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Comparative susceptibility of *Ostrinia furnacalis*, *Ostrinia nubilalis*, and *Diatraea saccharalis* (Lepidoptera: Crambidae) to *Bacillus thuringiensis* Cry1 toxins

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Abstract

Transgenic corn hybrids that express toxins from *Bacillus thuringiensis* (Bt) are highly effective against the European corn borer, *Ostrinia nubilalis* (Hübner), and the closely related Asian corn borer, *Ostrinia furnacalis* (Guenée). Since the registration of Bt corn hybrids in the U.S. in 1996, there has been a great deal of information generated on *O. nubilalis*. However, relatively little information exists for *O. furnacalis*. To help determine whether the information generated for *O. nubilalis* can be leveraged for decisions regarding the use of transgenic Bt corn against *O. furnacalis*, experiments were designed to determine whether the pattern of sensitivity to various Bt Cry1 toxins is similar between the two species. Test insects included laboratory-reared *O. furnacalis* originating from Malaysia, a Bt-susceptible laboratory colony of *O. nubilalis* maintained at the University of Nebraska-Lincoln (UNL) and an out-group consisting of the sugarcane borer, *Diatraea saccharalis* (F.), from Louisiana which represents a different genus from the same family. *O. furnacalis* and *O. nubilalis* exhibited a similar pattern of susceptibility to all the Cry1 toxins and were highly susceptible to the range of Bt toxins tested including Cry1Aa, Cry1Ab, Cry1Ac and Cry1F. Both of the *Ostrinia* species were more tolerant to Cry1Ba compared with *D. saccharalis*, although sensitivity of *O. furnacalis* was intermediate and did not differ significantly from that of *O. nubilalis* and *D. saccharalis*. *D. saccharalis* was also susceptible to the range of toxins tested but unlike the two *Ostrinia* species, was more tolerant to Cry1F and more susceptible to Cry1Ba. These results indicate that both of the *Ostrinia* corn borer species are similar in sensitivity to the Cry1Aa, Cry1Ab, Cry1Ac, Cry1Ba and Cry1F toxins, thus suggesting shared toxin receptors and mechanisms of toxicity for the two species.

Keywords: *Bacillus thuringiensis, Ostrinia furnacalis, Ostrinia nubilalis, Diatraea saccharalis,* Comparative patterns of susceptibility

1. Introduction

Since its first introduction in 1996, adoption of genetically modified crops has continued to increase globally with an 80-fold increase in biotech crop area and a total of 134 million ha planted in 2009 (James, 2010). This area is even greater when considering "trait or virtual hectares" which include the area that is planted with single and stacked traits (James, 2010). Of the total biotech crop area, 41.7 million ha (or 31.1%) was planted with genetically modified corn in 2009 (James, 2010). The rapid acceptance of this technology has benefited growers with yield increases due to reduction of insect damage especially during moderate and severe infestations (USEPA, 2001). The first Bt corn expressing a Cry1 toxin (Cry1Ab) was introduced in the United States (U.S.) in 1996 and is extremely effective against the European corn borer, *Ostrinia nubilalis* (Hübner), one of the most destructive pests in the U.S. Corn Belt (ILSI/HESI, 1998; Mason et al., 1996). Currently, additional Cry toxins have been stacked or pyramided in new events. Cry1Ab and Cry1F are expressed in both single and pyramided events, while Vip3Aa20, Cry1A.105, and Cry2Ab2 are available only as pyramided traits (USEPA, 2009). Gene pyramiding combines two different Bt Cry proteins with dissimilar target sites and is expected to delay pest resistance more effectively compared to crops that express single Bt toxins (Zhao et al., 2005). In addition to the U.S. which planted 22.2 million ha of Bt corn in 2009 (USDA NASS, 2009), Brazil, Argentina, Canada, South Africa, Uruguay, Spain, Honduras, Chile, Egypt, Romania, Slovakia, Czech Republic, Portugal and the Philippines also plant significant hectarage of Bt corn (James, 2010).

