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Establishment of the fungal entomopathogen *Beauveria bassiana* as an endophyte in sugarcane, *Saccharum officinarum*

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ABSTRACT

We investigated the ability of the fungal entomopathogen *Beauveria bassiana* strain GHA to endophytically colonize sugarcane (*Saccharum officinarum*) and its impact on plant growth. We used foliar spray, stem injection, and soil drench inoculation methods. All three inoculation methods resulted in *B. bassiana* colonizing sugarcane tissues. Extent of fungal colonization differed significantly with inoculation method ($\chi^2 = 20.112$, d. f. = 2, $p < 0.001$), and stem injection showed the highest colonization level followed by foliar spray and root drench. Extent of fungal colonization differed significantly with plant part ($\chi^2 = 33.072$, d. f. = 5, $p < 0.001$); stem injection resulted in *B. bassiana* colonization of the stem and to some extent leaves; foliar spray resulted in colonization of leaves and to some extent, the stem; and soil drench resulted in colonization of roots and to some extent the stem. Irrespective of inoculation method, *B. bassiana* colonization was 2.8 times lower at 14–16 d post inoculation (DPI) than at 7–10 DPI ($p = 0.020$). Spraying leaves and drenching the soil with *B. bassiana* significantly ($p = 0.01$) enhanced numbers of sett roots. This study demonstrates for the first time that *B. bassiana* can endophytically colonize sugarcane plants and enhance the root sett and it provides a starting point for exploring the use of this fungus as an endophyte in management of sugarcane pests.

1. Introduction

Sugarcane (*Saccharum officinarum*; Poaceae) is one of the world's most valuable crops. Although sugarcane originated in Polynesia, it is grown in approximately 120 tropical and subtropical countries with a global production of about 1.89 billion tonnes of crushed sugarcane in 2016 (FAOSTAT, 2018). The sugarcane ecosystem (phytobiome) comprises numerous weeds, arthropods and more than 50 plant pathogens (Ferreira and Comstock, 1993; Verma, 2004; Leach et al., 2017). Arthropod pests associated with the crop worldwide include complexes of stalk feeders, sap sucking insects (e.g., aphids, thrips, mealybugs), root feeders (e.g., white grubs, stemborers), and spider mites (Dittrich et al., 2005; Barker et al., 2006; Leslie, 2008, 2009; Goebel and Sallam, 2011; Goble et al., 2014; SASRI, 2014; Bharu, 2015).

The main arthropod pests infesting sugarcane in Africa include stemborers (*Chilo* and *Sesamia* spp.), black maize beetles (*Heteronychus* spp.), thrips (*Fulmekiola serrata*), scale insects (*Aulacaspis tegalensis*), mealybugs (*Saccharicoccus sacchari*) and spider mites (*Tetranychus urticae*) (Smith-Meyer, 1974; Conlong, 2001, 2008; Nuessly, 2014; SASRI, 2014; Language, 2015). The sugarcane yellow aphid (*Sipha flava*) was first recorded in southern Africa in 2013 (Conlong and Way, 2014; Way et al., 2014). Management of all these pests currently relies on cultural methods, host plant resistance, chemical insecticide application, and biological control focusing on use of insect predators and parasitoids (Akbar et al., 2010; Goebel et al., 2010; Bowling et al., 2016). Chemical insecticides provide rapid and effective control of many pests and reduce labour costs associated with mechanical pest removal. However, health and environmental problems, the development of insecticide resistance, and cost, limit their use (WHO, 2014; Kasambala Donga and Eklo, 2018). Host plant resistance may contribute to reduced pesticide load in the environment, but it might not be long lasting or practical in instances of a new virulent pest species (Humphries et al., 2010). Biological control agents are usually compatible with other pest control methods and are central in integrated pest

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management (IPM) programs of many crops.

Fungal entomopathogens belonging to the order Hypocreales (Ascomycota) or to the phylum Entomophthoramycota have been reported to protect plants from insect pests (Pell et al., 2009; Vega et al., 2012). Fungi in the Entomophthoromycota are generally associated with natural epizootics on foliar insect hosts and are mostly used in conservation biological control (Ekesi et al., 2005; Baverstock et al., 2008; Pell et al., 2009). The major disadvantage with Entomophthoromycota is that they are mainly biotrophic with a close association with their insect or mite host and many cannot be mass-produced on artificial media (Jaronski and Jackson, 2012). On the other hand, hypocrealean fungi such as *Beauveria* and *Metarhizium* are hemibiotrophic, cosmopolitan and ubiquitous in the soil but do not commonly cause natural, large-scale epizootics on foliar insects in annual crops (Pell et al., 2009; Jaronski, 2010). For instance, in a survey of natural enemies of *Chilo sacchariphagus* in sugarcane plantations in Moçambique, Conlong and Geobel (2002) found *Beauveria bassiana* infesting only three cadavers of *C. sacchariphagus* larvae. Hypocrealean fungi are traditionally employed in both inundation and inoculation biological control (Maniania et al., 2001; Meyling and Eilenberg, 2007; Remadevi et al., 2010; Klingen et al., 2014). Currently, large-scale inundation and inoculative biological control is being practiced in many countries including Austria, Brazil and South Africa (Lacey et al., 2015).

There is growing evidence that fungal entomopathogens occur naturally or can be established artificially as endophytes in various crop plants and that such establishment might adversely affect insect pests (Vega, 2008, 2018; Vega et al., 2009; Quesada-Moraga et al., 2014a; Greenfield et al., 2016). *Beauveria bassiana* artificially introduced as an endophyte in cotton (*Gossypium hirsutum*) negatively affected cotton aphid reproduction (Castillo Lopez et al., 2014) and endophytic *B. bassiana* in maize (*Zea mays*) resulted in all-season suppression of the European corn borer, *Ostrinia nubilalis* (Bing and Lewis, 1992a; 1992b). In banana (*Musa* spp.), endophytic *B. bassiana* significantly reduced damage caused by larvae of *Cosmopolites sordidus* by 42–87% depending on the plant tissue (Akello et al., 2007).

