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Research Paper

A non-pathogenic strain of *Fusarium oxysporum* and grape marc compost control Verticillium wilt of olive

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Summary. Verticillium wilt of olive (VWO), caused by the widespread soil-borne fungus *Verticillium dahliae* Kleb., is currently the most serious disease affecting olive trees (*Olea europaea* L.) in all production areas. An integrated management strategy using eco-friendly approaches such as genetic resistance and biological control is considered the most advisable approach for controlling the disease in commercial olive orchards. This study evaluated a non-pathogenic strain of *F. oxysporum* (FO12) and the grape marc compost CGR03 for reducing inoculum density of *V. dahliae* and the disease progress in two olive cultivars with different VWO resistance levels. The experiment was conducted under semi-controlled conditions, using a naturally infested soil with two inoculum densities of *V. dahliae*. The biocontrol treatments (FO12 and CGR03) were previously selected out of 220 natural products as two of the most effective treatments against the pathogen. FO12 and CGR03 treatments significantly reduced pathogen inoculum density in comparison with that of the control (P = 0.05), with minimum microsclerotium amounts of 0.13 g^{-1} for FO12 and 0.53 g^{-1} for CGR03, during the experimental period. CGR03 reduced the progression of the disease compared with that in the control (P = 0.05), and FO12 achieved complete control of VWO, since no plants treated with this biological control strain developed VWO symptoms. This study highlights the effectiveness of these biocontrol treatments, and the potential use of eco-friendly approaches for control of VWO.

Keywords. Biocontrol agents, *Olea europaea*, organic amendment, microsclerotia, *Verticillium dahliae*.

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ABBREVIATIONS:

ANOVA: Analysis of variance; BCAs: Biological control agents; DAP: Days after planting; HID: High inoculum density; ID: Inoculum density; LID: Low inoculum density; LSD: Least significant difference; MS: Microsclerotia; MSPA: Modified sodium polypectate agar medium; PDA: Potato dextrose agar; RAUDPC: Relative area under the disease progress curve; RAUIPC: Relative area under the inoculum progress curve; VWO: Verticillium wilt of olive.

INTRODUCTION

The widespread soil-borne fungus *Verticillium dahliae* Kleb. is known to cause vascular diseases in several crops with agronomic value (Pegg and Brady, 2002). Verticillium wilt of olive (VWO) is the most serious disease caused by this pathogen affecting olive trees (*Olea europaea* L.) in all olive-producing areas, causing significant economic losses and plant death (López-Escudero and Mercado Blanco, 2011). In the infested olive groves of the Guadalquivir valley (Andalusia, Spain), this disease has reached an average incidence of 20% (López-Escudero *et al*., 2010).

Since there is no efficient control of VWO when control measures are individually applied, an integrated management strategy is the most advisable approach to reducing the disease in commercial olive crops (López-Escudero and Mercado Blanco, 2011). Within this strategy, and due to current environmental concerns, eco-friendly approaches such as cultural practices, genetic resistance and biological control have acquired increased relevance. The genetic resistance levels of olive cultivars against VWO have been assessed in previous studies. López-Escudero *et al*. (2004) reported that cv. 'Picual' and 'Arbequina' are highly susceptible to the defoliating pathotype of *V. dahliae* when they are artificially inoculated by root dipping. Additionally, the relationship between inoculum density (ID) of *V. dahliae* in infested soils and the resistance among olive cultivars was confirmed in several studies. López-Escudero and Blanco-López (2007) reported that 'Picual' was extremely susceptible to *V. dahliae* when planted in soils with low ID, while 'Arbequina' was moderately resistant under the same conditions (Trapero *et al*., 2013b). This relationship was confirmed in a study carried out in commercial olive orchards (Roca *et al*., 2015).

