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Colletotrichum infections during flower development and fruit ripening in four olive cultivars

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Summary. Olive anthracnose, caused by *Colletotrichum*, is an important disease in olive-growing regions, with the most destructive symptoms being fruit rot and blossom blight. Susceptibility of fruit to *Colletotrichum* increases with maturity, but differences between cultivars and *Colletotrichum* species have been reported, still information on flower susceptibility during development is scarce. The susceptibility of the olive cultivars Arbequina, Coratina, Frantoio and Picual was evaluated during flower development and fruit maturity to *Colletotrichum acutatum* s.s., *C. nymphaeae*, *C. fioriniae*, *C. theobromicola* and *C. alienum*. Susceptibility to anthracnose begins in early stages during flower development and increases during blossoming. Flowers of Arbequina, Coratina and Picual were susceptible, whereas those of Frantoio were moderately susceptible. Green fruit developed less anthracnose than mature fruit. At the green fruit stage, Arbequina and Frantoio were the most susceptible, Coratina was intermediate, and Picual was moderately susceptible, while no differences were found among the cultivars at mature fruit stages. No mayor differences were found among the *Colletotrichum* species with exception of *C. theobromicola*, which caused greatest severity at the green fruit stage. Future research should focus on developing anthracnose management strategies to minimize the disease progress from early stages of flower development and fruit ripening, especially in the most susceptible olive cultivars.

Keywords. Olive anthracnose, cultivar susceptibility, blossom blight, soapy rot.

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INTRODUCTION

Anthracnose is an important olive disease in olive-growing regions (Cacciola *et al.,* 2012; Moral *et al.,* 2014; Talhinhas *et al.,* 2018; Azevedo-Nogueira *et al.,* 2020), especially in those with humid climates such as South Africa (Gorter, 1956), Australia (Sergeeva *et al.*, 2008), Brazil (Filoda *et al.,* 2021) and Uruguay (Moreira *et al.*, 2021). Eighteen *Colletotrichum* species of the species complexes *C. acutatum*, *C. gloeosporioides* and *C. boninense* have been reported to be associated with this disease (Talhinhas *et al.,* 2011; Schena *et al.,* 2014; Chattaoui *et al.,* 2016; Moral *et al.,* 2017; Talhinhas *et al.,* 2018; Moreira

et al., 2021). In Uruguay, *C. acutatum* s.s. was found as the prevalent species, followed by *C. nymphaeae* and *C. fioriniae* belonging to the *C. acutatum* species complex, and *C. theobromicola* and *C. alienum* belonging to the *C. gloeosporioides* species complex (Moreira *et al.,* 2021).

The most common host symptom caused by anthracnose is fruit rot at the ripening stage. Fruit rot causes yield losses and deterioration in oil quality (Moral *et al.,* 2014; Leoni *et al.,* 2018). Infections can also occur from flowering to fruit ripening. During bloom, species of *Colletotrichum* can infect host plant calices, petals, stamens, and pistils causing flower collapse known as blossom blight (Moral *et al.,* 2009; Talhinhas *et al.,* 2018; Filoda *et al.,* 2021; Moreira *et al.,* 2021). Each infected flower is usually covered with an orange gelatinous mass of *Colletotrichum* conidia (Sergeeva *et al.,* 2008; Moreira *et al.,* 2021). If the flower is not destroyed, *Colletotrichum* infects the fruit set, remaining as latent until fruits ripen, when the typical soapy fruit is expressed (Moral *et al.,* 2009; Talhinhas *et al.,* 2018), although the importance of these infections for yield losses is unknown.

Infected fruits show depressed brown lesions that are rapidly covered with abundant orange gelatinous masses of *Colletotrichum* conidia. Fruits can be infected from green stages, and these infections remain latent infections until fruit maturation. The infected fruit can fall or remain mummified on trees, serving as a primary inoculum sources for subsequent infections in the next year (Moral *et al.,* 2008; 2014; Mosca *et al.,* 2014; Talhinhas *et al.,* 2018).

The Uruguayan climate is characterized by persistent high humidity, frequent rainfall (approx. 1,100 mm per year) and moderate temperatures (Leoni *et al.,* 2018; Conde-Innamorato *et al.,* 2019). These conditions favour olive anthracnose development. In addition, 50% of the plantations (currently 2788 ha) are of the Arbequina cultivar (MGAP-DIEA, 2021), which is moderately susceptible to this disease (Moral, *et al*., 2014; 2017; Leoni *et al.,* 2018). The remaining 50% of olive groves is planted mostly with cultivars such as Coratina, Picual and Frantoio, which have shown no resistance under these climatic conditions (Leoni *et al.,* 2018). These cultivars are mainly used for oil production for export (MGAP-DIEA, 2020).

Studies have shown that susceptibility of olive cultivars to anthracnose can be variable (Moral and Trapero, 2009; Cacciola *et al.,* 2012; Moral *et al.,* 2014; 2017), and depends on the *Colletotrichum* species (Talhinhas *et al.,* 2015). Fruit susceptibility varies according to developmental stage (Moral *et al.,* 2008; Moral and Trapero, 2009; Talhinhas *et al.,* 2015; Moral *et al.,* 2017). Nevertheless, information is limited on the susceptibility of different fruit maturity stages and different cultivars to *Colletotrichum*. Although flowers can be infected by *Colletotrichum* (Moral *et al.,* 2014; Kolainis *et al.,* 2020; Moreira *et al.,* 2021), there have been no studies that indicate when in flower differentiation the flowers become susceptible. Knowing when the first infections occur at flowering and their potential incidence is important so anthracnose management strategies can be developed, including those based on fungicides.

The present study focused on evaluating the susceptibility to five *Colletotrichum* species of the Arbequina, Coratina, Picual and Frantoio olive cultivars, the major cultivars produced in Uruguay. The study concentrated on flower development and fruit ripening host stages.

MATERIAL AND METHODS

Plant and fungal material

Apparently healthy olive flower panicles and fruit were collected from commercial orchards of the cultivars Arbequina, Coratina, Picual and Frantoio with scarce anthracnose. The panicles were collected at three different flowering stages, 1- swollen bud (BBCH51), 2- final differentiation (BBCH55), or 3- beginning of flowering (BBCH61), and fruits were collected at two physiological maturity stages, 1- green fruit (BBCH80) or 2- ripe fruit (BBCH89) (Sanz-Cortes *et al.,* 2002). The collected samples were stored in nylon bags in coolers until processing in the laboratory.

