Phytopathol. Mediterr. (2010) 49, 370-380

Verticillium wilt of olive in the Guadalquivir Valley (southern Spain): relations with some agronomical factors and spread of *Verticillium dahliae*

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Summary. Verticillium wilt of olive (VWO) is now the most destructive olive disease in the Guadalquivir valley in Andalucía (southern Spain). Disease surveys, conducted to assess the association of agronomical and geographical factors with the current spread of the disease, have shown that VWO is widespread in the valley, with a mean disease incidence (DI) in infested plots reaching 20.4% (9000 inspected trees), but with significant differences among the provinces surveyed (25.7, 23.7 and 12%, for Jaén, Córdoba and Seville, respectively). The DI was significantly higher in irrigated (20.7%) than in dry-farming (18.3%) orchards, and also higher in non-tilled orchards (25.6%) than in regularly-tilled orchards (16.3%). The DI was likewise significantly lower for tree densities above 200 trees ha^{-1}; and it was higher (21.5%) when the orchards were located near areas where other *V. dahliae* host plants were cultivated, than if the orchards were surrounded by non-host plants (11.9%). Lastly, the DI was significantly higher in plots where the tree were less than 25 year old and in plantations close to the Guadalquivir River (less than 10 km). 'Picual' was the cultivar most often affected with the disease, reaching a DI of 41.9% in orchards where this cultivar was grown. Highly virulent defoliating (D) isolates in the plantation surveyed were significantly more common (67.7%) than non-defoliating (ND) isolates (32.3%). These factors could explain the substantial increase in incidence and severity of VWO seen in the valley during the last decade.

Key words: Andalucía, defoliating and non-defoliating pathotypes, disease incidence, epidemiology, *Olea europaea*.

Introduction

Olive (*Olea europaea* L.) is mainly grown in countries of the Mediterranean basin, and particularly in Spain, where 2.4 million ha of olive orchards are planted, accounting for 26% of the total world acreage (Barranco *et al.*, 2008). More than 60% of the Spanish olive tree plantations (near 1.5 million ha) is concentrated in the Guadalquivir valley (Andalucía region, southern Spain), the largest olive cultivation area and a leading producing area of both olive oil and table olives in the world (Barranco *et al.*, 2008). The main provinces in this valley are Seville, Córdoba and Jaén (Figure 1), which comprise 75% of the total olive acreage in Andalucía.

Since the 1970s, the number of olive tree orchards in the Guadalquivir valley has gradually increased thanks to subsidies from the European Community and to olive oil price rises. The expansion in acreage was also accompanied by yield increases per ha because of the improvement and modernization of cultural practices. As a consequence, olive orchards were established in many areas of the valley that had previously been cultivated with herbaceous crops, such as cotton, sugar-beet, sunflower, alfalfa, or vegetables, such

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as potato, tomato, pepper, eggplants or artichokes, because these were now less profitable. All these species are important hosts of *Verticillium dahliae* Kleb, a soil-borne pathogen that causes Verticillium wilt in many plants (Hiemstra and Harris, 1998, Pegg and Brady, 2002). The establishment of new olive plantations in soils with a history of *V. dahliae*-susceptible crops has been recognized as one of the most important causes of the current distribution and spread of VWO (Blanco-López *et al.*, 1984; López-Escudero and Blanco-López, 2001; Navas-Cortés *et al.*, 2008). In olive, *V. dahliae* causes Verticillium wilt of olive (VWO), which is currently the most injurious disease of this plant wherever it is grown (Rodríguez-Jurado, 1993; López-Escudero and Blanco-López, 2001).

