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Short communication

Development of a new method for characterizing olive cultivar resistance to *Verticillium dahliae*, the causal agent of Verticillium wilt

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ABSTRACT

Verticillium wilt of olive (*Olea europaea* L.), caused by the soil-borne fungus *Verticillium dahliae* Kleb., is one of the most important diseases affecting this crop. Using resistant cultivars is among the most effective control measures. Various inoculation methods have been used to assess olive cultivars resistance to Verticillium wilt under controlled conditions, but significant discrepancies often arise when comparing results with field conditions. This study aimed to develop a new method capable of detecting subtle differences in resistance or susceptibility among olive cultivars. Olive cultivars 'Picual', 'Arbequina', 'Koroneiki' and 'Frantoio' were inoculated using an artificial substrate containing *V. dahliae* microsclerotia at two doses (20 and 80 %). For comparison, root seedlings were also immersed in a conidial suspension of the pathogen. The 20 % substrate dose allow distinguished close levels of susceptibility ('Picual' and 'Arbequina'), while the 80 % dose can distinguish between close variations of resistance ('Koroneiki' and 'Frantoio'). To validate these findings under real conditions, a field experiment was conducted over 4 years, demonstrating alignment with the controlled environment results. 'Frantoio' consistently showed the highest resistance, 'Picual' the greatest susceptibility, and 'Arbequina' exhibited intermediate levels, thus confirming the differentiation achieved using the artificial inoculation method. Consequently, this newly developed method offers a significant advancement in the accuracy and reliability of resistance assessments for olive cultivars against Verticillium wilt within breeding programs.

1. Introduction

Verticillium wilt, caused by the soil-borne fungus *Verticillium dahliae* Kleb., is a major threat to many crops, including olive (*Olea europaea* L.) (Jiménez-Díaz et al., 2012; López-Escudero and Mercado-Blanco, 2011; Montes-Osuna and Mercado-Blanco, 2020). Verticillium wilt of olive (VWO) is particularly devastating in the Mediterranean basin, where this crop has economic, ecological, and cultural significance, causing tree death and frequently high fruit yield losses (Jiménez-Díaz et al., 2012). The pathogen, *V. dahliae*, is widely known for its ability to survive for long periods in crop debris and soil by producing microsclerotia (MS), which can remain dormant until they germinate stimulated by root exudates from a susceptible host (Fradin and Thomma, 2006; Keykhasaber et al., 2017). Disease severity (DS) depends mainly on the *V. dahliae* virulence with defoliating (D) and non-defoliating (ND) pathotypes distinguished by their ability to defoliate the tree (Keykhasaber et al., 2017; Klosterman et al., 2009; López-Escudero and Mercado-Blanco, 2011).

Due to the negative impact of VWO on the olive sector and the complexity and persistence of *V. dahliae*, managing this disease requires an integrated approach combining all available control measures. To date, genetic resistance is one of the most economical and effective long-term strategies against VWO (López-Escudero and Mercado-Blanco, 2011; Montes-Osuna and Mercado-Blanco, 2020; Trapero et al., 2013b). Unfortunately, most cultivars have shown high susceptibility to *V. dahliae* infections (López-Escudero and Mercado-Blanco, 2011; Trapero et al., 2015), highlighting the urgency of finding new tolerant/resistant genotypes to VWO. Long-term field evaluations provide valuable data on the resistance or susceptibility of these cultivars, but the variability of natural infection conditions (e.g. climate, soil type, microbial interactions, previous crop history in the plot, inoculum density) makes them time-consuming and costly, with evaluation