Although there are numerous studies related to the biological control of *V. dahliae*, few have addressed the use of biocontrol treatments against VWO. The use of *Pseudomonas* spp. (Mercado-Blanco *et al*., 2004; Triki *et al*., 2012; Gómez-Lama *et al*., 2017) and *Trichoderma* spp. (Lima *et al*., 2007; Jiménez-Díaz *et al*., 2009) have been reported as promising biological control agents (BCAs) against VWO. Preliminary studies on the use of organic amendments against VWO have been conducted (Vitullo *et al*., 2013). An extensive screening was conducted to evaluate the effectiveness of 220 natural compounds, including microorganisms (Lozano-Tovar *et al*., 2013; Varo *et al*., 2016), organic amendments (Varo-Suárez *et al*., 2018) and plant extracts (Varo *et al*., 2017), against *V. dahliae*, in *in vitro* and *in planta* experiments under controlled conditions. The most effective treatments were evaluated under field conditions (Mulero-Aparicio *et al*., 2020), as the only study that has evaluated biological control treatments against VWO in natural conditions. From these two studies, a non-pathogenic strain of *Fusarium oxysporum*, designated FO12, and a grape marc compost, labelled CGR03, were selected as two of the most effective treatments against the pathogen. However, further studies evaluating these products under semi-controlled and different field conditions are essential for demonstrating their effectiveness against VWO.

Knowledge of the interactions between different disease management strategies, such as genetic resistance or biological control, used for integrated control of VWO is important for achieving efficient control of the disease. However, the combined effects of these two strategies remains unknown. The main objective of the present study was to evaluate the effect of two biological treatments, the non-pathogenic strain of *F. oxysporum* FO12 and the grape marc compost CGR03, on the progression of ID of *V. dahliae*, and on the development of VWO, using two olive cultivars with different levels of genetic resistance to the disease.

MATERIALS AND METHODS

Plant material

One-year-old rooted olive plants of cv. 'Picual' and 'Arbequina' (respectively susceptible and moderately susceptible to VWO) (Trapero *et al*., 2013a) were used for the experiment. These plants were obtained from a commercial nursery producing plants that were certified free of *V. dahliae* and other olive pathogens. At planting time, the plants were 1 y old and 1.0 to 1.1 m high, each with a single trunk and three or four secondary branches.

Naturally infested soil and inoculum density estimation

The soil used in this study was collected from a commercial field previously cultivated with cotton over the last 50 years, located in Villanueva de la Reina (UTM coordinates X: 38.012845; Y: 3.909219) in Jaen Province (Andalusia, southern Spain).

To estimate the ID of *V. dahliae* in this soil, five soil sub-samples of ≈500 g were collected from the upper 30 cm of soil, using a cylindrical soil auger. Sub-samples were mixed, air-dried at room temperature, and sieved (0.8 mm mesh) to remove large particles. Inoculum density of *V. dahliae* in the soil was estimated by wet sieving (Huisman and Ashworth, 1974) onto modified sodium polypectate agar medium (MSPA) (Butterfield and DeVay,

1977). Three samples (25 g each) of the naturally infested soil were suspended in 100 ml of distilled water, shaken at 270 rpm for 30 min at room temperature and filtered through 150 and 35 µm sieves. The residue retained on the 35 µm sieve was recovered in 100 ml of distilled water. Part of this suspension was then plated across ten plates of MSPA (1 ml/plate), and the plates were incubated for 14 d at 24 ± 2°C in the dark. Soil residues were removed from the agar surfaces under running tap water, and colonies of *V. dahliae* were counted using a stereomicroscope (Nikon SMZ-2T). The ID in soil was estimated from the number of *V. dahliae* colonies counted per sample and was expressed as microsclerotia (MS) per gram of soil (MS g^{-1}) (López-Escudero and Blanco-López, 2005).

Biocontrol treatments

Two biocontrol treatments were evaluated in this study. The grape marc compost CGR03 and the non-pathogenic strain of *Fusarium oxysporum* FO12 were selected due to their suppressive effects on VWO demonstrated in previous *in vitro* and *in vivo* experiments (Varo *et al*., 2016; Varo-Suárez *et al*., 2018; Mulero-Aparicio *et al*., 2019a).

