Population dynamics of *Fusarium graminearum* in various crop residues using qPCR measurements

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**Abstract:** The influence of biotic (disinfected and non disinfect ed soil) and trophic (presence of crop residues or not) factors as well as the nature of residues (wheat, maize, rape) on the saprotrophic competence of *Fusarium graminearum* in soil was investigated in controlled conditions in soil microcosms for 3 months using qPCR to monitor the population dynamics of the plant pathogenic fungus. The main factor was soil disinfection establishing that the survival of *F. graminearum* was regulated by soil microbial communities. Crop residues provided a favourable habitat for *F. graminearum* but the growth of the pathogenic fungus was promoted to different extents according to the origin of the residues.

**Key words:** *Fusarium graminearum*, population dynamics, soil, crop residues, real-time PCR

**Introduction**

Fusarium Head Blight (FHB) is a disease affecting crop cultures worldwide and concerns mainly wheat (Parry et al., 1995). It leads to important yield losses and the fungi responsible for the disease also produce mycotoxins which are a major concern to human and animal health. The disease is mainly caused by *Fusarium graminearum* although other *Fusarium* sp. might be involved (Bottalico, 1998). The pathogen overwinters in soil and on infested crop residues providing primary inoculum for the infection (Sutton, 1982). The only alternative to prevent FHB is to control the development of this primary inoculum of *F. graminearum* in its natural habitat. The aim of the study was to better understand the saprotrophic competence of *F. graminearum* in soil and crop residues by monitoring its population dynamics in disinfected and non-disinfected soil in absence or in presence of different residues.

**Material and methods**

*Microcosms preparation*

*F. graminearum* (strain MIAE 00376; [http://www2.dijon.inra.fr/umrmse/](http://www2.dijon.inra.fr/umrmse/)) was inoculated into small microcosms (30g of soil, dry weight basis) of natural or previously disinfected soil, in presence or not of the following different crop residues: wheat, maize and rape. The residues were gamma-irradiated and when needed, the soil was disinfected by autoclaving. The experiments were performed during 3 months in a silt loam soil, pH 7.2, at 20°C and 80% water holding capacity. At each sampling day, 3 microcosms of each treatment were destroyed to quantify *F. graminearum*. 
**DNA extraction**

DNA was extracted from soil and soil-residues mixtures by physical disruption followed by a chemical extraction at 70°C (Edel-Hermann et al., 2004). DNA was purified twice using polyvinylpolypyrrolidone spin column to remove coextracted humic acids and once using Geneclean® Turbo kit (Q-BIOgene).

**Real-time PCR**

Quantity of *F. graminearum* was assessed by amplification of a 280 pb product in real-time PCR using specific primers Fg16N forward (ACAGATGACAAGATTCAGGCACA) and Fg16N reverse (TTCTTTGACATCTGTTCAACCCA). Standard curve was obtained by cloning the specific product in pGEM®-T easy vector (Kit pGEM®-T easy vector systems II, Promega). Three technical replicates were performed for each biological template.

**Statistical analysis**

Number of copies was analyzed using ANOVA on following independent variables: soil disinfection, nature of the residues and sampling date.

**Results and discussion**

**Role of biotic and trophic factors on the population dynamics of *F. graminearum***

The biotic capacity of the soil towards *F. graminearum* was significantly improved when the indigenous biota of the soil was removed by previous disinfection (Figure 1). It was also significantly improved by the addition of wheat straw in both the natural and the disinfected soils. Such results indicate that the development of *F. graminearum* was regulated by soil communities. In this experiment, microbial communities were probably mainly concerned since no macrofaunal communities (earthworms, insects) were present in the microcosms. However, although not observed or studied, the putative role of the microfauna (mites, enchytraeids or protozoa) cannot be discarded. Both in disinfected and in non disinfected soil, the presence of wheat residues allowed a better growth of the fungus what suggests that the residues provided a favourable habitat for the fungal development. The residues could supply either trophic resources by providing carbohydrates or structural resources by changing the soil environment. It is clear anyway that the presence of wheat straw in the soil allowed the pathogenic fungus to overcome only partially (non significant difference) but not totally the biotic regulation as the carrying capacity of the natural soil-residues mixture for the strain MIAE 00376 is significantly less than the one of the disinfected soil. This result supports the idea that multitrophic interactions in soil could be used to control soil-borne pathogenic inoculums. On the other way, it shows also that some exogenous resources such as wheat crop debris may promote the development of the pathogenic populations.

**Role of the origin of the crop residues on the population dynamics of *F. graminearum***

Statistical analysis revealed an influence of both the origin of residues and the sampling date. Owing to the qPCR based quantity of *F. graminearum* (Figure 2), maize provided a significantly better growth promotion to MIAE 00376 than wheat which in turn provided a significantly better growth promotion than rape, all crop residues providing a significantly better set of resources than the non amended soil. However none of soil-crop residues increased by 100 times the qPCR based quantity of MIAE 00376 as it was previously observed with soil disinfection. Maize and wheat are host plants of the disease and having these crops as preceding culture represents a risk of disease (Champeil et al., 2004).
Nevertheless maize residues are usually presented as a good promoter for inoculum production because of the quantity of residues produced. The results showed that the effect of maize is not only quantitative but also qualitative since for a same weight of residues, maize promoted fungal growth more than wheat did.

![Figure 1. Population dynamics of *F. graminearum* in disinfected and non disinfected soil, in presence or not of wheat straw as a crop residue. Numbers of * indicate significantly different patterns of population dynamics.](image)

Surprisingly, rape, which is not a host plant, also promoted the fungal growth but at a lesser extent than the cereal residues. Rape is a brassicaceae, known to have a deleterious action towards fungi and more generally on microbial communities (Morra & Kirkegaard, 2002). Therefore, it would be interesting to check if a legume which is richer in nitrogen than cereals and with a different chemical composition could supply a better set of resources to *F. graminearum* than cereals did, and at the opposite, if another brassicacea like mustard, which is used as an intermediate culture could limit the development of the pathogenic fungus.

The shapes of the curves were similar for all the treatments. First a priming effect was observed. It corresponded to an utilisation of resources directly available by the fungus in the environment. Then a decrease and a kind of stagnation could correspond to an adaptation to the habitat. This phase was followed by a second phase of development which could be explained by the activation of the enzymatic machinery of the fungus towards the complex trophic resources and eventually a decrease. Several factors could explain this final decrease: exhaustion of successive resources, competition with microbial communities or a technical bias linked to the moisture content as this parameter was not controlled.
Figure 2. Population dynamics of *F. graminearum* in soil in presence or not of different crop residues. Numbers of * indicate significantly different patterns of population dynamics.

**Conclusion**

Crop residues can indeed supply *F. graminearum* with a set of resources which have not yet been determined, but which are different according to the type of crop residues. Although some speculations were made about legumes or mustard leading to test the impact of these crops on the saprotrophic abilities of *F. graminearum*, the soil disinfection clearly showed that the biotic components of the soil is the reservoir to exploit to control the primary inoculums of this plant pathogenic fungus. Microbial communities were suggested but it is likely that the components of the soil fauna take part in the decomposition of the crop residues, limiting thus the beneficial influence the residues revealed in our controlled experimental conditions.

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**References**


