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Sensitivities of Fall Armyworm (*Spodoptera frugiperda*) Populations in Different Regions of China to Four Bt Proteins

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Abstract: Fall armyworm (FAW), *Spodoptera frugiperda*, invaded the south of China in December 2018 and has since posed a huge threat to crop production in China. However, transgenic *Bacillus thuringiensis* (Bt) corn can efficiently control the damage caused by FAWs. In fact, the Chinese government has issued biosafety certificates for several Bt corn hybrids expressing any one of four Bt proteins, Vip3A, Cry1F, Cry1Ab, and Cry2Ab, or combinations thereof, to control FAWs. These Bt corn events are soon to be commercialized in China. Therefore, it is necessary to monitor and evaluate whether the FAW has developed resistance to any of the Bt corn hybrids planted in fields in China. To address this issue, we collected 11 geographical populations of FAWs and determined the sensitivity of each to the aforementioned four purified Bt proteins as assessed by diet surface overlay bioassays. The ranges for the 50% lethal concentration (LC_{50}) of the four Bt proteins to all FAW populations were as follows: 11.42–88.33 ng/cm^2 (for Vip3A), 111.21–517.33 (Cry1F), 135.76–1108.47 (Cry1Ab), and 994.42–5492.50 (Cry2Ab). The corresponding ranges for the 50% growth inhibition concentrations (GIC_{50}) were 1.43–14.86, 2.35–138.97, 1.58–464.86, and 25.01–1266.07 ng/cm^2. The lethal effects and growth inhibition effects of the four Bt proteins on FAW were in the same order of Vip3A > Cry1F > Cry1Ab > Cry2Ab. A comparison with published LC_{50} values of Bt proteins towards sensitive FAW populations revealed that all 11 FAW populations in this study were sensitive to Vip3A, Cry1F, and Cry1Ab. This study provides foundational data for monitoring and controlling the resistance of Bt corn to FAW in China.

Keywords: fall armyworm; geographical population; Bt protein; resistance monitoring

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1. Introduction

The fall armyworm (FAW) *Spodoptera frugiperda* is a notoriously polyphagous pest native to tropical and subtropical regions of the American continent, with host plants comprising ~353 species distributed into 76 families [1]. Like other major migratory agricultural pests, FAWs have been described as having the characteristics "international, migratory, explosive, and destructive" [2]. FAW larvae feed on stems, leaves, tassels, ears, and other plant parts. FAW-related damage to seedlings can reduce yield by 10–25% [3]. Indeed, FAW-related damage caused a 20% reduction in corn production in the United States in 2018. In certain South American countries with underdeveloped agricultural technology and relatively low investment in prevention and control, FAW-related damage can reduce corn yield by more than 70% [3]. Therefore, FAW has become a major biological disaster that seriously threatens food security worldwide. Unfortunately, this invasive pest moved into the south of China in December 2018 and quickly spread to 26 provinces by October 2019 [4]. Globally, FAW has two different strains, namely the corn and rice strains; the strain that first invaded China was identified as the corn strain [5]. If left unchecked, FAW has the potential to cause substantial damage to China's agricultural economy.

Many methods such as synthetic insecticides and biopesticides, cultural control tactics including push–pull, and natural enemies can be used to minimize the damage caused by FAW [6], but some of these methods are time-consuming, laborious, pollute the environment, and can lead to resistance or other problems [7,8]. However, the development of molecular biotechnology techniques has fostered new ways of controlling FAW via the commercial planting of transgenic insect-resistant corn hybrids, which has become a widely adopted means of controlling FAW in the tropical and subtropical regions of the American continent. For example, transgenic *Bacillus thuringiensis* (Bt) corn events have been commercially planted abroad to control FAW; these hybrids include TC1507, which expresses the Bt protein toxin Cry1F, MON89034, which simultaneously expresses Cry1A.105 and Cry2Ab, and MIR162, which expresses Vip3Aa20, among other hybrids. Most hybrids express an individual Bt protein, i.e., Cry1Ab, Cry1F, Cry1A.105, Cry2Ab, or Vip3A, although some hybrids express two or more proteins [9–13]. With long-term and large-scale planting, however, FAW has developed field resistance to each of Cry1Ab, Cry2Ab, and Cry1F [14–19]. In Puerto Rico, for example, the FAW has developed 1000-fold field resistance to TC1507 corn [16]; consequently, TC1507 was voluntarily withdrawn from the territory by the technology's developers. Since then, many populations of FAW that are field-resistant to Cry1F have been reported in Brazil [17] and Argentina [18] as well as in the states of Florida and North Carolina in the United States [19]. Brazil first introduced the Bt corn MON810 in 2008 and MON89034 in 2010; however, 3 years later, field-resistant FAW populations were found since their first introduction [20]. Although a Vip3A-resistant FAW population (up to 9800-fold resistance) was identified and selected for lab culture [21], no Vip3A-resistant field population has been reported to date. As such, the FAW is still considered to be sensitive to the Bt corn variety expressing Vip3A, and the frequency of resistance is considered low [21,22]. Obviously, resistance monitoring and management are crucial to the successful control of FAW in planting areas.