A sibling species to *O. nubilalis*, the Asian corn borer, *Ostrinia furnacalis* (Guenée), is the most economically important corn stalk boring pest in Asia and is distributed throughout India, Southeast Asia, China, Korea, Japan, Australia, New Guinea, Solomon Islands, and western Micronesia (Lewvanich, 1973; Mutuura and Monroe, 1970). Bt corn (Event MON810) expressing Cry1Ab was approved for planting in the Philippines to manage *O. furnacalis* in 2002, and the first commercial plantings occurred in early 2003 (James, 2003). Excellent field performance of the Cry1Ab corn in the Philippines against *O. furnacalis* has been documented and Bt corn hybrids consistently out-yielded conventional hybrids by 41% in field trials and by 60% compared with traditional farmer practices (Gonzalez, 2002; James, 2003). The commercial production of MON810 in the Philippines increased rapidly, from only 120 ha in 2002 to 400,000 ha in 2009 (Koul, 2010). In addition to the studies performed in the Philippines, it has been well documented that *O. furnacalis* is susceptible to Bt corn and Bt cotton that express Cry1Ab or Cry1Ac in China (He et al., 2003a, 2003b). However, the Philippines is so far the only Asian country that plants Bt corn commercially. The Bt events approved for propagation against lepidopteran pests in the Philippines are those that express Cry1Ab (Bureau of Plant Industry, 2010a, 2010b), while Bt events undergoing field trials include those expressing Cry1F and the pyramided events that expresses Cry1A.105, Cry1F, and Cry2Ab (Bureau of Plant Industry, 2010c).

O. furnacalis is very similar to *O. nubilalis* in biology and morphology, and has been frequently misidentified as *O. nubilalis*. A revision of the genus *Ostrinia* confirmed the status of *O. furnacalis* as a distinct species separate from *O. nubilalis* (Mutuura and Monroe, 1970). Host preferences of *O. furnacalis* (Caasi-Lit, 2006; Nafus and Schreiner, 1991) and *O. nubilalis* (Mason et al., 1996) are almost identical. Both prefer corn and share a number of common hosts including cotton, tomato, sorghum, peppers and some beans. Interestingly, both species are found in different parts of China (Zhou et al., 1988). *O. furnacalis* is the dominant pest in most of the corn growing regions (central to west and southern portions of China) while *O. nubilalis* predominates in Yining of Xinjiang province and to a lesser extent in Inner Mongolia, Ning Xia, and other areas of northwest China (Zhou et al., 1988).

In view of the success of Bt corn in controlling *O. nubilalis* in the U.S. and the potential for Bt corn to be adopted in areas where *O. furnacalis* is a major pest, information that has been generated on the Bt corn technology as it relates to *O. nubilalis* may be useful to regulatory decisions regarding the use of transgenic corn against *O. furnacalis*. In the present study, bioassays were conducted to compare the pattern of susceptibility between *O. furnacalis* and *O. nubilalis* against five Bt Cry1 toxins: Cry1Aa, Cry1Ab, Cry1Ac, Cry1Ba and Cry1F. The sugarcane borer, *Diatraea saccharalis* (F.), was included in the experiments because it belongs to the same family as *Ostrinia* species but is taxonomically distant enough to be considered as an out-group. It is also a major corn stalk boring pest in the mid-southern region of the U.S. and South America (Huang et al., 2007). The results of this investigation provide a starting point to determine whether data generated with *O. nubilalis* can be applied to *O. furnacalis*.

