Mycopathologia (2007) 163:225–232
DOI 10.1007/s11046-007-9006-y

ORIGINAL PAPER

Pathogenicity of fungal isolates (Ascomycota: Hypocreales) against *Peregrinus maidis*, *Delphacodes kuscheli* (Hemiptera: Delphacidae), and *Dalbulus maidis* (Hemiptera: Cicadellidae), vectors of corn diseases

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Received: 1 September 2006 / Accepted: 20 February 2007 / Published online: 4 April 2007

Abstract Preliminary screening assays were carried out on 17 isolates from five fungal species *Beauveria bassiana*, *Lecanicillium muscarium*, *Metarhizium anisopliae*, *Isaria farinosa*, and *I. fumosorosea*. The three most effective isolates against *Peregrinus maidis* (Hemiptera: Delphacidae) were *B. bassiana* CEP 147, CEP 150, and CEP 189. There were no consistent differences found in males and females regarding fungal susceptibility. However, more females than males were proportionally infected. There was not a correlation between the percentage of conidial germination and the percentage of mortality caused by fungal infection in any of the treatments. Only *B. bassiana* CEP 147, which caused a cumulative mortality of 69.8 ± 6.4% after 7 days post-inoculation, was selected to be assayed against adults of *P. maidis*, *Delphacodes kuscheli* (Hemiptera: Delphacidae), and *Dalbulus maidis* (Hemiptera: Cicadellidae). In pathogenicity tests significant differences were observed among treatments. After 2 weeks post-inoculation, both *D. kuscheli* (cumulative mortality of 73.3 ± 9.0%) and *P. maidis* (cumulative mortality of 68.6 ± 6.7%) were significantly more susceptible than *D. maidis* (cumulative mortality of 49.9 ± 9.7%) to the selected isolate.

Keywords corn · *Dalbulus maidis* · *Delphacodes kuscheli* · entomopathogenic fungi · grooming behavior · *Paecilomyces farinosus* · *Paecilomyces fumosoroseus* · pathogenicity tests · *Peregrinus maidis*

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Introduction

Leafhoppers and planthoppers are known worldwide as vectors of different plant pathogens such as viruses and bacteria [1]. In tropical and subtropical areas, corn (*Zea mays* L.) can become infected with destructive viruses and spiroplasms; some of the primary vectors of these pathogens are *Peregrinus maidis* (Ashmead), *Delphacodes kuscheli* Fennah (Hemiptera: Delphacidae), and *Dalbulus maidis* (De Long & Wolcott) (Hemiptera: Cicadellidae). *Peregrinus maidis* is a pantropical species and has been recorded in most tropical regions [2]. In Argentina this species was frequently associated with corn [3], although it has been reported on sorghum (*Sorghum vulgare* L. and *Sorghum halepense* L.), millet (*Panicum*

milliaceum L.), some shrubs, and horticultural plants [4]. This vector transmits maize stripe tenuivirus (MStV), maize Iranian mosaic virus (MIMV), maize mosaic nucleorhabdovirus (MMV), maize sterile stunt virus (MSSV), and Mal de Río Cuarto virus (MRCV) [1, 5–11]. *Delphacodes kuscheli*, a native species that is widespread in central Argentina, is the principal natural vector of MRCV, which produces a severe endemic disease in corn crops in this country [12]. Another species of economic importance is the corn leafhopper *D. maidis*, principal vector of maize fine streak marafivirus (MRFV), corn stunt spiroplasma (CSS) and maize bushy stunt phytoplasma (MBSM). This vector has been recorded from the southern USA to the temperate regions of Argentina and it is considered the most important pest of corn crops in Latin America [13, 14]. Corn stunt is one of the most economically important diseases of maize in the USA, Mexico, and Central and South America [15].

A number of entomopathogenic fungi have been isolated from hopper pests in rice crops in China, Taiwan, Philippines, Thailand, Japan, Korea, and India [16–20], and several have been evaluated for the control of planthoppers and leafhoppers [13, 21–26]. *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin (Ascomycota: Hypocreales) appear to be the most useful because of the ease of their mass production, storage, virulence, and application [18].

Although *B. bassiana* is a well-known soilborne fungus, several studies have shown that it also forms an endophytic symbiosis with maize [27, 28] making it an excellent candidate for biocontrol of hemipteran pests of corn.

