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HETEROGENEITY OF TWO *BEAUVERIA BASSIANA* STRAINS REVEALED BY BIOCHEMICAL TESTS, PROTEIN PROFILES AND BIO-ASSAYS ON *LEPTINOTARSA DECEMLINEATA (COL. : CHRYSOMELIDAE)* AND *COLEOMEGILLA MACULATA LENGI (COL. : COCCINELLIDAE)* LARVAE

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Biochemical profiles on API Rapid CH* strips and protein profiles on polyacrylamide gels in the presence of sodium dodecyl sulfate were used to distinguish two strains of the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin, ARSEF 2991 and ATCC 44860. Next, the toxicity of these two strains was determined at concentrations of 10^2, 10^4, 10^6 and 10^8 blastospores/ml on larvae of the Colorado potato beetle *Leptinotarsa decemlineata* Say (Coleoptera : Chrysomelidae) and of its predator, the spotted ladybird beetle, *Coleomegilla maculata lengi* Timberlake (Coleoptera : Coccinellidae).

Both strains were highly toxic to *L. decemlineata* larvae. However, the two strains exhibited different levels of toxicity for *C. maculata* larvae : ARSEF 2991 was toxic, whereas ATCC 44860 caused little coccinellid larval mortality.

KEY-WORDS : Entomopathogenic fungus, strain, blastospores, larval mortality, *Coleomegilla maculata lengi, Leptinotarsa decemlineata.*

Among fungi, *Beauveria bassiana* (Balsamo) Vuillemin is one of the most widespread entomopathogen (Roberts & Yendol, 1971 ; Tanada & Kaya, 1993). Many different *B. bassiana* strains exist, some of these are toxic, at varying degrees, to the Colorado potato beetle *Leptinotarsa decemlineata* Say (Ferron, 1981 ; Anderson *et al.*, 1989).

B. bassiana has been experimentally tested for the control of *L. decemlineata* in ex-USSR, in Poland (Bajan *et al.*, 1987), in ex-Czecoslovakia (Weiser, 1987), in France (Fargues *et al.*, 1980, 1991) and in USA (Hajek *et al*, 1987 ; Groden & Lockwood, 1991). A commercial formulation made from conidiospores has been available since 1977 in Ukraine under the name Boverin™. The dose recommended by the manufacturer is $6x10^9$ conidiospores/g (Lippa, 1985).

In Quebec, Canada, the Colorado potato beetle, *Leptinotarsa decemlineata* Say (Coleoptera : Chrysomelidae), is the insect pest against which the greatest quantity of chemical insecticides is used (Chagnon *et al.* 1990). This insect, however, has rapidly developed a resistance to a great variety of insecticides (Martel, 1987) which has led to an increase in the dosage used in the field. In addition to the many negative consequences on the environment, insecticides seriously reduce populations of the Colorado potato beetle's natural enemies.

One of these natural enemies, the spotted ladybird beetle, *Coleomegilla maculata lengi* Timberlake (Coleoptera : Coccinellidae), should be regarded as a very important component in an integrated pest management system in Canada (Boiteau, 1983). It is a polyphagous predator that can significantly reduce Colorado potato beetle populations (Groden *et al.*, 1990). Each *C. maculata* adult can attack 20 eggs in 48 h (Hazzard & Ferro, 1991) and consume, on average, 11.2 young *L. decemlineata* larvae (Groden *et al.*, 1990).

