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ORIGINAL ARTICLE

Severity assessment of isolates of defoliating and nondefoliating *Verticillium dahliae* pathotypes on woody and herbaceous hosts

Begoña I. Antón-Domínguez[1,3] · **Ana López-Moral**[1] · **María Lovera**[2] · **Octavio Arquero**[2] · **Antonio Trapero**[1] · **Carlos Trapero**[1] · **Carlos Agustí-Brisach**[1]

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Abstract

Verticillium wilt, caused by the soilborne fungus *Verticillium dahliae* Kleb. is a major vascular disease affecting a wide range of woody and herbaceous hosts worldwide. The aim of this study was to assess the pathogenicity of defoliating (D) and nondefoliating (ND) *V. dahliae* pathotypes–isolated from pistachio (*Pistacia vera* L.) and olive (*Olea europaea* L.)–on two commonly used *Pistacia* rootstocks in the Mediterranean basin (*P. terebinthus* and UCBI hybrid) as well as on other major *V. dahliae*–susceptible hosts, such as olive, cotton (*Gossypium hirsutum* L.) and tomato (*Solanum lycopersicum* L.). Compared with the ND isolates, the D pathotype from olive induced greater disease progression in olive and cotton. However, no differences in the final disease severity in cotton were observed between D and ND isolates from olive. In terebinth plants, no significant difference in disease progression and severity was noted between the D and ND isolates, whereas UCBI remained symptomless and showed high resistance, although with a significant reduction in plant biomass in inoculated plants. Notably, the ND isolates from pistachio were significantly more aggressive on terebinth than on cotton or olive, highlighting host-specific interactions and the intraspecific variability of *V. dahliae*. These findings contribute to the understanding of *V. dahliae* host–pathogen dynamics and reinforce the importance of selecting resistant rootstocks, particularly in pistachio–growing regions where Verticillium wilt is prevalent in the soil.

Keywords *Gossypium hirsutum* · *Olea europaea* · *Pistacia* spp · *Solanum lycopersicum* · UCBI · Verticillium wilt

Introduction

Verticillium wilt, caused by the hemibiotrophic soilborne fungus *Verticillium dahliae* Kleb., is one of the most economically detrimental vascular diseases, causing severe damage to a broad diversity of woody and herbaceous hosts worldwide (Fradin and Thomma 2006). The most prominent hosts affected by the disease include economically relevant woody crops such as almond (*Prunus dulcis* [Mill.] D.A. Webb), olive (*Olea europaea* L.) and pistachio (*Pistacia vera* L.), as well as herbaceous crops such as cotton (*Gossypium hirsutum* L.), potato (*Solanum tuberosum* L.), eggplant (*S. melongena* L.), sunflower (*Helianthus annuus* L.) and tomato (*S. lycopersicum* L.), among others (Inderbitzin et al. 2011; Klosterman et al. 2009; Kowalska 2021; Pegg and Brady 2002).

This study focused on *Pistacia* species. Pistachio is currently experiencing significant expansion across the Mediterranean basin, where Verticillium wilt frequently occurs (Gusella et al. 2024). Iran leads in the global cultivated surface of pistachio (497,484 ha), followed by Turkey (408,709 ha), the USA (173,207 ha) and Spain (70,235 ha) (FAOSTAT 2022; MAPA 2022), whereas the USA leads in global production (400,070.00 t), followed by Iran (241,668.58 t) and Turkey (239,289.00 t) (FAOSTAT 2022). Spain ranks sixth in terms of production, with 19,889 t, with Castilla-La Mancha (south–central Spain) being the main pistachio growing area, with 53,823 ha of cultivated surface area and 14,161.00 t of production, followed by

✉ Carlos Agustí-Brisach
cagusti@uco.es

[1] Departamento de Agronomía, Universidad de Córdoba, Campus de Rabanales, Córdoba 14071, Spain

[2] Departamento de Fruticultura Mediterránea, IFAPA, Alameda del Obispo, Córdoba 14004, Spain

[3] Present address: CIMO, LA SusTEC, Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança 5300-253, Portugal

the Andalusia region (southern Spain; 6654 ha; 3013.00 t) (MAPA 2022).

During the last decade, pistachio has undergone intensive expansion from marginal to nontraditional growing areas across central–southern Spain, with these new orchards being established in intensive farming systems (Gusella et al. 2024). In addition, most of them have been established in soils previously used for growing herbaceous crops highly susceptible to Verticillium wilt, such as cotton, tomato, and potato, as well as in soils previously used for growing olive trees for decades (López-Escudero and Mercado-Blanco 2011). It is well known that *V. dahliae* survives for a long time in plant debris and in the soil by means of resistance structures called microsclerotia (MS), which germinate in response to root exudates from host plants, causing infections (Fradin and Thomma 2006; Mol and Van Riessen 1995). Notably, two populations of the pathogen, defoliating (D) and nondefoliating (ND) pathotypes, have been identified as causal agents of the disease in cotton and olive trees in southern Spain (Klosterman et al. 2009; López-Escudero and Mercado-Blanco 2011; Markakis et al. 2010). These two pathotypes differ in their ability to cause defoliation: pathotype D is much more aggressive than pathotype ND and causes severe defoliation and plant death, whereas pathotype ND does not cause defoliation and is generally not lethal (Bejarano-Alcázar et al. 1995; Göre et al. 2009; Jiménez-Díaz et al. 2012, 2017; Korolev et al. 2008; Markakis et al. 2010; Mercado-Blanco et al. 2003; Triantafyllopoulou et al. 2022). In addition, there is variability in the aggressiveness and defoliating ability of *V. dahliae* isolates within both pathotypes, depending on the host (Dadd-Daigle et al. 2020, 2022; Jiménez-Díaz et al. 2012).

