Test of the pathogenicity of two commercial Beauveria strains on third-instar larvae of the mango blossom gall midge, Procontarinia mangiferae (Felt) (Diptera: Cecidomyiidae)

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Test de pathogénicité de deux souches commerciales de Beauveria sur le troisième stade larvaire de la cécidomyie des fleurs du manguier, Procontarinia mangiferae (Felt) (Diptera: Cecidomyiidae).

Abstract – Introduction. The invasive gall midge, Procontarinia mangiferae (= Erosomyia mangiferae Felt), is one of the most important flowering pests of mango orchards worldwide. To achieve chemical input reduction, developing integrated pest management (IPM) strategies using bio-control agents is pertinent. Materials and methods. We tested the pathogenicity of two commercial strains of the entomopathogenic fungi Beauveria on non-diapausing 3rd-instar larvae of P. mangiferae. Results and discussion. Neither the Beauveria sp. commercial strain Betel nor the B. bassiana strain Bb 147 were effective, even though they proved their pathogenicity on the control, Galleria mellonella. Hypotheses to explain the inefficiency of the two strains on P. mangiferae are discussed. Conclusion. Other strains of Beauveria or other entomopathogenic fungi or nematodes should be tested on diapausing and non-diapausing larvae of P. mangiferae.

Réunion / Mangifera indica / pests of plants / Procontarinia mangiferae / integrated pest management / Beauveria

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Réunion / Mangifera indica / ravageur des plantes / Procontarinia mangiferae / gestion intégrée des ravageurs / Beauveria

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1. Introduction

Procontarinia mangiferae (Felt) is one of the species of gall midge (Diptera: Cecidomyiidae) that attack mango trees (Mangifera indica L.) [1]. This midge is considered to be indigenous to India and invasive in Thailand, Mauritius, Reunion Island, Iran, the West Indies and Brazil [2, 3]; it causes economic damage in many countries [4, 5]. Adults lay eggs on inflorescences and young leaves [6]. After hatching, larvae bore the plant tissues and cause the formation of galls. Larvae stay there for up to a week, completing their development. Then, third-instar larvae leave the mango tree to pupate in the soil. They can emerge about a week later or enter into diapause for up to several years. Adults stay alive for one to two days [7].

Gall midge populations have often been controlled in commercial farms with neonicotinoid, organophosphate, pyrethroid, organochlorine or carbamate insecticides [7–9]. Today, the use of some of these insecticides is prohibited because of their toxicity toward humans and the environment. Moreover, some new insecticides are ineffective against P. mangiferae [10] or need to be applied regularly during the blooming season [7]. Other ways have to be found to regulate populations of this pest without negative consequences for farmers, consumers or the environment [11]. Among these solutions, entomopathogens such as fungi [12] or nematodes [13] can be considered to control gall midge. Beauveria bassiana and B. brongniartii (Ascomycota: Hypocreales: Clavicipitaceae), naturally present in many soils around the world, are widely used as mycoinsecticides and considered safe for the environment and for living beings [14]. Some Beauveria species are known to infect some gall midges [15]. In France, two Beauveria strains are authorized for pest control: the B. bassiana strain Bb 147 (Ostrinil®, Arysta Lifescience) is authorized against the corn borer (Ostrinia nubilalis (Hübner), Lepidoptera: Pyralidae) on corn and the palm moth (Pyxidanisca archon (Burmeister), Lepidoptera: Castniidae) on palm trees, and Beauveria sp. (Betel®, Betel Reunion) is authorized against the white grub Hoplochelus marginalis (Fairmaire), Coleoptera: Scarabaeidae) on sugar cane. In our study, we tested the pathogenicity of these two commercial strains on P. mangiferae, with the aim of identifying a potential bio-control agent.

2. Materials and methods

2.1. Collection of P. mangiferae larvae

Third-instar P. mangiferae larvae were collected in orchards located in Saint-Gilles, Reunion Island (21°02’33" S, 55°13’45" E). Tarpaulin traps containing water were installed in the afternoon under trees with damaged inflorescences. Larvae fallen from the inflorescences during the night were collected the next morning, an hour before starting the pathogenicity test. Larval mortality was negligible with this collection technique. Dead or not very active larvae were discarded before the experiments.

2.2. Beauveria strains

Two commercial strains of Beauveria sp. were tested. The first one was the Beauveria sp. strain B 507 used in Reunion Island to control the sugar cane white grub Hoplochelus marginalis [16]. The commercial name of the fungal product is Betel® (Betel Reunion, Saint-Benoît, Reunion Island). The conidial suspension was directly obtained from the firm Betel Reunion. The initial concentration of 10^8 spores·mL^{-1} was also tested to assess the pathogenicity of the two Beauveria strains.

