

Control of *Verticillium* wilt of cotton by means of soil solarization and tolerant cultivars in southern Spain

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Eight field experiments (I–VIII) were conducted in clay soils naturally infested with a cotton-defoliating pathotype of *Verticillium dahliae* in the lower Guadalquivir Valley of Andalucía, southern Spain, during the period 1986–90. Experiments I–VI aimed to determine the efficacy of soil solarization in reducing populations of the pathogen in soil and eventually contributing to the control of *Verticillium* wilt of cotton. The population of *V. dahliae* in the 0- to 40-cm soil layer was reduced to undetectable or very low levels after solarization for 6 to 10 weeks. The final incidence of *Verticillium* wilt in the cotton crop following solarization was reduced to 13% or less in solarized plots, compared to 55–90.5% in unsolarized controls. The onset of disease incidence in the solarized plots was delayed by 2–7 weeks, increased at a lower rate, and had a smaller area under the disease progress curve, compared to that in unsolarized plots. Seed cotton yields in solarized plots increased by 11.3–130.9% depending upon experiments, cultivars and years. Experiments VII and VIII aimed to determine the use of the highly wilt-tolerant cotton cv. Acala GC 510 for control of the disease that had been cropped to susceptible cotton cultivars the year before in solarized soils. Solarized or unsolarized plots were first sown to susceptible cotton cvs Acala SJ2 and Coker 310, and the following year were sown to cv. Acala GC 510. The inoculum density of *V. dahliae* at the time of sowing cv. Acala GC 510 in previously solarized plots had increased to moderate levels, but remained considerably lower than that in unsolarized plots. The final disease incidence in cv. Acala GC 510 grown in unsolarized plots was lower than that in susceptible cultivars grown in the same plots the year before. Furthermore, the disease incidence in cv. Acala GC 510 grown in solarized plots was as high as that in susceptible cultivars grown the year before with much less initial inoculum.

INTRODUCTION

Approximately 70 000–90 000 ha of cotton (*Gossypium hirsutum*) are grown annually in Spain. About 90–95% is planted in the Guadalquivir Valley of Andalucía in the southern part of the country, of which 35 000–45 000 ha are grown intensively in the clay, fertile soils of the Lower Guadalquivir Valley (Anonymous, 1992).

Verticillium wilt, caused by *Verticillium dahliae*, is the most important disease of cotton in Spain. The disease occurs in 74–82% of the fields throughout the Guadalquivir Valley, with a disease incidence range of 15–26% (Blanco-López *et al.*, 1989). Disease incidence and severity are highest in the Lower Guadalquivir

Valley, and occurrence is associated with high inoculum densities of a cotton-defoliating pathotype of *V. dahliae* (Blanco-López *et al.*, 1989). This defoliating pathotype, which was first identified in the Lower Valley in 1983 (Blanco-López *et al.*, 1986), has since spread throughout this area. The occurrence of cotton-defoliating pathotypes of *V. dahliae* has been shown in Mexico, Peru and the USA, but the nature of the disease symptoms observed in cotton crops in other regions suggests that they might also be present elsewhere (Mathre *et al.*, 1966; Schnathorst, 1969; Kannan & Srinivasan, 1984).

Verticillium wilt of cotton can be controlled by reducing inoculum density and/or the efficacy of

the initial inoculum. Several control measures have been shown to be partially effective, but only integrated management can effectively control the disease (El-Zik, 1985). Crop rotation with non-host crops is unlikely to reduce high levels of inoculum density in the soil to a level sufficient for disease control, because of the length of rotation required (Huisman & Ashworth, 1976) and for economic reasons. Furthermore, the residual inoculum of the defoliating pathotype, even at low levels, can cause severe *Verticillium* wilt in susceptible cotton cultivars (Blanco-López *et al.*, 1989). Tolerant cotton cultivars are known to withstand a lower rate of development of *V. dahliae* within infected plants, but they seem to exert positive selection for highly virulent isolates of the pathogen (Ashworth, 1983). *V. dahliae* in soil can be controlled by soil fumigation and solarization. Soil solarization has successfully controlled *V. dahliae* in soil at different depths in several areas (Katan *et al.*, 1976; Pullman *et al.*, 1981a; Ben-Yephet *et al.*, 1988; Davis, 1990). In Andalucía, soil solarization is economically feasible and it has been practised successfully by growers for control of *Fusarium* wilt of watermelon (González-Torres *et al.*, 1993) and *Verticillium* wilt of cotton (Melero-Vara *et al.*, unpublished data; Alvarado & Durán, 1992). Soil solarization is an ecologically sound control measure that reduces recolonization risks, and has beneficial effects on soil properties and plant growth beyond the control of plant pathogens, weeds and insects. The principles and achievements of soil solarization have been thoroughly summarized (see, for example, Katan, 1981, 1987; Stapleton *et al.*, 1985; Stapleton & DeVay, 1986). Tolerance of *Verticillium* wilt has been reported in some Upland cotton cultivars, derived mostly from *G. barbadense* and *G. darwinii* (El Zik, 1985). However, most Acala cultivars that are tolerant of mildly virulent pathotypes of *V. dahliae* are not useful at the high inoculum levels of the defoliating pathotype that occur in the Lower Guadalquivir Valley of Andalucía (Blanco-López *et al.*, 1989; Bejarano-Alcázar, 1990). Recently, new cotton cultivars have been developed, such as Acala GC510, which are highly tolerant of the disease in fields with a high inoculum density of unspecified pathotypes of *V. dahliae* (Paplomatas *et al.*, 1992). These cultivars might be useful for control of *Verticillium* wilt of cotton under the conditions that prevail for cotton crops in the Lower Guadalquivir Valley.

