Seed-borne *Fusarium* pathogens in agricultural crops

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Abstract

Diseases caused by *Fusarium* spp. occur worldwide throughout all production areas of edible and ornamental plants as well as tree plantations. *Fusarium* spp. cause yield losses of up to 80% in both field- and greenhouse-grown crops, resulting in serious economic losses. It is known that plant material such as fruits and seeds as well as soil, can be a pathway of disease transmission; therefore, different strategies to control Fusarium have been used: planting new cultivars, grafting, using inorganic and organic substrates, soil solarization, biofumigation and seed treatments. Nonetheless, new *Fusarium* pathotypes are often detected or reported to be the causal agent of diseases. To determine the pathway of Fusarium transmission, specifically through seed, is challenging and scientific reports in this regard, are lacking. To prove that seed is the pathway of pathogen transmission is time-consuming and laborious, since pathogenicity tests with several *Fusarium* isolates must be conducted during several growth stages of the host. The isolates then have to be re-isolated from infected plant parts to confirm Koch's postulates and seeds from inoculated plants need to be obtained which in turn need to be tested again for the presence of the pathogen. To prevent the introduction and distribution of *Fusarium* spp., growers (for local and export markets), technical advisors, and seed producers need to use disease-free seed. The potential production of *Fusarium* mycotoxins during storage is an additional concern when using *Fusarium* infected seeds for food. Depending on the region, it is important to know which crops (hosts) are susceptible to a particular *Fusarium* sp., how this pathogen can be avoided, and how to produce and select healthy fruits and seeds. Efforts must be directed towards avoiding seed contamination with pathogens and developing or optimizing seed health testing methods to detect *Fusarium.*

Keywords: *Fusarium oxysporum*, *Zea mays*, cereal, vegetable*, Cucumis melo*, *Ocymum basilicum*

INTRODUCTION

In agricultural production areas, diseases caused by *Fusarium* species are common all over the world. *Fusarium* diseases affect the agricultural industry by reducing yield of edible vegetable and fruit crops (cucurbits, mango), ornamental plants, trees and cereal plant species on all continents. Disease incidence causes serious yield losses of 40 to 100% in fields or under greenhouse production areas.

Fusarium spp. are considered to be soil-borne and seed-borne with saprophytic and parasitic abilities. Seed-borne fungi are of considerable importance due to their influence on the overall health and germination of seeds and the final crop stand in the field (Neergaard, 1979; Richardson, 1991). Fungal pathogens may be externally or internally seed-borne, extra- or intra-embryal, or associated with the seeds as contaminants. Removal of external

seed-borne fungi by surface disinfection/sterilization creates an opportunity for the internal seed-borne fungi to emerge in greater numbers (Singh and Mathur, 2004).

Fusarium oxysporum (*Fo*) and *F. solani* (*Fs*) are two main species causing symptoms of wilt or yellows, crown and root rot and necrosis, which eventually causes the death of plants. Both *Fo* and *Fs* are able to produce chlamydospores, which enables these pathogens to survive in the soil or substrate under unsuitable conditions in the absence of a host. Other species such as *Fusarium verticillioides* (formerly *Fusarium moniliforme)* are also important.

In some horticultural cultivation systems the range of host specificity is so high that some populations of *Fo* and *Fs* cause disease on one plant species or family but not on others (known as *forma specialis* or f. sp.). Egel and Martyn (2007) summarized that there are over 100 *formae speciales* (ff. spp.) described for *Fo*, which are morphologically similar, but generally host-specific. *Formae speciales* are further differentiated into races based on their ability to cause disease to a particular set of differential host cultivars with varying disease resistance. Some fungus populations can cause disease on one cultivar but not on other cultivars (called races or pathotypes). This fact is used by seed companies to produce new cultivars resistant to new pathogens.

