



## Response of Olive (*Olea europaea*) Cultivars Against *Venturia oleaginea* Causing Olive Scab in Uruguay

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### Summary

In Uruguay, the area cultivated with olive (*Olea europaea*) has increased in recent years reaching approximately 10,000 hectares. Among the foliar diseases favored by mild temperatures, high relative humidity, and abundant rainfall, olive scab or peacock spot caused by the fungus *Venturia oleaginea* is the most important. In 2015 and 2016, with the objective of knowing the response of olive cultivars introduced in Uruguay against this fungus, field, greenhouse, and laboratory trials were carried out. Disease incidence and severity were recorded based on a scale of the affected leaf area and/or the number of lesions per leaf, considering both visible and latent lesions after NaOH treatment. Results from field data, greenhouse and laboratory trials indicated that 'Frantoio', 'Leccino' and 'Picual' were the cultivars with the best behavior against *V. oleaginea*. 'Arbequina' had intermediate values while 'Barnea', 'Coratina' and 'Manzanilla' were the most susceptible.

**Keywords:** fungi, Venturiaceae, phytopathogens, foliar diseases, fruit crops

## Comportamiento de cultivares de olivo (*Olea europea*) frente a *Venturia oleaginea*, causante de repilo en Uruguay

### Resumen

En Uruguay, la superficie cultivada con olivo (*Olea europaea*) se ha expandido en los últimos años, alcanzando las 10.000 hectáreas aproximadamente. Entre las enfermedades foliares favorecidas por las condiciones climáticas de temperaturas moderadas, humedad relativa alta y precipitaciones abundantes, se destaca la 'mancha ojo de pavo' o 'repilo' causada por el hongo *Venturia oleaginea*. En 2015 y 2016, con la finalidad de conocer el comportamiento de cultivares introducidos al país frente a este hongo se realizaron ensayos a campo, invernáculo y laboratorio. Se determinó la incidencia y la severidad de acuerdo a una escala elaborada en base al área foliar afectada y/o el número de lesiones por hoja tanto en lesiones visibles como latentes visualizadas mediante tratamiento con NaOH. Los resultados a campo con infecciones naturales y de los ensayos bajo condiciones controladas indicaron que 'Frantoio', 'Leccino' y 'Picual' fueron los cultivares de mejor comportamiento frente a *V. oleaginea*. 'Arbequina' mostró un comportamiento intermedio, mientras que 'Barnea', 'Coratina' y 'Manzanilla' fueron los más susceptibles.

**Palabras clave:** hongos, Venturiaceae, fitopatógenos, enfermedades foliares, frutales

## Introduction

The fungus *Venturia oleaginea* (Castagne) Rossman & Crous 2015, Ascomycetes, Pleosporales<sup>(1)</sup>, is the causal agent of the foliar disease called 'peacock spot' or 'olive scab' in olives. The Fungal Taxonomy International Commission proposed the use of *Venturia* instead of *Fusicladium* for the species that only present anamorphic phase, following the guidelines of The Amsterdam Declaration on Fungal Nomenclature<sup>(2)(3)</sup>. Among the foliar diseases of the olive, 'olive scab' is the most important. Moderate temperatures, high relative humidity, and abundant rainfall are favorable for this disease<sup>(4)(5)(6)</sup>. The severely affected plants present defoliation, weakening, and loss of productivity. In the upper face of the leaves, circular injuries of variable size are observed sometimes concentric, of dark brown-black coloration according to the amount of sporulation of the fungus, and surrounded by a yellowish halo in mature lesions (Figure 1A)<sup>(7)</sup>. The appearance of the injuries varies according to the cultivar, the age of the injury and the environmental conditions. The old injuries may have whitish colorations due to the cuticle detachment from the surrounding tissue<sup>(8)</sup>. Symptoms may also appear on the midrib on the underside of the leaves, on the petiole and on the peduncle of the fruits as discontinuous blackened areas corresponding to the sporulation of the fungus. In severe epidemics, the fruits show circular brownish lesion<sup>(8)</sup>.

