Eur J Plant Pathol (2010) 127:287–301
DOI 10.1007/s10658-010-9595-z

Verticillium wilt of olive in Turkey: a survey on disease importance, pathogen diversity and susceptibility of relevant olive cultivars

Sibel Dervis · Jesús Mercado-Blanco · Latife Erten · Antonio Valverde-Corredor · Encarnación Pérez-Artés

Accepted: 15 February 2010 / Published online: 10 March 2010

Abstract A comprehensive survey on the prevalence and incidence of Verticillium wilt of olive in Turkey has been conducted over 6 years (2003–2008). Vegetative compatibility group (VCG) assessment and PCR-based molecular pathotyping were used to evaluate the distribution of the defoliating (D) and nondefoliating (ND) pathotypes of *Verticillium dahliae* in surveyed areas. Pathogen prevalence was 35% of all olive orchards inspected and incidence of the disease reached 3.1%. VCG1A was predominant (29.3%) and infected all major cultivars grown in Turkey. The other two VCGs detected (2A and 4B) were of minor relevance (4.9% and 0.9%, respectively). Disease incidence caused by VCG1A infections was higher (ranging from 1.1% to 6.9%) than that caused by VCG2A and VCG4B in 10 provinces (Manisa, Aydin, Kahramanmaras, Izmir, Mugla, Kilis, Denizli, Gaziantep, Mardin and Balikesir). However, VCG2A and 4B were more prevalent (and responsible for higher disease incidence) than VCG1A in three provinces (Hatay, Osmaniye and Bursa). Finally, VCG1A isolates were found in all provinces except Canakkale, and simultaneous presence of the three VCGs was only verified in Hatay province. An artificial inoculation bioassay (19 representative *V. dahliae* isolates included) revealed that VCG1A (13) isolates as a group were more aggressive and caused defoliation, whereas VCG2A (5) and VCG4B (1) isolates induced milder symptoms. Within a VCG group, virulence varied among isolates infecting the same olive cultivar and this virulence was also related to the differential susceptibility of the cultivars ('Manzanilla', 'Ayvalik' and 'Gemlik') tested. Molecular pathotyping allowed the identification of D (VCG1A) and ND (VCG2A/4B) pathotypes, which correlated with results from pathogenicity tests. Remarkably, the *V. dahliae* VCG1A/D pathotype population infecting olive in Turkey was molecularly different from that one previously identified in Spain.

Keywords Molecular markers · *Olea europaea* · PCR · VCG · Virulence

S. Dervis (✉)
Department of Plant Protection, Faculty of Agriculture,
University of Mustafa Kemal,
31034 Antakya, Hatay, Turkey
e-mail: sdervis@mku.edu.tr

J. Mercado-Blanco · A. Valverde-Corredor ·
E. Pérez-Artés (✉)
Department of Crop Protection,
Instituto de Agricultura Sostenible, CSIC,
Alameda del Obispo s/n, Apdo. 4084,
14080 Córdoba, Spain
e-mail: eperezartes@ias.csic.es

L. Erten
Olive Research Institute,
Turkish Ministry of Agriculture and Rural Affairs,
35100 Bornova,
Izmir, Turkey

Introduction

The olive (*Olea europaea* L. var. *sativa*) is historically cultivated in most of the Mediterranean basin countries. Turkey is located in a geographical area considered to be the origin of this species. In recent years olive production has increased rapidly. This crop consists of about 143 million trees occupying over 753.000 ha in Turkey, mainly in the Western, Southern and Southeastern provinces of the country (ZAE 2008) (Table 1, Fig. 1). The rapid spread of olive cultivation to lowland areas of the subtropical basin of Turkey is explained because olive is more profitable than other crops and the government has supported its cultivation. As a result, it is currently one of the most important tree crops in these regions.

Verticillium wilt of olive, caused by the soil-borne fungus *Verticillium dahliae* Kleb., is a very serious disease, which has been reported in all important olive cultivation areas in the world (Tjamos 1993; Jiménez-Díaz et al. 1998). The first record of the disease in Turkey dates from 1972, when it was detected in Balikesir (Ayvalik district) and Mugla (Milas district) provinces (Saydam and Copcu 1972). The disease is responsible for considerable production loss in Turkey. Since olive groves have spread from mountainous to lowland areas of the western and southern part of the country during the last decade, changes in olive cultivation practices as well as substitution of cotton (*Gossypium hirsutum* L.) by olive might have contributed to the expansion of the disease and severity of pathogen attacks in these areas, as it has been suggested for other regions within the Mediterranean basin (Rodríguez et al. 2008).

V. dahliae isolates infecting olive can be classified as defoliating (D) and non-defoliating (ND) pathotypes according to their ability to defoliate the plant (López-Escudero et al. 2004). While infections by the D pathotype can be lethal to the plant, olive plants infected with the ND pathotype can recover from symptoms (Rodríguez-Jurado 1993; Jiménez-Díaz et al. 1998; Mercado-Blanco et al. 2001). In Europe, natural infections by the D pathotype have been only reported in Spain (López-Escudero and Blanco-López 2001; Mercado-Blanco et al. 2003; Navas Cortés et al. 2008; Rodríguez et al. 2008) and Western Turkey (Dervis et al. 2007).

Vegetative compatibility refers to the genetically controlled ability of individual fungal strains to undergo hyphal anastomosis and form viable heterokaryons; compatible isolates are therefore placed within the same vegetative compatibility group (VCG) (Korolev et al. 2008). For the strictly asexually-reproducing *V. dahliae*, isolates in different VCGs are thought to be genetically isolated populations that may differ in many traits, including those related to pathogenicity and virulence, adaptation to environments, and sensitivity to fungicides (Katan 2000; Rowe 1995). Out of all identified VCGs (VCG1, VCG2, VCG3, VCG4 and VCG6) and subgroups (A and B in VCG1, VCG2, and VCG4) from diverse host sources, only VCG1A, VCG2A, VCG2B, and VCG4B have been identified among olive *V. dahliae* isolates worldwide (Cherrab et al. 2002; Tsror and Levin 2003; Collado-Romero et al. 2006; Dervis et al. 2007). VCG groups and subgroups and D/ND pathotypes of *V. dahliae* isolates infecting olive (and cotton) can be differentiated by PCR-based procedures and specific primers (Pérez-Artés et al. 2000; Mercado-Blanco et al. 2003; Collado-Romero et al. 2006).

Olive cultivars show a broad range of genetic variability for a large number of agronomic traits (Owen et al. 2005), including resistance to Verticillium wilt. To date, screening for resistance has been mainly carried out by examining the susceptibility, tolerance or resistance of different olive cultivars in artificial inoculation bioassays under greenhouse or semi-controlled conditions (López-Escudero et al. 2004; Colella et al. 2008). In a study conducted in Turkey, cultivars Ayvalik, Memecik, Domat, Uslu and Manzanilla were reported to be extremely susceptible to the D pathotype of *V. dahliae*, but 'Gemlik' plants were tolerant to the same pathotype. Seven clones of 'Gemlik' and five clones of 'Ayvalik' displayed similar reactions to their own parents (Erten 2004).

