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# Heat treatments for sustainable control of soil viruses

Andrea Luvisi · Alessandra Panattoni · Alberto Materazzi

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**Abstract** Food contamination by toxic pesticides has induced intense research for alternative methods to control pests and diseases. For instance, the phasing out of methyl bromide used for soil disinfection has led to a reconsideration of heat-based methods to control soil-borne pathogens. Techniques such as soil steaming and soil solarization have been applied successfully. However, traditional steaming and solarization are unable to control viruses such as the *Tobacco mosaic virus*. Thus, methods for sustainable control of virus in soil are not available. Here, we tested the effect of short-duration soil steaming enhanced by the addition of exothermically reacting chemicals—potassium hydroxide or calcium oxide—and soil solarization. These methods were tested against three viruses having different stability: the highly stable *Tobacco mosaic virus* and the medium stable *Potato virus Y*, and the *Cucumber mosaic virus* as non-persistent control. Steaming was done in laboratory and open-field conditions after incorporation of exothermically reacting chemicals. Soils were solarized for 20 days using transparent polyethylene, ethylene–vinylacetate and high-effectiveness infrared films. Our results show that steaming with exothermic chemicals decreased the infectivity of the *Tobacco mosaic virus* below 3.0 %. We also found that the *Tobacco mosaic virus* was not controlled by solarization. The *Potato virus Y* was totally controlled by steam treatments and by soil solarization with ethylene–vinylacetate or high-effectiveness infrared films. Overall, our findings evidence promising ways to control soil viruses.

**Keywords** Soil disinfection · Solarization · Steaming · Virus

## 1 Introduction

Methyl bromide was phased out in 2005 pursuant to the Montreal Protocol on Substances that Deplete the Ozone Layer, imposing a re-thinking of techniques to control soil-borne pathogens (McSorley et al. 2009). The search for alternatives began a decade before the deadline, but it was soon clear that none of the available and registered chemical alternatives has the full spectrum of activity and versatility of methyl bromide as a pre-plant soil fumigant (Duniway 2002). Moreover, all of the chemical alternatives to methyl bromide are subject to continuing review and the risk that registered fumigants will not be available indefinitely for large-scale use in soil is very likely.

In this perspective, it seems appropriate to reconsider physical methods for soil disinfection, even if some common issues (i.e. costs, times of treatments, efficacy) may still cause concerns. Among physical methods, heat treatments, such as steam and solarization, represent traditional approaches to soil-borne pathogen and weed control, thanks to their potentially wide spectrum of action and lack of residues (Katan 2000; Gill and McSorley 2011).

Steam soil disinfestation is now being reconsidered in open-field and greenhouse horticulture for its efficiency, while it ensures low ecological impact (Gay et al. 2010). Short-duration steaming (3 min) may represent an effective approach for mobile steaming treatment and may reduce costs to control fungal pathogens, nematode pests and weeds (van Loenen et al. 2003). Moreover, the addition of exothermically reacting chemicals to steam able to release energy when reacting with water can improve the effectiveness of short-duration heat treatments (Luvisi et al. 2006, 2008) and it is economically competitive with chemical fumigation (Peruzzi et al. 2012). Tests showed the potential for this approach to control various soil-

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All authors contributed equally to this work.

A. Luvisi (✉) · A. Panattoni · A. Materazzi  
Department of Agriculture, Food and Environment, University of  
Pisa, Via del Borghetto 80, 56124 Pisa, Italy  
e-mail: aluvisi@agr.unipi.it

A. Panattoni  
e-mail: alessandra.panattoni@unipi.it

A. Materazzi  
e-mail: alberto.materazzi@unipi.it

borne pathogens, and it may therefore serve as an alternative to chemical soil disinfection for horticultural crops. The advantages of the combination of steam and exothermically reacting chemicals for management of soil-borne pathogens were associated to reduced labour and fuel consumption for open-field application in comparison with other physical soil disinfection systems (Peruzzi et al. 2011).

Soil solarization is a milder heat treatment. It has recently been re-evaluated, thanks to the development of novel plastic films able to reduce treatment time and enhance biological effects (Gill et al. 2009). Moreover, new techniques combining soil biofumigation and soil solarization (biosolarization) represent promising sustainable options for plant production (Domínguez et al. 2014; Mauromicale et al. 2010) and add beneficial soil microbes (Camprubí et al. 2007).