The bibliography highlights the variability of the response of olive cultivars against *Venturia oleaginea*<sup>(5)(9)</sup>. For example, 'Manzanilla' cultivar was cited as resistant or highly susceptible to *V. oleaginea*<sup>(9)</sup>. This is why the sanitary characterization of olive cultivars at a local level takes special importance because it considers at the same time the microorganism, the host, the orchard management and the environmental conditions.

The disease management is based largely on foliar applications of fungicides, mainly cupric, dithiocarbamates or phthalamides applied before the rainfall, although there are also records of IBE (inhibitors of ergosterol biosynthesis) and QoI (quinone outside inhibitors) for the control of this disease<sup>(10)</sup>. The relative humidity of the environment is a determining factor in the development of the disease, so cultural measures that favor good ventilation of the plants through pruning and choosing the plantation frame are also recommended<sup>(11)</sup>. In areas where agroclimatic conditions are highly predisposing, the choice of tolerant or resistant cultivars is suggested<sup>(12)(13)</sup>.

In Uruguay, previous studies have identified the 'Repilo' as one of the main foliar diseases of the olive<sup>(14)</sup>. Based on the observations of the typical symptomatology and the reproductive structures of the fungus, the authors determined that *V. oleaginea* was the causal agent of this disease. Likewise, the identity of this fungus was recently confirmed through the polymerase chain reaction (PCR) with specific primers from the 18S region of the rDNA developed by González-Lamothe<sup>(15)</sup> for this species<sup>(16)</sup>. However, there is no systematic national information on how the most planted cultivars behave according to their disease susceptibility. In addition, it is necessary to adjust and validate the inoculation techniques and severity scales developed for 'olive scab' in agroclimatic regions very different from Uruguay. For the producer is very useful to know which cultivars are the most sensitive to the disease and which scales can be used to quantify it in a simple and precise way, and for the breeding programs which inoculation technique is the most useful as well as the scales needed for disease assessment.

The objective of this work is to characterize the response of seven olive cultivars introduced in Uruguay against *V. oleaginea*, by natural infections and by inoculations under controlled laboratory and greenhouse conditions.

## Materials and Methods

### Response of commercial olive cultivars against natural infections of *Venturia oleagina*

The response to natural infections of *V. oleagina* was evaluated in the field in plants of 15 years (Figure 1B) of the cultivars 'Arbequina' (Spanish origin), 'Barnea' (Israeli), 'Coratina' (Italian), 'Frantoio' (Italian), 'Leccino' (Italian), 'Manzanilla' (Spanish) and 'Picual' (Spanish) in January-April 2015 and November 2015-April 2016. 'Picual' cultivar is cited as highly susceptible to this disease and is normally used as a susceptible control in the evaluation trials<sup>(5)</sup>. On the other hand, 'Frantoio' is considered highly resistant<sup>(5)</sup>. The studied cultivars were included in an experimental trial of olive germplasm introduction at INIA Las Brujas (34° 40' S, 56° 20' W, 32 m over sea level), Department of Canelones, Uruguay. The olive trees were planted at a density of 400 plants/ha, with irrigation and subsequent management according to the recommendations of the Good Agricultural Practices Guidelines for commercial production<sup>(17)</sup>.



**Figure 1.** A. Concentric foliar lesions in olive leaves (*Olea europaea*) caused by the *Venturia oleaginea* in the field after natural infections. B. Evaluation orchard of olive cultivars (*Olea europaea*) in the Experimental Station INIA Las Brujas, Canelones, Uruguay.