Few studies have been conducted to study the impact of entomopathogenic microorganisms on *C. maculata*. In a laboratory study, Giroux *et al.* (1993) have shown that *C. maculata* larvae and adults are not sensitive to M.One™, a commercial *Bacillus thuringiensis* var. *san diego* preparation, even at doses 10 times higher ($5.6x10^8$ Colorado Potato Beetle International Units (CPBIU)/l) than recommended by the manufacturer. Fungi, however, appear to be less selective than bacteria. A study of the activity of *B. bassiana* strain ARSEF 731 has shown that the $7.2x10^4$ conidiospores/mm^2 dose was not toxic to adults of *C. maculata* after contamination by ingestion, while this strain caused 60 % mortality following treatment by contact (Lord *et al.*, 1988). According to Goettel *et al.* (In Lord *et al.*, 1988), 16 coccinellid species can be infected by *B. bassiana*. Iperti (1966) has frequently observed dead *Semiadalia undecimnotata* Schneider (Coleoptera : Coccinellidae) adults infected by *B. bassiana* at hibernation sites, especially at altitudes of less than 1000 m.

The USDA-ARS Collection of Entomopathogenic Fungal Cultures (Humber, 1992) contains close to 1000 different *B. bassiana* isolates, some of which isolated from *L. decemlineata*, others from Coccinellidae. The study of the heterogeneity of the species either at the biochemical level or at the level of host specificity has received little attention. Clearly, some methods were needed to distinguish rapidly between *B. bassiana* isolates and to get a picture of the heterogeneity of the species. The aim of the current study was to determine first, whether two strains of *B. bassiana* could be distinguished using biochemical methods or protein profiles on SDS-PAGE and second, whether different strains of *B. bassiana* could exhibit different host toxicity against *C. maculata* and *L. decemlineata* larvae.

API Rapid CH* strips contain various carbon sources and are used to identify biochemical or nutritional characteristics of microorganisms (Anonymous, 1988). API Rapid CH* profiles are routinely used to distinguish between bacteria at the genus, species, variety and isolate level (Logan & Berkeley, 1984). API Rapid CH* strips were tested in this study for their ability to discriminate rapidly between fungal isolates. Protein profiles generated by electrophoresis on polyacrylamide gels in presence of sodium dodecyl sulfate have been used extensively to, among other things, discriminate between microorganisms. They were used here to determine whether they were sensitive enough to distinguish between two *B. bassiana* isolates.

We chose to work with blastospores because they usually germinate within 48 h following infection, compared with three to four weeks for conidiospores (Müller-Kögler & Samsinakova, 1969). *B. bassiana* strain ARSEF 2991 was chosen because it is indigenous to Quebec, Canada, and was originally isolated from *L. decemlineata*, the target insect to be controlled. The other strain chosen needed to be as different as possible from ARSEF 2991 in order to test the discriminatory level of API Rapid CH* test, protein profiles, and toxicity level on *C. maculata* and *L. decemlineata* larvae. ATCC 44860 appeared as a good candidate because it had been isolated from soil in Georgia, U.S.A.

MATERIALS AND METHODS

STRAINS

Two *Beauveria bassiana* strains were used. The ARSEF 2991 strain was obtained from T. Searle (Macdonald College, McGill University, Montreal, Canada). It was initially isolated from dead adult *L. decemlineata*, collected in the summer of 1988 in a potato field near Ste-Clothilde (Québec, Canada). The strain is registered at the USDA-ARS collection in Ithaca, New York (Humber, 1992). The ATCC 44860 strain was purchased from the American Type Culture Collection in Rockville, Maryland. It was initially isolated from a soil sample in Georgia, USA (Hammill, 1970).

BLASTOSPORE PRODUCTION

B. bassiana blastospores were used to contaminate spotted ladybird beetle larvae. Blastospores were produced in 300 ml of sporulation medium for three days, incubated at 25 °C with agitation, as described by Alioshina *et al.* (In Ferron, 1981) with some modifications. The pH was adjusted to 5 and sucrose was replaced with sorbitol to increase blastospore production (Samsinakova *et al.*, 1981). The culture was centrifuged at 8000 RPM in a Beckman T-865 rotor for 10 min at 4 °C in a Beckman J2-21 M high speed centrifuge. The supernatant was discarded and the pellet resuspended in 50 ml of 0.85 % NaCl. The blastospores were observed under a microscope and the titer determined by serial dilutions.

