Research Article

Received: 30 July 2022 Revised: 24 October 2022 Accepted article published: 3 November 2022 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/ps.7273

Performance of the domestic Bt corn event expressing pyramided Cry1Ab and Vip3Aa19 against the invasive *Spodoptera frugiperda* (J. E. Smith) in China

Shengyuan Zhao,[a,b] Xianming Yang,[a] Dazhong Liu,[a] Xiaoxu Sun,[c] Guoping Li[d] and Kongming Wu[a*]

Abstract

BACKGROUND: The invasive fall armyworm, *Spodoptera frugiperda* (J.E. Smith), has caused serious corn yield losses and increased the frequency of insecticide spraying on corn in Africa and Asia. Drawing lessons from the use of Bt corn to manage fall armyworm in the Americas, China released a certificate for the genetically modified corn event DBN3601T pyramidally expressing Cry1Ab and Vip3Aa19 for industrialization in 2021. Performance of the DBN3601T event against invasive fall armyworm in China was evaluated by plant tissue-based bioassays and field trials during 2019–2021.

RESULTS: In the bioassays, tissues and organs of DBN3601T corn differed significantly in lethality to fall armyworm neonates in the order: leaf > husk > tassel and kernel > silk. In field trials, compared with non-Bt corn, DBN3601T corn greatly suppressed fall armyworm populations and damage; larval density, damage incidence, and leaf damage scores for DBN3601T corn were significantly lower than for non-Bt corn at different vegetative stages, and efficacy against larval populations during the 3 years ranged from 95.24% to 98.30%.

CONCLUSION: A laboratory bioassay and 3-year field trials confirmed that DBN3601T corn greatly suppressed fall armyworm populations and has high potential as a control of this invasive pest, making it a key tactic for integrated management of fall armyworm in China.

Keywords: pyramided Bt corn; Cry1Ab; Vip3Aa19; fall armyworm; efficacy; China

1 INTRODUCTION

Fall armyworm, *Spodoptera frugiperda* (J. E. Smith), native to tropical and subtropical regions of the American continents, has caused enormous agricultural losses worldwide.[1,2] Its highly polyphagous nature (feeding on more than 350 host plants),[3] high fecundity (nearly 1500 eggs per female),[4] resistance to numerous classes of synthetic insecticides,[4] and remarkable migratory ability (flying approximately 1600 km within 30 h under a suitable atmosphere, and with a self-powered flight speed of 2.73 km h^{-1} measured by laboratory flight mill)[5,6] have enabled it to become a global super pest of more than 80 crops including corn, sorghum, sugarcane, wheat, soybean, cotton.[3,4,7,8] Fall armyworm was first detected Africa in 2016, having likely arrived in cargo,[9,10] beginning the invasion and spread of fall armyworm to Africa, Asia and Australia.[4,11–16] Fall armyworm larvae not only feed on most parts of the corn plant during all growth stages, directly reducing yields,[9,13] but also lead to serious fungal ear rot and high levels of mycotoxins causing serious health problems for humans and animals.[17,18] In Brazil, fall armyworm has reduced corn yields by 34% at a cost of nearly US $400 million per year.[19] In sub-Saharan Africa, fall armyworm infestations have caused mean annual yield losses of 21%–53% with an estimated annual economic loss of US $2.5 to 6.2 billion in 2017.[7] In China, potential economic loss of corn production caused by fall armyworm without any preventive controls has been estimated at US $5.6 to 48.8 billion by the random model @RISK in 2019.[20,21] Chemical pesticides as part of integrated pest management strategies have become the most important method to control fall armyworm infestation in China.[8] Historically in the Americas, before the

* Correspondence to: K Wu, State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 1000193, China. E-mail: wukongming@caas.cn

a State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China

b Guangdong laboratory for Lingnan Modern Agriculture, Guangzhou, China

c Zhengzhou Fruit Research Institute, Chinese Academy of Agricultural Sciences, Zhengzhou, China

d Key Laboratory of Integrated Pest Management on Crops in Southern Part of Northern China, Ministry of Agriculture and Rural Affairs, Institute of Plant Protection, Henan Academy of Agricultural Sciences, Zhengzhou, China

commercial planting of Bt corn, traditional synthetic insecticides, such as carbamate, organophosphorus, and pyrethroid were the main methods of control for fall armyworm, which has now developed resistance to 36 insecticides with seven different modes of action.[22] Fall armyworm populations in Puerto Rico have developed 14-fold resistance to spinosad and 160-fold resistance to chlorantraniliprole.[23] The Chinese government have adopted an integrated pest management strategy mainly based on chemical control, monitoring, and an early-warning system for emergency control of fall armyworm.[8] As expected, owing to the development of pesticide resistance, smallholders have had to increase the frequency of pesticide spraying and switch to novel pesticides with new insecticidal mechanisms, such as chlorantraniliprole and spinetoram. Hence, the economic cost of pesticide spraying for corn rose from \$81 ha^{-1} in 2018 to \$276 ha^{-1} in 2020.[24]

Commercial cultivation of genetically modified (GM) corn with insect-resistant traits has become the most widely used and effective technical method of fall armyworm management in the Americas.[25,26] The USA and Canada approved the commercial use of GM corn in 1996, and the planting area reached 6.09×10^7 ha in 2019, accounting for 32% of the total acreage planted with GM crops.[26] In 1995 and 1996, the first GM corn events Bt176, MON810, and Bt11, which express the Cry1Ab protein, were successively approved for commercial cultivation in the USA and Canada, and provided effective control of lepidopteran pests such as European corn borer (*Ostrinia nubilalis*) and southwestern corn borer (*Diatraea grandiosella*).[25,27–30] However, efficacy against fall armyworm in field trials was inadequate.[31,32] Subsequently, the commercial cultivation of TC1507 corn expressing Cry1F toxin, approved in the USA in 2001, provided excellent control against fall armyworm.[33] The efficacy of MIR162 corn expressing the novel Bt toxin Vip3A against fall armyworm was above 99%.[34] To expand the insecticidal spectrum in Bt corn and delay the evolution of resistance to Cry1A and Cry1F, multiple genes were stacked to generate insect-resistant corn that was planted in the USA. At present, most commercial Bt corn events have multiple pyramided Bt genes or modified Bt genes, such as MON89034 (expressing Cry1A.105/Cry2Ab2), Bt11 × MIR162 (Cry1Ab + Vip3A), MON89034 × TC1507 (Cry1A.105/Cry2Ab2 + Cry1F).[25,35–37] In the USA and Canada, pyramided genes with *cry1*, *cry3*, and *vip3A* in corn control not only various lepidopteran caterpillars, but also coleopteran beetles.[38–40] After invading South Africa in 2017, fall armyworm was listed as a target pest of MON810, MON89034 in 2018.[41]

Corn is the primary grain crop grown in China and is mainly used as food, feed, and an industrial raw material. In 2021 in China, 4.33×10^8 ha were planted with corn; the total yield was 2.73×10^8 tons, and 2.84×10^7 tons were imported (National Bureau of Statistics of China, https://data.stats.gov.cn/). Frequent infestations with native pests, such as *Helicoverpa armigera*, *Mythimna separata*, *O. furnacalis*, *S. litura*, and *Agrotis ipsilon*, have been reported to cause 10%–20% yield loss annually,[42] and the invasion of fall armyworm poses a further challenge to pest management in China.[8,37,42] To improve crop yield and quality, Chinese researchers and companies have developed varieties of GM corn resistant to several target pests. Up to March 2021, the Chinese government had issued seven safety certificates for GM corn events including Ruifeng125 (Cry1AB + Cry2Aj), DBN9858 (with glufosinate and glyphosate herbicide tolerance), DBN9936 (Cry1Ab), DBN9501 (Vip3A), DBN3601T (Cry1Ab + Vip3Aa19), ND207 (Cry1Ab + Cry2Ab), and Ruifeng8 (Cry1Ab + Cry2Ab) to promote the industrialization of GM corn (http://www.moa.gov.cn/ztzl/zjyqwgz/). Although not yet commercialized, GM transformants with sufficient insecticidal efficacy against major native pests have been developed in China, but their field efficacy has not been assessed against fall armyworm. We thus tested the efficacy of DBN3601T corn against fall armyworm in a laboratory bioassay and field trials to advance the development of GM corn for the management of fall armyworm populations in China.

2 MATERIALS AND METHODS

2.1 Bt corn and non-Bt near-isogenic corn

DBN3601T hybrid corn pyramidally expressing Cry1Ab and Vip3Aa19, which are active against lepidopteran pests, and hybrid non-Bt near-isogenic corn "Wugu 3861" (2019), "Dongdan 6531" (2020), and "Luodan566" (2021) were provided by Beijing DaBeiNong Biotechnology Co. Ltd.