Since 2019, the Ministry of Agriculture and Rural Affairs of China has issued safety certificates for several genetically modified Bt corn hybrids, such as DBN9936 expressing Cry1Ab, DBN9501 expressing Vip3A, DBN3601T simultaneously expressing Cry1Ab and Vip3A, BLF4-2 simultaneously expressing Cry1Ab and Cry1F, and ND207 expressing Cry1Ab and Cry2Ab. Some bioassay experiments have shown that certain Bt hybrids are highly effective for controlling FAW [23–26]. In 2022, for example, several hybrids mentioned above were pilot-planted in the provinces of Yunnan and Inner Mongolia, and the results showed significant resistance to FAW and other insects in the field. Currently, the Chinese government is vigorously promoting the commercialization of Bt corn cultivation, and several Bt hybrids will be approved for field planting in China soon. Data from other countries have shown that the long-term commercialization of Bt crops has brought enormous selection pressure to populations of targeted insects, leading to the rapid evolution of the resistance of target pests to Bt corn hybrids, thereby reducing the efficiency of pest control [27,28]. Therefore, it is necessary to monitor and evaluate the resistance of the FAW to specific Bt proteins produced by Bt corn hybrids in China.

In the present study, 11 field populations of FAW were collected from eight cities in seven provinces in four major corn-producing regions in China, i.e., the Northern, Huanghuaihai, Southern, and Southwest regions, from 2021 to 2022. We measured the sensitivity of these field populations to four purified Bt proteins, namely Vip3A, Cry1F, Cry1Ab, and Cry2Ab, which are likely to be approved for large-scale cultivation in China. Our work is expected to provide fundamental data for monitoring and controlling the resistance of transgenic Bt corn to FAW in China and perhaps other countries as well.

2. Materials and Methods

2.1. Purified Bt Proteins

Purified Cry1Ab and Cry1F proteins were purchased from Envirotest-China (agent for EnviroLogix Inc., Portland, ME, USA; www.envirotest-china.com; accessed on 10 March 2021). The protoxin from Bt had been expressed as a single-gene product in *Escherichia coli* at

Case Western Reserve University (Cleveland, OH, USA). The protoxin inclusion bodies were then dissolved, isolated, and purified, and the pure fractions were desalted and lyophilized. The purity was 94–96% (Marianne P. Carey, Case Western Reserve University, personal communication). Cry2Ab and Vip3A were purchased from Beijing Meiyan Agricultural Technology Co., Ltd. (Beijing, China; www.caasbuy.com/platform/shop.lf?shopid=2972; accessed on 10 March 2021). The production process of Cry2Ab and Vip3A was the same as that of Cry1Ab and Cry1F, and the purity was >90%. All protein samples were dissolved in distilled water at a concentration of 5 mg/mL and stored at −80 °C before use in the bioassay.

2.2. FAW Populations

Eleven different geographical populations of FAW were collected from eight cities in seven provinces in the four major corn production regions of China from 2021 to 2022 (Figure 1 and Table 1). These four regions make up more than 90% of the total corn acreage of China [29]. Information pertaining to FAW collection is given in Table 1.

Figure 1. Corn production regions and sampling locations where FAWs were collected. Percentage of corn grown in each region for the total area: I. Northern Spring Corn Region, 30%; II. Huang–Huai–Hai Summer Corn Region, 40%; III: Southwest Hilly Corn Region, 20%; IV: Southern Hilly Corn Region, 5%; V. Northwest Inland Corn Region, 3%; and VI. Qiang-Zang Plateau Corn Region, 2%. 1. Dehong; 2. Dali; 3. Sanya; 4. Nanchang; 5. Yuanyan; 6. Jinan; 7. Langfang; 8. Tongzhou.

The larvae were fed an artificial diet [30], which was cut into small pieces of ~1 g. Each well in a 24-well plate contained one piece of the diet and one larva. When the larvae grew to the 4th to 5th instar stage, they were transferred to a glass tube with an artificial diet; the tube was then closed with a cotton plug for pupation and adult emergence. The adults were placed in cages (4 × 25 × 25 cm) and fed a 10% w/v sugar solution to supplement nutrition and water. The top of each cage was covered with medical gauze for oviposition, which was changed every day and put into a zip-lock bag, and then the bag was placed in a climatic chamber at 26 ± 1 °C, 60 ± 10% relative humidity, with a photoperiod of 16 h:8 h (L:D). The resulting 0 to 24 h-old neonates after 2~3 generations were used for bioassays.

Table 1. Collection information for the studied FAW populations from corn fields in primary corn production regions in China.

