Hindawi Publishing Corporation
International Journal of Zoology
Volume 2012, Article ID 303589, 12 pages
doi:10.1155/2012/303589

Review Article

Pathogens Associated with Sugarcane Borers, *Diatraea* spp. (Lepidoptera: Crambidae): A Review

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Received 14 June 2012; Accepted 29 August 2012

Academic Editor: Thomas Iliffe

The objective of this paper was to analyze information related to entomopathogenic-associated *Diatraea* spp. Gaining a better understanding of the effects of these microorganisms will help in the development of successful microbial control strategies against stem borers that attack sugarcane plants.

1. Introduction

The *Diatraea* spp. (Lepidoptera: Crambidae) complex is only found in the American continent, and it is the most important group of stem borers that principally attack maize and sugarcane, as well as other gramineous crops, including rice, sorghum, and forage grasses [1]. The sugarcane borer (SCB) *D. saccharalis* Fab. is the most economically important pest in South America [2, 3], whereas the neotropical corn stalk borer (NCB) *D. lineolata* Walk is primarily found in Central America [4], *D. magnifactella* Dyar and *D. considerata* Heinrich are found in Mexico [1], and the southwestern corn borer *D. grandiosella* Dyar is found in the United States [5].

Unfortunately, commercially available insecticides are not efficient for the control of *Diatraea* spp. for a variety of reasons, mainly because of the continuous presence of the host plants in fields throughout the year, the concomitant presence of mature and immature forms of the insect, and the cryptic feeding habits of the insect [6]. An alternative strategy is integrated pest management with biological control as the first defense, which includes the use of parasitoids and entomopathogens.

In this paper, we present a perspective on the attempts to control *Diatraea* spp. using pathogens in the Americas. We also discuss the status of recent attempts to use pathogens in the field.

2. Fungi

One promising field for research is the use of entomopathogenic fungi as biological control agents of insect pests in sugarcane plants. Approximately 80% of the etiological agents involved in insect diseases are fungi, which encompass 90 genera and more than 700 species [7]. A number of fungi (Hypocreales: Clavicipitaceae) including *B. bassiana*, *B. brongniartii*, *M. anisopliae*, *P. fumosoroseus*, *Hirsutella* sp., *Cylindrocarpon* sp., and *Nomuraea rileyi* have been isolated from *Diatraea* spp. in the Americas from Argentina to the USA (Table 1). Under certain climatic conditions, *B. bassiana* has been reported to cause natural epizootics on *D. grandiosella* [8].

The life cycle of entomopathogenic fungi in the arthropod hosts is initiated with the germination of conidia that contacts the host integument and produces a germ tube that penetrates the host through a combination of physical pressure and enzymatic degradation of the cuticle. The fungus initially colonizes the host through a yeast phase. Host death usually results from a combination of nutrient

Table 1: Entomopathogens fungi from *Diatraea* spp.

Host species	Entomopathogen	Country	Reference
D. grandiosella *D. crambidoides*	*B. bassiana* (1–8%)	Mississippi, United States	Inglis et al., 2000 [5]
D. grandiosella	*B. bassiana* (epizootics)	Texas High Plains, United States	Knutson and Gilstrap, 1990 [8]
D. saccharalis	*B. bassiana* *M. anisopliae* *P. fumosoroseus*	Venezuela	Zambrano et al., 2002 [9]
D. saccharalis	*M. anisopliae*	PE, Brazil	Alves, et al. 2002 [10]
D. saccharalis	*B. bassiana*	Pinar del Río, La Habana, Matanzas, Villa Clara, Cienfuegos and Camagüey, Cuba	Estrada et al., 2004 [11]
D. saccharalis	*B. bassiana* *M. anisopliae* *Nomuraea rileyi* *Iseria* sp.	Tucumán, Argentina	Yasen de Romero et al., 2008 [12]
D. saccharalis	*M. anisopliae* (Ma2 and Ma3)	Mexico	Angel-Sahagún et al., 2005 [13]
D. saccharalis	*B. bassiana* ARSEF: 1489 1832 1834 2629 3020 3858 5500 5502	Pernambuco Brazil Brazil Brazil Pernambuco Brazil Pernambuco, Brazil Brazil Santa Fe, Argentina Santa Fe, Argentina	Humber et al., 2009 [14]
D. saccharalis	*B. brigniartii* ARSEF: 1830	Sao Paulo, Brazil	Humber et al., 2009 [14]
D. saccharalis	*Cylindrocarpon* sp. ARSEF: 8043 8044	Colima, Mexico	Humber et al., 2009 [14]
D. saccharalis	*M. anisopliae* ARSEF: 3290 3291 3292 3298	Colima, Mexico Colima, Mexico Colima, Mexico Colima, Mexico Colima, Mexico	Humber et al., 2009 [14]
D. magnifactella	*M. anisolpliae* *B. bassiana* *Hirsutella* sp.	Mexico	Hernández and Velázquez 2004 [15]
D. grandiosella	*Nosema* sp. isolate 167	Marshal County, (Mississippi, USA)	Inglis et al. 2000 [5]
D. grandiosella	*Nosema* sp. isolates 295, 504	Oktibbeha County, (Mississippi, USA)	Inglis et al. 2000 [5]

Table 1: Continued.

