Chemical Alternatives to Methyl Bromide in Spanish Strawberry Nurseries

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ABSTRACT

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Strawberry runners are a high-value cash crop in Spain that requires vigorous transplants free of pathogens. Preplant soil fumigation with methyl bromide, or with mixtures of methyl bromide and chloropicrin, is a standard practice for controlling soilborne diseases. Soil fumigants chloropicrin, 1,3-dichloropropene, dazomet, metam-sodium, metam potassium, and dimethyl disulfide were evaluated in combination with different plastic films as alternatives for methyl bromide soil fumigation of strawberry nurseries. The studies were conducted over a 4-year period, with fumigant applications prior to planting. Verticillium wilt (caused by *Verticillium* spp.) and crown rot (caused by *Phytophthora cactorum*) were the main diseases. Chloropicrin, 1,3-dichloropropene, and dazomet compared well with methyl bromide fumigation for control of strawberry nursery diseases. Furthermore, 1,3-dichloropropene and methyl bromide, applied at 50% rate under virtually impermeable film, provided effective disease control in strawberry nurseries. Fumigant effects on fungal soil populations are discussed.

Additional keywords: soilborne plant pathogens, VIF film

Both fruit and runners of strawberry (*Fragaria × ananassa* Duchesne) are important crops in Spain. In 1998, there were 9,400 ha of fruit production, yielding 312,000 metric tons (t) of strawberry fruit (91% of production was in Huelva, southwestern Spain, where 79% were exported), with a wholesale price of 1,193 euros/t. Overall, the 1998 fruit strawberry production was valued at 373 million euros in Spain (2). Strawberry runners (transplants) are produced in nurseries in Castilla-León in north-central Spain and then are shipped to the fruit-production fields throughout Spain and other countries (especially European Union [EU] countries) each year, where they are transplanted. More than 634 million strawberry runners (80% of which were cv. Camarosa) were produced in the 1999–2000 season on an area of about 1,100 ha, which is the most important runner production area in the EU. The high-elevation nurseries (800 to 1,100 m) are located in sandy, flat areas where plants are grown under continental climatic conditions (hot summers, colder winters, and dry conditions). The cultivation system

is annual, with planting dates between April and May and digging of fresh commercial runner plants throughout October. Of the mother plants for Spanish nurseries, 95% come from commercial nurseries in California.

In Spanish strawberry nurseries, preplant soil fumigation with mixtures of methyl bromide (MB) and chloropicrin (Pic) (MB:Pic, 50:50, at a rate of 40 g/m²) is a standard practice for controlling soilborne plant pathogens such as *Phytophthora cactorum* and *Verticillium* spp., and it is considered to be a critical part of current production practices. All Spanish strawberry growers depend on clean nursery stocks each year because there is a risk that pathogens can be transplanted from nurseries to fruit fields. However, MB is being phased out in the EU and globally because of impact on the ozone layer (1). By the year 2005, the use of MB will be banned in EU countries (3). Strawberry farmers in developed countries such as Spain will be at a competitive disadvantage when competing with products produced in developing countries where MB will be available for several years after it is phased out in the EU. The loss of MB could create potential trade barriers for Spanish runner strawberry plants in the export markets. Without MB or an acceptable alternative, Spain will not be able to export to those markets.

A great number of chemical, cultural, solarization, breeding, and organic alternatives to MB have been evaluated in strawberry fruit production (4,24). However only four fumigants, 1,3-dichloropropene

(1,3-D), iodomethane, metam sodium, and chloropicrin, either alone or combined with other products, have been suggested as short-term chemical alternatives to MB in American strawberry nurseries (10). The objective of this research was to evaluate certain soil fumigants in combination with different plastic films as possible alternatives for MB for controlling soilborne pathogens in Spanish strawberry nurseries.

MATERIALS AND METHODS

Treatments and experimental design.

