Relative Susceptibility of New Olive Cultivars to *Spilocaea oleagina*, *Colletotrichum acutatum*, and *Pseudocercospora cladosporioides*

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Abstract

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The evaluation of the relative susceptibility of new cultivars to the main diseases of a crop is a key point to consider prior to their release to the nursery industry. This study provides a rigorous characterization of the resistance of 15 new olive cultivars and their genitors ('Arbequina,' 'Frantoio,' and 'Picual') to the three main aerial diseases, peacock spot, anthracnose, and cercosporiosis caused by *Spilocaea oleagina*, *Colletotrichum acutatum*, and *Pseudocercospora cladosporioides*, respectively. To do so, developing leaves and detached green-yellowish fruit were inoculated in laboratory tests with *S. oleagina* and *C. acutatum*, respectively, using conidial suspensions of both pathogens. Additionally, a previously validated rating scale was used to assess the incidence of leaves with symptoms of *S. oleagina* or *P. cladosporioides*

and the fruit rot incidence of *C. acutatum* in the trees for four years under field conditions. As a result, only two of the cultivars were susceptible to peacock spot, most likely because these new cultivars were previously screened for resistance to the disease on previous phases of the breeding program. Conversely, the 15 cultivars were susceptible or moderately susceptible to cercosporiosis. Five of the 15 new cultivars were classified as resistant to anthracnose, with four of them descendants of 'Frantoio' × 'Picual' crosses. In addition, the cultivars resistance to *C. acutatum* showed a negative linear correlation with the total phenols content of olive oil. This information regarding disease reaction of the new olive cultivars is essential for nursery industry and growers.

Foliar and fruit fungal pathogens cause economically important diseases in olives (*Olea europaea* L.) in most olive-growing areas around the world (8,40,41). Within these pathogens, the most serious diseases in order of importance are peacock spot, anthracnose, and cercosporiosis caused by *Spilocaea oleagina*, *Colletotrichum* spp., and *Pseudocercospora cladosporioides*, respectively (41). These pathogens cause tree defoliation, branch dieback, premature fruit dropping, and fruit rot, which can devastate entire olive production under pathogen-favoring environmental conditions (8,31,41). In addition, these diseases can also negatively affect olive oil quality, particularly anthracnose disease. The olive oils from fruit that are affected by *Colletotrichum* spp. show low oxidative stability and polyphenol and α -tocopherol content and various organoleptic defects (32). In Spain, the overall loss in net income for the olive industry caused by these three diseases is approximately \$315 million per annum (8,31).

The management of aerial olive diseases in the field involves cultural and chemical practices, including preventative sprays with copper-based fungicides (8,41). The commercial control of aerial olive diseases requires 2 to 6 fungicide applications during the entire growth season, although a higher number of applications may be necessary in wet areas, in areas of super-high-density plantings (hedgerow orchards), or when susceptible cultivars are grown (25).

Successful olive breeding programs by crossing and progeny selection began in various countries at the end of the 1980s (5,15). In Spain, a breeding program to obtain new cultivars for oil production began in 1991 in Córdoba Province (Andalusian region,

southern Spain). Since then, more than 10,000 seedlings from open and controlled crosses between traditional cultivars have been screened and selected as new cultivars according to two main features: first, desirable agronomical traits, such as low tree vigor and exceptional oil quality profiles (11,16,17,36,37); and second, disease resistance, which offers an economically sound alternative to chemical control (1,23,32). To this end, we evaluated the relative susceptibility of new cultivars to peacock spot, anthracnose, and cercosporiosis, which were systematically evaluated under field and controlled conditions. Field evaluations were conducted for several seasons because the severity of these diseases depends on the specific weather conditions of each year (2,25). Moreover, severe epidemics are necessary for a correct classification of the susceptibility of olive cultivars to allow the observation of the complete range of olive resistance in the field (23). These long-term field evaluations also allow us to describe the susceptibility of the new cultivars to other, more sporadic diseases. For example, the olive cv. FS-17 is unusually susceptible to *Alternaria alternata* (28), and the cv. Barnea is extremely susceptible to olive knot caused by the bacterium *Pseudomonas savastanoi* pv. *savastanoi* under field conditions (24). Additionally, the relative susceptibility of new olive cultivars to these pathogens should be confirmed by artificial inoculation because the olive tree frequently may not be subjected to these pathogens under field conditions (27,44).

Olive fruit and, by extension, olive oils are rich in polyphenols, which are of significant physiological importance for both the plants and their human consumers (4,11). Phenolic compounds from olive may inhibit the growth of pathogens, such as *S. oleagina* (13) and species of genera *Phytophthora* and *Cylindrocarpum* (3,10). In addition, these compounds show a preventative role in olive fly (*Bactrocera oleae*) infestations (47). The greater susceptibility to *Colletotrichum* spp. of mature olive fruit may be related to the loss of one or several host resistance mechanisms that are present in immature fruit, including decreases or changes in the phenolic compounds (27,34). The relationship between phenolic compounds and olive resistance to *Colletotrichum* is unknown.