Location			Coordinates	Map Ref. No.	Collection Date	No. of Larvae Collected
Production Region	**Province**	**City**				
Southwest Hilly Corn Region	Yunnan	Dehong	24.01° N, 97.82° E	1	August 2021	120
		Dali	25.23° N, 100.24° E	2	May 2022	183
Southern Hilly Corn Region	Hainan	Sanya	18.38° N, 109.20° E	3	July 2021, July 2022	159, 188
	Jiangxi	Nanchang	28.33° N, 115.56° E	4	August 2021, August 2022	239
Huang–Huai–Hai Summer Corn Region	Henan	Yuanyan	35.06° N, 113.96° E	5	August 2021	130
	Shandong	Jinan	36.39° N, 117.01° E	6	August 2021	218
Northern Spring Corn Region	Hebei	Langfang	39.53° N, 116.70° E	7	September 2021, July 2022	76, 65
	Beijing	Tongzhou	39.90° N, 116.65° E	8	September 2022	138

2.3. Bioassay

The sensitivity of each FAW population to each Bt protein was determined by a diet surface overlay bioassay. Briefly, approximately 1 mL of liquefied diet was poured into each well of a 24-well plate. After the diet solidified, 50 µL Bt protein solution was added onto the diet surface in each well; each Bt protein was tested at 10 concentrations: 625, 312.50, 156.25, 78.13, 39.06, 19.53, 9.77, 4.88, 2.44, and 0 ng/cm^2 for Vip3A, or 5000, 2500, 1250, 625, 312.50, 156.25, 78.13, 39.06, 19.53, and 0 ng/cm^2 for Cry1F, Cry1Ab, and Cry2Ab. Once all wells on a plate had been treated, the plate was tilted from front to back and from left to right to ensure that the protein solution was evenly distributed on the diet surface. After the diet surface dried, a single 0 to 24 h-old neonate was transferred to each well with a fine brush (24 neonates per concentration of Bt protein). After infestation, the plate was covered with a sheet of blow-molded paper pad and then fastened with four rubber bands to prevent escape. The infested plates were placed in a climatic chamber under the following conditions: temperature, 26 ± 1 °C; light/dark cycle, 16/8 h; relative humidity, 60 ± 10%. The bioassays were technically repeated three times (insects from the same generation) for each Bt protein toxin, and thus the total number of neonates used was 720 for each FAW population and for each protein. Mortality was recorded at 7 days after treatment. Larvae that remained in the first instar stage throughout the experiment were counted as dead. The weight of surviving larvae was measured and recorded at the end of each experiment.

2.4. Data Analysis

The growth inhibitory rate of FAW larvae was calculated as growth inhibitory rate (%) = [(body weight of control group − body weight of treated group)/body weight of control group] × 100. The fold of 50% lethal concentration (LC_{50}) and 50% growth inhibition concentration (GIC_{50}) between the most susceptible and least susceptible populations for each Bt protein = LC_{50} or GIC_{50} of the least susceptible populations/LC_{50} or GIC_{50} of the most susceptible. The susceptibility of different populations to Bt protein toxins was analyzed by probit and logit analysis using PoloPlus [31], which generated LC_{50} and GIC_{50} values with 95% fiducial limits, chi-square (χ^2) data, slope with standard error (slope ± SE), and degrees of freedom. The difference between LC_{50} values was considered significant if the 95% fiducial limits of the values did not overlap. One-way analysis of variance followed by Duncan's multiple range test was used to compare the statistical significance of differences among the average values of LC_{50} or GIC_{50} among the 11 FAW populations for the four Bt proteins by using the SPSS software package (version 13, 2004); $p < 0.05$ was considered to reflect a statistically significant difference between values.

3. Results

3.1. Sensitivity of Different FAW Populations to Vip3A Protein

Table 2 lists dose-mortality data for FAW populations exposed to Bt Vip3A. Among the 11 FAW populations, the LC_{50} values ranged from 11.42 ng/cm^2 (Nanchang2021) to 88.33 ng/cm^2 (Dehong2021), and the GIC_{50} values ranged from 1.43 ng/cm^2 (Langfang2021) to 14.86 ng/cm^2 (Jinan2021). There were significant differences in susceptibility among some of the populations involved. The fold difference between the most susceptible and least susceptible populations was 7.73 (for LC_{50}) and 10.39 (for GIC_{50}).

Table 2. Susceptibility of *S. frugiperda* populations in different regions to Vip3A protein.