Host species	Entomopathogen	Country	Reference
D. grandiosella	*Nosema* sp. isolates 181, 513, 522	Washington County, (Mississippi, USA)	Inglis et al. 2000 [5]
D. saccharalis	*Nosema* sp.	Colombia	Lastra and Gómez 2000 [16]
D. saccharalis	*Granulovirus (DsGV)*	Southern United States	Pavan et al. (1983) [17]
D. saccharalis	*Densovirus (DsDNV)*	Guadeloupe	Meynadier et al. (1977) [18]
D. magnifactella	*B. thuringiensis*	Mexico	Fonseca-González et al. (2011) [19]

depletion, invasion of organs, and the action of fungal toxins. Hyphae usually emerge from the cadaver. The mummified corpse of the insect remains in the environment for several weeks and, in the case of stem borer, it keeps remains protected inside the stem. Therefore, it is more common to detect and isolate fungi compared to other pathogens that destroy the host, such as *Bacillus thuringiensis* (*Bt*), viruses, or nematodes.

Entomopathogenic fungi are widely distributed in all regions of the world; these species have wide genetic variation among the different isolates. Pathogenicity and virulence to different species, as well as enzymatic and DNA characteristics, vary among different isolates [14, 20, 21]. Therefore, it is important to evaluate as many geographic isolates as possible from different areas to select the most suitable isolate based on its virulence and growth at high temperatures. Several research groups have verified the pathogenicity and virulence of Hypocreales fungi, such as *M. anisopliae* and *B. bassiana* (Table 2), which have become important biocontrol agents used for the microbial control of *Diatraea* spp. It is possible that a limited selection of available isolates of *B. bassiana* could identify highly virulent strains from each of the different *Diatraea* species.

Field evaluations have been performed using *M. anisopliae* and *B. bassiana* against several insect pests of sugarcane, including *D. saccharalis* in Brazil. Application of *M. anisopliae* at a rate of 1×10^{13} spores per hectare caused 58% mortality of *D. saccharalis*, and *B. bassiana* at 3.7×10^{8} spores per milliliter reduced *D. saccharalis* damage by 45% (Alves et al., 1984 and 1985, cited in Legaspi et al. [22]). For effectiveness in the field, it is important to consider the contact between the spores and the host, formulation, and the virulence of the pathogens.

3. Microsporidia

Microsporidia (Eukaryota: Fungi) is the most ubiquitous group among insect populations [23, 24]. Microsporidia are tiny unicellular organisms (from 2 to 40 μm in diameter), that are opportunistic and obligate intracellular parasites and attack different groups of invertebrate and vertebrate animals. Microsporidia generally produce chronic diseases and reduce the physiological and reproductive ability of their hosts. Many species of microsporidia infect arthropods, especially insects such as Lepidoptera and Coleoptera [23–25].

In general, these microorganisms live as parasites in cells of the midgut epithelium, where they complete their development, and the cycle starts when the infective states of microsporidia (spores) arrive at the digestive tube and colonize this region all the way to the excretory system. The spores germinate because of the acid intestinal pH, the microorganisms penetrate the midgut cells, and the intestinal activity is paralyzed between 14 to 21 days later because the insect cannot assimilate nutrients. *Nosema locustae* infects the adipocytes of fat body, which interferes with the adequate function of the insect's intermediary metabolism and competes with the insect for energetic reserves. Microsporidia produce effects that depend on the species and concentration; however, they generally produce weakness and eventually lead to death [31, 32].

Only some species in this group have the possibility to be potentially relevant for natural or classical control. There are many studies worldwide on the pathogenic effects of the microsporidia *N. pyrausta* (Payllot) and *Vairimorpha necatrix* (Kramer) on borer organisms such as *Ostrinia nubilalis* (Hübner) (European corn borer), *Lymantria dispar* (Gypsy moth), and the grasshopper [5, 32]. In this group, only the microsporidium *N. locustae* has been registered as a microbial insecticide for the control of grasshoppers in grasslands [33].