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concentration effect on the pathogenicity of Beauveria sp. strain B 507.

The second strain tested was the Beauveria bassiana strain Bb 147, used in the commercial product Ostrinil® (Arysta LifeScience, France). The strain was cultivated from a freeze-dried culture obtained from the Centre de Biologie et de Gestion des Populations (Montpellier, France). It was cultivated on a medium containing antibiotics and anti-fungus: 18% agar, 10% glucose, 5% yeast extract, 1.4% Na$_2$HPO$_4$, 1% KCl, 0.7% NH$_4$NO$_3$, 0.6% MgSO$_4$, 0.5% chloramphenicol, 0.4% KH$_2$PO$_4$ and 0.25% cycloheximide ethanol. Once the fungi had sporulated, we prepared a 20-mL spore suspension and diluted it to prepare a C$_b$ suspension at a concentration of $9 \times 10^6$ spores-mL$^{-1}$. It was not possible to obtain a more concentrated suspension, and only this one was tested for B. bassiana strain Bb 147 since this concentration was intermediate between the two concentrations tested for Beauveria sp. strain B 507. Conidial suspension concentrations were determined using a Malassez hemocytometer [18].

2.3. Verification of the virulence of the Beauveria strains

Fourth-instar larvae of the wax moth Galleria mellonella L. (Lepidoptera: Pyralidae) were used as a control to test the virulence of the two Beauveria strains [19, 20]. The larvae came from a laboratory population reared on artificial media.

Sixty G. mellonella larvae were inoculated with the suspension C$_b$2 of the Beauveria sp. B 507 strain, and 30 larvae with the suspension C$_h$ of B. bassiana Bb 147. Inoculation consisted of immersing larvae in the suspension for two seconds. Two control groups of 30 larvae each were immersed in distilled water. Then, larvae were placed in individual 25-mL hermetic plastic boxes with 0.4 g beeswax and an 8-cm$^2$ piece of Whatman paper humidified with 0.5 mL distilled water. Larvae were stored at 25 °C. After 21 days, dead and alive larvae were counted.

2.4. Pathogenicity test on gall midge larvae

To test the effect of the two Beauveria strains on P. mangiferae, larvae were inoculated using the same method as for G. mellonella. Groups of 30 larvae were inoculated with suspensions of Beauveria sp. B 507 strain C$_h$1, C$_h$2, or with distilled water (control group). Then, each group was equally separated into three 50-mL hermetic plastic boxes containing 10 g of sand humidified with 1 g of distilled water. Three repetitions were performed to achieve a total of 90 larvae per treatment. For the B. bassiana Bb 147 strain, thirty larvae were inoculated with the suspension C$_b$ or with distilled water. Four repetitions were performed to achieve a total of 120 larvae per treatment. Pathogenicity tests were performed at 25 °C. At this temperature, adult emergence occurs between 5 and 6 days (data not shown). Ten days after the inoculation, the number of emerged adults was counted.

2.5. Statistical analysis

We performed the exact Fisher’s test to assess the significance of the differences in mortality rates between the inoculated and the control groups.

Generalized linear models with binomial distribution were used to test the effect of each Beauveria strain on the emergence of P. mangiferae. Statistical analyses were performed with the R software, version 2.15 [21].

3. Results and discussion

Mortality of G. mellonella larvae was significantly higher in groups inoculated with Beauveria sp. B 507 and B. bassiana Bb 147 than in control groups (table I), confirming the viability and virulence of both strains.

Analyses showed that neither Beauveria strain had a significant effect on the emergence of P. mangiferae (table II). None of the strains was efficient in controlling the
immediate emergence of *P. mangiferae* and they therefore showed no pathogenic effect on the larvae.

To explain these results, several hypotheses can be suggested. Firstly, another inoculation method could have been used. In other studies, the soil [22], the leaves [23] or the bark [24], which are shelters for the larvae, are drenched or sprayed with a conidial suspension, instead of spreading the conidia directly on the body of the larvae. Secondly, the life cycles of *P. mangiferae* and of these strains of *Beauveria* sp. might not be compatible because the fungus incubation period is longer than the non-diapausing larval development tested here [14]. Thereby, the spores would not have enough time to germinate and penetrate the larvae before the pupal moulting. However, these entomopathogenic fungi could infest larvae which enter diapause in the soil, or have an effect on the emerged adult flies and their fertility, as observed on the red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae) [25]. The third hypothesis is that the larvae were not susceptible to the fungus. The peptides and amino acids of the larval cuticle may not be recognized by the fungi, or, once the fungus has penetrated, the larval immune system may be efficient enough to enable the larvae to finish their development into adults [14]. From these experiments, we conclude that other strains need to be tested against *P. mangiferae* since, for example, specific strains of *Beauveria* sp. are effective against pine gall midges, *Thecodiplosis japonensis* Uchida et Inouye (Diptera: Cecidomyiidae) [15, 26]. Therefore, it is necessary to include these strains in future tests.

