Crop Protection 106 (2018) 190–200

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Potential efficacy of soil-applied disinfectant treatments against *Verticillium* wilt of olive

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ARTICLE INFO

Keywords:
Control
Verticillium dahliae
Defoliating pathotype
Inoculum density
Integrated management

ABSTRACT

Disinfestation of irrigation water has re-emerged the interest in including disinfectant treatments within the integrated management of Verticillium wilt of olive to reduce the pathogen spread and introduction through irrigation facilities. OX-VIRIN® (OV) and OX-AGUA AL25® (OA) are two disinfectants based on oxidizing and non-oxidizing agents, respectively, that have shown a potential efficacy reducing water infestations by *V. dahliae* under certain guidelines. This investigation was designed to evaluate now their effects on *V. dahliae* in the soil, the olive plant and the plant-pathogen interaction under growth chamber conditions. Seven disinfectant treatments were applied through watering to *V. dahliae*-infested soils sustaining 'Picual' or 'Arbequina' olives. The OV-w (weekly), OV-m (monthly) or OA-b (biweekly) treatments reported a significant deleterious effect in the total inoculum density in soil and reduced or tended to decrease the sclerotia survival of all *V. dahliae* isolates (three isolates used) in presence of both cultivars in all the experiments (two experiments per cultivar were carried out). 'Picual' olives exhibited a greater disease incidence than 'Arbequina' ones. The incidence of diseased plants was lower in olives subjected to disinfectant treatments in comparison with those under untreated control, with a maximum reduction of 21.1% and 43.4% in 'Picual' and 'Arbequina', respectively. OV-w or OA-b treatments applied to 'Picual' and OV-w treatment to 'Arbequina' decreased partially but solidly (all experiments per cultivar) the disease intensity index. Furthermore, values from the area under the disease progress curve were significantly reduced by OV-m and OA-b treatment in both 'Picual' experiments, depending on the isolate. The olive growth parameters were not significantly affected by the disinfectants and an absence of phytotoxicity was reported. Results from this work demonstrate that disinfection treatments reducing the fungus in water can also potentially reduce the fungus in soil and, partially, the Verticillium wilt in olive.

1. Introduction

Verticillium wilt of olive (VWO) is responsible for incalculable direct and indirect costs in olive and olive-oil production, becoming the main phytopathological issue in the major olive-growing regions worldwide (Jiménez-Díaz et al., 2012; Mercado-Blanco and López-Escudero, 2011). The main factors making this tracheomycosis hard to control are: i) the virulence variability among populations of the causal agent, *Verticillium dahliae* Kleb., ii) the broad range of herbaceous and woody hosts of the fungus (over 400; Pegg and Brady, 2002), iii) its capacity to produce survival structures (sclerotia) able to persist in soil for up to 14 years (Schnathorst, 1981), and iv) the difficulty of targeting the fungus within the xylem vessels (López-Escudero and Mercado-Blanco, 2011).

Soil fumigation, and its combination with soil solarization, has been used as an useful pre-planting tool for reducing *V. dahliae* inoculum within herbaceous agrosystems (Pegg and Brady, 2002). During the phasing out of methyl bromide in developed nations signatories to Montreal Protocol, existing fumigants have emerged as effective alternatives for controlling phytopathogens in the soil (Ajwa et al., 2002; Duniway, 2002; Gullino et al., 2005). Moreover, the improvement of application technologies has led to an increase in the efficacy of fumigants treatments, reduction in the application rates and an increase in the area that can be treated. Thus, the application of chemical

Abbreviations: OV, OX-VIRIN®; OA, OX-AGUA AL25®; VWO, Verticillium wilt of olive; D, defoliating; ND, non-defoliating; CWA, chlortetracycline water agar; PDA, potato dextrose agar; CSW, cornmeal; sifted sand, and distilled water; w, once a week (weekly); b, once every two weeks (biweekly); m, monthly in two consecutive irrigation events (monthly); total ID, total inoculum density; dpt, days post-transplant; MSPA, modified sodium polypectate agar; CFU, colony-forming units; SAUIPC, standardized area under the inoculum progress curve; SS, sclerotia survival; DII, disease intensity index; SAUDPC, standardized area under the disease progress curve; DFP, disease-free period; FDI, final disease incidence; FDII, final disease intensity index; II, infection incidence; CI, colonization index; ANOVA, analysis of variance; LSD, least significant difference

