Reaction of Selected Apple Cultivars to Wilt Pathogen *Verticillium dahliae*

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Abstract

Karajeh M.R., Owais S.J. (2012): **Reaction of selected apple cultivars to wilt pathogen** *Verticillium dahlia***.** Plant Protect Sci., **48**: 99–104.

The reaction of five apple cultivars to olive wilt caused by *Verticillium dahliae* Kleb was evaluated. Five apple cultivars (Royal Gala, Delicious Anabri, Double Red, Golden Delicious, Granny Smith) and one olive cultivar (Nabali Mohassan) were inoculated with *V. dahliae* and grown under partially controlled greenhouse conditions. Typical symptoms of the disease were developed on the inoculated olive seedlings. However, all tested apple cultivars did not show the typical symptoms of Verticillium wilt except cv. Delicious Anbari, which showed 8.31% disease severity. The average percentage of disease severity was 61.2% in Nabali Mohassan. The pathogen was positively re-isolated from the inoculated seedlings of cvs Nabali Mohassan and Delicious Anbari after 12 months from the inoculation date. Results indicate that an apple plantation to replace dead or severely diseased olive trees infested with Verticillium wilt is an appropriate low-cost and environmentally sound IPM alternative approach to control Verticillium wilt.

Keywords: control; *Malus domestica*; disease assessment

Olive (*Olea europea* L.) is the most widely grown fruit tree in Jordan, occupying more than 70% of the total area planted with fruit trees and covering around 64500 hectares (Anonymous 2006). Several fungal diseases attacking olive trees were reported in Jordan; Verticillium wilt caused by *V. dahliae* Kleb. (Naser 1996), Peacock eye spot caused by *Cycloconium oleaginum* Cast. (Mamluk *et al.* 1984), "Leaf spot" caused by *Alternaria* spp. (Mamluk *et al.* 1984), and Leaf mould caused by *Cladosporium* spp. (Qasem 1970).

Verticillium wilt of olive is one of the most serious diseases affecting olive worldwide and in Mediterranean countries causing severe annual economic losses to irrigated olive trees planted with high-yielding but susceptible olive cultivars (Thanassoulopoulos *et al.* 1979; Al-Ahmad & Hamidi 1984; Tjamos 1993; Naser 1996; Heale 2000; Tjamos *et al*. 2000; Karajeh 2006; Al-Momany 2010; Al-Taae & Al-Taae 2010).

Several approaches were used to control Verticillium wilt in olive groves including chemical control (Tawil *et al*. 1992; Tawil & Abdin 1994; Karajeh 1997), post-planting soil solarisation (Tjamos *et al*. 1991; Abu-Gharbieh *et al*. 1998; Karajeh & Al-Momany 2008), solar chamber (Al-Ahmad & Duksi 1994; Karajeh & Al-Momany 2008), cultural practices (Al-Ahmad 1993; Karajeh & Al-Momany 2008), integrated control (Abu-Qamar & Al-Momany 2001, 2002) and biological control (Tjamos 1993; Karajeh & Al-Raddad 1999). Recently, disease control has essentially relied on preventive measures such as using disease-free plant materials and soils that are free from pathogens especially when establishing new olive groves (Karajeh 2006; Karajeh

& Masoud 2006). Chemical fungicides such as benzimidazoles did not provide sufficient control (Biris & Thanaassoulopulos 1980; Karajeh 1997). Post-planting soil solarization was fairly effective in controlling the disease but limited to regions that receive high solar radiation (Tjamos *et al*. 1991; Tjamos 1993; Lopez-Escudero & Blanco-Lopez 1997; Karajeh & Al-Momany 2008). The management of Verticillium wilt is difficult and requires an integrated approach (Jimenez-Diaz *et al*. 1998). However, young olive groves are significantly more affected by *V. dahliae* than the old ones (Rodriguez *et al*. 2008). Therefore, replacement of diseased olive trees especially in young olive groves with non-host plants might be an environmentally friendly management solution to control Verticillium wilt.

There are a few conflicting reports that pointed out the genetic potential of apple tree in resisting Verticillium wilt such as some internet sites which indicate that "infections of apple and pear trees with Verticillium wilt are rare" (Anonymous 2010) and that "apple, pear, and quince are susceptible to the European strains of *V. albo-atrum* Reinke & Berthold" (Dykstra 1997). To the best of our knowledge, no documents were found that were aimed to evaluate the level of the genetic potential of apple tree to resist Verticillium wilt. Therefore, this study was carried out to evaluate the level of resistance of five apple cultivars to an olive isolate of *V. dahliae*.

