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STEAM PASTEURISATION AS AN ALTERNATIVE TO FUMIGATION FOR DESINFECTING CONTAINER MEDIA

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ABSTRACT

Two types of container medium differing in composition were either artificially infested with Phytophthora cinnamomi *and* Fusarium oxysporum, *or left un-infested. Infested and un-infested media were fumigated with methyl bromide at 200 g m^3, or exposed to aerated steam at 75 °C in the laboratory and in a commercial steam pasteuriser for periods of 30, 60 and 120 min. Steam treatment for 30 min in the commercial pasteuriser was sufficient for eliminating* P. cinnamomi and F. oxysporum *from pre-infested medium, and therefore comparable with methyl bromide regarding disinfective properties. Laboratory results nevertheless indicated a pasteurisation time of one hour for optimal efficiency. Light container medium (1 sand : 1 peat: 3 compost) appeared more conducive to recolonisation by fungi than heavy medium (1 composted sugar-cane fibre : 1 river sand) after pasteurisation, whereas the opposite was true for bacteria. However, heavy medium was colonised more readily by an antagonistic* Trichoderma hamatum *after artificial augmentation with the organism. Methyl bromide fumigated medium supported establishment of* T. hamatum *better than medium pasteurised with steam.*

INTRODUCTION

Methyl bromide (MBr) has been used for many years in South African avocado nurseries as a preplant fumigant (Kotzé *et al.,* 1987). However, the compound is to be phased out in the foreseeable future due to its harmful effect on the environment. Apart from being noxious to animals, plants, and the atmosphere, MBr is also a nonselective eradicant which renders treated substrates conducive to plant pathogens fortuitously introduced into the vacuum it creates (Baker & Cook, 1974; Sylvia & Schenck, 1984). Fumigation with MBr therefore provides only a transient absence of pathogens, and does not protect a substrate from reinfestation. It futher more is known to kill beneficial vesicular-arbuscular mycorrhizal fungi (Menge, 1982) which stimulate growth and reduce transplant injury (Menge *et al,* 1978).

A number of alternative methods are available for disinfecting container media, eg. radurisation, autoclaving, and steam pasteurisation. Although effective as sterilants, the former two Procedures are relatively expensive, and are known to affect soil chemical Properties to such an extent that it could result in phytotoxicity (Bowen &

Rovira, 1961; Baker & Olsen, 1962; Eno & Popenoe, 1964; Awuah & Lorbeer, 1991). Steam pasteurisation, on the other hand, has negligible effect on the composition of soil, but has been reported to selectively eliminate or suppress various soilborne pathogens (Baker, 1962; Eno & Popenoe, 1964; Bollen, 1968).

This paper reports on the efficacy of steam pasteurisation for treating container media artificially infested with avocado root pathogens, and on the effect of steam treatment and MBr fumigation on the microbial composition of the medium.

MATERIALS AND METHODS

Container media

Experiments were conducted with two container medium formulations. One consisted of a 1:1:3 (v/v) mixture of sand, peat, and composted milled pine bark (light medium), and the other of composted sugar-cane fibre and river sand mixed in a ratio of 1:1 (v/v) (heavy medium).

Artificial infestation with pathogens

The two avocado root pathogens used were *Phytophthora cinnamomi* Rands *(*Pc*),* and *Fusarium oxysporum* Schlecht. emend. Snyd. & Hans. *(Fo).* Mycelial inoculum of Pc was prepared as described by Duvenhage et al. (1991), and chlamydospores of the pathogen were induced according to the procedure of Tsao (1971) and Kadooka & Ko (1973). Fo was incubated for 5 wks at 25 °C on quarter strength potato-dextrose agar (PDA), after which conidia were harvested and suspended in 0,1 % sterile water agar. Light and heavy container media were infested separately with Pc or Fo at rates of 0,1 % (m/v) mycelium + three chlamydospores $m\ell^{-1}$, and 5,4 x 10^4 conidia $m\ell^{-1}$ respectively. To ensure even distribution of the inoculum, each infested medium was blended thoroughly in a concrete mixer.