In Argentina, few entomopathogenic fungi have been recorded from Hemiptera: *Cordyceps sobolifera* (Berkeley) Berkeley & Broome (Ascomycota: Pyrenomycetes) has been isolated from *Proarna bergi* (Distant) (Hemiptera: Cicadidae), *Isaria fumosorosea* Wize from *Oliarus dimidiatus* Berg (Hemiptera: Cixiidae), and *Clonostachys rosea* (Link: Fries) Schroers, Samuels, Seifert & Gams (Ascomycota: Hypocreales) from *Oncometopia tucumana* Schröder and *Sonesimia grossa* (Signoret) (Hemiptera: Cicadellidae) [29–31]. In this article, a selection of native hypocrealean fungal isolates was evaluated against adults of *P. maidis*, *D. kuscheli*, and *D. maidis* to test its pathogenicity under laboratory conditions.

Material and methods

Peregrinus maidis used in the assays were reared on corn spread in plastic flowerpots isolated with 24 × 9 cm polyethylene terephthalate (PET) plastic cages, in the greenhouse of Centro de Estudios Parasitológicos y de Vectores (CEPAVE, La Plata, Buenos Aires, Argentina) at 20 ± 5°C and under a photoperiodicity of 12:12 h (day: night).

Preliminary screening assays were carried out on 17 isolates from five fungal species *Beauveria bassiana Lecanicillium muscarium* (Petch) Zare & W. Gams, *Metarhizium anisopliae*, *Isaria farinosa* (Holmsk.: Fr.) Fr. and *I. fumosorosea*.

Fungal isolates were obtained from hosts in the Hemiptera, Coleoptera, and Dermaptera, as well as from soil samples from sorghum and corn crops. All isolates were collected from 2001 to 2005 from Buenos Aires, Corrientes, and Tucumán provinces northern Argentina, examined, and then stored at 4°C in the Mycological Collection of CEPAVE.

Entomopathogenic fungi were cultured on malt extract agar (MEA) in Petri dishes and incubated for 10 days at 25°C in darkness. After this period of time conidia were harvested with a disposable cell scraper (Fisherbrand®) and placed into test tubes containing 5 ml of 0.01% (v/v) Tween 80 (Merck). The suspension was vortexed for 1 min to homogenize it and afterwards was filtered through sterile muslin layer; the concentration of propagules in the resulting suspension was adjusted to 1×10^7 conidia/ml using a Neubauer hemacytometer. Conidial germination was calculated for each isolate according to Lane et al. [32].

For each fungal isolate 60 adults (approximately 10 d old) were collected from the greenhouse by aspiration and placed in groups of 10 males and 10 females (brachypterous) into glass tubes. They were then transferred in groups of 20 individuals to PET plastic bottles (500 ml capacity) whose bottom was cut off and then sealed with Parafilm® punched with small holes

to let air circulation before inoculation with 350 µl of a suspension of 1×10^7 conidia/ml using a sterilized glass nozzle of 26 cc of capacity. The bottle assemblies were placed upside down on 200 cc glass flasks containing young corn plants (3–4 leaf stage) with their roots surrounded by cotton and immersed in distilled water (Fig. 1A). Four assemblies, each of which contained 20 insects (10 males and 10 females), for a total of 80 insects were used as control. Controls were sprayed with 350 µl of 0.01% (v/v) Tween 80. Treated and control insects were maintained at 24 ± 2°C, 70% RH and a photoperiod of 14:10 h (light: dark). Young plants of corn were changed every 3 days. Insects were checked every 24 h up to 7 days. Dead insects were removed daily and superficially sterilized placing the specimens in 70% ethanol for a few seconds, washed in sterile distilled water, placed in 0.5% sodium hypochlorite for 1 min, and finally rinsed again in distilled water according to Lacey and Brooks [33] before being placed in Petri dishes with filter paper moistened with sterile distilled water and incubated at 25°C for 3–5 days. Mycelial emergence was confirmed microscopically. Only those insects showing external mycelial growth were considered to have died from fungal infection. After a week, the cumulative mortality (%) was calculated. The differences in mortality levels among treatments were analyzed on a weekly basis, and cumulative mortality data were analyzed by analysis of variance (ANOVA), and their means were separated by Fisher's least significant difference (LSD) multiple range test option ($P \leq 0.05$) using the Statgraphics software [34].