Population	N *	LC_{50} (95% FL, ng/cm^2) §	Slope ± SE	χ^2	GIC_{50} (95% FL, ng/cm^2) §	Slope ± SE	χ^2
Dehong2021	720	88.33 (41.64–139.19) a	2.42 ± 0.32	19.11	2.36 (1.21–3.79) de	0.85 ± 0.08	7.08
Dali2022	720	46.52 (24.54–79.45) abc	1.88 ± 0.18	28.26	7.05 (4.12–10.54) abc	1.26 ± 0.08	19.96
Sanya2021	720	61.94 (40.34–86.91) ab	2.49 ± 0.25	14.38	4.91 (0.37–13.34) abcde	0.78 ± 0.07	56.65
Sanya2022	720	13.63 (7.75–19.05) e	2.70 ± 0.35	10.09	13.66 (8.27–21.40) ab	1.64 ± 0.10	41.14
Nangchan2021	720	11.42 (4.59–21.30) ce	1.28 ± 0.10	33.03	7.37 (4.70–10.49) abc	1.07 ± 0.08	14.91
Nanchang2022	720	51.07 (20.81–77.24) abc	3.91 ± 0.55	24.44	1.94 (0.40–4.25) de	0.88 ± 0.08	22.08
Yuanyan2021	720	68.07(39.44–96.71) ab	2.25 ± 0.30	10.42	2.09 (0.98–3.51) de	1.24 ± 0.09	7.28
Jinan2021	720	20.12 (9.62–33.40) cde	1.96 ± 0.17	29.46	14.86 (9.16–22.53) a	1.43 ± 0.09	30.96
Tongzhou2022	720	73.83 (43.91–122.63) a	1.07 ± 0.13	7.19	14.03 (6.35–25.03) ac	0.66 ± 0.06	17.06
Langfang2021	720	25.73 (11.10–42.78) bcde	1.70 ± 0.17	20.71	1.43 (0.77–2.21) e	0.96 ± 0.10	5.75
Langfang2022	720	23.30 (7.26–40.11) cde	1.75 ± 0.23	14.33	5.06 (2.80–7.76) cd	0.98 ± 0.07	12.11

* Total number of larvae tested in the bioassay. § Values followed by different letters within the same column imply statistically significant differences ($p < 0.05$). 95% FL, 95% fiducial limits; LC_{50}, 50% lethal concentration; GIC_{50}, 50% growth inhibition concentration.

3.2. Sensitivity of Different FAW Populations to Cry1F Protein

Table 3 lists the sensitivities of 11 FAW populations exposed to Cry1F. The LC_{50} values ranged from 111.21 ng/cm^2 (Jinan2021) to 517.33 ng/cm^2 (Langfang2021), and the GIC_{50} values ranged from 2.35 ng/cm^2 (Nanchang2022) to 138.97 ng/cm^2 (Dehong2021). The fold difference between the most susceptible and most tolerant populations with respect to LC_{50} and GIC_{50} was 4.65 and 59.14, respectively.

Table 3. Susceptibility of *S. frugiperda* populations in different regions to Cry1F protein.

Population	N *	LC_{50} (95% FL, ng/cm^2) §	Slope ± SE	χ^2	GIC_{50} (95% FL, ng/cm^2) §	Slope ± SE	χ^2
Dehong2021	720	262.11 (109.78–469.77) abc	1.16 ± 0.14	8.77	138.97 (26.27–316.84) a	0.81 ± 0.14	35.41
Dali2022	720	174.71 (96.42–289.03) bc	1.19 ± 0.09	22.90	3.24 (0.25–10.61) bc	0.65 ± 0.08	10.59
Sanya2021	720	347.64 (196.91–547.35) ab	1.38 ± 0.12	14.50	2.50 (0.14–9.38) bc	0.53 ± 0.07	10.52
Sanya2022	720	445.59 (75.93–1017.25) abc	1.03 ± 0.14	22.46	67.65 (35.92–108.02) a	0.82 ± 0.07	13.59
Nangchan2021	720	281.19 (185.73–382.71) abc	1.61 ± 0.19	4.18	82.83 (41.98–137.16) a	1.21 ± 0.08	33.45
Nanchang2022	720	232.56 (61.70–551.02) abc	0.93 ± 0.09	28.42	2.35 (0.25–7.86) c	0.65 ± 0.08	6.39
Yuanyan2021	720	141.23 (51.50–283.01) bc	1.13 ± 0.09	33.16	17.05 (7.54–29.55) abc	0.76 ± 0.07	7.57
Jinan2021	720	111.21 (49.42–196.63) c	1.22 ± 0.11	18.66	42.73 (8.91–80.74) ab	1.07 ± 0.08	55.98
Tongzhou2022	720	308.56 (209.88–406.54) ab	2.24 ± 0.26	7.84	27.90 (7.66–57.27) abc	0.77 ± 0.07	20.52
Langfang2021	720	517.33 (379.99–699.84) a	1.38 ± 0.11	3.87	17.22 (9.94–25.94) b	0.86 ± 0.08	5.83
Langfang2022	720	212.57 (122.11–336.49) bc	1.08 ± 0.09	10.04	8.09 (3.59–14.23) bc	0.77 ± 0.08	5.30

* Total number of larvae tested in the bioassay. § Values followed by different letters within the same column imply statistically significant differences ($p < 0.05$). 95% FL, 95% fiducial limits; LC_{50}, 50% lethal concentration; GIC_{50}, 50% growth inhibition concentration.

3.3. Sensitivities of Different FAW Populations to Cry1Ab Protein

As for Cry1Ab, the LC_{50} values for the 11 FAW populations ranged from 135.76 ng/cm^2 (Yuanyan2021) to 1108.47 ng/cm^2 (Tongzhou2022), and the GIC_{50} values ranged from 1.58 ng/cm^2 (Sanya2022) to 464.86 ng/cm^2 (Tongzhou2022) (Table 4). The fold difference

between the most susceptible and least susceptible populations at the LC_{50} and GIC_{50} levels was 8.16 and 294.22, respectively.