Nevertheless, other important factors have most likely contributed to the expansion of VWO in the Guadalquivir Valley and other irrigated areas as well. These can be summarized as follows: 1) the planting of susceptible olive cultivars (Cirulli and Montemurro, 1976; Paplomatas and Elena, 2001; López-Escudero *et al.*, 2004; 2007; Martos-Moreno *et al.*, 2006; López-Escudero and Blanco-López, 2007); 2) the use of infected planting material (Thanassoulopoulos, 1993); 3) excessive watering and/or fertilization (Blanco-López *et al.*, 1984; Al-Ahmad and Mosli, 1993; Serrhini and Zeroual, 1995); 4) inadequate cultural practices, such as tilling in affected orchards or using infested organic amendments (López-Escudero and Blanco-López, 1999), which facilitate the spread of the pathogen within and between plantations; 5) the long-distance dispersal of *V. dahliae* infectious, resistant-structures (microsclerotia, MS), which are efficiently distributed by water pumping stations over large irrigated areas (Moraño-Moreno *et al.*, 2008; López-Escudero *et al.*, 2009a).

In particular, the spread of highly virulent isolates (defoliating [D] pathotype) of the pathogen throughout the Guadalquivir valley poses one of the most serious threats for olive. D isolates were first reported in 1983, when they were restricted to the Marismas area of Andalucía, in the lower Guadalquivir valley, where cotton was intensively grown (Blanco-López *et al.*, 1986; Bejarano-Alcázar *et al.*, 1995). Today, several studies have demonstrated that the D pathotype occurs in other cotton-growing areas as well (Bejarano-Alcázar *et al.*, 1995), and also in new olive groves planted close to those areas in the middle and upper valley (López-Escudero and Blanco-López, 2001; Mercado-Blanco *et al.*, 2003; Navas-Cortes *et al.*, 2008).

In Spain, the available data on the importance and distribution of VWO come from some partial geographical surveys in Andalucía, carried out at random, or in planned inspections at VWO affected olive plantations. These surveys revealed that both the disease incidence (DI, the percentage of diseased plants within an orchard) and the disease prevalence (DP, the percentage of olive orchards where pathogen is detected, divided by the total number of olive orchards inspected) are increasing in some areas both inside and outside the valley (Sánchez-Hernández et al, 1998; León, 2000, Rodríguez *et al.*, 2008). Nevertheless, broad and up-to-date surveys to assess the importance of VWO throughout the Guadalquivir valley provinces are lacking, and more extensive studies are necessary. The main objectives of this work were to update the data on the severity and distribution of VWO in the Guadalquivir valley, and to investigate some agronomical and geographical factors that seen to be associated with the spread of the disease. A third objective was to study the current distribution of virulence groups (D and ND pathotypes) of *V. dahliae* isolates.

Materials and methods

Disease surveys

During the period from April to June 2001 a VWO disease survey was conducted in the three major olive growing provinces of the Guadalquivir Valley, Jaén (upper Valley), Córdoba (central Valley) and Seville (lower Valley) (Figure 1). Surveys were planned in advance, by tracing on a map of Andalucía (scale 1:10.000) several routes in each region, having regard to the existence of roads and access tracks. Theses routes were traveled until wilt symptoms were sighted on one or more olive trees (mainly dieback of branches). The orchard was then geographically located with a global positional system (GPS eTrex SUMMIT, Garmin Europe Ltd., Romsey, UK), and indicated on the map. In this way, 90 olive orchards (27 in Jaén, 33 in Córdoba and 30 in Seville provinces), were inspected (Table 1). In each orchard, a rectangular plot containing 100 trees was chosen at random in the part of the orchard where the diseased trees were first seen. A total 9000 trees were inspected during the survey.

Parameters evaluated

For each olive orchard, a plot containg 100 trees was chosen, from which general, agronomical and phytopathological information was recorded. General information consisted of: the inspection date, municipality, geographical location of the orchard by GPS and its reference code, and where possible, the name and area of the farm and the name of the owner. The geographical location was also indicated by the distance from the plantation to the Guadalquivir River. Agronomical information comprised: plantation age bracket (1–10 years, 10–25, 25–50, older than 50), olive cultivar when it could be clearly identified, number of trees per ha, watering regime (irrigated or dry-farming), soil type according to the FAO classification (Alfisol, Entisol, Inceptisol or Vertisol) (Anonymous, 1998), tillage practices (tilling or no tilling), any crops growing nearby, and wherever possible, the history of previous crops on the plot. Phytopathological information comprised: the DI, the distribution of affected trees in the orchard (random, distribution on boundaries, in clusters, or grouped in areas where the soil profile was frequently waterlogged) and the type of symptom (dieback, defoliation, or both) was recorded in three affected olive trees per plot.