In many areas of the USA and Europe, *Nosema* is the main agent used for the control of grasshoppers; however, in Latin America, few studies have been performed with *Nosema* [33]. For example, Inglis et al. [5] reported six isolates of *Nosema* on larvae in the winter diapause stage from *D. grandiosella* Dyer collected during 1998 in corn stems from three locations in Mississippi, USA. However, the frequency of infection in the field was very low (1, 3, and 15% in the counties of Marshal, Oktibbeha, and Washington, resp.), and no isolates were found in *D. crambidoides* (Grote) (Table 3).

When the mortality produced by the *Nosema* isolates was assessed in the laboratory using larvae that had stayed in environmental-like natural winter diapause conditions, variations in mortality between 0 and 55% were observed in larvae, and variations between 7 and 29% were observed in pupae; homogenization of the dead larvae revealed a large amount of *Nosema* spores. However, in the surviving adults, a large number of larvae were positive for the *Nosema* spores when they were analyzed under light microscopy using staining techniques and electron microscopy.

Table 2: Entomopathogenic-fungi bioassays on *Diatraea* spp.

Host species	Pathogen	Bioassay	Result	Reference
D. saccharalis	*B. bassiana*	Immersing third instar larvae into a suspension of 10^8 conidia/mL	TL_{50} (conidia isolates Bb1 and 5): 4.3 days. Mortality dry mycelium preparations: 21.3% (Bb1) 82.5% (Bb5) at 7 days after inoculation	Arcas et al., 1999 [26]
D. saccharalis	*B. bassiana* Mycotrol strain GHA	Sprayed first, second and third instar larvae with 1 mL	LD_{50} in spores/mm^2 1st instar: 72.1 2nd instar: 384.3 3rd instar: 777.0 Mean days of survival 1st instar: 4.6 2nd instar: 4.8 3rd instar: 6.4	Legaspi et al., 2000 [27]
D. grandiosella	*B. bassiana* Eight isolates	Dipped in conidial suspension (dosage not specified)	Mortality from 10 to 21%	Inglis et al., 2000 [5]
D. saccharalis	*B. bassiana* ATCC 20872 from *Solenopsis invicta*	Third instar larvae sprayed with suspension of 10^8 conida/mL	TL_{50} 3.02 to 4.10 days	Marques et al., 2000 [28]
D. sacccharalis	*B. bassiana* ATCC 20872 from *Solenopsis invicta*	Third instar larvae sprayed with 3 mL of yeast-like cells or conidia	LC_{50} 5.6×10^6 yeast/mL and 4.8×10^6 conidia/mL	Alves et al., 2002 [29]
D. saccharalis	*B. bassiana* IBCB from Brazil	1 cm larvae sprayed with 1 mL	CL_{50} 1.58×10^7 conidia/mL at 6 days after inoculation	Wenzel et al., 2006 [30]

Table 3: Entomopathogenic-microsporidia bioassays on *Diatraea* spp.

Host specie	Pathogen	Bioassay	Results	Reference
D. grandiosella	*Nosema* sp isolate 167	Surface of diet (squares), concentration not mentioned. Larvae and pupae	Mortality of 1–15%	Inglis et al., 2000 [5]
D. grandiosella	*Nosema* sp isolate 295, 504	Surface of diet (squares), concentration not mentioned. Larvae and pupae	Mortality of 1–15%	Inglis et al., 2000 [5]
D. grandiosella	*Nosema* sp isolate 181, 513, 522	Surface of diet (squares), concentration not mentioned. Larvae and pupae	Mortality of 1–15%	Inglis et al., 2000 [5]
D. saccharalis	*Vairimorpha necatrix*	1st instar larvae, Surface of diet	Microsporidiosis LC_{50} = 48.4 spores/mm^2 Gut damage LC_{50} = 8941 spores/mm^2	Fuxa, 1981 [32]
D. grandiosella	*Nosema* sp isolate 506	5-day-old larvae and pupae, leaf pieces ~5 cm^2 with 10 μl inoculum (10^1 to 10^7 spores)	Median infective dose of 2 $\times$ 10^3 spores per larva, pupae small, decreased egg production	Inglis et al., 2003 [34]

In these experiments, the larvae were reared in the winter diapause stage, and the temperature for *Nosema* was not optimal for the development of infection; in the field during the winter, the prediapause larvae migrate to the base of the corn stem just below the surface of the soil [5], and it is possible that *Nosema* can produce infection in natural conditions as the temperature inside the cane is higher than the external temperature.

Phoofolo et al. [35] observed a similar behavior, and they reported that the *O. pyrausta* infection by *Nosema* is

chronic. Although no immediate mortality is produced, the longevity and fecundity of the adults are reduced. Likewise, Phoofolo mentioned the possibility of a response to other factors of mortality that regulate the population dynamics of borers, such as low temperature, the host plant, and crowding, which have chronic effects on *Nosema* infection. Therefore, although the pest did not die directly because of microsporidia infection, the population size was eventually reduced.