Fifteen *Colletotrichum* isolates belonging to *C. acutatum*. s.s (eight isolates), *C. nymphaeae* (three), *C. fioriniae* (one), *C. theobromicola* (two) and C. *alienum* (one isolate) were used in this study (Table 1). The previously identified isolates were selected from the olive *Colletotrichum* collection and deposited at the Plant Protection Department, Facultad de Agronomía, Universidad de la República, Uruguay (Moreira *et al.,* 2021).

For inoculum preparation, each isolate was grown on Potato Dextrose Agar (Oxoid Ltd) at 24°C under near UV-light with a 12-h photoperiod. After 7 d incubation, colony surfaces in culture plates were each flooded with 10 mL of sterile distilled water (SDW), and the scraped with a sterile spatel. The resulting conidium suspensions were filtered through layers of cheesecloth, and conidium concentration was adjusted to 1×10^6 conidia mL^{-1} with a hemacytometer.

Flower inoculation

Flowers in either the Stage 1- swollen bud (BBCH51) or Stage 2- final differentiation (BBCH55) were each surface-disinfected by dipping for 1 min in 1.0% NaClO

Table 1. Uruguayan *Colletotrichum* isolates used to evaluate the susceptibility of four olive cultivars at different phenological stages during flower development and fruit ripening.

Species Complex	Fungal species	Isolate	Olive cultivar	Geographical origin		Organ
C. acutatum species complex	*C. acutatum*	OL18	Arbequina	Maldonado	Garzón	Flower
		OL36	Arbequina	Montevideo	Melilla	Flower
		OL42	Arbequina	Treinta y Tres	Mendizabal	Flower
		OL51	Arbequina	Rocha	Nuevo Manantial	Leaf
		OL53	Arbequina	Rocha	Nuevo Manantial	Branch
		OL74	Picual	Rocha	Nuevo Manantial	Fruit
		OL92	Coratina	Maldonado	Garzón	Fruit
		OL97	Arbequina	Montevideo	La Paz	Fruit
	C. nymphaeae	OL28	Arbequina	Treinta y Tres	Mendizabal	Flower
		OL96	Arbequina	Montevideo	La Paz	Fruit
		OL113	Arbequina	Canelones	Las Brujas	Fruit
	C. fioriniae	OL23	Arbequina	Montevideo	La Paz	Flower
C. gloeosporioides species complex	*C. theobromicola*	OL110	Manzanilla	Canelones	Las Brujas	Fruit
		OL112	Arbequina	Canelones	Las Brujas	Fruit
	C. alienum	OL98	Arbequina	Montevideo	Melilla	Fruit

solution, and then rinsed three times with SDW. For the Stage 3- beginning of flowering (BBCH61) flowers, surface disinfection was not possible due the sensitivity of flower petals to NaClO. After air-drying, the flowers were dipped in respective isolate conidium suspensions for 30 s, placed in transparent plastic trays containing moistened filter paper, and then incubated at 24°C with a 12 h photoperiod. Control treatments inoculated with SDW were included for each cultivar and flower phenological stage. Three repetitions for each cultivar, phenological stage and *Colletotrichum* isolate were used. Each repetition consisted of at least eight swollen buds for Stage 1, two panicles each with at least 15 undeveloped flower buds for Stage 2, and two panicles with at least ten open or semi-open flowers for Stage 3. Each experiment was performed using a completely randomized design with factorial arrangement.

Anthracnose incidence was assessed periodically until the plant material was destroyed or until 100% incidence was achieved, after 12 d for swollen buds, 6 d for final differentiation, or days for beginning of flowering. Incidence was calculated as the percentage of affected buds or flowers in the total number of buds or flowers evaluated. In each evaluation, the initial symptoms and their evolution was recorded as the number of necrotic buds or flowers, and the presence of gelatinous mass of *Colletotrichum* conidia.

Fruit inoculation

Fruits at the Stage 1- green fruit (BBCH80) or Stage 2- ripe fruit (BBCH89) were surface-disinfected by dipping for 3 min in 1% NaClO solution, and then rinsed three times with SDW. After air-drying, the fruits were dipped in respective isolate conidium suspensions for 30 s, and were then placed into plug seedling trays, one fruit in each hole. The plug seedling trays were enclosed in moistened transparent nylon bags, and were then incubated at 24°C with a 12 h photoperiod. Control treatments inoculated with SDW were included for each cultivars and fruit phenological stages. Four repetitions of fruits per cultivar, phenological stages and *Colletotrichum* isolates were used. Each repetition consisted of five fruits, and each experiment was carried out using a completely randomized design with factorial arrangement.

Anthracnose severity was periodically assessed during 50 d for green fruit and 18 d for mature fruit. The 0–5 scale proposed by Moral *et al.,* (2008) was used, where 0 = no visible lesions, 1 = visible lesions affecting <25% of the fruit surface, 2 = 25–49%, 3 = 50–74%, 4 = 75–100% of fruit surface, and 5 = soapy fruit (fruit completely covered with gelatinous mass of *Colletotrichum* spores). Presence of gelatinous masses of *Colletotrichum* conidia was also recorded.

Statistical analyses

Flower anthracnose incidence was plotted for the three flower phenological stages of the four olive cultivars. A regression curve was fitted considering the significance of the regression and the coefficient of determination (R^2), based on average incidence of the 15 *Colletotrichum* isolates and evaluation time. Anthrac-

nose incidence values were also used to calculate the area under the disease progress curve (AUDPC) for each *Colletotrichum* species, using the following formula:

$$AUDPCi = \sum_{i=1}^{n}[(I_{i+1}+I)/2]\ (t_i+1-t_i),$$

where I = incidence (%) at i*th* observation, ti = time (d) at the i*th* observation, and *n* = the total of number of observations.

The AUDPC data were analyzed for normality with the Shapiro-Wilk test, and for homogeneity with the Levene test. These data were subjected to ANOVA with a factorial arrangement, with olive cultivar and *Colletotrichum* species as factors. the treatments means were compared using Tukey's test ($P \leq 0.05$).

Fruit anthracnose severity values were used to calculate the McKinney's Index (Moral *et al.,* 2017), using the following formula:

$$McKinney's\ Index = \frac{\sum(n_i\ x\ i)}{5\ x\ N} \times 100$$

where i = the severity of symptoms (0 to 5), n_i = the number of fruits with the severity of i, and N = the total number of evaluated fruits.

