Crop Protection 30 (2011) 554–559

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Monitoring Cry1Ab susceptibility in Asian corn borer (Lepidoptera: Crambidae) on Bt corn in the Philippines

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ARTICLE INFO

Article history:
Received 23 September 2010
Received in revised form
14 December 2010
Accepted 19 December 2010

Keywords:
Ostrinia furnacalis
Diagnostic concentration
Diet surface-overlay assay

ABSTRACT

Between 2002 and 2004, collections of egg masses of Asian corn borer (ACB), *Ostrinia furnacalis* (Guenée) were made from corn-planting sites on the major Philippine islands of Luzon (Laguna, Pangasinan, Camarines Sur and Isabela provinces) and Mindanao (Bukidnon and South Cotabato provinces). The resulting neonates were bioassayed for susceptibility to *Bacillus thuringiensis* (Bt) Cry1Ab protein. The median lethal concentrations (LC_{50}s) for the different collections ranged from 0.42 to 2.37 ng/cm^2. The bioassay results suggest that Philippine corn borer populations were highly susceptible to Cry1Ab protein prior to the widespread deployment of Bt corn. The upper limit of the estimated LC_{99} (104 ng/cm^2) from the pooled bioassay data was selected as a candidate diagnostic concentration and subsequently tested on eleven ACB populations. Results of the validation assays showed that the mortality response of all the tested ACB populations was higher than the expected mortality (99%). Therefore, the concentration of 104 ng/cm^2 was used to monitor susceptibility in ACB populations in the Philippines. Monitoring of field populations during 2009 in areas where Bt corn had been grown for 3 years found some enhanced survival of neonates at the diagnostic concentration but progeny of the diagnostic-concentration survivors did not survive on Bt corn, indicating that ACB populations in the Philippines remain susceptible to Cry1Ab-containing Bt corn hybrids.

1. Introduction

The development of transgenic corn expressing the Cry1Ab protein from *Bacillus thuringiensis* (Bt) represents a novel approach to controlling potentially destructive populations of Asian corn borer (ACB) *Ostrinia furnacalis* (Guenée). An important prerequisite to commercial deployment of Bt corn is a monitoring scheme for detecting the early evolution of ACB resistance to Bt corn. This, in turn, requires the estimation of baseline susceptibility of Philippine ACB populations to Cry1Ab, which can be compared with future estimates of ACB susceptibility obtained after deployment of Cry1Ab corn. These baseline susceptibility estimates also can be used as the basis for selecting a diagnostic concentration for resistance monitoring, which is less labor intensive than using a quantal response bioassay and allows for the testing of the large number of insects required to detect shifts in susceptibility (Reed and Halliday, 2001). A dose of toxin that kills 99% of susceptible individuals has been proposed for diagnostic bioassays (ffrench-Constant and Roush, 1990).

The objectives of this study were 1) to quantify baseline susceptibility of Philippine ACB populations to Cry1Ab protein prior to the deployment of Bt corn, 2) to develop a diagnostic concentration for monitoring of ACB resistance to Bt corn, and 3) to use the diagnostic concentration to monitor field populations for changes in susceptibility to Cry1Ab toxin in locations where multiple Bt corn crops have been grown.

2. Materials and methods

2.1. Study overview

This study was completed in several stages between 2002 and 2010. Baseline susceptibility was assessed between 2002 and 2004. Bt corn was first approved for propagation in the Philippines in December 2002 and by 2004 constituted only approximately 1% of the country's corn area (Ebora et al., 2005). Thus, all baseline data were collected prior to Bt corn being a significant component of the agricultural system and prior to any potential widespread selection

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doi:10.1016/j.cropro.2010.12.019

Table 1
Cry1Ab baseline susceptibility estimates of Asian corn borer populations from the Philippines, 2002–2004.