Our knowledge on pathogen prevalence and disease incidence is still limited. Because Verticillium wilt is an increasing problem for olive production in all major olive-producing areas of Turkey, this study was aimed to: (i) explore the prevalence of the pathogen and the incidence of the disease in all important olive-growing areas and olive cultivars present in these regions; (ii) document the genetic (VCG and PCR-based pathotyping) diversity of a broad and representative collection of *V. dahliae* isolates infecting olive trees; and (iii) identify differences in virulence of representative isolates on three

Table 1 *Verticillium dahliae* prevalence and Verticillium wilt incidence in the main olive cultivation provinces in Turkey

Province	Production area (× 1000 ha)	Number of olive orchards surveyed	Prevalence (%)				Incidence (%)				Cultivars from which major percentages (>1%) of *V. dahliae* isolation was achieved
			V. dahliae[a,b]	VCG1A[a,c]	VCG2A[a,c]	VCG4B[a,c]	*V. dahliae*[a,b]	VCG1A[a,c]	VCG2A[a,c]	VCG4B[a,c]	
Manisa	81.0	140	52.9a	50.0a	2.9c	0.0b	6.9a	6.5a	0.4cde	0.0b	Ayvalik (9.9%), Domat (5%), Uslu (3.4%), Gemlik (3.1%), Memecik (1.6%)
Aydin	152.8	140	46.4a	45.7a	0.7c	0.0b	4.2b	4.0b	0.2ef	0.0b	Memecik (6.5%), Yamalak Kabasi (5.9%), Domat (3.1%), Manzanilla (2.5%), Gemlik (1.9%)
Kahramanmaras	5.7	45	44.4ab	44.4a	0.0c	0.0b	1.1cd	1.1cde	0.0f	0.0b	Kilis Yaglik (1.6%), Maras (1.2%)
Izmir	92.6	110	40.9abc	37.3ab	0.0c	3.6ab	4.1b	3.8b	0.0f	0.4a	Memecik (4.7%), Ayvalik (4.3%), Domat (2.5%), Gemlik (1.9%)
Mugla	88.9	80	38.8abc	33.8abc	5.0c	0.0b	4.4b	3.8b	0.7bc	0.0b	Memecik (8.7%)
Hatay	46.3	80	36.3abcd	12.5de	18.8ab	5.0a	1.6c	0.5cdef	0.9ab	0.1b	Gemlik (5.6%), Ayvalik (1.2%)
Kilis	17.4	20	25.0bcde	25.0bcd	0.0c	0.0b	1.2cd	1.2cd	0.0f	0.0b	Kilis Yaglik (1.6%)
Bursa	38.1	44	22.7cde	2.2e	20.5a	0.0b	1.3cd	0.2ef	1.1a	0.0b	Gemlik (3.1%)
Osmaniye	7.0	30	20.0cde	6.7de	13.3b	0.0b	0.8cd	0.2ef	0.6cd	0.0b	Ayvalik (1.9%)
Denizli	3.4	40	20.0cde	17.5cde	2.5c	0.0b	1.3cd	1.1cd	0.3def	0.0b	Gemlik (1.6%)
Gaziantep	38.8	60	16.7de	16.7cde	0.0c	0.0b	0.9cd	0.9cde	0.0f	0.0b	Nizip Yaglik (1.2%), Gemlik (1.2%)
Mardin	1.9	30	16.7de	16.7cde	0.0c	0.0b	1.4c	1.4c	0.0f	0.0b	Halhali (1.6%)
Balikesir	80.0	50	14.0e	14.0de	0.0c	0.0b	0.4d	0.4def	0.0f	0.0b	Ayvalik (2.2%)
Canakkale	30.1	50	14.0e	0.0e	14.0ab	0.0b	0.6cd	0.0f	0.6cd	0.0b	Ayvalik (2.2%)
Mean	683.9	919	35.0	29.3	4.9	0.9	3.1	2.7	0.3	0.1	
			χ^2:82.3, df:1,13, P:0.0001; F :6.188, df:13,905, P:0.0001				χ^2:54953.4, df:1,13, P:0.0001; F :78.507, df:13,62466, P:0.0001				

[a] Numbers within a column not followed by the same letter are significantly different ($P<0.05$) by chi-square and Duncan tests

[b] Prevalence of the pathogen was calculated as the percentage of olive orchards where *V. dahliae* was detected relative to the total number of olive orchards inspected in a province. Disease incidence was calculated as the percentage of olive trees with wilt symptoms relative to the total number of olive trees inspected in a province (for each orchard, 30–150 trees were inspected depending on the size of the orchard)

[c] VCG prevalence (%) calculations were estimated by multiplying the pathogen prevalence (%) values with the presence ratio of each VCG among all *V. dahliae* positive orchards in a province. Disease incidence (%) for VCGs was calculated by multiplying the disease incidence (%) values with isolation ratio of each VCG among all isolates from a province

Fig. 1 Provinces (colored) surveyed to assess Verticillium wilt of olive incidence in Turkey. These provinces are the most important for olive cultivation in the country. 1A, 2A and 4B refer to *Verticillium dahliae* vegetative compatibility groups VCG1A, VCG2A, and VCG4B, respectively, infecting olive orchards within each surveyed province. VCG abbreviations are written in the order of VCG prevalence found in each province

important olive cultivars with different susceptibility to the disease.

Materials and methods

Verticillium wilt and *Verticillium dahliae* survey

Commercial olive orchards from all main olive-growing areas in the Aegean (Aydin, Denizli, Izmir, Manisa and Mugla provinces), Mediterranean (Hatay, Kahramanmaras and Osmaniye provinces), Marmara (Balikesir, Bursa and Canakkale provinces) and the Southeastern Anatolia (Gaziantep, Kilis and Mardin provinces) regions of Turkey (Fig. 1) were surveyed to assess the prevalence of *V. dahliae* and the incidence of Verticillium wilt. Inspections were always carried out from March to November from 2003 through 2008. A total of 919 orchards in 14 provinces was inspected (Table 1). Surveyed areas represent 91% of the total olive acreage in Turkey (753.000 ha).

To evaluate the influence of geographical and host (cultivars) origin on the occurrence of the disease, coordinates of the sites (orchards) from which the pathogen was isolated and cultivars present in them were recorded. Therefore, duplicate sampling from the same orchard was avoided during the six-year survey period. Prevalence of the pathogen was calculated as the percentage of olive orchards where *V. dahliae* was detected relative to the total number of olive orchards inspected in a province. Disease incidence was calculated as the percentage of olive trees with wilt symptoms (for each orchard, 30–150 trees were inspected depending on the size of the orchard) relative to the total number of olive trees inspected in a province (Rodríguez et al. 2008). For pathogen isolation, stem pieces (15–25 1-cm-pieces/tree) from two to five affected branches of each tree were surface sterilized with NaClO (5%) for 1 min and subsequently rinsed with sterile distilled water. Stem segments were then transferred onto potato dextrose agar (PDA) plates amended with streptomycin sulphate (Sigma-Aldrich, St. Louis, Missouri, USA; 100 μg ml^{-1}) and incubated at 24°C in the dark for 5 to 7 days, and the colonies grown were identified as *V. dahliae* on the basis of morphological features. All *V. dahliae* isolates were stored on sterile Whatman filter papers at −20°C. Single-spore cultures from each *V. dahliae* isolate were used for VCG

testing, molecular characterization and pathogenicity tests.

Data on the pathogen prevalence and disease incidence were analyzed by chi-square test and analysis of variance (ANOVA); means were separated using Duncan Multiple Comparison Tests ($p<0.05$) using SPSS software (version 13.0; SPSS Inc., Chicago, IL, USA).

Prevalence and incidence of VCGs

In the present study, 1.087 *V. dahliae* isolates originating from all regions/districts where olive is relevant were assessed for VCG using nitrate non-utilizing (*nit*) mutants. Additionally, 207 *V. dahliae* isolates previously characterized for VCG (Dervis et al. 2007) were also considered for prevalence and incidence estimations. *Nit* mutants were generated from all *V. dahliae* isolates on water–agar–chlorate (WAC) medium (2% agar, 0.02% glucose, and 2.5–5% potassium chlorate) (Korolev and Katan 1997). The mutants were phenotyped (Correll et al. 1987) and then used in complementation tests as described by Korolev et al. (2000); *nit* mutants derived from international reference strains 70–21 and 131 M of *V. dahliae*, kindly provided by Dr. Korolev, were used as testers to identify VCG3 and VCG4A, respectively (Joaquim and Rowe 1990). Turkish local *nit* testers CotVd19, OVd211, OVd60 (Dervis et al. 2007) and Ch03 (Dervis and Bicici 2005) were used to identify VCG1A, VCG2A, VCG4B and VCG2B, respectively. These latter testers were previously developed from local isolates and complemented with the American (VCG1A, T9 from cotton) (Joaquim and Rowe 1990) and the Israeli (VCG2A, ep8 and ep52 from eggplant; VCG2B, cot11 and cot256 from cotton; and VCG4B, Pt15M and Pt9G from potato) (Korolev et al. 2000) *nit* testers of *V. dahliae* VCGs.