2.2 Bioassays using plant tissues

The fall armyworm population used in the bioassay was collected from a corn field (101°39′3.72″ N; 22°40′53.19″ E) in Jiangcheng County, Pu'er City, Yunnan Province in January 2019 and reared on artificial diet (100 g corn flour, 40 g soybean flour, 50 g wheat bran, 30 g yeast, 30 g white sugar, 3 g sorbic acid, 40 g casein, 22 g agar, 3.5 g ascorbic acid, 0.15 g vitamin B complex, 1000 ml water) in a controlled environment at 25 ± 1°C, 75% ± 5% relative humidity, and a 16:8 h light/dark photoperiod. Different plant tissues at various growth stages (based on the code in Abendroth et al.[43])—V5 leaves, VT leaves, VT tassel (closed, 5 cm long), R1 leaves, R1 silks (unpollinated, 5 cm long), R2 husks, R2 kernels—were cut from DBN3601T and non-Bt corn plants from a field trial and taken immediately to the laboratory for bioassay against fall armyworm neonates. One type of corn tissue was placed in a transparent plastic box (diameter 10 cm, height 6 cm) containing moistened cotton to delay tissue dehydration; on the same day, 40 newly hatched (within 1 day) larvae were placed on the tissues using a brush. Three boxes were set up for each tissue type, with three pieces of the tissue in each box (seven tissues per trial × three boxes per tissue type × three pieces per box). Dead and surviving larvae in each treatment were counted daily for 5 days after the start of the infestation. Larvae were regarded as dead if they did not move when touched lightly with a brush. The acceptable background mortality was 20%. Fresh corn tissues of the same variety and growth stage in the field trial were collected to supply the larvae with fresh, healthy samples daily.

2.3 Field trials

In Yunnan Province, the main area in China with annual infestations of fall armyworm, three field trials (JC-2019, September to November 2019; JC-2020, January to April 2020; JC-2021, November 2020 to March 2021) were set up at Jiangcheng Experimental Station, Chinese Academy of Agricultural Sciences (JC station, 22°41′13.13″N, 101°38′40.63″E) in Jiangcheng County. A further field trial (LC-2019, August to November 2019) was undertaken at Lancang Experimental Station, Institute of Plant Protection, Chinese Academy of Agricultural Sciences (LC station, 22°30′24.64″N, 99°53′22.29″E) in Lancang County. A randomized block design with three replicates per treatment was adopted in all field trials. The plot size for each treatment was 200 m^2 (10 m × 20 m), and each plot was set with a 1.5-m isolated path. The planting density was about 52 000 plants ha^{-1} with a row spacing of 60 cm and a plant spacing of 32 cm. Standard agronomic practices were used, but no insecticides were applied to ensure natural fall armyworm infestation. Monthly precipitation at the study sites (Jiangcheng County and Lancang County) from 2019 to 2021 was collected from the National Oceanic and Atmospheric Administration (https://www.ncei.noaa.gov/maps/daily/) (Fig. 1). Field trial metrics, such as egg mass density (EMD), larval density (LD), instar proportion, plant damage incidence, and leaf damage score (based on a severity score suggested by CIMMYT[4]), were assessed using a W-type five-point sampling method (20 plants per point) every 7–10 days, and the number of egg masses, number of larvae of each instar, and leaf damage score for each plant was recorded by screening the leaves, whorl, tassel, and ear. Plant damage incidence was screened by checking the whole corn plant, especially leaf and whorl at the V stage, silk, and kernels on the ear tip at the R stage. Fall armyworm larval development was assessed according to a description of the morphology[1,4] (Table 1). To estimate differences in EMD between DBN3601T and non-Bt corn, the fold-change (FC) in EMD was calculated as $(EMD_{DBN3601T} + 1)$ / $(EMD_{non\text{-}Bt} + 1)$. Ear damage incidence of husk, silk, and kernel was assessed for 20 plants in JC-2021 using a three-point sampling method. Taking into account differences in the population density of fall armyworm neonates caused by female oviposition bias for Bt corn in this study, the weighted efficacy of DBN3601T corn against fall armyworm larvae at each stage was calculated as:

$$\text{Weighted efficacy}\,(\%) = \frac{FC \times LD_{Non-Bt} - LD_{DBN3601T}}{FC \times LD_{Non-Bt}} \times 100$$

2.4 Statistical analyses

The corrected mortality of fall armyworm neonates on the different tissues was analyzed using an analysis of variance (ANOVA) and Kruskal–Wallis nonparametric test with Duncan's multiple comparisons. Data from field trials were analyzed with an ANOVA and Kruskal–Wallis nonparametric test with Duncan's multiple comparisons to test for significant differences in fall armyworm variables (EMD, LD) and damage variables (leaf damage score, damage incidence) at each corn growth stage for DBN3601T and non-Bt corn, respectively. A two-sample *t*-test and Mann–Whitney *U*-test were used to test the significance of fall armyworm variables (EMD, LD) and damage variables (leaf damage score, damage incidence) between DBN3601T and non-Bt corn at each growth stage in the field trials. All statistical analyses were performed with SAS 9.4 (SAS Institute).

3 RESULTS

3.1 Bioassay of DBN3601T tissues against fall armyworm neonates

The ANOVA and Kruskal–Wallis test indicated that the corrected mortality of fall armyworm neonates feeding on different tissues of DBN3601T corn differed significantly among the treatment durations (day 1, $F_{6,\,20} = 13.48$, $p < 0.0001$; day 2, $F_{6,20} = 59.22$, $p < 0.0001$; day 3, $H = 19.40$, $df = 6$, $p = 0.0035$; day 4, $H = 19.38$, $df = 6$, $p = 0.0036$; day 5, $H = 19.51$, $df = 6$, $p = 0.0034$). Corrected mortalities of fall armyworm neonates feeding on leaves at stages V5, VT, or R1 were significantly higher than on other tissues from day 1 to day 3, and mortality on these three tissues was always above 60% on day 1 (respectably, V5 leaf, 76.67% ± 7.95%; VT leaf, 68.64% ± 6.62%; R1 leaf, 64.91% ± 9.28%) and 100% on day 3 (Fig. 2). Husk yielded a significantly higher corrected mortality against neonates compared with kernel, tassel, or silk from day 3 to day 4, and reached 100% by day 4 (Fig. 2). The corrected mortality on silk on day 5 was 41.67% ± 4.17%, which was significantly lower than that on kernel (73.96% ± 8.14%) and tassel (86.30% ± 4.94%) (Fig. 2). Overall, the corrected mortality of the neonates differed significantly among the various tissues of DBN3601T corn, with the tissues in increasing order of lethality: V5 leaf, VT leaf, R1 leaf > husk > tassel or kernel > silk.

3.2 Field differences in EMD and LD of fall armyworm on Bt- and non-Bt corn plants

Generally, the EMD of fall armyworm on DBN3601T and non-Bt corn differed for oviposition behavior in different locations and years. At JC station, corn was heavily infested with fall armyworm, especially in JC-2019 (26.00 ± 2.31 egg masses per 100 plants at V5 on DBN3601T, and 8.33 ± 0.88 egg masses per 100 plants on non-Bt corn; Figure 3A). The *t*-test results for EMD at each whorl stage showed that fall armyworm had an oviposition bias for DBN3601T corn following natural infestation in the three field trials at JC station, but not in LC-2019. At JC station, the EMD of fall armyworm on DBN3601T corn was significantly higher than on non-Bt corn at V5 ($t = 7.15$, $P = 0.0020$), V8 ($t = 8.84$, $p = 0.0009$), V9 ($t = 5.38$, $p = 0.0058$), and V11($t = 6.12$, $p = 0.0036$) in 2019;

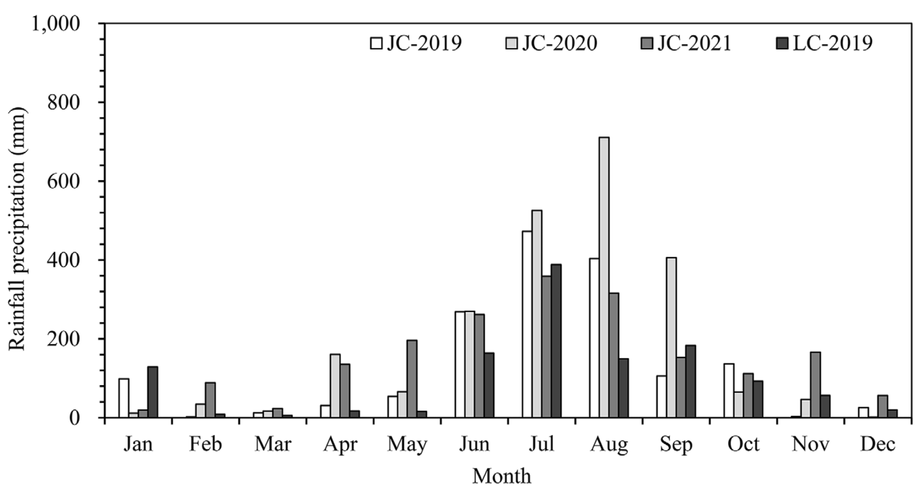

Figure 1. Monthly rainfall precipitation of JC station and LC station from 2019 to 2021.