Fo f. sp. *niveum* (on *Citrullus lanatus* -watermelon); *Fo* f. sp. *melonis* (on *Cucumis melo* -muskmelon) and *Fo* f. sp. *cucumerinum* (on *Cucumis sativus* -cucumber) are the most economically important species on cucurbits causing wilts. *Fo* can also be found on the Solanaceae, for example, *Fo* f. sp*. lycopersici* on *Solanum lycopersicum* (tomato), *Fo* f. sp*. melongeae* on *Solanum melongea* (aubergine, eggplant) and *Fo* f. sp. *capsicum* on *Capsicum annuum* (pepper). When *Fo* causes root rot on tomato, pepper and cucumber roots it is named *Fo* f. sp. *radicis-lycopersici* (Jarvis and Shoemaker, 1978), *Fo* f. sp. *radicis-capsicum,* and *Fo* f. sp*. radicis-cucumerinum* (Vakalaunakis 1996), respectively. The *formae speciales* are morphologically the same, but differ physiologically.

To be able to advise producers on how to control *Fusarium* spp., identification of the pathogen not only to species level but to the level of *formae speciales* and races or pathotypes, is essential. Morphological identification of *Fusarium* spp. is not easy even with the aid of books, papers and manuals such as those written by Booth (1971), Messiaen and Cassini (1981), Gerlagh and Niremberg (1982), Nelson et al. (1983), Summerell et al. (2003), Leslie and Summerell (2006). Identification up to race level is even more difficult since it requires the pathogen to be inoculated onto a set of differential cultivars or genotypes. By means of different molecular or vegetative compatibility groups (VCG) techniques, researchers try to identify the pathogenicity of the isolates and differentiate races from each other, but not always with success (Carbonell et al., 1994; García-Alcázar et al., 2006). Genetic differences among *Fo forma speciales* and races are evaluated through the analyses of pathogenicity, VCG, chromosomal feature, ribosomal DNA (rDNA) and other molecular techniques, including, isozyme polymorphisms and various types of DNA markers, but no one method has proven to be totally reliable for all *Fusarium* spp. (Egel and Martyn, 2007; Saikia and Kadoo, 2010).

Database information on seed-borne Fusarium

References referring to seed and *Fusarium* related topics can be obtained from the American Phytopathological Society (APS: www.apsnet.org), the Agricultural Research Service (ARS) of United States Departement of Agriculture (USDA), and the Centre for Agricultural Bioscience International (CABI) webpages. If a search is done in the Ars-Grin Gov USDA web page for *Fusarium oxysporum*, over 737 references relating to seed can be found (Farr and Rossman, 2014).

CABI contains one of the first references where Elliot and Crawford (1922) demonstrated that tomato seeds transmitted *Fo* f. sp. *lycopersici* in the USA, proving that *Fusarium* isolates were pathogenic on tomato. Already in the 1930's, Leach (1936) showed

that *Fusarium* was on/in the seed of the diseased fruit coming from diseased plants, explaining that this fungus was transmitted through the placental tissue to the seed. More recently, Thippeswamy et al. (2011) reported that *Fo* was predominantly associated with tomato seeds, and similar findings were obtained by Al-Askar et al. (2014) in 2011-12 in Saudi Arabia. On the webpage of the International Seed Federation (ISF, 2016) there is a database containing documents with references about *Fusarium* on horticultural crops as *Spinacea oleracea* (spinach) (Bassi and Goode, 1978). Literature on *C. melo,* such as the reference of Leach (1936) indicating internal seed transmission of *Fusarium,* is also available. The ISF webpage also gives information concerning the presence of *Fusarium* on seed, survival on seed or in the soil, treatments (chemical, organic) with references for risk mitigation by treatment of the seed for organic management of the pathogens, etc.

Fusarium in horticultural crops

It is known that fruit, seeds and seedlings can be a pathway of pathogen transmission. The Association of Official Seed Annalists (AOSA, 2016) and other international associations such as the International Seed Testing Association (ISTA, 2016) have developed methods for seed health testing on some horticultural crops but not for *Fusarium* in some specific host species.

Fo was the most dominant species among all *Fusarium* species associated with tomato seeds on potato dextrose agar (PDA) medium and deep-freeze blotter (DFB) technique (Al-Askar et al., 2014). Mathur and Kongsdal (2003) recommend three methods to detect *Fusarium* on seeds: agar plate method, blotter method and DFB method, because these methods are able to reveal spores/conidia on the seed surface, and as mycelium in the seed coat and/or in the internal tissues of the seed.

