

Insecticidal potential of *Brevibacillus laterosporus* against dipteran pest species in a wide ecological range

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1 **Insecticidal potential of *Brevibacillus laterosporus* against dipteran pest species in a wide**  
2 **ecological range**

3

4

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23

24 **Abstract**

25 In order to increase our understanding of the insecticidal potential of the entomopathogenic  
26 bacterium *Brevibacillus laterosporus* strain UNISS 18 against insect pests, investigations were  
27 conducted on a selection of dipteran species including fruit flies, house flies, blow flies, and  
28 mosquitoes, characterized by adaptations to very diverse habitats.  
29 According to lethal concentration (LC<sub>50</sub>) values, the common house mosquito *Culex pipiens* (LC<sub>50</sub>  
30 = 0.10 x 10<sup>6</sup> spores/mL) and the yellow fever mosquito *Aedes aegypti* (LC<sub>50</sub> = 0.18 x 10<sup>6</sup>  
31 spores/mL) were significantly more susceptible than the flies. The blow flies were the second taxon  
32 in term of susceptibility to *B. laterosporus* spores, with *Calliphora vomitoria* achieving a higher  
33 mortality (LC<sub>50</sub> = 78.84 x 10<sup>6</sup> spores/mL) than *Lucilia caesar* (LC<sub>50</sub> = 148.30 x 10<sup>6</sup> spores/mL). The  
34 effectiveness of *B. laterosporus* spores was reduced by half in the case of the house fly *Musca*  
35 *domestica* (LC<sub>50</sub> = 82.41 x 10<sup>6</sup> spores/mL), while, the lowest susceptibility was observed in the fruit  
36 flies among which the Spotted wing drosophila (SWD) *Drosophila suzukii* was the most susceptible  
37 (LC<sub>50</sub> = 217.51 x 10<sup>6</sup> spores/mL) in comparison with the medfly *C. capitata* and the olive fly *B.*  
38 *oleae* (LC<sub>50</sub> = 2567.32 and 2567.36 x 10<sup>6</sup> spores/mL, respectively). The present study demonstrated  
39 that significantly different degrees of susceptibility are associated with diverse dipteran species  
40 including plant and animal parasites, that suggest that *B. laterosporus* established different  
41 relationships with dipteran species living in different ecosystems.

42  
43 Key words: bioinsecticide; pest management; flies, mosquitoes, bacteria.

44

## 45 **Introduction**

46 *Brevibacillus laterosporus* is an emerging biological control agent with significant potential against  
47 invertebrate pests and several phytopathogens affecting various crop species (Ruiu, 2013). The  
48 pesticidal and antimicrobial activity of this bacterium is due to the production of an arsenal of  
49 toxins, antibiotics and other virulence factors. Despite remarkable differences among strains, most  
50 of the pesticidal potential appears to be well conserved in the genome of this species (Glare et al.,  
51 2020). Within a complex insecticidal mechanism of action, an important role is played by certain  
52 enzymes (i.e., chitinases, proteases) (Marche et al., 2018), Cry toxin homologues (Bowen et al.,  
53 2017), spore-associated proteins (Marche et al., 2017), polyketides, nonribosomal peptides, and  
54 other putative toxins (Djukic et al., 2011). Normally, after ingesting bacterial spores, the insect goes  
55 into progressive midgut epithelium deterioration, followed by paralysis and death (Ruiu et al.,  
56 2012). Although there are differences between *B. laterosporus* strains, the target range includes  
57 pests in the orders Coleoptera, Lepidoptera and Diptera (Ruiu, 2013). On the other hand, this  
58 bacterium has been observed to have no detrimental activity against certain Hymenopteran species  
59 like the honeybee with which it has an endosymbiotic relationship (Marche et al., 2016), or parasitic  
60 wasps (Ruiu et al., 2007a). A lack of significant toxicity was also observed against chrysopids  
61 (Ruiu et al., 2020). The insecticidal activity so far found against dipterans includes some species of  
62 mosquitoes and muscoid flies (Pereira et al., 2018; Zubasheva et al., 2010). However, several  
63 studies on different bacterial strains and preparations, including vegetative or sporulating cells,  
64 sporangia and spores, led to a high variability of results in terms of specificity and effectiveness.  
65 Moreover, a narrow range of dipteran species has so far been considered, while this order is wide  
66 and includes a variety of families characterized by crop pests living in very different ecosystems  
67 and species of medical and veterinary interest. Due to this still limited knowledge, the pathogen-  
68 host relationship between *B. laterosporus* and Diptera remains unclear. In order to increase our  
69 understanding of *B. laterosporus* target range, investigations were conducted on a selection of  
70 dipteran species characterized by adaptations to very diverse habitats. These include house flies and

71 blow flies adapted to develop on organic matter, polyphagous and monophagous fruit flies, and  
72 mosquito species.

