available at www.sciencedirect.com

SciVerse ScienceDirect

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

Diversity of indigenous *Beauveria* and *Metarhizium* spp. in a commercial banana field and their virulence toward *Cosmopolites sordidus* (Coleoptera: Curculionidae)

R.B. LOPES[a], A.L.M. MESQUITA[b], M.S. TIGANO[a], D.A. SOUZA[a], I. MARTINS[a], M. FARIA[a,*]

[a]EMBRAPA *Genetic Resources and Biotechnology, CP 02372, Brasilia, DF 70770-917, Brazil*
[b]EMBRAPA *Tropical Agroindustry, Rua Dra. Sara Mesquita 2270 – Planalto do Pici, Fortaleza, CE 60511-110, Brazil*

ARTICLE INFO

Article history:
Received 10 October 2012
Revision received 21 May 2013
Accepted 14 June 2013
Available online 19 August 2013
Corresponding editor:
Ann E. Hajek

Keywords:
Banana weevil
Conservation biological control
Entomopathogenic fungi
Fungal ecology
Microbial control
Molecular characterization

ABSTRACT

Metarhizium anisopliae and *Beauveria bassiana sensu lato* were isolated, from 7 and 41 % of soil samples from a commercial banana field, with average fungal density of 4.3×10^3 and 8.2×10^3 CFU g^{-1} soil, respectively. Twenty-one morphologically distinct *B. bassiana* and four *M. anisopliae sensu lato* isolates from different plots within the field were further characterized. ISSR fingerprinting revealed six different clusters for *B. bassiana*, whereas gene sequencing revealed three *M. anisopliae sensu stricto* and one unclassified *Metarhizium* sp. Bioassays with one or more representative isolates from each *Metarhizium* species and *B. bassiana* cluster showed that all indigenous isolates had lower virulence and significantly greater ST_{50}s than reference (exotic) isolates. The data suggest that the low virulence of most indigenous isolates toward *Cosmopolites sordidus* adults and their relatively low density in soil samples, may help explain the low occurrence of epizootics caused by entomopathogenic fungi in populations of this pest, also known to burrow under the soil surface in banana plantations.

Introduction

The banana weevil, *Cosmopolites sordidus* (Coleoptera: Curculionidae), is one of the most important pests in commercial plantain and banana plantations in tropical areas (Gold et al., 2001). Laboratory assays have shown that isolates of *Beauveria* and *Metarhizium* spp. may be virulent to *C. sordidus* adults and larvae (Kaaya et al., 1993; Krauss et al., 2004; Lopes et al., 2011). However, natural prevalence of *C. sordidus* infected by entomopathogenic fungi (EF) under field conditions is low, usually below 3–6 % (Gold et al., 2001). Except for one report in which natural infection reached 34 % (Pena et al., 1995), epizootics by indigenous *Beauveria bassiana* (Ascomycota: Hypocreales) or other EF on banana weevil populations have not been reported. Despite a few field studies reporting good results under some circumstances (Godonou et al., 2000; Tinzaara et al., 2004), and the availability of commercial *B. bassiana*-based mycoinsecticides in some countries (Li et al.,

* *Corresponding author*. Tel.: +55 61 3448 4674; fax: +55 61 3448 4673.
E-mail addresses: marcos.faria@embrapa.br, marcosfaria1000@gmail.com (M. Faria).
1754-5048/$ – see front matter
http://dx.doi.org/10.1016/j.funeco.2013.06.007

2010), inundation biocontrol strategies by use of EF has not resulted in consistently satisfactory control levels of *C. sordidus*. The negative effects of abiotic factors and the lack of efficient and cost-effective application methods are mentioned as the main limiting factors for broader adoption of microbial control strategies (Gold et al., 2001; Tinzaara et al., 2004; Akello et al., 2009).

Agricultural practices or modification of the environment to favor the performance of EF in banana plantations are desirable strategies, known as 'conservation biological control'. These strategies could benefit from advances in our knowledge of ecological processes related to EF (Meyling and Eilenberg, 2007; Pell et al., 2010). Regarding *C. sordidus*, factors that explain the low natural infection of this pest under field conditions are not well understood. Since the adult stage of this pest has a cryptic lifestyle, during which contact with soil takes place, the transmission of EF from soil to *C. sordidus* needs a closer look. Soils play an important role as reservoirs for some EF (Klingen and Haukeland, 2006). Dynamics of fungal propagules in soil environments are affected by numerous biotic and abiotic factors, including dissemination of conidia by insects (Pauli et al., 2011), agricultural practices (Klingen et al., 2002; Meyling and Eilenberg, 2006; Jabbour and Barbercheck, 2009), crop and soil types (Bidochka et al., 2001; Quesada-Moraga et al., 2007; Bruck, 2010) and season (Rath et al., 1995; Sun et al., 2008).

