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Research article

GEOGRAPHICAL DISTRIBUTION OF *PHYTOPHTHORA PALMIVORA* IN DIFFERENT OLIVE GROWING REGIONS IN MOROCCO

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ABSTRACT. *Phytophthora palmivora*, causative agent of olive wilting was searched during the spring 2012 and summer 2013 in different olive-growing regions of Morocco: Meknès, Souk Larbaa, Kénitra, Sefrou, Ouazzane, Marrakech (Azouzia, Aataouia, Ait Aourir, Sraghna, Tamnsourt, Jaidate, Tassaout…) and in the nurseries of Sidi Taibi. It was isolated from the roots and stems of olive plants and trees growing in nurseries and in the fields of different regions with varying percentages: Souk Larbaa (85 %), Sidi Taibi nurseries (73.6%), Aattaouia (64%), Jaidate (47 %), Sraghna (40 %) and Tassaout (32 %). The importance and the origin of this disease have been discussed in this study.

Keywords: *Phytophthora palmivora*, Olive tree, Morocco, Souk Larbaa, Sidi Taibi, Aattaouia, Jaidate, Sraghna, Tassaout.

INTRODUCTION

Olive-tree (*Olea europaea* L.) is one of the most ancient domesticated fruit trees and the most extensively cultivated fruit crop in the world [18]. The olive is native of the Mediterranean region, tropical and central Asia and various parts of Africa. *O. europaea* may have been cultivated independently in two places, Crete and Syria. Archaeological evidences suggest that olives were being grown in Crete as long ago as 2,500 B.C. From Crete and Syria, olives spread to Greece, Rome and other parts of the Mediterranean area. Olives are also commercially cultivated in California, Australia and South Africa [34]. It is concentrated between latitudes 30°–45° at both the northern and southern hemispheres in Mediterranean-type climatic regions. Thus, 97% of the global cultivation area (approximately 10.5 millions ha) is located in the Mediterranean Basin, 0.8% in the Americas, 1.5% in Asia and 0.01% in Oceania [19]. Morocco occupies the 4th place behind Spain, Italy, Greece [2], with an olive-growing area that amounts to 784 000 ha [25], with a production of 1,483,510 tons of olives per year [2]. Plus, it actively contributes to the establishment of the rural population by creating more than 11 million working days [32]. 5.6% of the global area [33] distributed on three main zones: the Rif (Taounate Chefchaoune), the center (Fez, Meknès, Taza) and the south (Haouz, Tadla and coastal region between Safi and Essaouira) [23]. In Morocco, the olive trees cultivation knows several problems related to pests and diseases [51] and to a various environmental stress under a Mediterranean climate, characterized by long drought periods [27]. One of the important foliar diseases affecting olive trees in humid regions in the world is peacock spot disease caused by *Cycloconium oleaginum*, also known as olive leaf spot and bird's-eye spot [47], *Verticillium dahliae* responsible to defoliation and wilting of olive trees and death of young trees [48], *Fusarium solani* that provokes the root rots to the olive trees [38] and *Phytophthora palmivora* that provokes leaf chlorosis, defoliation, wilting and twig dieback in the olive plants [7].

