Original article

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

Eléonore S. Bricca[1,2], Samuel Nibouche[3], Hélène Delatte[3], Frédéric Normand[1], Paul Amouroux[1,4]*

[1] CIRAD, Persyst, UPR HortSys, F-97455 Saint-Pierre, La Réunion, France, paul.amouroux@cirad.fr

[2] Montpellier SupAgro, F-34060 Montpellier, France

[3] CIRAD, Bios, UMR PVBMT, F-97410 Saint-Pierre, La Réunion, France

[4] Univ. la Réunion, UMR PVBMT, F-97410 Saint-Pierre, La Réunion, France

* *Correspondence and reprints*

Received 13 September 2013
Accepted 3 December 2013

Fruits, 2014, vol. 69, p. 189–194

DOI: 10.1051/fruits/2014008
www.fruits-journal.org

***Resumen Español**, p. 194*

Test of the pathogenicity of two commercial *Beauveria* strains on third-instar larvae of the mango blossom gall midge, *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*

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).

Résumé – Introduction. La cécidomyie des fleurs, *Procontarinia mangiferae* (= *Erosomyia mangiferae* Felt), est une espèce invasive et l'un des plus importants ravageurs de la floraison du manguier dans le monde. L'utilisation d'organismes de bio-contrôle est une stratégie pertinente de gestion intégrée des ravageurs pour réduire l'emploi de produits phytosanitaires. **Matériel et méthodes**. La pathogénicité de deux souches commerciales du champignon entomopathogène *Beauveria* a été testée sur des larves de *P. mangiferae* du troisième stade non diapausantes. **Résultats et discussion**. Malgré leur pathogénicité sur l'espèce témoin, *Galleria mellonella*, la souche commerciale Betel de *Beauveria* sp. et la souche Bb 147 de *B. bassiana* ne se sont pas montrées pathogènes sur les larves de *P. mangiferae*. Des hypothèses pour expliquer l'inefficacité des deux souches testées sont discutées. **Conclusion**. D'autres souches de *Beauveria*, ou d'autres champignons ou nématodes entomopathogènes, doivent être testés sur des larves diapausantes et non-diapausantes de la cécidomyie des fleurs du manguier.

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

Article published by EDP Sciences

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[1]: 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 (*Paysandisia 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.

[1] Anon., e-phy, le catalogue des produits phytopharmaceutiques et de leurs usages, des matières fertilisantes et des supports de culture homologués en France, Minist. Agric. Agroalim., access. 21 Dec 2013, http://e-phy.agriculture.gouv.fr/.

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 tests. The 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 Betel® suspension, C_h2, had a concentration of 10^8 spores·mL^{-1}. We diluted this suspension to obtain a second suspension, C_h1, concentrated at 10^6 spores·mL^{-1}, a concentration used to assess the biological activity of *Beauveria* sp. against *Hoplochelus marginalis* [17]. The initial concentration of 10^8 spores·mL^{-1} was also tested to assess the

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 medium containing antibiotics and anti-fungus: 18% agar, 10% glucose, 5% yeast extract, 1.4% Na_2HPO_4, 1% KCl, 0.7% NH_4NO_3, 0.6% $MgSO_4$, 0.5% chloramphenicol, 0.4% KH_2PO_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 9.10^6 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_h2 of the *Beauveria* sp. B 507 strain, and 30 larvae with the suspension C_b 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-cm² 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_h1, C_h2, 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

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

Strains	Treatment	Number of larvae	
		Total	Dead at 21 days
Beauveria sp. B 507	Dipped in water for control	30	5
	Inoculated with 10^8 spores·mL^{-1} (C_h2)	60	59
B. bassiana Bb 147	Dipped in water for control	30	12
	Inoculated with 9×10^6 spores·mL^{-1} (C_b)	30	30

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.

