ATTI Giornate Fitopatologiche
1992, 2, 407-416

EFFECT OF METHYL BROMIDE AND CHLOROPICRIN ON THE SOIL MYCOFLORA IN GREENHOUSE TOMATO

BOURBOS, V.A. and SKOUDRIDAKIS M.T.

Institute of Subtropical Plants and Olive Trees.
Plant Pathology Laboratory, 73100 Chania, Greece

SUMMARY

After disinfestation with methyl bromide and chloropicrin, the whole soil mycoflora of greenhouse tomato showed profound qualitative and quantitative disturbances till a soil depth of 30cm. From the 107 species in the soil mycoflora of the control soil, 7-9 were isolated 4 days after disinfestation. The soil layer 31-40cm was not influenced by disinfestation. The re-colonisation of the disinfected soil two months after disinfestation covered only the 35-40% in species and the 60-63% in density of the primary mycoflora. The saprophytic fungi *Aspergillus alutaceus, Paecilomyces lilacinus, Penicillium chrysogenum, P. funiculosum, P. herquei, Trichoderma harzianum* and *T. viride* seem to have developed a certain degree of tolerance to the fumigant. Similar behaviour can be observed also for the pathogen *Verticillium dahliae*. This fact, together with the quick contamination of the disinfected soil by certain pathogens (*Fusarium oxysporum* f. sp. *radicis-lycopersici, F. solani* and *Pythium* spp.) and the possibility of re-infection from the lower soil layers beyond the effect of the biocide, makes difficult, under certain conditions, the control of soil-borne diseases in greenhouse tomato with these fumigants.

INTRODUCTION

A primary point in greenhouse crops is disease-free soil. As a general rule, soil diseases are controlled before planting with a broad-spectrum disinfestant or vapour. The disinfestants, known also as biocides, cause profound disturbances of the biological equilibrium in the soil which can lead to disastrous ecological phenomena, like alternating diseases, trophobiosis, "boomerang" and concentration of plant toxic substances (Bourbos and Skoudridakis, 1989; Coleno et *al*, 1965; Davet, 1981; Kreutzer, 1965).

Many researchers observed microflora development in soils after disinfestation with physical (vapour) or chemical (biocides) methods. They all agree that these techniques create a biological vacuum with unfavourable consequences for the physio-biological characteristics of the treated soil (Bourbos, 1983; Bourbos, 1986; Domsch, 1963, Rapilly and Coleno, 1967; Warcup, 1951; Messiaen and Lafon, 1970).

Soil microflora in tomato crops which had been previously disinfected was studied for the first time in 1943 (Katzenelson and Richardson, 1943). Useful information is also given in the studies of Bollen (1966, 1974), Marois and Mitchell (1979) and Munnecke et *al.* (1982). The last two studies mention the effect of methyl bromide on the chlamydospores of *Fusarium*

oxysporum f.sp. *lycopersici* and the sclerotia of *Sclerotium rolfsii* and *Verticillium albo-atrum.*

The present research deals with the effect of methyl bromide on the soil mycoflora in greenhouse tomato. Special subjects are the qualitative and quantitative presence of the different species as well as the re-colonization of the soil within two months after disinfestation.

MATERIALS AND METHODS

To study the effect of the chemical soil disinfestation on the quantitative presence and composition of mycoflora, greenhouse soils were used with the following presumptions:
- To cultivate tomato during three subsequent seasons.
- No application of any chemical or physical soil disease control method.
- The soils must be as homogeneous as possible towards their physio-chemical properties.
- To have been applied with similar fertilizers.

The fumigant applied is the trade mark Dowfume MC_2 of the company Dow Chemical at a dose of 75g/m2. This product with methyl bromide and 2% chloropicrin as active substance is the most widely used soil disinfestant in protected crops in Greece. From each experimental plot (total area 25 m^2) and at four soil layers (0-10, 11-20, 21-30 and 31-40 cm). 10 samples were taken under aseptic conditions. On the whole, 5 samplings were done. The first one shortly before the application of the disinfestant and the rest 4, 12, 30 and 60 days after the disinfestation.