73

## 74 **2 Materials and methods**

### 75 **2.1 Bacterial preparations**

76 The bioassays were conducted employing pure spore suspensions of *Brevibacillus laterosporus*  
77 strain UNISS 18 (= NCIMB 41419), an entomopathogenic strain previously reported to be active  
78 against dipters (Marche et al., 2018). Pure spores were selected as the bacterial fraction to be used  
79 in bioassays for standardization purposes and because they are known to have a significantly higher  
80 insecticidal activity, in comparison with vegetative cells and culture supernatant (Ruiu et al., 2007b;  
81 Marche et al., 2017). The strain was cultured on LB broth in a shaking incubator (180 rpm) at 30  
82 °C. In order to produce pure spore suspensions, culture synchronization was obtained as described  
83 by Marche et al. (2017). Briefly, heat-activated spore suspension (1 mL) was inoculated in a pre-  
84 culture rich medium (25 mL, LB broth). An aliquot of this culture at the exponential phase was then  
85 used to inoculate a sporulation medium (200 mL, T3). After 48 h of growth, the spores were  
86 harvested by centrifugation at  $15,000 \times g$  at 4° C for 15 min and resuspended in water to adjust the  
87 concentration at  $2 \times 10^9$  or at  $1 \times 10^9$  spores/mL to be stored at -20 °C until being used in insect  
88 bioassays.

89

### 90 **2.2 Bioassays**

91 Bioassays were conducted exposing dipteran larvae or adults to bacterial spores added to their diet  
92 or developmental substrate. Based on the feeding behaviour, a different bioassay method was  
93 employed for each target species, in order to simulate their natural intake of a spore suspension  
94 possibly applied to their habitat. In this way, all insects were forced to feed spores while satisfying  
95 their daily food needs. The bioassays were performed under laboratory conditions at 25° C, 65%

96 RH and natural photoperiod with insects provided by University rearing facilities or collected in the  
97 field. Four replicates were performed for each experiment that was repeated twice.

98

### 99 2.2.1 Muscoid flies

#### 100 *House fly*

101 Because the insecticidal activity of the strain on *Musca domestica* (L.) (Diptera: Muscidae) is  
102 known (Ruiu et al., 2007b), these bioassays had the main purpose to confirm the bioinsecticidal  
103 potential of bacterial preparations used in the experiments. Lab reared flies (0-24 h after emergence)  
104 were fed with 30% saccharose solution drops incorporating *B. laterosporus* spores at a dose of 10  
105  $\mu\text{l}/\text{fly}/\text{day}$ . Flies were exposed to different concentrations ranging between  $10^7$  and  $10^9$  spores/mL.  
106 Treated and untreated flies (control) were maintained in groups of 10 individuals inside plastic  
107 cages and mortality was assessed after 72 h (Mura and Ruiu, 2017).

108

#### 109 *Blow fly*

110 Newly emerged (0-24 h) *Lucilia caesar* (L.) and *Calliphora vomitoria* (L.) (Diptera: Calliphoridae)  
111 were fed with 30% saccharose solution drops incorporating *B. laterosporus* spores at a dose of 20  
112  $\mu\text{l}/\text{fly}/\text{day}$ . Control was performed with drops of 30% saccharose water solution. Bacterial drops  
113 were administered into plastic cages containing 10 individuals that were checked daily for 72 h to  
114 assess mortality. The following range of bacterial concentrations was assayed:  $1.7 \times 10^9$ ,  $9.0 \times 10^8$ ,  
115  $4.5 \times 10^8$ ,  $2.3 \times 10^8$ ,  $9.0 \times 10^8$ ,  $1.0 \times 10^8$ ,  $5.8 \times 10^7$ ,  $2.9 \times 10^7$  spores/mL.

116

### 117 2.2.2 Fruit flies

#### 118 *Spotted wing drosophila (SWD)*

119 *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) was reared under laboratory conditions  
120 following the method described in Bedini et al. (2020). Briefly, ten newly emerged adults (0-24 h)  
121 were exposed to drops including bacterial spores at different concentrations:  $2.0 \times 10^8$ ,  $1.0 \times 10^8$ ,

122  $5.0 \times 10^7$ ,  $2.0 \times 10^7$ ,  $2.0 \times 10^6$ ,  $2.0 \times 10^5$  spores/mL. Control was performed with drops of 30%  
123 saccharose water solution. The bioassay was conducted into plastic cages containing 10 individuals  
124 that were checked daily for 72 h to assess mortality.