The McKinney's Index values at the two maturity stages and for the four olive cultivars were plotted. A regression model was adjusted for each *Colletotrichum* species considering the statistical significance of the regression and the coefficient of determination (R^2). Anthracnose severity values were used to calculate the AUDPC for each *Colletotrichum* species using the formula outlined above. The AUDPC data were analyzed for normality with the Shapiro-Wilk test, and for homogeneity with the Levene test. The AUDPC was transformed to when necessary, to comply with normality assumptions. Then, the AUDPC data were subjected to ANOVA, with a factorial arrangement, with olive cultivar and *Colletotrichum* species as factors. The treatments means were compared using by Tukey's test ($P \leq 0.05$). Statistical analyses were carried out using the programs InfoStat version 2016 (http://www.infostat.com.ar) and SigmaPlot version 12.0 (http://www.sigmaplot.co.uk).

RESULTS

Flower infections

Typical anthracnose symptoms and signs developed in flowers at the three phenological stages in the four cultivars inoculated with the *Colletotrichum* species. No symptoms were observed in control treatments (Figure 1, a to c). Initial symptoms consisted of brownish colouration of the swollen buds or the flower buds at final differentiation, and necrotic lesions on flower petals (Figure 1, a.2 to c.2). The affected organs then quickly blighted, and were covered with orange-salmon coloured gelatinous masses containing abundant *Colletotrichum* conidia (Figure 1, a.3 to c.3). Accelerated detachment of swollen buds, flower buds and open flowers was observed in comparison with the control treatments, where the buds or flowers remained attached for longer periods.

Almost all graphs of blossom blight incidence fitted exponential curves (Figure 2 and Supplementary Table 1). For the AUDPC variables, statistically significant interactions were found between olive cultivars and *Colletotrichum* species inoculated in the three flowering phenological stages (Table 2). The lowest anthracnose incidence was observed at the swollen bud stage, and no major differences were recorded among the four olive cultivars. In this stage, the first symptoms appeared approx. 4 to 7 d after inoculation, whereas the greatest anthracnose incidence was recorded at 12 d, and ranged between 20 and 40% (Figure 2).

At final differentiation stage, the first symptoms were observed at 3 d after inoculation, with the least average incidence (2.0%) in the Frantoio cultivar and the greatest (17%) in the Arbequina cultivar. Incidence then progressed rapidly, and 6 d after inoculation reached 59 for Frantoio and 100% for Arbequina. At Stage 3-beginning flowering greatest anthracnose incidence was recorded. Two d after inoculation, incidence ranged from 8% for Frantoio to 50% for Picual, and 2 d later between 45% for Frantoio and 91% for Arbequina (Figure 2, Table 3).

Except for *C. alienum* inoculated onto cv. Arbequina at bud swollen stage, all five *Colletotrichum* species infected flowers at all three phenological stages. Nevertheless, in some specific species-cultivar-phenological stage combinations, anthracnose symptoms were not visible until the second evaluation, for example, the culivars Coratina and Picual inoculated with all *Colletotrichum* species at the swollen bud stage (Figure 2).

Fruit infections

Characteristic anthracnose symptoms and signs developed in fruits at the two phenological stages (green and ripe) of the four inoculated cultivars, and also in some fruit not inoculated with *Colletotrichum* species (Figure 1, d and e). Symptoms in uninoculated fruits could have been from natural latent *Colletotrichum* infections. At the green fruit stage, initial symptoms consisted of small, depressed, 1-2 mm necrotic lesions scattered

