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Relationship between *Trichoderma* Soil Populations and Strawberry Fruit Production in Previously Fumigated Soils

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Abstract. **Several preplant soil fumigation treatments were repeated over a period of three years on strawberry (*Fragaria* ×*ananassa* Duchesne) crops, at two different places in the province of Huelva (southwestern Spain). The influence of these treatments on *Trichoderma* soil populations and on populations of soilborne pathogens was examined every year by isolating soil onto selective media. No strawberry pathogens were detected but *Trichoderma* soil populations increased each year after the treatment. Significant differences were noted between the treatments and also compared to the control. The largest populations were observed after treatments with methyl bromide and chloropicrin, and so resulting in a higher production. Chemical name used: trichloronitromethane (chloropicrin).**

More than half of the strawberry acreage in Spain is situated in Huelva (southwestern Spain) (Hancock, 1999). The majority of the hectarage is under small polyethylene film tunnels, which allow early production of fresh-market fruit. Plants are grown in intensively managed annual systems on raised beds with pre-plant fumigation (López-Aranda and Bartual, 1999). Broad-spectrum fumigants such as methyl bromide (MB) and chloropicrin are used routinely. Due to the use of MB for many years, the soil in Huelva contains low levels of soil pathogens and crop yields are better from fumigated soils than from non-fumigated soils. Fumigation of the soil affects the environment and produces variations in *Trichoderma* populations since these fungi can reproduce rapidly after MB treatments (Munnecke et al., 1981). Species of *Trichoderma* are primarily studied for their ability to control plant disease through mycoparasitism, the production of antimicrobial compounds, or both (Bailey and Lumsden, 1998). These species could affect plant growth positively (Lindsey and Barker, 1967; Wright, 1956). Determination of these effects depends on many interactions that take place in the soil between *Trichoderma* spp., other microorganisms, changes in the soil environment, and the plant root (Bailey and Lumsden, 1998). The aim of this report is to study the effects of repeated fumigation treatments on crop yields and *Trichoderma* populations over a period of 3 years.

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Materials and Methods

Field trials were carried out at two sites in Huelva, Spain: Moguer (east coast) and Cartaya (west coast), for three consecutive years (1998–1999, 1999–2000, and 2000–2001). Strawberries were grown in Cartaya on sandy soil fumigated with MB for the 5 years previous to the start of the trials. The sandy soil at Moguer had not been previously fumigated and strawberry had been grown there only one year previous to the start of the trials. Both experiments were repeated three years running on the same plot, in randomized complete block with three replicates. Each year in the last week of September the soil was fumigated (Table 1). Each year in the last week of October strawberry plants (cv. Camarosa) were planted. Conventional practices for annual strawberry production were followed. Plants were grown on raised beds with black plastic mulch under small polyethylene film tunnels (López-Aranda and Bartual, 1999).

Each year, 10 transplants per plot were analyzed to detect infections. Crowns and 5-cm-long roots tissues were surface disinfected and plated on potato dextrose agar. Plates were placed at 25 °C for 7 d in the dark.

Soil samples (five per plot) were taken 30 days after fumigation, each year. Samples were taken between two plants on the same row by a vertical calibrated drill (0–20 cm depth). Soil samples were air-dried and processed as follows.

One gram of soil per sample was suspended in 99 mL of water agar (0.3%). Aliquots of 1 mL were spread on petri dishes with semi-selective media: P_5ARP(H) to determine the presence of *Phytophthora* and *Pythium* spp. (Jeffers and Martin, 1986); and KO for *Rhizoctonia* spp. (Ko and Hora, 1971).

The *Trichoderma* isolations were carried out as follows. Ten grams of soil per sample were suspended in 90 mL of water agar (0.3%), shaken for 15 min, and 0.1-mL aliquots were spread on petri dishes with *Trichoderma* Selective Medium (TSM) with a glass rod (Elad et al., 1981). Plates were placed at 25 °C in the dark for 7 d. Analysis of variance (Statistix 7, Analytical Software for Windows) was performed for *Trichoderma* colony-forming units (cfu). Mean separation was conducted using the Newman and Keuls test at $P < 0.05$. The data were square root (cfu + 0.5)-transformed.

