Scientific Research and Essays Vol. 5(12), pp. 1561-1565, 18 June, 2010
Available online at http://www.academicjournals.org/SRE
ISSN 1992-2248 ©2010 Academic Journals

Full Length Research Paper

Resistance of olive cultivars to *Verticillium dahliae*

M. Sesli[1]*, E. Onan[2], S. Oden[1], H. Yener[3] and E. D. Yegenoglu[4]

[1]College of Tobacco Expertise, Celal Bayar University, Republic of Turkey, 45210 Akhisar, Manisa, Turkey.
[2]Alasehir Vocational College, Celal Bayar University, Republic of Turkey, Alasehir, Manisa, Turkey.
[3]Sarigol Vocational College, Celal Bayar University, Republic of Turkey, Sarigol, Manisa, Turkey.
[4]Akhisar Vocational College, Celal Bayar University, Republic of Turkey, 45210 Akhisar, Manisa, Turkey.

Accepted 21 May, 2010

Seventeen important olive cultivars have been evaluated for resistance to *Verticillium dahliae* under controlled conditions. One-year-old nursery olive plants were inoculated with cotton defoliating (D) (Mn 16) isolate (VCG1) of V*. dahliae*. Resistance was evaluated by assessing symptom severity using a 0 - 4 rating scale and estimating the area under disease progress curves. Besides, additional parameters for including cultivars into a defined category were used such as the percentage of plants killed and those which recovered from the disease. All cultivars were susceptible to isolate of V*. dahliae* at different levels. 'Wild 6' and 'Gemlik 2' were moderately susceptible to isolate of V*. dahliae*. Their resistance was evident by the plant ability to recover from infection with isolate.

Key words: *Verticillium* wilt, olive, root dip inoculation, resistant cultivars.

INTRODUCTION

Wilting disease of olive caused by *Verticillium dahliae* Kleb. is widespread wherever this crop is grown. Verticillium wilt of olive was first reported in Turkey in 1970 (Saydam and Copçu, 1972), and subsequently in many other European countries (Cirulli, 1975, 1981) and the Middle East and the USA (Jiménez-Diaz et al., 1998). The disease is most damaging on young plants, while on older plants, including those over 100-year-old, the disease does not normally kill the plant, but reduces vegetation and causes partial defoliation of one or more branches.

There has been a remarkable increase in the olive tree inventory in recent years in Turkey. Verticillium wilt of olive has been seen more widely and severely since last 15 - 20 years especially in areas where cotton, tomatoes, peppers and eggplants were planted previously (Provincial Directorate of Agriculture, 2005; MOARA, 2006).

In commercial olive orchards, natural recovery has been observed as a phenomenon that could play an important role in overcoming seasonal infections by *V. dahliae*, especially in young olive orchards, being more prevalent in soils with lower inoculum densities of non-defoliating (ND) pathotypes (Blanco-Lopez et al., 1990; Lopez-Escudero and Blanco-Lopez, 2001; Martos-Moreno et al., 2001). There has been a spread risk of defoliating (D) isolates of the pathogen throughout new olive orchards (Lopez-Escudero and Blanco-Lopez, 2001; Lopez-Escudero et al., 2004).

At present, control is based essentially on preventive methods such as the use of pathogen-free plants and soil when planting new olive orchards. Chemical fungicides such as benzimidazoles are not effective (Biris and Thanassoulopulos, 1980; Cirulli, 1981). Soil solarization with the crop in progress, whether alone or followed by applications of the natural antagonist *Talaromyces flavus* (Klöcker) Stolk Samson to the soil, has been fairly effective in containing the disease (Skoudridakis and Bourbos, 1989; Tjamos et al., 1991; Tjamos, 1993; Lopez-Escudero and Blanco-Lopez, 1997).

Use of resistant olive varieties are the most effective, economically feasible and ecologically sustainable means of control. Research in Italy and elsewhere have shown that the cvs Frantoio, Coratina, Frangivento, Oblonga and Kalamon have interesting resistance properties (Wilhelm and Taylor, 1965; Hartmann et al., 1971; Schnathorst and Sibbett, 1971; Cirulli and Montemur-ro, 1976; Tjamos, 1993; Lopez-Escudero et al., 2004), while cvs Ascolana,

*Corresponding author. E-mail: meltem.sesli@bayar.edu.tr. Tel: (+90) 236 412 68 96. Fax: (+90) 236 413 70 58.