Table 4. Susceptibility of *S. frugiperda* populations in different regions to Cry1Ab protein.

Population	N *	LC_{50} (95% FL, ng/cm^2) §	Slope ± SE	χ^2	GIC_{50} (95% FL, ng/cm^2) §	Slope ± SE	χ^2
Dehong2021	720	541.85 (273.04–1059.28) abc	0.96 ± 0.11	17.40	2.59 (0.25–8.88) b	0.36 ± 0.06	4.88
Dali2022	720	434.79 (300.40–594.88) bc	2.18 ± 0.19	16.43	14.22 (3.45–31.35) b	0.71 ± 0.07	14.31
Sanya2021	720	148.07 (68.38–255.29) de	1.13 ± 0.12	8.82	12.56 (3.28–27.40) b	0.65 ± 0.07	10.83
Sanya2022	720	179.58 (127.54–240.41) e	1.82 ± 0.26	3.28	1.58 (0.038–7.65) b	0.42 ± 0.68	4.83
Nangchan2021	720	758.16 (496.30–1039.59) ab	1.40 ± 0.19	4.11	4.85 (0.10–34.08) b	0.31 ± 0.07	9.12
Nanchang2022	720	697.35 (492.16–926.19) ab	1.56 ± 0.23	1.52	11.77 (1.33–33.58) b	0.51 ± 0.08	6.66
Yuanyan2021	720	135.76 (71.85–212.63) e	1.11 ± 0.11	8.40	10.34 (0.99–35.52) b	0.69 ± 0.10	4.88
Jinan2021	720	401.30 (240.87–642.05) bcd	1.61 ± 0.12	28.36	31.72 (5.87–77.57) b	0.70 ± 0.08	12.41
Tongzhou2022	720	1108.47 (780.35–1438.36) a	2.15 ± 0.32	8.08	464.86 (323.66–635.77) a	0.90 ± 0.07	8.38
Langfang2021	720	943.00 (457.67–1474.74) ab	1.61 ± 0.12	17.64	372.56 (203.35–602.15) a	1.26 ± 0.08	33.53
Langfang2022	720	181.74 (32.08–406.36) cde	1.12 ± 0.14	18.46	28.36 (0.10–134.23) b	0.60 ± 0.06	28.46

* Total number of larvae tested in the bioassay. § Values followed by different letters within the same column imply statistically significant differences ($p < 0.05$). 95% FL, 95% fiducial limits; LC_{50}, 50% lethal concentration; GIC_{50}, 50% growth inhibition concentration.

3.4. Sensitivity of Different FAW Populations to Cry2Ab Protein

As for Cry2Ab, the LC_{50} values for the 11 FAW populations ranged from 994.42 ng/cm^2 (Nangchan2021) to 5492.50 ng/cm^2 (Dali2022), and the GIC_{50} values ranged from 24.17 ng/cm^2 (Langfang2022) to 1266.07 ng/cm^2 (Yuanyan2021) (Table 5). The fold difference between the most susceptible and most tolerant populations with respect to LC_{50} and GIC_{50} was 5.52 and 50.62 respectively.

Table 5. Susceptibility of *S. frugiperda* populations in different regions to Cry2Ab protein.

Population	N *	LC_{50} (95% FL, ng/cm^2) §	Slope ± SE	χ^2	GIC_{50} (95% FL, ng/cm^2) §	Slope ± SE	χ^2
Dehong2021	720	2781.24 (1557.35–7184.05) abc	1.27 ± 0.17	18.35	296.41 (178.72–440.95) bc	0.85 ± 0.07	10.69
Dali2022	720	5492.50 (4087.30–9106.10) a	1.86 ± 0.34	5.72	304.57 (221.58–419.87) bc	1.19 ± 0.09	15.45
Sanya2021	720	1008.61 (54.11–1357.48) de	2.02 ± 0.29	7.46	117.94 (82.22–161.32) de	0.89 ± 0.06	8.16
Sanya2022	720	1506.56 (1231.76–1776.26) cde	2.95 ± 0.39	5.23	67.33 (48.86–88.39) de	1.14 ± 0.08	7.77
Nangchan2021	720	994.42 (725.19–1290.45) e	2.86 ± 0.28	14.33	96.18 (67.49–118.71) de	1.16 ± 0.08	8.42
Nanchang2022	720	2380.72 (1033.92–4711.16) abcde	1.81 ± 0.36	15.22	490.15 (326.75–693.32) b	0.76 ± 0.06	7.74
Yuanyan2021	720	4489.60 (2067.40–22081.00) ab	1.02 ± 0.10	25.67	1266.07 (877.72–1817.75) a	1.27 ± 0.07	30.75
Jinan2021	720	3231.40 (1905.90–7364.20) ab	1.10 ± 0.10	14.55	224.54 (52.33–493.20) bcd	0.75 ± 0.06	35.06
Tongzhou2022	720	1409.79 (990.00–1871.47) cde	1.59 ± 0.26	6.99	152.95 (60.22–277.95) ce	0.75 ± 0.07	15.36
Langfang2021	720	1089.20 (382.21–2194.60) cde	1.19 ± 0.17	19.49	25.01 (6.42–54.09) df	0.55 ± 0.06	12.08
Langfang2022	720	1816.53 (1350.92–2782.32) bcd	2.17 ± 0.39	6.86	24.17 (13.35–37.12) f	0.77 ± 0.07	8.47

* Total number of larvae tested in the bioassay. § Values followed by different letters within the same column imply statistically significant differences ($p < 0.05$). 95% FL, 95% fiducial limits; LC_{50}, 50% lethal concentration; GIC_{50}, 50% growth inhibition concentration.

