Review

Key pests and diseases of citrus trees with emphasis on root rot diseases: An overview

Manal JAOUAD[1], Alieu MOININA[1], Said EZRARI[1], Rachid LAHLALI[1]*

[1] *Department of Crop Protection, National School of Agriculture of Meknès, Morocco*

* *Corresponding author rlahlali@enameknes.ac.ma*

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Abstract

Citrus fruits are among the most consumed fruits in the world. However, the world production of citrus fruits is faced with several constraints which hinder its development. Furthermore, the spread of pests such as mealybugs, mites and Mediterranean fruit fly has impacted negatively on citrus quality and yield. Therefore, the main objectives of this review were to identify the key harmful organisms to the citrus crops and to highlight the appropriate methods to manage them. In this paper, more attention was given to root rot and dry root rot diseases caused by *Phytophthora spp*. and *Fusarium spp*., respectively. It was concluded that the control of citrus pests relied on the use of chemicals. The use of integrated orchard management methods remains the best practice to minimize the adverse effects of pesticides on the environment. Two fungicides, Fosetyl-Al and Metalaxyl, were widely curatively used to control *Phytophthora* root rot. Moreover, the control of dry root rot disease due to *Fusarium spp.* requires the optimization of irrigation and fertilization inputs. In addition to the use of sanitation practices, scouting and monitoring the appearance and development of key pests and diseases help to strengthen the control methods and reduce the use of pesticides.

Keywords: Citrus, pests and diseases, root rot, *Phytophthora spp.*, *Fusarium spp.*, integrated pest management strategy, Morocco

INTRODUCTION

Citrus is the most extensively cultivated fruit crop worldwide. The citrus industry is clearly divided into the fresh fruits and the processed juice markets. The most commonly grown species are *Citrus aurantifolia* (lime), *Citrus aurantium* (sour orange), *Citrus grandis* (pomelo, shaddock), *Citrus limon* (lemon), *Citrus medica* (citron), *Citrus paradisi* (grapefruit), *Citrus reticulata* (mandarin orange) and *Citrus sinensis* (sweet orange). The sweet orange is the predominant species for both markets (Ollitrault and Navarro, 2012). Citrus species are known to have a sustainable growth rate. In 2016, the global citrus production was about 131 million tons of fresh fruits; including 52% of orange, 29% of mandarin, 12% of lime and lemon and 7% of grapefruit (FAOSTAT, 2016).

As many tropical and subtropical crops, citrus is a host of numerous pests and diseases. These crops are also one of the few crops that are susceptible to a large number of destructive diseases, which are continuously emerging and can severely hinder or completely destroy the entire production. Citrus fruits production destined for processing may not require stringent managements of pests and diseases when compared to those for the fresh fruit market. Nevertheless, a clear understanding of the biology of the pathogen is crucial to the development of a certification program and maintenance of productive citrus orchards (Tennant, 2009)·

Diseases present in all citrus-growing regions are nematodes diseases, *Phytophthora* diseases, and Citrus tristeza virus (CTV). These diseases precludes the use of some rootstocks that has an excellent horticultural behavior and reduces fruit production as well as the quality of some specific varieties. Some of these diseases can just lower the yield quality, while others have the potential to raze the citrus industry (Ollitrault and Navarro, 2012). Furthermore, the citrus gummosis caused by *Phytophthora spp.* and the citrus Tristeza were outlined as main diseases in 30-50% of the Mediterranean countries (Franco, 2006). Citrus crops have been reported susceptible to a large number of fungal pathogens and oomycetes. Among them, *Fusarium spp*., *Phythophtora spp*., and postharvest pathogens (*Penicillium spp*., *Aspergillus spp*., *Altenaria citri*, *Geotrichum candidum*, ...etc.) are considered as the most important pathogens of citrus fruits during storage and are responsible for considerable losses in some developing countries (OEPP, 2004).

The key pests of citrus trees in the Mediterranean countries include the medfly (*Ceratitis capitata*), the citrus red scale (*Aonidiella aurantii*), the citrus leafminer (*Phyllocnistis citrella*) and the citrus mealybug (*Planococcus citri*) (Francis, 2015). The use of pesticide to control pests has enabled farmers to modify production systems and to increase crop productivity without enduring higher losses likely to occur due to an increased susceptibility to the damaging effect of pests. The concept of integrated pest/crop management includes a threshold concept for the application of pest control measures and reduction in the amount/frequency of pesticides applied to an economically and ecologically acceptable level. Often, minor crop losses are economically acceptable. The key to successful citrus production relies on the effectiveness of pest management strategies. However, an increase in crop productivity without adequate crop protection does not make sense, because an increase in attainable yields is often associated with an increased vulnerability to damage inflicted by pests (Oerke, 2006).

WORLD SITUATION OF CITRUS FRUITS

Citrus fruit production and consumption worldwide have increased significantly since the mid-1980s. Production of oranges, mandarins, lemons and limes has increased rapidly, and even faster growth has been observed for processed citrus products. Partially, thanks to the improvements in transport and packaging that have led to lower costs and improved quality (FAO, 2004).

However, the rapid expansion of production and the slower growth in demand for oranges and grapefruits have led to lower prices of fresh and processed oranges and grapefruits. The rate of new plantings has slowed and the expected growth rates of production and consumption over the next ten years are expected to be lower than those recorded over the past ten years (FAO, 2004).