Viruses are rarely investigated with regard to heat treatments for soils, but soil-borne inoculum of viruses can be very important in primary infection. Shoots and roots or the remains of both in soil have been considered the principal reservoir of high-stability viruses such as *Tobamovirus* responsible for *Tobacco mosaic virus* initial infections (Candemir et al. 2012). Considerable quantities of soil-borne viruses that are excreted into the rhizosphere from infected plants could serve as inoculum (Allen 1981), and transmission presumably occurs when root hairs are damaged in the presence of infected debris or when virus leached from infected soil is disseminated to leaves through splashing or other means. In this context, the stability of the virus particles plays a key role, contributing to its survival in the field and facilitating its dissemination by human actions, which could explain its spread without a biological vector (Saraiva et al. 2006). The elimination of a high-stability virus such as *Tobacco mosaic virus* was investigated with traditional soil disinfection methods during the twentieth century but without satisfactory results. Broadbent et al. (1965) reported how virus in undiluted tomato leaf sap was inactivated by steam treatments at 88–82 °C for 5–20 min, while root tissues need 90–85 °C for 10–20 min. Most commercial soil fumigants are ineffective in inactivating the virus directly or increase its survival, presumably by killing microorganisms that denature the virus (Broadbent et al. 1965). Moreover, attempts at soil solarization using first-generation films were unable to reduce virus inoculum in soil (Triolo and Materazzi 1992).

Thus, to evaluate the spectrum of activity of enhanced heat treatments such as short-duration soil steaming plus exothermically reacting chemicals or soil solarization with novel plastic films, three viruses having different stability to environmental factors (Luvisi et al. 2012; Mehle and Ravnkar 2012) were chosen: *Tobacco mosaic virus* as high-stability virus, *Potato virus Y* as mid-stability virus and *Cucumber mosaic virus* as low-stability virus, the latter was used as non-persistent control.

## 2 Materials and methods

### 2.1 Inoculum preparation

A collection of virus isolates of *Tobacco mosaic virus*, *Potato virus Y* and *Cucumber mosaic virus* were maintained in tobacco plants (*Nicotiana tabacum* L. cv. Turkish) under greenhouse conditions (18–24 °C), free from insects. The plants, systematically infected by an isolate of virus type-strain identified by RT-PCR (Luvisi et al. 2011), were used as the inoculum source of viruses. Crude sap was obtained from leaves of infected plants by grinding leaves with 0.03 M phosphate buffer at pH 7.2 (1:4 w/v, g/mL). The crude sap was uniformly mixed with air-dried soil samples (5 g of soil collected from the plots used for open-field tests) in the proportion of 1:5 (v/w). The resulting mixtures were arranged in nylon screen (50 mesh) bags (about 8×8×2 cm high). Three bags per treatment were buried in the soil at depths of 20 and 40 cm (±1 cm) 12 h before treatments.

Stability in sap of the tested virus strains was evaluated by determining its longevity in vitro and thermal inactivation point using infected tobacco of the collection as the source of virus (Nascimento et al. 2010). The physical properties were determined by inoculation of nine young tobacco plants with each treatment. The thermal inactivation point was estimated using the temperature range 45 to 95 °C per 10 min, and the longevity in vitro was determined using the periods of 0, 100, 200 and 300 days for TMV; 0, 30, 33 and 36 days for *Potato virus Y*; and 0, 3, 5 and 7 days for *Cucumber mosaic virus*. Stability data and other physical characteristics of viruses (size, genome partition, RNA %, CG %) were used to evaluate the virus variables with statistical methods (Luvisi et al. 2012).

### 2.2 Heat treatments

#### 2.2.1 Soil steaming treatments

To secure information about the effects of the addition of exothermically reacting chemicals to short-duration steaming against viruses, two trials were carried out: a laboratory test using a specially constructed apparatus for soil steaming and an open-field test using a self-propelling soil steaming machine. The soil characteristics for both trials were sandy loam (62.5 % sand, 21.0 % silt, 16.5 % clay, 1.0 % organic matter, moisture holding capacity 14.8 %, pH 7.4, electrical conductivity 188 mS cm<sup>-1</sup>, exchangeable potassium 63.22 ppm, active calcium carbonate 2.35 %). In each trial, virus samples were buried at 20- and 40-cm depth. The treatments performed for each trial were steam and steam with exothermically reacting chemicals: calcium oxide (CaO) or potassium hydroxide (KOH), at 1000 kg ha<sup>-1</sup> or equivalent dose

for laboratory test. After each treatment, the soil was covered with a 40- $\mu\text{m}$ -thick black polyethylene film for 24 h. Untreated virus samples were used as control.

For laboratory tests, the apparatus was devised to simulate the thermal effects caused by a mobile steam generator. The specific adopted target for designing the apparatus was the time–temperature profiles obtained by open-field soil steaming (Luvisi et al. 2006). The apparatus consisted of a 100 $\times$ 80 $\times$ 80-cm box containing a steam injection system composed of two pipes as steam dispenser 20 cm space-out at 40-cm depth. The injection system was 100 cm wide with 20 holes for steam dispensing. The steam discharge was 500 kg h<sup>-1</sup> with a pressure of 1.0 MPa. The box was filled with soil, or, for soil steaming treatments with exothermically reacting chemicals, CaO or KOH (0.08 kg, the equivalent of 1000 kg ha<sup>-1</sup> used for open-field treatments) was manually mixed with soil up to 40-cm depth. Virus samples were buried between the two pipes at 20- and 40-cm depth. Steam was dispensed for 60 s by the first pipe and for 60 s by the second pipe in order to achieve the target time–temperature profiles obtained by mobile

steam generator. Each treatment/virus combination was replicated three times.

