatment on fungal and actinomycete communities, using steam / KOH or only steam, compared to untreated plots, were evaluated. Soil samples were collected from a plow layer at a depth of 0-10 cm, immediately before planting, from three points in each plot and mixed well to make one composite sample. The composite samples were eased through a 4-mm mesh sieve and kept at 4°C until the microbial analysis. Sub-samples of soil (1 gr) suspended in sterile water (10^{-3} w / v) were used to evaluate total fungi, *F. oxysporum*, *Trichoderma* spp. and actinomycetes, sprinkling 1 ml of suspension in petri dishes prepared with 20 ml of selective medium (VDYA for fungi, Komada for *F. oxysporum*, P190 for *Trichoderma* and water-agar for actinomycetes) according to standard methods (Papavizas and Davey, 1959; Komada, 1975; Ho and Ko, 1979). Incubation was conducted at 25°C and the colonies were counted after seven days. For each soil sample / microbial group, three replicates were used. Results were expressed as colony forming units (cfu) per gr of soil.

In 2004 – 2006 we assessed the yield of basil in non-inoculated plots, expressed as an increase in fresh weight and dry weight in plots treated with steam / KOH or only steam, compared to untreated plots. The measure of fresh weight was evaluated removing all plants in each plots from soil one month after treatments, washing off any lose soil, blotting plants with gently with soft paper towel to remove any free surface moisture, cutting off roots and weighing immediately. The dry weight was performed

drying the plants previously used for determining fresh weight in a oven set at 100°C overnight, and cooling the plants in a closed plastic bag.

The effect of field steaming on weed development was investigated each year in non-inoculated plots, evaluating species and number of weeds m^{-2}, in order to evaluate the herbicidal effect of treatments.

Data analysis

The effects of soil treatments were compared by analysis of variance in a split-plot design. The least significant difference (LSD) at the 5% level was calculated for the comparison of treatments for disease incidence, microbial and growth assessment, and weed control. The differences of temperatures among the treatments were analyzed by Duncan's Multiple Range test at the 5% level.

3. RESULTS AND DISCUSSION

Temperatures

We report the duration of the thermal periods according to depth and treatments, for evaluate the time of permanence of useful temperature for pest control. Considering the soil at 0 – 10 cm depth, temperatures were maintained at 40 – 60°C for at least 155 min after treatment, on three hours of recording (Table 1), with no significant differences between duration of thermal periods according to treatments, even if

combining steam with KOH determined longer time of permanence at higher temperature (40 – 60°C) compared to steam only in each year of trial.

Table 1. Duration of the thermal periods at 0-10 cm depth according to treatments (means data from three replications per treatment).

Duration of the thermal periods (min) at 0-10 cm depth						
Thermal periods	2003		2004		2005	
	Steam	Steam/KOH	Steam	Steam/KOH	Steam	Steam/KOH
<40	25 a[1]	10 a	16 a	6 a	12 a	7 a
40-60	155 a	170 a	164 a	174 a	168 a	173 a
60-80	-	-	-	-	-	-
>80	-	-	-	-	-	-

[1]Values in the same row (in the same year) followed by the same letter do not differ significantly according to Duncan's Multiple Range test (P=0.05).

At 11 – 20 cm depth (Table 2) we report duration of thermal periods at higher range of temperature compared to 0 – 10 cm depth, and this results can be due to the position of the steam dispenser in the soil profile (at 15 cm depth). At higher thermal periods (above 60°C), significant differences between treatments are showed during the three years of experimental trials, with longer duration determined by the use of KOH combined with steam in comparison to only steam.

Table 2. Duration of the thermal periods at 11-20 cm depth according to treatments (means data from three replications per treatment).

Duration of the thermal periods (min) at 11-20 cm depth						
Thermal periods	2003		2004		2005	
	Steam	Steam/KOH	Steam	Steam/KOH	Steam	Steam/KOH
<40	-	-	-	-	-	-
40-60	156 a[1]	146 a	157 a	146 a	155 b	133 a
60-80	22 a	28 b	20 a	24 a	21 a	32 b
>80	2 a	6 b	3 a	10 b	4 a	15 b

[1]Values in the same row (in the same year) followed by the same letter do not differ significantly according to Duncan's Multiple Range test (P=0.05).