The objective of this work was to determine the effectiveness of soil solarization, alone and in

combination with tolerant cultivars, for control of *Verticillium* wilt of cotton in two consecutive cotton crops following solarization in the Lower Guadalquivir Valley of southern Spain. Preliminary reports of part of this work have been published elsewhere (Jiménez-Díaz *et al.*, 1991; Blanco-López *et al.*, 1992).

MATERIALS AND METHODS

Eight field experiments (I–VIII) were carried out in vertic soils (c. 61% clay, 0.9–1.4% organic matter, pH 8.3–8.8) in the Lower Guadalquivir Valley, southern Spain, during the period 1986–90. The soil in these fields was naturally infested with a defoliating pathotype of *V. dahliae*, as indicated by the disease reaction of cotton PI 70–110 and cvs Acala SJ5 and Acala SJC-1 to artificial inoculation with a sample of monoclinal isolates of the pathogen obtained from affected cotton, and/or the severe defoliation and death of infected cotton plants observed in previous years (Blanco-López *et al.*, 1989; Bejarano-Alcázar, 1990). Seedbed preparation, fertilization and irrigation were performed in accordance with farmers' practices.

Effect of soil solarization on *Verticillium* wilt in a cotton cultivar highly susceptible to the disease

All experiments consisted of two treatments (soil solarized or unsolarized) and were conducted according to a randomized complete block design. Experiments I and II were carried out during the period 1986–87 and consisted of six replicate plots 10 m long and 6 or 4.5 m wide, respectively. Two additional experiments (III and IV) were conducted during the period 1987–88 in order to confirm the results of the previous experiments and to obtain information about the practicality and efficacy of soil solarization by cotton growers. Therefore, two replications of larger plots (15–30 m long and 6.5 m wide) were used.

In all cases, the soil was thoroughly disked after harvest of the previous wheat crop, then rotovated and furrow irrigated to field capacity in the upper 30–40 cm layer. Plots to be solarized were covered, 1–2 days after irrigation, with transparent 6.5-m-wide, 37.5- μ m-thick (experiments I, III and IV) or 25- μ m-thick (experiment II) polyethylene sheets. Plots were solarized from 17 July to 31 August in 1986. In 1987 solarization was extended from 17 July to 30 September because the weather had been cloudy and rainy, with lower temperatures than usual during the early solarization period.

After solarization was complete, the polyethylene sheets were removed and plots remained in place until early spring of the year following that of solarization. Plots were then disked lengthwise to reduce mixing of soil and sown with cotton cv. Coker 310, highly susceptible to *V. dahliae*, in late March 1987 (experiments I and II) and early April 1988 (experiments III and IV). Plots consisted of four (experiment II) or six (experiments I, III and IV) rows 0.95 m apart with plant stand density adjusted to ca. 120 000 plants/ha. In all experiments the increase in disease incidence, expressed as the percentage of plants showing foliar symptoms characteristic of *Verticillium* wilt (Schnathorst, 1981; Blanco-López *et al.*, 1989), was determined over time in the two (experiment II) or four (experiment I, III and IV) central rows of each plot at approximately 2-week intervals from June to September. Final percentage values were transformed into $\arcsin (Y/100)^{1/2}$ for analyses of variance. Plots were harvested by hand-picking and seed cotton yields were determined for experiments II–IV. Analyses of variance were made and treatment means were compared using Fisher's protected least significant difference (LSD) at $P = 0.05$.

