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Fusarium oxysporum f. sp. *niveum*: Causal Agent of Vascular Wilt of Watermelon

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ABSTRACT

Objective: To describe *Fusarium oxysporum* f. sp. *niveum* (*Fon*) as the causal agent of vascular wilt in the watermelon crop (*Citrullus lanatus* (Thunb.) Matsum and Nakai).

Design/Methodology/Approach: A review of scientific literature (scientific books, notes and articles) was carried out about *Fon* as the causal agent of vascular wilt in the watermelon crop; biology, symptoms, disease cycle, isolation and management alternatives.

Results: *Fusarium* wilt in watermelon is the main fungal disease of this crop worldwide. Necrosis of the vascular tissue and wilt of the plant are the most characteristic symptoms of the disease. There are four races of this fungus (*Fon* race 0, 1, 2 and 3); the commercial varieties of watermelons have different degrees of resistance to these races. Practices such as the correct diagnosis, use of grafts, solarization, fungicides, biological products and genetic resistance can significantly reduce the impact of the disease on the production.

Findings/Conclusions: In Mexico, knowledge about vascular wilt of watermelon is scarce despite this disease being one of the factors that limits commercial production. The greatest knowledge about the symptoms, the fungus's biology, presence and distribution of races, diagnosis and alternatives of *Fon* management, will allow integrating appropriate management practices that favor the commercial production of the crop.

Keywords: *Citrullus lanatus*, *Fusarium oxysporum* f. sp. *niveum*, vascular wilt.

INTRODUCTION

Cultivating watermelon (*Citrulllus lanatus* (Thunb.) Matsum. & Nakai) at the national level has great importance because of the amount of labor that it requires annually. Mexico is one of the eleven top watermelon producing countries in the world, with a production of 1,345,705 t, and an approximate surface of 40,000 ha (SIAP, 2020). The diseases that affect the crop constitute a limitation for its production in many parts of the world, particularly when cultivars with resistance are not used. Among these diseases, vascular wilt caused by *Fusarium oxysporum* f. sp. *niveum* (*Fon*) stands out due to its importance, since it

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is the main fungal disease that affects the watermelon crop in the whole world, with the ability to cause losses of up to 100% when cultivars with fungus resistance are not used (Dau *et al*., 2009). It was observed for the first time at the beginning of the decade of the 1890s, in southern United States, where it caused severe losses to farmers (Martyn, 2014). Presently this disease has been described in every continent of the world where watermelon is cultivated, except in the Antarctica (Egel & Martyn, 2007). In Mexico, the scientific information about *Fon* is scarce; it has been generated in its majority in other countries, particularly the United States. Therefore, and taking into consideration that vascular wilt from *Fusarium* in watermelon cultivation continues to be a limiting factor for commercial production, there is the need to generate updated, clear and accurate information that allows a greater comprehension about this disease.

MATERIALS AND METHODS

A review of scientific literature (scientific books, notes and articles) was carried out, about the importance of *Fon* as the causal agent of vascular wilt in the watermelon crop: biology, symptoms, disease cycle, isolation and control.

RESULTS AND DISCUSSION

Biology of *Fusarium oxysporum* f. sp. *niveum*

Fusarium oxysporum is a cosmopolitan pathogen capable of causing vascular necrosis, withering and death of plants in more than 100 plant species of agronomic importance (Rana *et al*., 2017). *Fon* does not have a known sexual phase, and it produces three types of asexual spores: microconidia, macroconidia and chlamydospores (Figure 1A, 1B, 1C), in addition to short phialides (Figure 1D). Microconidia play a role of low importance in the initial infections in the field due to their short-lived nature, while macroconidia have a more relevant role in the survival of the fungus, since they have the capacity of forming chlamydospores (resistance structures) (Egel & Martyn, 2007), which can survive in the soil for many years, making cultivation in fields infested with the fungus difficult (Kang *et al*., 2014).