Phytophthora and other oomycetous micro-organisms were long included within the fungi, but today, because of evolutionary phylogeny and structure of biflagellate zoospores, they are grouped in the kingdom Chromista, which includes e.g. brown algae [3, 17]. At least 60–80 *Phytophthora* species has been described and most of them are soil-borne pathogens causing damping off, root rot, collar and stem rot and foliar blight on different woody plant species [28]. *Phytophthora* species have limited saprophytic ability and do not grow and compete in soil with other microorganisms [17]. Most *Phytophthora* species attack only healthy, intact plant tissue or freshly made wounds and do not invade plant tissue previously invaded by other microorganisms; that is, they are primary, not secondary, invaders [17]. *Phytophthora species* are responsible for most of the crown rots of woody plants. Tsao [46] argues that if 90 % of the crown disease of woody plants is attributable to *Phytophthora*. *Phytophthora palmivora* is a ubiquitous pathogen causing many different diseases on a wide range of plants. The pathogen is believed to have originated in Southeast Asia but is now pantropical. It causes significant losses to farmers of tropical fruit and vegetable crops [15]. *P. palmivora* infects more than 200 species of ornamental, shade and hedge plants, mostly from tropical areas. In Argentina, for instance, *P. palmivora* was first recorded in *Citrus* spp. in 1937 [29]. One of the most common tropical species is *P. palmivora*, with more than 150 plant hosts. Some of the most important hosts are Citrus (Zitko et al., 1991), black pepper (*Piper nigrum*), rubber (*Hevea brasilensis*), durian (*Durio zibethinus*), coconut (*Cocos nucifera*), cocoa (*Theobroma cacao*), breadfruit (*Artocarpus altilis*), African oil palm (*Elaeis guineensis*) in Colombia (Torres et al., 2010) and papaya (*Carica papaya*). In American Samoa, the last three hosts are attacked by *P. palmivora* [26]. Concerning olive tree, this fungi attacks young olive trees in southern Spain and causes wilt or dieback and death (Sanchez Hernandez *et al.*, 1998), pathogen of olive tree in Italy that provoked leaf chlorosis, defoliation, wilting, twig dieback and eventual plant collapse associating the symptoms with the root rot [5], same symptoms on young olive trees in Sicily [30].

The objective of this study was to know the geographical distribution of *Phytophthora palmivora* in the olive regions of Morocco and to discuss the importance and the gravity of this responsible fungus of the olive wilting.

MATERIALS AND METHODS

Surveys were conducted in different olive regions of Morocco exactly in Meknès, Souk Larbaa, Kénitra, Sefrou, Ouazzane, some olive fields in Marrakech province (Azouzia, Aataouia, Ait Aourir, Sraghna, Tamnsourt, Jaidate, Tassaout …) and in some nurseries in Sidi Taibi during the spring 2012- summer 2013 (Figure 1).

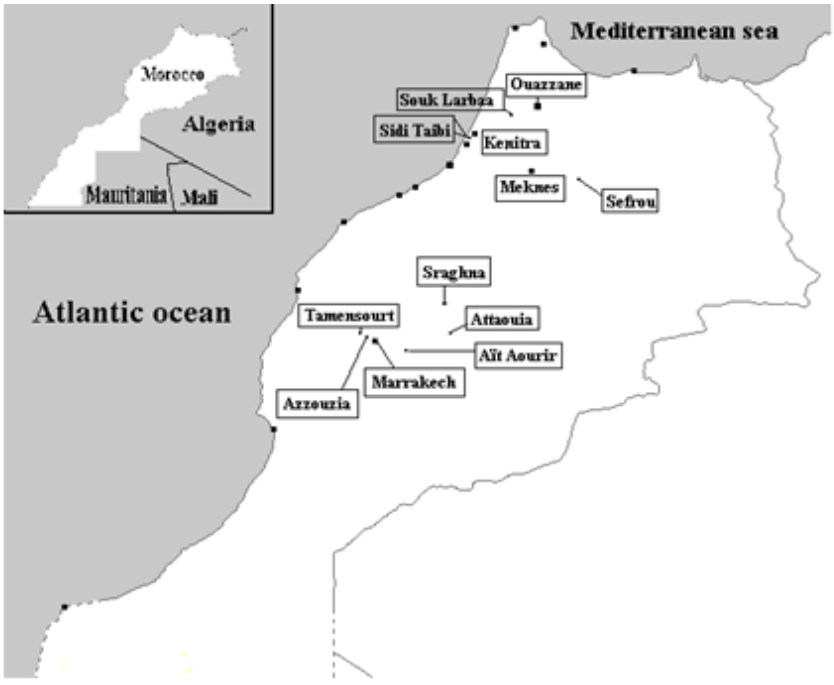

Figure 1: Geographical locality map of the studied sites in Morocco.

Olive Stems showing symptoms of the disease thus the roots of attacked trees were sampled and returned to the laboratory for analysis. Isolation of fungal species was realized on the roots and the stems of the olive trees showing decline, leaf chlorosis and wilting symptoms (Figure 2). The samples were cut into small pieces, washed with water, disinfected with alcohol for five minutes, put on sterile distilled water and then dried with sterile filter paper. Then, they were put on PSA agar plates (Potato Sucrose Agar: 200 g potato, 20 g sucrose, 15 g Agar-agar, and 1000 ml distilled water) and incubated on darkness at 28 ^{0}C.

