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Survey and evaluation of *Olive Leaf Spot* caused by *Venturia oleaginea* (Castagne) Rossman & Crous (2015) on olive trees in Algeria

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ABSTRACT
Objective: Olive Leaf Spot fungal disease caused by *Venturia oleaginea* (Castagne) Rossman & Crous (2015). It has a significant negative impact on certain olive growing regions of Algeria.
*Methodology and results***:** A survey was conducted from 2013 to 2015 during periods at risk of contamination (i.e. autumn, winter and early spring) to map the geographical distribution of this pathogen. The results obtained showed that *Olive Leaf Spot* is present in all olive- growing regions in Algeria but not in all states. Twenty seven (27) states out of the 35 surveyed states were reported to be infested, with a total of 1163 infected orchards out of the 1696 orchards surveyed, and a percentage of infection also varying from one region to another and depending on the year. The results of the statistical analyses based on Tukey HSD test at $P<0.05$ show very highly significant differences between the Wilayas affected. The western regions are very highly significant, whereas in the east the infection is less important. This difference may be explained by the more favourable climatic conditions, the dominance of a single olive variety "Sigoise" which seems to be more susceptible to the disease and the lack of a technical itinerary.
Conclusion and application of results: This survey is the first on the situation of olive leaf spot in the olive growing regions located in the East, West and Center of the country, allowing to map the distribution of this pathogen, and the use of resistant varieties as biological control agents in the treatment of OLS disease in Algeria.
Keywords: Infected, *Venturia oleaginea*, Olive Leaf Spot, survey, variety.

INTRODUCTION

The olive tree (*Olea europaea L.*) is a symbol tree of the Mediterranean Basin, and a source of wealth and prosperity for all regions of the Mediterranean. In Algeria, growing olive trees has-been encouraged by the Algerian State since the implementation of the National Plan for Agricultural Development (NADP) in 2000. The spatial distribution of olive trees is in constant evolution, and more than three quarters of orchards are situated in the Central east of the Country (Bejaia, Tizi Ouzou and Bouira) and in the Eastern part (Bordj Bou Arreridj, Jijel and Setif). In the Western part of the country (Mascara, Sidi Bel Abbes, Relizane and Tlemcen), olive orchards are intended for the production of table fruits. Currently, the olive tree is cultivated in practically all the province of Algeria (Karboua, 2003). The olive tree is a subject for several pests and diseases causing important economic and agronomic impacts (A yield loss of up to 20% has been around the world, in addition to the deterioration of the quality of the oil) (Viruega *et al.,* 2013; Friday *et al.,* 2010). One of the fungal diseases is caused by *Venturia oleaginea* (Castagne) (Rossman & Crous, 2015), called, *Cycloconium oleagineum* (Castagne, 1845), *Spilocaea oleagina* (Castagne) Hughes 1953 or *Fusicladium oleagineum* (Castagne), (Ritschel & Braun, 2003), known as the Olive Leaf Spot (Viruega *et al.,* 2013) is the most widespread. It has been reported in Mediterranean olive growing regions (Obanor *et al.,* 2005). This scab, which has been described for almost a century, causes dark circular spots being later surrounded by a yellow or pale green halo on the upper surface of the leaves. Each circle corresponds to a season of development of the fungus. When the pathogen develops its conidia, the spots become dark brown; sometimes black (Obanor *et al.,* 2005). Attacks on the fruit and peduncle are rare, (Graniti, 1993), but if they are severe, they can prematurely cause total defoliation of the tree. The management strategy against the Olive Leaf Spot consists of monitoring the disease and protecting plants early in the season to control its spread within orchards of susceptible varieties, by applying fungicide treatments, mostly based on Copper (Obanor *et al.,* 2008; Salman *et al.,* 2011). This work, will present the different olive-growing regions (Central, Eastern and Western) prospected in Algeria in order to draw up a distribution map of this pathogen for a better control strategies. Also, to evaluate the tolerance of cultivars towards *Venturia oleaginea.*

MATERIALS AND METHODS

Survey of olive growing regions: A survey was conducted during three successive campaigns (2013, 2014 and 2015), a questionnaire survey was drawn up which takes into consideration several factors including the department, the commune, the climatic conditions, the data on the crop (age, variety, origin, phytosanitary treatments applied, soil type and irrigation system used) (Information sheet below).