This effect has been previously observed by Fuxa [32], when he evaluated the susceptibility of larvae in the first and third instars of six species of Lepidoptera, including *D. saccharalis*, to the microsporidium *Vairimorpha necatrix*. He described two routes of infection that resulted in mortality; one resulted from the chronic effects produced by the exposure of larvae to low doses of spores, which led to lethal septicemia (microsporidiosis) just before pupating, and the other route was due to the intake of a large number of spores, which apparently damaged the gut because of the introduction of a large number of spore polar filaments. In this study, Fuxa [32] concluded that this pathogen could be promising for the remaining five species, although not for *D. saccharalis*, as he had observed direct mortality by damage to the gut and indirect mortality by septicemia and because a high concentration of spores was necessary.

Inglis et al. [34] reported that one strain of *Nosema* (506), which had been previously isolated [5], can infect other species of insects in the Crambidae family, including *O. nubilalis* and *D. crambidoides* (Grote), but not other species of Noctuidae. However, they also observed that the infection can be transmitted transovarially, although at a very low frequency (a difference with other *Nosema* species that can be transmitted frequently in the vertical rout, such as the genus *Ostrinia*).

Solter et al. [36] researched the vertical and horizontal transmission of seven species of microsporidia, including two strains of *Nosema* sp. (isolated from their natural hosts in the field *D. saecharalis* and *Eoreuma loftini*); although five of these strains were transmitted at a low percentage horizontally and vertically, two *Nosema* strains did not behave similarly.

In assessing the infectivity percentage under laboratory conditions when applying high concentrations of spores, they observed very low mortality in the larvae of *D. sacharalis* and *E. loftini* (2 and 4%, resp.), although the same strains produced high mortality at low concentrations in the other species studied, including *O. nubilalis*. They explained the low mortality, even at high doses, as a function of low horizontal and vertical transmission because few infective or abnormal spores were produced.

In this case, it is important to clarify that they could obtain live infected larvae without mortality; however, they only measured mortality and did not measure other parameters, such as fecundity or larvae hatching in the next generation. As mentioned by Fuxa [32] and Inglis et al. [5], infection does not always lead to mortality, and occasionally the effects are observed long term and can be inferred from a reduction in fecundity and susceptibility to other stress situations.

Lastra and Gómez [16] implemented a system to produce natural enemies of *D. saccharalis* in CENICAÑA (Clombian Sugarcane Research Center, Colombia) and began their colony with larvae collected in the field. Their most significant result was the detection of one "protozoa," possibly *Nosema*, identified as the causal agent for the diminution in larvae production. They observed refractile spores in the fluids of the malpighi tubes or hemolymph using phase contrast microscopy in healthy adults. Macroscopically, the diseased larvae were dwarfed and white.

In addition, different pathogenic microorganisms have been isolated from *Diatraea* sp. in experimentally transmitted infections in the laboratory; however, there is little information about the natural presence of entomopathogens belonging to the microsporidia group in this species of borer. It is important to perform a systematic search for infected larvae, pupae, or adults of *Diatraea*, and studies need to include microscopy techniques to complement the mortality bioassays because infections could be asymptomatic. As many microsporidia do not produce insecticide activity quickly and because many species have complex life cycles that involve more than one host, very few attempts have been made to implement microsporidia and use them as agents of biological control.

4. Nematodes

Pathogenic nematodes of the Heterorhabditidae and Steinernematidae families live in the soil where they are parasitic to certain soil-dwelling insects. The free-living third juvenile stage (infective juveniles, IJs) locates a suitable host and penetrates the host in different ways. Once inside the insect, the IJs initiate its development, the nutritional tract becomes functional, and the symbiotic bacterium *Xenorhabdus* or *Photorhabdus* is released through the anus and begins to multiply in the hemocoel, which kills the insect by septicemia and creates suitable conditions for the reproduction of the nematode. Nematodes feed on bacteria and the dead tissue of the host, and they pass through several generations until new IJs are produced, which emerge from the cadaver [37]. They have a ubiquitous distribution [37]; therefore, it is not unusual to find them in the soil of sugarcane plantations (Pizano et al., 1985 cited in Khan et al. [38, 39]).

However, there are few reports of nematodes infecting stem borers of the *Diatraea* genus in natural and experimental situations. In Costa Rica, several entomopathogenic species, including nematodes, have been isolated from *D. tabernella* [40]; however, the author does not mention the nematode species.