In every case, the trend of soil temperature registered in all the trials at 11 – 20 cm depth, is characterized by few minutes at 80°C or above (a "thermal flash"), then decreased and stayed above 60°C at least for 20 min, and in the following 133 min or more, the soil temperature is above 40 °C: the use of KOH cause longer duration of the higher thermal periods, even if in 2004 significant differences by the use of KOH are registered only above 80°C. Otherwise, at 0 – 10 cm depth, we report a more homogeneous trend, maintaining temperature in one range (40 – 60°C) for almost three hours.

Management of F. oxysporum *f.sp.* basilici *and* S. minor

Symptoms of *Fusarium* wilts became manifest about two weeks after transplanting in each year, appearing as slight vein clearing on the outer portion of the younger basil leaves, followed by epinasty of the older leaves. After one month from transplanting, symptomatic plants were stunted, marginal necrosis of leaves and yellowing of the lower leaves was reported. Observation of vascular tissue was performed at the end of trial (four weeks after transplanting) and portions of tissue token from all symptomatic plant and 30% of non-symptomatic plants per plot were used for identification on Komada medium.

The treatments performed reduce the incidence of *Fusarium* wilt (Table 3), with better results obtained using KOH. In fact, the combination of steam and exothermic reaction chemicals reduced the incidence of disease of 76.9 – 95.9%, better than using only steam, with a reduction of 69.6 – 88.9%. The results confirm that a combination of

steam and exothermic reaction is effective in reducing disease incidence, even if the improvement of the combination is limited: the major effect it is due to the use of steam only, able to reduce the disease of 69.6% or more, while the KOH seems to increase the efficacy of 6.8 – 7.3%. A different steam dispenser was used from 2004 trials, optimizing the treatments and obtaining better results: an increase of 18.5 – 19.3% considering steam treatments and of 18.0 – 19.0% in steam / KOH treatments. No infection by *F. oxysporum* f.sp. *basilici* were registered in non-symptomatic plants.

Table 3. Effectiveness of treatments (steam or steam / KOH) on incidence of *F. oxysporum* f.sp. *basilici*, expressed as disease reduction (%) compared to the untreated control (means data from three replications per treatment).

F. oxysporum f.sp. *basilici* / basil				
Year		Control	Steam	Steam / KOH
2003	Diseased plants	23.0±3.0 a[1]	7.3±1.2 b	5.3±1.2 b
	Disease reduction (%)	-	69.6 a	76.9 a
2004	Diseased plants	25.3±0.6 a	3.0±0.0 b	1.3±0.6 c
	Disease reduction (%)	-	88.1 a	94.9 b
2005	Diseased plants	24.3±0.6 a	2.7±0.6 b	1.0±0.0 c
	Disease reduction (%)	-	88.9 a	95.9 b

[1]Values in the same row followed by the same letter do not differ significantly according to the LSD test (P=0.05); arc sin square root was used prior to % data analysis, non transformed means are presented.

Symptoms stem rot caused by S*clerotinia minor* became manifest about three weeks after transplanting in each year, and the stems develop water-soaked spots which later were covered with a cottony white growth. As the disease progresses, affected portions of the stem develop a bleached appearance, appearing as an browning of basil stem and epinasty of the leaves with marginal necrosis. After one month from transplanting, symptomatic plants were stunted with visible mycelial on the basal part of the stem and on the surface of the soil near the plant. Observation of vascular tissue was

performed at the end of trial (four weeks after transplanting) and portions of tissue token from symptomatic plant were used for identification.

Steam treatments and the combination of steam / KOH reduced disease incidence caused by S*clerotinia minor* (Table 4). Control of *S. minor* was better with KOH than with steam only, but as showed in *F. oxysporum* f.sp. *basilici* / basil trials, the beneficial improvement of the combination is limited. In fact, using steam / KOH, we obtain a disease reduction of 93.8 – 96.1%, compared to 83.1 – 87.0% obtained with only steam, with an increase in *S. minor* control of 9.1 – 10.7% caused by the use of the exothermic reaction chemical. No infection by *S. minor* were registered in non-symptomatic plants.