Recent studies have shown that such variability is not strictly determined by the vegetative compatibility group (VCG) or pathotype classification, as isolates from both D and ND pathotypes can exhibit a range of virulence and host preferences. Indeed, certain ND isolates have been reported to be highly aggressive in specific hosts, and the adaptability of *V. dahliae* populations to different hosts is well documented (Dadd-Daigle et al. 2020, 2022). For example, Dadd-Daigle et al. (2020) demonstrated that certain isolates of *V. dahliae* ND pathotypes caused higher levels of disease severity in cotton than isolates of *V. dahliae* D pathotypes did, underscoring the variability of *V. dahliae* populations in terms of severity depending on the host species.

The new pistachio orchards established in central–southern Spain during the last decade have been severely affected by Verticillium wilt. Surveys conducted across this geographical area revealed that only *V. dahliae* ND pathotype isolates have been isolated and identified from diseased pistachio trees (Antón-Domínguez et al. 2024a; Gusella et al. 2024; López-Moral et al. 2023). In these scenarios, we must also consider that disease severity depends not only on the history of previous crops and the inoculum density of the pathogen in the soil of the orchard but also on the rootstock used (Antón-Domínguez et al. 2024a; Epstein et al. 2004; Gusella et al. 2024; López-Moral et al. 2023; Vahdati et al. 2021). *Pistacia terebinthus*, a species native to the Mediterranean area, is widely used as a rootstock for pistachio trees in Spain and Italy, as well as in various other pistachio-growing regions. Its ability to withstand drought makes it adaptable to the agronomic conditions of the main growing areas in central–southern Spain. However, its susceptibility to Verticillium wilt is a major limitation for its use as a pistachio rootstock, despite the genetic diversity within *P. terebinthus* (terebinth) populations (Guney et al. 2021; Gusella et al. 2024; Moral et al. 2010; Teviotdale et al. 1995). Currently, the most widely used pistachio rootstock is the seed-propagated hybrid UCBI, a cross between *P. atlantica* KAC and *P. integerrima* KAC (Kearney Agricultural Center, Parlier, California) × *P. integerrima* KAC. This hybrid has shown high resistance to Verticillium wilt (Antón-Domínguez et al. 2024a; Epstein et al. 2004; Gusella et al. 2024; Morgan et al. 1992).

We hypothesize that the incidence of Verticillium wilt in pistachio in Spain has increased due to its coexistence with olive, cotton, tomato, and other *V. dahliae* hosts, in addition to the widespread use of the susceptible rootstock *P. terebinthus* in pistachio orchards. This coexistence facilitates the movement and persistence of the pathogen in the soil and surrounding susceptible crops, thereby increasing the risk of infection and disease incidence. Thus, the aim of this study was to assess the severity of *V. dahliae* ND pathotype isolates recovered from pistachio in terebinth and UCBI *Pistacia* rootstocks as well as on other *V. dahliae*–susceptible hosts, such as olive, cotton, and tomato. Isolates of D and ND *V. dahliae* pathotypes from olive were included for comparative purposes. The assessment of the severity of isolates of *V. dahliae* D and ND pathotypes in two of the most widely used *Pistacia* rootstocks in Spain, as well as in olive, cotton, and tomato, will shed light on the possible cross–pathogenicity effects between isolates within pathotypes and between pathotypes and their hosts. This aspect is particularly relevant in areas where several *V. dahliae*–susceptible crops coexist.

Materials and methods

Fungal isolates

Four isolates of *Verticillium dahliae* recovered from olive (V323 and V004 isolates) and pistachio (V478 and V484 isolates) showing Verticillium wilt symptoms were used in this study (Table 1). Isolate V323 was characterized as a D pathotype, while isolates V004, V478 and V484 were characterized as ND pathotypes by nested PCR, following the protocols described by Mercado-Blanco et al. (2001, 2002, 2003). These isolates have been deposited in the fungal collection of the Department of Agronomy of the University of Cordoba (DAUCO, Spain).

Prior to conducting the experiments of this study, all the isolates were first grown on potato dextrose agar (PDA; Difco® Laboratories, MD, United States) acidified with lactic acid [0.1% (vol/vol); pH = 4.0–4.5; APDA] and incubated at 24 ± 2 °C in the dark for 7 days. The growing fungal colonies were transferred to PDA and incubated under the same conditions described above.