2. Materials and methods

2.1. Insects

A Bt-susceptible colony of *O. furnacalis* was established from a collection of ca. 300 late instar larvae and pupae obtained from a commercial field in Tanjung Karang, Selangor, Malaysia in June 2008. The colony was maintained in the Department of Crop Protection, University Putra Malaysia, using the standard rearing techniques established for *O. nubilalis* (Guthrie et al., 1965; Lewis and Lynch, 1969) as described by Siqueira et al. (2004b), except that a commercial meridic diet (Southland Products Incorporated, Lake Village, AR) was used for larval rearing. A pupation-ring made up of waxed-corrugated-cardboard (Custom Bioproducts, Maxwell, IA) was placed in each larval rearing pan to collect the pupae of *O. nubilalis*. When sufficient number of pupae were obtained, the corrugated-cardboard was transferred into a screened-cage (Bioquip Products, Rancho Dominguez, CA) and eggs were collected daily by using wax papers. A Bt-susceptible *O. nubilalis* colony maintained at the University of Nebraska-Lincoln (UNL) was established from a field collection of ca. 400 larvae from northern Italy. This colony was chosen because it has been maintained in the absence of selection for over 140 generations. Although the colony has been shown to represent both *E* and *Z* pheromone races (Marçon et al., 1999a), previous studies have shown that the two strains are similar in susceptibility to Cry1 toxins (Marçon et al., 1999b).

A Bt-susceptible laboratory population of *D. saccharalis* was established from a field collection of >300 larvae from non-Bt corn fields near Winnsboro in Franklin Parish, LA during December 2008. A meridic diet specific for *D. saccharalis* (Southland Products Incorporated, Lake Village, AR) was used for larval rearing. At third instar, larvae were transferred individually into 32-cell rearing trays (Bio-Serv) and provided with 5 ml of diet. Diet was moistened on alternate days until pupation to prevent desiccation. Pupae were transferred from the rearing trays into an oviposition cage. The cage set-up and egg harvesting method were similar to those previously described for *O. nubilalis* (Marçon et al., 1999b).

2.2. Bt toxin preparation

Cry toxins were prepared from fermentation of recombinant *Escherichia coli* strains transformed to express Cry1Aa (ECE52), Cry1Ab (ECE53), Cry1Ac (ECE54), and Cry1Ba (ECE128) obtained from the *Bacillus* Genetic Stock Center of the Ohio State University (Columbus, OH). The four recombinant *E. coli* cultures were grown at 37 °C for 48 h in Terrific broth media (Tartof and Hobbs, 1987) except the Cry1Ab producing strain which was grown in Luria-Bertani Media. Protoxins were obtained from *E. coli* fermentation products following the method described by Lee et al. (1992). The solubilized protein was digested with trypsin from bovine pancreas (Sigma–Aldrich®) and insoluble material was removed by centrifugation. The protoxin preparations were dialyzed against 50 mM $NaCO_3$/$NaHCO_3$ buffer (pH 10.0) using Snake-Skin™ pleated dialysis tubing, 10 k molecular weight cut off (Thermo Scientific, Rockford, IL).

Lyophilized Cry1F toxin was supplied by Dow AgroSciences LLC, Indianapolis, IN. The purified toxin was produced through fermentation of recombinant *Pseudomonas fluorescens* (Flügge) strain MR872, proteolytically activated and chromatographically purified. Except for the Cry1F toxin which was 13.7% by weight, other Cry1 toxin preparations were quantified by densitometric quantification (Crespo et al., 2008) of the 60–65 kDa peptides after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Laemmli, 1970) and compared to a standard curve for bovine serum albumen (BSA). These endotoxins were lyophilized and stored at −80 °C.