Khan et al. [38] reviewed the world bibliography on rice stem borers and found five reports of entomopathogenic nematodes associated with *D. saccharalis*, two reports of *Steinernema* (=*Neoaplectana*) *glaseri* in Brazil and three reports of *Steinernema* (=*Neoaplectana*) *carpocapsae* in the USA and Guadeloupe. However, in one study, they report that the aim of the research was the use of *D. saccharalis* to produce *S. glaseri* in controlled conditions because of its susceptibility to the nematode. Another study by Folegatti et al. [41] used entomopathogenic nematodes and *D. saccharalis*

Table 4: Entomopathogenic-nematode bioassays on *Diatraea* spp.

Host specie	Pathogen	Bioassay	Result	Reference
Diatraea saccharalis	*Steinernema feltiae* [*Neoaplectana carpocapsae*]	Several concentrations for all species	100% mortality with 5000 nematodes/larvae	Sosa et al., 1993 [42]
	Heterorhabditis heliothidis		100% mortality with 5000 nematodes/larvae	
	S. glaseri		30% mortality with 5000 nematodes/larvae	
Diatraea saccharalis	*S. feltiae* *S. rarum* *Heterorhabditis bacteriophora*	500 IJs/5 larvae in Petri dishes with two moistened filter papers disks	Adults and IJs produced. Larval mortality >90%	De Doucet et al., 1999 [43]
Diatraea saccharalis	*H. bacteriophora JPM4* isolated from soil in Larvae MG, Brazil	Larvae were exposed to 25 ± 5 IJs/100 μL for 48 h in sand-filled Petri dishes	100% larval mortality within 5 days. LT_{50} 2.1 d and LT_{95} 4.4 d	Molina et al., 2007 [44]
Diatraea saccharalis	*Heterorhabditis bacteriophora*	500 mL/L applied to larvae	93% of larvae mortality	Aguila et al., 2008 [45]

in laboratory conditions to produce *S. carpocapsae in vivo*, using larvae of the sugarcane borer as the host.

We believe that pathogenic nematodes have good potential for the biological control of *Diatraea* species because of their presence in sugarcane-producing regions (as mentioned above) and because the species *S. feltiae, S. glaseri, S. rarum, Heterorhabditis heliothis*, and *H. bacteriophora* have demonstrated high infectivity in *D. saccharalis* [42–45] (Table 4) as well as in *E. loftini* (some of them) [46, 47] in experimental studies. We consider that performing a systematic search for infected larvae or pupae of *Diatraea* could be helpful in finding new nematode strains and species adapted to local environmental conditions and pest species that could be used in the future.

Although we could not find any report on field trials, we are aware that there have been mass rearings of *H. bacteriophora* in Cuba since 1987 to control soil pests, such as *D. saccharalis* [45].

5. Bacteria

Bacillus thuringiensis (Bt) is a Gram-positive bacterium that has been isolated from several sources, including soil, water, phylloplane, and insect cadavers. *Bt* produces various insecticidal crystal proteins during the onset of sporulation that are toxic to insects, acari, and nematodes [48]. The steps involved in the mode of action of the proteins after their ingestion are as follows: (a) solubilization of the crystals by the highly alkaline pH of the midgut, (b) activation of the proteins by proteases, (c) binding of the toxins to specific receptors located on the microvilli membrane of the midgut columnar epithelium cells, and (d) the insertion of the toxin into the membrane, which forms a pore and induces cell lysis. There are no reports from the American continent on the isolation of Bt strains from *Diatraea* spp. larvae cadavers; however, some Bt strains and pure proteins have been evaluated against these pests.

Bohorova et al. [49] evaluated Cry1Aa, Cry1Ab, Cry1Ac, Cry1B, Cry1C, Cry1D, Cry1E, and Cry1F Bt pure proteins against four species of Lepidoptera that are pests of maize, including *D. saccharalis*. The proteins were diluted in water and added to the diet at doses of 10 and 100 mg/mL of diet, and mortality was recorded after seven days. *D. saccharalis* was susceptible to Cry1B protein at 10 mg/g, and the LC_{50} was 113.6 mg/g of meridic diet (Table 5).

Twelve Bt strains were evaluated by Rosas García et al. [50] at a dosages of 50 and 500 μg of total protein and spores per milliliter against 2-day-old *D. saccharalis* larvae. The strains used were HD1, HD2, HD9, HD29, HD37, HD59, HD133, HD137, and HD559, as well as the GM7, GM10, and GM34 native strains. The strains that killed more than 50% of larvae were selected to obtain the LC_{50}. Strains HD133, HD559, GM7, GM10, and GM34 were toxic; however, GM34 was the most toxic with an LC_{50} of 33.21 μg/mL (Table 5). PCR analysis was performed to determine the *cry1* genes of the toxic strains: HD133 *cryAa*, *cry1Ab*, *cry1C*; HD559 and GM7 *cry1Aa*, *cry1Ab*, and *cry1B*; GM10 *cry1Aa*, *cry1Ab*, *cry1Ac*, and *cry1C*; GM34 *cry1Aa*, *cry1Ab*, and *cry1Ac*.