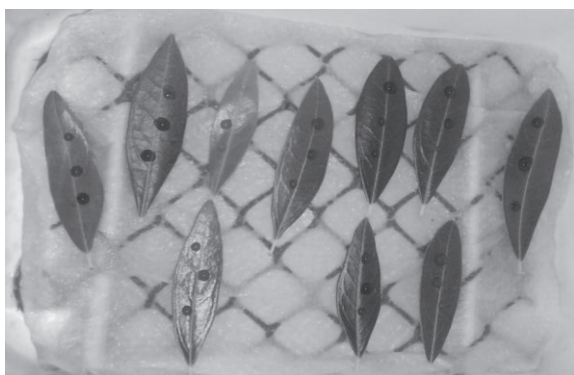
A randomized complete block design with four replications was used. The experimental unit consisted of 100 young leaves per olive tree selected randomly from the middle portion of the sprout, at a height of 1.5 meters. Each block included three trees per cultivar, and the central one was used for evaluation. The field evaluations were made monthly. In each evaluation, incidence and severity were recorded in two instances: first quantifying the visible symptoms and then by developing the latent lesions of leaves with 5 % NaOH for 30 minutes<sup>(18)</sup>. All leaves were scanned for assessing the disease the same day. The incidence was estimated as the number of symptomatic leaves over the total leaves. Severity was determined using the scales developed by López-Doncel and others<sup>(6)</sup> and Salman and others<sup>(18)</sup>. In order to calculate the severity index (SI) this formula was used:  $SI = \sum (n_i * s_i) / N$ , where  $n_i$  is the number of leaves in each category,  $s_i$  the severity value of the class and  $N$  the total number of evaluated leaves. The values of incidence and SI were analyzed with generalized linear models from the statistical program InfoStat<sup>(19)</sup>. The means were compared with the Bonferroni Test with an alpha value of 0.05. The climatic data of air temperature and precipitations were registered in the automatic weather station installed at the Experimental Station INIA Las Brujas (data available at <http://www.inia.org.uy/online/site/69264611.php>).

#### Response of olive cultivars against inoculations with *Venturia oleaginea* in greenhouse and laboratory trials

The experiments were conducted in 2016 and 2017. The inoculum used was obtained from naturally infected olive leaves, in two orchards, one located in Melilla, Montevideo (34° 48' 48" S, 56° 16' 10" W), and the second one in Rincón del Colorado, Canelones (34° 40' 05" S, 56° 20' 35" W). The leaves with sporulating lesions were dried at room temperature for 24 h. Then, they were transferred into paper bags inside airtight containers and kept at 5 °C and darkness until their use<sup>(6)</sup>. The conidia were obtained after shaking the infected leaves submerged in deionized water and Tween 20 for 2 h. The morphological characteristics of the conidia were observed under an optical microscope. After agitation, the leaves were discarded, the conidial suspension was centrifuged at 3000 rpm for three minutes, and finally the conidial concentration was adjusted to  $1.5 \times 10^5$  conidia/mL using a Neubauer chamber. To verify the conidia viability, 100  $\mu$ L of the suspension was plated in agar-water and incubated at 17 °C in the dark for 24 h. After this time, the percentage of germinated conidia was recorded to ensure 45 to 60 % germination, as suggested by López-Doncel and others<sup>(6)</sup>. In the 2016 greenhouse trial, plants of the cultivars 'Arbequina', 'Leccino' and 'Manzanilla' growing in pots, were inoculated. In 2017, 'Coratina', 'Frantoio' and 'Picual' cultivars were added. A randomized block design with three replications was used, with the experimental unit consisting of four young sprouts per plant. A suspension of conidia with two drops of Tween 20 was sprayed on the sprouts at

the point of run-off, according to the technique described by López-Doncel and others<sup>(6)</sup>. The control plants were inoculated with sterile water and two Tween 20 drops. The inoculated buds were covered with moistened polyethylene bags to assure conditions close to 100 % of humidity. The plants were incubated in a growth chamber at 17 °C and darkness for 48 h and then maintained in the greenhouse until symptoms appeared. Weekly evaluations were made recording the estimated severity according to two scales: a) the percentage of affected foliar area: 0: 0 %; 1: < 12,5 %; 2: 12,5- 25 %; 3: 25-50 %; 4: 50-75 %; 5: > 75<sup>(6)</sup> and b) the number of injuries by leaf<sup>(18)</sup>. One hundred and thirty two days after inoculation, the inoculated and the control leaves were submerged in a solution of NaOH to 5 % by 30 minutes according to Salman and others<sup>(18)</sup>. Incidence and severity were estimated before and after the treatment with NaOH 5 %.