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requiring at least 3 years and large field plots (Trapero et al., 2013b; Valverde et al., 2023). Thus, setting up a method under controlled conditions that can reliably discriminate among close levels of resistance and susceptibility is required. Some of the available methods to characterize the resistance of olive cultivars and genotypes to VWO under controlled conditions are root dipping, stem injection or pot immersion (Cirulli et al., 2008; García-Ruiz et al., 2014, 2015; López-Escudero et al., 2004, 2007; Martos-Moreno et al., 2006; Trapero et al., 2013a). However, some of these studies have reported discrepancies between resistance levels observed in field trials and those observed under controlled environments within the same cultivar or genotype (Cirulli et al., 2008; López-Escudero et al., 2007; López-Escudero and Mercado-Blanco, 2011; Trapero et al., 2013a, 2013b; Wilhelm and Taylor, 1965). Moreover, molecular (Ramírez-Tejero et al., 2021) and morphological/anatomical (Cardoni et al., 2022) studies have also provided insight into the resistance mechanisms. However, due to the large number of genes and pathways likely involved, these methods are not currently able to accurately predict the level of resistance of a given cultivar or genotype.

Considering these insights, this study aimed to set up a new inoculation method that closely replicates field conditions, allowing for precise differentiation of resistance levels among cultivars and genotypes to VWO under semi-controlled conditions. Since *V. dahliae* infects its host through MS as the primary inoculum source in nature, the development of a method that replicates it would be a step forward in the search for VWO-resistant cultivars and genotypes. As Antoniou et al. (2008) noted, the use of different MS densities could be a strategy in host resistance screening experiments. Field observations show that MS density can greatly vary within the soil of the same field, affecting disease severity (Johansson et al., 2006). Therefore, building on the artificial substrate inoculation method developed by Varo et al. (2016), this study validates its use with two substrate doses. To this end, four olive cultivars with different resistance levels to VWO were inoculated with two doses of artificial substrate and was validated by comparison with the results from the root dipping method and the experimental field under natural field conditions. This novel approach increases the accuracy of cultivar characterization and the efficiency in olive breeding against Verticillium wilt.

2. Materials and methods

2.1. Inoculation experiment in controlled conditions

2.1.1. Plant materials

The cultivars 'Picual', 'Arbequina', 'Koroneiki' and 'Frantoio' were selected according to their level of resistance, susceptible, moderately susceptible, moderately resistant and resistant, respectively, based on the previous study conducted by Trapero et al. (2013b). All of them are traditional and widely used cultivars in their areas of origin ('Picual' and 'Arbequina' in Spain, 'Koroneiki' in Greece and 'Frantoio' in Italy). Plant material for propagation was collected from the World Olive Germplasm Bank of the University of Cordoba (Cordoba, Spain), where all accessions have been previously genetically authenticated. These cultivars were propagated by rooting of soft-wood cuttings in trays with sterile perlite under mist following the methodology described by Caballero and Del Río (2010). When the cuttings rooted, they were transplanted into sterile PVC pots with peat moss and kept in a greenhouse at constant 23 ± 2 °C under natural photoperiod conditions until use.

2.1.2. Fungal isolates and inoculum preparation

Verticillium dahliae isolates V323 (D pathotype isolated from olive; López-Moral et al., 2022) and V117 (D pathotype isolated from cotton; Blanco-López et al., 1984) were used in all experiments in this study. They were characterized as D pathotypes by nested PCR following the protocols described by Mercado-Blanco et al. (2003), and they are preserved in the fungal collection of the Department of Agronomy at the University of Cordoba (DAUCO; Spain). The isolates were first grown on potato dextrose agar (PDA; Difco® Laboratories, MD, United States) acidified with lactic acid [APDA; 0.1 % (vol/vol); pH = 4.0–4.5] and incubated at 24 ± 2 °C in the dark for 7 days. Growing fungal colonies were transferred to PDA by streaking and incubated under the same conditions described above.

The inoculum was performed in 2 L Erlenmeyer flasks containing 1 kg of sterile sand, cornmeal and distilled water mixture (9:1:2; w:w:v; CMS). Then, flasks were double sterilized (120 °C for 50 min; and 24 h later, 120 °C for 20 min) and inoculated by adding 50 mycelial plugs (5 mm diameter each) of *V. dahliae* isolate V323 growing on PDA at 23 °C in the dark for 14 days (Varo et al., 2016). After 1 month from inoculation, inoculum density was estimated using the serial dilution method in PDA and expressed as colony forming units (CFUs) (Mulero-Aparicio et al., 2019).