Table 1. Morphological description of fall armyworm larval instars for assessment of larvae population density and instar structure in the field trials

Larval instar	Head capsule width range (mm)	Body length range (mm)	White inverted 'Y' on the face	White stripes on the head	Description of main morphological characteristics of head and body
First	≤ 0.4	≤ 1.7	No	No	Black head; body whitish before feeding and greenish white afterwards, cylindrical, no other color markings.
Second	(0.4, 0.5]	(1.7, 3.5]	No	No	Amber head; body pale whitish with a tinge of brown on the dorsum, the dorsal and subdorsal white lines faintly outlined.
Third	(0.5, 0.8]	(3.5, 6.4]	Yes	No	Amber head; body light brown on the dorsum, greenish on the venter, dorsal and subdorsal white lines plainly visible.
Fourth	(0.8, 1.3]	(6.4,10]	Yes	Yes	Reddish-brown head; body dark brown on the dorsum, with pale venter and subventer; the subventer mottled with pale brown; dorsal and subdorsal white lines conspicuous.
Fifth	(1.3, 2.0]	(10,17.2]	Yes	Yes	Dark brown head, ocular area amber, mottled with patches of white on entire epicranium; body grayish brown on the dorsum, venter and subventer greenish, the latter mottled with pink; suprastigmatal band dark brown, almost black; substigmatal band pale whitish, filled in with pale reddish-brown mottling.
Sixth	(2.0, 2.6]	(17.2, 34.2]	Yes	Yes	Reddish-brown head mottled with patches of white; body grayish brown on the dorsum, greenish on the venter and subventer, the latter being mottled with reddish-brown; dorsal and subdorsal white lines conspicuous.

at V8 (t = 3.67, p = 0.0213), V10 (t = 4.01, p = 0.0160), and V12 (t = 2.91, p = 0.0438) in 2020; and at V8 (t = 3.48, p = 0.0254), V9 (t = 3.16, p = 0.0341), and V14 (t = 2.83, p = 0.0474) in 2021 (Fig. 3A,C,D). However, EMD did not differ between the two corn types at V3 (t = −0.53, p = 0.6213) in 2019; at V3 (t = 0.27, p = 0.8025) and V5 (t = 1.58, p = 0.1890) in 2020; or at V3 (t = −0.50, p = 0.6433), V5 (t = 0.32, p = 0.7676), V7 (t = 1.58, p = 0.1890), and V11 (t = 2.21, p = 0.0913) in 2021 (Fig. 3A,C,D). At LC station, fall armyworm infestation was relatively low in 2019. The highest density of fall armyworm egg masses was 1.67 ± 0.88 at V3, and the Mann–Whitney U-test showed no significant difference between DBN3601T and non-Bt corn at any stage in 2019 (Fig. 3B). The fold-change in EMD showed a significant difference between various growth stages in the field trials in 2019 ($F_{4, 14}$ = 3.81, p = 0.0391), 2020 ($F_{4, 10}$ = 4.28, p = 0.0283), and 2021 ($F_{6, 20}$ = 4.03, p = 0.0149) at JC station (Fig. 3A,C,D). The highest fold-change in egg masses by year at the JC station was 3.66 ± 0.63 in JC-2019 (Fig. 3A), 3.42 ± 0.82 in JC-2020 (Fig. 3C), and 3.17 ± 0.60 in JC-2021 (Fig. 3D). The fold-change in EMD showed no significant difference among all growth stages in LC-2019 (Kruskal–Wallis test: H = 3.7969, df = 11, p = 0.9755) (Fig. 3B).

The results of the t-test and Mann–Whitney U-test showed that the fall armyworm LD at each growth stage on non-Bt corn was significantly higher than on DBN3601T corn in all field trials (Fig. 4A–D). LD at each growth stage on non-Bt corn differed significantly among the field trials in different years at JC station (2019, $F_{8, 26}$ = 116.52, P < 0.0001; 2020, $F_{7, 23}$ = 15.62, p < 0.0001; 2021, $F_{11, 35}$ = 21.28, p < 0.0001) (Fig. 4A,C,D), but not at LC station in 2019 ($F_{12, 38}$ = 0.91, p = 0.5462) (Fig. 4B). For LD of fall armyworm on non-Bt corn, the most serious infestation

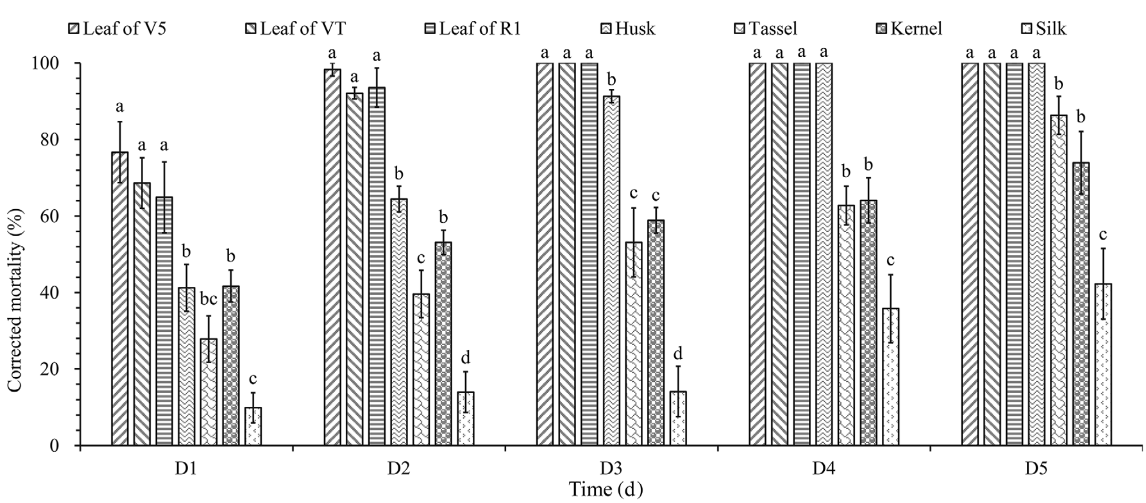

Figure 2. Corrected mortality of *Spodoptera frugiperda* neonate that fed on different tissues of DBN3601T corn (expressing Cry1Ab and Vip3Aa19) for different durations. Error bars represent SE. Different lowercase letters above error bars for the same treatment time indicate a significant difference in mortality among tissues by Duncan's multiple range test at the 0.05 level.

