FACTORS AFFECTING RESISTANCE OF TWO OLIVE CULTIVARS TO LEAF SPOT DISEASE IN THE NORTH WEST OF TUNISIA

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ABSTRACT

Evaluation of the incidence of the leaf spot disease caused by the biotrophic pathogen *Fusicladium oleagineum* on two olive cultivars (*Olea europaea* L.) was carried out during six years (2009/2015) in groves located at Touiref (North West of Tunisia). The studies focused on Meski and Chetoui cultivars. The evaluated foliar incidence of disease was analyzed in relationship with some factors. Disease incidence varied greatly among the tow cultivars submitted to similar conditions. Meski is the susceptible olive cultivar grown but Chetoui is more resistant to these pathogen infections ($R^2 = 0.94$). The obtained results revealed that disease incidence was correlated with the height of the trees ($R^2 = 0.80$), with the direction ($R^2 = 0.82$), with the leaf age ($R^2 = 0.78$) and the relative humidity ($R^2 = 0.91$). During our studies, we were attempted to collect the fallen leaves under trees to survey the development of the fungus *Fusicladium oleagineum*, but no sexual form was found.

Keywords: Disease incidence, Fusicladium oleagineum, olive, resistant, susceptible.

INTRODUCTION

The olive (Olea europaea) is the most important olive-growing in Tunisia country where occupied around 30 % of its cultivated land (1.68 million ha). The annual production is recently estimated to 790,000 t of olives in 2014 (FAO, 2014). The olive leaf spot disease (peacock spot) is caused by the fungus *Fusicladium oleagineum* (syn *Spilocaea oleaginea*) causes severe premature defoliation of the olive tree (*Olea europaea*), and sometimes the death of the whole plant (Miller, 1949; Azeri, 1993). Table olive cultivar Meski and oil olive cultivar Chetoui are the most growing in the North West of Tunisia. The first one is susceptible to leaf spot but Chetoui is less susceptible. The disease is widespread in the world and in specialized areas of cultivation of olive trees with yield losses estimated at more than 20% (Wilson and Miller, 1949). Moist weather conditions favor fungus sporulation, conidia germination and infection, and young leaves are more susceptible to infection than older ones (Graniti, 1993). Conidia germination and infection required continuous moisture for 12-24 h and temperatures ranging from 5 to 25 °C (Obanor et al., 2008).

This foliar disease is very common in the humid regions of Tunisia and widespread in many olive-growing regions, including North West of the country. Infections are normally associated with high humidity and winter conditions, where high temperatures restrict spore germination and growth (Al-khatib et al., 2010). Symptoms were quickly determined according to the method of (Zarco et al., 2007).

Fusicladium oleagineum (syn= *S. oleagina*) is a specific pathogen of olive (*Olea europaea L.*), whose development is restricted to tissues cuticle affected (Tenerini and Loprieno, 1960), as with the causative fungi the scabs of fruit (Wheeler, 1969). The name refers pathogen only the asexual state fungus. The sexual stage, although it has been subject of some research

(Graniti and Laviola, 1968; Laviola, 1966; 1968; Miller, 1949; Viruega et al, 1999), it is not known, but could correspond with *Venturia* by analogy with other species *Spilocaea*, as with S. pomi, anamorph of *Venturia inaequalis*, causing the 'scab' or 'Mottled' apple (MacHardy, 1996). The life cycle of the causative agent, Spilocaea oleagina is dependent on climatic conditions, which the most important are temperature and humidity (Obanor et al, 2008).

According to results of previous studies (Obanor et al, 2011; Viruega et al, 2011), the main risk period occurs during early spring due to the presence of conidia of the pathogen, favorable weather conditions temperatures from 15 to 20° C and wetness duration >24 h), and the presence of developing leaves (Viruega and Trapero, 1999).

Other authors have suggested that infected fallen leaves are an important inoculum source but they did not provide any epidemiological evidence to support this claim (Guechi and Girre, 1994; Hudson, 1971), we cannot exclude the possibility that they might function as an inoculums source under different weather conditions, with different populations of the pathogen, or in the presence of different saprophytic microorganisms.

