Phytoprotection

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S.I. Todorova, D. Coderre and J.-C. Côté

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Article abstract

Ten isolates of *Beauveria bassiana* from different sources and geographical sites were evaluated under laboratory conditions at a concentration of 107 conidia ml^{-1} for their pathogenicity against two insect pests, the Colorado potato beetle (*Leptinotarsa decemlineata*) and the green peach aphid (*Myzus persicae*), and their predator, the spotted ladybird beetle (*Coleomegilla maculata lengi*). Six isolates were highly virulent to all three insect species. Four others showed different degrees of specificity. The isolates 49, 233 and 210087 were the most interesting for their potential development as biological control agents because they were highly virulent for the two insect pests and caused low mortality in the coccinellid.

Pathogenicity of *Beauveria bassiana* isolates toward *Leptinotarsa decemlineata* [Coleoptera : Chrysomelidae], *Myzus persicae* [Homoptera : Aphididae] and their predator *Coleomegilla maculata lengi* [Coleoptera : Coccinellidae]

Silvia I. Todorova[1], Daniel Coderre[1] and Jean-Charles Côté[2]

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Ten isolates of *Beauveria bassiana* from different sources and geographical sites were evaluated under laboratory conditions at a concentration of 10^7 conidia ml^{-1} for their pathogenicity against two insect pests, the Colorado potato beetle (*Leptinotarsa decemlineata*) and the green peach aphid (*Myzus persicae*), and their predator, the spotted ladybird beetle (*Coleomegilla maculata lengi*). Six isolates were highly virulent to all three insect species. Four others showed different degrees of specificity. The isolates 49, 233 and 210087 were the most interesting for their potential development as biological control agents because they were highly virulent for the two insect pests and caused low mortality in the coccinellid.

[Effet pathogène de différents isolats de *Beauveria bassiana* sur *Leptinotarsa decemlineata* [Coleoptera : Chrysomelidae], *Myzus persicae* [Homoptera : Aphididae] et leur prédateur *Coleomegilla maculata lengi* (Coleoptera : Coccinellidae]]

L'effet pathogène de dix isolats de *Beauveria bassiana* de différentes sources et provenances géographiques a été évalué au laboratoire à une concentration de 10^7 conidies ml^{-1} sur deux insectes ravageurs, le doryphore de la pomme de terre (*Leptinotarsa decemlineata*) et le puceron vert du pêcher (*Myzus persicae*), et leur prédateur, la coccinelle maculée (*Coleomegilla maculata lengi*). Six isolats ont provoqué une mortalité élevée sur les trois espèces d'insectes. Les quatre autres ont démontré un différent degré de spécificité pour les insectes visés. Les isolats 49, 233 et 210087 se sont avérés les plus intéressants comme agents de lutte biologique parce qu'ils ont démontré une forte virulence pour les insectes nuisibles mais ne causant qu'une faible mortalité pour la coccinelle.

1. Université du Québec à Montréal, Département des Sciences biologiques, C. P. 8888, Succ. Centre-Ville, Montréal, Quebec, Canada H3C 3P8; e-mail : todorova@total.net
2. Agriculture and Agri-Food Canada, 430 Gouin Blvd., Saint-Jean-sur-Richelieu, Quebec, Canada J3B 3E6

INTRODUCTION

The entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin is a promising control agent against many insect pests (McCoy 1990). *B. bassiana* occurs naturally in populations of the Colorado potato beetle (CPB), *Leptinotarsa decemlineata* Say [Coleoptera : Chrysomelidae], economically the most important insect pest of potatoes in North America (Radcliffe *et al.* 1991). Several studies have demonstrated the high potential of this fungus for CPB control with foliar or soil application (Anderson *et al.* 1988; Hare and Andreadis 1983; Watt and LeBrun 1984). A second insect pest in the potato crop system is the green peach aphid (GPA), *Myzus persicae* (Sulzer) [Homoptera : Aphididae] (Boiteau *et al.* 1995). Different strains of *B. bassiana* were also found to be pathogenic to several species of aphids such as cereal aphids (Feng and Johnson 1990; Feng *et al.* 1990; Wang and Knudsen 1993) and hop aphids (Dorschner *et al.* 1991).

The spotted ladybird beetle (SLB), *Coleomegilla maculata lengi* Timberlake [Coleoptera : Coccinellidae], a polyphagous predator, is another important biological control agent of CPB and GPA (Giroux *et al.* 1996; Groden *et al.* 1990; Hazzard and Ferro 1991).