For the laboratory tests, carried out in 2016 and 2017, asymptomatic leaves from plants of 'Arbequina', 'Barnea', 'Coratina', 'Frantoio', 'Leccino', 'Manzanilla' and 'Picual'



**Figure 2** Backside of olive leaves (*Olea europaea*) inoculated with *Venturia oleaginea* by drops of conidia and incubated in humid chamber.

kept in the greenhouse, were used. A randomized complete block design with three repetitions was used, with the experimental unit being 10 leaves per cultivar. The adaxial side of each leaf was inoculated with three drops of 5  $\mu$ l of the conidial suspension. For incubation in a humid chamber, the inoculated and the control leaves were placed in 19 x 12 cm base and 12 cm deep plastic containers. The leaves were placed over filter paper which was over a plastic mesh. The mesh was held on four points at a height of 3 cm above the bottom of the container, which was covered with sterile distilled water. The filter paper was communicated with the water of the bottom of the container by the four sides, this system allowed the leaves to be in a humid atmosphere during the course of the tests (Figure 2). The moist chambers were kept at 17 °C and in the dark until the symptoms appeared. Leaves were evaluated weekly for disease symptoms according to the severity scale described by Salman and others<sup>(18)</sup> for number of injuries per leaf. The scale goes from 1 to 5 where 1: 1 injury, 2: 2 injuries, 3: 3-5 injuries, 4: 6-10 injuries and 5: more than 11 injuries. After 40 days the inoculated and the control leaves were first evaluated for scab lesions and then immersed in 5 % NaOH solution for 30 minutes to determine latent infections («development» of the injuries)<sup>(18)</sup>. The incidence and severity were registered for both evaluations.

## Results

### Field response of olive cultivars against natural infections of *Venturia oleaginea*

The estimated average values of disease incidence are indicated in Table 1, for the seven evaluated olive cultivars. In both years the March and April evaluations distinguished three groups in increasing order of susceptibility: A-

**Table 1.** Incidence of *Venturia oleaginea* on leaves of seven olive cultivars after de development with NaOH evaluated between January-April of 2015 and November 2015-April 2016.

Cultivars	Incidence									
	Jan-15	Feb-15	Mar-15	Apr-15	Nov-15	Dec-15	Jan-16	Feb-16	Mar-16	Apr-16
Leccino	0,50 a	0,56 a	0,31 a	0,20 a	0,79 c	0,87 c	0,70 b	0,53 a	0,20 a	0,30 a
Frantoio	0,57 a	0,52 a	0,39 a	0,14 a	0,65 ab	0,80 b	0,64 b	0,53 a	0,40 b	0,27 a
Picual	0,55 a	0,56 a	0,39 a	0,17 a	0,57 a	0,68 a	0,51 a	0,52 a	0,39 b	0,42 b
Arbequina	0,71 b	0,57 a	0,37 a	0,12 a	0,57 a	0,88 c	0,82 c	0,73 b	0,59 c	0,48 b
Barnea	0,85 c	0,81 b	0,84 b	0,54 b	0,85 c	0,92 cd	0,85 cd	0,67 b	0,61 c	0,60 c
Coratina	0,87 c	0,87 b	0,95 c	0,76 c	0,97 d	0,91 cd	0,94 d	0,86 c	0,61 c	0,70 cd
Manzanilla	0,78 bc	0,77 b	0,85 b	0,51 b	0,68 b	0,97 d	0,88 d	0,90 c	0,58 c	0,73 d

Incidence: proportion of affected leaves varies between 0 and 1

Different letters indicate significative differences between cultivars within the same column ( $p < 0.05$ ).



'Frantoio', 'Leccino' y 'Picual', B- 'Arbequina' and C- 'Barnea', 'Coratina' and 'Manzanilla'. The severity data indicated that the cultivars were different from each other only in some of the evaluated months, each year. Between the months of January and April 2015, significant

differences were observed between the cultivars for the two severity scales (Tables 2 and 3). On the other hand, in 2015/2016 only data of January and for the scale based on the number of injuries by leaf, showed differences between cultivars (Table 3). Regardless of the used scale, the

**Table 2.** Severity index, López-Doncel and others<sup>(6)</sup> (SI-LD) Scale, on leaves of seven cultivars naturally infected with *Venturia oleaginea* and after the treatment with NaOH evaluated between January-April 2015 and November 2015-April 2016.