Root dipping (RD) method was included for comparative purposes. To this end, V323 and V117 isolates conidial suspensions were obtained from 14-day-old colonies growing as described above, by adding sterile distilled water (SDW) to the Petri dishes and scraping the mycelial colony with a sterile scalpel. Then, they were adjusted at 10^7 conidia/ml using a hemocytometer.

2.1.3. Plant inoculation and experimental design

Prior to inoculation, peat was manually removed from the roots of the 4-month-old olive seedlings, and the roots were rinsed under running tap water. To CMS method, seedlings were transplanted into 0.8 L sterile PVC pots filled with a substrate mixture at two different inoculum densities 1) CMS20: 20 % (w:w) of the previously described inoculum and 80 % sterile peat moss, 2) CMS80: 80 % of inoculum substrate and 20 % sterile peat moss. The theoretical final substrate densities were 10^7 and 10^8 CFU/g for CMS20 and CMS80, respectively. Seedlings from each cultivar transplanted in sterile peat moss were included as control.

RD inoculation was conducted by immersing the roots in the conidial suspensions of the described isolates for 30 min. Seedlings from each cultivar soaked in SDW were included as control. After then, the seedlings were transplanted into 0.8 L sterile PVC pots filled with sterile peat moss.

Plants were incubated in the dark at 19 °C and 100 % relative humidity (RH), maintained using a humidifier, for 3 days after inoculation. Subsequently, the growth chamber conditions were adjusted to 23 ± 2 °C, 70 % RH, 12 h day/night photoperiod of fluorescent light (10,000 lux) and plants were irrigated three times per week (López-Moral et al., 2022).

For each cultivar, a randomized complete block design with three blocks and three replicate plants per inoculation method (or control) and block were used (3 × 3 × 5 = 45 plants per cultivar; 45 × 4 = 180 in total). The experiment was conducted twice under the same biological conditions, in March of 2021 and 2023.

2.2. Field experiment in infested soil

On March 30, 2015, an experimental plantation was carried out on an experimental orchard located in Villanueva de la Reina (Jaen province, southern Spain; UTM coordinates X: 38.01374, Y: 3.91000).

Seven-month-old olive plants of the cultivars 'Picual' (susceptible), 'Arbequina' (moderately susceptible) and 'Frantoio' (resistant) were used, propagated and grown as described in section 2.1.1. A randomized complete block design with five blocks and three replicate plants (clones) of each cultivar per block were used (5 × 3 = 15 plants per cultivar; 15 × 3 = 45 in total). Prior to planting, soil sampling was carried out to determine the amount of inoculum present in the experimental plot. A representative sampling was conducted of the plot area, with a 100 g soil sub-sample taken every 20 m along the two planting lines. Subsamples were collected using a cylindrical auger from a depth of 25–30 cm, then mixed, bulked, and crumbled. Samples were then air-

dried under ambient conditions for 4 weeks. In the laboratory, each sample was analyzed four times (replications) through the wet sieving method (Huisman and Ashworth, 1974).

For the five blocks included in the experiment, an average inoculum density of 38 MS of *V. dahliae* per gram of soil was determined as described before.

