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**PATHOGENICITY OF *BEAUVERIA BASSIANA* (DEUTEROMYCOTINA: HYPHOMYCETES) AGAINST THE WHITE GRUB *LANIIFERA CYCLADES* (LEPIDOPTERA: PYRALIDAE) UNDER FIELD AND GREENHOUSE CONDITIONS**

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ABSTRACT

The white grub *Laniifera cyclades* Druce of prickly pear cactus or nopal is a pest that limits the commercial production of *Opuntia*. The gregarious larvae perforate the cladodes devouring the inner part, thereby forming large galleries until reaching the central axis of the plant; during their movement through the inner part of the cactus, the larvae make orifices to the exterior to expel their excrements. In this investigation, the virulence of strains BbZ3 and BbZ4 of the entomopathogenic fungus *Beauveria bassiana* was determined by introducing infested cadavers of *Galleria mellonella* L. through the orifices on the stem pads of the nopal plant. Both strains of *B. bassiana* were highly pathogenic causing 100% mortality in the larvae of *L. cyclades* inside the nopal cladodes in the greenhouse as well in the field. BbZ3 was more virulent with a LT50 of 5.1 d in the greenhouse and 6.4 d in the field, while the LT50 of BbZ4 was 6 and 7.5 d, respectively. The application of larval cadavers of *G. mellonella* infested with the fungus *B. bassiana* was an effective control strategy against larvae of *L. cyclades*.

Key Words: *Opuntia* spp. microbial control, entomopathogenic fungi, larval cadavers.

RESUMEN

El gusano blanco del nopal *Laniifera cyclades* Druce es una plaga limitante de la producción comercial de *Opuntia*. Las larvas gregarias perforan los cladodios devorándolos en la parte interna, formando grandes galerías hasta alcanzar el eje central de la planta; durante su traslado a través de la parte interna del nopal, las larvas hacen orificios al exterior para expulsar sus excrementos. En esta investigación se determinó la virulencia de las cepas BbZ3 y BbZ4 del hongo entomopatógeno *Beauveria bassiana* mediante la introducción de un cadáver micosado de *Galleria mellonella* L. a través de los orificios en las pencas de nopal. Ambos aislados de *B. bassiana* fueron altamente patogénicos al originar el 100% de mortalidad sobre las larvas de *L. cyclades* dentro de los cladodios de nopal tanto en invernadero como en campo, BbZ3 fue más virulento con un TL50 de 5.1 días en invernadero y 6.4 días en campo, mientras que los TL50 de BbZ4 fueron de 6 y 7.5 días respectivamente. La aplicación de cadáveres de larvas de *G. mellonella* micosadas con el hongo *B. bassiana* fue una estrategia efectiva de control sobre las larvas de *L. cyclades*.

Translation by the authors.

The prickly pear cactus or nopal *Opuntia* spp. is one of the most important plants of Mexico, especially in semi-arid and arid regions where few crops can be cultivated. The main production of the cactus is for fruits and vegetables for human consumption, forage for livestock, and for industrial products such as cosmetics and dyes (Vigueras & Portillo 2001). The white grub *Laniifera cyclades* Druce of nopal is one of the pests limiting the production of *Opuntia* spp. (Badii & Flores 2001). The adults deposit eggs in groups of 30 to 50 on the cladodes, and the gregarious larvae perforate the cladodes devouring the inner part and gradually penetrating the tissue forming large galleries until reaching the central axis of

the plant, where inside they pass through larval stages and pupate. During their movement through the inner part of the nopal, the larvae make orifices to the exterior in order to expel their excrements, forming on the ground what growers call "little mountains of rice." These wastes serve as signs for detecting the presence of the pest, which enables destroying larvae mechanically, but often a major part of the plant must be destroyed. Strategies for control of *L. cyclades* recommended by governmental institutions consist in application of chemical insecticides through the orifices made by the larvae (Saenz 1998), thereby contaminating the whole plant and its fruits. Thus, an alternative biological control