Experiments were carried out in 1999, 2000, 2001, and 2002, each year in a different experimental field not fumigated with MB for at least 6 years prior to the experiment. In 1999, 2000, and 2001, the fields were located in different nurseries at Navalmanzano (Segovia, Spain), where horticultural crops were grown the year before each experiment. In 2002, the experiment was located at Arévalo (Ávila, Spain), where a cereal was grown the year before. A randomized complete block design with three replications (blocks) was used, and the size of each treatment plot was 400 m² (5.5 m wide by 72 m long). Treatments were applied when soil temperature was at least 7°C, with a soil moisture content of approximately 60%. If necessary, water was applied to the soil before treatments. Treatments were applied on 9 April 1999, 5 April 2000, 30 March 2001, and 4 April 2002 (Table 1). All fumigants except Dazomet were applied at a depth of 20 to 25 cm using eight injection chisels spaced 33 cm apart. Dazomet was applied to the soil surface and incorporated to 14 to 15 cm with a rototiller. Transparent, low-density polyethylene (PE; Reyenvas, Sevilla, Spain) film or co-extruded three-layer virtually impermeable film (VIF; Reyenvas, Sevilla, Spain) was used after fumigations and left in place for at least 14 days. Hand weeding was used to control weeds.

Treatment effects on fungal soil population.

Populations of soil fungi were estimated in each plot before (17 March 1999, 14 March 2000, 28 March 2001, and 25 March 2002) and after (29 April 1999, 28 April 2000, 26 April 2001, and 6 May 2002) treatments as CFU per gram of dry soil to evaluate the effectiveness of the treatments. Twenty samples were taken from the first 15-cm depth of soil in each plot and mixed to make a composite sample. Aliquots (10 g) of the soil mixture were suspended in 90 ml of sterile distilled

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water in 250-ml flasks and shaken for 30 min at 150 rpm; 10- to 100-fold dilutions were made, and 100-µl aliquots from undiluted and diluted suspensions were spread onto petri dishes containing potato dextrose agar amended with streptomycin at 0.5 g/liter (PDA). Three replicate dishes were used for each plot and dilution. Petri dishes were maintained at 20 to 25°C for 5 to 7 days and the fungal colonies were counted (16). Dry weight of soil samples was determined using 5-g subsamples.

In addition, qualitative studies of fungal soil populations were carried out. Fungi on PDA were identified to genus in 2001 and 2002, before and after each treatment, with the aid of a microscope at ×100 to ×400 and using appropriate taxonomic keys (8). In each plate, the number of colonies of each different genus was counted and the frequency of each genus for each treatment was calculated as a percentage using the formula frequency = (number of colonies of a given genus × 100)/(total number of colonies). For each treatment, the similarity coefficient between soil samples before and after application of treatments was calculated (6,14). For each pair of samples being compared, only frequencies of fungal genera present in both samples were

used in the calculation of the similarity coefficient. The similarity coefficient was calculated as

$$\frac{\left(\sum_{i=1}^n W_i\right)}{100}$$

where W_i is the lowest frequency of the i th genus in the two samples, and n = the total number of genera common to both samples. A similarity coefficient of 1.0 indicates that samples had identical compositions, whereas a similarity coefficient of 0 indicates that two samples had no species in common.

Treatment effects on strawberry diseases. Mother strawberry cv. Camarosa plants were obtained from California nurseries and planted on 6 May 1999, 18 April 2000, 7 May 2001, and 9 May 2002. Runner plants were harvested on 14 October 1999, 7 October 2000, 16 October 2001, and 23 October 2002. Three rows of 115 strawberry plants each per row were planted in each plot. In all, 360 additional strawberry mother plants were transferred to humid chambers and the presence or absence of fungal pathogens on the crowns (cut lengthwise and internal tissues examined) and roots of the plants was determined after

7 to 15 days of incubation at 20 to 25°C. Pathogens were identified by observing fungal structures under a light microscope (Zeiss Axioplan; Carl Zeiss, Inc., Oberkochen, Germany). Fungal structures were taken from vegetative tissues, placed on slides, stained with methylene blue, squashed when necessary, and examined microscopically. When necessary, fungi were isolated on PDA. In addition, immature carnation petals were immersed in distilled water together with strawberry crowns and roots to bait Pythiaceae fungi.