Gitahy et al. [51] evaluated a spore-crystal complex *in vitro* from five native Bt strains (S48, S76, S90, S105, and S135) and HD1, which served as a positive control, on second instar larvae of *D. saccharalis* and mortality was recorded after 5 days. The strain S76 caused 100% mortality at 72 h, HD1 caused 69% mortality, and 3% of the other native strains caused mortality at 500 $\mu g\,L^{-1}$ of the spore-crystal complex. The LC_{50} values of the S76 and HD1 strains were determined, and S76 was 11-fold more toxic (13.06 μg/L) than HD1 (143.88 μ/L) (Table 5). The S76 strain carries *cryAa*, *cry1Ab*, *cryAc*, *cry2Aa*, and *cry2Ab* genes, which is similar to HD1.

There are other species of bacteria with potential insecticide activity; Carneiro et al. [52] evaluated *Photorhabdus temperata,* which is a bacterium associated with *Heterorhabditis* entomopathogenic nematodes. Cells were injected with

Table 5: Entomopathogenic-bacteria bioassays on *Diatraea* spp.

Host species	Pathogen	Bioassay	Result	Reference
Diatraea saccharalis	*Bacillus thuringiensis*	Diet incorporated spores-crystals	LC_{50} 113.6 mg/g diet	Bohorova et al., 1997 [49]
D. saccharalis	*B. thuringiensis*		LC_{50} 33.21 μg/mL	Rosas-Garcia et al., 2004 [50]
D. saccharalis	*B. thuringiensis*	Diet incorporated spores-crystals complex	LC_{50} 13.06 μg/L	Gitahy et al., 2007 [51]
D. saccharalis	*Photorhabdustemperata*	Cells injected	LD_{50} 16.2 bacterial cells LT_{50} 33.8 h	Carneiro et al., 2008 [52]
Diatraea grandiosella	*Bacillus thuringiensis*	Diet incorporated spores-crystals	LC_{50} 5.2 mg/g diet	Bohorova et al., 1997 [49]
Diatraea grandiosella	*Bacillus thuringiensis* Biotrol BTB 183-25 Nutrilite Products Incorporated, Kansas City, Missouri, USA	Immersing stem seedling in spores suspensions: 1.25×10^8, 6.25×10^7, 2.5×10^7 and 1.25×10^7	Mean mortality on 5-day-old larvae: 32, 31, 25, 15 out of 40. Control mean mortality 0.01 Mean mortality on 10-day-old larvae: 15, 15, 12 and 6 out of 20.	Sikorowski and Davis, 1970 [53]

a volume of 10 μL of phosphate-buffered saline directly into the hemocoel of fourth instar *D. saccharalis* larvae, and the LD_{50} was 16.2 bacterial cells with an LT_{50} of 33.8 h.

Sikorowski and Davis [53] determined the susceptibility of 5- and 10-day-old *D. grandiosella* larvae with a Bt commercial product (Biotrol BTB 183-25) using 1.25×10^8, 6.25×10^7, 2.5×10^7, and 1.25×10^7 spores per milliliter (Table 5) where two-inch stem seedlings were dipped for 30 min in spore suspensions. One 10-day-old or two 5-day-old larvae were allowed to feed on each stem. In addition, a known number of spores were placed or injected into 5-day-old stem seedlings at 1/4 inch sections. Stems dipped in water were used as controls. Mortality was recorded after 48 h. With 10-day-old larvae, five replicates with 20 larvae for each treatment were used; for the 5-day-old larvae, two replicates with 40 larvae per treatment were used. With 5- and 10-day-old larvae at 1.25×10^8 spores per milliliter, a mean of 32 dead larvae out of 40 and 15 dead larvae out of 20 were recorded (Table 5), respectively. Thus, it was concluded that this species is highly susceptible to *B. thuringiensis*.

6. Viruses

Viruses that infect insects have received great attention as biological control agents because of their specificity on insect populations; they have little or no impact on the environment and are an ecological friendly alternative to chemical pesticides [58]. Entomopathogenic viruses are grouped in 33 genera within fifteen families [59]. However, only a few of these families, such as *Baculoviridae*, *Poxviridae,* and *Reoviridae,* have potential as biological control agents and have been successfully used in microbial control programs. A common characteristic among these entomopathogenic viruses is that the virions (infective unit) are occluded within a crystalline protein matrix to form an occlusion body (OB) [60], which is a unique characteristic of viruses that infect insects. In these entomopathogenic viruses, OBs have independently evolved as a protective mechanism to environmental factors, which gives the viruses a great advantage as biological control agents [61]. Baculoviruses (BVs) and entomopoxviruses (EPVs: subfamily *Entomopoxvirinae*) have a large double-stranded DNA genome, and cypoviruses (CPVs: family *Reoviridae*, genera *Cypovirus*) contain segmented double-stranded RNA viruses.