Table 4. Effectiveness of treatments (steam or steam / KOH) on incidence of *Sclerotinia minor*, expressed as disease reduction (%) compared to the untreated control (means data from three replications per treatment).

		S. minor / basil		
Year		Control	Steam	Steam / KOH
2003	Diseased plants	16.0±1.0 a[1]	2.7±0.6 b	1.0±0.0 c
	Disease reduction (%)	-	83.1 a	93.8 b
2004	Diseased plants	17.7±1.2 a	2.3±0.6 b	0.7±0.6 c
	Disease reduction (%)	-	87.0 a	96.1 b

[1]Values in the same row followed by the same letter do not differ significantly according to the LSD test (P=0.05); arc sin square root was used prior to % data analysis, non transformed means are presented.

Effects of treatments on fungal and actinomycete populations

Additional experiments in 2004 revealed no reductions after treatments in the number of total fungi and actinomycetes developed on specific selective medium, without significant differences between treatments. Otherwise, the treatments caused

significant reductions in the number of *Fusarium oxysporum* colonies (samples taken from inoculated plots) compared to the untreated control (cfu per gr of soil reduction of more than 99%). Moreover, steam / KOH or only steam treatmens cause an increase in the number of cfu per gr of soil of *Trichoderma* spp. of 197.3% and 211.8%, respectively (Table 5).

Table 5. Effects of treatments (steam or steam / KOH) on fungal and actinomycete populations expressed as colony forming units (cfu) per gr of soil (means data from three replications per treatment).

Treatment	Total Fungi	Actinomycetes	*Trichoderma* spp.	*F. oxysporum* in inoculated plots
Control	28.3±2.1 a[1]	55.0±5.0 a	11.0±1.0 b	317.7±5.7 a
Steam	31.3±3.1 a	63.7±5.7 a	34.3±1.2 a	1.0±0.0 b
Steam/KOH	31.7±1.5 a	56.3±4.6 a	32.7±1.5 a	1.0±0.0 b

[1]Values in the same column followed by the same letter do not differ significantly according to the LSD test (P=0.05).

These results show as a mobile steam generator, combined or not with exothermic reaction chemicals, do not cause biological vacuum, but it is able to increase a beneficial biota as *Trichoderma* spp. and reduce drastically a potentially pathogen as *F. oxysporum*.

Effects on yield

Steam treatments and the combination of steam and exothermic reaction produced positive effects on yield of basil in non-inoculated plots (Table 6). Basil fresh weight increased from 6.9 – 7.5 gr to 23.0 – 25.3 gr in steam treatments and to 23.7 –

25.5 gr in steam / KOH treatments, while basil dry weight increase from 1.5 – 1.7 gr to 4.8 – 5.1 gr in steam treatments and to 5.0 – 5.1 gr in steam / KOH treatments.

Table 6. Effects of treatments (steam or steam / KOH) on yield of basil as fresh weight or dry weight (g) compared to untreated controls (means data from three replications per treatment).

Basil productivity				
Year		Control	Steam	Steam / KOH
2003	Fresh weight	6.9±0.4 a[1]	23.0±4.5 b	23.7±5.5 b
	Dry weight	1.5±0.3 a	5.0±1.0 b	5.1±0.9 b
2004	Fresh weight	7.0±0.6 a	23.2±5.4 b	24.9±6.6 b
	Dry weight	1.6±0.2 a	4.8±1.3 b	5.1±1.5 b
2005	Fresh weight	7.5±0.9 a	25.3±4.4 b	25.5±3.6 b
	Dry weight	1.7±0.3 a	5.1±1.5 b	5.0±1.6 b

[1]Values in the same row followed by the same letter do not differ significantly according to the LSD test (P=0.05).

Therefore, the treatments increased drastically plant fresh and dry weights in all the trials, but the exothermic reaction chemical did not cause significant differences compared to the use of steam only during all three years of trials.