The incidence of crown and root diseases in each plot was recorded three times after transplanting throughout the season. Twenty plants (mother and its runners) in the center row of each plot were sampled arbitrarily on 22 July, 29 September, and 14 October 1999; 5 July, 8 September, and 16 October 2000; 3 July, 7 August, and 9 October 2001; and 2 July, 29 August, and 25 September 2002. The plants were examined visually and those with any external disease symptoms (e.g., collapse, wilting, rot, necrosis, or spots) were recorded and taken to the laboratory, where the causal agents were determined as described above for mother plants. The incidence of diseased plants was calculated as

Table 1. Description of treatments applied to strawberry nursery soils prior to planting in 1999, 2000, 2001, and 2002

Treatment	Active ingredient	Application rate	Plastic film ^z	Formulated product, manufacturer
1,3-D:Pic + PE	1,3-dichloropropene:chloropicrin (61:35)	35 cm ³ /m ²	PE	Telopic, Dow Agrosciences Ibérica, Sevilla, Spain
1,3-D:Pic + VIF	1,3-dichloropropene:chloropicrin (61:35)	17.5 cm ³ /m ²	VIF	Telopic, Dow Agrosciences Ibérica, Sevilla, Spain
Pic + PE	chloropicrin (trichloronitromethane)	40g/m ²	PE	Chloropicrin, Atofina Chemicals, Paris, France
Dazomet + PE	Dazomet (tetrahydro-3,5-dimethyl-2H-1,3,5-thiadiazine-2-thione)	50 g/m ²	PE	Basamid Granulado, BASF Española, Barcelona, Spain
Metam sodium + PE	Metam sodium (sodium N-methyldithiocarbamate)	125 cm ³ /m ²	PE	Trimaton 50%, Desarrollo Químico Industrial, Valencia, Spain
Metam potassium + PE	Metam potassium (potassium N-methyldithiocarbamate)	160 cm ³ /m ²	PE	Raisan K-50, Lainco, Barcelona, Spain
DMDS	Dimethyl disulphide	80 g/m ²	PE	DMDS, Atofina Chemicals, Paris, France
MB:Pic (30:70) + VIF	Methyl bromide:chloropicrin (30:70)	20 g/m ²	VIF	Bromothirty, Eurobrom España, Barcelona, Spain
MB:Pic (50:50) + VIF	Methyl bromide:chloropicrin (50:50)	20 g/m ²	VIF	Bromofifty, Eurobrom España, Barcelona, Spain
MB:Pic (50:50) + PE	Methyl bromide:chloropicrin (50:50)	40 g/m ²	PE	Bromofifty, Eurobrom España, Barcelona, Spain
Untreated	...	...	...	...

^z PE = polyethylene and VIF = virtually impermeable film.

Table 2. Fungal populations (CFU × 10³/g dry weight of soil) before and after fumigation treatments in strawberry nursery soils during 4 years^y

Treatment ^z	1999		2000		2001		2002	
	Before	After	Before	After	Before	After	Before	After
1,3-D:Pic + PE	24.4 a	0.2 b	15.7 a	10.6 b	29.9 a	7.7 b	80.4 a	3.9 b
1,3-D:Pic + VIF	...	...	...	...	16.9 a	1.9 b	54.2 a	4.4 b
Pic + PE	27.7 a	1.6 b	41.5 a	6.6 b	...	...	...	...
Dazomet + PE	21.5 a	2.3 b	6.0 a	13.3 b	8.2 a	1.5 b	41.2 a	0.2 c
Metam sodium + PE	...	...	17.4 a	8.7 ab	29.6 a	5.7 b	77.6 a	9.3 b
Metam potassium + PE	...	...	71.1 a	9.3 b	21.7 a	2.9 b	47.0 a	6.2 b
DMDS	...	...	...	...	...	...	44.8 a	40.6 a
MB:Pic (30:70) + VIF	...	...	...	...	...	...	24.3a	1.9 b
MB:Pic (50:50) + VIF	16.5 a	0.4 b	30.2 a	23.4 ab	27.3 a	2.1 b	23.0 a	2.4 b
MB:Pic (50:50) + PE	19.7 a	0.6 b	10.4 a	5.6 b	33.0 a	0.5 b	49.3 a	5.1 b
Untreated	27.7 a	36.6 a	16.1 a	128.1 a	22.7 a	123.8 a	25.1 a	105.5 a
MSE	1.03 × 10 ⁸	(0.28)	(0.13)	(0.24)	2.08 × 10 ⁸	(0.17)	(0.06)	(0.11)