Plant and seed material

Healthy one-month-old terebinth and UCBI hybrid plants and six-month-old olive plants of cv. Picual were obtained from a commercial nursery and transplanted into 1 L PVC pots filled with peat moss. The plants were preconditioned for one month in a culture chamber at 22 ± 2 °C with a 14:10 h (light:dark) photoperiod of white fluorescent light (10,000 lux), 60% relative humidity (RH) and irrigated three times per week.

One-month-old cotton seedlings of cv. Siokra 1–4 and tomato seedlings of cv. Tres Cantos were used in this study as herbaceous hosts. To obtain the seedlings, commercial seeds of each plant species were germinated and grown in PVC pots filled with sterile peat moss. The plants were maintained in a culture chamber at 25 ± 2 °C and 80% RH and irrigated three times per week. Before sowing, the seeds were surface disinfected by immersion in a 10% (vol/vol) solution of sodium hypochlorite for 20 min, followed by rinsing in sterile distilled water (SDW) to remove any residual sodium hypochlorite. Seeds were held in SDW for 24 hours until sowing, and those that floated were discarded to ensure further germination.

Inoculum preparation, plant inoculation and experimental design

Conidial suspensions were obtained from 14-day-old colonies of the four *V. dahliae* isolates growing as described above by adding SDW to Petri dishes and scraping the mycelial colonies with a sterile scalpel. Their concentrations were subsequently adjusted to 10^6 conidia/ml using a haemocytometer.

Prior to plant inoculation, the original peat was manually removed from the roots of the plants or seedlings, and they were rinsed with running tap water. The inoculation was subsequently conducted by dipping the roots in the conidial suspensions for 30 min. Plants from each host soaked in SDW were included as a control. The plants or seedlings were transplanted into 1 L PVC pots previously disinfected in a commercial sodium hypochlorite solution (20%) for 2 h, which were subsequently filled with sterilized peat moss [double sterilization: 1) 120 °C for 50 min; 24 h later, 2) 120 °C for 20 min; (López-Moral et al. 2021)]. The inoculated plants were kept in the dark at 19 °C and 100% RH for 3 days or 24 h after inoculation for woody or herbaceous hosts, respectively. The plants were incubated at 23 ± 2 °C, 70% RH, and a 12 h day/night photoperiod with 10,000 lux photosynthetically active radiation and watered three times per week with equal amounts of water per plant (López-Moral et al. 2021).

For each host, a randomized complete block design with three blocks and five replicate plants per isolate (or control) and block was used ($3 \times 5 \times 5 = 75$ plants per host; $75 \times 5 = 375$ in total). The experiment was conducted twice.

Disease assessment and fungal reisolation

Disease severity (DS) was assessed weekly from three to nine weeks after inoculation using a 0 to 4 scale with 17 values to estimate the percentage of affected tissue. Thus, this severity rating scale is composed of 5 categories: 0 = no symptoms, 1 = 25%, 2 = 50%, 3 = 75% affected foliar surface, and 4 = dead plant (100%), with three intermediate values (0.25, 0.50, and 0.75) between the main categories (Antón-Domínguez et al. 2024b; López-Moral et al. 2022). For each host, each plant was individually rated using this scale at each evaluation, and the DS values were recorded over time. At the end of the experiment, DS data were used to estimate the area under the disease progression curve (AUDPC) using the formula described by López-Moral et al. (2022). The AUDPC was subsequently expressed as a relative percentage (RAUDPC; %) using the trapezoidal integration method (Campbell and Madden 1990). The percentages of affected plants (disease incidence, DI) and dead plants (mortality) were estimated at the end of the experiment for each host. Additionally, at the end of the experiment, the fresh and dry

Table 1 *Verticillium dahliae* isolates used in this study and their origin

Isolate	Pathotype	Host	Cultivar/rootstock	Tissue/Substrate	Sample location
V004	Nondefoliating	*Olea europaea*	Picual	Soil	Cádiz (Cádiz)
V323	Defoliating	*O. europaea*	Picual	Soil	Villanueva de la Reina (Jaén)
V478	Nondefoliating	*Pistacia vera*	Kerman/*P. terebinthus*	Xylem	Alhama de Granada (Granada)
V484	Nondefoliating	*P. terebinthus*	*P. terebinthus*	Xylem	Alhama de Granada (Granada)

weights (g) of the aerial and root parts and the total plant, as well as the length (cm) of each plant, were measured for both terebinth and UCBI hybrid rootstocks.

To verify pathogen infection, at the end of the experiment, three plants (one per block) per host and *V. dahliae* isolate combination were randomly selected for reisolation. To achieve this goal, basal stems were rinsed under running tap water, followed by bark removal and the cutting of small fragments from the affected xylem. These fragments were surface disinfected by soaking them in a 10% (vol/vol) commercial bleach solution (Cl at 50 g/l) for 1 min. The fragments were then rinsed with SDW, air dried on sterile filter paper at room temperature, plated on APDA (7 fragments per Petri dish and 2 Petri dishes per plant, isolate and host combination), and incubated under the conditions described above. The frequency of isolation was estimated as a percentage (%) considering the coefficient between the total successful isolations and the isolation attempts (total fragments of tissue seeded in APDA) of the samples processed in combination (Antón-Domínguez et al. 2023).