Isolation percentage (Pi %) was obtained by applying the following formula:

$$Pi = NsX/ N_T \times 100$$

N_SX: Number of segments containing the fungal species X
N_T: Total number of used segments.

Figure 2. Leaf chlorosis, defoliation, wilting and twig symptoms on the olive tree.

On the PSA plates, some colonies appeared four to six days later on the segments of stems and roots of the diseased olive trees. The observation of different cultures and fragments under the optical microscope has allowed us to identify the fungal species by using the identification keys of Gilman [22], Tarr [43], Ellis [16], Chidambaram [8], Domsch [14], Ho [24], Champion [6], Cacciola [5], Lucero [29] and Gallegly and Hong [20].

RESULTS AND DISCUSSION

We have noted that in the field that the attacked Olive trees by *Phytophthora* can be distinguished from other through out the year. They have less dense foliage and relatively small leaves, as a result of prolonged drought**.** Indeed, at the end of April, leaves of the affected branches change their color; they take a dull green color and curl longitudinally towards the underside. Then, the color changes to brown and green to brown. A month and a half after the first few events, the leaves dry up and remain attached to branches for long. The stem of affected parts gradually takes the yellow-brown color, buds become mummified and fall at the slightest touch and the symptoms always evolve in end of the branches to the trunk of the tree. The isolations results showed that on the PSA plates, some colonies appeared four days later on the stems and roots segments of the diseased olive trees (Figure 3 A and B). *P. palmivora* morphological characterization showed that all isolates produced papillate sporangia on the soil extract medium (Figure 3c), which were ellipsoid to ovoid with a length of 15 to 20.1 µm and a larger of 11 to 13µm. Some isolates produced subglobose, non-papillate sporangia and abundant chlamydospores, sexual forms were absent (heterothallic species).

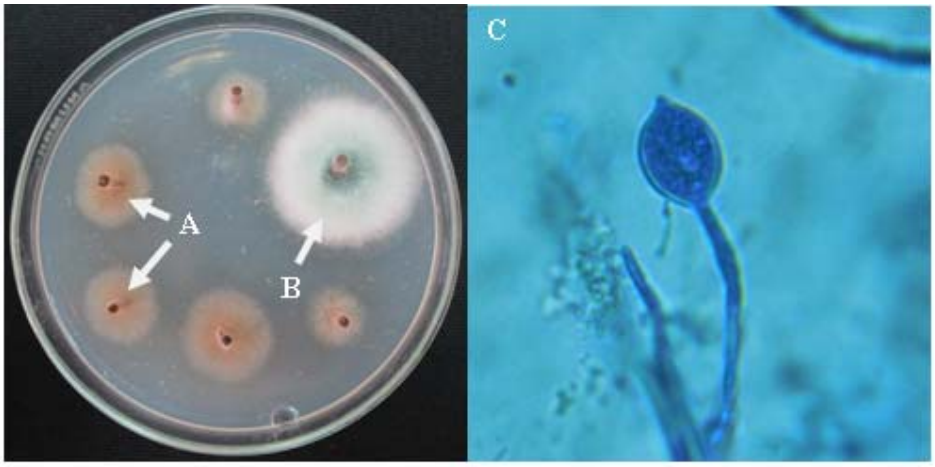

Figure 3. *Phytophthora palmivora* (A), *Penicillium* sp. (B) isolated from the olives stem segments on PSA agar; (C): Papillate sporangia of *P. palmivora*.

Phytophthora palmivora was widespread in six regions with different isolations percentages (Table 1). Souk Larbaa was the station where *P. palmivora* existed more (85 %), followed by the nurseries of Sidi Taibi (73.6 %), Aattaouia (64 %) and Jaidate (47 %). *P. palmivora* existed in the region of Tassaout with the lowest isolation percentage (32 %). Several species were isolated with *P. palmivora* (Table 1); *Alternaria alternata* was widespread in the all location sites (13 % in Aattaouia and 37 % in Sraghna). Other species were isolated in different regions: *Colletotrichum gloeosporioides* (10.33%) and *Circinella* sp. (4%) in Tassaout, *Ulocladium* sp. (13%) in Jaidate and *Penicillium* (10%) in Sraghna (Figure 3 B). The disease may it was introduced in the different regions through the circulation of infected plants. In fact, olive plants in the nurseries of Sidi Taibi are attacked with the pathogen and probably farmers provide olive plants from these nurseries. In the region of Marrakech, cultivated olive varieties are diversified addition to the Moroccan Picholine. We find other varieties imported from other countries. The disease can be introduced from these varieties after incubation periods of development and multiplication necessary to produce inoculum for the appearance of the disease. Lucero [29] have reported that *P. palmivora* may be introduced in Argentina from olive varieties cultivated in the Mediterranean region.

