Selection of *Beauveria bassiana* (Bals.) Vuill. isolates for controlling *Sitophilus zeamais* (Mots.) (Coleoptera: Curculionidae)

Seleção de isolados de *Beauveria bassiana* (Bals.) Vuill. para o controle de *Sitophilus zeamais* (Mots.) (Coleoptera: Curculionidae)

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ABSTRACT

*Sitophilus zeamais* (Mots.) (Coleoptera: Curculionidae) is considered a major pest of maize, responsible for reducing grain quality and making the corn inappropriate for industrial use and human consumption. *S. zeamais* has been controlled exclusively with chemical products. The objective of this research was to select isolates of *Beauveria bassiana* (Bals.) Vuill. to control *S. zeamais*. Beetles were immersed in conidia suspensions of each isolate for five seconds and placed in a gerbox container with maize grains. In pathogenicity tests, the isolates that caused the highest mortality to the maize weevil were ESALQ-447 (68.0%), CCA-UFES/Bb-36 (57.3%) and CCA-UFES/Bb-31 (51.3%). ESALQ-447 was the most virulent, with an LC50 of 1.7 x 10⁷ conidia/ml and shows promise for controlling maize weevils. These isolates of *B. bassiana* can be used as effective substitutes for conventional chemical control, normally carried out with phosphine. Further tests should be performed under field and semi-field conditions to develop an appropriate strategy for the use of this entomopathogen to manage *S. zeamais*.

Key words: maize weevil, microbial control, entomopathogenic fungus, stored grains.

RESUMO

*Sitophilus zeamais* (Mots.) (Coleoptera: Curculionidae) é considerado uma das principais pragas do milho, responsável pela redução da qualidade dos grãos, tornando-os impróprios para indústria e consumo humano. Para seu controle tem-se utilizado exclusivamente produtos químicos. Assim, o objetivo deste trabalho foi selecionar isolados de *Beauveria bassiana* (Bals.) Vuill. para o controle de *S. zeamais*. Besouros foram imersos em suspensões de conídios de cada isolado por cinco segundos e acondicionados em gerbox contendo grãos de milho. Nos testes de patogenicidade os isolados que causaram maior mortalidade confirmada ao gorgulho-do-milho foram ESALQ-447 (68,0%), CCA-UFES/Bb-36 (57,3%) e CCA-UFES/Bb-31 (51,3%), sendo o primeiro mais virulento e promissor ao controle desta praga com CL50 de 1,7 x 10⁷ conídios/ml. A utilização desses isolados de *B. bassiana* pode ser um substituto eficaz ao controle convencional realizado, normalmente, com fosfina. Testes devem ser feitos em campo e semi-campo para elaboração de uma estratégia adequada para utilização deste entomopatogênico no manejo de *S. zeamais*.

Palavras chave: gorgulho-do-milho, controle microbiano, fungo entomopatogênico, grãos armazenados.

Introduction

Ten percent of total grain production is lost to insect pests, causing serious economic losses (Trevizan & Baptista, 2000). If grain storage pest control is not improved, efforts to increase grain production could be fruitless (Fontes et al., 2003).

The maize weevil, *Sitophilus zeamais* (Mots.) (Coleoptera: Curculionidae), is considered one of the most important corn pests (Gallo et al., 2002), responsible for a large amount of grain damage; it reduces grain quality and in turn makes grains inappropriate for industrial use and human consumption (Caneppele et al., 2003). The most frequently used
method to control the maize weevil is to purge the insect from grains with phosphine (aluminum phosphide or magnesium phosphide) (Nakakita et al., 1974; Potrich et al., 2006). However, this product has an elevated persistence in the food supply and environment, in addition to being toxic to those applying the product and promoting the selection of resistant insect populations (Collins et al., 2002; Daglish, 2004; Potrich et al., 2006).

Thus it is necessary to develop new control methods, such as biological control using entomopathogenic fungi, which show great potential as control agents against *Sitophilus* spp. The mode of action of entomopathogenic fungi against pests occurs primarily through their contact with conidia (Alves & Lecuona, 1998; Potrich et al., 2006), which show an elevated capacity for horizontal dispersal, being able to be transported by diverse means over large distances, forming foci of dissemination (Alves, 1998b; Castrillo et al., 2005).