Based on morphological characteristics *Trichoderma* species were identified (Gams and Bissett, 1998).

Fruits were picked for the fresh market from the beginning of February until May. Fruit yield was determined as tons per hectare. Analysis of variance (Statistix 7, Analytical Software for Windows) was performed for crop yield, followed by a Newman–Keuls test at $P < 0.05$.

The correlation between *Trichoderma* soil population and fruit production was determined using Kendall and Spearman rank correlation coefficients (Statistix 7, Analytical Software for Windows).

Results and Discussion

Results of the analyses of variance (ANOVAs) on fruit yield from studying the effects of treatment, site, and [treatment × site] interaction showed that no significant experimental effects were found. The effects of site and

Table 1. Fumigant treatments in field experiments.

Treatments	Description
Control	No fumigation
MB-VIF	Methyl bromide/Chloropicrin (50:50) dose 20 g·m^{-2} bed fumigation, black VIF (virtually impermeable film, 40 μm thickness)
MB	Methyl bromide/Chloropicrin (50:50) dose 40 g·m^{-2}, preformed beds (black polyethylene, 35 μm thickness)
Telone	1,3-Dichloropropene/Chloropicrin (61:35); dose 40 cc/m^2, preformed beds (black polyethylene, 35 μm thickness)
Metham-S	Metham Sodium; 1998 dose 125 cc/m^2. 1999 and 2000 dose 175 cc/m^2. Applied under preformed beds (black polyethylene, 35 μm thickness)
Dazomet	Tetrahydro-3, 5-dimethyl-2*H*-1,3,5-tiadizin-2-tione; dose 50 g·m^{-2}. 1998 and 1999 applied broadcast. 2000 applied under preformed beds (black polyethylene, 35 μm thickness)
Chloropicrin	Trichloronitromethane, Chloropicrin; dose 40 g·m^{-2}. 1998 applied broadcast. 1999 and 2000 under preformed beds (black polyethylene, 35 μm thickness)

treatment were significant while the effect of [treatment × site] interaction was not significant in any of the three years (Table 2). Results of the analyses of variance on *Trichoderma* soil population showed that the effect of the site was not significant, however a significant effect of the treatment was observed each year (Table 3). Since the effect of [treatment × site] interaction was not significant in any case, we carried out an ANOVA for both sites, followed by a Newman–Keuls test in order to rank the treatments according to their effectiveness. Results are shown in Table 4.

In general, the highest crop yields were obtained after fumigation with MB, Telone or Chloropicrin, with significant differences to the control. Crop yields were also at their highest after three applications of the above mentioned fumigants. In treatments with MB, no significant differences were found when comparing the use of virtually impermeable film with the commonly black polyethylene film used by growers in this area. In contrast crop yields were significant lower from plots treated with Metham-S and Dazomet.

Strawberry pathogens were not detected in soil or tissue samples. *Aspergillus*, *Penicillium*, and low levels of *Pythium* spp. were isolated from soil samples on semi-selective media. In the absence of strawberry pathogens, the effect of various fumigation treatments on crop yield (Table 4) may be attributed to the involvement of other soil microorganisms. Yuen et al. (1991) reported the lack of efficacy of Metham-S, whereas MB increased yields in soils with low levels of pathogens.

In 1998–99, the number of *Trichoderma* spp. propagules was the lowest, without significant differences between the control and the other treatments. After one crop and two fumigation treatments, soils treated with MB or Telone showed a considerable increase in *Trichoderma* spp. populations, with significant differences in all cases between MB as well as Telone and the untreated control. There were no significant differences when using different doses of MB. *Trichoderma* populations were even higher after the third application of soil fumigant.

Soil fumigations with Metham-S and Dazomet reduced *Trichoderma* populations to very low levels. Metham-S and Dazomet both generate methyl isothiocyanate in soil (Szczygiel, 2002). The results suggest that this compound could be responsible for the decrease in *Trichoderma* populations observed after these treatments.

Three species of *Trichoderma* were detected at both locations: *T. inhamatum*, *T. harzianum*, and *T. viride*.

