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Short Communication

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Colonization of Olive Inflorescences by *Verticillium dahliae* and its Significance for Pathogen Spread

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Abstract

Verticillium wilt of olive, caused by *Verticillium dahliae* Kleb., is the most severe disease affecting this crop in most olive growing countries. In this study, the presence of viable structures of *V. dahliae* in dried inflorescences from wilted olive shoots was investigated. The pathogen was found inside peduncles and flowers, by assessing the number of typical star-shaped microsclerotial colonies formed onto the modified sodium polypectate agar medium. Microsclerotia of *V. dahliae* were observed inside the peduncles under the stereoscopic microscope. The presence of microsclerotia in these easily decomposable olive tissues shows that infected inflorescences can act as a source of inoculum for Verticillium wilt epidemics.

Introduction

Verticillium wilt of olive, caused by *Verticillium dahliae* Kleb., is a devastating disease widespread in all olive growing countries, including the Guadalquivir Valley in Andalucía (southern Spain) (Hiemstra and Harris 1998; López-Escudero and Mercado-Blanco 2010). Recent disease surveys in affected orchards reveal a mean disease incidence of nearly 20% of trees (López-Escudero and Mercado-Blanco 2010). One of the main epidemic factors involved in the rapidity and extent of the disease is probably the effective spread of the pathogen, due to: (i) infected planting material; (ii) infested soil or plant debris; (iii) irrigation water; or (iv) cultural practices, such as the use of infested animal manure as amendment in olive plantations (López-Escudero and Mercado-Blanco 2010). Microsclerotia, the survival and infective vegetative structure of the pathogen, are produced in the senescent or died plant tissues, before being incorporated back into the soil (Hiemstra and Harris 1998).

Leaf wilting, defoliation and necrosis of immature inflorescences are characteristic Verticillium wilt of olive symptoms (Blanco-López et al. 1984). While infected leaves have been shown to be an important inoculum source of the pathogen (Tjamos and Tsougriani 1990; Navas-Cortés et al. 2008; López-Escudero and Mercado-Blanco 2010), the presence of *V. dahliae* in olive inflorescences has not been studied before.

The objective of this research was to determine the presence of *Verticillium dahliae* in dried inflorescences of wilted olive trees and its potential as an inoculum source for pathogen dispersal and infection.

Materials and Methods

Fourteen affected olive trees of the susceptible cultivar 'Picual' were selected in five olive orchards in the Guadalquivir Valley (Fig 1a, Table 1). Samples consisted of several symptomatic shoots bearing dried inflorescences, which were collected from early summer to fall 2010 (Fig 1b). Infection of trees was confirmed by isolation of *V. dahliae* from affected twigs.

From collected necrotic inflorescences, desiccated peduncles and flowers were separated, weighted and stored in 20 ml flask tubes. The amount of collected tissues per sample ranged from 0.77 to 3.09 g for peduncles and from 1.39 to 6.98 g for flowers (Table 1).

The potential of both tissues as inoculum source of the pathogen was assessed using the modified sodium polypectate agar (MSPA) medium (Butterfield and DeVay 1977). This medium is currently used for determining the inoculum density of *V. dahliae* in soil samples (López-Escudero and Mercado-Blanco 2010).

Water suspensions of flower and peduncle tissues were prepared in 100-ml Erlenmeyer flasks, by grinding tissues using a homogenizer (Polytron PT 10/35; Kinematica, Lucerne, Switzerland). The amount of 70 mg of peduncle or flower tissue per ml of water was previously estimated as the optimum density for grinding. One microliter aliquots of the suspensions were sown onto the MSPA medium plates, using 10