Open-field soil steaming was carried out in San Piero a Grado (PI), central Italy, in July over a 2-year period. Plots of 1 m<sup>2</sup> were prepared, and randomized layouts with three replicates for a treatment/virus combination were used. Soil steaming was carried out using a self-propelling machine (Peruzzi et al. 2011) able to spread the exothermically reacting chemicals (1000 kg ha<sup>-1</sup> of CaO or KOH) on the soil surface from a hopper, and then, they were incorporated into the soil by means of a rotor blade (Fig. 1). Behind the rotor, a steam injection system was positioned and connected to the steam generator via a feed pipe: this steam dispenser was filled at 40-cm depth by a system for the adjustment of rotor working depth. After the release of steam and exothermically reacting chemicals, a roller and ridging–mulching machine covered the soil with a plastic film. The steam discharge was 600 kg h<sup>-1</sup>, with a pressure of 1.2 MPa. The injection system was 160 cm wide with 70 holes for steam dispensing. The treatments were carried out with a feeding speed of 60 m h<sup>-1</sup>.



**Fig. 1** Soil solarization (a) was carried out using three plastic films: transparent polyethylene film, ethylene–vinylacetate thermic clear film and high-effectiveness infrared film. Soil steaming (b) was carried out

using a self-propelling machine able to spread exothermically reacting chemicals (1000 kg ha<sup>-1</sup> of CaO or KOH) on the soil from a hopper, and then, they were incorporated into the soil by means of a rotor blade

### 2.2.2 Soil solarization treatments

Soil solarization (Kasirajan and Ngouajio 2012) was carried out in San Piero a Grado (PI), central Italy, in July over a 2-year period using three plastic films: transparent polyethylene film (50- $\mu\text{m}$ -thick), ethylene–vinylacetate film (30- $\mu\text{m}$ -thick) and high-effectiveness infrared film (30- $\mu\text{m}$ -thick). Plots of 1 m<sup>2</sup> were prepared, and randomized layouts with three replicates for each treatment/virus combination were used. The soil characteristics were sandy loam (63.0 % sand, 21.5 % silt, 16.2 % clay, 1.3 % organic matter, moisture holding capacity 15.1 %, pH 7.4, electrical conductivity 190 mS cm<sup>-1</sup>, exchangeable potassium 62.85 ppm, active calcium carbonate 2.31 %). There have not been heavy rainfall during the trials (below 10 mm during each trial), the mean max temperature was 31 °C, and the mean min temperature was 22 °C. Total visible light emission was  $\geq 88$ , 89 or 91 (test method EN 2155-5) for the polyethylene film, ethylene–vinylacetate film or high-effectiveness infrared film, respectively. Diffused light was  $\leq 30$ , 18 or 20 (test method EN 2155-5) for the polyethylene film, ethylene–vinylacetate film or high-effectiveness infrared film, respectively. Infrared effectiveness was  $\geq 30$ , 82 or 95 (test method EN 13206) for the polyethylene film, ethylene–vinylacetate film or high-effectiveness infrared film, respectively. Films covered the soil for 20 days. Full details regarding field preparation are presented elsewhere (Stapleton 2000).

### 2.2.3 Soil temperature

The box and plots were monitored for temperature using a dedicated system made up of PT100 bifilar sensors (PT100-420 degree B, in compliance with IEC 751) connected to a data logger (Peruzzi et al. 2012). Sensors, two per box or plot positioned in the central area, were placed vertically along the soil profile until the desired depth (20 or 40 cm). The highest, lowest and average soil temperatures after the various treatments were measured and calculated. For soil steaming treatments, the measured temperatures were divided into four classes ( $T \leq 40$  °C,  $40$  °C  $< T \leq 60$  °C,  $60$  °C  $< T \leq 80$  °C,  $T > 80$  °C (min)). The length of time each class persisted in the soil was taken into account, along with the highest, average and the final (after 3 h) temperature values. The thermal addition parameter ( $\Sigma T$ ) was calculated as the sum of the individual temperatures (measured every minute) for the 180 min following steam treatment.

### 2.3 Virus recovery and infectivity tests

Purified viruses were utilized to construct a calibration curve for quantitative determinations by means of a dilution series of stocks of purified viruses (Noordam 1973). Virus concentration was estimated spectrophotometrically by using a specific extinction coefficient ( $E^{0.1\%}_{1\text{cm}, 260\text{nm}}$ ) of 3.1 for *Tobacco*

*mosaic virus*, 5.0 for *Cucumber mosaic virus* and 1.21 for *Potato virus Y* (Brunt et al. 1996). With regard to the inoculum source of virus and its dilution, quantification of virus can be carried out by DAS-ELISA (Candemir et al. 2012; Luvisi et al. 2011; Yang et al. 2012). Three bags per treatment were recovered 24 h after soil steaming or soil solarization. Each soil sample was separately processed for virus extraction according to the procedure of Cheo (1980) with minor modifications. Soil was extracted with 0.075 M phosphate buffer at pH 7.5 in the proportion of 1:10 (w/v). After being thoroughly stirred, the suspension was subjected to 10 min of ultrasonic vibration (Branson 221). This was followed by two cycles of differential centrifugation at 4000g for 30 min and at 15,000g with PEG for 15 min. The recovered viruses in the final pellets were suspended in 0.01 M phosphate buffer at pH 7.2 for quantitative assessment and for infectivity tests.