Prevalence and incidence of *V. dahliae* VCGs were obtained after determining the VCG of each isolate. VCG prevalence (%) calculations were estimated by multiplying the pathogen prevalence (%) values with the presence ratio of each VCG among all *V. dahliae*-positive orchards in a province. Disease incidence (%) for VCGs was calculated by multiplying the disease incidence (%) values by the isolation ratio of each VCG among all isolates from a province.

Molecular pathotyping of *Verticillium dahliae* isolates

Verticillium dahliae isolates from infected olive trees were characterized to the pathotype (D or ND) level by specific PCR assays. Active cultures of isolates were obtained on PDA plates incubated at 24°C in the dark for 5–6 days. The HotSHOT method described by Truett et al. (2000) was used for rapid, small-scale DNA extraction as carried out by Collado-Romero et al. (2006). Molecular assessment of pathotypes was based according to the amplification of specific PCR markers. Thus, primer pair DB19/DB22 (Carder et al. 1994) yields a *V. dahliae*-specific polymorphic DNA band of either 539 bp (amplified in cotton and olive Spanish D isolates) or 523 bp (associated with ND isolates) (Mercado-Blanco et al. 2003). Primer pairs INTD2f/INTD2r (Mercado-Blanco et al. 2002) and INTND2f/INTND2r (Mercado-Blanco et al. 2001) produce PCR markers of 462 or 824 bp associated with the cotton and olive D and ND *V. dahliae* pathotypes, respectively. These two primer pairs were used jointly in duplex PCR assays. Finally, primer pair DB19/espdef01 amplifies a 334-bp PCR marker which is present, among other genetic/molecular groups (Collado-Romero et al. 2006), in cotton and olive Spanish D isolates (Mercado-Blanco et al. 2003). *V. dahliae* isolates were then assigned to any of the PCR patterns described by Collado-Romero et al. (2006). Amplification reactions (25 μl) consisted of 100 nM each primer, 200 nM each dNTP, 2 mM $MgCl_2$, 2.5 μl of 10× reaction buffer, 0.75 U of EcoTaq polymerase (Ecogen S.R.L., Barcelona, Spain), and 5 to 8 μl of freshly obtained mycelium lysate. PCR parameters were 94°C for 4 min; 35 cycles of 94°C for 1 min, 64°C for 1 min, and 72°C for 1 min; and a final step of 6 min at 72°C. For primer pair DB19/DB22 the annealing step was set at 54°C for 30 s; for primer pair DB19/espdef01 the annealing step was set at 62°C for 1 min. For each single or duplex primer pair combination, and for all isolates tested, PCR assays were performed at least two times, using always new, freshly obtained lysates (see above). Each PCR assay always included control samples from D (V138I) and ND (V176I) *V. dahliae* representatives, which have been previously characterized by PCR markers (Mercado-Blanco et al. 2003), as well as a negative control (no template DNA). These controls were submitted to the above-mentioned rapid, small-scale DNA extraction proce-

dure. Results of PCR assays were visualized by agarose gel electrophoresis according to standard procedures. In cases in which molecular pathotyping of isolates was not possible by the rapid, small-scale protocol, specific PCR assays using purified DNA as template were carried out as described previously (Mercado-Blanco et al. 2003; Collado-Romero et al. 2006).

Additionally to specific-PCR assays, RAPD-PCR analyses were performed to investigate the possibility of genetic diversity among the selected VCG1A isolates included in the pathogenicity test. For this purpose, DNA was extracted from lyophilized mycelia using the DNeasy Plant Kit (Quiagen, Hilden, Germany). Amplifications were carried out using random primers OPH19 and OPH20 from Operon (Operon Technology, Alameda, CA) as described by Pérez-Artés et al. (2000).

Olive-*Verticillium dahliae* bioassay

To assess pathogenicity and virulence of selected *V. dahliae* isolates, a bioassay including 17 Turkish *V. dahliae* isolates, representatives of all VCGs found and originating from diverse olive cultivars and different geographical origins (Table 2), was carried out. Eleven of these isolates belonged to VCG1A, five to VCG2A, and one to VCG4B. In addition, two VCG1A isolates (V937I from Spain, olive and PCR pattern A, and V640I from Greece, cotton and PCR pattern B) (Collado-Romero et al. 2006) were used for comparison. Turkish cultivars Ayvalik and Gemlik were chosen because of their economic relevance and commonness in Turkish agriculture. These cultivars have been previously characterized as susceptible (Ayvalik) and tolerant (Gemlik) to the D pathotype *V. dahliae* isolate OVd007 (Erten 2004), which was also included in the bioassay. Cultivar Manzanilla, originally from Spain and cultivated in Turkey since 1984, was also included in the bioassay because of its well known susceptibility to Verticillium wilt (López-Escudero et al. 2004; Erten 2004). Six-month-old rooted cuttings of these cultivars, obtained from an olive accession collection established at the campus of the Institute of Olive Research in Izmir and grown in sterilized propagation mix (soil: sand: farmyard manure, 1: 1: 1) in black plastic bags (2 l), were inoculated by a stem-injection method. Approximately 5 cm above the soil layer, plant tissue was sterilized by swabbing 70% EtOH-soaked cotton and inoculated by injecting 500 µl of spore suspension (10^7 conidia ml^{-1}) into the stem through an incision made by a sterile scalpel. The inoculated plants were then maintained on benches in a greenhouse at 22-26°C, with a relative humidity ranging from 60 to 90%, in a randomized block design of three blocks (replicates) with four plants per block. Severity of the disease in olive trees was scored at 1, 2 and 3 months after inoculation on the basis of external symptoms using a 0 to 5 scale: 0 = healthy plant; 1 = up to 25% of plant with symptoms; 2 = up to 50% of plant affected; 3 = up to 75% of plant affected; 4 = up to 90% of plant affected; 5 = plant dead. Mean disease severity index (DSI%) was calculated by summing the scores of 12 plants, and expressing the value as a percentage using the formula: $DSI = [\sum (\text{rating number} \times \text{number of plants in rating})] \times 100]/(\text{total number of plants} \times \text{highest disease rating})$. The percentage of dead olive plants was also calculated for each treatment 6 months after inoculation. Statistical analysis of the data on disease severity index was carried out using SPSS software (version 13.0; SPSS Inc., Chicago, IL, USA). When necessary, arcsine transformation was performed on data before statistical analysis. Analysis of variance was followed by mean separation using the Student-Newman-Keuls (SNK) multiple comparison tests ($P<0.05$).

Results

Pathogen prevalence and disease incidence

From 2003 through 2008, olive orchards in the main 14 olive-growing provinces in Turkey were surveyed (Fig. 1). Thirty-five percent of the 919 olive orchards inspected showed wilt symptoms and yielded positive isolation of *V. dahliae*, whereas in 8.2% of the orchards symptoms were observed but the pathogen could not be isolated. Finally, 56.8% of olive orchards surveyed did not show any wilt symptoms. Isolation of *V. dahliae* was tried but not achieved from these symptomless orchards. Table 1 summarizes data on pathogen prevalence (%) and disease incidence (%) for the inspected provinces. *V. dahliae* prevalence varied among regions, and reached the highest values in Manisa (52.9%) and Aydin (46.4%) provinces. In contrast, the lowest pathogen prevalence percentage

Table 2 Disease reaction in cultivars Manzanilla, Ayvalik and Gemlik upon artificial inoculation (see "Materials and Methods" for details) with representative *Verticillium dahliae* isolates belonging to different VCGs and from diverse cultivars and geographical origins