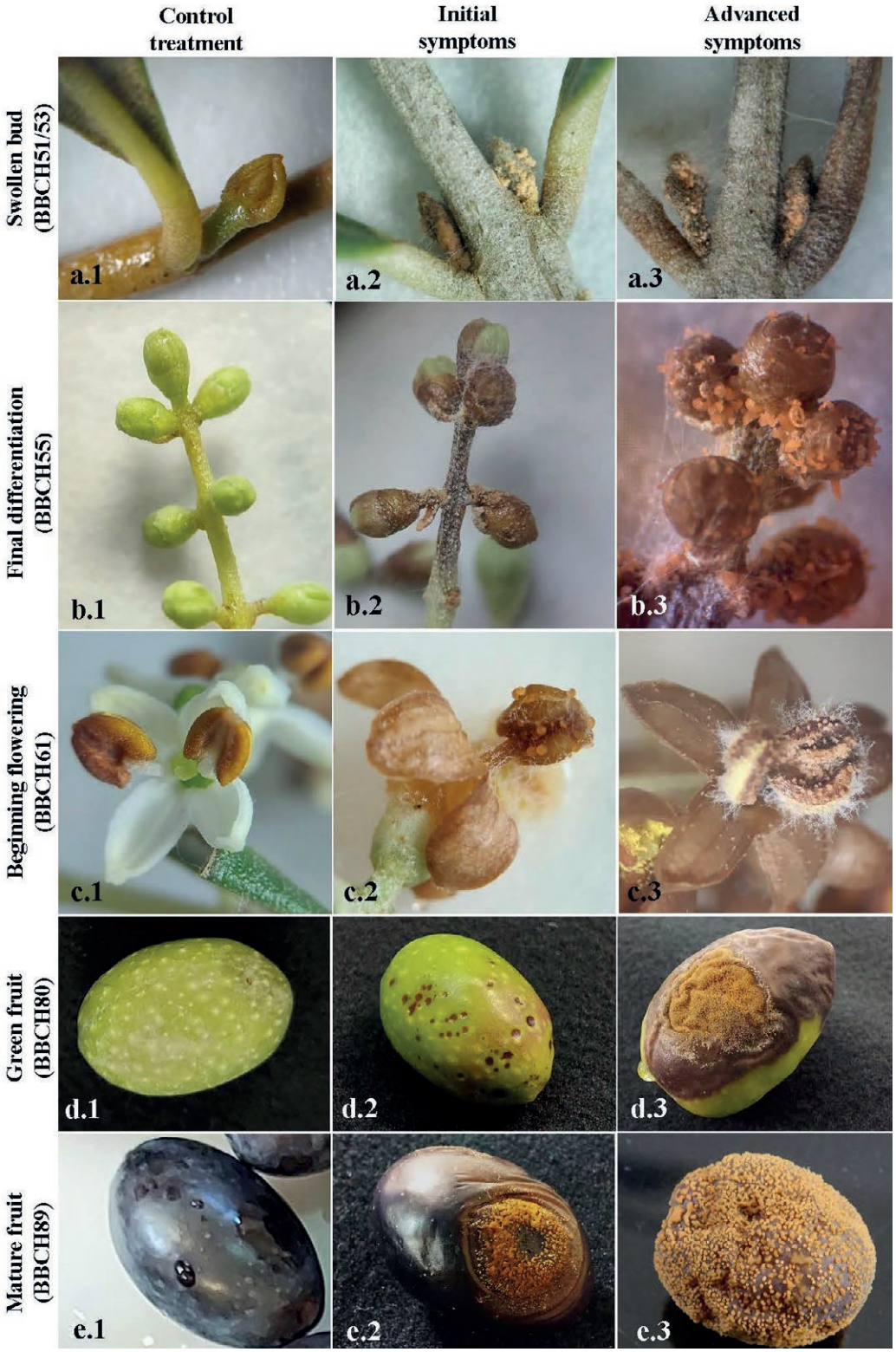

Figure 1. Initial and advanced anthracnose symptoms developed on olive flower and fruits at different phenological stages, after inoculation with species of *Colletotrichum*. **a,** swollen buds (BBCH51/53) 7 and 12 d after inoculation. **b,** final differentiation (BBCH55), 3 and 6 d after inoculation. **c,** beginning of flowering (BBCH61), 2 and 4 d after inoculation. **d,** green fruit (BBCH80) 7 and 26 d after inoculation. **e,** mature fruit (BBCH89) 6 and 12 d after inoculation.

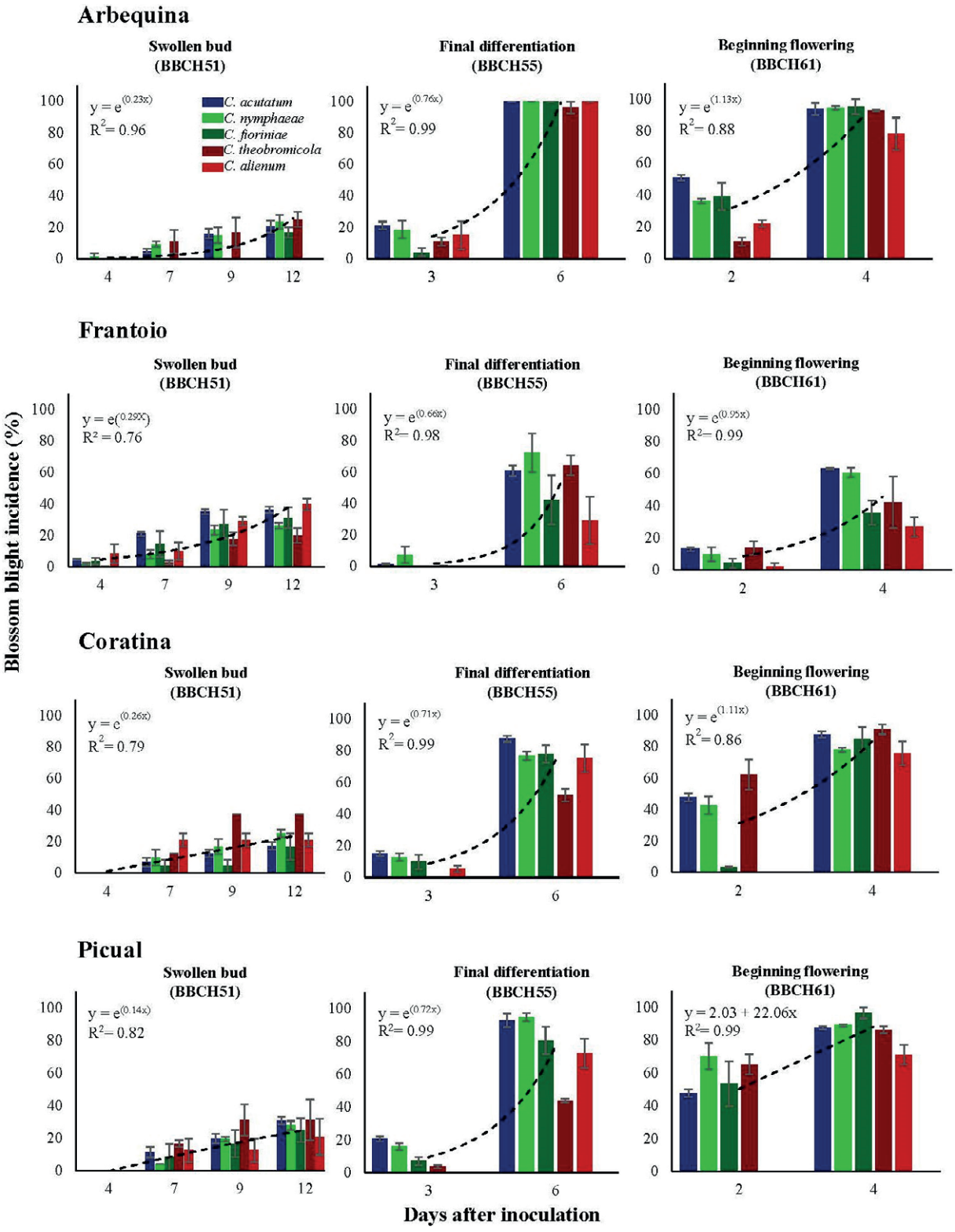

Figure 2. Mean incidences of olive blossom blight in flowers of four olive cultivars inoculated with five *Colletotrichum* species, at swollen buds (BBCH51/53), final differentiation (BBCH55), and beginning of flowering (BBCH61). Vertical bars indicate standard errors of means, each calculated from three replicates. Trend curves were graphed based on the average incidence at each evaluation time. The R^2 and estimations of the trend curves were obtained using SigmaPlot version 12.0 software.

throughout each fruit (Figure 1, d.2). Symptoms then progressed into depressed brown lesions, which were quickly covered with orange gelatinous masses of *Colletotrichum* conidia, known as "soapy fruit" (Figure 1, d.3). On ripe fruit, symptoms developed more quickly and consisted of typical soapy fruit (Figure 1, e.2 and e.3).

Table 2. Analysis of variance of data of Areas Under Disease Progress Curves (AUDPCs) estimated based on anthracnose incidence and severity developed in olive flower and fruits, for four olive cultivars inoculated with five *Colletotrichum* species. The flowers were inoculated at three phenological stages and fruits at two stages.

