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Effect of latent and symptomatic infections by *Colletotrichum godetiae* on oil quality

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Abstract Anthracnose, caused by *Colletotrichum* spp., is the main olive fruit disease. *Colletotrichum* can severely infect olive fruit with a negative impact on the oil quality. However, the relationship between visible infections of *Colletotrichum* spp. and olive oil quality is unclear and the influence of latent infections is unknown. This study considers *Colletotrichum* spp. latent infections and visible infections as factors affecting the quality of olive oil. IAbsorbance in UV (K232 and K270), free acidity, and peroxide index were evaluated in oils from fruit with latent and symptomatic infections by *Colletotrichum godetiae* of the cvs. Arbequina, Hojiblanca, and Picual. Olive oil samples from i) latent infected fruit at three maturity stages and after two incubation periods and from ii) sets of oils from mixtures of healthy and infected symptomatic fruit were used to determine the impact of the disease on oil quality. Oils from latent infected fruit of cv. Arbequina showed higher acidity than control oils (P = 0.012). Linear and exponential models were fitted to relate the oil quality parameters to the proportion of symptomatic fruit. Acidity was the most affected parameter, mainly in oils from cv. Arbequina. The thresholds of the percentage of affected fruits causing the loss of category in the quality of the oil varied greatly according to cultivar, with 'Arbequina' being the most sensitive.

Keywords Acidity · *Colletotrichum* spp. · K232 · K270 · Olive oil · Peroxide index

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Introduction

Anthracnose of olive (*Olea europaea* subsp. *europaea* L.) is the main fruit disease of this crop worldwide (Cacciola et al. 2012; Moral et al. 2014; Talhinhas et al. 2018). Spain is the leading olive producer, with 23.6% of the world production area (11.5 million ha) and 31.4% of the global production (FAO, 2019). Economic losses are correlated to the concurrence of factors eliciting disease development, which can reach up to 50% of fruit affected in the southern Iberian Peninsula, including the Andalusia region (southern Spain; Moral et al. 2014) and Portugal (Talhinhas et al. 2018). In Spain, it has been estimated that olive anthracnose can cause economic losses of $100 million per year due to fruit drop anda negative impact on the oil quality obtained from infected fruits (Moral et al. 2018).

Olive anthracnose is caused by at least 13 *Colletotrichum* species that belong to the complexes *Cerastium acutatum* J.H. Simmonds, *C. gloeosporioides* (Penz.) Penz. & Sacc., and *C. boninense* Moriwaki, Toy. Sato & Tsukib. (Cacciola et al. 2012; Moral et al. 2014, 2018; Moreira et al. 2021). Among the species belonging to *C. acutatum* complex, *C. godetiae* Neerg. is the

dominant species in Greece, Italy, Montenegro, and Spain (Cacciola et al. 2012; Moral et al. 2014); *C. nymphaeae* (Pass.) Aa is the major species in Portugal (Talhinhas et al. 2005; Materatski et al. 2018); and *C. acutatum* sensu stricto is the most prevalent species in Tunisia (Chattaoui et al. 2016) and is replacing *C. godetiae* as the prevalent species in Italy (Schena et al. 2017). The pathogen mainly affects fruit at maturity, causing rot and subsequent mummification as well as fruit drop (Schena et al. 2017; Moral et al. 2009). Moreover, olive oil extracted from symptomatic fruit has an undesirable quality based on physicochemical parameters (Carvalho et al. 2008; Iannotta et al. 1999; Leoni et al. 2018).

Under Mediterranean conditions, olive anthracnose is a polycyclic disease with few secondary cycles (oligocycle). The disease cycle starts with the infection of inflorescences and developing fruit through water-splashed conidia during spring-summer (Moral et al. 2009). Infections in developing fruit remain latent until fruit ripening (fall-winter). Therefore, the epidemic development highly depends on weather conditions (Moral et al. 2012), cultivar resistance (Moral and Trapero 2009; Moral et al. 2017), and the degree of fruit ripening (Moral et al. 2008). Dramatic increase of anthracnose is expected when warm and wet conditions concur with ripened fruit of susceptible olive cultivars (Moral and Trapero 2012).

Disease control is challenging, and several measures should be combined in an integrated pest management strategy (Boller et al. 2004; Nigro et al. 2018a). There is a wide range of cultivar resistance/susceptibility to the pathogen. However, cultivar resistance is only one criterion among others in the choice of a cultivar for a concrete olive plantation (Moral et al. 2017). Cultural practices, such as a balanced fertilization, wide planting frames, and selective pruning (eliminating dead tissue and mummies), are usually recommended (Cacciola et al. 2012; Moral et al. 2014). Early harvesting is considered an effective way to avoid the exponential growth of olive anthracnose incidence, but it has some inconveniences such as low oil content in fruit and strong fruit retention) (Moral et al. 2017). The most effective strategy of chemical control is based on protective fungicides at the beginning of the epidemic. However, few active ingredients effectively control olive anthracnose, and fungicide applications must be frequently repeated, leading to an environmental and economic impact (Moral et al. 2018). Biological control is a useful tool to manage olive anthracnose, but it is still not applied in commercial orchards (Nigro et al. 2018b; Pesce et al. 2018).

Olive anthracnose is a well-known disease affecting oil quality, mainly increasing the free acidity (Leoni et al. 2018; Moral et al. 2014). The disease causes a shortage of extra virgin olive oil qualification based on International Olive Council standards (IOC, 2018; Table 1). Oils from symptomatic fruit show a reddish colour, get high free acidity and peroxide level, low oxidative stability and phenolic composition, and poor organoleptic attributes (Carvalho et al. 2008; Iannotta et al. 1999; Moral et al. 2014). When anthracnose incidence exceeds 4–20% and 40–45%, it might lose the extra virgin or virgin oil qualification, respectively; although these parameters vary depending on the cultivar (Carvalho et al. 2008; Iannotta et al. 1999; Leoni et al. 2018). Anthracnose also affects the sterol composition of the oils that may prevent compliance with international trade standards for olive oils; meanwhile, the fatty acid composition remains stable (Iannotta et al. 1999; Moral et al. 2014). Runcio et al. (2008) reported an increase in the content of some aldehydes (e.g., heptanal, octanal, and nonanal) in oils from fruit affected by anthracnose. Nevertheless, no studies have been carried out with olive oils extracted from infected but asymptomatic fruit (from now on fruit with latent infection). This fact might be due to the lack of an easy and economical method to quantify the incidence of latent infections caused by *Colletotrichum* spp. (Romero et al. 2017). Thus, the objectives of this study were to: i)

Table 1 Thresholds of four physicochemical parameters that categorize the olive oil quality based on International Olive Council

Olive oil	K232	K270	Acidity (%)	Peroxide Index (meq O^2 Kg^{-1})
Extra virgin	≤ 2.50	≤ 0.22	≤ 0.8	≤ 20.0
Virgin	≤ 2.60	≤ 0.25	≤ 2.0	≤ 20.0
Ordinary virgin	-[a]	≤ 0.30	≤ 3.3	≤ 20.0

[a] Non determined

evaluate the impact of the *C. godetiae* on the quality of the oils of the three dominant Spanish cultivars ('Arbequina", Hojiblanca', and 'Picual'); and ii) elucidate the impact of latent infections of *Colletotrichum* spp. on the olive oil quality.