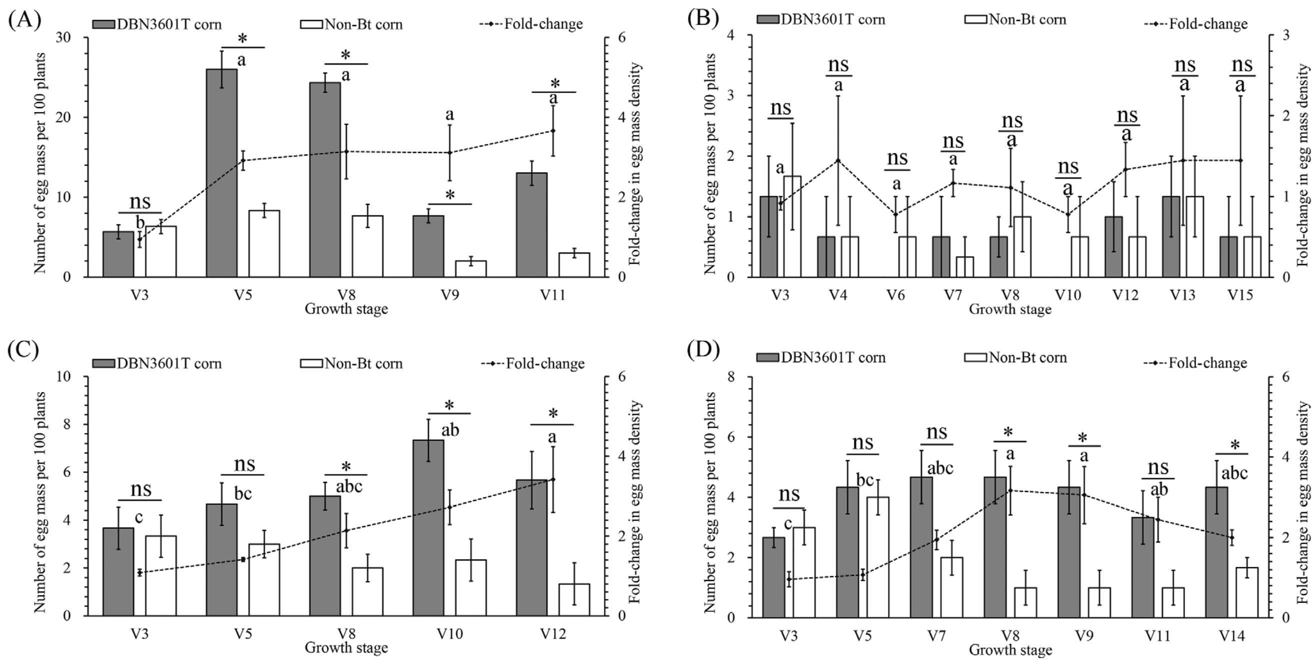

Figure 3. Egg mass density and fold-change in egg mass density for *Spodoptera frugiperda* in different growth stages of DBN3601T corn (expressing Cry1Ab and Vip3Aa19) and non-Bt corn. Field trial (A,B) in 2019 at (A) JC station from September to November and (B) at LC station from August to November, and (C, D) in 2020–2021 at JC station (C) from January to April 2020 and (D) from December 2020 to March 2021. Error bars represent SE. The asterisk represents a significant difference in egg mass density between DBN3601T corn and non-Bt corn in a two-sample *t*-test at the 0.05 level. Different lowercase letters above the error bars indicate a significant difference in the fold-change in egg mass density among the different stages (Duncan's multiple range test, 0.05 level).

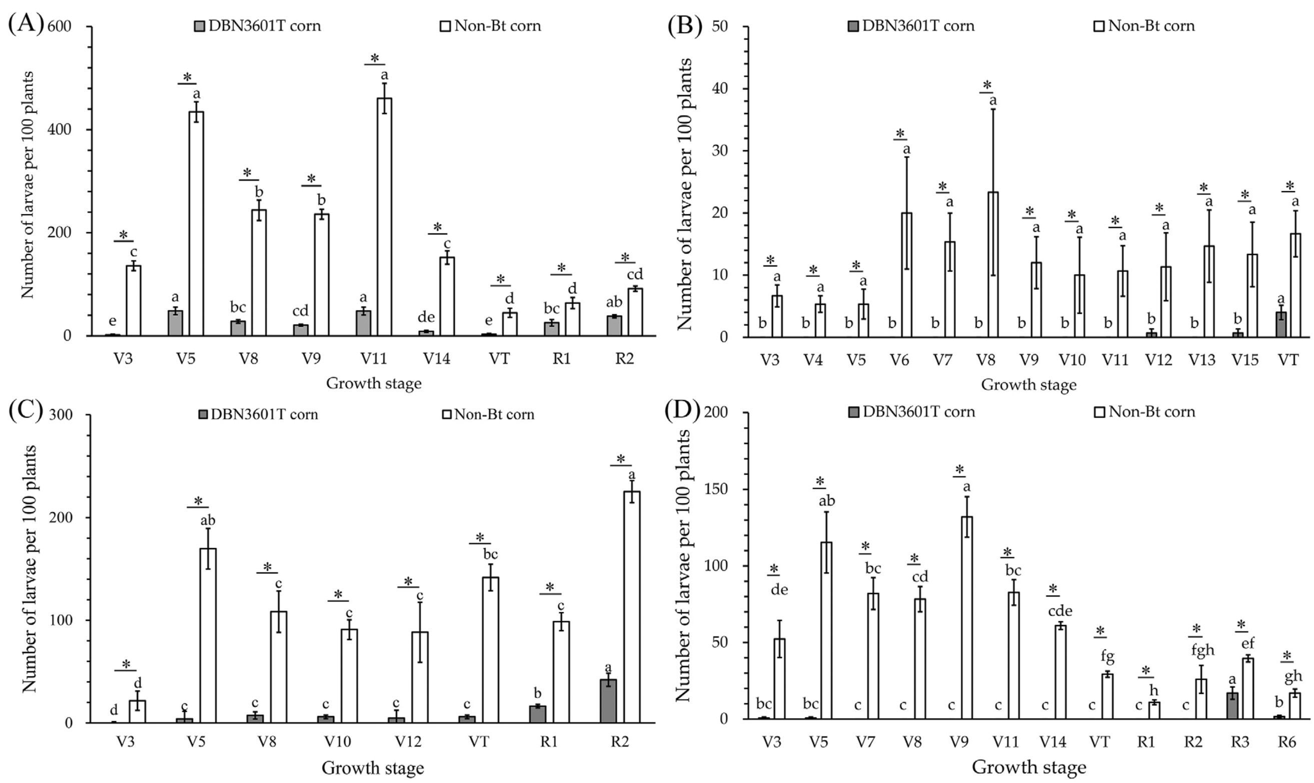

Figure 4. Larval densities of *Spodoptera frugiperda* at different growth stages of DBN3601T corn (expressing Cry1Ab and Vip3Aa19) and non-Bt corn in field trials in different locations and years: (A) JC station from September to November 2019; (B) LC station from August to November 2019; JC station in (C) January to April 2020 and (D) December 2020 to March 2021. Asterisk represents a significant difference in the number of larvae between DBN3601T corn and non-Bt corn at different stages (two-sample *t*-test or Mann–Whitney *U*-test, 0.05 level). Different lowercase letters above the error bars indicate a significant difference in the number of larvae at different stages of the same variety (one-way ANOVA for 2019 and 2020 at JC station; Kruskal–Wallis test for 2019 at LC station and 2021 at JC station) in Duncan's multiple range test at the 0.05 level.

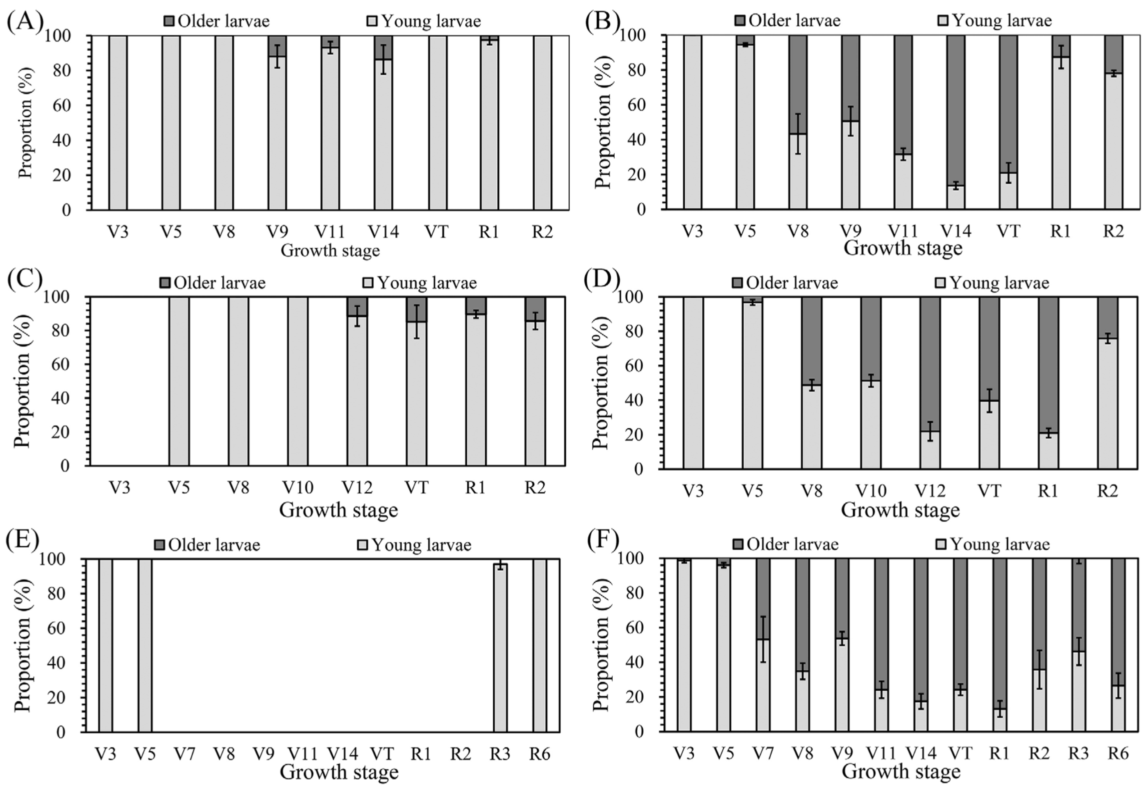

Figure 5. Instar structure of *Spodoptera frugiperda* larval population on DBN3601T corn (expressing Cry1Ab and Vip3Aa19) and non-Bt corn at different growth stages in field trials: (A) DBN3601T corn in field trial at JC station in 2019; (B) non-Bt corn at JC station in 2019; (C) DBN3601T corn at JC station in 2020; (D) non-Bt corn at JC station in 2020; (E) DBN3601T corn at JC station in 2021; (F) non-Bt corn at JC station in 2021.