Province/ Country	Olive cultivar	Isolate	VCG	Manzanilla				Ayvalik				Gemlik			
				DSI(%)[a,b]			DP(%)[c]	DSI(%)[a,b]			DP(%)[c]	DSI(%)[a,b]			DP(%)[c]
				1[d]	2[d]	3[d]	6[d]	1[d]	2[d]	3[d]	6[d]	1[d]	2[d]	3[d]	6[d]
Hatay	Ayvalik	OVd241	1A	6.7	71.7	95.0 a	83.3	5	31.7	66.7 abcd	41.7	0	20	35.0 abc	0
Manisa	Domat	OVd141	1A	31.7	66.7	91.7 ab	83.3	15	66.7	90.0 a	50.0	15	28.3	41.7 a	0
Gaziantep	Nizip yaglik	OVd191	1A	20	76.7	91.7 ab	75.0	15	41.7	65.0 abcd	50.0	8.3	35	43.3 a	0
Spain	Arbequina	V937I	1A	13.3	55	91.7 ab	58.3	15	38.3	73.3 abc	33.3	6.7	20	28.3 abcd	0
Mardin	Halhali	OVd245	1A	15	63.3	81.7 ab	66.7	10	51.7	78.3 ab	33.3	6.7	16.7	26.7 abcd	0
Mugla	Memecik	OVd007	1A	21.7	43.3	73.3 abc	58.3	3.3	40	56.7 bcd	16.7	0	26.7	46.7 a	0
Balikesir	Ayvalik	OVd227	1A	3.3	36.7	73.3 abc	50.0	0	26.7	63.3 abcd	25.0	1.7	21.7	33.3 abc	0
K.maras	Maras	OVd253	1A	6.7	58.3	73.3 abc	33.3	5	48.3	65.0 abcd	25.0	3.3	15	18.3 bcde	0
İzmir	Memecik	OVd023	1A	13.3	43.3	68.3 bc	33.3	0	31.7	55.0 bcd	25.0	0	18.3	38.3 ab	0
Greece	Cotton isolate	V640I	1A	8.3	31.7	68.3 bc	25.0	13.3	28.3	46.7 cde	16.7	6.7	20	28.3 abcd	0
Kilis	Kilis yaglik	OVd072	1A	0	35	58.3 c	25.0	0	23.3	48.3 cde	0	0	15	43.3 a	0
Aydin	Memecik	OVd104	1A	6.7	46.7	55.0 c	41.7	5	33.3	40.0 def	8.3	1.7	11.7	15.0 cde	0
Denizli	Gemlik	OVd265	1A	1.7	13.3	30.0 d	8.3	0	6.7	26.7 efg	0	1.7	5	10.0 de	0
Canakkale	Ayvalik	OVd261	2A	1.7	8.3	26.7 d	0	0	6.7	21.7 efg	0	0	1.7	18.3 bcde	0
Osmaniye	Ayvalik	OVd243	2A	1.7	11.7	23.3 d	0	0	6.7	21.7 efg	8.3	1.7	5	3.3 e	0
Mugla	Memecik	OVd211	2A	1.7	6.7	18.3 d	0	0	3.3	11.7 g	0	1.7	1.7	3.3 e	0
Manisa	Uslu	OVd151	2A	5	8.3	13.3 d	0	0	6.7	11.7 g	0	0	0	6.7 e	0
Bursa	Gemlik	OVd206	2A	3.3	3.3	13.3 d	0	0	1.7	5.0 g	0	0	0	3.3 e	0
Izmir	Ayvalik	OVd060	4B	0	6.7	18.3 d	0	0	5	15.0 fg	0	0	3.3	3.3 e	0

[a] A mean disease severity index (DSI) was calculated from each treatment by summing the score of the 12 plants (three replicates of 4 plants per isolate) by using the 0–5 scale, and expressing the value as a percentage. Arcsine transformation was performed prior to statistical analysis

[b] Means, obtained at each time after inoculation, followed by different letters are significantly different according to Student-Newman-Keuls (SNK) Multiple Comparison Tests ($P< 0.05$)

[c] Percentage of dead plants (DP) in each treatment was calculated by counting the number of the dead plants after 6 months of inoculation and expressing the value as a percentage

[d] Month(s) after inoculation

(14%) was scored in Balikesir and Canakkale provinces. Overall, Verticillium wilt incidence in Turkey was 3.1% (62,480 trees inspected) (Table 1), which can be extrapolated to 3.2 million infected olive trees in the country for the duration of the survey. Disease incidence ranged from 0.4% (Balikesir) to 6.9% (Manisa). In general, the highest disease incidence values corresponded with the highest pathogen prevalence percentages. For instance, Verticillium wilt incidence was significantly higher in Manisa, Mugla, Aydin and Izmir provinces compared to the remaining provinces, which correlated with the high pathogen prevalence percentages found in the former provinces (Table 1). However, there were some exceptions to this rule. For example, Kahramanmaras had high prevalence (not significantly different from Manisa, Aydin, etc.) but incidence was significantly lower compared to that found in those provinces.

With regards to cultivars in surveyed areas, 'Memecik' was the cultivar from which *V. dahliae* was predominantly isolated (23.3% of the total number of isolates). Infected 'Memecik' trees were amply represented in the provinces of Mugla (8.7% of the total number of isolates), Aydin (6.5%), Izmir (4.7%) and Manisa (1.6%) (Table 1).Cultivar Ayvalik was the second in importance regarding the number of *V. dahliae* isolates collected (22.4% of the total). It was distributed throughout the provinces of Manisa (9.9%), Izmir (4.2%), Balikesir (2.2%), Canakkale (2.2%), Osmaniye (1.9%) and Hatay (1.2%) (Table 1). Cultivar Gemlik was the third in the ranking from which pathogen isolates were sampled (19.3%). Infected 'Gemlik' trees were found in Hatay (5.6%), Bursa (3.1%), Manisa (3.1%), Izmir (1.9%), Aydin (1.9%), Denizli (1.6%) and Gaziantep (1.2%). *V. dahliae* isolates originating from cultivar Domat represented 11.2% of the total number. This cultivar is mainly grown in the provinces of Manisa (5.0% of the total number of isolates), Aydin (3.1%) and Izmir (2.5%). Finally, cultivars Yamalak Kabasi (5.9%; all from Aydin), Uslu (4.0%; 3.4% from Manisa), Kilis Yaglik (3.4%; 1.6% from Kilis and 1.6% from Kahramanmaras), Halhali (3.1%; 1.6% from Mardin and 1.5% from Hatay, Kahramanmaras and Gaziantep), Manzanilla (2.5%; all from Aydin), Nizip Yaglik (1.9%; 1.2% from Gaziantep) and Maras (1.2%; all from Kahramanmaras) were other olive cultivars from which *V. dahliae* isolates were also rescued. Smaller numbers of isolates (<1%) which were recovered from other provinces or cultivars were not shown in Table 1. Interestingly, some pathogen isolates (0.3%) were also obtained from wild-type olives in some orchards.

Diversity of *Verticillium dahliae* VCGs infecting olive cultivars in Turkey

Isolates of *V. dahliae* examined in this study were assigned to VCG1A (887 isolates), VCG2A (172 isolates) or VCG4B (28 isolates). In the previous study, 189 isolates belonged to VCG1A, eight to VCG2A and four to VCG4B; one isolate was heterokaryon-self-incompatible (HSI) and five isolates could not be characterized (NC) (Dervis et al. 2007). HSI and NC isolates from the previous study were not included in VCG prevalence and incidence calculations. Prevalence of VCG1A and disease incidence caused by isolates of this group were higher than those found for VCG2A and VCG4B in most (10 out of 14) of the provinces inspected (Table 1). Only in Bursa, Canakkale, Hatay and Osmaniye provinces was VCG1A prevalence lower than that of VCG2A or VCG4B. VCG2A was detected in eight provinces (Bursa, Hatay, Canakkale, Manisa, Aydin, Mugla, Denizli and Osmaniye), with the highest pathogen prevalence and disease incidence in Bursa (Table 1). Isolates of VCG4B showed the lowest prevalence value and were detected only in Izmir and Hatay provinces. In some provinces (Manisa, Aydin, Mugla, Denizli, Bursa and Osmaniye) both VCG1A and VCG2A isolates were found. Simultaneous presence of VCG1A, VCG2A and VCG4B was only detected in Hatay province (Table 1 and Fig. 1).

