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Full Length Research Paper

Effects of soil solarization and some amendments to control verticillium wilt in established olive orchards

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The effects of solarization treatments, alone or with organic amendments and urea against verticillium wilt on olives were studied. Trials were carried out during the 2005 - 2007 seasons in an olive orchard consisting of 700 4 - 6 year old trees (cv Gemlik) in Germencik region of Aydin. Five treatments were carried out: Solarization (S), solarization + chicken manure (SCM) (1 kg m^{-2}), solarization + olive processing waste (SOPW) (2 kg m^{-2}), solarization + urea (SU) (100 g m^{-2}) and an untreated control (C). Maximum soil temperatures reached in solarized plots were 54.7 and 43.9°C and in the non-solarized plots were 44.4 and 37.4°C at 5 and 20 cm soil depth, respectively. At the end of two years, the disease incidence and severity index decreased to zero in the treatments that included S. In the SCM treatment and in the control, the highest severity index (8.0) was recorded while the disease incidence slightly decreased from 66.7 to 44.4%. In SOPW plots, the disease severity index decreased to 2.0 and the disease incidence remained the same. In SU plots, the disease severity index decreased from 5.6 to 4.5 and disease incidence slightly decreased to 44.4%. As a result, the trees treated with solarization alone and solarization with olive processing waste showed an increased recovery and symptom remission compared with the initial disease severity and incidence.

Key words: *Olea europaea*, *Verticillium dahliae*, amendments.

INTRODUCTION

In Turkey, like in most Mediterranean countries, olive (*Olea europaea* L.) is a traditional, high value woody crop with an estimated 10 million trees. Aegean region (western Turkey), which contains 56% of olive trees in the country, supplies 80% of olive oil and 20% of pickled olive production of Turkey (Anonymous, 2006). Verticillium wilt of olive caused by *Verticillium dahliae* (Kleb.) was first detected in Turkey in 1972 (Saydam and Copçu, 1972). Until the 1990's, the disease was not so wide-spread in the olive growing areas of Turkey. However, the establishment of new orchards in fields previously cropped with cotton and the use of infected propagating material caused widespread of olive wilt throughout Aegean region (Benlioğlu et al., 2001; Çelebi and Benlioğlu, 2004). Recent surveys showed that the disease was widespread in 5 major olive growing provinces (Aydın, Çanakkale, İzmir, Manisa, and Muğla) of Turkey and the disease incidence in these areas were 0.8 and 1% in 1998 and 1999, respectively, (Yolageldi et al., 2003). The disease also limited the production of high yielding and high quality olive cultivars grown extensively and intensively in the Mediterranean basin countries (Thanassoulopoulos et al., 1979; Rodriguez-Jurado et al., 1993; Al-Ahmad and Mosli, 1993; Serrhini and Zeroual, 1995; Levin et al., 2003a).

There are no effective control measures for verticillium infected trees. The most effective management strategies to protect trees from verticillium wilt are those taken before planting. Soil solarization is a pre-planting technique in reducing inoculum density of soil-borne pathogens

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Abbreviations: S, Solarization; **SCM,** solarization and chicken manure; **SOPW,** solarization and olive processing waste; **SU,** solarization and urea; **C,** control; **MS,** microsclerotia.

such as *V. dahliae* and controls verticillium wilt in annual plants (Katan and DeVay, 1991). However, there are alternative attempts of soil solarization to control verticillium wilt of woody plants (Ashworth and Gaona, 1982; Stapleton et al., 1993). Similarly, Tjamos et al. (1991) suggested that olive trees with verticillium wilt could be recovered after soil solarization of individual 10- to 15-year-old trees. In a study conducted in three commercial olive orchards infested with *V. dahliae* in southern Spain, soil solarization did not result significant differrences in disease incidence and severity, but improved recovery of trees from the disease in orchards with low inoculum densities while the disease severity was reduced only in orchards with medium or high initial inoculum densities (Lopez-Escudero and Blanco-Lopez, 2001).