Table 1. Provinces, district and villages where wild and cultivated olives are obtained.

Province	District	Village	Olive types
Mugla	Milas	Pınarcık	Wild 1
Manisa	Akhisar	Harlak	Wild 2
Manisa	Akhisar	Sabancılar	Wild 3
Manisa	Akhisar	Hasköy	Wild 4
Manisa	Akhisar	Caglak	Wild 5
Manisa	Akhisar	Yayakırıldık	Wild 6
Manisa	Soma	Karacakas	Wild 7
Izmir	Bornova	Bornova	Wild 8
Izmir	Dikili	Bademli	Wild 9
Izmir	Bornova	ORI*	Manzanilla 1
Izmir	Bornova	ORI*	Manzanilla 3
Izmir	Bornova	ORI*	Gemlik 2
Izmir	Bornova	ORI*	Gemlik 5
Manisa	Akhisar	Supplier	Edremit
Manisa	Akhisar	Supplier	Uslu
Izmir	Bornova	ORI*	Domat
Izmir	Bornova	ORI*	Memecik

*ORI: Olive Research Institute Turkey.

Cellina, Leccino, Manzanillo, Chemlalie, Konservolia, Mission and Picual are susceptible (Wilhelm and Taylor, 1965; Cirulli and Montemurro, 1976; Wilhelm, 1981, Tjamos, 1993; Rodriguez-Jura-do et al., 1993; Lopez-Escudero et al., 2004). Also, it has been determined susceptibility of some economically important olive cultivars and clones to V. *dahliae* in Turkey. The severity of the disease has varied between 35.40 and 100% in the tested cultivars. The best results have been obtained from Gemlik cultivars with the values of 35.40 - 36.25% (Erten and Yildiz, 2008).

The integrated control of Verticillium wilt of olive are also mainly based on the use of resistant cultivars. For this reason, selection of resistant cultivars are of great importance in controlling the disease.

The aim of this research was to assess the level of resistance of some cultivated and wild type olives to a cotton defoliating (D) isolate (VCG1) of *V. dahliae* due to spread risk so that resistant cultivars can be identified and thus used for resistance in future breeding programmes.

MATERIALS AND METHODS

Seventeen olive cultivars (8 cultivated and 9 wild olives) were evaluated for resistance to *V. dahliae* in four blocks containing five plants in controlled conditions in a growth chamber in three experiments. Plant material consisted of twelve-month-old rooted cuttings, obtained from different provinces and districts of Aegean Region (Table 1).

Twelve (12) cm long cuttings were taken from the selected wild and cultivated olives; and two pairs of leaves were remained at the ends of cuttings. Bottoms of cuttings were treated with 0.4% indole butyric acid, 0.4% naphthalene acetic acid and 15% Captan, and were planted in perlite setting. Rooting was performed under fogging in a way to ensure proportional moisture of 80 - 100% under atmospheric temperature of 20°C (Abu-Qamar and Al-Raddad, 2001).

Plants were inoculated with isolate of *V. dahliae* (VCG1), Mn 16, from the collection of Plant Protection Research Institute, Bornova. Isolate Mn 16 represents a highly virulent, cotton D isolate (Göre, 2007).

The technique for plant inoculation was based on Rodriguez-Jurado et al. (1993). Inoculum was prepared from single-spore cultures of Mn 16 isolate, maintained on potato dextrose agar (PDA) slants at 4°C. Plants were inoculated by dipping their bare root systems in a suspension of 10^7 conidia/ml for 30 min. They were transplanted to steril soil (1:1:1, peat: sand: lime) in pots and incubated in a growth chamber adjusted to 22 ± 2°C. Plants remained in darkness and at 95% RH for 3 days after inoculation to reduce losses due to the transplanting and the inoculation process. Then, light and humidity were adjusted to a photoperiod of 16 (216 $\mu Em\text{-}2s^{-1}$ fluorescent light) and 80% HR.