The range of hosts of *Fusarium oxysporum* is very broad at the species level, since there are more than 120 special forms that affect plants from different botanical families (Michielse & Rep, 2009). In turn, these special forms are subdivided into races, which are described in function of their virulence or pathogenicity to specific varieties of the same plant species which vary in resistance to the

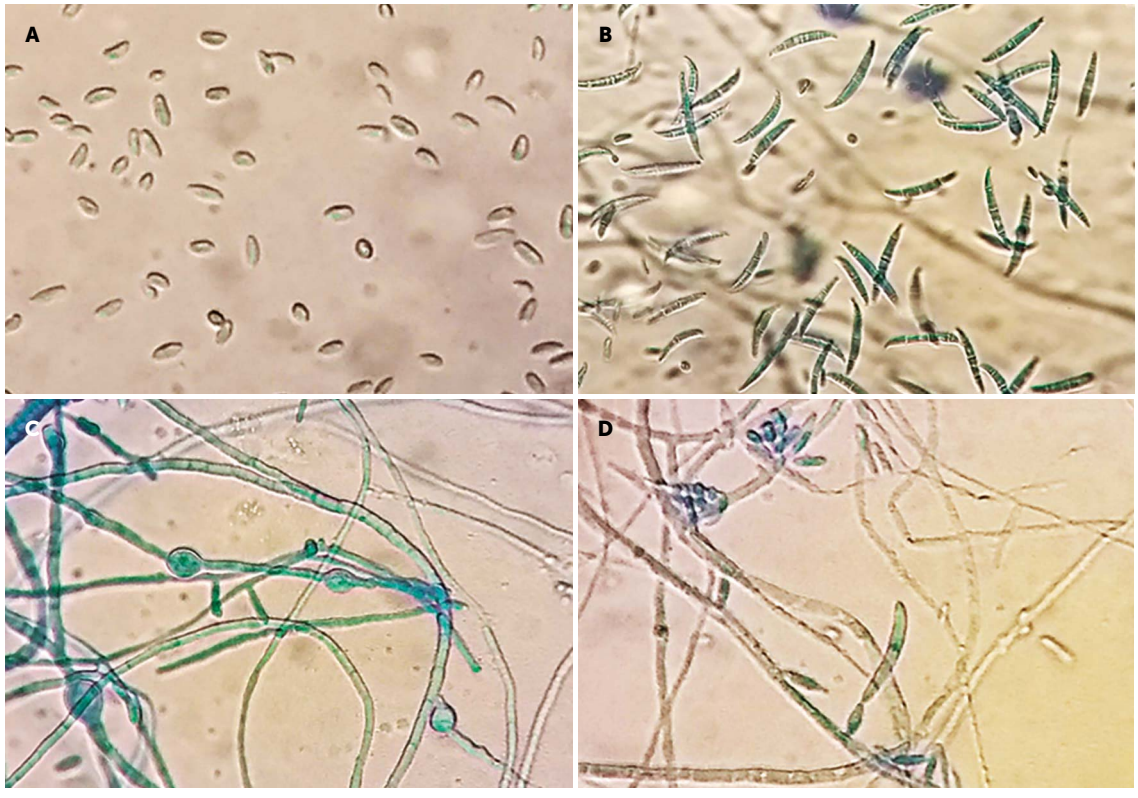

Figure 1. Morphology of *F. oxysporum* f. sp. *niveum* isolated from watermelon plants with vascular necrosis. A) Microconidia, B) macroconidia, C) chlamydospores, D) short phialides.

disease (Correll, 1991; Garcés de Granada *et al*., 2001). *F. o.* f. sp. *niveum* is the special form that is only pathogenic to watermelon, although under greenhouse conditions some isolates of *Fon* can also infect other Cucurbitaceae (Zhou & Everts, 2007).