The analysis of soil mycoflora was carried out with the technique of "dilution-suspension" (Waksman, 1927). The species in the soil mycoflora were determined after their cultivation in selective substrates recommended by Tsao (1970), Rapilly (1968), Komada (1975), Bouhot and Rouxel (1971). The quantitative presence of each species is expressed in propagules/g of soil.

RESULTS

Soil layer 0-10 cm

In this soil layer the disinfestation lead to a significant reduction of the whole mycoflora. The analysis 4 days after disinfestation and directly after the raising of the plastic cover showed a reduction of the whole mycoflora by 95,02 to 95,14% (Tabl. 1, 2). From the 105 species participating in the whole mycoflora, only 9 were isolated in the first analysis: *Aspergillus alutaceus, Paecilomyces lilacinus, Penicillium chrysogenum, P. funiculosum, P. nigricans, P. herquei, Trichoderma harzianum, T. viride* and *Verticillium dahliae.* The frequency of isolation fluctuates from 6 to 100% (Tabl. 3).

The pathogenic mycoflora of tomato which before disinfestation included parasites of the above-ground part *(Alternaria chlamydospora, Fulvia fulva, Phytophthora infestans, Pleospora herbarum, Ulocladium chartarum*), of the above ground part and the crown (*Alternaria alternata, A. solani, Botrytis cinerea, Phoma lycopersici, Phytophthora nicotiana* var. *parasitica, Sclerotinia sclerotiorum*) and of the underground part (*Colletotrichum coccodes, Fusarium moniliforme, F. oxysporum* f.sp. *lycopersici, F.o.* f.sp. *radicis-lycopersici, F. solani, Pyrenochaeta lycopersici, Pythium debaryanum, P. ultimum, Rhizoctonia solani, V. dahliae*) is represented only by *V. dahliae* (Tabl. 4, 5, 6, 7).

Eight days after the first sample taking, the soil mycoflora is represented by 14 new species. Its population with 1290 propagules/g of soil presents an increase by 230,77% compared to the first analysis. Isolated pathogens are the *Fusarium oxysporum* f.sp. *radicis-lycopersici* and *V. dahliae.*

One month after the disinfestation the whole mycoflora consists of 37 species with 3000 propagules/g of soil. Except the species already existing, the analysis isolated also the pathogens *Alternaria alternata,A. chlamydospora, A. solani, Botrytis cinerea, Colletotrichum coccodes, Phoma lycopersici, Pythium debaryanum* and *Rhizoctonia solani.* The dominant species is *Fusarium oxysporum* f.sp. *radicis-lycopersici* with 18 propagules/g of soil.

In the fourth analysis 60 days after disinfestation, the total mycoflora covered 62,90-65,06% of the control mycoflora with 43 species. The isolated new pathogens are: *Fusarium oxysporum* f.sp. *radicis-lycopersici* and *Pythium ultimum.* The fungi *F. o.* f.sp. *radicis-lycopersici* and *Alternaria alternata* are the dominant species. The total pathogen population in the mycoflora includes the 35, 65-38, 95% of the control soil with 113 propagules/g of soil.

Soil layer 11-20 cm

In this layer, the reduction of the whole mycoflora in the disinfected soil amounts to 97.70% in the first analysis, compared with the control soil. From the 107 species of the total mycoflora in this layer, the analysis 4 days after disinfestation showed the same fungus species as in the previous case (0-10cm) with *Verticillium dahliae* as pathogen representant. In the second analysis, 23 fungus species are determined. The population amounts to 760 propagules/g of soil. The pathogen fungus *Verticillium dahliae* doubled its inoculum (4 propagules/g of soil).