Serological verification of virus was carried out using polyclonal antibodies against *Tobacco mosaic virus*, *Cucumber mosaic virus* and *Potato virus Y* (LOEWE Biochemica GmbH, Germany). Absorbance values (405 nm) were determined with a Titertek Multiskan photometer (Flow Lab., Switzerland). Optical density was correlated to concentration by calibration curve. Virus recovery was expressed as the concentration of virus extracted from buried samples compared to concentration of virus in crude sap of infected plants (%).

At the end of each trial, the levels of infectivity of viruses from the soil extracts were assessed by mechanical inoculation of leaves of *Chenopodium quinoa* plants. Each soil extract was used to inoculate ten leaves of *C. quinoa*. Inoculated plants were placed in a climatic chamber at a temperature of  $23 \pm 2$  °C, light intensity of 200  $\mu\text{Em}^{-2} \text{s}^{-1}$  and photoperiod of 16 h and monitored for 10 days to evaluate the development of chlorotic lesions. Infectivity recovery was expressed as the number of chlorotic lesions caused by virus extracted from buried samples compared to those caused by crude sap of infected plants (%).

### 2.4 Microbial tests

Soil samples were collected after treatments at 20-cm depth to evaluate total fungi, *Trichoderma* spp. and actinomycetes as CFU per gramme of soil, using potato dextrose agar, P190 and water–agar medium, respectively. Community-level physiological profiles of soil microbial communities, using EcoPlates (Biolog Inc., CA, USA) incubation, were carried out by calculating the average well colour development, richness and Shannon–Weaver index (Chen et al. 2013).

### 2.5 Statistical analysis

Virus and infectivity recovery were elaborated using SigmaPlot software (version 11; Systat Software, San Jose, CA, USA). Effectiveness of steam treatments (alone or with

CaO or KOH) or solarization treatments (three plastic films: polyethylene film, ethylene–vinylacetate film or high-effectiveness infrared film) on virus samples buried at 20–40-cm depth was compared. The software was used to perform three-way analysis of variance (ANOVA) in a random design and pairwise multiple comparisons on significant effects and interactions using the Holm–Sidak method. Normality and equal variance tests were performed. Data expressed in percent were converted to arcsin values.  $P$  lower than 0.05 was considered to be significant. Principal component analysis, a multivariate approach, was performed to evaluate the virus variables.

### 3 Results and discussion

Exothermically reacting chemicals increased the  $\sum T$ ,  $T$  max,  $T$  mean and  $T$  final achieved after soil steaming in laboratory tests (Table 1). With regard to depth (20 or 40 cm), CaO caused an increase of 10.0–14.0 % in  $\sum T$  and kept the temperature above

80 °C for longer (+33.3–66.7 %) compared to soil steaming. KOH caused an increase of 6.1–9.3 % in  $\sum T$  and kept the temperature above 80 °C for periods greater than 25.5–54.2 % compared to soil steaming. With regard to depth (20 or 40 cm), CaO caused an increase of 8.2–9.7 % in soil pH compared to soil steaming, while KOH caused an increase of 10.0–10.8 %. After 20 days, the pH of soil was normalized, with a final increase of 1.5 % at 20-cm depth and 1.2 % at 40-cm depth after soil steaming with exothermically reacting chemicals. Similar temperature behaviour (within 2.8 % of variance) was recorded in open-field tests. Soil solarization led to different temperature profiles depending on the film applied to soil (Table 1).  $\sum T$  was increased by 11.0 and 12.6 % by ethylene–vinylacetate film and high-effectiveness infrared film compared to polyethylene film, respectively. According to depth (40 or 20 cm), both ethylene–vinylacetate film and high-effectiveness infrared film increased maximum soil temperature by 5.2–9.3 and 5.5–10.1 % compared to polyethylene film, respectively.

To estimate virus concentration, a calibration curve ( $r=99$  %) was constructed in order to correlate optical density with concentration. In laboratory tests, soil steaming was very

**Table 1** Soil temperature, pH and electrical conductivity at two depths (20 and 40 cm) in treated soil with steam (alone or with CaO or KOH) or solarization (three plastic films: polyethylene film, ethylene–vinylacetate film or high-effectiveness infrared film)