occurred in JC-2019, and LD reached 473.00 ± 24.19 per 100 plants at stage V11 and 435.00 ± 19.47 per 100 plants at V5 (Fig. 4A). In JC-2020, the highest LD was 225.33 ± 39.57 per 100 plants at R2 (Fig. 4C). In JC-2021, the highest LD was 132.00 ± 13.23 per 100 plants at V9 (Fig. 4D). In LC-2019, the natural infestation of fall armyworm was slight, with a highest LD of only 23.33 ± 13.38 at V8 (Fig. 4B). On DBN3601T corn, the density of surviving fall armyworm larvae was extremely low in all field trials, and the highest LD values were 45.00 ± 4.36 (V5), 42.00 ± 10.97 (R2), 17.00 ± 14.04 (R3) and 4.00 ± 1.15 (VT) per 100 plants in JC-2019 (Fig. 4A), JC-2020 (Figure 4C), JC-2021 (Fig. 4D) and LC-2019 (Fig. 4B). By instar structure of the fall armyworm larval population on DBN3601T corn, younger instars (first to third) accounted for 100% of larvae at V3–V8, VT, and R2 in JC-2019 (Fig. 5A), V5–V10 in JC-2020 (Fig. 5C), and V3–V5 and R3–R6 in JC-2021(Fig. 5E). On DBN3601T corn, fourth-instar larvae accounted 11.95% ± 6.47% at V8, 6.86% ± 3.43% at V9, 13.69% ± 8.268% at V11, and 2.56% ± 2.56% at R1 in JC-2019 (Fig. 5A), and also 11.43% ± 5.95% at V12, 14.81% ± 9.80% at VT, 10.33% ± 2.27% at R1, and 14.36 ± 5.01% at R2 in JC-2020 (Fig. 5C). In JC-2021, fourth-instar larvae were only screened at R3 (3.03% ± 3.03%) (Fig. 5E) on DBN3601T corn. There was only one fifth-instar larva screened by field survey, which accounted for 0.55% ± 0.55% at R2 stage on DBN3601T corn in JC-2020 (Fig. 5D), and no sixth-instar larvae was screened on DBN3601T corn in all field trials. However, the proportion of older larvae (fourth to sixth instar) on non-Bt corn fluctuated with the growth of corn in each field trial at the JC station (maximum proportion, 86.32% at V14 in JC-2019, 78.99% at R1 in JC-2020, 86.88% at R1 in JC-2021) (Fig. 5B,D,F).

3.3 Differences in damage on Bt- and non-Bt corn plants

Generally, leaf damage scores for non-Bt corn at various stages differed significantly in each of the field trials: JC-2019 ($F_{5,\ 17} = 134.43$, $p < 0.0001$), JC-2020 ($F_{4,\ 14} = 108.03$, $p < 0.0001$), and JC-2021 ($F_{6,\ 20} = 19.42$, $p < 0.0001$). During the initial period of fall armyworm infestation, leaf damage scores on non-Bt corn in three field trials at the JC station were below level 2 (JC-2019, 1.26 ± 0.03; JC-20201.11 ± 0.01; JC-2021, 1.56 ± 0.08) (Fig. 6A–C). The highest leaf damage score of non-Bt corn reached 8.82 ± 0.4 in JC-2019 (V14), 5.44 ± 0.33 in JC-2020 (V12), and 5.27 ± 0.58 in JC-2021 (V14) (Fig. 6A–C). ANOVA indicated that the leaf damage score of DBN3601T corn in JC-2019 and JC-2020 also differed significantly among different growth stages (2019, $F_{5,\ 17} = 108.03$, $p = 0.0004$; 2020, $F_{4,\ 14} = 6.62$, $p = 0.0072$), but not in JC-2021 ($F_{6,\ 20} = 11.18$, $p = 0.3726$). All leaf damage scores were extremely low (no more than level 2) (Fig. 6A–C). The two-sample *t*-test showed that the leaf damage score for DBN3601T corn was significantly lower than for non-Bt corn at the same growth stage (Fig. 6A–C).

Plant damage incidence for non-Bt corn differed significantly among the different stages in each field trial at JC station (JC-2019, $H = 21.00$, $df = 8$, $p = 0.0071$; JC-2020, $F_{7,\ 20} = 47.82$, $p = 0.0071$; JC-2021, $F_{11,\ 35} = 3.58$, $p = 0.0044$) (Fig. 7A,C,D), but not at LC station in 2019 ($H = 14.43$, $df = 12$, $p = 0.2189$) (Fig. 7B). At JC station, the highest plant damage incidence was 100% (V5-R2) in JC-2019, 99.00% (R2) in JC-2020 and 82.33% (V9) in JC-2021 (Fig. 7A,C,D). At LC station, the highest plant damage incidence was only 17.33% at VT (Fig. 7B). On DBN3601T corn, plant damaged incidence differed significantly among the various stages in all field trials (JC-2019, $F_{8,26} = 9.43$, $p < 0.0001$; JC-2020,

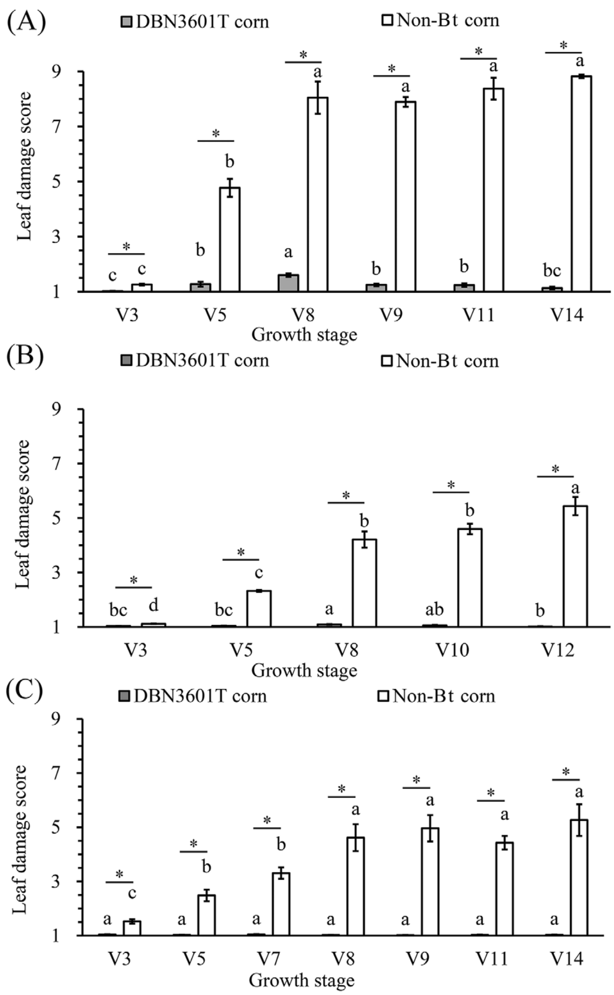

Figure 6. Leaf damage score caused by *Spodoptera frugiperda* larvae at different growth stages of DBN3601T corn and non-Bt corn in field trials at JC station in different years: (A) 2019, (B) 2020, (C) 2021. Error bars represent SE. The asterisk represents a significant difference in damage between DBN3601T corn and non-Bt corn at that growth stage (two-sample *t*-test or Mann–Whitney *U*-test at the 0.05 level). Different lowercase letters above the error bars indicate a significant difference in damage among different stages on DBN3601T corn and non-Bt corn severally in a one-way analysis of variance with Duncan's multiple range test at the 0.05 level.

$F_{7,\ 20} = 24.81$, $p < 0.0001$; JC-2021, $F_{11,\ 35} = 17.94$, $p < 0.0001$; LC-2019, $H = 23.08$, $df = 11$, P = 0.0025). The highest plant damage incidence in each field trial was 65.33% at R2 (JC-2019), 55.67% at R2 (JC-2020), 41.53% at R6 (JC-2021), and 7.33% at VT (LC-2019) (Fig. 7A–D). The two-sample *t*-test and Mann–Whitney *U*-test indicated that the plant damage incidence for non-Bt corn was significantly higher than for DBN3601T corn at the same growth stage, except for stages R6 (JC-2021), V8 (LC-2019), and V12 (LC-2019).

