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ORIGINAL PAPER

Screening of Argentine native fungal strains for biocontrol of the grasshopper *Tropidacris collaris*: relationship between fungal pathogenicity and chitinolytic enzyme activity

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Abstract *Tropidacris collaris* (Orthoptera: Romaleidae) is a large and voracious grasshopper, which, in recent years, has become a recurrent pest in increasingly extensive areas of Argentina's northern provinces. In the present work chitinase activity was measured in 59 entomopathogenic fungal isolates native to Argentina, and the relationship between enzymatic activity and fungal virulence was assessed. Isolate LPSC 1067 caused the highest mortality on *T. collaris* nymphs (97.7 ± 1.22%). Nine isolates caused no mortality, while the remaining 49 caused mortalities ranging from 6.6 ± 0.3% (LPSC 770) to 91.06 ± 1.51% (LPSC 906). Several isolates revealed chitinolytic capabilities on test plates, although the activities differed with respect to the ratio of the chitin-decay–halo and fungal-colony diameters. A principal component analysis indicated that isolate LPSC 1067, obtained from a long-horned grasshopper (Orthoptera: Tettigoniidae), would be a potential candidate for *T. collaris* biocontrol because the strain exhibited the highest mortality, a shorter median lethal time, and a high enzymatic activity and growth rate.

Keywords Biocontrol · Chitinase activity · Entomopathogenic fungi · *Tropidacris collaris* · Grasshopper

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Introduction

In different regions of Argentina, as in other parts of the world, certain species of grasshoppers and locusts are major agricultural pests (Carbonell et al. 2006). The romaleid *Tropidacris collaris* (Stoll), one of the largest grasshoppers (♂ = 73–101 mm, ♀ = 92–126 mm), is voracious and strongly gregarious during juvenile development. Although adults tend to prefer the leaves of hardwood trees and bushes, *T. collaris* is a polyphagous species. The crops known to be affected include sugar cane, cotton, olive, jojoba, corn, soybean, and wheat (Barrera and Paganini 1975; Carbonell 1986). The marching bands of nymphs consume virtually all the plant material available within their vicinity. In recent years, *T. collaris* has become an increasingly recurrent pest in progressively extensive areas of some northern provinces of Argentina (Catamarca, Córdoba, Chaco, and Santiago del Estero). There have been no reports of pathogens specific

for *T. collaris* (Lange et al. 2005). *Paranosema locustae*, a microsporidium developed in the USA as a grasshopper biocontrol agent and established in Argentina in some areas (Lange and Azzaro 2008; Lange 2010), did not produce encouraging results when tested against *T. collaris* under laboratory conditions (Lange et al. 2008). At the present time, chemical insecticides are still the only means of grasshopper control available, but their use is of significant environmental concern.

Unlike entomopathogenic bacteria, viruses, and most protists that invade insects through the alimentary canal, fungi can also actively penetrate the host exoskeleton (Charnley 1984; Hajek and St. Leger 1994). Fungal input through the cuticle is mediated by a combination of mechanical processes and enzymatic lysis (Hassan and Charnley 1989). The relative contribution of each process depends on several factors, including the chemistry of the cuticle, the intrinsic enzymatic capabilities of the entomopathogenic fungi, and the structure and composition of the exoskeleton (Charnley 1984). Among the enzyme systems involved in the fungal attack on insects, different chitinases have been shown to be better determinants of fungal virulence within the complex and multifactorial insect host-pathogen relationship (Fang et al. 2007; Yang et al. 2007; Gillespie et al. 2000).

Chitin, a structural polysaccharide that occurs in the exoskeleton and gut linings of insects, is a key target for selective pest-control agents (Binod et al. 2007). Accordingly, entomopathogenic fungi such as *Metarhizium anisopliae* (Mesch.) Sorokin, *Beauveria bassiana* (Bals.) Vuill., and *Nomurea rileyi* (Farlow) (Ascomycota: Hypocreales) can overcome those exoskeletal molecular barriers by elaborating multiple extracellular degradative enzymes, including chitinases and proteases, which enable the pathogens to penetrate the exoskeleton and expedite infection (Krieger de Moraes et al. 2003; Binod et al. 2007).