	Effect of soil steaming treatment					
	Soil depth					
	20 cm			40 cm		
	Steam	Steam CaO	Steam KOH	Steam	Steam CaO	Steam KOH
Temperature						
$\sum T$	7.382a	8.131b	7.832c	8.231a	9.385b	8.896c
$T$ max (°C)	83.2a	90.4b	89.2c	89.3a	93.5b	91.6c
$T$ mean (°C)	51.3a	54.3b	53.3b	58.3a	62.4b	61.5b
$T$ min (°C)	42.5a	43.3a	42.5a	43.2a	43.3a	43.4a
Temperature classes						
$T \leq 40$ °C (min)	–	–	–	–	–	–
$40 < T \leq 60$ °C (min)	101a	86b	90c	91a	62b	71c
$60 < T \leq 80$ °C (min)	58a	66b	64c	61a	71b	67c
$T > 80$ °C (min)	21a	28b	26c	28a	47b	42c
Soil parameters						
pH	7.41a	8.02b	8.15b	7.40a	8.12b	8.20b
Electrical conductivity (mS cm <sup>-1</sup> )	225a	238b	235b	241a	281b	279b
	Effect of soil solarization treatment					
	Soil depth					
	20 cm			40 cm		
	PEF	EVA	IRF	PEF	EVA	IRF
Temperature						
$T$ max (°C)	44.1	48.2	48.6	24.2	25.0	25.1
$T$ mean (°C)	35.3	37.2	36.7	23.2	24.1	24.0
$T$ min (°C)	25.3	25.2	25.3	22.1	22.2	21.9

Within a soil depth, means followed by the same letter are not significantly different at  $P=0.05$  by Duncan's multiple range test

$\sum T$  thermal addition, PEF polyethylene film, EVA ethylene–vinylacetate film, IRF high-effectiveness infrared film

effective against all viruses (Table 2), achieving no virus or infectivity recovery for *Potato virus Y* or *Cucumber mosaic virus*, regardless of depth of inoculum or exothermically reacting chemical addition. Conversely, more than 60 % of inoculated *Tobacco mosaic virus* was recovered after soil steaming and more than 50 % thanks to exothermically reacting chemical addition. However, *Tobacco mosaic virus* infectivity was drastically reduced by the short-duration steaming, while exothermically reacting chemicals caused a further significant decrease, setting virus recovery below 3.0 %, without significant differences among exothermically reacting chemicals. Open-field treatments achieved the same effects against the three viruses (data not shown). The best results were achieved at 40-cm depth, in proximity to the steam dispenser.

As expected, the low stability of *Cucumber mosaic virus* was underlined by its elimination after 20 days in untreated control plots; thus, soil disinfection was naturally achieved by short set-aside (Table 2). *Potato virus Y* was totally controlled by soil solarization with ethylene–vinylacetate film or high-effectiveness infrared films at 20-cm depth. Polyethylene film did not eliminate the infectivity of recovered virus even if it was greatly reduced. Solarization did not cause any effect against *Tobacco mosaic virus*, with virus and infectivity recovery comparable to untreated plots. Solarization did not cause any effect on viruses at 40-cm depth.

Factorial analysis of variance of virus recovery and infectivity after soil solarization treatments or soil steam treatments is reported in Table 3.

Longevity in vitro was esteemed higher than 300 for *Tobacco mosaic virus*, 36 for *Potato virus Y* and 5 for *Cucumber mosaic virus*, according to literature (Luvisi et al. 2012). Considering the principal component analysis performed on the nine chosen parameters (Fig. 2), the first principal component axis accounted for 81.61 % of the observed variation. It was strongly and positively correlated with steam–exothermically reacting chemicals and solarization with ethylene–vinylacetate film or high-effectiveness infrared film. The second principal component axis (18.39 %) was strongly correlated to genome partition and RNA % and negatively correlated to size and GC %. Treatments were not substantially correlated to the second principal component axis. The first principal component axis separated *Tobacco mosaic virus* from *Potato virus Y* and *Cucumber mosaic virus*; the second principal component axis separated *Potato virus Y* from *Cucumber mosaic virus*. A strong negative correlation was calculated between longevity in vitro or thermal inactivation point and all soil treatments. As reported by Nascimento et al. (2010), longevity in vitro may represent an important parameter in order to choose or optimize the disinfection methods. Anyway, tests report difference between the longevity in vitro found for some viruses and their survival in

**Table 2** Virus recovery (virus recovered after treatment out of total virus inoculum, %) and infectivity recovery (chlorotic lesions caused by viruses recovered compared to control (untreated virus inoculum), %) considering three viruses (*Tobacco mosaic virus*, *Potato virus Y* and *Cucumber*

*mosaic virus*) buried at two depths (20 and 40 cm) in treated soil with solarization (three plastic films: polyethylene film, ethylene–vinylacetate film or high-effectiveness infrared film) or steam (alone or with CaO or KOH)

