SHORT NON-REFEREED PAPER

PLANT-ENDOPHYTE-PEST INTERACTIONS: INVESTIGATING THE BIOLOGICAL CONTROL OF *E. SACCHARINA* AND STEM ROT DISEASE IN SUGARCANE

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Abstract

Endophytic *Beauveria bassiana* reduces insect and pathogen damage in several important crops. To find alternative control methods against both the stalk borer *Eldana saccharina* and the stem rot disease agent, *Fusarium* spp., 28 sugarcane genotypes and natural host plants of *E. saccharina* were surveyed for the presence of endophytic *B. bassiana*. Collectively, 326 plant samples (roots, stems or leaves) were sampled from five locations in sugarcane-producing areas. Following the disinfection of the plant surfaces, 130 fungal colonies were confirmed as *B. bassiana* by using a DNA sequence analysis of the Internal Transcribed Spacer region (ITS). Of those, 119 were recovered from 22 of the 28 sugarcane genotypes. The number of *B. bassiana* isolates obtained from the upper internodes were significantly higher ($P = 0.003$, $df = 27$) than those from the lower internodes. Furthermore, varieties N31 and N41 were found to favour endophytic colonisation, with *B. bassiana* being isolated from all the plant parts that were sampled. The potential of two *B. bassiana* isolates (*TL-leaf* and N41S1TI) as biocontrol agents against the *Fusarium* strains (PNG40, MN57a, SC17 and ZN7) were also investigated. *In vitro* plate co-culture assays revealed that both genera (*Fusarium* and *Beauveria*) had the ability to antagonise and limit the growth of the other. When *B. bassiana* strains were grown in the *Fusarium* culture filtrate of a 2000 ppm concentration, and *vice versa*, both genera had a significantly lower biomass. The dynamics that may exist between the plant, the endophytic biological control agent, the pathogen and the pest are important to understand if biological control strategies are to be adopted effectively.

Key words: *Eldana saccharina*, *Beauveria bassiana*, *Fusarium* spp., endophytes, biological control, microorganism interaction.

Introduction

Eldana saccharina Walker (Lepidoptera: Pyralidae) (Eldana) is a problematic sugarcane stem borer pest in South Africa (Singels *et al*., 2016). It owes its success to its cryptic life cycle and its intricate relationship with the *Fusarium* species. Its larvae bore into the lower parts of the stalk, completing all their larval stages by feeding on the internal tissues. The larvae remain protected within the stem from traditional pest management methods (Keeping, 1995). The feeding damage and larval survival is compounded by wound infections from some *Fusarium* spp. (McFarlane *et al*., 2009). *Fusarium* species form complex interactions with plants and insects. Some species improve the larval development and survival (*Fusarium pseudonygamai*: SC17), while others form antagonistic associations (*F. sacchari*: PNG40).

Microorganisms, such as *Fusarium* spp., form complex ecological interactions with plants, insects and each other in agricultural systems (Ishaq, 2017). In plants, they can occur as beneficial endophytes, living asymptomatically and defending against insects. Yet they also can form pathogenic associations (Malcolm *et al*., 2013), which cause plant infections and diseases. A similar association was observed between sugarcane *Fusarium* spp. and Eldana. Therefore, in order to effectively control both Eldana and *Fusarium* spp. infections, a deeper understanding is required of the interactions existing between the plant-pathogen-pest and the biological control agent.

Microorganisms colonise plant tissues by competing for space and nutrients. They also produce secondary metabolites, which may limit and antagonize other microorganisms present (Dara, 2019) thus forming good biological control agents. The reported potential of endophytic *Beauveria bassiana* as a dual biological control agent against pest and pathogens, makes it a potential candidate for alleviating the Eldana-Fusarium problem in sugarcane. Limited research has been conducted on *B. bassiana* for controlling sugarcane pest and disease (Kasambala *et al*., 2021). Hence, this study aims to survey for endophytic *Beauveria* spp. isolates in sugarcane ecosystems and to investigate the interactions that exist between *Fusarium-Beauveria*, using *in vitro* techniques.

Materials and Methods

Sample collection and isolations

Collectively, 326 plant materials, comprising of either leaf, stem and/or roots, were collected from sugarcane-growing regions in KwaZulu-Natal, from natural host plants of *E. saccharina* and sugarcane (28 genotypes). The plant material was cleaned, cut into sections (for sugarcane: top node, top internode, bottom node and bottom internode) and disinfected, as described by Reay *et al*. (2010). It was then transferred onto Sabouraud Dextrose Agar (SDA), supplemented with 50 mg/l Dodine, 50 mg/l Chloramphenicol and 50 mg/l Rifampicin. The plates were incubated at 25°C for two weeks (Rehner *et al*. 2011). DNA was extracted from the mycelia by using a PrepMan® Ultra buffer. The ITS1-5.8S rRNA gene-ITS2 region was PCR-amplified, sequenced and subjected to an NCBI-BLAST search, to confirm its identity.