Information sheet

➢ **Orchard data**

• Municipality: …… Wilaya: ……..……
• Area (ha): ……
• Control system: ……..……
• Type of soil: ……
• Geographical coordinates:……

➢ **Culture data**

• Specified the culture of the olive tree either: Orchard ☐ In masses ☐ Others ☐
• Origin: ……
• Variety: ……..……
• Planting date: ……
• Vegetative stage: ……
• Date of prospecting: ……..……..…
• Cultural practices used……

➢ **Presence of scab**

• Year of appearance: ……
• Infection rate (%):…. ……
• Phytosanitary treatments used against Olive Leaf Spot
(Period of application, number of uses and dose): ……

In 35 olive growing areas, a total of 1,696 canvas was recovered for the three campaigns: in 2013 (517 Survey sheet), 2014 (452 Survey sheet) and in 2015 (727 Survey sheet), in periods at risk of contamination, from late September to December, and from late February to early April, as recommended by Sanei and Razavi (2011) who considered that the best periods of infections are Fall and the Winter.

Surveys and sampling: Sampling was carried out at the level of the orchards reached using the technique of complete random blocks with four repetitions where each block contains 5 trees taken at random. A total of 100 leaves/tree were collected at the four cardinal points (Eastern, Western and Central), for a total of 2000 leaves taken (symptomatic or asymptomatic) by region and by campaign to evaluate the incidence of the disease. Each sample collected from the orchard was put in a paper bag with the variety, age, site, and date of collection. The study was carried out on 16 varieties of olive trees: Sigoise, Terrella, Khodeiri, Sorani, Zaïti, Chemlal, Sylvaine, Rougette, Blanquette, Arbequina, Manzanilla, Azerradj, Moroccan, Bounguergueb, Beef Heart and Limli. Field trips are made twice per region during all Fall/winter and early spring infection periods and during the three successive seasons. The symptomatic leaves (Figure 1a) are calculated in relation to the total number of leaves collected. The asymptomatic leaves were analysed using the Soda method. This technique is used for the detection of latent Olive Leaf Spot not visible in the field (Salman *et al.*, 2011). It consists of soaking the asymptomatic leaves in a 5% Sodium Hydroxide (NaOH) solution for 20 minutes at room temperature (Lopez Doncel *et al.*, 2000). Visible lesions appeared as black spots on the majority of the analysed leaves (Figure 1b). These black spots are due to the reaction of the alkaline solution with phenolic compounds produced. This solution caused deformations under the epidermis; the upper epidermal cells infected leaves will become more visible (Lops *et al.*, 1993; Lopez Doncel *et al.*, 1999).

Figure 1. Typical symptoms of *F. oleagineum* (Var. Sigoise) at the Center of Algeria.
a: Symptoms on tree;
b : Symptoms after 20 minutes in the solution of Hydroxide of Sodium in 5%.

Monthly average temperatures and precipitation are recorded from September to April for the 3 seasons (2013, 2014 and 2015). These data will show us the most favourable periods for *Venturia oleaginea* infections.

Symptomatological diagnosis of the infection: All the collected leaves were visually examined. Infection was reported based on symptoms using the description already published by several authors Mac Donald *et al.,* (2000); Gonzalez- Lamothe *et al.,* (2002); Obanor *et al.,* (2005); Trapero and Lopez- Doncel (2005); Trapero and Blanco (2008); Viruega *et al.,* (2013): appearance of circular spots of an oily dark and pale yellow or green halo on the upper leaf surface.

Statistical analysis: The results of the statistical analyses were carried out on the percentage of infected leaves compared to the total number of leaves collected with the XLSTAT 1994 software (ANOVA).

RESULTS

Geographical distribution: In the light of the results obtained during the surveys in the different olive-growing regions, a geographical distribution map of the peacock eye was drawn over 2013, 2014 and 2015 campaigns. Out of 35 surveyed states including 514 communes, 27 states revealed the existence of the peacock eye in 1163 orchards (a rate of 68.6%) out of the 1696 orchards surveyed, these states are (Figure 2):

- **Western province:** Relizane, Mostaganem, Oran, Chlef, Tlemcen, Sidi Bel Abbes, Ain Temouchent, Mascara, Ain Defla;
- **Center province:** Djelfa, Blida, Medea, Tizi Ouzou , Algiers, Boumerdes, Bejaia , Bouira , Tipaza;
- **Eastern province:** Souk Ahras, Annaba, Setif, El Tarf, Mila, Bourdj Bou Arriradj, Oum El Bouaghi, Skikda and Jijel.