^y Values are means of three replicates. Values of mean square error (MSE) from analysis of variance (in parentheses) were subjected to log₁₀ transformation prior to analysis. Untransformed means were presented but statistical analysis was performed on log-transformed data when necessary for homogeneity of variance. Means followed by the same letter in each column were not significantly different ($P > 0.05$) by the Student-Newman-Keul test; ... = not determined.

^z Details of treatments are described in Table 1.

the percentage of symptomatic plants in the 20 sampled in each plot.

Data analysis. Total fungal populations and percent disease incidence in each year were subjected to a two-way analysis of variance (STATGRAPHICS, Version Plus 4.1; Manugistics, Rockville, MD; 27). Prior to analysis, fungal populations were log₁₀ transformed to improve homogeneity of variances. Sources of variation were treatment and block. When the *F* test was significant at *P* = 0.05, treatment means were compared by the Student-Newman-Keul test (26).

RESULTS

Fungal populations in soil (CFU/g) in experimental fields before treatments were not significantly different (*P* > 0.05) among treatments (Table 2). Most fumigation treatments reduced the soil fungal

population with the exception of metam sodium + PE, and MB:Pic (50:50) + VIF in 2000; and dimethyl disulfide (DMDS) in 2002 (Table 2). No differences were observed among plots covered with the different plastic films (Table 2).

Frequencies of fungal genera isolated before applications with MB:Pic (50:50) under PE or VIF were different from those isolated after treatment in both 2001 and 2002 (similarity coefficients < 0.5; Table 3). Differences in frequencies of fungal genera isolated before and after treatments also were observed with 1,3-D:Pic (61:35) under VIF and metam potassium applications in 2001 and 1,3-D:Pic application under PE in 2002. Frequencies of genera of fungi isolated from nonfumigated plots on the first soil sampling date (before treatment application to other plots) were

similar to those isolated on the second soil sampling date (after treatment application to other plots; Table 3). The most common genus of fungi isolated from nonfumigated plots was *Penicillium*, followed by *Alternaria*, *Fusarium*, *Mortierella*, and *Cladosporium* (Table 4). However, in those plots where similarity coefficients were <0.5 (frequencies of fungal genera were different before and after treatments), an increase in frequency of *Trichoderma* spp. and a reduction in the frequency of *Penicillium* spp., *Mortierella* spp., or *Alternaria* spp. were observed.

Analysis of mother plants from California showed infections by fungal pathogens such as *Phytophthora cactorum*, *Botrytis cinerea*, *Fusarium* spp., *Pythium* spp., *Verticillium* spp., and *Colletotrichum* spp. (Table 5). *Phytophthora cactorum* was the most prevalent; this pathogen was detected each year of the study on 1.9 to 6.4% of transplants. *B. cinerea* was detected in 1999, 2000, and 2001 on 0.9 to 2.7% of transplants, and the other fungi occasionally. In 2000 and 2001, 2% of transplants were freeze damaged.

With the exception of metam sodium and metam potassium in 2001, all fumigant treatments reduced incidence of disease in runner plants harvested in 1999, 2000, and 2001 (Table 6). In 2002, all treatments, including the untreated control, showed little disease development. In 1999 and 2001, infections were caused mostly by *P. cactorum*, and by *Verticillium* sp. in 2000 (Table 6).

