The effects of soil solarization and soil fumigation on fusarium wilt of watermelon grown in plastic houses in south-eastern Spain

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The effects of pre-planting solarization or fumigation with metham-sodium of sand-mulched soil on fusarium wilt of watermelon in plastic house culture were investigated at Almería, south-eastern Spain. In two trials, 2 months' solarization increased the average maximum soil temperature by c. 5 C to 44-48°C at 10 cm depth and by 4-5°C to 40-42°C at 20-30 cm. The amount of Fusarium oxysporum in the upper 15 cm of a naturally infested soil was reduced by solarization and by furnigation. During the 9 months following treatment, the F. oxysporum population stabilized at a low level in soil solarized for 2 months, but fluctuated in soil solarized for 1 month and increased in fumigated soil. The amount of wilt in watermelon sown into this soil after treatment was generally low; plants growing in solarized or fumigated soil suffered less wilt than plants in untreated soil but the differences were not significant. In a soil artificially infested with the highly pathogenic race 2 of F. oxysporum f. sp. niveum, F. oxysporum populations were greatly reduced following solarization or fumigation, and fluctuated erratically thereafter. Solarization for 2 months completely controlled wilt in watermelon and gave a fruit yield almost five times that of plants in untreated soil. Solarization for 1 month only slowed disease development slightly but gave a yield more than twice that in untreated soil. Fumigation with methamsodium retarded disease development considerably and tripled fruit yield. Plant performance was significantly better in soil solarized for 2 months than in uninfested control soil, suggesting beneficial effects of this treatment additional to wilt control.

INTRODUCTION

Almeria, in south-eastern Spain, now has the highest provincial income from agricultural production in the country as a result of the expansion of intensive horticulture in sand-mulched soil under plastic (polyethylene) houses. Sand-mulching is practised to increase soil temperature and the rate of plant growth. Natural soil is amended with manure, overlaid with a 10-cm layer of sand and planting is done just under the sand layer. The nutrient requirements of crops are met by standard fertigation practices. About 3800 ha of plastic houses are sown annually to watermelon (*Citrullus lanatus*) with an average yield of 35 t/ha amounting to 25% of the total plastic house area in Almeria (Anon, 1988).

Disease surveys of watermelons in Almeria during 1985-87 indicated that crops in 22-54% of plastic houses were affected by the wilt caused by Fusarium oxysporum f. sp. niveum, with an incidence of 10-100% (González-Torres et al., 1988). These surveys also showed that both races 0 and 2 of the pathogen were present, race 2 being the more prevalent. Race 2 is the most pathogenic of the three races of F. oxysporum f.sp. niveum described (Netzer, 1976; 1982). The extensive and repeated use of watermelon cultivars susceptible to fusarium wilt (such as cv. Sugarbaby), the prevalence of race 2, and the longevity of the pathogen in the soil have undoubtedly combined to make fusarium wilt a major limiting factor for watermelon production in Almeria.

Because commercially acceptable watermelon cultivars resistant to fusarium wilt are not available, control of the disease in Almeria is mainly by soil fumigation with methyl bromide or a methyl isothiocyanate + DD (1,2 dichloropropane-1,3 dichloropropene) mixture (Ditrapex) and/or the use of the resistant F_1 hybrid RS-841 rootstock. However, all these measures have disadvantages such as high cost, toxicity, recolonization risks and environmental hazards, and more acceptable control measures are needed. Soil solarization, a hydrothermal process in which heat from solar radiation is absorbed by moist soil beneath a thin layer of transparent plastic (Katan, 1981; Stapleton & DeVay, 1982), has proved effective for controlling fusatium wilt

plastic (Katan, 1981; Stapleton & Devay, 1982), has proved effective for controlling fusarium wilt in field-grown watermelon in Israel (Greenberger *et al.*, 1986) and in Texas (Martyn & Hartz, 1986). This paper describes experiments designed to compare the efficacy of soil solarization with soil fumigation in plastic houses at Almería for controlling fusarium wilt of watermelon. A summary of part of this work has been published elsewhere (González-Torres *et al.*, 1989).

MATERIALS AND METHODS

Two experiments (I and II) were carried out in 1985/86 and 1988/89, respectively.

