Efficacy of *Beauveria bassiana* against the strawberry pests, *Lygus lineolaris*, *Anthonomus signatus* and *Otiorhynchus ovatus*

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Abstract

There are several insect species causing serious economic losses in strawberry, *Fragaria vesca* L., productions. In Quebec, Canada, the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), the strawberry bud weevil clipper, *Anthonomus signatus* (Say) and the strawberry root weevil, *Otiorhynchus ovatus* (L.) are the most important pests. We tested the susceptibility of these pests to the entomopathogenic fungus *Beauveria bassiana* under laboratory conditions. Sixteen isolates were evaluated for their insecticide potential against these insects. Adults of each species were infected by the immersion method. All isolates were pathogenic to adults of all three species, causing mortality rates between 23.3% and 100% at a concentration of 1×10^7 conidia/ml. Based on the screening results, isolate INRS-CFL was selected for its insecticide potential and then used for further analyses against *L. lineolaris, A. signatus* and *O. ovatus* adults. Bioassays were performed to evaluate the lethal concentration (LC_{50}) and the average survival time (AST) of this isolate against both insect species. Results of dose–response mortality bioassays using four concentrations – 1×10^4, 1×10^6, 1×10^8 and 1×10^9 conidia/ml – indicated a LC_{50} values of 5.3×10^5, 1.8×10^7 and 9.9×10^7 conidia/ml at 7 days after inoculation for *L. lineolaris, A. signatus and O. ovatus* respectively. Using a dose of 1×10^8 conidia/ml, the AST values were estimated at 4.41, 7.56 and 8.29 days, respectively, at a concentration of 1×10^8 conidia/ml. This study demonstrated the potential of *B. bassiana* for the management of *L. lineolaris, A. signatus* and *O. ovatus*. Results also suggest that the heteropteran species is more susceptible than coleopteran species to *B. bassiana*.

Introduction

The tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois) (Het.: Miridae), the strawberry bud weevil clipper, *Anthonomus signatus* (Say) (Col.: Curculionidae), and the strawberry root weevil, *Otiorhynchus ovatus* (Linnaeus) (Col.: Curculionidae) are major pests of strawberry culture in Quebec (Mailloux and Bostanian 1993; Vincent and Bostanian 2005).

The tarnished plant bug is the most important pest of strawberries and can be found in several vegetable crops in North America (Handley and Pollard 1993). Nymphs and adults feed on flowers and developing fruit, causing malformation, what results in fruit downgrading. More than 50% of fruits can be downgraded as a result of *L. lineolaris* feeding on unsprayed fields (Young 1986; Easterbrook 2000).

With time, the tarnished plant bug populations have developed resistance to insecticides (Steinkraus and Tugwell 1997). It is therefore essential that alternative bio-based management strategies be developed to replace these compounds and reduce risks of environmental contamination. *L. lineolaris* natural enemies such as hymenopteran and dipteran parasitoids (Day et al. 1990; Day 1995) and polyphagous predators (Bostanian and Mailoux 1994) have been tested against this pest and it appears that they do not have the potential to become biocontrol agents.

The strawberry bud weevil clipper causes the most serious damage at the time of flowers pollination. *A. signatus* female lays their eggs in holes pierced in staminate buds. After oviposition, the affected bud stalk is girdled below the bud. The stem wilts and the bud droops and may later fall off (Vincent et al. 1990). Crop losses caused by *A. signatus* can be important when infestations are severe (Pritts et al. 1999).

Chemical insecticides are currently used in the management of *A. signatus* populations. Chlopyrifos (Lorsban) treatment reduces the number of weevils in the summer generation and the quantity of floral buds clipped in fields the following year (Chagnon et al. 1990). This control tactic has several deficiencies and is not completely effective. There is a need for alternative, biologically based insecticide materials that can be used to complement or replace labelled existing chemical insecticides. To our knowledge, no biological agent has been identified to control the strawberry bud weevil populations.

Buried in the soil, the larvae of the strawberry root weevil feed on the roots of their host, what reduces plant growth and eventually cause them to completely wither (Rutherford et al. 1987; Bostanian et al. 2003; Bruck 2004). *O. ovatus* adults may also feed on strawberry leaves causing a characteristic notching on the leaf edges. The leaf injury is not serious, but it serves as an alert to growers and scouts when adult root weevils are present (Bouchard et al. 2005).