BIOCHEMICAL PROFILES

Blastospores from the *B. bassiana* strains were transferred on 2YT agar plates (10 g Yeast extract, 16 g Bacto-tryptone, 5 g NaCl, 15 g Agar per litre) and incubated at 30 °C for 48 h. The cultures were resuspended in 3 ml 0.85 % NaCl. The biochemical profiles were determined on API Rapid CH* strips following the manufacturer's recommendations (Anonymous, 1988).

PROTEIN PROFILES

Total proteins were separated by SDS-PAGE electrophoresis (Laemmli, 1970). The gel (20 cm x 20 cm x 1.5 mm) contained 10 % acrylamide for separation and 4 % acrylamide for protein concentration. The electrophoresis was carried out under continuous current at 60 mA for 5 h. The proteins were stained with Coomassie Blue R-250 (methanol : acetic acid : water, 50 :10 :40) for 1 h, then unstained with a mixture of methanol : acetic acid : water (7 :5 :88).

REARING OF COCCINELLIDS

Adults *C. maculata* were collected in the spring of 1992 at hibernation sites situated in the vicinity of corn fields in St-Hyacinthe, Quebec, Canada (45°39'N, 72°56'W). They were placed in cages with a diet designed to stimulate egg laying. The diet comprised pollen, aphids *Aphis fabae* (Scop.) and *Anagasta kuehniella* (Zeller) eggs. To avoid cannibalism, each larva, upon hatching, was individually reared in Petri dishes on a pollen diet. The conditions were kept constant at 25 °C, 70 % humidity and a 16L :8D photoperiod.

REARING OF COLORADO POTATO BEETLES

L. decemlineata larvae were obtained from a mass rearing maintained from individuals collected in June 1990 in Trois-Pistoles, Quebec, Canada (48°07'N,69°10'W). Rearing was done on potato plants of the "Kennebec" variety under controlled conditions (23 °C, 40 % RH, 16L :8D).

BIOASSAYS

Blastospore concentrations of 10^2, 10^4, 10^6 and 10^8 colony forming units (CFU)/ml of each strain were used for bioassays. The two strains were diluted in 0.85 % NaCl and Triton X-100 was added to the solution at a final concentration of 0.1 %.

The contaminated pollen, an easily accessible and inert food, was offered as a diet to first instar *C. maculata* larvae. A 25 g quantity of wildflower pollen was mixed with 25 ml of the blastospore solution. After a vacuum filtration, the pollen was dried at 60 °C for 3 h. This procedure was repeated for all concentrations. To observe the toxicity of the *B. bassiana* samples on *L. decemlineata*, the larvae were transferred to Petri dishes. A Whatman 3M filter paper moistened with 0.5 ml distilled water was placed at the bottom of each Petri dish. Potato leaves were sprayed with 1 ml of each *B. bassiana* concentration, then dried and placed on the filter paper. In each Petri dish, 30 first or second instar larvae were placed on a contaminated leaf which was replaced daily. Two Petri dishes were used for each blastospore concentration.

Mortality was noted for each blastospore concentration every 24 h over a ten day period. Results were analysed with a χ^2 test (Statview, version 1.03, Macintosh®) (Abacus Concepts Inc., 1988). The percentage mortality was corrected following Abbott's (1925) method.

RESULTS

COMPARISON OF THE TWO *B. BASSIANA* STRAINS

After a three day incubation period in the sporulation medium at 25 °C, sporulation of the two *B. bassiana* strains occurred. Blastospores from both strains appear indistinguishable from each other under the microscope (800X) based on size and shape or from other *B. bassiana* blastospores as reported in the literature (Weiser, 1972).