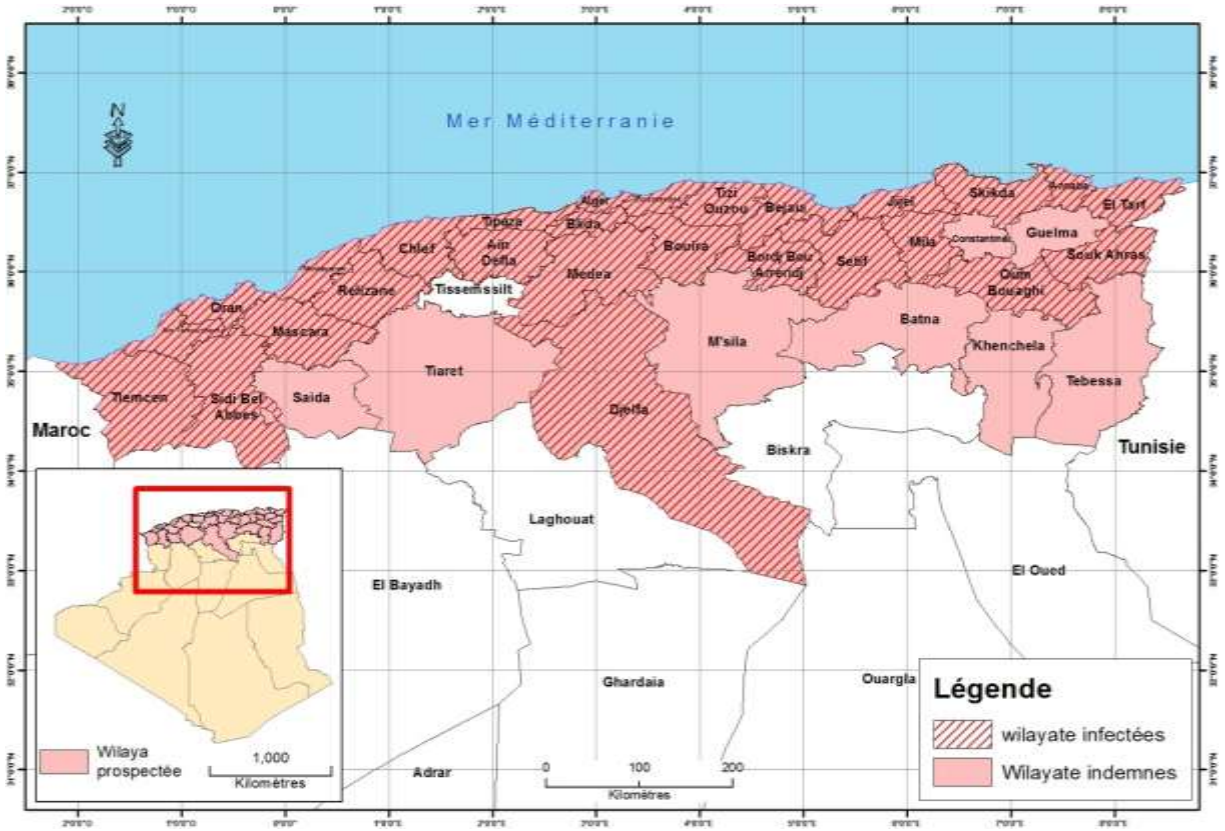

Figure 2: Geographical distribution of the Olive Leaf Spot in Algeria (Campaigns 2013, 2014 and 2015).

Orchard infection rate by campaign: The infection varies from one campaign to another, from a rate of 81% in 2013 to less than 74.6% in 2014 and 56.2% in 2015. The most infected orchards are those from the West (87.80% in 2013, 81.90% in 2014 and 70.50% in 2015). In the Central of the country, this rate is lower (8.60%, 13.60% and 19.3%). In contrast, the Eastern regions recorded significantly lower rates (3.6%, 4.40% and 10%) (Figure 3 a, b, c). The south of the country is for the moment unscathed by this pathogen.