Early studies by Coudron et al. (1984) demonstrated that chitinolytic activity in several entomopathogens is necessary for growth and potentially needed for penetration. Brandt et al. (1978) proposed that chitinases cause perforations in the external membranes, thus facilitating the entry of pathogens into the tissues of susceptible insects.

Knowledge of the fungal enzymatic activities involved in insect penetration and a selection of virulent fungal strains are required for the development of an efficient fungal agent for the biological control of grasshoppers. Accordingly, in this study, we analyzed the effectiveness of 59 fungal strains, isolated both from several insects and from soil collected in Argentina, in the control of *T. collaris* under laboratory conditions and determined the relationship between the chitinase levels of these fungi and their insecticidal activities.

Materials and methods

Collection of insects

Nymphs of *T. collaris* were collected in February 2010 with sweep nets in dense woodland vegetation at a site (27°8′21.9″S; 61°34′23.8″W) near Tres Estacas, in the western Chaco Province, Argentina. The area is part of the Chaqueña Biogeographic Province (Cabrera and Willink 1973), where the annual rainfall is 600 mm and the average temperature ranges from 19 to 21°C, with summer maxima reaching 42–46°C. Samples were immediately taken to the laboratory where they were kept in groups in wire-screened cages in a rearing room under controlled conditions (30°C; 14:10, L:D photoperiod; 60% relative humidity).

Fungal isolates

The fungal strains used were anamorphic Ascomycota belonging to the culture collection of the Spegazzini Institute (LPSC), which had been isolated from a broad spectrum of insects and soil within several locations throughout Argentina (Table 1). The conidia of different isolates were obtained from cultures on potato-dextrose-agar medium after incubation for 10 days at 25°C in the dark.

Pathogenicity assays

Conidia were harvested with disposable cell scrapers (Fisherbrand®) from 10-day-old cultures and placed in test tubes containing 0.01% (v/v) Tween 80 (Merck). Suspensions were vortexed for 2 min, filtered through four layers of sterile muslin, and adjusted to 1×10^8 conidia/ml according to Prior et al. (1995) after cell enumeration in a Neubauer hemocytometer. The viability of the conidia from all isolates used in the tests was determined after 24 h, as described by Lane et al. (1988). This germination test was repeated for each stock suspension to maintain the fidelity of the viability assessments.

Three replicates (on different dates), of 15 third-instar *T. collaris* nymphs each, were sprayed, through the use of a 35-ml glass atomizer, with about 1 ml of a suspension containing 1×10^8 conidia/ml (in 0.01% [v/v] Tween 80). Three additional replicates of 15 grasshoppers each for use as controls were sprayed in the same fashion, but with 1 ml of 0.01% [v/v] Tween 80 only. The grasshoppers were kept in groups of 15 individuals in acetate tubes (50 × 9-cm; Henry 1985) and fed with "quebracho" (*Aspidosperma, Schipnosis*) leaves. Treated and control insects were maintained at 30°C, a 60% relative humidity, and a 14:10-h light:dark photoperiod. The cumulative mortality was

Table 1 Characteristics of the fifty-nine entomopathogenic fungi used and the results obtained for mean lethal time, percent mortality (means ± SD), enzymatic activity, and growth on chitin-azure