Relationship between the parameters evaluated and DI

The relation of the geographical and agronomical factors to the DI from the fields was studied for all the findings and for the irrigation regime, also by province. Statistical analysis was performed by Statistix 9.0 (Analytical Software, Tallahassee, FL, USA). The Kruskal-Wallis non-parametric AOV test was used for differences of the DI of plots between provinces. The statistical significance of the relationships between the DI and the agronomical and cultural factors was determined by a Chi-square test that compared observed and expected values.

Tree sampling and pathogen isolation.

To isolate the pathogen from the olive tissues and to build up a collection of *V. dahliae* isolates from the Guadalquivir valley area, three 20 cm-long and 1–2 cm diameter stem pieces were taken from wilted branches of each of the three olive trees per field evaluated for symptom type. Stem samples were washed in running tap water, stripped of their bark and surface-disinfected in 0.5% sodium hypochlorite for 1 min. Six wood chips per sample were placed on a Petri dish containing potato dextrose agar (PDA) and incubated at 24°C in the dark for 5–6 days. In this way, a collection of 102 *V. dahliae* isolates was obtained from trees growing in 46 out of the 90 surveyed fields.

PCR-based molecular pathotyping of *V. dahliae* isolates.

Molecular identification of *V. dahliae* pathotypes (D or ND) of the isolates collected was done by specific PCR assays. A representative sample (65) of the isolates was analyzed. Active cultures of isolates were obtained on PDA plates incubated at 24ºC for 5–6 days in the dark. The HotSHOT method (Truett *et al.*, 2000) was used for rapid, small-scale DNA extraction as described by Collado-Romero *et al.* (2006). Briefly, a small amount of pathogen mycelium was scrapped off the PDA colonies with the tip of a sterile pipette, and broken up in 20 μL of a solution of 25 mM NaOH, and 0.2 disodium EDTA, pH 12, in 0.2-ml PCR tubes. The suspensions were incubated for 1 h at 95°C and for 5 min at 4°C in a thermocycler. Then, 20 μL of 40 mM Tris-HCl, pH 5, was added. From the lysate obtained, 5–8 μL was used directly for the PCR assays. Several PCR primer pair combinations were used to identify the pathotype (D or ND) according to the PCR markers that were amplified. Primer pair DB19/DB22 (Carder *et al.*, 1994) yielded a *V. dahliae*-specific polymorphic DNA band of either 539 bp (amplified in D isolates of Spanish cotton and olive) or 523 bp (associated with ND isolates) (Mercado-Blanco *et al.*, 2003). The primer pairs INTD2f/INTD2r (Mercado-Blanco *et al.*, 2002) and INTND2f/INTND2r (Mercado-Blanco *et al.*, 2001) produced PCR markers of 462 or 824 bp previously associated with the cotton and olive D and ND pathotypes of *V. dahliae* pathotypes, respectively. These last two primer pairs were used jointly in duplex PCR assays. Lastly, primer pair DB19/espdef01 amplified a 334-bp PCR marker which is present, among other genetic/molecular groups (Collins *et al.*, 2005; Collado-Romero *et al.*, 2006), in D isolates of Spanish cotton and olive (Mercado-Blanco *et al.*, 2003). Amplification reactions consisted of (25 μL final volume): 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 SRL; Barcelona, Spain), and 5–8 μL of fresh mycelium lysate. The 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, the PCR assays were repeated at least once, using new, fresh lysates every time(see above). Each PCR assay always included control samples from D (V138I) and ND (V176I) for representatives *V. dahliae*, which had 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 procedure. Results of the PCR assays were visualized by agarose gel electrophoresis according to standard procedures.