A one-way ANOVA of the damage incidence on the three ear tissues in JC-2021 at stage R4 indicated that the damage incidence differed significantly among silk, husk, and kernel for DBN3601T corn ($F_{2,\ 44} = 115.15$, $p < 0.0001$) and for non-Bt corn ($F_{2,\ 44} = 116.67$, $p < 0.0001$) (Fig. 8). Among the non-Bt ear tissues, silk had the highest incidence of damage (63.11% ± 6.54%), followed by kernel (48.89% ± 1.60%) and husk (7.56% ± 2.47%). The susceptibility of the different ear tissues of DBN3601T was similar (silk, 39.00% ± 3.72%; kernel, 17.78% ± 3.79%; husk, 1.78% ± 0.44%), but the damage incidence on each tissue of DBN3601T corn was significantly lower than those of non-Bt corn (Fig. 8).

3.4 Efficacy of DBN3601T event corn against fall armyworm during 2019–2021

Overall the weighted efficacy of DBN3601T event corn against fall armyworm was 95.24% ± 0.37% in JC-2019, 97.11% ± 0.83% in JC-2020, 97.71% ± 1.69% in JC-2021 and 98.16% ± 1.17% in LC-2019 (Fig. 9E). Results of the Kruskal–Wallis test showed that the weighted efficacy for each growth stage differed significantly in each field trial (JC-2019, $H = 21.08$, $df = 8$, $p = 0.0069$; JC-2020, $H = 18.77$, $df = 7$, $p = 0.0009$; JC-2021, $H = 25.05$, $df = 11$, $p = 0.0009$; LC-2019, $H = 26.23$, $df = 12$, $p = 0.0099$). The weighted efficacy of each stage from V3 to VT was above 96% and was significantly higher than that of R1 and R2 in JC-2019 and JC-2020 (Fig. 9A,C), but not in JC-2021 (Fig. 9D). The weighted efficacy of most growth stages in LC-2019 and JC-2021 reached 100%, except for V12, V15, and VT in LC-2019, and V3, V5, R3, and R6 in JC-2021 (Fig. 9B,D). In JC-2021, the weighted efficacy values at stages R3 and R6 were significantly lower than at other stages.

4 DISCUSSION

The bioassay results indicated that DBN3601T corn leaves at different growth stages achieved 100% corrected mortality against fall armyworm neonates by day 3, were significantly more lethal than the husk, tassel, silk, and kernel, which is consistent with a previous study,[44] and were higher than DBN9936 corn.[45] Significant differences in corrected mortality among different tissues of DBN3601T corn in this study showed that the concentration of Bt toxins might differ among tissues and growth stages, as found in DBN9936 (Cry1Ab),[45] MON810 (Cry1Ab),[46] MON88017 (Cry3Bb1),[47] and MIR162 (Vip3Aa20).[48] In our study, we took into account only larvae that were dead within 5 days without testing the weight of surviving larvae, which is considered part of scoring mortality in Bt toxins.[49] Overall, the lethality against fall armyworm neonates was ranked as follows: leaf > husk > tassel and kernel > silk.

DBN3601T corn effectively suppressed the occurrence of fall armyworm in our field trials from 2019 to 2021 in Yunnan. EMD in the three JC field trials indicated that fall armyworm had an obvious oviposition bias for DBN3601T corn, but this was not seen not in LC-2019 where the fall armyworm infestation was sparse, which is consistent with previous findings.[50–52] Oviposition bias for Bt corn can be used as a death trap to protect other crops,[52] but the negative impact on insect resistance management (IRM) requires more attention, especially if facing a heavy infestation.[50,53] LD was significantly lower on DBN3601T corn than on non-Bt corn and most of the surviving larvae on DBN3601T corn were young instars (first to third). No survival of fifth- or sixth-instar larvae was screened on DBN3601T corn, indicating extremely low survivability to complete a full generation for fall armyworm. Both leaf damage score and damage incidence for DBN3601T corn remained lower than for non-Bt corn, and the leaf damage score for DBN3601T corn was always below level 2 in all field trials, which is similar to pyramided event Bt11 × MIR162 × GA21 (Cry1Ab and Vip3Aa20) in Brazil.[48] DBN3601T corn could be assessed as a highly resistant germplasm against fall armyworm.[4] At the R stage, high damage incidence was shown by checking the silk and kernels on the ear

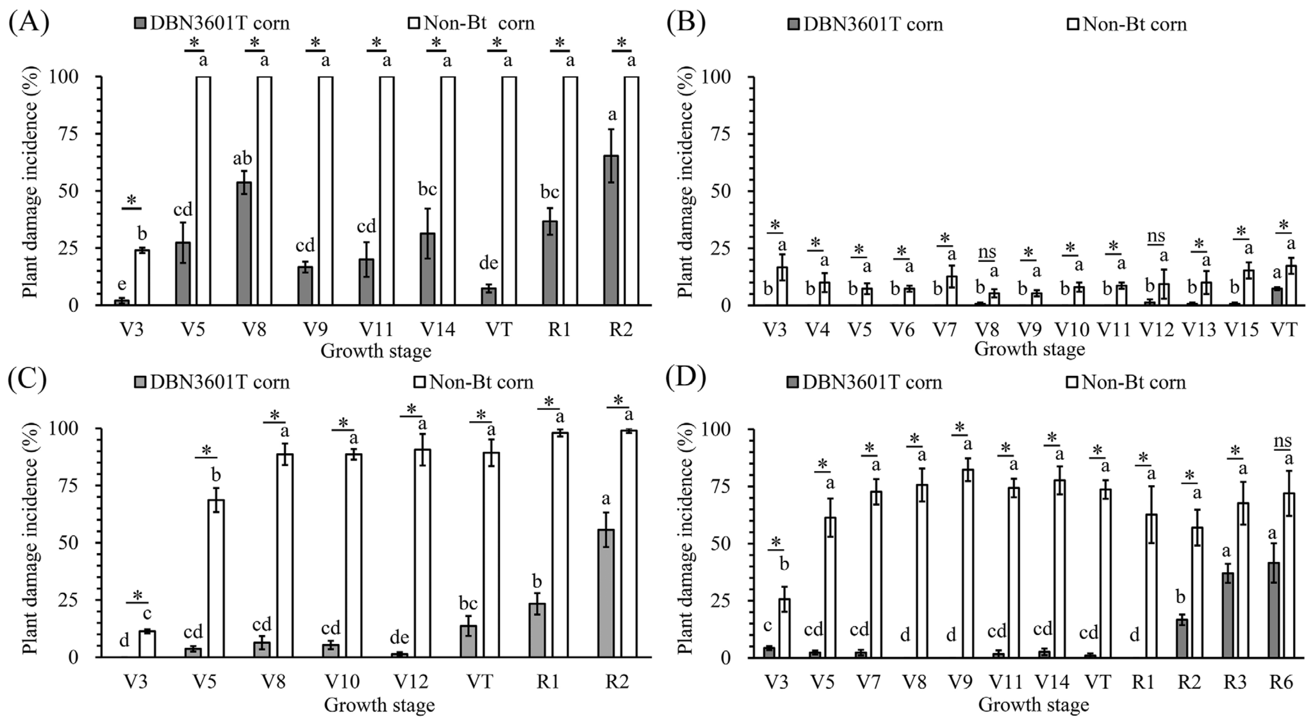

Figure 7. Plant damage incidence of DBN3601T corn (expressing Cry1Ab and Vip3Aa19) and non-Bt corn damaged by *Spodoptera frugiperda* at different stages in field trials at two locations and different years: (A) 2019 at JC station; (B) 2019 at LC station; (C) 2020 at JC station; (D) 2021 at JC station. Error bars represent SE. The asterisk represents a significant difference in damage between DBN3601T corn and non-Bt corn at that growth stage (two-sample *t*-test or Mann–Whitney *U*-test at the 0.05 level). Different lowercase letters above the error bars indicate a significant difference in damage among different stages between DBN3601T corn and non-Bt corn (one-way analysis of variance with Duncan's multiple range test at the 0.05 level).

tip of DBN3601T corn in three field trials at JC station, which is the field performance of the bioassay results.