After evaluating the biological activity of the different fungal isolates against *P. maidis*, the single best isolate against this host was assayed against adults of *P. maidis*, *D. maidis*, and *D. kuscheli*. This assay was run twice over time under the same conditions above-mentioned, although daily mortality was recorded for up to 14 days. After 2 weeks the cumulative mortality was noted, and the median lethal time [MLT] were calculated using the formula reported by Lecuona and Díaz [35]: MLT = Σ [$\text{Days}_n \times \text{dead insect}_n$] / total of dead insects. The differences in mortality levels among treatments were analyzed by one-way ANOVA or Kruskal–Wallis analysis, and

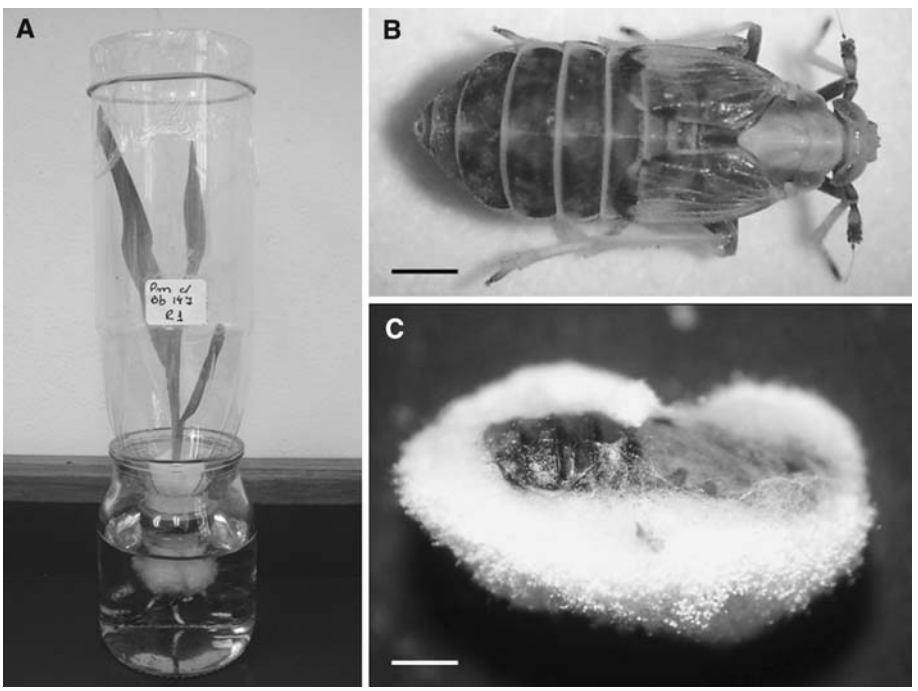

Fig. 1 (**A**) Bottle placed upside down on glass flask containing distilled water. (**B**) Healthy adult of *Peregrinus maidis*. (**C**) Adult of *P. maidis* 72 h after its death caused by *Beauveria bassiana* CEP 147

means were separated by LSD test ($P \leq 0.05$) using the Statgraphics software.

Results and discussion

Seventeen isolates of five fungal species, their CEPAVE collection accession numbers, host insect, substrate, location, province, and date of collection are given in Table 1. The results of the ANOVA applied to the cumulative mortality of *P. maidis* and fungal isolates conidial germination percentages are given in Table 2.

After 7 days post-inoculation significant differences were observed among treatments ($F = 2.38$; df = 17; 37; $P = 0.014$). Percentages of fungal infection were 18.5–69.8%, although mortalities exceeded 20% in the majority of isolates tested. The three most effective isolates against *P. maidis* (Fig. 1B) were *B. bassiana* CEP 147, CEP 150, and CEP 189, all of whose cumulative mortalities exceeded 50%. *B. bassiana* CEP 147 (cumulative mortality of 69.8 ± 6.4%) was selected for comparative pathogenicity tests against *P. maids*, *D. kuscheli*, and *D. maidis*. Proportionally more females than males were infected, but the differences were not statistically significant ($F = 0.73$; df = 1; 108; $P = 0.39$). Mortality percentages were similar for males

Table 1 List of fungal isolates screened against adults of P. maidis. Note: CEPAVE = Centro de Estudios Parasitológicos y de Vectores, La Plata, Buenos Aires, Argentina

