© 2015 IJSRST | Volume 1 | Issue 6 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
1st International Symposium of Applied Biology CIBA 2015 USTO-MB, Algeria in association with IJSRST

Isolation of monosporal strains of *Fusicladium oleagineum*, the fungal causal agent of olive leaf spot from North-Western Algerian groves

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ABSTRACT

Olive scab also called "peacock eye" desease caused by *Fusicladium oleagineum* (FO) is among the most important pathology of the olive tree (*Olea europaea*). Symptoms are initiated by the appearance of blackish circular spots on leafs, which turn to yellow and drop prematurely. Drastic foliar volume drop, shoots-death and general weakness of the tree leads to yield reduction. Under Algerian climate, the fruit is also infected. The scabbed olives eventually fall. This phytopathology known since 1923 was first considered of minor incidence. However, current intensive olive groves extension provides an ideal habitat for the FO development making it a major concern for farmers. Chemical or copper-based nature available treatments are source of pollution in ecosystems, although their limited effectiveness. Being biotrofic, FO has been poorly studied since *in vitro* cultures are hard to achieve. This work is related to isolation and conservation methods of Algerian FO native strains. This work would be a first step to a better understanding of the local FO agent.

Keywords: *Fusicladium oleagineum*, olive scab, isolation, identification.

I. INTRODUCTION

The olive tree (*Olea europaea* subspecies *oleaceae*) is one of the main fruit species planted in Algeria with about 208 000 ha (33% of the trees area) [1]. The plantations are concentrated in the North, particularly in areas of the Tell. There are numerous olive varieties in Algeria (Var.), representing the basis of the livelihoods of several rural communities: Var. "Siguoise" found from Oued Rhiou to Tlemcen. Its expansion zone reaches the Mitidja. It is used mainly for the production of table green and black olives and for oil production. Var. “Sevillanas”, of Hispanic origin, with her very large fruits, is located in sub-coastal plains of Oran, used for olive green table production. Oil Var. “Rougette” is common in the Mitidja plain and the foothills of the Atlas, at low altitudes. Var. "Chemlal" is the most famous in Algeria, for its oil, her distribution goes from the Atlas of Blida to Bibans and Guergour regions. Thanks to her great vigour poor soils became profitable and provide oils of high quality. In Algeria there are also low extension varieties such as “Azeradj”, “Bouchouk”, “Aguenaou”, “Guergour”, “Limli” and “blanquette” of Guelma. Olive trees can be affected by many pests and diseases. They require a Mediterranean climate-type, characterized by cold (mean monthly t° ≥-3°C), wet but short winters and long, dry and hot (below 30°C) summer conditions. These diverse climatic conditions are favourable for olive leaf spot (OLS), which is actually the most significant leaf disease in olive-growing countries [2], [3], [4].

Occurrence and importance:

OLS is wide spread in the Mediterranean region (Fig. 1) such as Algeria, Morocco, Tunisia, Iran, Greece, France, Italy, and Spain, but also in the major olive-growing areas of the world; USA, South America, Australia, and New Zealand [6], [7], [8], [3].

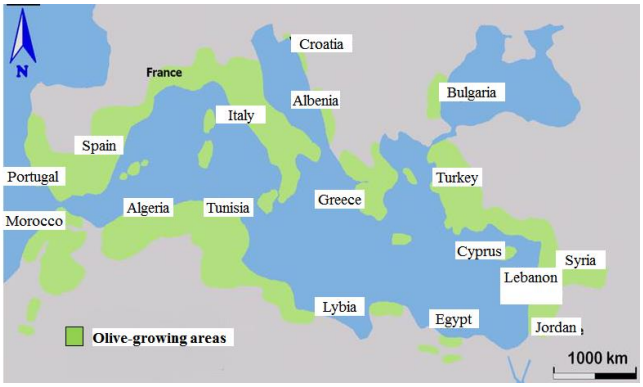

Figure 1: Mediterranean olive groves expansion [5].

The damage that causes is the dieback of twigs, defoliation of branches and finally reduction of leaf coverage. Since OLS has been known for over a century in the Mediterranean countries, it is likely that these are the sources of this pathogen.