Effect of soil solarization on epidemic components of *Verticillium* wilt and yield in susceptible cotton cultivars

Two experiments (V and VI) were conducted during the period 1988–89 with four treatments, as follows: (1) solarized soil sown to cv. Coker 310; (2) solarized soil sown to moderately susceptible cv. Acala SJ2 (Ashworth, 1983); (3) unsolarized soil sown to cv. Coker 310; and (4) unsolarized soil sown to cv. Acala SJ2. The experiments were conducted according to a split-plot treatment design within randomized complete blocks, with soil treatment and cultivars as the main plot and subplot treatments, respectively. There were three or four blocks, respectively, for experiments VI and V. Soil preparation, irrigation and plastic tarping were performed as for the previous experiments I–IV using 37.5- μ m-thick transparent polyethylene sheets. The main plots in experiments V (6×100 m) and VI (6×85 m) were solarized from 12 July to 2 September, and from 26 July to 16 September 1988, respectively. After solarization was complete, the polyethylene sheets were removed and plots remained in place until March 1989. The main plots were then divided into two halves across their longest

side so that two subplots (6×50 m for experiment V and 6×42.5 m for experiment VI) were obtained from each main plot, and cultivars were randomly allocated to them. Plots were sown in mid-March 1989, and consisted of six rows 0.95 m apart and either 50 m (experiment V) or 42.5 m (experiment VI) long, with a stand adjusted to 88×10^3 and 156×10^3 plants/ha, respectively.

For both experiments the increase in disease incidence, expressed as the percentage of plants showing foliar symptoms characteristic of *Verticillium* wilt, was determined over time in the two central rows of each plot at 2-week intervals from June to September 1989, and the area under the disease progress curve (AUDPC) was calculated. Yields were determined by combine-harvesting the four central rows of each plot. Afterwards, 50 plants per plot were randomly selected and cut 4–6 cm above the soil level to determine the incidence (percentage) of plants showing vascular discoloration. Disease incidence values over time for each treatment were transformed by $\ln [1/(1-Y)]$ (VanderPlank, 1963). Incidence values of 0 or 100% disease were not included in these transformations. Linear regression analyses were performed with transformed and non-transformed data. The coefficients of determination (R^2) and distribution patterns of residuals against expected values were used to indicate appropriateness and to select the best regression model (Daniels & Wood, 1980). Comparisons of linear regressions were made in order to detect the influence of soil treatment and cultivar used on the increase in disease incidence. The final incidence of foliar symptoms was transformed into $\arcsin (Y/100)^{1/2}$ and, together with the AUDPC and yield data, was subjected to analysis of variance. Treatment means were compared using Fisher's protected LSD at $P = 0.05$.

Combined effect of soil solarization and cultivar on *Verticillium* wilt of cotton

At the end of March 1990, plots from experiments V and VI, which had remained untreated since harvest in September 1989, were sown to cotton cv. Acala GC510, which is highly tolerant of *Verticillium* wilt (Paplomatas *et al.*, 1992), at a stand of 157×10^3 and 215×10^3 plants/ha, respectively. Thus plots from experiments V and VI became plots for experiments VII and VIII, respectively. The final incidence of foliar symptoms was determined in the two central rows of each plot at the end of September 1990

Table 1. The effect of soil solarization on the population of *Verticillium dahliae* in soil, development of *Verticillium* wilt and seed cotton yield of cotton cv Coker 310 during the period 1986–87

Soil treatment ^a	Inoculum density (propagules/g dry soil) ^b						Final incidence of foliar symptoms ^c (%)	Seed cotton yield (kg/ha) ^d
	Before treatment		After treatment		After sowing			
	mean	CV(%)	mean	CV(%)	mean	CV(%)		
Experiment I								
Unsolariized	23.3	87.9	16.1	125.5	51.3	35.6	90.5 a	NA
Solarized	27.3	68.3	0.0	—	NA ^e	NA	5.0 b	NA
Experiment II								
Unsolariized	2.4	85.2	2.8	96.4	6.1	47.2	60.1 a	2093 a
Solarized	1.8	85.4	0.3	167.3	0.1	244.9	4.5 b	4833 b

^a Soil was solarized from 17 July to 31 August 1986.

^b Determined by means of an Andersen sampler in four 500-mg aliquots of soil collected from the 0–20-cm soil layer of each of six replicate plots immediately before and after solarization. Samples of soil the year after solarization were collected in early May, about 1 month after sowing. CV = coefficient of variation.

^c Determined from plants in the four (experiment I) and two (experiment II) central rows of each plot that were affected by symptoms characteristic of *Verticillium* wilt at the end of September, 23 weeks after sowing. For each experiment, values followed by the same letter are not significantly different ($P = 0.05$) according to LSD test.