Experiment I

A plastic house with a history of severe fusarium wilt of watermelon was used. Experimental plots ($6 \times 6 \text{ m}^2$) were treated in one of four ways: 1 solarized for 1 month (17 July-17 August); 2 solarized for 2 months (17 July-17 September); 3 fumigated with metham-sodium on 17 July; and 4 control. There were four replicates in a 4×4 latin square design with plots 1 m apart. Plots were first drip-irrigated to field capacity. Plots to be solarized were covered with transparent polyethylene sheets, 6 m wide and $37.5 \,\mu\text{m}$ thick. For fumigation, 4.3 1 of Vapam (40% methamsodium) were diluted with 25.71 of water and the mixture was uniformly distributed over each plot (equivalent to 1200 | Vapam/ha). Fumigated plots were irrigated with 12.5 l/m² immediately after treatment to provide a seal for the fumigant. Control plots had no cover or fumigant but otherwise were treated similarly to other plots. Except for access, the plastic house was closed during the 2-month solarization period. Maximum and minimum soil temperatures were recorded daily during the period 17 July-17 September 1985 in solarized and non-solarized plots at depths of 10 and 30 cm by means of maxmin soil thermometers. In mid-December, plots were sown to the watermelon cv. Resistent which is susceptible to fusarium wilt. Observations on disease development were made at 2-week intervals up to 5 June 1986.

Experiment II

In order to provide better conditions for disease development than in Experiment I (see Results), soil in a plastic house was fumigated with methyl bromide at a rate of 500 kg/ha in April 1988, and then artificially infested with a mixture of three isolates of race 2 of F. oxysporum f.sp. niveum. The inoculum was prepared by growing the pathogen in flasks of potato dextrose broth (PDB) continuously agitated on a rotary shaker with a 12 h/day photoperiod of fluorescent light (45.0 μ E/m².s) at room temperature (c. 24°C). After 10 days, the contents of the flasks were added to talcum powder (at the rate of 2 ml/g) which was then allowed to dry at room temperature. The infested talcum powder was spread (200 g/m) in furrows 15 cm wide, 10 cm deep and 4 m long (three per plot for four treatments, see below) in the plastic house, and the furrows were then covered with a 10-cm deep sand layer. In some plots a PDB/talc preparation was incorporated to provide uninfested control plots. Immediately after soil infestation, watermelon cv. Sugarbaby was sown in the infested strips to amplify the pathogen population in soil through natural disease development.

In June, after harvest, plant residues were removed and treatments were applied. The experiment consisted of five treatments: 1 soil solarized for 1 month (28 June-28 July); 2 soil solarized for 2 months (28 June-28 August); 3 soil fumigated with metham-sodium in early July: 4 infested control; and 5 uninfested control. There were four replicate plots ($6 \times 4 \text{ m}^2$) 1 m apart in a randomized complete block design. Irrigation, covering with plastic and fumigation were performed as for Experiment I. During solarization, the plastic house was again closed in order to promote soil temperature increase. Soil temperatures were recorded every 5 min in solarized and nonsolarized plots at depths of 10 and 20 cm by means of four thermistors linked to a data-logger. Mean hourly temperatures were determined from these data. In early April 1989, plots were sown to watermelon cv. Sugarbaby in three rows with two seeds at nine equally spaced stations. Plots were thinned 43 days after sowing. The percentages of wilted plants were recorded at 1-week intervals from 17 to 60 days after sowing. However, the increase in disease incidence was negligible after thinning, possibly as a result of the high temperatures prevailing in the plastic house. Therefore, the comparison of disease development is restricted to the period up to 38 days after sowing. Epidemic progress lines were computed by plotting percentage disease against time (transformed to natural logarithms). The influence of treatments on crop was assessed by the dry weight of apparently healthy plants removed when thinning the plots, as well as by the weight of fruits harvested per plot 3 months after sowing.