The citrus sector has changed significantly in recent decades. Italy was the leading producer until the 1970s, while Spain is now the leading producer in Europe and accounts for a quarter of world citrus fruit production. The new competitors are Australia, Morocco, Egypt, South Africa, Turkey, Portugal, Florida (USA), California (USA) and Uruguay (Anonymous, 2018).

World citrus production decreased slightly in 2015, influenced by a continued decline in orange production in the United States. This accounted for a decrease from about one million tons to 4.8 million tons. After growing by more than 10 percent per year for almost thirty years, citrus production in China increased by only about one percent in 2015. In Brazil, production increased by 3% (16.7 million tons) due to higher yields, but remains about 20% below its record level (FAO, 2016).

In South Africa, citrus production increased slightly to 1.5 million tons (+4.5%). This country is the main exporter of oranges in the world with 1.1 million tons, with the main destinations being the European Union and Russia. Egypt is the 6th largest orange producer in the world, its production for the 2012/2013 season is estimated at 2.5 million tons, with an increase of 4% compared to the previous season. Egypt is the world's 2nd largest exporter of oranges with a volume of 1.1 million tons in 2012/13 (+10% compared to 2011/12) (MAPMDREF, 2013).

CITRUS FRUIT PRODUCTION AND AREA DISTRIBUTION IN MOROCCO

The citrus fruit sector in Morocco plays an important socio-economic role. This crop covers a surface area of 126,600 ha and the average production is around 2.6 million tons per year. Since the program contract signed in 2008 between the government and the profession under the Green Morocco Plan came to effect, the citrus fruit sector has undergone significant development (Anonymous, 2018).

The main production regions are Souss Massa, Gharb, Moulouya, Tadla and Haouz, which alone account for more than 93% of the national area. The distribution of the surface area of citrus crops citrus by region is shown in figure 1.

A wide range of varieties characterizes the national citrus orchards, the three most cultivated varieties are the Clementine group (35%), Maroc-Late (21%) and Navels (18%). Table 1 shows the evolution of citrus production and the distribution of citrus fruits by variety group.

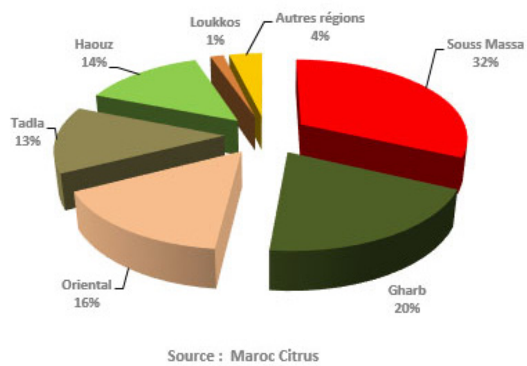

Figure 1: Distribution of surface area of citrus crops by region in Morocco (Maroc citrus, 2018)

Table 1: Distribution of citrus area by variety (Morocco citrus, 2018)

Varieties	Surface (ha)	Importance (%)
Total small fruits (SF)	**64 047**	**50.6**
Clementine	30 151	23.8
Nules	8 361	6.7
Nour	7 697	6.1
Nadorcott	6 998	5.5
Other SF	10 840	8.5
Total oranges	**57 734**	**45.6**
Navels	19 918	15.7
Maroc Late	25 663	20.3
Sanguines	3 620	2.8
Other oranges	8 533	6.7
Lemon	**4 819**	**3.8**
Total citrus fruits	**126 600**	**100**

With most of the harvest now completed, Moroccan citrus production for 2018/19 attained about 2.6 million metric tons, an 18% increase over the previous marketing year. Mandarin production increased by 14% over the previous year to 1.35 MMT, while orange production increased by 18% to 1.2 MMT and lemon/lime production by 45,000 tons. Much of the increase in production is due to favorable weather conditions and an increase in the area harvested, as young trees begin to bear fruit. Exports of mandarins are expected to amount to about 585,000 tons, while exports of oranges are expected to reach about 190,000 tons (Anonymous, 2019).

KEY PESTS IN CITRUS ORCHARDS

Scale insects

In Morocco, mealybugs cause very serious damage to citrus fruits. The scale insect species inventoried in Moroccan citrus orchards are: *Aonidiella aurantii* Mask, *Parlatoria pergandii*, *Lepidosaphes beckii* Newman, *Parlatoria ziziphus* Lucas (Benziane *et al.*, 2003).

Californian red scale, *Aonidiella aurantii* Maskell (Hemiptera: Diaspididae), is a major pest of citrus worldwide. The presence of scales on fruits reduces considerably their market value, resulting in important economic losses. Currently, integrated pest management, especially rational pesticide application, mineral oil sprays are used to control Californian red scale infestations in citrus orchards (Jacas *et al.*, 2010).

The Mediterranean fruit fly (*Ceratitis capitata*)

In Morocco, the Mediterranean fruit fly causes significant damage to citrus fruits. It is considered a great challenge for researchers and farmers all over the world. *C. capitata* control programs have used conventional insecticides (organophosphates and pyrethroids). The combination of bait and insecticide sprays for fruit fly control has been used since the early 20th century (Roessler, 1989). Other control strategies, such as sterile insect techniques, have been used in the past, but their effectiveness was often limited (Mitchell and Saul, 1990). The use of synthetic insecticides has resulted in insecticide resistance, toxicity to non-target organisms, food residues and environmental pollution (Desneux *et al.,* 2007).