DISCUSSION

Preplant soil fumigation of strawberry nurseries with 1,3-D:Pic, Pic alone, or

Table 3. Similarity coefficients between soil fungal populations isolated before and after fumigation treatments in 2001 and 2002^y

Treatment ^z	2001	2002
1,3-D:Pic + PE	0.720	0.245
1,3-D:Pic+ VIF	0.410	0.665
Pic + PE	...	...
Dazomet + PE	0.545	0.510
Metam sodium + PE	0.765	0.655
Metam potassium + PE	0.440	0.585
DMDS	...	0.520
MB:Pic (30:70) + VIF	...	0.475
MB:Pic (50:50) + VIF	0.390	0.455
MB:Pic (50:50) + PE	0.230	0.435
Untreated	0.620	0.840

^y Similarity coefficient = $\left(\frac{\sum W_i}{n}\right) / 100$, where W_i is the lowest frequency of the *i*th genus in the two

samples, and *n* = the total number of genera common to both samples. Frequency = (number of colonies of a given genus × 100)/total number of colonies; ... = not determined.

^z Details of treatments are described in Table 1.

Table 4. Frequency (%) of *Penicillium* spp. (P), *Alternaria* spp. (A), *Fusarium* spp. (F), *Cladosporium* spp. (C), *Mortierella* spp. (M), and *Trichoderma* spp. (T) isolated from strawberry nursery soils before and after fumigation treatments in 2001 and 2002^y

Treatment ^z	Before treatment						After treatment					
	P	A	F	C	M	T	P	A	F	C	M	T
2001												
1,3-D:Pic + PE	56	12	10	7	5	1	84	0	0	0	0	16
1,3-D:Pic+ VIF	62	9	7	3	4	8	36	0	3	0	0	60
Dazomet + PE	32	14	13	5	15	5	57	8	0	0	0	17
Metam sodium + PE	63	11	8	4	5	5	74	1	6	6	1	12
Metam potassium + PE	55	13	10	6	7	8	45	2	5	1	0	44
DMDS	...	...	...	...	...	...	...	...	...	...	...	...
MB:Pic (30:70) + VIF	...	...	...	...	...	...	...	...	...	...	...	...
MB:Pic (50:50) + VIF	54	16	10	4	7	5	42	0	0	0	0	53
MB:Pic (50:50) + PE	73	4	7	0	3	12	15	0	0	2	0	81
Untreated	61	4	7	10	11	0	78	5	5	7	1	3
2002												
1,3-D:Pic + PE	39	26	14	0	19	0	37	3	10	6	0	43
1,3-D:Pic + VIF	39	19	16	5	18	1	31	30	12	8	3	13
Dazomet + PE	36	25	17	1	19	1	50	26	12	0	0	12
Metam sodium + PE	43	23	13	2	15	4	30	22	34	0	8	6
Metam potassium + PE	31	31	14	6	13	5	27	39	19	2	3	5
DMDS	38	23	15	3	16	3	66	9	17	0	2	5
MB:Pic (30:70) + VIF	31	37	11	4	15	1	48	9	6	8	3	24
MB:Pic (50:50) + VIF	32	29	19	5	9	1	69	6	2	2	0	19
MB:Pic (50:50) + PE	53	24	16	1	5	0	34	6	9	5	6	38
Untreated	21	34	23	1	17	2	26	43	20	5	3	2

^y Values are the means of three replicates; ... = not determined.

^z Details of treatments are described in Table 1.

dazomet resulted in significant reduction of disease incidence, similar to that obtained with MB:Pic, whereas metam sodium and metam potassium only reduced disease incidence for 1 year. Nevertheless, all fumigation treatments effectively reduced populations of soil fungi (Table 2). Duniway (10) reported that MB:Pic, 1,3-D:Pic (65:35), and Pic alone appeared to be effective in reducing soil populations of *V. dahliae* in strawberry nurseries, especially when fumigants were applied in the fall before a spring planting. Furthermore, Lopez-Aranda et al. (18) showed that fumigations with 1,3-D:Pic resulted in the same runner plant production as MB:Pic in Spanish nurseries. Experiments to identify alternatives to MB in terms of strawberry fruit production showed that fumigation with 1,3-D:Pic (61:35%), Pic alone, or dazomet resulted in yields similar to those obtained with MB:Pic (5,18). 1,3-D initially was developed as a nematicide, but its known spectrum of activity includes certain plant-pathogenic fungi and bacteria (9). Chloropicrin was first used for strawberry crops in California to control *Verticillium* wilt and other fungal diseases; however, it is less nematocidal than MB or 1,3-D and is less active on dormant weeds and seed in soil (29). More recent trials of chloropicrin (336 kg/ha) as a stand-alone soil fumigant for strawberry production in California showed that it can be as effective