However, the API Rapid CH* profiles of the two strains are different (table 1). The ARSEF 2991 strain is able to acidify the following components. L-arabinose, galactose, sorbose, mannitol, sorbitol, salicin, melibiose, melezitose, gentiobiose and D-turanose. Strain ATCC 44860, however, shows a negative reaction for all these tests. It differs in the lack of acidification of erythritol, adonitol, α-methyl-D-glucoside and lactose, while the ARSEF 2991 strain possesses slight activity. Both strains acidify ribose, arbutin, and cellobiose, yet strain ARSEF 2991 is more active in this respect.

Protein electrophoretic analysis indicates significant differences between both strains (fig. 1). Strain ARSEF 2991 possesses a predominant protein band with a low molecular weight, estimated at 34 kDa, while strain ATCC 44860 differs in the presence of a protein of 70 kDa absent in strain ARSEF 2991.

TOXICITY OF THE TWO *B. BASSIANA* STRAINS ON *L. DECEMLINEATA* LARVAE

After 10 days, the two strains resulted in mortality significantly different to the control at the following concentrations: 10^4, 10^6 and 10^8 blastospores (blsp)/ml (χ^2 ; df = 3 ;

TABLE I

Carbon sources utilisation profiles of B. bassiana *strains determined on API Rapid CH* strips*

	CTRL	GLY	ERY	DARA	LARA	RIB	DXYL	LXYL	ADO	MDX	GAL	GLU	FRU	MNE	SBE	RHA	DUL	INO	MAN	SOR	MDM	MDG	NAG	AMY	ARB
ATCC 44860	0	1	0	0	0	3	0	0	0	0	0	5	5	5	0	0	0	0	0	0	0	0	5	0	3
ARSEF 2991	0	1	1	0	5	5	1	0	1	0	5	5	5	5	5	0	0	0	5	5	0	1	5	0	5

	ESC	SAL	CEL	MAL	LAC	MEL	SAC	TRE	INU	MLZ	RAF	STA	GLG	XLT	GEN	TUR	LYX	TAG	DFUC	LFUC	DARL	LARL	GNT	2KG	5KG
ATCC 44860	5	0	3	5	0	0	5	5	0	0	0	5	5	0	0	0	0	0	0	0	0	0	0	0	0
ARSEF 2991	5	5	5	5	1	5	5	5	0	5	0	5	5	0	5	5	0	0	0	0	0	0	0	0	0

Composition of the strip : ConTRoL, GLYceroL, ERYthritol, D-ARAbinose, L-ARAinose, RIBose, D-XYLose, L-XYLose, ADOnitol, ß-Methyl-D Xyloside, GALactose, GLUcose, FRUctose, MaNnosE, SorBosE, RHAmnose, DULcitol, INOsitol, MANitol, SORbitol, α-Methyl-D Mannoside, α-Methyl-D Glucoside, N-Acetyl Glucosamine, AMYgdalin, ARButin, ESCulin, SALicin, CELiobiose, MALtose, LACtose, MELibiose, SACcharose, TREhalose, INUlin, MeLeZitose, RAFfinose, STArch, GLycoGen, XyLiTol, GENtiobiose, D-TURanose, D-LYXose, D-TAGatose, D-FUCose, L-FUCose, D-ARabitoL, L-ARabitoL, GlucoNaTe, 2-KetoGluconate, 5-KetoGluconate.

Interpretation of results : 0 = negative ; 1 = slightly positive ; 3 = positive ; 5 = strongly positive.