2.3. Disease assessment

DS was evaluated using a 0 to 4 scale with 17 levels to estimate the percentage of affected tissue. This scale consists of 5 categories (0 = no symptoms, 1 = 25 %, 2 = 50 %, 3 = 75 %, and 4 = 100 %, dead plant) with three intermediate values (0.25, 0.50, and 0.75) between the main categories (Antón-Domínguez et al., 2024; Varo et al., 2018). In the inoculation experiment, the DS was evaluated weekly for 3 months from 1 month after inoculation, for a total of 10 assessments. In the field experiment, the DS was evaluated for 4 years from 1 month after planting, for a total of 11 assessments, especially corresponding with the greatest expression of symptoms (spring and autumn). DS data were used to calculate the relative area under the disease progression curve (RAUDPC) using the trapezoidal integration method (Campbell and Madden, 1990) according to the following formula:

$$RAUDPC = \frac{100}{DS_{max} \times t_e} \times \left(\sum_{i=1}^{n} \frac{(DS_i + DS_{i+1})}{2} \times (t_{i+1} - t_i) \right)$$

where n is the number of observations, DS_i is the DS value for observation number i, DS_{max} is the maximum value of severity (4), t_e is the total evaluation period and t_i is the number of days between planting and observation i.

Disease incidence (DI; % of affected plants) was estimated for each cultivar and inoculation method at the end of the experiment. In addition, plant height was measured with a tape measure at the beginning of the experiment and at the end (3 months later) to determine the relative growth rate (RGR) using the following equation:

$$RGR\ (\%\,/\,día) = 100 \times \frac{(final\ height - initial\ height)}{experiment\ time}$$

RGR was only measured for CMS inoculation method to compare the two doses with control plants.

2.4. Data analysis

In all cases, data were tested for homogeneity of variance, normality and residuals pattern prior to analysis of variance (ANOVA), and logarithmic transformation of the data was performed when necessary. In the experiment under controlled conditions, the data from the two repetitions were combined after verifying that there were no significant differences between them ($P \geq 0.05$).

For each inoculation method, one-way ANOVA was performed with olive cultivars as the independent variable and RAUDPC, DS or DI as dependent variables. For height development, a two-way ANOVA was performed with CMS dose, cultivar, and their interaction as independent variables, and RGR (%/day) as 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). For the DI and mortality analysis in the field experiment, Chi-Square test at $P = 0.05$ was used. Data analyses were performed using Statistix 10 software (Analytical Software, 2013).

3. Results

3.1. Inoculation experiment in controlled conditions

Plants inoculated with *V. dahliae* showed typical disease symptoms (i.e. chlorosis, wilting and defoliation of green leaves) approximately 30 days after inoculation. Noninoculated control plants showed no disease symptoms. There were significant differences in RAUDPC, DS and DI among cultivars for inoculation with CMS20 ($P = 0.0010$, $P = 0.0002$ and $P \leq 0.0001$, respectively), CMS80 ($P = 0.0098$, $P = 0.0014$ and $P = 0.0001$), RD-V323 ($P \leq 0.0001$ in all three cases) and RD-V117 ($P = 0.0013$, $P = 0.0002$ and $P = 0.0033$) (Supplementary Table S1).

For inoculation with CMS20, 'Picual' showed a significantly higher RAUDPC, DS and DI (32.1 ± 5.7 %, 81.1 ± 12.5 % and 100 ± 0.0 %, respectively) than the rest of the cultivars. The rest of the cultivars obtained statistically equal values of RAUDPC and DS, while 'Frantoio' was the one that showed the lowest DI (0.0 ± 0.0 %) (Supplementary Table S1; Fig. 1).

For inoculation with CMS80, 'Picual' and 'Arbequina' showed statistically equal values of RAUDPC (40.7 ± 7.9 % and 32.3 ± 11.3 %, respectively). 'Koroneiki' and 'Frantoio' showed lower and significantly different RAUDPC values, allowing to distinguish the level of resistance between them (RAUDPC = 8.3 ± 1.9 % and 0.0 ± 0.0 %, respectively) (Supplementary Table S1; Fig. 1).

In the case of the RD-V323, 'Picual' showed the highest RAUDPC and DS values (46.9 ± 7.5 % and 86.8 ± 7.5 %, respectively), followed by 'Arbequina' (RAUDPC = 21.2 ± 9.5 %; DS = 41.1 ± 17.1 %). 'Koroneiki' and 'Frantoio' obtained statistically equal values of RAUDPC and DS (Supplementary Table S1; Fig. 2).