Estimation of F. oxysporum populations in experimental plots

The populations of F. oxysporum in treated and untreated soil of Experiments I and II were monitored over time. Six soil samples were collected with a shovel to a depth of 10-15 cm from each plot just before and immediately after the 2-month solarization period, and at 3-month intervals thereafter. The six samples from each plot were bulked, thoroughly mixed, and used for soil dilution plating. Different dilution factors were used depending on sampling date and treatment. A 1-g air-dried sample of soil was placed into a vessel containing 100, 150 or 200 ml of sterile 0.1% water agar and stirred in a blender for 1 min. One ml of this suspension was spread on each of three plates of the V8 juice-oxgall-PCNB agar medium selective for Fusarium (Bouhot & Rouxel, 1971). The plates were incubated at 26 C and a 12-h/day photoperiod of fluorescent light (45 µE/m².s¹) for 7 days. Colonies of Fusarium that grew on the selective medium were identified as F. oxysporum based on the presence of short monophialides bearing microconidia in false heads, chlamydospores, and characteristically shaped macroconidia (Booth, 1971).

RESULTS

Experiment I

Mean maximum and minimum daily temperatures at 10 cm depth in solarized soil were 5.4 C and 8.8° C higher, respectively, than those recorded at the same depth in non-solarized soil (Table 1). At 30 cm depth, mean maximum and minimum temperatures in solarized soil were 4.8 C and 4.3 C higher than at the same depth in non-solarized soil. Average minimum temperatures at 10 and 30 cm depths in solarized soil were similar to the average maximum temperatures in non-solarized soil at the same depths.

Estimates of F. oxysporum in soil showed high variability (Table 2), but some population trends are apparent. The overall mean population level of F. oxysporum in mid-July before treatments

Table 1. The effect of solarization on the mean maximum (T_{max}) and minimum (T_{min}) temperatures (°C) of soils in closed plastic houses at Almería, south-castern Spain in 1985 and 1988

Year of experiment	Soil treatment	Soil depth (cm)	T max ⁴	
1985	Control	10	42·3	32.8
	Solarized	90 10	30°8 7.7	30-8 A1-6
	Solarized	30	41-6	35-1
1988	Control	10	38 ∙7	32-0
		20	36 ·2	33·0
	Solarized	10	43-9	34-7
		20	40·0	36 I

^a Averages of temperature extremes during 2-month solarization periods, 17 July-17 September 1985 (recorded daily) and 28 June-28 August 1988 (recorded hourly).

was 367 propagules per g of soil. By September, the amounts of F. oxysporum in soil fumigated or solarized for 1 or 2 months were 19, 17 and 21% respectively of their initial populations (Table 2). However, the amount of the fungus in control soil had decreased to 29% during this period. During the next 9 months F. oxysporum declined further in soil that had been solarized for 2 months, apparently stabilizing at about 5% of the initial population. In soil solarized for 1 month, levels of F. oxysporum fluctuated between 8 and 25% of that before solarization, and in soil treated with metham-sodium F. oxysporum levels increased steadily during this time to 68% of the original estimate; amounts of F. oxysporum in control soil were between 39 and 67% of the pretreatment figure.

Symptoms of fusarium wilt did not develop until about 5 months after sowing, and 2 weeks after harvest. Because the spreading growth of plants made it difficult to identify plants individually, the severity of disease was assessed by the percentage of foliage showing wilt symptoms. At the final assessment on 5 June, mean disease severities in plots solarized for 1 or 2 months ware 28.1% and 25.0% respectively, compared with 40.6% in plots fumigated with metham-codium and 46.9% in untreated controls. However, the differences in mean disease severity were act statistically significant, as indicated by the LSD (P=0.05) test.

	1985			1986		
Treatment	17 July ^b	17 Sept.	20 Dec.	21 March	20 June	
Control	400	117	250	267	156	
Soil solarization (1 month)	400	67	33	100	44	
Soil solarization (2 months)	400	83	33	17	22	
Soil fumigation (metham-Na)	267	50	100	167	181	
C.V. (%)	62	103	41	80	106	

 Table 2. Estimates of Fusarium oxysporum in naturally infested soil in a plastic house before and after various treatments

^a Means of four replicate plots.

^b Determined just before treating soil.

 Table 3. Estimates of Fusarium oxysporum in artificially infested soil in a plastic house before and after various treatments

	Colony-forming units of F. oxysporum per g of soil ^a				
	1988			1989	
Soil/Treatment	28 June ^b	28 Sept.	30 Dec.	6 April	3 July
Uninfested/control	100	258	567	500	517
Infested/control	1500	317	767	350	367
Infested/soil solarization (1 month)	1888	109	300	617	133
Infested/soil solarization (2 months)	1813	17	67	283	333
Infested/soil fumigation (Metham-Na)	2088	58	67	50	150
C.V. (%)	28	55	60	59	88

^a Means of four replicate plots.