125

#### 126 *Mediterranean Fruit Fly*

127 Lab reared *Ceratitis capitata* Wied (Diptera: Tephritidae) adults aged of 0-24 h were maintained in  
128 groups of 10 individuals and fed daily by capillary tubes (50  $\mu$ l) containing a saccharose solution  
129 (30%) including bacterial spores at different concentrations:  $2.0 \times 10^9$ ,  $1.0 \times 10^9$ ,  $7.5 \times 10^5$ ,  $5.0 \times$   
130  $10^8$  spores/mL. Mortality was assessed after 72 h.

131

#### 132 *Olive fly*

133 Field collected *Bactrocera oleae* (Diptera: Tephritidae) olive fly pupae were maintained in the  
134 laboratory up to emergence of adults that were employed in bioassays. Newly emerged adults (0-24  
135 h) were kept in plastic containers in groups of 10 and were administered daily by capillary tubes (50  
136  $\mu$ l) a saccharose solution (30%) including bacterial spores at the following concentrations:  $2.0 \times$   
137  $10^9$ ,  $1.0 \times 10^9$ ,  $7.5 \times 10^5$ ,  $5.0 \times 10^8$  spores/mL. Control was performed with capillary tubes (50  $\mu$ l)  
138 containing only 30% saccharose water solution. Mortality was assessed after 72 h.

139

#### 140 2.2.3 Mosquitoes

##### 141 *Common house mosquito* and *Yellow fever mosquito*

142 Groups of 10 coetaneous second instar larvae of *Culex pipiens* L and *Aedes aegypti* L. (Diptera:  
143 Culicidae), obtained from laboratory reared colonies, were maintained in plastic cups containing 50  
144 mL of the appropriate dilution in dechlorinated tap water (Lacey, 1997). The following range of  
145 concentrations was assayed:  $1.0 \times 10^6$ ,  $7.5 \times 10^5$ ,  $5.0 \times 10^5$ ,  $2.5 \times 10^5$ ,  $1.2 \times 10^5$ ,  $0.6 \times 10^5$ ,  $0.3 \times$   
146  $10^5$ . Control was performed with dechlorinated tap water only. Mortality was assessed after 48 h.

147

## 148 **2.3 Statistical analysis**

149 Fly adults and mosquito larvae median and lethal concentration to 95% (LC<sub>50</sub>, LC<sub>95</sub>) were  
150 calculated by probit regression. The differences among species susceptibility were assessed by  
151 relative median potency analyses (rmp) and considered statistically significant when values in the  
152 95% confidence interval of were  $\neq 1.0$ . Data were processed by SPSS 22.0 software (IBM SPSS  
153 Statistics, Armonk, North Castle, New York, USA)

154

## 155 **3. Results**

156

### 157 3.1 Muscoid flies

158 The *B. laterosporus* spores were found to have a clear toxic effect on the blow flies *C. vomitoria*  
159 and *L. caesar*, and on the house fly *M. domestica*. According to the Probit analysis, *C. vomitoria*  
160 was the most susceptible species with an LC<sub>50</sub> value of  $78.836 \times 10^6$  spores/mL while the most  
161 resistant appeared to be *L. caesar* whose LC<sub>50</sub> value was  $148.296 \times 10^6$  spores/mL (Table 1). The  
162 RMP analysis showed no significant difference in toxicity between *C. vomitoria* and *M. domestica*,  
163 while *L. caesar* was significantly more resistant than *C. vomitoria* and *M. domestica*, (*L. caesar* vs  
164 *C. vomitoria* RMP = 1.881 (1.332-2.807) (Table 2). The effectiveness of *B. laterosporus* spores  
165 appeared to be reduced by half in the case of *L. caesar* compared to *C. vomitoria* and *M. domestica*  
166 (Fig. 1).

167

### 168 3.2 Fruit flies

169 Similarly to what has been observed on muscoid flies, significant insecticidal action was also  
170 observed on the fruit flies *D. suzukii*, *C. capitata*, and *B. oleae*, fed a saccharose suspension  
171 containing different amounts of *B. laterosporus* spores. The most susceptible species was *D.*  
172 *suzukii*, with an LC<sub>50</sub> value of  $217.508 \times 10^6$  spores/mL, while *B. oleae* and *C. capitata* showed  
173 about the same LC<sub>50</sub> value ( $2567.364$  and  $2567.320 \times 10^6$  spores/mL for *B. oleae* and *C. capitata*,

174 respectively) (Table 3). As expected, the RMP analysis showed no significant difference in toxicity  
175 between *B. oleae* and *C. capitata*, while *D. suzukii* was significantly more susceptible (Table 4)  
176 (Fig. 2).