Spearman correlation coefficients for the relationship between *Trichoderma* populations and fruit yield are shown in Table 5. In the first year there was no significant correlation. However, after the second treatment the correlation became significant. The closest relationships were obtained after 3 years of fumigation, when *Trichoderma* populations were at their highest.

The results indicate a significant correlation between *Trichoderma* populations and fruit

Table 2. Analyses of variance in strawberry yield after soil fumigation at two different sites in Huelva, Spain.

Year	Source of variation	df	MS[z]	*P*
1998/1999	Blocks	2	13094.35	0.1801^{NS}
	Treatment	6	25050.94	0.0002***
	Site	1	144546.45	0.0000***
	Treatment × site	6	5199.77	0.2323^{NS}
	Error	26	3572.79	
	Total	41		
1999/2000	Blocks	2	2803.50	0.2549^{NS}
	Treatment	6	40381.45	0.0000***
	Site	1	1269873.46	0.0000***
	Treatment × site	6	3582.69	0.1298^{NS}
	Error	26	1945.15	
	Total	41		
2000/2001	Blocks	2	1595.07	0.6592^{NS}
	Treatment	6	90531.54	0.0000***
	Site	1	481714.38	0.0000***
	Treatment × site	6	7942.67	0.0866^{NS}
	Error	26	3766.61	
	Total	41		

[z]Mean squares.

$^{NS,\ ***}$Nonsignificant at $P = 0.05$ or significant at $P = 0.001$, respectively.

Table 3. Analyses of variance in *Trichoderma* spp. soil populations after soil fumigation at two different sites in Huelva, Spain.

Year	Source of variation	df	MS[z]	*P*
1998/1999	Blocks	2	25.64	0.8692^{NS}
	Treatment	6	578.56	0.0165*
	Site	1	15.18	0.7737^{NS}
	Treatment × site	6	403.29	0.0697^{NS}
	Error	26	179.00	
	Total	41		
1999/2000	Blocks	2	24.85	0.9799^{NS}
	Treatment	6	15811.97	0.0000***
	Site	1	1391.27	0.2962^{NS}
	Treatment × site	6	497.19	0.8682^{NS}
	Error	26	1224.22	
	Total	41		
2000/2001	Blocks	2	10302.48	0.1852^{NS}
	Treatment	6	38171.31	0.0002***
	Site	1	18121.41	0.0868^{NS}
	Treatment × site	6	6536.79	0.3664^{NS}
	Error	26	5722.54	
	Total	41		

[z]Mean squares.

$^{NS,\ *,\ ***}$Nonsignificant at $P = 0.05$ or significant at $P = 0.05$ or 0.001, respectively.

Table 4. Effects of repeated soil fumigation using different fumigants on *Trichoderma* spp. soil populations and strawberry yield at two sites, in Spain.

	Trichoderma populations cfu/g[z]			Yield t·ha^{-1}[y]		
Treatments	1998/1999	1999/2000	2000/2001	1998/1999	1999/2000	2000/2001
Control	36.6 a	36.6 b	19.8 b	22.1 b	21.4 c	25.9 c
MB-VIF	49.5 a	346.5 a	2668.3 a	32.4 a	34.0 a	40.9 a
MB	42.9 a	351.5 a	2625.5 a	31.3 a	35.0 a	42.2 a
Telone	42.9 a	264.0 a	1188.1 ab	33.5 a	34.7 a	40.7 a
Metham-S	0 a	0 b	0 b	30.5 a	29.2 b	28.5 c
Dazomet	0 a	9.9 b	113.85 b	31.8 a	33.7 a	35.3 b
Chloropicrin	26.4 a	66.0 b	797 b	32.7 a	32.3 ab	40.7 a

[z]*Trichoderma* colony-forming units per gram of soil. Each value is the average of three plots from each of two locations. Treatments followed by the same letter within a column are not significantly different according to the Newman–Keuls test ($P < 0.05$) square root transformed data.

[y]Fruit production. Each value is the average of three plots from each of two locations. Treatments followed by the same letter within a column are not significantly different according to the Newman–Keuls test ($P < 0.05$).