^b Determined just before treating soil.

Experiment II

Mean maximum and minimum hourly temperatures at 10 cm depth in solarized soil were 5.2 C and 2.7°C higher, respectively, than those recorded at the same depth in non-solarized soil. At 20-cm depth, mean maximum and minimum hourly temperatures in solarized soil were 3.8° C and 3.1° C higher respectively than in the corresponding non-solarized soil (Table 1).

Estimates of F. oxysporum were again highly variable (Table 3). The overall mean population density of F. oxysporum in the artificially infested plots was about 1800 propagules/g soil before treatment. In infested, untreated plots there was a decline to 21° in the level of the pathogen from the mean initial value of these plots by September 1988; the amounts of *F. oxysporum* had increased substantially by December and returned to the September 1988 level in 1989. There were more marked reductions in pathogen levels following two soil solarization and fumigation treatments (to 6. 1 and 3% of the original populations). In soil receiving the shorter solarization treatment, the population fluctuated markedly thereafter; in soil solarized for 2 months, it increased steadily; while in soil treated with metham-sodium, it remained low until April 1989 and increased threefold by July. There was a significant level of *F. oxysporum* before treatments in soil which had



Fig. 1. The effect of soil solarization or fumigation with metham-sodium on the development of fusarium will in watermelon cv Sugarbaby in a plastic house artificially infested with *Fusarium oxysporum* f. sp. *nucum* race 2. Linear regression of disease incidence $\binom{9}{1}$ over ln of time period after sowing for plots untreated (\blacksquare) , fumigated (\Box) or solarized for 1 (+) or 2 (*) months.

not been deliberately infested and this increased steadily in 1988 to a value which was maintained through 1989.

No disease developed in plants growing in soil solarized for 2 months or in plants growing in uninfested, untreated soil. Fusarium wilt progressed rapidly in plants in the infested untreated soil, reaching a mean incidence of 34.5% at 38 days after planting. Thereafter, disease increase was negligible. There was a good linear relationship between disease progress and log-transformed time after sowing for the three treatments in which wilt developed (coefficients of determination 0.96-0.97) (Fig. 1). Fumigation with metham-sodium reduced the rate of disease progress to 32% of that in the artificially infested control, and the disease incidence at 38 days was 9.6%. Solarization for 1 month reduced the rate of disease progress to 79% of that in the infested control, and the mean disease incidence at the final record was 24%. The levels of disease attained in the various treatments were reflected in the weight of thinnings and the yield of fruits (Table 4). Soil solarization for 2 months was the most beneficial treatment for cropping. It significantly increased yield as well as the dry weight of thinnings compared with both infested and uninfested controls. The effects of solarization for 1 month were not statistically significant but this treatment gave a yield more than twice that of untreated infested soil and similar to that of the uninfested control. A significant increase of both the weight of thinnings and yield resulted from fumigation with metham-sodium as compared with that of the infested control, but these increases were lower than those resulting from 2 months' solarization (Table 4).

DISCUSSION

Soil solarization has given control of a number of diseases caused by soilborne fungal pathogens including vascular wilt fusaria (c.g. Katan, 1980; Pullman *et al.*, 1981; Stapleton & DeVay, 1982; Martyn & Hartz, 1986). The heating caused by solarization affects pathogen propagules directly and may also result in enhanced activity of microbial antagonists in soil (Katan *et al.*, 1976; Tjamos & Paplomatas, 1988).