Data analysis

Data from the two replicates of each experiment were combined after confirming that there were no significant differences between them ($p \geq 0.05$). In all the cases, the data were tested for homogeneity of variance and normality prior to conducting ANOVA, and logarithmic transformation of the data was conducted when necessary.

For each host, one-way ANOVA was conducted with *V. dahliae* isolates as the independent variable and RAUDPC, DS, length, or weight as the dependent variables. All treated means were compared according to Fisher's protected least significant difference (LSD) test at $p = 0.05$ (Steel and Torrie 1985). Absolute data for DI (% plants affected) and mortality (% plants dead) were analysed using multiple comparisons tests for proportions at $p = 0.05$ (Zar 2010). Data analyses were performed using Statistix 10 software (Analytical Software 2013).

Results

Typical symptoms of Verticillium wilt were observed in inoculated olive and terebinth plants and in cotton seedlings at 23, 15, and 11 days after inoculation, respectively, with the DS varying among *V. dahliae* isolates depending on the host. The main symptoms observed in these three hosts were leaf chlorosis and wilting, regardless of the pathotype. Defoliation was very common in olive plants and cotton seedlings inoculated with pathotype D. Intense defoliation was also observed in cotton inoculated with pathotype ND (especially the V004 isolate) (Fig. 1). Thus, there were significant differences in RAUDPC and DS between *V. dahliae* isolates and controls for cotton ($p \leq 0.0001$ in both cases), olive ($p \leq 0.0001$ in both cases), and terebinth ($p = 0.0397$ and $p = 0.0164$ for RAUDCP and DS, respectively) plants (Figs. 1, 2; Table 2). However, tomato (Fig. 1B) seedlings and UCBI (Fig. 3) plants did not exhibit disease symptoms for any of the *V. dahliae* isolates evaluated.

Cotton seedlings

Compared with the other isolates, the D *V. dahliae* V323 isolate from olive led to a significantly greater RAUDPC (66.8 ± 1.4%), followed by the ND isolates V478 from pistachio, V004 from olive (RAUDPC = 26.1 ± 2.7% and 23.5 ± 1.7%, respectively), and V484 (RAUDPC = 8.1 ± 2.2%). Compared with the other isolates, the V323 and V004 isolates caused greater DS (94.2 ± 0.4 and 86.3 ± 5.7%, respectively) and mortality (66.7 and 60.0%, respectively). Finally, cotton seedlings inoculated with the V323 and V478 isolates showed 100% DI, followed by those inoculated with the V004 (DI = 93.3%) and V484 (DI = 46.7%) isolates (Table 2; Figs. 1A, 2).

Olive plants

For olive plants, the results were similar to those observed in cotton seedlings. Compared with the other isolates, the V323 isolate was the most aggressive, with significantly greater values for all disease–related parameters (RAUDPC = 55.3 ± 6.6%, DS = 75.8 ± 9.9%, mortality = 43.3%, and DI = 100.0%). In contrast, the ND isolate V484 from pistachio exhibited the lowest disease–related parameters (RAUDPC = 11.3 ± 2.8%, DS = 15.6 ± 3.6%, and mortality = 3.3%), with no significant differences from those of noninoculated control plants (Table 2; Fig. 1C, 2).

Terebinth plants

Although there were significant differences in RAUDPC and DS between the isolates and the noninoculated control terebinth plants, no significant differences were observed among the evaluated isolates. RAUDPC ranged from 36.4 ± 8.0 to 50.1 ± 13.6% for the V323 and V478 isolates, respectively. The DS values ranged from 46.1 ± 10.7% to 61.9 ± 13.9% for the V484 and V004 isolates, respectively. Compared with the V484 isolate, the V478 isolate had significantly greater mortality (33.3%) (mortality = 13.3%) and a significantly greater DI (100.0%) than the other isolates did (DI = 93.3%) (Table 2; Fig. 1D, 2).

