

Incidence and Prevalence of Verticillium Wilt of Olives in Jordan

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ABSTRACT

A study of the incidence of Verticillium wilt disease in olive groves and the prevalence of *V. dahliae* races in the olive major producing regions in Jordan was started in 1997. For the race typing of *V. dahliae*, three tomato cultivars were used as differential hosts. From irrigated fields, 48% of the samples collected showed Verticillium infections, while only 7.4% of the samples collected from non-irrigated olive groves showed positive fungal isolations. Some olive groves showed symptoms of Verticillium wilt during spring months, but leaf and vascular necrosis was mainly clear in October and November. Both races of *V. dahliae* were reported for the first time in Jordan. Race 2 appeared in 63% of the total collected isolates, while race 1 constituted 32% among the tested isolates. The ratio of race 2 to race 1 isolates obtained from infected olive fields was nearly 2:1. The highest disease severity was observed in irrigated fields. The proportion of infected petioles sampled from the bottom of tomato plants was greater than that of infected stem segments sampled from the mid part of inoculated seedlings. For precise disease evaluation, isolation must be done from the lower petioles of the infected or inoculated plants. Tomato seedlings cv. AR Tom 001 inoculated with the isolates of *V. dahliae* race 1 produced no fruits during the whole experimental period. Isolation of the pathogen was successful from fallen olive leaves but unsuccessful from the seed flesh or embryo of olive fruits collected from infected trees.

Keywords: *Verticillium*, *Olea europea*, Disease Survey, Pathogen Races.

INTRODUCTION

Olives are cultivated over large areas in Jordan; the total number of olive trees was about 8.6 million, covering 645324 dunums in 2006 (Agricultural Statistics, 2006). Olive cultivation has expanded during the last decade, especially since 1980, into the eastern area between Zarka and Azraq, in the villages Dulayl, Hallabat, Khaldieh and Mafraq. New olive groves were established on fields previously cultivated with

vegetables. Olive trees are affected by many fungal pathogens and the most important one is *Verticillium* species which is the most difficult problem in olive tree pathology. The impact of the disease was particularly severe in intercropped orchards during the first years after the establishment of the groves, but tended to become milder with aging. This is true in irrigated groves of susceptible olive cultivars (Rodriguez et al., 2008). Verticillium wilt of olives caused by *Verticillium albo-atrum* Reinke and Berth was first reported to occur in Sicily in 1946 and 1948 (Ruggieri, 1946; 1948).

Verticillium wilt of olive is a limiting factor for the cultivation of high yielding, excellent quality cultivars in Greece (Thanassouloupoulos, et al., 1979). It caused severe damage to olive groves in Idleb and Aleppo districts in

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Syria (Al-Ahmad and Mosli, 1993). Verticillium wilt of vegetables was detected in 23 localities of the Jordan Valley and the uplands mainly on eggplant, potato and tomato. Eleven isolates were identified as *V. dahliae* (Mamluk and Skaria, 1979). An increase in the number of affected trees has occurred in irrigated olive groves in Jordan since 1993 (Naser and Al-Momany, 1998; Karajeh and Al-Momany, 1999). In contrast to the rapid spread of the disease in the desert-irrigated districts, very few olive trees have become diseased in Irbid County of north Jordan (non-irrigated districts).

The objectives of this study were to evaluate the incidence of Verticillium wilt and the prevalence of *V. dahliae* races in the major olive producing regions in Jordan and to determine the most aggressive races on olives trees.

Materials and Methods

A-Field Surveys

About 8000 dunums of land were surveyed in this investigation. Disease symptoms were observed during several field visits covering all olive growing areas in Jordan. Many fields were visited at the same location and in every field; fifty trees were selected randomly for pathogen isolations. Visual symptoms were recorded; in addition to that, semi-hard wood cuttings were taken from each tree. Normally, one to two year old branches were selected and taken to the laboratory for further isolation. Foliar symptoms, including dull green, internally rolled, or necrotic leaves and defoliated twigs were evaluated to record disease incidence and severity. Disease severity was rated as 1 = no symptoms, 2 = vascular necrosis of lower branches, 3 = necrosis in less than 50% of the plant, 4 = necrosis in more than 50% of the plant, 5 = death or complete defoliation of tree (McHugh and Schreiber, 1984).