Otiorhynchus ovatus larvae feed underground and because of their cryptic habitat, they are protected from most pesticide treatments. The application of chemical pesticides against the adult stage is the only suppression method currently used against this pest, but it is mainly larvae that cause most crop damage (Bruck 2004). However, chemical treatments against *O. ovatus* increase production costs, reduce populations of beneficial species, and may contribute to the development of insect resistance (Wright and Chandler 1991).

The use of biological agents to control pests, coupled with the loss of many insecticide registrations and increased environmental awareness, has simulated renewed interest in the development of alternate and environmentally compatible materials (Wright and Chandler 1991). The entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin have shown great potential for the management of various insect pests (Goettel et al. 1990; McCoy 1990; Feng et al. 1994; Adane et al. 1996; Jaronski and Goettel 1997; Rice and Cogburn 1999; Chikwenhere and Vestergaard 2001; Faria and Wraight 2001; Inglis et al. 2001; Tafoya et al. 2004). If microbial control agents are effective, one of the chief criticisms directed at these organisms remains the length of time they take to kill the target when compared with commonly used chemical insecticides. On the other hand fungi, and especially *B. bassiana*, often cause a long-term effect because of the persistence of the spores in the soil and their recovery after host infection (Inglis et al. 2001).

Beauveria bassiana is currently under investigation for the management of *Lygus* bugs populations (Bidochka et al. 1993; Snodgrass and Elzen 1994; Steinkraus and Tugwell 1997; Kouassi et al. 2003; Liu et al. 2003; Leland and McGuire 2006). Currently, there is no study evaluating the efficacy of *B. bassiana* against *A. signatus*. However, some isolates of this entomopathogenic fungus are pathogenic to certain related species of the Curculionidae family. Wright and Chandler (1992) indicated that boll weevil, *Anthonomus grandis* (Boheman), could become infected by *B. bassiana* either by direct spray contact or indirect contact with treated leaf.

For *Otiorhynchus* species, the larvae of both *O. ovatus* and *Otiorhynchus dubius* (Ström) have been shown to be susceptible to a *B. bassiana* formulation under laboratory conditions (Vainio and Hokkanen 1993). Furthermore, Moorhouse et al. (1993) and Bruck (2004) demonstrated that some *Metarhizium anisopliae* (Metchnikov) and *B. bassiana* isolates were pathogenic to the black vine weevil, *Otiorhynchus sulcatus* (Fabricius), larvae. In contrast, *Metarhizium flavoviride* and *Metarhizium album* isolates were not pathogenic to weevil larvae. Some *B. bassiana* isolates were pathogenic to adults and larvae of the clover root weevil, *Sitona lepidus* (Gyllenhal), causing high mortality within 2–4 weeks of inoculation (Willoughby et al. 1998).

This study was designed to select a highly virulent isolate of *B. bassiana* against adult populations of *L. lineolaris*, *A. signatus* and *O. ovatus* in hope to develop a biological strategy in Quebec strawberry crops.

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Materials and Methods

Insects rearing

Lygus lineolaris adults were collected with sweep nets and *A. signatus* adults were collected with a pot following striking from a floral pole from strawberry plantations in Mirabel (45°08′N; 74°05′W) Quebec, Canada. Potato sprouts and celery petiole were provided to *L. lineolaris* adults as food sources and oviposition sites, which were reared in a 30 × 30 × 60 cm meshed cages while *A. signatus* adults were kept on strawberry plants in 60 × 60 × 60 cm meshed cages. A Petri dish containing soaked cotton balls was placed in the cages to supply water. *O. ovatus* adults were collected using pitfall traps by burying a cup shaped-tray placed in the ground of infested strawberry greenhouses in Portneuf (47°00′ N and 72°00′ W) Quebec, Canada. *O. ovatus* adults were reared on strawberry plants in a greenhouse. All insect species were reared in growth chambers (MLR-350, Sanyo, Osaka, Japan) at 26°C, 70% relative humidity (RH), and 16 : 8 h [light : dark (L : D)] photoperiod for a period of 2 weeks prior to the laboratory bioassays.