Grape marc compost CGR03

The organic amendment CGR03 was provided by the agri-food cooperative "Cooperativa San Acacio", Montemayor, Córdoba (southern Spain). To produce the compost, grape marc wastes consisting of grape skins, seeds and stems of the grapevine cv. 'Pedro Ximénez' from the wine industry were used as feedstock. Grape marc is collected annually from August to September and composted in insulated bins each of 15 m^3 for 8 months. When the compost temperature fell below 60°C at a depth of 50 cm, the compost was turned to promote aeration and homogeneity and to renew the composting process. Compost was collected for use when the temperature permanently fell below 40°C (maturation/recolonization phase). The pH of the compost was 6.9, determined in 1:5 (v:v) compost/water extract. Before use, the compost was proven to be mature and stable by measuring its temperature (30-35°C for mature compost) to avoid phytotoxicity (Mehta *et al*., 2014; Varo-Suárez *et al*., 2018).

Non-pathogenic strain of Fusarium oxysporum

The non-pathogenic *F. oxysporum* strain FO12, from to the fungal collection of the Department of Agronomy, University of Córdoba (Spain), was used as a BCA. The isolate was prepared from a single-spore stock culture maintained on potato dextrose agar (PDA; Difco® Laboratories) slants at 4°C. Seven-d-old cultures of *F. oxysporum* incubated on PDA at 25°C under a 12-h photoperiod of fluorescent light were used as the inoculum source. To prepare the inoculum suspension of FO12 needed for the treatment, a 2 L Erlenmeyer flask containing 1 L of potato dextrose broth (PDB; Difco® Laboratories) was inoculated with a conidium suspension from a 7 d PDA-mycelium culture of the BCA. The conidium concentration of the inoculated PDB flask was adjusted to 2×10^5 conidia mL^{-1} (assessed by haemocytometer) and incubated at 25°C on an orbital shaker (Grant bio PSU-20i, Grant Instruments) at 90 rpm for 7 d. After incubation, the FO12 inoculum was adjusted to 10^6 conidia mL^{-1} prior to application.

Semi-controlled conditions and experimental design

The experiment was conducted under semi-controlled conditions in a set of microplots located at the 'Campus de Rabanales' at the University of Córdoba (UCO, Córdoba Province, Andalusia region, southern Spain. UTM coordinates X: 37.919056; Y: -4.724306) from March 2015 to January 2017. The setup consisted of a line of 18 cement and brick containers (microplots) built on the ground. Each container was of 1 m^3 capacity and was open at the bottom, and the line of containers was oriented north to south and protected from rain and excessive sun by a metal rooftop structure. This microplot system has been used previously in epidemiological studies of VWO (López-Escudero and Blanco-López, 2007; Pérez-Rodriguez *et al*., 2015).

The ID of *V. dahliae* in the original naturally infested soil (above) and used in this experiment was estimated at 168 MS g^{-1}. To study the influence of the initial ID of *V. dahliae* on the effectiveness of the two biocontrol treatments, the soil was diluted to obtain two different IDs. The original naturally infested soil was mixed with *V. dahliae*-free sand at two rates: 1:2 and 1:10 (v:v; infested soil:sand). Both mixtures were separately homogenized by mixing using a motor hoe (Zeppelin® 111 7HP) at the experimental site. The initial ID of the two mixtures was determined (as above) to be 83.6 MS g^{-1} for the high inoculum density (HID) mixture and 23.6 MS g^{-1} for low inoculum density (LID) mixture.

Each microplot was filled with 800 kg of the required soil mixture (bulk density = 1,300 kg m^3), and eight olive plants were planted in each microplot, four of each of the two cultivars. A total of 18 microplots were used in this experiment, which was carried out in a split-split-

plot design with three blocks, each block composed of six microplots, two levels of ID (HID and LID) as the main plot, three treatments (FO12, CGR03 or water treated control) as the subplot and two olive cvs. ('Arbequina' or 'Picual') as the sub-subplot, with a total of 144 olive plants (72 of each cultivar).

Plant establishment and treatment application

Olive plants were transplanted in March 2015, and microplots were treated with the grape marc compost (CGR03) or with the non-pathogenic strain of *F. oxysporum* (FO12). To prevent damage to the roots of the olive plants, CGR03 was incorporated into the soil just before planting as an organic amendment, by tillage (to 30 cm depth) with a manual hoe at a rate of 20% (v:v) (i.e., 60 L/microplot). Subsequently, microplots treated with CGR03 were planted and watered with 30 L of tap water. The treatment with FO12 was applied just after planting by watering each microplot with 30 L of the inoculum suspension (10^6 conidia mL^{-1}). Microplots treated with 30 L of tap water were used as the experimental controls. Treatments were applied twice a year at the beginning of each spring and autumn season, until the end of the experiment in January 2017. The subsequent treatments with CGR03 were applied at less than the initial dose (i.e., 30 L/microplot) by superficial tillage to prevent damage to the plants. The microplots were irrigated each week during spring, summer and autumn and biweekly during the winter season, according to Pérez-Rodríguez *et al*. (2015).