In watermelon there are numerous varieties with resistance to withering from *Fon*; however, resistance is not universal and in some agricultural regions, the varieties succumb to withering, due to the great physiological specialization of *F. oxysporum* in races (Martyn, 2014). Currently, there are four races of *Fon*: 0, 1, 2 and 3. The four races are present in the United States and in some European countries, although detailed information about the distribution and prevalence of these physiological races in different watermelon producing zones of the world, including Mexico, is still lacking. Race 0 only causes wilt in susceptible cultivars (Sugar Baby), without resistance genes to this disease. Race 1 causes severe wilt in susceptible cultivars and mild to moderate in most of the cultivars that are considered resistant to wilt from *Fon*, as is the case of the Charleston Grey watermelon variety. This race is the one of greatest distribution in the world, and it is present in every production area of the United States (Zhou & Everts, 2007). Race 2 is highly aggressive and causes severe wilt in all the known resistant cultivars; it has been isolated from withered watermelons in Texas, Florida and Indiana, in the United States (Egel *et al*., 2005). Race 3, reported for the first time in the United States, is highly virulent to all the resistant varieties and genotypes of watermelon (Zhou *et al*., 2010).

Symptoms caused by *Fusarium oxysporum* f. sp. *niveum* in watermelon

The initial symptoms of vascular wilt from *F. oxysporum* include an opaque appearance of grey green color, loss of turgidity, and leaf chlorosis, lower growth, wilt and finally plant death. These symptoms are more severe when the infection happens in early seasons of growth. Foliage yellowing generally begins with the inferior leaves and progresses toward the superior part of the plant (Egel & Martyn, 2007). In addition to wilt (Figure 2A), another typical symptom is necrosis and discoloration of the vascular tissue (Figure 2B) that could be observed, especially in the region of the neck or inferior part of

Figure 2. Symptoms caused by *F. oxysporum* f. sp. *niveum* on watermelon plants. A) Wilt under field conditions, B) vascular necrosis in stem, C) wilt on one side of the plant, D) necrosis in the hypocotyl, E) mycelial emerging from infected watermelon plant.

the plant stem, through a longitudinal or transversal cut. Frequently, the wilt symptom can be observed from a single side of the plant (Figure 2C), although as the disease advances the whole plant can wither and die. The watermelon seedlings can also be affected, causing wilt, chlorosis and death of the seedlings, even before the formation of true leaves. In these cases, the hypocotyl of the infected seedlings is seen to be necrotic (Figure 2D) and with fungal mycelium when humidity is high (Figure 2E) (Kleczewski & Egel, 2011).

Biological cycle of *Fusarium oxysporum* f. sp. *niveum*

Vascular wilt caused by *Fon* is a monocyclic disease (Keinath & Hassell, 2014). The initial infection of seedlings generally takes place from chlamydospores that have spent the winter in the soil. The chlamydospores germinate and produce infection hyphae that penetrate the root's cortex, often where lateral roots emerge; wounded roots favor infection by the fungi (Egel & Martyn, 2007).

The progress of the infection by *Fon* in watermelon plants was documented recently by Zhang *et al*. (2015). These authors indicate that, at the beginning of the infection, the fungus spores bind to the root epidermis, germinate and the mycelium grows on the main root, to later penetrate the tissue of the cortex until reaching the xylem vessels, where mycelium grows in both directions and the fungus growth is restricted to the xylem. Within a xylem, the fungus moves upward together with the transpiration current, sporulates and produces microconidia that reach the end of the vessels, where it germinates and mycelium grows in the next vessel, to produce more microconidia (Gordon, 2017). At this point, the characteristic symptom of withering appears as the result from severe hydric stress due to the obstruction of the xylem vessels, product of a combination of the defense response of the host (production of gum, gels and tylose) (Agrios, 2005). As long as the plant is alive, the fungus stays limited to xylem and only when it dies, the fungus sporulates abundantly on the surface of the plant (Di Pietro *et al*., 2003).

F. oxysporum is a pathogen that is easily transmitted through contaminated soil or infected tissue, such as seeds. Tools and machinery contribute to a high percentage of dispersion of the pathogen from one field to another, especially when the implements are used in an infected field and the hygiene of the tools is not verified before using them in a clean field. Air and water also play a role in the propagation of this fungus, especially floods and irrigation water (Joshi, 2018). *F. o.* f. sp. *niveum* could infect watermelon seeds latently, which can be an important source of inoculum and contribute to severe outbreaks of the disease (Petkar & Ji, 2017).