B- Isolation of *V. dahliae* from Olive Plant Tissue

Stem pieces (4cm long and 4-10 mm in diameter)

were surface disinfected in 0.5% solution of sodium hypochlorite (2-3 min). Stems were debarked, and 3 mm from each end of the stem was discarded. The remaining de-barked stem was sectioned into sections 4-5mm thick and sections were placed with the cut ends on selective media (Ausher et al., 1975), and incubated at 22°C in the darkness. Six sections were placed on each Petri- dish. After one to two weeks, sections were examined for white fluffy mycelium. Dark sclerotia formed after 10 days of incubation. Examination of the colonies of *V. dahliae* under a dissecting microscope revealed the presence of microsclerotia in a radial pattern.

C. Race Testing:

Isolates were obtained from olive branch sections collected during the course of this study. Tomato cv. AR Tom 001 susceptible to race 1 and 2, AR Tom 002 (resistance unknown) and Farida No. 076 F1 HYB resistant to race 1 were used as differential cultivars. Tomato seeds were obtained from Yousif Marto company-Jordan. Inoculum was prepared from pure cultures of *Verticillium* obtained from infected olive trees and started from mycelial and microsclerotial stock cultures. Conidia and microsclerotia were produced in abundance on selective medium for *Verticillium* after 2 weeks from incubation. Inoculum suspensions were prepared by gently flooding the dishes with distilled water, filtering the suspensions through cheesecloth and adjusting the inoculum to 1.0×10^8 spores /ml.

Tomato seeds were germinated in peat moss placed under muslin covers in separate trays with concave pits. Sixty days after seeding, the seedlings were transferred into plastic pots of 7cm depth and 7cm diameter. One seedling was planted into each pot, 10 plants were inoculated with each fungal isolate. Inoculation was done 10 days after transplanting. Each pot received 5ml from the inoculum (1.0×10^8 spores /ml). Each inoculum from 46 fungal isolates was gently distributed around the

root zone with a 5-ml pipette. Each pot was placed in a Petri-dish to avoid any inoculum leaching during watering. Inoculated seedlings were grown under greenhouse conditions for additional 12 weeks. Isolates that did not cause disease symptoms on Farida cv. but infected AR Tom 001 cv. were typed as race 1. Isolates were typed as race 2 when they produced disease symptoms and positive isolations on both cultivars (AR Tom 001 and Farida) and as non-pathogenic on tomato if no symptoms were produced on both cultivars. *Verticillium* was isolated by taking three petioles from each plant from the lower portion of the inoculated tomato transplants. After one month from inoculation, plant height, number of leaf petioles/plant, plant yield and disease severity were recorded. Stem segments from the mid part of the stem of each plant were taken for

further isolation to verify infections by *V. dahliae* (Dobinson *et al.*, 1996). The leaf petioles and stem sections were surface sterilized for 2 minutes in 0.5% NaOCL. Six sections were transferred from each plant into Petri-dishes containing *Verticillium* selective medium. Plates were incubated in the dark at 22°C for 2 weeks, and the presence of *V. dahliae* in the petiole and stem cross sections was examined microscopically.

Results:

The percent incidence of infected olive trees in fields infested with verticillium wilt, varied from 4 to 80%. Verticillium wilt was found in 48% of the irrigated fields, in comparison to only 7.4% in the non-irrigated olive groves and the average disease incidence was 20.5 and 14.8%, respectively (Table 1).

Table 1. Occurrence of disease incidence and severity of *Verticillium* wilt in olive groves in Jordan.

