Phenolic Responses of Resistant and Susceptible Olive Cultivars Induced by Defoliating and Nondefoliating *Verticillium dahliae* Pathotypes

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ABSTRACT

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Verticillium wilt is the most serious olive disease worldwide. The olive-infecting *Verticillium dahliae* pathotypes have been classified as defoliating (D) and nondefoliating (ND), and the disease is mainly controlled in olive orchards by using resistant or tolerant cultivars. Limited information is available about the nature of resistance in most of the olive cultivars. In the present study, the phenolic responses of the susceptible to *V. dahliae* olive cv. Amfissis and the resistant cv. Koroneiki upon D and ND *V. dahliae* infection were monitored in relation to the fungal DNA levels in the vascular tissues with the purpose to explore the defense mechanisms of olive trees against *V. dahliae*. Quantitative polymerase chain reaction revealed that the decrease in symptom severity shown in Koroneiki trees was associated with significant reduction in the growth of both *V. dahliae* pathotypes in the vascular tissues compared with Amfissis. In Koroneiki trees, the levels of *o*-diphenols and verbascoside were positively associated with the DNA levels of the D and ND pathotypes. In addition, a positive association was observed between the levels of verbascoside and the fungal DNA level in Amfissis trees, whereas a negative association was revealed between the fungal DNA level and the total phenols and oleuropein content in both cultivar. The levels of verbascoside were clearly higher in Koroneiki trees compared with Amfissis trees, indicating for the first time in the literature the involvement of verbascoside in the defense mechanism of olive trees against *V. dahliae*.

Verticillium wilt, caused by the soilborne fungus *Verticillium dahliae* Kleb., is one of the most serious diseases affecting olive (*Olea europea* L.) worldwide (20) and may cause severe losses and plant death (1,39). Microsclerotia, the resting structure of *V. dahliae*, constitute the main potential infective inoculum of the pathogen in the field and persist in the soil for more than 20 years. The use of fungicides, in addition to having potentially toxic consequences and representing an environmental threat, has little effect on them (14,45).

The olive-infecting *V. dahliae* pathotypes have been classified as defoliating (D) and nondefoliating (ND) according to their ability to defoliate the tree (38). The D pathotype is considered to be highly virulent and the ND one to be moderately severe, as has been documented in artificial inoculation studies (26,35,38). Although infections by the D pathotype can be lethal to the plant, olive plants infected by the ND pathotype can show complete remission from symptoms (20,35).

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V. dahliae is generally controlled by a combination of preventive measures; among these is the use of resistant cultivars or root stocks (40,41). The use of host resistance is considered to be the most effective and ecologically sound method for managing *V. dahliae* in olive orchards (23). For this purpose, a large number of olive cultivars has been evaluated for resistance to Verticillium wilt (3,9,22,28). In these studies, 'Koroneiki' exhibited a high level of resistance against the D and ND *V. dahliae* pathotypes, showing less disease symptoms and vascular colonization by the pathogen compared with other olive cultivars; implying, in this way, the trigger of defense mechanisms against *V. dahliae* (10,25,27,33).

It is known that phenolic compounds accumulate in plant tissues and are challenged by fungal pathogens (14,35,43). Phenolics play an important role in the resistance of plants to pathogen attack because they belong to the antimicrobial defense arsenal (37,42,44). It has been shown that some phenolic substances of olive trees may inhibit the growth of fungi belonging to the genus *Phytophthora* (11,12). Furthermore, Cayuela et al. (7) reported enhanced levels of verbascoside in olive shoots infected by *Pseudomonas savastanoi* pv. *savastanoi* and Baidez et al. (4) reported the antifungal activity of oleuropein (an *o*-diphenol present in olive trees) against *V. dahliae* in vitro. In the same study, a higher accumulation of total phenols was observed in the stems of *V. dahliae*-infected olive cv. Picual trees compared with the noninfected; however, these data were not correlated with symptom development and the actual time of pathogen invasion in the olive trees.

On the other hand, in the few published studies (3,22,24,26,28) about the colonization pattern of the D and ND pathotypes in resistant and susceptible olive cultivars, the biochemical mechanisms involved in these interactions were not investigated. Nicholson and Hammerschmidt (29) have remarked that future research on defense responses should address the time, location, and concentration of phenols relative to the sequential development of stages of disease that lead ultimately to the resistance expression. Taken together, the aforementioned studies (3,4,7,22–24,26–28) conclude that, even if *V. dahliae* is a devastating pathogen for olive trees, limited information is available in the literature correlating the biochemical aspects of this plant–pathogen interaction with the symptom development and rate of colonization of the D and ND pathotypes in the susceptible and resistant olive cultivars.

In view of all the above, the main objective of this study was to investigate the involvement of phenolic compounds in the defense mechanisms underlying the observed resistance in Koroneiki compared with the susceptible 'Amfissis', in relation to the root colonization rate of the D and ND *V. dahliae* pathotypes and symptom development.

MATERIALS AND METHODS

Plant material. Plant material consisted of 3-month-old rooted cuttings of Amfissis and Koroneiki olive trees. Amfissis is susceptible to D and ND *V. dahliae* pathotypes while Koroneiki is resistant to both pathotypes (3,24,26,27,32).

***V. dahliae* isolates.** *V. dahliae* isolates PA and A6, representatives of the ND and D pathotypes, respectively, were used. These isolates have been characterized by biological and molecular pathotyping in a previous study (26).

Inoculum preparation. Microsclerotia of the PA (ND) *V. dahliae* strain were prepared in sucrose sodium nitrate (SSN) liquid culture according to Antoniou et al.