Fungal isolates	Isolate number LPSC	Isolation substrate	Media lethal time	Percent mortality (means ± SD)	Chitinase activity (means ± SD)	Growth (cm) on chitin-azure (means ± SD)
Beauveria bassiana	893	Hemiptera: Cicadellidae	–	0	1.83 ± 0.47	6.8 ± 0.3
Beauveria bassiana	883	Hemiptera: Cicadellidae	–	0	0	7.13 ± 0.32
Beauveria bassiana	885	Hemiptera: Cicadellidae	–	0	0	6.76 ± 0.3
Beauveria bassiana	900	Coleoptera: Lampyridae	–	0	2.53 ± 0.5	7.63 ± 0.37
Beauveria bassiana	803	Soil	–	11.1 ± 0.46	3.53 ± 0.47	8 ± 0
Beauveria bassiana	888	Hemiptera: Pentatomidae	–	13.3 ± 0.5	0	8 ± 0
Beauveria bassiana	894	Hemiptera: Cicadellidae	–	13.3 ± 0.4	3.13 ± 0.32	6 ± 0.5
Beauveria bassiana	169	Lepidoptera: Pyralidae	–	17.7 ± 0.58	2.96 ± 0.75	8 ± 0
Beauveria bassiana	881	Soil	–	20 ± 0.7	1.6 ± 0.43	6.33 ± 0.49
Beauveria bassiana	890	Hemiptera: Cicadellidae	–	26.6 ± 0.69	0	6.4 ± 0.4
Beauveria bassiana	771	Hemiptera: Reduviidae	–	26.6 ± 0.81	1.6 ± 0.43	5.56 ± 0.3
Beauveria bassiana	884	Hemiptera: Cicadellidae	–	31.06 ± 0.86	0	5.5 ± 0.3
Beauveria bassiana	901	Coleoptera: Lampyridae	–	33.3 ± 0.93	2.73 ± 0.75	8 ± 0
Beauveria bassiana	886	Hemiptera: Membracidae	–	37.7 ± 1.21	0	6.46 ± 0.41
Beauveria bassiana	897	Coleoptera: Chrysomelidae	–	39.96 ± 0.93	0	8 ± 0
Beauveria bassiana	782	Soil	–	42.2 ± 0.66	2.6 ± 0.36	6.3 ± 0.43
Beauveria bassiana	889	Hemiptera: Cicadellidae	–	48.8 ± 0.86	5.2 ± 0.2	5.46 ± 0.25
Beauveria bassiana	902	Hemiptera: Cixiidae	7.28	51.06 ± 1.16	1.16 ± 0.05	4.33 ± 0.2
Beauveria bassiana	899	Hemiptera: Cicadellidae	6.95	53.3 ± 1.09	6.36 ± 0.47	7.56 ± 0.49
Beauveria bassiana	896	Coleoptera: Coccinellidae	6.08	55.5 ± 0.87	2.03 ± 0.2	6.36 ± 0.35
Beauveria bassiana	887	Hemiptera: Cicadellidae	5.68	57.7 ± 1.1	5 ± 0.5	3.03 ± 0.73
Beauveria bassiana	183	Lepidoptera: Pyralidae	5.2	60 ± 0.99	4.63 ± 0.55	8 ± 0
Beauveria bassiana	1063	Orthoptera: Acrididae	5.27	71.06 ± 1.01	2.5 ± 0.4	7.2 ± 0.5
Beauveria bassiana	895	Hemiptera: Cicadellidae	6.31	75.5 ± 1.07	4.43 ± 0.4	6.63 ± 0.55
Beauveria bassiana	892	Hemiptera: Cicadellidae	5.92	77.76 ± 1.36	5.46 ± 0.45	6.96 ± 0.05
Beauveria bassiana	898	Dermaptera: Forficulidae	4.9	80 ± 1.15	2.66 ± 0.4	6.33 ± 0.73
Beauveria bassiana	882	Soil	5.39	82.2 ± 1.1	4.26 ± 0.25	5.3 ± 0.3
Beauveria bassiana	1066	Heteroptera: Cimicidae	4.29	84.4 ± 1.31	3.03 ± 0.25	3.13 ± 0.2
Beauveria bassiana	1067	Orthoptera: Tettigoniidae	3.19	97.7 ± 1.22	6.13 ± 0.05	8 ± 0
Clonostachys rosea	785	Hemiptera: Cicadellidae	–	0	4.6 ± 0.26	8 ± 0
Clonostachys rosea	780	Hemipetra: Cicadellidae	–	28.8 ± 0.62	0	8
Clonostachys sp.	1075	Soil	–	0	0	8
Fusarium verticillioides	1057	Acridoidea: Romaleidae	7.02	53.3 ± 0.8	6.6 ± 0.45	8 ± 0
Isaria javanica	1071	Hemiptera: Aleyrodidae	–	15.5 ± 0.5	2.5 ± 0.45	8 ± 0
Lecanicillium lecanii	1070	Hemiptera: Aphididae	–	22.2 ± 0.71	0	5.23 ± 0.25
Lecanicillium muscarium	1079	Hemiptera: Aphididae	–	11.1 ± 0.59	0.86 ± 0.05	5.43 ± 0.2
Lecanicillium sp.	1069	Hemiptera: Aphididae	–	17.7 ± 0.58	0	5.36 ± 0.23
Metarrhizium anisopliae	908	Hemiptera: Cercopidae	–	19.96 ± 0.59	0	4.43 ± 0.25
Metarrhizium anisopliae	503	Soil	–	22.2 ± 0.71	3.76 ± 0.68	6.4 ± 0.52
Metarrhizium anisopliae	84	Soil	–	37.7 ± 1.16	2.3 ± 0.43	4.7 ± 0.36
Metharrizium anisopliae	904	Soil	–	40 ± 1.09	0	5.46 ± 0.45
Metarrhizium anisopliae	963	Soil	–	46.6 ± 1.05	6.5 ± 0.1	8 ± 0
Metarrhizium anisopliae	905	Hemiptera: Cercopidae	5.72	55.5 ± 0.87	2.76 ± 0.25	8 ± 0
Metarrhizium anisopliae	909	Hemiptera: Cercopidae	6.39	64.4 ± 1.18	2.16 ± 0.28	5.73 ± 0.2
Metarrhizium anisopliae	907	Hemiptera: Cercopidae	5.82	73.3 ± 1.24	4.9 ± 0.75	7.5 ± 0.43
Metarrhizium anisopliae	906	Hemiptera: Cercopidae	3.8	91.06 ± 1.51	5.06 ± 0.5	5.93 ± 0.51