Laboratory optimisation of steam treatment

Pathogen-infested container media were moistened, dispensed in 100 g quantities into 250 mℓ erlenmeyer flasks, and placed in an oven at 75 °C. Three flasks of each treatment were removed from the oven after 15, 30, 45, 60, 75, 90, 105, and 120 minutes respectively. The incidence of Pc and Fo in the chronologically exposed media was determined by the leaf disc method of Grimm & Alexander (1973), and by diluting and plating on the medium of Van Wyk *et al.* (1986).

Treatment with steam and MBr

Container media were transferred to linen bags permeable to steam and MBr. Bags with medium were subjected to aerated steam at *ca* 75°C for 30, 60, and 120 min in an Agrelek pasteuriser (Bungay, 1994). Fumigation was performed by exposure for 48 h to 200 g m^3 MBr in a polyethylene container. Controls consisted of container medium not pasteurised or fumigated.

Four and eight days after treatment with steam and MBr respectively, container medium of each treatment was dispensed into six 250 ml polystyrene cups, and five aseptically pregerminated blue lupin *(Lupinus angustifolius* L.*)* seeds were planted to each cup (Darvas, 1979). Cups were arranged randomly in a greenhouse at 25-32 °C, and each cup received 30 ml of sterile distilled water thrice a week. Lupin seedlings which

capsised were recorded, and their crowns plated on PARP medium (Solel & Pinkas', 1984) and the medium of Van Wyk *et al.* (1986) for recovery of PC and Fo.

Container media pot artificially infested with PC and Fo were also left for 10 d after treatment to establish the degree of saprophytic recolonisation. This was determined by diluting and plating in triplicate on Standard 1 agar containing 300 mg ℓ^{1} cycloheximide, and on PDA supplemented with 50 mg ℓ^{1} rifampicin. Bacteria and fungi which developed on the respective media were counted, and representative isolates were screened for antibiosis towards PC according to the dual culture technique (Fokkema, 1976).

Post-treatment augmentation with *Tríchoderma hamatum*

Un-infested container medium was also inoculated with millet seed inoculum of *Tríchoderma hamatum* (Bonard.) Bain at one unit $m\ell^{-1}$ immediately after steam pasteurisation and MBr fumigation, using six replicates per treatment. Population densities of *T. hamatum* were determined after 10 d by diluting and plating on PDA supplemented with 250 mg ℓ^{-1} chloromycetin.

TABLE 1. Survival of *P. cinnamomi* and *F. oxysporum* in container medium exposed to 75 °C in the laboratory.

Pathogen[1]	Medium[2]	Population density[3]					
		0	15	30	45	60	120
Pc	LM	100	0	0	0	0	0
	HM	100	100	90	40	0	0
Fo	LM	2500	50	30	0	0	0
	HM	2000	0	0	0	0	0

[1] Pc: *P. cinnamomi;* Fo: *F. oxysporum*
[2] LM = 1:1:3 (v/v) sand:peat:composted milled pine bark; HM = 1:1 (v/v) composted sugar-cane fibre:river sand
[3] Mean of three replicates; incidence of Pc calculated according to the leaf disc technique of Grimm & Alexander (1973)

RESULTS

In the laboratory, heat treatment for 15 min at 75 °C was sufficient for eradicating PC from light medium, whereas an exposure time of 60 min was necessary to achieve this in heavy medium (Table 1). Conversely, propagule numbers of Fo declined to zero after 15 min in heavy medium, but did so only after treatment for 45 min in light medium. In the Agrelek pasteuriser, steam treatment for 30 min proved effective for reducing PC and Fo to undetectable levels in both light and heavy medium (Table 2). Fumigation with MBr also eliminated the two pathogens.

Quantitatively, no significant differences were evident in natural fungal populations between the light and heavy medium, although the latter contained higher numbers of bacteria (Table 3). In general, pasteurised or fumigated light medium appeared more conducive than heavy medium to survival of, or recolonisation by fungi, whereas the opposite was true for bacteria. Apart from *Aspergillus fumigatus, Botryotrichum piluliferum* and *Trichurus spiralis* which occurred naturally in heavy medium, and

Paecilomyces variotii and *Verticillium fungicola* which were native to light medium, most identified fungi isolated from treated medium appeared to be recolonisers, and not survivors (Table 4). Be that as it may, heavy medium seemed to favour *A. fumigatus,* whereas light medium supported *P. variotii* and *V. fungicola.* The only *Tríchoderma sp.* isolated, viz. *T. hamatum,* occurred in medium fumigated with MBr. MBr-disinfested heavy medium was the only substrate recolonised by a potential avocado pathogen, viz. *F. oxysporum.* Virtually all fungal isolates from container medium exhibited *in vitro* antibiosis towards PC, whereas none of the bacterial isolates inhibited growth of the pathogen. Fungal incidence (Table 3); therefore also reflects the antagonistic potential of the respective media.