These agents are not toxic to humans or other animals, do not select for resistant pest populations, do not cause environmental pollution and do not cause damage to the grain mass (Alves, 1998a; Pereira et al., 1998). However, the action of entomopathogenic fungi is slow and adequate conditions are needed to maintain their viability and pathogenicity (Lord, 2005).

Because of the vast genetic variability shown by entomopathogenic fungi, various studies have emphasized the necessity of bioassays to screen for isolates that are highly virulent, persistent and with a high reproductive capacity (Alves, 1998b; Silva et al., 2003; Neves & Hirose, 2005). Thus there is increased potential for the use of entomopathogenic fungi as microbiological insecticides (Dal Bello et al., 2001; Potrich et al., 2006).

Among entomopathogenic fungi, *Beauveria bassiana* (Bals.) Vuill. possesses the greatest potential for controlling pest insects that damage stored grains (Moino Júnior & Alves, 1997; Kassa et al., 2002; Potrich et al., 2006). The objective of this study was to select isolates of *B. bassiana* to control *S. zeamais*.

**Materials and Methods**

The experiment was conducted in the Entomology sector at the Núcleo de Desenvolvimento Científico e Tecnológico em Manejo Fitossanitário (NUDEMAFI), at the Centro de Ciências Agrárias of the Universidade Federal do Espírito Santo (CCA-UFES) in Alegre, Espírito Santo, Brazil, and consisted of the following steps:

**Maize weevil rearing**

Insect strains from the stocks of the Entomology Laboratory at NUDEMAFI were tested. These insects were reared in maize kernels in glass containers (12 cm diameter and 17 cm height) with a perforated top to allow gas exchange. The containers were kept in a climatized room with a temperature of 25 ± 2 °C, relative humidity of 70 ± 10%, and 12-hour photophase (Coitinho et al., 2006).

**Obtaining and maintenance of isolates**

The study tested eleven isolates of the fungus *B. bassiana* (extracted from agricultural soils) and the standard isolate ESALQ-447, registered and selected for the control of other pests (Alves, 1998b) (Table 1). These isolates were stored in the entomopathogen stocks at NUDEMAFI in plastic Eppendorf-type tubes containing potato, dextrose, agar, yeast extract and tetracycline antibiotic (PDAY+A) at 8 °C. The fungi were incubated for 7 days on Petri dishes (10 x 2.0 cm) containing PDAY+A medium. Coffee borer beetles, *Hypothenemus hampei* (Ferr.) (Coleoptera: Scolytidae), were inoculated with the fungus, and after conidiogenesis the fungi were reisolated in Petri dishes with PDAY+A medium. The fungi were then incubated for another 7 days (Leite et al., 2003).

Conidia viability was monitored by the germination method (Silva et al., 2003), in which conidia were considered viable when they showed a germination rate above 90%. The fungus was grown in a climatized chamber at a temperature of 26 ± 1 °C, relative humidity of 70 ± 10%, and 12-hour photophase.

**Pathogenicity evaluation**

The fungi were multiplied again for 10 days, using a Drigalski handle to produce conidia. Fungal suspensions were obtained by adding 10 ml of sterilized distilled water plus Tween® 80 adhesive spreader (0.05%) (SDW+S) to plates containing the culture medium and the fungus. After rapid manual shaking and scraping with a sterilized soft bristle brush, they were filtered in sterilized gauze,
Selection of Beauveria bassiana (Bals.) Vuill. isolates for controlling Sitophilus zeamais (Mots.)…

quantified in a Neubauer chamber and adjusted to a concentration of 1 x 10⁸ conidia/ml (Silva et al., 2003).

S. zeamais adults between the ages of 1 and 30 days were immersed for 5 seconds in conidia suspensions of each isolate (housed in glass tubes), then removed by filtration in Voil-type tissue and placed in plastic gerbox containers (6.3 cm in diameter and 2.2 cm in height) containing one corn kernel per insect. The control treatment consisted of the immersion of insects in SDW+S. The containers were placed in a climatized chamber at a temperature of 26 ± 1 ºC, relative humidity of 70 ± 10% and a 12 hour light cycle.