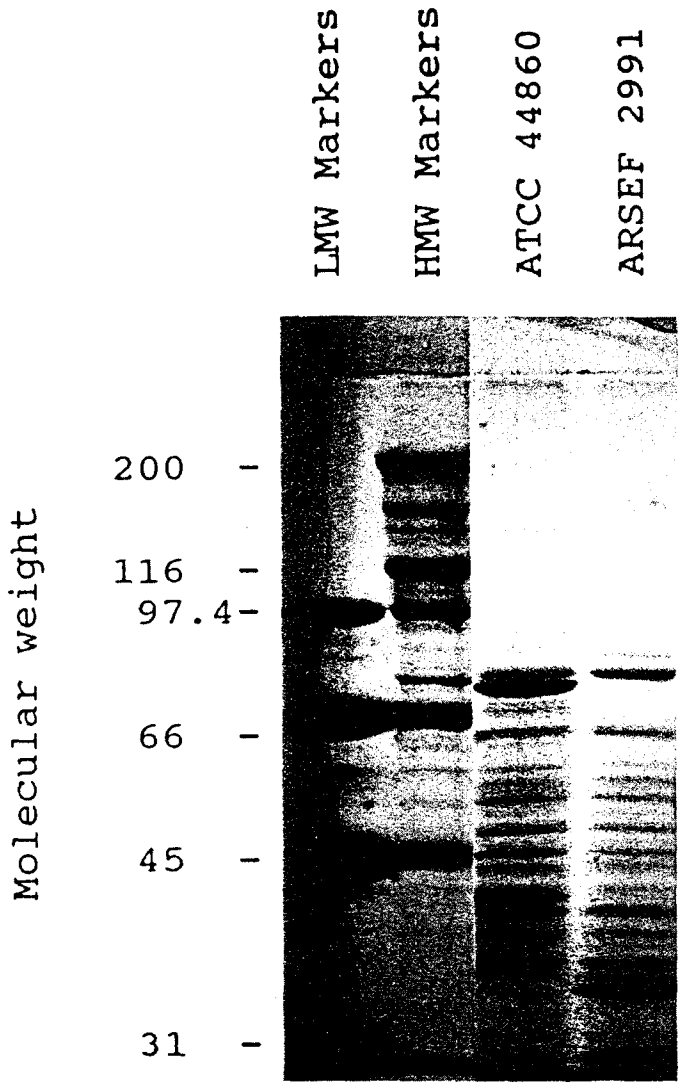

Fig. 1. Protein profiles of two *B. bassiana* strains on sodium dodecyl sulfate-polyacrylamide gel ;
lanes 1 and 2 - molecular weight markers ;
lanes 3 and 4 - strains ATCC 44860 and ARSEF 2991

P = 0.0001) (fig. 2). Strain ATCC 44860 caused 29.5 % larvae mortality at the 10^4 blsp/ml dose ($\chi^2 = 20.76$; P = 0.0001). This strain caused 90.9 % and 98.9 % mortality at concentrations of 10^6 blsp/ml ($\chi^2 = 99.85$; P = 0.0001) and 10^8 blsp/ml ($\chi^2 = 117.23$; P = 0.0001), respectively. Strain ARSEF 2991 showed a weaker toxicity by causing mortality rates of 37.6 % ($\chi^2 = 27.84$; P = 0.0001), 52.9 % ($\chi^2 = 43.08$; P = 0.0001) and 67.1 % ($\chi^2 = 60.68$;

$P = 0.0001$), at concentrations of 10^4, 10^6 and 10^8 blsp/ml, respectively (fig. 2). No significant effect was observed for either strain at a concentration of 10^2 blsp/ml ($P = 0.05$).

The two strains gave rise to similar effects on *L. decemlineata* larvae at concentrations of 10^2 and 10^4 blsp/ml (χ^2 ; df = 3 ; $P = 0.05$). At concentrations of 10^6 and 10^8 blsp/ml strain ATCC 44860 showed a higher toxicity than strain ARSEF 2991 (χ^2 ; df = 3 ; $P = 0.0001$).