TABLE 2.
Survival of *P. cinnamomi* and *F. oxysporum* in container medium after steam pasteurisation at 75 °C and fumigation with methyl bromide

Pathogen[1]	Medium[2]	Incidence after treatment[3]		
		Control	Pasteurisation	Methyl bromide
Pc	LM	+	-	-
	HM	+	-	-
Fo	LM	+	-	-
	HM	+	-	-

[1] Pc: *P. cinnamomi;* Fo: *F. oxysporum*
[2] LM = 1:1:3 (v/v) sand:peat:composted milled pine bark; HM = 1:1 (v/v) composted sugar-cane fibre:river sand
[3] Mean of six replicates; incidence of Pc and Fo determined according to the seedling technique (Darvas, 1979), and plating on the medium of Solel & Pinkas (1984) and Van Wyk et al (1986)

TABLE 3. Recolonisation of unaugmented pasteurised and methyl bromide fumigated container medium by fungi and bacteria

Taxon	Medium[1]	Propagule numbers 10 d after treatment (x 10^2 g^{-1})[2]				
		Control	Steam-30	Steam-60	Steam-120	Mbr
Fungi	LM	270Aa	560Ab	10033Bb	3866Ab	503Ab
	HM	107Ca	17Aa	35Aa	4Aa	75Ba
Bacteria	LM	5ABb	2Ab	10Bb	1Ab	2Ab
	HM	8800Aa	16633Ba	3000Aa	30000Ca	3000Aa

[1] LM = 1:1:3 (v/v) sand:peat:composted milled pine bark; HM = 1:1 (v/v) composted sugar-cane fibre:river sand
[2] Mean of three replicates; enumeration of fungi was on PDA supplemented with 50 mgℓ^{-2} rifampicin, and of bacteria on Standard 1 agar containing 300 mg ℓ^{-1} cyclohexamide; in columns (lower case) and rows (upper case) , for each taxon, values followed by the same letter do not differ significantly according to Tukey's multiple range test (P = 0,05)

Contrary to the situation with unaugmented substrates, heavy medium was colonised more readily by *T. hamatum* than light medium following artificial incorporation of the antagonist (Table 5). Although steam pasteurisation enhanced conduciveness to

colonisation, establishment of *T. hamatum* was supported best in medium fumigated with MBr.

TABLE 4
Incidence of fungal species in unaugmented container medium 10 d after pasteurisation or fumigation with methyl bromide.

Fungal species	Propagule numbers 10 d after treatment (x10² g⁻¹)[1]									
	LM[2]					HM[2]				
	Control	Steam-30	Steam-60	Steam-120	Methyl Bromide	Control	Steam-30	Steam-60	Steam-120	Methyl Bromide
Aspergillus fumigatus	O	O	O	O	O	5b	2ab	33a	1a	3ab
Aspergillus niger	30a	O	O	O	O	O	O	O	O	1a
Botryotrichum piluliferum	O	O	O	O	O	7b	1a	O	O	O
Doratomyces microsporus	O	O	O	O	O	O	O	O	O	11a
Fusarium oxysporum	O	O	O	O	O	O	O	O	O	1a
Fusarium subglutinans	O	16a	3a	O	O	O	O	O	O	O
Gliocladium catenulatum	21a	O	O	O	O	O	O	O	O	O
Paecilomyces variotii	4a	10a	262b	125ab	164ab	O	O	1a	1a	2a
Penicillium brevicompactum	O	O	O	O	O	O	O	O	O	21a
Penicillium canencens	O	O	O	O	O	1a	O	O	O	O
Penicillium cyclopium	O	O	O	O	6a	O	O	O	O	O
Penicillium frequentans	1a	O	O	O	O	O	O	O	O	O
Penicillium cf. funiculosum	O	O	O	O	O	24a	O	O	O	O
Penicillium lanosum	O	O	O	O	36a	O	O	O	O	21a
Penicillium simplicissimum	O	O	O	O	O	O	O	O	2a	O
Trichoderma hamatum	O	O	O	O	20a	O	O	O	O	1b
Trichurus spiralis	O	O	O	O	O	15a	O	O	O	15a
Verticillium fungicola	28a	11ab	943b	1826a	O	O	O	O	O	O
Unidentified	186	523	8825	1915	277	55	14	1	O	O