Our previous study showed that the concentration of Cry1Ab and Vip3A expressed in DBN3601T (DBN9936 × DBN9501 event) corn leaves at stage V4 was 74.51 and 6.78 μg g^{-1} respectively, and was significantly lower than that in Bt11 × MIR162 corn (86.64 μg g^{-1} for Cry1Ab, 24.83 μg g^{-1} for Vip3Aa20),[54] which may be the key factor in the high survival LD and high plant damage incidence in our field trials. Different expression of Bt toxins in

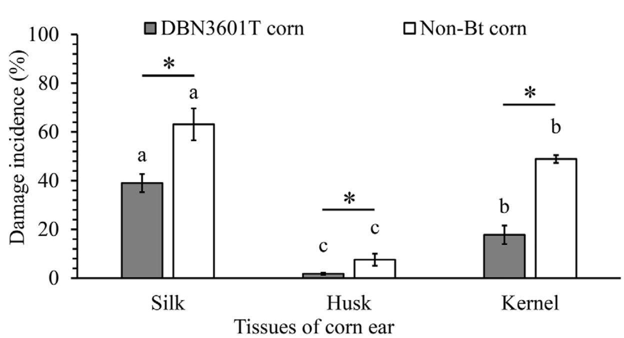

Figure 8. Damage incidence of silk, husk, and kernel caused by *Spodoptera frugiperda* at stage R4 of DBN3601T corn and non-Bt corn in the field trial of JC-2021. Error bars represent SE. The asterisk represents a significant difference in damage of a tissue type between DBN3601T corn and non-Bt corn (two-sample *t*-test at the 0.05 level). Different lowercase letters above the error bars indicate a significant difference among the ear tissue types for DBN3601T corn and non-Bt corn (one-way analysis of variance with Duncan's multiple range test at the 0.05 level).

different corn ear tissues may be the reason for the high damage incidence at the R stages like DBN9936,[45] and is a serious risk for IRM of fall armyworm. With multiple genes pyramided, DBN3601T corn expressing Cry1Ab and Vip3Aa has two insecticidal modes of action.[55–57] DBN3601T corn showed high resistance against fall armyworm at vegetative growth stages in three field trials (LC-2019, JC-2020, and JC-2021), but seemingly provided inadequate control when faced with heavy infestation in JC-2019, especially at the R stages. Rapid invasion and colonization by fall armyworm, and inadequate prevention and control by smallholders led to high-pressure fall armyworm infestation in 2019. Rare drought in spring and autumn occurred in Jiangcheng County in 2019 (Fig. 1), which aggravated the occurrence of fall armyworm and led to heavy infestation. In addition, drought stress resulted in a decrease in total water-soluble protein, protease, and peptidase activities in transgenic crops (rice, cotton, and so on), which led to a decrease in Bt protein expression.[58,59] This is probably one of the main reasons for the inadequate efficacy against fall armyworm in JC-2019, and needs further assessment. Considering the whole growth stage of corn in all field trials, the weighted efficacy of DBN3601T corn against fall armyworm larvae was above 95% and is the most direct performance of its resistance to the pest.

Commercial cultivation of Bt corn has been adopted as a pest management strategy for decades globally, not only reducing economic losses and pesticide usage, but also promoting biocontrol services and improving environmental benefits.[26,60–64] The long-term commercial cultivation of Bt corn has led to the evolution of Bt resistance in fall armyworm to Cry1F and Cry1Ab in the Americas, and even Vip3A in Brazil.[18,25,65–70] High pest pressure, long-distance immigration, cross-resistance among Bt toxins,

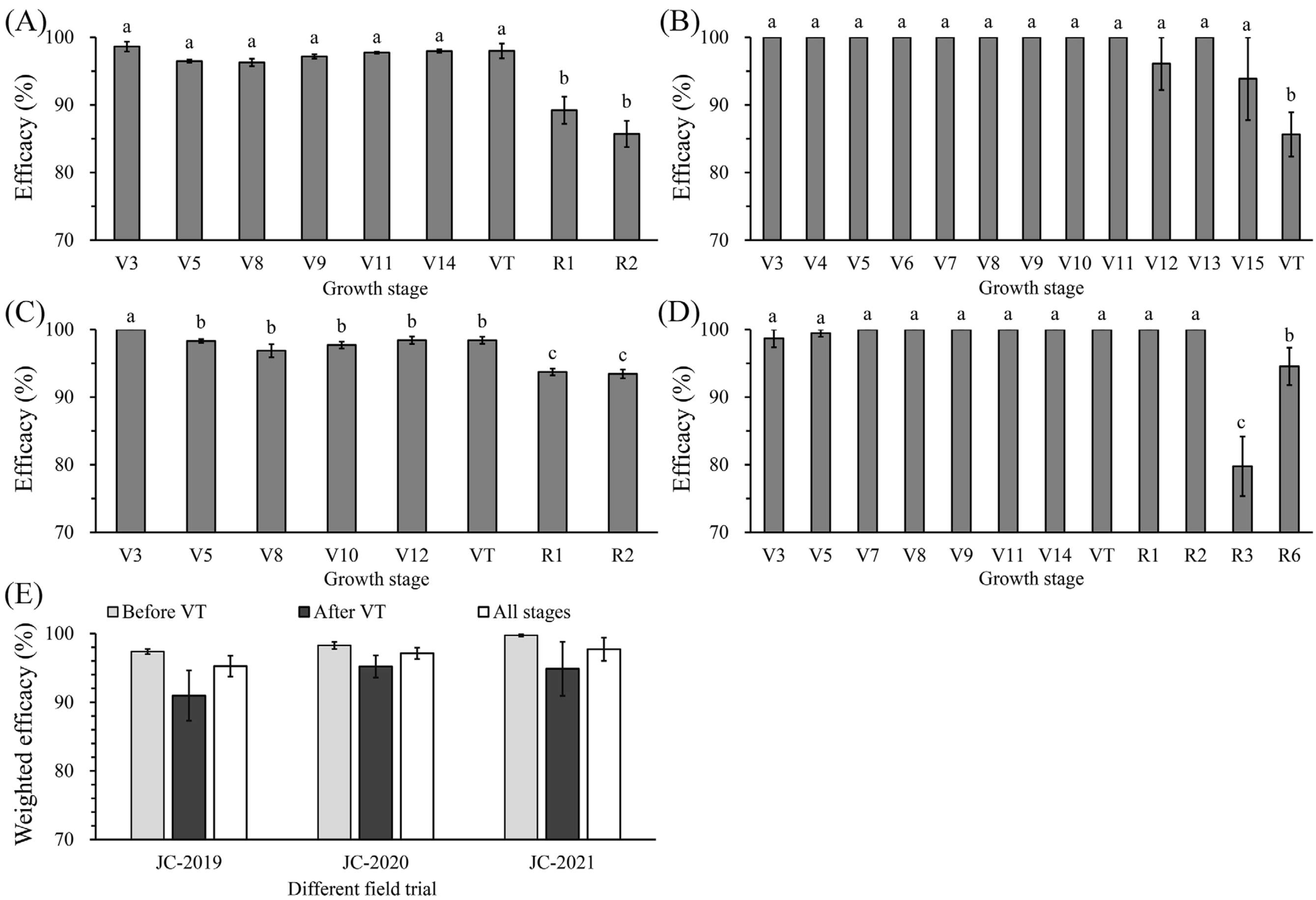

Figure 9. Weighted efficacies of DBN3601T corn against *Spodoptera frugiperda* larvae in field trials at two locations during 2019–2021: (A) 2019 at JC station; (B) 2019 at LC station; (C) 2020 at JC station; (D) 2020–2021 at JC station. (E) Weighted efficacy of DBN3601T corn against *S. frugiperda* at V3-VT, VT-R or all stages. Error bars represent SE.

multiple initial resistance alleles, usage of nonhigh-dose products, and lack of/recessive fitness costs are the main reasons for Bt resistance in fall armyworm[40,71] More than 20 years' experience of planting Bt corn shows that establishing and implementing a sound pest resistance management plan is indispensable to delay the escalation of Bt resistance.[71] A high-dose/refuge strategy has been shown to be effective in mitigating the evolution of resistance in fall armyworm in the USA.[25,39,40] Our previous study showed that the relative susceptibility indexes of fall armyworm to Cry1Ab, Cry1Ac, Cry1F, Cry2Ab, and Vip3A proteins ranged from 0.28 to 3.76 compared with a susceptible population in the USA,[42,72] and genetic characterization analysis also found no reported Bt resistance mutation loci for the fall armyworm population in China,[73] which is favorable for fall armyworm management using Bt corn. Given the performance of DBN3601T corn against fall armyworm in field trials, it seems likely that DBN3601T event corn provides inadequate control to meet the criteria of high-dose (concentration causing mortality at least at the 25× lethal concentration of 99% (LC99) level[74]). As a pyramided event, DBN3601T corn did not kill all fall armyworm larvae on all tissues, which will lead to a certain number of Cry1Ab- or Vip3A-resistant survivals in the Bt area, especially at the R stage; the risk of Bt resistance also increases. The function of refuge is to dilute the frequency of resistance genes in natural populations based on random mating between resistant and non-resistant individuals.[75,76] In practice, inadequate refuge is the main reason for fall armyworm Bt resistance in Brazil.[40,71] Although the pyramid strategy significantly reduces refuge size,[76] a large refuge for DBN3601T corn should be considered based on an accurate concentration and resistance gene frequency of Cry1Ab and Vip3A. We believe that trapping resistant individual adults in the Bt area using sex hormone/food attractants/light will be a key approach in Bt resistance management of fall armyworm, and the sterile insect technique, which also provides a new avenue for target pest resistance management, has been applied successfully in the USA.[77]