Despite its importance, there is little information on some factors affecting their development of this disease in our country. Thus, we conducted this attempt of study in order to contribute in the understanding of the development the leaf spot disease and the effects of various factors environmental and host on infection and development of disease and olive cultivars resistance to leaf spot disease.

METHODOLOGY Plant material

The olive groves were located in Touiref (in El-Kef at the North West of Tunisia: $36^{\circ}.12$ 'E; $8^{\circ}.7'$ N, 516 m above sea level)). Fresh leaves of two cultivars (Chetoui, and Meski) were collected in spring time, the main period of olive attack by the leaf spot disease, from orchards planted in 2001, during the seasons beginning 2009 and finished on 2015.

A total of 100 Leaves were collected from three trees of each variety selected and then stored at 4°C in Crop disease laboratory of ESA Kef. The determination of the percentage of latent infections of olive cultivars by the leaf spot disease was based on the method of (Zarco et al., 2007). It consists on immersing leaves in a 5 % sodium hydroxide solution for 20 minutes at 22 °C. After this treatment, visible lesions were more prominent and latent infections appeared as black circular spots, clearly differentiated from healthy green tissue.

Assessment of incidence disease for different cultivars

100 leaves, visibly infected, were randomly collected from 20 trees of the same age (planted in 2001) for each cultivar (Meski and Chetoui). Latent infections were determined by immerging leaves in a NaOH 5% solution for 20-30 min (Zarco et al., 2007). The number of infected leaves was recorded. For each variety, three repetitions were done.

Assessment of incidence disease for different level of relative humidity

Trials were conducted on Meski and Chetoui cultivars, the first which is highly susceptible to OLS (Graniti, 1993); the second is moderately susceptible (Triki et al., 2003). Measurements of relative humidity were taken randomly in each spring for all 6 years by means of a Lutron HT-3001F Digital Humidity / Temperature Meter.

Assessment of incidence disease for different heights of trees

A total of 90 leaves were collected from the 40 trees of 2 cultivars (Meski and Chetoui). For each tree 30 leaves were collected from the bottom, 30 from the middle and 30 from the down. The percentage of infected leaves.

Assessment of incidence disease for different ages of leaves

90 leaves from each variety were chosen randomly: 30 leaves new formed (for the current year), 30 leaves from the last year and 30 oldest leaves were collected and evaluated for the latent infection. Evaluation of latent infections was carried out by method described by Zarco et al. (2007).

Assessment of incidence disease for different directions

For the field trials, latent infections were based on a total of 100 leaves taken randomly from each cultivar with 20 leaves on per branch on five branches selected in various directions. The sampling was repeated three times and data were transformed to meet the requirement for normal distribution.

Fungal material

Leaves fallen down the trees were collected in two seasons in order to study the cycle of the fungus Fusicladium oleagineum. In Phytomycology laboratory of El-Kef High School of Agriculture conidia were obtained from fresh diseased leaves of olive tree cv Meski (susceptible) and cv Chetoui (less susceptible). In order to obtain the suspension, the infected leaves were dried at ambient temperature for 2 weeks and then stored at 5°C in darkness (Trapero et al., 1994) and then were agitated in sterile distilled water for 2 h at 80 rpm at ambient temperature. The conidial suspension was then filtered through a double layer of cheesecloth to eliminate vegetable matter (Alonso et al., 2006). Observations of germination were done on slides for each sample.

Data analysis

A data incidence diseases was applied on the analysis of variance and the Mean values were compared using Fisher protected LSD test at P = 0.05 (SAS, 1985). All measurements were repeated at least once and results show the average values of experiments.

The results were analyzed using analysis of variance (ANOVA) and Duncan's multiple range tests (Software SAS System, 1985).

RESULTS

Evaluation of latent infections of cultivars

Since the symptoms are invisible before 176 days after inoculation, it was important to use latents infections technique. The results shown in Fig.1 reveal an important variation in the susceptibility of the cultivars to the disease. The analysis of variance and the means comparison revealed significant differences between the cultivars. Indeed, the Meski cultivar was the most susceptible and Chetoui the most resistant to the disease since 8 % latent infections were observed. The However, cv. Meski was the most susceptible to the disease, with the percentage of latent infections ranging from 33 % to 52 % (figure 1).