Results

Importance of VWO in the Guadalquivir valley

Verticillium wilt of olive is widespread in the Guadalquivir Valley, with a mean DI in the infected plots of 20.4% of the 9000 trees inspected in the 90 orchards surveyed. The Kruskal-Wallis test confirmed that the mean DI in the infected plots was significantly higher in the Córdoba and Jaén provinces (23.7 and 25.7% respectively), than in Seville province (12%) (Table 1). A lower DI, falling within the 1–22% bracket of inspected trees affected, was found in 77% of the orchards surveyed in Seville province. Meanwhile, high DI values, in the brackets 45–63% and 64–84%, were recorded in 11% of Jaén orchards and in 12% of Córdoba olive fields, respectively (data not shown).

Association of agronomical, cultural and geographical factors with VWO incidence in the Guadalquivir valley

Some agronomical and cultural factors were studied to evaluate their influence on VWO incidence. As regards the irrigation regime, the DI was significantly higher in irrigated (20.7%) than in dry-farming (18.3%) olive orchards (Table 2). These differences were particularly marked in the central valley (Córdoba province) and the upper valley (Jaén province) valley where the DIs were about 40% higher in irrigated orchards than in dry-farming orchards (Table 2). In olive orchards that were not tilled, the mean DI of VWO was significantly higher (25.6%) than in regularly tilled orchards (16.3%) (Table 3). The planting density of olive trees was also related to the DI. Chi-square analysis showed that the DI was significantly lower when the number of trees per ha was above 200 and higher when it was below 200 (Table 3).

The DI of Verticillium wilt was also related to the proximity of *V. dahliae* susceptible crops to the orchard. When nearby *V. dahliae*-susceptible crops were present, the DI was significantly higher (21.5%) than if the olive orchards were surrounded by non-susceptible crops (11.9%) (Table 3).

Other factors examined for their relation to DI were (i) orchard age, where it was found that plots with trees less than 25-years-old had a significant-

Table 1. Incidence of Verticillium wilt of olive in some orchards in three provinces of the Guadalquivir Valley (southern Spain).

Province	No. inspected fields	Mean DI[a] (%)
Seville (Lower Valley)	30	12 0 a
Córdoba (Middle Valley)	33	23.7 b
Jaén (Upper Valley)	27	25.7 b
Guadalquivir Valley mean	90	20.4

[a] DI, Disease incidence. Values with different letters were significantly different at P=0.05 according to the Kruskal-Wallis one-way nonparametric AOV test.

Table 2. Irrigation regime and incidence (DI) of Verticillium wilt of olive in the Guadalquivir Valley.

Factor	Sub-factor	Fields		DI (No. of trees)	
		No.	Mean DI (%)	Observed	Expected
Seville province	Irrigated	22	12.6	277	251
	Dry-farming	8	10.2	82	91
				$P\,(c^2) = 0.05304$	
Córdoba province	Irrigated	13	28.8	374	320
	Dry-farming	20	20.4	408	492
				$P\,(c^2) = 0.0000$	
Jaén province	Irrigated	6	33	198	170
	Dry-farming	21	23.7	498	594
				$P\,(c^2) = 0.00001$	
Guadalquivir Valley	Irrigated	41	20.7	849	799
	Dry-farming	49	18.3	897	955
				$P\,(c^2) = 0.00837$	

Table 3. Agronomical and cultural factors and the incidence (DI) of Verticillium wilt of olive in the Guadalquivir Valley.