Another approach to improve the efficiency of solarization is to combine organic or inorganic amendments with the soil solarization (Stapleton et al., 1991; Gamliel and Stapleton, 1997). Animal manure, specifically chicken manure and composted plants combined with solarization resulted in few degrees higher soil temperatures compared to unamended soil solarization (Gamliel and Stapleton, 1993; Benlioğlu et al., 2005). Tenuta and Lazarovits (2002) demonstrated that ammonia and nitrous acid from nitrogenous amendments kill the microsclerotia of *V. dahliae*. In microcosmos, addition of meat and bone meal (2.5%) to an acidic loamy sand resulted in the accumulation of ammonia and death of microsclerotia within 2 weeks. At lower concentrations (0.5 and 1%), microsclerotia were killed after 2 weeks when nitrous acid accumulated (> 0.03 mM). In an alkaline loam soil, microsclerotia survived at 3% meat and bone meal and neither ammonia nor nitrous acid accumulated. The toxicity of ammonia to the pathogen was verified by increasing the concentration of meat and bone meal to 4% or addition of urea (1,600 mg of N per kg) to the loam soil resulting in the accumulation of ammonia (> 35 mM) and death of microsclerotia. The form of N, either NO_3 or NH_4, is also important. Tenuta and Lazarovits (2004) also studied the effectiveness of a nitrogenous organic amen-dment to kill microsclerotia of *V. dahliae* in several soils and the soil properties associated with this effectiveness. They found that NH_3 is effective in killing *V. dahliae* microsclerotia only in soils where it accumulates above the concentration of 25 mM. HNO_2 is also able to kill microsclerotia, but in this case, the soil pH has to be acid.

The purpose of the study was to investigate the effects of chicken manure, oil processing waste and urea in combination with solarization and alone to control verticillium wilt in an established olive orchard.

MATERIALS AND METHODS

Plot establishment and disease assessment

This study was conducted in an olive orchard, which had been previously used as a cotton field, with 700 4 - 6-year-old trees (cv Gemlik) planted 6 x 6 m in Germencik town of Aydin province, Turkey. The orchard soil was sandy loam with a pH of 7.5 and similar in the orchard. The orchard was cultivated by grower using standard cultural practice. Before designing experiments, all trees in orchard were visually inspected for the verticillium wilt symptoms. Before treatments, disease severity of each infected tree in the plots was evaluated based on a scale of 0 to 5; 0 = healthy tree; 1 = up to 25% of tree with symptoms, including chloritic leaves and dead twigs; 2 = up to 50% of tree affected, including symptoms on lateral branches; 3 = up to 75% of tree affected including most branches; 4 = up to 95% of tree affected; 5 = tree dead (Levin et al., 2003b). After visual inspection and laboratory confirmation, 45 infected trees of similar age and rated as 1-3 were selected for treatments in the orchard. A randomized experimental design with 5 treatments and nine replicates per treatment are detailed in Table1.

Once trees are classified according to this rating, infected branches were removed before treatments (18/July/2005). After pruning the trees, they were classified as symptomless (Levin et al., 2003b).

In 2005 and 2006 all trees were pruned to remove dead and dying branches in February. Trees were evaluated two times (on 28 November 2005 and 9 July 2006) in the first year and finally at the end of the second year (on 12 July 2007). After each evaluation, samples of diseased trees were confirmed by pathogen isolations from affected twigs during the observation period. Isolations from shoots and branches were also made from all dead plants at the end of the experiments. Pieces of affected tissues were washed in running tap water, bark was removed and woody tissues surface disinfected in 0.5% sodium hypochlorite for 1 min. Chips of wood were placed onto potatoe dextrose agar (PDA). Plates were incubated at 24°C in the dark for 5 - 6 days (Lopez-Escudero and Blanco-Lopez, 2001). The severity index (on a scale of 2 - 10) took into consideration the number of diseased trees with the different levels of symptoms and was calculated as: SI = [(number of trees in level 1 X 2) + (number of trees in level 2 X 4) + (number of trees in level 3 X 6) + (number of trees in level 4 X 8) + (number of trees in level 5 X 10)] / total diseased trees (Levin et al., 2003b). Disease incidence was determined as the percentage of plants with above ground wilt symptoms.

