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Résumé de l'article

La vulnérabilité des adultes et des larves du scarabée japonais aux isolats des champignons *Beauveria bassiana, B. brongniartii, Metarhizium anisopliae, Lecanicillium longisporum* et *L. muscarium* (Ascomycota : Hypocreales) a été évaluée lors de tests biologiques en laboratoire. La présence de variations intra- et interspécifiques concernant le pouvoir pathogène des isolats a été étudiée. Les résultats montrent que la plupart des isolats fongiques provoquent de la mortalité chez les adultes et les larves. Les isolats *M. anisopliae* INRS 705 et *B. bassiana* INRS 236 ont mené à des taux de mortalité de 70,3 % et 65,2 % chez les adultes de *P. japonica*, respectivement, et les deux isolats ont provoqué la mort d'environ 37 % des larves. Des 17 isolats testés, 13 ont causé de la mortalité larvaire. Cependant, aucune différence importante n'a été notée entre la vulnérabilité des larves aux isolats de différentes espèces et à ceux de la même espèce. Les espèces *Lecanicillium* n'ont clairement pas de pouvoir pathogène chez les adultes et semblent n'avoir que peu d'effet chez les larves. En se fondant sur les résultats obtenus avec les isolats choisis, des différences intra- et interspécifiques concernant le pouvoir pathogène des isolats semblent être présentes. Dans son ensemble, l'étude a permis d'accroître les connaissances sur la vulnérabilité de *P. japonica* aux champignons pathogènes de l'ordre Hypocreales. Les conséquences de cette étude pour le développement d'un biopesticide sont présentées.

Susceptibility of the Japanese beetle, *Popillia japonica* (Newman) (Coleoptera: Scarabaeidae), to entomopathogenic Hypocreales fungi

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The susceptibility of adults and larvae of the Japanese beetle to isolates of the fungi *Beauveria bassiana, B. brongniartii, Metarhizium anisopliae, Lecanicillium longisporum* and *L. muscarium* (Ascomycota: Hypocreales) was evaluated in laboratory bioassays. The presence of intra- and interspecific variations regarding the pathogenicity of the isolates was investigated. Results show that most of the fungal isolates caused mortality in adults and larvae. Isolates *M. anisopliae* INRS 705 and *B. bassiana* INRS 236 induced 70.3% and 65.2% of mortality in *P. japonica* adults, respectively, and both caused the death of about 37% of larvae. Of the 17 tested isolates, 13 caused larval mortality. However, no significant difference was found between the susceptibility of larvae to isolates from different species and those from a same species. *Lecanicillium* species are undoubtedly not pathogenic to adults and seem to have few effects on larvae. Based on the results obtained with the selected isolates, intra- and interspecific differences relative to the pathogenicity of the isolates appeared to be present. Overall, this study expanded the knowledge about *P. japonica* susceptibility towards entomopathogenic Hypocreales fungi. The implications of this study regarding the development of a biological control agent are discussed.

Keywords: *Beauveria bassiana*, Japanese beetle, *Lecanicillium longisporum, Lecanicillium muscarium, Metarhizium anisopliae*, microbial control, *Popillia japonica*.

[Vulnérabilité du scarabée japonais, *Popillia japonica* (Newman) (Coleoptera : Scarabaeidae), aux champignons pathogènes de l'ordre Hypocreales]

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Mots-clés : *Beauveria bassiana, Lecanicillium longisporum, Lecanicillium muscarium*, lutte microbienne, *Metarhizium anisopliae, Popillia japonica,* scarabée japonais.

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INTRODUCTION

The Japanese beetle, *Popillia japonica* (Newman), was first reported in Canada in 1938 (CFIA 2009). Since then, this insect has never stopped spreading northward. Japanese beetle is becoming an increasing problem in tree nurseries in Canada, and it particularly affects the provinces of Ontario and Quebec. Adults feed on more than 300 species of plants and are thus considered among the most aggressive polyphagous plant-feeding insects (Potter and Held 2002). Turf roots are the preferred host of the larvae (grubs), but other host plants may also be attacked.

Tree nurseries significantly contribute to the success of reforestation programs. In the province of Quebec, 130 million seedlings are produced annually by nurseries (MRN 2014). The use of chemical insecticides is necessary to reduce the damage caused to nursery plants and to decrease the risk of insect dispersal. However, as the populations of Japanese beetle will most likely increase in Canada, more environmentally acceptable approaches to *P. japonica* population control are required.