OLS is a foliar disease widespread in all olive growing regions of the world [9], [7], [6] and is also called peacock spot (because of the circular dark lesions with a chlorotic halo on the leaf) [10], [11] is caused by the fungus *Fusicladium oleagineum* (FO) (Castagne, Ritschel and U. Braun comb. nov., syn. *Spilocaea oleagina,* Castagne (Hughes)). This fungus (Emb. *Ascomycota,* S.Emb. *Pezizomycotina,* Cl. *Dothideomycetes,* S.Cl. *Pleosporomycetidae,* Ord. *Pleosporales,* Fam. *Venturiaceae,* Gen. *Cycloconium*) is mitosporic, since no sexual stage has been demonstrated [12]. But recent phylogenetic analysis has shown that FO is an anamorphic phase of a yet unidentified *Venturia* species [13]. FO strains *in vitro* cultures are hard to obtain, since the fungus is endogenous parasite and requires particular culture conditions. Consequently, this phytopathology is poorly studied, despite its economic importance damages.

FO Symptoms

FO causes a leaf-spot (2,5 to 12,5 mm in diameter) disease with symptoms on the upper surface of the leafs (Fig. 2).

Figure 2 : FOL symptoms on Var. "Siguoise" of Algeria.

The infective hypha enters the leaf by piercing and enzymatically degrading the thick cuticle, and then grows parallel to the leaf surface as hyaline, septate, branched subcuticular mycelium. Lesions are primary inconspicuous then scarcely detectable, later enlarge to form dark-brown, circular, mostly annular that become lightly velvety and are often surrounded by concentric, faint yellow, violent or pale brown halos [6]. The colonies remain localized in a cutinised layer of the epidermal cell wall until the leaf tissues decay. The effected leafs fall prematurely. Occasionally, under very wet conditions, small, sunken brown lesions can be found on the petioles, fruit peduncles and fruit. Once trees are infected, fungal spread is caused by rain-splashed [14], [15], or insects and wind carried conidia [16], [17]. The disease causes severe defoliation resulting in both reduced flower bud differentiation and fruit set in subsequent years. Serious yeald losses have been reported [7].

Disease Cycle

The olive is an evergreen perennial crop, leafs live generally for 2-3 years, dropping when the tree is putting on new growth, or when they are shaded. Under Algerian climates, the trees produce few or no new leafs in mid-summer, and resume growth again in the autumn, giving two peaks of leaf production [18]. It is this new leafs that are most susceptible to OLS disease [19].

An incubation period precedes the infection (Fig. 3). Its duration depends on the olive cultivar, environmental conditions, leaf age and trees seasonal growth. In our region, she is about 15 days under favourable temperature (below 20°C) and moisture conditions [20].

The inoculum of the primary infection comes from sporulating spots on the over wintered hanging leafs or aestivated on trees. When detached from their conidiophores, conidia lose their germinability in less than one week.

During infection early stages, the germ tubes of the conidia develop appressoria, to attach the pathogens to the leaf surface. Leaf infection is through the cuticle, which is pierced and enzymatically degraded by the hyphae. Further growth by radiating mycelia, composed of branched hyaline, septate hyphae, expand to form round, flat submerged colonies between the outermost portions of the epidermal cell wall and the cuticular layer. Conidiophores develop at this phase producing easily dispersible conidia [6], [20].

There are two periods of main infections during first-autumn and last-spring. The existence of a sexual stage of FO has not been reported, and thus the role of sexual reproduction in the infection process of this pathogen remains unknown [21], [22], [23].

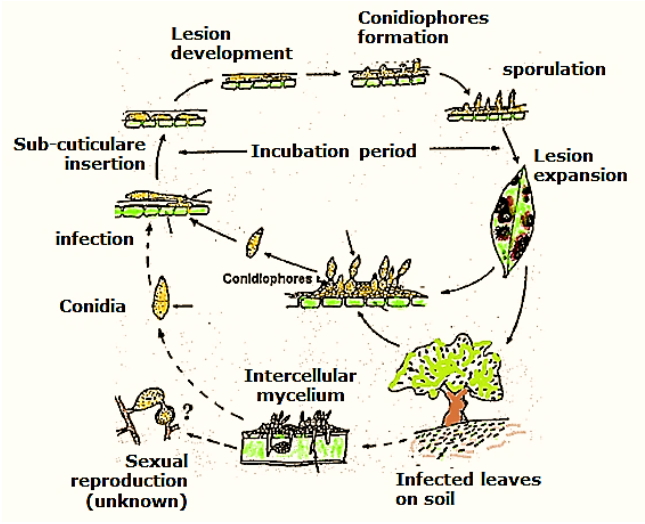

Figure 3 : Suggested life cycle of FO (Art by M., Mohamed Benkada).