Fungus species	CEPAVE access No.	Host insect / substrate	Locality of origin and collection date
Beauveria bassiana	CEP 001	Soil / *Zea mays*	Magdalena, Buenos Aires (35°13' S–57°43' W) 05/30/2003
B. bassiana	CEP 002	Soil / *Z. mays*	Los Hornos, Buenos Aires (34°52' S–57°58' W) 07/03/2003
Metarhizium anisopliae	CEP 003	Soil / *Sorghum vulgare*	San Vicente, Buenos Aires (35°01' S–58°27' W) 07/13/2003
Isaria farinosa	CEP 004	Soil / *Z. mays*	Los Hornos, Buenos Aires (34°52' S–57°58' W) 07/03/2003
I. fumosorosea	CEP 031	*Oliarus dimidiatus* Berg (Hemiptera: Cixiidae) / gramineous	La Plata, Buenos Aires (34°54' S–57°55' W) 11/2001
Lecanicillium muscarium	CEP 063	*Delphacodes kuscheli* Fennah (Hemiptera: Delphacidae) / *Hordeum vulgare* L.	La Plata, Buenos Aires (34°54' S–57°57' W) 08/26/2003
B. bassiana	CEP 074	*Balacha melanocephala* (Signoret) (Hemiptera: Cicadellidae) / *Eryngium* sp.	Berazategui, Buenos Aires (34°51' S–58°06' W) 12/30/2003
B. bassiana	CEP 080	*Kronides sp*. (Hemiptera: Membracidae) / *Eryngium* sp.	San Vicente, Buenos Aires (35°01' S–58°26' W) 03/20/2004
M. anisopliae	CEP 086	Unidentified species of Cercopidae/ *Eryngium* sp.	La Plata, Buenos Aires (34°57' S–58°04' W) 04/07/2004
B. bassiana	CEP 137	*B. melanocephala* / *Eryngium* sp.	Berazategui, Buenos Aires (34°51' S–58°06' W) 07/21/2004
B. bassiana	CEP 142	*Pawiloma victima* (Germar) (Hemiptera: Cicadellidae) / *Eryngium* sp.	Berazategui, Buenos Aires (34°51' S–58°06' W) 08/25/2004
B. bassiana	CEP 147	*Cycloneda sanguinea* L. (Coleoptera: Coccinellidae) / *Z. mays*	El Manantial, Tucumán (26°49' S–65°16' W) 03/11/2004
B. bassiana	CEP 150	*Diabrotica speciosa* (Germar) (Coleoptera: Chrysomelidae) / *Zea mays*	El Manantial, Tucumán (26°49' S–65°16' W) 03/11/2004
B. bassiana	CEP 151	*Doru lineare* (Eschscholtz) (Dermaptera: Forficulidae) / *Z. mays*	El Manantial, Tucumán (26°49' S–65°16' W) 04/2004.
M. anisopliae	CEP 160	Unidentified species of Cercopidae/ *Eryngium* sp.	Esteros del Iberá, Corrientes (28°24' S–57°07' W) 10/06/2004
M. anisopliae	CEP 178	*Kanaima fluvialis* (Lallemand) (Hemiptera: Cercopidae) / *Eryngium* sp.	Bella Vista, Corrientes (28°26' S–58°55' W) 11/15/2004
B. bassiana	CEP 189	*O. dimidiatus* / *Oryza sativa* L.	Los Hornos, Buenos Aires (34°52' S–57°58' W) 03/28/2005

Table 2 Results of conidial germination and cumulative mortality of 17 fungal isolates evaluated against *P. maidis*

Treatment	Conidial viability	Cumulative mortality
Control	–	6.3 ± 3.2 a
B. bassiana CEP 001	100	20.2 ± 8.3 ab
B. bassiana CEP 002	100	32.6 ± 15.4 abcd
B. bassiana CEP 074	98.0 ± 0.7	20.0 ± 11.6 ab
B. bassiana CEP 080	100	45.5 ± 2.3 bcde
B. bassiana CEP 137	99.2 ± 0.8	24.4 ± 4.9 abc
B. bassiana CEP 142	99.6 ± 2.7	23.3 ± 13.3 abc
B. bassiana CEP 147	96.5 ± 1.6	69.8 ± 6.4 e
B. bassiana CEP 150	98.2 ± 0.5	59.9 ± 11.5 de
B. bassiana CEP 151	80.5 ± 5.5	42.2 ± 10.2 bcde
B. bassiana CEP 189	99.5 ± 0.3	52.9 ± 5.3 cde
L. muscarium CEP 063	100	36.3 ± 12.6 bcd
M. anisopliae CEP 003	100	18.5 ± 8.3 ab
M. anisopliae CEP 086	100	35.2 ± 18.6 abcd
M. anisopliae CEP 160	100	26.9 ± 7.9 abc
M. anisopliae CEP 178	100	31.8 ± 12.6 abcd
I. farinosa CEP 004	99 ± 0.6	19.4 ± 10.0 ab
I. fumosorosea CEP 031	100	44.1 ± 17.2 bcde

Data are given in mean ± standard error; values followed by the same letters do not differ significantly according to LSD test ($P \leq 0.05$)

(31.0 ± 3.3%) and females (35.0 ± 3.4%). However, under field conditions natural infections by *B. bassiana* were observed in a larger number of females than males of Cicadellidae (Toledo, unpublished data). A recent study showed a sex-based differential mortality among thrips (Thysanoptera) treated with different isolates of *B. bassiana* [36].