Treatments

At the onset of the study, shallow trenches were dug around each tree trunk having about 6 X 6 m dimensions. Soil surfaces around the trees were cleared from weeds, debris and stones to avoid damaging plastic sheets and then the soil was saturated with water at a rate of 350 - 500 l $tree^{-1}$ (40 - 50 cm deep). When solarization was applied alone, the area surrounding each tree was covered with transparent 6 X 6 m 100 µm polyethylene sheets tightly sealed both near the trunks and around the edges (Tjamos et al., 1991). When solarization was combined with other treatments, the amendments were spread over the soil surface and incorporated into soil and covered with polyethylene as described above. Control plants were only watered at the same rate. During the study period, soil temperatures were recorded hourly by thermocouples connected to a data logger (Hobo) at a depth of 5 and 20 cm. To avoid dissemination of microsclerotia of *V. dahliae* from untreated to soil solarization treatments, herbicides were used to control weeds and no tillage was done. Solarization treatments were completed between July 15 and September 1, 2005.

Estimation of microsclerotia

The population density of *V. dahliae* in soil was determined twice,

Table 1. Treatments, disease incidence and severity of the trees in each experimental plot at the onset of the trial.

Treatments[1]	Disease rating			Replicates	SI[2]	DI[3]
	1	2	3			
S (45 days)	5	3	1	9	3.1	100
SCM (1 kg m^{-2})	6	2	1	9	2.9	100
SOPW (2 kg m^{-2}) m2)	6	2	1	9	2.9	100
SU (100 g m^{-2})	6	2	1	9	2.9	100
C	7	2	-	9	2.4	100

[1]S, Solarization alone; SCM, solarization with chicken manure; SOPW, solarization with olive processing waste; SU, solarization with urea; C, untreated control (nonsolarization), [2]SI, severity index; [3]DI, disease incidence.

Table 2. Soil temperatures recorded in solarized and nonsolarized plots in 2005.

Solarization period	Depth (cm)	Soil Temperature (°C)						
		Solarized plots		Nonsolarized plots		Cumulative hours in solarized plots		
		Mean	Max	Mean	Max	> 40°C	> 45°C	> 50°C
15 July - 1 Sept. 2005	5	36.8	54.7	32.7	44.4	503	331	182
	20	35.9	43.9	32.0	37.4	335	0	0

immediately after solarization and 24 months later. Soil subsamples were taken from the surface and 20 cm below the surface with a cylindrical (4 x 25 cm) auger, at 75 - 100 cm from the trunk. The subsamples were taken four side of each tree. Each sample consisted of the mix of 36 subsamples collected from the 9 trees of each treatment. Soil samples from each treatment then were air-dried for 4 weeks at room conditions. The soil was assayed by the modified Anderson sampler technique, with plating on petri plates containing NP-10 sodium polypectate agar selective medium. Briefly, air-dried soil was passed through a 435 µm sieve. Sieved 10 g of soil from each sample was placed in a screw capped plastic vial containing 2.5 ml of a DL- methionine (to break fungistasis and to significantly improve detection percentages of microsclerotia) solution (0.0075 g ml^{-1}). The vials were capped and incubated at 30°C for a week and air dried at room temperature (23 ± 1°C) for a week. All air dried soil from the vial was poured into a mortar and pulverized gently to break clods. The pulverized 100 mg soil from each treatment was distributed onto five petri plates each, containing NP 10 semi-selective medium. The plates were incubated for three weeks in the dark at 23±1°C. Following incubation, the surface of each plate was gently washed under a stream of water to remove soil. The numbers of microsclerotial colonies of *V. dahliae* on each plate were counted under a stereoscope at x 10 to x 20. The density of microsclerotia (MS) was expressed as the number of propagules per gram of dry soil (Kabir et al., 2004). Data were first analyzed by analysis of variance, followed by mean separation using Fisher's protected least significance difference test. All analysis was performed with the JMP IN program (SAS Institute, Cary, NC, release 5.1) at P ≤ 0.05.