177

### 178 3.3 Mosquitoes

179 A clear mosquitocidal activity was associated with *B. laterosporus* spores, achieving significant  
180 larval mortality at low concentrations against different species. More specifically, the LC<sub>50</sub> values  
181 for *Ae. aegypti* and *C. pipiens* were 0.179 and  $0.097 \times 10^6$  spores/mL, respectively (Table 5). When  
182 compared (Fig. 3), *C. pipiens* appeared significantly more susceptible to *B. laterosporus* spores than  
183 *Ae. aegypti* larvae (*Ae. aegypti* vs *C. pipiens* RMP = 1.839; CI, 1.288 – 2.739).

184

## 185 4. Discussion

186 *Brevibacillus laterosporus* is a ubiquitous bacterium that has developed different ways to interact  
187 with invertebrates and soil-dwelling microorganisms including plant pathogens (Ruiu, 2013). Due  
188 to its remarkable antimicrobial properties, the antagonistic potential of this bacterium is also  
189 considerable with regard to several human pathogenic agents (Choopan et al., 2008; Desjardine et  
190 al., 2007).

191 Several studies have highlighted the entomopathogenicity of various *Brevibacillus* strains, and this  
192 bacterial genus is emerging as an important source of bacterial toxin genes with potential against  
193 noxious pests (Glare et al., 2020). Among the putative virulence factors there are a variety of  
194 enzymes like chitinases and proteases, homologous Cry proteins, polyketides, and nonribosomal  
195 peptides (Marche et al., 2018). These molecules are implied in midgut epithelium degeneration,  
196 following ingestion of bacterial spores (Ruiu et al., 2012). Although the involvement of specific  
197 bacterial compounds against certain targets has been documented, the insecticidal action appears to  
198 be complex and not fully understood. Alongside the availability of *B. laterosporus* strains especially

199 promising for applications in pest management, the evolutionary relations of this entomopathogen  
200 with the arthropod world are still little known.

201 *B. laterosporus* has been reported to have insecticidal activity against insects belonging to different  
202 orders, and in particular Coleoptera (De Oliveira et al., 2004), Lepidoptera (Narciso et al., 2019),  
203 and Diptera (Zubasheva et al., 2010), in addition to other invertebrates like nematods (Singer, 1996)  
204 and mollusks (Ruiu et al., 2013). Although the wide range of targets, *B. laterosporus* appears to be  
205 a selective microbe, being not significantly active against non-target Hymenoptera (Ruiu et al.,  
206 2007a) and chrysopids (Ruiu et al., 2020). The lack of toxicity observed in these cases makes it  
207 even more complex to understand the ecological role of this species. Moreover, *B. laterosporus* is a  
208 common resident of the honeybee body in which it appears to play a role in health preservation,  
209 alongside the well-known bacterial symbiont community of this insect species (Marche et al., 2016;  
210 Marche et al., 2019).

211 Based on the information available, it could be inferred that the insect species belonging to certain  
212 taxa tend to be more susceptible, while others have possibly established beneficial relationships  
213 with this bacterium. Although the effectiveness of an entomopathogenic agent may in many cases  
214 be ascribed to one or a few orders of insects, specific studies should be conducted for a more  
215 appropriate assessment of actual activity within each taxon. Diptera is a good example of an insect  
216 order characterized by species living in distinct habitats and establishing significantly diverse  
217 relationships with the species communities inhabiting the ecosystems of which they are part (Pape  
218 et al., 2011). Accordingly, the present study demonstrated that significantly different degrees of  
219 susceptibility are associated with diverse dipteran species including plant and animal parasites (Fig.  
220 4). Young larvae of the common house mosquito *C. pipiens* and of the yellow fever mosquito *Ae.*  
221 *aegypti* appeared to be the most affected by *B. laterosporus* treatment, achieving median lethal  
222 concentrations below one million spores per mL. This is in line with studies employing crystal  
223 forming strains of this bacterium against mosquitoes, in which the insecticidal activity was  
224 associated with crystal proteins (Zubasheva et al., 2010). On the other hand, the susceptibility of the