Citrus mites

In Morocco, the main mites associated with citrus trees are the red mite (*Panonychus citri*), the yellow mite (*Tetranuchus urticae*) and the bud mite (*Aceria sheldoni*, Ewing) (Mazih, 2008). *Panonychus citri* is one of the most serious citrus pests. In addition to citrus, the mite inhabits many other plants, such as deciduous fruit trees, ornamental trees, vegetables and herbs (Bowman and Bartlett, 1978; Osakabe, 1987). It causes severe damage to citrus fruits, rapidly developing harmful populations in early spring, late spring and mid-fall. Acaricides have been widely used to control pest mites in orchards. However, occurrence of resistance problems are mostly caused by frequent acaricide use (Gerson and Cohen, 1989; Van Leeuwen *et al.*, 2010) and have become more toxic to natural enemies. The use of synthetic acaricides has caused enormous concern for the environment and human health. Therefore, it is urgent to seek an effective acaricide against spider mites while preserving auxiliary fauna.

Snails

Snails are considered as one of the most important agricultural pests in humid regions of the world (Kiss *et al.*, 2005). Molluscs cause enormous damage in Moroccan citrus orchards. They are well adapted to variations in air humidity. Therefore, it is important to continuously inspect the orchard, especially after rainfall or rising humidity (Ouzine, 2012).

There is a wide variety of methods for controlling citrus snails, but a strategy that combines several control methods is the most effective way to reduce snail damage. Regular application of 5% granulated metaldehyde after any increase in humidity from the environment to the plants, as well as tillage around the edges of the plots to destroy the pest and weed outbreaks is an effective method for snail control (Ouzine, 2012).

Aphids associated with citrus

Many aphid species attack citrus fruits. Among them, *Aphis spiraecola*, *Aphis gossypii* Glover and *Toxoptera aurantii* Boyer are widely distributed in citrus orchards (Kalaitzaki *et al.*, 2019). Aphids cause considerable damage to trees directly by removing sap from the phloem of the plant while injecting saliva and this causes the plant to become phytotoxic. In addition, aphids cause indirect damage by secreting honeydew during sap feeding and moulds frequently develop on the honeydew, thus hindering photosynthetic activity (Blackman and Eastop, 2000). Aphids indirectly damage plants by transmitting the citrus virus Tristeza (Delkhosh and Tousi, 2009).

KEY DISEASES ASSOCIATED WITH CITRUS ORCHARDS

In agriculture, plant diseases are mainly responsible for the reduction of production, which leads to economic losses.

Tristeza

The Citrus tristeza Virus (CTV) is one of the most dangerous viruses infecting citrus trees. Its most dramatic expression is a quick decline, a syndrome in which a tree with normal appearance starts showing wilt symptoms and completely collapses in a few weeks. Commonly, infected trees show dull green or yellow thin foliage, leaf shedding and twig dieback, small chlorotic leaves resembling the effects of nitrogen deficiency, and small pale-coloured fruits are unmarketable (Moreno *et al.*, 2008). CTV induces obliteration, collapse and necrosis of sieve tubes and companion cells close to the bud union, producing an excessive amount of non-functional phloem (Schneider, 1959). This causes progressive reduction of the root system with deficient supply of water and minerals, which results in wilting, chlorosis and dieback symptoms. As this specific interaction does not occur with many other citrus species, the tristeza syndrome can be avoided using decline-tolerant species as rootstocks (Schneider, 1959).

The ability to control disease damage depends to a large extent on the CTV incidence and on the virus strains and citrus varieties predominant in each region (Garnsey *et al.*, 1998). Effective quarantine and budwood certification programmes are the best measures to avoid CTV introduction or dispersal in CTV-free areas where citrus are still propagated on sour orange rootstock (Navarro *et al.*, 2002).

Citrus Greening

Citrus greening (Huanglongbing [HLB]) is one of the most serious and destructive diseases of citrus worldwide. It is also called Yellow Dragon caused by a bacterium (*Candidatus Liberibacter asiaticus*) and transmitted by two species of psyllids *Diaphorina citri* in Asia and *Trioza erytreae* in Africa. The bacterium invades conductive tissues and causing citrus fruits decline by making them unproductive (Da Graça, 1991). Zinc deficiency and several other conditions can cause similar symptoms to citrus Greening, such as yellowing of the veins and adjacent tissues, followed by yellowing or staining of the entire leaf. These symptoms are sometimes accompanied by clogging of the veins, followed by premature defoliation, twig dieback, root rots, decreased in plant vigour and finally the plant die (Da Graça, 1991).

Greasy spot disease

Greasy spot is a disease caused by *Mycosphaerella citri* Whiteside. Its symptoms manifest on leaves or fruits. The disease begins with the appearance of yellow mottling on the leaf surface. Often, he infected surfaces turn a dark brown to black colour and take on a greasy appearance (Timmer *et al.*, 2000). Affected leaves often fall before the dark black colour develops. The application of $CaCO_3$, dolomites and urea helps to reduce inoculum at ground level (Mondal and Timmer, 2003). Copper-based fungicides are very effective in disease control. The application of benomyl is also effective in disease management, except that resistance to it is developing rapidly (Timmer *et al.*, 2000).

Diseases caused by *Alternaria spp.*

The genus *Alternaria* can cause two diseases on citrus fruits: brown spot disease and black rot. These diseases begin with necrotic spots of a dark brown to black coloration on the fruit and manifests as black necrotic spots on leaves that can follow the veins of the leaves, and the stems also produce blackened lesions (Timmer, 1998). To limit the spread of the infection, it is recommend to avoid injuries during picking and dispose of contaminated fruit. For chemical control, the application three times of copper fungicides t are recommended. In rainy season, more frequent applications may be necessary for disease control (Timmer, 1998).