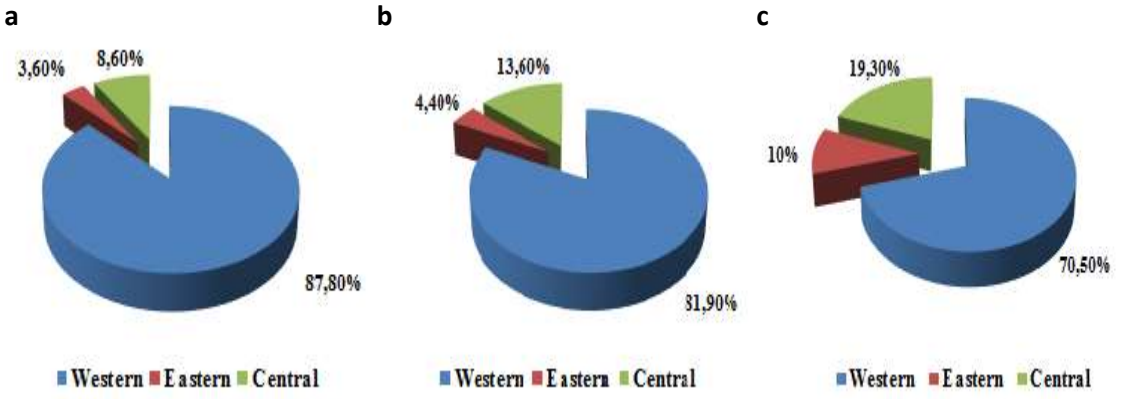

Figure 3: Distribution of the infection rate of affected orchards collected by region during the campaigns 2013 (a), 2014 (b) and 2015 (c).

Symptomatological estimation of the infection: The rates of visible and latent infections were calculated on 2,326,000 olive leaves taken at random from 1163 orchards reached during the three campaigns 2013, 2014 and 2015. The results obtained in the table 1, based on field observations, show visible infections (symptomatic leaves), revealed by the method of soda were variable (45.84% and 38.50%), a rate of 84.34% compared to the total number of leaves collected, asymptomatic leaves is around 54.16%. A total of 364,284 leaves remaining healthy, representing a low rate of around 15.66%, were recorded during the 2013, 2014 and 2015 campaigns.

Table 1: Number of leaves collected and leaves tested by the Soda method (5% NaOH solution).

Campaigns	Total number of collected leaves		Leaves tested with the Soda method	Healthy leaves
	Symptomatic leaves	Asymptomatic leaves		
2013	364 300	471 700	387 648	84 052
2014	297 120	376 880	225 864	151 016
2015	404 760	411 240	282 024	129 216
Total	1 066 180 (45.84%)	1 259 820 (54.16%)	895 536 (38.50%)	364 284 (15.66%)

According to symptomatological estimates, the cultivars react differently to the disease and thus show a very highly significant difference between the different cultivars for incidence ($F=19.02$, $df=8$, $P<0.000$ in 2013; $F=19.56$, $df=8$, $P<0.000$ in 2014 and $F=29.06$, $df=5$, $P<0.000$ in 2015) and severity ($F=4.23$, $df=8$, $P<0.000$ in 2013; $F=2.27$, $df=8$, $P<0.000$ in 2014 and $F=14.00$, $df=5$, $P<0.000$ in 2015). In the Sigoise, Terrella, Khodeiri, Sorani and Zaiti varieties, the incidence is significantly higher compared to the "Chemlal" cultivar, which is resistant. While Manzanille, Azerradj, Moroccan Picholine, Bounguergueb, beef Heart and Limli had infection rates of less than 50%, they are considered semi-resistant. No infection was recorded among the four cultivars: Sylvaine, Rougette, Blanquette, Arbéquina throughout the period of the survey, they seem to be the most resistant. It has also been observed that the highest level of infection has resulted in defoliation of the trees. Most of the affected leaves seem to fall prematurely around the olive tree (Figure 3a). The presence of the pathogen *Venturia oleaginea*, has been reported in young plantations from 1 to less than 30 years old and older orchards from 30 to over 50 years. The age of the orchards was counted and classified as follows: A (1 to 10 years), B (10 to 30 years), C (30 to 50 years) and D (>50 years). Following the age scale, we found four (04) groups; the highest degree of infection affected was in-group A and B, then group C and group D. This situation leads us to believe that the intensity of the attacks in most of the western regions may also be linked to the criterion of the age of the orchards surveyed.