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https://doi.org/10.1016/j.cropro.2018.01.002
Received 26 September 2017; Received in revised form 12 December 2017; Accepted 3 January 2018

compounds such as chloropicrin, 1,3-dichloropropene, and the methyl isothiocyanate generators, metam-sodium and dazomet, via irrigation (chemigation) has reported some effectiveness against *V. dahliae* in some pre-planting studies (Ben-Yephet et al., 1983; Moens and Ben-Aicha, 1986; Ślusarski and Spotti, 2016; Taylor et al., 2005; Woodward et al., 2011). The efficacy of chemigation as a post-planting measure to deal with the pathogen in soil of olive orchards has been scarcely studied. Sánchez-Alcalá (2005) evaluated the effect of several chemicals on the inoculum density of *V. dahliae* in soil. Although carbendazim, fosetyl-aluminium and kresoxim-methyl showed some efficacy reducing the inoculum density in laboratory assays, none of the tested products provided a decline in the *V. dahliae* populations respecting the control treatment after 8 biweekly chemigations in field experiments.

Aside from chemical control directed against inoculum levels in the soil, other methods focused on the containment or elimination of the pathogen *in planta* (chemotherapy) have been studied. Since the late 70's, the benzimidazoles systemic fungicides have been available for plant chemotherapy against *Verticillium* infection. While their application has reported favorable results in studies with herbaceous plants such as strawberry, tomato, cotton, cacao or tomato, benzimidazoles have been found to be of little use in controlling Verticillium wilt in trees (Pegg and Brady, 2002, and references therein). Control of VWO by systemic fungicides has been addressed in several studies including soil drenching, foliar spraying and trunk injection (Jiménez-Díaz et al., 2012). López-Escudero and Blanco-López (1996) reported no success when evaluated sprayed applications of carbendazim, prochloraz, quinosol and the mixtures carbendazim + quinosol and carbendazim + prochloraz upon 1-year-old inoculated 'Picual' trees. In contrast, Abu-Qamar and Al-Raddad (2001) observed a decrease on the development of Verticillium wilt on 'Nabali' olive trees when applied a soil drench treatment with cryptonol (8-hydroxyquinoline) in comparison with untreated control.

The variability of results in the field, the limited number of authorized chemicals, their environmental impact, as well as the development of breeding programs aimed to get resistant cultivars, have made that efforts aimed to find effective chemical control measures against VWO take second place. However, the interest in chemical control has emerged again as a result of the occurrence of *V. dahliae* in water sources for irrigation of olive orchards in the last years. In Andalusia, southern region of Spain that hosts above 1.5 million ha dedicated to olive cultivation (CAP-JA, 2015), it has been demonstrated that irrigation facilities provide a dispersal means for propagules of *V. dahliae* from wells, ponds and natural water streams, enabling a long-distance dissemination and an introduction into free-pathogen soils (García-Cabello et al., 2012; Rodríguez-Jurado and Bejarano-Alcázar, 2007). In this context, the treatment of irrigation water with disinfectants is a measure that is currently under consideration, since the affordable cost and user-friendliness could facilitate its implementation. Some products based on oxidizing and non-oxidizing substances, namely OX-VIRIN® and OX-AGUA AL25®, showed promising results in previous studies. In laboratory experiments, water infestations by conidia or sclerotia of various *V. dahliae* isolates differing in virulence were effectively reduced when OX-VIRIN® and OX-AGUA AL25® were applied at some concentrations (Santos-Rufo and Rodríguez-Jurado, 2016; Gómez-Gálvez et al., submitted for publication). Additionally, a significant reduction of conidia in ponded infested water was reported when the disinfectants were applied under certain guidelines during spring natural conditions (Santos-Rufo and Rodríguez-Jurado, 2016). Accordingly, these authors proposed treating irrigation water with disinfectants as a potential measure to be integrated in the management of the disease.