Strain	Treatment	Number of larvae	Mean of adult emergence[1] at 10 days (% ± standard error)	p-value
Beauveria sp. B 507	Dipped in water for control	90	74.4 ± 11.3	0.088
	Inoculated with 10^6 spores·mL^{-1} (C_h1)	90	80.0 ± 14.1	
	Inoculated with 10^8 spores·mL^{-1} (C_h2)	90	65.7 ± 18.1	
B. bassiana Bb 147	Dipped in water for control	120	85.8 ± 13.8	0.564
	Inoculated with 9×10^6 spores·mL^{-1} (C_b)	120	88.3 ± 22.9	

[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.

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

strains of *Beauveria* sp. on diapausing individuals. Other biological solutions could be tested, such as the fungus *Metarhizium anisopliae* [27].

Acknowledgments

The authors would like to thank Richard Tibere and Magali Hoarau for their technical assistance in the laboratory and the rearing of *G. mellonella* larvae and *B. bassiana*, Mr. Zuin for providing the Betel® suspension, and Mr. Law-Yat for allowing us to visit his orchards and collect larvae frequently. This work was funded by the European Agricultural Fund for Rural Development (EAFRD, *Bilan de Santé de la PAC*, No 11111D974000019) and CIRAD.

References

[1] Kolesik P., Rice A.D., Bellis G.A., Wirthensohn M.G., *Procontarinia pustulata*, a new gall midge species (Diptera: Cecidomyiidae) feeding on mango, *Mangifera indica* (Anarcadiaceae), in northern Australia and Papua New Guinea, Aust. J. Entomol. 48 (2009) 310–316.

[2] Gagné R.J., Update for a catalog of the Cecidomyiidae (Diptera) of the world, Digit. vers. 1, USDA, Washington, 2010, http://www.ars.usda.gov/SP2UserFiles/Place/12754100/Gagne_2010_World_Catalog_Cecidomyiidae.pdf.

[3] Anon., Crop Protection Compendium, CAB Int., Wallingford, U.K., 2004.

[4] Amouroux P., Normand F., Survey of mango pests on Réunion Island, with a focus on pests affecting flowering, Acta Hortic. 992 (2013) 459–466.

[5] Whitwell A.C., The pest/predator/parasitoid complex on mango inflorescence in Dominica, Acta Hortic. 341 (1993) 421–432.

[6] Amouroux P., Normand F., Nibouche S., Delatte H., Invasive mango blossom gall midge, *Procontarinia mangiferae* (Felt) (Diptera: Cecidomyiidae) in Reunion Island: ecological plasticity, permanent and structured populations, Biol. Invasions 15 (2013) 1677–1693.

[7] Prasad S.N., The mango midge pests, Cecidological Soc. India, Allahabad, India, 1971.

[8] Abbas S.R., Verghese M., Fasih M., Studies on the mango inflorescence midge, *Erosomyia indica* Grover, Acta Hortic. 231 (1989) 593–596.

[9] Wu Q.J., Zhao J.Z., Taylor A.G., Shelton A.M., Evaluation of insecticides and application methods against *Contarinia nasturtii* (Diptera : Cecidomyiidae), a new invasive insect pest in the United States, J. Econ. Entomol. 99 (2006) 117–122.

[10] Barbosa F.R., Gonçalves M.E., De Souza E.A., Chemical control of *Erosomyia mangiferae* on mango trees at the Sao Francisco river valley, Brazil, in: de Queiros Pinto A.C., Lederman I.E., Fernandes Bezerra J.E., Canto Pereira M.E., Elesbão Alves R., Barbosa de Souza V.A. (Eds.), Program and Abstracts, VII Int. Mango Symp., Recife, Brasil, 2002, pp. 261–261.

[11] Malézieux E., Designing cropping systems from nature, Agron. Sustain. Dev. 32 (2012) 15–29.

[12] Keller S., Wilding N., *Entomophthora brevinucleata* sp. nov. [Zygomycetes, Entomophthoraceae], a pathogen of gall midges [Dip.: Cecidomyiidae], Entomophaga 30 (1985) 55–63.

[13] Corlay F., Boivin G., Bélair G., Efficiency of natural enemies against the Swede midge *Contarinia nasturtii* (Diptera: Cecidomyiidae), a new invasive species in North America, Biol. Control. 43 (2007) 195–201.