Several approaches have been used in establishing *B. bassiana* as an endophyte in target plants. Lewis and Bing (1991), Bing and Lewis (1992a; 1992b) and Wagner and Lewis (2000) successfully established *B. bassiana* as an endophyte in maize using foliar application at the two-leaf or whorl stage. *Beauveria bassiana* was also established as an endophyte in cocoa (*Theobroma cacao*; Posada and Vega, 2005) and coffee (*Coffea arabica*; Posada and Vega, 2006) by inoculating the main radicle of seedlings. Posada et al. (2007) also established *B. bassiana* in coffee seedlings using stem injections, foliar sprays, and soil drenches, with highest endophytic recovery obtained in plants whose stems had been injected with a *B. bassiana* spore suspension. Tefera and Vidal (2009) reported that *B. bassiana* could be established as an endophyte in different sorghum (*Sorghum bicolor*) tissues through seed dressing, foliar sprays, and soil inoculation, with foliar sprays being the best method. Brownbridge et al. (2012) introduced *B. bassiana* into pine seedlings (*Pinus radiata*) using seed coating and root dipping. Quesada-Moraga et al. (2014b) established *B. bassiana* as an endophyte in opium poppy (*Papaver somniferum*) tissue via seed soaking and found that *B. bassiana* was vertically transmitted via seeds from endophytically colonized maternal plants. Evaluating the potential of an entomopathogenic fungal species to establish as an endophyte in a given plant species is the first step in the process of determining whether this fungus might protect the plant from insect pests or mites. The most common method for evaluating endophytic establishment is the fragment plating method (Torres et al., 2011). This method involves the elimination of epiphytes, by surface sterilizing plant tissue sections, and plating the sterilized sections on selective growth media (Vega, 2018). Post-inoculation time for performing this step varies. Ten days were enough to confirm that *B. bassiana* could establish endophytically in artichoke, *Cynara scolymus* (Guesmi-Jouini et al., 2014). Greenfield et al. (2016) evaluated *B. bassiana* endophytic colonization of cassava (*Manihot esculenta*) at 7–9 and 47–49 d. Renuka et al. (2016) traced post-inoculation persistence of *B. bassiana* in maize (*Z. mays*) for 90 d.

Information on the ability of *B. bassiana* to endophytically colonize sugarcane and the effects of *B. bassiana* on sugarcane plant growth is not available. We report that *B. bassiana* can become established as an endophyte in sugarcane using foliar spray, stem injection and soil drench and that endophytism with *B. bassiana* resulted in enhanced sugarcane plant growth.

2. Materials and methods

2.1. Treatments, study location, and experimental design

The experiment was conducted in a greenhouse at the ILLOVO Malawi sugarcane quarantine facility at Bvumbwe Agricultural Research Station, Thyolo District, Malawi (15°55′27.1″S 35°04′12.5″E, 1174 m a.s.l). The experiment was set up as a completely randomized design with subsampling, and treatments consisted of three different fungal inoculation methods (foliar spray, stem injection, soil drench) and the control. The experiment was repeated four times. Each replicate had 36 plants: 9 foliarly-sprayed plants, 9 stem-injected plants, 9 soil-drenched plants, and 9 control plants. Therefore, the experiment consisted of 144 plants. Destructive sampling of plant tissue (leaves, stems, roots) to evaluate endophytic colonization by *B. bassiana* was done 7 and 14 d post-inoculation (DPI). For method, see below. Evaluation of plant growth was done 16 DPI.

2.2. Plants

The sugarcane variety MN1 was used. This is a commonly grown variety in Malawi (Kasambala Donga and Eklo, 2018). Sugarcane stems free from pests and diseases were collected from 7 to 10-month-old irrigated seedcane growing at the ILLOVO Nchalo Sugar Estate (Chikwawa District, Malawi). The stems were cut into smaller sections approximately 13.5 cm long. Each of these sections had two buds. These stem cuttings are referred to as 2-bud cane-setts (Fig. 1A). To prevent ratoon stunting disease and other bacterial sugarcane pathogens, cane-setts are routinely dipped in 50 °C water for 2 h. This treatment could have negative effects on germination (McFarlane, 2013); therefore, surface sterilization in alcohol and sodium hypochlorite was used as described below. Two-bud cane-setts were washed for 1 min in running tap water to remove any debris before surface sterilizing by immersing for 3 min in 1% sodium hypochlorite followed by 1 min in 70% ethanol (Parsa et al., 2013; McKinnon et al., 2016). The tissues were then rinsed in sterile distilled water three times. The sterilized plant tissues were dried on sterile filter paper for 30 min before plating. Effectiveness of the sterilization process was evaluated by plating 100 μl of the last rinse water on Sabouraud dextrose agar (SDA) and incubating the plate for 10 d at 25 °C. Imprints of sterilized plant tissue were also prepared to ensure that the sterilization was successful. This was done by momentarily placing and pressing a surface sterilized plant tissue on SDA and incubating the plate for 10 d at 25 °C.

Two surface sterilized two-bud cane-setts were horizontally planted in each 10 L plastic bucket (height 235 mm, upper diameter 265 mm, lower diameter 170 mm) containing a steam-sterilized mixture (2:1:1) of sandy loam soil, bagasse and sand from the