'Ayvalik' was the only olive cultivar from which representatives of all VCGs were isolated: VCG1A (19.6% of the total isolates), VCG2A (1.6%), and VCG4B (1.2%) (Table 1). Representatives of VCG1A and VCG2A were recovered from cultivars Memecik (23% and 0.3%, respectively), Gemlik (10% and 9.3%, respectively), Uslu (1.9% and 2.2%), and Halhali (2.5% and 0.6%). From cultivar Domat, both VCG1A (9.9%) and VCG4B (1.2%) representatives were recovered. Only VCG1A representatives were found in cultivars Yamalak Kabasi, Kilis Yaglik, Manzanilla, Nizip Yaglik, Maras, Buyuk Topak Ulak (<1%, cultivar was not shown in Table 1), Izmir Sofralik (<1%) and some wild-type olives. Finally, only VCG2A isolates (<1%) were recovered from cultivar Saurani.

Molecular characterization of Turkish *Verticillium dahliae* isolates infecting olive

A group of selected (115) isolates, representative of geographical origin, VCG diversity and olive cultivars, was used for PCR tests. The number of *V. dahliae* isolates selected from the Aegean Region was higher compared to those from other surveyed areas because of the higher incidence of Verticillium wilt in that region (Table 1). Eighty-eight isolates yielded the PCR pattern B and were molecularly identified as D pathotype. This pattern is characterized by the amplification of the 334-bp PCR marker (defined by primer pair DB19/espdef01), and by the absence of amplification products when using primer pairs INTD2f/INTD2r or INTND2f/INTND2r (Collado-Romero et al. 2006). These isolates corresponded to VCG1A (86), NC (1) or HSI (1), and were recovered from 11 olive cultivars (Memecik, Ayvalik, Domat, Gemlik, Yamalak Kabasi, Kilis Yaglik, Halhali, Manzanilla, Nizip Yaglik, Uslu and Maras) in 12 provinces (Aydin, Balikesir, Bursa, Denizli, Gaziantep, Hatay, Izmir, Kahramanmaras, Kilis, Manisa, Mardin and Mugla). Sixteen isolates yielded the PCR pattern C and were therefore molecularly identified as ND pathotype. This pattern is characterized by amplification of the 824-bp PCR marker yielded by primer pair INTND2f/INTND2r, and by the absence of amplification products when using primer pairs INTD2f/INTD2r, or DB19/espdef01 (Collado-Romero et al. 2006). This group comprised 12 VCG2A isolates, three VCG4B isolates, and one NC isolate, from seven cultivars (Ayvalik, Domat, Gemlik, Halhali, Memecik, Saurani and Uslu) in seven provinces (Aydin, Bursa, Canakkale, Hatay, Izmir, Manisa and Mugla) (Table 3). Eleven isolates

Table 3 *Verticillium dahliae* isolates used in this study with indication of geographic origin, olive cultivar from which they were isolated, vegetative compatibility grouping (*VCG*), pattern of specific polymerase chain reaction amplicons (*PCR*), and pathotype

Country/ Region[a]	Province	Cultivar	Isolate(s)[b]	VCG	PCR Pattern[c]	Pathotype[d]
Spain	–	Arbequina	V937I	1A	A	D
Greece	–	Cotton	V640I	1A	B	D
Me	Hatay	Ayvalik	OVd241[b], 244	1A	B	D[b]
		Gemlik	OVd235, 267	1A	B	–
		Gemlik	OVd070, 236, 274, 275	2A	C	–
		Halhali	OVd271	2A	C	–
		Halhali	OVd259	2A	–	–
		Saurani	OVd246	2A	C	–
	Kahramanmaras	Gemlik	OVd249	1A	B	–
		Kilis Yaglik	OVd198, 247, 254	1A	B	–
		Maras	OVd251, 253[b], 257, 277	1A	B	D[b]
		Nizip Yaglik	OVd256	1A	B	–
	Osmaniye	Ayvalik	OVd242, 243[b]	2A	–	ND[b]
Ae	Aydin	Gemlik	OVd044, 223	1A	B	–
		Manzanilla	OVd100, 124	1A	B	–
		Manzanilla	OVd129	1A	–	–
		Memecik	OVd039, 045, 094, 104[b], 116, 118, 119, 121, 122, 128, 205, 231	1A	B	D[b]
		Yamalak Kabasi	OVd105, 110, 115	1A	B	–
		Memecik	OVd126	2A	C	–
		Wild type	OVd038	NC	–	–
	Denizli	Gemlik	OVd265[b], 280	1A	B	ND[b]
		Memecik	OVd199, 224	1A	B	–

Table 3 (continued)

Country/ Region[a]	Province	Cultivar	Isolate(s)[b]	VCG	PCR Pattern[c]	Pathotype[d]
	Izmir	Ayvalik	OVd026, 074, 219, 266	1A	B	–
		Domat	OVd069, 080	1A	B	–
		Memecik	OVd021, 023[b], 028, 030, 034, 076, 077, 079, 082, 083, 214, 215, 218	1A	B	D[b]
		Uslu	OVd062	1A	B	–
		Ayvalik	OVd031	4B	–	–
		Ayvalik	OVd057, 060[b]	4B	C	ND[b]
		Domat	OVd056	4B	C	–
		Domat	OVd055	HSI	B	–
		Domat	OVd019	NC	–	–
	Manisa	Ayvalik	OVd006, 022, 047, 050, 054, 143, 156, 157, 160, 163, 166, 222	1A	B	–
		Domat	OVd133, 136, 141[b], 146, 177	1A	B	D[b]
		Uslu	OVd151	2A	C	ND
		Domat	OVd053	NC	–	–
	Mugla	Domat	OVd187	1A	B	–
		Memecik	OVd0007[b], 009, 017, 186, 207	1A	B	D[b]
		Memecik	OVd211	2A	C	ND
		Memecik	OVd016	NC	B	–
		Memecik	OVd018	NC	C	–
S	Gaziantep	Nizip Yaglik	OVd191[b], 238	1A	B	D[b]
	Kilis	Kilis Yaglik	OVd072[b],192	1A	B	D[b]
	Mardin	Halhali	OVd197	1A	–	–
		Halhali	OVd245	1A	B	D
Ma	Balikesir	Ayvalik	OVd227[b], 262	1A	B	D[b]
	Bursa	Gemlik	OVd206[b], 232	2A	C	ND[b]
		Gemlik	OVd234	1A	B	–
	Canakkale	Ayvalik	OVd261	2A	C	ND
		Ayvalik	OVd264	2A	–	–

[a] *S* South-East Anatolian, *Me* Mediterranean, *Ae* Aegean, *Ma* Marmara

[b] Pathotype information for corresponding isolate in each line

[c] 115 representative isolates were used for PCR tests out of 1,294 isolates tested for VCG. PCR markers pattern: *A*=334 bp (+), 824 bp (–), 462 bp (+); *B*=334 bp (+), 824 bp (–), 462 bp (–); *C*=334 bp (–), 824 bp (+), 462 bp (–) (Collado-Romero et al. 2006); – = not determined

[d] *D* olive defoliating, *ND* olive nondefoliating, – = not determined

[VCG1A (3), VCG2A (5), VCG4B (1) and NC (3)] could not be molecularly characterized since no PCR marker could be obtained after PCR assays (Table 3).

In addition to the specific PCR analyses, the group of selected isolates (17) used in the pathogenicity/ virulence tests was also subjected to RAPD-PCR analysis with primers OPH19 and OPH20. The aim was to investigate whether differences in virulence found among isolates in VCG1A (see below) were associated with differences in the RAPD patterns. Results indicated that all VCG1A isolates yielded the same RAPD-PCR profile, being similar to that previously described (Pérez-Artés et al. 2000) for *V. dahliae* D pathotype isolates infecting cotton and olive (results not shown).