^d Determined by hand-picking all plants in the four central rows of each plot. Average of six replicate plots. Data were not available for experiment I.

^e Not determined.

for both experiments. Plots were harvested as in the previous year, and seed cotton yields were determined. Afterwards, 50 consecutive plants per plot were selected to determine the incidence (percentage) of plants showing vascular discoloration, as described for experiments V and VI. Analysis of variance and mean comparisons were performed on the data.

Estimation of inoculum density of *V. dahliae* in experimental plots

The populations of *V. dahliae* in solarized and unsolarized soil of experiments I–VIII were assessed immediately before and after solarization, and about 1 month after sowing either 1 year (experiments I–VI) or 2 years (experiments VII and VIII) following that of solarization. Depending upon the plot size, seven or 12 soil samples (200 g) were collected with an Edelman sampler (Eijkelkamp, Giesbeek, The Netherlands) to a depth of 0–20 cm (experiments I–IV, and experiments VII and VIII) or 0–20 and 20–40 cm (experiments V and VI) at equally spaced sites in the plot. The soil samples from each plot were bulked, thoroughly mixed and air dried at room temperature for 4–6 weeks. The soil was then crumbled by hand and with a wooden hammer, and milled in a rotating drum with steel cylinders for 20 min. Four aliquots

of 500 mg each were processed per plot. Each aliquot was divided into five 100-mg subsamples and each subsample was plated on to a Napolypectate semi-selective medium (Butterworth & DeVay, 1977) by means of an Andersen sampler (Andersen Sampler Inc., Atlanta, GA, USA). Plates were incubated at 25°C in the dark for 12–14 days, and were then washed free from soil with tap water. The fungal colonies growing on the semi-selective medium were observed with a stereoscope at $\times 15$ magnification. Colonies of *V. dahliae* were identified on the basis of microsclerotia formed in the medium (Butterfield & DeVay, 1977). The number of colonies of *V. dahliae* formed from each 500-mg aliquot was determined. Estimates of *V. dahliae* populations in solarized and unsolarized soils showed high variability and consequently were not subjected to analyses of variance; instead, the standard deviation and coefficient of variation of estimates were calculated.

RESULTS

Effect of soil solarization on *Verticillium* wilt in a cotton cultivar highly susceptible to the disease

Maximum and minimum daily air temperatures

Table 2. The effect of soil solarization on the population of *Verticillium dahliae* in soil, development of *Verticillium* wilt and seed cotton yield of cotton cv. Coker 310 during the period 1987–88

Soil treatment ^a	Inoculum density (propagules/g dry soil) ^b						Mean final incidence of foliar symptoms(%) ^c	Mean seed cotton yield (kg/ha) ^d
	before treatment		after treatment		after sowing			
	mean	CV(%)	mean	CV(%)	mean	CV(%)		
	mean	CV(%)	mean	CV(%)	mean	CV(%)		
Experiment III								
Unsolarized	20.8	42.6	68.3	46.1	28.8	20.9	87.7 a	1562 a
Solarized	20.0	10.6	0.0	—	1.5	94.3	13.5 a	3279 b
Experiment IV								
Unsolarized	3.0	23.6	34.3	108.4	15.3	118.2	55.0 a	3425 a
Solarized	3.5	60.6	0.0	—	0.3	141.4	1.8 a	4575 b

^a Soil was solarized from 17 July to 30 September 1987.

^b Determined by means of an Andersen sampler in four 500-mg aliquots of soil collected from the 0–20-cm soil layer of each of two replicate plots immediately before and after solarization. Samples of soil the year after solarization were collected in early May, about 1 month after sowing. CV = coefficient of variation.

^c Determined from plants in the four central rows of each plot that were affected by symptoms characteristic of *Verticillium* wilt at the end of September, 23 weeks after sowing. For each experiment, values followed by the same letter are not significantly different ($P = 0.05$) according to LSD test.

^d Determined by hand-picking all plants in the four central rows of each plot. Average of two replicate plots. For each experiment, values followed by the same letter are not significantly different according to LSD test.

at the experimental sites during the solarization period were in the ranges 26–39°C and 13–20°C, respectively, in 1986, and 24–42°C and 14–21°C in 1987.