Assessment of inoculum density progress

The ID of *V. dahliae* in the soil of each microplot was periodically quantified to evaluate the ID progression over time in each treatment. A total of nine soil samples were collected during the experiment. Initially, soil samples were collected at 15, 30 and 60 d after planting (DAP). From 60 d until the end of the experiment, samples were collected at the beginning of each season, which corresponded to 100, 180, 250, 390, 470 or 570 DAP. At each sampling time, four soil sub-samples (100 g) per microplot were collected, using a cylindrical (3.5 cm × 22 cm) auger at a soil depth of 20 to 30 cm. The sub-samples from each microplot were mixed to obtain a homogenous sample, which was then processed to estimate the ID using the wet sieving method described above. The randomized area under the inoculum progress curve (RAUIPC) for each treatment was calculated by the trapezoidal integration method (Campbell and Madden, 1990) from all ID values obtained from the nine soil sampling times.

Disease assessments

The first symptoms of VWO were observed 4 months after planting, in July 2015. The plants in the experiment were surveyed each week for wilt symptoms, from disease onset until the end of the experiment in January 2017. Disease severity was estimated using a 0 to 16 rating scale, according to the proportions of plant tissue affected by chlorosis, necrosis or defoliation. The scale estimated the percentage of affected tissue using four main categories (≤ 25, 26–50, 51–75, or 76–100%), with four ratings per category. Each scale value represented the number of sixteenths of affected plant area. The scale values (X) were linearly related to the percentage of affected tissue (Y) using the equation $Y = 6.25X - 3.125$ (Varo-Suárez *et al*., 2018). At the end of the disease assessments, the relative area under the disease progress curves (RAUDPC) were calculated from the disease severity values, using the trapezoidal integration method (Campbell and Madden, 1990). In addition, plant infection by *V. dahliae* was confirmed by isolating the fungus from the affected shoots of diseased plants, as described by Varo-Suárez *et al*. (2018).

Data analyses

Analyses of variance (ANOVA) were carried out for the disease parameters (final disease severity and RAUDPC) and of the inoculum progress data (final ID and RAUIPC). Values of these parameters met the assumptions of normality and homogeneity of variances for these analyses. Final disease severity and RAUDPC data were analysed according to a split-split-plot experimental design, with the initial IDs as the main plots, treatments as the subplots and the cultivars as the sub-subplot factors. The data of final ID and RAUIPC were arranged in a split-plot design with the initial ID levels as the main plots and treatments as the subplots. When the ANOVA showed statistically significant differences among treatments, values were compared using Fisher's protected least significant difference (LSD) at $P = 0.05$. All statistical analyses were carried out using Statistix 10 (Analytical Software, 2013).

RESULTS

The ID progress of *V. dahliae* in naturally infested soils after the application of CGR03 and FO12 is repre-

sented in Figure 1. The effectiveness of CGR03 in reducing the ID of *V. dahliae* was observed at the first sampling time (15 DAP) for both initial inoculum densities (HID and LID). At this time, in the case of a high initial pathogen ID (HID), CGR03 reduced (P = 0.0006) the ID in comparison with that in the control and from the FO12 treatment, with an ID of 2.3 MS g^{-1}. A more pronounced effect from CGR03 compared with that from FO12 was observed at the first stages of the experiment (Figure 1a). On the other hand, FO12 gave a significant reduction in ID at 100 DAP (P = 0.0059) compared with the control, with a concentration of 23.9 MS g^{-1} (Figure 1a). Fluctuations in the ID of *V. dahliae* during the sampling period were observed for all treatments tested, reaching minimum values of 39.2 for the control (15 DAP), 0.8 from CGR03 (470 DAP), and 0.53 MS g^{-1} (470 DAP) from FO12. At the last sampling (570 DAP), differences (P < 0.0001) were found among treatments, with CGR03 resulting in the greatest reduction in ID, with a final ID of 1.9 MS g^{-1}.