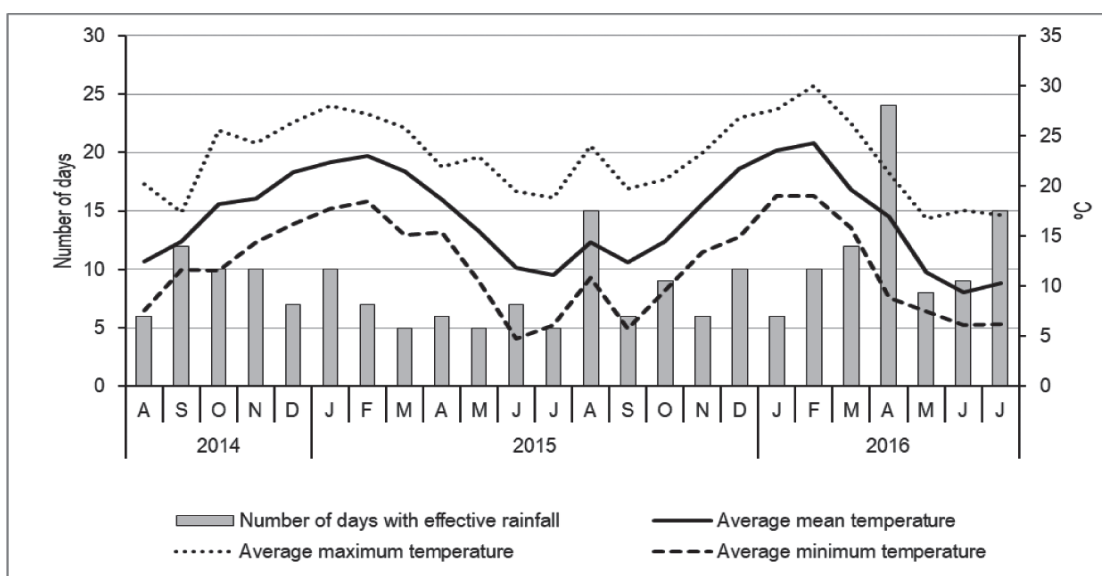
Cultivars	Severity Index									
	Jan-15	Feb-15	Mar-15	Apr-15	Nov-15	Dec-15	Jan-16	Feb-16	Mar-16	Apr-16
Leccino	0,52 a	0,58 a	0,31 a	0,2 a	1,01 a	0,98 a	0,73 a	0,55 a	0,27 a	0,31 a
Frantoio	0,72 a	0,68 ab	0,39 a	0,14 a	0,93 a	1,12 a	0,75 a	0,61 a	0,43 a	0,29 a
Picual	0,74 a	0,79 ab	0,40 a	0,17 a	0,87 a	0,86 a	0,54 a	0,73 a	0,87 a	0,49 a
Arbequina	1,68 ab	0,78 ab	0,38 a	0,13 a	0,72 a	1,68 a	1,25 a	0,70 a	0,71 a	0,53 a
Barnea	2,66 b	2,21 b	1,14 b	0,54 b	2,56 a	2,83 a	1,27 a	0,75 a	0,89 a	0,84 a
Coratina	1,76 ab	1,80 ab	1,15 b	0,88 b	2,11 a	2,64 a	1,20 a	1,81 a	1,21 a	0,99 a
Manzanilla	2,49 b	1,30 ab	0,89 b	0,51 c	1,18 a	3,56 a	1,70 a	1,89 a	1,29 a	0,87 a

Different letters indicate significative differences between cultivars within the same column ( $p < 0.05$ ).

**Table 3.** Severity index (SI-S) according to the Salman Scale and others<sup>(18)</sup> after the treatment with NaOH on leaves of seven olive cultivars naturally infected and evaluated between January-April of 2015 and November 2015-April 2016.