225 house fly and the blow flies *C. vomitoria* and *L. caesar* was much lower than mosquitoes,  
226 corroborating previous studies conducted with *M. domestica* (Marche et al., 2017.) and different  
227 Calliphoridae species, including *Chrysomya megacephala* (Fabricius, 1794) (Carramaschi et  
228 al.,2015) and *Lucilia cuprina* (Wiedemann, 1830) (Pessanha et al., 2015). The present study also  
229 demonstrated the susceptibility of different fruit fly species to *B. laterosporus*. Among them *D.*  
230 *suzukii* appeared to be significantly more susceptible, than *B. oleae* and *C. capitata*. However, the  
231 susceptibility of fruit flies to the bacterium appeared to be lower when compared to muscoid flies  
232 and mosquitoes. The reasons for these differences are not known, but it can be assumed that the  
233 bacterium may express different degrees of virulence against diverse targets as a consequence of the  
234 differential action of a variety of insecticidal factors (Glare et al., 2020). Although different  
235 susceptibility among targets is reported in the literature, the data were obtained from different  
236 studies conducted with different bacterial strains and preparations (i.e, vegetative cells, sporangia,  
237 sporulated cultures, pure spores, parasporal bodies, purified toxins) and therefore such a different  
238 susceptibility may be biased by many variables. In this study, all target insect species were exposed  
239 to the same spore preparation, which allowed to highlight the species different susceptibility to the  
240 strain that it is known to express several insecticidal protein genes and virulence factors (Marche et  
241 al., 2018). On the other hand, we cannot exclude that a different comparative susceptibility could be  
242 associated with diverse bacterial stages of growth. Increased resistance to spore ingestion may result  
243 from different conditions in the insect intestine, that is the first barrier to *B. laterosporus* infection  
244 (Mura and Ruiu, 2017) and the environment in which the activation of bacterial toxins normally  
245 takes place (Bravo et al., 2002). Different diets and eating habits could be the first causes of such  
246 differences. Accordingly, the highest susceptibility was found in mosquito larvae normally feeding  
247 on solid particles suspended in water, followed by muscoid fly species that feed on organic matter.  
248 A lower susceptibility has been detected in insects that feed on plant species, and in particular on  
249 fruit. Such result is in line with the previously observed reduced susceptibility of fruit fly species to  
250 other entomopathogenic bacteria, including *Bacillus thuringiensis* and other *Bacillus cereus* group

251 species (Ruiu et al., 2015), although the potential of some strains against Tephritid fruit flies has  
252 been reported (Alberola et al., 1999; Robacker et al., 1996).  
253 Based on previous knowledge and the results in this study, *B. laterosporus* appears to be a selective  
254 microbial species that show a high potential only against specific targets, supporting its use in their  
255 management. However, further research is needed to understand the specific mechanisms leading to  
256 different degrees of susceptibility of insects belonging to the same order and the lack of  
257 pathogenicity against certain non-target species.

258

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266

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351

352 **Table 1 - Virulence of *Brevibacillus laterosporus* to the synanthropic flies *Calliphora vomitoria*,**  
 353 ***Lucilia caesar*, and *Musca domestica*.**

354

Species	LC <sub>50</sub> <sup>a</sup>	95% CI	LC <sub>95</sub> <sup>b</sup>	95% CI	Intercept ± SE	P <sup>d</sup>
<i>C. vomitoria</i>	78.836	65.105 - 96.440	689.865	494.168 - 1045.522	-3.312 ± 0.214	< 0.001
<i>L. caesar</i>	148.296	117.060 - 187.274	1297.685	939.709 - 1919.676	-3.791 ± 0.267	< 0.001
<i>M. domestica</i>	82.408	63.705 - 105.758	721.119	522.050 - 1061.960	-3.345 ± 0.249	< 0.001

355 <sup>a</sup> concentration of *B. laterosporus* spores that kills 50% of adult flies.

356 <sup>b</sup> concentration of *B. laterosporus* spores that kills 95% of adult flies; CI, confidence interval. Data are calculated on the  
 357 number of dead flies after 72 h from contact with the pathogen by Probit regression analysis and given as 10<sup>6</sup> spores/mL.  
 358 Model: Slope = 1.746 ± 0.114;  $\chi^2 = 53.142$ ; df = 64; Pearson Goodness-of-Fit Test  $P = 0.832$ ; Parallelism Test  $P < 0.001$ .

359

360

361 **Table 2 - Relative susceptibility of the synanthropic flies *Calliphora vomitoria*, *Lucilia caesar*,**  
 362 **and *Musca domestica* to *Brevibacillus laterosporus***

363

Species (X) \ Species (Y)	<i>C. vomitoria</i>	<i>L. caesar</i>	<i>M. domestica</i>
<i>C. vomitoria</i>	-	<b>1.881(1.332-2.807)</b>	1.045(0.762-1.465)
<i>L. caesar</i>	<b>0.532 (0.356-0.751)</b>	-	<b>0.556 (0.378-0.790)</b>
<i>M. domestica</i>	0.957 (0.682-1.313)	<b>1.800(1.266-2.648)</b>	-

364 <sup>a</sup> Relative median potency analyses (rmp) values of probits (Species in column vs Species in row): Values  
 365 < 1 indicates lower susceptibility Values > 1 indicates higher susceptibility. Bold indicates significant  
 366 values (95% CI ≠ 1).  
 367  
 368

369 **Table 3 - Virulence of *Brevibacillus laterosporus* to the fruit flies *Batrocera oleae*, *Ceratitis***  
 370 ***capitata*, and *Drosophila suzukii*.**

Species	LC <sub>50</sub> <sup>a</sup>	95% CI	LC <sub>95</sub> <sup>b</sup>	95% CI	Intercept ± SE	P <sup>d</sup>
<i>B. oleae</i>	2567.364	1776.508- 4140.198	28769.216	13897.229- 94327.171	-5.344 ± 0.652	< 0.001
<i>C. capitata</i>	2567.320	1776.480- 4140.110	28768.718	13897.033- 94325.008	-5.344 ± 0.652	< 0.001
<i>D. suzukii</i>	217.508	167.818- 313.975	2437.338	1231.512- 7625.594	-3.664± 0.426	< 0.001

371 <sup>a</sup> concentration of *B. laterosporus* spores that kills 50% of adult flies.