Isolation and diagnosis of *Fusarium oxysporum* f. sp. *niveum*

In the field, the necrosis of vascular tissue is the most characteristic symptom of the disease and which is used for their preliminary diagnosis (Figure 3A). However, the isolation of *F. oxysporum* in non-selective cultivation medium (PDA) or selective (Komada), is necessary for the morphological identification of the pathogen. During isolation, sections of the tissue affected (~1 cm) are disinfected superficially with sodium hypochlorite at 0.5-1%, they were rinsed three times with sterilized distilled water and dried to be placed in Petri dishes with cultivation medium at 28 °C. *Fon* grows rapidly within the next 48 hours in PDA medium and slowly in Komada medium (Figure 3B, 3C). The isolates generally present pink to purple coloration in PDA medium, and a milky aspect in Komada medium. This "traditional" identification of *F. oxysporum* presents the disadvantages of requiring a lot of time, effort and broad knowledge of the fungus for a reliable identification. On the contrary, the molecular identification by PCR presents advantages on the traditional methods of diagnosis, since it is faster, more sensitive and reliable in complement with pathogenicity tests.

Zhang *et al*. (2005) developed a specific PCR assay for the fast and reliable detection of *F. o.* f. sp. *niveum* in tissues of sick plants and soil. These specific primers, Fn-1 (5´-TACCACTTGTTGCCTCGGC-3´) and Fn-2 (5´-TTGAGGAACGCGAATTAAC-3´), amplified a fragment of 327 pb only in *Fon* isolates and not in isolates from other fungi (ascomycetes, basidiomycetes, deuteromycetes and oomycetes). For their part, Lin *et al*. (2010) developed the primers Fon-1 and Fon-2 which amplified a fragment of 174 pb of isolates from *F. o.* f. sp. *niveum*, even from the tissue collected in early stages of the disease development. However, these pairs of primers do not allow identification of *Fon* at the level of race.

Identifying the race of the *Fon* isolates was done based on the virulence toward different watermelon genotypes (Zhou *et al*., 2010). This set of differential plants should include Sugar Baby, Charleston Gray, Calhoun Gray and

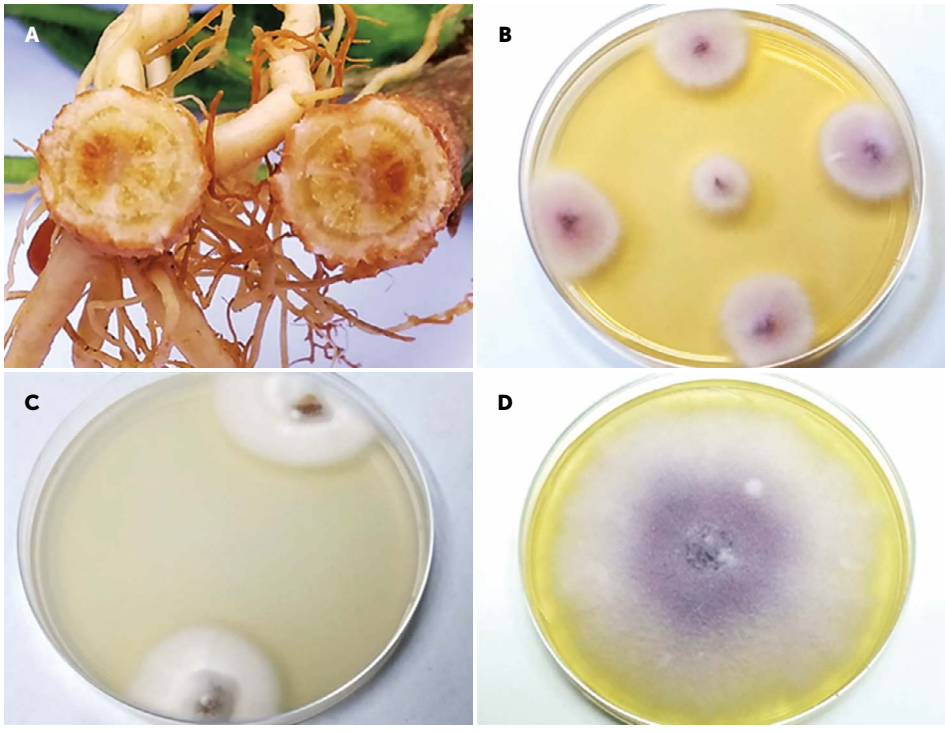

Figure 3. Isolation of *F. oxysporum* f. sp. *niveum* from wilting watermelon plants. A) Vascular necrosis, b) colony in potato dextrose agar (PDA) medium after 72 h of incubation, C) colony in Komada medium after 10 days of incubation, D) colony in PDA medium after 7 days of incubation.