Citrus anthracnose

The genus *Colletotrichum* was recently named the eighth most important group of plant pathogenic fungi worldwide, based on its scientific relevance and economic importance (Dean *et al.,* 2012). *Colletotrichum* species are responsible for anthracnose disease and post-harvest rot of many tropical, subtropical and temperate fruits, crops and ornamentals (Bailey and Jeger, 1992; Freeman and Shabi, 1998; Damm *et al.*, 2012).

Two species, *C. acutatum* and *C. gloeosporioides*, are the main causal agents of citrus diseases (Damm *et al.*, 2012). Post-harvest anthracnose was initially attributed to *C. gloeosporioides*, which was also reported responsible for preharvest symptoms such as shoot wilting, sunken spots or lesions (blight) of various colours in leaves and fruit decay (Benyahia *et al.*, 2003). More recently, *C. gloeosporioides* has been more frequently associated with anthracnose in preharvest orange crops (Ramos *et al.,* 2016; Rhaiem and Taylor, 2016; Guarnaccia *et al.*, 2016). *C. acutatum* causes Postbloom fruit drop (PFD) of citrus and lime anthracnose (KLA) on citrus fruits. PFD is a disease restricted to flowers of sweet orange and most other citrus, and symptoms include petal necrosis, abscission of developing fruit, and the formation of persistent calyces (Pers *et al.*, 2008; Lima *et al.*, 2011). KLA is a complex of diseases related to leaves, flowers and fruits. Symptoms of KLA include necrotic lesions on leaves, fruits, twigs, flowers and shoot blight (Brown *et al.*, 1996; Peres *et al.*, 2008).

The most key symptoms of anthracnose on the leaves are the appearance of flat, light tanned areas that are more or less circular and slightly mottled. Brown to black spots, sometimes turning silvery grey, appears on ripe fruit. These lesions had a diameter of approximately 1.5 mm or more. Black fruiting are observed on the surface of the lesions and on the dried tips of infected twigs (Rhaiem and Taylor, 2016). Decay often progresses slowly and withers leaves eventually fall (Aiello, 2014). On fruits, symptoms appear as small, slight, irregular and sunken lesions, with a diameter of 0.5 cm or more, which could turn black (Aiello, 2014).

Orchard crop control methods mainly involve the use of appropriate sanitation techniques during processing of harvested fruits, transport, packaging and storage to avoid exposure of the fruits to the pathogen. As well as proper handling to avoid abiotic factors such as mechanical injuries and extreme temperatures, which can predispose fruit to pathogenic infection. The availability of primary inoculum of *Colletotrichum spp.* leads often rapid to epidemic development of the disease under appropriate conditions and reduces the effectiveness of many pre-harvest chemical treatments. However, general sanitation practices in orchard are essential in integrated disease control as the elimination of obvious sources of inoculum, such as diseased leaves and fruits, can increase the effectiveness of chemical control (Waller, 1972).

Overall, *Colletotrichum* diseases can be controlled by a wide range of chemicals such as copper compounds, dithiocarbamates, benzimidazole, triazole compounds, and other fungicides such as chlorothalonil, imazalil and prochloraz (Waller, 1993). In addition, other fungicide groups can be used to effectively control *Colletotrichum* species, including external quinone inhibitors "Strobilurins" (e.g. Azoxystrobin and pyraclostrobin) that interfere with mitochondrial respiration (Piccirilloa *et al.,* 2018).

Phythophthora spp.

Phythophthora spp. diseases are the most threatening fungal diseases on citrus fruits under Moroccan conditions (Vanderweyen *et al.*, 1982; Benyahia *et al.,* 1998). *Phythophthora* pathogens cause extensive damage to the tree by attacking all parts and causing root rot, collar rot often with gum exudation on the trunk near ground level, and brown rot on fruit (Whiteside, 1988; Timmer, 2002). Both *P. citrophthora* R.E. Sm & E.H. Sm and *P. parasitica* Dastur are both cited as causal agents of gummosis and root rot of citrus (Javaherdehi *et al.*, 2008). *P. nicotianae* is more common in subtropical regions of the world and causes foot rot and root rot. This species sometimes attacks the aerial parts of the tree and the fruit. *P. citrophthora* induces also brown rot on fruit (Graham *et al.*, 2003). In addition, other *Phytophthora* species such as *P. boehmeriae, P. cactorum, P. cinnamoni, P. citricola, P. dreschleri, P. hibernalis, P. megasperma, P. palmivora* and *P. syngiae* have been reported pathogenic on citrus from different citrus-producing areas of the world (Erwin *et al.,* 1996).

It was noticed that severely damaged and infected trees become dry in 1 or 2 months and then die. However, a careful monitoring allows usually early stages of the disease to be detected quickly and easily, such as wilting branches, which are generally observed two weeks after rainfall (Mahdavian, 2014). Furthermore, diseased trees show leaf chlorosis and shoot decline with reduced fruit production (Francis *et al.*, 2015).

Phytophthora spp. infects the root cortex and causes degradation of fibrous roots. The cortex becomes soft, a little discoloured and appears to be soaked in water. The fibrous roots cover their cortex, giving the root system a stringy appearance. In the advanced stages of the decline, the production of new fibrous roots cannot keep pace with root death. Therefore, the tree is unable to maintain adequate absorption of water and minerals, and repeated fungal attacks deplete the root's nutrient reserves (Graham and Timmer, 2003).