[1]Mean of three replicates; identified after enumeration on PDA supplemented with 50 mg ℓ⁻¹ rifampecin;
[2]LM = 1:1:3 (v/v) sand:peat:composted milled pine bark; HM = 1:1 (v/v) composted sugar-cane fibre:river sand; in rows, values followed by the same letter do not differ significantly according to Tukey's multiple range test (P = 0,05)

TABLE 5. Population densities of *T. hamatum* in pasteurized and methyl bromide fumigated media 10 d after artificial augmentation with the organism.

Medium [1]	Propagule numbers of *T. hamatum* after 10 d (x10² g⁻¹)[2]				
	Control	Steam-30	Steam-60	Steam-120	Mbr
LM	5Aa	3Aa	80Aa	400Aa	1400Ba
HM	120Ab	170Ab	800Cb	600Bb	200Db

[1]LM = 1:1:3 (v/v) sand : peat : composted milled bark; HM = 1:1 (v/v) composted sugar- cane fibre : river sand
[2]Mean of six replicates; values in rows (uppercase) and columns (lower case), followed by the same letter, do not differ significantly according to Tukey's multiple range test.

DISCUSSION

Results presented here indicate that steam pasteurisation is a feasible alternative to MBr fumigation for disinfecting container medium used in avocado nurseries. The exposure time of 30 min at 75 °C found effective in the Agrelek pasteuriser corresponds

with treatment regimes reported previously as sufficient for eliminating pathogenic organisms like *Fusarium, Phytophthora, Cylindrocarpon, Rhizoctonia, Verticillium* and *Thielaviopsis* (Baker, 1962; Bollen, 1969; Sylvia & Schenck, 1984). However, in view of the laboratory screening which showed that PC survived in heavy medium after exposure to heat for 45 min, ¡t is recommended that the pasteurisation process should last for at least one hour. This period could increase even further once information is available on the survival of PC oospores, as well as of other avocado root pathogens. *Pythium* spp., which are commonly associated with avocado roots (Darvas, 1978), are especially resistant to pasteurisation (Campbell, 1989).

In the absence of sufficient evidence, it is not known whether the variously treated media differ in conduciveness or resistance to reinfestation by PC or other root-invading fungi. In accordance with various previous reports (Martin, 1950; Bollen, 1969; Awuah & Lorbeer, 1990), results nevertheless showed that the biological vacuum created by pasteurisation and fumigation was rapidly occupied by microbial communities. Although these communities are obviously determined by specific environments, the composition of a medium appears to affect its conduciveness towards inhabitation by higher taxa, eg. Preferential colonisation of heavy medium by bacteria, and light medium by fungi. In terms of sustained biotic disease suppression, unaugmented light medium therefore seems to be a more suitable substrate, since it preferentially supported colonisation by fungi antagonistic to PC, and not bacteria, which were not antagonistic. On the other hand, artificial augmentation with an antagonistic isolate of *T. hamatum* was more effective in heavy medium, especially after fumigation with MBr. The question therefore arises whether treated medium should be supplemented with a "foreign" antagonist, or whether disinfestation procedures should be adapted to benefit naturally-colonising antagonistic organisms. In the present situation, the principal fungal species in light medium comprised *P. variotii* and *V. fungicola,* and in heavy medium *A. fumigatus.* All three are ubiquitous thermotolerant inhabitants of organic substrates with known antagonistic properties (Domsch et al., 1980). Utilisation of these fungi as antagonists in pasteurised substrates should be considered since they obviously survived steam treatment and, for that matter, fumigation with MBr.

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