as a standard mixture of MB:Pic (9). Different results were obtained after application of dazomet and metam sodium, although both of them are methyl isothiocyanate generators. The lack of efficacy of metam sodium in some years could be due to problems of distribution in soil after application because of conditions of soil temperature and moisture (7,17,30). Dazomet, formulated as a granular product, is used effectively for control of soilborne pests and weeds in tree nurseries (11); although optimum soil moisture for full activation following application, without residual phytotoxicity, is difficult to achieve (9).

Although 1,3-D and chloropicrin are registered and available for use as soil fumigants in Spain, there is resistance by regulators in some countries to the use of relatively high rates (4,9). However, it is possible to use 1,3-D at one-half the recommended dosage under VIF film to achieve effective disease control in strawberry nurseries. This VIF benefit has been demonstrated for MB:Pic by other authors (12,13,15,20). The PE films used commonly for MB fumigation provide a poor barrier, and enable the escape of fumigant to the atmosphere during the fumigation process, whereas VIF films retain fumigants in soil for longer periods (13). VIF films are more expensive than PE, but the subsequent fumigant dosage reduction

may compensate for the additional cost (20).

Diseases caused by soilborne plant pathogens such as *P. cactorum* and *Verticillium* spp. cause serious losses of strawberry crops in Spanish nurseries without preplant soil fumigation, especially in fields with a history of horticultural crops (21). In this study, we observed significant numbers of diseased runner plants in 1999, 2000, and 2001 in soils previously planted in horticultural crops. These runner plants were infected with *P. cactorum* and *Verticillium* spp. However, fewer diseased runner plants were recorded in 2002, in a field where cereal crops previously were grown. Although we detected some level of *P. cactorum* or *Verticillium* spp. on mother plants, there was no relationship between these frequencies and the disease incidence of runner plants each year. For example, *Verticillium* spp. were isolated from mother plants only in the year 2002 and yet none of the diseased runner plants were infected by *Verticillium* spp. These results suggest that disease was not initiated by infected transplants. There could be other inoculum sources, such as pathogens in soils or vegetative host tissues. *Verticillium* spp. have a wide host range that includes tomato, potato, chrysanthemum, and various weeds (23), and their microsclerotia can survive in the soil for many years; therefore, rotation is less effective for control. *P. cactorum* can also survive in host-free soil for 2 years (8). Therefore, it is necessary to fumigate the soil in which the runner plants are to be cultivated. *Verticillium* wilt also caused devastating losses in California strawberry fields before the development of chloropicrin and MB soil fumigation as a standard practice in the early 1960s (23,28).

Although only 1,3-D:Pic, Pic alone, or dazomet resulted in significant reduction of disease incidence caused by *Verticillium* spp. and *P. cactorum* every year, similar to that obtained with MB:Pic, most of the

Table 5. Percentages of strawberry mother plants (cv. Camarosa) obtained from California nurseries in 1999, 2000, 2001, and 2002 from which specific fungi were isolated or on which freeze damage was detected

Pathogen or disorder	Plants (%)			
	1999	2000	2001	2002
<i>Phytophthora cactorum</i>	6.48	7.00	1.94	3.83
<i>Botrytis cinerea</i>	1.94	0.90	2.70	0.00
<i>Fusarium</i> sp.	0.00	3.00	0.50	0.00
<i>Pythium</i> sp.	2.51	0.00	0.00	0.00
<i>Verticillium</i> sp.	0.00	0.00	0.00	0.16
<i>Colletotrichum</i> sp.	0.00	0.00	0.00	0.16
Freeze damage	0.00	2.00	2.00	0.00

Table 6. Percentage of strawberry runner plants infected with *Verticillium* sp. (VER), *Phytophthora cactorum* (PC), or other unidentified crown or root rot pathogens (O)^y