Virulence of Turkish *Verticillium dahliae* isolates on olive cultivars with different susceptibility levels to Verticillium wilt

The virulence of representative isolates from each province and VCG was examined on susceptible cultivars Manzanilla and Ayvalik and tolerant cultivar Gemlik. Virulence of isolates varied depending on the susceptibility of the cultivars tested (Table 2 and Fig. 2). VCG1A isolates induced severe symptoms, including defoliation, in 'Manzanilla' and 'Ayvalik' plants, and thus were confirmed as D pathotype. First symptoms (chlorosis, grayish-pale green leaves, rolling inward of leaves, necrosis of affected shoots) on 'Manzanilla' and 'Ayvalik' VCG1A-inoculated plants were scored at 16–21 days after inoculation. First defoliation events induced by VCG1A isolates were observed 3 weeks after inoculation. Falling of first green leaves in most of the plants inoculated with the most virulent isolates (OVd141, OVd241 and OVd191), took place without any previous symptom. Severe defoliation was evident at 5–6 weeks after inoculation and, in some cases, the bark of affected branches turned to purple color above 15 cm of the inoculation point. Eleven percent of dead plants were scored 7 weeks after inoculation. On the contrary, only slight defoliation was observed in VCG1A-inoculated 'Gemlik' plants. These plants showed first visible symptoms (chlorosis) about 1 month after inoculation, only mild symptoms were observed during the first 2 months, and partial defoliation rarely occurred during the third month. VCG2A/4B isolates induced mild symptoms (mainly chlorosis) in all cultivars, without defoliation, and therefore were characterized as ND pathotype. However, some 'Manzanilla' and 'Ayvalik' inoculated plants showed necrotic leaves which remained attached to the twigs. Cultivar Gemlik was highly resistant to VCG2A/4B isolates, with the only exception being isolate OVd261 (VCG2A) (Table 2).

Within a VCG, virulence varied among isolates infecting the same cultivar. For instance, VCG1A isolates OVd241, OVd141, and OVd191 showed DSI values >91% 3 months after inoculation on cultivar Manzanilla. In contrast, at the same scoring time, VCG1A isolates OVd72, OVd104 and OVd265 showed DSI values <59% on the same cultivar (Table 2).

Overall, VCG2A isolates were less virulent than VCG1A isolates, and DSI values were significantly lower ($P<0.05$) for most of the VCG2A-olive interactions analyzed 3 months after inoculation (Table 2). Nevertheless, exceptions to this general rule were observed. For instance, VCG1A isolate OVd265 was as virulent as VCG2A/4B isolates on cultivar Manzanilla. Similarly, VCG2A isolates OVd261 or OVd243 did not differ in virulence from some VCG1A isolates (i.e., OVd72, OVd104 or OVd265) on cultivar Ayvalik. Finally, virulence displayed by all VCG2A isolates (and VG4B isolate OVd60) was not significantly different from that of the less virulent VCG1A isolates OVd104 or OVd265

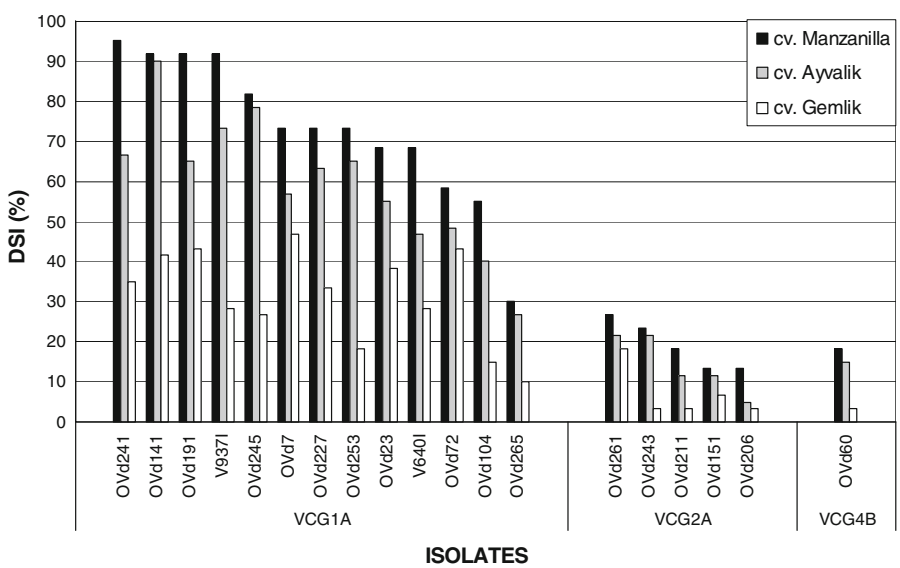

Fig. 2 A continuum of virulence scored in olive cultivars Manzanilla, Ayvalik and Gemlik inoculated with *Verticillium dahliae* isolates belonging to different VCGs 3 months after inoculation

on cultivar Gemlik. Interestingly, virulence of VCG2A isolate OVd261 was not significantly different from that of the vast majority of VCG1A isolates on 'Gemlik' plants. As shown in Table 2, the only VCG4B isolate assayed in this study (OVd60) did not show significant differences in virulence to that scored for VCG2A isolates, regardless of the olive cultivar tested.

Finally, growth of new shoots was observed in 'Gemlik' plants 6 months after inoculation (including VCG1A-inoculated plants that showed severe symptoms 12 weeks after inoculation). On the contrary, no recovery phenomenon (including emergence of new shoots) was observed in 'Manzanilla' and 'Ayvalik' plants inoculated with VCG1A isolates. Mortality in 'Manzanilla' and 'Ayvalik' plants inoculated with VCG1A isolates was high, ranging from 8.3 to 83.3%. However, among VCG2A/4B isolates, only OVd243 caused the death of 8.3% of inoculated 'Ayvalik' plants. No mortality was observed in 'Gemlik' plants inoculated with either VCG1A or VCG2A/4B isolates (Table 2).

Discussion

In this study, all important provinces for olive cultivation in Turkey were surveyed to assess the current importance of Verticillium wilt and the diversity and prevalence of *V. dahliae* VCGs. The surveyed area represented nearly 91% of the total olive acreage in the country. Olive orchards are present in other provinces, comprising an area of about 69.000 h. However, these orchards are smaller in size and geographically dispersed, and were therefore considered as negligible, not representative, or with minor importance within olive cultivation in Turkey.

V. dahliae was present in 35% of the olive orchards examined, and mean disease incidence was 3.1%. In general, values of pathogen prevalence and disease incidence were directly related in all inspected provinces. The survey showed that olive *V. dahliae* isolates in Turkey belonged to VCG1A, VCG2A, or VCG4B. Remarkably, VCG1A (D pathotype) was the most prevalent (29.3%) VCG in Turkey, followed by VCG2A (ND pathotype, 4.9%) and VCG4B (ND pathotype, 0.9%). Accordingly, disease incidence caused by VCG1A was higher than that caused by VCG2A and VCG4B in 10 out of 14 provinces inspected. So far, Canakkale province is the only olive cultivation area in Turkey free of VCG1A infections. VCG2A isolates were recovered from all regions except Southeastern Anatolia. Finally, VCG4B was only found in Izmir and Hatay provinces. Even though VCG2B has been reported as commonly as VCG1A on cotton in Turkey (Dervis et al. 2008), the present survey revealed that the former group was not found to naturally infect olive. Overall, the prevalence of each VCG was associated to a certain extent with specific provinces.

An important conclusion of this study is that Turkey is the second country, besides Spain, in which *V. dahliae* D pathotype (VCG1A) infecting commercial olive orchards is reported, confirming previous observations (Dervis et al. 2007). Moreover, the alarmingly high prevalence of VCG1A isolates in Turkey is in accordance with reports informing of a continuous spread of the D pathotype to new olive-cultivation regions in Spain. In this case, early studies showed that the D pathotype was localized in marsh areas of the Lower Guadalquivir Valley (Southern Spain), where cotton was intensively grown (Bejarano-Alcázar et al. 1995). From that well-defined area, a steady dispersal of the D pathotype to new, distant olive cultivation regions has been reported (Mercado-Blanco et al. 2003; Navas Cortés et al. 2008; Rodríguez et al. 2008). It is tempting to suggest the existence of similar scenarios where the same factors (i.e., previous susceptible crops, changes from dry-farming to irrigation, very susceptible cultivars, use of infected planting material, etc.) might be contributing to the spread of the D pathotype in distant countries of the Mediterranean basin.