Location	Date of Sampling	No. of Tested fields	Disease incidence %	Disease severity %
Ar Ramtha	August	6	4	2
*Qasr Al Hallabat	September	4	28	16
Jureinat Eshawabkeh	October	1	18	8
*Ad Dulayl	October	1	34	18
*Al Hallabat	November	3	33	12
*Al Azraq	November	6	12	4
Hartha	November	2	4	1.5
Kufor Jayez	November	5	17	5
Jubeiha	November	3	22	7
Jureinat Eshawabkeh	December	2	12	4

*Thugret Eljob	February	5	20	16
*Ad Dulayl	February	6	17	12
*Al Hallabat	March	3	60	38
Altaibeh-Madaba	March	4	30	17
Zyzya	March	4	18	8
Bouidah	March	1	80	60
* Irrigated fields		56 Fields		

Two hundred eleven different fields were tested and found disease free in the following locations: Imraweh, Al Turra, Ash Shajarah, Jarash, Balila, Jordan University farm, Wadi al Yabis, Qasr al Hallabat, Al Khalidiyya, Manja, Jureinat Eshawabkeh, Ar Rafeed, Hubras, Baqaa, Wadi Assalt, Al Azraq, Naur, Madaba, Alteem, Lub, Bushra, Alshalaleh, Ar Ramtha, Jordan University of Science and Technology farm, Ad Dulayl, Hood

Khraisha, Al-Mufradat, Mafraq, Um Elamad, Al Mamoneeh, Hisban, Um Elgotain, Alkitech, Sakib, Ajlun, Aydun, Hakama, Marou, Irbid, Al Huson, An Nuaimmah, Qafqafa, Al Qatrana, Adir, Alkarak, Al-Manshiyyah, Debbein, Al Gadeer Alabiad, Rihab and Aldugmoseh. Forty six isolates were obtained from Balka, Mafraq, Amman, Alkarak, Irbid and Madaba districts (Tables 2, 3 and 4).

Table 2: Incidence of infected petioles of the three tomato cultivars inoculated with *V.dahliae* isolates.

Isolate/location	Number	AR	AR	Farida 076	Race type
		Tom002	Tom001	F1	
Al -Ramtha	1	-ve	20*	0	R1
	2	-ve	10	0	R1
Al-khalidiyya	3	-ve	20	17	R2
	4	+ve	20	17	R2
	5	-ve	40	20	R2
	6	-ve	40	17	R2
Al-Dulayl	7	-ve	10	20	R2
Al-hallabat	8	-ve	40	33	R2

Isolate/location	Number	AR Tom002	AR Tom001	Farida 076 F1	Race type
Al-Azraq	9	-ve	20	17	R2
	10	-ve	10	17	R2
	11	-ve	40	33	R2
Al-Raffeed	12	-ve	20	0	R1
	13	-ve	10	0	R1
	14	-ve	20	0	R1
Kufor jayez	15	-ve	0	0	Non-
	16	-ve	10	0	pathogenic R1
Jureinet eshawabkeh	17	-ve	20	0	R1
	18	-ve	40	0	R1
Jubeiha	19	-ve	20	33	R2
	20	-ve	60	66	R2
Baq'a'a	21	-ve	80	40	R2
	22	-ve	100	40	R2
	23	-ve	80	60	R2
Thugret eljob	24	-ve	20	17	R2
	25	-ve	40	17	R2
	26	-ve	40	33	R2
	27	-ve	20	17	R2
	28	-ve	60	17	R2

Isolate/location	Number	AR Tom002	AR Tom001	Farida 076 F1	Race type
Al-dulayl	29	+ve	40	33	R2
	30	-ve	60	33	R2
	31	-ve	100	66	R2
	32	-ve	40	17	R2
	33	-ve	20	17	R2
	34	-ve	40	33	R2
	35	-ve	40	33	R2
Al hallabat	36	-ve	20	17	R2
	37	-ve	40	17	R2
	38	-ve	0	0	Non-pathogen
Altaibeh-Madaba	39	-ve	20	0	R1
	40	-ve	60	0	R1
	41	-ve	60	0	R1
	42	-ve	40	0	R1
Al-Qatraneh	43	-ve	100	0	R1
	44	+ve	80	0	R1
	45	-ve	80	0	R1
Algadeer -alabiad	46	-ve	100	80	R2

* = % of segments showing positive results of *Verticillium* isolation.

-ve = negative isolation.