Fungal production

The 16 isolates of *B. bassiana* used in this study are from the Institut National de la recherche scientifique (INRS)-Institut Armand-Frappier (Laval, Canada) and they have been isolated from a variety of hosts and geographical origins (table 1). Stock cultures of each isolate were kept frozen at −70°C in 70% glycerol. Isolates from diverse hosts and geographic regions were selected. The selected isolates were grown on sabouraud dextose agar (10% neopeptone, 40% dextrose, 15% agar, pH 5.6) (Difco Laboratories, Augsburg, Germany) in a growth chamber (MLR-350) under controlled conditions at 25°C, 80% RH, and darkness.

Mass production was attempted to produce suspensions of *B. bassiana* conidia for isolate screening and evaluation of insecticide potency. *B. bassiana* isolates were used to inoculate sterilized pearled barley cereal (Clic Import Export Inc., Montreal, QC, Canada) in plastic fungal spawn bags containing a filter of 0.22 μm (Fungi Perfecti, Olympia, WA). For each isolate, 100 ml of a concentration of 1×10^7 conidia/ml was added to 200 g of pearled barley, and the bags were sealed and placed in growth chambers for 14 days at 25°C in darkness. The cereal was manually crumbled within bags each other day to provide aeration throughout the culture substrate. For each bag, conidia were harvested by adding and mixing 200 ml of deionized water. The suspension was then filtered through two layers of cheesecloth to remove mycelia and barley beads. The concentration of conidia suspension was determined using a haemacytometer (Bright-Line Improved Neubauer; Hausser

Table 1 Host and place of origin of *Beauveria bassiana* isolates

Isolate	Host	Geographic origin
INRS-CFL	*Tomicus piniperda* (Col.: Scolytidae)	Quebec, Canada
INRS-IP	*Lygus* sp. (Het.: Miridae)	Quebec, Canada
INRS-2001	Hemiptera	Quebec, Canada
ARSEF 1322	*Lygus lineolaris* (Het.: Miridae)	Senneville, France
ARSEF 1516	*Adelphocoris* sp. (Het.: Miridae)	Senneville, France
ARSEF 2988	*Leptinotarsa decemlineata* (Col.: Chrysomelidae)	Quebec, Canada
ARSEF 1395	N.A.	Seine-St Denis, France
ARSEF 2991	*Leptinotarsa decemlineata* (Col.: Chrysomelidae)	Quebec, Canada
DAOM 196605	*Melanopus bivittatus* (Orth.: Acrididae)	Saskatchewan, Canada
DAOM 210087	*Leptinotarsa decemlineata* (Col.: Chrysomelidae)	Quebec, Canada
DAOM 210569	Metallic green beetle, under plants on ground	British Columbia, Canada
LRS 12	*Hypera postica* (Col.: Curculionidae)	Drome, France
LRS 20	Soil	Benin, Africa
LRS 33	Soil	Alberta, Canada
LRS 49	*Galerucella calmariensis* (Col.: Chrysomelidae)	Alberta, Canada
IPP 206	Heteroptera	Bulgaria

INRS: Institut National de la recherche scientifique, Institut Armand Frappier, Entomopathogenic Fungi Collection, Laval, QC, Canada.
ARSEF: Agriculture Research Service of Entomopathogenic Fungi, USDA, Ithaca, NY, USA.
DAOM: Eastern Cereal and Oilseed Research Centre, Ottawa, ON, Canada.
LRS: Lethbridge Research Station, Agriculture and Agri-Food Canada, AB, Canada.
IPP: Institute of Plant Protection, Sofia, Bulgaria.

Scientific, Horsham, PA) under light microscope. Viability of conidia of each suspension was also evaluated by measuring the per cent 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 fungal suspensions were kept at 4°C until use.