2.3. Bioassays

O. nubilalis and *D. saccharalis* bioassays were conducted at UNL during March 2008 and May 2009, respectively. Because of quarantine issues, *O. furnacalis* bioassays were conducted in Malaysia at Universiti Putra Malaysia from June–July 2008. Bioassay methods were consistent for both species, and identical toxin preparations were used for all bioassays. Neonates

of each species (<24 h after eclosion) were exposed to artificial diet (Marçon et al., 2000) in 128-well trays (each well 16 mm diameter, 16 mm height, CD International, Pitman, NJ). Approximately 1 ml of diet was dispensed into each well and allowed to solidify. Serial dilutions of seven increasing concentrations were made in 0.1% Triton-X 100 non-ionic detergent (v/v) to obtain uniform coverage on the diet surface. The diet surface in each well was topically treated with 30 µl of the appropriate dilutions. The bioassay diet that was developed for the tobacco budworm, *Heliothis virescens* (F.), (King et al., 1985) and adapted for *O. nubilalis* (Marçon et al., 1999b) was used in the bioassays for the three insect species. Control treatments consisted of diet surface treated with 0.1% Triton X-100 only. Treatments were allowed to air dry, and one neonate was transferred into each well. The wells were covered with vented lids (CD International). Trays for assaying *O. nubilalis* and *D. saccharalis* were held in an incubator at 27 °C, 24 h scotophase, and 80% RH. For *O. furnacalis*, bioassays were conducted under ambient laboratory conditions (24–28 °C and 60–80% RH). Larval mortality was recorded after 7 d and included both the number of dead insects and living larvae that had not grown beyond first instar and weighing ≤ 0.1 mg (Marçon et al., 1999b). Therefore, the mortality data combined both death and severe growth inhibition. Control mortality never exceeded 10%. Bioassays were replicated three to seven times for each combination of insect species and Cry toxin, with 16–32 larvae per replicate.

2.4. Data analysis

Probit analyses (Finney, 1971) of the mortality data were performed using POLO-PC (LeOra Software, 2003) to estimate LC_{50} and LC_{90} values and the slopes of the dose-response curves. The analyses were performed on both pooled mortality data of individual insect species and Bt Cry toxin and separately on each replication. The latter values were treated as observations from a 3 × 5 factorial experimental design and analyzed with a two-way analysis of variance (ANOVA) using the GLMMIX procedure (SAS Institute Inc., 2009). The two main factors were insect species and Bt Cry toxin. ANOVA tests indicated a significant simple interaction between these factors ($P < 0.01$). Comparisons between insect species within each Bt Cry toxins were conducted with a total of 15 combinations. Treatment means were separated using Fisher's LSD at $a = 0.05$ level implemented using the LSMEANS option in PROC GLIMMIX (SAS Institute Inc., 2009). To control the experiment-wise Type I error in the interpretation, a Tukey adjustment for multiple comparisons was applied whereby differences between means were significant at $P < 0.05$.

3. Results

3.1. Pooled mortality

The results of probit regression of pooled dose-response mortality data for the bioassays of the five Bt Cry toxins with neonates of *O. furnacalis*, *O. nubilalis* and *D. saccharalis* are shown in Table 1. For all species, the probit model was a good fit for the mortality data as confirmed by the Pearson Chi-square test for goodness-of-fit (Table 1). *O. furnacalis* and *O. nubilalis* were highly susceptible to Cry1Aa, Cry1Ab, Cry1Ac, and Cry1F. The LC_{50} values ranged from 1.2 (Cry1Ac) to 13.9 (Cry1F) ng/cm^2 for *O. furnacalis* and 5.4 (Cry1Ac) to 12.7 (Cry1F) ng/cm^2 for *O. nubilalis*. However, both corn borer species were more tolerant to Cry1Ba compared to the other Cry toxins with a LC_{50} value of 24.0 for *O. nubilalis* and 37.2 ng/cm^2 for *O. furnacalis*. *D. saccharalis* was also susceptible to Cry1Aa, Cry1Ab, and Cry1Ac, but unlike the two *Ostrinia* species, it was significantly more tolerant to Cry1F with a LC_{50} value of 73.4 ng/cm^2 and more susceptible to Cry1Ba (LC_{50} = 12.5 ng/cm^2).