Both OX-VIRIN® and OX-AGUA AL25® consist of hydrogen peroxide (H_2O_2), either as primary substance or mixed with other main non-oxidizing substances, respectively (see below). Peroxygen-based products are currently attracting particular interest for its biocidal properties together with the fact their by-products are eco-friendly (Carrasco and Urrestarazu, 2010; Kitis, 2004; Stampi et al., 2002). This oxidative compound presents also an inhibitory effect against *V. dahliae* sclerotia in soil as demonstrated by Kim et al. (1987) when they studied the role of a metabolite produced by the antagonist agent *Talaromyces flavus*. Thus, OX-VIRIN® and OX-AGUA AL25® could affect the inoculum density of *V. dahliae* in soil. Once it is known the high efficacy of OX-VIRIN® and OX-AGUA AL25® in reducing the fungus in water, additional effects once the treated water gets the soil and the olive plant should be evaluated.

OX-VIRIN® at 3.2 ml L^{-1} and OX-AGUA AL25® at 0.4175 ml L^{-1} are concentrations that reduced above 90% of *V. dahliae* inoculum in *in vitro* assessments, and showed a high or moderated efficacy, respectively, against conidia in infested ponded water in outdoor experiments during spring (Santos-Rufo and Rodríguez-Jurado, 2016; Gómez-Gálvez et al., submitted for publication). The aim of this study was to assess the potential efficacy of treatments with OX-VIRIN® and OX-AGUA AL25® at these concentrations in reducing the inoculum density of different isolates of *V. dahliae* in soil and the VWO development on olive cultivars differing in resistance. Concurrently, disinfectant treatments were evaluated for their phytotoxicity and effect on the plant growth.

2. Materials and methods

2.1. Plant material

Four-month-old nursery-propagated rooted cuttings of 'Picual' or 'Arbequina', the most widely grown olive cultivars in Spain and worldwide, respectively (Barranco, 2010), were used. Both cultivars are extremely susceptible to the defoliating (D) pathotype of *V. dahliae* when inoculated by dipping their bare root system in a conidial suspension (López-Escudero et al., 2004). Furthermore, it has been reported that 'Picual' and 'Arbequina' have high and moderate susceptibility, respectively, when grown in heavily D-infested soil (Trapero et al., 2013).

2.2. Fungal inoculum and plant inoculation

Three D isolates of *V. dahliae* were used: VO153-D, VO145-D and VO161-D (from less to more virulence). Isolates were obtained from the culture collection of the Area of Sustainable Crop Protection, IFAPA "Alameda del Obispo" Centre (Cordoba, Spain). They were selected for having been collected from irrigation water of olive orchards in southern Spain and having shown the D phenotype and the three highest virulence levels when characterized on olive plants in previous works.

Inoculum was prepared from stored cultures that were refreshed on chlortetracycline water agar (CWA) and subcultured on potato dextrose agar (PDA). *V. dahliae*-colonized PDA plugs (0.5 mm in diameter) were transferred to 1L-flasks containing 450 g of sterilized substrate composed of cornmeal, sifted sand and distilled water (CSW) in a ratio of 1:9:2, respectively, by weight. The infested CSW substrate was incubated at 24 ± 1 °C in darkness for 40 days, shaking the flask every 4–5 days to get a redistributed growth. Non-infested CSW substrate was similarly incubated prior to be included as controls.

For inoculation, plants were transplanted into 0.5 L pots filled with a mixture of autoclaved soil (lime: peat, 2:1, v/v) and infested CSW at a rate of 20% (w/w) (Santos-Rufo et al., 2017; Varo et al., 2016a). As negative controls, plants in pots with the mixture of soil and non-infested CSW were included. Plants were incubated in a controlled growth chamber at 22 ± 2 °C, 45–85% relative humidity, and 14-h photoperiod of fluorescent light (360 μmol m^{-2} s^{-1}) for 16 weeks (Rodríguez-Jurado, 1993). They were watered (70 ml) three times a week (see below) and neither herbicides nor pesticides were applied.