Isolate screening

To select the most virulent *B. bassiana* isolates against the three pests, screening tests were performed using 16 isolates. In our bioassay, the fungus–host contact has been maximized by immersion of the host in a conidial suspension. The inoculation of insects was then performed by dipping individually 20 adults of each species for 5 s in 50 ml suspensions at 1×10^7 conidia/ml (Butt et al. 1994). The insects were then kept individually on wet filter paper (Whatman International Ltd, Maidstone, England, UK) in a 9 cm diameter Petri dish for incubation in a growth chamber at 25°C, 70% RH and 16 : 8 h (L : D) photoperiod. Control insects were dipped in deionized water. Pieces of strawberry buds and flowers were added to Petri dishes as food source and filter papers were kept moist throughout the experiments. Mortality was recorded daily over a period of 7 days for *L. lineolaris* and over a period of 14 days for *A. signatus* and *O. ovatus*. Dead insects were kept in a growth chamber at 25°C, 90% RH and darkness for 2 weeks to promote fungal outgrowth. *B. bassiana* was identified based on macro- and microscopic characteristics (Humber 1997). The experiment was repeated three times. The replicated treatments were made from different culture bugs. The most virulent isolates were identified based on the percentage of mortality obtained with *L. lineolaris*, *A. signatus* and *O. ovatus* adults.

Insecticide potency

The INRS-CFL isolate was selected based on isolate screening on the three insect species, and according to their geographic origin. The isolate Agriculture Research Service of Entomopathogenic Fungi (ARSEF) 2988 was discarded for further evaluation based on its detrimental effect on *Coleomegilla maculata lengi* (Timberlake) (Sabbahi et al., in press), a beneficial insect of strawberry crops. The lethal concentrations ($LC_{50,\ 90}$ values) and the average survival time (AST) of the INRS-CFL isolate were estimated on *L. lineolaris*, *A. signatus* and *O. ovatus* adults. Conidia mass production was homogenized and a serial dilution was performed to generate concentrations of 1×10^4, 1×10^6, 1×10^8 and 1×10^9 conidia/ml. A haemacytometer was used to confirm those concentrations. For each concentration, 20 adults of each species were individually immersed for 5 s in 50 ml suspensions and transferred on a wet filter paper in a 9 cm diameter Petri dish, and then incubated in a growth cabinet at 25°C, 70% RH and 16 : 8 h (L : D) photoperiod. Pieces of strawberry buds and flowers were added to Petri dishes as food source. Dead insects were kept in a growth chamber at 25°C, 90% RH and darkness for 2 weeks to promote fungal outgrowth. *B. bassiana* was identified based on macro- and microscopic characteristics (Humber 1997). There were four replicates per treatment. The replicated treatments were made from different culture bugs. The same experiment was conducted separately to estimate the AST values using a unique concentration of 1×10^8 conidia/ml.

Statistical analysis

Data are expressed as means ± SD. Mortality data from the screening tests were corrected for natural mortality (Abbott 1925), arcsine square-root transformed and subjected to analysis of variance using the general linear model procedure (PROC GLM) (SAS Institute 2002). Means were then subjected to Tukey's studentized range test (HSD) to determine if there was evidence of significant differences between isolates. Estimates of the $LC_{50,\ 90}$ values at 7 days post-inoculation were computed using probit analysis (PROC PROBIT, SAS). Estimates of the AST values at a concentration of 1×10^8 conidia/ml were performed by using the survivorship analysis (PROC LIFETEST, SAS). Estimates of LC_{50} values were compared between insect species on the basis of overlapping 95% confidence limits. Pair-wise comparisons between insect species were performed on AST data using a log-rank chi-squared test.

Results

Isolate screening

Our results demonstrated that all *B. bassiana* isolates tested caused significant mortality (based on macro- and microscopic characteristics) in adults of *L. lineolaris* ($F = 48.19$; d.f. = 16, 50; $P < 0.01$) (fig. 1), *A. signatus* ($F = 26.83$; d.f. = 16, 50; $P < 0.01$) (fig. 2), and *O. ovatus* adults ($F = 44.51$; d.f. = 16, 50; $P < 0.01$) (fig. 3) when compared with the control insect cohorts. As time progressed, cumulative