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The objective of this study was to evaluate the phenotypic expression of resistance of 15 new olive cultivars and their genitors ('Arbequina,' 'Frantoio,' and 'Picual') to three major aerial pathogens of olive, *S. oleagina*, *Colletotrichum acutatum*, and *P. cladosporioides*. We evaluated the incidence of leaves and fruit that were affected by the pathogens for four seasons in an experimental orchard where the three diseases are endemic. In addition, standard inoculation tests were performed under controlled conditions. Finally, we established the relationship between the phenols of olive oil and the resistance of olive fruit to *C. acutatum*.

Materials and Methods

Plant material. The 15 evaluated genotypes are new cultivars from the first set of 748 seedlings from reciprocal crosses among the cvs. Arbequina, Frantoio, and Picual that were conducted by the Agronomy Department of the University of Córdoba and the Andalusian Institute for Research and Formation in Agriculture and Fishery (IFAPA in Spanish) in Córdoba during the springs of 1991 and 1992 (35,37). The results from previous studies regarding the productivity, fruit characteristics (e.g., removal force, size, and ripening time), and olive oil characteristics (e.g., fatty acid composition and phenolic profile) of these and additional genotypes have been reported (9,11,16,17). The 15 new cultivars and their genitors, which were used as controls, were vegetatively propagated (rooted semi-hardwood stem cutting) and planted in the field in July 2001 (Table 1). The experimental orchard was located in a 1.2-ha flat, uniform field at the IFAPA Alameda del Obispo Agricultural Research Station, Córdoba, southern Spain (37.5°N, 4.8°W, altitude 110 m). The soil of the orchard was classified as a Typic Xerofluvent with a sandy-loam texture, and the climatic conditions were typical of the Mediterranean area. The experimental orchard is located 703 m from the main river of Andalucía, Guadalquivir River, in a humid area where anthracnose, peacock spot, and cercosporiosis are endemic diseases. Initially, a randomized block design with 16 replications and one tree per plot was used with a 5 m distance between olive trees in a row and 6 m between rows. Currently, only 10 blocks remain due to the removal of six blocks for road construction. Because of damage by rabbits, however, there was one tree less of the cvs. Picual, UC-I 7-34, and UC-I 7-60. The trees in the orchard were drip-irrigated, and the experimental or-

chard was managed according to cultural practices of commercial olive orchards in Andalusia (4). Copper-based fungicides (copper sulfate, 3.5 kg Cu per ha) were applied during the spring and autumn to partially control the fungal foliar and fruit diseases (41). No fungicide treatments, however, were applied to allow for the development of an anthracnose epidemic in 2011.

The relative susceptibility of the genitors to *S. oleagina*, *C. acutatum*, and *P. cladosporioides* was previously characterized as follows: cv. Frantoio is resistant (R), resistant (R), and susceptible (S), respectively; cv. Picual is susceptible (S), resistant (R), and moderately susceptible (M), respectively; and cv. Arbequina is moderately susceptible (M) to the three pathogens (2,23,42; Table 2). The three genitors were used as controls in both the field and controlled trials.

Susceptibility of the new cultivars in artificial inoculations. The relative susceptibility to peacock spot and anthracnose of the 15 new cultivars was evaluated under controlled conditions. The relative susceptibility to cercosporiosis was not evaluated because the long incubation period of the disease (up to 18 months) does not allow for differentiating the infected leaves from those with natural senescence symptoms (2).

Spilocaea oleagina. Developing leaves of the 15 new cultivars were collected from the trees in the experimental orchard during the spring of 2003. The detached leaves were placed in plastic trays between two layers of moistened filter paper immediately after the leaves were removed from the plants. Before inoculation, the detached leaves were preconditioned for 24 h at the same temperature to which they would be exposed after inoculation. The inoculum was obtained from naturally infected leaves with sporulating peacock spot lesions that were collected from December to March (44). The affected olive leaves of cv. Manzanilla de Antequera located in Málaga Province (Andalucía region) were used as the inoculum source. The detached leaves were sprayed with a conidial suspension of 10^5 conidia per ml or sterile water for the control and incubated as described in the previous section. The inoculated and control leaves were assessed for disease severity 50 days after inoculation. To reveal the latent infections, we immersed the inoculated leaves in a 5% sodium hydroxide solution for 30 min at room temperature ($22 \pm 2^\circ\text{C}$). After this treatment, the visible lesions were more prominent, and the latent infections ap-