Table 5. Correlation coefficients between the number of colony-forming units of *Trichoderma* spp. in the soil and strawberry fruit yield at two sites in Spain.

	1998/1999		1999/2000		2000/2001	
Location	Spearman coefficient	*P* value	Spearman coefficient	*P* value	Spearman coefficient	*P* value
Cartaya	–0.21	0.3539[NS]	0.46	0.0356[*]	0.79	0.0006[***]
Moguer	0.28	0.2098[NS]	0.44	0.0435[*]	0.72	0.0002[***]

[NS, *, ***]Nonsignificant at $P = 0.05$ or significant at $P = 0.05$ and 0.001, respectively.

yield production after repeated soil fumigation of the same site.

The effect of MB on *Trichoderma* spp. has been reported previously in soils treated to control *Armillaria mellea*. *Trichoderma* spp. were more resistant to the fumigant than *A. mellea*, had few competitors, and could reproduce rapidly. Thus, the antagonist may build up very rapidly in fumigated soils (Munnecke et al., 1981).

Plant growth promotion by species of *Trichoderma* has been reported for many years (Lindsey and Barker, 1967; Wright, 1956). Plant growth promotion was demonstrated in plants grown in semi-sterile solution, suggesting the effect was not entirely associated with suppression of minor pathogen (Bailey and Lumdsen, 1998), which may explain the beneficial effect of MB and other fumigants like Telone on strawberry crop in pathogen-free soils.

The results of this work show a generalized presence of wild *Trichoderma* species in the soil in Huelva where strawberries are grown, even thought the location was not a variable factor. The repeated applications of MB and other fumigants produce a significant increment in this population and in the crop yield. MB will be banned in Spain in the very near future, however the repetitive applications of Telone or chloropicrin will obtain similar yields to those obtained when using MB and it will produce an increment in the native population of *Trichoderma*.

Literature Cited

Bailey, B.A. and R.D. Lumsden. 1998. Direct effects of *Trichoderma* and *Gliocladium* on plant growth and resistance to pathogens, p. 185–204. In: G.E. Harman and C.P. Kubicek (eds.). *Trichoderma* and *Gliocladium*. Vol. 2. T.J. Intl. Ltd. Padstow, U.K.

Elad, Y., I. Chet, and Y. Henis. 1981. A selective medium for improving quantitative isolation of *Trichoderma* spp. from soil. Phytoparasitica 9: 59–67.

Gams, W. and J. Bissett. 1998. Morphology and identification of *Trichoderma*, p. 3–34. In: G.E. Harman and C.P. Kubicek (eds.). *Trichoderma* and *Gliocladium*. Vol. 1. T.J. Intl. Ltd. Padstow, U.K.

Hancock, J.F. 1999. Strawberries. CABI Publishing, New York.

Jeffers, S.N. and S.B. Martin. 1986. Comparison of two media selective for *Phytophthora* and *Pythium* species. Plant Dis. 70:1038–1043.

Ko, W.H. and F.K. Hora. 1971. A selective medium for the quantitative determination of *Rhizoctonia solani* in soil. Phytopathology 61:707.

Lindsey, D.L. and R. Barker. 1967. Effect of certain fungi on dwarf tomatoes grown under gnotobiotic conditions. Phytopathology 57:1262–1263

López-Aranda, J.M. and R. Bartual. 1999. Strawberry production in Spain. Cost Action 836: W.G. 2 and M.C. Meetings. Málaga, Spain.

Munnecke, D.E., M.J. Kolbezen, W.D. Wilbur, and H.D. Ohr. 1981. Interactions involved in controlling *Armillaria mellea*. Plant Dis. 65: 384–389.

Szczygiel, A. 2002. Alternatives to methyl bromide in strawberries in Poland. Proc. Intl. Conf. on Alternatives to Methyl Bromide 1:58–61.

Wright, J.M. 1956. Biological control of a soil-borne Pythium infection by seed inoculation. Plant and Soil 8:132–140.

Yuen, G.Y., M.N. Schroth, A.R. Weinhold, and J.G. Hancock. 1991. Effects of soil fumigation with methyl bromide and chloropicrin on root health and yield of strawberry. Plant Dis. 75: 416–420.