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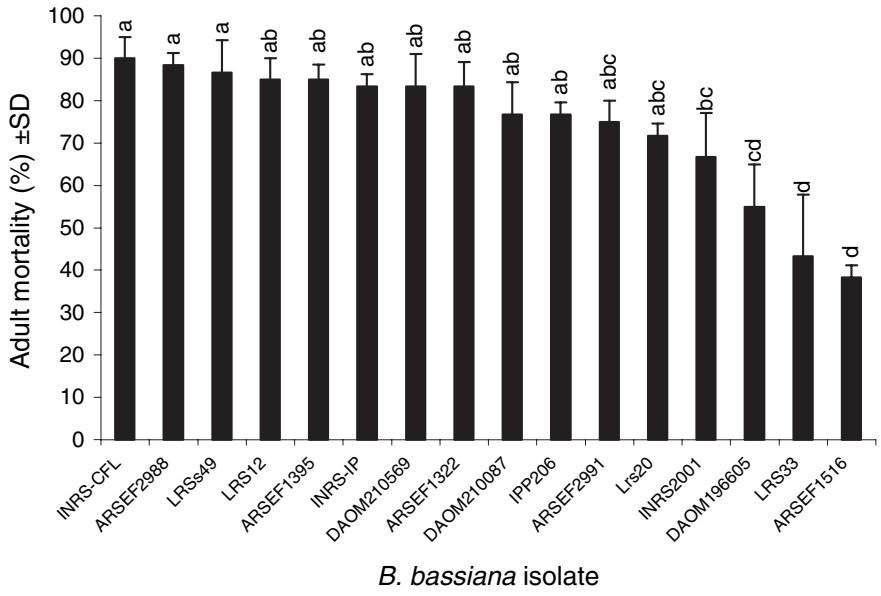

Fig. 1 Per cent mortality of adult *Lygus lineolaris* following treatment with 16 *Beauveria bassiana* isolates after 7 days post-treatment. Results are expressed as a percentage of the number of adults at the beginning of the bioassay (n = 60). Bars with different letters are significantly different (Tukey's HSD test: $P < 0.05$).

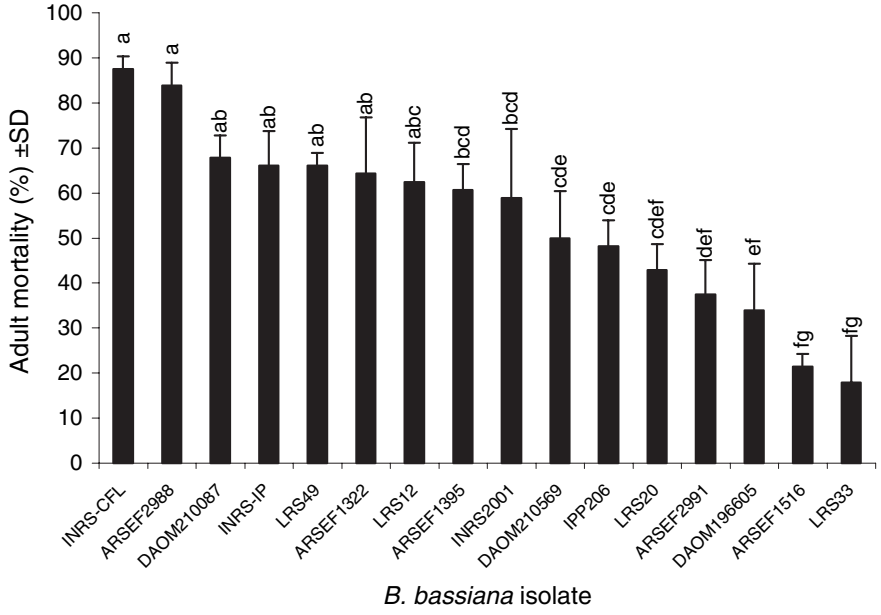

Fig. 2 Per cent mortality of adult *Anthonomus signatus* following treatment with 16 *Beauveria bassiana* isolates after 14 days post-treatment. Results are expressed as a percentage of the number of adults at the beginning of the bioassay (n = 60). Bars with different letters are significantly different (Tukey's HSD test: $P < 0.05$).