There was no correlation between the conidial germination percentage and the insect infection percentage ($r = -0.21$; df = 1; $P = 0.41$) in any treatment. The lack of correlation between these parameters was also observed among isolates of *M. anisopliae* and of *Nomuraea rileyi* (Farlow) Samson by de Faria et al. [37] and Tigano–Milani et al. [38], respectively.

Analyzing comparative pathogenicity tests against adults of *P. maidis*, *D. kuscheli*, and *D. maidis* after 7 d post-inoculation indicated significant differences among treatments. Both *D. kuscheli* (cumulative mortality of 59.5 ± 7.9%) and *P. maidis* (cumulative mortality of 54.4 ± 6.6%) were significantly more susceptible to *B. bassiana* CEP 147 than *D. maidis* (cumulative mortality of 14.0 ± 3.9%) ($K = 28.71$; $P = 0.0000$).

The grooming behavior of *Beauveria*-infected *D. maidis* deserves mention. In contrast to the other two species of insects, *D. maidis* tried to clean its body by repeatedly rubbing the third pair of legs on its tegmine. Few studies describe defensive behaviors induced in response to pathogens because defensive reactions of hosts to pathogens are usually considered only from an immunological perspective [39]. However, grooming is a common defense used by ants for the continuous removal from their bodies of bacteria, fungi, and other disease-inducing organisms found in the soil [40]. In addition, recent studies indicate that termites are able to detect the presence of conidia of the fungal pathogen *M. anisopliae* and exhibit a striking vibratory display [41] that might directly reduce the number of propagules on the insect cuticle, thus decreasing the probability of fungal infection.

After 7 d post-treatment no significant differences in mortality levels according to the sex of the insect were recorded ($F = 0.50$; df = 1; 46; $P = 0.48$). Similar percentages of cumulative mortality for males and females were observed. Average percent mortality values for the three insect species were 37.2 ± 6.2% for females and 31.0 ± 6.1% for males.

After 2 weeks post-inoculation significant differences were also observed among treatments ($K = 22.69$; $P = 0.0000$). Coincidently with results already given for 7 d post-inoculation, *D. kuscheli* and *P. maidis* were the most susceptible species to fungal infection, presenting cumulative mortality percentages of 73.3 ± 9% and 68.6 ± 6.7%, respectively, although *D. maidis* mortality was 49.9 ± 9.7%. After this period of time males and females were equally affected by *B. bassiana* CEP 147. In this case the mortalities were 55.1 ± 6.7% for females and 46.7 ± 7.7% for males. No significant difference between the sexes were recorded ($F = 0.67$; df = 1; 46; $P = 0.42$). The MLT was 6.1 days for *P. maidis* and 6.2 days for *D. kuscheli*, while it was 8.6 days for *D. maidis*. The results of the Kruskal–Wallis test applied to the cumulative mortality of the three species treated, and the MLT are shown in Table 3.

Twenty-four hours after death, mycelia began to grow out through the spiracles and intersegmental membranes of the host cadavers. The time

Table 3 Cumulative mortality and median lethal time (MLT) caused by *B. bassiana* CEP 147

Treatment	Cumulative mortality		Median lethal time
	7 days	14 days	
Control	8.3 ± 2.1 a	11.7 ± 2.1 a	
D. maidis	14.0 ± 3.9 a	49.9 ± 9.7 b	8.6
P. maidis	54.4 ± 6.6 b	68.6 ± 6.7 bc	6.1
D. kuscheli	59.5 ± 7.9 b	73.3 ± 9.0 c	6.2

MLT data are given in days calculated at one- and two-weeks post-inoculation, while cumulative mortality values are given in percentage (mean ± standard error); values followed by the same letters do not differ significantly according to Kruskal–Wallis test ($P \leq 0.05$)

after death for hyphal emergence by *B. bassiana* was similar in all insect species treated. Mycelium was observed to cover all the surfaces of dead insects within 48 h after death. Mycelium was completely sporulated at 72 h after death (Fig. 1C).

