Reduction of *Phytophthora cactorum* in Strawberry Fields by *Trichoderma* spp. and Soil Solarization

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ABSTRACT

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Field experiments were conducted in southwest Spain for three consecutive years from 2000 to 2003 to evaluate the effectiveness of solarization and *Trichoderma* spp., alone and combined, in reducing *Phytophthora cactorum* soil populations and consequently leather rot on fruit of strawberry plants. Plots (12.5 by 3.3 m), never treated with methyl bromide, were naturally infested by *P. cactorum*. Solarization was conducted during the summer, using clear 50-µm low-density polyethylene mulch. *Trichoderma* spp. were applied via drip and dip, adding to the soil 7 days before planting (10^8 conidia/m^2), and strawberry roots were dipped in a suspension of *Trichoderma* spp. (10^6 conidia/ml) prior to planting. Solarization reduced the soil *P. cactorum* population 100% in year 1, 47% in year 2, and 55% in year 3 relative to the untreated control. *Trichoderma* spp. applications reduced soil populations of *P. cactorum* and reduced leather rot incidence 76.6% in year 1 and 33.8% in year 2 compared with the untreated control. The combination of solarization and *Trichoderma* spp. reduced *P. cactorum* soil population the most each year, 88.9% in January 2001, 97.6% in 2002, and 99.0% in 2003. The very promising effect of *Trichoderma* spp. and solarization against *P. cactorum* indicates that there may be future alternatives to traditional chemicals for disease control.

Additional keywords: antagonism, biological control

Huelva (southwestern Spain) is the most important area of strawberry (*Fragaria* × *ananassa* Duch.) production in Europe (27). In 2001, 7,500 ha was cultivated, yielding 281,000 metric tons of strawberry fruit, 80% of which was exported (2).

Phytophthora cactorum (Lebert & Cohn) Schröt. is one of the most destructive pathogens of strawberry. It causes leather rot on fruit and crown rot and wilt of plants. Leather rot first was reported by Rose (39) in the United States and has since been found in Europe and Asia (15). The disease usually occurs only sporadically; however, when it does occur, losses may be considerable (15). Crown rot has caused economic losses in Europe since 1960 (40). In southwestern Spain, it was first reported in 2002 (10).

The principal procedures for control of *Phytophthora* spp. on strawberry include cultural practices and the use of fungicides (30,40). Methyl bromide (MB) is being phased out globally because it is considered an ozone-depleting compound (1). Since 2005, the use of MB has been banned in European Union countries (5). Chemical, physical, and biological alternatives to MB have been evaluated in strawberry fruit production (9,12,31,35,38).

Solarization is a process that employs solar radiation to heat soil under a transparent plastic film to temperatures that are detrimental to soilborne pathogens. Increased soil temperatures can decrease populations of plant pathogens (24,33). Solarization can enhance the effectiveness of other pest management approaches (7,24,37) and has the additional advantages of being a nonchemical alternative method for pathogen control (5). *Trichoderma* spp. are among the microorganisms that survive solar heating (7,11). Fumigation of the soil affects the environment and also produces variations in *Trichoderma* populations because these fungi can reproduce rapidly after MB treatments (32). *Trichoderma* spp. have been shown to inhibit the growth of *Phytophthora* spp. and reduce the diseases caused by them (19,25,43,44,49).

Baker and Cook defined biological control as "the reduction of inoculum density or disease-producing activities of a pathogen or parasite in its active or dormant state, by one or more organisms, accomplished naturally or through manipulation of the environment, host, or antagonist, or by mass introduction of one or more antagonists" (8). Disease suppression by biocontrol agents is the sustained manifestation of interactions between the plant, the pathogen, the biocontrol agent, and the microbial community on and around the plant and the physical environment (18). Species of *Trichoderma* are studied primarily for their ability to control plant disease through antagonism, rhizosphere competence, enzyme production, induction of defense response in plants, metabolism of germination stimulants, and beneficial growth of the host following root colonization (4,6,13,14,17,21,26,44,46–48,50,51). Determination of these effects depends on many interactions that take place in the soil among *Trichoderma* spp., other microorganisms, the plant root, and the soil environment (4).

The objective of this research was to evaluate the use of soil solarization, *Trichoderma* spp., or both for reducing populations of *P. cactorum* in the soil, thus enhancing plant health.