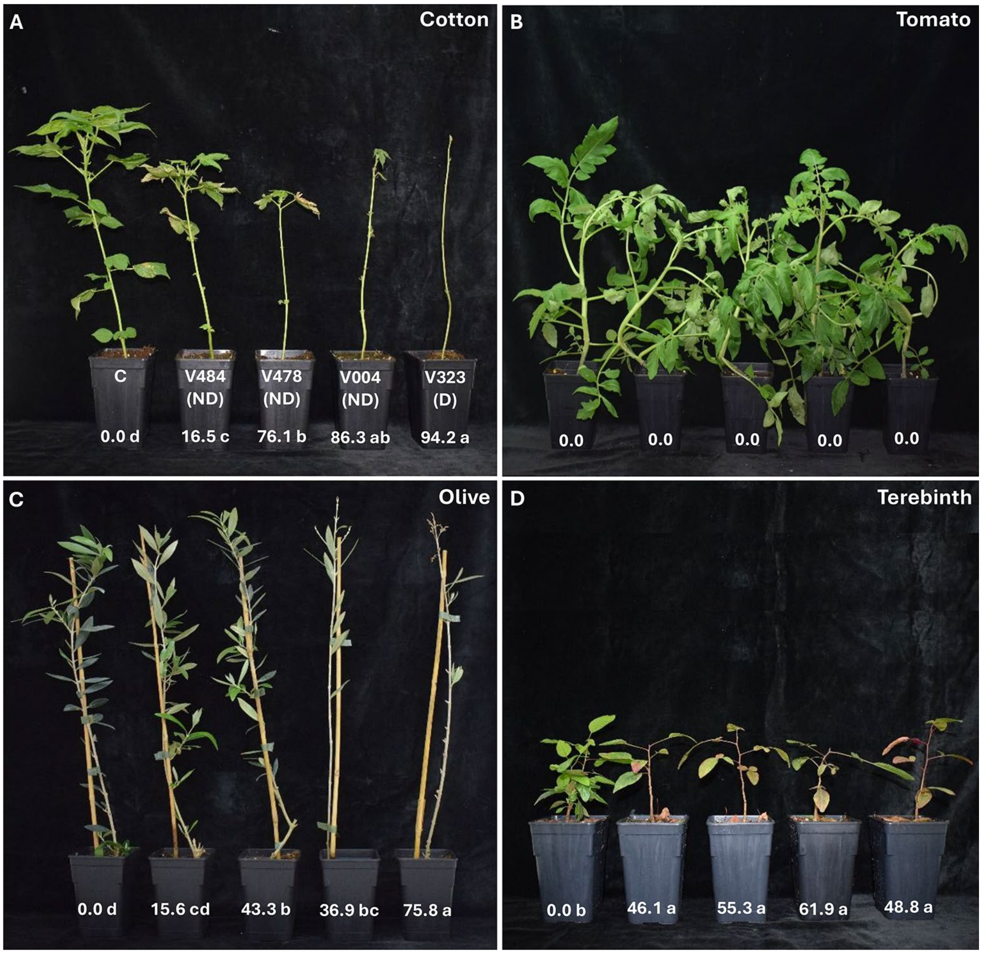

Fig. 1 Wilt symptoms and disease severity (DS) rates of cotton (cv. Siokra 1–4) (**A**), and tomato (cv. Tres cantos) (**B**) seedlings, and olive (cv. Picual) (**C**), and terebinth (**D**) plants at 1 month after inoculation with different isolates and pathotypes of *V. dahliae*. In each image, from left to right: noninoculated control plants (**C**), and then plants inoculated with the isolates V484, V478, V004 or V323. For each host, the mean values of disease severity (DS, %) for the control or inoculated plants are indicated below, and the mean DS values followed by the same letter do not differ significantly according to Fisher's protected LSD test at $p=0.05$ (Steel and Torrie 1985)

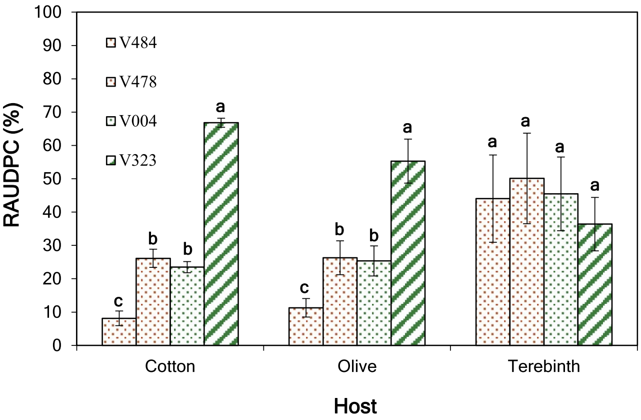

Fig. 2 Disease severity progression (relative area under the disease progression curve, RAUDPC; %) in cotton (cv. Siokra 1–4), olive (cv. Picual) and terebinth plants at 1 month after inoculation with *V. dahliae* isolates V484, V478, V004 or V323. The columns represent the means of two sets of experiments with 15 replicated plants each ($n=30$), and the vertical bars represent the standard error of the means. For each host, columns with common letters do not differ significantly according to Fisher's protected LSD test at $p=0.05$ (Steel and Torrie 1985). The orange and green columns represent *V. dahliae* isolates from pistachio and olives, respectively, and the dotted and diagonal lines represent nondefoliating (ND) and defoliating (**D**) pathotypes, respectively

Weight and length parameters

There were significant differences between *V. dahliae* isolates in terms of their effect on the fresh weight (g) of aerial part ($p=0.0347$) but not in terms of the length or other weight parameters ($p \geq 0.05$) of terebinth plants (Supplementary Table S1). However, there were significant differences among *V. dahliae* isolates in UCBI plants in terms of their effects on plant growth development for all the weight (g) parameters measured ($p \leq 0.05$). There were no significant differences in plant length (cm; $p=0.1153$) among the isolates. Compared with noninoculated control plants, plants inoculated with V484, V478 or V323 presented a significant reduction in plant fresh weight (g), with a maximum of 33.0% for the V323 isolate (data not shown). Compared with noninoculated control plants, inoculated plants presented a significantly lower dry weight (g), with a maximum reduction of 34.4% for the V484 and V323 isolates (Fig. 3).