Several species of *Fusarium* [*Fo, Fs, Fusarium moniliforme (Fm)*] have been detected on muskmelon seeds and other horticultural plants even *formae speciales* as *Fo melonis* and some *f. sp.* specific to other hosts (Gómez Vázquez and Tello Marquina, 2000; Gómez et al., 2014). The citation describing *Fo* on seed on *Capsicum annuum* (pepper) in 2008 (Ali et al., 2008) is also available on the ISF webpage. In Spain, *Fo* was first detected in sweet pepper seedlings in 2013 (Lomas-Cano et al., 2014).

Example 1: Cucurbits

A systematic analysis of commercial muskmelon seeds from 1989 to 1994 of 32 cultivars from 7 different regions of Spain was carried out by Gómez Vázquez and Tello Marquina (2000). A total of 106 seed samples were healthy as tested by the Ulster method (Muskett and Malone, 1940) on both PDA and the semi-selective medium, Komada. *Fo* was isolated from 16% of the seed samples with 1 to 14% of seeds contaminated in each sample. The detection of the pathogen was possible even on thiram (TMTD) treated seeds. The pathogenicity of the *Fo* isolates obtained from seeds coming from diseased fruit was proven by inoculating more than 82 isolates onto melon plants. *Fo* was detected on diseased melon plants and following re-inoculation of the pathogen it was found that the causal agent was *Fo melonis*. Artificial inoculations of the *Fo* isolates on differential cultivars of melon showed that *Fo melonis* belonged to the race 1-2. The authors reported that before the 1980's the cultivars of this crop were produced by farmers in Spain who produced their own seed. The method of cultivation changed with the introduction of new hybrid material where seeds were produced outside the country and imported. Results demonstrated that seeds were pathogen carriers, and the pathotype/race 1-2 was introduced into Spain on the commercial seeds (Gómez Vázquez and Tello Marquina, 2000). In this manner new *Fo* f. sp. *melonis* races appeared. Pathogenic *Fo* isolates were obtained from commercial seeds (treated) and plants which had been cultivated and grown on soilless perlite and from seeds obtained from diseased fruit.

Egel and Martyn, (2007) showed that vascular wilt was caused by *Fo* f. sp. *niveum* and that the fungus was transmitted by seed of watermelon and was isolated from both the external seed coat and from within the seed.

All these results show that with the new agronomic techniques used for producing plants in nurseries, the use of contaminated seeds will result in quick dissemination of the pathogen to distant points from where the plants had been produced. Thus seed can be a pathway for pathogenic fungi such as *Fo*, a very dangerous situation in an agronomic region such as the southern east of Spain, with almost 30.000 ha of continuous plastic greenhouses or other new areas and needs to be controlled.

Example2: *Ocymum basilicum* (basil)

Another research study carried out in Spain reported on *Fo* detection from diseased plants of *Ocymum basilicum* (basil) showing symptoms of *Fusarium* wilt and necrosis (Guirado et al., 2004). Artificial inoculation of the fungus onto different hosts showed that the causal agent was pathogenic to basil but not to other horticultural crops or aromatic herbs, and thus it was possible to conclude that the pathogen was a new *formae special*, *Fo* f. sp. *basilici*. The source of the pathogen was determined in the study and included the perlite substrate, the nursery, commercial seeds and seeds from diseased basil and polypropylene trays used for seedling production (Table 1).

Table 1.- Origin of *Fusarium oxysporum* isolates inoculated and pathogenic to basil.

Origin of the isolates	Number of inoculated isolates	Number of pathogenic isolates
Diseased basil growing in Soil	17	12
Perlite substrate	7	1
Nursery	9	1
Commercial seeds	16	13
Seeds from diseased basil	4	2
Polypropylene trays	10	3
Total	63	32

Source Guirado et al. (2004)

From the results summarized in Table 1, it is remarkable that from a total of 63 inoculated isolates from different origins, Guirado et al. (2004) found that half of them were shown to be pathogenic on basil. Of the 16 isolates coming from commercial seeds, 13 of them were pathogenic and of the 4 isolates obtained from diseased basil, 2 were pathogenic to basil. Once again it was demonstrated that a pathogenic *Fusarium* was seed-borne.