DISCUSSION

In light of our results, there is a strong infection of the disease, which should appear the spring in the western regions and the central countries. These results are similar to those of some authors (Gorter, 1943; Asawah, 1967) which showed that a high infection of the disease appears in spring in the land of Egypt, Kobras and North Africa. The factors

favouring the development of this pathogen concern favourable climatic conditions (Tajnari, 1999), a temperature in the range of 5 to 25°C (Sistani *et al.*,2009) with an optimum between 15 to 20°C and optimal humidity 80-85% (Guechi and Girre,1994). Indeed according to Obanor *et al.*,(2008) and Trapero *et al.*,(2001), infection can occur in two periods of the year, especially in spring (late February to early April) and in autumn - winter , particularly in autumn (late September to December) (Sistani *et al.*, 2009). In North America, average temperatures are between 17°C and 24°C in autumn and early winter in the states of Tipaza, Boumerdes, Algiers, Blida and the Kabylie Mountains (Bejaia, Tizi Ouzou and Bouira). The end of winter (end of February) and the beginning of spring (March and April) have a temperature of 19 to 24°C on average. In summer, high temperatures halt the development of the fungus (Al-khatib *et al.*, 2010). Graniti (1993) observed that all infected leaves that fell after infection (Figure 4a), with the remaining infected leaves become a source of infection during the next season (Figure 4b), which explains the appearance of the disease in three successive seasons. It has been shown that this pathogen can survive adverse conditions: dry and warm weather in dead leaves, as well as in infected leaves on the tree (Figure 4c, d, e).