To evaluate wilt resistance, disease severity was assessed weekly for 12 weeks, starting 2 weeks after inoculation. A scale 0 - 4 was used according to the percentage of plant tissue affected by chlorosis, leaf and shoot necrosis or defoliating (0 = healty plant or plant without symptoms; 1 = affected plant in 1 – 33%; 2 = 34 – 66%; 3 = 67 – 99%; 4 = dead plant). The percentage of dead plants, recovery from the disease and other symptoms such as marginal spots of leaves and irregular growth of twigs were also considered to estimate the severity of reactions (Lopez-Escudero et al., 2004). The estimate for the area under the disease progress curve (AUDPC) was based on Campbell and Madden (1990). It was estimated for each cultivar considering its percentage with regard to the maximum possible value that could be reached in the 10 weeks period of assessment:

$$AUDPC = \left[(t/2 * (S_2 + 2S_3 + \cdots + 2S_{i-1} + S_i) / 4n \right] * 100$$

Where:

t = interval in days between observations;

Table 2. AUDPC and PDP of olive cultivars inoculated with D isolate[1].

Cultivars	AUDPC[2]	PDP[2]
Manzanilla 3	73.3 a[3]	100 a
Edremit	73.3 a	100 a
Memecik	73.3 a	100 a
Uslu	73.3 a	100 a
Gemlik 5	73.3 a	100 a
Domat	73.3 a	100 a
Manzanilla 1	68.3 ab	90 ab
Wild 1	63.3 ab	70 b
Wild 2	63.3 b	70 b
Wild 8	63.3 b	70 b
Wild 3	63.3 b	70 b
Wild 7	56.6 c	60 c
Wild 4	56.6 c	60 c
Wild 5	56.6 c	60 c
Wild 9	56.6 c	60 c
Wild 6	43.3 d	20 d
Gemlik 2	43.3 d	20 d

[1]Twelve- month-old olive plants were inoculated with cotton D isolate of *V. dahliae*. Symptom severity was assessed weekly from 2 to 12 weeks after inoculation.
[2]AUDPC = area under the disease progress curve; PDP = percentage of dead plants.
[3]The differences between groups shown with different letters in same column are important ($P < 0.001$).

S_i = final mean severity;
4 = maximum disease rating;
n = number of observations).

At the end of the experiments, isolations from shoots and branches were made from all dead plants. 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 on PDA. Plates were incubated at 24°C in the dark for 5 – 6 days.

Plants were arranged according to completely randomised block design. Three blocks were inoculated and fourth block was reserved as control group. The roots of control plants were immersed in steril water simultaneously. The blocks were separated randomly in polyethylene trays and conveyed to climate rooms. The analysis of variance was performed to determine the variability among cultivars by JMP 6.0 program (JMP User Guide, 2005).

RESULTS

The cultivars did not result in statistical differences between experiments and so the results were evaluated together.

Disease symptoms

Chlorosis was associated with cultivars showing certain level of resistance. Defoliation was the most common symptoms observed. It occurred in all susceptible cultivars, starting at 3 weeks after inoculation and intensifying from the sixth week after inoculation. Defoliation was intensive in plants from extremely susceptible cultivars: Manzanilla 3, Edremit, Memecik, Uslu, Gemlik 5, Domat and Manzanilla 1, and slight and restricted to the middle of the main shoots of the plants in moderately resistant cultivars: Wild 6 and Gemlik 2.

Apoplexy or sudden wilt, characterized by the progressive rolling inward and chlorosis of leaves, was also observed in plants. Leaves became necrotic and remain attached to the twigs.

Cotton defoliating (D) (Mn 16) isolate caused between 90 and 100% of dead plants in 7 out of the 17 cultivars inoculated (Table 2).

No Verticillium wilt symptoms were observed in non-inoculated control plants, nor was the pathogen reisolated from any of them.

Presence of *V. dahliae* in olive shoots

The pathogen was reisolated from 70% of affected shoots in living plants at the middle of experiments and from 80% of dead plants at the end of them. The fungus was also isolated from petioles of green leaves collected immediately after defoliation.

Disease progress

From the eight week after inoculation, some cultivars

Table 3. Progress of the severity of symptoms in olive cultivars inoculated with the D isolate.