Weed control

The effects of treatments on main weed development are show in table 7. The field used in reported trials was naturally infested by various weed, as *Amaranthus retroflexus*, *Portulaca oleracea*, *Hordeum vulgare*, *Chenopodium* spp. and other species. The results achieved were very interesting with a satisfactory weed control: in fact, using steam / exothermic reaction caused significant reduction in plants m^{-2} in *Amaranthus retroflexus* (93.9 – 94.7%), *Portulaca oleracea* (96.1 – 98.9%), *Chenopodium* spp. (92.7 – 93.8%) and *Hordeum vulgare* (85.6 – 90.0%), even if no

statistical differences between steam treatment and steam / KOH treatment have been reported

Table 7. Effects of treatments (steam or steam / KOH) on weed control expressed as plants m^{-2}, compared to untreated controls (data from three replications per treatment).

		Weed control		
Year	Weed (plants m^{-2})	Control	Steam	Steam / KOH
2003	*Amaranthus retroflexus*	583.3±29.0 a[1]	96.0±11.5 b	35.3±10.0 b
	Portulaca oleracea	97.7±10.8 a	0.0±0.0 b	2.0±1.0 b
	Chenopodium spp.	582.3±16.6 a	25.0±1.0 b	39.7±2.1 b
	Hordeum vulgare	67.0±3.5 a	5.0±3.0 b	9.0±1.0 b
	Other	3.1±1.3 a	1.0±0.0 a	2.1±1.1 a
2004	*Amaranthus retroflexus*	560.5±25.1 a	80.1±10.0 b	30.2±6.0 b
	Portulaca oleracea	90.1±10.8 a	2.1±1.0 b	1.0±1.5 b
	Chenopodium spp.	452.5±10.3 a	20.2±1.5 b	31.3±1.1 b
	Hordeum vulgare	60.1±3.0 a	6.0±2.0 b	6.0±4.2 b
	Other	4.2±1.1 a	2.0±1.0 a	2.2±1.0 a
2005	*Amaranthus retroflexus*	550.5±20.0 a	75.0±8.4 b	28.7±4.3 b
	Portulaca oleracea	80.2±9.8 a	1.0±0.0 b	3.1±1.5 b
	Chenopodium spp.	500.5±15.3 a	21.5±1.3 b	36.4±2.2 b
	Hordeum vulgare	62.2±4.4 a	4.5±2.1 b	7.1±2.1 b
	Other	2.5±1.5 a	1.5±.1.5 a	2.0±1.0 a

[1]Values in the same row followed by the same letter do not differ significantly according to the LSD test (P=0.05).

4. CONCLUSION

To improve the efficacy of a soil steaming treatment characterized by a short period of exposure to the required temperature, we combined exothermic reaction chemicals with steam to achieve a slightly higher treatment temperature, and to change soil thermal levels through the distribution of temperatures in the soil profile as well as to avoid dramatic shifts in the soil's microbial equilibrium. Experimental trials showed that the combination of steam and exothermic reaction chemicals reduced the incidence of *F. oxysporum* f.sp. *basilici* and *S. minor* in basil, with better results compared to the

use of only steam, even if the improvement is limited: in fact, the major effect it is due to the use of steam only, able to reduce *Fusarium* disease of 69.6% or more and *S. minor* disease of 83.1%, while the KOH seems to increase the efficacy of 6.8 – 7.3% for *Fusarium* and of 9.1 – 10.7% for *S. minor*. Therefore, the use of exothermic reaction chemicals, even if cause positive effects on disease control, need to be evaluate considering the cost of application in relation to the level of inoculum in the soil, and considering the incremental control of soil inoculum that could happen in repeated treatments on the same land. The 2002 results on microbial assessment (Triolo et al., 2004) were confirmed in 2004 trials, with a dramatic reduction in the number of *Fusarium oxysporum* colonies (cfu per gr of soil reduction of more than 99%) and with minimal effects on total fungi and actinomycetes, while the number of *Trichoderma* cfu per gr of soil was increased about 200%. Thus, one of the main concern about steam treatments, biological vacuum, seems to be limited, preserving or increasing population of various microorganisms and decreasing potential pathogens. The effects of treatment on weeds are also encouraging, with a reduction in plants m^{-2} above 85.6% for the four weeds monitored during the trials, according to the results obtained on seed germination (Peruzzi et al., 2004). Moreover, dramatic increases in yield are reported: in a basil plant treated with steam / KOH or steam only there was an increase in fresh weight of above 300%, and this positive effect is confirmed considering the dry weight. The use of a combination of KOH or CaO with steam is safe and does not cause hygiene-toxicological and environmental issues, but generally has undesirable agronomic effects, such as an increase in soil pH, exchangeable K (KOH), active calcium carbonate (CaO) and conductivity. If a low amount of exothermic reaction chemicals is used, the variations are mild and disappear in 15 to 30 days after treatment (Mazzoncini et al. 2002). Concluding, the use of exothermic reaction chemical seems to be useful for