Factor	Sub-factor	Fields		DI (No. of trees)	
		No.	Mean DI (%)	Observed	Expected
Tilling	Tillage	68	16.3	1108	1421
	Non-tillage	22	25.6	563	460
				$P\,(c^2) = 0.00000$	
Trees/ha	> 200	26	16.9	439	502
	< 200	64	21.8	1395	1235
				$P\,(c^2) = 0.0000$	
Neighboring *V. dahliae* host crops	Yes	80	21.5	1720	1336
	No	10	11.9	119.0	167
				$P\,(c^2) = 0.0000$	
Plant age	1–10	14	24.2	339	288
	10–25	30	21.8	654	618
	25–50	21	16.7	351	433
	>50	25	19.7	492	515
				$P\,(c^2) = 0.0000$	
Soil type	Alfisol	21	12.9	271	435
	Entisol	14	24.4	342	290
	Inceptisol	36	21.3	767	745
	Vertisol	19	24.1	458	393
				$P\,(c^2) = 0.0000$	
Distance to Guadalquivir river (km)	<10	28	28.1	787	568
	10–30	35	20.5	717	710
	>30	27	12.4	335	548
				$P\,(c^2) = 0.0000$	

ly higher DI; ii) soil type: orchards established on Entisol and Vertisol soils had a with significantly higher mean DI, accounting for 24% of wilted plants (Table 3); and iii) the olive cultivar: the cv. Picual was most often affected by the disease. In the 24 plots in which this cultivar could be unequivocally identified, the mean DI reached 41.9%.

Lastly, those orchard located closer to the Guadalquivir River (less than 10 km away) had a significantly higher mean DI, accounting for 28.1% (Table 3).

Disease symptoms and pathogen isolation

Two hundred and seventy olive trees (3 per olive orchard) were inspected for VWO symptoms and the pathogen was isolated from affected aerial tissue. Apoplexy (dieback) was seen in 48.5% of symptomatic trees, green leaf defoliation in 32.6% of trees, and 18.8% of trees showed both apoplexy and defoliation.

Symptomatic trees were randomly distributed in most plots (65 out of 90), but in some plots diseased trees were grouped in clusters (focal points or waterlogged soil areas) (16 orchards) or on the boundaries adjoining plots that were cultivated with herbaceous plants susceptible to *V. dahliae* (9 orchards).

Verticillium dahliae was isolated in at least one out of the three sampled trees in 71.1% of the 90 olive orchards surveyed. Pathogen isolation was positive in 49.6% of the 270 symptomatic plants sampled, and in 48.9% of the analyzed tissues. The pathogen was isolated more often from affected plant tissues in irrigated olive orchards than in dry-farming plots or plots with young trees (<10 years old). The other factors were not correlated with pathogen isolation.

PCR-based pathotyping.

The D pathotype occurred in 75% of the 46 fields from where *V. dahliae* was isolated, whereas the ND pathotype was only found in 33% of these fields. In five olive fields, both the D and the ND pathotypes were detected infecting either different trees (four fields) or the same tree (one field) (Figure 1).