Control

Pruning and chemical control schedules are the main measures. Chemical controls include the application of fungicides before and during the main infection seasons. In many areas with a dry Mediterranean climate, three spays are suggested.

II. METHODS AND MATERIAL

FO strains were isolated from infected olive trees of "Sigoise" variety from the region of Sig (50 km Eastern of Oran, Algeria). Leafs with sporulating lesions were collected from trees chosen at random at least 10m apart, selecting only one lesion per tree to reduce the likelihood of clones. The leafs were dried for 3 days on the bench at room temperature and then stored at 4 °C until required for producing the single-spore isolates, which were grown on a mixture of three antibiotics-amended media; malt extract agar (MEA), potato dextrose agar (PDA) and our "special olive leaf extract " (SOLE) without and agar-amended at 15°C. Isolates were identified by their morphological and cultural characteristics when grown on agar SOLE medium. Colony morphology and pigmentation were compared with the published descriptions of Graniti [6].

III. RESULT AND DISCUSSION

The fungal colonies are visible to the naked eye as dark, olive-brown spots after 2 to 3 weeks of growth at 15°C. After just one month of growth, the aerial mycelium of the colony appears as a hemispherical greyish green, felt-like stromatic body. The older colonies are differentiated into three layers: a basal submerged layer of loosely interwoven hyphae rich in chlamydospores, a medial layer above the surface of the agar formed of dark-brown closely interwoven hyphae, and an upper aerial layer of light brown felt-like mycelium (Fig. 4 a).

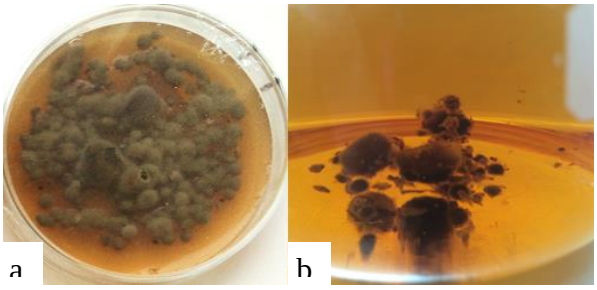

Figure 4 : Mycelial cultures of monospora FO on (a) agar and (b) liquid SOLE medium.

Mycelium growth was also obtained in static liquid cultures of SOLE (Fig. 4b). Monosporal fungal growth is practically absent on PDA and MEA media (Fig. 5a and b) compared to solidified SOLE (Fig. 5c).

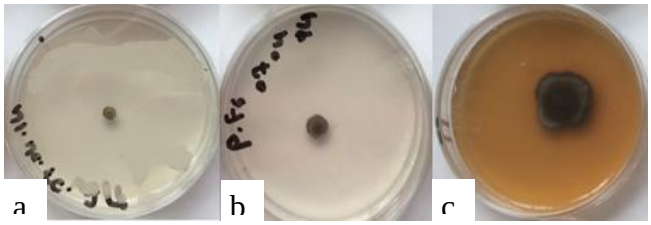

Figure 5 : Culture of FO on solidified (a) MEA, (b) PDA and (c) SOLE.

Culture conditions described in this work using a simple technic and an easy low-coast culture medium permit the reduction of incubation period from 6 months [24] to less than 2 months allowing a precious win of time.

IV. CONCLUSION

These encouraging results open the way to comparative studies of different population genotypes of FO from Algeria and of neighbouring countries for a better knowledge of the biology of this pathogen. Due to increasing labour costs of harvesting of olives, the worldwide olive industry, including Algeria, is moving towards easily mechanized planting systems (i.e., ultra-high density plantings). These new systems, however favour disease occurrence with susceptible cultivars easily becoming infected by OLS [6]. The development of biological control research against FO would limit the environmental pollution due to fungicide use. In fact, environmental conditions favouring OLS development, copper-containing fungicides may neither be sufficiently effective nor sustainable for complete dependence on them alone because of the ecological impact of copper deposits in the soil. One of the main implications arising from the use of any fungicides is the behaviour of their residues, which give rise to important health considerations, especially in products destined for human consumption (Obanor, 2006). This would be an important component of the project that we already started to prepare with colleagues from Tunisia, Morocco and France.

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