Table 1. Isolation percentage of *Phytophthora palmivora* and of other fungal species

Regions / Fungal species	Aattaouia	Jaidate	Tassaout	Sidi Taibi (nurseries)	Souk Larbaa	Sraghna
Phytophthora palmivora	64 ^{c}	47 ^{d}	32 ^{e}	73.6 ^{b}	85 ^{a}	40 ^{d}
Alternaria alternata	13 ^{b}	33 ^{a}	25 ^{a}	24.6 ^{a}	15 ^{b}	37 ^{a}
Colletotrichum gloeosporioides	-	-	10.33 ^{a}	-	-	-
Ulocladium sp.	-	13 ^{a}	-	-	-	-
Circinella sp.	-	-	4 ^{a}	-	-	-
Penicillium sp.	-	-	-	-	-	10.11^{a}

Numbers in the same line followed by the same letter (a, b, c, d) are significantly different at the 5% level of significance.

Phytophthora palmivora is known as a pathogenic agent of Citrus, notably in Japan [44], in Florida [49] and in Egypt [1]. But in Morocco, *P. palmivora* had never been reported on Citrus. We can also admit that the primary inoculum is already present in the soil, especially in parcels containing both olive and Citrus. This inoculum, probably constituted of non-pathogenic strains against olive, may subsequently present adaptation faculties on the olive tree. In fact, the ability to adapt to a new culture is known in *Verticillium dahliae* [12, 13]. This hypothesis seems to be very important if we consider the absence of the disease in the olive groves of Sefrou, Meknes and Ouazzane. Indeed, these regions are not known by Citrus cultivation. To verify this hypothesis, it is so important to realize some isolations of *Phytophthora palmivora* from olive tree and Citrus cultivate in the same parcels.

As with all cryptogamic diseases, field dissemination of olive wilting due to *P. palmivora* depends on the extent of the sites where the parasite survives or multiplies, on the abundance of primary and secondary inoculum in these sites, on factors which ensure their effectiveness as sources of contamination, and on vectors of the reproductive organs of the parasite. The pathogen is disseminated through rain splash, insects and human activity into the canopy of trees, where symptoms appear. Secondary inoculum spreads rapidly through wind and rain splash, contact and vector activity in humid weather [15]. Muller [35] reported that the secondary inoculum of cocoa black pod caused by *Phytophthora palmivora* is triggered once the wet season begins. In the case of the olive trees, zoospores may scattering on to healthy olive roots through the impact of rain drops falling on diseased olive trees or on the ground which acts as a reservoir of the parasite.

The control of this fungus, based on agronomic, genetic and chemical measures [9, 10, 21, 42] is difficult and frequently ineffective. The failure of the control measures may be due to combinations of high density and virulence of the inoculum [4], the ability of the fungus to rapidly increase its population in the presence of rainfall or irrigation [39], and to select strains resistant to fungicides [36, 37, 40] or able to overcome the genetic resistance introduced to the commercial lines.

CONCLUSION

The geographic distribution of *Phytophthora palmivora* the causal agent of wilting, dieback and leaf chlorosis of the olive trees was widespread in different regions in the Moroccan olive fields as in the nurseries. In the long-term, it may constitute a real danger to this culture and to other cultures. So, due to the severe symptoms and the increasing incidence recorded; *P. palmivora* should be considered as a potential threat to olive cultivation and nurseries in Morocco that it must be controlled. The inoculum may be present from the start strains that were subsequently shown difference in details of adaptation to the climatic and cultural conditions in different regions of Morocco. Or, non pathogen strains that developed some faculties of adaptation to the olive trees.

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