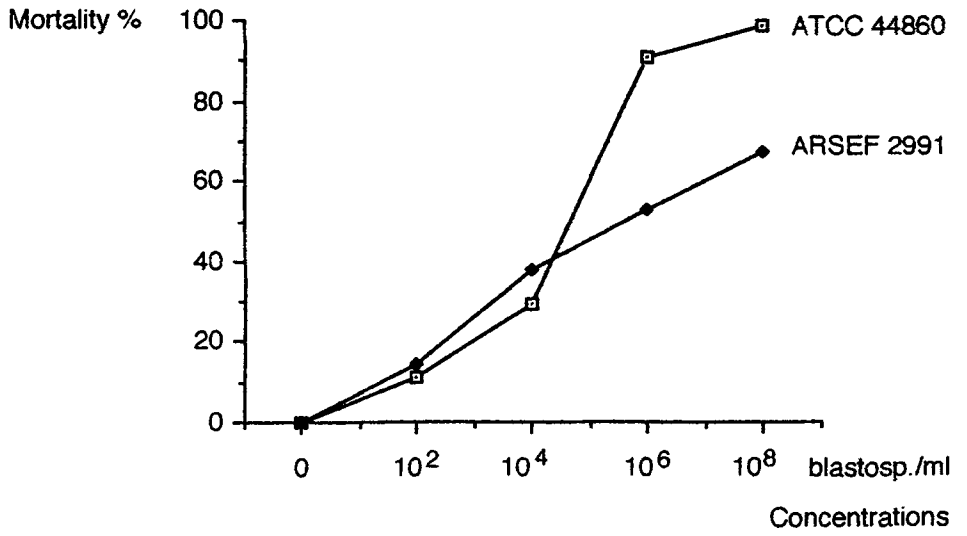

Fig. 2. Mortality, after 10 days, of *L. decemlineata* subjected to different doses of *B. bassiana*

TOXICITY OF THE TWO *B. BASSIANA* STRAINS ON *C. MACULATA* LARVAE

Every tested concentration of strain ARSEF 2991 caused heavy mortality among young larvae which had consumed contaminated pollen (fig. 3). The slope of the mortality-concentration curve is very pronounced at a concentration of 10^2 blsp/ml, and 55.6 % of the larvae died during the first 10 days following treatment ($\chi^2 = 35.71$; df = 3 ; $P = 0.0001$). Concentrations of 10^4 and 10^8 blsp/ml caused 66.7 % mortality while a concentration of 10^6 blsp/ml caused 77.8 % mortality of individuals. The effect did not differ significantly among all doses used ($P = 0.05$) but was significantly different relative to the control ($P = 0.0001$).

Strain ATCC 44860 gave rise to little mortality in *C. maculata* larvae (fig. 3). Concentrations of 10^2, 10^4 and 10^6 blsp/ml did not differ significantly from the control ($\chi^2 = 3.33$; $P = 0.07$), causing 11.1 % mortality. The 10^8 blsp/m concentration was significantly more toxic than the control resulting in 28.7 % mortality ($\chi^2 = 10.00$; $P = 0.002$), but did not differ from the 10^2, 10^4 and 10^6 blsp/ml concentrations ($\chi^2 = 2.00$; $P = 0.16$).

The toxicity of the two *B. bassiana* strains, however, were very different relative to each other in regard to all doses used ($P = 0.001$).