Finally, for RD-V117, 'Picual' showed significantly higher RAUDPC and DS values (18.6 ± 7.0 % and 40.3 ± 13.0 %, respectively) than the rest of the cultivars. The rest of the cultivars obtained statistically equal values of RAUDPC and DS, while 'Frantoio' and 'Koroneiki' showed the lowest DI values (0.0 ± 0.0 %, in both cases) (Supplementary Table S1;

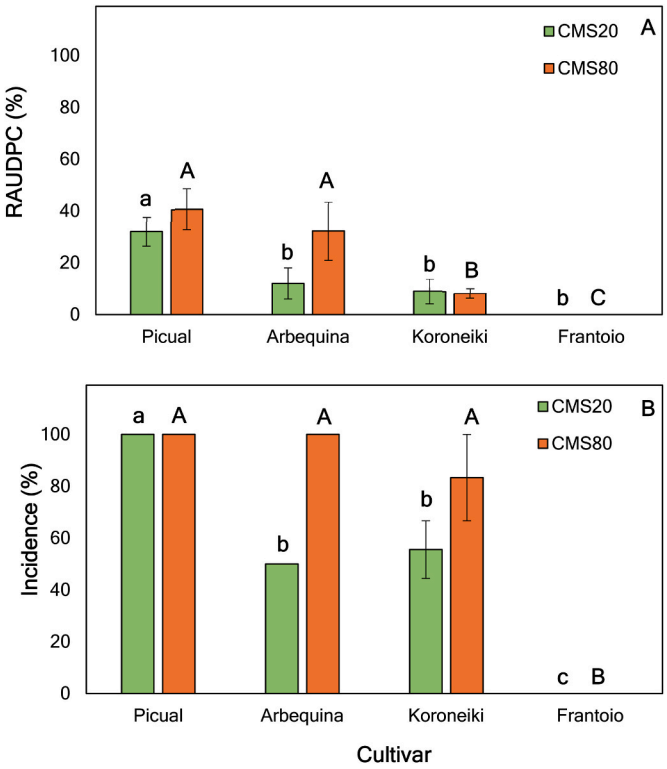

Fig. 1. Relative area under the disease progression curve (RAUDPC; %) (**A**) and disease incidence (DI) (**B**), in olive plants of cvs. 'Picual', 'Arbequina', 'Koroneiki' and 'Frantoio' at 3 months after inoculation with cornmeal-sand mixture (CMS) containing microsclerotia of *V. dahliae* isolate V323 at two doses (20 % and 80 %). Data are the mean of three blocks with six replicated plants per cultivar and inoculation dose and vertical bars the standard error of the means. For each graph and treatment combination (CMS20 or CMS80), columns with common letters do not differ significantly according to Fisher's protected LSD test at $P = 0.05$ (Steel and Torrie, 1985).