would be of great utility. Microbial pathogens offer possibilities as biopesticides, but little is known about the microbial enemies of *L. cyclades*. *Beauveria bassiana* Vuillemin is the entomopathogenic fungus most widely distributed in the world, and it infects insects in tropical, temperate, humid, and desert areas (Zimmermann 2007). Various products based on *B. bassiana* are commercially available for controlling insect pests of agricultural importance, such as the coffee berry borer *Hypothenemus hampei* Ferrari and various species of Curculionidae (Adane et al. 1996; de la Rosa et al. 1997). Lepidoptera are also important hosts of this fungus, including several species of agricultural importance (Abdel-Razek et al. 2006). One factor that affects the efficacy of *B. bassiana* is sunlight; persistence and infectivity are reduced within a few minutes after exposure to sunlight (Fargues et al. 1996). Reduced environmental humidity also affects the efficacy and survival of the fungus because the most effective germination of the spores on the insect cuticle requires a relative humidity (RH) range of 92 to 100%, but there are reports of *B. bassiana* infection at 60-70% RH (Zimmermann 2007). The relative humidity of the semi-arid region in the municipality of Noria de Ángeles, Zacatecas is on average 49%, but the use of infested cadavers might afford protection to *B. bassiana*. Koppenhofer et al. (1997) and Shapiro-Ilan et al. (2001) reported that entomopathogenic nematodes could survive under dry conditions for long periods of time if they remained inside cadavers of host insects. Larvae of the wax moth *Galleria mellonella* L. have been used as cadavers infested with the entomopathogenic nematode *Heterorhabditis bacteriophora* Poinar for controlling the sweetpotato weevil *Cylas formicarius* (Fabricius) (Jansson & Lecrone 1994). Cadavers of *G. mellonella* infested with *B. bassiana* and placed in the holes of nopal plants infested with *L. cyclades* could prevent exposure to sunlight and protect the fungus from the low RH outside the plant. The aim of this investigation was to evaluate the pathogenicity of the strains BbZ3 and BbZ4 of *Beauveria bassiana* to *L. cyclades* after introducing infested cadavers of *G. mellonella* into the excretion orifices in the nopal stem pads, under greenhouse and field conditions.

## MATERIALS AND METHODS

### Biological Material

Third instars of *L. cyclades* to be used in the bioassays in the greenhouse were collected directly from nopal plants in Noria de Ángeles, Zacatecas, Mexico. These were transported to the Entomology and Biological Control Laboratory of the Academic Unit of Agronomy at the Universidad Autónoma de Zacatecas, maintained for a

week at  $23 \pm 1^\circ\text{C}$ ,  $33 \pm 5\%$  RH in Petri dishes, and fed daily with pieces of fresh nopal. Holding the larvae in this manner enabled us to rule out the presence of disease.

Strains BbZ3 and BbZ4 of *B. bassiana* were originally isolated from soil samples from 2 orchards of "nopal tunero" in the municipalities of Noria de Ángeles and Pinos, Zacatecas, Mexico, and they are part of the collection of entomopathogenic fungi of the Academic Unit of Agronomy at the Universidad Autónoma de Zacatecas. Pathogenicity bioassays were conducted on third instars of *L. cyclades*. The isolated strains of *B. bassiana* were grown in Sabouraud dextrose agar (SDA) with yeast extract (2g/L) (SDAY) at  $24 \pm 1^\circ\text{C}$ . After 3 weeks, the conidia were collected in 10 mL sterile distilled water with 0.01% Tween-80 to reduce surface tension. Conidia were counted in a Neubauer chamber and dilutions were made to a final concentration of  $1 \times 10^8$  conidia/mL. Whatman No.1 filter paper was placed on the bottom of 40 Petri dishes and 10 fifth instars of *G. mellonella* were inoculated in each Petri dish with 1 mL spray of a conidial suspension of each fungal isolate. Two weeks later, 100% of the larvae were dead and infested, and each of the cadavers of *G. mellonella* was utilized for application of the fungus during the bioassays with *L. cyclades* in the field and in the greenhouse. The method for applying the fungus consisted of the introduction of a *G. mellonella* cadaver into the orifices made by the *L. cyclades* larvae in the nopal plants. In the greenhouse, the orifices were made manually and larvae of *L. cyclades* were inserted into the orifices. The conidial density applied per orifice was estimated by vortexing an infested *G. mellonella* larva with conidia in 10 mL of 0.01% Tween-80 solution for 3 min, and then counting spores and colony forming units (Tang & Hou 2001). The dose employed was  $10^{13}$  conidia or CFU per cadaver.

### Bioassays in the Greenhouse

For the inoculation of fungus in the greenhouse, 30 healthy nopal plants were selected, and in 1 cladode per plant an orifice was made that was 20 cm long and 1 cm in diameter, similar to those made by the larvae in the field. Two days later, 10 third instars of *L. cyclades* were introduced, and after a week the larvae were established as evidenced by the external mounds of excrement. At that time an infested cadaver of *G. mellonella* was introduced, so that *L. cyclades* larvae had to crawl over the wax moth larva when exiting to expel excrements. Starting on the fourth day, mortality was recorded daily. On ninth day, larvae were extracted from all the plants and placed in Petri dishes to continue to monitor them for mortality. For control treatments, 10 plants were selected that had mounds of excrement, and

maintained without introduction of wax moth cadavers.