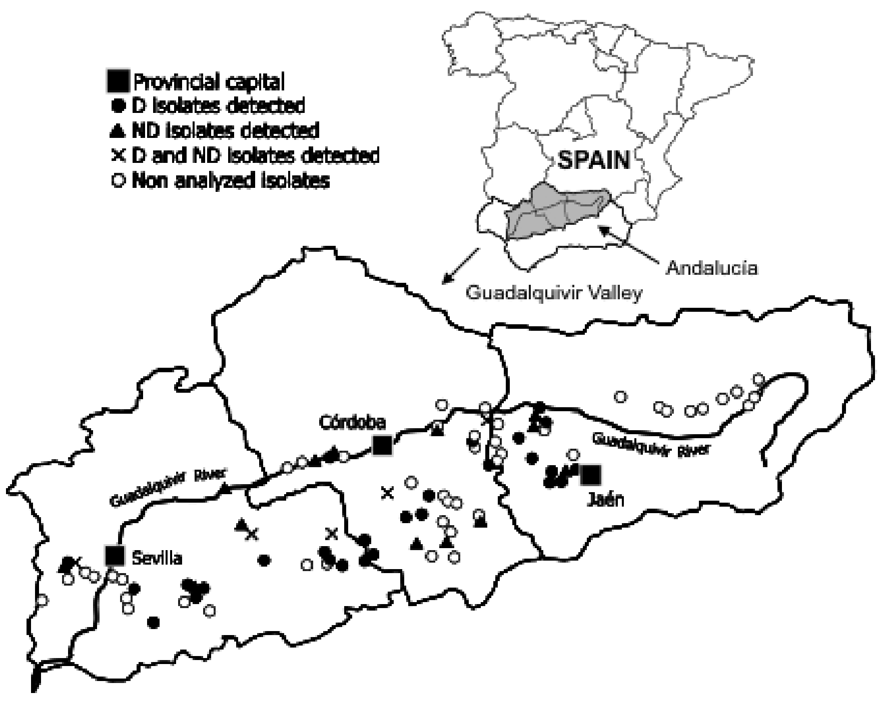

Figure 1. Distribution of *Verticillium dahliae* isolates in olive orchards surveyed throughout the Guadalquivir Valley (Southern Spain). D. Defoliating isolates; ND, non- defoliating isolates.

The percentage of D isolates (67.7%) recovered was significantly higher than the percentage of ND isolates (32.3%) (Figure 1). These differences were particularly great in the Seville and Jaén provinces, where D isolates occurred in 75% of the fields where isolations were conducted but ND isolates only in 25% of fields. In the orchards of Córdoba province both pathotypes were equally common (50%) in the isolates examined (Figure 1).

Finally, while the D pathotype was more frequent in trees from irrigated orchards (79%) than in trees from dry-farming orchards (43%), the ND pathotype was equally common (32%) in irrigated and dry-farming orchards.

Discussion

Surveys during the study revealed that the DI in olive orchards affected by VWO is increasing at an alarming rate in the Guadalquivir Valley, which is the largest olive cultivation area in the world. In this study the DI of orchards was used to evaluate the severity of the disease, instead of the DP. The DI has also been used in other VWO surveys in Andalucía (Rodríguez *et al.*, 2008; 2009). In our opinion the DI is less variable over time and provides more reliable and practical information than the DP. The importance of a disease in a wide region changes continuously over time, since new olive orchards are always being established in soils infested with the pathogen, and established orchards are transformed from old dry-farming plantations into intensive and modern irrigated orchards, and this usually favors disease appearance (Blanco-López *et al.*, 1984; Al-Ahmad and Mosli, 1993, Serrhini and Zeroual, 1995).

Nevertheless, the DP and DI values are more relevant for olive orchards within the valley than for those outside. Sánchez-Hernández *et al.* (1998) reported on extensive surveys in southern Spain during 1989–1996, mainly in the three provinces of the Guadalquivir Valley, in order to determine the relative importance of VWO in relation to the occurrence and etiology of the so-called Drying Syndrome of young olive trees. These authors concluded that nearly 20% of the 372 fields they inspected showed trees with this syndrome, which in fact however was an infection caused by *V. dahliae*. In contrast, the north of Cádiz province (located in south Andalucía, but out of the Guadalquivir valley region here studied), was reported to have a DP of 34% (León, 2000), but the mean DI of the fields examined accounted for only 1%. Similarly, in surveys carried out exclusively in Granada province (located at the east of the valley), 14.1% of 873 olive orchards inspected showed some wilted trees, but the mean DI within olive orchards reached 9.5% of trees (Rodríguez *et al.*, 2009).