It is interesting to note that not all dead insects always presented external mycelial growth, and such individuals were excluded the statistical analysis, thus indicating that the actual mortalities were potentially slightly higher than what has been reported here. Only 26.2% of *D. kuscheli*, 28.2% of *P. maidis*, and 29.7% of *D. maidis* adults, respectively, that died by 14 d post-inoculation did not present external growth of mycelia. In some individuals of *D. maidis* (25%), cuticular darkening after death was noted, and 50% of insects with this change of coloration failed to present external growth of mycelia. This could be a consequence of the defense mechanisms used by insects such as production of melanin pigments [42] or the interaction between fungus and bacteria after the insect death.

Although the mortality of *D. maidis* was 49.9 ± 9.7%, which 19–23% less compared with the two other insect species at 14 d post-inoculation, the mortality was higher than the 8.6–22.5% mortality reported for this host by Ibarra Aparicio et al. [13] after 25 d post-inoculation. These authors also reported that adult leafhoppers died from fungal infection in an average of 10.5 days whereas our results indicated an average time to mortality of 8.6 days.

No relationship between pathogenicity and the isolates' original hosts was observed during this research, even though it is often hypothesized that the more virulent fungal strains are isolated from the test organism or a closely related species [43–46]. Our results do not support this hypothesis since the most pathogenic isolate, *B. bassiana* CEP 147, was isolated from adults of *Cycloneda sanguinea* L. (Coleoptera: Coccinellidae) living on corn crops. The results from this study indicate that the screening of entomopathogenic fungi should not be limited only to isolates from the target host or its close relatives.

The *B. bassiana* strain selected here seems to be a good candidate for further development as a potential biological control agent against leafhopper and planthopper pests of corn in Argentina. Further studies under field conditions are needed to confirm whether the laboratory results with this isolate will accurately predict its performance in the field.

Acknowledgments The authors wish to thank Dr. Paradell (Entomology Division, Natural Science Museum of La Plata, Buenos Aires, Argentina) for identifying the cicadellids, Dr. Virla (PROIMI, Tucumán, Argentina) who for kindly providing some infected material from Tucumán province, and Dr. Schneider (CEPAVE, Buenos Aires, Argentina) for helping with statistical analysis. This study was partially supported by the National Research Council of Argentina (CONICET).

References

1. Nault LR, Ammar ED. Leafhopper and planthopper transmission of plant viruses. Ann Rev Entomol 1989;34:503–529.
2. Tsai JH, Wilson SW. Biology of *Peregrinus maidis* with descriptions of immature stages (Homoptera: Delphacidae). Ann Entomol Soc Am 1986;79:395–401.
3. Kunkel LO. Insect transmission of yellow stripe disease. Hawaii Plant Rec. 1922;26:58–64.
4. Cadwell JS, Martorell LF. Review of the Auchenorrhynchous Homoptera of Puerto Rico. Part II. The Fulgoroidea. J Agric Univ PR. 1950;34:133–270.
5. Harpaz I. Maize rough dwarf. A planthopper virus disease affecting maize, rice, small grains and grasses. Israel Universities Press, Jerusalem. 1972:p. 1–251.
6. Harris KF. Leafhopper and aphid as biological vectors: vector-virus relationships. In: Maramoroch K, Harris KF, editors. Plant diseases and vectors. Academic Press, S. Francisco, London. 1979. p. 217–308.
7. Remes Lenicov AMM de, Virla EG. Delfácidos asociados a cultivos de maíz en la República Argentina (Insecta-Homoptera-Delphacidae). Revista de la Facultad de Agronomía 1999;104:1–15.