MATERIALS AND METHODS

Field experiments. Experiments were conducted in an experimental strawberry farm located in Moguer (Huelva, southwestern Spain; 6ºW, 37ºN), for three consecutive growing seasons from October to May (2000–01, 2001–02, and 2002–03). The soil (90% sand, 2.8% slime, and 7.2% clay) never had been previously fumigated, and strawberry had not been grown there prior to the start of this work. A randomized complete block design with four replications (blocks) was used. Each plot was 12.5 by 3.3 m and had three raised beds. Treatments were soil solarization and applications of *Trichoderma* spp., alone or combined (Solarization, *Trichoderma,* Solarization+*Trichoderma*), and the untreated control. Treatments were done in the same plots each year. The plots were solarized from 10 July to 12 September 2000, from 11 July to 18 September 2001, and from 11 July to 27 September 2002, using clear 50-µm low-density polyethylene mulch. Soil temperatures at 5, 10, and 20 cm were measured daily in single solarized and nonsolarized plots at 8:00 a.m., 1:00 p.m., and 6:00 p.m., using soil thermometers, to estimate the thermal inactivation potential of solarization.

A premix of *Trichoderma harzianum* and *T. viride* (Tusal, 5×10^8 conidia/g; Newbiotechic, Seville, Spain) was applied via drip and dip. Drip irrigation (10^8 conidia/m^2) was done once (7 days before planting) for 10 min. Furthermore, the roots of strawberry plants were dipped, for

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10 min, in a suspension of *T. harzianum* and *T. viride* (10^6 conidia/ml) immediately prior to planting.

Plots were not inoculated with *P. cactorum*.

Each year in the last week of October, strawberry cv. Camarosa was planted. Plants were grown in an intensive annual system on drip-irrigated raised beds (35 cm high and 50 cm wide) with black plastic mulch. Plants were set 25 cm apart within each of two rows per raised bed, and the rows were spaced 25 cm apart. Beds were covered with 70-cm-tall polyethylene perforated film tunnels from late November to late March (28).

Quantifying of *Trichoderma* spp. and *P. cactorum* propagules. Soil samples were collected in July (prior to solarization), in October (10 days before planting), in January (80 days after planting), and in May (at the end of the trials) each year. Samples consisted of three cores (4.5 cm in diameter by 20 cm deep) collected between two plants on the same row of the central raised bed. Soil samples were held at 4ºC for 1 to 7 days and processed as follows.

Aliquots (10 g) of the soil mixture were suspended in 100 ml of sterile water agar (0.1%), shaken for 15 min at 150 rpm, and 100-µl aliquots from undiluted suspensions were spread on petri dishes with *Trichoderma* selective medium adapted by Askew and Laing (3). Dry weight of soil samples was determined using 50-g subsamples.

Air-dried soil (1 g per sample) was suspended in 99 ml of sterile water agar (0.3%), and 1-ml aliquots were spread onto petri dishes containing semiselective medium P_5ARP to determine the presence of *P. cactorum* (22).

Ten replicate dishes were used for each plot and medium. Plates were incubated at 25ºC in the dark for 7 days. Based on morphological characteristics, *Trichoderma* spp. and *P. cactorum* colonies were identified and counted (16,17,45). For *Phytophthora* spp., colonies were transferred to V8 juice agar to induce growth of diagnostic structures (16,41). Results were expressed as CFU/g of dry soil.

Disease assessment. All ripe and diseased fruit were harvested once per week from each plot from February to May. The number of leather rot-infected fruit was recorded for each replication. Potato dextrose agar and V8 juice agar were used to isolate fungi from diseased fruit (41).

Statistical analysis. Analysis of variance (ANOVA; Statistix 8, Analytical Software for Windows) was performed for *P. cactorum* and *Trichoderma* spp. CFU. The data were square root (CFU + 0.5) transformed. Mean separation was conducted using the Tukey highly significant difference (HSD) comparison method at $P < 0.05$.

ANOVA was used to determine the effects of treatments on the percentage of fruit with leather rot symptoms caused by *P. cactorum*. Arc sine transformation of percentage data was used to stabilize variances. The Tukey HSD comparison method was used to separate means after ANOVA at $P = 0.05$.

RESULTS

Soil temperatures. Mean soil temperatures at 6:00 p.m. in solarized plots averaged 46ºC at 5 cm, 43ºC at 10 cm, and 38ºC at 20 cm, compared with 33, 32, and 28ºC, respectively, in nonsolarized plots (Table 1).

