

Fusarium musae infected banana fruits as potential source of human fusariosis: May occur more frequently than we might think and hypotheses about infection

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ABSTRACT

The banana fruit infecting fungus *Fusarium musae* was originally known as a distinct population within *Fusarium verticillioides*. However, recently, *Fusarium musae* was installed as a separate species and the first cases of human infection associated with *Fusarium musae* were found. In this article, we report an additional survey indicating that human pathogenic *Fusarium musae* infections may occur more frequently than we might think. Moreover, we evaluate the hypotheses on how infection can be acquired. A first hypothesis is that banana fruits act as carriers of *Fusarium musae* spores and thereby be the source of human infection with *Fusarium musae*. Acquisition is likely to be caused through contact with *Fusarium musae* contaminated banana fruits, either being imported or after traveling of the patient to a banana-producing country. An alternative hypothesis is that *Fusarium musae* is not only present on banana fruits, but also on other plant hosts or environmental sources.

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Banana plants (*Musa sapientum*) can be affected by several fungal diseases of which *Fusarium* wilt caused by *Fusarium oxysporum* is considered the most important and responsible for severe economic losses in banana-producing countries.^{1,2} Less known, however, are the post-harvest diseases caused by *Fusarium* spp., such as *Fusarium* fruit and crown rot.^{2,3} These are a major cause of ripe fruit losses in banana-consuming countries, with crown and fruits of bananas being subject to decay during import, storage and marketing.^{2,4} *Fusarium* spp. often associated with these post-harvest diseases include, among others, *Fusarium verticillioides* and *Fusarium musae*.²⁻⁵

The banana infecting fungus *Fusarium musae* is also a human pathogen

Both *Fusarium verticillioides* and *Fusarium musae* are members of the *Fusarium fujikuroi* species complex (FFSC), a species-rich group containing many other important plant pathogens.⁵⁻⁸ Originally, *Fusarium musae* was known as a distinct population within *Fusarium verticillioides*.^{5,9} However, in 2011, Van Hove et al.⁵ showed, by multilocus phylogeny and mating experiments, that this population represents a unique lineage

in the FFSC and consequently they installed the new species *Fusarium musae*, being closely related to (i.e. sister species) but distinct from *Fusarium verticillioides*.

Whereas *Fusarium musae* has, until now, only been recovered from banana fruits and seems restricted in its plant pathogenicity, *Fusarium verticillioides* has much broader host specificity than only banana fruits and is mostly found associated with maize.^{4,5} Also, *Fusarium musae* strains appear to have a significantly greater ability to cause infection on banana fruits than *Fusarium verticillioides* strains.² Another difference with typical *Fusarium verticillioides* strains is that *Fusarium musae* strains are unable to produce the mycotoxin fumonisin due to the absence of most fumonisin biosynthesis genes and the characteristic presence of a fumonisin gene cluster excision site (Δ FGC).^{5,10}

In contrary to the differences between *Fusarium musae* and *Fusarium verticillioides* on the molecular level, pathogenicity and secondary metabolism, these species are practically impossible to distinguish morphologically.⁵ In combination with the rather recent installment of *Fusarium musae* as a separate species, it is therefore not surprising that several strains previously identified as *Fusarium verticillioides* are in fact *Fusarium*

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musae. The latter was also observed during re-identification of the *Fusarium* strains in the Belgian fungal culture collection BCCM/IHEM.¹¹ Five strains collected between 2001–2008 and identified upon preservation as *Fusarium verticillioides*, using morphology, appeared to be *Fusarium musae* according to multilocus phylogeny.¹¹ Interestingly, 4 of these strains were isolated from blood samples or biopsies of immune-suppressed patients and the other strain was isolated from the clinical environment.¹¹ Moreover, all 5 strains originated from different hospitals in Belgium or France and showed differences in their amplified DNA marker sequences, indicating they are not clones or cases of common source contamination.¹¹