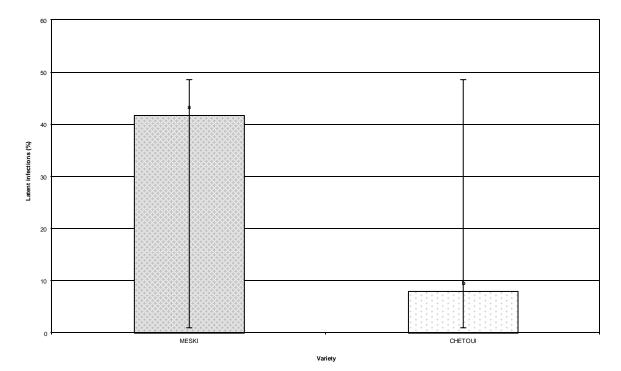


Figure 1. Effects of relative humidity of collected leaves on percentage of latent infections of the leaf spot disease

Incidence disease for different levels of relative humidity

The analysis of results showed that there is a high significant difference in the percentage of latent infections of leaf spot disease between the two cvs and different levels of relative humidity. The relative humidity is raising more than the percentage of infestation by olive leaf spot is high. The Meski variety presents the highest percentage of latent infections with about 65% at RH>80%. If is less than 60%, leaves of the two cultivars presented a minimum lesions.

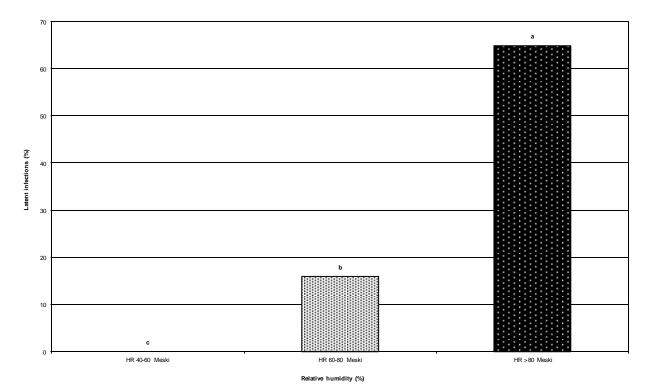


Figure 2. Effects of relive humidity of collected leaves from Meski cv. on percentage of latent infections of the leaf spot disease

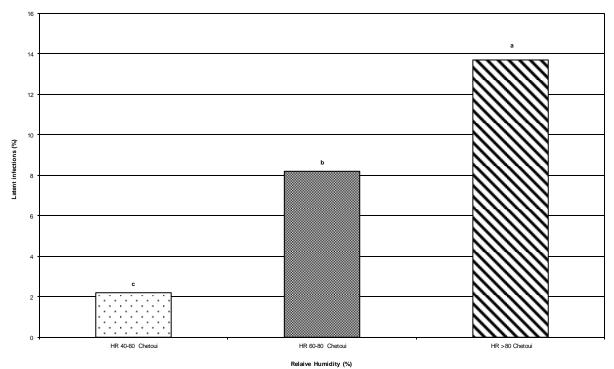


Figure 3. Effects of relative humidity of collected leaves from Chetoui cv. on percentage of latent infections of the leaf spot disease

Incidence disease for different heights of trees

Measurements of latent infections percentage for three different level of tree height showed significant differences among the same cultivar and between cultivars. Latent infections ranged between 3 and 11 % for the cv. Chetoui and from 20 to 60% for cv. Meski. In all cases, Chetoui cv. had the lowest latent infections percentage. Meski has the highest percentage of infected leaves for all levels of height.

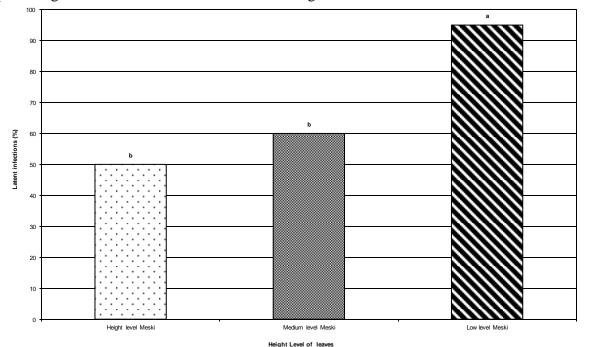


Figure 4. Effects of height level of collected leaves from Meski cv. on percentage of latent infections of the leaf spot disease.