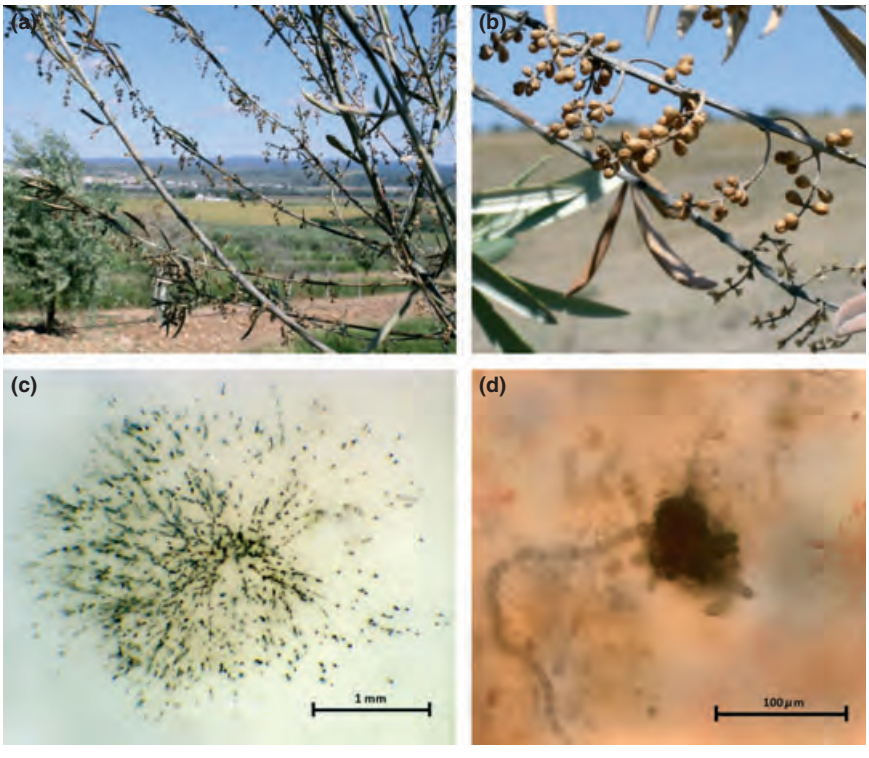

Fig. 1 (a) Desiccation and death of inflorescences in an olive tree affected by *Verticillium dahliae*. (b) Detail of necrotic inflorescences in a wilted tree. (c) Microsclerotial colony of *V. dahliae* formed onto modified sodium polypectate agar medium. (d) Microsclerotium of *V. dahliae* formed inside a peduncle and observed under the stereoscopic microscope

Table 1
Inoculum density of *Verticillium dahliae* in tissues of dried inflorescences of olive trees affected by Verticillium wilt[a]

Orchard location	Sample	Sampling time[b]	Sample weight (g)		*V. dahliae* colonies on MSPA (N°)[c]		Inoculum density (ppg of sample)	
			Peduncles	Flowers	Peduncles	Flowers	Peduncles	Flowers
Higuera de Arjona, Jaén	1	55	1.785	5.614	51	5	73.5	7.2
Arjona, Jaén	1	55	0.911	4.458	1	0	1.4	0.0
	2	55	2.168	3.400	12	3	17.3	4.4
Montilla, Córdoba	1	150	–	5.204	–	2	–	1.9
	2	150	3.087	6.987	7	1	6.7	0.9
Palenciana, Córdoba	1	170	2.421	5.913	1	0	1.4	0.0

[a]Only positive analyses are shown in the table; additional analyses from another eight affected trees did not yield the pathogen.
[b]Days after flowering date of olive trees in each location.
[c]Each modified sodium polypectate agar (MSPA) plate was sown with 1 ml aliquot of a suspension obtained by diluting plant tissue samples (peduncles or flowers) in sterilized water at a concentration of 70 mg of the tissue per ml. Ten replications (plates) of the MSPA media were used per sample.

replicates per sample. After 14 days of incubation at 23 ± 2°C in the dark, plates were washed to remove residues and the number of typical star-shaped microsclerotial colonies of *V. dahliae* formed were counted under a stereoscopic microscope (Nikon SMZ-2T, Tokyo, Japan) (Fig 1c). The inoculum density in each sample was estimated by the number of colonies of *V. dahliae* and expressed as propagules (microsclerotia) per gram (ppg) of peduncles or flowers.

Microscope slides were prepared from samples of ground tissues from the peduncle and flower suspensions, and from colonies produced onto MSPA medium. Images of microsclerotia were taken by the analytical program Analysis (Olympus Soft Imaging Solutions, Münster, Germany), connected to a microscope Nikon Optiphot-200 trough a video camera Kappa (CF 20/4 DX; K-Vision BV, Huizen, The Netherlands).

Results and Discussion

Verticillium dahliae was found in 43% (six of fourteen) of the wilted olive trees. According to Green (1980), the survival of mycelium and/or conidia in a dried necrotic plant tissue is expected to be very limited because of these severe weather conditions. However, the pathogen was easily isolated from inflorescences on wilted branches even 170 days after flowering (Table 1). This means that such inflorescences remained dried on infected trees for a long period at high temperature (> 35°C) and low moisture (< 40% RH, Relative Humidity) in the summer. Indeed, presence of *V. dahliae* microsclerotia was subsequently

demonstrated by the direct microscopic observation of ground tissues of some of the peduncle samples, which were photographed (Fig. 1d). Therefore, *V. dahliae* not only is able to colonize olive peduncles and flowers and to form microsclerotia, but also to survive in these tissues the hot conditions during summer in Andalucía.

Epidemiological consequences of the presence of the pathogen in affected inflorescences may be similar to those attributed to defoliated leaves (Tjamos and Tsougriani 1990). The presence and survival of the pathogen in defoliated leaves from wilted olive trees has been demonstrated in several studies, as well as the dispersal and infective potential of this inoculum source (López-Escudero and Mercado-Blanco 2010). In the present study, *V. dahliae* microsclerotia were found inside the colonized inflorescences, with inoculum densities that ranged from 1.4 to 73.5 ppg and from 0.9 to 7.2 ppg, for ground peduncles and flowers, respectively. A single olive tree can produce more than 20 000 inflorescences with approximately 500 000 single flowers (Lavee 1986). Considering the peduncles and flowers weight (10 and 3 mg, respectively), and the maximum inoculum density found in them (Table 1), a severely wilted olive tree would produce approximately 25 000 microsclerotia. Although the amount of microsclerotia produced is not negligible, the most important fact is that wilted inflorescences become a dry and fragile material, which can be easily powdered and distributed to a short or medium distance by wind or rain. Thereafter, these residues are likely to decompose rapidly, acting as a source of inoculum in other areas of the plantation or in nearby plots.

These evidences strengthen the importance of quickly removing infected plant debris from olive trees affected by Verticillium wilt as a control measure to reduce incoming inoculum into the soil and to prevent its spread.

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