The estimates of the population level of *V. dahliae* in soil by mid-July, before solarization, indicated that plots of experiments I and III, and those of experiments II and IV, were heavily (>20 propagules/g dry soil) and slightly (<5 propagules/g dry soil) infested with the pathogen, respectively (Tables 1 and 2). Soil solarization of these plots over a period of 6 weeks in 1986 and 10 weeks in 1987 brought about a reduction in the *V. dahliae* population to undetectable levels in the 0–20 cm soil layer in experiments I–IV (Tables 1 and 2). By contrast, the inoculum density of *V. dahliae* in unsolarized soil had either increased or remained at the same level during the period of solarization (Tables 1 and 2). Although some soil mixing might have occurred during the 6-month period that elapsed between removal of the polyethylene sheets and sowing, the inoculum density of *V. dahliae* in the soil of solarized plots was still very low at the time of seedling emergence (Tables 1 and 2).

The reduction in the *V. dahliae* population in solarized soil was associated with a highly significant degree of control of the disease. The

final incidence of disease in solarized plots was 5% or less, except for experiment III (13.5%), in contrast to the high disease incidence occurring in unsolarized plots, which ranged from 55 to 90.5% (Tables 1 and 2). Although the mean values of final disease incidence in solarized and unsolarized plots for experiments III and IV were quite different (Table 2), the difference was not statistically significant as revealed by analysis of variance. This was possibly due to the low number of replicates (two) in those experiments. The levels of disease control achieved by soil solarization were reflected by significant increases in the seed cotton yield of cv. Coker 310, dependent upon year and experiment (Tables 1 and 2). Thus, while the seed cotton yield in solarized plots was increased by 131% and 110% of that in unsolarized plots in experiments II and III, respectively, there was only a 34% increase in experiment IV for which the yield in unsolarized plots was 1.6–2.2 times that in experiments II and III.

Effect of soil solarization on epidemic components of *Verticillium* wilt and yield in susceptible cotton cultivars

Maximum and minimum daily air temperatures

Table 3. The effect of soil solarization on inoculum of *Verticillium dahliae* in soil during the period 1988–89

Soil treatment ^a	Soil layer (cm)	Inoculum density (propagules/g dry soil) ^b					
		before treatment		after treatment		after sowing	
		mean	CV(%) ^c	mean	CV(%)	mean	CV(%)
Experiment V							
Unsolarized	0–20	39.5	57.9	71.6	59.9	39.0	32.7
	20–40	19.4	37.1	47.9	51.3	15.2	21.9
Solarized	0–20	42.1	30.4	0.0	—	2.0	79.1
	20–40	38.3	41.4	0.5	200.0	1.7	30.0
Experiment VI							
Unsolarized	0–20	64.0	8.9	79.9	18.6	34.0	78.8
	20–40	22.0	37.8	106.0	20.4	5.7	41.3
Solarized	0–20	90.2	28.0	0.0	—	1.2	89.2
	20–40	27.8	32.2	0.2	173.2	0.8	47.1

^a Soil was solarized from 12 July to 2 September and from 26 July to 16 September 1988, for experiments V and VI, respectively.

^b Determined by means of an Andersen sampler in four 500-mg aliquots of soil collected from each of four (experiment V) or three (experiment VI) replicate plots immediately before and after solarization. Samples of soil the year after solarization were collected in mid-April 1989, about 1 month after sowing.

^c CV = coefficient of variation.

at experimental sites during the solarization period were in the ranges 27–42°C and 12–22°C, respectively, in 1988.

The estimates of inoculum density of *V. dahliae* in the 0–20-cm soil layer prior to solarization were much higher in experiments V and VI than the estimates for experiments I–IV, and were in the range 40–90 propagules/g dry soil (Table 3). Furthermore, for the two experiments, the population of *V. dahliae* in the 20–40-cm soil layer was consistently lower than that in the upper soil layer (Table 3). As for the previous experiments, in experiments V and VI soil solarization reduced *V. dahliae* populations to undetectable levels both in the 0–20-cm and in the 20–40-cm soil layers. Also, in these two experiments there was a marked increase in inoculum density in the two layers of unsolarized soils during the solarization period (Table 3). *V. dahliae* populations in solarized soils remained at a low level in the upper and lower soil layers sampled, regardless of the time period that elapsed between solarization and sowing. Inoculum densities in plots sown to cvs Acala SJ2 and Coker 310 were similar within each soil treatment and experiment (e.g. 37.1 and 40.9 propagules/g soil in the 0–20-cm soil layer in

unsolarized plots, and 1.9 and 2.0 propagules/g soil at the same depth in solarized plots, for plots sown to cvs Acala SJ2 and Coker 310 of experiment V, respectively). Therefore, values for the two cultivars were combined (Table 3).