Materials and methods

Materials

Detached olive fruits of the cvs. Arbequina, Hojiblanca, and Picual, which are considered moderately susceptible, highly susceptible, and resistant to olive anthracnose, respectively, were used in this study (Moral et al. 2017). Fruits were collected from two olive orchards without historical reports of anthracnose and were located in the province of Cordoba (Andalusian region, southern Spain). Collected fruit were surface disinfested by dipping in an ethanol solution (96% v/v) for 2 min, and subsequently in a solution of commercial bleach (10% v/v from 50 g of chlorine/l) for 7 min. Finally, disinfested fruit were rinsed with sterile water and air dried on a laboratory bench for 30 min. Fruits were stored for no more than 3 days at 5 ± 1 °C until the beginning of the experiments.

Fungal isolate, inoculation, and incubation

The *C. godetiae* isolate COL-420 from infected olive fruit of cv. Hojiblanca from a commercial orchard located in Cordoba province in the fungal collection of the Department of Agronomy at he UCO (Spain) was used. The isolate was cultured in Petri dishes (9 cm) containing potato dextrose agar (PDA; Difco Laboratories, Detroit) acidified with lactic acid (25% [vol/vol] at 2.5 ml l^{-1} of medium) (PDAA). Cultures were incubated at 23 ± 2 °C with a 12-h photoperiod of fluorescent light (350 μmol m^{-2} s^{-1}) for 10 days. Conidial suspensions were prepared and adjusted (10^5 conidia ml^{-1}) with a haemocytometer. In order to ensure conidial viability, germination of each batch of inoculum before inoculation was evaluated by placing drops of the conidial suspensions on slides, which were placed on covered water agar plates to maintain humidity (Viruega et al., 2011).

The inoculation was conducted by fruit-immersion in conidial suspension to guarantee a homogeneous infection on all fruit surface for 5 min. Non-inoculated fruit were immersed in distilled water as controls. Inoculated and non-inoculated control fruit were placed on plastic trays (80 × 60 × 15 cm) and incubated at 19 ± 2 °C in darkness at 100% RH. Each plastic tray contained 1.5 kg of fruit. At the end of the incubation period, which depended on the experiment treatment (see below), infected fruit were rinsed with distilled water, dried on a laboratory bench, and stored at 5 ± 1 °C for no more than one day until the beginning of the oil extraction procedure (see the section of extraction and evaluation of olive oil).

Experiment I. effect of latent infections on olive oil quality

Green-yellowish, veraison, and black fruit [maturity stages 1, 3 and 5, respectively (Barranco et al. 2017)] of cv. Arbequina were used to study the impact of the latent infection on oil quality. Veraison fruit of cvs. Hojiblanca and Picual with latent infection of the pathogen were also used to compare with cv. Arbequina. Two incubation periods (3 and 6 days) were studied, with the exception of the black fruit of cv. Arbequina, which were incubated only for 3 days to avoid the appearance of symptoms (Table 2). In all the cases, non-inoculated fruit were used as control treatments. A split-plot design with cultivar or maturity stages as main plot and inoculation type (inoculated or non-inoculated fruits) as subplot was used. There were three replicates (i.e., oil extractions) per treatment (i.e., combinations of maturity, cultivar, and incubation period, with 1.5 kg of detached olive fruit per treatment combination), with plastic trays as repetitions. The experiment was conducted twice.

Experiment II. Effect of visible infections on olive oil quality

Black fruit (stage 5) of cvs. Arbequina, Hojiblanca, and Picual were used to evaluate the impact of different proportions (0, 5, 10, 20, 40 and 80%) of artificially infected symptomatic fruit on the oil quality (Table 2). Inoculated fruit remained in the controlled environment chambers for 14 days (cvs. Arbequina and Hojiblanca) or 21 days (cv. Picual), until the fruit was rotted completely. A randomized design with three replicates (i.e., oil extractions) per treatment (i.e. combinations of cultivar and proportion of symptomatic fruit, with 1.5 kg

Table 2 Experimental design of experiments I and II, relatives to the study of the effect of *Colletotrichum godetiae* infection on olive oil quality

	Asymptomatic infections (*Experiment I*)			Symptomatic infections (*Experiment II*)		
Cultivar	Arbequina	Hojiblanca	Picual	Arbequina	Hojiblanca	Picual
Fruit maturity stage[x]	1, 3 and 5	3	3	5	5	5
Days of latency	3, 6[y]	3	3	14[z]	14	21
Infected fruit (%)	100	100	100	0, 5, 10, 20, 40, 80	0, 5, 10, 20, 40, 80	0, 5, 10, 20, 40, 80

[x] Rating scale based on Barranco et al. (2017)

[y] The treatment of six latency-days was conducted only on stages 1 and 3 to avoid the onset of olive anthracnose symptoms

[z] Number of days until 100% of symptomatic fruit were in a soapy stage due to the beginning of the conidial production

of detached olive fruit per treatment combination) was used. The experiment was done twice.

Disease severity was assessed by using a 0–5 rating scale (Moral and Trapero 2009). Only olive fruit with their entire surface affected by the disease were used (value 5 on the scale). The healthy fruit of each cultivar were weighed to get the different proportions of symptomatic fruit (i.e. 0.15 kg of affected fruits and 1.35 of healthy fruits for the treatments with 10% of infected fruits).

Extraction and evaluation of olive oil

An Abencor system (MC2 Ingeniería y Sistemas, Sevilla; Martínez et al. 1975) was used for oil extraction. The process was performed, reproducing the oil factory process without using any chemical substances. As the Abencor system has eight thermo-mixers, we used four mixers for control oils. Thermo-mixers were cleaned and disinfested with an ethanol solution (70% v/v) between samples. Sets of fruit (i.e., 1.5 kg of fruit per repetition) were ground to a paste using the hammer mill, stirred in the thermo-mixer for 30 min at 28 ± 1 °C and centrifuged for 2 min to separate the oil, which was collected, and decanted in graduated cylinders. Finally, the oil was stored in darkness at 5 °C until their evaluation, no more than 7 days after the extraction.