When the initial ID was low (LID), both biocontrol treatments reduced (P = 0.0005) the ID of *V. dahliae* in comparison with that in the control at 15 DAP, with IDs of 13.6 MS g^{-1} for the control, 3.4 MS g^{-1} from the FO12 treatment, and 0.5 MS g^{-1} from CGR03 (Figure 1b). The ID of *V. dahliae* in soil treated with each of the biocontrol products remained less (P = 0.0003) than that in the control until the end of the experiment, reaching final IDs of 17.2 MS g^{-1} for the control, 3.6 MS g^{-1} from FO12, and 1.2 MS g^{-1} from CGR03. The CGR03 treatment also gave a greater reduction in the viable inocula of *V. dahliae* in this case (Figure 1b). In LID soil, fluctuations in ID during the experiment occurred from all the treatments, with minimum ID values of 13.6 MS g^{-1} for the control (15 DAP), 0.53 MS g^{-1} from CGR03 (15 DAP) and 0.13 MS g^{-1} from FO12 (470 DAP) (Figure 1b).

The initial ID had no effect on RAUIPC (P = 0.9224), but statistically significant differences were found between treatments (HID, P = 0.0004 and LID, P < 0.0001). In both cases (HID and LID), CGR03 was the most effective treatment for reducing the ID, giving significantly lower values of RAUIPC than those from FO12 and the control (respectively, 7.6 and 7.4% for HID and LID; Figure 2a and b).

VWO symptoms were first observed in control plants 17 weeks after planting (Figure 3a). A delay of symptom onset was observed in plants treated with CGR03. For microplots containing LID of *V. dahliae*, VWO were first observed in the control and CGR03-treated plants 7 months after planting (Figure 3b). Reisolations from shoots of diseased plants confirmed their infection by *V. dahliae*. There were no significant differences between the two initial pathogen ID levels and final disease severity (P = 0.4557) or RAUDPC (P = 0.1993). Similarly, no significant differences in final disease severity were

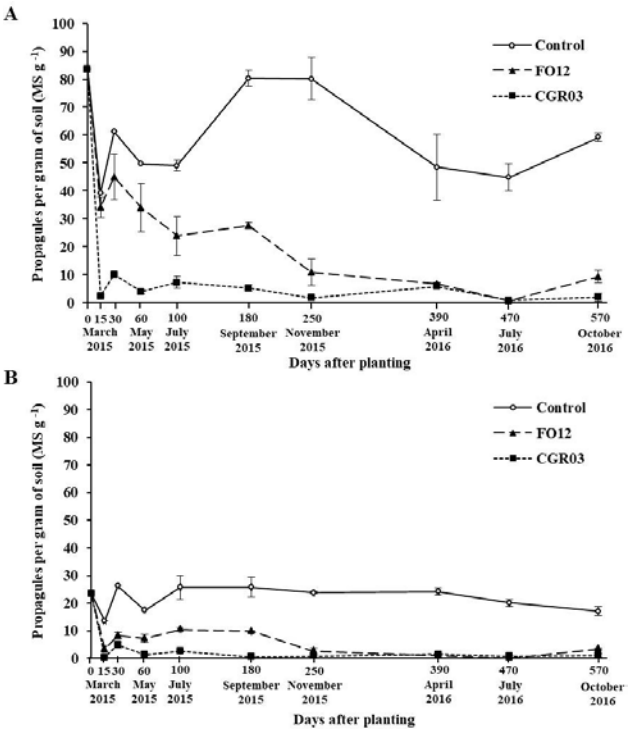

Figure 1. Progress of inoculum density (ID) of *Verticillium dahliae* at nine sampling times (from 15 to 570 days after planting), in naturally infested soils with a) high inoculum density (HID) or b) low inoculum density (LID), after treatment with FO12 or CGR03. For each sampling time, the means of three soil samples per treatment are shown, and the bars are standard errors of the means.