RESULTS

Soil temperatures

Maximum soil temperatures were 54.7 and 43.9°C in solarized plots and 44.4 and 37.4°C in non-solarized plots at 5 and 20 cm depth of soil, respectively. The maximum soil temperatures recorded in the upper 5 and 20 cm of soil around the unshaded parts of trees were 10.3 and 6.5°C higher in solarized plots than in nonsolarized plots (Table 2). The daily maximum soil temperatures in tarped and nontarped soil from July 15 to September 1 in 2005 are presented in Figure 1. To estimate the thermal dose accumulation during the solarization period, the calculated cumulative hours of temperatures greater than 40, 45 and 50°C in solarized plots at 5 and 20 cm depth of soil are shown in Table 2. The cumulative hours of temperatures greater than 40, 45 and 50°C in solarized plots at 5 cm depth were 503, 331 and 182, respectively. However, the cumulative hours of temperatures greater than 40°C at 20 cm depth were 335 while there was no cumulative hour of temperature greater than 45 and 50°C.

Estimation of microsclerotia

Viable microsclerotia counts proved that soil solarization almost completely destroyed the natural population of microsclerotia at the upper 20 cm of soil around the treated trees while 20 microsclerotia per gram of nonsolarized soil remained viable (Table 3). However, the values of microsclerotia recorded 24 months later in the solarization (S), solarization + olive processing waste (SOPW) and solarization + urea (SU) treated plots increased slightly but remained significantly lower than