Table 1 continued

Fungal isolates	Isolate number LPSC	Isolation substrate	Media lethal time	Percent mortality (means ± SD)	Chitinase activity (means ± SD)	Growth (cm) on chitin-azure (means ± SD)
Metarrhizium sp.	1077	Soil	–	0	6.16 ± 0.28	7.1 ± 0.1
Metarhizzium sp.	1073	Soil	–	13.3 ± 0.5	1.06 ± 0.11	4.3 ± 0.26
Metharrizium sp.	1074	Soil	–	39.96 ± 1.03	2.5 ± 0.3	8 ± 0
Nomurea rileyi	779	Lepidoptera: Noctuidae	–	0	1.53 ± 0.35	3.5 ± 0.2
Paecilomyces farinosus	910	Soil	–	8.8 ± 0.43	0	5.9 ± 0.36
Paecilomyces fumosoroseus	770	Hemiptera: Aleyrodidae	–	6.6 ± 0.3	3.76 ± 0.41	8 ± 0
Paecilomyces fumosoroseus	769	Hemiptera: Auchenorynca	–	19.96 ± 0.53	2.2 ± 0.1	4.93 ± 0.15
Paecilomyces lilacinus	1076	Arachnida: Araneae	–	11.06 ± 0.46	3.16 ± 0.15	3.46 ± 0.32
Paecilomyces lilacinus	827	Hemiptera: Reduviidae	–	24.43 ± 0.62	4.73 ± 0.2	8 ± 0
Paecilomyces lilacinus	952	Soil	–	28.8 ± 0.62	5.06 ± 0.49	8 ± 0
Paecilomyces marquandi	1078	Soil	–	0	3.16 ± 0.3	8 ± 0
Paecilomyces marquandi	1072	Arachnida: Araneae	–	35.5 ± 0.93	5.06 ± 0.11	8.2 ± 0.26
Tolypocladium cylindrosporum	1065	Soil	6.11	66.6 ± 1.25%	2.3 ± 0.45	6.46 ± 0.41