Source	SS[1]	df	MS	F	*P*-value	CV
Swollen bud (BBCH51/53)						
Model	441.44	19	23.23	3.77	0.0003	42.61
Cultivar	118.23	3	39.41	6.4	0.0013	
Species	103.16	4	25.79	4.18	0.0068	
Cultivar × species	211.8	12	17.65	2.86	0.0069	
Error	228.01	37	6.16			
Total	669.45	56				
Final differentation (BBCH55)						
Model	4518.11	19	237.8	14.51	<0.0001	17.46
Cultivar	2425.35	3	808.45	49.31	<0.0001	
Species	1264.55	4	316014	19.28	<0.0001	
Cultivar × species	828.22	12	69.02	4.21	0.0003	
Error	655.76	40	16.39			
Total	5173.87	59				
Beginning flowering (BBCH61)						
Model	15598.22	19	820.96	23.28	<0.0001	16.92
Cultivar	8356.56	3	2785.5	78.99	<0.0001	
Species	2926.24	4	731.56	20.75	<0.0001	
Cultivar × species	4315.43	12	356.62	10.2	<0.0001	
Error	1410.57	40	35.26			
Total	17008.79	59				
Green fruit (BBCH80)						
Model	2.1	19	0.11	138.49	<0.0001	5.34
Cultivar	1.08	3	0.36	451.37	<0.0001	
Species	0.84	4	0.21	261.74	<0.0001	
Cultivar × species	0.17	12	0.01	18.18	<0.0001	
Error	0.04	53	8.0E-0.4			
Total	2.14	72				
Mature fruit (BBCH89)						
Model	5114.24	19	269.17	8.51	<0.0001	15.98
Cultivar	924.51	3	308.17	9.74	<0.0001	
Species	1593.17	4	398.29	12.59	<0.0001	
Cultivar × species	2453.88	12	204.49	6.49	<0.0001	
Error	1866.42	59	31.63			
Total	6980.66	78				

[1] SS: sum of squares, df: degrees of freedom, MS: mean squares, F: teste F, CV: coefficient of variation.

Fruit anthracnose severity at both green and ripe fruit phenological stages, fitted sigmoidal curves (Figure 3 and Supplementary Table 1). For AUDPC variables, significant interactions were found between olive cultivars and inoculated *Colletotrichum* species inoculated, at both green and ripe fruit phenological stages (Table 2). On green fruit, the first symptoms were observed between 5 and 14 d after inoculation. In this stage, Arbequina and Frantoio reached severity values close to 50% at 30 d after inoculation, and Coratina 35 d after inoculation, whereas Picual reached 50% severity at 50 d after inoculation (Figure 3). At the mature fruit stage, the first symptoms were observed 4 d after inoculation. Anthracnose evolution occurred more quickly compared to that at the green fruit stage. For all cultivars and *Colletotrichum* species, severity was an average of 77% at 12 d after inoculation, and 92% at 18 d after inoculation (Figure 3).

The five *Colletotrichum* species were able to infect fruits at two phenological stages in the four olive cultivars. *Colletotrichum theobromicola* caused the greatest AUDPC values at the green fruit stage (Table 3). Green fruit inoculated with this species developed symptoms earlier (5 d after inoculation) compared with the other *Colletotrichum* species (14 d after inoculation), and reached almost 100% severity about 15 d earlier than those inoculated with the other *Colletotrichum* spp. (Figure 3). In ripe fruit, the behaviour among the five *Colletotrichum* species was similar. Anthracnose indices close to 100% were reached at about 15 d after inoculation, except for the cultivars Arbequina and Frantoio inoculated with *C. alienum*, where severity indices were close to 50%.

DISCUSSION

This study assessed anthracnose susceptibility during flower development and fruit ripening in the four main olive cultivars grown in Uruguay, Arbequina, Frantoio, Coratina and Picual. That study used artificial inoculations with five *Colletotrichum* species, of detached olive panicles and fruits. The results indicated that the four olive cultivars assessed were are susceptible to *Colletotrichum* at all the evaluated phenological stages, although differences in phenological stages, olive cultivars, and *Colletotrichum* species were detected.

The swollen bud stage was susceptible to *Colletotrichum* species. Although susceptibility of this phenological stage to anthracnose was low, we demonstrated that *Colletotrichum* can infect flowers from early stages during flower development. In later stages of flower develop-

Table 3. Mean Areas Under Disease Progress Curves (AUDPCs) estimated based on anthracnose incidence and severity developed in flower and fruits of four olive cultivars inoculated with five *Colletotrichum* species. The flowers were inoculated at three phenological stages and fruits at two stages.

Cultivar	Species	Phenological stage				
		Flowers			Fruit	
		Swollen bud (BBCH51/53)[1]	Final differentition (BBCH55)	Beginning flowering (BBCH61)	Green Fruit (BBCH80)	Mature Fruit (BBCH89)
Arbequina	*C. acutatum*	4.66 abcd[2]	35.62 h	48.93 cde	35.94 ef	41.44 de
	C. nymphaeae	5.35 abcd	34.29 gh	34.47 bcd	29.28 de	38.90 cde
	C. fioriniae	1.39 ab	27.09 defgh	43.40 cde	46.70 gh	44.38 e
	C. theobromicola	5.94 abcd	29.53 efgh	31.16 bc	67.78 j	44.30 e
	C. alienum	0.00 a	32.49 fgh	47.90 cde	36.26 fg	13.05 a
Frantoio	*C. acutatum*	11.94 d	15.83 abcd	22.23 ab	24.77 cd	43.24 de
	C. nymphaeae	6.85 abcd	21.82 bcdefg	20.13 ab	35.72 ef	37.72 bcde
	C. fioriniae	4.58 abcd	10.62 ab	11.06 a	23.60 cd	44.44 e
	C. theobromicola	4.55 abcd	16.31 abcd	17.44 ab	61.98 ij	37.72 bcde
	C. alienum	6.66 abcd	4.90 a	7.85 a	17.97 cd	26.5 abc
Coratina	*C. acutatum*	4.11 abcd	29.91 efgh	45.75 cde	30.04 de	32.13 bcde
	C. nymphaeae	5.75 abcd	25.59 defgh	40.91 cde	29.28 de	31.33 bcde
	C. fioriniae	2.55 abc	24.49 cdefgh	22.27 ab	20.04 bc	40.72 cde
	C. theobromicola	10.07 cd	13.00 abc	53.81 e	51.39 hi	40.59 cde
	C. alienum	7.52 abcd	21.58 bcdef	18.95 ab	16.96 bc	39.88 cde
Picual	*C. acutatum*	6.90 abcd	34.62 h	55.61 e	6.26 a	23.11 ab
	C. nymphaeae	5.60 abcd	31.72 fgh	56.98 e	11.95 b	31.35 bcde
	C. fioriniae	5.56 abcd	23.75 cdefgh	50.89 de	4.56 a	31.55 bcde
	C. theobromicola	9.26 bcd	12.84 abc	54.24 e	34.13 ef	31.35 bcde
	C. alienum	7.82 abcd	18.21 bcde	17.78 ab	5.64 a	23.22 bcd

[1] Phenological scale according to Sanz-Cortes *et al.* (2002).
[2] In each column, mean values followed by the same letter are not significantly different (Tukey's HSD test; $P = 0.05$).

ment, susceptibility increased, and symptoms and signs progressed rapidly. Symptoms included brown colouration of the flower organs that progressed to collapse of inflorescences, known as blossom blight. Affected organs were rapidly covered by typical orange-salmon coloured gelatinous masses, corresponding to *Colletotrichum* conidia (Sergeeva *et al.,* 2008; Iliadi *et al.,* 2018; Moreira *et al.,* 2021).