mortality for all isolates increased but at differing rates. No mortality attributable to *B. bassiana* infection occurred in the controls. Inoculated dead insects showed mycelial growth and this fungal outgrowth was used to validate the death by fungal infection (Moorhouse et al. 1993; Vandenberg et al. 1998). Each isolate had different effects on the three pests; however, the screening performed on *L. lineolaris* adults revealed that more than 86% of mortality was obtained with the isolates INRS-CFL, ARSEF 2988 and LRS 49 7 days after inoculation. No significant difference was observed between *L. lineolaris* and *A. signatus* adults exposed to INRS-CFL and ARSEF 2988 isolates 7 days after inoculation. On the other hand, *O. ovatus* adults seemed to be more susceptible to ARSEF 2988 isolate, followed by INRS-CFL and ARSEF 1395 isolates, 14 days after inoculation.

Insecticide potency

During the dose–response mortality bioassays using the INRS-CFL isolate, there was a proportional increase in the insect mortality with time and with

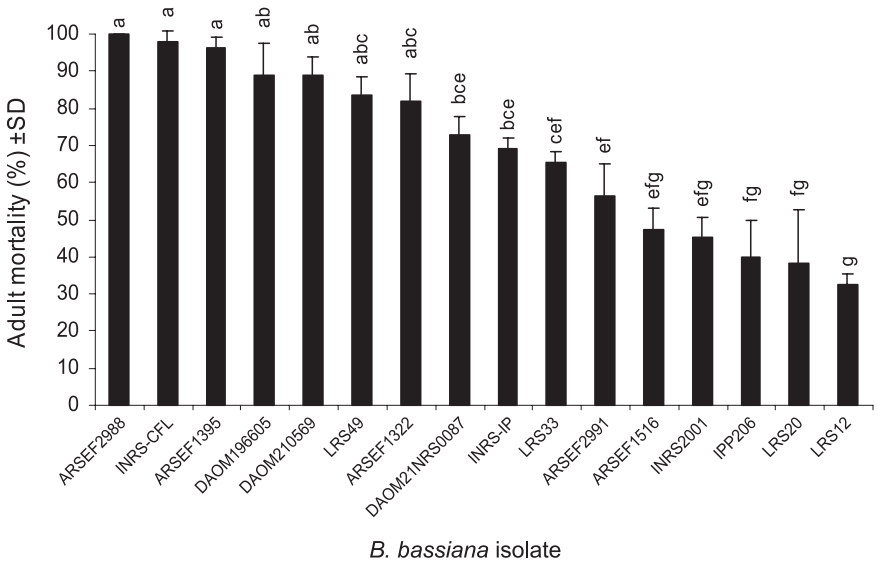

Fig. 3 Per cent mortality of adult *Otiorhynchus ovatus* following treatment with 16 *Beauveria bassiana* isolates after 14 days post-treatment. Results are expressed as a percentage of the number of adults at the beginning of the bioassay (n = 60). Bars with different letters are significantly different (Tukey's HSD test: $P < 0.05$).

the increase of inoculum concentrations. The LC_{50} values at 7 days were 5.3×10^5, 1.8×10^7 and 9.9×10^7 conidia/ml for *L. lineolaris*, *A. signatus* and *O. ovatus* adults respectively (table 2). In addition to its efficacy, the INRS-CFL isolate also revealed higher vegetative growth and spore yield (personal communication).

Results of the lethal time tests showed that INRS-CFL isolate is highly virulent to adults of the three studied species (mortality ranged from 54% to 77%). Precisely, the AST values at a concentration of 1×10^8 conidia/ml were 4.37, 6.58 and 7.80 days for *L. lineolaris*, *A. signatus* and *O. ovatus* adults respectively (table 3). The variation of AST values was statistically significant (P-values from log-rank chi-squared test all <0.0004).

Discussion

For the first time, our results clearly indicated the susceptibility of *A. signatus* and *O. ovatus* to the fungus *B. bassiana*. For *L. lineolaris*, however, this experiment allowed to confirm the susceptibility of this pest to *B. bassiana* isolates. Following the screening of several *B. bassiana* isolates, Todorova (1998) identified certain pathogenic isolates against *L. lineolaris*. The pathogenicity of some *B. bassiana* isolates was also reported for *Lygus rugulipennis* (Poppius) (Bajan and Bilewicz-Pawinska 1971).