MATERIAL AND METHODS

Verticillium dahliae (MU6) isolate was obtained from an infested olive grove in Karak Province, Jordan. The isolate was well-characterised by morphological and molecular diagnostic methods (Masoud 2002; Masoud & Karajeh 2003; Karajeh & Masoud 2006) and was highly aggressive to olive causing extensive defoliation and also was highly aggressive to tomato and cucumber under laboratory conditions (Masoud & Karajeh 2003). The isolate was maintained in pure cultures on potato dextrose broth (PDB) at 22°C in darkness in an orbital incubator at 120 rpm for one month and used for artificial pathogen inoculation in this study. After culturing, mycelia, conidia and microsclerotia were microscopically examined to confirm the identity of the pathogen with olive wilt pathogen (*V. dahliae*) using taxonomic keys (Issac 1967; Heale 2000). The concentration of the fungal inoculum was adjusted to 10^7 propagules/ml using a haemocytometer slide (Rodriguez-Tudela *et al*. 2003). The pathogenicity of the isolates was previously confirmed by testing on different crop species (Masoud & Karajeh 2003).

One-year-old healthy seedlings of apple cultivars (Royal Gala, Delicious Anbari, Double Red, Golden Delicious, Granny Smith) grafted on Anbari seedling rootstock were individually transplanted into 9-l plastic pots filled with a virgin soil. The olive cultivar Nabali Mohassan, which was previously found to be susceptible to *V. dahliae* (Naser 1996), was used as a positive control. Thereafter, the seedlings were subsequently allowed to grow under greenhouse conditions at 25 ± 3°C in the spring season (early March) at the Agricultural Research Station of the Faculty of Agriculture, Mutah University, Karak, Jordan. Three weeks later, apple cultivars and olive seedlings were individually inoculated with the fungal isolate by pouring 100 ml of the fungal inoculation suspension into 4 holes made in the rhizospheric zone. The control treatments were performed by adding the same concentration of PDB but without the fungal culture.

Pots were arranged according to a completely randomised block design with 6 replicates (6 pots per treatment) on well-drained metal benches inside the glasshouse. Six blocks were inoculated and six blocks were reserved as control group. The experiment received all agricultural practices including regular irrigation and recommended fertilisation.

A scale of visual estimation of disease severity (Tjamos *et al*. 1991), depending on foliar symptoms such as leaf yellowing, wilting, rolling, and defoliation of infected branches, was monthly monitored to assess disease severity % of Verticillium wilt. The following score was used to evaluate the resistance of the different cultivars: 0 = seedling free of infection (immune), 1 = 1–20%: very slight infection (highly resistant), 2 = 21–40%: slight infection (slightly resistant), 3 = 41–60%: intermediate infection (susceptible), 4 = 61–80%: severe infection (susceptible), 5 = 81–100%: nearly dead to dead seedling (susceptible). The overall assessment of disease severity was calculated using the following equation:

$$\text{Disease servity (\%)} = \frac{\text{Sum of all numerical ratings per a treatment}}{\text{Total number of trees per a treatment} \times \text{Mean disease servity category (5)}} \times 100$$

At the end of the experiments, the pathogen was re-isolated from the crown areas of seedling scion and rootstock. Stem pieces of 2 cm in length were taken and subsequently washed in running tap water, debarked, surface-sterilized in 0.5% sodium hypochlorite solution for 1 min, double-washed in sterilised distilled water for 5 min and cultured on a selective medium (Ausher *et al.* 1975). Afterward, cultured samples were incubated in darkness at 22°C for two weeks. Fungal growth was microscopically examined to confirm the identity of *V. dahliae* (Issac 1967; Heale 2000). Two additional traits were recorded as horticultural parameters: seedling shoot and root fresh and dry weights were recorded.

Data was statistically analysed using the general linear model (GLM) procedure (SPSS software version 11.5; SPSS Inc., Chicago, USA). Least significant difference (*LSD*) test was used for mean separation at the 0.05 probability level (Steel *et al.* 1997).

RESULTS

Microscopic examinations and pathogenicity test indicated that *V. dahliae* was the causal agent of olive wilt. White cottony mycelial growth was observed after one week after incubation of infected tissues on the selective medium. As a consequence of microsclerotia and dauermycelia formation, the colony colour converted to dark black within 2 weeks after inoculation.

Early symptoms of infection appeared on the leaves located on young shoots during the period extending from late winter to early spring. Leaves rolled downward along the main axis. Two weeks later, the leaves turned brown. Defoliation occurred within one month. Inoculated seedlings of olive cultivar showed the typical symptoms including leaf wilting, rolling and defoliation of the seedling branches. Slight to moderate yellow to tan brown vascular discoloration was observed inside the seedling main stems. The percentage of disease severity among inoculated seedlings ranged from 32% to 78% with an average of 61.2%. The average infestation rate in Delicious Anbari was 8.3% with slight disease symptoms including defoliation of some leaves without visible vascular discoloration.