Fusarium verticillioides is a well-known causative agent of opportunistic human infections (i.e., after *Fusarium solani* and *Fusarium oxysporum*, probably the third most common *Fusarium* spp. involved in superficial and/or invasive human infections), however *Fusarium musae* is not and the BCCM/IHEM strains implied the first report of invasive human infection associated with *Fusarium musae*.^{11–15} In addition, by performing sequence similarity searches in public databases, other cases of human infection were, retrospectively, found to be associated with *Fusarium musae*.¹¹ These were keratitis cases, associated with the use of contact lenses in the United States of America (i.e. cases from the multistate *Fusarium* keratitis outbreak reported by Chang et al.¹⁶), as well as other superficial infections (i.e., skin infection and sinusitis).¹¹

Occurrence of human pathogenic *Fusarium musae* infections

In a more recent survey performed by our laboratory, testing the feasibility of an in-house developed MALDI-TOF MS identification assay and thereby using 390 fungal isolates collected between July 2012 and July 2013 from 2 hospitals located in Brussels, one *Fusarium musae* strain was found among the 20 *Fusarium* isolates identified.^{17,18} This *Fusarium musae* strain was isolated from a blood sample of an immune-suppressed patient, whereas majority of the other fusarioses were onychomycosis cases associated with members of the *Fusarium solani* species complex and *Fusarium oxysporum* species complex.¹⁸ In this survey, *Fusarium verticillioides* was not found to be the causative agent in any of the fusariosis cases.¹⁸ These observations suggest that human infections by *Fusarium musae* are not that uncommon as originally suspected. Based upon our studies, we estimate that about 20% of the reported cases of human pathogenic *Fusarium verticillioides* infections are actually

human pathogenic *Fusarium musae* infections or even more when taking into account our 2012–2013 survey.^{11,18}

Hypotheses about infection

Human pathogenic *Fusarium musae* infections may thus occur more frequently than we think and several hypotheses on how infection can be acquired are proposed.¹¹ A first is that banana fruits, according to our present knowledge the only known habitat of *Fusarium musae*, act as carriers of *Fusarium musae* spores and thereby be the source from which an opportunistic human infection with *Fusarium musae* is acquired.¹¹ Since all reported cases of human infection by *Fusarium musae* involve patients hospitalized in Belgium, France or the United States of America (Fig. 1), acquisition is likely to be caused through contact with imported banana fruits carrying *Fusarium musae* spores. Indeed, it has been shown that *Fusarium musae* contaminated banana fruits have been imported and marketed, for example in Japan and Hungary.^{4,19} However, it cannot be excluded that the patients referred to acquired their *Fusarium musae* infection by contact with contaminated banana fruits in a banana producing-country (Fig. 1), although no cases of *Fusarium musae* infected patients have yet been described in those countries. To evaluate this hypothesis, the travel history of *Fusarium musae* infected patients needs to be investigated (this was not possible for our cases found by retrospective analysis).

Worthwhile to notice is that *Fusarium musae* strains originating from banana fruits of ‘Neotropical’ countries (Ecuador, Panama, ...) can be distinguished from those originating from banana fruits of Asian countries (Philippines) based upon their Δ FGC sequence (Fig. 2).⁵ However, until now, only one *Fusarium musae* strain (i.e. MUCL 51371) has been isolated from banana fruits of Asian origin.⁵ Also 3 of the clinical *Fusarium musae* BCCM/IHEM strains showed this Δ FGC sequence indicating Asian origin (Fig. 2). In addition, no *Fusarium musae* strains have yet been found originating from banana-producing African countries.⁵ Nevertheless, this difference in Δ FGC sequence between *Fusarium musae* strains originating from ‘Neotropical’ and Asian banana-producing countries, may provide evidence whether the first hypothesis concerning the acquisition of a *Fusarium musae* infection (i.e., contact with contaminated banana fruits), involving the patient travel history to a banana-producing country or not, is likely to be valid.