Results of the present study are in similar to those of Kolainis *et al.,* (2020), who observed that in the olive cultivars Koroneiki and Kalamon, the first anthracnose symptoms appeared 2 d after inoculation of detached flowers at beginning of flowering stage. In contrast, Moral *et al.,* (2009) inoculated flowers of Arbequina, Hojiblanca and Picual cultivars at the same phenological stage, but the first symptoms were visible 5 d after inoculation. These differences could be because Moral *et al.,* (2009) utilized attached flowers, whereas in the present study and that of Kolainis *et al.* (2020), detached flowers were used.

For the four olive cultivars evaluated, some differences were observed in anthracnose susceptibility at different flowering stages. No major variations were observed at the swollen bud stage, but at final differentiation and beginning of flowering, Frantoio was the least susceptible cultivar to *Colletotrichum*, Arbequina was the most susceptible, and Coratina and Picual were of intermediate susceptibility. Similar results were obtained by Moral *et al.,* (2009), who found that Arbequina was more susceptible than Hojiblanca and Picual when inoculated at the beginning of flowering.

In previous research, we demonstrated that species *C. acutatum* s.s., *C. nymphaeae*, *C. fioriniae*, *C. theobromicola* and *C. alienum* caused typical blossom blight at beginning of flowering (Moreira *et al.,* 2021). In the present study, although the incubation period could be variable, isolates of these five *Colletotrichum* species infected olive from the early stages of flower development.

Results recorded here for behaviour of inoculated green and ripe olive fruit were generally in agreement

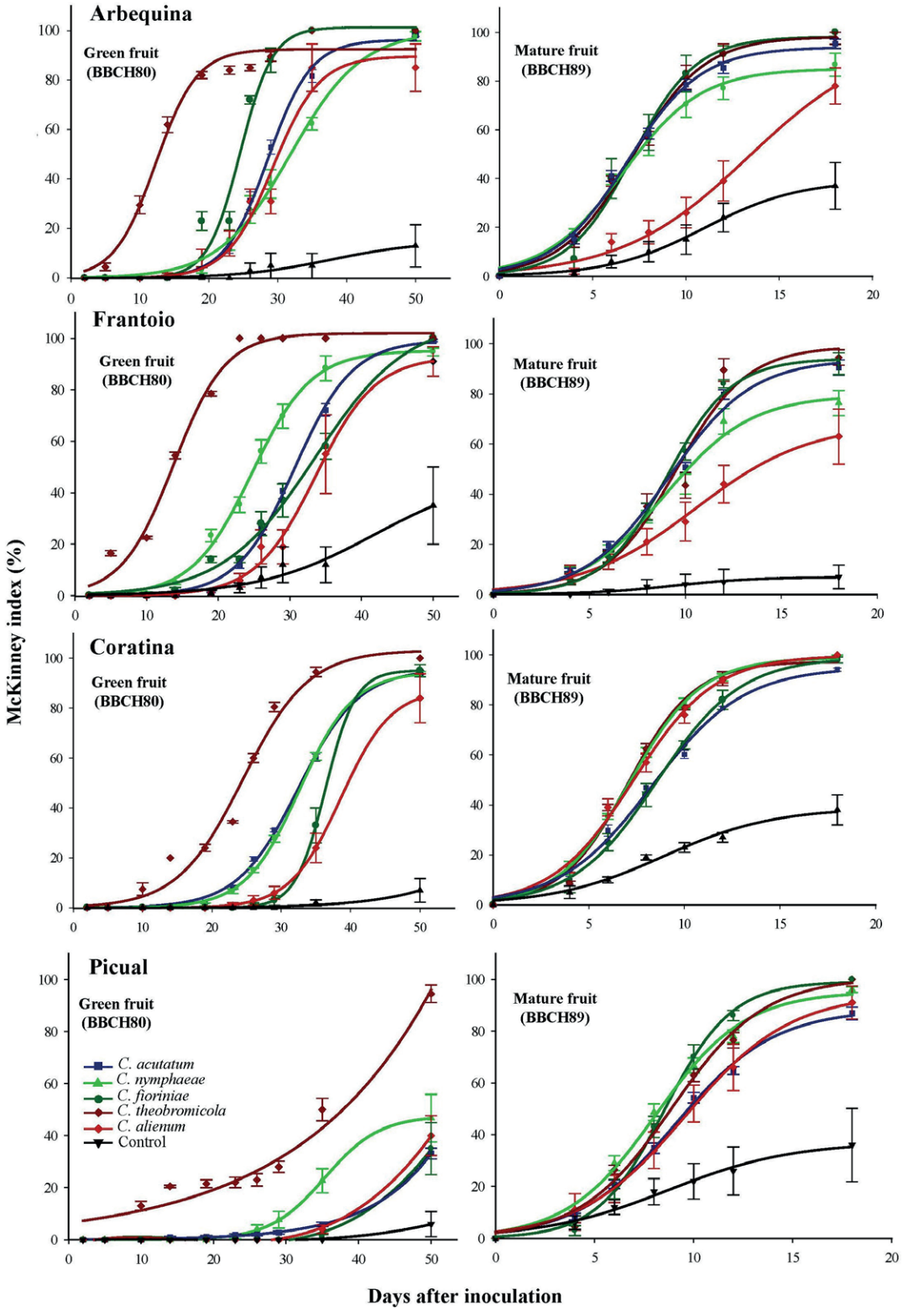

Figure 3. Mean anthracnose severity indices in fruit of four olive cultivars inoculated with five *Colletotrichum* species at green (BBCH80) and ripe fruit (BBCH89) phenological stages. Severity values were used to calculate the McKinney indices. The incidence registered in the control treatments can be attributable to the latent infection of *Colletotrichum* spp. expression. Vertical bars correspond are standard errors of the means calculated based on four replicates. The R^2 and the estimations of trend curves were obtained using SigmaPlot version 12.0 software.