Cultivars	Severity Index									
	Jan-15	Feb-15	Mar-15	Apr-15	Nov-15	Dec-15	Jan-16	Feb-16	Mar-16	Apr-16
Leccino	1,52 a	1,87 a	0,74 a	0,42 ab	2,78 a	3,22 a	2,14 ab	1,57 a	0,51 a	0,69 a
Frantoio	1,72 ab	1,79 a	1,08 a	0,40 ab	2,05 a	2,52 a	1,68 a	1,50 a	0,98 a	0,53 a
Picual	1,69 ab	1,93 a	0,91 a	0,30 ab	1,59 a	2,30 a	1,26 a	1,60 a	1,38 a	1,01 a
Arbequina	2,84 abc	2,08 a	1,01 a	0,34 ab	2,05 a	3,28 a	2,96 ab	2,20 a	2,13 a	1,38 a
Barnea	3,64 c	3,53 ab	3,17 bc	1,43 c	3,89 a	3,99 a	2,71 ab	2,07 a	1,89 a	1,74 a
Coratina	3,92 c	4,04 b	4,04 c	2,74 d	4,45 a	4,01 a	3,98 b	3,63 a	2,64 a	2,77 a
Manzanilla	3,10 bc	2,76 ab	2,66 b	1,27 bc	2,82 a	4,39 a	3,32 ab	3,55 a	2,34 a	2,27 a

Different letters indicate significative differences between cultivars within the same column ( $p < 0.05$ ).



**Figure 3.** Number of days with effective rainfall and monthly average maximum, mean and minimum temperatures from 2014 to 2016 when natural infections of *Venturia oleaginea* were evaluated in the field in Canelones, Uruguay.

cultivars 'Frantoio', 'Leccino' and 'Picual' presented the best behavior, 'Barnea', 'Coratina' and 'Manzanilla' were the ones that presented more incidence of the disease and 'Arbequina' presented an intermediate behavior between these two groups. The climatic conditions registered were favorable for the development of the disease during the sprouting of spring, time of greater susceptibility of the plants. In the spring of 2014, average temperatures were warmer than in 2015 and there were more days (August-November) with rainfall (Figure 3).

#### Response of olive cultivars against inoculations with *Venturia oleaginea* greenhouse trials

After 132 days of incubation in the greenhouse, the plants showed no visible symptoms of the disease. There was also no fall of the leaves in the inoculated plants nor in

the control. After the «development», latent infections were detected only in the inoculated leaves, which showed that the treatment with 5 % sodium hydroxide was effective to detect latent infections. However, the levels of latent infections were very low, both in incidence and severity (Table 4). All the same, it was possible to detect significant differences among cultivars. Cultivars 'Leccino' and 'Frantoio' presented significantly lower incidence than 'Coratina' and 'Manzanilla'. On the other hand, 'Arbequina' and 'Picual' showed intermediate records (Table 4). In terms of severity, although the obtained data were very low, in 2017 'Leccino' and 'Frantoio' also presented the lowest values and statistically different from 'Manzanilla' and 'Coratina', and also in this case 'Arbequina' and 'Picual' showed an intermediate behavior (Table 4). The severity index according to the percentage of diseased foliar area (Scale of López-Doncel and others<sup>(6)</sup>, data not shown) was

**Table 4.** Incidence (I) and severity index (SI) according to injuries per leaf (SI-S), Scale of Salman and others<sup>(18)</sup>, in young sprouts of six olive cultivars inoculated and incubated in the greenhouse.

Cultivar	Incidence		Severity Index (SI-S)	
	2016	2017	2016	2017
Leccino	0 a	0,02 a	0 a	0,02 a
Frantoio	-	0,06 a	-	0,06 a
Arbequina	0,22 b	0,13 ab	0,14 a	0,15 ab
Picual	-	0,23 abc	-	0,25 ab
Coratina	-	0,3 bc	-	0,54 bc
Manzanilla	0,33 b	0,35 c	0,31 a	0,71 c

Means with different letters are significantly different ( $p < 0.05$ ) according to Bonferroni Test.