3.2. Patterns of susceptibility

The overall pattern of susceptibility among the three species is described in Figure 1. Both *O. furnacalis* and *O. nubilalis* exhibited a similar pattern of susceptibility in terms of the relative LC_{50} values. In most cases, the *D. saccharalis* response was similar to that of the two corn borer species, except that an inverse relationship was observed with respect to the relative LC_{50} values for Cry1Ba and Cry1F. *O. furnacalis* and *O. nubilalis* were more tolerant of Cry1Ba relative to *D. saccharalis*, while *D. saccharalis* was more tolerant to Cry1F. In general, the slopes for probit regressions of *O. nubilalis* mortality were steeper than those observed with *O. furnacalis* and *D. saccharalis*. This may reflect reduced genetic variability in the lab colony of *O. nubilalis* as a result of long-term lab rearing relative to the other two species.

Patterns of the susceptibility were analyzed using ANOVA, and the interaction between insect species by Bt toxins was significant ($F = 18.99$; df = 36; $P = <0.0001$). Therefore, the simple interactions between insect species within each Bt toxin were examined. Individual comparisons between Cry1 toxins were

Table 1. Lethal concentrations of five *Bacillus thuringiensis* Cry1 toxins to *Ostrinia furnacalis*, *O. nubilalis* and *Diatraea saccharalis* with Probit analyses on combined mortality data.

Insect species	Cry toxin	n[a]	Slope (SE)	LC_{50} (95% FL)[b]	LC_{90} (95% FL)[b]	χ^{2c}	df
Ostrinia furnacalis	Cry1Aa	770	2.1 (0.1)	10.7 (9.3–12.3)	44.2 (36.3–56.1)	0.7	4
	Cry1Ab	1009	1.7 (0.1)	4.0 (3.4–4.8)	23.9 (19.4–30.8)	4.4	5
	Cry1Ac	667	1.4 (0.1)	1.2 (0.9–1.6)	10.7 (8.3–14.8)	2.8	5
	Cry1Ba	1020	3.1 (0.3)	24.0 (21.3–26.8)	62.0 (53.8–74.1)	3.1	5
	Cry1F	669	2.4 (0.2)	13.9 (12.0–15.9)	47.5 (39.4–59.9)	1.8	4
Ostrinia nubilalis	Cry1Aa	781	2.3 (0.2)	9.6 (8.3–11.0)	34.1 (28.3–43.0)	3.7	4
	Cry1Ab	890	2.5 (0.2)	11.5 (9.1–14.0)	37.4 (28.9–54.6)	5.1	4
	Cry1Ac	754	2.6 (0.2)	5.4 (4.7–6.1)	16.1 (13.6–19.8)	2.1	5
	Cry1Ba	478	5.4 (0.7)	37.2 (33.3–41.1)	64.0 (56.5–76.9)	1.7	3
	Cry1F	379	2.8 (0.3)	12.7 (9.1–17.2)	35.9 (25.2–64.9)	3.9	3
Diatraea saccharalis	Cry1Aa	764	1.9 (0.1)	5.6 (4.6–6.7)	26.9 (21.0–36.6)	3.2	5
	Cry1Ab	670	1.7 (0.1)	10.4 (8.3–12.9)	61.7 (46.9–87.0)	1.4	4
	Cry1Ac	575	1.8 (0.1)	2.8 (1.7–4.4)	14.8(8.4–39.3)	6.0	3
	Cry1Ba	574	1.8 (0.2)	12.5 (6.2–20.5)	66.0 (38.3–173.6)	5.4	3
	Cry1F	480	1.5 (0.2)	73.4 (35.2–163.3)	546.5 (217.6–14691.0)	5.0	3

a. Total number of larvae tested in bioassay.
b. ng of Cry toxin/cm^2 of treated artificial diet surface with 95% fiducial limits in parentheses.
c. χ^2 values from the goodness-of-fit test indicate a significant ($P < 0.05$) fit of the probit model.