3.5. Sensitivity Comparison of the Three Populations from the Same Location Collected in Different Years

We also compared the sensitivity of the three populations (Sanya, Nanchang, and Langfang) collected from the same locations in different years. The LC_{50} values of the three populations of the four proteins in 2022 did not increase or decrease simultaneously when compared with those of 2021 (Figure 2). There were no significant differences between most of the LC_{50} values of the same protein for the different years except those of Vip3A (Sanya), Cry1F (Langfang), and Cry1Ab (Langfang).

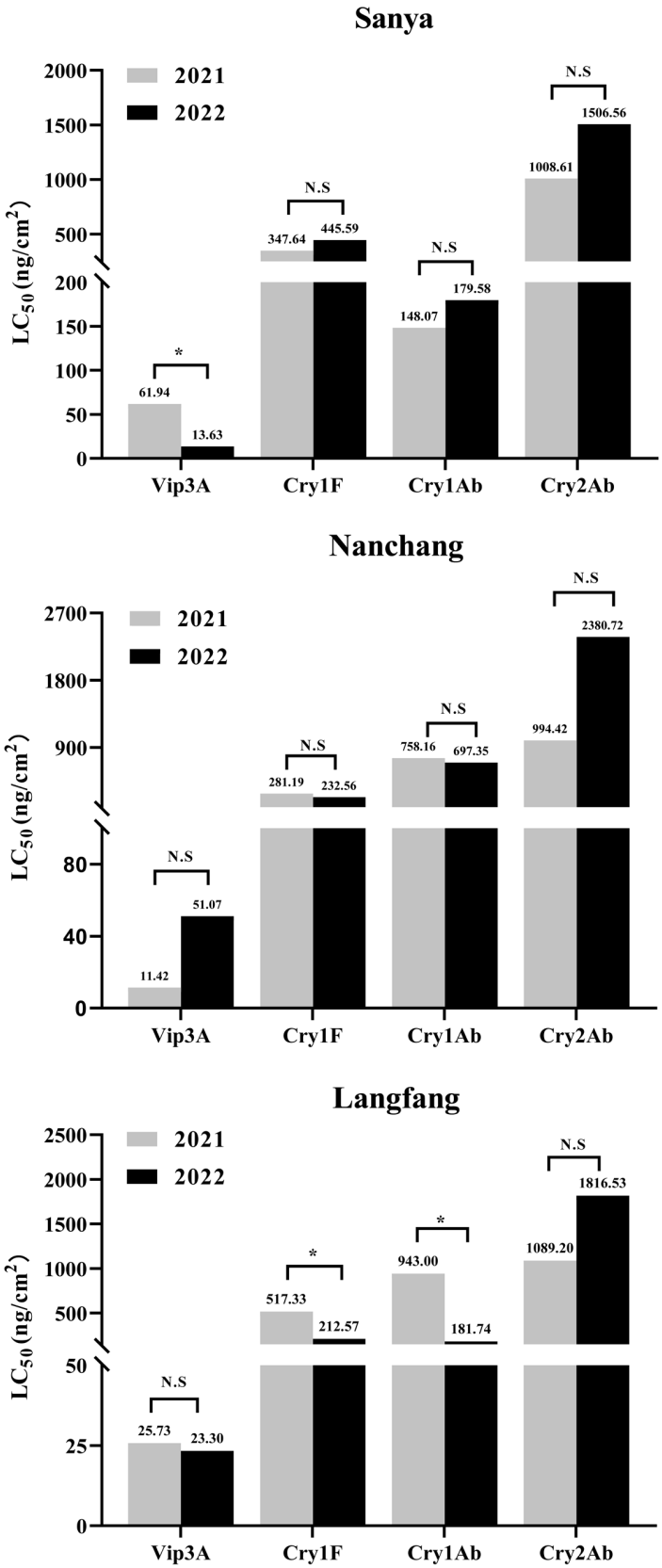

Figure 2. Sensitivity comparison of the 3 populations from the same locations collected in different years. The difference between LC_{50} values was considered significant if the 95% fiducial limits of the values did not overlap. The data of the 95% fiducial limits are shown in Tables 2–5. N.S., not significant. An asterisk indicates a significant difference between two groups.