An alternative hypothesis assumes that the habitat as well as distribution of *Fusarium musae* is not as narrow as currently described and *Fusarium musae* is also present on other plant hosts or environmental sources than

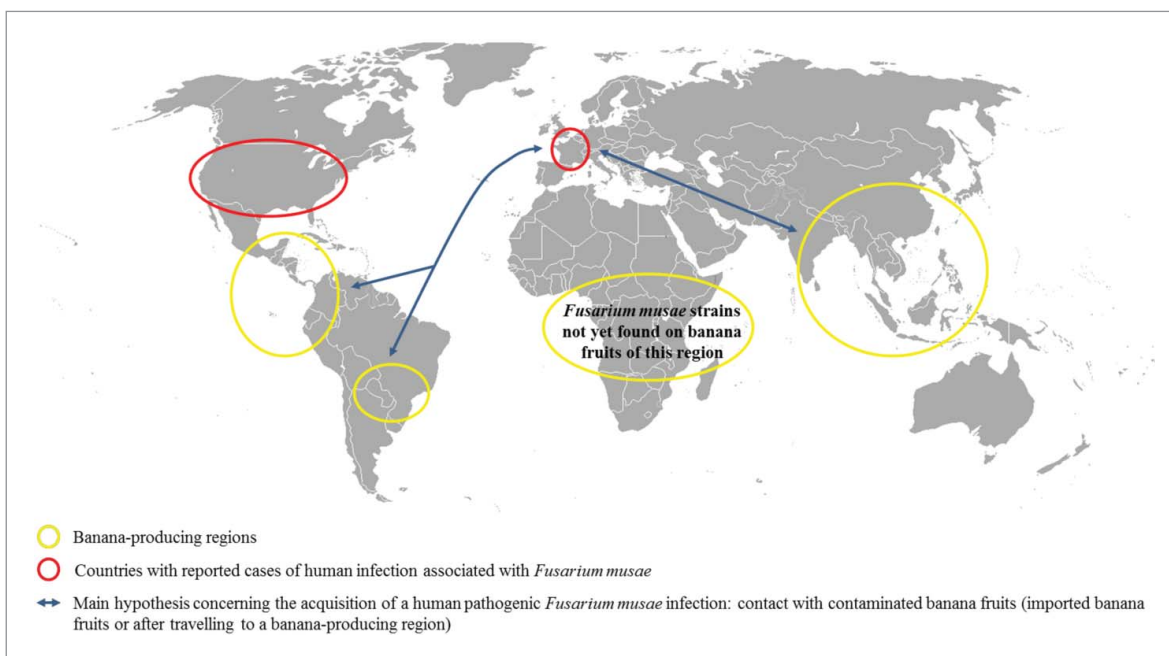


Figure 1. Map indicating the banana-producing regions as well as the countries (i.e. Belgium, France and the United States of America) with reported cases of human pathogenic *Fusarium musae* infections.