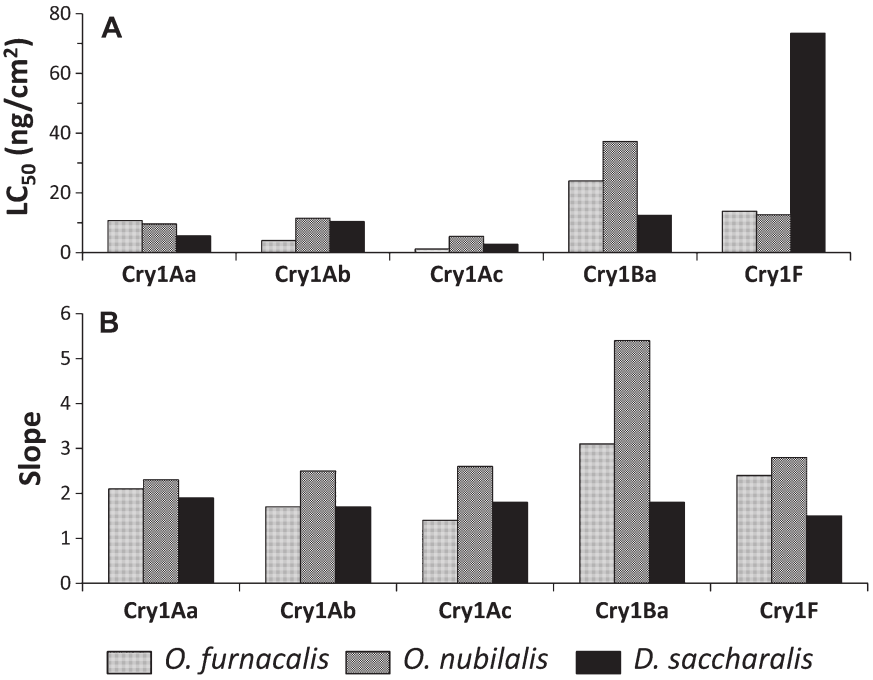

Figure 1. LC_{50} (A) and slope of probit regressions (B) for *O. furnacalis*, *O. nubilalis* and *D. saccharalis* against five Bt Cry1 toxins.

not reported because we were interested only in comparing the insect species among the Bt toxins tested. Table 2 indicates that out of 15 pairwise comparisons, significant differences ($P < 0.05$) were observed with Cry1Ba and Cry1F toxins. *O. nubilalis* (LC_{50} = 37.88 ng/cm^2) was significantly less susceptible (3-fold) to Cry1Ba compared to *D. saccharalis* (LC_{50} = 12.98 ng/cm^2). The sensitivity of *O. furnacalis* (LC_{50} = 24.0 ng/cm^2) to Cry1Ba was intermediate between *O. nubilalis* and *D. saccharalis,* and the pairwise comparisons were not significantly different (0.0255 and 0.554 respectively at $P > 0.05$). Cry1F was 5-fold more toxic towards both *Ostrinia* species (*O. furnacalis*: LC_{50} = 17.15; *O. nubilalis*: LC_{50} = 18.09 ng/cm^2) compared to *D. saccharalis* (LC_{50} = 78.14 ng/cm^2). None of the pairwise comparisons of LC_{90} values were significantly different except for the differences between both *Ostrinia* species and *D. saccharalis* when exposed to Cry1F ($P < 0.05$).

4. Discussion

The results presented here provide the first comparison of susceptibility to Bt toxins between *O. nubilalis* and *O. furnacalis*. Although the two species were bioassayed at different times and in different laboratories, the materials and methods were kept as similar as possible to enable comparison between insect species. *D. saccharalis* was used as a positive control where differences in the pattern of susceptibility among the

Table 2. Simple effect comparisons between insect species among the five Bt toxins at LC_{50} and LC_{90} values.