#### Bioassays in the Field

In an orchard of nopal of 2,500 m<sup>2</sup> situated in the municipality of Noria de Angeles, Zacatecas, 30 plants with orifices and "little mountains of rice" indicating the presence *L. cyclades* larvae were selected. Each treatment was evaluated in 10 isolated plants. A *B. bassiana*-infested cadaver of *G. mellonella* was introduced into the natural excretion orifices, and starting on the fourth day and continuing to the eighth day, the diseased larvae of *L. cyclades* that exited to die outside of the colony were collected. On the ninth d all remaining larvae were extracted from the plant and held in the laboratory in Petri dishes to continue to monitor mortality. Dead larvae were placed in moist chambers to determine if sporulation of the fungus occurred. For control treatments, 10 plants showing evidence of excrement from larvae of *L. cyclades* were selected but were not inoculated with a waxmoth larval cadaver.

#### Statistical Analyses

The percentages of mortality were arcsine transformed and analyzed in a complete randomized block design (each repetition considered as a block), and analysis of variance (ANOVA) was utilized, followed by means separations by Tukey's test ( $P < 0.05$ ) (SAS Institute 1998). The means of mortality are presented with the original data. The LT50 was determined by probit analysis in the program ED50 plus v1.0.

#### RESULTS

The strains BbZ3 and BbZ4 of *B. bassiana* produced 100% mortality in the larvae of *L. cyclades* inside the cladodes of nopal by d 9 in the greenhouse and by d 10 in the field. Daily mean mortality data from d 4 to d 12 in the laboratory and field are presented in Tables 1 and 2, respectively. The data for mortality in the greenhouse indicated that by d 4, and continuing through d 10, strain BbZ3 of *B. bassiana* was statistically more patho-

genic than strain BbZ4 to the larvae of *L. cyclades* ( $F = 115.89$ ;  $df = 2, 27$ ;  $P = 0.0001$ ) after exposure to 1 infested larva of *G. mellonella*. By d 11 and 12 in the greenhouse, essentially 100% mortality was caused by both strains of the fungus. Similarly, in the treatment in the field, strain BbZ3 was more pathogenic than BbZ4, with a statistically significant difference starting at d 5 ( $F = 174.87$ ;  $df = 2, 27$ ;  $P = 0.0001$ ) and continuing through d 11 ( $F = 1699.68$ ;  $df = 2, 27$ ;  $P = 0.0001$ ). On d 12 in the field both strains of the fungus had caused 100% mortality in *L. cyclades* larvae. All the cadavers of *L. cyclades* gave rise to sporulation of the fungus, indicating that death was caused by infection and that the strains were capable of sporulating under existing humidity conditions. The LT50 was determined by linear regression in which percentage of mortality with each treatment was plotted against time for the days of evaluation. The LT50 occurred at 5.1 and 6.4 d in the greenhouse and field, respectively, for strain BbZ3, and at 6.0 and 7.5 d, respectively, in greenhouse and field for strains BbZ4.

#### DISCUSSION

Strains BbZ3 and BbZ4 of *B. bassiana* infesting cadavers of *G. mellonella* larvae were pathogenic to larvae of *L. cyclades* when the cadavers were placed inside nopal plants. The LT50 value was slightly lower for strain BbZ3 than for strain BbZ4, but both eventually killed 100% of *L. cyclades* larvae within 11 to 12 d in the greenhouse and field, respectively. Samuels et al. (1989) from their study noted that an LT50 longer than 14 d indicated non-pathogenicity. Sprenkel & Brooks (1975) demonstrated that the conidia of *Nomuraea rileyi* could remain infective on the surface of cadavers of the tobacco budworm *Heliothis virescens* (F.) for at least 256 d, causing epizootics and reduction in the populations of Lepidoptera pests in the soybean crop. The gregarious larvae *L. cyclades* have favorable conditions to develop induced epizootics inside the plants. Tanada & Kaya, (1993) argue that infection and sporulation of several entomopathogenic fungi are influenced by environmental factors, especially temperature and humidity, and to lesser extent photoperiod. In

TABLE 1. MEAN CUMULATIVE PERCENT MORTALITY  $\pm$ SD OF *LANIIFERA CYCLADES* LARVAE EXPOSED TO LARVAL CADAVERS OF *G. MELLONELLA* INFESTED WITH THE FUNGUS *B. BASSIANA* IN THE GREENHOUSE.