The wilt causing pathogen (*V. dahliae*) was positively re-isolated from the crown at the end of the experiment from Nabali Mohassan cultivar, from scion and rootstock of Delicious Anbari and from

Table 1. Reactions of apple and olive cultivars against olive wilt caused by *Verticillium dahliae*

Cultivar	Treatment	SFW (g)	RFW (g)	SDW (g)	RDW (g)	DS (%)	RI (+/–)	
							rootstock	scion
Royal Gala	inoculated	$113.3^{ad} \pm 30.2$	$92.5^{a} \pm 18.9$	$63.5^{a} \pm 11.9$	$59.2^{c} \pm 10.0$	0^{a}	+	–
	control	$128.0^{ab} \pm 29.6$	$113.6^{bc} \pm 26.6$	$74.3^{ab} \pm 17.7$	$40.6^{ab} \pm 5.4$	0^{a}	+	–
Delicious Anbari	inoculated	$138.3^{ab} \pm 13.7$	$125.7^{bc} \pm 31.9$	$73.4^{ab} \pm 11.3$	$48.3^{b} \pm 7.9$	$8.3^{a} \pm 1.4$	+	+
	control	$141.6^{ab} \pm 25.3$	$121.1^{bc} \pm 29.5$	$88.1^{abc} \pm 15.7$	$61.2^{c} \pm 17.8$	0^{a}	+	–
Double Red	inoculated	$145.3^{ab} \pm 19.0$	$88.4^{a} \pm 20.1$	$70.5^{ab} \pm 13.1$	$38.7^{ab} \pm 3.5$	0^{a}	+	–
	control	$147.5^{ab} \pm 25.7$	$102.4^{bc} \pm 17.3$	$84.9^{abc} \pm 11.0$	$49.4^{bc} \pm 7.2$	0^{a}	+	–
Golden Delicious	inoculated	$134.5^{ab} \pm 26.2$	$92.5^{ab} \pm 9.6$	$72.0^{ab} \pm 12.6$	$41.9^{b} \pm 4.9$	0^{a}	+	–
	control	$154.9^{abc} \pm 38.1$	$133.9^{c} \pm 34.7$	$100.2^{bc} \pm 20.2$	$59.2^{c} \pm 7.1$	0^{a}	+	–
Granny Smith	inoculated	$141.8^{ab} \pm 30.9$	$78.8^{a} \pm 10.3$	$75.9^{ab} \pm 14.7$	$34.5^{ab} \pm 13.5$	0^{a}	+	–
	control	$150.3^{abc} \pm 20.8$	$71.5^{a} \pm 14.5$	$88.4^{bc} \pm 7.0$	$36.1^{ab} \pm 8.7$	0^{a}	+	–
Nabali Mohassan	inoculated	$157.7^{bc} \pm 25.1$	$67.8^{a} \pm 19.3$	$80.9^{ab} \pm 14.1$	$24.8^{a} \pm 7.5$	$61.2^{b} \pm 6.1$	+	+
	control	$208.1^{d} \pm 35.4$	$111.7^{bc} \pm 21.4$	$112.3^{c} \pm 8.0$	$45.9^{bc} \pm 8.4$	0^{a}	–	–

SFW – shoot fresh weight; RFW – root fresh weight; SDW – shoot dry weight; RDW – root dry weight; DS – overall % of disease severity; RI – re-isolation result

All these cultivars are apple cultivars except the last one which is an olive cultivar

Average and standard deviation of six plants/treatment

Means followed by the same letter within columns are not significantly different according to least significant difference (*LSD*) test ($P \leq 0.05$)

Table 2. Coefficients of correlation among the parameters of apple and olive cultivar reactions against olive wilt caused by *Verticillium dahliae*

Parameter	SFW	RFW	SDW	RDW	DS
SFW	1				
RFW	0.25	1			
SDW	0.84**	0.39**	1		
RDW	0.35**	0.88**	0.47**	1	
DS	0.19	–0.29*	0.09	–0.32*	1

SFW – shoot fresh weight; RFW – root fresh weight; SDW – shoot dry weight; RDW – root dry weight; DS – disease severity (%)

*correlation is significant at the 0.05 probability level; **correlation is significant at the 0.01 probability level

the rootstocks of the other inoculated apple cultivars (Table 1). The pathogen was not successfully isolated from the seedling of olive cultivar in the control and from scions of the four inoculated apple cultivars: Royal Gala, Double Red, Golden Delicious, and Granny Smith. Therefore, these apple cultivars were highly resistant to *V. dahliae* while the cultivar Delicious Anbari was moderately resistant.