In Biological Control and Integrated Pest Management programs, it is essential to know not only the effects of biological agents on pest insects but also the possible interactions between different control agents. *B. bassiana* is considered as a pathogen with broad host range, infecting different insect orders (Goettel *et al.* 1990). Laboratory and field experiments had demonstrated different susceptibility of *C. maculata* to different isolates of *B. bassiana* (Goettel *et al.* 1990; Magalhaes *et al.* 1988; Pingel and Lewis 1996; Todorova *et al.* 1994).

The objective of this study was to test in the laboratory the effect of 10 highly pathogenic isolates of *B. bassiana* toward *L. decemlineata* on *Myzus persicae* and their predator *C. maculata lengi*.

MATERIALS AND METHODS

Fungal isolates

Ten isolates of *B. bassiana* were used in this study. Their original host and country of origin are listed in Table 1. The fungal isolates were selected because they caused more than 61% mortality among CPB adults at 10^7 conidia ml^{-1} concentration from among 60 isolates tested in preliminary bioassays (Todorova 1998). The isolates were stored at -80°C on slopes of Sabouraud dextrose agar (SDA; Difco).

Rearing of insects

CPB adults and GPA were collected from a potato field in L'Epiphanie, Quebec, Canada (lat. 45°50' N, long. 73°25' W). Insects were maintained in cages with

Table 1. Isolates of *B. bassiana* used in this study, their number and their provenance

Original collection number	Host or source	Country of origin
ARSEF[a] 252, 353	Coleoptera : Chrysomelidae	USA, Maine
ARSEF 2990, 210087	Coleoptera : Chrysomelidae	Canada, Quebec
INRA[b] 28	Coleoptera : Chrysomelidae	France
LRS[c] 49	Coleoptera : Galerucinae	Canada, Alberta
IPP[d] 226, 233	Coleoptera spp.	Bulgaria
IPP 46	Lepidoptera spp.	Bulgaria
LRS 20	soil	Benin

[a] ARS Collection of Entomopathogenic Fungi, USDA, Ithaca, New York, USA.
[b] Institut National de la Recherche Agronomique, Paris, France.
[c] Lethbridge Research Station, Agriculture and Agri-Food Canada, Alberta, Canada.
[d] Institute of Plant Protection, Bulgaria.

potato plants ("Kennebec" cv.) under controlled conditions (23°C, 40% RH, 16 L : 8 D).

C. maculata lengi were collected, from hibernation sites in Saint-Hyacinthe, Quebec, Canada (lat. 45°39' N, long. 72°56' W). They were reared at 24°C, 60% RH and a photoperiod of 16 L : 8 D on a diet of flower pollen and liver-based artificial diet (Coderre, unpublished data).

Bioassays

Conidia from 20 day-old sporulating cultures, grown on SDA, were gently harvested using a sterile swab and added to the saline solution (0.85% NaCl; 0.1% Triton X-100). The concentration was adjusted to 1 x 10^7 conidia ml^{-1} based on preliminary bioassays. Groups of 10 CPB, 20 GPA or 10 SLB were inoculated by immersion for 5 s in 10 ml of conidial suspension of each isolate as described by Butt *et al.* (1994). Control insects were treated as above, but without fungus. Each assay consisted of three replicates.

After inoculation, each group of 10 CPB adults was transferred to 150 mm x 10 mm Petri dishes lined with moist Whatman no. 1 filter paper and were supplied with fresh potato plant leaves. Leaves were replaced daily. Twenty-five grams of flower pollen per 150 mm x 10 mm Petri dish were used as diet for each group of treated SLB adults. Each group of GPA adults were placed on a potato leaf in 50 mm x 10 mm Petri dishes. All treated insects were incubated at 24°C and a 16 L : 8 D photoperiod. Mortality was noted daily for 8 d.

The results were arcsin-transformed and analysed using a one-way ANOVA followed by Fisher's PLSD test. Statistical tests were done using SuperANOVA^{tm} 1.1 for Macintosh (Abacus Concepts Inc. 1989).

RESULTS

Pathogenecity of *B. bassiana* isolates

The effects of *B. bassiana* on CPB mortality differed significantly between isolates (ANOVA; $F = 41.3$; df = 10, 28; $P = 0.0001$) (Fig. 1a). All 10 isolates tested caused a mortality significantly different from the control group by 8 d post-treatment (Fisher's Protected LSD test, $P = 0.0001$). External signs of infection were first visible at 4 d post-infection and were characterised by mycelial growth over the insect cadaver. The highest mortality by 8 d post-treatment were 100, 93.3, 90 and 86.7% for isolates 353, 46, 210087 and 252, respectively.