Olive oil samples were transported to the Laboratorio Agroalimentario of Cordoba (Junta de Andalucía), where analytics of absorbance in UV (K232 and K270), free acidity, and peroxide index were performed based on the protocols of the National Accreditation Entity of Spain (ENAC). The analytics was repeated twice and the mean of the two averaged. K232 and peroxides indicate the primary oxidation; while K270 represents the degree of secondary oxidation. Acidity measures the amount of free fatty acids (Barranco et al. 2017). The magnitude of K232, K270, acidity and peroxide values were interpreted according to the thresholds of physicochemical parameters that categorize the olive oil quality based on the International Olive Council (Table 1).

Data analysis

Data were analyzed using Statistix 10 (Analytical Software, Tallahassee, FL). Box and whisker plots were performed to check the data distribution and perform subsequent statistical analysis. Analysis of variance (ANOVA) was used to analyse the data from Experiment I to test the interaction between oil from latent infection for each cultivar, phenological stage and incubation period on oil quality parameters of K232, K270, acidity and peroxides. ANOVA was done on the data from Experiment II to evaluate the interaction between cultivar and percentage of symptomatic fruit on the four oil parameters. In experiment 1, K232, K270, acidity and peroxide data were analysed according to a split-plot design. When the ANOVA showed significant differences among treatments, values were compared using Fisher's protected least significant difference (LSD) test at $P = 0.05$. ANOVA was applied after checking data for normality, homogeneity of variances and pattern of residuals. In experiment 2, the effect of olive cultivar on K232, K270, acidity and peroxides values of oils from symptomatic fruit was examined using an analysis of covariance (ANCOVA), in which the percentage of symptomatic fruit was used as a covariable since there was a linear correlation between the percentage of symptomatic fruit and the original or

transformed values of oil quality parameters. Treatments were compared by LSD test at $P = 0.05$.

Pearson's correlation coefficients (r) were calculated between the parameters of the oils from asymptomatic and symptomatic fruit in both experiments. Linear and non-linear regression models were used to relate oil parameters with the proportion of symptomatic fruit in oil. The best regression model was chosen based on the coefficient of determination (R^2) and pattern of residuals over predicted and independent variables. For linear regression analysis, the inverse of variance was used as a weight variable. Based on these regressions, the thresholds of symptomatic fruit that may be present in the olive oil without causing loss of extra virgin, virgin and ordinary virgin categories were calculated per each cultivar. These thresholds were compared by the ANOVA and LSD test ($P = 0.05$).

Results

Experiment I. effect of latent infections on olive oil quality

The average acidity of oils from latent infected fruit slightly exceeded the threshold 0.8% to get the extra virgin olive oil qualification. The average acidity of oils from non-inoculated fruit (control) was 0.627%. The 29.6, 18.5 and 3.7% of the samples of oils from latent infected fruit exceed the 0.8, 2 and 3.3% thresholds of acidity, respectively (with respect to 18.5, 14.7 and 0% of control samples). Only control oil samples exceeded the 2.5 and 2.6 K232 thresholds (11.11 and 7.4%, respectively) (Fig. 1). There was no effect of the incubation period or interaction between incubation period and inoculation type on K232, K270, acidity and peroxides. The K232 parameter was positively correlated with peroxides in oil from asymptomatic fruit ($r = 0.868$; $P < 0.001$) (Table 3).

For the K232 parameter, there were significant differences ($P < 0.001$) in oils from the three olive cultivars in the veraison stage, but there was no difference between inoculated and non-inoculated fruit of each cultivar ($P = 0.230$) Oils from cv. Arbequina fruit showed the highest K232 values (2.5) while cv. Picual (1.5) the lowest values (Fig. 2A). In cv. Arbequina, maturity stage and inoculation type influenced K232. Oils from latent infected fruit of cv. Arbequina showed lower K232 than control oils in all the three maturity stages (Fig. 2B).

For the K270 values, interactions between cultivar and inoculation type (inoculated or non-inoculated) and between maturity stage and inoculation type were found ($P < 0.05$). Moreover, cultivar and maturity stage significantly influenced on K270 ($P < 0.05$). Oils from cv. Hojiblanca fruit at the veraison stage, both inoculated or not (0.1), and from non-inoculated veraison fruit (0.1) showed the highest values of K270 (Fig. 3A, B).

In relation to acidity, there were interactions between cultivar and inoculation type and between phenology and inoculation type ($P < 0.05$). Oils from fruit with latent infection of cv. Arbequina at the veraison stage (1.7%) showed a highest acidity than controls ($P \leq 0.01$) (Fig. 4A, B). Oils from green-yellowish inoculated fruit reached the same acidity level than oils from black fruit (Fig. 4B).

Finally, there were no interactions between cultivar and inoculation type, and phenology and inoculation type ($P > 0.0.05$) for peroxides index. Cultivar variable and phenology had an effect on peroxides ($P \leq 0.01$). Oils from veraison fruit of cv. Picual (7.2–8.0) and from green-yellowish fruit of cv. Arbequina (5.2–5.23) showed the lowest values of peroxides (Fig. 5A, B).

Experiment II. Effect of symptomatic fruit on olive oil quality

The percentage of symptomatic fruit affected the intensity of the red colour of the extracted oil, with oils from affected fruits of 'Arbequina' showing the highest red intensities (Fig. 6A, B). The interaction between the percentage of symptomatic fruit and the cultivar significantly affected the four quality parameters ($P < 0.001$). Subsequently, the impact of the percentage of symptomatic fruit in olive oil quality parameters was individually studied for each cultivar. The values of K232 and K270 were lower in cv. Picual (1.8 and 0.1, respectively) than in cvs. Arbequina (2.1 and 0.1, respectively) and Hojiblanca (2.0 and 0.1, respectively) ($P < 0.035$). There were also differences in acidity among cultivars ($P < 0.001$). Thus, cultivar Arbequina oils showed the highest values (2.18% of free oleic acid), while these were 0.7 and 0.8% for the cvs. Hojiblanca and Picual, respectively. Peroxides were highest in the cv. Hojiblanca (11.7 meq O_2/kg of oil) and lowest in cv. Picual (8.8), with cv. Arbequina showing an intermediate value (10.0) ($P = 0.037$).