Bt toxins and comparisons[a]	LC_{50} (SE) Insect species 1	Insect species 2	$Pr > \|t\|$ [b]	LC_{90} (SE) Insect species 1	Insect species 2	$Pr > \|t\|$ [b]
Cry1Aa						
Of × On	10.66(3.53)	9.77(4.07)	0.9448	44.7(72.7)	30.8(83.9)	0.9915
Of × Ds	10.66(3.53)	5.54(4.07)	0.6116	44.7(72.7)	27.2(83.9)	0.9864
On × Ds	9.77(4.07)	5.54(4.07)	0.7448	30.8(83.9)	27.2(83.9)	0.9755
Cry1Ab						
Of × On	3.76(3.53)	12.06(4.07)	0.2848	24.2(72.7)	37.6(83.9)	0.9995
Of × Ds	3.76(3.53)	12.64(4.07)	0.2396	24.2(72.7)	108.8(83.9)	0.7283
On × Ds	12.06(4.07)	12.64(4.07)	0.9945	37.6(83.9)	108.8(83.9)	0.8211
Cry1Ac						
Of × On	2.42(2.67)	5.43(4.99)	0.8567	13.4(55.0)	16.1(102.8)	0.9997
Of × Ds	2.42(2.67)	3.35(4.07)	0.9803	13.4(55.0)	25.8(83.9)	0.9916
On × Ds	5.43(4.99)	3.35(4.07)	0.9443	16.1(102.8)	25.8(83.9)	0.9970
Cry1Ba						
Of × On	23.65(3.53)	37.88(4.99)	0.0642	72.7(72.7)	66.1(102.8)	0.9985
Of × Ds	23.65(3.53)	12.98(4.07)	0.1391	72.7(72.7)	66.0(83.9)	0.9980
On × Ds	37.88(4.99)	12.98(4.07)	0.0013	66.1(102.8)	66.0(83.9)	1.0000
Cry1F						
Of × On	17.15(3.16)	18.09(4.99)	0.9860	51.6(65.0)	53.1(102.8)	0.9999
Of × Ds	17.15(3.16)	78.14(4.07)	<0.0001	51.6(65.0)	1098.9(83.9)	<0.0001
On × Ds	18.09(4.99)	78.14(4.07)	<0.0001	53.1(102.8)	1098.9(83.9)	

a. Pairwise comparisons between 2 insect species with LSMeans where *Of, On* and *Ds* represent *O. furnacalis*, *O. nubilalis* and *D. saccharalis* respectively.

b. LC_{50} and LC_{90} values are not significantly different when $P > 0.05$.

Bt toxins are more likely given the greater taxonomic distance between the two genera, and thus increasing the sensitivity of the analyses. In general, *D. saccharalis* grew more slowly compared to both *Ostrinia* species on the bioassay diet which may indicate that the diet composition is less suitable for this species relative to the two *Ostrinia* species. However, similar feeding behavior was observed among these three insect species in that initial feeding involved scraping the diet surface before tunneling it. This suggests a similar uptake of the Bt Cry toxins. In addition, all control mortality was less than 10% which confirms the response to Bt toxin exposure in all species.

Cry1Aa, Cry1Ab, and Cry1Ac toxins were very active against all three species and their sensitivity was not significantly different between species at LC_{50} and LC_{90} values (Table 2). In general, Cry1Ac was more active than Cry1Ab for both *Ostrinia* species which contrasts with previous results for *O. nubilalis* in which Cry1Ab has been reported as being more toxic than Cry1Ac (Denolf et al., 1993). Cry1Ac might be more active compared to Cry1Ab because the recombinant *E. coli* that expressed Cry1Ac was grown in a different media. Nutrient broth variation used for fermentation and crystal-harvesting time could influence the levels of attached sugars, a product of non-enzymatic glycosylation, which may in turn affect the specificity and toxicity of the crystals produced (Bhattacharya et al., 1993).