Diseased trees show cankers and gum exudations on the above-ground parts on the scions, especially on the major limbs, whereas rootstocks generally remain healthy (Francis *et al.*, 2015). Infected bark remains firm with the formation of small cracks through which an abundant exudation of gum occurs. Citrus gum, which is water-soluble, disappears after heavy rains but is persistent on the trunk under dry conditions (Graham *et al.*, 2003). Diseased bark becomes darker than the surrounding healthy tissues (Francis *et al.*, 2015). It is often cracked and detached as the disease progresses. Lesions extend around the circumference of the trunk, slowly girdling the tree, and when they surround the trunk, trees eventually die (Francis *et al.*, 2015).

Factors contributing to the *Phytophthora* disease and its control strategies

The irrigation system: In fact, there are significant correlations between the prevalence of the disease and a set of agronomic factors, particularly the irrigation system (Vincent *et al.*, 2012). Inoculum density is higher in regions adopting surface irrigation systems, as soils in these regions are saturated with water due to the large amounts of water applied during each irrigation (Yaseen *et al.*, 2010). It has been shown that the high populations of *Phytophthora spp.* and the incidence of the disease may be due to the number of zoospores released by sporangia when the soil becomes saturated (Lutz and Menge, 1986).

Good drainage and irrigation system management are essential for the regeneration of replacement roots. If *Phytophthora spp.* populations reach harmful levels, i.e. more than 10 to 20 propagules/cm^3 of soil, their numbers will increase during wet periods, due to increased root infection (Graham and Menge, 1999). Infection in saturated soils is due to the fact that roots become damaged by reduced mineral forms and toxic metabolites, which predispose the tree to infection (Graham and Menge, 1999). So, farmers are advised to use the drip irrigation system, which gradually applies water to the soil, and only the soil directly under the dripper is saturated (Yasseen *et al.*, 2010).

Soil salinity: Soil and irrigation water salinity has become a major constraint in Morocco's citrus-growing regions (Chetto *et al.*, 2015), and sour orange is the most widely used rootstock in because of its adaptation to most soils and its positive effect on the fruit quality (Omari *et al.*, 2012). Although, its assets are numerous, its resistance to *Phytophthora spp.* attacks is drastically weakening in saline soil or by irrigation water (Sulistywati and Keane, 1992; Benyahia *et al.*, 2003).

The climate: Environmental conditions are a key factor of infection rate of *Phythophtora* rot. *P. citrophthora* is more active during the coldest periods and probably survives as dormant sporangia or as chlamydospores and oospores (Graham *et al.*, 2003). This pathogen develops well at moderate to low temperatures in the Mediterranean areas. It is more active during the winter when rainfall occurs. In addition, soil clogging increases the risk of *Phytophthora* propagules being dispersed by rainfall splashes from the soil to the tree fork (Vicent *et al.,* 2012). For effective management of *Phytophthor*a diseases, it is essential to integrate cultural practices such as the use of *Phytophthora*-free plant material and resistant rootstocks as well as the adoption of adequate watering system (Graham *et al.*, 2003).

Resistant rootstocks: The use of resistant rootstocks is one of the most effective cultural methods for controlling *Phytophthora spp.* diseases. Although, this resistance is not absolute, but it was considered acceptable by Moroccan's citrus growers (Benyahia *et al.*, 2003). However, Benyahia *et al.* (2003) indicated that this resistance might be affected by the soil and water salinity observed in most citrus-growing regions. According to Benyahia *et al.* (2003) resistance to *Phytophthora spp.* is present in most citrus rootstock including trifoliate oranges, lemons, rough lemons, tangelos, macrophylla and volkameriana.

Preventive measures: To achieve exclusion, several measures are needed, including:

• Use of propagating material from listed and certified pathogen-free sources.

• Use of well water *Phytophthora spp.*-free for plant watering.

• Choice of selection site to avoid runoff from surrounding areas entering the nursery.

• Copper fences and footbaths at the entrances.

• Plants grown in containers containing soil-free from *Phytophthora spp.*, nematodes and other harmful pathogens to citrus.

• Propagation must be carried out on benches 40 cm at least above the ground.

• Other recommended sanitation measures in nursery include frequent washing and disinfestation of soil, walls and benches (Graham and Feichtenberger, 2015). In case of susceptible rootstocks, foot rot on young trees can be mitigated by cultural practices such as ensuring adequate soil drainage (Feichtenberger, 2000; Feichtenberger *et al.*, 2005; Graham and Menge, 1999).

Chemical control: Chemical control is necessary when other control methods are unable to effectively control *Phytophthora* root rot (Graham *et al.*, 2011). Fungicide treatments should begin after the appearance of foot rot lesions. Fungicides applied as a spray to the trunk are the most effective (Feichtenberger, 1997).

Preventive treatments: Some fungicides, applied at high concentrations as a trunk injection, have long-term activity on zoospores of *Phytophthora spp.*; Captafol is a *Phytophhtora spp.* inhibitor at concentrations well below those of Captan or copper, but some copper-based fungicides are effective as captafol for long-term control. Captafol or some copper-based fungicides applied at high concentrations are much more effective than the equipment currently used for long-term control of *Phytophthora* infection (Timmer *et al.*, 1977). The persistence of fungicidal activity varies considerably. Liquid copper formulations performed well than wettable powders. In addition, materials containing a high proportion of large particles, such as basic copper sulphate, were quickly washed off (Timmer *et al.*, 1977). Pyroxychlor applied as a trunk paint has significant activity 13 days post-application on *Phytophthora* disease. In treated trees with pyroxychlor, the control activity covered only the bark surface in the treated area. Since the development of the disease is often limited to the bark and cambium tissue, the limited movement of the fungicide should theoretically reduce the growth of *Phythophthora* in infected trees. In addition, when pyroxychlore was applied on infected trees as a trunk paint, a curative action was observed. However, the product did not succeed in reducing the spread of the lesion on infected trees when it was applied to the ground (Timmer *et al.*, 1977).