One month after disinfestation the total mycoflora increased its population to 2320 propagules/g of soil. Thirty one species were determined. Pathogen mycoflora, apart from *Verticillium dahliae* includes *Alternaria chlamydospora, Fusarium solani* and *Pythium debaryanum.*

In the fourth analysis, one month after the third, the whole mycoflora with 43 species reached 62,90-65,06% of the control soil. New pathogens are added like: *Fusarium oxysporum* f.sp. *lycopersici, Pyrenochaeta lycopersici, Pythium ultimum, Phoma lycopersici.* The pathogen population amounts already to 17, 1-17, 42% of the control soil

Soil layer 21-30 cm

The first analysis in this layer isolated only 7 species from the 89 existing in the total mycoflora of the control soil. Furthermore, a reduction in inoculum up to 96,90% can be observed. The only pathogen isolated is *V. dahliae.* In the second analysis, 12 days after disinfestation, 11 species with 380 propagules/g of soil were determined. Moreover, the pathogen *F. oxysporum* f.sp. *lycopersici* was isolated.

One month after disinfestation, the total mycoflora increased qualitatively and quantitatively. It included already 24 fungus species with a population of 1010 propagules/g of soil. For the first time, the following pathogens appeared: *Colletotrichum coccodes, Fusarium moniliforme, F. oxysporum* f.sp. *radicis-lycopersici, F. solani, Pyrenochaeta lycopersici.*

The fourth analysis indicated a continuous increase of the population and the species of the total mycoflora. 31 species were isolated. Their population reached 56, 53-59,62% of the control soil. Concerning the pathogen mycoflora there are 10 species and a quantity of 45 propagules/g of soil which corresponds to 56.25-57, 69% of the control soil.

Soil layer 31-40cm

None of the analyses in this soil layer showed a statistically significant differentiation in quality and quantity of the total and pathogenic mycoflora of disinfected soil compared with the control soil.

Table 1. Quantitative (propagules/g of soil), evolution of the total mycoflora in soil disinfected with methyl bromide + chloropicrin (a = control soil, b = disinfected soil).

Depth	0		4		12		30		60	
	days after the soil disinfestation									
in cm	a	b	a	b	a	b	a	b	a	b
0–10	7825	7817	7840	390	7855	1290	7890	3000	7990	4910
11–20	6940	6932	6960	160	6955	760	7085	2320	7170	4910
21–30	3225	3237	3250	100	3285	380	3325	1010	3414	1930
31–40	1625	1631	1694	1635	1725	1678	1745	1703	1750	1721

Table 2. Qualitative (number of species) evolution of the total mycoflora in soil disinfected with methyl bromide + chloropicrin (a = control soil, b = disinfected soil).

Depth	0		4		12		30		60	
	days after the soil disinfestation									
in cm	a	b	a	b	a	b	a	b	a	b
0–10	105	105	105	9	105	23	105	37	105	50
11–20	107	107	106	9	107	23	107	31	107	43
21–30	89	89	89	7	89	11	89	24	89	31
31–40	62	62	62	62	62	62	62	62	62	62

Table 3. Frequency of fungi isolation in soil disinfected with methyl bromide + chloropicrin directly after raising of plastic cover.

Species	0–10	11–20	21–30	31–40
	Frequency %			
Aspergillus alutaceus	00–05	31–50	06–15	00–05
Paecilomuyces lilacinus	81–100	81–100	81–100	51–80
Penicillium chrysogenum	51–80	31–50	31–50	16–30
Penicilllium funiculosum	51–80	51–80	51–80	16–30
Penicillium herquei	81–100	81–100	81–100	31–50
Penicillium nigricans	81–100	51–80	00–05	00–05
Trichoderma harzianum	81–100	81–100	81–100	81–100
Trichoderma viride	81–100	81–100	81–100	51–80
Verticillium dahliae	06–15	16–30	16–30	06–15

Table 4. Quantitative (propagules/g of soil) evolution of the pathogen mycoflora of tomato in soil disinfected with methyl bromide + chloropicrin. (a = control soil, b = disinfected soil).

Depth	0		4		12		30		60	
	days after the soil disinfestation									
in cm	a	b	a	b	a	b	a	b	a	b
0–10	303	294	307	1	277	6	326	56	317	113
11–20	158	147	149	2	156	4	158	11	155	27
21–30	78	78	69	2	78	6	83	20	80	45
31–40	45	42	42	37	42	38	43	37	46	40

Table 5: Quantitative (propagules/g of soil), evolution of the pathogens in the above ground and underground part of greenhouse tomato in soil disinfected with methyl bromide + chloropicrin.
(a=control soil, b= disinfected soil; 0, 4, 12, 30, 60 = 0, 4, 12, 30, 60 days after disinfestation).