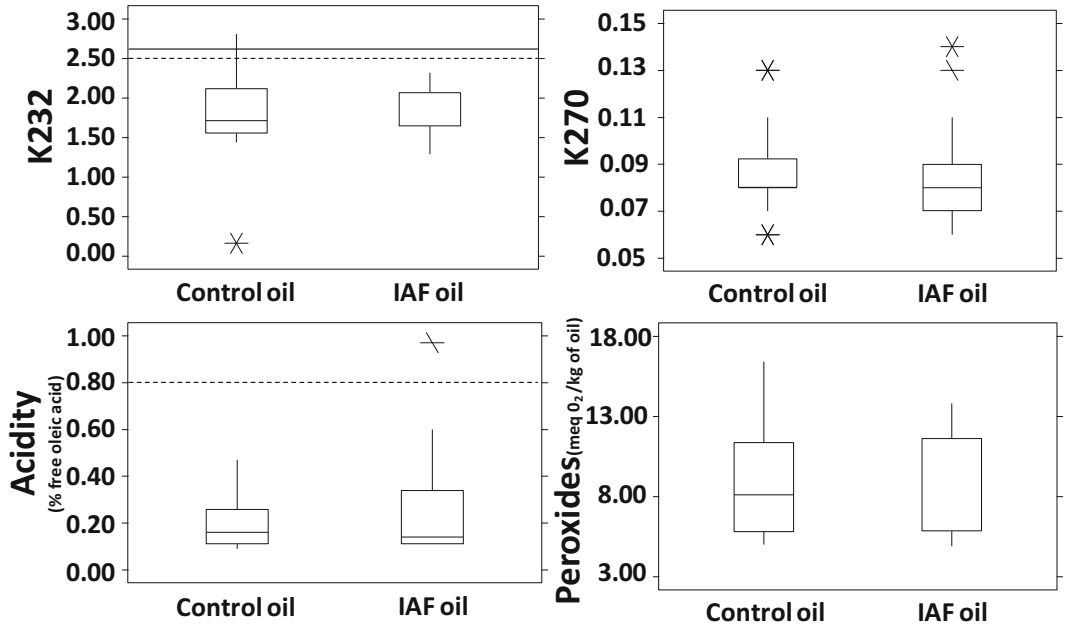

Fig. 1 Box and whisker plots of physicochemical parameters of the olive oil from healthy fruit (control oil) and from infected asymptomatic fruit by *Colletotrichum godetiae* (IAF oil). The horizontal line inside the box represents the median. The box represents the interquartile range, and the whiskers extend to the maximum and minimum values of the series or up to 1.5 times the interquartile range; * represents outliers (up to 3 times the interquartile range). The dashed and continuous lines represent the loss thresholds of extra virgin and virgin oil qualification, respectively (Table 1)

Overall, the K270 and acidity values increased with an increasing percentage of symptomatic fruits, a decreasing value of the K232 parameter and the peroxide index (Fig. 7, Table 4). Acidity levels higher than 0.8% (threshold of extra virgin qualification) were obtained with more than 2 6% of symptomatic fruit in oil from the cv. Arbequina. This percentage was lower than those obtained with cvs. Hojiblanca and Picual: 30.0 and 26.5%, respectively ($P < 0.001$). There were also threshold differences between the percentage of symptomatic fruit to obtain the virgin and ordinary virgin qualifications in every cultivar ($P < 0.001$ and $P = 0.003$, respectively) (Table 5). Based on Pearson's coefficients, K270 was positively correlated with acidity ($r = 0.737$; $P < 0.001$); and peroxides was correlated positively with K232 ($r = 0.883$; $P < 0.001$) and negatively with acidity ($r = -0.329$; $P = 0.019$) (Table 3).

Discussion

This study added new information on the effect of anthracnose infection on the oil quality of Spanish olive

Table 3 Pearson correlation coefficients (r) and P-values among physicochemical parameters of olive oils from fruit with latent infections or symptomatic infections by *Colletotrichum godetiae.*

Parameter	Oil from latent infected fruit								Oil from symptomatic fruit							
	K232		K270		Acidity		Peroxides		K232		K270		Acidity		Peroxides	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
K232	1.00								1.00							
K270	0.04	0.83	1.00						0.12	0.42	1.00					
Acidity	0.31	0.11	−0.27	0.17	1.00				0.04	0.77	0.73	<0.001	1.00			
Peroxides	0.87	<0.001	0.28	0.16	0.13	0.52	1.00		0.88	<0.001	−0.08	0.59	−0.33	0.019	1.00	

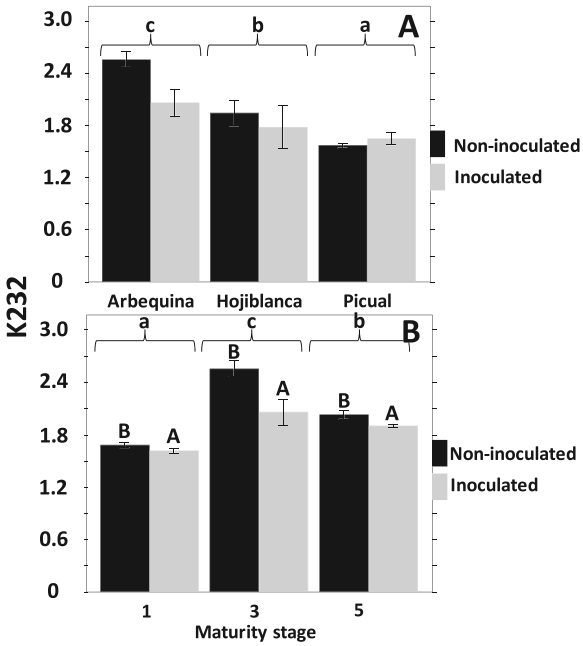

Fig. 2 Effect of fruit with latent infections by *Colletotrichum godetiae* (inoculated) vs. healthy fruit (non inoculated) on K232 of olive oil coming from **A)** three different cultivars at the veraison stage and **B)** cultivar Arbequina at three maturity stages (1: green-yellowish, 3: veraison, and 5: black fruit). Bars with the same letter are not significantly different according to Fisher's protected LSD test at $P = 0.05$. In Fig. B, lowercase letters show differences between maturity stages and uppercase letters between inoculation type for each maturity stage