372 <sup>b</sup> concentration of *B. laterosporus* spores that kills 95% of adult flies; CI, confidence interval. Data are calculated on the  
 373 number of dead flies after 72 h from contact with the pathogen by Probit regression analysis and given as 10<sup>6</sup>  
 374 spores/mL. Model: Slope = 1.567 ± 0.208;  $\chi^2 = 26.077$ ; df = 36; Pearson Goodness-of-Fit Test  $P = 0.888$ ; Parallelism  
 375 Test  $P = 0.268$ .

376

377

378 **Table 4 - Relative susceptibility of the fruit flies *Batrocera oleae*, *Ceratitis capitata*, and**

379 ***Drosophila suzukii* to *Brevibacillus laterosporus***

380

Species (X)		<i>B. oleae</i>	<i>C. capitata</i>	<i>D. suzukii</i>
Species (Y)				
<i>B. oleae</i>	-		1.00(0.595-1.680)	<b>0.085(0.014-0.250)</b>
<i>C. capitata</i>	1.00(0.595-1.680)		-	<b>0.085(0.014-0.250)</b>
<i>D. suzukii</i>	<b>11.804 (3.995-71.181)</b>	<b>11.803 (3.995-71.179)</b>		-

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<sup>a</sup>, Relative median potency analyses (rmp) values of probits (Species in column vs Species in row): Values < 1 indicates lower susceptibility Values > 1 indicates higher susceptibility. Bold indicates significant values (95% CI ≠ 1).

385

386 **Table 5 - Virulence of *Brevibacillus laterosporus* to the mosquitoes *Aedes aegypti*, and *Culex***  
 387 ***pipiens***

Species	LC <sub>50</sub> <sup>a</sup>	95% CI	LC <sub>95</sub> <sup>b</sup>	95% CI	Intercept ± SE	P <sup>d</sup>
<i>Ae. aegypti</i>	0.179	0.139 – 0.229	1.935	1.326 – 3.152	-7.934 ± 0.658	< 0.001
<i>C. pipiens</i>	0.097	0.074 - 0.125	1.052	0.746 – 1.629	-8.355± 0.672	< 0.001

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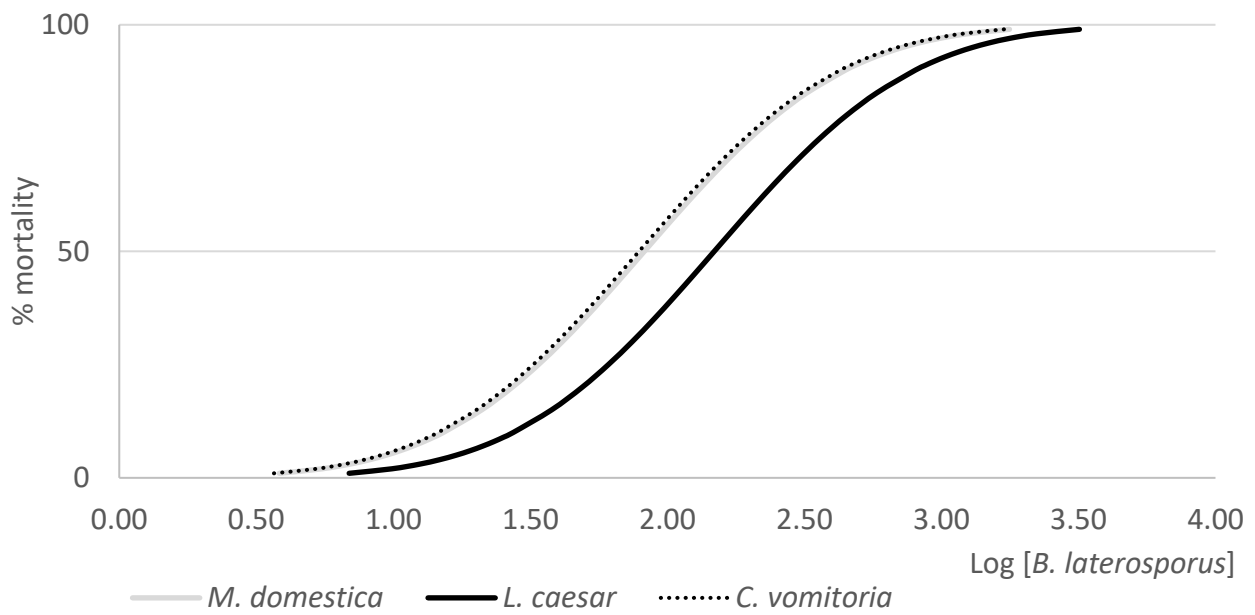
389 <sup>a</sup>, concentration of *B. laterosporus* spores that kills 50% of mosquito larvae. <sup>b</sup>, concentration of *B. laterosporus* spores  
 390 that kills 95% of larvae; CI, confidence interval. Data are calculated on the number of dead larvae after 24 h from  
 391 contact with the pathogen by Probit regression analysis and given as 10<sup>6</sup> spores/mL. Model: Slope = 1.591 ± 0.126;  $\chi^2$  =  
 392 26.077; df = 56; Pearson Goodness-of-Fit Test *P* = 0.969; Parallelism Test *P* = 0.007.