Evaluations were performed daily for a period of 10 days by verifying the number of dead insects. Those that died were transferred to humid chambers to verify the cause of death. A completely randomized design (CRD) was used with thirteen treatments and five repetitions containing 30 insects in each repetition, totaling 150 insects per treatment.

**Virulence evaluation**

The isolates which caused the highest confirmed mortality rate in S. zeamais adults were selected for the virulence evaluation phase. These isolates were newly multiplied for the production of conidia. Suspensions were prepared at concentrations of 1 x 10⁴, 1 x 10⁵, 1 x 10⁶, 1 x 10⁷ and 1 x 10⁸ conidia/ml for each isolate, in addition to the control using SDW+S. The suspension preparation, the inoculation of insects with fungal isolates, and the evaluations used the same procedures described for the evaluation of pathogenicity. Five repetitions were used per treatment with 20 insects in each repetition, totaling 100 insects per concentration for each isolate.

**Statistical Analysis**

The corrected mortality was calculated with the formula of Abbott (1925). Corrected and confirmed mortality data were checked for normality by the Shapiro-Wilk test, and for homogeneity of variance by the Bartlett test. Data were subjected to analysis of variance and means were compared by the Scott-Knott test using a significance level of 5%. The lethal concentration (LC₅₀) was calculated using probit analysis.

**Results and Discussion**

**Pathogenicity evaluation**

All isolates were pathogenic in S. zeamais adults. The percentages of corrected mortality varied from 72.8% to 19.7% and the confirmed mortality varied from 68.0% to 20.7% (Table 2). The control group had 2.0% mortality. The isolate ESALQ-447 showed the highest percentage of corrected mortality. The confirmed mortality of isolates ESALQ-447, CCA-UFES/Bb-36, and CCA-UFES/Bb-31 did not show significant differences, with 69.8%, 57.3%, and 51.3%, respectively. These isolates could be potential control agents for the maize weevil, since entomopathogenic fungi are considered effective when they show mortality values above 40% (Lecuona et al., 1996).

Using the same inoculation technique with the fungi B. bassiana and Metarhizium anisopliae (Metsch.) Sorok., Kassa et al. (2002) were able to

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Source</th>
<th>Host or substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCA-UFES/Bb-31</td>
<td>Ibatiba-ES</td>
<td>Soil, coffee plants</td>
</tr>
<tr>
<td>CCA-UFES/Bb-32</td>
<td>Ibatiba-ES</td>
<td>Soil, coffee plants</td>
</tr>
<tr>
<td>CCA-UFES/Bb-33</td>
<td>Ibatiba-ES</td>
<td>Soil, coffee plants</td>
</tr>
<tr>
<td>CCA-UFES/Bb-34</td>
<td>Ibatiba-ES</td>
<td>Soil, coffee plants</td>
</tr>
<tr>
<td>CCA-UFES/Bb-35</td>
<td>Alege-ES</td>
<td>Soil</td>
</tr>
<tr>
<td>CCA-UFES/Bb-36</td>
<td>São José do Calçado-ES</td>
<td>Soil, coffee plants</td>
</tr>
<tr>
<td>CCA-UFES/Bb-37</td>
<td>São José do Calçado-ES</td>
<td>Soil, coffee plants</td>
</tr>
<tr>
<td>CCA-UFES/Bb-38</td>
<td>São José do Calçado-ES</td>
<td>Soil, coffee plants</td>
</tr>
<tr>
<td>CCA-UFES/Bb-39</td>
<td>São José do Calçado-ES</td>
<td>Soil, coffee plants</td>
</tr>
<tr>
<td>CCA-UFES/Bb-40</td>
<td>Alege-ES</td>
<td>Soil</td>
</tr>
<tr>
<td>CCA-UFES/Bb-41</td>
<td>Ibatiba-ES</td>
<td>Soil, coffee plants</td>
</tr>
<tr>
<td>ESALQ-447</td>
<td>Cuiabá-MS</td>
<td>Solenopsis invicta (Buren)</td>
</tr>
</tbody>
</table>