Curative treatments: It was reported that applying systemic fungicides likes Metalaxyl and Fosetyl-Al increased the weight of the fibrous roots of citrus rootstocks and reduced the propagules of *P. parasitica* (Sandler *et al.,* 1989). Their applications of Fosetyl-Al and Metalaxyl should coincide with the onset of *Phytophthora* activity. This generally occurs at the beginning of the rainy season, from September to October (Feichtenberger, 1990). In major cases, one application of Fosetyl-Al/Metalaxyl is effective only in reducing the size of lesions on the trunk. Therefore, several applications of these systemic fungicides are necessary for adequate control of citrus gummosis. It was indicated that at least two foliar sprays of Fosetyl-Al during the rainy season are required for the complete control of *P. citrophthora* and *P. parasitica* (Feichtenberger, 1990). It should be stressed that adequate control of *Phytophthora* diseases on citrus relies on integrated strategy combining systemic fungicides with other control measures that includes the use of resistant or tolerant rootstock and other biological and sanitation practices (Feichtenberger *et al.*, 1990). Consequently, the use of fungicides should be alternated in order to minimize the risk of developing resistant strains of the pathogen (Graham *et al.*, 2011). Furthermore, applying fungicides after planting, i.e. metalaxyl and phosphite-based fungicides, should be determined by:

• The sensitivity of rootstocks;

• The probability of infection in the nursery;

• The history of *Phytophthora* diseases on the site (Feichtenberger *et al.*, 1990).

Foliage or soil surface applications are less effective against root rot diseases. Fungicide treatments must be conducted for at least one growing season for tolerant rootstocks and beyond two growing seasons for susceptible rootstocks (Feichtenberger *et al.*, 1990). Moreover, disease incidence can be lowered by adopting an integrated management approach that includes sanitation practices at the orchard level (i.e. prophylactic methods, pre-harvest fungicide application (e.g. copper-based fungicides) (Adaskaveg *et al.*, 2015).

Dry root rot

Dry root rot of citrus (DRR), caused by *Fusarium spp.*, is commonly widespread in nurseries and orchards. In nurseries, symptomatic infections can occur on citrus seedlings by producing dry root rot (Khouja *et al.,* 2008). Several species of Fusarium have been identified and associated to dry root rot disease, including *Fusarium solani* which is considered the main species isolated from diseased citrus trees. Other species such as *Fusarium oxysporum* was also isolated and characterized (Hafizi *et al.*, 2013). *F. oxysporum* is a ubiquitous pathogens of agricultural importance. It is widely distributed throughout the world, it has been isolated from diseased roots and rhizosphere (Hafizi *et al.*, 2013). *Fusarium equiseti* (Corda) Saccardo, is a phytopathogenic fungus that produces secondary metabolites that can be toxic for cultivated plants (Kosiak *et al.*, 2005). *F. equiseti* and *F. semitectum* Berkeley & Ravenel are rarely isolated from citrus roots (Smith *et al.*, 1988); they are economically important species because they have been reported responsible for vascular wilt and root rot in various crops (Hafizi, 2013).

The phytopathogenic fungus *F. equiseti* can cause damage to various crops, including maize, rice and wheat in fields and during storage (Hasem *et al.*, 2010). Palmero *et al.* (2011) reported that all *Fusarium spp.* isolates tested were shown pathogenic to tomato and melon. Abu Bakar *et al.* (2013) concluded that *Fusarium* species are among the common pathogens of post-harvest diseases causing rot on tomatoes and other perishable fruits and vegetables. A total of 180 *Fusarium* isolates were obtained from 13 locations throughout Selangor. *F. solani* was the most abundantly isolated (34%) followed respectively by *F. semitectum* (31%) and *F. oxysporum* (31%), *F. subglutinans* (3%) while the latter was *F. equiseti* (1%). In Tunisia, this pathogen has infected potato plants (Ayed *et al.*, 2005).

The disease is commonly chronic, with symptoms beginning with a prolonged decline, accompanied with slight wilting and low vegetation outbreak (Khouja *et al.,* 2008). At advanced decline stage, the canopy appears thin and sparse. In some cases, there is also the production of an unusually high number of fruits, resulting from flowering delay (Khouja *et al.*, 2008).

Roots: Dry rot of citrus acts by invading the root system; the infected root turns from purple to greyish black (Sakovich, 2003). Kunta *et al.* (2015) observed that symptoms may include a reddish-purplish to greyish colour. One or more major roots structures are often blackened or dead, and brown discoloration extends into and through the trunk of the tree, and stopping at the union of buds (Figure 2).

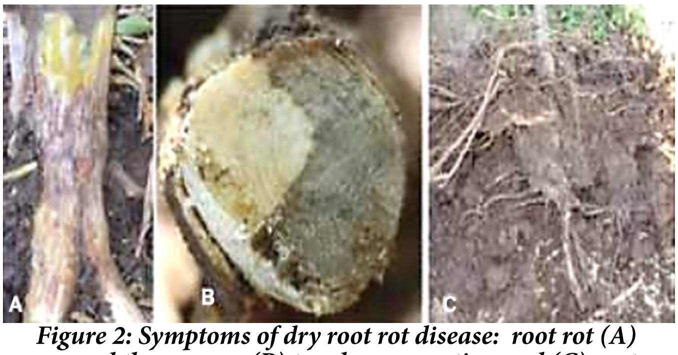

Figure 2: Symptoms of dry root rot disease: root rot (A) around the canopy, (B) trunk cross-section and (C) root lesions (Kunta et al., 2015).