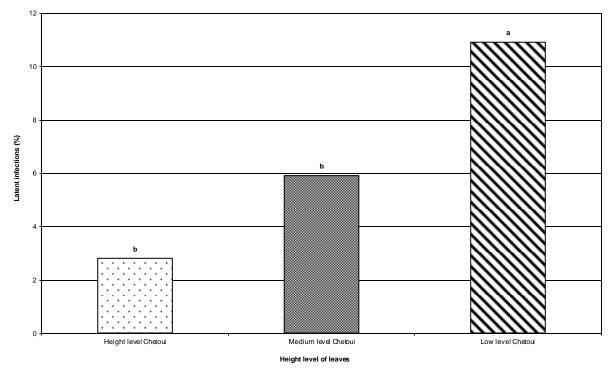


Figure 5. Effects of height level of collected leaves from Meski cv. on percentage of latent infections of the leaf spot disease.

Incidence disease for different ages of leaves

The main effects of age were highly significant (P <0.05), and the means of measurements repeats along 6 years differ significantly. For each cv. young leaves showed the highest infection, but oldest had significantly fewer leaf lesions (figure 6, 7). The effect of leaf age on the rate of infected leaves was highly significant. Oldest leaves presented the lowest latent infections percentage which reduced by 7.3% for Chetoui cv. And 45% for Meski cv. This increase of degree of susceptibility of the olive tree to OLS disease with decrease of leaf age might be due to the cuticle thickness.

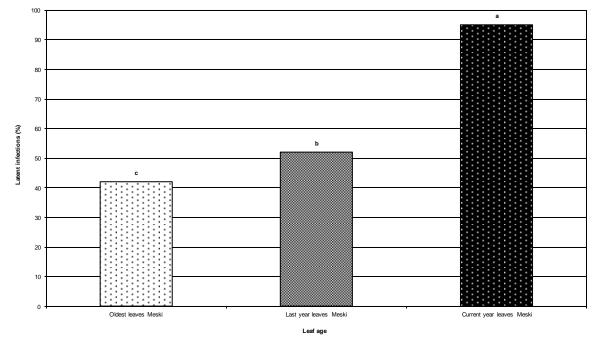


Figure 6. Effects of age of leaves from Meski cv. on percentage of latent infections of the leaf spot disease.

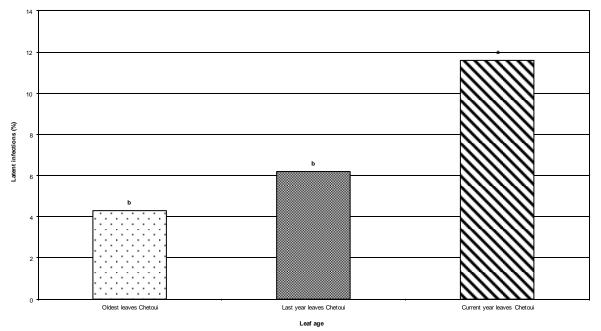


Figure 7. Effects of age of leaves from Chetoui cv. on percentage of latent infections of the leaf spot disease.

Incidence disease for different directions

The efficacy of the different directions varied was shown in fig 5. At all trees, the leaves submitted to the north direction were the most affected by leaf spot disease with the mean of latent infection percentages ranging from 16 to 67% for Meski cv. Latent infections for Meski cv. was approximately reduced by 50% on trees that exposed to south and eastern directions, compared with about 6% latent infections for those of Chetoui cv. in the same directions (Figure 8, 9).