The marked decrease in inoculum density of the pathogen in solarized soil had a significant effect on *Verticillium* wilt epidemics. The linear fit for curves of disease incidence increase over time in 1989 was better for non-transformed than for transformed data, as indicated by the higher R^2 values and the lack of a discernible pattern in the distribution of residuals plotted against expected values. Therefore, only linear regressions with non-transformed incidence values are presented (Table 4). Epidemics in solarized plots were delayed, increased at a significantly lower rate, and had a significantly lower AUDPC, compared to epidemics in unsolarized plots (Table 4). Epidemic onset was delayed by 2 weeks in experiment V and by 6–7 weeks in experiment VI. For each of cvs Acala SJ2 and Coker 310, the rate of incidence increase was significantly lower ($P < 0.001$) in solarized compared to unsolarized soils, but there were no differences ($P = 0.05$) between cultivars within soil treatments. Similarly, no significant ($P = 0.05$) differences in AUDPC

Table 4. The effects of soil solarization on the epidemics of *Verticillium* wilt and yield of two cotton cultivars in 1989

Soil treatment*	Cultivar	R ^{2b}	Rate of increase ^c	Time to Y _i = 0 (days) ^d	Final incidence of foliar symptoms (%) ^e	AUDPC ^f	Seed cotton yield (kg/ha) ^g
Experiment V							
Unsolariized	Coker 310	0.94	0.829	89	85.0	4458	3214
	Acala SJ2	0.90	0.729	93	74.6	3609	3571
Solarized	Coker 310	0.76	0.062	103	8.2	199	4935
	Acala SJ2	0.72	0.108	106	12.9	290	4550
LSD (P = 0.05)	Soil treatments	—	—	—	—	977	859
LSD (P = 0.05)	Cultivars	—	—	—	—	703	272
Experiment VI							
Unsolariized	Coker 310	0.93	0.703	84	82.4	4151	3946
	Acala SJ2	0.92	0.637	78	79.4	4161	3517
Solarized	Coker 310	0.69	0.048	127	4.7	80	4529
	Acala SJ2	0.79	0.038	124	3.3	79	3915
LSD (P = 0.05)	Soil treatments	—	—	—	—	1074	390
LSD (P = 0.05)	Cultivars	—	—	—	—	791	460

* Soil was solarized from 12 July to 2 September and from 26 July to 16 September 1988, for experiments V and VI, respectively.

^b Incidence (%) of plants affected by *Verticillium* wilt (Y) was determined at 2-week intervals from June to September, and regression analyses vs. time in days were performed. All coefficients of determination (R²) are significant at P = 0.01.

^c Slope of linear regression of disease incidence vs. time in days. Values for soil treatments within each cultivar and experiment are significantly different at P < 0.001. Values for cultivars within each soil treatment and experiment are not significantly different at P = 0.05.

^d Axis t intercept of linear regression vs time.

^e Average values for soil treatments are significantly different (P < 0.05). There were no significant effects by cultivar of cultivar × soil treatment interaction as determined by analysis of variance of arcsin (Y/100)^{1/2} transformed data.

^f Disease progress curves were drawn by plotting disease incidence values, and the area under these curves (AUDPC) was calculated. There was no significant cultivar × soil treatment interaction.

^g Determined by combine-harvesting. Average of four (experiment V) or three (experiment VI) replicate plots. There was no significant cultivar × soil treatment interaction.

Table 5. Long-term effect of soil solarization on the population of *Verticillium dahliae* in soil, development of *Verticillium* wilt and seed cotton yield of cotton wilt-tolerant cv. Acala GC 510 grown in 1990 after a crop of susceptible cotton cvs Acala SJ2 and Coker 310 in 1989^a

Soil treatment	Inoculum density after sowing (propagules/g dry soil) ^b		Final incidence (%) ^c		Seed cotton yield (kg/ha) ^d
	mean	CV(%)	foliar symptoms	vascular discoloration	
Experiment VII					
Unsolarized	84.0	33.4	25.8 a	71.3 A	2555 A
Solarized	13.0	36.0	12.4 b	53.5 B	2867 B
Experiment VIII					
Unsolarized	85.5	37.7	14.9 a	64.7 a	2375 a
Solarized	7.5	89.3	4.7 b	41.7 b	2684 b

^a Soil solarized from 12 July to 2 September (experiment V) and from 26 July to 16 September (experiment VI), 1988, was sown to *Verticillium* wilt-susceptible cotton cvs Acala SJ2 and Coker 310 in mid-March 1989. Plots were harvested in September 1989 and sown to cv. Acala GC 510 at the end of March 1990 (experiments VII and VIII).

^b Determined by means of an Andersen sampler in four 500-mg aliquots of soil collected from the 0–20-cm soil layer of each of four (experiment VII) or three (experiment VIII) replicate plots at the end of April 1990. CV = coefficient of variation.