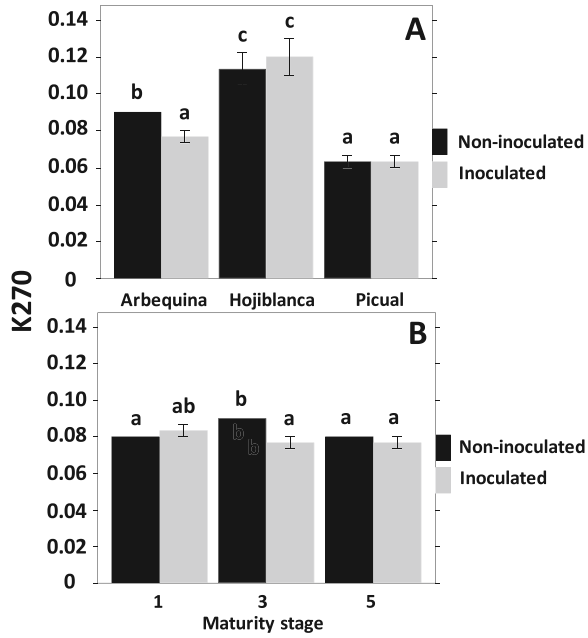

Fig. 3 Effect of fruit with latent infections by *Colletotrichum godetiae* (inoculated) vs. healthy fruit (non-inoculated) on K270 of olive oil coming from **A)** three different cultivars at the veraison maturity stage and **B)** cultivar Arbequina at three maturity stages (1: green-yellowish, 3: veraison, and 5: black fruit). Bars with the same letter are not significantly different according to Fisher's protected LSD test at $P = 0.05$

cultivars infected by *C. godetiae*, one of the prevailing *Colletotrichum* species associated to olive anthracnose in the Mediterranean basin. The acidity and K232 values were consistently high in all the oil samples and exceeded quality thresholds of olive oil. It could be justified by the incubation period to which fruit were subjected for a fair comparison among inoculation type. The incubation period may facilitate the action of bacteria and yeasts (Barranco et al. 2017; García-Figueres 1998). Although the previous disinfestations of the material in contact with the fruit and bacteria and yeast growth was no visually detected, they can cause the deterioration of the oil. The maturity stage and the cultivar affected the physicochemical oil parameters. There was a noteworthy increase of the acidity values of cv. Arbequina oils from latent infected fruit, which agrees with the well-known instability of cv. Arbequina oil, especially compared to the cv. Picual (Barranco et al. 2017) The highly positive correlation between K232 and peroxides can be easily explained since both measure primary oxidation (Barranco et al. 2017).

Cultivar has an effect on the oil quality of symptomatic fruit. Percentage of symptomatic fruit was related to the four physicochemical parameters. Reddish coloration intensity was directly correlated to the proportion of symptomatic fruit. This discoloration was previously noticed by Moral et al. (2014) as a sign of acidity increase and poor organoleptic characteristics. Linear and exponential equations highly explained the relationship between the percentage of symptomatic fruit and the physicochemical parameters in the oil derived from them. K270 and acidity values of the oil were positively related to the percentage of symptomatic fruit. The thresholds on percentage of symptomatic fruit causing the loss of olive oil commercial categories were lower in cv. Arbequina than in cvs. Hojiblanca and Picual. A threshold of 2.6% was obtained for 'Arbequina', and 29.9% for 'Hojiblanca' and 26.5% for 'Picual, to lose the extra virgin category. These results agree with the constitutive instability of the oil of cv. Arbequina aspreviously found (Barranco et al. 2017). All the results of this study were obtained with fruit with 100% disease severity. This severity was selected because the time elapsed between the appearance of the first anthracnose

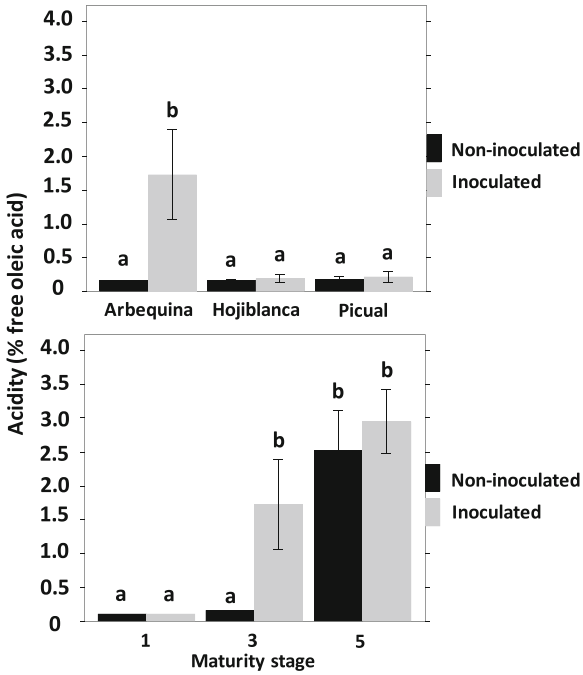

Fig. 4 Effect of fruit with latent infections by *Colletotrichum godetiae* (inoculated) vs. healthy fruit (non-inoculated) on acidity of olive oil coming from **A)** three different cultivars at the veraison maturity stage and **B)** cultivar Arbequina at three maturity stages (1: green-yellowish, 2: veraison, and 3: black fruit). Bars with the same letter are not significantly different according to Fisher's protected LSD test at $P = 0.05$

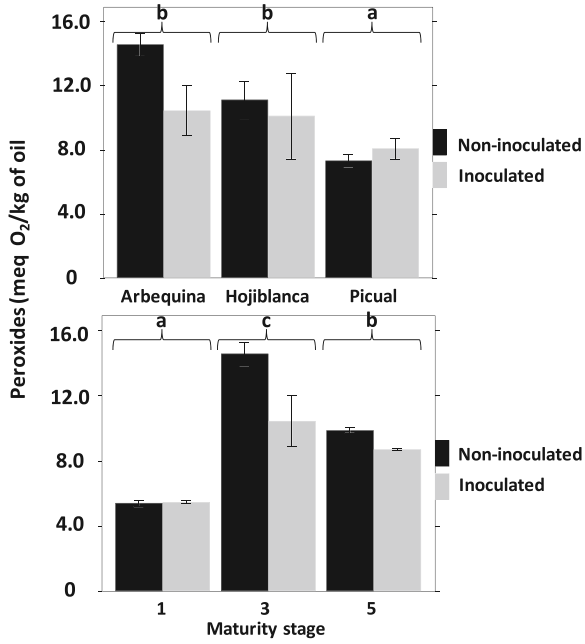

Fig. 5 Effect of fruit with latent infections of *Colletotrichum godetiae* (inoculated) vs. healthy fruit (non-inoculated) on peroxide index of olive oil coming from **A)** three different cultivars at the veraison stage and **B)** cultivar Arbequina at three maturity stages (1: green-yellowish, 3: veraison, and 5: black fruit). Bars with the same letter are not significantly different according to Fisher's protected LSD test at $P = 0.05$