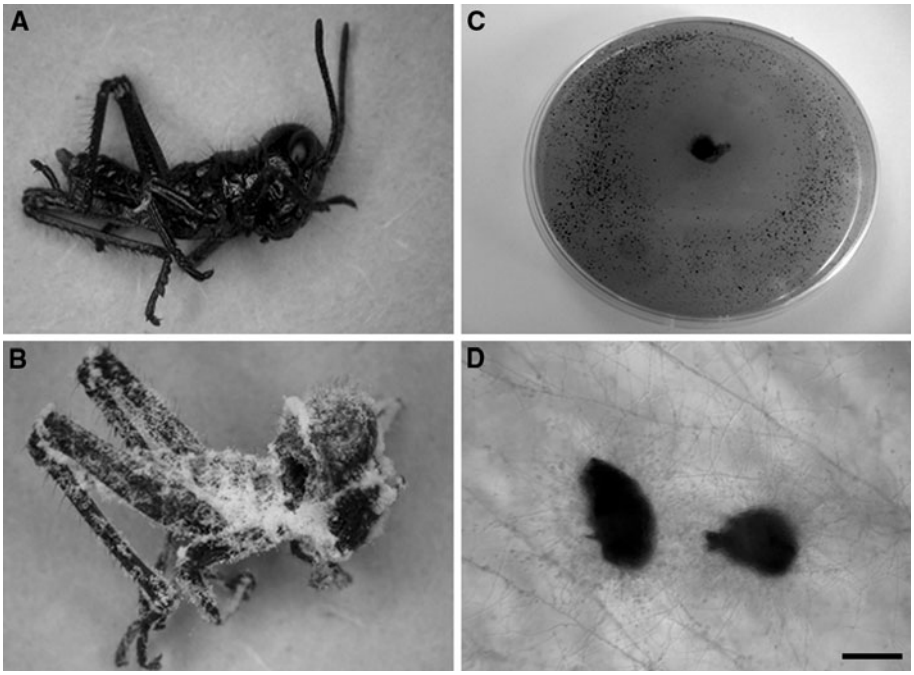

Fig. 1 Third instar *Tropidacris collaris* dead, without external mycelia **a**, and 48 h after its death caused by *Beauveria bassiana* LPSC 1067 **b**. Zone of clearance on chitin azure by *Beauveria bassiana* LPSC 1067 **c** and chitin flake colonization **d**. *Bar scale* = 500 μm

recorded daily for 10 days. Dead grasshoppers with no external mycelium (Fig. 1a) were surface-sterilized by dipping them successively in 70% ethanol (10–15 s), 0.5% sodium hypochlorite solution (1 min), and sterile distilled water (1 min, two consecutive baths) according to Lacey and Brooks (1997). Next, they were placed in a sterile culture chamber consisting of a Petri dish (60 mm diameter) with a filter-paper disk that was periodically moistened with sterile distilled water and incubated at 25°C in the dark. Mycosis was confirmed by microscopic examination of the dead grasshoppers. When mortality was higher than 50%, the median lethal time (MLT) was calculated according to Lecuona and Diaz (2001).

Fungal chitinolytic activity (chitinase)

The levels of chitinolytic activity of the fungal strains was assayed on an agar medium containing 500 ml of mineral-containing saline—$(NH_4)_2SO_4$, 2 g/l; KH_2PO_4, 4 g/l; Na_2HPO_4, 6 g/l; $FeSO_4$ $7H_2O$, 0.2 g/l; $CaCl_2$, 1 mg/l;

H_3BO_3, 10 μg/l; $MnSO_4$, 10 μg/l; $ZnSO_4$, 70 μg/l; $CuSO_4$, 50 μg/l; MoO_4, 10 μg/l) in 500 ml of distilled water—supplemented with 0.02% (v/v) of yeast extract, 15 g of agar (Hankin and Anagnostakis 1975), and 0.08% (w/v) of Chitin Azure®, according to Howard et al. (2003).