Table 1. Incidence of peacock spot caused by *Spilocaea oleagina*, cercosporiosis caused by *Pseudocercospora cladosporioides*, and anthracnose caused by *Colletotrichum* spp. in 3 traditional and 15 new cultivars of olive in an experimental orchard in southern Spain

Genotype	Trees (No.)	Origin ^z	Disease									
			Peacock spot ^y					Cercosporiosis				
			2007	2008	2010	2011	Average	2007	2008	2010	2011	Average
Arbequina	10		4.5 b	1.3 b	1.6 b	3.1 b	2.62	2.3 b	2.4 b	2.4 b	2.2 b	2.32
Frantoio	10		0.1 c	0.0 c	0.0 c	0.1 c	0.05	4.1 b	1.9 b	4.8 a	5.6 a	4.10
Picual	9		5.7 b	0.8 b	1.3 b	3.9 b	2.92	3.6 b	0.7 b	1.3 b	2.4 b	2.00
Chiquitita	10	P × A	0.1 c	0.0 c	0.2 c	0.1 c	0.10	2.8 b	2.6 b	2.7 b	2.8 b	2.80
UC-I 2-68	10	P × A	2.6 c	0.1 c	0.0 c	0.2 c	0.72	5.3 a	3.3 b	4.3 b	5.9 a	3.63
UC-I 5-44	10	P × A	0.3 c	0.0 c	0.0 c	0.2 c	0.12	3.7 b	4.1 b	5.0 a	5.9 a	4.67
UC-I 7-34	9	P × A	1.0 c	0.0 c	0.0 c	0.2 c	0.30	4.2 b	2.7 b	2.2 b	4.5 a	3.42
UC-I 8-20	10	P × A	0.8 c	0.0 c	0.3 b	0.0 c	0.27	3.5 b	0.9 b	4.7 a	5.5 a	3.65
UC-I 11-16	10	P × A	4.5 b	0.4 b	0.2 c	1.6 b	1.67	2.8 b	1.5 b	3.9 b	4.7 a	3.22
UC-I 6-9	10	A × P	4.7 b	0.9 b	0.1 c	1.3 c	1.75	3.3 b	1.9 b	5.8 a	5.3 a	4.08
UC-I 7-8	10	A × P	6.2 b	0.8 b	0.5 b	2.3 b	2.45	3.6 b	3.7 b	5.5 a	5.7 a	4.62
UC-I 9-67	10	A × P	0.0 c	0.0 c	0.1 c	0.3 c	0.10	2.9 b	2.4 b	0.9 b	1.8 b	2.00
UC-I 10-54	10	A × P	9.9 a	6.2 a	5.9 a	9.4 a	7.85	-	0.3 b	3.2 b	-	2.32
UC-I 11-10	10	A × P	0.9 c	0.3 b	0.1 c	0.0 c	0.32	1.4 b	1.6 b	2.5 b	4.1 b	2.40
UC-I 1-19	10	F × P	2.3 c	0.2 c	0.3 c	1.0 c	0.95	2.0 b	0.8 b	5.5 a	5.0 a	3.30
UC-I 4-62	10	F × P	6.5 a	1.1 b	4.5 a	6.9 a	4.75	2.5 b	1.0 b	2.4 b	3.4 b	2.32
UC-I 7-60	9	F × P	3.0 b	0.1 c	0.1 c	1.4 b	1.15	1.5 b	0.6 b	0.7 b	4.0 b	1.75
UC-I 10-30	10	F × P	1.0 c	0.1 c	0.2 c	0.3 c	0.40	3.3 b	3.3 b	4.3 b	6.2 a	4.27
Average			3.0	0.7	0.9	1.8		3.1	2.0	3.5	4.5	

^y The leaf or fruit rot incidence was estimated using a 1 to 10 rating scale in which binary data (proportion of affected fruits) are normalized by applying the logit transformation of proportion (22). The scale values were directly subjected to an analysis of variance and mean comparison tests. For each year, the mean values with the letters a, b, or c are significantly higher, equal, or lower, respectively, than the moderately susceptible control 'Arbequina' according to Dunnett's test at $P = 0.05$.

^z The new cultivars come from crosses between the cvs. Arbequina (A), Frantoio (F), and Picual (P).

peared as black circular spots or rings, clearly differentiated from the healthy green tissue (45). Disease severity was assessed using a 0 to 8 rating scale. The scale considers the percentage of the affected leaf surface similar to the rating scale of Viruega et al. (44): 0 = no symptoms, 1 = <12.5%, 2 = 12.5 to 25%, 4 = 26 to 50%, 6 = 51 to 75%, and 8 = >75% of the upper surface covered by black spot lesions. The evaluators of the disease were trained using the software ASSESS (14) to obtain a good relationship between the scale value and the pathogen-affected leaf surface. The disease severity index (DSI) was calculated in each replication and was expressed as the relative severity with respect to the susceptible parent cv. Picual. There were three replicates (moist chambers) per treatment with 20 leaves per replicate arranged in a completely randomized design. The experiment was repeated twice using two populations of the pathogen from different orchards.