Table 3: Incidence of infected stem segments of the three tomato cultivars inoculated with *V. dahliae* isolates.

Isolate/location	Number	AR Tom002	AR Tom 001	Farida 076 F1	Race type
Al_Ramtha	1	0	10*	0	R1
	2	0	0	0	-
Al-khalidiyya	3	0	20	10	R2
	4	0	10	10	R2
	5	0	0	0	-
	6	0	0	0	-
Al-Dulayl	7	0	34	0	-
Al-Hallabat	8	34	0	10	R2
Al-Azraq	9	0	0	0	-
	10	0	10	10	R2
	11	0	0	0	-
Al-Raffeed	12	0	20	0	R1
	13	0	20	0	R1
	14	0	0	0	-
Kufor Jayaz	15	0	0	0	-
	16	0	0	0	-
Jureint-eshawabkeh	17	0	20	0	R1
	18	0	0	0	-
Jubeiha	19	0	17	10	R2
	20	0	17	10	R2
Baq'a'a	21	0	10	17	R2
	22	0	20	10	R2
	23	0	0	0	-

Isolate/location	Number	AR Tom002	AR Tom 001	Farida 076 F1	Race type
Thugret eljob	24	0	0	0	-
	25	0	20	17	R2
	26	0	0	0	-
	27	0	10	10	R2
	28	0	0	0	-
Al-Dylayl	29	0	10	10	R2
	30	0	0	0	-
	31	0	10	20	R2
	32	0	0	0	-
	33	0	0	0	-
	34	0	0	0	-
Al-Hallabat	35	0	0	0	-
	36	0	0	0	-
	37	0	20	20	R2
Altaibeh-Madaba	38	0	0	0	-
	39	0	0	0	-
	40	0	20	0	R1
	41	0	10	0	R1
Al_ Qatraneh	42	0	0	0	-
	43	0	0	0	-
	44	10	10	0	R1
	45	0	20	0	R1
Algadeer Alabiad	46	0	40	10	R2

* % of segments showing positive results of *Verticillium* isolation.

Table 4: The incidence of *Verticillium* wilt on olives and distribution of *Verticillium dahliae* races among the different counties of Jordan.

County	Race 1	Race 2
Amman	0	2
Balqa	0	3
Mafraq	0	24
Irbid	6	0
Ajlun	0	0
Madaba	5	0
Alkarak	4	0
Total	15	29
%	32.6	63.0

The mycelium of *Verticillium* grew from both cut ends of stem pieces (Fig 1).



Fig 1: *Verticillium* growth from cut vascular tissues.

Colonies of *Verticillium dahliae* were followed later by the formation of black microsclerotia (Figures 2 and 3). Race 1 showed circular growth pattern while race 2 showed irregular growths of sclerotia. Some olive groves showed symptoms of infection by *V. dahliae* during spring months, but leaf and vascular necrosis symptoms were most pronounced in October and November.

Leaves of affected branches become yellow then desiccated quickly and assume a light tan color, but remained attached for an extended period of time. Xylem necrosis is a pronounced internal symptom of the disease and extends well out into small upper branches by the time foliage symptoms appear.

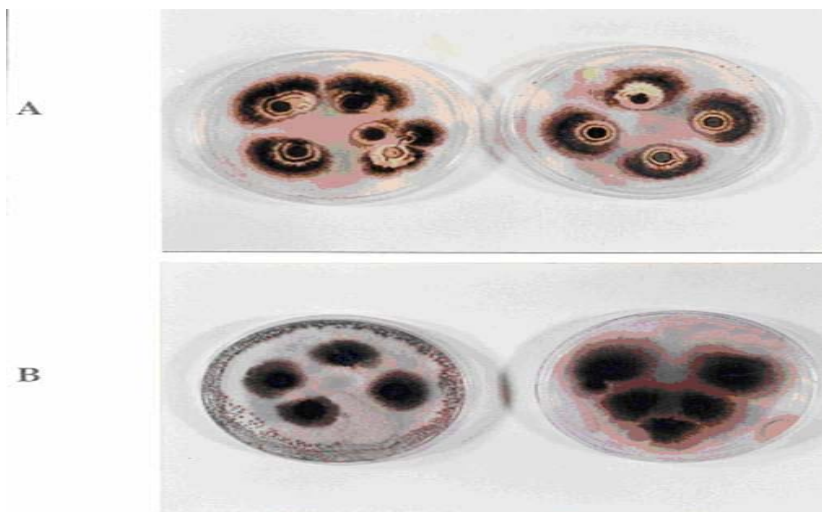


Fig.2. Colonies of *Verticillium dahliae* (A = race1, B = race 2)