Pathogenicity of *B. bassiana* for GPA differed significantly between isolates (ANOVA; $F = 5.18$; df = 10, 25; $P = 0.0004$) (Fig. 1b). Mortality caused by fungal infection was first observed at 2 d post-treatment for all fungal isolates except for isolate 2990. However, for this period, only isolates 28 and 252 caused a mortality significantly different from the control (LSD, $P < 0.045$). During the next 2 d, the mortality due to isolate 353 increased sharply up to 87.8%. Isolates 353 and 252 were the only ones that caused a mortality significantly different from the control by 4 and 6 d post-infection period (LSD, $P = 0.001$). By 8 d post-infection, the isolates 49 and 353 caused 100% mortality of GPA, significantly different from the control (LSD, $P = 0.0001$). Isolates 252, 20, 210087, 46 and 28 caused also a mortality significantly different from the control, respectively of 98.8, 92.7, 89, 85.4 and 75.6% (LSD, $P < 0.015$).

C. maculata lengi adults were less susceptible to *B. bassiana* isolates tested than CPB and GPA (Fig. 1c). Four d after treatment, only isolate 353 caused a mortality different from the control (20%) (LSD, $P = 0.01$). After this period, the effect of *B. bassiana* on SLB differed significantly between isolates (ANOVA; at 6 d post-treatment : $F = 5.95$, df = 10, 28; $P = 0.0001$; at 8 d post-treatment : $F = 9.33$; df = 10, 28; $P = 0.0001$). At 8 d post-treatment, isolate 353 caused the highest mortality (76.7%). Mortality caused by isolates 252, 2990, 28, 226, 46 and 20 were between 20 and 70%, significantly higher than the control (LSD, $P < 0.008$). Isolates 233, 210087 and 49 caused a mortality to SLB adults not different from the control (LSD, $P > 0.052$).