Colletotrichum acutatum. Olive fruit from the 15 new cultivars were collected at the onset of ripening from trees in the experimental orchard in 2005. The fruit were green-yellowish and had a value of 1 on the ripening scale of 0 (green fruit) to 4 (black fruit) (4). The fruit were washed, disinfested, air-dried, and sprayed with a conidial suspension (10^5 conidia per ml or sterile water for the control) from pure cultures of the pathogen grown on potato dextrose agar (PDA) as described by Moral et al. (27). To assure that the conidia were viable, their germination was evaluated and ranged from 50 to 80%. The inoculated and control fruit were incubated in moist chambers (plastic containers, 22 × 16 × 10 cm with 100% RH) at 23 ± 2°C under fluorescent lights (12-h photoperiod, 350 μmol m⁻² s⁻¹). The disease severity was assessed weekly for 1 month after inoculation using a 0 to 5 rating scale where 0 = no visible symptoms, 1 = visible symptoms affecting less than 25% of the fruit surface, 2 = 25 to 50%, 3 = 50 to 75%, 4 = 75 to 100%, and 5 = fruit completely rotted (soapy fruit) with abundant conidia in a gelatinous matrix. A disease severity index (DSI) was calculated for each replication using the following formula: $DSI = (\sum n_i \times i) / N$, where i represents severity (0 to 5), n_i is the number of fruits with a severity of i , and N is the total number of all inoculated fruit (27). The area under the disease progress curve (AUDPC) was calculated via the trapezoidal integration of DSI values over time. There were three replicates (moist chambers) per treatment with 25 fruit per replicate arranged in a completely randomized design. The experiment was repeated twice using the isolates Col-87 and Col-94. Both of these isolates were identified

as *C. acutatum*-group A4 according to their internal transcribed spacer 5.8S and β-tubulin regions. According to Damm et al. (7), group A4 corresponds to *C. godetiae*, although the latter name is rarely used. The *C. acutatum* group A4 is the dominant group in the Andalusia region (32) and is the only isolate that was detected in the experimental orchard.

Susceptibility of the new cultivars under field conditions. The disease severity of anthracnose, peacock spot, and cercosporiosis was assessed in each olive tree by estimating the percentage of affected fruit or leaves using a 0 to 10 rating scale where 0 = no affected fruits or leaves per tree, 1 = one to three affected fruits or leaves per tree, and 2 = one to three affected fruits or leaves per each quadrant of the tree canopy. Higher rating values were obtained by directly estimating the percentage of affected leaves or fruit with 5 = 10%, 6 = 25%, 7 = 50%, 8 = 75%, 9 = 90%, and 10 = >94% of the affected tissues. Then, the data of the percentage affected leaves or fruits were transformed in scaling rating values using the logistic equation (23):

$$\text{Logit}(Y) = \ln \frac{Y}{100 - Y} = -1.2 \times (X - 7) \quad (1)$$

where X is the scaling rating value and Y is the percentage of affected leaves or fruit. The transformed scale data are normally distributed so that they can be directly subjected to an analysis of variance and other parametric analyses. This rating scale is a useful and rapid method to estimate the incidence of affected leaves or fruit in a tree based on the binary nature of the data and the logistic growth of the epidemics (23).

During the disease evaluations, the assessors circled the canopy of each olive tree looking for affected leaves or fruit in a 1- to 2-m band above ground. The area checked was approximately 1/4 (25%) of the total canopy. The assessment took approximately 3 to 10 min, depending on the size of the olive canopy. Overall, the incidence of fruit with symptoms of anthracnose or leaves with symptoms of cercosporiosis or peacock spot were assessed while these tissues were still attached to the tree, but in some cases, the assessment included the fruit or leaves on the soil surface. With cercosporiosis, both the upper and lower surfaces of the leaves were observed because the pathogen, at times, sporulated abundantly on the lower surface of the asymptomatic leaves. Conversely, the chlorotic leaves without signs of pathogen infection were incubated in a humid chamber for 14 days to induce fungal sporu-

Table 2. Relative susceptibility of 18 olive genotypes (3 traditional cultivars and 15 new cultivars) to peacock spot caused by *Spilocaea oleagina*, cercosporiosis caused by *Pseudocercospora cladosporioides*, and anthracnose caused by *Colletotrichum* spp.