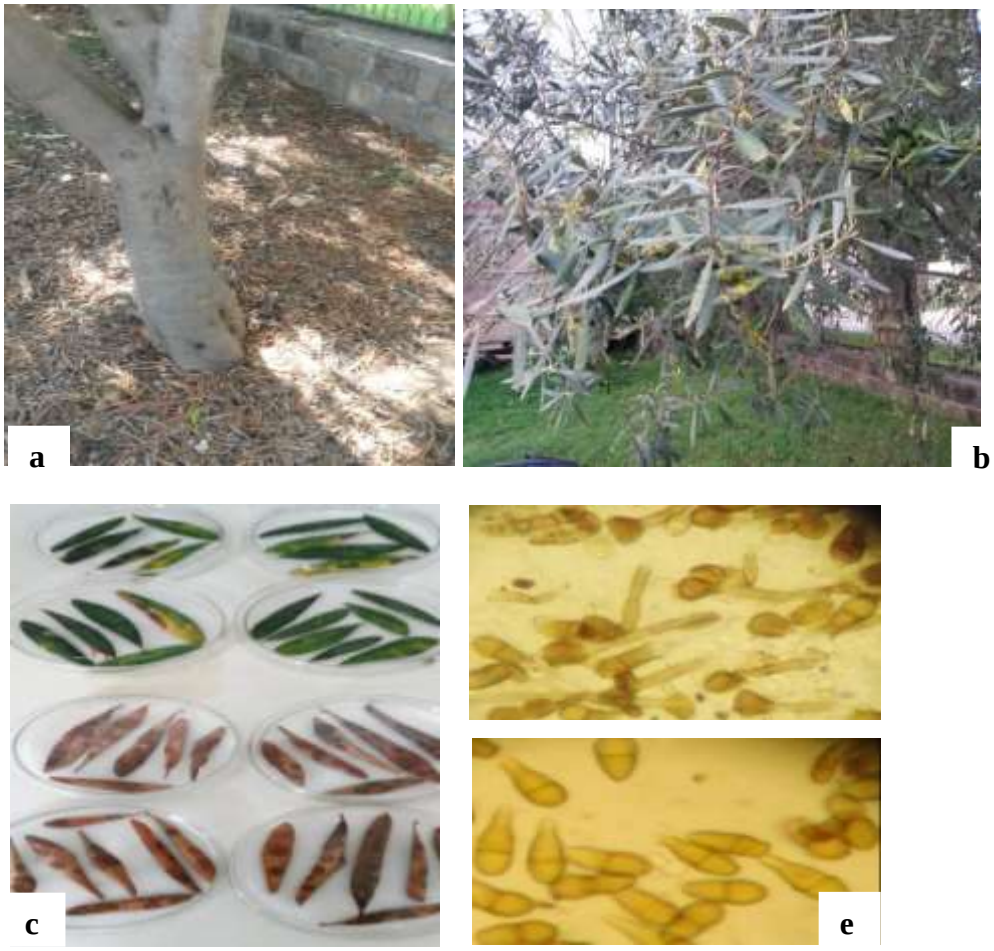

Figure 4: Macroscopic and microscopic observations of Olive Leaf Spot of the olive tree caused by *Venturia oleaginea*; **a:** Affected leaves detached from the tree. **b:** Leaves remaining expectations on the trees; **c:** Humid leaf chamber in the New Year and the year preceding; **d** and **e:** *Venturia oleaginea* conidia after 24h at 48h incubation at 20°C.

According to the symptomological estimates realized on the field, cultivars respond differently to this disease. In the cultivars "Sigoise, Terrella, Khodeiri, Sorani and Zaiti", the incidence assessment was significantly higher in the western and the central olive groves compared to the Chemlal" cultivar. These results are consistent with those obtained by Abuamsha *et al.*, (2013), who consider that the "Chemlal" variety was resistant to the pathogen. The varieties "Sylvaine, Rougette, Blanquette, Arbéquina" were the most resistant varieties to the disease. After Cyril Msimango (2015), the cultivar "Sylvaine" which was the subject of a tolerance study at the *Venturia oleaginea* seems sensible. On the other hand, Manzanille, Azerradj, Marocain, Bounguergueb, Heart of beef and Limli show a weak appearance of the disease; they appear resistant, since they have a lower incidence compared to all other varieties. In a similar study, Mekurie *et al.*, (2001), consider that the cultivar "Manzanille" was semi-resistant. In the Eastern regions, it is less than 10%, which does not conform to Guechi's results (2001). The latter notes a very strong contamination in several sites of the Center including Bejaia, Tizi Ouzou and Bouira and East to Setif. In the Northwest, especially in Oran, Mostaganem, Tlemcen, Relizane, the temperatures vary between 19°C and 24°C. The pathogen has become darker and produces a very high rate of conidia. In Algeria, olive groves differ in several parameters: cultural practices such as size, fertilization, the irrigation system (seguia and drip) and the type of soil (sandy, clay, loam, limestone or a complex between the two types of soil) that characterizes all regions surveyed. But these cultural practices have no influence on the appearance of the disease, unless the abiotic conditions are favourable, a temperature in the range of 5 to 25°C (Sistani *et al.*, 2009) with an optimum between 15 and 20°C and humidity with an optimum of 80-85% (Guechi and Girre, 1994). During the survey conducted on 35 states during 3 consecutive campaigns, from 2013 to 2015, it was established a distribution map of the Olive Leaf Spot is an economically important disease in some regions of the West and the Center. But the infection in the olive orchards achieved differs from one region to another and from one season to the next. However, the use of susceptible varieties facilitates the appearance of the peacock eye with a higher rate of infection in the presence of favourable climatic conditions to the development of the disease. It should be noted that orchards affected by the Olive Leaf Spot are irrigated either by seguia or by drip, or irrigated affecting much more the colonial orchards or planted in the mountainous areas. Our results show the constant regression of the disease in Algeria.

CONCLUSION AND APPLICATION OF RESULTS

During our survey of 35 province for 3 consecutive campaigns, from 2013 to 2015, a distribution map of the Olive Leaf Spot was established which shows how much it is growing in Algeria. The Olive Leaf Spot is an economically important disease in parts of the West and Center. However, the infection in affected olive orchards differs from region to another and from campaign to another countryside. However, the use of susceptible varieties facilitates the appearance of the Olive Leaf Spot with a higher infection rate in the presence of climatic conditions favourable to the development of the disease. It should be noted that orchards affected by the Olive Leaf Spot are irrigated either by seguia or by drip or non-irrigated affecting much more colonial orchards or planted in mountainous areas. Our results show the constant progression of the disease in Algeria.

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