SPECIES		0 - 10					11-20					21-30					31-40 cm				
		0	4	12	30	60	0	4	12	30	60	0	4	12	30	60	0	4	12	30	60
Alternaria alternata	a	55	50	30	58	60	15	14	16	15	16	5	5	6	5	6	-	-	-	-	-
	b	58	-	-	5	20	13	-	-	-	-	6	-	-	-	-	-	-	-	-	-
Alternaria solani	a	20	25	25	27	26	14	14	14	12	13	5	6	5	6	5	-	-	-	-	-
	b	22	-	-	4	16	12	-	-	-	-	5	-	-	-	-	-	-	-	-	-
Botrytis cinerea	a	40	45	40	45	50	10	10	9	10	9	-	-	-	-	-	-	-	-	-	-
	b	40	-	-	10	10	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phoma lycopersici	a	15	15	14	13	12	5	6	7	6	5	-	-	-	-	-	-	-	-	-	-
	b	14	-	-	3	4	4	-	-	-	2	-	-	-	-	-	-	-	-	-	-
Phytophthora nicotianae var. parasitica	a	10	10	10	11	10	1	2	1	2	1	-	-	-	-	-	-	-	-	-	-
	b	10	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sclerotinia sclerotiorum	a	8	10	8	10	9	2	2	1	2	1	-	-	-	-	-	-	-	-	-	-
	b	9	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-

- = not isolated

Table 6: Quantitative (propagules/g of soil), evolution of the pathogens in the above ground part of greenhouse tomato in soil disinfected with methyl bromide + chloropicrin.
(a=control soil, b= disinfected soil; 0, 4, 12, 30, 60 = 0, 4, 12, 30, 60 days after disinfestation).

SPECIES		0 -10					11-20					21-30					31-40 cm				
		0	4	12	30	60	0	4	12	30	60	0	4	12	30	60	0	4	12	30	60
Alternaria chlamydospora	a	10	8	9	10	8	4	3	4	3	4	-	-	-	-	-	-	-	-	-	-
	b	9	-	-	1	2	4	-	-	1	1	-	-	-	-	-	-	-	-	-	-
Fulvia fulva	a	20	22	24	23	25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	b	18	-	-	5	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phytophthora infestans	a	15	13	12	14	10	2	3	2	3	2	-	-	-	-	-	-	-	-	-	-
	b	14	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pleospora herbarum	a	8	7	6	8	7	2	1	2	2	3	-	-	-	-	-	-	-	-	-	-
	b	7	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ulocladium chartarum	a	10	8	8	9	7	3	2	3	4	3	-	-	-	-	-	-	-	-	-	-
	b	9	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-

- = not isolated

Table 7: Quantitative (propagules/g of soil), evolution of the pathogens in the above underground part of greenhouse tomato in soil disinfected with methyl bromide + chloropicrin.
(a=control soil, b= disinfected soil; 0, 4, 12, 30, 60 = 0, 4, 12, 30, 60 days after disinfestation).