[14] Zimmermann G., Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*, Biocontrol Sci. Technol. 17 (2007) 553–596.

[15] Shin S.C., Park S.J., Lee S.M., Choi K.S., Lee S.G., Lee C.Y., Pathogenicity of *Beauveria* spp. isolated from Korea against the pine needle gall midge (*Thecodiplosis japonensis* Uchida and Inouye) and their physiological characterization, FRI J. For. Sci. (Seoul) 59 (1998) 57–63.

[16] Neuvéglise C., Brygoo Y., Riba G., 28s rDNA group-I introns: a powerful tool for identifying strains of *Beauveria brongniartii,* Mol. Ecol. 6 (1997) 373–381.

[17] Neuvéglise C., Brygoo Y., Vercambre B., Riba G., Comparative analysis of molecular and biological characteristics of strains of *Beauveria brongniartii* isolated from insects, Mycol. Res. 98 (1994) 322–328.

[18] Rodriguez-Tudela J.L., Chryssanthou E., Petrikkou E., Mosquera J., Denning D.W., Cuenca-Estrella M., Interlaboratory evaluation of hematocytometer method of inoculum preparation for testing antifungal susceptibilities of filamentous fungi, J. Clinic. Microbiol. 41 (2003) 5236–5237.

[19] Hussein K.A., Abdel-Rahman M.A.A., Abdel-Mallek A.Y., El-Maraghy S.S., Joo J.H., Pathogenicity of *Beauveria bassiana* and *Metarhizium anisopliae* against *Galleria mellonella*, Phytoparasitica 40 (2012) 117–126.

[20] Powell J.R., Webster J.M., Target host finding by *Steinernema feltiae* and *Heterorhabditis bacteriophora* in the presence of a non-target insect host, J. Nematol. 36 (2004) 285–289.

[21] Anon., R: A language and environment for statistical computing, R foundation for statistical computing, R Development Core Team, Vienna, Austria, 2012.

[22] Wraight S.P., Ramos M.E., Synergistic interaction between *Beauveria bassiana* and *Bacillus thuringiensis* tenebrionis-based biopesticides applied against field populations of Colorado potato beetle larvae, J. Invert. Pathol. 90 (2005) 139–150.

[23] Wraight S.P., Carruthers R.I., Production, delivery, and use of mycoinsecticides for control of insect pests on field crops biopesticides: use and delivery, in: Hall F.R., Menn J.J. (Eds.), Humana Press, Totowa, N.J., U.S.A., 1999.

[24] Dean K.M., Vandenberg J.D., Griggs M.H., Bauer L.S., Fierke M.K., Susceptibility of two hymenopteran parasitoids of *Agrilus planipennis* (Coleoptera: Buprestidae) to the entomopathogenic fungus *Beauveria bassiana* (Ascomycota: Hypocreales), J. Invert. Pathol. 109 (2012) 303–306.

[25] Dembilio Ó., Quesada-Moraga E., Santiago-Alvarez C., Jacas J.A., Potential of an indigenous strain of the entomopathogenic fungus *Beauveria bassiana* as a biological control agent against the Red Palm Weevil, *Rhynchophorus ferrugineus*, J. Invert. Pathol. 104 (2010) 214–221.

[26] Seo J.B., Jin B.R., Shin S.C., Park H.Y., Lee B.Y., Chang K.L., Kang S.K., Isolation of entomopathogenic fungi for infection to the pine gall midge, *Thecodiplosis japonensis*, from the forest soil in Korea, Korean J. Appl. Entomol. 34 (1995) 368–372.

[27] Leles R.N., D'Alessandro W.B., Luz C., Effects of *Metarhizium anisopliae* conidia mixed with soil against the eggs of *Aedes aegypti,* Parasitol. Res. 110 (2012) 1579–1582.

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

Resumen – Introducción. La cecidomia de las flores, *Procontarinia mangiferae* (= *Erosomyia 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 testeó 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 diapausantes y no diapausantes de la cecidomia de las flores del mango.

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