controlling pathogens with a constant increase in disease control in all the experiments, even if the major effects were caused by steam treatments, in particular considering microbial assessment, yield and weeds control. Thus, based on our research with soil steaming, the exothermic reaction can improve the effectiveness of treatment carried out by mobile steam generators, adopting a short treatment exposure time, improving a steam-based method. Our conclusion is, therefore, that a combination of steam and exothermic reaction can be included in integrated pest management programs.

5. REFERENCES

Bollen G.J. (1969) The selective effect of heat treatment on the microflora of a greenhouse soil, Neth. J. Path. 75, 157-163.

Bollen G.J. (1985) Lethal temperatures of soil fungi, in: Parker (Eds.), Ecology and management of soilborne plant pathogens, pp. 191-193.

Dawson J.R., Johnson R.A.H. (1965) Influence of air mixture, when used for heating soil, on biological and chemical properties that affect seedling growth, Ann. Appl. Biol. 56, 243-251.

Ho W.C., Ko W.H. (1979) Alkalized water agar as a selective medium for enumerating actinomicetes, Phytopathology 69, 1031.

Imolehin E.D., Grogan R.G., Duniway J.M. (1980) Effect of temperature and moisture tension on growth, sclerotial production, germination and infection by *Sclerotinia minor*, Phytopathology 70, 1153-1157.

Katan J. (1984) The role of soil disinfestation in achieving high production in horticultural crops, Proc. Brit. Crop. Prot. Conf., Brighton 1984, 1189-1196.

Katan J. (2000) Soil and substrate disinfestation as influenced by new technologies and constraints, Acta Horticolturae 532, 29-35

Koike S.T., O'Brien R. D. (1995) Basil as a host of *Sclerotinia minor*, Plant Disease 79, 859.

Komada H. (1975) Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil, Rev. Plant Prot. Res. 8, 114-125

Mazzoncini M., Risaliti R., Ginanni M., Mainardi M. (2002) Effetto del vapore e di sostanze a reazione esotermica sulle principali caratteristiche chimiche del terreno: risultati di un quadriennio di sperimentazione, in: Proc. "Vapor d'acqua e sostanze a reazione esotermica: una combinazione a ridotto impatto ambientale per la disinfezione e la disinfestazione del terreno", Forlì, 30 Ottobre, pp. 79-88.

Papavizas G.C., Davey C.B. (1959) Evaluation of various media and antimicrobial agents for isolation of soil fungi, Soil Science 88, 112-117.

Peruzzi A., Raffaelli M., Ginanni M., Mainardi M., Toniolo S., Marchi D. (2003) Le macchine per la disinfezione e disinfestazione termica del terreno, L'Informatore Agrario 46, 51-56.

Peruzzi A., Borelli M., Mazzoncini M., Raffaelli M., Ginanni M., Barberi P. (2004) Weed seeds control by steam and substances in exothermic reaction, in: Proc. 6th EWRS Workshop on Physical and Cultural Weed Control, Lillehammer, Norway, 8-10 March, pp. 128-138.

Triolo E., Materazzi A., Luvisi A. (2004) Exothermic reactions and steam for the management of soil-borne pathogens: five years of research, Adv. Hort. Sci. 18(2), 89-94.

UNEP (1998) Report of the Methyl Bromide Technical Options Committee (1998), Assessment of alternatives to Methyl Bromide, United Nations Environmental Programme, Nairobi, Kenya.