Cultivars	Mean severity of symptoms /week*										
	2	3	4	5	6	7	8	9	10	11	12
Manzanilla 3	0.3	0.8	2.0	3.2	3.5	3.7	3.7	3.7	3.7	4.0	4.0
Edremit	0.5	0.9	2.1	3.1	3.4	3.6	3.7	3.7	3.7	4.0	4.0
Memecik	0.4	0.7	2.2	3.2	3.4	3.7	3.7	3.7	3.7	4.0	4.0
Uslu	0.6	1.0	2.3	3.3	3.6	3.6	3.7	3.7	3.7	4.0	4.0
Gemlik 5	0.7	1.2	2.1	2.6	3.5	3.6	3.7	3.7	3.7	4.0	4.0
Domat	0.6	1.3	2.5	3.1	3.2	3.4	3.7	3.7	3.7	4.0	4.0
Manzanilla 1	0.6	0.9	2.4	3.0	3.2	3.3	3.4	3.5	3.5	3.8	3.8
Wild 1	0.2	0.7	0.9	1.0	2.2	2.8	3.0	3.1	3.1	3.6	3.6
Wild 2	0.3	0.6	0.8	1.2	2.3	2.8	3.0	3.1	3.1	3.7	3.7
Wild 8	0.4	0.8	1.2	1.6	2.0	2.4	2.8	3.0	3.1	3.5	3.5
Wild 3	0.3	0.7	0.9	1.3	2.5	2.7	2.9	3.0	3.1	3.7	3.7
Wild 7	0.3	0.8	1.0	1.6	2.0	2.3	2.8	3.0	2.7	2.6	2.6
Wild 4	0.2	0.6	0.8	1.0	1.4	1.8	2.0	2.4	2.7	2.5	2.5
Wild 5	0.4	0.7	0.9	1.2	1.3	1.8	2.4	2.6	2.7	2.7	2.7
Wild 9	0.4	0.8	1.2	1.2	1.4	1.8	2.0	2.4	2.7	2.6	2.6
Wild 6	0.2	0.8	1.0	1.0	1.3	1.6	2.4	2.2	2.1	1.6	1.6
Gemlik 2	0.3	0.7	0.9	1.2	1.6	2.0	2.6	2.4	2.1	1.8	1.8

(Wild 6 and Gemlik 2) showed recovery from the disease, expressed as a reduction in disease severity (Table 3).

Severity of plants symptoms was weekly assessed for 12 weeks, starting at 2 weeks after inoculations, on a 0 - 4 rating scale according to percentage of plant tissue affected by chlorosis, leave and shoot necrosis, or defoliation (0 = healty plant or plant without symptoms; 1 = affected plant in 1 - 33%; 2 = 34 - 66%; 3 = 67 - 99%; 4 = dead plant)

Recovery of the disease over time was characterised by the production of new vegetative growth of twigs and leaves with no symptoms that developed from healthy buds close to old affected tissues.

Categories of resistance

Cultivars were classified into resistance categories or susceptibility categories as shown in Table 4. Resistance categories correspond to following interval of values of AUDPC for the D isolate of V.*dahliae*: HR = 0 - 10%; R = 11 - 30%; MS = 31 - 50%; S = 51 - 70%; E = 71 - 100% (Lopez-Escudero et al.,2004). Most of the evaluated cultivars were susceptible to Mn 16 (D) isolate of *V. dahliae*. Seven of the 17 cultivars were extremely susceptible to the isolate of the pathogen. This group includes Manzanilla 3, Edremit, Memecik, Uslu, Gemlik 5, Domat and Manzanilla 1 (Table 4).

The second group including wild 1, wild 2, wild 8, wild 3, wild 7, wild 4, wild 5 and wild 9 were susceptible to the pathogen (Table 4). The third group including wild 6 and Gemlik 2 was moderately susceptible (Table 4).

DISCUSSION

In this study, all the evaluated cultivars have been listed as extremely susceptible, susceptible, and moderately susceptible to the D isolate of *V. dahliae* (Table 4). Especially it was remarkable that cultivated cultivars including Manzanilla 3, Edremit, Memecik, Uslu, Gemlik 5, Domat, Manzanilla 1 were extremely susceptible to the pathogen. The fact that the new olive orchards established in Turkey during the last 15 -20 years have increased the importance of Verticillium wilt of olive may be due to extremely susceptible cultivated cultivars.