Bark: DRR symptoms on the bark appear as a dark tinted colour, observation of the cross-section of infected trunk shows a greyish brown discolouration in the wood tissue (Sakovich, 2003). Nemec *et al.* (1980) demonstrated that in an advanced stage of the disease, leaves suddenly fade and dry out in a few days while remaining attached to the tree, which ends in a total collapse (Khouja *et al.*,2008). The discoloration of infected tissue may be limited to one section and not the entire tissue. Diseased trees become weak and lose their ability to produce good fruit (Adesemoye *et al.*, 2013). In addition, leaves turn yellow and then brown, and followed immediately by a quick dieback (Kunta *et al.*, 2015). Moreover, some trees apparently healthy wither and die suddenly, and their roots are blackened and rotten, with brown vascular discoloration in the rootstock trunk (Kunta *et al.*, 2015).

Morphological and molecular characterization

In most cases, morphological identification is performed by preparation of fresh fungal cultures on a PDA medium in order to assess certain morphological traits, such as colour of the colony, growth rate, type of fructification, arrangement of spores and pigmentation. With regard to molecular characterization, colonies with same traits are grouped together and, in each group, a representative isolate is identified by DNA sequencing to confirm the exact identity (Khouja *et al.*, 2008). Previous studies have shown that morphological traits are not reliable for species identification due to their variability under varying environmental conditions. Hence the need to use molecular tools to determine species by using specific primers T1 (5’ -AACATGCGTGTGAGATTGTAAGT-3’) and T2 (5’ -TAGTGACCCTTGGCCCAGTTGTTGTTG-3’) (Khouja *et al.*, 2008).

Fusarium solani: Leslie *et al.* (2006) showed that colonies of *F. solani* on PDA medium developed abundant mycelium with purple-brown pigmentation, macroconidia are broad, straight, generally with 5 septate, with a basal cell in the shape of a poorly developed foot (Figure 3). The abundant, oval microconidia show 0 to 1 septate produced in false heads of long monophialidic conidiogenous cells (Leslie and Summerell 2006; Nelson *et al.*, 1983). Hafizi *et al.* (2013) reported that, on palm trees, *F. solani* isolates have a brown to greenish-brown cottony mycelium with dark brown areas, as well as other isolates have a creamy white to greyish-white mycelium. The macroconidia were 27.0 to 37.3 µm long and 3.1 to 4.3

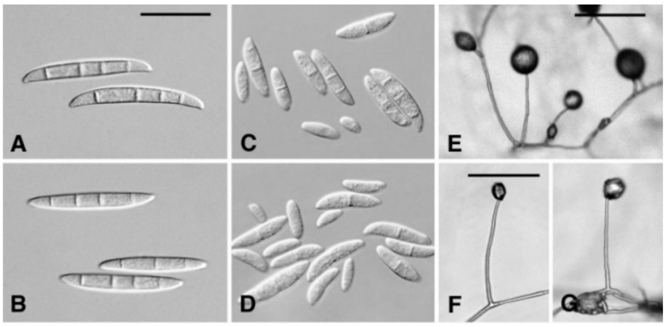

Figure 3: Fusarium solani. A-B: Macroconidia.C-D: Microconidia. Scale-bar: r =25µm; (Leslie and Summerell, 2006)

µm wide. Conidiophores are characterized by branched and long monophyalids. Chlamydiospores production is depending on the isolate. It was abundant in some isolates, while in others it was rare, even a- four-week post-incubation (Kunta *et al.*, 2015). Kunta *et al.* (2015) found that the size of macroconidia ranged from 13-15×3-4 µm to 27-29×4-5 µm and that of microconidia ranged from 3-4×1-2 µm to 9-10×1-3 µm. The number of septa in macroconidia and microconidia is 3-5 and 0-1, respectively, and conidia are hyaline. Macroconidia are sickle-shaped with blunt ends and microconidia are round to oval. Intercalar and terminal chlamydospores were observed in all isolates of *F. solani*.

Fusarium oxysporum: Hafizi *et al.* (2013) described *F. oxysporum* as fungus with a cottony mycelium and pigmentation of colonies ranging from pale to dark purple or from white to pale purple. Macroconidia have a width of 30 to 40 µm and have 3 to 7 septate (Figure 4). Fungal isolates on PDA medium showed variable appearances with purple-peach colony colour.

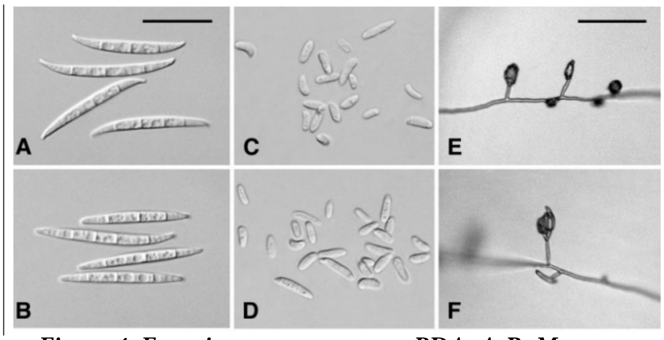

Figure 4: Fusarium oxysporum on PDA. A-B: Macroconidia; C-D: Microconidia. Scale-bar =25µm, (Leslie and Summerell, 2006).