Interestingly, Cry1Ba and Cry1F appeared to exhibit contrasting patterns of toxicity in the two corn borer species compared with *D. saccharalis*. Cry1Ba was less toxic to *O. nubilalis* (LC_{50} = 37.88 ng/cm^2) than *O. furnacalis* (LC_{50} = 23.65 ng/cm^2), and was significantly more toxic to *D. saccharalis* (LC_{50} = 12.98 ng/cm^2) ($P < 0.05$) (Table 2). The low Cry1Ba toxicity observed in both *Ostrinia* species is similar to results from bioassays of other lepidopterans including *H. virescens* (Karlova et al., 2005), *Spodoptera exigua* (Hübner) (de Maagd et al., 2000) and *Manduca sexta* (Linnaeus) (Bradley et al., 1995) which all exhibited relatively low susceptibility. It was reported that trypsin activity against Domain I during Cry1Ba production and activation generated a less active 55 kDa protein that may have decreased the toxicity (de Maagd et al., 2000). In general, both *Ostrinia* species were more tolerant to Cry1Ba compared to *D. saccharalis*. In contrast, Cry1F was significantly more toxic to both *Ostrinia* species than to *D. saccharalis* (Table 1 & Table 2).

The slopes of probit regressions for all toxins tested against *O. nubilalis* were steeper than those for *O. furnacalis* and *D. saccharalis*. The steeper slopes are indicative of a genetically homogeneous population (Eaton and Klaassen, 2001) and are not unexpected for a laboratory colony which in this case had been inbred for 141 generations. Although *O. nubilalis* exhibited higher slopes for response curves, the pattern of susceptibility to the Cry toxins tested was similar to *O. furnacalis*.

In general, the high sensitivity of both *Ostrinia* species towards Cry1 toxins shown in this study concurs with previous reports for *O. nubilalis* (Chaufaux et al., 2001; Crespo, 2008; Marçon et al., 1999b; Siqueira et al., 2004a) and *O. furnacalis* (He et al., 2005; Wen et al., 2005). However, our results indicate that Cry1Ba is less toxic to *O. nubilalis* compared to the Cry1A toxins, while Li et al. (2005) reported that a susceptible strain of *O. nubilalis* was equally sensitive to Cry1Aa, Cry1Ab, Cry1Ac and Cry1Ba, although different exposure methods were used. In addition, our results suggest similar susceptibility to Cry1Ab among the three species. However, Huang et al. (2006) reported *D. saccharalis* was 10-fold more tolerant to Cry1Ab than either laboratory or field strains of *O. nubilalis*. Such differences might have occurred due to differences in toxin sources (corn leaf tissue vs. toxin preparations from recombinant *E. coli* strains) as well as the differences in exposure methods (surface-treated diet vs. diet incorporation). It is apparent from these conflicting results that direct comparisons of bioassay results using different methods and sources of toxins should be avoided. Importantly, field populations of *O. nubilalis* remain susceptible after more than a decade since Bt corn introduction (Siegfried et al., 1995, 2007). Similarly, excellent field control on the *O. furnacalis* has been demonstrated in combinations of field evaluations and laboratory bioassays using plant tissues of corn expressing Cry1Ab (He et al., 2003a, 2003b; Wen et al., 2005) and cotton expressing Cry1A, Cry1A and CpTI, Cry1Ac, Cry1Ie (He et al., 2004, 2006; Song et al., 2003).

In general, these results provide strong evidence of similarity in sensitivity patterns between both *Ostrinia* species towards Cry1Aa, Cry1Ab, Cry1Ac, and Cry1F toxins which suggests a common target site among the two species and potentially common resistance mechanisms that might evolve in response to selection from Bt corn. Documenting this similarity may assist regulatory agencies in Asia to leverage existing data sets that have been developed for resistance management of *O. nubilalis* in North America for decisions regarding registration, regulation, cultivation, and resistance management of *O. furnacalis*. However, deployment of transgenic corn against *O. furnacalis* will need to consider other factors such as the differences in biology and ecology of two species, as well as differences in maize cultivation systems.

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