^c Determined from plants in the two central rows of each plot that were affected by symptoms characteristic of *Verticillium* wilt at the end of September, 24 weeks after sowing. Vascular discoloration in the stem was determined in 50 consecutive plants. For each experiment, values followed by the same letter are not significantly different at $P = 0.05$ (lower case) or $P = 0.1$ (upper case) according to LSD test.

^d Determined by combine harvesting. Average of four (experiment VII) or three (experiment VIII) replicate plots. For each experiment, values followed by the same letter are not significantly different at $P = 0.05$ (lower case) or $P = 0.1$ (upper case) according to LSD test.

occurred for cultivars or cultivar \times soil treatment interactions (Table 4). Furthermore, there was a highly significant reduction in final disease incidence, which was in the range 3.3–12.9% in solarized plots compared with 74.6–85% in unsolarized plots (Table 4). However, neither cultivar nor soil treatment \times cultivar interaction had statistically significant effects ($P < 0.05$). The final incidence of plants showing vascular discoloration at harvest was 12–19.3% higher than that of foliar symptoms, regardless of cultivar and soil treatment, except in solarized plots of experiment V, for which the incidence of foliar symptoms was similar to that of vascular discoloration (data not shown).

Soil solarization determined a significant ($P < 0.05$) increase in seed cotton yield, which averaged 40.5 and 13.1% in experiments V and VI, respectively (Table 4). Although the effects of cultivar and cultivar \times soil treatment interaction on seed cotton yield were not statistically significant, there was a trend towards slightly

higher yields for cv. Coker 310 compared with Acala SJ2.

Combined long-term effect of soil solarization and cultivar tolerance on *Verticillium* wilt of cotton

The estimates of inoculum density of *V. dahliae* in soil in May 1990, about 1 month after sowing the second consecutive crop, were much higher (Table 5) than those made for the same plots the previous year (Table 3). Populations of *V. dahliae* in plots sown to cvs Acala SJ2 and Coker 310 in 1989 were very similar (e.g. 83.9 and 84.3 propagules/g soil in unsolarized plots, and 13.6 and 12.3 propagules/g soil in solarized plots, for plots sown to cvs Acala SJ2 and Coker 310 of experiment VII, respectively, regardless of the soil treatment and experiment. Therefore, mean values for the two cultivars were combined (Table 5). These estimates indicated that *V. dahliae* populations in soil unsolarized or

solarized in 1988 had increased by about 2.1–2.5- or 6.3–6.5-fold, respectively, after a susceptible cotton crop in 1989 (Tables 3 and 5). When the highly wilt-tolerant cv. Acala GC510 was grown in these plots in 1990, the final disease incidence in unsolarized plots was very low compared to that in susceptible cvs Acala SJ2 and Coker 310 grown the year before. By contrast, the final disease incidence in solarized plots was as high as that observed in those susceptible cultivars with much less initial inoculum (Tables 4 and 5). However, the incidence of plants showing vascular discoloration was much higher than when assessed by foliar symptoms, regardless of the experiment, soil treatment and cultivar (Table 5). Seed cotton yield was increased in plots that were solarized in 1988 compared to that in unsolarized plots, for both experiment VII (12.2%) and experiment VIII (13%) (Table 5).

DISCUSSION

Soil solarization has been shown to control a number of diseases caused by soilborne fungi, including *Verticillium* wilt (Katan, 1980; Pullman *et al.*, 1981a; Ben-Yephet *et al.*, 1988; Davis, 1990). The increased temperature in humid soils caused by solarization affects pathogen propagules directly, and may also result in enhanced activity of microbial antagonists in the soil (Katan *et al.*, 1976; Tjamos & Paplomatas, 1988).

Soil temperatures were not recorded in our experiments. Although air temperatures were available during the experiments, they could not be used for Mahrer's forecasting system to estimate soil temperatures because solar irradiation data were lacking. A daily maximum temperature of 35–39°C was recorded at a depth of 25 cm in another location of the Guadalquivir Valley, at a distance of 5 km from the experimental site, in 1989 and 1990 (J. M. M.-V. *et al.*, unpublished data), which is lower than the temperatures reported from several locations in California (Pullman *et al.*, 1981a), but within the range of those reported in a cooler climate in Idaho (Davis & Sorensen, 1986). Similarly, comparison of the maximum daily air temperatures during the periods of solarization with those reported from some areas of Israel and California indicate that the environment of the experiments reported here was usually less extreme in relation to thermal inactivation of fungal pathogens (Katan *et al.*, 1976; Pullman *et al.*, 1981a). Nevertheless, the present results show that soil solarization successfully