Controlling gall midge populations in the soil is a way to develop IPM strategies. Controlling populations in diapause may be another solution, and we may test these

### Table I.
Mortality of *Galleria mellonella* larvae inoculated with the two strains of *Beauveria* sp. or dipped in distilled water (*p*-value < 0.01).

<table>
<thead>
<tr>
<th>Strains</th>
<th>Treatment</th>
<th>Number of larvae</th>
<th>Total</th>
<th>Dead at 21 days</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Beauveria</em> sp. B 507</td>
<td>Dipped in water for control</td>
<td>30</td>
<td>30</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Inoculated with $10^6$ spores·mL$^{-1}$ (C$_h$2)</td>
<td>60</td>
<td>60</td>
<td>59</td>
</tr>
<tr>
<td><em>B. bassiana</em> Bb 147</td>
<td>Dipped in water for control</td>
<td>30</td>
<td>30</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Inoculated with $9 	imes 10^6$ spores·mL$^{-1}$ (C$_l$)</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
</tbody>
</table>

### Table II.
Emergence of *Procontarinia mangiferae* adults 10 days after exposure of larvae to *Beauveria* sp. strain B 507, *B. bassiana* strain Bb 147 and distilled water.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Treatment</th>
<th>Number of larvae</th>
<th>Mean of adult emergence$^1$ at 10 days (% ± standard error)</th>
<th><em>p</em>-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Beauveria</em> sp. B 507</td>
<td>Dipped in water for control</td>
<td>90</td>
<td>74.4 ± 11.3</td>
<td>0.088</td>
</tr>
<tr>
<td></td>
<td>Inoculated with $10^6$ spores·mL$^{-1}$ (C$_h$1)</td>
<td>90</td>
<td>80.0 ± 14.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inoculated with $10^8$ spores·mL$^{-1}$ (C$_h$2)</td>
<td>90</td>
<td>65.7 ± 18.1</td>
<td></td>
</tr>
<tr>
<td><em>B. bassiana</em> Bb 147</td>
<td>Dipped in water for control</td>
<td>120</td>
<td>85.8 ± 13.8</td>
<td>0.564</td>
</tr>
<tr>
<td></td>
<td>Inoculated with $9 	imes 10^6$ spores·mL$^{-1}$ (C$_l$)</td>
<td>120</td>
<td>88.3 ± 22.9</td>
<td></td>
</tr>
</tbody>
</table>

$^1$ In each treatment, adult emergence is expressed as the mean percentage of emerged adults (for the three batches of 30 larvae) relative to the number of larvae initially present.
strains of Beauveria sp. on diapausing individuals. Other biological solutions could be tested, such as the fungus Metarhizium anisopliae [27].

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References


Test de patogenicidad de dos cepas comerciales de Beauveria en el tercer estado larvario de la cecidomía de las flores del mango, Procontarinia mangiferae (Felt) (Diptera: Cecidomyiidae).

Resumen – Introducción. La cecidomía de las flores, Procontarinia mangiferae (= Erosmiya mangiferae Felt), es una especie invasiva y una de las plagas mundiales más importantes de la floración del mango. El uso de organismos de control biológico representa una estrategia pertinente de gestión integrada de las plagas para reducir el uso de productos fitosanitarios. Material y métodos. La patogenicidad de dos cepas comerciales del hongo entomopatógeno Beauveria se testó en las larvas de P. mangiferae del tercer estado no diapausante. Resultados y discusión. A pesar de su patogenicidad en la especie testigo, Galleria mellonella, la cepa comercial Betel de Beauveria sp. y la cepa Bb 147 de B. bassiana no resultaron ser patógenas en las larvas de P. mangiferae. Se discutieron varias hipótesis para explicar la ineficacia de las dos cepas testeadas. Conclusión. Se deben testear otras cepas de Beauveria, u otros hongos o nematodos entomopatógenos, en larvas diapausanas y no diapausanas de la cecidomía de las flores del mango.

Reunión / Mangifera indica / plagas de plantas / Procontarinia mangiferae / gestión de lucha integrada / Beauveria