Several microbiological agents have been studied for their potential to regulate larval populations of the Japanese beetle, including the bacteria *Bacillus thuringiensis* serovar *japonensis* strain *Buibui* (Ohba *et al.* 1992; Alm *et al.* 1997; Koppenhofer *et al.* 2000; Bixby *et al.* 2007), *B. thuringiensis* subsp. *galleriae* SDS-502 (Yamaguchi *et al.* 2013) and *Paenibacillus popilliae* (formely *Bacillus popilliae*) (Bulla *et al.* 1975; Klein 1992; Redmond and Potter 1995; Matsuki *et al.* 1997; Rippere *et al.* 1998), the nematodes *Steinernema glaseri* Steiner and *Heterorhabditis bacteriophora* Poinar, and the fungi *Metarhizium anisopliae* (Lacey *et al.* 1994; Villani *et al.* 1994; Lacey *et al.* 1995b; Abalos *et al.* 2001; Cappaert and Smitley 2002; Petty *et al.* 2012) and *Beauveria bassiana* (Hanula and Andreadis 1988; Hanula *et al.* 1991; Klein 1992; Lacey *et al.* 1995a, b; Bixby *et al.* 2007).

There are few conclusive studies on the effectiveness of entomopathogenic fungi against *P. japonica*. This study was conducted to determine and compare the impact of different species of Hypocreales fungi with the goal of identifying the one possessing maximum virulence against larvae and adults of *P. japonica*. Several fungal isolates were screened to verify their entomopathogenic potential against both developmental stages of the Japanese beetle.

MATERIAL AND METHODS

Insects

Adults and larvae of the Japanese beetle were collected from a public tree nursery located near Berthierville, approximately 50 km northeast of Montreal, QC, Canada (45.99° N, -73.19° W), in 2010.

At the end of April, about 2 mo before adult emergence, larvae were collected for bioassays. Larvae were harvested in grass near an ash tree (*Fraxinus* spp.) plantation, an area seriously affected by Japanese beetles. The extraction was facilitated using a tractor that cut ground slices (approximately 15 cm thick). Ground slices were transferred to an elevated shaking conveyor that let the larvae fall to the ground. Japanese beetle larvae were differentiated from other insect larvae present in the soil by their typical creamy white colour and C-shaped body. Larvae collected in the field were brought to the laboratory for subsequent experiments. In order to ensure the viability of the larvae until the bioassay, they were kept in plastic boxes filled with soil and covered by a turf layer originating from the sampling site.

Adults were collected using Japanese beetle traps (Trécé Inc., Adair, OK, USA) previously placed in the field at the Berthierville tree nursery. The traps were baited with a mixture of two compounds: japonilure ($C_{14}H_{24}O_2$, CSA# 64726-91-6), a sexual pheromone (Tumlinson *et al.* 1977), and an attractant food-type lure [eugenol ($C_{10}H_{12}O_2$, CSA# 97-53-0), 2-phenylethyl propionate ($C_{11}H_{14}O_2$, CSA# 122-70-3); and geraniol ($C_{10}H_{18}O$, CSA# 106-24-1) in a ratio of 7:3:3] (Ladd *et al.* 1984). The adults were caught the day before the bioassay.

Fungal isolates

The entomopathogenic isolates used in the subsequent bioassays originated from the microfungal collection of the INRS–Institut Armand-Frappier. The selected fungi were associated with three different genera (*Beauveria, Metarhizium* and *Lecanicillium*) and related to the order Hypocreales. A total of 19 isolates belonging to five species (*Beauveria bassiana* (Bals.-Criv.) Vuill., *Beauveria brongniartii* (Sacc.) Petch, *Metarhizium anisopliae* Metschn.) Sorokin, *Lecanicillium longisporum* R. Zare & W. Gams and *Lecanicillium muscarium* R. Zare & W. Gams) were used for the bioassays (Table 1).

Inoculum preparation and solid-stage culture conditions

Fungal isolates were first incubated at 25 ± 1 °C for 7 d in Petri dishes containing Sabouraud Dextrose Agar (SDA). The conidia and the mycelium were harvested and dispersed in 10 mL of sterile distilled water by scraping the surface with a sterile scalpel blade under strictly aseptic conditions. The suspensions thus obtained were subsequently submitted to a solid-state fermentation production using barley (*Hordeum vulgare* L.) as substrate and spawn bags. For each isolate tested, three bags were prepared. In each of them, 200 g of barley were mixed thoroughly with 100 mL of water, then autoclaved at 121 °C for 20 min. Once the solid medium was cooled, 10 mL of a fungal suspension was added to each bag, and the conidia and mycelium were methodically mixed for a better dispersion within the substrate. After the incubation period, 400 mL of deionized water was added to each bag and mixed thoroughly to liberate the conidia. The suspension was then filtered through three layers of sterile cheesecloth, and the concentration of conidia was assessed using a standard (improved Neubauer) hemocytometer. The viability of conidia of each suspension was also evaluated by measuring the percentage of germination as described by Inglis *et al.* (1993). At least 200 conidia were examined for each germination test. For all isolates, the germination rate was between 97% and 100% after 24 h. All conidia suspensions were kept at 4 °C until their use in bioassays.