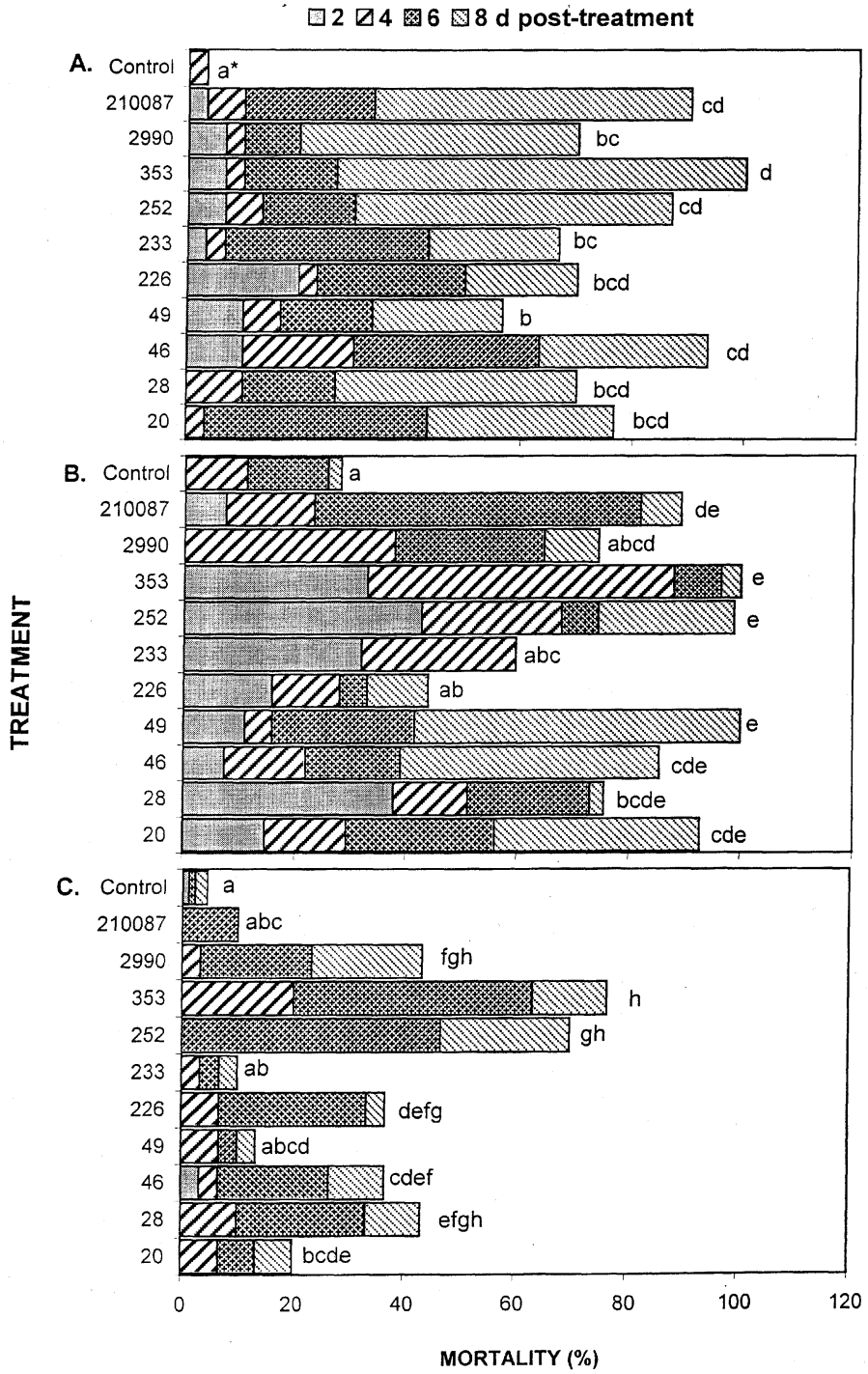

Figure 1. Effect of *B. bassiana* isolates at 10^7 conidia ml^{-1} concentration on *L. decemlineata* (A), *M. persicae* (B) and *C. maculata lengi* (C) mortality. *Different letters indicate that means are significantly different from each other (Fisher's Protected LSD tests; $P < 0.05$).

Virulence of selected *B. bassiana* isolates

Isolates 49, 233 and 210087 were selected on the basis that they were the only ones that cause a mortality of SLB not significantly different from the control by 8 d post-infection (Fig. 1c). Cumulative percentage of mortality was plotted against days post-infection (Fig. 2). The slopes of each curve provided additional information on isolate virulence.

Only isolate 49 caused a mortality on CPB significantly different from the control at 2 and 4 d post-infection (LSD, $P = 0.03$ and $P = 0.02$) (Fig. 2a). However, after this period, the mortality due to the three isolates increased sharply to reach 56.7, 66.7 and 90% for isolate 49, 233 and 21007, respectively, by 8 d post-infection.

At 6 d post-infection, mortality of GPA caused by all three isolates were significantly not different from the control (LSD, $P > 0.18$) (Fig. 2b). However, isolate 233 caused 59.7% mortality in only 4 d. Later, this isolate stopped its activity. Isolates 49 and 210087 caused 100 and 89% mortality, respectively, by 8 d post-infection. For SLB, the highest mortality by 8 d post-treatement was 13.3% for isolate 49, but not significantly different from control (LSD, $P = 0.052$) (Fig. 2c).

DISCUSSION

Several studies have shown that the same insect host can be resistant to certain strains of *B. bassiana* and very sensitive to others (Butt *et al.* 1994; Fargues 1972; Todorova *et al.* 1994, 1996). Certain strains may show no pathogenicity to one host and cause high mortality on other insects in the same order (Fargues 1976; Todorova *et al.* 1994).

Significant differences were found in mortality between the 10 *B. bassiana* isolates tested on *L. decemlineata, M. persicae* and *C. maculata lengi* (Fig. 1). Isolates 353, 252, 2990, 46, 28 and 20 caused high mortality among the three insect species. However, the other isolates demonstrated a different degree of specificity.

The isolates 49 and 210087 appear the most promising for biological control utilisation because they were highly virulent by 8 d post-infection to the two pests but not to their predator. Mortality due to isolate 49 was 56.7%, 100% and 13.3% for CPB, GPA and SLB, respectively. Isolate 210087 exhibited 100% mortality against CPB, 89% against GPA and only 10% on SLB.

The isolate 233 showed also an interesting potential for future investigations. Though this isolate caused a mortality on GPA not significantly different from the control, it appears to act more quickly than isolates 49 and 210087. Increasing concentration higher than 10^7 conidia ml^{-1} might give very high mortality of the pest species in a shorter time than the two other strains.

The fungal pathogenesis is a complex process and is dependent upon the attributes of both, the pathogen and the host. The cuticle appears to influence all stages of the infection process : adhesion, germination and appressorium differentiation (Butt 1990). In our experiments, the aphids died more quickly than the beetles, presumably because their soft bodies posed a weaker barrier to infection than the hard, sclerotinized beetle cuticle (Butt *et al.* 1995).