***P. cactorum* and *Trichoderma* spp. soil populations.** Before solarization, soil populations of *P. cactorum* and *Trichoderma* spp. (CFU/g) were not significantly different ($P > 0.05$) among plots (Tables 2 and 3, respectively). Solarization reduced the soil *P. cactorum* population 100% in year 1, 47% in year 2, and 55% in year 3 (Table 2). No differences were observed in *Trichoderma* spp. soil population between solarized plots and the untreated control in year 1 (Table 3). Solarization reduced the soil *Trichoderma* spp. population 44% in year 2, and 52% in year 3 (Table 3).

The combination of solarization and *Trichoderma* spp. applications reduced *P. cactorum* soil population the most each year and, at most sampling dates, the combination was significantly less than the individual treatments (Table 4). This combination significantly reduced *P. cactorum* in soil relative to the untreated control and the solarization-alone treatment in January and May of each year (Table 4; Fig. 1). Furthermore, solarization alone reduced *P. cactorum* in soil relative to the control each sampling date, except in May 2002 (Table 4). *Trichoderma* alone reduced significantly *P. cactorum* in soil relative to the untreated control each year (Table 4).

Solarization reduced the number of CFU of *Trichoderma* spp. in soil compared with the *Trichoderma* alone treatment in January and May of each year, and differences were observed in May 2003 relative to the untreated control (Table 5; Fig. 1). Nevertheless, *Trichoderma* applications increased their soil population and differences were observed in January and May of each year relative to the untreated control (Table 5). The combination of solarization and *Trichoderma* applications produced a recovery of *Trichoderma* spp. from soil that fluctuated between the *Trichoderma* spp. soil population recovered from the solarized plots and the *Trichoderma*-treated plots. On all the dates, *Trichoderma* spp. increased in soil, and differences were observed among the combination of treatments and the untreated control (Table 5).

Table 1. Soil temperatures (Temp) in solarized and nonsolarized plots at the experimental strawberry farm located in Moguer (Spain)[z]

Treatment, depth (cm)	Hour	Temp (ºC)
Solarized		
5	8:00 a.m.	23.3
	1:00 p.m.	46.4
	6:00 p.m.	45.6
10	8:00 a.m.	25.2
	1:00 p.m.	38.5
	6:00 p.m.	43.3
20	8:00 a.m.	27.4
	1:00 p.m.	31.2
	6:00 p.m.	37.6
Nonsolarized		
5	8:00 a.m.	17.2
	1:00 p.m.	31.4
	6:00 p.m.	33.3
10	8:00 a.m.	20.1
	1:00 p.m.	27.2
	6:00 p.m.	32.4
20	8:00 a.m.	22.4
	1:00 p.m.	23.8
	6:00 p.m.	28.3

[z] Temperature was recorded at 8:00 a.m., 1:00 p.m., and 6:00 p.m. Temperature data were collected from 10 July to 12 September 2000, from 11 July to 18 September 2001, and from 11 July to 27 September 2002. Shown are mean temperatures, averaged for the years 2000, 2001, and 2002.

Table 2. *Phytophthora cactorum* propagule densities (CFU/g soil) before (July) and after (October) solarization[z]

	2000–01		2001–02		2002–03	
Treatment	Before	After	Before	After	Before	After
Control	10.9	7.4	1,292.0	857.6	989.3	594.6
Solarization	11.0	0.0	1,113.8	454.8	831.3	269.8
P	0.92254	0.04802	0.27351	0.02802	0.48915	0.00752

[z] Data are means of four replications (plots). *P* = significant probability values associated with *F* tests.

Table 3. *Trichoderma* spp. propagule densities (CFU/g soil) before (July) and after (October) solarization[z]

	2000–01		2001–02		2002–03	
Treatment	Before	After	Before	After	Before	After
Control	0.0	0.0	143.9	1,199.4	859.8	2,379.9
Solarization	0.0	1.3	149.3	669.1	444.5	1,139.8
P	…	0.16617	0.79730	0.01351	0.12800	0.01292

[z] Data are means of four replications (plots). *P* = significant probability values associated with *F* tests.

Table 4. *Phytophthora cactorum* propagule densities (CFU/g soil) 80 days after planting (January) and at the end of the trials (May)[z]

Treatment	2000–01		2001–02		2002–03	
	January	May	January	May	January	May
Control	111.4 a	2,321.6 a	1,054.8 a	1,356.3 a	843.6 a	1,315.5 a
Solarization	57.8 b	1,559.3 b	570.1 b	1,079.1 ab	207.0 b	908.3 b
Trichoderma	33.0 bc	1,391.0 b	272.3 b	763.5 bc	41.3 c	722.7 b
Solarization+*Trichoderma*	12.4 c	1,017.2 c	24.8 c	659.6 c	8.3 c	391.1 c
P	0.00659	0.00001	0.00058	0.00636	0.00001	0.00568

[z] Data are means of four replications (plots). Mean values within the same columns followed by the same letter are not significantly different according to the Tukey highly significant difference test ($P < 0.05$). P = significant probability values associated with F tests.