Fungal reisolation

The percentage of *V. dahliae* isolated from the basal stem tissues of symptomatic plants ranged from 33.3% to 95.2%

Table 2 Disease–related parameters for cotton, olive and terebinth plants inoculated with *Verticillium dahliae* isolates

Isolate	Cotton (cv. Siokra 1–4)			
	Incidence (%)[a]	**Mortality (%)**[a]	**Disease severity (%)**[b]	**RAUDPC (%)**[b]
V004	93.3 b	60.0 a	86.3 ± 5.7 ab	23.5 ± 1.7 b
V323	100.0 a	66.7 a	94.2 ± 0.4 a	66.8 ± 1.4 a
V478	100.0 a	20.0 b	76.1 ± 3.9 b	26.1 ± 2.7 b
V484	46.7 c	13.3 b	16.5 ± 6.4 c	8.1 ± 2.2 c
Control	0.0 d	0.0 c	0.0 ± 0.0 d	0.0 ± 0.0 d
Isolate	**Olive (cv. Picual)**			
	Incidence (%)[a]	**Mortality (%)**[a]	**Disease severity (%)**[b]	**RAUDPC (%)**[b]
V004	90.0 b	23.3 b	36.9 ± 7.1 bc	25.3 ± 4.5 b
V323	100.0 a	43.3 a	75.8 ± 9.9 a	55.3 ± 6.6 a
V478	86.7 b	16.7 b	43.3 ± 10.3 b	26.3 ± 5.1 b
V484	70.0 c	3.3 c	15.6 ± 3.6 cd	11.3 ± 2.8 c
Control	0.0 d	0.0 c	0.0 ± 0.0 d	0.0 ± 0.0 c
Isolate	**Terebinth**			
	Incidence (%)[a]	**Mortality (%)**[a]	**Disease severity (%)**[b]	**RAUDPC (%)**[b]
V004	93.3 b	26.7 ab	61.9 ± 13.9 a	45.5 ± 11.1 a
V323	93.3 b	26.7 ab	48.8 ± 6.9 a	36.4 ± 8.0 a
V478	100.0 a	33.3 a	55.3 ± 15.2 a	50.1 ± 13.6 a
V484	93.3 b	13.3 b	46.1 ± 10.7 a	44.0 ± 13.1 a
Control	0.0 c	0.0 c	0.0 ± 0.0 b	0.0 ± 0.0 b

[a] In each column, data are the mean of two sets of experiments with 15 replicated plants each ($n = 30$) ± SE and, means followed by the same letter do not differ significantly according to multiple comparisons tests for proportions at $p = 0.05$ (Zar 2010)

[b] In each column, data are the mean of two sets of experiments with 15 replicated plants each ($n = 30$) ± SE and, means followed by the same letter do not differ significantly according to Fisher's protected LSD test at $p = 0.05$ (Steel and Torrie 1985)

in cotton, 38.1% to 90.5% in olive, 64.3% to 75.0% in terebinth and 42.9% to 85.7% in UCBI plants, confirming infection with the pathogen. *Verticillium dahliae* was not isolated from the control plants (Table 3).

Discussion

The cultivated area and production of pistachios have increased markedly during the last decade worldwide, with notable impacts on countries in the Mediterranean Basin, such as Italy and Spain (Gusella et al. 2024; Kaska 2002). As a consequence of the expansion of cultivation of this tree nut in Spain, there has been an increase in the incidence of Verticillium wilt in pistachio due to the establishment of new plantations on land previously cultivated with *V. dahliae*–susceptible hosts, which harbour high densities of the pathogen inoculum in the soil, especially when terebinth is used as a rootstock (Antón-Domínguez et al. 2024a; Gusella et al. 2024). Owing to the growing concern about this phytosanitary problem in the nut sector, field surveys have been conducted across new pistachio–growing regions in Spain (central–southern Spain), revealing that only the *V. dahliae* ND pathotype is being isolated from affected pistachio trees as well as from the soils of pistachio orchards (Antón-Domínguez et al. 2024a; López-Moral et al. 2023). This diagnosis contrasts with that previously described in the most common scenarios of Verticillium wilt in Spain, where olive and cotton are affected by both D and ND *V. dahliae* pathotypes, and the D pathotype is significantly more aggressive than the ND pathotype in both hosts regardless of the origin of the isolates (Bejarano-Alcázar et al. 1995; Jiménez-Díaz et al. 2012, 2017; Korolev et al. 2008; Markakis et al. 2010; Mercado-Blanco et al. 2003; Triantafyllopoulou et al. 2022). Similarly, the associations of Verticillium wilt in pistachio with only ND pathotypes causing tree death need to be studied by cross–pathogenicity tests. The phenomenon we are dealing with may be related to the fact that the differences in DS rates between *V. dahliae* pathotypes are due to their adaptive nature to the host (Dadd-Daigle et al. 2020, 2022; Jiménez-Díaz et al. 2012). Therefore, the present study focused on the pathogenic role of *V. dahliae* pathotypes recovered from olive or pistachio – on the most common pistachio rootstocks used in Spain: terebinth (susceptible) and the UCBI hybrid (resistant) (Antón-Domínguez et al. 2024a). In parallel, their pathogenic role was also evaluated in olive, cotton and tomato plants for comparative purposes.