Genotype	Trees (No.)	Origin ^w	Disease		
			Peacock spot ^x	Cercosporiosis ^y	Anthracnose ^x
Arbequina	10		M ^z	M	M
Frantoio	10		R	S	R
Picual	9		S	M	R
Chiquitita	10	P × A	M-R	M	S-M
UC-I 2-68	10	P × A	M-R	S	S-M
UC-I 5-44	10	P × A	R	S	M-R
UC-I 7-34	9	P × A	M-R	S	R
UC-I 8-20	10	P × A	M-R	S	S-M
UC-I 11-16	10	P × A	M-R	S	M-R
UC-I 6-9	10	A × P	M-R	S	M
UC-I 7-8	10	A × P	M-R	S	M-R
UC-I 9-67	10	A × P	M-R	M	M
UC-I 10-54	10	A × P	S	M	M-R
UC-I 11-10	10	A × P	R	M	S-M
UC-I 1-19	10	F × P	R	S	R
UC-I 4-62	10	F × P	S-M	M	R
UC-I 7-60	9	F × P	R	M	R
UC-I 10-30	10	F × P	R	S	R

^w The new cultivars come from crosses between the cvs. Arbequina (A), Frantoio (F), and Picual (P).

^x The relative susceptibility according to the disease incidence in the field and the disease severity in artificial inoculation.

^y The relative susceptibility according to disease incidence in the field.

^z The disease reaction: susceptible (S), moderately susceptible (M), and resistant (R); the cultivars with different reactions under the controlled and field conditions show two letters.

lation and to avoid confusion with the senescent leaves. For every disease, each tree was rated by two individuals, and the means were calculated from all of the ratings. The evaluations were carried out from 2006 to 2011 from December to March.

Relationship between the phenolic content and fruit resistance to *Colletotrichum*. To study the relationship between the phenolic compounds of fruit and the anthracnose resistance of genotypes, we used the recently published data of the phenolic profile of olive oil from nine of the new cultivars and the three genitors (11). These authors (11) collected olive fruit in 2009, a non-epidemic year, and characterized the phenolic profile of the oil of each cultivar by liquid-liquid extraction with 60:40 (v/v) methanol-water and subsequent chromatographic analysis with absorption and fluorescent detection in a sequential configuration. In this study, the concentration of the following compounds was determined: apigenin, hydroxyl-tyrosol, luteolin, *p*-coumaric acid, *o*-coumaric acid, tyrosol, vanillic acid, 3,4-DHPEA-EDA (dialdehydic form of elenolic acid linked to hydroxytyrosol), and total phenols. Because the phenolic profile of olive oil was determined for each cultivar and ripening scale value from 0 (green fruit) to 4 (black fruit), we correlated the total or specific phenol contents of the oils from fruits on each ripening scale value with the severity of the symptoms (AUDPC). In addition, we used the average total or specific phenol contents of the oil for this correlation.

Data analysis. The data analysis was performed using Statistix software (version 10; Statistix, Tallahassee, FL). In the inoculation experiments, the effects of the olive genotype on anthracnose and peacock spot severity were determined by an analysis of variance (ANOVA) because these data satisfied the normality and homogeneity of the variance requirements of ANOVA. The *C. acutatum* isolate or *S. oleagina* pathogen population was used as a block because the effects of the isolate or pathogen population and its interaction with the olive genotype were not significant ($P > 0.05$). The relationship between anthracnose severity (AUDPC) and the concentration of each phenolic compound of olive oil was analyzed by Pearson's correlation test and then by linear regression analysis.

For the field experiments, the ANOVA was performed on the rating scale data of fruit rot incidence and incidence of infected leaves for each year because of the important differences among the years. When none of the trees of the same cultivar showed any disease symptoms, the cultivar was considered significantly different from the diseased cultivars. Dunnett's test was used to determine significant differences between each genotype and the cultivar control ('Arbequina') at $P < 0.05$. The parental 'Arbequina' was selected as the control because it is moderately susceptible to the three pathogens and enables the separation of the genotypes, such as resistant, moderately susceptible, or susceptible. The relationship between the average rating scale data under field conditions and the severity under artificial inoculations was studied using a linear regression analysis forced through the origin. Finally, the relationship among the resistance to the three pathogens of the olive genotypes was studied by Pearson's correlation.