We conducted laboratory and field studies to investigate the genetic diversity, frequency (recovery rate) and density of indigenous *Beauveria* and *Metarhizium* spp. isolates in soil from a commercial banana plantation in a tropical region. We also assayed the virulence of selected indigenous isolates towards *C. sordidus*. Based on our results and published data we discuss the potential for occurrence of natural epizootics caused by entomopathogenic fungi to control adults of this banana pest.

Materials and methods

Fungal isolation from soil samples

Soil samples were collected in Feb. 2009 from a commercial banana plantation (*Musa* sp., genomic group AAB, cv. Prata-Anã) cultivated for 10 yr in Quixeré, Ceará state, Northeastern Brazil. The studied area was flat, apparently homogeneous in terms of soil characteristics (moisture, organic matter and soil type) and submitted to an intensive agricultural regime, including irrigation and pesticide applications. In 2009, the only chemical used in this field prior to sampling was a carbofuran-based bait. A sampling area of 1.8 ha was initially divided into 100 rectangular 180 m^2 (18 × 10 m) plots. In each plot, four soil subsamples (50 g from the top 10 cm) were randomly collected and mixed. Two grams of each soil sample (water content not determined) were suspended in 18 ml of sterile water and vortexed for 2 min, followed by plating 0.1 ml of a 10^{-1} dilution onto a selective medium (Chase et al., 1986). Colony forming unit (CFU) determinations were performed 12 d following incubation at 25 ± 0.5 °C. From each plot in which *Beauveria* or *Metarhizium* spp. were isolated, only morphologically distinct colonies, displaying significant differences in traits such as size, pigmentation or form, and spores/conidiophores features, were considered as different isolates. Likewise, isolates from adjacent plots showing identical features were considered as one isolate. Purified cultures were cultivated on potato dextrose agar (PDA) for 10 d at 25 ± 0.5 °C and 12 hr photophase. All isolates were assigned a code number and stored at −80 °C in 10 % glycerol until use.

Fungal isolation from banana weevil adults

In each plot mentioned in the previous section and at the same date, one pseudostem trap was set up according to Mesquita (2003) and kept in the field for 7 d. *C. sordidus* adults collected in each trap were transferred to the laboratory and kept in the same plastic cup (10 cm in diameter and 15 cm high), half-filled with sterilized vermiculite and pseudostem pieces and capped with a screened lid. During a 40 d observation period, dead insects were removed and incubated in moistened chambers.

Assessment of the genetic variability of Beauveria *and* Metarhizium *spp. isolates*

Inter-simple sequence repeats (ISSR) fingerprinting and phylogenetic analyses were used to assess the genetic variability of monosporic isolates of *B. bassiana sensu lato* (s.l.) and *Metarhizium anisopliae* (s.l.), respectively, which are species complexes comprising genetically distinct but morphologically similar taxa. For both species, 5 d old culture discs were inoculated in 500 ml Erlenmeyer flasks containing 250 ml of Sabouraud dextrose broth (1 % glucose, 0.3 % malt extract, 0.5 % peptone, and 0.3 % yeast extract), followed by incubation for 4 d at 25 ± 0.5 °C in a rotary shaker incubator at 250 rev min^{-1}. Mycelial samples were harvested on filter paper by vacuum filtration, frozen at −70 °C, and then lyophilized and stored at −20 °C until use. For each sample, approximately 25 mg of lyophilized mycelia were crushed in a mortar under liquid nitrogen, and the total genomic DNA extracted using a slight modification of the cetyltrimethylammonium bromide (CTAB) DNA extraction protocol described by Boucias et al. (2000). Modifications of this protocol included adoption of CTAB 2× (5 ml) instead of an extraction buffer (2 ml), incubation at 65 °C for 20 min instead of 1 hr, enzymatic digestion with RNase for 1 hr instead of 30 min, and precipitation of DNA in a 1:0.1 ratio (v/v) for isopropanol and NaCl instead of 1:2.5 for the ammonium acetate and ethanol mixture. PCR reactions were performed in a total volume of 13 μl, containing 9 ng of each template, using the PTC-100 programmable thermal controller (MJ Research Inc., Watertown, MA, USA). The temperature profile for all reactions was 94 °C for 2 min, followed by 40 cycles of 94 °C for 45 s, 48 °C for 45 s, 72 °C for 2 min, with a final extension of 72 °C for 5 min. Amplifications were performed using the following reaction mix: 1 μM of primer, 250 μM of each deoxyribonucleoside triphosphate, one unit of Taq DNA polymerase and 1× of the recommended polymerase buffer.

For *B. bassiana*, 24 ISSR primers were selected for this study, based on their ability to generate clearly distinguishable polymorphisms (Table 1). Amplified products were separated by electrophoresis in 2 % agarose gels prepared in 0.5×