	Effect of soil steaming treatment								Effect of soil solarization treatments							
	Control		Steam		Steam–CaO		Steam–KOH		Control		PEF		EVA		IRF	
	Inoculum depth															
Virus	20 cm	40 cm	20 cm	40 cm	20 cm	40 cm	20 cm	40 cm	20 cm	40 cm	20 cm	40 cm	20 cm	40 cm	20 cm	40 cm
<b>TMV</b>																
Virus recovery	98.4a	97.9a	71.4b	62.3b	64.3c	52.6c	66.0c	54.5c	97.4a	99.1a	98.3a	97.4a	99.1a	98.0a	98.9a	98.3a
Infectivity recovery	98.2a	97.8a	16.7b	13.6b	2.9c	1.2c	3.0c	1.3c	97.2a	97.2a	96.9a	97.0a	98.2a	97.3a	97.3a	98.0a
<b>PVY</b>																
Virus recovery	97.9a	98.2a	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b	75.2a	73.4a	33.4b	70.3a	24.2c	73.4a	23.2c	75.2a
Infectivity recovery	97.3a	97.8a	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b	64.3a	61.3a	4.4b	58.2a	0.0c	60.0a	0.0c	62.0a
<b>CMV</b>																
Virus recovery	85.3a	86.2a	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
Infectivity recovery	76.4a	75.4a	0.0b	0.0b	0.0b	0.0b	0.0b	0.0b	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a

Within a soil depth, means followed by the same letter are not significantly different at  $P=0.05$  by Duncan's multiple range test

TMV *Tobacco mosaic virus*, PVY *Potato virus Y*, CMV *Cucumber mosaic virus*, PEF polyethylene film, EVA ethylene–vinylacetate film, IRF high-effectiveness infrared film

**Table 3** Three-way factorial analysis of variance of virus recovery (virus recovered after treatment out of total virus inoculum, %) and infectivity recovery (chlorotic lesions caused by viruses recovered compared to untreated virus inoculum, %) considering three viruses (*Tobacco mosaic virus*, *Potato virus Y* and *Cucumber mosaic virus*) buried at two depths

(20 and 40 cm) in treated soil with solarization (three plastic films: polyethylene film, ethylene–vinylacetate film or high-effectiveness infrared film) or steam (alone or with CaO or KOH) compared to control (untreated virus inoculum)

Solarization			Steam		
Source of variation	Virus recovery	Infectivity recovery	Source of variation	Virus recovery	Infectivity recovery
<b>Main effect</b>			<b>Main effect</b>		
Soil treatment (A)	<0.001	<0.001	Soil treatment (A)	<0.001	<0.001
Depth (B)	<0.001	<0.001	Depth (B)	<0.001	<0.001
Virus (C)	<0.001	<0.001	Virus (C)	<0.001	<0.001
<b>Interactions</b>			<b>Interactions</b>		
A×B	<0.001	<0.001	A×B	<0.001	<0.001
A×C	<0.001	<0.001	A×C	<0.001	<0.001
B×C	<0.001	<0.001	B×C	<0.001	<0.001
A×B×C	<0.001	<0.001	A×B×C	<0.001	<0.001
<b>Comparison for A</b>			<b>Comparison for A</b>		
PEF vs. EVA	<0.001	<0.001	Steam vs. steam CaO	<0.001	<0.001
PEF A vs. IRF	<0.001	<0.001	Steam vs. steam KOH	<0.001	<0.001
EVA vs. IRF	<0.001	<0.001	Steam CaO vs. steam KOH	<0.001	<0.001
Control vs. PEF	<0.001	<0.001	Control vs. steam	<0.001	<0.001
Control vs. EVA	<0.001	<0.001	Control vs. steam CaO	<0.001	<0.001
Control vs. IRF	<0.001	<0.001	Control vs. steam KOH	<0.001	<0.001
<b>Comparison for B</b>			<b>Comparison for B</b>		
20 vs. 40 cm	<0.001	<0.001	20 vs. 40 cm	<0.001	<0.001
<b>Comparison for C</b>			<b>Comparison for C</b>		
TMV vs. CMV	<0.001	<0.001	TMV vs. CMV	<0.001	<0.001
TMV vs. PVY	<0.001	<0.001	TMV vs. PVY	<0.001	<0.001
CMV vs. PVY	<0.001	<0.001	CMV vs. PVY	NS	NS
<b>Comparison for A within TMV</b>			<b>Comparison for A within TMV</b>		
PEF vs. EVA	NS	NS	Steam vs. steam CaO	<0.001	<0.001
PEF A vs. IRF	NS	NS	Steam vs. steam KOH	<0.001	<0.001
EVA vs. IRF	NS	NS	Steam CaO vs. steam KOH	NS	NS
Control vs. PEF	NS	NS	Control vs. steam	<0.001	<0.001
Control vs. EVA	NS	NS	Control vs. steam CaO	<0.001	<0.001
Control vs. IRF	NS	NS	Control vs. steam KOH	<0.001	<0.001
<b>Comparison for A within CMV</b>			<b>Comparison for A within CMV</b>		
PEF vs. EVA	NS	NS	Steam vs. steam CaO	NS	NS
PEF A vs. IRF	NS	NS	Steam vs. steam KOH	NS	NS
EVA vs. IRF	NS	NS	Steam CaO vs. steam KOH	NS	NS
Control vs. PEF	NS	NS	Control vs. steam	NS	NS
Control vs. EVA	NS	NS	Control vs. steam CaO	NS	NS
Control vs. IRF	NS	NS	Control vs. steam KOH	NS	NS
<b>Comparison for A within PVY</b>			<b>Comparison for A within PVY</b>		
PEF vs. EVA	<0.001	<0.001	Steam vs. steam CaO	NS	NS
PEF A vs. IRF	<0.001	<0.001	Steam vs. steam KOH	NS	NS
EVA vs. IRF	NS	NS	Steam CaO vs. steam KOH	NS	NS
Control vs. PEF	<0.001	<0.001	Control vs. steam	<0.001	<0.001
Control vs. EVA	<0.001	<0.001	Control vs. steam CaO	<0.001	<0.001
Control vs. IRF	<0.001	<0.001	Control vs. steam KOH	<0.001	<0.001