This study demonstrated the susceptibility of terebinth to all *V. dahliae* isolates regardless of the pathotype and the host from which the isolates were obtained. The susceptibility of terebinth to the *V. dahliae* ND pathotype was greater than that of olive and cotton plants. Therefore, we can hypothesize that the high susceptibility to *V. dahliae* shown

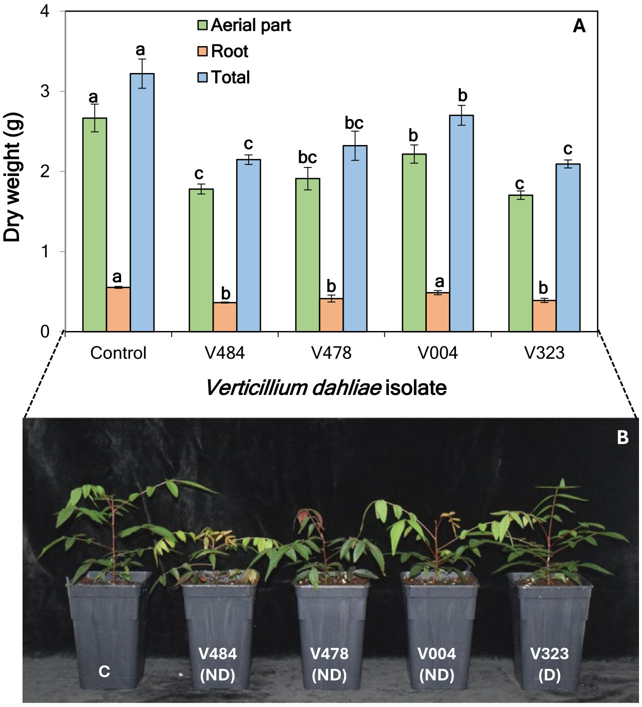

Fig. 3 Aerial (green columns), root (orange columns) and total (blue columns) dry weights (g) (**A**) and effects on plant growth development (**B**) of the UCBI hybrid at 1 month after inoculation with *Verticillium dahliae* isolates V484, V478, V004 or V323. The columns represent the means of two sets of experiments with 15 replicated plants each ($n=30$), and the vertical bars represent the standard error of the means. For each plant part, columns with common letters do not differ significantly according to Fisher's protected LSD test at $p=0.05$ (Steel and Torrie 1985)

Table 3 Frequency of isolation (%) of *Verticillium dahliae* isolates for each host[a].

Host	*Verticillium dahliae* isolates			
	V004	V323	V478	V484
Cotton	81.0	95.2	33.3	66.7
Olive	57.1	90.5	38.1	66.7
Terebinth	75.0	71.4	64.3	71.4
UCBI	64.3	42.9	85.7	60.7

[a] Reisolations were conducted in APDA from three plants per host and isolate combination or control (7 fragments per Petri dish and 2 Petri dishes per plant, isolate and host combination). The percentage of isolation was calculated as [(number of positive inoculated fragments/number of total inoculated fragments) × 100]

by terebinth can be partially explained by its susceptibility to both pathotypes, especially the ND. A prevailing hypothesis suggests that the highly virulent, defoliating pathotype of *V. dahliae* present in Andalusian olive groves originated in North America and was likely introduced through cotton cultivation (Jiménez-Díaz et al. Jiménez-Díaz et al. 2012; Jiménez-Díaz and Albajes 2025). First detected in 1983 in cotton fields in southern Seville (Andalusia region, southern Spain), this aggressive pathotype has since displaced the native, less virulent, nondefoliating strain and is now widespread in these regions (Jiménez-Díaz and Albajes 2025). However, the ND pathotype is more prevalent in other areas of Spain (Rodríguez et al. 2009), and it is believed to be the main pathotype in pistachio–growing regions in inland Spain; therefore, it is critical for the cultivation of this crop.

There are only a few previous studies of *V. dahliae* pathotypes affecting pistachios, most of which reported that the ND pathotype was infecting pistachios, similar to our study. These findings have been reported in Greece (Elena and Paplomatas 1998), Iran (Hadizadeh and Banihashemi 2007; Rafiei et al. 2018) and Spain (Antón-Domínguez et al. 2024). In these studies, some D isolates were found in Iran, but the majority of the isolates were identified as the ND pathotype. Some studies have demonstrated the high susceptibility of terebinth to Verticillium wilt, although intraspecific variability has been reported (Guney et al. 2021; Gusella et al. 2024; Moral et al. 2010; Raabe and Wilhelm 1978). Antón-Domínguez et al. (2024a) reported the susceptibility of terebinth even in soils with a low level of *V. dahliae* inoculum. In contrast, the UCBI rootstock showed high resistance to all the isolates evaluated in this study, as the plants did not develop disease symptoms despite showing significant growth reduction. To assess the impact of the pathogen on plant vigour, we measured both fresh and dry weights at the end of the experiment. Compared with those of the noninoculated plants, the fresh and dry weights of the inoculated UCBI plants were significantly lower. Previous studies have demonstrated the high resistance conferred by the UCBI rootstock under field conditions (Antón-Domínguez et al. 2024a; Epstein et al. 2004; Morgan et al. 1992). For these reasons, the use of UCBI rootstock is recommended to prevent Verticillium wilt in pistachio orchards established in soils infested with *V. dahliae*, regardless of the inoculum density of the pathogen in the soil.

