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 Burton, 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. Fumigant action on 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.

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).

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 mm thickness) |
| Telone | 1,3-Dichloropropene/Chloropicrin (61:35); dose 40 ccm/m², preformed beds (black polyethylene, 35 mm thickness) |
| Metham-S | Metham Sodium; 1998 dose 125 ccm/m²; 1999 and 2000 dose 175 ccm/m². Applied under preformed beds (black polyethylene, 35 mm thickness) |
| Dazomet | Tetrahydro-2H-1,3,5-tiadizin-2-tione; dose 50 g·m⁻², 1998 and 1999 applied broadcast. 2000 applied under preformed beds (black polyethylene, 35 mm thickness) |
| Chloropicrin | Trichloronitromethane, Chloropicrin; dose 40 g·m⁻²; 1998 applied broadcast. 1999 and 2000 under preformed beds (black polyethylene, 35 mm thickness) |

Each year, 10 transplants per plot were analyzed to detect infections. Crowns and 5-cm-long root 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 (0.3%). Aliquots of 1 mL were spread on petri dishes with semi-selective media P, ARPH (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.

Results and Discussion

Results of the analyses of variance (ANO-VAs) 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
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 Pithium 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 yield at two sites, in Spain.

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

| Year       | Source of variation | df | MS    | P     |
|------------|---------------------|----|-------|-------|
| 1998/1999  | Blocks              | 2  | 25.64 | 0.8692 |
|            | Treatment           | 6  | 575.56| 0.0165 |
|            | Site                | 1  | 15.18 | 0.0737 |
|            | Treatment × site    | 6  | 403.29| 0.0697 |
|            | Error               | 26 | 179.00|       |
| Total      |                     | 41 |       |       |
| 1999/2000  | Blocks              | 2  | 24.85 | 0.7979 |
|            | Treatment           | 6  | 1581.97| 0.0000 |
|            | Site                | 1  | 1391.27| 0.2962 |
|            | Treatment × site    | 6  | 497.19 | 0.8682 |
|            | Error               | 26 | 1224.22|       |
| Total      |                     | 41 |       |       |
| 2000/2001  | Blocks              | 2  | 10302.48| 0.1852 |
|            | Treatment           | 6  | 38171.31| 0.0002 |
|            | Site                | 1  | 18121.41| 0.0088 |
|            | Treatment × site    | 6  | 6536.79| 0.3664 |
|            | Error               | 26 | 5722.54|       |
| Total      |                     | 41 |       |       |

### 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    | P     |
|------------|---------------------|----|-------|-------|
| 1998/1999  | Blocks              | 2  | 25.64 | 0.8692 |
|            | Treatment           | 6  | 575.56| 0.0165 |
|            | Site                | 1  | 15.18 | 0.0737 |
|            | Treatment × site    | 6  | 403.29| 0.0697 |
|            | Error               | 26 | 179.00|       |
| Total      |                     | 41 |       |       |
| 1999/2000  | Blocks              | 2  | 24.85 | 0.7979 |
|            | Treatment           | 6  | 1581.97| 0.0000 |
|            | Site                | 1  | 1391.27| 0.2962 |
|            | Treatment × site    | 6  | 497.19 | 0.8682 |
|            | Error               | 26 | 1224.22|       |
| Total      |                     | 41 |       |       |
| 2000/2001  | Blocks              | 2  | 10302.48| 0.1852 |
|            | Treatment           | 6  | 38171.31| 0.0002 |
|            | Site                | 1  | 18121.41| 0.0088 |
|            | Treatment × site    | 6  | 6536.79| 0.3664 |
|            | Error               | 26 | 5722.54|       |
| Total      |                     | 41 |       |       |

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

| Treatments | Trichoderma populations cfu/g | Yield t/ha |
|------------|-------------------------------|------------|
| Control    | 36.6 a 36.6 b 19.8 b          | 22.1 b 21.4 c 25.9 c |
| MB-VIF     | 49.5 a 3465.5 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 |

*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.

*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.

| Location | 1998/1999 | 1999/2000 | 2000/2001 |
|----------|-----------|-----------|-----------|
|          | Spearman coefficient | Spearman coefficient | Spearman coefficient | Spearman coefficient |
|          | P value | P value | P value | P value |
| Cartaya  | -0.21   | 0.3539*  | 0.46     | 0.0356* |
|          | NS      |          | 0.79     | 0.0006*** |
| Moguer   | 0.28    | 0.2098** | 0.44     | 0.0435* |
|          | NS      |          | 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 Lumsden, 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.

Lindsay, 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.