SPECIES		0 - 10					11-20					21-30					31-40 cm				
		0	4	12	30	60	0	4	12	30	60	0	4	12	30	60	0	4	12	30	60
Colletotrichum coccodes	a	10	15	13	12	14	6	5	4	6	3	5	5	4	6	5	4	5	6	5	5
	b	10	-	-	2	4	5	-	-	-	-	5	-	-	3	4	4	4	5	4	5
Fusarium moniliforme	a	3	4	4	5	4	8	7	8	8	6	5	6	5	6	5	5	4	4	4	4
	b	3	-	-	-	-	7	-	-	-	-	4	-	-	2	3	4	3	4	3	4
Fusarium oxysporum	a	15	13	12	15	13	20	18	19	18	20	10	12	13	14	13	8	7	8	9	8
f.sp. lycopersici	b	13	-	-	-	4	18	-	-	-	2	8	-	2	4	6	8	6	7	7	6
Fusarium oxysporum	a	25	26	25	27	25	10	8	10	12	10	8	7	8	9	10	8	6	5	6	7
f.sp. radicis-lycopersici	b	24	-	5	18	23	9	-	-	-	-	7	-	-	2	6	7	6	5	5	7
Fusarium solani	a	12	10	9	10	10	18	16	17	15	17	8	8	7	7	7	6	5	7	6	6
	b	10	-	-	4	6	15	-	-	4	8	7	-	-	2	4	5	5	6	6	5
Pyrenochaeta lycopersici	a	-	-	-	-	-	15	14	13	14	13	5	5	4	4	4	5	6	4	5	6
	b	-	-	-	-	-	15	-	-	-	3	4	-	-	1	2	5	5	4	5	5
Pythium debaryanum	a	10	10	11	12	11	5	4	6	5	6	2	3	2	3	2	-	-	-	-	-
	b	9	-	-	2	6	5	-	-	1	4	2	-	-	-	1	-	-	-	-	-
Pythium ultimum	a	7	8	7	6	7	2	3	3	2	2	2	3	2	1	1	-	-	-	-	-
	b	6	-	-	-	2	2	-	-	-	1	2	-	-	-	10	-	-	-	-	-
Rhizoctonia solani	a	5	4	5	5	4	4	5	3	4	5	3	2	3	2	2	3	2	3	2	3
	b	4	-	-	-	-	4	-	-	-	-	2	-	-	-	1	3	2	2	2	3
Verticillium dahliae	a	5	4	5	6	5	12	12	14	15	16	20	18	19	20	20	6	7	5	6	7
	b	5	1	1	2	2	13	2	4	5	6	21	2	4	6	8	6	6	5	5	5

- = not isolated

DISCUSSION

Soil disinfestation of greenhouse tomato with methyl bromide + chloropicrin causes profound disturbance in the total mycoflora till a soil depth of 30cm. In detail, 4 days after disinfestation and directly after the raising of the plastic covers, a great decrease till 95-97,7% in soil-fungus population could be observed. Only 7-9 species were isolated, among them the pathogen *Verticillium dahliae*. The other species found in the first analysis (*Aspergillus alutaceus, Paecilomyces lilacinus, Penicillium chrysogenum, P. funiculosum, P. herquei, P. nigricans, Trichoderma harzianum, T. viride*) are known for their competitivity with many soil tomato pathogens. The presence of these fungi directly after the raising of plastic covers is probably due to their tolerance or resistance to the disinfestant. This conclusion is drawn also by other researchers for different species of the genus *Penicillium* and *Trichoderma* (Messiaen and Lafon, 1970).

The re-colonization of the disinfected soils proceeds with different rates in the three soil layers 0-10, 11-20 and 21-30cm. The lowest rate can be observed in the layer 11-20cm. This is due to the fact that the surface and the lower layer of 21-30cm are subject to the inoculum that comes from the atmosphere and from the deeper soil layer 31-40cm.

The contamination after disinfestation is probably caused by transport of inoculum by air, irrigation water, people, cultivation tools and by the soil layer 31-40cm which is not influenced by the disinfestant and may re-supply the above layers with inoculum.

In general, the re-colonization of the disinfected soil by saprophytic fungi proceeds with slower rate. Two months after disinfestation, the composition and the density of the total mycoflora is still reduced in species by 60-65% and in population by 37-40%, compared with the primary mycoflora. However, some pathogens multiply very quickly in disinfected soil (effect "boomerang"). This can be observed for the species *F. oxysporum* f.sp. *radicis-lycopersici, F. solani, Pythium* spp. and *V. dahliae*.

Based on these results it can be concluded that soil disinfestation in greenhouse tomato with methyl bromide and chloropicrin, under certain conditions, does not control satisfactorily certain pathogens like *V. dahliae* and those which contaminate speedily the disinfected soil (*Fusarium oxysporum* f.sp. *radicis-lycopercisi* and *Pythium* spp.). Moreover, the fumigant action does not reach beyond a soil depth of 30 cm, so that inoculum may be transferred from deeper layers under certain cultivation conditions (deep ploughing, soil washing with water for removing bromide salts).