Fusarium on accessions in seed genebanks.

Another aspect to consider is whether *Fusarium* on/in seeds is in germplasm bank collections (base and active collections).

In one of our studies at the CRF-INIA (*Plant Genetic Resource*, in Spanish Centro de Recursos Fitogenéticos-INIA), seed accessions were cleaned, evaluated for viability, and

desiccated before storage in hermetically sealed containers at -4ºC. *Fusarium* spp. were detected on several legumes and cereal species.

Mycobiota on *Pisum sativum* (peas) seeds from 10 accessions after storage (from 1982-1987) and the same accessions after multiplication (1994) without disinfection were analyzed on PDA and Komada media. On Komada medium *Fusarium* was detected in almost 90% of the samples but not on PDA (60%). On PDA, *Rhizopus* grew on most of the samples and interfered with the identification of *Fusarium*. On *Lupinus hispanicus* (lupin) seeds, 14 accessions were incubated on PDA and Komada media**.** Species of 19 fungal genera were isolated. *Aspergillus, Alternaria, Penicillium, Cladosporium* and *Fusarium verticillioides* were detected in more than 50% of the accessions (Blanco et al. 1999). The presence of pathogenic fungi on *Triticum* spp. (wheat species) from 290 accessions of *T. aestivum* and 420 of *T. turgidum* was analyzed by Blanco et al. (2004) after 10 years of storage by plating onto PDA and Komada media. After incubation (17-26ºC) for 6-20 days identification of fungi including *Fusarium* was carried out by periodical examination with a stereomicroscope. Good seed germination probably indicated that the microbiota was external contamination that did not affect the germination significantly.

On *Phaseolus vulgaris* (bean) detection of *Fusarium* after 10 years of storage was done by Blanco et al. (2010). Figure 1 shows *Fusarium* colonies growing on seeds germinated between filter paper, on paper in Petri dishes and on PDA.

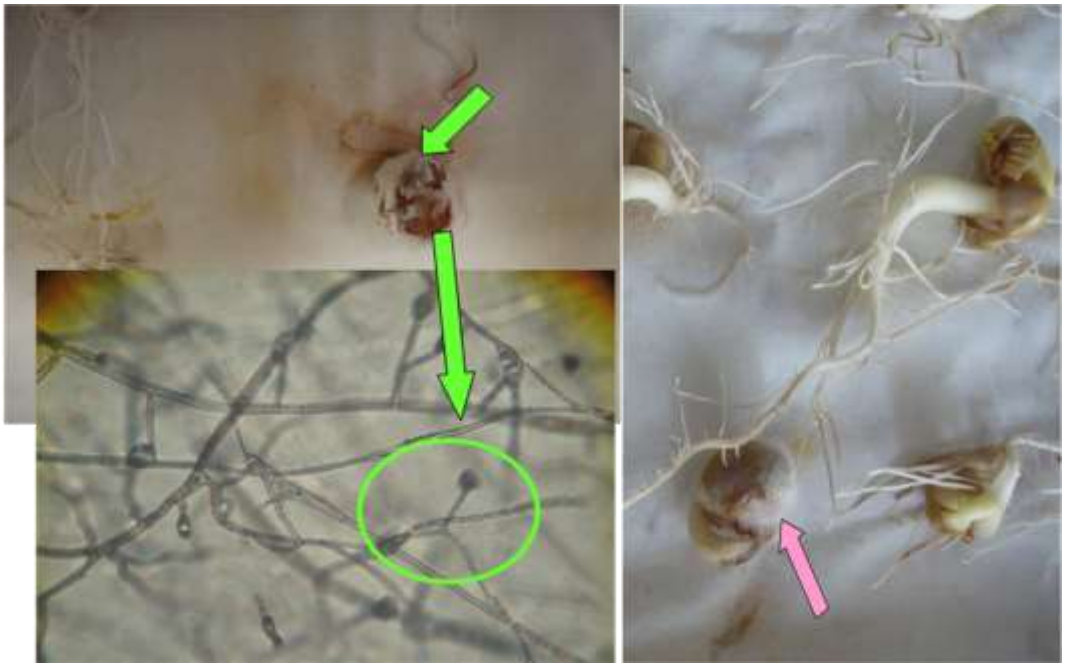

Figure 1. White colonies of *Fusarium* (arrows) on bean seeds after incubation between moist filter paper. Microconidia and false heads (in the circle) of *Fusarium verticillioides.*