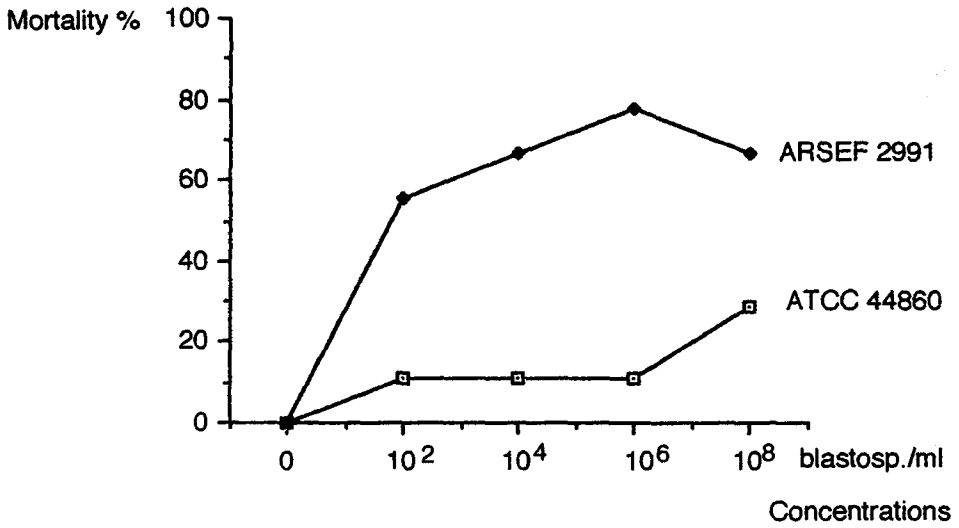

Fig. 3. Mortality, after 10 days, of *C. maculata* subjected to different doses of *B. bassiana*

DISCUSSION

Two *B. bassiana* strains were compared and differentiated, based firstly, on their differential use of carbon sources as determined on API Rapid CH* strips and, secondly on their protein profiles on sodium dodecyl sulfate-polyacrylamide gels.

The ARSEF 2991 strain possesses a capacity to acidify a wider variety of substrates. Following protein analysis, the two strains were distinguished by the presence of different protein bands.

It seems then, that the two strains are very different with respect to biochemical reactions and protein profiles. It was further necessary to determine whether the two strains presented different toxicities to *L. decemlineata* and *C. maculata*.

The toxicity study of the different *B. bassiana* strains on the insects shows a selectivity by the fungus that varies according to the host strain. Some authors report a correlation between the quantity of spores and the cumulative mortality rate (Müller-Kögler, 1967). Other studies (Fargues, 1972) have shown that larvae of the same host can be resistant to certain strains of *B. bassiana* and be very sensitive to other strains of the same pathogen. Often a strain presents no activity on a host while it causes a high mortality rate on other insects of the same family (Fargues, 1976).

Lord *et al.* (1988) have shown that the spotted ladybird beetle is susceptible to certain *B. bassiana* strains. Our study indicates that the *B. bassiana* strains used were both toxic to *L. decemlineata* larvae and yielded a different toxicity on young *C. maculata* larvae. Coccinellid larvae were very susceptible to every concentration of ARSEF 2991 ($P = 0.0001$). However, ATCC 44860 caused negligible mortality in spotted ladybird beetle larvae.

B. bassiana ATCC 44860 presents serious advantages over ARSEF 2991 for use in an integrated pest management program because of its high toxicity towards *L. decemlineata* and its low toxicity towards *C. maculata*. We plan to extend this line of research to a

greater number of *B. bassiana* strains in conjunction with a greater number of insects so as to identify the fungal strains that are potentially toxic to the target insect species and that present minimal negative effects on auxiliaries and predators.

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RÉSUMÉ

Hétérogénéité de deux souches de *Beauveria bassiana* caractérisées par tests biochimiques, profils des protéines et bio-essais sur larves de *Leptinotarsa decemlineata* (Col. : Chrysomelidae) et *Coleomegilla maculata lengi* (Col. : Coccinellidae)

Les profils biochimiques sur galeries API Rapid CH* et les profils protéiques sur gels de polyacrylamide ont été utilisés pour distinguer deux souches du champignon entomopathogène *Beauveria bassiana* (Balsamo) Vuillemin. La toxicité de ces deux souches a été déterminée à des concentrations de 10^2, 10^4, 10^6 et 10^8 blastospores/ml sur des larves du doryphore, *Leptinotarsa decemlineata* Say (Coleoptera : Chrysomelidae) et de la coccinelle maculée *Coleomegilla maculata lengi* Timberlake (Coleoptera : Coccinellidae).

Les deux souches de *B. bassiana* se sont avérées actives à l'égard des larves de *L. decemlineata*. Toutefois la souche ARSEF 2991 s'est avérée pathogène pour les larves de *C. maculata*, alors que la souche ATCC 44860 a provoqué une faible mortalité des larves.

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