symptoms and the presence of a severity of 100% takes on average 36 days according to Moral and Trapero (2009), which is usually less than the time elapsed between the disease outbreak and the harvest under Mediterranean conditions. So, fruits with an affected surface of 100% are the most common when reaching the oil mills. Ianotta et al. (1999) previously cited that the extra virgin oil qualification is lost with > 20% of affected fruit (cv. Sinopolese) due to acidity and >40% due to peroxides (anthracnose severity was unknown). Meanwhile, Carvalho et al. (2008) defined a threshold of 16% of symptomatic fruit (with anthracnose severity > 50%) in 'Galega' oil to lose this category due to acidity. Leoni et al. (2018), working with a cultivar also tested in the present study (cv. Arbequina), obtained a slightly higher threshold: 7 and 13%, when affected fruit showed 50–100% or 0–25% of affected fruit surface, respectively. These higher values may be due to a different *Colletotrichum* species used to inoculate the fruit in their study (*C. acutatum* instead of *C. godetiae*), or the use of symptomatic fruits but not completely rotted. In addition, Leoni et al. (2018) calculated the thresholds for the highly resistant cv. Frantoio. In this case, when anthracnose severity was 0–25%, more than 50% of symptomatic fruit were necessary to lose the extra virgin category. When the severity was higher (50–100%), only ≈4% of affected fruit was required. Although the

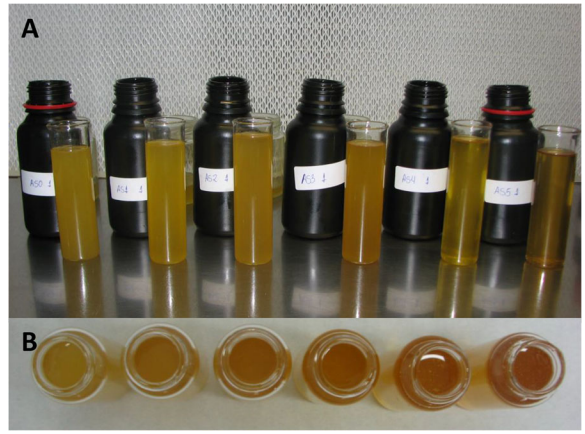

Fig. 6 Reddish coloration of cv. Arbequina olive oil extracted from an increasing percentage of fruit with symptoms of olive anthracnose (0, 5, 10, 20, 40 and 80%, from left to right). Olive oil filled tubes in a front view (A) or a top view (B)

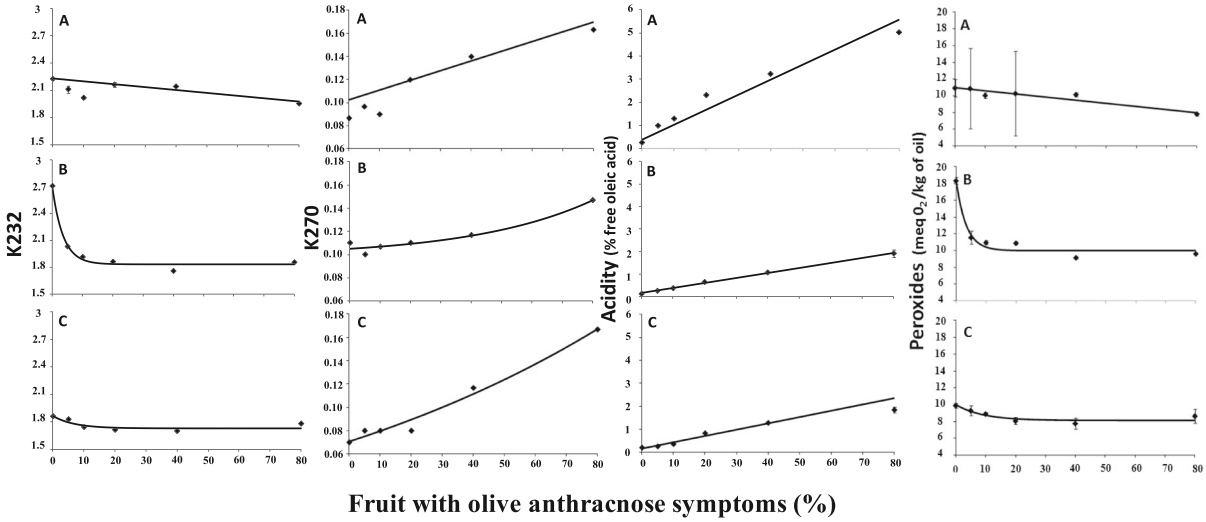

Fig. 7 Effect of the percentage of fruit with olive anthracnose symptoms on four quality parameters (K232, K270, acidity, peroxides) of the olive oil from three cultivars: Arbequina (A), Hojiblanca (B) and Picual (C). Regression lines represent the equations showed in Table 3. Points are the average of six repetitions with their standard errors

cv. Frantoio was not included in our study; our results agree with those of Leoni et al. (2018) regarding the fact that the olive cultivars most susceptible to anthracnose are not necessarily the most sensitive to alterations in the quality of their oils due to the disease.

The indexes K232 and peroxides were negatively related to the percentage of symptomatic fruit from which the oil was derived and these indexes are highly correlated with each other. Ianotta et al. (1999) obtained opposite results with cv. Sinopolese with oil from fruit symptomatic and naturally infected. These oils were less stable and had a higher peroxide index with increasing concentrations of symptomatic fruit, even losing the extra virgin category due to this parameter. The index K232 also increased in Ianotta et al. (1999) study, although without the loss of commercial category.

Table 4 Lineal and exponential regressions adjusted to relate the effect of the percentage of fruit with anthracnose symptomsonfour physicochemical parameters of olive oil

Parameter	Cultivar	Equation[a]	Equation parameters[b]			R^2
			a	b	c	
K232	Arbequina	1	$2.23\pm5.33\times10^{-2}$	$-3.18\times10^{-2}\pm9.55\times10^{-4}$	–	0.73
	Hojiblanca	2	$1.83\pm2.94\times10^{-2}$	$0.88\pm5.81\times10^{-2}$	$-0.28\pm5.00\times10^{-2}$	0.98
	Picual	2	$1.73\pm2.76\times10^{-2}$	$0.14\pm4.82\times10^{-2}$	$-0.15\pm1.20\times10^{-2}$	0.74
K270	Arbequina	1	$0.10\pm3.91\times10^{-4}$	$8.35\times10^{-4}\pm1.60\times10^{-4}$	–	0.90
	Hojiblanca	2	$9.90\times10^{-2}\pm1.23\times10^{-2}$	$6.52\times10^{-3}\pm9.87\times10^{-3}$	$2.58\times10^{-2}\pm1.60\times10^{-2}$	0.96
	Picual	2	$-4.10\times10^{-2}\pm0.16$	0.11 ± 0.16	$7.83\times10^{-3}\pm8.20\times10^{-3}$	0.98
Acidity	Arbequina	1	0.37 ± 0.12)	$6.59\times10^{-2}\pm7.84\times10^{-3}$	–	0.95
	Hojiblanca	2	-74.09 ± 599.22	74.25 ± 599.20	$3.00\times10^{-4}\pm2.40\times10^{-3}$	0.99
	Picual	1	$0.16\pm4.68\times10^{-2}$	$2.77\times10^{-2}\pm2.70\times10^{-3}$	–	0.96
Peroxides	Arbequina	1	10.98 ± 0.42	$-3.78\times10^{-2}\pm7.52\times10^{-3}$	–	0.86
	Hojiblanca	2	9.99 ± 0.46	8.31 ± 0.93	-0.30 ± 0.10	0.96
	Picual	2	8.15 ± 0.30	1.83 ± 0.484	$-0.11\pm6.94\times10^{-2}$	0.83