Shoot and root fresh and dry weights were significantly lower in the inoculated olive pots than that in the non-inoculated (control) pots (Table 1), with reduction percentages of 24.2, 39.3, 28.0, and 46.0%, respectively.

Normal leaf defoliation for apple, as a deciduous tree, occurred in autumn. No vascular discoloration was observed either in scions or in rootstocks.

Shoot fresh and dry weights did not significantly differ between *V. dahliae*-inoculated and non-inoculated apple seedlings regardless of the cultivar type. Root fresh and dry weights of cvs Royal Gala and Golden Delicious were significantly higher in the non-inoculated than in the inoculated plants.

Disease severity % and root fresh (–0.29) and dry weights (–0.32) were significantly and negatively correlated at the 0.05 probability level. Shoot and root fresh and dry weights were positively and significantly correlated at the 0.01 probability level (Table 2).

DISCUSSION

Olive wilt disease caused by *V. dahliae* is a limiting factor for the cultivation of high-yielding, excellent quality cultivars in Greece (Thanassoulopoulos *et al*. 1979) and Spain (Rodriguez *et al*. 2008). The disease causes severe damage to olive plantations in Syria (Al-Ahmad & Hamidi 1993; Al-Ahmad & Mosli 1993) and in Iraq (Al-Taae & Al-Taae 2010). In Jordan, the disease was first reported in the 1980s. In the last two decades, it has spread throughout the main olive growing areas of Jordan (Mamluk *et al*. 1984; Naser 1996; Masoud & Karajeh 2003). The increased number of infested trees occurred in irrigated olive groves in Jordan since 1993 (Naser & Al-Momany 1998; Karajeh & Al-Raddad 1999). In this study, the high disease severity and the successful re-isolation of *V. dahliae* indicated that the olive cultivar Nabali Mohassan was very susceptible. The inoculation procedure used in this study was successful, the disease developed normally under the experimental conditions and the *V. dahliae* (MU6) isolate used was highly virulent to olive. These results are in line with Naser (1996), who listed the olive cultivar Nabali Mohassan among the most susceptible olive cultivars to Verticillium wilt and with Masoud & Karajeh (2003), who cultured the *V. dahliae* isolate from a wilted olive tree, tested its pathogenicity on tomato, cucumber and eggplant and found it highly virulent. Other Jordanian *V. dahliae* isolates were also found virulent on olives (Karajeh & Al-Raddad 1997) and tomatoes (Al-Momany 2010). The isolate was able to greatly reduce the olive seedling growth compared to the normal growth of apple seedlings. The infection with *V. dahliae* was slightly detected in the rootstock and scion parts in cv. Delicious Anbari, however scions of the other four apple cultivars (Royal Gala, Double Red, Golden Delicious, and Granny Smith) did not show any disease symptoms. Similarly, the stem associated apple tree viruses such as A*pple stem pitting virus* (ASPV) and *Apple stem grooving virus* (ASGV) were detected frequently in the rootstock and scion parts

of Jonagold and Early Smith cultivars and to the lower extent in Gala cultivar (Kůdela *et al*. 2009). Therefore, cv. Delicious Anbari was moderately resistant to *V. dahliae* while the other apple cultivars were highly resistant. To our knowledge, this is the first study that determines the level of apple resistance to Verticillium wilt and that can provide a control measure for the disease management in olives since commercially, resistant olive cultivars to *V. dahliae* are not available even though no considerable level of tolerance was recorded among olive cultivars. Some studies showed that very few olive cultivars have resistance properties (Wilhelm & Taylor 1965; Hartmann *et al*. 1971; Schnathorst & Sibbett 1971; Tjamos 1993; Sesli *et al.* 2010). In Spain, studies on olive resistance to Verticillium wilt (Ciccarese *et al*. 2002; López-Escudero *et al*. 2004) led to establishment of different levels of susceptibility among olive cultivars. No significant differences in pathogen prevalence among olive cultivars were found (Rodriguez *et al*. 2008). Resistant olive cultivars once detected, they should be integrated with other control measures in an integrated management program in order to be economically feasible.

Verticillium wilt disease causes severe damage and yield losses to young olive trees. In old olive trees including those over 100-years old the disease does not normally kill the plant, but reduces vegetation and causes partial defoliation of one or more branches (Sesli *et al*. 2010). Therefore, the replacement of dead *Verticillium*-infested olive trees with apple plantations in newly established olive groves would be highly recommended to increase the farm income. Moreover, apple trees could replace *V. dahliae*-susceptible vegetable crops (e.g. cucurbits, solanaceous crops, brassicaceous crops, etc.) in *V. dahliae*-infested fields.

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Received for publication November 29, 2011
Accepted after corrections April 13, 2012

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