Pairwise multiple comparison analysis with Holm–Sidak test is reported. Numbers tabulated are levels of statistical significance

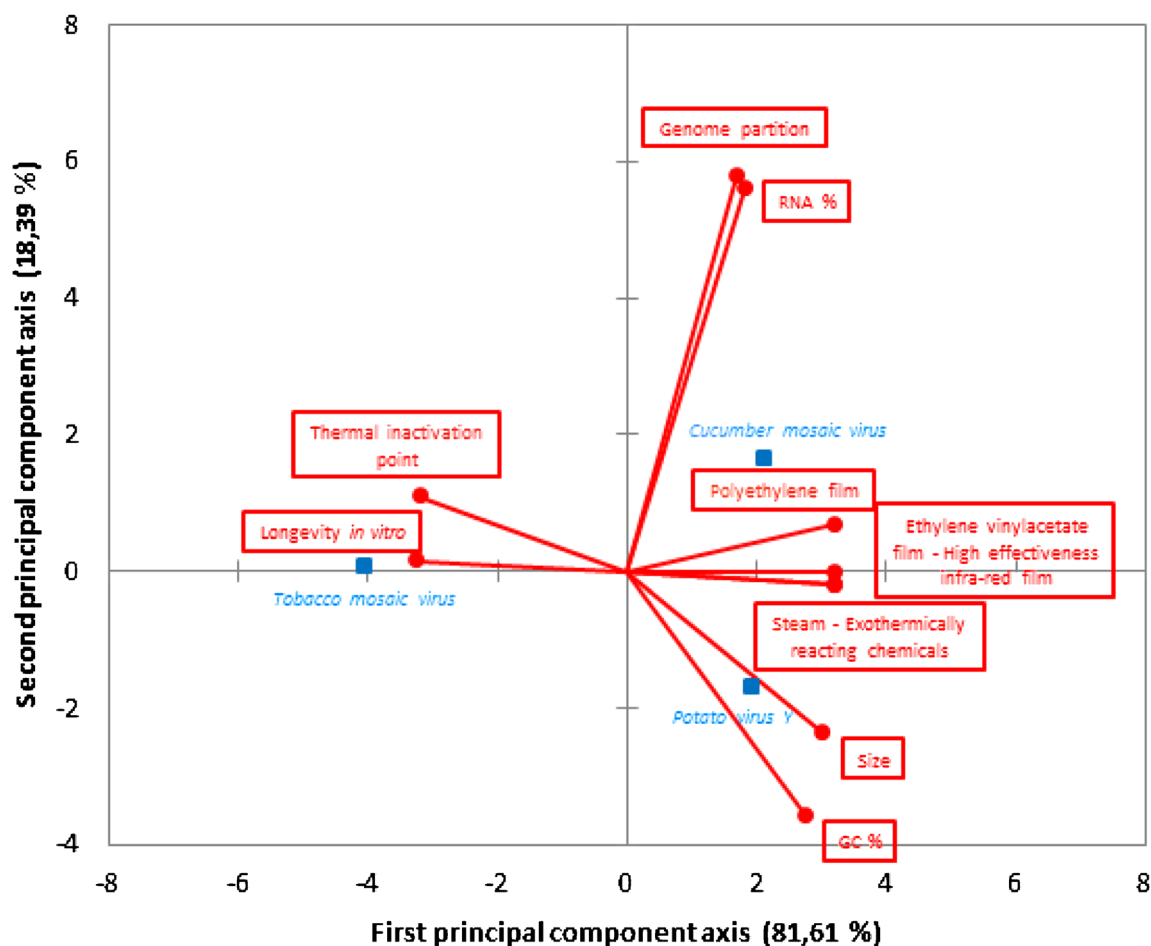
NS not significant, TMV *Tobacco mosaic virus*, PVY *Potato virus Y*, CMV *Cucumber mosaic virus*, PEF polyethylene film, EVA ethylene–vinylacetate film, IRF high-effectiveness infrared film



infected tissue due to the environment in which the virus particles were maintained. Our findings are in agreement with those obtained against Papaya lethal yellowing virus (Nascimento et al. 2010): high longevity in vitro (60 days) did not prevent heat treatment activity that demonstrated to be an efficient agricultural practice for virus inactivation. With regard to relevance of thermal inactivation point in virus elimination, our results are in agreement with those reported by van Loenen et al. (2003): steaming at 50 or 60 °C for 3 min, followed by an 8-min resting period in the steamed soil, resulted in a complete kill of weeds, fungal pathogens and nematodes. In our tests, temperature achieved by steam or solarization at 20-cm depth is able to control efficiently a mid-stability virus such as *Potato virus Y*. Even if *Tobacco mosaic virus* is highly resistant to thermal inactivation, the use of exothermically reacting chemicals combined with steam could lead to higher treatment temperature and increased soil heat