Another risk may be planting olive trees in field previously used for growing susceptible hosts to the pathogen, or in those close to cotton fields infested by *V. dahliae*. There has been a spread risk of defoliating (D) isolates of the pathogen throughout new olive orchards (Lopez-Escudero and Blanco-Lopez, 2001; Lopez-Escudero et al., 2004). The spread risk of the D pathotype of *V. dahliae* makes it necessary to determine which olive cultivars have higher resistance to *V. dahliae*. It is known that plants of wild and primitive populations are successful in transferring qualitative characteristics such as resistance against diseases; and that in addition, wild olives may be used in enriching gene pools of cultivated olives in plant breeding (Şehirali and Ozgen, 1987). In this study, generally it was determined that wild types of olive were less susceptibility to the pathogen than cultivated cultivars. Wild 6 and Gemlik 2 was moderately susceptible to the pathogen. Their recovery from Verticillium wilt has been clearly associated with the level of susceptible, as demonstrated by Resende et al. (1995) in

Table 4. Resistance or susceptibility levels of olive cultivars to Verticillium wilt caused by the D isolate of V.*dahliae.*

Susceptibility*	Cultivars
Extremely susceptibility (E)	Manzanilla 3, Edremit, Memecik, Uslu, Gemlik 5, Domat, Manzanilla 1
Susceptible (S)	Wild 1, Wild 2, Wild 8, Wild 3, Wild 7, Wild 4, Wild 5, Wild 9
Moderately susceptible (MS)	Wild 6, Gemlik 2

*Susceptibility has been determined according to values of AUDPC, PDP at 12 weeks after inoculation and other complementary critaria such as shape of AUDPC and recovery from the disease.

cocoa. Percentage of recovery in moderately susceptible diseased plants was twice or three times higher than in susceptible and extremely susceptible diseased plants. In olive, both pathogen virulence and cultivar resistance seem to be connected with the capability of the plant to slow down pathogen development during colonisation, as suggested by Rodriguez-Jurado (1993) and Lopez-Escudero et al. (2004).

Probably, these differential reactions may make them appropriate candidates as rootstocks in soil infested with low inoculum densities of *V. dahliae*. Finally, our results indicate that there are differential reactions to *V. dahliae* between wild types of olive and that detailed studies may be performed aiming at defining molecular markers associated with the resistance characteristics of trees against *V. dahliae*.

ACKNOWLEDGEMENT

This research was supported by the Turkish Republic State Planning Organization.

REFERENCES

Abu-Qamar M, Al-Raddad A (2001). Integrated control of Verticillium wilt of olive with Cryptonol in combination with a solar chamber and fertilizer. Phytoparasitica, 29: 3.

Biris DA, Thanassoulopoulos CC (1980). Field trials for chemical control of Verticillium wilt of olives. In: Proceedings of the 5 Congress of the Mediterranean Phytopathological Union, Patras: pp. 54-55.

Blanco-Lopez MA, Rodriguez-Jurado D, Jimenez-Diaz RM (1990). Incidence and seasonal variation of Verticillium wilt in olive orchards. In: Proceedings of the 5th International Verticillium Symposium, Leningrad, USSR, p. 5.

Campbell CL, Madden LV (1990). Introduction to Plant Disease Epidemiology, John Wiley and Sons, New York.

Cirulli M (1975). Il deperimento dell'olivo da *Verticillium dahliae* Kleb. L'Italia Agricola, 112 (6): 120-124.

Cirulli M, Montemurro G (1976) A comparation of pathogenic isolates of *Verticillium dahliae* and sources of resistance in olive. Agriculturae Conspectus Scientificus, 39: 469-476.

Cirulli M (1981). Attuali cognizioni sulla Verticilliosi dell'olivo. Informatore Fitopatologico, 31(1-2): 101-105.

Erten L, Yıldız M (2008). Susceptibility of some economically important olive cultivars and clones to *Verticillium dahliae* Kleb in Turkey. ISHS Acta Horticulture, V. Int. Symposium on olive growing, 791: 2.