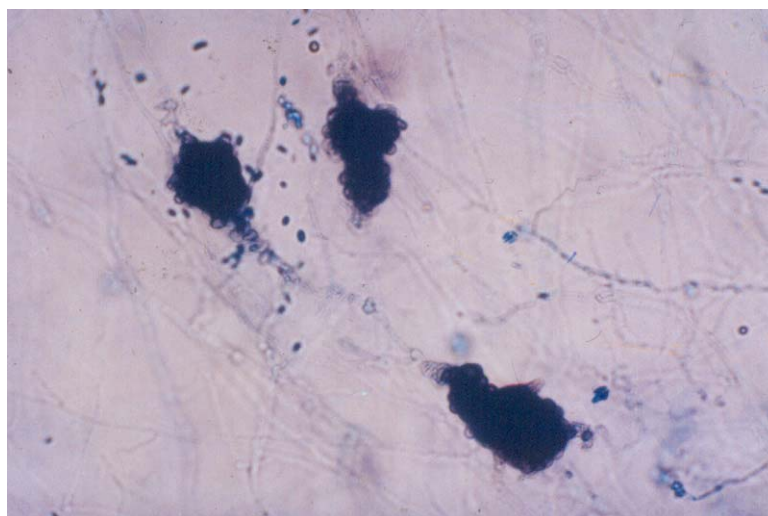


Fig 3: Sclerotia for *Verticillium dahliae* on Potato Dextrose Agar.

The pathogen appeared to spread rapidly in infected trees killing them during the first year of infection, although some trees survived beyond the first year. The pathogen was isolated successfully from fallen leaves but not from the flesh and the seed embryo of olive fruits collected from infected trees.

The ratio of infected petioles sampled from the bottom of tomato plants was significantly greater than that of infected stem segments sampled from the mid part of plants (Tables 2 and 3). The data in this study indicated that the pathogen colonized the lower petioles of the plant and the level of the disease could be underestimated if leaves were not sampled from the lower part of the infected plant.

Two races of *V. dahliae* were reported for the first time in Jordan. Race 2 isolates appeared in 63% of the collected isolates. Fifteen isolates out of 46 were typed as race 1 and two were non-pathogenic on the tomato cultivars used in this study (Table 4). The ratio of race 2 to race 1 isolates obtained from infected olive plant tissues were nearly 2:1. These results indicated that *Verticillium dahliae* race 2 prevailed in the infected olive trees. The highest disease severity was observed in Mafraq County. Disease severity of inoculated tomato seedlings cv. AR Tom 001 with different *V. dahliae* isolates varied from 0 to 40 %. No significant differences in disease severity were found between the isolates of both races. No clear disease severity effects were observed on plant height among the different isolates. Tomato plants inoculated with isolates of race 1 were fruitless at the end of the experiment. Tomato cv. AR Tom 002 was tolerant to both *Verticillium* races (Tables 2 and 3).

Discussion:

The results of this study provided some baseline information on the incidence of *Verticillium* wilt in the

major olive growing regions in Jordan and the prevalence of *V. dahliae* races in the affected orchards. This indicates the need for continued monitoring and further investigations on the impact of races 1 and 2 on various olive cultivars and their effects on production. Of the 267 fields checked, 56 showed wilted trees from which *Verticillium* isolates were obtained. This explained the observed fact that the disease was mainly present in young irrigated olive groves and not in the old trees pre-existing in the area. There are two reasons, which may account for the higher incidence of the disease in the irrigated area. First, the nursery growers may have used infested or improperly sterilized soils. Second, they selected hard wood cuttings from old trees that were already infected but asymptomatic. It was also very interesting to note that the disease was prevalent in irrigated areas, where over-irrigation was in most cases a common practice. This facilitates disease spread which is more pronounced in groves where the previous crop was susceptible to *V. dahliae* (Thanassoulopoulos and Kitsos, 1972). Similar findings were reported previously (Thanassoulopoulos, 1993; Naser, 1996 and Karajeh 1997).