An important research subject remains the creation of desirable composition of antagonistic mycoflora with the stimulation of the tolerant to the soil disinfestant antagonists. Such an orientation could counterbalance the disadvantage of the appearance of pathogens tolerant to methyl bromide and chloropicrin and the danger of the "boomerang" phenomenon.

REFERENCES

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

BOLLEN G.J. (1974). Fungal recolonization of heat-treated glasshouse soil. Agro. Ecosystems, 1, 139-155.

BOURBOS E.A. (1983). Contribution a l' etude de la mycoflore du sol de culture de tomate en serre non chauffee. These Ing. Doct. Univers. Paris VI, 108 p.

BOURBOS E.A. (1986). Etude de l' evolution de la mycoflore du sol de tomate en serre non chauffee apres desinfection. Bulletin OEPP, 16, 321-325.

BOURBOS E.A., SKOUDRIDAKIS M.T. (1989). Sun energy for the control of the soil diseases of the cultivated plants. 2nd European Symposium on soft energy sources and systems at the local level. 16-21 October 1989, Chania, Greece.

BOUHOT D., ROUXEL F. (1971). Technique selective et quantitative d' analyse des *Fusarium oxysporum* et *Fusarium solani* dans le sol. Mode d' emploi. Ann. Phytopathol., 3(2), 251-254.

COLENO A., RAPILLY F., PIONNAT J.C., AUGG C. (1965). Incidence des variations de la microflore d' un sol desinfecte sur la genese de certaines maladies des plantes. I. Variations quantitatives et qualitatives de la microflore d' un sol traite a la vapeur. Ann. Epiphyties, 16 (3), 267-278.

DAVET P. (1981). Effets non intentionnels des traitements fongiques sur les microflores aerienne, tellurique et aquatique. III colloque sur les effets non intentionnels des fongicides, 41-45.

DOMSCH K.H. (1964). Soil fungicides. Ann. Rev. Phytopathol., 2, 293-320.

KATZENELSON H., RICHARDSON L.T. (1943). The microflora of the rhizosphere of tomato plants in relation to soil sterilization. Can. J. Res. C., 21, 249-255.

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

KREUTZER W.A. (1965). The reinfestation of treated soil in ecology of soil borne plant pathogens. prelude to biological control. Univ. of California Press. Berkeley.

MAROIS J.J., MITCHELL D.J. (1979). The relationship of density of chlamydospores of *Fusarium oxysporum* f.sp. *lycopersici* to infection of tomato in fumigated and non fumigated soil. Phytopathology, 69 (9), 1037.

MESSIAEN C.M., LAFON R. (1970). Les maladies des plantes maraicheres. Ed. INRA, 442 p.

MUNNECKE D.E., KOLBEZEN N.M., BRICKER J.L. (1982). Effect of moisture chloropicrin and methyl bromide singly and in mixtures on sclerotia of *Sclerotium rolfsii* and *Verticillium albo-atrum*. Phytopathology, 72, 1235-1278.

RAPILLY F., COLENO A. (1967) Incidenee des variations de la microflore d' un sol desinfecte sur la genese de certaines maladies des plantes. II. Etude du comportement de germes introduits dans un sol desinfecte a la vapeur. Ann. Epiphyties, 18(4), 495-507.

RAPILLY F. (1968). Les techniques de mycologie en pathologie vegetale. Ann. Epiphyties, 19, hors serie, 102 p.

TSAO P.H. (1970). Selective media for isolation of pathogenic fungi. Ann. Rev. Phytopathol., 8, 157-186.

WAKSMAN S.A. (1927). Principles of soil microbiology. Baltimore, Williams et Wilkins Co.

WARCUP J.H. (1951). Effect of partial sterilization by steam or formalin on the fungus flora on old forest nursery soil. Trans. Br. Mycol. Soc., 34, 519-532.