Results

Susceptibility of the new olive cultivars to artificial inoculations. *Spilocaea oleagina*. All of the genotypes showed latent or visible symptoms of peacock spot 50 days after inoculation. The DSI varied among the new cultivars ($P < 0.001$), whereas the population of the pathogen and its interaction with the genotype were not significant ($P > 0.05$). Among the genitors, the cv. Picual was the most susceptible to the pathogen, and the cv. Frantoio was the most resistant (Fig. 1). The DSI of the new cultivars ranged from 15.13% for UC-I 1-19 to 96.36% for UC-I 10-54. No other genotype was as resistant to the pathogen as was the cv. Frantoio. Five new cultivars were significantly ($P > 0.05$) more resistant to *S. oleagina* than was the cv. Arbequina, while UC-I 10-54 was as susceptible as cv. Picual. The other genotypes did not differ significantly ($P > 0.05$) in susceptibility compared with the control cultivar. Three out of five resistant new cultivars descended from the 'Frantoio' × 'Picual' crosses (Fig. 1).

***Colletotrichum acutatum*.** All of the genotypes developed fruit rot symptoms 28 days after inoculation. The cv. Frantoio, however, showed only two fruit (1.3%) with anthracnose symptoms 28 days after inoculation, but 60 days after inoculation 40% of the fruit exhibited disease symptoms. The disease severity (AUDPC) varied greatly among the genotypes ($P < 0.001$), but the effect of isolates and the genotype-isolate interaction did not significantly influence the disease severity ($P > 0.05$). The genitors exhibited different responses to *C. acutatum*, with the cvs. Frantoio and Picual being more resistant than the cv. Arbequina. Four of the 15 new cultivars were significantly ($P < 0.05$) more susceptible to *C. acutatum* than the cv. Arbequina, while another four were as susceptible as this cultivar. Conversely, seven new cultivars were more resistant than the cv. Arbequina, with four descending from 'Frantoio' × 'Picual' crosses (Fig. 2).

Susceptibility of the new cultivars under field conditions. The leaves affected by *S. oleagina* or *P. cladosporioides* and the incidence of fruit with symptoms of *C. acutatum* in the new cultivars and their genitors varied greatly among years and genotypes (Table 1). During the four years of this study (2007, 2008, 2010, and 2011), two years (2007 and 2011) were favorable for peacock spot epidemics, two other years (2010 and 2011) were favorable for cercosporiosis epidemics, and only one year (2011) was favorable for anthracnose epidemics. However, some trees of the new cultivars UC-I 8-20 and UC-I 9-67 and the cvs. Chiquitita and Arbequina showed a small number of infected fruits in previous seasons. During the favorable years for peacock spot epidemics, nine new cultivars were significantly ($P < 0.05$) more resistant to *S. oleagina* than was the cv. Arbequina, four new cultivars showed a similar disease incidence, and only the new cultivars UC-I 4-62 and UC-I 10-54 were more susceptible to *S. oleagina* than cv. Arbequina (Table 1). The latter two new cultivars were even more susceptible to peacock spot than the susceptible genitor 'Picual' (data not shown), although this cultivar did not differ significantly from the moderately susceptible cv. Arbequina in these experiments.

With regard to cercosporiosis, the genitors 'Frantoio' and 'Picual' and all new cultivars were equally or more susceptible than cv. Arbequina. When considering only the most favorable year (2011),

Fig. 1. Effect of olive genotype on the peacock spot severity (disease severity index) of olive leaves that were inoculated with *Spilocaea oleagina* conidia obtained from naturally infected trees. The cvs. Arbequina (A), Frantoio (F), and Picual (P) were the genitors of the new genotypes. The bars represent the average of 120 leaves. For each genotype, the mean values with the letters a, b, or c are significantly higher, equal, or lower, respectively, than the moderately susceptible control 'Arbequina' according to Dunnett's test at $P = 0.05$.

nine and six of the new cultivars were respectively more or equally susceptible to *P. cladosporioides* than this control cultivar (Table 1). In 2007 and 2011, the high susceptibility of UC-I 10-54 to peacock spot hindered the correct evaluation of the severity of *P. cladosporioides*, because most of the leaf surface was affected by *S. oleagina*.

Under field conditions, none of the new cultivars were more susceptible to *C. acutatum* than was the cv. Arbequina; seven were more resistant to the disease than was the cv. Arbequina, and the remaining showed a range of resistance that was similar to that of this cultivar (Table 1).

The disease severity based on both artificial inoculation and field observations for the 15 new cultivars and their genitors were compared by linear regression analysis. With peacock spot, a good linear regression ($Y = 0.04X$; $R^2 = 0.677$; and $P < 0.001$) between the susceptibility of new cultivars and their genitors under artificial and field conditions was observed. In the case of anthracnose, this relationship was highly accurate ($Y = 0.10X$; $R^2 = 0.855$; and $P < 0.001$) (data not shown).

Finally, when the correlations among the susceptibility of the olive genotypes to the three diseases were studied, we only observed a low but significant correlation ($r = 0.481$; $P = 0.043$) among the resistance to peacock spot and anthracnose.