Table 1. Origins and hosts of Beauveria bassiana isolates tested in Sitophilus zeamais.
elevate the mortality rate of *S. zeamais* from 92% to 100%. Potrich et al. (2006) evaluated thirteen isolates of *B. bassiana* at a concentration of 1 x 10⁹ conidia/ml with *S. zeamais* adults immersed in a 1 ml suspension of conidia and shaken for 10 seconds, with confirmed mortality varying from 8.3% to 98.3%. However, at a concentration of 1 x 10⁸ conidia/ml for 10 days, the best isolates (Unioeste 4, Unioeste 39 and ESALQ-643), showed confirmed mortality of 48.3%, 45.0% and 45.0%, respectively, while the isolate ESALQ-447 showed a mortality rate of 68.0% (Table 2).

The mortality of the control group was 2.0%. This affected negatively the results of corrected mortality. Thus for some isolates for which all deaths were confirmed, e.g. CCA-UFES/Bb-36 and CCA-UFES/Bb-38, the confirmed mortality was slightly greater than the corrected mortality.

### Evaluation of virulence

The lowest lethal concentration (LC₅₀) was observed using the isolate ESALQ-447 (1.7 x 10⁷ conidia/ml), followed by the isolates CCA-UFES/Bb-31 (7.9 x 10⁷ conidia/ml) and CCA-UFES/Bb-36 (1.5 x 10⁸ conidia/ml); however, the LC₅₀ of these last isolates did not differ within a 95% confidence interval (Table 3). A high LC₅₀ value indicates less toxicity; therefore a less toxic isolate needs a greater concentration of conidia to cause the same insect mortality as a more toxic isolate.

The isolate *B. bassiana* PPRC-HH showed high virulence to *S. zeamais* beetles immersed in suspended conidia, with an LC₅₀ of 2.04 x 10⁶ conidia/ml (Kassa et al., 2002). By contrast, isolate 604 *B. bassiana*, inoculated with rice grains, showed an LC₅₀ of 0.0067 g of conidia per 100 g of rice after ten days of exposure (Moino Júnior & Alves, 1997).

The ratios of LC₅₀ toxicity for isolates CCA-UFES/Bb-36 and ESALQ-447; CCA-UFES/Bb-31 and ESALQ-447; and CCA-UFES/Bb-36 and CCA-UFES/Bb-31 were 8.8, 4.6 and 1.9, respectively. This indicates that the isolate CCA-UFES/Bb-36 should

### Table 2. Corrected and confirmed mortality (%) of adult maize weevils, *Sitophilus zeamais*, subjected to different isolates of *Beauveria bassiana*, 10 days after inoculation (temperature: 26 ± 1 °C, RH: 70 ± 10%, and 12-hour photophase).

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Mortality(1)</th>
<th>Corrected</th>
<th>Confirmed</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESALQ-447</td>
<td>72.8 ± 8.40 a</td>
<td>68.0 ± 7.79 a</td>
<td></td>
</tr>
<tr>
<td>CCA-UFES/Bb-36</td>
<td>56.5 ± 5.53 b</td>
<td>57.3 ± 5.42 a</td>
<td></td>
</tr>
<tr>
<td>CCA-UFES/Bb-31</td>
<td>51.7 ± 6.12 b</td>
<td>51.3 ± 5.54 a</td>
<td></td>
</tr>
<tr>
<td>CCA-UFES/Bb-38</td>
<td>43.5 ± 9.95 c</td>
<td>44.0 ± 9.39 b</td>
<td></td>
</tr>
<tr>
<td>CCA-UFES/Bb-35</td>
<td>41.5 ± 7.25 c</td>
<td>42.7 ± 7.10 b</td>
<td></td>
</tr>
<tr>
<td>CCA-UFES/Bb-32</td>
<td>40.8 ± 6.14 c</td>
<td>42.0 ± 6.02 b</td>
<td></td>
</tr>
<tr>
<td>CCA-UFES/Bb-34</td>
<td>38.1 ± 5.10 c</td>
<td>39.3 ± 4.99 b</td>
<td></td>
</tr>
<tr>
<td>CCA-UFES/Bb-37</td>
<td>38.1 ± 7.33 c</td>
<td>39.3 ± 7.18 b</td>
<td></td>
</tr>
<tr>
<td>CCA-UFES/Bb-41</td>
<td>33.3 ± 2.76 c</td>
<td>34.7 ± 2.71 b</td>
<td></td>
</tr>
<tr>
<td>CCA-UFES/Bb-40</td>
<td>29.3 ± 7.33 c</td>
<td>30.0 ± 6.91 b</td>
<td></td>
</tr>
<tr>
<td>CCA-UFES/Bb-33</td>
<td>24.5 ± 4.74 c</td>
<td>26.0 ± 4.64 b</td>
<td></td>
</tr>
<tr>
<td>CCA-UFES/Bb-39</td>
<td>19.7 ± 3.97 c</td>
<td>20.7 ± 3.71 b</td>
<td></td>
</tr>
<tr>
<td>CV(2) (%)</td>
<td>35.61</td>
<td>33.63</td>
<td></td>
</tr>
</tbody>
</table>