Inocula were obtained from cultures grown on 2% (v/v) malt-extract agar. A 6-mm agar plug with mycelium was inoculated onto the agar surface in each plate and incubated at 24 ± 1°C for 20 days (Saparrat et al. 2008). Three replicates were carried out for each strain, and the results represent the mean values ± the standard deviation of the mean (SD). Fungal growth was estimated by measuring the diameter of the colonies. The extracellular chitinolytic activity was assayed according to Saparrat et al. (2008) on colonies for 20 days and expressed as the ratio between the discoloration-halo and the colony diameters.

Statistical analysis

To determine whether the differences among isolates with respect to each of the variables studied (i.e. percentage of mortality, chitinase activity, and colony diameter) were significant, a Univariate Analysis of Variance was performed.

Thereafter, a principal-component analysis (Johnson and Wichern 1992), in which the original variables were mortality rate, enzyme activity, colony diameter, and the observations made on the isolated fungi, was conducted. To eliminate possible biases caused by different interpretations of measurement scales, data were standardized as

$$z_j = \frac{x_j - \bar{x}_j}{S_j}$$

where x_j is the variable j-ecime and $\bar{x}_j$ and S_j are the mean and standard deviation of that variable in the sample respectively. We used only the measurements of the observations, and only eigenvalues of ≥ 1 were retained for the principal-component analysis. This statistical method works with the correlation matrix; therefore, to measure the association between the original variables, X_k, and the principal component, Y_j, we used the correlation coefficient between the two, given by:

$$r(Y_j, X_k) = \frac{e_{kj}\sqrt{\lambda_j}}{\sqrt{S_k}}$$

where e_{kj} is the coefficient of the eigenvector standardized, λ_j is the eigenvalue j-ecime, and S_k is the variance of the variable k-ecime. To discuss joint relations between observations and variables (i.e., fungal isolates and mortality, enzyme activity, and colony diameter) biplot graphs were constructed (Gabriel 1971) from the principal-component analysis on the correlation matrix.

The principal-component analysis was performed with the software Version InfoStat 2007 (InfoStat 2001).

Results

In assessing the pathogenicity of 59 fungal isolates on third-instar nymphs of *T. collaris,* we observed that isolate LPSC 1067 caused a higher mortality and had a lower MLT with nymphs at 97.7 ± 1.22% and 3.19 days, respectively (Fig. 1b). Nine isolates caused no grasshopper mortality, while the remaining 49 showed mortalities ranging from 6.6 ± 0.3% (LPSC 770) to 91.06 ± 1.51% (LPSC 906) (Table 1). The average viability of the conidia from the different fungal isolates was 95%.

Table 1 summarizes the results of the chitinase activity of the isolates. These strains exhibited differences with respect to the diameters of the chitin-degradation halos in culture (Fig. 1c, d). *B. bassiana* (LPSC 892), *B. bassiana* (LPSC 889), *B. bassiana* (LPSC 899), *B. bassiana* (LPSC 870), *B. bassiana* (LPSC 183), *B. bassiana* (LPSC 895), *B. bassiana* (LPSC 882), *B. bassiana* (LPSC 1067), *Clonostachys rosea* (LPSC 785), *Fusarium verticillioides* (LPSC 1057), *Metarrhizium* sp. (LPSC 1077), *M. anisopliae* (LPSC 963), *M. anisopliae* (LPSC 907), *M. anisopliae* (LPSC 906), *Paecilomyces lilacinus* (LPSC 827), *P. lilacinus* (LPSC 952), and *P. marquandi* (LPSC 1072) had the highest activities (>4.2). By contrast, *Clonostachys* sp. (LPSC 1075), *B. bassiana* (LPSC 883), *B. bassiana* (LPSC 885), *B. bassiana* (LPSC 888), *Lecanicillium* sp. (LPSC 1069), *M. anisopliae* (LPSC 908), *Lecanicillium lecanii* (LPSC 1070), *B. bassiana* (LPSC 890), *Clonostachys rosea* (LPSC 780), *B. bassiana* (LPSC 884), *B. bassiana* (LPSC 886), *B. bassiana* (LPSC 897), and *M. anisopliae* (LPSC 904) exhibited no extracellular chitinase activity under the culture conditions tested.