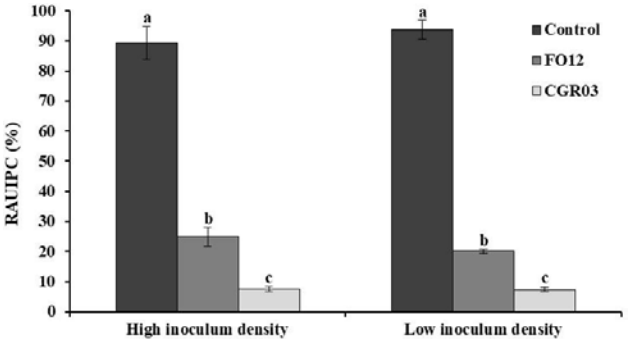

Figure 2. Mean relative area under inoculum progress curves (RAUIPC, %) in naturally infested soils with high *Verticillium dahliae* inoculum density (HID) or inoculum density (LID) for each treatment at 570 d after planting. For each treatment, the histogram is the mean of three replications. Different letters indicate statistically significant differences (P = 0.050, according to Fisher´s protected LSD test, and the bars are the standard errors of the means.

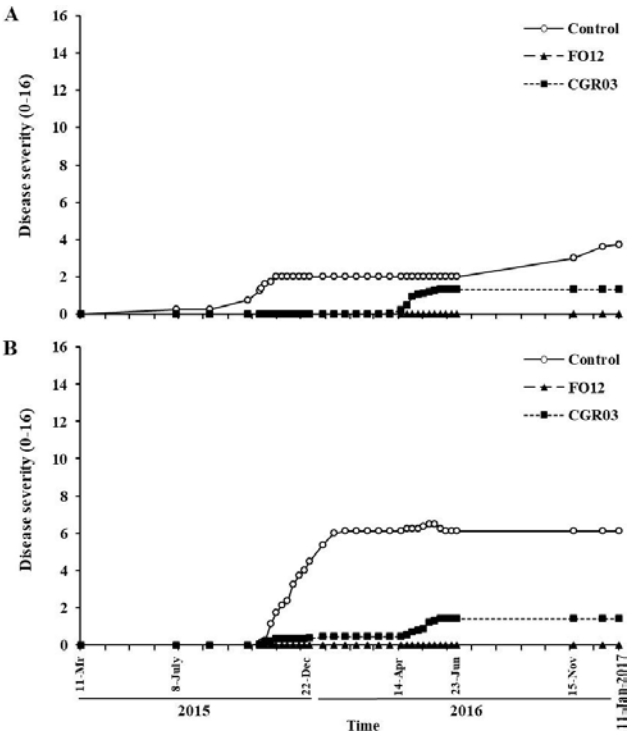

Figure 3. Progress of Verticillium wilt severity of in olive plants grown in naturally infested soils with a) high inoculum density (HID) and b) low inoculum density (LID) of *Verticillium dahliae* during 2 years under semi-controlled conditions, after treatment with the non-pathogenic *Fusarium oxysporum* strain FO12 and the grape marc compost CGR03. Disease severity was estimated based on a 0-16 scale (0 = no lesions, 16 = 94-100% of canopy with symptoms). Each datum is the mean of 24 plants.

found between cv. 'Picual' and 'Arbequina' (P = 0.2632), or RAUDPC (P = 0.5483). Data for each disease parameter (severity and RAUDPC) for both cultivars were grouped for statistical analyses (Table 1) and significant differences between treatments were observed. The plants grown in the microplots treated with CGR03 or FO12 showed had reduced disease severity (P = 0.0190) and RAUDPC (P = 0.0054) compared with those of the control (Table 1). The treatment with FO12 achieved complete control of the disease, as no plants treated with this BCA showed VWO symptoms (Table 1).

DISCUSSION

Verticillium wilt of olive is the most important disease affecting olive groves in Mediterranean countries. To date, there is no effective control measure for this disease when the control is individually applied. In this study, the non-pathogenic *F. oxysporum* strain FO12 and the suppressive grape marc compost CGR03 have been evaluated for effectiveness in controlling this major disease, under semi-controlled conditions. These biocontrol products were selected from previous studies since they were the most effective at suppressing *V. dahliae* growth *in vitro* and *in planta* (Varo *et al*., 2016; Varo-Suárez *et al*., 2018; Mulero-Aparicio *et al*., 2019a), and under field conditions (Mulero-Aparicio *et al*., 2020). This study is a final step indicating the potential of these treatments before their evaluation under different scenarios of natural infections or field conditions, where olive producers urgently require feasible control strategies.

