

Short Research Article

Olfactory attraction of banana tree (Coleoptera: Curculionidae) to banana genotypes inoculated with entomopathogenic fungus

ABSTRACT

The species: *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) stands out as one of the main pests of banana. The damage to the crop is caused by the larvae of this pest, when they feed on the plant tissues. The aim of this study was to investigate the olfactory responses of *C. sordidus* for different varieties and the possible olfactory interference after application of *B. Beauveria*-bassiana fungus on the crop. The research was conducted at the Phytosanitary Clinic in the Agriculture Sector of the Center for Humanities, Social and Agrarian Sciences of the Federal University of Paraíba, located in Bananeiras - PB, from January to July 2017. The experimental design was completely randomized, with four treatments (banana varieties) and 11 repetitions each. The bioassays were distributed in two stages; ~~The~~ the first stage was an evaluation of the attractiveness of banana genotypes and rhizome and pseudostem tissues. In the second, the attractiveness of *C. sordidus* to the tissues contaminated with the fungus *B. bassiana* was investigated. The Prata banana plantation was less attractive to *C. sordidus*, the highest preference was to the Nanica banana plantation. The most attractive tissue was pseudostem. Given the conditions under which the study was conducted, the banana plantation and the plant tissues analyzed present an olfactory influence under *Cosmopolites sordidus*. The Nanica banana plantation is the most susceptible to insect attack. The application of the fungus *Beauveria bassiana* on banana baits does not interfere with the power exerted by the nanica banana plantation under *C. sordidus*.

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Keywords: Plant resistance, Coleobrocas, Olfactometer, Beauveria bassiana

1. INTRODUCTION

The species beetle: *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) stands out as one of the main banana pests, occurring in practically every region of the planet [1]. Injuries to the crop are caused by the larvae of this pest, which when feeding on plant tissues build galleries causing the interruption of the sap and the rotting of the plant and thus leading to decreased productivity-[2].

The use of systemic insecticides is considered one of the main forms of control for this pest [3]. However, one of the major problems encountered in chemical control is the translocation of the active ingredient of these insecticides via sap to the fruits, which are mostly marketed "in natura" [4]. In this context another form of control which has been widely used is the use of attractive baits, produced with pieces of pseudostem split in half. These baits are based

28 on the attraction exerted by the volatile substances present in the banana pseudostem and
29 rhizome. [5].

30 Use of attractive baits may be enhanced after spraying with entomopathogenic fungus:
31 *Beauveria bassiana* (Bals.) Vuill, this way the fungus acts against insects which served as
32 parasite spreading agents to other parts of the banana plantations. The fungus *B. bassiana*
33 is one of the most effective fungi and studied in biological control (reference). This fungus as
34 well as other entomopathogenic fungi penetrate the host via the integument, causing the
35 death of insects due to mycotoxin production, and due to vegetative growth promoting
36 mechanical blockage of the digestive tract and other physical damage due to mycelial
37 growth. [6].

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38 Several studies have been performed to detect *C. sordidus* olfactory preference to different
39 banana varieties (references). However, information on the olfactory preference of these
40 insects for varieties treated with entomopathogenic fungi is rare or almost nil.

41 Thus, the objective of this study was to investigate the *C. sordidus* olfactory responses for
42 different varieties and the possible olfactory interference after *B. bassiana* fungus application
43 in the culture.

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45 | 2. MATERIALS AND METHODS

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47 The research was carried out at the Phytosanitary Clinic in the Agriculture Sector of the
48 Center of Human, Social and Agrarian Sciences of the Federal University of Paraíba, located
49 in Bananeiras - PB. At the same site, *C. sordidus* specimens were obtained. The capture
50 took place by means of tile baits, made from pieces of banana pseudostems. The insects
51 were kept in the laboratory in plastic containers, measuring approximately 10 cm high by 80
52 cm wide, with 1 cm radius perforations on the sides, containing as a food source and shelter
53 fresh pseudostem pieces, changed every 05 days. The four banana genotypes analyzed
54 were: Pacovan, Nanica, Maçã and Prata (these are kinds of banana), from which two parts
55 of the plant (pseudostem and rhizome) were used. Later the most preferred variety was
56 inoculated with the fungus: *B. bassiana* for possible verification of the preference after
57 inoculation by the banana tree coleobroca. The strain of the entomopathogenic fungus *B.*
58 *bassiana* was isolated from a mummified specimen of *C. sordidus*, found in the Rural
59 community of Roma, Bananeiras district (Add some data on the isolation and cultivation of
60 *Beauveria bassiana*).