1. Means (± SE) followed by the same letter in the columns do not differ by the Scott-Knott test at 5% probability.
2. Coefficient of variation.

### Table 3. Slope of the concentration-mortality curves (mean ± standard error) and lethal concentration (LC₅₀) of *Beauveria bassiana* on adult maize weevils, *Sitophilus zeamais* (temperature: 26 ± 1 °C, RH: 70 ± 10%, and 12-hour photophase).

<table>
<thead>
<tr>
<th>Isolate</th>
<th>n(1)</th>
<th>Slope ± SE(2)</th>
<th>LC₅₀ (conidia/ml) (95% CI)(3)</th>
<th>df(4)</th>
<th>χ²(5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESALQ-447</td>
<td>500</td>
<td>0.73 ± 0.089</td>
<td>1.7 x 10⁷ (1.0 x 10⁷-3.0 x 10⁷)</td>
<td>3</td>
<td>2.1</td>
</tr>
<tr>
<td>CCA-UFES/Bb-31</td>
<td>500</td>
<td>0.44 ± 0.061</td>
<td>7.9 x 10⁷ (3.2 x 10⁷-2.9 x 10⁸)</td>
<td>3</td>
<td>2.4</td>
</tr>
<tr>
<td>CCA-UFES/Bb-36</td>
<td>500</td>
<td>0.46 ± 0.066</td>
<td>1.5 x 10⁸ (5.7 x 10⁷-6.7 x 10⁸)</td>
<td>3</td>
<td>2.9</td>
</tr>
</tbody>
</table>

1. Number of insects used in the test.
2. Standard error.
3. Confidence interval of the CL₅₀ at 95% probability.
4. Number of degrees of freedom.
5. Chi-square value.
be applied at a concentration 8.8 times that of the isolate ESALQ-447 to cause the same mortality of adult *S. zeamais*.

The mortality concentration curve of the ESALQ-447 isolate presented the steepest slope (0.73), while the slopes of the other isolates (0.44 for CCA-UFES/Bb-31 and 0.46 for CCA-UFES/Bb-36) were not as steep and were similar to each other (Table 3). High slope values of curves indicate that small variations in fungal concentration promote large variation in the mortality of adult *S. zeamais*.

In the field, environmental conditions are the principal limitation for using entomopathogenic fungi for the control of insect pests (Alves, 1998a). For example, solar radiation prevents the development of conidia (Fernandes et al., 2007). Fungi such as *B. bassiana* need temperatures between 23 and 28°C with 90% relative humidity for adequate development (Alves, 1998b). Conditions under grain storage are more stable (Moino Júnior & Alves, 1997) and grains are not exposed to solar radiation, thus such conditions are favorable for the use of this method to control storage grain pests.

Therefore, microbial control with *B. bassiana* can be an effective substitute for conventional control, which is normally done with phosphine. However, studies should be carried out under the field and semi-field conditions to develop an adequate strategy to control *S. zeamais* with entomopathogenic fungi.

**Conclusion**

The isolate ESALQ-447 was the most virulent for adult *S. zeamais*; however, the isolates originating in soils also showed satisfactory mortality rates for this pest.

**Acknowledgements**

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