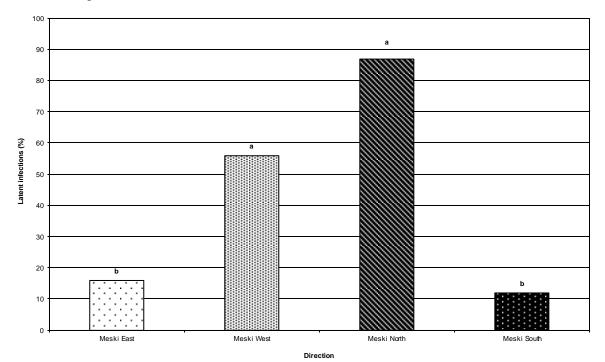


Figure 8. Effects of direction on percentage of latent infections of the leaf spot disease on Meski cv.

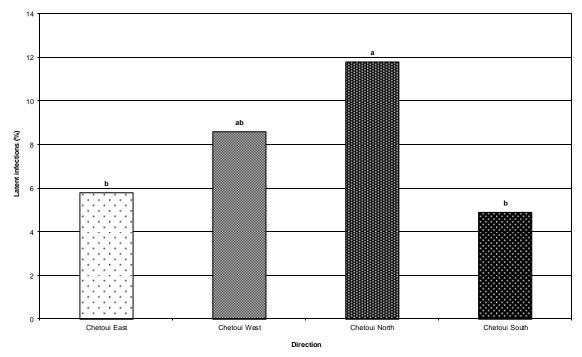


Figure 9. Effects of direction on percentage of latent infections of the leaf spot disease on Chetoui cv.

Fungal development

Observations on slides containing *Fusicladium oleagineum* showed conidia germination on fallen leaves until 12 days in spring time, after that conidia development was stopped in culture media. Studies carried out on different cultivars and in various seasons showed that no sexual form was observed on infected leaves or in culture media.

DISCUSSION

Our studies were carried out on two olive cultivars in the north west of Tunisia in order to survey the effects of some biotic and abiotic factors on their resistance to olive leaf spot disease.

The olive leaf spot disease, caused by the fungus *Fusicladium oleagineum* is the disease most common olive in many countries, causing severe defoliation that weaken progressively the tree and cause a crop losses estimated at more than 6%, although in some years and places can be catastrophic. Most infected leaves and fruit fall to the ground. Olive oil obtained from fallen fruit is of poor quality because various saprophytic fungi colonize the fruit and change the acidity and organoleptic characteristics of the oil (Andrés, 1991).

Although symptoms may vary depending on the variety olive, age and conditions of the lesion environment in which they develop, usually are easily identified (Trapero and Blanco, 1999).

It is known that climatic conditions influence the development of the olive leaf spot. The disease is very grave in humid areas and causes very significant yield losses (Wilson et Ogawa, 1979). The fruit rot caused by *Spilocaea oleagina* was observed in the humid zones, case of Calabria region (Southern Italy) characterized by environmental conditions favoring the development of the epidemic (Graniti,1993) depends on other factors: cultivar types, density and age of plantations (Agostino , 2007; Graniti, 1993; De Marzo et al., 1993; Laviola et Scarito, ,1993; Macdonald et al., 2000).

Latent infections of disease were measured in spring and autumn seasons, the main periods of development of leaf spot in Tunisia. López-Doncel et al. (1994, 1998, and 1999) has used the same method in laboratory to evaluate the resistance olive cultivars to olive leaf spot disease and to determine the influence of factors environmental and host the infection and leaf spot disease development (Viruega and Trapero, 1999). Our results showed that Meski is susceptible than Chetoui to this disease. Many studies showed that Meski cultivar is the most susceptible and Chetoui is less susceptible but Picholine variety is more resistant to OLS (Triki et al., 2003).

Measurements carried on the leaf age, height and direction of trees on two olive cultivars Meski and Chetoui showed a difference between varieties. The relative humidity effects were significantly different for the cultivars on the disease incidence. Weather conditions and the presence of developing leaves during early spring are usually adequate for leaf infection in olive growing areas in southern Spain (Viruega et al, 2011; Obanor et al, 2011; Roubal et al, 2012).