Pathogenicity

Some authors attempted to reproduce the symptoms of citrus dry rot by artificial inoculation of healthy citrus seedlings with sporal suspension from different Fusarium species. The results differed from experiment to another. In Morocco, Bouhart (1993) inoculated citrus seedlings after their predisposition to nitrogen excess, leading to rapid development of the disease and the death of some inoculated plants. In Texas, Kunta *et al.* (2015) underlined that *F. solani* caused decline symptoms in inoculated seedlings 9 months post-inoculation; the inoculated seedlings showed reduced growth compared to the uninoculated control. In Tunisia, the pathogenicity test performed on olive seedlings showed the appearance of Fusarium disease symptoms within 20 to 25 days after inoculation (Rhaiem *et al.*, 2017). Furthermore, some researchers' successfully inoculated different citrus varieties by *Fusarium spp.* after weakening the plant material by various factors other than nematodes. Malikoutsaki-Mathioudi *et al.* (1987) performed the pathogenicity of *F. solani* by exposing plants to abiotic stresses such as excess nitrogen, water stress, and excessive watering. Surprisingly, the highest mortality rates occurs in plants weakened by excess nitrogen and having mechanical injuries in the roots.

Factors favouring dry root rot disease of citrus

Water stress: The disease can spread over the field due to water stress, which predispose the plant to infection by *Fusarium spp.* However, the pathogen can colonize roots in the absence of stress, giving rise to an asymptomatic infection (Dandurand and Menge, 1992). *Fusarium spp.* has been shown to act by depleting the tree's reserves of starch due to stresses conditions. Furthermore, some factors including poor drainage, excessive irrigation and poor aeration, might increase the possibility of *Fusarium spp.* infections on citrus trees (Kunta *et al.*, 2015). Trees stressed due to environmental factors have shown a higher incidence of root rot disease than trees growing without stress (Polizzi *et al.,* 1992). In addition, it was outlined that trees weakened by frost are prone to infection by *F. solani* (Nemec, 1987). Graham et al. (2003) found that *F. solani* infects only weakened citrus trees with reduced vigour. Intrinsically, *F. solani* seems to be a primary colonizer of citrus roots with less starch reserves in the tree due to stressful conditions.

Climate: The climate strongly affects the disease dispersal; a study carried out by Ippolito (1992) showed a peak of the disease within May and July, probably related to the temperature increase. Pathogen population increase may also result from root activity (Sandler *et al.*, 1989), by starting to develop on May and ends on November; the presence of abundant nutrient in roots can stimulate the growth and provide a suitable substrate for the proliferation of the pathogen. This is in line with findings of Malikoutsaki *et al.* (1987) who reported that symptoms of dry root rot are more obvious and severe in summer likely due to high temperatures.

Resistance to dry root rot disease

Screening of a large number of citrus rootstocks has shown that resistance to *F. solani* is quasi-absent (Krueger and Bender, 2015). It is therefore necessary to seek sources of resistance by avoiding stressful conditions described above (Adesemoye *et al.*, 2013). Other possible stresses include invasion by other pathogens, such as *Phytophthora*, which predisposes citrus trees to *F. solani* infections (Dandurand and Menge,1994), Tristeza virus (CTV), injuries caused by rodents, insects or weed control operations strongly influence the spread of the disease (Adesemoye *et al.*, 2013).

Dry rot management

The management of dry root rot of citrus relies mainly on integrated control method. This strategy includes different measures such as eliminating the stressful factors that predispose citrus to sudden death; careful site selection with good surface drainage, and accurate monitoring of irrigation to avoid excessive watering. The use of inter-row cover crops can also improve soil structure (Donovan *et al.*, 2007). Irrigation should be carried out with care, the trunk should be kept dry, and care should be taken with drainage because water should not be into contact with the treetops for an extended period of time

(Adesemoye *et al.*, 2013). The movement of equipment facilitates the spread of the pathogen, so the equipment must be thoroughly cleaned before moving it between orchards (Donovan *et al.*, 2007).

There is a need for applying integrated disease management based directly on knowledge of the pathogen and its life cycle. This approach is an eco-friendly alternative to chemicals. Therefore, integrating sanitation practices and biological methods might help to overcome the dry rot disease on citrus. Furthermore, calcium is an important nutrient that has been used to control *Phytophthora* by reducing the release of zoospore, could be applied for dry rot control (Donovan *et al.,* 2007). Sanitation practices is very useful and permit to clean plantations from remaining plants debris, which might harbour the pathogen primary *inoculum*. In addition, managing other devastating pathogens, which predispose the trees to DRR likes *Phythophtora spp.* is also essential. Finally, rational fertilization helps to minimize stress and disease occurrence; the application of insufficient or excessive nutrients should be avoided. The rational use of herbicides and other chemicals will also be useful (Donovan *et al.*, 2007).

CONCLUSION

The citrus industry plays an important socio-economic role in Morocco. However, the development of this sector is hampered by a series of constraints, in particular pest and diseases problems. It appears that the most serious phytosanitary problems are caused by Mediterranean fruit fly, mites and mealybugs, *Phytophthora* root rot and dry root rot diseases. Managing of these constraints in the field relies on the adoption of integrated management practices, which include the use of pesticides, chemical copper treatments, and Fosetyl-Al for managing *Phytophthora* root rot.

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