A number of authors have reported that several agronomical and edaphic factors affect wilts caused by *V. dahliae* in herbaceous and woody hosts (Blanco-López *et al.*, 1984; Hiemstra and Harris, 1998; Pegg and Brady, 2002; Rodríguez *et al.*, 2008). In this study, we examined the relation between the DI of VWO in the Guadalquivir valley and factors such as tilling, orchard age, neighboring crops, distribution of symptomatic trees and distance from the Guadalquivir River. The DI in the valley was significantly correlated with some of these factors. For instance, the DI was higher in irrigated orchards than in dry-farming orchards. Such differences have been reported in earlier surveys carried out in Andalucía (Blanco-López *et al.*, 1984), in olive-growing areas of Morocco (Al-Ahmad and Mosli, 1993) and in Syria (Serrhini and Zeroual, 1995). Semi-continuous irrigation may enhance wilt disease incidence and severity in herbaceous hosts (Pegg and Brady, 2002). The increase of VWO in olive orchards could be related to an increase of the pathogen population in watered soil within the wet areas produced by the drippers, as demonstrated by López-Escudero and Blanco-López (2005a). An additional to explain the higher DI of VWO in irrigated plantations could be the dispersal of the pathogen by water as reported by a number of authors (Thanassoulopoulos *et al.*, 1981; Rodríguez-Jurado and Bejarano-Alcázar, 2007; Moraño-Moreno *et al.*, 2008; López-Escudero *et al.*, 2009a).

Tilling is reported to be an important way for *V. dahliae* dispersal on herbaceous and woody hosts within and between cultivated plots (Hiemstra and Harris, 1998; Pegg and Brady, 2002). Unexpectedly, however, it was found that DI was significantly higher in untilled olive orchards. The reason for this is unclear, but probably some of these untilled orchards had a high initial pathogen population in the soil, so that the DI increased regardless of tilling practices.

Olive orchards with plant densities over 200 trees ha^{-1} had significantly lower DI values than orchards with fewer than 200 trees ha^{-1}. Nevertheless, this finding should not be extrapolated automatically to all orchards, since olive is planted under a wide range of tree densities and other studies have reported different results (Navas-Cortés *et al.*, 2008; Rodríguez *et al.*, 2008; López-Escudero *et al.*, 2009b). It appears that factors such as inoculum density and distribution in the soil, the type of irrigation, the existence of mechanical or natural pathogen dispersal mechanisms within an orchard, the virulence of the isolates prevailing in the soil, the cultivar resistance, the number of years that other herbaceous hosts were grown in the plot before olive was planted on it, or orchard age also have a decisive role here. Surveys performed by Rodríguez *et al.* (2008) in Granada province (southern Andalucía but part of the Guadalquivir valley area studied here) showed that, when the olive tree density increased, the DI of VWO decreased in non-irrigated orchards, but increased exponentially in irrigated orchards. On the other hand, López-Escudero *et al.* (2009b) found that plant density did not affect final disease incidence, when they compared hedgerow olive of 'Arbequina' or 'Picual' density (around 2000 trees ha^{-1}) with intensive orchards of the same cultivars density (around 200 trees ha^{-1}). An important factor would also seen to be that olive trees should be established for a long period of time. After some years their roots extend over large soil volumes and this causes plant density to become less decisive.

The incidence of VWO was significantly higher in olive plots surrounded by fields cultivated with herbaceous hosts that also harbored the pathogen, mainly cotton. The proximity of these other host plants may enable pathogen propagules, attached to soil particles or carried on infected plant debris, to invade the adjacent olive plots. This supposition is supported by several studies that have stressed the importance of the movement of infested soil or infected plant debris, such as fallen leaves, by machinery, equipment, wind, rainfall, watering runoff, soil slopes, etc., as favoring pathogen dispersal to and between plots (Wilhelm and Taylor, 1965; Cirulli and Montemurro, 1976; Thanassoulopoulos *et al.*, 1979; Blanco-López *et al.*, 1984; Navas-Cortés *et al.*, 2008).