During this experiment, isolates of *B. bassiana* exhibited variations in virulence and pathogenicity against the tested insect species. Similar observations, with other species, were also previously reported (Fargues 1972; Prior 1990; Tanada and Kaya 1993). Such variations have been observed by Butt et al. (1992) with six isolates of *M. anisopliae* against the chrysomelid beetles *Psylliodes chrysocephala* L. and *Phaedon cochleariae* F. Furthermore, Kassa et al. (2002) also noted variations with different *B. bassiana* strains from Ethiopia against the storage pests, *Sitophilus zeamais* (Motsch) and *Prostephanus truncates* (Horn). Previous studies have shown that the physiological characteristics and enzyme production among fungal isolates could be responsible for the observed variation in their virulence (Bidochka and Khachatourians 1990; Feng and Johnson 1990;

Table 2 Lethal concentration (LC_{50}) values of *Beauveria bassiana* INRS-CFL isolate against *Lygus lineolaris*, *Anthonomus signatus* and *Otiorhynchus ovatus* adults at 7 days post-treatment

Insect species	LC_{50} (conidia/ml) (95% fiducial limits)	LC_{90} (conidia/ml) (95% fiducial limits)	Slope ± SE	χ^{2*}
L. lineolaris	5.33×10^5 (9.48×10^4–1.84×10^6)	4.16×10^9 (9.04×10^8–4.35×10^{10})	0.33 ± 0.04	19.3
A. signatus	1.86×10^7 (3.50×10^6–6.30×10^7)	3.90×10^{11} (6.10×10^{10}–8.10×10^{11})	0.32 ± 0.05	18.3
O. ovatus	9.99×10^7 (1.22×10^7–8.15×10^8)	2.80×10^{12} (1.11×10^{11}–3.41×10^{12})	0.29 ± 0.03	26.5

*$P < 0.01$.

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Table 3 Per cent mortality and average survival time (AST) ± SE for adults of *Lygus lineolaris*, *Anthonomus signatus* and *Otiorhynchus ovatus* after inoculation with *Beauveria bassiana* INRS-CFL isolate at a concentration of 1×10^8 conidia/ml

Insect species	Per cent mortality ± SE	AST in days ± SE
L. lineolaris	77.47 ± 4.1	4.41 ± 0.3
A. signatus	60.35 ± 3.9	7.56 ± 0.6
O. ovatus	54.50 ± 3.0	8.29 ± 0.6

Butt et al. 1992; Varela and Morales 1996). In addition, the higher mortality induced by most efficient *B. bassiana* isolates might be attributable to the arrays of mycotoxins produced by each isolate (Leland et al. 2005).

Many researchers have noted that the highest levels of isolate virulence were observed when isolates were used on the same insect species or a closely related species on which they have been collected (Latch 1976; Soares et al. 1983; Poprawski et al. 1985). This contrasts with the results obtained in the current study, as there is no relationship between the level of pathogenicity and the origin of isolates. All *B. bassiana* isolates performed well against the tarnished plant bug, although they were not originally isolated from *Lygus* bugs (table 1; fig. 1).

In this study, *B. bassiana* isolates had shown to be pathogenic to the three insect species, which belong to two orders. *B. bassiana* has an extensive host range extended to several orders (Willoughby et al. 1998). While individual isolates are not pathogenic to all recorded hosts for the species, isolates will often infect multiple hosts to varying degrees (Willoughby et al. 1998). In the present study, among the sixteen *B. bassiana* isolates tested, INRS-CFL isolate was highly virulent against all the strawberry pests tested (figs 1–3). The choice of this isolate to conduct further analyses was justified by its efficacy against the three pests and the fact that this isolate is native from Quebec, what minimizes the environmental risks related to field application.

With other *B. bassiana* isolates, Noma and Strickler (1999) and Steinkraus and Tugwell (1997) obtained a LC_{50} value of 1.9×10^6 and 2.2×10^6 conidia/ml respectively on *Lygus hesperus* and *L. lineolaris* adults at 5 days post-treatment. Variations of LC_{50} values can be explained by the virulence variations of different isolates to a species of host insect, or the virulence variations of a single isolate to related species of the host insect (Khachatourians 1992).