**Table 5.** Incidence and severity index according to number of injuries per leaf (SI-S), Scale of Salman and others<sup>(18)</sup>, of detached leaves of seven olive cultivars inoculated and incubated in vitro

Cultivar	Incidence		Severity Index (SI-S)	
	2016	2017	2016	2017
Leccino	0,04 a	0,21 a	0,31 a	0,50 a
Frantoio	0,30 ab	0,55 ab	0,30 a	0,87 a
Picual	0,44 bc	0,70 bc	1,04 ab	1,67 a
Arbequina	0,67 bc	0,63 bc	1,25 ab	1,10 a
Barnea	0,79 c	0,74 bc	1,15 ab	1,33 a
Coratina	0,71 c	0,93 c	1,55 ab	2,17 a
Manzanilla	0,75 c	0,93 c	2,04 b	1,97 a

Means with different letters are significantly different ( $p < 0.05$ ) according to Bonferroni Test.

not significant since lesions were very small or tiny dots, therefore the affected leaf area was minimal in all cases.

### Response of detached leaves of olive cultivars against inoculation *in vitro* with *Venturia oleaginea* in laboratory trials

In none of the two tests (2016, 2017) symptoms of the disease were observed in the leaves detached from the plants. 40 days after inoculation, the leaves were removed from the humid chamber because they were starting to become brown and decaying. The leaves developed by immersion in NaOH 5 % showed dark injuries in the inoculation points, confirming the infection of the fungus. The control leaves did not show injuries after the development. The average incidence of latent infections in all cultivars was higher in the 2017 test (0.21-0.93) than in 2016 (0.04-0.79) (Table 5). The cultivars showed differences in behavior with great variability between years (Table 5). 'Leccino' presented the lowest incidence (0.04 and 0.21 for 2016 and 2017 respectively), differing significantly from the other cultivars except for 'Frantoio'. While 'Frantoio' differed from 'Barnea', 'Coratina' and 'Manzanilla' in 2016, and from 'Coratina' and 'Manzanilla' in 2017. 'Arbequina', 'Barnea', 'Coratina' and 'Manzanilla' presented incidence values greater than 0.5 in both years. 'Picual' presented the most erratic behavior between years with 0.44 and 0.70 for 2016 and 2017, respectively. Considering the severity index, no significant differences were observed between the cultivars. 'Leccino' and 'Frantoio' presented values between 0.3 and 0.87 in the two trials. 'Arbequina', 'Barnea' and Picual between 1.04 and 1.67, and 'Manzanilla' presented an average value of 2.04 in 2016 and of 1.97 in 2017. 'Coratina' showed similar behavior to 'Manzanilla' but inversely.

## Discussion

There are numerous studies on olive cultivars susceptibility to *V. oleaginea*<sup>(6)(9)(12)(20)</sup>. The vast majority of these studies refer to evaluations of different cultivars under field conditions and others to evaluations by artificial inoculations. The fact that *V. oleaginea* shows a great difficulty to sporulate *in vitro*, determines that cultivar evaluation by artificial inoculations must be carried out with inoculum coming from the field. As a result of what has been explained above, an enormous variability of susceptibility responses has been reported according to the cultivar, climatic conditions and year of evaluation<sup>(20)</sup>.

Even the same cultivar has been classified in all existing resistance categories, such as the cultivar 'Manzanilla'. Moral and others<sup>(9)</sup> mention that these contradictions found in the literature may be due to incorrect identification of the plant material, differences in virulence between pathogen populations, differences in behavior among clones of the same cultivar or to different climatic conditions in the geographical areas under study.