All the strains grown on agar medium revealed different levels of development on the plates. About 65% produced colonies with diameters greater than 6 cm after 20 days of growth on a medium supplemented with chitin.

Although the fungi with chitinolytic activity evidenced this capacity on the plates at an early time after inoculation and subsequent growth (data not shown), the largest halos were reached only after 20 days of culture.

The analysis of variance indicated highly significant differences between the mortality rates, the enzyme activities, and the colony diameters of all the fungal isolates analyzed in this bioassay (Table 2).

The first component in the principal-component analysis explained 50% of the total variability in the model, the second 32%, and the third 18% (Table 3). Variables with a high degree of correlation with the first principal component (CP1) were the mortality rate and the enzyme activity. The CP1 separated the fungal isolates with a high percentage of mortality and a high enzymatic activity from those that had a low percentage of mortality and little enzymatic activity.

Table 2 Results of analysis of variance (ANOVA) for the variables mortality rate, enzymatic activity, and colony diameter

Variables studied	*df*	F Value	*P*
Mortality	58,118	29.61	<0.0001
Enzymatic activity	58,118	100.04	<0.0001
Diameter of colony	58,118	65.38	<0.0001

Table 3 Eigenvalues in the principal-components analysis and their contribution to overall variation of the model

Eigenvalue	Value	Proportion of total variation	Cumulative proportion
1	1.50	0.50	0.50
2	0.95	0.32	0.82
3	0.55	0.18	1.00

Table 4 Correlation coefficients among the variables for colony diameter, enzymatic activity, and percent mortality, and principal components (CP1 and CP2)

Variables	CP1	CP2
Diameter of colony	−0.39	0.92
Enzymatic activity	0.84	0.12
Percent mortality	0.80	0.31

The diameter of the colonies indicated a high correlation with the second principal component (CP2), segregating the fungal isolates with larger colony diameters from those of smaller diameters. Therefore, isolates with high mortality, high enzyme activity, and a larger colony diameter were in the first quadrant of the biplot, with isolate LPSC 1067 being the most representative of this group (Table 4, Fig. 2).

In summary, cluster 1 contained the isolates with the lowest values among the variables studied, cluster 2 the strains with higher values of the three variables, and clusters 3 and 4 the isolates with intermediate values.

Discussion

Entomopathogenic fungi are an important group of pathogens of insect pests. Some of the advantages offered for use in microbial control programs of insects are: their high specificity, contact transmission, natural dispersion, safety for non-target organisms and the ability to maintain lasting control once established in the environment (Van Driesche et al. 2007).

The pathogenesis of insects by fungi is governed by many different conditions related to the host involved, the pathogen itself, and the environment. Several mechanisms, such as the mechanical action of the fungal hyphae and the activity of enzymes, promote the fungal invasion into the body of the host (St. Leger et al. 1986a, b). Through their chitinases, entomopathogenic fungi break the structural barrier of the exoskeleton in insects. Such activities are thus effective screening criteria for selecting entomopathogenic fungi for pest biocontrol (Binod et al. 2007). The catalytic and regulatory properties of these enzymes, as well as their potential for development as biopesticide and microbial biological control agents have been investigated for several pest insects (Kramer et al. 1997). Chitinolytic enzymes play a role in depolymerizing the insect exoskeleton during the attack by entomopathogenic fungi