[a] 1: 1: $y = a + b \times x$; 2: $y = a + b\,(\mathrm{Exp}(c \times x))$

[b] Equation parameters estimated from six repetitions. Each average is complemented by its standard error

Table 5 Percentage of symptomatic olive fruit infected by *Colletotrichum godetiae* which cause the loss of the extra virgin, virgin and ordinary virgin oil quality categories, based on the acidity (% free oleic acid) parameter

Cultivar	Symptomatic fruit (%) causing the loss of category		
	Extra virgin	Virgin	Ordinary virgin
Arbequina	2.6 a[a]	22.8 a	44.7 a
Hojiblanca	29.9 b	98.3 c	>100.0 b
Picual	26.5 b	78.9 b	>100.0 b

[a] For each column, means with the same letter are not significantly different according to Fisher's protected LSD test at $P = 0.05$

Carvalho et al. (2008) corroborated the data related to the decrease in oxidative stability using the Portuguese cv. Galega. Conversely, following a methodology like that carried out in this study, Leoni et al. (2018) also obtained a negative relationship between the percentage of symptomatic fruit in oil and K232 and peroxides by using fruit artificially infected in their study with the cvs. Arbequina and Frantoio. However, the linear relationships fitted in their work with both physicochemical parameters were not significant ($P > 0.05$), except cv. Arbequina oil and peroxide values; and these linear regressions had a lower negative slope than those obtained in this study. Apart from using a *Colletotrichum* isolate of a different species (*C. acutatum*), these results can be explained in different ways. The absence of significant regressions could be due to not having tested non-linear regressions to relate percentages of symptomatic fruit in oil and the values of the physicochemical parameters. Besides, the lower negative slope might be explained by the lower latency period (5–7 days), and the use of no completely rotten fruit implying a level of primary oxidation compounds still reduced in oils extracted from lots with a low number of symptomatic fruits. Therefore, an increase in oxidation of primary compounds is still possible as the percentage of symptomatic fruit in the oil increases. The level of primary oxidation compounds (i.e., K232 and peroxides) could be reduced if all of the oxidation of these compounds has already occurred, which exclusively undergo secondary oxidation (i.e., K270) (Barranco et al. 2017). In other words, the primary compounds may decrease from a certain level of oxidation despite the gradual deterioration of oil quality, which the number of secondary oxidation compounds would only reflect. It is in line with cv. Hojiblanca oils coming from a low percentage of symptomatic fruits showed higher levels of K232 and peroxides than those of the other cultivars, one i.g. 'Arbequina' being more unstable (Barranco et al. 2017) and the other i.g. 'Picual' suffering a period of longer latency due to its higher resistance to anthracnose. In this way and due to the methodology used, based on a latency period after artificial inoculation, the K270 and acidity parameters represent the gradual deterioration of oil caused by *Colletotrichum godetiae*.

This study shows the relevance of anthracnose as an explanatory variable of olive oil quality, demonstrating the adverse effects of both latent and symptomatic *Colletotrichum* infections. Similar results have been recently obtained by Peres et al. (2021), who have confirmed thenegative impact of the dominant species of *Colletotrichum* spp. in Portugal (*C. acutatum*, *C. godetiae* and *C. nymphaeae*) on oils of 'Galega Vulgar' and 'Cobrançosa'. Overal, the finalincidence of affected fruit may be relevant to decide the strategy of chemical control of the disease or even come early the olive harvesting time, once it has been demonstrated that there is a clear deterioration of the oil quality when the affected fruit show visible symptoms. As a result, a decrease in fat content may be desirable if the deterioration of oil quality is avoided. These management practices could be especially recommended in high-density plantations with favourable conditions to epidemic development and the appearance of symptomatic fruit (Moral et al., 2012). Furthermore, the development of a decision support system integrating models predicting the incidence of olive anthracnose (Romero et al., 2021) would facilitate the decision making of farmers and technicians because it could be estimated the risk of getting low quality oils.

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Declarations

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Research involving human participants and/or animals This research is focused on the study of the effect of fungi on oil quality. This article does not contain any experiments with human participants or animals.

Informed consent Please be informed that authors are satisfied to publish this work in European Journal of Plant Pathology.

References

Barranco, R., Fernández-Escobar, & Rallo, L. (2017). *El cultivo del olivo*. Mundi-Prensa.

Boller, E. F., Avilla, J., Joerg, E., Malavolta, C., Wijnands, F. G., & Esbjerg, P. (2004). *Guidelines for integrated production - principles and technical guidelines*. IOBC/WPRS Bulletin.

Cacciola, S. O., Faedda, R., Sinatra, F., Agosteo, G. E., Schena, L., Frisullo, S., et al. (2012). Olive anthracnose. *Journal of Plant Pathology, 94*, 29–44.

Carvalho, M. T., Simoe-Lopes, P., & Monteiro da Silava, M. J. (2008). Influence of different olive infection rates of *Colletotrichum acutatum* on some important olive oil chemical parameters. *Acta Horticulturae, 791*, 555–559.

Chattaoui, M., Raya, M. C., Bouri, M., Moral, J., Pérez-Rodriguez, M., Trapero, A., et al. (2016). Characterization of a *Colletotrichum* population causing anthracnose disease on olive in northern Tunisia. *Journal of Applied Microbiology, 20*, 1368–1381.

García-Figueres, F. (1998). Micosis de las aceitunas y su incidencia en la calidad del aceite. *Phytoma España, 102*, 171–175.

FAO, 2019. http://www.fao.org/faostat/en/#data/QC. Accesed 20 October 2019.