In this study, all the evaluated cultivars had the disease to a lesser or greater degree in the different experiments. In the field evaluations, the incidence varied between 0.12 and 0.97, the Severity Index according to the percentage of affected area (SI-LD) was between 0.13 and 3.56 and the Severity Index according to the number of injuries (SI-S) was between 0.3 and 4.45. No discrepancies were observed among the obtained results in the different field evaluations, in detached leaves and in potted plants, showing that the inoculation techniques are valid to evaluate olive cultivars against *V. oleaginea*. The severity scales used to quantify the disease were equally useful in determining the level of both visible and latent infections in the evaluated cultivars. The Salman and others scale<sup>(18)</sup> based on the number of injuries per leaf stands out due to its lower requirement in the training of the evaluator for being simpler and faster. The validation of these scales in local conditions allows us to know to what extent the pathogen is affecting the host, which is of utmost importance for producers who, through field scouting, can decide the control management. At the same time, these scales allow the evaluation of the disease management applied, and are a useful tool to compare cultivars for their resistance-tolerance to the disease as well as to perform epidemiological studies on the progress of the disease and associate it with loss prediction systems, among other tasks.

In contrast to the high susceptibility of 'Picual' to *V. oleaginea*<sup>(11)</sup> reported internationally, in this work no differences were observed in susceptibility between this cultivar and 'Leccino' and 'Frantoio', both in the artificial inoculation tests and in the field evaluations. 'Arbequina', the most planted cultivar in the country, presented an intermediate behavior in terms of incidence and severity. 'Coratina', one of the most planted cultivars after 'Arbequina', was very susceptible to the disease, showing incidence levels higher than 0.6 in the field evaluations. Studies conducted in South Africa and Chile<sup>(12)(21)</sup> classified 'Coratina' as a cultivar highly susceptible to olive scab. Msimango<sup>(12)</sup> also evaluated the cultivars 'Leccino' and

'Frantoio' classifying them as moderately tolerant and highly tolerant respectively. On the other hand, Henríquez and Alarcón<sup>(21)</sup> considered 'Leccino' and 'Frantoio' as moderately susceptible based on artificial inoculations and evaluations of natural infections. In this work, 'Leccino', 'Frantoio' and 'Picual' were the most tolerant, without significant differences among them in most of the evaluated dates.

Among the possible causes of the differences found in this study with respect to what has been reported, the genetic variability of the local population of *V. oleaginea*, the different reaction of the cultivars and the climatic conditions appear as the variables that could explain these differences. Although the genetic variability of *V. oleaginea* was not determined in this study, there are precedents that report its existence. López-Doncel and others<sup>(22)</sup> demonstrated the existence of genetic variability between populations of this fungus from different olive farms while Alsalmiya cited by Moral and others<sup>(9)</sup> identified six virulence groups among different populations of the fungus. This variability could explain the differences of 'Picual' behavior between this work and other studies. This behavior has been observed for other cultivars, as it is the case of 'Lechín de Granada', which was very susceptible to olive scab but is mentioned as resistant<sup>(9)</sup> and of 'Frantoio' and 'Arbequina' that are very susceptible in their original regions (Italy and Catalonia respectively) and were resistant and moderately resistant to the populations of the fungus in Cordoba, Spain<sup>(6)</sup>.

The variability in the degree of tolerance of the olive to *V. oleaginea* has sometimes been associated with errors in the identification of the cultivars<sup>(9)</sup>. In this study, the identity of the plant material used in the field evaluation was confirmed by molecular markers (Jorge Pereira, *com pers*) so that an incorrect identification of the cultivars as a cause of the differences found is ruled out. On the other hand, the mechanisms of resistance of different olive cultivars against this pathogen have not yet been studied<sup>(23)</sup>. The response of the different olive cultivars is not separated into discrete categories of resistant or susceptible but shows a wide range of behaviors<sup>(5)</sup>. This aspect, together with the variability of agroclimatic conditions in which the olive is grown, indicates that the behavior of the cultivars against *V. oleaginea* is highly dependent on the evaluation site.

The agro-climatic conditions of Uruguay are very different from the geographic zones where the olive originated and where it is traditionally planted. In Uruguay, high relative humidity, high annual rainfall, and moderate temperatures favor the development of this type of disease<sup>(14)</sup>. These environmental conditions are determinant for

the conidia germination, the establishment of the infection and the symptom development<sup>(6)(8)</sup>. In this context, the choice of cultivars with better behavior against the pathogen is of utmost importance to achieve a sustainable management of the plantation.

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## Author's contribution

All the authors contributed equally to the content.

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