In earlier studies, the genetic and molecular analyses of *V. dahliae* isolates from different geographic origins and different host sources made it possible to categorize all VCG1A isolates as the D pathotype, displaying either the so-called PCR pattern A (amplification of 334-bp and 462-bp markers) or PCR pattern B (amplification of a 334-bp marker). Similarly, all VCG2A and VCG4B isolates were described as the ND pathotype and displayed the PCR pattern C (amplification of the 824-bp marker) (Pérez-Artés et al. 2000; Korolev et al. 2001, 2008; Collado-Romero et al. 2006). This notable correlation between the VCG of isolates and their PCR patterns was also found in the present study (Table 1). Indeed, Turkish *V. dahliae* isolates infecting olive have shown, regardless of olive cultivar source or province

of origin, that: i) all VCG1A isolates yielded the PCR pattern B and were molecularly qualified as D pathotype; and ii) all VCG2A and VCG4B isolates produced the PCR pattern C characteristic of the ND pathotype. Collado-Romero et al. (2006) demonstrated that isolates within a VCG subgroup are molecularly similar and can be clearly differentiated from those in other groups, but also that some molecular variability can be detected within a VCG subgroup associated with the geographic origin of isolates. For instance, they found that all VCG1A cotton and olive isolates from Spain yielded the PCR pattern A (334-bp and 462-bp PCR markers), whereas VCG1A cotton isolates from Greece and Turkey yielded the PCR pattern B (334-bp PCR marker). Our results using a large number of olive isolates corroborate the previously reported molecular difference between VCG1A/D pathotype isolates from Spain (PCR pattern A) and Turkey (PCR pattern B). Therefore, two distinct VCG1A/D pathotype, olive-infecting populations can be found in the Mediterranean basin: one associated with the PCR pattern A and located in Spain and another yielding the PCR pattern B and found in the Eastern Mediterranean basin. Interestingly, both PCR patterns have been found in VCG1 (no information on subgroup A or B reported) isolates in Israel (Korolev et al. 2008). These differences would suggest that new strains of *V. dahliae* might develop in different geographic areas or, alternatively, they might have spread from a common center from where VCG1A originated (Collado-Romero et al. 2006).

The present survey has also explored the distribution of *V. dahliae* VCGs among the most economically-relevant olive cultivars in Turkey. Cultivar Memecik was the one from which most *V. dahliae* isolates (23.3% of the total number) were obtained, followed by cultivars Ayvalik, Gemlik and Domat. Almost all isolates sampled from 'Memecik' trees in this present study were VCG1A and predominantly (about 75% of the samples) originated from olive orchards on land formerly devoted to cotton cultivation (Aydin, Izmir and Mugla provinces), and rarely (about 5% of the samples) from orchards in mountainous areas (Cine and Karacasu districts in Aydin province). The high *V. dahliae* prevalence in cultivar Memecik, as well as the high disease severity observed under natural conditions in 'Memecik' trees (dead trees were commonly observed in our surveys) are in full agreement with its previous qualification as a very susceptible cultivar to Verticillium wilt under greenhouse conditions (Erten 2004). The high prevalence of VCG1A could be explained by newly-established 'Memecik' olive orchards in former cotton fields (Jiménez-Díaz et al. 1998), where presence of VCG1A isolates had been reported previously (Göre 2007). Cultivar Ayvalik was the only cultivar from which the three VCGs detected in Turkey were isolated. The high *V. dahliae* prevalence value in this cultivar also corresponded with its previous qualification as very susceptible to Verticillium wilt under greenhouse conditions (Erten 2004).

Results from the artificial inoculation bioassay showed that virulence varied among isolates according to VCG and Verticillium wilt susceptibility of cultivars tested, and confirmed all VCG1A isolates as D pathotype and all VCG2A/4B isolates as ND pathotype. More interestingly, differences in virulence among isolates within a VCG were also observed, and disease severity induced by representatives of different VCGs sometimes did not significantly differ on a specific olive cultivar. Differences in virulence were evident among isolates in VCG1A. For instance, DSI (%) values scored on the susceptible cultivar Manzanilla ranged between 30% (isolate OVd265) and 95% (isolate OVd241) 3 months after inoculation. In fact, disease severity induced by the latter isolate was not significantly different from that induced by VCG2A/4B isolates. Moreover, overlapping in disease severity scores among isolates was even more evident in cultivars Ayvalik and Gemlik. As expected, VCG1A isolates were the most virulent. However, a continuum of virulence within this group was revealed, and the boundary between the virulence caused by some VCG1A isolates and that by VCG2A/4B isolates was vague (Fig. 2). A broad range of virulence has also been reported for *V. dahliae* isolates in VCG2B from cotton in Israel (Korolev et al. 2000; Korolev et al. 2001). Highly virulent *V. dahliae* VCG1A isolates from Turkey were as virulent as the VCG1A olive isolate (V937I) from Spain. This suggests that molecular differences between VCG1A olive isolates from Turkey (PCR pattern B) and Spain (PCR pattern A) are not related to virulence phenotype.

Data from this present study provide relevant information on the relationship between *V. dahliae* pathotypes/VCGs and olive cultivars present in a defined area, particularly if the D pathotype is prevalent and very susceptible cultivars are grown.

When the ND pathotype (VCG2A or VCG4B) was present and very susceptible cultivars to the D pathotype such as Ayvalik, Memecik and Uslu (Erten 2004) were grown, then disease symptoms were less severe and only a low number of dead trees was recorded (for instance, some orchards in Aydin, Izmir, Mugla, etc.). It was also noted that in several cases (orchards in Hatay province, diseased trees were observed in year 2004, and then recovery was recorded in year 2007), trees infected with the ND (VCG2A or 4B or both) pathotype showing Verticillium wilt symptoms eventually recovered from the disease, a phenomenon described earlier (Rodríguez-Jurado 1993; Jiménez-Díaz et al. 1998; Mercado-Blanco et al. 2001), and here recorded for 'Gemlik' plants in artificial-inoculation bioassays. According to the distribution and prevalence of *V. dahliae* VCGs in Turkey here reported, and the results from the pathogenicity test, planting of susceptible cultivars (Memecik, Ayvalik, Domat, etc.) should be avoided since most of the olive orchards here examined are contaminated with the D pathotype.

The first report of Verticillium wilt of olive in Turkey informed of infections in 'Memecik' (Mugla province) and 'Ayvalik' (Balikesir province) trees (Saydam and Copcu 1972). The disease was not considered a serious threat for olive cultivation at that time. Diverse factors have likely contributed to the spread of the D pathotype within Turkey, configuring the disease as the most serious phytopathological problem for olive growers. Firstly, recent incentives of the Turkish government promoting olive production shifted the traditional olive orchard pattern from arid/semi-arid conditions to irrigated lowlands where the previous crop pattern was mostly cotton and/or rarely vegetables. Because of the demonstrated cross pathogenicity to olives, VCG1A strains from cotton (Göre 2007; Dervis et al. 2008) could have caused severe wilt in olives. Secondly, since olive trees have a long unproductive period (up to 3–6 years), farmers usually perform intercropping with cotton and susceptible vegetable hosts (eggplant, pepper, cucumber, etc.) which may increase pathogen inoculum potential in soils (Jiménez-Díaz et al. 1998). Additionally, propagation of symptomless but infected planting material by nurseries and uncontrolled seedling production and distribution of 'Gemlik' to the other provinces might have contributed to the introduction of Verticillium wilt to newly established orchards (Rodríguez-Jurado 1993).

Results obtained in this work contribute to the progress in the knowledge of the distribution and diversity of *V. dahliae* in olive plantations in Turkey, and would be of much interest for implementing potential disease management strategies for Verticillium wilt.

Acknowledgements This study was partially supported by The Scientific and Technical Research Council of Turkey (TUBITAK).

References

Bejarano-Alcázar, J., Blanco-López, M. A., Melero-Vara, J. M., & Jiménez-Díaz, R. M. (1995). Influence of inoculum density of defoliating and nondefoliating patothypes of *Verticillium dahliae* in epidemics of Verticillium wilt of cotton in southern Spain. *Phytopathology, 85*, 1474–1481.