EPVs have been reported to infecting insects of a variety of orders, such as Coleoptera, Lepidoptera, Orthoptera, and Diptera. Some EPVs have two distinct OBs spheroids and spindles. The spheroids occlude virions, whereas the spindles do not [62]. The most abundant proteinaceous component of spheroids and spindles is proteins called spheroidin and fusolin, respectively [63–66]. EPV fusolin is an enhancing factor (EF) that increases BVs infection and has been characterized as a chitin-binding protein [67]. The fusolin mechanism of action is similar to that of Calcofluor, which facilitates BV infection by disrupting or preventing the formation of the peritrophic membrane [68]. EPVs are pathogenic but are scarcely virulent; infected larvae exhibit extreme longevity and take up to 70 days to die. However, EPVs have potential as biological control agents for pest insects where BVs have not been isolated [61]. Because of the activity of spindles, the EF of EPVs can be used as a synergistic agent to increase BV infectivity or generate genetically modified organisms, such as BVs and transgenic plants.

CPVs have been mainly isolated from Lepidoptera insects. OBs are dissolved in the midgut, and virions only infect epithelial cells; therefore, CPVs are very pathogenic but act slowly and frequently to produce chronic infections [69]. At this time, no commercial bioinsecticides based on CPVs have been developed. However, Caballero and Williams [61] suggest that their greatest potential as biological control agents is through inoculative or augmentative releases.

Table 6: Entomopathogenic-virus bioassays on *Diatraea* spp.

Host species	Pathogen	Bioassay conditions	Result	Reference
D. grandiosella	*Autographa californica* MNPV (*Ac*MNPV)	4 and 7 day-old larvae were fed strips of corn leaf soaked in different dilutions of virus.	DL_{50}: 1.32×10^3 PIB*/larvae (for 4-day-old larvae) DL_{50}: 1.32×10^4 PIB/larvae (for 7-day-old larvae).	Davis and Sikorowsy, 1978 [54]
D. saccharalis	*Diatraea saccharalis* Granulosis Virus (DsGV)	3rd instar larvae feeding individually on small artificial diet discs treated with 2.7 μL of virus dilutions.	LD_{50}: 42 PIB/larva at 26°C LT_{50}: From 29 to 63 for 10^7 and10^2 PIB/larvae, respectively.	Lastra and Gómez et al., 1983 [16]
D. saccharalis	*Anticarsia gemmatalis* MNPV (*Ag*MNPV)	3rd instar larvae fed with artificial diet discs treated with 5 different doses of PIB through 20 serial passages.	LD_{50}: From 7.9×10^5 to 5.3×10^2 for 1 and 20 serial passages, respectively.	Pavan and Ribeiro, 1989 [55]
D. saccharalis	*Ag*MNPV wt	3rd instar larvae individually fed small artificial diet discs containing 10^5 PIB of virus and kept individually at 10 different temperatures.	LT_{50}: From 10 to 13 for 39 and 30°C, respectively	Ribeiro and Pavan, 1994 [56]
	*Ag*MNPV-D10		From 9 to 29 for 39 and 22°C, respectively	
D. saccharalis	*Trichoplusia ni* MNPV (*Tn*MNPV) wt	3rd instar larvae fed individually with small artificial diet discs containing 10^5 PIB of virus and kept individually at 10 different temperatures.	LT_{50}: From 9 to 16 for 39 and 24°C, respectively	Ribeiro and Pavan, 1994 [56]
	*Tn*MNPV-D11		From 6 to 46 for 39 and 17°C, respectively	
D. sacccharalis	*Ag*MNPV	3rd instar larvae fed on formalin free diet disc (3 mm) inoculated with 2.7 μL of the viral solution from 10 clonal isolates of the AgMNPV-Ds20	LD_{50}: From 5.3×10^3 PIB/larvae (7A genotypic variant) to 8×10^4 PIB/larvae (33B genotypic variant)	Ribeiro et al., 1997 [57]

* PIB: Polyhedral inclusion bodies.

BVs predominantly infect insects within the Lepidoptera order, which includes important agricultural insect pests [70]. The *Baculoviridae* family includes two genera: *nucleopolyhedrovirus* (NPV), which forms large, polyhedric OBs where many enveloped virions are occluded [71], and *Granulovirus* (GV), which forms small, granular OBs that occlude only one enveloped virion each [72]. BVs are safe for humans and wildlife. Their specificity is usually very narrow and often is species specific. Because of their specificity and other characteristics, such as elevated virulence and pathogenicity, BVs are by far the most studied and extensively used as commercial biopesticides for the control of a variety of insect pests in many countries around the world [60, 61, 73].