8. Slykuis JT. Virus and virus like diseases of cereal crops. Ann Rev Phytopathol 1977;14:189–210.
9. Trujillo G, Guevara, Colina R. ''*Peregrinus maidis* (Ashm.), transmisor de enfermedades virales al maíz''. Revista de la Facultad de Agronomía (Maracay) 1978;9:51–61.
10. Tsai JH, Falk BW. Tropical maize pathogens and their associated insect vectors. In: Harris KF, editor. Advances in disease vector research. Springer-Verlag, New York, 1988. p. 177–201.
11. Virla E, Miotti I, Giménez Pecci MP, Carpane P, Laguna G. *Peregrinus maidis* (Hem.: Delphacidae), new experimental vector of the ''Mal de Río Cuarto'' disease to corn. Biocell 2004;28:54 (abs. 31) .
12. Remes Lenicov AMM de, Tesón A, Dagoberto E, Huguet N. Hallazgo de uno de los vectores del ''Mal de Río Cuarto'' del maíz. Gac Agronóm 1985;5:251–8.
13. Ibarra-Aparicio G, Moya-Raygoza G, Berlanga-Padilla A. Efecto de *Beauveria bassiana* y *Metarhizium anisopliae* sobre la chicharrita del maíz (*Dalbulus maidis*) (Delong & Wolcott, 1923) (Hemiptera: Cicadellidae). Folia Entomol Mex 2005;44:1–6.
14. Virla E, Remes Lenicov AMM de, Paradell S. Presencia de *Dalbulus maidis* (Insecta-Homoptera-Cicadellidae) sobre maíz y teosinte en la Argentina. Rev Fac Agronomía 1990/91;66/67:23–30.
15. Nault LR, Bradfute OE. Corn stunt: Involvement of a complex of leafhopper-borne pathogens. In: Maramorosch K, Harris KF, editors. Leafhopper vectors and plant disease agents. Academic Press, New York, NY. 1979. pp 654.
16. Chu YI, Hirashima Y. Survey of Taiwanese literature on the natural enemies of rice leafhoppers and planthoppers. Esakia Occas. Pap Hikosan Biol Lab Entomol 1981;16:33–7.
17. Reissig WH, Heinrichs EA, Litsinger JA, Moody K, Fiedler L, Mew TW, Barrion AT. Illustrated guide to integrated management in rice in tropical Asia. Intern. Rice Res. Inst. Los Baños, Laguna, Philippines. 1986.
18. Rice WC, Choo HY. Rice Pests. In: Lacey LA, Kaya HK, editors. Field manual of techniques in invertebrate pathology. Application and evaluation of pathogens for control of insects and other invertebrate pests. Kluwer Academic Publishers, Dordrecht, Boston, London. 2000. p. 425–446.
19. Rombach MC, Humber RA, Evans HC. Metarhizium album, a fungal pathogen of leaf-and planthoppers of rice. Trans Brit Mycol Soc 1987;88:451–9.
20. Rombach MC, Rombach GM, Roberts DW. Pathogens of insect pests of rice: A bibliography Insect Sci. Appl. 1987;8:197–210.
21. Aguda RM, Rombach MC, Im DJ, Shepard BM. Suppression of populations of brown planthopper, *Nilaparvata lugens* (Stål) (Hom.; Delphacidae) in field cages by entomogenous fungi (Deuteromycotina) on rice in Korea. J Appl Entomol 1987;104:167–172.
22. Holdom DG, Taylor PS, Soper RS. Activity of Entomophthoran fungal isolates (Zygomycetes) against *Nilaparvata lugens* and *Sogatodes orizicola* (Homoptera: Delphacidae). J Invert Pathol 1988;52:221–230.
23. Kanga LHB, Jones WA, Humber RA, Boyd Jr. DW. Fungal pathogens of the glassy-winged sharpshooter *Homalodisca coagulata* (Homoptera: Cicadellidae). Fla Entomol 2004;87:225–8.
24. Lane BS, Trinci APJ, Gillespie AT. Influence of cultural conditions on the virulence of conidia and blastospores of *Beauveria bassiana* to the green leafhopper, *Nephotettix virescens*. Mycol Res 1991;95:829–833.
25. Rombach MC, Aguda RM, Roberts DW. Biological control of the brown planthopper, *Nilaparvata lugens* (Homoptera: Delphacidae) with dry mycelium applications of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes). Philipp Ent 1986;6:613–9.
26. Rombach MC, Aguda RM, Shepard BM, Roberts DW. Infection of rice brown planthopper, *Nilaparvata lugens* (Homoptera: Delphacidae), by field application of entomopathogenic Hyphomycetes (Deuteromycotina). Environ Entomol 1986;15:1070–3.
27. Lewis LC, Cossentine JE. Season long intraplant epizootics of entomopathogens, *Beauveria bassiana* and *Nosema pyrausta*, in a corn agroecosystem. Entomophaga 1986;31:363–9.
28. Lewis LC, Bing LA. *Bacillus thuringiensis* Berliner and *Beauveria bassiana* (Balsamo) Vuillemin for European corn borer control: Program for immediate and season-long suppression. Can Entomol 1991;123:387–393.
29. López Lastra CC. Primera cita de Cordyceps sobolifera (Ascomycotina Pyrenomycetes) patógeno de insectos en la República Argentina. Bol Soc Argent Bot 1989;26:59–60.
30. Toledo AV, Scorsetti AC, Dikgolz VE, López Lastra CC. *Paecilomyces fumosoroseus* y *Nomuraea rileyi* (Deuteromycotina: Hyphomycetes), hongos patógenos de insectos plaga de la agricultura en la Argentina. Bol Soc Argent Bot 2004;39:21–6.
31. Toledo AV, Virla E, Humber RH, Paradell SL, López Lastra CC. First record of *Clonostachys rosea* (Ascomycota: Hypocreales) as an entomopathogenic fungus of *Oncometopia tucumana* and *Sonesimia grossa* (Hemiptera: Cicadellidae) in Argentina. J Invert Pathol 2006;92:7–10.
32. Lane BS, Humphreys AM, Thompson K, Trinci APJ. ATP Content of stored spores of *Paecilomyces farinosus* and the use of ATP as a criterion of spore viability. Trans Brit Mycol Soc 1988;90:109–148.
33. Lacey LA, Brooks WM. Initial handling and diagnosis of diseased insects. In: Lacey LA, editor. Manual of techniques in insect pathology. Academic Press, San Diego. 1997. p. 1–15.
34. STSC Statgraphics User's Guide, Version 4.0. Graphic Software System, STSC, Rockville, MD, 1987.
35. Lecuona RE, Díaz BM. Susceptibilidad de *Spodoptera frugiperda* (J. E. Smith) a los hongos entomopatógenos *Nomuraea rileyi*, *Metarhizium anisopliae* y *Beauveria bassiana*. Revista de Investigaciones Agropecuarias 2001;30:25–42.
36. Abe M, Ikegami T. Susceptibility of five species of thrips to different strains of the entomopathogenic fungus *Beauveria bassiana*. Appl Entomol Zool 2005;40:667–674.