3.6. Sensitivity Comparison among the Four Bt Proteins

We next compared the sensitivity differences among the four Bt proteins (Figure 3). The mean ± SE values of LC_{50} for all 11 FAW populations for each Bt protein were as follows: 44.00 ± 8.05 ng/cm^2 (Vip3A), 275.88 ± 37.53 (Cry1F), 502.73 ± 102.17 (Cry1Ab), and 2381.87 ± 130.05 (Cry2Ab), and the corresponding GIC_{50} values were 6.80 ± 1.55, 37.32 ± 3.75, 86.86 ± 49.94, and 278.67 ± 30.92 ng/cm^2. Significant differences were apparent among the average LC_{50} or GIC_{50} values for the four Bt proteins (Figure 3). The lethal effects and growth inhibition effects of the four Bt proteins on FAW were in the same order: Vip3A > Cry1F > Cry1Ab > Cry2Ab.

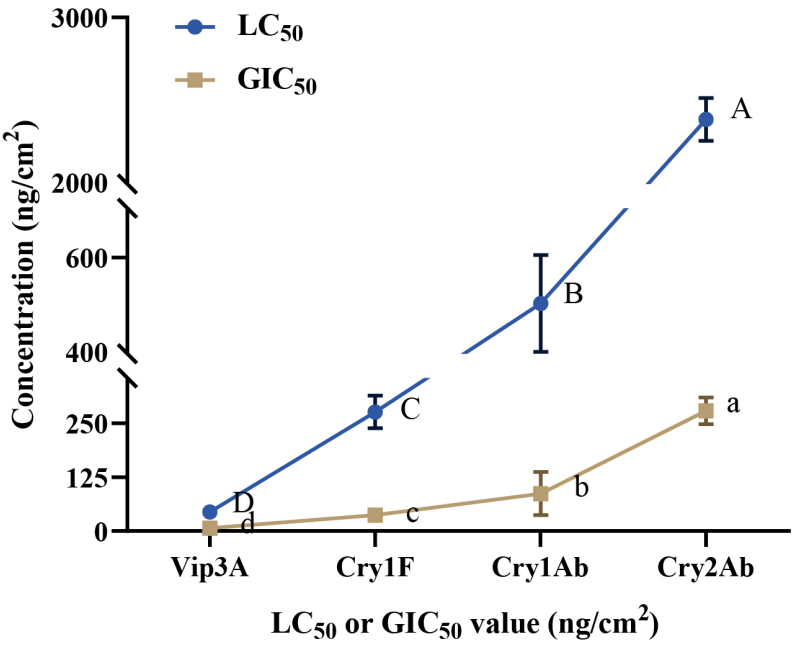

Figure 3. Mean ± SE values of LC_{50} and GIC_{50} for 11 FAW populations for four Bt proteins. Different letters indicate significant differences among LC_{50} values (uppercase letters) and among GIC_{50} values (lowercase letters) for different Bt proteins ($p < 0.05$, Duncan's test).

4. Discussion

Using genetically modified crops is one of the most effective ways of controlling FAW in the Western Hemisphere. Results from Chinese researchers showed that the larval density, the percentage of damaged plants, and the average damage ratings of GM corn (expressing Cry1Ab) were significantly lower than those of the control group [32]. At present, insect-resistant corn used to control FAW mainly expresses Cry1 proteins such as Cry1Ab, Cry1A.105, and Cry1F, Cry2 proteins such as Cry2Ab, or Vip3 proteins such as Vip3Aa [9–13]. Although some of these proteins have been expressed alone, they are now typically expressed in combinations with each other. Therefore, in the present study, we evaluated the sensitivity of FAW to these four commonly used and likely commercially applicable Bt proteins in China. China has six main corn production regions [29], and we collected 11 FAW populations from four of these regions (Table 1), which together comprise more than 90% of the total corn acreage of China. A diet incorporation assay [15,17,25,33] or surface overlay bioassay [11,18,34–39] is generally used to determine the sensitivity of FAW to Bt proteins. In our present study, we evaluated the sensitivity of 11 FAW populations to four Bt proteins based on results from a diet surface overlay method.

Among the various FAW populations we tested, the LC_{50} values for Vip3A, Cry1F, Cry1Ab, and Cry2Ab proteins ranged from 11.42 to 88.33, 111.21 to 517.33, 135.76 to 1108.47, and 994.42 to 5492.50 ng/cm^2, respectively, representing fold differences of 7.73, 4.65, 8.16, and 5.52 among several populations. This indicated clear inter-population variation in susceptibility to the same Bt protein. In fact, variations between FAW populations [13,14,25,39]—and

between certain populations of other Lepidoptera pests [40,41]—have been frequently reported. For example, the median effective concentration (i.e., EC_{50}) of Cry1Ab in field populations of FAW in Brazil was reported to range from 0.30 to 3.67 µg/mL, implying a 12.23-fold difference among populations [14]. The LC_{50} for Vip3Aa20 varied from 92.38 to 611.65 ng/cm^2, implying a 6.6-fold difference among populations [22]. It was assumed that the broad range of EC_{50} and LC_{50} values reflects natural variability among populations rather than selection pressure [25,41].