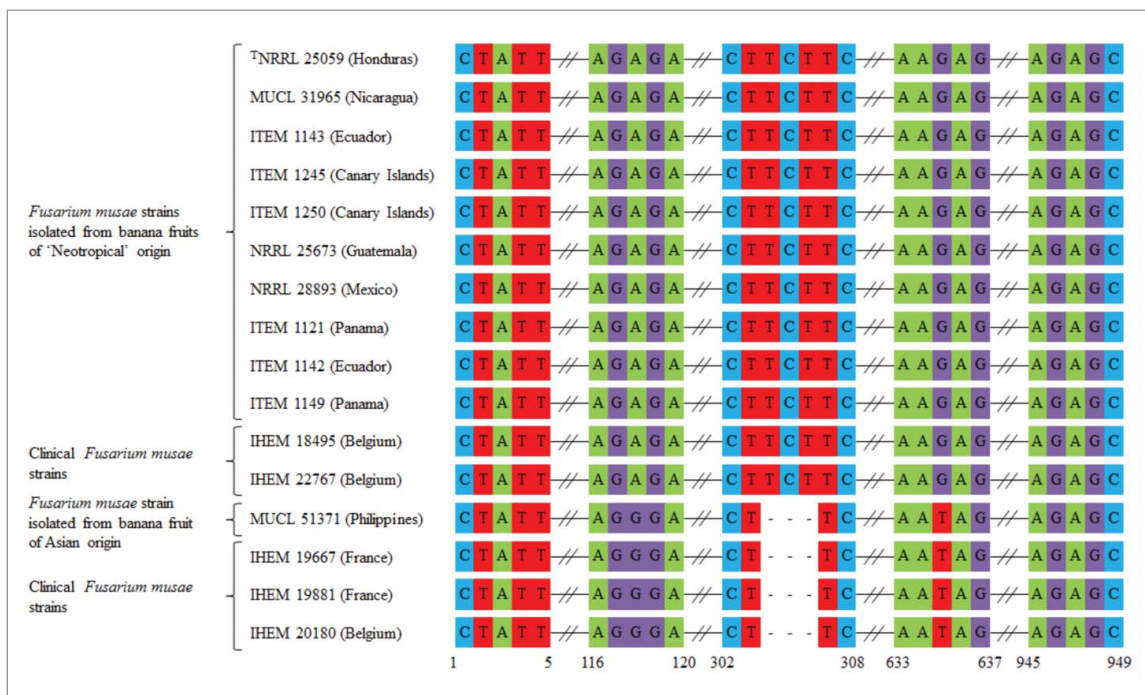


Figure 2. Aligned Δ FGC sequences (949 nucleotides long) of *Fusarium musae* strains obtained by Van Hove et al.⁵ and Triest et al.¹¹ The sites showing differences between the strains are represented. The countries from which these strains originate are indicated in parentheses. A distinction can be made between the Δ FGC sequence of strains originating from banana fruits of 'Neotropical' countries and those of Asian origin. The clinical *Fusarium musae* strains found in the BCCM/IHEM fungal culture collection (Brussels, Belgium) are also indicated. ^T, type strain; NRRL, Northern Regional Research Laboratory (Peoria, Illinois, United States of America); MUCL, Mycothèque de l'Université Catholique de Louvain (Louvain-la-Neuve, Belgium); ITEM, Agri-Food Toxigenic Fungi Culture Collection (Bari, Italy).

only banana fruits.¹¹ An argument in favor for this hypothesis is the fact that in multilocus phylogenies, *Fusarium musae* appears to have its evolutionary origin within the African clade of the FFSC.⁵ For the FFSC, a monophyletic taxon, O'Donnell et al.⁷ defined 3 biogeographic clades, i.e. the African, the American and the Asian clade, based upon the biogeographical origins of the plant hosts to which the species in the FFSC are mostly found associated to. For example, *Fusarium fujikuroi*, a member of the Asian clade, is a pathogen of rice, which has its evolutionary origin in Asia. However, there are some exceptions in the FFSC biogeographic definition of O'Donnell et al.,⁷ such as with *Fusarium verticillioides*, a member of the African clade. *Fusarium verticillioides* is mostly found associated to maize, which has a 'Neotropical' evolutionary origin.^{7,8} Therefore, O'Donnell et al.⁷ theorized that a host switch occurred for *Fusarium verticillioides* on the African continent once maize was introduced in Africa by humans and *Fusarium verticillioides* became a widespread pathogen from thereof. Also for *Fusarium musae* the biogeographic definition of O'Donnell et al.⁷ is inconsistent, since banana plants have an Asian origin. Van Hove et al.⁵ therefore suggested that *Fusarium musae* evolved in Africa on some unknown host or hosts and then moved onto banana, its current preferential host, once Asian banana plants were introduced in Africa. Since banana plants were only introduced in 'Neotropical' countries after being transferred from West Africa to the Canary Islands in the early 1500s, the latter would also explain the current distribution of *Fusarium musae* in 'Neotropical' countries.^{5,20} Nevertheless, this hypothesis doesn't explain the observation of one *Fusarium musae* strain, isolated from an Asian banana variety (i.e., MUCL 51371).⁵ Also, *Fusarium musae* has not yet been isolated from banana fruits of African countries (Fig. 2), nor has *Fusarium musae* been found on other plant substrates than banana fruits. Therefore, additional sampling is required in order to search for *Fusarium musae* strains on banana fruits of African (as well as Asian) countries or on other plant substrates.

Abbreviations

ΔFGC	fumonisin gene cluster excision site
FFSC	<i>Fusarium fujikuroi</i> species complex
MALDI-TOF MS	matrix-assisted laser desorption ionization-time of flight mass spectrometry.

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No potential conflicts of interest were disclosed.

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