persistence, thus producing an effect known as “thermal flash” (Peruzzi et al. 2011), able to overcome the difficulty of controlling *Tobacco mosaic virus* with steam only, in particular adopting a mobile steam generator with a short treatment exposure time. Moreover, the exothermically reacting chemicals raise the soil pH which may affect virus stability (Kegel and van der Schoot 2006). Various aggregation states of the viral coat protein subunits are stable as a function of the ionic strength and pH. Alkali caused the protein subunits to be stripped from *Tobacco mosaic virus* RNA beginning at the 5' end of the RNA, the concave end of the virus rod. Moreover, factors influencing the rate of virus decline in soil, such as temperature, moisture, pH or presence of antibiotics, have been reported (Cheo 1980), and it has been suggested that microbial activity may contribute to the instability of *Tobacco mosaic virus* in soil.

**Biplot (First and Second principal component axis: 100,00 %)**



**Fig. 2** Biplot of principal component analysis performed to evaluate the virus variables. The first principal component axis separated *Tobacco mosaic virus* from *Potato virus Y* and *Cucumber mosaic virus*, and it was strongly and positively correlated with steam–exothermically reacting chemicals and solarization with ethylene–vinylacetate film or high-

effectiveness infrared film. The second principal component axis separated *Potato virus Y* from *Cucumber mosaic virus*. A strong negative correlation was calculated between longevity in vitro or thermal inactivation point and all soil treatments

None of the treatments induced biological vacuum with regard to the investigated microbial community. Soil solarization did not significantly alter total fungi and actinomycetes, while steam treatments led to a reduction in total fungi (−18.3 %) without detrimental effects caused by exothermically reacting chemicals. The community of *Trichoderma* spp. was slightly increased with solarization by polyethylene film (+6.7 %), ethylene–vinylacetate film (+8.2 %) and high-effectiveness infrared film (+11.4 %). Short-duration steaming also increased *Trichoderma* spp. (+4.2 %) as did CaO (+6.3 %) and KOH (+5.7 %). Cumulative carbon metabolic activity was the integration of average well colour development over incubation time, relatively indicating the total carbon utilized by soil microbial communities. The soil microbial community of soils treated with solarization exhibited a slight reduction (9.3 %) of cumulative carbon metabolic activity compared to control, while a significant reduction was calculated for steam (33.3 %), steam plus CaO (38.9 %) and steam plus KOH (37.3 %). This implies the soil microbial community established after solarization was capable of consuming carbon substrates at nearly the same efficiency as untreated soils unlike to steam and exothermically reacting chemicals. Carbon source utilization richness expressed the number of usable substrates by a soil microbial community and reflected the diversity of microbial metabolism. This measurement was not significantly affected by solarization, steam or steam plus exothermically reacting chemicals. Shannon's index of carbon source utilization was not influenced by heat treatments either, suggesting no effect on functional diversity. With regard to microbial tests, our findings following steam treatments are in agreement with those reported by Gelsomino et al. (2011) in which soil steaming reduced the microbial biomass while the genetic structure of soil bacterial and actinobacterial communities was largely unresponsive to the treatments.

#### 4 Conclusion

Up to now, steaming and solarization methods had not yet been valued considering non-living pathogens such as viruses while one of the most resistant representative—the *Tobacco mosaic virus*—was known to be almost uncontrollable by traditional solarization or steaming treatments. Our results show that the mid-stability virus *Potato virus Y* was totally controlled by short-duration steaming charged with exothermically reacting chemicals and the residual infectivity of the high-stability *Tobacco mosaic virus* was found to be very lower in the treated soil compared to control. Thus, results from this study demonstrate that improved steaming methods, based on high-temperature and short-duration application, tested here seem effective against the most resistant soil-borne virus. *Potato virus Y* was totally controlled by soil solarization with ethylene–vinylacetate or high-effectiveness

infrared films, while polyethylene film did not eliminate the infectivity of recovered virus even if it was greatly reduced. Conversely, *Tobacco mosaic virus* was unaffected by solarization treatments regardless of films.

Study of a model system such as *Tobacco mosaic virus* makes it possible to evaluate mobile steam treatments as a valuable method to control important viruses that can infect soils. Moreover, considering the results obtained against various pathogens, this method can be considered a promising approach to address pathological problems that may occur particularly in monoculture practices. On the other hand, even if improved plastic films for solarization are useless to control high-stability viruses, they may represent a very effective tool against mid-stability viruses. Moreover, techniques able to preserve the natural soil biota could be useful for controlling even high-stability viruses, not only in terms of inactivating the virus but also by conserving antiviral biological factors.

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