Carder, J. H., Morton, A., Tabrett, A. M., & Barbara, D. J. (1994). Detection and differentiation by PCR of subspecific groups within two *Verticillium* species causing vascular wilts in herbaceous hosts. In A. Schots, F. M. Dewey, & R. Oliver (Eds.), *Modern assays for plant pathogenic fungi* (pp. 91–97). Oxford: CAB International.

Cherrab, M., Bennani, A., Charest, P. M., & Serrhini, M. N. (2002). Pathogenicity and vegetative compatibility of *Verticillium dahliae* isolates from olive in Morocco. *Journal of Phytopathology, 84*, 703–709.

Colella, C., Miacola, C., Amenduni, M., D'Amico, M., Bubici, G., & Cirulli, M. (2008). Sources of Verticillium wilt resistance in wild olive germplasm from the Mediterranean region. *Plant Pathology, 57*, 533–539.

Collado-Romero, M., Mercado-Blanco, J., Olivares-García, C., Valverde-Corredor, A., & Jiménez-Díaz, R. M. (2006). Molecular variability within and among *Verticillium dahliae* vegetative compatibility groups determined by fluorescent amplified fragment length polymorphism and polymerase chain reaction markers. *Phytopathology, 96*, 485–495.

Correll, J. C., Klittich, C. J. R., & Leslie, J. F. (1987). Nitrate nonutilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. *Phytopathology, 77*, 1640–1646.

Dervis, S., & Bicici, M. (2005). Vegetative compatibility groups in *Verticillium dahliae* isolates from cotton in Turkey. *Phytoparasitica, 33*, 157–168.

Dervis, S., Erten, L., Soylu, S., Tok, F. M., Kurt, S., Yildiz, M., et al. (2007). Vegetative compatibility groups in *Verticillium dahliae* isolates from olive in Western Turkey. *European Journal of Plant Pathology, 119*, 437–447.

Dervis, S., Kurt, S., Soylu, S., Erten, L., Soylu, E. M., Yildiz, M., et al. (2008). Vegetative compatibility groups of

Verticillium dahliae from cotton in the Southeastern Anatolia region of Turkey. *Phytoparasitica, 36*, 74–83.

Erten, L. (2004). Determination of susceptibility of some olive and rootstocks against Verticillium wilt (*Verticillium dahliae* Kleb.). Ph.D. Thesis, Aegean University, Turkey.

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

Jiménez-Díaz, R. M., Tjamos, E. C., & Cirulli, M. (1998). Verticillium wilts of major tree hosts. In J. Hiemstra & D. Harris (Eds.), *Compendium of Verticillium wilt in tree species* (pp. 55–57). Wageningen: Ponsen and Looijen.

Joaquim, T. R., & Rowe, R. C. (1990). Reassessment of vegetative compatibility relationships among strains of *Verticillium dahliae* using nitrate-nonutilizing mutants. *Phytopathology, 80*, 1160.

Katan, T. (2000). Vegetative compatibility in populations of Verticillium—an overview. In E. C. Tjamos, R. C. Rowe, J. B. Heale, & D. R. Fravel (Eds.), *Advances in Verticillium research and disease management* (pp. 69–86). St. Paul: APS.

Korolev, N., & Katan, T. (1997). Improved medium for selecting nitrate nonutilizing (*nit*) mutants of *Verticillium dahliae*. *Phytopathology, 87*, 1067–1070.

Korolev, N., Katan, J., & Katan, T. (2000). Vegetative compatibility groups of *Verticillium dahliae* in Israel: their distribution and association with pathogenicity. *Phytopathology, 90*, 529–566.

Korolev, N., Pérez-Artés, E., Bejarano-Alcázar, J., Rodríguez-Jurado, D., Katan, J., Katan, T., et al. (2001). Comparative study of genetic diversity and pathogenicity among populations of *Verticillium dahliae* from cotton in Spain and Israel. *European Journal of Plant Pathology, 107*, 443–456.

Korolev, N., Pérez-Artés, E., Mercado-Blanco, J., Bejarano-Alcázar, J., Rodríguez-Jurado, D., Jiménez-Díaz, R. M., et al. (2008). Vegetative compatibility of cotton defoliating *Verticillium dahliae* in Israel and its pathogenicity to various hosts. *European Journal of Plant Pathology, 122*, 603–617.

López-Escudero, F. J., & Blanco-López, M. A. (2001). Effect of a single or double soil solarization to control Verticillium wilt in established olive orchards in Spain. *Plant Disease, 85*, 489–496.

López-Escudero, F. J., del Río, C., Caballero, J. M., & Blanco-López, M. A. (2004). Evaluation of olive cultivars for resistance to *Verticillium dahliae*. *European Journal of Plant Pathology, 110*, 79–85.

Mercado-Blanco, J., Rodríguez-Jurado, D., Pérez-Artés, E., & Jiménez-Díaz, R. M. (2001). Detection of the nondefoliating pathotype of *Verticillium dahliae* in infected olive plants by nested PCR. *Plant Pathology, 50*, 609–619.

Mercado-Blanco, J., Rodríguez-Jurado, D., Pérez-Artés, E., & Jiménez-Díaz, R. M. (2002). Detection of the defoliating pathotype of *Verticillium dahliae* in infected olive plants by nested-PCR. *European Journal of Plant Pathology, 108*, 1–13.

Mercado-Blanco, J., Rodríguez-Jurado, D., Parrilla-Araujo, S., & Jiménez-Díaz, R. M. (2003). Simultaneous detection of the defoliating and nondefoliating *Verticillium dahliae* pathotypes in infected olive plants by duplex, nested polymerase chain reaction. *Plant Disease, 87*, 1487–1494.

Navas Cortés, J. A., Landa, B. B., Mercado-Blanco, J., Trapero-Casas, J. L., Rodríguez-Jurado, D., & Jiménez-Díaz, R. M. (2008). Spatiotemporal analysis of spread of infections by *Verticillium dahliae* pathotypes within a high tree density olive orchard in Southern Spain. *Phytopathology, 98*, 167–180.

Owen, C. A., Bita, E. C., Banilas, G., Hajjar, S. E., Sellianakis, V., Aksoy, U., et al. (2005). AFLP reveals structural details of genetic diversity within cultivated olive germplasm from the Eastern Mediterranean. *Theoretical and Applied Genetics, 110*, 1169–1176.

Pérez-Artés, E., García-Pedrajas, M. D., Bejarano-Alcázar, J., & Jiménez-Díaz, R. M. (2000). Differentiation of cotton-defoliating and nondefoliating pathotypes of *Verticillium dahliae* by RAPD and specific PCR analyses. *European Journal of Plant Pathology, 106*, 507–517.

Rodríguez, E., García, P. A., García-Garrido, J. M., García, P. A., & Campos, M. (2008). Agricultural factors affecting Verticillium wilt in olive orchards in Spain. *European Journal of Plant Pathology, 122*, 287–295.

Rodríguez-Jurado, D. (1993). Interacciones huésped-parásito en la Verticilosis del olivo (*Olea europaea* L.) inducida por *Verticillium dahliae* Kleb. PhD Thesis, University of Córdoba, Spain.

Rowe, R. C. (1995). Recent progress in understanding relationships between Verticillium species and subspecific groups. *Phytoparasitica, 23*, 31–38.

Saydam, C., & Copcu, M. (1972). Verticillium wilt of olives in Turkey. *Journal of Turkish Phytopathology, 9*, 235–252.

Tjamos, E. C. (1993). Prospects and strategies in controlling Verticillium wilt of olives. *Bulletin OEPP/EPPO Bulletin, 23*, 505–512.

Truett, G. E., Heeger, P., Mynatt, R. L., Truett, A. A., Walker, J. A., & Warman, M. L. (2000). Preparation of PCR-quality mouse genomic DNA with hot sodium hydroxide and Tris (HotSHOT). *BioTechniques, 29*, 52–54.

Tsror, L., & Levin, A. G. (2003). Vegetative compatibility and pathogenicity of Verticillium dahliae Kleb. isolates from olive in Israel. *Journal of Phytopathology, 151*, 451–455.

ZAE. (2008). Olive Research Institute Statistical Database, http://www.zae.gov.tr/.