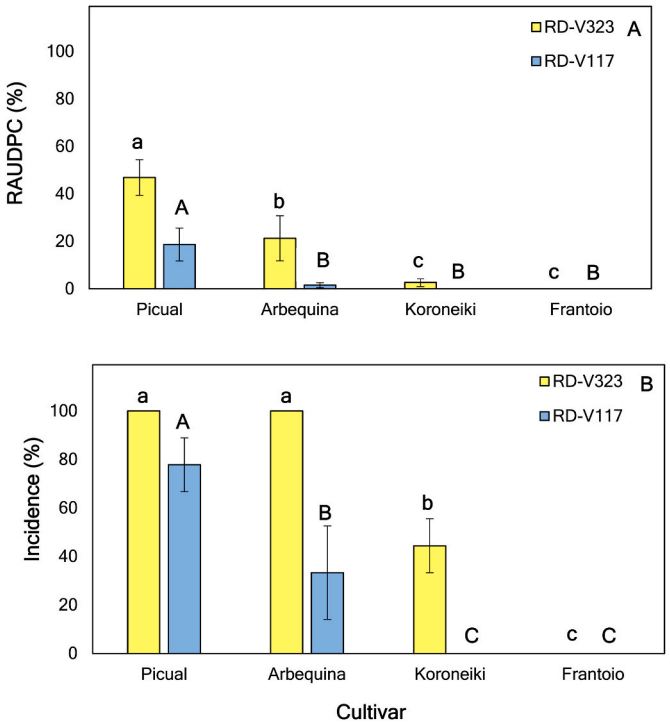

Fig. 2. Relative area under the disease progression curve (RAUDPC; %) (**A**) and disease incidence (DI) (**B**), in olive plants of cvs. 'Picual', 'Arbequina', 'Koroneiki' and 'Frantoio' at 3 months after inoculation by using the root dipping (RD) method with *V. dahliae* V323 and V117 isolates. Data are the means of three blocks with six replicated plants per cultivar and inoculation dose and vertical bars the standard error of the means. For each graph and treatment combination (RD-V323 or RD-V117), columns with common letters do not differ significantly according to Fisher's protected LSD test at $P = 0.05$ (Steel and Torrie, 1985).

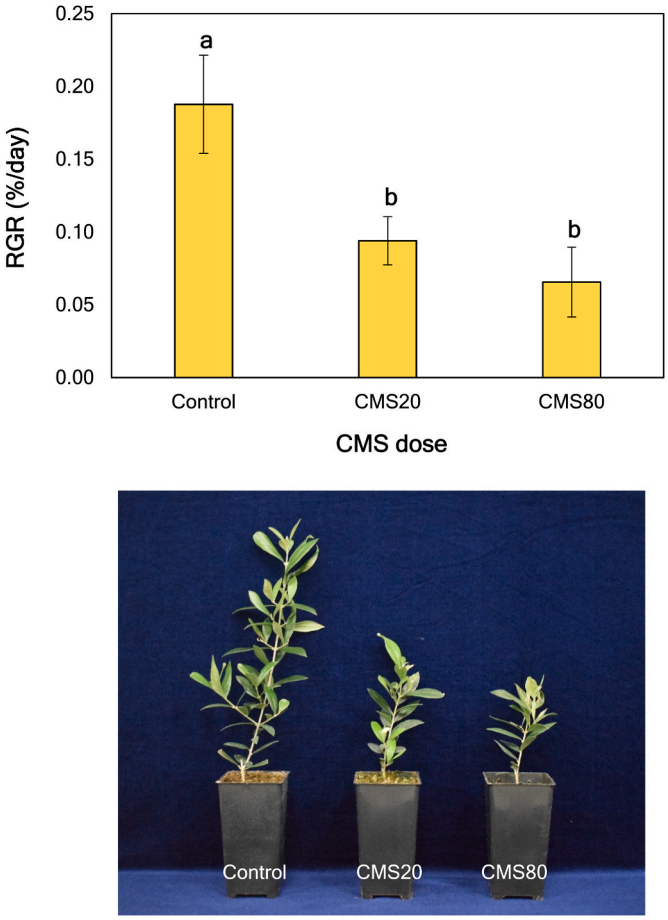

Fig. 3. Relative growth rate (RGR; %/day) of olive plants at 3 months after inoculation with cornmeal-sand mixture (CMS) containing microsclerotia of *V. dahliae* isolate V323 at two doses (20 % and 80 %), and control plants. Data are the mean of three blocks with six replicated plants per cultivar and inoculation dose and vertical bars the standard error of the means. Columns with common letters do not differ significantly according to Fisher's protected LSD test at $P = 0.05$ (Steel and Torrie, 1985).

Fig. 2).