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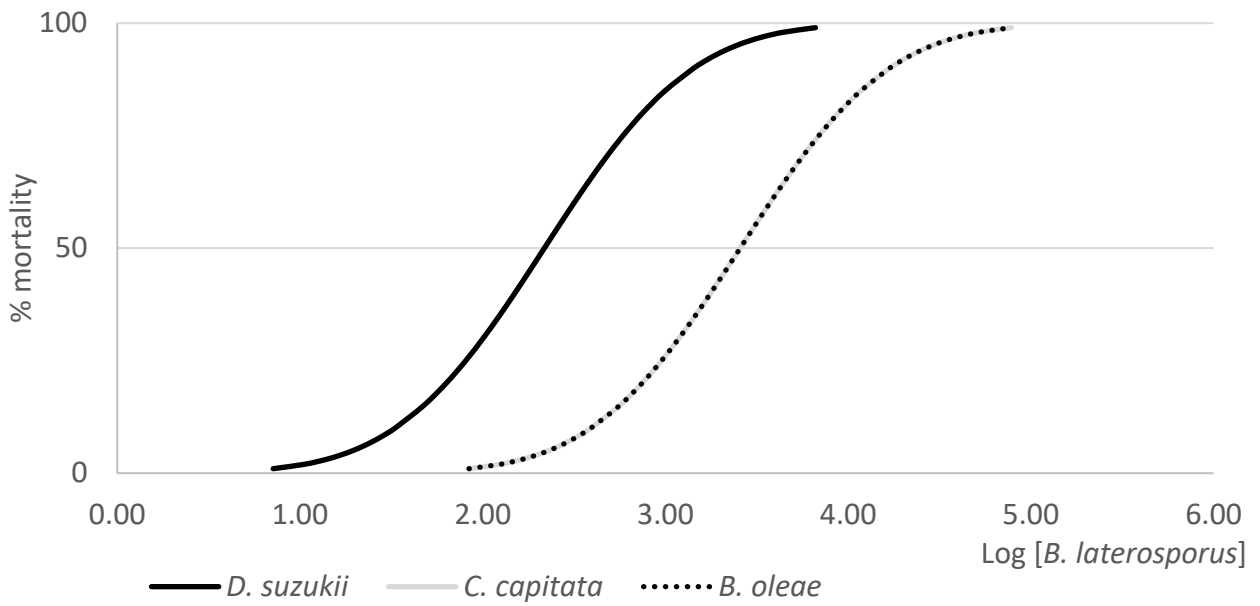
397

398 Fig. 1 - Probability of mortality (mortality %) of the synanthropic flies *Calliphora vomitoria*,  
399 *Lucilia caesar*, and *Musca domestica* exposed to *Brevibacillus laterosporus* spores calculated by  
400 Log-Probit regression.