with previous research. Olive fruits can be infected by *Colletotrichum* at different stages during fruit ripening, but the susceptibility increases with ripening (Moral *et al.,* 2008; 2009; Sergeeva, 2014; Chattaoui *et al.,* 2016; Moral *et al.,* 2017). We also observed differences in symptom development. While on ripe fruit the typical soapy fruit symptoms were observed from the beginning, on green fruit initial symptoms consisted of small and depressed necrotic lesions that later progressed into the typical soapy fruit. In addition, at the green stage, the first symptoms were visible between 5 and 7 d after inoculation, whereas on ripe fruit symptoms were seen earlier, between 1 and 5 d post inoculation. Similar results were obtained by Moral *et al.,* (2008), who observed first symptoms at 7 d on detached leaves, and at 4 d on ripe fruit, after *Colletotrichum* inoculation.

For susceptibility of the different olive cultivars, at the green fruit stage Picual was the least susceptible and Arbequina and Frantoio were the most susceptible cultivars to *Colletotrichum* spp., while Coratina developed disease with intermediate susceptibility. These results are similar to other studies, where Arbequina was susceptible to moderately susceptible, Coratina was moderately susceptible (Andres 1991; Moral and Trapero, 2009; Bartolini and Cerreti, 2013), and Picual was moderately resistant or resistant to anthracnose (Moral and Trapero, 2009; Talhinhas *et al.,* 2015; Moral *et al.,* 2017).

On mature fruit, the four olive cultivars were all highly susceptible to anthracnose. These results showed some discrepancies with other studies, where Frantoio was found to be highly resistant to anthracnose in Spain (Moral *et al.,* 2008; Moral and Trapero, 2009; Moral *et al.,* 2017). Nevertheless, in accordance with the present results, Frantoio showed high susceptibility to anthracnose in Argentina (Andres, 1991) and Italy (Loprieno and Tenerini, 1960).

Although the differences in anthracnose susceptibility found among cultivars at the green fruit stage were not recorded for ripe fruit, this was not surprising. Moral and Trapero (2009) reported that green fruits were more resistant to *Colletotrichum* than ripe fruit, probably because of greater concentrations of phenolic compounds in green than ripe fruits. However, when maturity is reached, all olive cultivars can become diseased, and develop complete rot regardless of their early fruit susceptibilities (Moral *et al.,* 2008).

Colletotrichum theobromicola differed substantially from the other *Colletotrichum* species inoculated in this study, being the most aggressive pathogen at the green fruit stage. This was surprising since this species, together with *C. alienum*, was isolated from a low proportion of olives with typical anthracnose symptoms in Uruguay (Moreira *et al.,* 2021). In Australia, inoculated detached green stage fruit was more affected by *C. theobromicola* and *C. gloeosporioides* s.s than *C. aenigm*a, *C. cigarro*, *C. queenslandicum*, *C. siamense* and *C. karstii* (Schena *et al.,* 2014). Regarding the international occurrence of *C. theobromicola*, This pathogen has been reported affecting olive in Argentina, Australia, and Uruguay (Schena *et al.,* 2014; Lima *et al.,* 2020; Moreira *et al.,* 2021), but not in countries of the Mediterranean basin, where most olives are cultivated. Schena *et al.,* (2014) mentioned that new diseases are expected to emerge as consequence of climate change, and suggested that *C. theobromicola* could play an important role in olive anthracnose disease with these changes.

For ripe fruit, behaviour of the five *Colletotrichum* species was similar, except for *C. alienum* which was less aggressive when it was inoculated on the cultivars Arbequina and Frantoio. In Portugal, Talhinhas *et al.,* (2015) found that *C. acutatum* s.s. and *C. nymphaeae* were more aggressive than *C. gloeosporioides* s.s. and *C. rhombiforme* on ripe fruit. In Italy Schena *et al.* (2017) observed that *C. acutatum* s.s. was more aggressive than *C. godetiae* on ripe fruit. In this work, *C. acutatum* s.s., *C. nymphaeae* and *C. fioriniae* were of similar aggressiveness.

The present study is the first in which anthracnose susceptibility has been evaluated at different host flower development stages. Based on these results, the risk of anthracnose occurrence starts at early stages of flower development. This would allow development of improved disease management decisions. For example, the most opportune time to initiate preventive fungicide applications should be at early stages of flower development, to minimize yield losses caused by olive anthracnose. For olive cultivars, Frantoio was moderately susceptible to anthracnose, whereas Picual, Coratina and Arbequina were susceptible cultivars during flowering. However, the present study results indicate that at the green fruit stage, Frantoio and Arbequina were the most susceptible cultivars, Coratina had an intermediate susceptibility, and Picual was least susceptible. In mature fruit, no differences were found among the assessed cultivars. In this study we confirmed that Arbequina, the main olive cultivar produced in Uruguay, was one of the most susceptible to *Colletotrichum* during flowering and fruit ripening. Future research should focus on improving anthracnose management strategies to minimize the impacts of this disease during flower development and fruit maturity, especially in those olive cultivars that are most susceptible to this disease.

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AUTHOR CONTRIBUTIONS

VM was responsible for performing the assays, data analyses and drafted the manuscript of this paper. MJC assisted in experimental assays, data analyses, and made contributions to the manuscript. PM and SA supervised the assays, interpretation of the results, and performed critical revisions of the manuscript. All authors approved the final manuscript.

LITERATURED CITED

Andrés F., 1991. *Enfermedades y Plagas del Olivo*. 2nd Ed. Riquelme y Vargas Ediciones, S.L., Jaén

Azevedo-Nogueira F., Martins-Lopes P., Gomes S., 2020. Current understanding of Olea europaea L. – *Colletotrichum acutatum* interactions in the context of identification and quantification methods. *Crop Protection* 132, 105106. https://doi.org/10.1016/j.cropro.2020.105106

Bartolini G., Cerreti S., 2013. Olive germplasm (*Olea europaea* L.). Available at: http://www.oleadb.it Accessed on January 20, 2022.

Cacciola S.O., Faedda R., Sinatra F., Agosteo G.E., Schena L., Frisullo S., San Lio G., 2012. Olive anthracnose. *Journal of Plant Pathology* 94: 29-44p. http://dx.doi.org/10.4454/JPPFA2011001

Chattaoui M., Raya M.C., Bouri M., Moral J., Pérez- Rodríguez M., … Rhouma A., 2016. Characterization of a *Colletotrichum* population causing anthracnose disease on olive in northern Tunisia. *Journal of Applied Microbiology* 120: 1368–1381. https://doi.org/10.1111/jam.13096.