The correlation of some other factors with VWO has also been investigated, providing useful information for further studies or confirming previous knowledge. Thus, VWO seems to develop in a similar way in all soil types, except Alfisol soils, where the mean DI was significantly lower (12.9%). However, to determine the relation between soil type and DI would require of a more in-depth investigation, which was beyond the scope of this work. On the other hand, plots planted with 'Picual' trees showed a higher DI than plots where other olive genotypes were grown. The high susceptibility of 'Picual' to *V. dahliae* has been widely reported in controlled (López-Escudero *et al.*, 2004; 2007; Martos-Moreno *et al.*, 2006), semi-controlled (López-Escudero and Blanco-López, 2007), and field conditions (López-Escudero and Blanco-López, 2001). Lastly, plantations located closer to the Guadalquivir River (at a distance less than 10 km) showed a significant higher DI of WVO. The association of high DI with proximity to the river is probably linked to the fact that many herbaceous hosts of *V. dahliae* are cropped throughout the fertile lowland of the Guadalquivir valley. The existence of susceptible neighboring crops and the dispersal of pathogen propagules through irrigation water are likely contributing factors increasing the inoculum density in soils located at a short distance from the river (see below).

The highly-virulent D pathotype was isolated significantly more often from wilted olive trees than the ND pathotype, especially in the lower and upper Guadalquivir valley. The abundance of highly virulent *V. dahliae* isolates in our survey could explain the alarming increase of VWO incidence as well as the increase in severity seen along the Guadalquivir valley over the last decade (López-Escudero and Blanco-López, 2001; Mercado-Blanco *et al.*, 2003; Navas-Cortés *et al.*, 2008). However, in provinces of Andalucía located outside the valley, such as Granada, ND isolates are more common than D isolates (Rodríguez *et al.*, 2008), probably because this area normally was not cropped with cotton in the past. Interestingly, the D pathotype was clearly prevalent in a broad survey recently carried out in Turkey (Dervis *et al.*, 2007; 2010). This suggests that the most aggressive *V. dahliae* pathotype is rapidly spreading to different olive growing areas, and that similar (agronomical, cultural) factors can be contributing to this process in widely separated geographical areas within the Mediterranean basin.

Little is known so far about the distribution of the D and ND pathotypes of *V. dahliae* in Andalucian soils. However, it seem likely that D isolates have become more common than ND isolates over the last few years. This could be explained by assuming that D isolates in the soil are better able to infect and colonize olive, as has been reported under controlled conditions (Rodríguez-Jurado, 1993; López-Escudero and Blanco-López, 2005b). As a result, larger amounts of D pathotype biomass are released into the soil from infected plant debris after the infective phase. Moreover, various epidemiological studies seem to confirm that D isolates are now spreading throughout the valley. For instance, *V. dahliae* propagules are efficiently dispersed over long-distances through watering systems in large irrigated areas (López-Escudero *et al.*, 2009a). Moreover, Moraño-Moreno *et al.* (2008) found that irrigation water contains a significantly higher proportion of D isolates than of ND isolates. Consequently, D isolates, dispersed by irrigation systems could be causing the rapid outbreaks of severe VWO epidemics which are increasingly being observed. On the other hand, D isolates from infested soils could also be dispersed by human activity to nearby soils previously colonized by ND isolates, particularly soils repeatedly cropped by cotton before. Such human activity would include the movement of machinery and equipment with infested soil and/or infected fallen leaves adhering to them. Navas-Cortés *et al.* (2008) suggested that mechanical operations were a more efficient dispersal method for D than for ND isolates.

In conclusion, positive evidence is presented that VWO, predominantly caused by the D pathotype, is spreading at an alarming rate throughout the most important olive-growing areas of the Guadalquivir valley in southern Spain. Various agronomical and geographical factors are significantly associated with the current spread of the disease. Within the study area, the DI is significantly related to the type of irrigation, the planting density of trees, the proximity to *V. dahliae*-susceptible crops, the young age of the trees, the proximity to the Guadalquivir River and, unexpectedly, to the absence of tillage. Findings here presented are of interest from an epidemiological point of view, as well as with a view to implementing control measures in the different olive-growing areas in which VWO is undergoing a dramatic upsurge.

Acknowledgements

Research was partially supported by a grant CAO-00-011-C12-03 from the Comisión Interministerial de Ciencia y Tecnología (Spain). Thanks are due to two anonymous referees for interesting suggestions on how to improve an earlier version of the manuscript.

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Accepted for publication: December 11 , 2010