Göre ME (2007). Vegetative compatibility and pathogenicity of *Verticillium dahliae* isolates from the Aegean Region of Turkey. Phytoparasitica, 35: 222-231.

Hartman H, Schnathorst, WC, Whysler J (1971). Oblonga, a clonal olive rootstock resistant to Verticillium wilt. California Agric., 25(6): 13-15.

Jiménez-Diaz RM, Tjamos EC, Cirulli M (1998). Verticillium wilt of major tree hosts. In: Hiemstra JA, Haris DC (editors), A Compendium of Verticillium wilts in Tree Species. Ponsen and Looijen, Wageningen, the Netherlands, p. 80.

JMP ver. (2005). 6.0 User's Guide, SAS Institute.

Lopez-Escudero FJ, Blanco-López MA (1997). Control of Verticillium wilt by soil solarization in established olive orchards in Andalucía (southern Spain). In: Tjamos E.C,. Rowe J.B., Fravel D. (eds). Advances in Verticillium. Research Disease Management, APS Press, St. Paul, MN, USA. pp. 332-335.

Lopez-Escudero FJ, Blanco-Lopez, MA (2001). Effect of a single or double soil solarization to control Verticillium wilt in established olive orchards in Spain. Plant Dis., 85: 489-496.

Lopez-Escudero FJ, Del Rio C, Caballero JM, Blanco-Lopez MA (2004). Evaluation of olive cultivars for resistance to *Verticillium dahliae*. Europ. J. Plant. Pathol., 110: 79-85.

Martos-Moreno C, Caballero JM, del Rio, C, Blanco-Lopez MA (2001). Epidemiological behaviour of olive cultivars in orchards infested with mixtures of defoliating and non-defoliating isolates of *Verticillium dahliae*. In: Proceedings of the 8th International Verticillium Symposium Cordoba, Spain. p. 67.

MOARA, Republic of Turkey, Ministry of Agriculture and Rural Affairs (2006). Presidency of Strategy Development. Agriculture in Turkey, Ankara. p. 144.

Provincial Directorate of Agriculture, Republic of Turkey, Governorship of Manisa. Verticillium Wilt in Olive trees. Branch Directorate of Plant Protection. Brochure, Manisa., (2005).

Resende MLV, Flood J, Cooper, RM (1995). Effect of method of inoculation, inoculum density and seedling age at inoculation on the expression of resistance of cocoa (*Theobroma cacao* L.) to *Verticillium dahliae* Kleb. Plant Pathol., 43: 104-111.

Rodriguez-Jurado D, Blanco-Lopez MA, Rapoport HF, Jimenez-Diaz RM (1993). Present status of Verticillium wilt of olive in Andalusia (southern of Spain). EPPO Bull., 23: 513-516.

Saydam C, Copçu M (1972). Verticillium wilt of olive in Turkey. J. Turkish. Phytopat. 1:45-49.

Schnathorst WC, Sibbett GS (1971). The relations of strains of *Verticillium albo-atrum* to severity of Verticillium wilt in *Gossypium hirsutum* and *Olea europaea* in California. Plant Disease Reporter. 55: 780-782

Skoudridakis MT, Bourbos VA (1989). Il riscaldamento solare del terreno mediante pacciamatura con films di poli-etilene trasparente nella lotta contro la verticilliosi dell'olivo. Rivista di Patologia Vegetale. 25: 46-49.

Şehirali S, Ozgen M (1987). Transfer of the characters. In: Plant Genetic Resources. Ankara University Faculty of Agriculture Publications, Ankara, p. 212.

Tjamos EC, Biris DA, Paplomatas EJ (1991). Recovery of olive trees from Verticillium wilt after individual application of soil solarization in established olive orchards. Plant Dis., 75: 557-562.

Tjamos EC (1993). Prospects and strategies in controlling Verticillium wilt of olive. Bulletin OEPP/EPPO Bull., 23: 505-512.

Wilhelm S, Taylor JB (1965). Control of Verticillium wilt in olive trough natural recovery and resistance. Phytopathol., 55: 310-316.

Wilhelm S (1981). Sources and genetics of host resistance in field and fruit crops. In: Mace M.E., Bell A.A., Beckman C.H. (eds.). Fungal Diseases of Plants. Academic Press, New York, NY, USA, pp. 299-376.