Relationship between phenolic content and resistance to *Colletotrichum*. The phenolic composition and the total phenol content varied greatly among the 12 studied cultivars. The highest phenol content was obtained from the cv. Frantoio [598 mg gallic acid equivalent (GAE) kg^{-1} oil]. Conversely, the olive oil of the cv. UC-I 9-67 showed the lowest total phenol content (132 GAE kg^{-1} oil). The anthracnose severity (AUDPC) of the inoculated fruit showed negative linear correlations ($r > 0.500$; $P < 0.05$) with the total phenol content of oil from the fruits in each ripening scale value. This correlation showed the best fit ($r = 0.762$; $P = 0.004$) when we used the phenolic content of olive oil from the green-yellowish fruit of each cultivar (Fig. 3), coinciding with the ripening stage of the inoculated fruit. Conversely, the disease severity was not correlated (Pearson correlation; $r > 0.05$) with any of the eight (apigenin, hydroxyl-tyrosol, luteolin, *p*-coumaric acid, *o*-coumaric acid, tyrosol, vanillic acid, and 3,4-DHPEA-EDA) specific phenols of olive oil.

Fig. 2. Effect of olive genotype on the anthracnose severity (area under disease progress curve) of olive fruit that were inoculated with *Colletotrichum acutatum*. The cultivars Arbequina (A), Frantoio (F), and Picual (P) were the genitors of the new genotypes. The bars represent the average of 150 fruits. For each genotype, the mean values with the letters a, b, or c are significantly higher, equal, or lower, respectively, than the moderately susceptible control 'Arbequina' according to Dunnett's test at $P = 0.05$.

Discussion

We evaluated the response of 15 new olive cultivars to the main aerial olive pathogens *S. oleagina*, *C. acutatum*, and *P. cladosporioides* under field and controlled conditions. As a result, we found wide variability in the response of these cultivars to the diseases, ranging from resistant to very susceptible. This large variation agrees with the variability that has been found for other horticultural characteristics, such as yield per tree, ripening date, and oil content (9,16,17). This diverse response might be a result of the different agronomical performances of the genitors (cvs. Arbequina, Frantoio, and Picual) to these diseases (1,2,23,26). Additionally, the generally high heterozygosity that is exhibited by olive cultivars may explain the broad segregation that is observed in their offspring (35,37).

The relative susceptibility to *S. oleagina* and *C. acutatum* was tested under field and controlled conditions using inoculation methods that were previously applied to assess cultivar resistance (18,19,23), pathogenic variability of fungi (18,29), and efficacy of biological and chemical control products (38). The relative susceptibility to *P. cladosporioides* was evaluated only under field conditions because the pathogen showed more than 1 year of latency. This feature does not allow for the differentiation between the infected leaves and those with natural senescence symptoms (2). Field evaluations were carried out for 4 years using a previously validated rating scale (23).

Resistance and susceptibility are two extremes of a continuum of olive reactions to aerial fungal diseases (24); consequently, olive reactions do not separate into discrete categories (6,26). Nevertheless, descriptions and comparisons of cultivars are more pragmatic and easily understood if the disease reactions are placed into distinct ordinal classes (33). To this end, olive cultivar reactions are often grouped into three or five groups from resistant to susceptible based on the severity of the disease symptoms (1,2,23,26,42). In this study, the new cultivars were compared with the genitor 'Arbequina,' which has been extensively studied and is considered moderately susceptible to the three pathogens (2,23,26,42). The genotypes were classified as resistant, moderately susceptible, or susceptible when they were less, equally, or more susceptible, re-

Fig. 3. Linear correlation between the total phenol content [mg gallic acid equivalent (GAE) kg^{-1} oil] of olive oil and the disease severity (area under disease progress curve) of fruit that were inoculated with *Colletotrichum acutatum*. The symbols represent 12 olive genotypes (Table 2).

spectively, to a pathogen than was the control cultivar. Several new cultivars were classified for their resistance to *S. oleagina* or *C. acutatum* into two different resistance groups depending on field or controlled trial, although none of them showed the opposite reaction (e.g., R and S or S and R) in both trials (Table 2). Because the resistance to anthracnose and peacock spot is correlated with the used genitor 'Frantoio' (23,26,42), this correlation was also observed with the new cultivars.