37. de Faria MR, Tigano MS, Fontes EMG, Lecuona RE. Characterization and virulence of *Metarhizium anisopliae* (Metsch.) Sorok. Isolates to eggs of *Deois flavopicta* (Stål) (Homoptera: Cercopidae). Am Soc Entomol Brasil 1995;24:659–663.
38. Tigano–Milani MS, de Faria MR, Lecuona RE, Sartori MR, Arima EY, Díaz BM. Análise de patogenicidade e germinação do fungo *Nomuraea rileyi* (Farlow) Samson isolado no Distrito Federal. An Soc Entomol Brasil 1995;24:53–60.
39. Lord JC, Howard RW. A proposed role for the cuticular fatty amides of *Liposcelis bostrychophila* (Psocoptera: Liposcelidae) in preventing adhesion of entomopathogenic fungi with dry-conidia. Mycopathologia 2004;158:211–7.
40. Siebeneicher SR, Vinson SB, Kenerley CM. Infection of the red imported fire ant by *Beauveria bassiana* through various routes of exposure. J Invert Pathol 1992;59:280–5.
41. Rosengaus RB, Jordan C, Lefebvre ML, Traniello JFA. Pathogen alarm behavior in a termite: A new form of communication in social insects. Naturwissenschaften 1999;86:544–8.
42. Boucias DG, Pendland JC. Insect immune defense system, Part III: Prophenoloxidase cascade and post-attachment processes of phagocytosis. In: Boucias DG, Pendland JC, editors. Principles of insect pathology. Kluwer Academic Publishers, Massachusetts, USA. 1998. p. 499–537.
43. Latch GCM. *Metarhizium anisopliae* (Meschnikoff) Sorokin strains in New Zealand and their possible use for controlling pasture-inhabiting insects. NZJ Agric Res 1965;8:384–396.
44. Latch GCM. Studies on the susceptibility of *Orycetes rhinoceros* to some entomogenous fungi. Entomophaga 1976;21:31–8.
45. Poprawski TJ, Marchal M, Robert PH. Comparative susceptibility of Otiorhynchus sulcatus and Sitona lineatus (Coleoptera: Curculionidae) early stage to five entomopathogenic hyphomycetes. Environ Entomol 1985;14:247–253.
46. Soares GG Jr, Marchal M, Ferron P. Susceptibility of Otiorhynchus sulcatus (Coleoptera: Curculionidae) larvae to *Metarhizium anisopliae* and *Metarhizium flavoviridae* (Deuteromycotina: Hyphomycetes) at two different temperatures. Environ Entomol 1983;12:1886–1890.