Results obtained in this study agree with those reported by Varo-Suárez *et al*. (2018) and Mulero-Aparicio *et al*. (2019a), where CGR03 and FO12 were two of the most effective products for reducing the ID of *V. dahliae* in two different soils in an *in vitro* experiment. Similarly, these results agree with those of Mulero-Aparicio *et al*. (2020), where these treatments reduced ID of the pathogen in natural conditions. Results from the present study also indicate that the CGR03 treatment, compared with FO12, achieved a greater reduction in ID during the first stages of the experiment when the initial ID was greatest (HID). However, when the initial ID was least, both biocontrol treatments showed similar efficacy for ID reduction at the first sampling (15 DAP). In addition, the

Table 1. Disease-related parameters for olive plants grown in naturally infested soil by *Verticillium dahliae* during along 2 years under semi-controlled conditions, after treatments with the non-pathogenic strain of *Fusarium oxysporum* FO12 and the grape marc compost CGR03.

Initial ID[a]	Treatment	Disease severity (%)[b]	RAUDPC (%)[c]
High inoculum density (HID, 83.6 MS g^{-1})	Control	23.0 ± 15.2 a	16.1 ± 11.8 a
	CGR03	8.1 ± 5.2 b	5.0 ± 3.2 b
	FO12	0.0 ± 0.0 c	0.0 ± 0.0 c
Low inoculum density (LID, 23.6 MS g^{-1})	Control	37.1 ± 15.1 a	36.2 ± 14.5 a
	CGR03	8.6 ± 5.2 b	6.2 ± 3.9 b
	FO12	0.0 ± 0.0 c	0.0 ± 0.0 c

[a] Initial inoculum density (ID) of *V. dahliae* in the two soil mixtures used in the experiment.
[b] Final disease severity (%) ± standard error 22 months after planting based on a 0-16 rating scale (0 = no lesions, 16 = 94-100% of canopy with symptoms).
[c] Relative area under the disease progression curve (RAUDPC, %) ± standard error developed over the assessment period (22 months).
[b, c] In each column, data represent the mean of 24 replicated plants per treatment. Mean values followed by the same letter do not differ significantly according to Fisher's protected LSD test at P = 0.05.

minimum values of ID reached during the assessment period were similar for the CGR03 and FO12 treatments. This confirms the effectiveness of these two biological treatments for reducing viable inoculum of *V. dahliae* in naturally infested soils, regardless of the initial ID of the pathogen. Nevertheless, neither of the two biocontrol treatments tested in this study eliminated inoculum of *V. dahliae* from the soil, and the pathogen was detected at all sampling times. Fluctuations in *V. dahliae* ID during the sampling period were observed. The increases in ID observed in July 2015 and July 2016 in the HID control microplots were similar to the results of López-Escudero and Blanco-López (2001). They attributed these ID changes in July probable decreases in the superficial soil microbiota due to the high temperatures at that time of year. This change in microbiota could lead to high *V. dahliae* ID due probably to low microbial competition against the pathogen in the natural soil, or in the MSPA Petri dishes used for the assessment.

The similar levels of resistance to VWO of 'Picual' and 'Arbequina' observed in this study have been reported. Previous studies under controlled conditions reported that resistance of 'Arbequina' was overcome when plants were artificially inoculated by root dipping with a large amount of the pathogen (10^6 conidia mL^{-1}), with this cultivar showing the same level of resistance to VWO as the susceptible 'Picual' (López-Escudero *et al*., 2004). Nevertheless, in field studies carried out in naturally infested soils with ID levels ranging from 5 to 21 MS g^{-1}, 'Arbequina' had greater resistance to VWO than 'Picual' (Trapero *et al*., 2013b). Similarly, an earlier study conducted under semi-controlled conditions in brick containers and using a naturally infested soil with moderate *V. dahliae* inoculum densities (9.8 MS g^{-1}) confirmed the greater VMO resistance of 'Arbequina' compared to 'Picual' (Pérez-Rodríguez *et al*., 2015). In the present study, with much greater initial pathogen IDs (83.6 and 23.6 MS g^{-1})), both cultivars may have shown similar resistance to VWO due to the high inoculum pressure of the pathogen at the beginning of the experiment which overcame the genetic resistance of 'Arbequina'.