Table 1. Species of Hypocreales fungi used in this study.

Species	Isolates	Bioassays performed on		Host or other reference
		Larvae	Adults	
Beauveria bassiana	INRS 236	Yes	Yes	Daom[1] 210569
	INRS 261	Yes	Yes	LRS[2] 100
	INRS 209	No	Yes	Arsef[3] 1956
	INRS 227	No	Yes	Daom 71453
	INRS 200	Yes	Yes	Arsef 252
	INRS 242	Yes	Yes	*Tomicus piniperda* L.
	INRS 243	Yes	Yes	*Leptinotarsa decemlineata* (Say)
Beauveria brongniartii	INRS 600	Yes	Yes	Arsef 659
	INRS 603	Yes	Yes	LRS 24
	INRS 604	Yes	Yes	LRS 25
	INRS 602	Yes	Yes	LRS 23
Metarhizium anisopliae	INRS 706	Yes	Yes	UAMH[4] 1674
	INRS 701	Yes	Yes	UAMH 4450
	INRS 705	Yes	Yes	UAMH 9198
	INRS 707	Yes	Yes	UAMH 2801
	INRS 704	Yes	Yes	UAMH 9197
	INRS 700	Yes	Yes	UAMH 421
Lecanicillium longisporum	INRS 1105	Yes	Yes	Vertalec, Koppert Biological Systems
Lecanicillium muscarium	INRS 1106	Yes	Yes	Mycotal, Koppert Biological Systems

1 National Mycology Herbarium of the Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada.
2 USDA-ARS Collection of Entomopathogenic Fungi.
3 Lethbridge Research Centre, Agriculture and Agri-Food Canada.
4 University of Alberta Microfungus Collection and Herbarium.

Bioassays

Bioassays of all fungal isolates were carried out against larvae and adults of the Japanese beetle at an inoculum concentration of 8 x 10^{10} conidia/mL. For each fungal isolate, bioassays were replicated three times. For adults, 20 individuals per replicate were used to evaluate the pathogenicity of the different Hypocreales fungi (Table 1). For larvae, each bioassay was performed using 10 individuals per replicate. Adults and larvae were individually inoculated by immersion in a vial containing 10 mL of fungal suspension. The vial was capped and inverted five times over a 5 s period to increase the probability of the insect being completely drenched with fungal suspension. The suspension and insect were filtered through a plastic strainer (7 cm diam). For controls, both stages of the insect were treated with distilled sterile water. Treated and untreated adults were individually transferred into a 29.5 mL cup (P100-0100; Solocup, IL, USA) containing sterile filter papers on the bottom. The larvae, which are more sensitive to desiccation, were placed into cups containing sterilized soil to provide support and avoid death by desiccation. In both cases, the cups were incubated at 25 ± 1 °C. The mortality of both adults and larvae were assessed after 14 d.

Statistical analysis

The bioassay data does not follow a normal distribution. Therefore, a non-parametric Kruskal-Wallis one-way analysis of variance was performed to see if there was any difference in the mortality associated with isolates. When the difference was statistically significant, a Mann-Whitney test was performed to analyse the response of specific pairs of isolates.

RESULTS AND DISCUSSION

The bioassays conducted on adults of the Japanese beetle show considerable variability among the 19 isolates of Hypocreales fungi (Fig. 1). Significant intra- and interspecific variability was found between the tested isolates (Wilcoxon-Mann-Whitney test, p = 0.0023). Both *Lecanicillium* isolates do not have any impact on the mortality of adults. On the other hand, *M. anisopliae* INRS 705 and *B. bassiana* INRS 236 isolates induced high levels of mortality in adults (70.3% and 65.2%, respectively). These results are in accordance with those of previous studies, which showed mortality rates near 60% and 79% after exposure of *P. japonica* adults to *M. anisopliae* and *B. bassiana* isolates, respectively (Lacey *et al.* 1994, 1995b; Klein and Lacey 1999). However, for each fungal species, variation in susceptibility to isolates was observed in adults. Mortality rate varied from 0 to 70.3% for *M. anisopliae*, from 0 to 65.2% for *B. bassiana*, and from 10.5 to 38.8% for *B. brongniartii*.