The number of CFU of *Trichoderma* spp. in soil increased over the three consecutive years (Table 5; Fig. 1).

Disease incidence. *Trichoderma* applications reduced the incidence of leather rot by 76.6% relative to the untreated control in year 1 (Table 6). *Trichoderma* and solarization, alone or combined, reduced leather rot incidence by 30.8 to 36.9% relative to the untreated control in year 2. In the third growing season, reduction of the incidence of leather rot was not significant ($P \geq 0.05$), presumably due to the low incidence of disease (Table 6).

DISCUSSION

Potential use of biological control agents as replacements or supplements for chemical fungicides has been addressed in many reports (4,6,13,17,21,26,44,46–48,50,51); however, few agents have been tested under field conditions. In this study, the potential of *Trichoderma* spp. and soil solarization, alone or combined, for controlling *P. cactorum* in strawberry field trials was evaluated over a 3-year period.

In our experiment, solarization suppressed the survival of *P. cactorum* in soil, but it did not totally eliminate the pathogen. Similarly, Hartz et al. (20) found that solarization reduced pathogen propagules. *P. cactorum* was killed within 30 min at 45ºC (23). In field studies, solarized plots reached a maximum temperature of 45ºC at the 15-cm depth, and *P. cactorum* was killed within 2 weeks at the 15-cm depth but withstood the effects of soil solarization at depths of 30 and 45 cm (23). The results indicate that solarization may serve as a component in an integrated, sustainable approach to the management of *P. cactorum* in strawberry.

In the study described here, solarization reduced *Trichoderma* spp. in soil, except in October 2000, presumably due to the low propagule densities because the soil had not been inoculated previously with *Trichoderma* spp. and strawberry had not been grown there prior to the start of this work. Nevertheless, *Trichoderma* spp. are among the microorganisms that survive solar heating (7,11) or rapidly recolonize. We observed that the number of CFU of *Trichoderma* spp. in soil increased over the three consecutive years in solarized plots.

Sivan and Chet (42) recovered only *Trichoderma* spp. from plots treated with the antagonist. *Trichoderma* spp. failed to become established in the soil and, by the end of the season, they could not be recovered from most of the soil samples (42). A requirement for an efficient biological control agent is the ability to survive and to become established in the rhizosphere (29). We observed that the inoculated strains of *Trichoderma* favored its development in this environment (34,36), as also was suggested by the higher population detected in soils treated with the an-