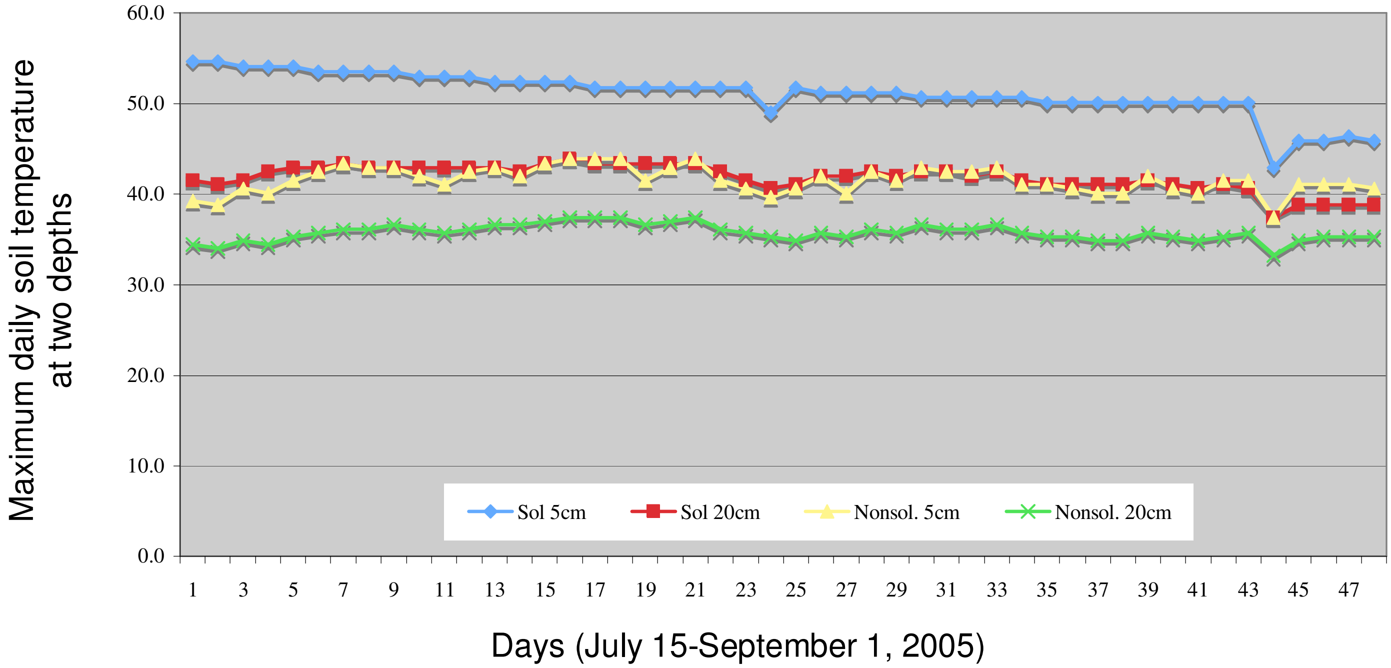

Figure 1. Maximum daily soil temperatures at two depths in solarized and nonsolarized plots at Germencik town of Aydin province from 15 July to 1 September 2005.

Table 3. Effect of various treatments on microsclerotia population density after solarization.

Treatments	Mean microsclerotial density at record dates (propagules/g soil)	
	September 2005	June 2007*
S	0	0.6 c
SCM	0	4.2 b
SOPW	0	0.8 c
SU	0	1.2 c
C	20	14.2 a

*Values followed by the same letter are not significantly different at P=0.05 according to Fisher's protected least significant difference test.

those in solarization + chicken manure (SCM) treated and untreated control plots.

Disease progress

Before the experiment started, the disease severity index averaged 2.9 for SCM, SOPW and SU, 3.1 for S and 2.4 for C plots while the initial disease incidence was 100% in all plots (Table 4). After 45 days solarization period, the disease severity index slightly decreased in solarization plots except untreated control and the disease incidence decreased to 55.5, 33.3, 55.5, 22.2 and 33.3% in plots S, SCM, SOPW, SU and C, respectively. Immediately after pruning diseased branches in January 2006, trees were considered symptomless again. Seven months later, the severity index increased to 3.3, 7.0, 4.0, 5.6 and 5.6 in plots S, SCM, SOPW, SU and C, respectively. But the disease incidence decreased from 55.5 to 33.3% in S and SOPW treatments. A year after the third pruning of diseased branches (January 2007), the disease incidence and severity index decreased to zero in S treatments. In SCM and C, the highest disease severity index (8.0) was recorded while the disease incidence slightly decreased from 66.7 to 44.4%. In SOPW plots, the severity index decreased to 2.0 and the disease incidence remained the same. In SU plots, the severity index decreased from 5.6 to 4.5 and disease incidence slightly decreased to 44.4%. At the end of the second year, the trees treated with solarization plus olive processing waste and alone showed an increased recovery and symptom remission compared with the initial disease severity and incidence.

DISCUSSION

Mediterranean climate is characterized as extremely hot and dry in the summer. Also, the summer temperatures are less variable than winter temperatures. In our soil solarization experimental plots, the maximum soil temperatures recorded at 5 and 20 cm depth of covered soil

Table 4. Incidence and severity of *V. dahliae* in cv. Gemlik in four solarization and nonsolarization plots during 2005-2007.

Treatments[1]	28.11.2005		09.07.2006		12.07.2007	
	SI	DI (%)	SI	DI (%)	SI	DI (%)
S	2.4 a	55.5	3.3 b	33.3	0.0 b	0.0
SCM	2.0 a	33.3	7.0 a	66.7	8.0 a	44.4
SOPW	2.0 a	55.5	4.0 b	33.3	2.0 ab	33.3
SU	2.0 a	22.2	5.6 ab	55.5	4.0 ab	44.4
C	4.0 a	33.3	5.6 ab	66.7	8.0 a	44.4

*Values followed by the same letter are not significantly different at P = 0.05 according to Fisher's protected least significant difference test.[1]S, Solarization alone; SCM, solarization with chicken manure; SOPW, solarization with olive processing waste; SU, solarization with urea; C, untreated control (nonsolarization); [2]SI, severity index; [3]DI, disease incidence.

were 54.7 and 43.9°C. The soil temperatures reported in our study is in keeping with previous results regarding the eradication of populations of *V. dahliae* in soil (Katan, 1980; Pullman et al., 1981; Ashworth and Gaona, 1982). Similarly, in a solarization experiment conducted in established olive orchards in Greece, the maximum soil temperatures in covered soil were recorded as 58 and 48°C at depths of 10 and 20 cm, respectively (Tjamos et al., 1991). In another solarization experiment carried to control verticillium wilt of olive in Spain, Lopez-Escudero and Blanco-Lopez (2001) reported that the maximum soil temperatures at depths of 15 cm were 45 and 40.7°C in 1995 and 1996, respectively.