Several factors, such as the dose used (Vandenberg *et al.* 1998), food sources and the presence of fungal inhibitors (Hare and Andreadis 1983; Ramoska and

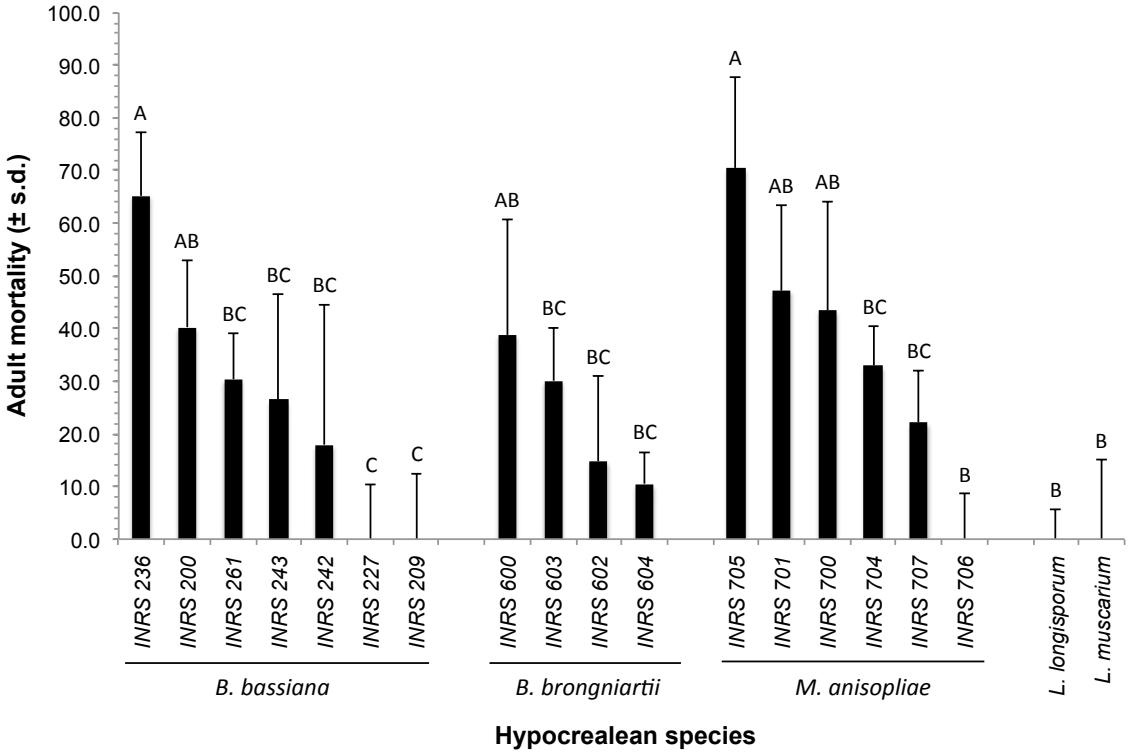

Fig. 1. Percentage of mortality of Japanese beetle adults exposed to 19 isolates of Hypocreales fungi (*Beauveria bassiana, Beauveria brongniartii, Metarhizium anisopliae, Lecanicillium longisporum* and *Lecanicillium muscarium*). Means with the same letter are not significantly different.