Isolate	Days after each treatment									
	4	5	6	7	8	9	10	11	12	
BbZ3	39 $\pm$ 8.7 a	57 $\pm$ 8.8 a	74 $\pm$ 6.9 a	89 $\pm$ 7.3 a	98 $\pm$ 4.2 a	100 $\pm$ 0 a	100 $\pm$ 0 a	100 $\pm$ 0 a	100 $\pm$ 0 a	100 $\pm$ 0 a
BbZ4	26 $\pm$ 5.1 b	45 $\pm$ 12.6 b	54 $\pm$ 5.1 b	68 $\pm$ 4.2 b	83 $\pm$ 6.7 b	88 $\pm$ 6.3 b	90 $\pm$ 6.6b	97 $\pm$ 4.8 a	100 $\pm$ 0 a	100 $\pm$ 0 a
Control	1 $\pm$ 3.1 c	1 $\pm$ 3.1 c	1 $\pm$ 3.1 c	1 $\pm$ 3.1 c	1 $\pm$ 3.1 c	1 $\pm$ 3.1 c	1 $\pm$ 3.1 c	1 $\pm$ 3.1 b	1 $\pm$ 3.1 b	1 $\pm$ 3.1 b

Means followed by the same letter within days (columns) are not significantly different (Tukey test,  $P > 0.05$ ).

TABLE 2. MEAN CUMULATIVE PERCENT MORTALITY  $\pm$ SD OF *LANIIFERA CYCLADES* LARVAE EXPOSED TO LARVAL CADAVERS OF *G. MELLONELLA* INFESTED WITH THE FUNGUS *B. BASSIANA* IN THE FIELD.

Isolate	Days after each treatment									
	4	5	6	7	8	9	10	11	12	
BbZ3	12 $\pm$ 10.3 a	32 $\pm$ 10.3 a	41 $\pm$ 11.0 a	60 $\pm$ 6.6 a	75 $\pm$ 7.0 a	82 $\pm$ 10.3 a	100 $\pm$ 0 a	100 $\pm$ 0 a	100 $\pm$ 0 a	100 $\pm$ 0 a
BbZ4	7 $\pm$ 6.7 a	11 $\pm$ 5.6 b	20 $\pm$ 4.7 b	32 $\pm$ 9.1 b	51 $\pm$ 5.6 b	68 $\pm$ 10.3 b	77 $\pm$ 8.2 b	91 $\pm$ 7.3 b	100 $\pm$ 0 a	100 $\pm$ 0 a
Control	0 $\pm$ 0.0 b	0 $\pm$ 0.0 c	0 $\pm$ 0.0 c	0 $\pm$ 0.0 c	0 $\pm$ 0.0 c	0 $\pm$ 0.0 c	0 $\pm$ 0.0 c	0 $\pm$ 0.0 c	0 $\pm$ 0.0 c	0 $\pm$ 0.0 b

Means followed by the same letter within days (columns) are not significantly different (Tukey-test,  $P > 0.05$ ).

this study, we have demonstrated that the introduction of sporulating *G. mellonella* cadavers into nopal plants infested with *L. cyclades* is an effective strategy to maintain pathogenicity of the fungus under the semi-arid conditions of the region. Movement of *L. cyclades* larvae over the infested *G. mellonella* cadavers may be an important factor in promoting infection with pathogens. For example, Baverstock et al. (2005) demonstrated that aphid movement could indirectly influence transmission of *Pandora neoaphidis* (Remaudière & Hennebert) to *Acyrtosiphon pisum* (Harris) by allowing the aphids to come into contact with the conidia. Shimazu (2004) noted that adhesion of dry conidia to the pine borer *Monochamus alternatus* from contact provided effective control of the insect. Our results demonstrate that it is easy to infect larvae of *L. cyclades* inside nopal plants with inoculation of *B. bassiana* sporulating in cadavers of *G. mellonella* larvae, and that it can be useful in controlling *L. cyclades*. Meyling et al. (2006) reported that, even though aphids *Microlophium carnosum* (Bukton) are likely to only contact inoculum briefly, they apparently become contaminated upon such encounters and distribute the inoculum on the host plant. Although the method we used of introducing an infected cadaver into an opening in nopal plant to control *L. cyclades* larvae is labor intensive, the use of infected insect cadavers may be a control strategy for other pests that develop inside cladodes such as *Cactoblastis cactorum* Berg (Lepidoptera: Pyralidae), the zebra worm *Olycella nephelepasa* (Lepidoptera: Pyralidae), and *Moneilema variolaris* Thompson (Coleoptera: Cerambycidae). Further investigations are necessary to evaluate the effectiveness of additional *B. bassiana* strains under laboratory and field conditions and to examine the potential impact on other species.

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