In the experiments described here, soil solarization caused substantial and sustained increases of soil temperature at 10 and 20-30 cm depth in The temperature increases houses. plastic recorded are similar to those reported under field conditions in Israel (Greenberger et al., 1986) and Texas (Martyn & Hartz, 1986), and higher than those recorded in South Australia (Walker, 1989). This soil heating controlled fusarium wilt and increased yield of watermelon in the second experiment described here. The incidence of wilt that developed in cv. Sugarbaby in infested control plots in Experiment II was much lower than that obtained by Martyn & Hartz (1986) with the same cultivar in a similar trial in Texas. However, the level of soil infestation achieved by Martyn & Hartz (1986) was about seven times that achieved here. The complete disease control obtained in Experiment II with 2 months' solarization, compared with the delayed wilt development and reduced final disease incidence observed by Martyn & Hartz (1986), may be explained in the same way. The control of wilt achieved in this experiment was associated with a significant increase in plant growth and yield of watermelon. These beneficial effects are probably more than can be explained by control of fusarium wilt alone, as suggested by the significant improvement of plant performance over the uninfested control (Katan, 1981, Stapleton & DeVay, 1982). Similar effects were obtained in

Soil/Treatment	Dry weight (g) of watermelon plants ^a	Yield/plot (fruit fresh weight kg)		
Uninfested/control	111.9	16.6		
Infested/control	78-2	65		
Infested/soil solarization (1 month)	116-2	15-1		
Infested/soil solarization (2 months)	213.9	32.0		
Infested/soil fumigation (metham-Na)	178-2	21.9		
LSD ($P = 0.05$)	75 -7	12.5		

 Table 4. The effects of pre-planting treatments of soil artificially infested with race 2 of

 Fusarium oxysporum f. sp. niveum on growth and yield of watermelon cv. Sugarbaby in a

 plastic house at Almeria, southeastern Spain

^a Thinnings (15 plants) removed from each plot 43 days after planting.

solarization experiments in Israel (Greenberger *et al.*, 1986). By contrast, solarization for 2 months did not control fusarium wilt of watermelon satisfactorily in South Australia, where several plant parasitic nematodes occurred together with the wilt pathogen and relatively low temperatures prevailed during the solarization period (Walker, 1989).

Fumigation with metham-sodium was less effective for wilt control than 2 months' solarization, but more effective than 1 month's solarization, although yields from the two treatments were not significantly different. The relatively poor performance of metham-sodium can be attributed to high soil temperatures (over 30 C) when soil was treated in the summers of 1985 and 1988. By contrast, methyl bromide fumigation was carried out in April 1988, when soil temperatures were lower and more conducive to efficient disinfestation. In addition, the sealing of soil with a plastic cover after methyl bromide treatment provides more effective containment of the fumigant than the water seal used with the methamsodium treatment.

In contrast to Experiment II, no effects of soil treatments were detected in Experiment I against a background of slow disease development and low incidence. Disease was unlikely to have been limited by soil inoculum concentration, since a similar level was associated with 95-100% wilt incidence in cv. Sugarbaby in Israel (Netzer, 1976). A more likely explanation is the time of planting: fusarium wilt would be more favoured by the higher temperatures prevailing at planting time in Experiment II (April) than in Experiment I (December).

Results of analysis of F. oxysporum in soil were highly variable, and they give only an indication of the effect of treatments on the fungus. This problem was compounded by the lack of information on the relative proportion of pathogenic and non-pathogenic F. oxysporum isolates obtained on soil plates, and by the possibility that some isolates could be antagonistic to the disease (Lemanceau & Alabouvette, 1991). F. oxysporum sensu lato in naturally infested soil was reduced to c. 20% by soil solarization for 1 or 2 months in Experiment I. Greater destruction of the pathogen by soil solarization was achieved in artificially infested soil. This may be explained by the relatively superficial location of inoculum in the soil in the latter case and or by a greater sensitivity of the artificial inoculum. Similar reductions of inoculum in artificially infested microplots were reported by Martyn & Hartz (1986). The erratic fluctuations of F. oxysporum populations after solarization may reflect the influence of roots of susceptible watermelon plants which were growing at the time of the later soil samplings. Martyn & Hartz (1986) observed a significant increase of the fungus 8 months after solarization, which they attributed to the formation of multiple chlamydospores.

The results reported here indicate that soil solarization in sand-mulched plastic houses can provide satisfactory control of fusarium wilt of watermelon under the climatic conditions prevailing in south-eastern Spain. Furthermore, following these experiments the practicality and efficacy of soil solarization for watermelon growers have been demonstrated in non-replicated trials in commercial plastic houses (R.G.-T. *et al.*, unpublished data): control of wilt following solarization was associated with an average yield increase of 17 t/ha over an average yield of 38 t ha in untreated plastic houses.

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