Fusarium-Beauveria interaction

To gain an understanding of the interactions existing between *Beauveria bassiana* (*TL-leaf* and N41S1TI) and the *Fusarium* spp. strains (PNG40, MN57a, SC17 and ZN7), two *in vitro* assays were conducted, namely: (a) co-culture assays; and (b) culture filtrate assay.

a. *Co-culture assays*

Co-culture assays were carried out in 90 mm Potato Dextrose Agar Petri dish plates, as described in Rahman *et al*. (2009) and Thanh *et al*. (2014). Using a pipette, 10µl of a 10^6 conidia/ml suspension of *B. bassiana TL-leaf* or N41S1TI were placed on adjacent sides to the *Fusarium* strain, PNG40, MN57A, SC17 or ZN7, and allowed to grow concurrently. For the controls, each fungal strain was grown alone. Eighteen replicates were conducted for each combination. All plates were incubated at 25°C and the radial growth was measured every three days, until Day 12, when the growth covered the plate and ceased.

b. *Culture filtrate assay*

A Culture Filtrate (CF) of *Fusarium* isolates SC17 and PNG40 was produced, and the concentration was quantified, as described by Mahlanza *et al*. (2013). *Beauveria bassiana* isolates *TL-leaf* and *N41S1TI* were cultured in Potato dextrose broth containing 0, 1000

and 2000ppm *Fusarium* CF. After seven days, the fresh and dry weights of *B*. *bassiana* were recorded to assess the effect of CF on the *B. bassiana*. This procedure was conducted to test the effect of *B. bassiana* CF on the growth of *Fusarium* SC17 and PNG40.

Results and Discussion

Isolations

A total of 130 *Beauveria bassiana* fungal colonies were isolated from 22 sugarcane genotypes and seven natural host plant species of *E. saccharina*, which indicated a natural occurrence of endophytic *B. bassiana* within the sugarcane ecosystem. Genotypes N41, N31 and N28 had the highest number of *B. bassiana* (Figure 1), while NCo376 and N11 had zero isolates. Mahlanza *et al*. (2015) also reported a higher endophytic colonisation of N41 than NCo376. Finkel *et al*. (2017) reported that different genotypes possess different nutritional content, genetics and biochemical profiles, which contribute to the plant microbiome assemblages. This suggests that different genotypes can favour high, middle, low or/and no endophytic colonisation by fungi.

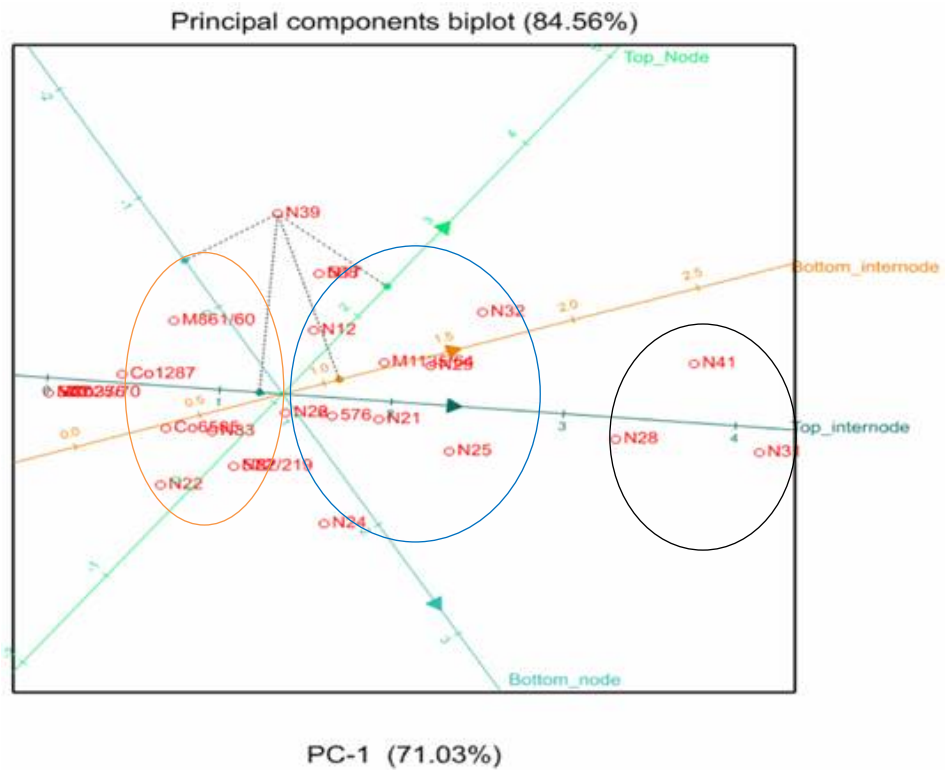

Figure 1. Biplot diagram of Principal Component Analysis (PCA), describing *B. bassiana* isolated from 22 of the 28 sugarcane varieties from the top (internodes and nodes) and bottom (internodes and nodes). Different circle colours represent the clustering of varieties with similar isolations of *B. bassiana*: orange: low, blue: medium and black: varieties with highest numbers of *B. bassiana* in the bottom internode, top internode, top node and bottom node

