Biological Control 83 (2015) 20–28

Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

Analysis of microbial taxonomical groups present in maize stalks suppressive to colonization by toxigenic *Fusarium* spp.: A strategy for the identification of potential antagonists

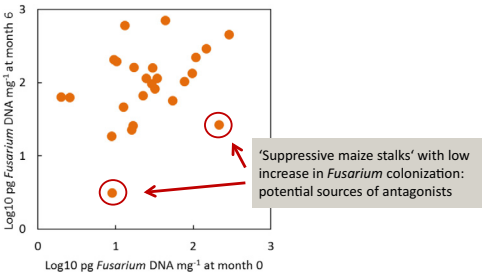
Jürgen Köhl [a,*], Carin Lombaers [a], Antonio Moretti [b], Ranajit Bandyopadhyay [c], Stefania Somma [b], Pieter Kastelein [a]

[a] Wageningen UR – Plant Research International, Droevendaalsesteeg 1, P.O. Box 69, 6700 AB Wageningen, The Netherlands
[b] Institute of Sciences of Food Production – Research National Council, Bari, Italy
[c] International Institute of Tropical Agriculture, Ibadan, Nigeria

HIGHLIGHTS

- Colonization of maize stalk residues by toxigenic *Fusarium* spp. was analyzed by qPCR.
- The level of colonization of individual stalks differed significantly.
- 'Suppressive stalks' with low *Fusarium* spp. colonization levels were found.
- Certain microbial groups were more abundant in 'suppressive stalks'.
- 'Suppressive stalks' are proposed as source of new antagonists.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 22 April 2014
Accepted 11 December 2014
Available online 18 December 2014

Keywords:
Toxigenic *Fusarium* spp.
Maize pink ear rot
Crop residues
Competition
Biological control

ABSTRACT

Pink ear rot of maize caused by *Fusarium verticillioides*, *Fusarium proliferatum* and *Fusarium graminearum* can lead to severe yield losses and contamination of grain with a range of mycotoxins. Maize stalks colonized by *Fusarium* spp. are the main primary inoculum source for *Fusarium* incited epidemics in maize or other susceptible crops grown in rotation.

The colonization of individual maize stalks originating from fields in The Netherlands, Italy and Nigeria by *Fusarium* spp. was quantified using specific TaqMan-PCR assays. Highest values were found for *F. graminearum* and *Fusarium avenaceum* in Dutch samples, for *F. graminearum* and FUM producing species (*F. verticillioides* and *F. proliferatum*) in Italian samples and FUM producing *Fusarium* spp. in Nigerian samples. The increase in *Fusarium* spp. colonization under field conditions during a period of 3–6 months after harvest of the maize crops varied considerably between individual stalks. The fungal and bacterial diversity was analyzed for sub-sets of stalks with high versus low increase of *Fusarium* colonization. Bacterial taxonomic groups such as *Bacillus*, *Curtobacterium*, *Erwinia*, *Flavobacterium*, *Novosphingobium*, *Pantoea*, *Sphingomonas*, *Rahnella* and *Staphylococcus* and fungal taxonomic groups such as *Acremomium sp.*, *Cryptococcus flavescens*, *Cryptococcus zeae*, *Leptosphaeria* sp. and *Microdochium bolleyi* were more abundant in the stalks with lower increase in pathogen colonization. Such fungal and bacterial groups associated with 'suppressive stalks' may be antagonistic to *Fusarium* spp. and a source of candidate strains for the selection of new biological control agents.

© 2015 Elsevier Inc. All rights reserved.

* Corresponding author. Fax: +31 317 418094.
E-mail addresses: jurgen.kohl@wur.nl (J. Köhl), carin.lombaers@wur.nl (C. Lombaers), antonio.moretti@ispa.cnr.it (A. Moretti), R.Bandyopadhyay@CGIAR.ORG (R. Bandyopadhyay), stefania.somma@ispa.cnr.it (S. Somma), pieter.kastelein@wur.nl (P. Kastelein).

http://dx.doi.org/10.1016/j.biocontrol.2014.12.007
1049-9644/© 2015 Elsevier Inc. All rights reserved.

1. Introduction

A broad range of *Fusarium* spp. such as *Fusarium graminearum*, *Fusarium verticillioides*, and *Fusarium proliferatum*, cause ear rot in maize leading to yield losses (Logrieco et al., 2002). These pathogens also produce mycotoxins such as deoxynivalenol, fumonisin, zearalenone and moniliformin in the infected grain during the pre- and post-harvest stages. Contamination of grains produced for food and feed with mycotoxins leads to severe quality losses. Main infection route of *Fusarium* spp. in maize is via the silks during flowering (Munkvold, 2003). *F. verticillioides* also invades maize plants as intracellular endophyte often without causing any symptom and can switch its nature becoming pathogenic in ears (Bacon et al., 2008). Potentially, *Fusarium* spp. can infect other parts of the maize plant including stalks leading to the symptom of maize stalk rot (Cotten and Munkvold, 1998). After harvest, *Fusarium* spp. survive in stalks on the surface of the field soil or buried in soil for at least 1 year and infected stalks act as long term source of inoculum (Tamburic-Ilincic and Schaafsma, 2009). Maize stalks colonized by *Fusarium* spp. are considered as the main primary inoculum source of *Fusarium*-incited epidemics in maize (Munkvold, 2003) and other susceptible crops grown in rotation with maize, especially wheat (Blandino et al., 2010; Scauflaire et al., 2011).