Treatment ^z	1999			2000			2001			2002		
	O	VER	PC	O	VER	PC	O	VER	PC	O	VER	PC
1,3-D:Pic + PE	0.0 a	0.0 b	0.0 b	1.7 a	1.7 b	0.0 b	1.7 a	0.0 a	3.3 b	0.0 a	0.0	0.0 a
1,3-D:Pic + VIF	...	...	...	...	...	...	1.7 a	0.0 a	6.7 b	0.0 a	0.0	1.7 a
Pic + PE	0.0 a	0.0 b	0.0 b	0.0 a	0.0 b	0.0 b	...	...	...	...	...	...
Dazomet + PE	0.0 a	0.0 b	0.0 b	1.7 a	3.3 b	3.3 b	7.0 a	0.0 a	8.2 b	0.0 a	0.0	0.0 a
Metam sodium + PE	...	...	...	1.7 a	5.0 b	0.0 b	4.4 a	0.0 a	24.5 ab	1.7 a	0.0	3.3 a
Metam potassium + PE	...	...	...	0.0 a	1.7 b	1.7 b	5.9 a	1.8 a	28.1 ab	1.7 a	0.0	1.7 a
DMDS	...	...	...	...	...	...	...	...	...	5.0 a	0.0	0.0 a
MB:Pic (30:70) + VIF	...	...	...	...	...	...	...	...	...	0.0 a	0.0	0.0 a
MB:Pic (50:50) + VIF	0.0 a	0.0 b	0.0 b	0.0 a	0.0 b	0.0 b	0.0 a	0.0 a	3.3 b	1.7 a	0.0	1.7 a
MB:Pic (50:50) + PE	0.0 a	0.0 b	0.0 b	0.0 a	0.0 b	0.0 b	1.7 a	1.7 a	3.3 b	0.0 a	0.0	0.0 a
Untreated	1.7 a	11.7 a	3.3 a	3.3 a	45.0 a	13.3 a	0.0 a	7.7 a	40.1 a	0.0 a	0.0	0.0 a
MSE	1.4	18.1	1.3	7.3	25.0	6.2	27.2	11.0	165.1	6.7	0.0	3.3

^y Values are means of three plots or replicates. Twenty plants (mother + its runners) in the center row of each plot were arbitrarily sampled and examined visually. From these plants those with any disease external symptoms (e.g., collapse, wilting, rot, necrosis, spots) were recorded and taken to the laboratory, where the causal agents were determined. Means followed by the same letter in each column is not significantly different ($P > 0.05$) by the Student-Newman-Keul test; ... = not determined. MSE = mean square error from analysis of variance.

^z Details of treatments are described in Table 1.

fumigation treatments, except DMDS, had significant effects on total fungal populations. *Penicillium* spp., *Alternaria* spp., and *Mortierella* spp. were the most numerous fungi isolated in PDA and reductions of more than 80% of their populations were observed. However, only MB:Pic (similarity coefficient <0.5) qualitatively changed the soil fungal population: *Trichoderma* spp. increased in soils after treatment and *Penicillium* spp., *Alternaria* spp., and *Mortierella* spp. decreased. Apart from *Verticillium* spp. and *Phytophthora* spp. or other soil fungal pathogens, a number of other soil-inhabiting fungi also can contribute to significant losses when strawberry is grown in nonfumigated fields (19). Russell (25) suggested that the increase in strawberry yields in MB-fumigated soils probably was due more to reduced populations of soil microorganisms, which were widespread in cultivated soils (where they inhibited plant growth and reduced yields without inducing any visible symptoms), than to an effect of soil nutrient content. Speculating further, because *Trichoderma* spp. are well known antagonistic fungi (22), their presence in soil could contribute to a beneficial effect in health of plants.

Strawberry production in Spain depends on preplant soil fumigation in a sequence of nursery propagation steps. The use of VIF films with reduced dosages of MB will help Spanish strawberry nurseries to conform to Montreal Protocol requirements in the short term, but reliable and long-term solutions such as 1,3-D:Pic, Pic, or dazomet under VIF films are possible. Although VIF films are costly, the increase in costs is offset by the use of reduced doses of fumigants.

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