Blanco et al. (2010) applied several treatments which included dry heat, humid heat and saturation to bean seeds from stored accessions. After the seed was treated, the seed mycobiota was tested using three incubation methods: 1) seeds between filter paper, 2) on filter paper in Petri dishes, and 3) on PDA. The best seed treatment for decreasing *Fusarium* and other fungi was the dry heat seed treatment. In a study by Tylkowska et al. (2010), microwave radiation of bean reduced *Penicillium* spp. on/in seeds; however, the treatment was not effective in controlling *Fusarium* spp.

In summary, in germplasm banks of seeds, good conservation practices involves avoiding pathogens distribution through exchanges as proposed by Hewett (1987), and seed health control (FAO/IPGRI, 1994), taking care of the interchange of seeds and conservation and storage practices. Because of the presence of propagules surviving at subzero storage temperatures, and the low moisture contents seeds, cleaning of the machinery, containers and storage chambers are essential. Seed treatments are used to protect the seed from pests and pathogens, and have been used with much success (Bradley et al., 2001); nevertheless, chemical seed treatment is not always recommended for seed accessions since it can result in reduced germination if seed is stored for a long time.

MANAGEMENT AND CONTROL

The need for seed health control of cultivated species of plants in order to control the introduction of pathogens by means of infected seed and to protect the producer is clear. Efforts to control *Fusarium,* not only on seeds but other plant material such as seedlings, planting substrates, trays, plastics, etc have to be made in nurseries and in greenhouses.

The use of soilless media for cultivation is considered one of the best means to avoid diseases caused by soil-borne microorganisms such as *Fusarium*. By using an artificial substrate such as rock wool in perlite bags it seems logical to expect no *Fusarium* related disease development since *Fusarium* is considered a soil-borne pathogen. Nevertheless, despite the use of perlite as substrate in soilless cultivation of *C. sativus* (cucumber), disease symptoms of *Fo* crown rot, root rot, fruit rot and even death of some plants was observed. The pathogen was identified as *Fo* f. sp. *radicis-cucumerinum* and was detected in Almería (Spain) in 1999 (Moreno et al., 2001). This fungus was reported by Vakalounakis in Greece in 1996 (Vakalounakis, 1996) and has since then been distributed around the world from Australia to China, and the USA. Studies demonstrating whether seeds were the origin of this pathogen have not been done, although, the distribution of the diseased plants on the various continents can give an indication that seed could be a pathway. Therefore, transplants and seeds should be obtained from reliable sources and be disease-free.

Crop rotation could be a possible solution for controlling *Fusarium* diseases but the plants used in the rotation program may also host *Fusarium.* It is therefore suggested that plants from different families should be inoculated with the *Fusarium* pathogen to determine if they could be used for crop rotation. New races of pathogens continually appear in many production areas all over the world. Consequently, the same cultivar should never be grown in succession on the same land or in a greenhouse. The fungus can develop pathogenic abilities by different means, for instance by generating genetic variability by anastomosis (Figure 2). Furthermore, chlamydospores (spores with a thick wall produced under different conditions by some species of *Fusarium*) are able to remain viable in the soil, sea bed, rainwater, artificial substrates, dust, etc., for many years (Palmero et al., 2011b).

Genetic resistance to Fusarium wilt is the best and economical method of control; however, complete resistance to all races of *Fusarium* is not available in some commercial lines. In 2002, symptoms of Fusarium wilt on melon at Colima State (México), was observed which resulted in a total loss of the yield during the second year of cultivation. De Cara et al. (2004) tested the Fusarium isolates obtained from the diseased plants with differential cultivars, and they found that race 1 of *Fo* f. sp. *melonis* was the causal agent of the diseased muskmelons in this region of Mexico. It was important to determine the presence of race 1 because there were resistant cultivars available in the market that could be commercialized and that had not yet been used in the zone.