Iannotta, I., Perri, E., Siriani, R., & Tocci, C. (1999). Influence of *Colletotrichum gloeosporioides* (Penzing) and *Camarosporium dalmatica* (Thum) attacks on olive oil quality. *Acta Horticulturae, 474*, 399–401.

IOC – International Olive Council, 2018. Trade Standard Applying to Olive Oils and Olive-pomace Oils. COI/T.15/NC No 3/Rev. 12. http://www.internationaloliveoil.org/estaticos/view/222-standards, Accessed 19 September 2019.

Leoni, C., Bruzzone, J., Villamil, J. J., Martínez, C., Montelongo, M. J., Betancur, O., et al. (2018). Percentage of anthracnose (*Colletotrichum acutatum* s.s.) acceptable in olives for the production of extra virgin olive oil. *Crop protection, 108*, 47–53.

Martínez, S. J., Muñoz, A. E., Alba, M. J., & Lanzon, R. A. (1975). Informe sobre utilización del Analizador de Rendimientos "Abencor". *Grasas y Aceites, 26*, 379–385.

Materatski, P., Varanda, C., Carvalho, T., Bento, A., Doroteia, M., Rey, F., et al. (2018). Diversity of *Colletotrichum* species associated with olive anthracnose and new perspectives on controlling the disease in Portugal. *Agronomy, 8*. https://doi.org/10.3390/agronomy8120301

Moral, J., Agustí-Brisach, C., Agalliu, G, Rodrigues de Oliveira, Pérez-Rodríguez, M., Roca, L.F.et al. (2018). Preliminary selection and evaluation of fungicides and natural compounds to control olive anthracnose caused by *Colletotrichum* species. *Crop Protection*, *114*, 167–176.

Moral, J., Bouhmidi, K., & Trapero, A. (2008). Influence of fruit maturity, cultivar susceptibility, and inoculation method on infection of olive fruit by *Colletotrichum acutatum*. *Plant Disease, 92*, 1421–1426.

Moral, J., Jurado-Bello, J., Sánchez, M. I., Oliveira, R., & Trapero, A. (2012). Effect of temperature, wetness duration, and planting density on olive anthracnose caused by *Colletotrichum* spp. *Phytopathology, 102*, 974–981.

Moral, J., Oliveira, R., & Trapero, A. (2009). Elucidation of disease cycle of olive anthracnose caused by *Colletotrichum acutatum*. *Phytopathology, 99*, 548–556.

Moral, J., Xavién, C., Roca, L. F., Romero, J., Moreda, W., & Trapero, A. (2014). La Antracnosis del olivo y su efecto en la calidad del aceite. *Grasas y Aceites, 65*(2), e028. https://doi.org/10.3989/gya.110913

Moral, J., & Trapero, A. (2009). Assessing the susceptibility of olive cultivars to anthracnose caused by *Colletotrichum acutatum*. *Plant Disease, 93*, 1028–1036.

Moral, J., & Trapero, A. (2012). Mummified fruit as a source of inoculum and disease dynamics of olive anthracnose caused by *Colletotrichum* spp. *Phytopathology, 102*, 982–989.

Moral, J., Xaviér, C., Viruega, J. R., Roca, L. F., Caballero, J., & Trapero, A. (2017). Variability in susceptibility to anthracnose in the world collection of olive cultivars of Cordoba (Spain). *Frontiers in Plant Science, 8*. https://doi.org/10.3389/fpls.2017.01892

Moreira, V., Mondino, P., & Alaniz, A. (2021). Olive anthracnose caused by *Colletotrichum* in Uruguay: Field symptoms, species diversity and flowers and fruits pathogenicity. *European Journal of Plant Pathology, 160*, 663–681.

Nigro, F., Antelmi, I., Labarile, R., Sion, V., & Pentimone, I. (2018b). Biological control of olive anthracnose. *Acta Horticulturae, 1199*, 439–444.

Nigro, F., Antelmi, I., & Sion, V. (2018a). Integrated control of aerial fungal diseases of olive. *Acta Horticulturae, 1199*, 327–332.

Peres, F., Talhinhas, P., Afonso, H., Alegre, H., Oliveira, H., & Ferreira-Dias, S. (2021). Olive oils from fruits infected with different anthracnose pathogens show sensory defects earlier than chemical degradation. *Agronomy, 11*, 1041.

Pesce, V. M., Nally, M. C., Carrizo, G. P., Rojo, C., Pérez, B. A., Toro, M. E., Castellanos de Figueroa, L. I., & Vazquez, F. (2018). Antifungal activity of native yeasts from different microenvironments against Colletotrichum gloeosporioides on ripe olive fruits. *Biological Control, 120*, 43–51.

Romero, J., Agustí-Brisach, C., Santa-Bárbara, A. E., Cherifi, F., Oliveira, R., Roca, L. F., et al. (2017). Detection of latent infections caused by *Colletotrichum* spp. in olive fruit. *Journal of Applied Microbiology, 124*, 209–219.

Romero, J., Moral, J., González-Domínguez, E., Agustí-Brisach, C., Roca, L. F., Rossi, V., et al. (2021). Logistic models to predict olive anthracnose under field conditions. *Crop Protection, 148*, 105714.

Runcio, A., Sorgona, L., Mincione, A., Santacaterina, S., & Poiana, M. (2008). Volatile compounds of virgin olive oil obtained from Italian cultivars grown in Calabria. Effects of processing methods, cultivar, stone removal, and anthracnose attack. *Food Chemistry, 106*, 735–740.

Schena, L., Abdelfattah, A., Mosca, S., Nicosia, M. G. L. D., Agosteo, G. E., & Cacciola, S. O. (2017). Quantitative detection of *Colletotrichum godetiae* and *C. acutatum sensu stricto* in the phyllosphere and carposphere of olive during four phenological phases. *European Journal of Plant Pathology, 149*, 337–347.

Talhinhas, P., Loureiro, A., & Oliveira, H. (2018). Olive anthracnose: A yield-and oil quality-degrading disease caused by several species of *Colletotrichum* that differ in virulence, host preference and geographical distribution. *Molecular Plant Pathology, 19*, 1797–1807.

Talhinhas, P., Sreenivasaprasad, S., Neves-Martins, J., & Oliveira, H. (2005). Molecular and phenotypic analyses reveal association of diverse *Colletotrichum acutatum* groups and a low level of *C. gloeosporioides* with olive anthracnose. *Applied and Environmental Microbiology, 71*, 2987–2998.

Viruega, J. R., Roca, L. F., Moral, J., & Trapero, A. (2011). Factors affecting infection and disease development on olive leaves inoculated with *Fusicladium oleagineum*. *Plant Disease, 95*, 1139–1146.