Conde-Innamorato P., Arias-Sibillotte M., Villamil J.J., Bruzzone J., Bernaschina Y., … Leoni C., 2019. It is feasible to produce olive oil in temperate humid climate regions. *Frontiers in Plant Science* 10: 1544. https://doi.org/10.3389/fpls.2019.01544.

Filoda P.F., Arellano A.D.V., Dallagnol L.J., Chaves F.C., 2021. *Colletotrichum acutatum* and *Colletotrichum nymphaeae* causing blossom blight and fruit anthracnose on olives in southern Brazil. *European Journal Plant Pathology* 161: 993–998 https://doi.org/10.1007/s10658-021-02372

Gorter G.J., 1956. Anthracnose fungi of olives. *Nature* 178: 1129–1130.

Iliadi M.K., Tjamos E., Antoniou P., Tsitsigiannis D.I., 2018. First report of *Colletotrichum acutatum* causing anthracnose on olives in Greece. *Plant Disease* 102: 820. https://doi.org/10.1094/PDIS-09-17-1451-PDN.

Kolainis S., Koletti A., Lykogianni M., Karamanou D., Gkizi D., … Aliferis K.A., 2020. An integrated approach to improve plant protection against olive anthracnose caused by the *Colletotrichum acutatum* species complex. *PLOS One* 15;15. https://doi.org/10.1371/journal.pone.0233916

Leoni C., Bruzzone J., Villamil J.J., Martínez C., Montelongo, M.J., Bentancur O., Conde-Innamorato P., 2018. Percentage of anthracnose (*Colletotrichum acutatum* s.s.) acceptable in olives for the production of extra virgin olive oil. *Crop Protection* 108: 47–53. https://doi.org/10.1016/j.cropro.2018.02.013.

Lima N.B., Pastor S.E., Maza C.E., Conforto C., Vargas-Gil S., Roca M., 2020. First report of Anthracnose of olive fruit caused by *Colletotrichum theobromicola* in Argentina. *Plant Disease* 104: 589. https://doi.org/10.1094/PDIS-06-19-1207-PDN

Loprieno N., Tenerini I., 1960. Indagini sul *Gloeosporium olivarum* Alm., agente della "lebbra" delle olive. *Phytopathology* 39: 262–290.

MGAP–DIEA, 2020. Censo de productores de olivos 2020. Serie de trabajos especiales n° 364 Available at: https://www.gub.uy/ministerio-ganaderia-agricultura-pesca/sites/ministerio-ganaderia-agricultura-pesca/files/2021-03/PUBLICACION_Olivos2020_Final.pdf Accessed on January 20, 2023.

MGAP–DIEA, 2021. Encuesta a productores de olivos 2021. Available at: https://www.gub.uy/ministerio-ganaderia-agricultura-pesca/sites/ministerio-ganaderia-agricultura-pesca/files/documentos/noticias/Comunicado_encuesta%20olivos%202021_0.pdf Accessed on January 20, 2023.

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(10): 1421–1426. https://doi.org/10.1094/PDIS-92-10-1421.

Moral J., Trapero A., 2009. Assessing the susceptibility of olive cultivars to anthracnose caused by *Colletotrichum acutatum*. *Plant Disease* 93: 10.

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

Moral J., Xaviér C., Roca L.F., Romero J., Moreda W., Trapero A., 2014. La Antracnosis del olivo y su efec-

to en la calidad del aceite. *Grasas Aceites* 65: 2. Doi: http://dx.doi.org/10.3989/gya.110913.

Moral J., Xavier V.J., 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: 1892. https://doi.org/10.3389/fpls.2017.01892

Moreira V., Mondino P., Alaniz S., 2021. Olive anthracnose caused by *Colletotrichum* in Uruguay: symptoms, species diversity and pathogenicity on flowers and fruits. *European Journal Plant Pathology* 160: 663–668. https://doi.org/10.1007/s10658-021-02274-z

Mosca S., Nicosia M.G., Cacciola S.O., Schena L., 2014. Molecular analysis of *Colletotrichum* species in the carposphere and phyllosphere of olive. *PLoS One* 9: e114031. https://doi.org/10.1371/journal.pone.0114031

Sanz-Cortés F., Martínez-Calvo J., Badenes, Bleiholder H., Hack H., Llacer G., Meier U., 2002. Phenological growth stages of olive trees (*Olea europaea*). *Annals of Applied Biology* 140: 151–157. https://doi.org/10.1111/j.1744-7348.2002.tb00167.x

Schena L., Mosca S., Cacciola S.O., Faedda R., Sanzani S.M., … San Lio G.M., 2014. Species of the *Colletotrichum gloeosporioides* and *C. boninense* complexes associated with olive anthracnose. *Plant Pathology* 63: 437–446. https://doi.org/10.1111/ppa.12110

Schena L., Abdelfattah A., Mosca S., Li Destri Nicosia M.G., 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 Plant Pathology* 149: 337–347 https://doi.org/10.1007/s10658-017-1185-x

Sergeeva V., Nair N.G., Spooner-Hart R., 2008 Evidence of early flower infection in olives (*Olea europaea*) by *Colletotrichum acutatum* and *C. gloeosporioides* causing anthracnose disease. *Australasian Plant Disease Notes* 3: 81–82. https://doi.org/10.1071/DN08032

Sergeeva V., 2014. The Role of Epidemiology Data in Developing Integrated Management of Anthracnose in Olives - a Review. VII International Symposium on Olive Growing, ISHS *Acta Horticulturae* 1057. 6p

Talhinhas P., Mota-Capitão C., Martins S., Ramos A.P., Neves- Martins J., … Oliveira H., 2011. Epidemiology, histopathology and etiology of olive anthracnose caused by *Colletotrichum acutatum* and *C. gloeosporioides* in Portugal. *Plant Pathology* 60: 483–495. https://doi.org/10.1111/j.1365-3059.2010.02397.x.

Talhinhas P., Gonçalves E., Sreenivasaprasad S., Oliveira H., 2015. Virulence diversity of anthracnose pathogens (*Colletotrichum acutatum* and *C. gloeosporioides* species complexes) on eight olive cultivars commonly grown in Portugal. *European Journal Plant Pathology* 142: 73–83

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* 8: 1797–1807. https://doi.org/10.1111/mpp.12676.