The number of *B. bassiana* strains isolated from different sugarcane parts (top internode, top node, bottom internode and bottom node) was significantly different (P = 0.002, df =3), with a significantly higher number (P = 0.003, df = 27) of *B. bassiana* strains isolated from the upper internodes than from the lower internodes (Figure 1). This may be explained by the higher nitrogen content levels in the upper stalk regions (Mahlanza *et al*., 2014). Nitrogen is essential for endophyte-microbial growth (Wei *et al*., 2020) and protein synthesis for actively-growing plant tissue (Mattson, 1980).

Fusarium-Beauveria interaction

a. *Co-culture assays*

Both the *Fusarium* spp. and *B. bassiana* radial growth increased over time, whether they were grown in a co-culture or alone. A regression analysis revealed the gradients of *B. bassiana* strains to be significantly different (p <0.001) from those of *Fusarium* spp. *B. bassiana TL-leaf*, and N41S1TI had half (0.10031; 0.10019, respectively) the gradients of *Fusarium* MN57 (0.24598), PNG40 (0.24370) SC17 (0.23174) and ZN7 (0.22148). The *Fusarium* strains grew faster and had larger radial growth measurements (cm), when compared to *B. bassiana*.

There was a reduction in the colony radius when *Fusarium* spp. and *B. bassiana* strains were grown in co-culture, compared to the controls (i.e. those grown alone). However, there was a significant interaction (F <.001; d.f.= 3) between the days and fungal strains (grown alone or in co-culture) for all the *Fusarium* spp. strains tested. This indicated that the reduction of radial growth in co-culture is dependent on the day's post-inoculation. Culebro-Ricaldi *et al.* (2017) found that the radial growth of *F. oxysporum* was only lower than *B. bassiana* when *F. oxysporum* was applied on agar plates two days after *B. bassiana* inoculation. Thus, if this endophytic method of colonisation is to be used, *B. bassiana* must colonise the plant before colonisation by other opportunistic fungi.

b. *Culture filtrate assay*

A dose-dependent response was observed for all the strains tested. Higher culture filtrate concentrations (2000ppm) had a significant effect on *B. bassiana* (P = 0.043, d.f. = 1) and on *Fusarium* spp. (P = 0.031; d.f. = 1), always resulting in reduced weight for the tested strains. Similar results were observed by Yun *et al*. (2017) when they exposed *Botrytis cinerea* to different concentrations of *B. bassiana* filtrate. Both *Fusarium* and *Beauveria* species produce secondary metabolites, enzymes that are able to degrade the fungal cell walls, and have antifungal, antibacterial and/or insecticidal activity (Künzler, 2018; Woźniak *et al*., 2019; Raad *et al*., 2019; Venkatesh and Keller, 2019).

There was no significant interaction (F = 0.226, d.f. = 1) between *B. bassiana* strains, concentration and the Fusarium culture filtrate used. This means that *B. bassiana* strain *TL-leaf* always weighed more and grew better than strain N41S1TI when grown in *Fusarium* spp. culture filtrates of different concentrations. Similar results (F= 0.798, d.f. = 1) were observed when Fusarium SC17 or PNG40 were grown in *B. bassiana* CF. *Fusarium* PNG40 always weighing more than *Fusarium* SC17. These results indicate that the production of mycotoxins is both species- and strain-dependent, with some species needing the production to be triggered by different factors or extremes (Duarte and Archer, 2003; McFarlane *et al*., 2009).

Conclusion

In conclusion, the results revealed that *B. bassiana* is prevalent in sugarcane tissues. These native *B. bassiana* strains could be ecologically-compatible biocontrol agents for *E. saccharina*, or against the plant pathogens of sugarcane. Both *B. bassiana* and *Fusarium* spp. have the ability to reduce each other's radial growth *in vitro*. *Fusarium* spp., however, grew faster and localized *B. bassiana* growth on Petri dishes. Therefore, for the potential application of *B. bassiana* as a dual control against *E. saccharina* and *Fusarium* spp., insect bioassays must be conducted to investigate their interaction. Furthermore, the inoculation of tissue culture plants with *B. bassiana,* followed by *Fusarium* spp., must be investigated to mitigate these challenges. These results emphasise the importance of understanding plant-pathogen and insect pest interactions, as these factors contribute to the effectiveness of biological control agents.

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