Province	Locality[a]	Slope ± SE	LC$_{50}$ (ng/cm^2)[b]	LC$_{90}$ (ng/cm^2)[b]	LC$_{99}$ (ng/cm^2)[b]	χ^2
2002						
Bukidnon	Cabanglasan (1)	1.54 ± 0.29	1.60 (0.73–2.59)	10.84 (6.10–35.51)	51.66 (19.81–522.39)	15.13*
		2.58 ± 0.33	1.44 (1.15–1.75)	4.54 (3.54–6.55)	11.55 (7.73–21.98)	6.78
		1.79 ± 0.20	2.37 (1.82–3.00)	12.30 (8.92–19.38)	47.09 (27.88–103.95)	8.28
	Maramag (2)	1.47 ± 0.19	1.53 (1.03–2.05)	11.22 (7.77–19.37)	57.04 (29.98–161.88)	5.29
South Cotabato	Koronadal (6)	1.17 ± 0.17	1.46 (0.87–2.11)	1.83 (11.42–38.95)	143.57 (60.29–648.17)	10.75
Isabela	Aurora (3)	1.63 ± 0.21	1.50 (1.05–1.98)	9.14 (6.53–14.95)	39.84 (22.35–100.41)	8.81
		2.28 ± 0.32	1.29 (0.96–1.62)	4.71 (3.58–7.12)	13.52 (8.58–28.95)	4.08
		1.68 ± 0.21	1.37 (0.95–1.80)	8.02 (5.77–12.98)	33.92 (19.22–84.83)	9.5
		1.65 ± 0.22	1.24 (0.83–1.65)	7.41 (5.33–12.06)	31.94 (17.95–82.14)	10.72
	Echague (4)	2.22 ± 0.30	1.18 (0.87–1.49)	4.47 (3.41–6.70)	13.23 (8.39–28.18)	8.09
		2.05 ± 0.28	1.16 (0.83–1.48)	4.87 (3.67–7.43)	15.73 (9.71–35.03)	1.71
	Luna (5)	1.28 ± 0.31	1.48 (0.35–2.87)	14.81 (6.82–131.77)	96.97 (25.62–8902)	21.89*
		1.41 ± 0.19	1.28 (0.82–1.77)	10.33 (7.08–18.25)	56.67 (28.83–175)	21.38*
2003						
Isabela	Angadanan (7)	1.56 ± 0.20	1.45 (0.99–1.94)	9.67 (6.81–16.19)	45.37 (24.69–121.26)	11.12
		1.70 ± 0.22	1.36 (0.95–1.79)	7.71 (5.57–12.38)	31.70 (18.15–77.84)	13.32
Laguna	Los Baños (8)	1.76 ± 0.35	1.41 (0.76–2.11)	7.53 (4.72–17.91)	29.50 (13.63–156.69)	3.84
2004						
Isabela	Ilagan (11)	1.39 ± 0.15	0.99 (0.73–1.31)	8.29 (5.58–14.53)	46.67 (24.15–125.72)	12.17
	Cauayan (10)	1.51 ± 0.16	0.42 (0.32–0.54)	2.98 (2.06–5.01)	14.73 (8.04–35.95)	9.41
		1.18 ± 0.14	0.97 (0.71–1.34)	11.76 (6.68–27.68)	90.08 (36.15–374.82)	6.68
		1.10 ± 0.13	0.97 (0.70–1.37)	14.01 (7.54–36.66)	123.87 (45.17–621.38)	6.95
Pangasinan	Laoac (12)	1.47 ± 0.25	1.15 (0.62–1.92)	8.54 (4.43–31.41)	43.72 (15.36–439.63)	21.6
		1.22 ± 0.14	1.48 (1.07–2.00)	16.70 (10.26–34.29)	120.51 (53.57–420.68)	13.85
		1.28 ± 0.14	1.49 (1.09–1.98)	14.95 (9.45–29.12)	98.07 (45.98–310.17)	11.55
		1.48 ± 0.16	0.94 (0.70–1.23)	6.86 (4.75–11.49)	34.59 (18.84–85.45)	13.86
		1.77 ± 0.18	0.85 (0.65–1.07)	4.47 (3.27–6.90)	17.38 (10.47–36.54)	9.64
		1.21 ± 0.14	0.95 (0.66–1.29)	10.97 (6.92–21.68)	80.67 (36.56–277.84)	13.76
Camarines Sur	Tigaon (9)	1.06 ± 0.13	0.89 (0.64–1.27)	14.54 (7.61–40.30)	141.55 (48.83–796.49)	11.15
		1.21 ± 0.14	0.64 (0.47–0.86)	7.31 (4.40–15.54)	53.40 (23.13–194.74)	8.73