During its life cycle, *Fusarium* spp. colonize different niches: (i) the host plant before and after infection in an epiphytical, endophytical or pathogenic life style, (ii) crop residues as saprophyte and (iii) the soil as saprophytes after decomposition of the residues. During these stages, *Fusarium* spp. interact with the microbiota present in the particular niches. The complex microbiota of soils, especially of the rhizosphere soil, but also on aerial plant parts have been extensively studied. Substantial progress has been made in understanding the role of rhizosphere inhabitants and plant epi- and endophytes after culture independent methods including metabolomics became available for characterization of the microbial biodiversity (Mendes et al., 2013; Vorholt, 2012; Porras-Alfaro and Bayman, 2011). In contrast, the microbiome of crop residues is still poorly understood. Compared to the relatively stable environment in the rhizosphere, crop residues go through a decomposition process, characteristically starting with senescing tissue of aboveground plant parts and ending as components of the stable organic matter in soil. During this process, successions of microorganisms of different trophic groups occur, depending on the available nutrient sources at different stages of decomposition (Frankland, 1998).

Pathogenic *Fusarium* spp. producing cellulases, hemicellulases and pectinases can be considered as early colonizers utilizing organic matter before and after tissue senescence. However, endophytic and epiphytic bacteria and fungi also may invade rapidly senesced tissue and compete with *Fusarium* spp. leading to competitive exclusion and disease suppression (Leplat et al., 2013). The build-up of disease suppressiveness in soils has frequently been reported and in some cases beneficial microbes playing an important role in disease suppressiveness could be identified (Berendsen et al., 2012). For example, multiple statistical approaches have been applied to analyze community fingerprints of suppressive soils to reveal bacterial populations associated with disease suppressiveness (Benítez et al., 2007).

We hypothesized that the colonization of maize stalks by *Fusarium* spp. varies between individual stalks and that early colonizing microbial communities in maize stalks may suppress colonization by *Fusarium* spp. resulting in a slower development of pathogen populations. The objectives of our study were: (i) to determine if stalks exhibiting reduced colonization by *Fusarium* spp. ('suppressive stalks') could be detected, and (ii) to identify bacterial and fungal groups associated with the hypothesized putative competitive exclusion of *Fusarium* spp. in 'suppressive stalks' detected. Individual maize stalks collected from different fields in the Netherlands, Italy and Nigeria were collected after harvest of the maize crops. We assessed the natural increase in colonization by toxigenic *Fusarium* spp. in individual maize stalks exposed to field conditions for 3–6 months. Specific TaqMan-PCR assays were used for the quantification of *Fusarium* spp. to find 'suppressive stalks' with a lower increase of colonization by *Fusarium* spp. compared to 'non-suppressive stalks'. Fungal and bacterial diversity present in such individual stalks was analyzed by Denaturing Gradient Gel Electrophoresis (DGGE).

2. Materials and methods

2.1. Maize stalks

The aboveground parts of 25 arbitrarily chosen maize stalks cut 2 cm above soil surface were sampled in each of 15 fields. In 2009, samples were collected from 3 commercial fields in Amerongen (cv Nerissa, Syngenta), Elst (cv F58, Pioneer), and Veenendaal (cv Adenzo, DSV) in the neighborhood of Wageningen, the Netherlands, and from 3 commercial fields in Gariga di Podenzana (cv KC 6309, Dekalb), Cremona (cv P33A46, Pioneer), and Chiavenna (cv DKC 6818, Pioneer), in the neighborhood of Piacenza, Italy. Stalks were sampled within 7 days after harvest of the maize crops in September or October. A similar sampling was conducted in October 2010 on different commercial fields in Rhenen (1) (cv PR39F58, Pioneer), Rhenen (2) (cv PR39F58, Pioneer), and Rhenen (3) (cv Salgado, Barenbrug) in the neighborhood of Wageningen, the Netherlands; 3 commercial fields in Gariga di Podenzana, Cremona and Chiavenna, in the neighborhood of Piacenza, Italy; and 3 experimental fields (varieties/lines in parenthesis) 'B3' (TZL Comp 1), 'C59' (Inbred Quality Protein Maize, early lines) and 'CX' (Open pollinated variety), all located at the International Institute of Tropical Agriculture, Ibadan, Nigeria. Sampled fields had been planted with maize for at least 5 preceding years. From each stalk, the lower 10 cm segment was used with a node in the middle of the segment. Segments were cut longitudinally in 2 parts. One half of a stalk segment was stored at −18 °C. The remaining half was kept cool at 10 °C and placed with the intact epidermis on the soil surface of a field at Wageningen (Dutch samples), a field in Gariga di Podenzano (Italian samples) and a field in Ibadan (Nigerian samples) within 7 days after sampling. No maize or other cereals had been grown in the fields in the preceding year and the soil had been cultivated to remove crop residues and weeds from the surface. The distance between stalk segments on the field soil was approximately 10 cm. Stalk segments were covered by iron nettings to fix their positions and labeled individually. Segments of maize stalks were collected after 3 months in Nigeria and after 6 months in The Netherlands and Italy. Soil loosely attached to stalk residues was removed and the partly cleaned samples were stored at −18 °C.

2.2. Sample processing and DNA extraction

The 750 individual samples consisting of half stalk segments without or with 3 or 6-month field exposure were shredded in frozen condition, freeze dried and pulverized in a laboratory mill (Cyclotec 1093 Sample Mill with a 1-mm mesh sieve, Helsinki, Finland, for Dutch and Nigerian samples; MF 10 basic Microfine grinder drive, IKA-WERKE, Staufen, Germany, for Italian samples). Directly before and after freeze drying, samples were weighed to assess the dry matter content. Powdered samples were stored at −18 °C until DNA extraction. The portion of the samples not needed for DNA extraction was used to determine the organic

Download English Version:

https://daneshyari.com/en/article/4503768

Download Persian Version:

https://daneshyari.com/article/4503768

Daneshyari.com