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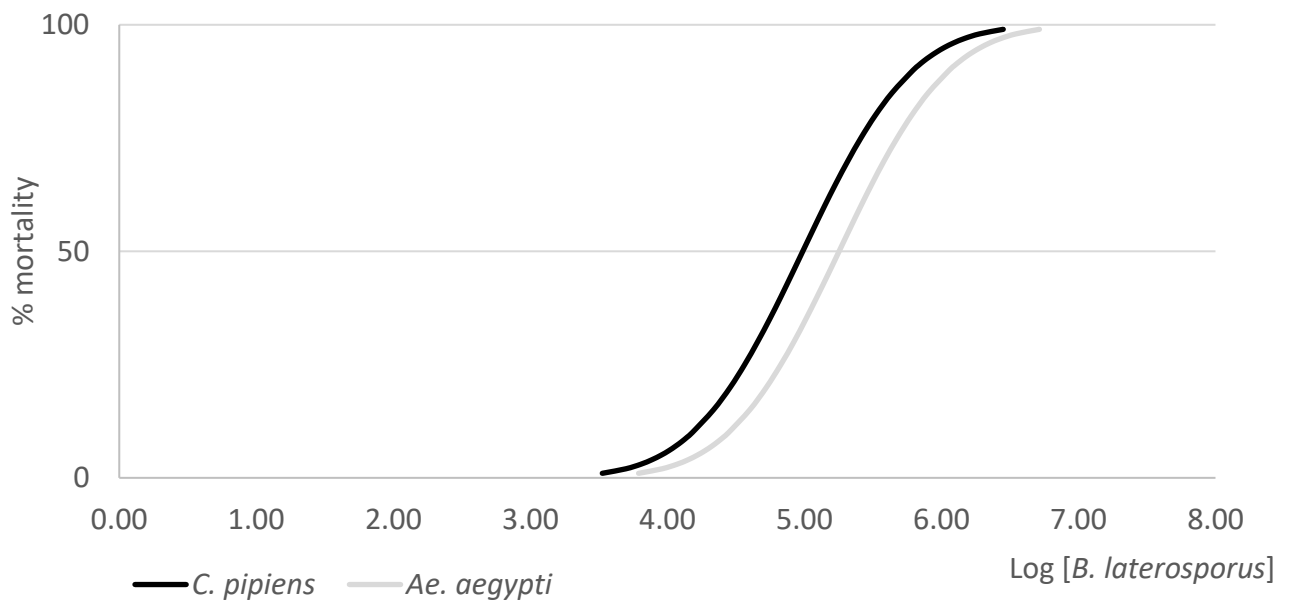
406 Fig. 2 - Probability of mortality (mortality %) of the fruit flies *Batrocera oleae*, *Ceratitidis capitata*,  
407 and *Drosophila sukuzii* exposed to *Brevibacillus laterosporus* spores calculated by Log-Probit  
408 regression.

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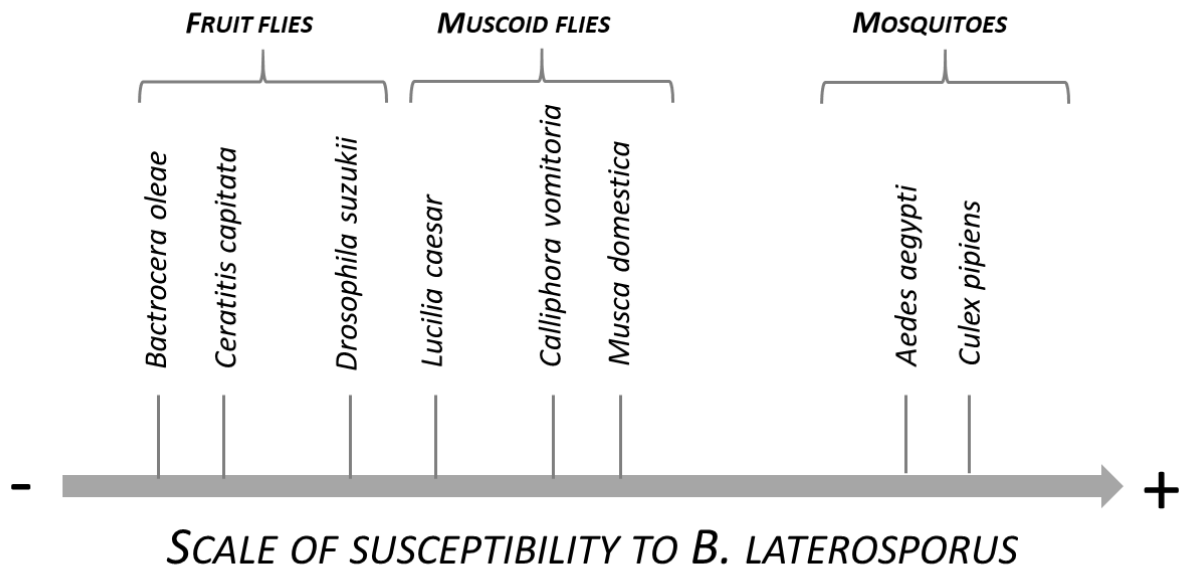


413

414 Fig. 3 - Probability of mortality (mortality %) of the larvae of the mosquitoes *Culex pipiens* and  
415 *Aedes aegypti* exposed to *Brevibacillus laterosporus* spores calculated by Log-Probit regression.

416

417



419

420 Fig. 4 - Relative susceptibility (increases to the right) of different Diptera species exposed to

421 *Brevibacillus laterosporus* spores.

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