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61 For evaluation of *C. sordidus* olfactory response to banana genotypes, the rhizome and
62 pseudostem of each genotype were reduced in dimensions of approximately 2 cm, arranged
63 in a multiple arena similar to that described by Botelho *et al.* [7] (Figure 1). Which features a
64 central arena and four side arenas as options to choose from. The bioassays were
65 performed during the night, a time associated with greater activity of the banana tree- [8].

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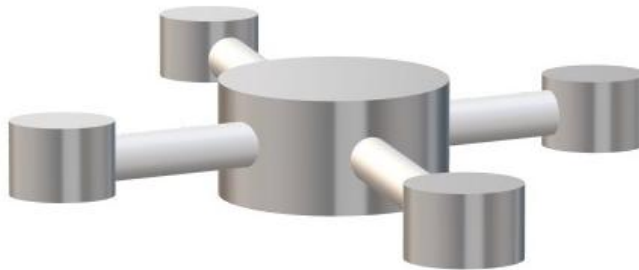
66 The experimental design used was a completely randomized design. The bioassays were
67 composed of four treatments (banana species) containing 11 repetitions and distributed in
68 two stages; where the first one evaluated the attractiveness to the banana genotypes and
69 the rhizome and pseudostem tissues. In this evaluation the tissues and each vegetable were
70 grouped in isolation on the olfactometer, where two arenas were filled with fresh tissue of
71 one genotype and the other two by cotton wicks soaked with distilled water as a control.
72 Subsequently, four *C. sordidus* adults were placed in the central arena and remained for 40
73 minutes exposed to the volatiles released by the tissues of the analyzed plants.

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74 | In the second stage to verify the attractiveness of *C. sordidus* to tissues contaminated with
 75 | the fungus: *B. bassina*, the arenas were filled with fresh tissue (pseudostem) of the Nanica
 76 | banana plantation, this time using only, detected in the first stage of the bioassays as the
 77 | genotype and the tissue with the highest preference for the insect. For the contamination of
 78 | banana tissues, the fungus was diluted in water and the tissues dipped in this solution for
 79 | conidia adherence. [\(Specify the technique adopted for the production of fungal inoculum and](#)
 80 | [the inoculation of plant tissues\)](#)

81
 82 | The parameters evaluated were preference, non-preference and individuals with no
 83 | response to odors. For this, the insects found in three conditions were quantified: on fresh
 84 | rhizome and pseudostem tissues, found on the control and those that remained in the
 85 | release arena. Insects not in these conditions were disregarded.

86 | The results for banana olfactory response to volatile banana genotypes were analyzed using
 87 | the non-parametric X² test (chi-square), which is composed by the formula: $X^2 = \sum(O-E)^2 / E$.
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 90 | **Figure 1. Multiple choice arena for olfactometric analysis of *C. sordidus***

91 92 | 3. RESULTS

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 94 | The results for *C. sordidus* olfactory preference (Table 1) showed a low insect attractive
 95 | effect on maçã banana variety plant tissues, and the observed frequency was lower than
 96 | expected in both tissues analyzed.

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 99 | **Table 1. Attractiveness of *C. sordidus* to maçã banana variety plant tissues offered in a**
 100 | **separated way**

Maçã banana plantation						
Observed Frequency (Expected) (%)						
Insect	Choice option	Preference	No preference	No answer	X ²	Value de (P)
<i>Cosmopolites sordidus</i>	Pseudocaule	36,36 (40)	18,18 (40)	45,45 (20)	44,6	(0,001)
	Rizoma	18,18 (40)	9,09 (40)	72,72 (20)	174,8	(0,001)

102 For the prata banana plantation the results indicate a high influence of pseudostem on *C.*
 103 *Sordidus*, when they showed an olfactory preference of 81.81%, while the expected was
 104 around 40%. However, the rhizome of this same variety was not attractive to the insect, and
 105 the expected frequency was higher than the observed frequency (Table 2).

106 **Table 2. Attractiveness of *C. sordidus* to prata banana variety plant tissues offered in a**
 107 **separated way**

Prata banana Plantation						
Observed Frequency (Expected) (%)						
Insect	Choice option	Preference	No preference	No answer	X ²	Value of (P)
<i>Cosmopolites sordidus</i>	Pseudocaulis	81,81 (40)	9,09 (40)	9,09 (20)	73,53	(0,001)
	Rizoma	9,09 (40)	18,18 (40)	72,72 (20)	174,8	(0,001)

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109 Both tissues of Nanica banana plantation were attractive to *C. Sordidus*, obtaining
 110 preference values higher than the observed frequency. The pseudostem showed a
 111 preference of 81.81% and the rhizome 54.54%, the expected frequency for the tissues was
 112 40% (Table 3).