Plants treated with FO12 did not develop VWO symptoms over the experimental period. These results agree with those of Varo *et al*. (2016) and Mulero-Aparicio *et al*. (2019a), in which this *F. oxysporum* strain gave complete control of VWO in experiments conducted under controlled conditions. In studies evaluating the suppressive effects of organic amendments against *V. dahliae,* reductions of inoculum were correlated with reductions of disease progress in 80% of the cases (Bonanomi *et al*., 2007). In the present study, treatment with CGR03 reduced disease development in comparison with that in the control, but complete prevention of VWO was not achieved This was probably due to the presence of remaining inoculum to infect the plants. This is similar to the results of López-Escudero *et al*. (2007), who reported an initial ID of 0.04 MS g^{-1} was enough to infect olive plants of the susceptible 'Picual' and cause development of the disease.

Understanding the mechanisms of action of organic amendments in their effects against fungal diseases has become a challenge for researchers, due to their biological and chemical complexity. Organic compost based on cotton wastes reduced severity of Verticillium wilt of eggplant by triggering induced systemic resistance in host plants (Markakis *et al*., 2016). Furthermore, studies evaluating different olive mill waste composts showed that their suppressive effects against *V. dahliae* were due to biotic and abiotic factors (Papasotiriou *et al*., 2013). The mechanisms involved in the suppressive effects of the grape marc compost CGR03 remain unknown. Further research evaluating the roles of biotic and abiotic factors is required to improve the efficacy of this organic amendment against VWO.

Although FO12 was not the most efficient treatment for reducing ID of *V. dahliae*, its capability to (i) colonize the host plant rhizosphere, (ii) produce volatile organic compounds with antifungal effects, and (iii) produce chlamydospores that remain attached within host root systems (Mulero-Aparicio *et al*., 2019b). These could all play roles in its antagonistic effects against the pathogen by competing in the rhizosphere for infection sites, thus preventing the infection of olive roots by *V. dahliae* and achieving effective control of VWO. These traits have been previously observed in other non-pathogenic strains of *F. oxysporum* used as BCAs in herbaceous crops (Fravel *et al.,* 2003). For instance, the non-pathogenic *F. oxysporum* strain F2 competed with *V. dahliae* for nutrients and space on root surfaces of eggplants (Pantelides *et al*., 2009). Similarly, the widely studied non-pathogenic *F. oxysporum* strain Fo47 has been reported to use different modes of action against *V. dahliae*, including induction of systemic resistance and competition for infection points on pepper plants (Veloso *et al.,* 2016).

The effectiveness of these biocontrol products against *V. dahliae* shown in the present study confirms the consistency of these treatments, since they were previously reported as effective biocontrol treatments against *V. dahliae* in studies conducted under controlled conditions (Varo *et al*., 2016; Varo-Suárez *et al*., 2018; Mulero-Aparicio *et al*., 2019a) and in the field (Mulero-Aparicio *et al*., 2020). Consistency is considered one of the most important traits for biocontrol products (Deketelaere *et*

al., 2017), since they are often known for their contradictory results between laboratory, semi-controlled and field conditions. Further research on the modes and times of application is needed to evaluate the efficacy and consistency of these products under different commercial field conditions, as well as further research on the mechanisms responsible for the suppressive effects of these agents and products.

In conclusion, the present study highlights the effectiveness of these two biocontrol treatments for reducing *V. dahliae* ID and progress of VWO under semi-controlled conditions. This study also expands knowledge about the use of biocontrol strategies as an eco-friendly approach to effective control of VWO, within an integrated disease management strategy. Although further research is needed to study the interactions of different biocontrol treatments with olive cultivars with different resistance levels in field conditions, the results obtained here indicate potential for applying these biocontrol treatments in *V. dahliae*-infested commercial fields, both before planting and during cultivation, to reduce initial pathogen inoculum pressure. This strategy, in combination with the use of moderately resistant olive cultivars, could be effective for the control of VWO.

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