reduced the population of the defoliating pathotype of *V. dahliae* within at least 40 cm depth of heavily infested clay soils. This effect was consistent over a range of inoculum levels of the pathogen in soil, years and locations. The effectiveness of soil solarization under our conditions agrees with the results obtained by Ben-Yephet *et al.* (1988), who suggested that *V. dahliae* is highly sensitive to suboptimal temperatures during solarization. Similarly, the present results relate to those of Davis & Sorensen (1986), who effectively controlled *Verticillium* wilt of potato in a cool climate, even though *V. dahliae* was not significantly reduced within a soil depth of 15–30 cm. As the non-defoliating pathotype of *V. dahliae* is more temperature-sensitive at 37°C than the defoliating pathotype (Pullman *et al.*, 1981b), soil solarization should be a useful control measure for reducing *V. dahliae* in soils of the Guadalquivir Valley, which are highly infested mainly with either the defoliating or the non-defoliating pathotype (Blanco-López *et al.*, 1989; Bejarano-Alcázar, 1990).

The effect of solarization in reducing *V. dahliae* populations in soil resulted in a corresponding highly significant control of the disease in *Verticillium*-wilt-susceptible cotton cultivars grown the next season, regardless of differences in the environment conditions during the period 1987–89. Control was achieved by delaying the time of epidemic onset as well as by reducing the rate of disease progress, which brought about a large decrease in the final incidence of disease and in AUDPC values. Because of the strong effect of early infections by *V. dahliae* in reducing cotton yield (Pullman & DeVay, 1982b), the 2- to 7-week delay of epidemic onset in solarized soils observed in these experiments is of major significance in terms of reducing yield losses. In this study, no attempts were made to establish a quantitative relationship between initial inoculum density of *V. dahliae* and final *Verticillium* wilt incidence, or seed cotton yield. A significant non-linear correlation was found between initial inoculum density and final disease incidence in susceptible cotton cultivars (Pullman & DeVay, 1982a; Paplomatas *et al.*, 1992).

Two other beneficial effects, in addition to control of *Verticillium* wilt, were observed in the course of this study, including a good control of weeds (other than *Portulaca oleracea* and *Solanum* spp.) and of *Rhizoctonia* damping-off. Since *Rhizoctonia* damping-off occurs in most cotton-growing areas of the world, including

Spain (Melero-Vara & Jiménez-Díaz, 1990), the potential of soil solarization for its control should also be taken into consideration.

The overall effect of soil solarization in our experiments was a significant increase in seed cotton yield compared to that in unsolarized controls, which is in agreement with previous results (Pullman *et al.*, 1981a). However, the extent of this increase varied with differences between experiments in the various factors that influence *Verticillium* wilt development (El-Zik, 1985), including differences in the amount of initial inoculum (experiments III and IV), tolerance of cultivars (experiments V and VI with susceptible cvs Acala SJ2 and Coker 310 in 1989, as compared to experiments VII and VIII with highly wilt-tolerant cv. Acala GC510 in 1990), and favourableness of weather conditions for disease development (severe disease with low inoculum in experiment II associated with mild summer temperatures). Nevertheless, in four out of five experiments (Tables 1, 2 and 4) soil solarization resulted in a seed cotton yield of cv. Coker 310 above 4.5 t/ha, which is about 0.5–0.8 t/ha greater than the average seed cotton yield of this cultivar in the Lower Guadalquivir Valley (Guerrero, 1984).

The results reported here indicate that soil solarization in the Lower Guadalquivir Valley of southern Spain provides satisfactory control of *Verticillium* wilt caused by the defoliating pathotype of *V. dahliae*. However, this control measure does not seem to have a long-term effect under these experimental conditions. When a susceptible cotton cultivar was grown in solarized soils, the inoculum level of the pathogen for the next crop increased to an extent that would cause severe disease in susceptible cultivars. Although highly wilt-tolerant cultivars such as Acala GC510 would not be severely affected by such a high inoculum, they would allow for a twofold increase in the inoculum left for the following crop. Therefore, the use of soil solarization should be integrated with that of tolerant cotton cultivars and rotation with non-host crops in order to avoid a sustained increase in inoculum level in solarized soils.

ACKNOWLEDGEMENTS

This research was supported by grants INIA 7637 and DGA/JA 3621 from Instituto Nacional de Investigaciones Agrarias and Dirección General de Agricultura, Ganadería y Montes, Junta de Andalucía, respectively. The authors wish to

thank J. Cobos, F. Martínez, M. Riera, J. Romero and L. Saavedra for technical assistance and Cooperative Algodoneras Trajano and Trobal at Marismas for allowing us to use their field plots and facilities.

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