The efficacy of soil solarization is dependent on the thermal dose (Pulman et al., 1981). Microsclerotia of *V. dahliae* are sensitive to moist heat. At a constant temperature of 36°C or greater for 41 days, this treatment will result in at least percentage mortality of microsclerotia in soil (Pullman et al., 1981; Katan and DeVay, 1991). In our study, the cumulative hours of temperatures above 40°C at 5 and 20 cm depth of soil were calculated as 503 and 335 h, respectively, (Table 2). This is in the range of temperatures necessary to eradicate or reduce populations of *V. dahliae* in soil (Katan, 1980; Pullman et al., 1981; Pinkerton et al., 2000). Indeed, we observed that the populations of microsclerotia at 20 cm soil depth were almost eradicated after 45 days solarization (Table 3). However, viable microsclerotia were detected in all solarized plots after two years. The fact that we have observed some increase in microsclerotia counts in solarized treatments could be attributed to microsclerotia originally located on the shady side of the trees and reinfestation of the soil with olive leaves from diseased twigs (Tjamos et al., 1991). However, significant increase of populations of microsclerotia in SCM treated plots compared to the other solarized plots during the second year after solarization could be due to the soil pH of our experimental orchard. Conn and Lazarovits (2000) reported that the efficacy of swine manure to kill *V. dahliae* microsclerotia was mainly dependent upon soil pH, and in acidic soils swine manure killed *V. dahliae* within a day after application, but had no effect in neutral or alkaline soils. Taking into consideration of the increase of inoculum density, the disease severity index was also found to be very high in SCM plots (Table 4). *V. dahliae* microsclerotia die within 7 days when in the presence of soils amended with high nitrogen-containing organic amendments, even though they were suspended in the head space above the soils. Thus, rapid death of MS results from the release of toxic volatile gases from the organic product. When the soil and amendments are sterilized, MS are unaffected indicating that the gases are derived from microbial degradation of the organic material (Tenuta and Lazarovits, 1998). Lopez-Escudero and Blanco-Lopez (2007) suggested a positive relationship between the initial inoculum density of *V. dahliae* and wilt disease progression in olive plants after a long time, and they also proved that inoculum densities greater than 3 propagules in the soil resulted in final disease incidence greater than 50% for the trees after 2.5 years.

Amendments used in our study did not show improvements over solarization in controlling disease progress and recovery. However, application of soil solarization to 6-year-old olive trees showing verticillium wilt symptoms at different severity levels (five 25%, three 50% and one 75%) resulted in complete recovery and symptom remission by corresponding to a significant reduction in inoculum density. Similar results were obtained from the soil solarization of individual olive trees of which the initial disease index was low (25% mild symptoms) in Greece (Tjamos et al., 1991). In four field experiments carried out during 1994 to 1997 in Spain, Lopez-Escudero and Blanco-Lopez (2001) reported that decrease inoculum density in soil by solarization did not correspond to a similar reduction in disease severity and soil solarization did not result in significant differences in disease incidence and severity, but improved recovery of trees from the disease in orchards with low inoculum densities.

Most importantly, there are currently no effective control measures available for verticillium wilt in established olive groves. Soil solarization and its combination with organic amendments could be effective tool not only to kill microsclerotia in soil but also reduce weed population in the olive orchards. We know that diseased trees are also able to recover and escape the disease after pruning, provided that future reinfestation through the roots can be avoided (Tjamos, 1991) and recovery from verticillium wilt is an important natural mechanism occurring in a high percentage of infected olive trees (Lopez-Escudero and Blanco-Lopez, 2005). Further investigations should be necessary to find the effective combination of amendments with soil solarization in terms of integrated control of verticillium wilt of olive.

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