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113 **Table 3. Attractiveness of *C. sordidus* to plant tissues of the Nanica banana variety**
 114 **offered in isolation.**

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Nanica banana Plantation						
Observed Frequency (Expected)(%)						
Insect	Choice option	Preference	No Preference	No answer	X ²	Value of (P)
<i>Cosmopolites sordidus</i>	Pseudocaulis	81,81 (40)	9,09 (40)	9,09 (20)	73,53	(0,001)
	Rizoma	54,54 (40)	0,09 (40)	36,36 (20)	58,48	(0,001)

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117 PTo cultivate Pacovan, the results indicate that the Insects presented a higher Preference
 118 for the pseudostem, which obtained 45.45% in the observed frequency against 40% of the
 119 expected frequency (Table 4).

120 **Table 4. *C. sordidus* attractiveness to plant tissues of the Pacovã variety offered alone**

Pacovan Plantation						
Observed Frequency (Expected)(%)						
Insect	Choice option	Preference	No Preference	No answer	X ²	Value of (P)
<i>Cosmopolites sordidus</i>	Pseudocaulis	45,45 (40)	9,09 (40)	45,45 (20)	57,01	(0,001)
	Rizoma	36,36 (40)	0,09 (40)	54,54 (20)	99,80	(0,001)

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122 As it was observed in Table 5, *C. sordidus* showed Preference by pseudostem tissues when
 123 they were inoculated by the fungus *B. bassiana*.

124 **Table 5. Attractiveness of *C. sordidus* in tissues of inoculated Nanica Banana**
 125 **plantation and without application of the fungus *Beauveria bassiana*.**

Insect	Observed Frequency (Expected)(%)		Pseudostem without application	X ²	Value of (P)
	Pseudostem with application of <i>B. bassiana</i>	X ²			
<i>Cosmopolites sordidus</i>	60,0 (50)	2,0	40,0 (50)	2,0	(0,001)

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127 **4. DISCUSSION**
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129 Among the various managements used for the control of agricultural pests, varietal
 130 resistance has been researched and pointed as a viable alternative in the control of *C.*
 131 *sordidus* [9, 10]. We evidenced in this study the olfactory influence exerted by different plant
 132 tissues and genotypes, analyzed on *C. sordidus*. The attractiveness of Insect by the plant is
 133 produced due to the secondary volatile compounds released by the plant as semiochemicals
 134 present in the rhizome and pseudostem- (11, 12].

135 Facundo *et al.* [13] states that the various banana genotypes have different volatile
 136 compounds that vary according to variety. According to Oliveira *et al.*, [14], the volatiles
 137 found in banana genotypes, whether varieties or hybrids, have the same attractiveness for
 138 *C. sordidus*. However, there is variation in the composition and concentration of volatiles that
 139 may vary among banana genotypes, reflecting the responses of *C. sordidus* adults to each
 140 genetic material [13, 15].

141 We corroborate in this study the high attractiveness of the volatile compounds of Nanica
 142 Banana plantation for both plant tissues analyzed. The Nanica Banana Plantation is
 143 considered one of the most susceptible to attack by this insect, with losses of up to 80% of
 144 production, these losses are also affected in the Prata banana plantation, with a smaller
 145 proportion around 30% [16].

146 Infestations of this pest in banana plants can be reduced by planting less attractive
 147 plantations, which resulted in lower insects demand for the plant and consequently less
 148 oviposition and emergence of new individuals [1]. Another efficient type of control widely
 149 used by producers is the use of entomopathogenic fungi traps. The use of these fungi for the
 150 biological control of banana broth as with other agricultural pests is a viable alternative that
 151 reduces environmental impacts caused by the use of chemicals such as insecticides [16].
 152 The use of alternative pest controls also reduces the use of chemicals, reducing the amount
 153 of toxic waste to humans in banana consumption and at the time of application [17].

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 155 **5. CONCLUSION**
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157 Given the conditions under which the study was conducted, it can be concluded that the
 158 banana plantations and plant tissues analyzed have an olfactory influence under
 159 *Cosmopolites sordidus*. The Nanica banana plantation is the most susceptible to Insect
 160 attack. The application of the fungus *Beauveria bassiana* in banana baits does not affect the
 161 power exerted by the nanica banan plantation under *C. sordidus*.
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164 **COMPETING INTERESTS**

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166 Authors have declared that no competing interests exist.

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