In integrated pest management programs, it is important to use compatible biological agents. *C. maculata lengi* favours shaded humid microhabitats (Coderre and Tourneur 1986; Ewert and Chiang 1966). These conditions increase the probability of contact with *B. bassiana* compared to other coccinellids (Pingel and Lewis 1996) and therefore suggest a more cautious approach in the selection of *B. bassiana* isolates. The present study indicated that *B. bassiana* isolates 49, 233 and 210087 are compatible with *C. maculata lengi* and present an interesting potential for the control of *L. decemlineata* and *M. persicae*. Further studies are however needed to define the environmental factors as temperature, humidity, etc., influencing the infection and effects of *B. bassiana* isolates on CPB, GPA and SLB in nature.

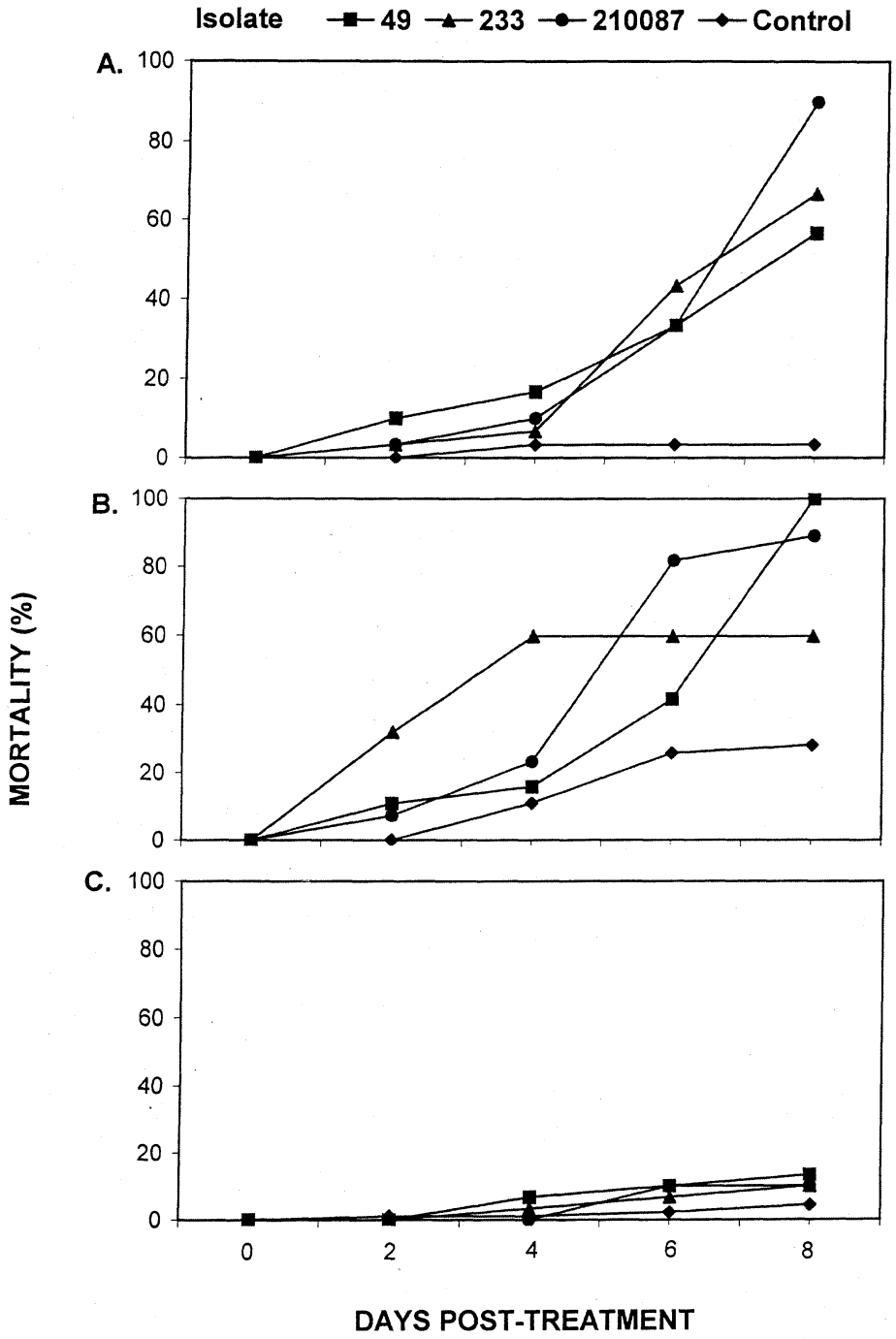

Figure 2. Mortality curves of *L. decemlineata* (A), *M. persicae* (B) and *C. maculata lengi* (C) caused by isolates 49, 233 and 210087.

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