PI-296341-FR. However, Calhoun Gray and PI-296341-FR are not commercially available, which is why the first can be substituted by Allsweet or Dixielee (Zhou *et al*., 2003; Kleczewski & Egel, 2011) and the second by Super Pollinizer-6 (SP-6), developed by Syngenta Seeds, Inc., which derives its resistance to the race 2 of PI-296341-FR (Everts & Himmelstein, 2015).

Management of vascular wilt of watermelon

The management of this disease is difficult when there is not resistant plant material, although there are several methods that individually or combined could help to decrease the disease in a significant way.

Solarization. In watermelon, Martyn & Hartz (1986) reported that solarization of the soil during 30 or 60 days reduces significantly the amount of propagules of *F. oxysporum* f. sp. *niveum* in comparison to the non-solarized soils, thus reducing the total impact of the disease in the susceptible Sugar Baby cultivar.

Grafts. The use of grafted watermelon plants on squash rootstock (*Cucurbita moschata*) was used for the first time at the end of the 1920s in Japan. In Turkey, the use of grafted plants of watermelon reduced in 100% the incidence of withering caused by *Fon* race 0, 1 and 2 (Yetişir *et al*., 2003), and 88% in the United States, compared to the non-grafted plants (Keinath & Hassell, 2014).

Biological control numerous studies have been performed on the control of vascular wilt in watermelon caused by *Fon*; however, most are under laboratory or greenhouse conditions, and very few in the field. Ling *et al*. (2010) reported that the application of a bio-organic fertilizer (*Paenibacillus polymyxa* -SQR21-) reduced between 60-100% the impact of withering caused by *Fon* in experiments in pot and 59-73% under field conditions, when the product was applied to greenhouse seedlings and to soil during field transplant. On the contrary, Himmelstein *et al*. (2014) indicated that the use of the biofungicide Actinovate (*Streptomyces lydicus* -WYEC 108-) for the control of *Fusarium* wilt, did not reduce significantly the disease severity.

Genetic resistance. This method is the most profitable and environmentally safe for the management of *Fusarium* wilt. The cultivars of triploid watermelon (without seeds) are generally more susceptible to wilt from *Fusarium* than diploid cultivars (with seeds) (Zhou *et al*., 2010). At the beginning, management of vascular wilt of watermelon was carried out with the use of diploid cultivars (with resistance to race 1); however, the

preference of consumers for triploid watermelons has made these be cultivated increasingly more. Currently, the development of triploid cultivars with high levels of resistance to *Fon* race 1 and 2 (Everts & Himmelstein, 2015) has not been achieved, while for race 3 of *Fon* there is no resistant commercial variety.

Use of agrochemicals. Fungicides represent an additional management option for the control of *Fusarium* in watermelon. In this regard, Everts *et al*. (2014) studied the effect of 14 fungicides for the management of vascular wilt of watermelon caused by *Fusarium*, finding that Protioconazol and methyl thiophanate reduce the damage caused by this pathogen; these agrochemicals were applied on their own or in combination through drip irrigation at 0, 2 and 4 weeks after transplant.

CONCLUSIONS

In Mexico, despite watermelon cultivation being of economic importance, knowledge about *Fusarium wilt* (*F. o.* f. sp. *niveum*) is scarce. In other countries, there have been substantial advances in the understanding of this disease; however, caused by *Fon* continues to be a limiting factor in commercial production. Greater knowledge about the symptoms, the fungus's biology, the presence of races, and the diagnosis and management alternatives of *Fon*, will enable integrating management practices that will allow reducing the losses caused by the disease.

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