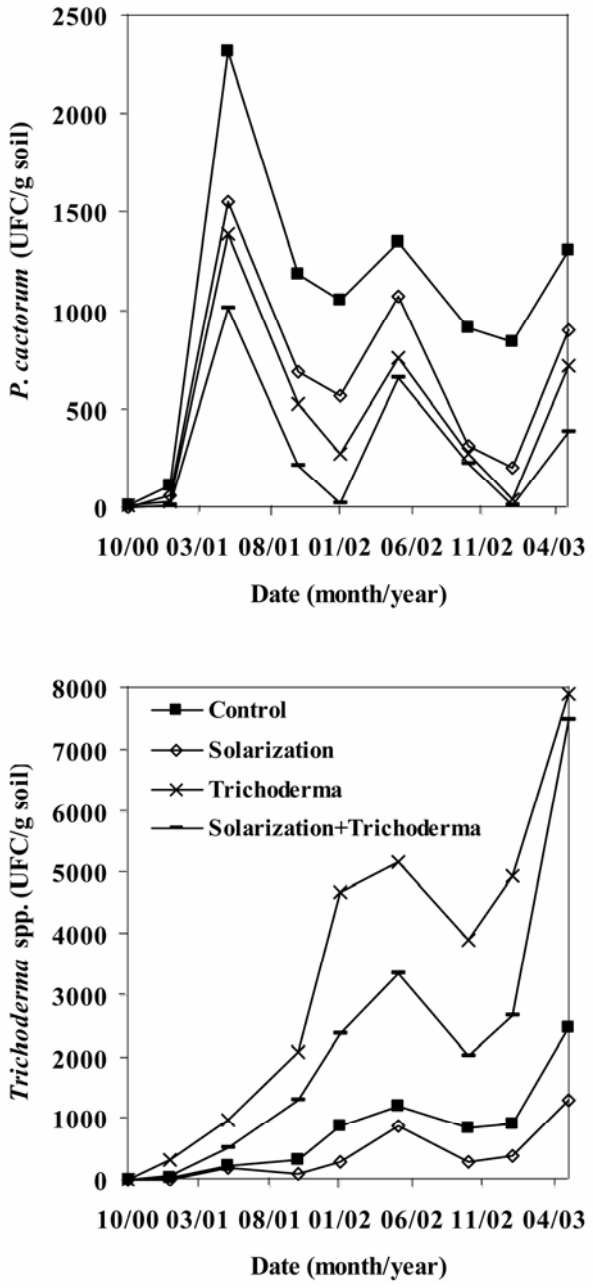

Fig. 1. Effect of solarization, *Trichoderma* spp. applications, or both on *Phytophthora cactorum* and *Trichoderma* spp. propagule densities (CFU/g soil).

Table 5. *Trichoderma* spp. propagule densities (CFU/g soil) 80 days after planting (January) and at the end of the trials (May)[z]

	2000–01		2001–02		2002–03	
Treatment	**January**	**May**	**January**	**May**	**January**	**May**
Control	35.0 c	233.2 c	868.1 c	1,218.1 c	925.4 c	2,455.9 b
Solarization	5.7 c	195.6 c	286.3 c	873.2 c	401.3 c	1,305.4 c
Trichoderma	318.7 a	962.4 a	4,696.1 a	5,166.2 a	4,939.7 a	7,889.6 a
Solarization+*Trichoderma*	72.4 b	520.6 b	2,384.5 b	3,360.9 b	2,661.1 b	7,468.1 a
P	0.00072	0.00170	0.00455	0.00208	0.00227	0.00000

[z] Data are means of four replications (plots). Mean values within the same columns followed by the same letter are not significantly different according to the Tukey highly significant difference test ($P < 0.05$). P = significant probability values associated with F tests.

Table 6. Leather rot (%) caused by *Phytophthora cactorum*[z]

Treatment	**2000–01**	**2001–02**	**2002–03**
Control	4.7 a	6.5 a	0.3
Solarization	3.6 a	4.5 b	0.6
Trichoderma	1.1 b	4.3 b	0.2
Solarization+*Trichoderma*	3.8 a	4.1 b	0.0

[z] Data are means of four replications (plots). Mean values within the same columns followed by the same letter are not significantly different according to the Tukey highly significant difference test ($P < 0.05$).

tagonist. *Trichoderma* spp. are known biocontrol agents of *P. cactorum* and other plant pathogens (19,43). These results help to explain the smaller number of CFU of *P. cactorum* that we registered in soils treated with *Trichoderma* applications compared with the untreated control. Antagonistic soil fungi may have contributed to reduce disease in our study by direct parasitism, competition, and antibiosis (21,25,44,49).

These experiments demonstrated that solarization and applications of *Trichoderma* spp. can reduce leather rot in strawberry. The smaller number of CFU of *P. cactorum* in treated soils helps to explain the lower incidence of leather rot in the treated plots relative to the untreated control. Nevertheless, there was low disease pressure, presumably due to various cultural practices to reduce or eliminate standing water, planting on raised beds with black plastic mulch, avoiding cultural operations that result in the formation of ruts between rows, and orienting rows to facilitate the runoff of surface water. In addition, beds were covered with perforated film tunnels to ensure good air circulation and be fully exposed to sunlight to reduce periods of free moisture on fruit.

We proved that solarization and *Trichoderma* spp., alone or combined, significantly increased strawberry yield relative to the untreated control after repeated treatments of the same site (34,36). The combination of soil solarization and *Trichoderma* applications increased marketable yield the most each year (34,36).

In addition, solarization and *Trichoderma* treatments, combined, reduced the soil *P. cactorum* population the most each year. Therefore, the joint action of solarization and *Trichoderma* should be considered if the soil is infested by *P. cactorum*. Sivan and Chet (42) had similar results on *Fusarium* spp. populations. The greatest reduction in *Fusarium* spp. counts was obtained by the combination of the antagonist with soil solarization (42).

The very promising effect of *Trichoderma* spp. and solarization against *P. cactorum*, along with cultural practices to reduce or eliminate standing water, the ability of the biocontrol agent to proliferate in the field soil, and the increase in marketable yield, indicate that there may be future alternatives to traditional chemicals for strawberry disease control.

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