The pathogen isolation was mainly successful from 2-year-old branches and the percentage of *Verticillium* recovery varied from one county to another. The disease incidence and severity were high in Mafraq County due to intercropping with many vegetables and the heavy use of drip irrigation. No serious disease incidence was observed in non-irrigated groves. *V. dahliae* was described as moisture loving fungus (Street, 1974).

We monitored the pathogenicity of the isolates and the disease incidence on the three tomato cultivars by collecting petioles from the lower part of inoculated plants. The ratio of infected petioles sampled from the lower part of inoculated tomato plants was significantly greater than that of infected stem segments from the top

of plants (Tables 2 and 3). These data indicated that the level of disease could be underestimated if leaves are not consistently sampled from the lower portions of the plant. Later in the growing season, the pathogen had already colonized the entire plant. These findings were in agreement with those of Dobinson *et al.* (1996).

Race 2 was more prevalent in the surveyed groves, constituting 63% of the total isolates. These data were not surprising, since the commercially grown tomato in Jordan (Farida 076) is resistant to race 1. The incidence of *V. dahliae* races in Crete was more in favor of race 1. Of the ninety two isolates tested, eighty nine were identified as race 1, one was race 2 and the remaining two were non-pathogenic (Ligoxigakis and Vakalounakis, 1994). Grogan *et al.*, (1979) reported that the ratios of race 1 and race 2 isolates obtained from infected plant tissue did not necessarily reflect the occurrence of *V. dahliae* soil populations. Masoud (2002) reported that the PCR primers of nuclear repetitive DNA showed variable band intensities for the different isolates of *V. dahliae* isolated from olive trees in Jordan. These results were in agreement with our findings. Polymerase chain reaction is an accurate technique for the precise identification of fungal pathogens. The nested PCR allowed more specific amplification of *V. dahliae* than did the single PCR resulting in detecting the fungus in all heights of the asymptomatic trees. The single or nested PCR-based assays could be used as a powerful diagnostic tool to detect *V. dahliae* latent infection in olive trees before the development of symptoms and disease spread to newly established orchards (Karajeh and Masoud, 2006).

The major method of dissemination of *Verticillium* is through semi-hard wood-cuttings obtained from infected old olive trees. All the *Verticillium* isolates from Jordan formed true microsclerotia. In general, the growth rate of the Jordanian isolates ranged between 43.0 and 52.1. These are very similar to those of *V. dahliae* isolates from other Near Eastern countries and Europe. Based on the growth habits and the formation of only microsclerotia as resting bodies, it could be concluded that all *Verticillium* isolates from Jordan are *V. dahliae* Kleb (Skadow, 1969; Singleton *et al.*, 1992).

All the Jordanian isolates except isolate 15 and 38 proved to be pathogenic on tomato cv. AR Tom 001. The majority of these isolates caused moderate to severe wilt when reinoculated into this cultivar. The preferred hosts for the Jordanian *Verticillium* isolates were okra, tomato, eggplant and sweet melon, while the less preferred hosts were pepper, potato and cucumber (Mamluk and Skaria, 1979). These hosts may play an important role in the epidemiology of *Verticillium* wilt of olive and might be of major importance in the survival and increase of inoculum affecting disease incidence. Of all the hosts tested, only chickpea remained disease-free, no wilt symptoms were observed on the above ground plant parts nor was there any vascular discoloration noticed (Naser, 1996).

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<i>Verticillium dahliae</i>			.1997
% 7.4			% 48
(2)			
	% 32	(1)	% 63
			.1:2
AR Tom 001			
		<i>Verticillium dahliae</i>	1
			:

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