Stem borers of the Lepidoptera order attack gramineous crops throughout the world [74, 75]. *Diatraea* stem borers (DSB) are widely distributed in the Americas and attack a wide variety of host plants, including maize and sugarcane [1]. Prediapause larvae of southwestern corn borers migrate to the base of the stalk of the corn plant below the soil surface to survive the winters [76]; cryptic habits make the chemical control of these insect pests difficult. Degaspari et al. [77] have argued that chemical control of *D. saccharalis* in Brazil is not economically feasible. To solve this problem, surveys to isolate endemic entomopathogens of stem borer populations in maize and sugarcane crops have been developed.

Inglis et al. [5] developed an exhaustive survey to isolate entomopathogens from the southern corn borer, *D. grandiosella*, and the southern corn stalk borer, *D. crambidoides*, and larvae in the diapause stage were collected from crops located in Mississippi and North Carolina. These authors did not observe OBs in any of the collected larvae and concluded that there are no naturally occurring viruses in these *Diatraea* populations [5]. According to Inglis et al. [5], Pavan and Ribeiro [55] mentioned that natural populations of the SCB in Brazil do not exhibit endemic viral pathogens.

Currently, there are only two records of endemic entomopathogenic viruses isolated from *Diatraea* spp. larvae. Pavan et al. [17] isolated a GV from the sugarcane borer (SCB), *D. saccharalis*, from sugarcane crops in the southern United States (Table 6). These authors developed bioassays

with *D. saccharalis* third instar larvae (Table 6). External symptoms of GV-infected larvae were similar to those reported for other lepidopterous larvae, and the symptoms and ultrastructures were determined using electronic microscopy as well as replication of DsGV, which are typical of GVs. The LD_{50} for the third instar larvae was 42.3 OBs/larva (Table 6) with 14.5 and 123.6 OBs/larva as the lower and upper limits at 95% probability, respectively [16]. These authors stated that this work was the first of a series of publications; however, there have been no additional studies produced to date, such as the molecular and biological characterization, of this GV, DsGV was introduced into Brazil [16], and Moscardi [78] mentioned that this virus is currently being applied at a small scale as an experimental product on sugarcane.

Because of their high pathogenicity combined with a limited host range, Densoviruses (DNVs) have potential as effective insecticides [79]. Meynadier et al. [80] isolated a DNV of *D. saccharalis* (DsDNV) from the Guadeloupe sugarcane borer (Table 6). Kouassi et al. [81] tested the pathogenicity of DsDNV on its host. These authors observed that the infected larvae exhibited infection symptoms from the fourth day postinfection, such as anorexia and lethargy followed by flaccidity and inhibition of molting and metamorphosis. Larvae became paralyzed and stopped feeding after 7 days. The cumulative mortality of infected larvae increased significantly and reached 60% after 12 days and 100% at 21 days postinfection [81]. Although DNVs have no potential for large scale use as a biological control agent because they have no OBs and are related to vertebrate pathogenic viruses, the genes involved with anorexia and paralysis could be used to produce transgenic BVs or plants.

Most BVs have a limited host range and may be species specific in some cases, although there are several NPVs, such as *Autographa californica* MNPV, *Anagrapha falcifera* MNPV, and *Anticarsia gemmatalis* MNPV, which have broader host ranges within the Lepidoptera order [60]. Because of the scarcity of reports on natural isolates of entomopathogenic viruses from *Diatraea* spp. populations, cross-infectivity of AgMNPV, TnMNPV, and AgMNPV has been evaluated in *D. saccharalis* and *D. grandiosella* larvae (Table 6).

Although only two reports of entomopathogenic viruses isolated from *Diatraea* stem borers exist in the Americas, entomopathogenic viruses, such as NPVs and GVs, have been reported to occur in Africa and Asia in cereal stem borers within the Crambidae family, including maize and sugarcane borers such as *Chilo* sp. [82], *Chilo sacchariphagus* [83, 84], *Ch. infuscatellus* [84, 85], *Ch. partellus* [86], and *Eldana saccharina* [87]. As the Mexican territory is the origin of some *Diatraea* stem borer species, we hypothesize that a great diversity of pathogenic viruses in *Diatraea* populations that attack sugarcane crops exists in Mexico.

Acknowledgments

This study was financed in part by the project "Selección de enemigos naturales de barrenadores de la caña de azúcar de Colima y Morelos con potencial como agentes de control biológico (SEP-PROMEP)," of Mexico. The authors also thank Mrs. Ingrid Masher for reviewing the paper and for editorial assistance.

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