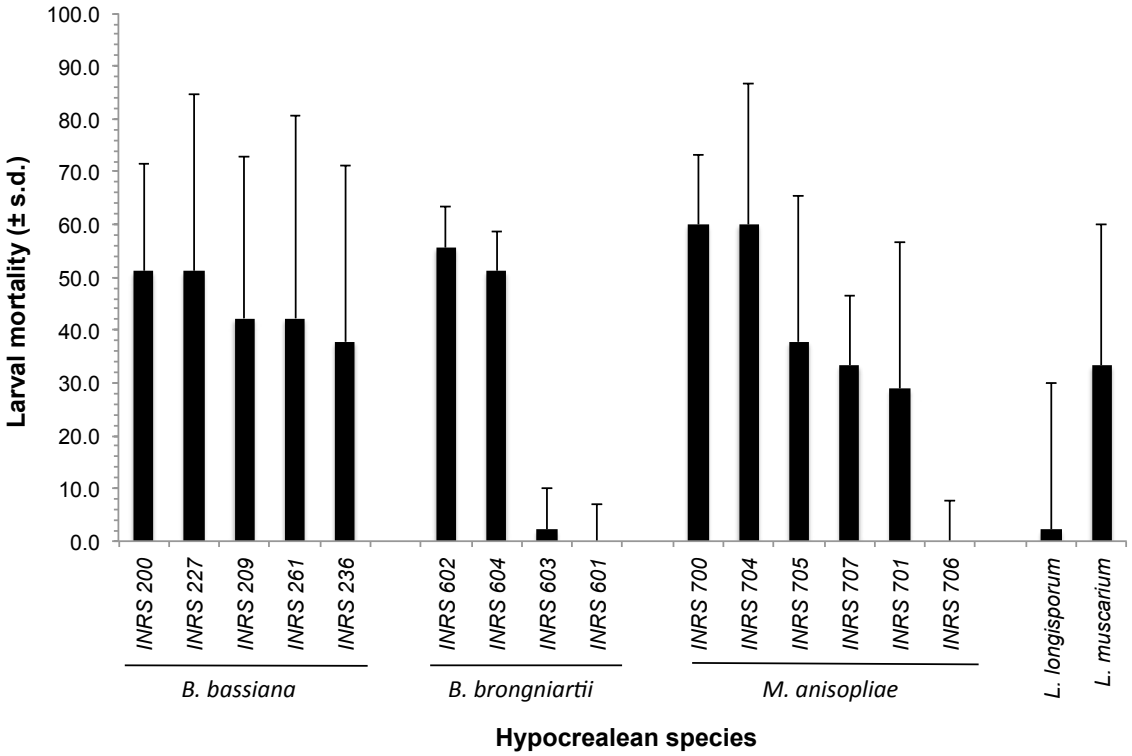

Fig. 2. Percentage of mortality of Japanese beetle larvae exposed to 17 isolates of Hypocreales fungi (*Beauveria bassiana, Beauveria brongniartii, Metarhizium anisopliae, Lecanicillium longisporum* and *Lecanicillium muscarium*). No significant difference was found between isolates.

Todd 1985), temperature (Vandenberg *et al.* 1998), and insect developmental stage (Vandenberg *et al.* 1998), may affect the pathogenicity of an entomopathogenic fungal isolate. However, these factors cannot explain the variation in mortality observed within the species tested. An individual isolate can exhibit a substantially restricted host range (Inglis *et al.* 2001). Moreover, isolates recovered from a closely related species are generally more virulent than isolates from non-related species (Sabbahi *et al.* 2008). Out of all the isolates tested, our results demonstrate the effectiveness of *M. anisopliae* INRS 705, which was isolated from soil sample, and *B. bassiana* INRS 236, which comes from a green metallic coleopteran insect. Both isolates seem to be good candidates for the biological control of adults of the Japanese beetle.

As with adults, an important variability in the susceptibility of Japanese beetle larvae exposed to 17 Hypocreales isolates was observed (Fig. 2). The highest mortality rates, which were superior to 55%, were recorded for *B. bassiana* INRS 200 and INRS 227, and for *M. anisopliae* INRS 602 and INRS 604. The susceptibility of larvae to *B. brongniartii* strongly varies between isolates, ranging from 0 (INRS 601) to 55.6% (INRS 602). Finally, the mortality rates recorded for isolates belonging to the *Lecanicillium* genus were 2.2 and 33.3% for *L. longisporum* and *L. muscarium*, respectively. However, no significant differences were detected when the Wilcoxon-Mann-Whitney test was applied to the results of all isolates ($p \geq 0.05$).

Only a few studies have demonstrated the potential of Hypocreales fungi for larval control (Krueger *et al.* 1991; Villani and Krueger 1994). The variability in mortality observed for each isolate and the lack of significant results allowing the identification of the most effective isolate may be attributed to the low number of larvae used for each replicate (n = 10). Experiments using less isolates and a higher number of larvae could be repeated with the most effective *B. bassiana*, *B. brongniartii* and *M. anisopliae* isolates. Although no statistically significant difference was observed, a positive trend in larval mortality rates can be distinguished in the bioassay using these three Hypocreales species. More specifically, the results obtained with *B. bassiana* INRS 200 and INRS 227, *B. brongniartii* INRS 602 and INRS 604, and *M. anisopliae* INRS 700 and INRS 704 suggest that biological approaches using these Hypocreales fungi can be effectively implemented to control populations of Japanese beetle larvae.

Larvae of *P. japonica* should be considered an important target in biological control programs in order to minimize the damage they induce to crops. The low mobility of larvae may facilitate the control of these populations (Potter and Held 2002). This study shows the potential of some Hypocreales isolates to control population of Japanese beetles. However, further studies will be needed to confirm the pathogenicity of the selected isolates and to implement efficient integrated management programs against larvae and adults of the Japanese beetle.

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