In our study, the smallest and largest LC_{50} values for Vip3A among the different FAW populations were 11.42 (range 4.59–21.30) and 88.33 (41.64–139.19) ng/cm^2, respectively. Some studies reported that the LC_{50} values for the most Vip3A-sensitive FAW population ranged from 8.96 to 611.65 ng/cm^2 [21,22,42,43]. Compared with values in this range, our FAW populations were highly susceptible to Vip3A.

Based on reported studies, the LC_{50} values for Cry1F ranged from 10.64 to 420 ng/cm^2 among susceptible FAW populations [11,18,35–39,43]. In our present study, the smallest and largest LC_{50} values for Cry1F were 111.21 (range 49.42–196.63) and 308.56 (209.88–406.54) ng/cm^2, respectively, which fall within the aforementioned reported range; this indicates that the FAW populations we studied were also susceptible to Cry1F.

Xu et al. measured the susceptibility of FAW populations collected from different planting areas of the Anhui Province of China to Cry1Ab, and the LC_{50} values ranged from 17.99 to 537.60 ng/cm^2 [39]. Results from Ingber et al. indicated that LC_{50} values for Cry1Ab exceeded 2000 ng/cm^2 with two "corn" strain FAW populations, whereas Cry1Ab LC_{50} values for the rice strain and their hybrid populations ranged from 1341 to 1912 ng/cm^2 [44]. In our present study, the LC_{50} values for Cry1Ab ranged from 135.76 to 1108.47 ng/cm^2, which is consistent with the aforementioned ranges. Many reports have confirmed that Cry1Ab has relatively low toxicity for Noctuidae pests such as FAW, which generally is not regarded as a target pest for this Bt protein [45,46]. For example, Bt corn hybrids such as "Bt11", "Bt176", and "MON810", which express Cry1Ab, are highly toxic to the corn borer, with field control efficiencies exceeding 99%; however, these hybrids also can help control FAW by reducing the damage rate of corn at the heart-leaf stage by 90% and at the ear stage by 50–80% [47,48].

As for Cry2Ab, the reported LC_{50} values for susceptible FAW populations ranged from 160.35 to 840 ng/cm^2 [15,36,43]. In the present study, the LC_{50} values for Cry2Ab ranged from 994.42 to 5492.50 ng/cm^2. Thus, the Cry2Ab LC_{50} values measured in our study are larger than those reported previously. The difference may be attributable to differences in the activity of Cry2Ab from different sources.

Our results also revealed that the lethality and growth inhibition effects of the four Bt proteins on FAW were in the same order, i.e., Vip3A > Cry1F > Cry1Ab > Cry2Ab. Li et al. determined the lethality and growth inhibition effects of five Bt proteins on FAW collected from Yunnan province and found that the relative lethality was Vip3A > Cry1Ab > Cry1F > Cry2Ab > Cry1Ac and that growth inhibition was Cry1Ab > Cry1F > Vip3A > Cry1Ac > Cry2Ab [41]. Our results are partially consistent with these results. Many reports have confirmed that Cry1Ab has relatively low toxicity toward Noctuidae pests such as FAW [43,44], whereas Cry1F is more toxic and thus can better control FAW [12]. Xu et al. [39] and Zhang et al. [49] conducted a bioassay on a susceptible FAW population and found that the activity of Cry1F was superior to that of Cry1Ab. Thus, their results tend to support ours.

In summary, our results indicate that Vip3A, Cry1F, Cry1Ab, and Cry2Ab are clearly effective for controlling FAW, yet their effectiveness varied among different FAW populations. Based on reported LC_{50} values for Bt proteins toward susceptible and resistant FAW populations, we infer that the FAW populations tested in the present study are indeed susceptible to Vip3A, Cry1F, and Cry1Ab. To date, there has been no report of the emergence of Bt-resistant FAW populations in China [25,39,43]. Existing studies suggest that the population of FAWs that invaded China is likely to have originated from the

United States [32], and this population is relatively sensitive to Bt biopesticides and Bt plants [32,50].

Based on the experience of Bt corn planting and FAW control in countries other than China, a multi-gene strategy should be followed to avoid planting corn hybrids that express a single Bt gene, especially Bt corn that only expresses Cry1Ab [14]. Multivalent Bt maize with a high-dose expression of Cry1 and Vip3A should be planted to control the number of FAWs at their source. The FAW has completed the colonization process in China and surrounding Southeast Asian countries. Bt corn or Bt cotton has been approved for planting in Vietnam, the Philippines, Myanmar, and other FAW source countries. If FAW develops resistance locally, individuals with resistance genes will likely invade the southern corn-producing areas of China, which is bound to pose a threat to the effectiveness and yield of Bt maize in China. Therefore, we recommend that the Bt protein bioassay be augmented for use in assessing the effectiveness of Bt proteins and that a unified resistance monitoring standard be formulated to facilitate the continued monitoring of the sensitivity of different geographical populations of FAWs to different Bt proteins and the frequency of the appearance of resistance genes [4], which will have important theoretical and practical significance for the rational layout and long-term use of transgenic corn in China.

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