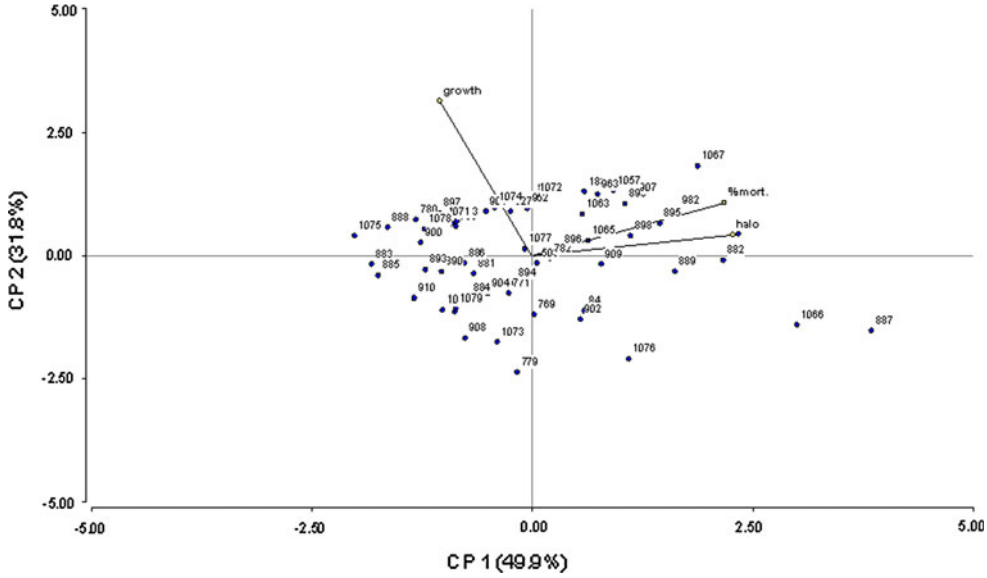

Fig. 2 Graphic biplot showing correlations between different fungal isolates and the observed variables percent mortality, enzyme activity (halo), and colony diameter (growth)

(Sahai and Manocha 1993). Brandt et al. (1978) proposed that chitinases cause perforations in the membranes, thus facilitating the entry of the pathogens into the tissues of the susceptible insects. Microbial chitinases have been used in mixing experiments to increase the potency of entomopathogenic microorganisms. Synergistic effects between chitinolytic enzymes and microbial insecticides have been known since the early 1970s (Binod et al. 2007).

Under the experimental conditions of this study, the results suggest a direct relationship between a high chitinolytic activity and an efficient virulence of the fungal strain. The isolate *B. bassiana* LPSC 1067 was not only the most virulent (97 ± 1.22% mortality) but also exhibited the lowest MLT (3.19 days) against nymphs of *T. collaris*. Prior et al. (1995) reported a similar percentage of mortality with an MLT of 4 days after applying the same fungal dose to *Schistocerca gregaria* (Forskal).

Beauveria bassiana LPSC 1067 was isolated from a katydid (Orthoptera: Tettigoniidae), a close relative of the long-horned grasshopper, collected at Salinas de Bustos (30°18′9.4″S, 67°34′40.6″W), La Rioja province, Argentina, where the high temperatures and low humidity are thought to be unfavorable for fungal development (Roberts and Hajek 1992; Sivasankaran et al. 1998). Thus, this fungal isolate appears to be well adapted to live under the type of stressful conditions that would normally constitute one of the main limitations for using fungi in the biological control of grasshoppers.

This is the first time that a wide intraspecific variability in chitinase activity as well as in grasshopper virulence has been reported in a representative sample of different entomopathogenic fungal strains from Argentina. These results suggest that chitinase activities in particular could be used as a diagnostic criterion for the selection of candidates for the biological control of pests such as grasshoppers and locusts.

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