The AST value obtained in this study for *L. lineolaris* adults was similar to those reported by Kouassi et al. (2003) and Bidochka et al. (1993) when tested on *L. lineolaris* and *Lygus borealis* (Kelton) adults at a concentration of 1×10^8 conidia/ml.

There are few reports in the literature on the susceptibility of related species of *A. signatus* and *O. ovatus* to *B. bassiana*. When tested on adult boll weevils, *A. grandis* (Boheman), a lower LC_{50} value of 7.94×10^5 conidia/ml was obtained (Wright and Chandler 1991) compared with our results on *A. signatus*. With different *M. anisopliae* isolates, Moorhouse et al. (1993) obtained similar LT_{50} values ranging from 6.09 to 8.72 days for *O. sulcatus* larvae, at a concentration of 1×10^7 conidia/ml compared with our results on *O. ovatus*. Furthermore, different *M. anisopliae* and *B. bassiana* isolates were also virulent to *S. zeamais* (92–100% mortality, AST ranged from 3.58 to 6.28 days) (Kassa et al. 2002).

For the control of pest insect populations in strawberry crop, it is essential to select highly virulent isolates to limit damage as the aesthetic criteria prevailing in these crops are very important. The isolate INRS-CFL appears to be an interesting biocontrol agent as it was virulent and induced high mortality of the three pests in 7 days at a concentration of 1×10^7 conidia/ml. Furthermore, adults' cadavers of each species supported fungal sporulation. This may be important for any control strategy aimed at attracting bugs or beetles to fungus contaminated traps, and subsequent transfer to adults or larvae in strawberries.

The differences observed in LC_{50} and AST values in the isolate INRS-CFL for different insects tested in this study could reflect characteristics of the insect host. The level of virulence of different *M. anisopliae* isolates for two chrysomelid beetles (*P. chrysocephala* and *P. cochleariae*) varied considerably (Butt et al. 1992). Our results suggested that INRS-CFL isolate is more effective against *L. lineolaris* than *A. signatus* and *O. ovatus*. It has been previously observed that soft-bodied insects have a weaker resistance to the fungus, unlike those with a harder cuticle (Butt et al. 1995). There was an observable fungal outgrowth onto the cuticle of the three species tested. Further researches must be carried out with several species belonging to different orders to verify if *B. bassiana* is more effective against heteropteran than coleopteran species.

This is the first time that the effect of several *B. bassiana* isolates on the mortality of three strawberry pests has been investigated. The bioassay results provide clear indications that isolate INRS-CFL induce high mortality in these pests. Our results

suggest that *B. bassiana* has a great insecticide potential for the management of the principal insect pests of strawberry in Quebec, Canada. The isolate INRS-CFL, selected following the screening of 16 *B. bassiana* isolates, would constitute a good candidate in a biological control programme against *L. lineolaris*, *A. signatus* and *O. ovatus*.

The use of *B. bassiana* is considered a potentially efficient component of integrated pest management strategies, but little is known with respect to the best ways to apply it in a microbial control context. It seems that multiple applications of *B. bassiana* conidia in strawberries, during the blooming period, provide control of nymph *L. lineolaris* and adult *A. signatus* populations (Sabbahi et al., in press; R. Sabbahi and C. Guertin, unpublished data). Little is known about *O. ovatus* populations control by *B. bassiana* in the strawberry field conditions. However, applying a prophylactic treatment of the root systems of the plants with *M. anispopliae* conidia at the time of planting is a potentially useful method of protection against this pest (Vainio and Hokkanen 1993).

Experiments are now required to determine the effects of *B. bassiana* applications for the control of populations of *L. lineolaris*, *A. signatus* and *O. ovatus* in strawberries. Furthermore, the possible effects of *B. bassiana* against non-target agricultural arthropods associated to strawberry crop needs also to be explored. Other fungal characteristics such as spore production, germination, and hyphal growth rates and effects of varying environmental conditions that influence persistence must also be evaluated, so that the further development of the most appropriate strain can proceed.

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