All assays used 540 neonate larvae per assay.
*χ^2 significant ($p < 0.05$).
[a] Numbers in brackets for each location are the location numbers on Fig. 1.
[b] Values in parenthesis are 95% fiducial limits.

of Bt resistant genes. During 2007, three potential concentrations for diagnostic bioassays were evaluated and the candidate concentration was validated later in 2007 and during 2008. In 2009, ACB populations which had been exposed to three Bt corn crops were evaluated with the diagnostic concentration. A number of these populations had higher survival than expected and so the progeny of the survivors were evaluated on Bt and non-Bt corn plants in 2010.

2.2. Field collection and laboratory rearing of ACB

Between 2002 and 2004, ACB egg masses were collected from corn-planting sites on the major Philippine islands of Luzon (Cagayan, Pangasinan, Camarines Sur and Isabela provinces) and Mindanao (Bukidnon, Sultan Kudarat and South Cotabato provinces) for susceptibility testing. These sites were chosen based upon their history of ACB infestation and because they span the important corn-growing regions of the Philippines. ACB egg masses collected from Laguna province served as the reference population because this location was easily accessible to the bioassay laboratory compared to the major corn-growing areas on northern Luzon and on Mindanao and is not a major Bt corn production region.

In all years of this study and for all populations, 25 to 50 egg masses (MacIntosh, 2009) that were laid by ACB on the leaves of vegetative stage corn were collected from the field and placed on plastic cups that were lined with moist filter paper. The cups were covered with plastic lids and transported to the laboratory. Upon arrival in the laboratory, the egg masses were allowed to develop to the blackhead stage before transfer to the surface of artificial corn borer diet (Camarao, 1976; Ceballo and Morallo-Rejesus, 1983) for bioassay.

2.3. Bioassay of Cry1Ab against ACB larvae

Artificial diet surface-overlay assays were carried out to estimate LC$_{50,}$ LC$_{90}$ and LC$_{99}$ for Cry1Ab in neonates from each collected population of ACB between 2002 and 2004. Glass drum vials (20 ml each) were used for the bioassay. The caps used to cover the vials were provided with holes (1 cm diameter on top) covered with copper wire mesh to facilitate air flow, thus preventing accumulation of moisture inside the vial during the duration of the experiment. Approximately 1.5 ml of liquefied artificial diet was dispensed in each vial. The diet was allowed to solidify before aliquots (50 μl/vial) of solubilized, activated Cry1Ab were spread on the surface of the solidified artificial diet and air dried before larval infestation. The surface area of the artificial diet was 4.52 cm^2. One neonate larva was placed inside each vial. At least six doses of Cry1Ab were used for each assay, with three replicates of 30 larvae at each dose. Each assay was repeated twice. All bioassays were performed under ambient condition. Mortality was recorded after 7 days, with the criterion for death being the absence of larval reaction when prodded and/or severe growth inhibition (i.e., weight is $\leq$0.1 mg). Data were analyzed by probit analysis (Finney, 1971) using Priprobit software (Sakuma, 1998). Two assays were determined to be significantly different when the 95% fiducial limits of the LC estimates did not overlap.

A series of linear mixed model (LMM) analyses were performed in GenStat V12 (Payne et al., 2009) to evaluate whether there was significant variation across years and provinces in the baseline estimates of slope, LC$_{50}$, LC$_{90}$ and LC$_{99}$ to provide a basis for decisions on pooling data for calculating pooled estimates of diagnostic doses of Cry1Ab toxin. The data (Table 1) was truncated to remove localities