The two-way ANOVA showed significant differences in RGR for CMS dose or control ($P = 0.0110$) but not for cultivar and their interaction ($P > 0.05$). Therefore, one-way ANOVA was performed with inoculation method as the independent variable and RGR as dependent variables. There were significant differences in RGR among CMS dose or control ($P = 0.0062$). The inoculated seedlings had a lower RGR than the non-inoculated seedlings, thus demonstrating the effect of fungal colonization on plant development (Fig. 3).

3.2. Field experiment in infested soil

The first symptoms were observed in 'Picual' cultivar less than 12 months after planting, and progress quickly over the next 3 years. On the other hand, disease incidence in 'Frantoio' was very low, with only 1 plant out of 15 showing light symptoms. There were significant differences in RAUDPC, DS, DI and mortality among cultivars for the field experiment ($P < 0.05$). The RAUDPC, DS, DI and mortality values showed that 'Picual' cultivar is the most susceptible (41.8 ± 4.1 %; 77.3 ± 5.3 %; 92.3 ± 8.5 %; and 61.5 ± 12.8 %, respectively). 'Arbequina' (13.1 ± 5.6 %; 18.4 ± 7.7 %; 28.6 ± 11.2 %; and 21.4 ± 10.1 %) shows intermediate resistance values and 'Frantoio' appears to be the most resistant with incidence and mortality values of 6.7 ± 6.7 % and 0.0 ± 0.0 % (Table 1). DS progression throughout the experiment at each assessment is shown in Supplementary Fig. S1.

Table 1
Disease-related parameters for olive plants of cvs. 'Picual', 'Arbequina' and 'Frantoio', 4 years after planting in a field naturally infested with *Verticillium dahliae* (38 microsclerotia per gram of soil).

Cultivar	Incidence (%)[a]	Mortality (%)[a]	Disease severity (%)[b,c]	RAUDPC (%)[b,d]
Picual	92.3 ± 8.5 a	61.5 ± 12.8 a	77.3 ± 5.3 a	41.8 ± 4.1 a
Arbequina	28.6 ± 11.2 b	21.4 ± 10.1 b	18.4 ± 7.7 b	13.1 ± 5.6 b
Frantoio	6.7 ± 6.7 c	0.0 ± 0.0 c	0.2 ± 0.2 c	0.1 ± 0.1 c

[a] In each column, data are the mean of five blocks with three replicated plants each ± SE and means followed by the same letter do not differ significantly according to Chi-Square test at $P = 0.05$.
[b] In each column, data are the mean of five blocks with three replicated plants each ± 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).
[c] Final disease severity ± SE at 4 years after planting. Disease severity was assessed using a 0 to 4-rating scale (0 = no lesions, 4 = 100 % of affected plant tissues) and the scale values are expressed as a percentage relative to the control.
[d] Relative area under the disease progression curve (RAUDPC) ± SE developed over the assessment period.

4. Discussion

Previous studies under controlled conditions reveal that 'Picual' and 'Arbequina' are considered susceptible or extremely susceptible to *V. dahliae* (López-Escudero et al., 2007; Trapero et al., 2013a). However, in trials conducted under field conditions, Trapero et al. (2013b) demonstrated that 'Arbequina' is significantly more resistant to the

disease than 'Picual', and some other cultivars show intermediate resistance levels undetected by the root dipping inoculation method (López-Escudero et al., 2004; Martos-Moreno et al., 2006). Serrano et al. (2021) confirmed that 'Picual' is more susceptible than 'Arbequina' using CMS inoculation although under semi-controlled conditions in open-air microplots. However, the present study under fully controlled conditions, clearly distinguishes the difference in susceptibility to *V. dahliae* between 'Picual' and 'Arbequina' as observed in the field.