It is possible that in the presence of new hosts, some saprophytic Fusarium strains can develop the ability to penetrate root tissue and invade the xylem causing disease in new hosts as suggested by Gordon and Martyn (1997) and Egel and Martyn (2007). If no resistance cultivars are available on the market, grafting cucurbits, tomato, etc. can provide

good control of Fusarium. The use of other plant species, which are not susceptible to *Fusarium*, as rootstocks for grafting watermelon, cucumber (C*ucurbita maxima* x *Cucurbita moschata)* (Añaños-Berdriñana et al., 2009) and tomato has been successful in controlling Fusarium wilt or root rot. This technique is widespread in the Mediterranean and Asian areas in horticultural crops (Añaños-Berdriñana et al., 2009; Palmero et al., 2011a)

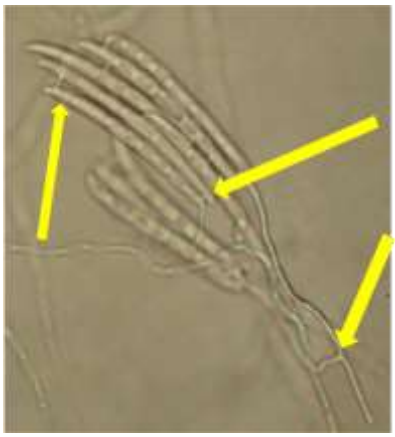

Figure 2. Anastomosis between hyphae and cells from macroconidia spores (arrows) of *Fusarium* can produce genetic variability. Direct observation on potato dextrose agar (PDA) under a stereomicroscope (x 400).

Long-term survival of Fusarium in the soil and organic and inorganic substrates, make the control of this pathogen difficult. Solarization, biofumigation, chemical and physical disinfection of substrates are common agricultural practices that aim to reduce pathogenic inoculum. Soil solarization involves the use of a clear plastic mulch placed over fallow soil in the field or in a closed greenhouse during the hot months of the year. The purpose of soil solarization is to maintain the heat from the sun, in the soil, so that temperatures become lethal to the pathogen. This technique has been as effective as chemical control in terms of reducing the inoculum in areas where appropriate climatic conditions exist; however, it can be laborious and expensive. Biofumigation, incorporating organic matter, is recommended in integrated pest management (Añaños-Berdriñana, 2006; Egel and Martyn, 2007; Palmero et al. 2011a). Re-colonization and/or re-infestation of the soil by the pathogen can occur however, because of improper application and management of the fumigants. Other methods such as biological control of Fusarium are promising in laboratory and greenhouse trials; although, few have shown significant control at the field level. Hairy vetch and other cover crops can be used as soil amendments and may reduce Fusarium wilt (Egel and Martyn, 2007).

Identification of the pathogenicity of *Fo* by molecular techniques can help us to understand other management approaches of the pathogen as suggested by Masachis et al. (2016).

CONCLUSIONS

Fusarium is a common seed-borne pathogen of important horticultural crops. Continuous management of the *Fusarium* pathogen by classic methods (crop rotation, resistant cultivars, grafting, seed health testing and inspections of the seedlings in nurseries, soil, substrates, water and in the field) is imperative in order to prevent the introduction or spread of the disease in producers' fields or greenhouses.

While no one control method gives adequate or complete control in the field or greenhouse, it may be useful when used in combination with other management strategies such as solarization, biofumigation and/or disinfection of substrates.

The seed industry must avoid using contaminated seed in fields or greenhouses with no history of the disease to avoid any potential primary inoculations. Extracting seeds from diseased fruits is not recommended since seed may be carriers of the *Fusariu*m pathogen. Seed health testing could also help prevent Fusarium infections. The development of an ISTA/AOSA validated and accepted technique to be used nationally and internationally for the detection of *Fusarium* spp., such as *Fusarium verticillioides* on maize seed and other *Fusarium* spp. such as *Fo* and *Fs*, is needed. Thompson et al. (2013) developed a PCNB free selective medium due to the toxicity of. Future research should also focus on improving seed treatments so that they are more effective against these pathogens.

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