Studies conducted on olive inoculated with *S. oleagina* have revealed that the severity of infections is correlated linear and negatively with age leaves, being very young leaves extremely susceptible. Because the mature olive leaves have important resistance to infection

by *S. oleagina*, the main infection periods occur during the leaf growth (Obanor et al, 2011; Viruega et al, 2011). These results are consistent with those of López-Doncel et al. (2000) who found that younger leaves were more susceptible to olive leaf spot than older leaves.

The obtained results definitely clarify the controversy existing between authors assigned increased susceptibility to the old leaves (Andreucci and Bonifacio, 1962; Petri, 1913; Prota, 1958; Wilson and Miller, 1949) and those who thought otherwise (D'Armini and Raggai, 1966; Lavee, 1990; Tenerini, 1964), based mainly on observations country. Typically, disease severity on the youngest leaves was about three to five times higher than the oldest leaves, and there was a more rapid increase in the severity between 6 and 24 h of wetness for the youngest leaves than for the other two leaf ages (Obanor, 2011).

MacHardy (1996) reported that the decrease of olive scab severity with increasing leaf age is a case of resistance, or age-related resistance, which has been reported for many plant diseases including apple scab. The pathosystems has been associated with various physical and chemical features of the leaf cuticle in California (Miller, 1949; Graniti, 1993; Guechi and Girre, 1994).

The differences could also be associated with the water-repellent waxes that are built up on leaf surfaces as they age. The waxes prevent formation of water films in which pathogens might germinate (Agrios, 1997). In addition, the thicker cuticle of older leaves can better impede penetration than in young leaves (Jeyaramraja et al., 2005), an important factor for *S. oleagina* which infects by direct cuticle penetration (Graniti, 1993).

The results of this study showed that the leaves exposed to north and west directions presented more lesions on upper surface of Meski variety and then were more susceptible to the OLS then those in the eastern and south fronts. In Morocco, Besri and Outassourt (1984) have worked on the olive leaf spot distribution on two faces (North and South) of the some trees canopy in two olive-growing regions: Béni Mellal and Marrakech. Both authors noted that the percentage of leaves infection exposed to North is significantly higher than leaves exposed to South. Tajnari (1999) also studied the disease distribution in the region of Kelaa Sraghna in Morocco. He noted that the exposed face of the olive tree in the North is the most contested, followed by the West, South and East. The interior of the canopy is more attacked than the outside and the bottom of the canopy is more infested than the top.

For The height of tree influences, obtained results showed that percentage of latent infections decreases with increasing distance from the land. Graniti (1993) indicated that environmental factors such as temperature and humidity are the driving forces of infection and spread of the disease. The dispersal distance and its response to weather conditions have not been reported for olive scab as it has been for the ascosporic phase of similar pathogens, such as *Venturia inaequalis* and *V. pirina* (Aylor, 1995).

Conidial germination occurs during a cold and rainy weather (Tajnari, 1999). The excess of free water increases the humidity in the foliage of the olive trees; the leaf surface becomes wet which promotes the dissemination, contamination and germination of conidial (Laviola et Scarito, 1993). In the absence of rain and when the humidity is above 70%, there is a dispersion of conidia by wind which affect severity of the olive leaf spots (Tajnari, 1999; Besri et Outassouta, 1984).

The conidia germination was stopped after less than week. These results are in concordance with those obtained by Vruega and Trapero (1999) which reported that the conidia formed in leaves on fallen leaves can survive for several months; although once they have separated from the conidiophores they lose their germination ability in less than week. Sexual fruiting bodies of *S. oleagina* were not observed in this study or found in previous research done in California (Miller, 1949; Graniti, 1993; Guechi and Girre, 1994). According to other authors, however, the most important inoculum sources are infected leaves that have fallen to the soil surface (Assawah, 1967; Guechi and Girre, 1994; Laviola, 1968). Under humid conditions, the pathogen could not be detected on fallen leaves after 3 months because the leaves were colonized by saprophytic fungi (Viruega et al., 2011).

CONCLUSIONS

Finally, our study demonstrated that in North West of Tunisia, autumn and spring survey of development of OLS on Meski and Chetoui cultivars in the similar field conditions, indicated that there are correlation between some parameters like cultivar, leaf age, height of tree, direction of leaves and relative humidity and the severity disease of leaf spot.

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