Our study extends the work by Varo et al. (2016), who developed and validated a controlled inoculation technique to assess biological treatment efficacy against VWO, emphasizing the critical role of inoculation methods that replicate field-like conditions in producing reliable resistance assessments. Here, we demonstrate that a 20 % CMS dose effectively distinguishes between these two cultivars consistent with field observations. Likewise, we discriminated resistance levels using the root dipping method with both the olive V323 isolate and the cotton V117 isolate. In the field experiment, we observed resistance patterns consistent with previous field studies: 'Frantoio' (most resistant), 'Picual' (most susceptible) and 'Arbequina' (intermediate) (Trapero et al., 2013b). Moreover, 'Koroneiki' is considered moderately resistant and 'Frantoio' is resistant when inoculated by stem injection or root dipping (López-Escudero et al., 2004, 2011; Markakis et al., 2022; Martos-Moreno et al., 2006).

In this study, 'Frantoio' demonstrated consistent high resistance across inoculation methods, showing no disease symptoms, corroborating Bubici and Cirulli (2012). However, Trapero et al. (2013b) reported that this cultivar did not show complete resistance in a naturally infested soil, although it is one of the best options for new olive plantations in soils with high densities of the pathogen. In the same study, the moderate resistance of 'Koroneiki' cultivar was reported, aligns with the present study.

Our approach to CMS-based inoculation in controlled environments offers practical advantages for olive breeding programs. Field conditions introduce numerous variables, including soil composition, temperature fluctuations, and pathogen density, which complicate consistent resistance assessments (Roca et al., 2016). This means that long periods of time are required to evaluate the resistance of olive cultivars or genotypes to VWO under field conditions (Trapero et al., 2013b; Valverde et al., 2023). Although the method developed in this study is more laborious than the commonly used root dipping method, it more accurately reproduces natural field infection conditions. The 20 % CMS dose in our study effectively distinguished 'Picual' as having significantly higher RAUDPC values, while 'Arbequina' and 'Koroneiki' exhibited statistically similar RAUDPC values, supporting field-based resistance categorization (Trapero et al., 2013b). Higher CMS doses (80 %) allowed the distinction between moderately resistant ('Arbequina'), intermediate ('Koroneiki'), and most resistant ('Frantoio') cultivars. Such fine-tuned differentiation under controlled conditions ensures that resistance levels observed in field conditions can be approximated without the constraints of long-term field trials.

It appears that the root dipping inoculation method may be overcoming some resistance mechanisms that are normally in place when plants are grown in field conditions. The artificial substrate introduced by Varo et al. (2016) and further optimized in this study using two doses allowed effective characterization of olive cultivars resistance to Verticillium wilt, similarly to other species where it has been successfully used (Bae et al., 2011; Steventon et al., 2002). This difference in effectiveness in characterizing VWO resistance between the two methods, root dipping and CMS, is mainly due to the *V. dahliae* structures present in them, conidia and MS, respectively. Considering the biology of the fungus, its life cycle and the mode of infection through MS germination, the use of different doses of CMS seems to more closely reproduce the natural mode of infection (Antoniou et al., 2008). This new method allowed us to differentiate between subtle susceptibility and resistance levels, depending on the dose of CMS used. Validating our findings in a multi-year field experiment demonstrated that the CMS inoculation method, particularly at specific dose levels, can emulate the complex interactions observed under natural conditions. Particularly, the lower inoculum dose (20 %) allowed distinguishing between highly susceptible and intermediate levels, while the higher dose (80 %) allowed the differentiation of the moderately resistant to resistant levels, which may be more suitable for breeding programs looking for *V. dahliae* resistance (Valverde et al., 2023). This method could potentially be adapted for screening the cultivar susceptibility in other pathosystems with similar infection dynamics, specially to *V. dahliae* hosts. In conclusion, this reproducible methodology aligns with field conditions, enabling efficient evaluation of the resistance of olive cultivars and genotypes against VWO and reducing evaluation time compared to field trials.

CRediT authorship contribution statement

Begoña I. Antón-Domínguez: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Pedro Valverde:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Carlos Agustí-Brisach:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Formal analysis, Conceptualization. **Carlos Trapero:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

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Declaration of 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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cropro.2025.107396.

Data availability

Data will be made available on request.

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