This study also demonstrated the pathogenicity of the different *V. dahliae* D and ND isolates on crops that coexist with pistachio, such as olive and cotton, while none of them showed pathogenicity in tomato. Our results suggest that both olive and cotton are significantly more susceptible to the *V. dahliae* D pathotype used in this study than to the ND pathotype, regardless of the origin of the isolates. These findings are consistent with the study conducted by López-Escudero et al. (2004), who reported greater susceptibility of olive cultivars to the D pathotype of the fungus than to the ND pathotype. Specifically, in our study, the RAUDPC values for the D and ND (V004) pathotypes were similar to those reported by López-Escudero et al. (2004) for other D and ND pathotype isolates (66.6% and 22.1%, respectively) in olive plants cv. Picual. Similarly, Göre et al. (2009) reported that almost all cotton cultivars tested were susceptible or extremely susceptible to both *V. dahliae* pathotypes, although there was a greater overall susceptibility to the D pathotype than to the ND pathotype. Furthermore, Dadd-Daigle et al. (2020) reported that *V. dahliae* ND isolates sometimes caused high levels of DS in infected cotton fields in Australia, similar to the D pathotype. Specifically, ND VCG2A has been reported to cause complete defoliation in cotton fields throughout New South Wales (NSW), despite its classification as an ND pathotype (Dadd-Daigle et al. 2020, 2022). They determined that those ND isolates, especially those that were virulent, were genetically different from the "standard" ND. This highlights the high variability of pathotypes of this pathogen in terms of severity and the need for studies that address the differences between *V. dahliae* isolates within and between pathotypes to improve the understanding of its pathogenicity. In our study, there were no significant differences in the final DS between the D and the ND isolates from olive, although disease progression was lower than that caused by the D isolate. Notably, one of the ND isolates from pistachio caused statistically the same DS level in both cotton and olive plants as the ND isolate from olive, suggesting that some ND isolates may also have considerable pathogenic potential on these hosts.

None of the isolates caused disease symptoms in tomato in this study. With respect to the effects of the D and ND pathotypes of *V. dahliae* in tomato plants, only ND isolates were detected in plants affected by Verticillium wilt in South Africa, but they did not cause severe defoliation (Retief et al. 2023). Nevertheless, more recent studies conducted by Akar et al. (2024) detected both D and ND pathotypes in tomato plants in Turkey. A study conducted by Jiménez-Díaz et al. (Jiménez-Díaz et al. 2017) revealed that *V. dahliae* D isolates from olive and cotton have significantly low virulence in tomato plants, but the pathogenic effect of *V. dahliae* isolates from pistachio in tomato was uncertain before the present study. The absence of pathogenicity of both D and ND pathotypes of *V. dahliae* isolated from olive and pistachio on tomato may reflect host specialization, as selection pressure can lead to the emergence of host-specific pathotypes. Similar patterns have been observed in *V. dahliae* populations from horticultural crops, where isolates adapt to specific hosts or even cultivars, losing pathogenicity to unrelated species (Jiménez-Díaz et al. 2012). Certain isolates of *V. dahliae* carry the effector gene *Ave1*, which causes avirulence in tomato or lettuce cultivars that carry the resistance gene *Ve1* or its homologue *Vr1* (Jiménez-Díaz et al. Jiménez-Díaz et al. 2017). In addition, the nonaggressiveness of any of the *V. dahliae* isolates in tomato plants may be related to the absence of the effector gene (*Tom1*) in all these isolates, as this gene has been shown to mediate the pathogenicity of *V. dahliae* in tomato (Li et al. 2022). Further studies are needed to clarify the contribution of these factors to the nonpathogenic behaviour of *V. dahliae* isolates from olive and pistachio in tomato.

In conclusion, the present study is the first to demonstrate the high susceptibility of terebinth to the two pathotypes of *V. dahliae* regardless of their origin and the resistance conferred by the UCBI rootstock to both pathotypes. The differences observed in DS between pathotypes in olive and cotton are in line with previous reports, although further studies including a wider representation of isolates are needed to confirm these trends. These findings provide new insights into the cross–pathogenicity between *V. dahliae* pathotypes and the host, confirming the intraspecific variability of this pathogen and its host-adaptive nature. These findings provide a better understanding of the aetiology of Verticillium wilt in pistachio. Therefore, further investigation into the mechanisms of pathogenesis involved in *V. dahliae* infection is necessary to improve the understanding of this challenging disease.

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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate Not applicable.

Competing interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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