Based on the combined laboratory and field evaluations, 14 new cultivars were classified as resistant or moderately susceptible to *S. oleagina*. Only the cv. UC-I 10-54 was classified as susceptible to peacock spot. This high proportion of resistant genotypes could be explained by the early screening test for peacock spot resistance that is applied to seedlings in this olive breeding program (1,26,35,37). This test takes advantage of the strong correlation between the resistance to *S. oleagina* of seedlings and that of adult plants, although some susceptible seedlings might escape this screening, such as the new cultivars UC-I 4-62 and UC-I 10-54 (1). The latter cultivar was extremely susceptible to *S. oleagina* under field conditions, showing a heavy defoliation (average severity = 7.85) even with the weather conditions of the year being unfavorable for the disease. Under the same conditions, the infected trees of the susceptible parental 'Picual' showed slight defoliation (average severity = 2.92). For this unusual susceptibility, the cv. UC-I 10-54 is currently being used to study the influence of several factors on leaf infection caused by *S. oleagina*.

Four of the 15 new genotypes (cvs. Chiquitita, UC-I 2-68, UC-I 8-20, and UC-I 11-10) were susceptible to *C. acutatum* in the controlled inoculations. The other 11 new cultivars were classified as resistant or moderately susceptible to this pathogen. Under field conditions, there was only an anthracnose epidemic during the fall and winter of 2011, and no cultivar was more susceptible to *C. acutatum* than was the cv. Arbequina. This overall resistance could be due to the resistance of two of the genitors, the cvs. Frantoio and Picual (23,26). The weather conditions of each season strongly influenced the development of anthracnose, highlighting the need to evaluate disease resistance for several years (23). Remarkably, there was a good correlation between the reactions of the genotypes under the controlled and field conditions, as has been previously reported (23). The selection of new genotypes with an elevated resistance to *C. acutatum* is essential because (i) diseased fruit, even with a low incidence, adversely affect the quality of the olive oil (32); (ii) fungicides have a limited use because *C. acutatum* has low sensitivity to copper fungicides, and the use of organic fungicides in olive orchards is very scarce (38); and (iii) the disease is particularly severe in orchards that are densely planted, such as new super-intensive olive growing systems (29). In addition, several fruit of the cvs. UC-I 9-67 and UC-I 0-54 were affected by *Botryosphaeria dothidea*, the causal agent of dalmatian disease (30), while *Phlyctema vagabunda*, the causal agent of fruit leprosy (41), affected fruits of the cv. UC-I 4-62 in the field. These diseases, however, have not been extensively evaluated because they were not homogeneously distributed in the orchard and affected only some of the cultivars and trees.

Some studies support the idea that the resistance to pathogens and pests might be related to certain types of phenolics of olive tissues (3,10,13,46), but data are often lacking for olive anthracnose. To establish this relationship, we used the phenolic profile of olive oil from the same cultivars of our study, which were recently published (11). Our results showed that the resistance of olive fruit to *C. acutatum* is related to the constitutive phenolic content of fruit, similar to other diseases that are caused by this pathogen (21,22). Conversely, Gomes et al. (12) did not find a relationship between the total phenolic compounds of olive fruits of the cvs. Galega vulgar and Cobrançosa and resistance to *Colletotrichum*, although this can be explained by the fact that both cultivars are highly and moderately susceptible to the pathogen, respectively (32). Olive fruit susceptibility increases with increasing fruit maturity (27), which in turn decreases the total phenol content (11,39). In olive anthracnose, previous studies carried out on the

antifungal properties of extracts from the exocarp and mesocarp of unripened fruit of the susceptible cv. Barnea indicated that the main antifungal compounds that are present in unripened fruit are phenolics (J. Moral, A. Trapero, and D. Prusky, *unpublished data*). These results argue for the importance of additional outreach and additional research on the role of phenolic compounds in the resistance to olive anthracnose.

Currently, screening tests of olive seedlings for anthracnose and cercosporiosis are not available. In the first case, adult plants are needed because the fruit is required for inoculation (23,27), and in the second case, the latent period of cercosporiosis is too long to be applied in early screening (2). None of the new cultivars were classified as resistant to *P. cladosporioides*. This lack of resistance could be due to the susceptibility of the three genitors to the pathogen (2,26). In contrast, the effect of the cv. Frantoio in conferring high resistance to *S. oleagina* and *C. acutatum* to its progeny was evident. The additional high resistance of the cv. Frantoio to *Verticillium dahliae*, which is considered the main soilborne disease threatening olive production worldwide, makes this cultivar especially valuable as a genitor in breeding programs (20,43). Other resistant cultivars to *V. dahliae* (cvs. Changlot Real and Empeltre), *C. acutatum* (cv. Koroneiki), and *S. oleagina* (cv. Lechín de Sevilla) are being used as genitors in the olive breeding program of Córdoba (37).

Overall, the majority of the cultivars, especially the cv. UC-I 7-60, showed a good level of resistance to the three pathogens, and remarkably, none of the cultivars was susceptible to all three of the pathogens. This study also provides a helpful guideline for the evaluation of olive cultivars and the main aerial pathogens of this crop. The results of these tests are key points to consider prior to the release of any new cultivars into the nursery industry.

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