Studies have shown that the biological characteristics of the target pest, such as generation occurrence and migration habits, exacerbate the diffusion and increase the frequency of Bt resistance alleles.[71,78–80] Fall armyworm has exhibited a north–south migration in latitude[8,16,37] and annual breeding with more than seven theoretical generations at low latitude (subtropical–tropical regions), such as Yunnan, Guangdong, Guangxi, and Hainan in China,[81,82] which poses a challenge to Bt resistance management of fall armyworm in the future. Suggestions based on the performance of DBN3601T corn and the actual occurrence of fall armyworm are as follows. First, the susceptive baseline and corresponding Bt resistance gene frequency of major Bt toxins, such as Cry1Ab, Cry2Ab, Cry1Ac, and Vip3A, in China as a benchmark for Bt resistance monitoring should be established as soon as possible. Second, a comprehensive and diverse IRM program based on a high-dose/refuge and pyramid strategy is required, supplemented by migratory population monitoring and intercepting, sex hormone/food attractants and a light trapping strategy, biological control techniques, agricultural control, and other integrated pest management techniques in accordance with the reality of conditions in China. At the same time, the scientific popularization and implementation of IRM by growers, especially smallholders, should be strengthened, together with increasing the supervision of seed quality (for example, purity) of seed production enterprises. Third, there should be an increase in research on the evolutionary mechanism of fall armyworm Bt resistance and refuge design for major Bt corn events according to the planting patterns and cropland ecosystem in China, especially Vip3A toxin-relevant events, and rapid accurate molecular detection technology for the resistance gene in fall armyworm. Fourth, there is a need for increased research and the development of a subsequent new generation of Bt maize products with novel toxin target sites, insecticidal mode of action, such as a pyramid Bt event with no cross-resistance toxins, application of RNA interference and gene editing in Bt corn. Last but not least, there should be increased cooperation with border countries (Laos, Myanmar, Vietnam, Thailand, and so on) in fall armyworm population dynamics and Bt resistance monitoring and also with the Americas (the USA, Brazil, Mexico, and so on) in research into Bt resistance management.

5 CONCLUSION

This evaluation of the efficacy of DBN3601T corn against fall armyworm using a laboratory bioassay and assessments of EMD and LD, infestation levels and damage in the field confirmed that DBN3601T corn could play an important role in fall armyworm management in China based on comprehensive and effective IRM.

AUTHOR CONTRIBUTIONS

Kongming Wu designed the experiments. Shengyan Zhao, Xianming Yang, Dazhong Liu, and Xiaoxu Sun did the experiments. Shengyan Zhao, Xianming Yang, and Dazhong Liu analyzed the data. Shengyan Zhao, Kongming Wu, Xianming Yang, and Guoping Li wrote and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

ACKNOWLEDGMENTS

This study was funded by Lingnan Modern Agriculture Project (NT2021003) and National Key R&D Program of China (2021YFD1400702) and National Modern Agricultural Industry Technology System Construction Fund of China (CARS-02). We thank Beijing DaBeiNong Biotechnology Co., Ltd. for providing materials for Bt corn and advice on field planting.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

REFERENCES

1 Luginbill P, The fallarmy worm. *USDA Tech Bull* **34**:91 (1928).
2 Overton K, Maino JL, Day R, Umina PA, Bett B, Carnovale D *et al.*, Global crop impacts, yield losses and action thresholds for fall armyworm (*Spodoptera frugiperda*): a review. *Crop Prot* **145**:15 (2021). https://doi.org/10.1016/j.cropro.2021.105641.
3 Montezano DG, Specht A, Sosa-Gomez DR, Roque-Specht VF, Sousa-Silva JC, Paula-Moraes SV *et al.*, Host plants of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in the Americas. *Afr Entomol* **26**:286–300 (2018). https://doi.org/10.4001/003.026.0286.
4 Prasanna BM, Huesing JE, Eddy R and Peschke VM eds, *Fall Armyworm in Africa: A Guide for Integrated Pest Management*, 1st edn. CIMMYT, Mexico (2018).
5 Johnson S, Migration and the life history strategy of the fall armyworm, *Spodoptera frugiperda* in the western hemisphere. *Int J Trop Insect Sci* **8**:543–549 (1987). https://doi.org/10.1017/S1742758400022591.
6 Ge S, He L, He W, Yan R, Wyckhuys KAG and Wu K, Laboratory-based flight performance of the fall armyworm, Spodoptera frugiperda. *J Integr Agric* **20**:707–714 (2021). https://doi.org/10.1016/S2095-3119(20)63166-5.
7 Day R, Abrahams P, Bateman M, Beale T, Clottey V, Cock M *et al.*, Fall armyworm: impacts and implications for Africa. *Outlooks Pest Manage* **28**:196–201 (2017). https://doi.org/10.1564/v28_oct_02.
8 Wu K, Management strategies of fall amryworm (*Spodoptera frugiperda*) in China. *Plant Prot* **46**:1–5 (2020) (in Chinese).
9 Goergen G, Kumar PL, Sankung SB, Togola A and Tamo M, First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (J E Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in West and Central Africa. *PLoS One* **11**:e0165632 (2016). https://doi.org/10.1371/journal.pone.0165632.
10 Cock MJW, Beseh PK, Buddie AG, Cafá G and Crozier J, Molecular methods to detect *Spodoptera frugiperda* in Ghana, and implications for monitoring the spread of invasive species in developing countries. *Sci Rep* **7**:4103 (2017). https://doi.org/10.1038/s41598-017-04238-y.
11 Rwomushana I, Bateman M, Beale T, Beseh P, Cameron K, Chiluba M *et al.*, *Fall Armyworm: Impacts and Implications for Africa Evidence Note Update, October 2018*. CABI, Wallignford, UK, pp. 1–53 (2018) https://www.invasive-species.org/wp-content/uploads/sites/2/2019/02/FAW-Evidence-Note-October-2018.pdf.
12 Gwan-Seok L, Bo Yoon S, Jongho L, Hyunju K, Jeong Heub S and Wonhoon L, First report of the fall armyworm, *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera, Noctuidae), a new migratory pest in Korea. *Korean J Appl Entomol* **59**:73–78 (2020). https://doi.org/10.5656/KSAE.2020.02.0.006.
13 Prasanna B, Huesing JE, Peschke VM, Nagoshi RN, Jia X, Wu K *et al.*, *Fall armyworm in Asia: invasion, impacts, and strategies for sustainable management, in Fall armyworm in Asia: A Guide for Integrated Pest Management*. CIMMYT, Mexcio (2021). https://www.cgiar.org/research/publication/fall-armyworm-in-asia-a-guide-for-integrated-pest-management/.
14 Sun X, Hu C, Jia H, Wu Q, Shen X, Zhao S *et al.*, Case study on the first immigration of fall armyworm, *Spodoptera frugiperda* invading into China. *J Integr Agric* **20**:664–672 (2021). https://doi.org/10.1016/S2095-3119(19)62839-X.
15 Secretariat IPPC, *Prevention, Preparedness and Response Guidelines for Spodoptera frugiperda*. FAO on behalf of the Secretariat of the International Plant Protection Convention, Rome (2021). https://doi.org/10.4060/cb5880en.
16 Wu Q, He L, Shen X, Jiang Y, Liu J, Hu G *et al.*, Estimation of the potential infestation area of newly-invaded fall armyworm *Spodoptera frugiperda* in the Yangtze River valley of China. *Insects* **10**:298 (2019). https://doi.org/10.3390/insects10090298.
17 Ni X, Wilson JP, Buntin GD, Guo B, Krakowsky MD, Lee RD *et al.*, Spatial patterns of aflatoxin levels in relation to ear-feeding insect damage in pre-harvest corn. *Toxins* **3**:920–931 (2011). https://doi.org/10.3390/toxins3070920.
18 Farias CA, Brewer MJ, Anderson DJ, Odvody GN, Xu WW and Setamou M, Native maize resistance to corn earworm, *Helicoverpa zea*, and fall armyworm, *Spodoptera frugiperda*, with notes on aflatoxin content. *Southwest Entomol* **39**:411–425 (2014). https://doi.org/10.3958/059.039.0303.
19 Lima MS, Silva PSL, Oliveira OF, Silva KMB and Freitas FCL, Corn yield response to weed and fall armyworm controls. *Planta Daninha* **28**:103–111 (2010). https://doi.org/10.1590/S0100-83582010000100013.

20 Wan M, Tai H, Gu R, Wang G, Liu Z, Mi Q *et al*., Econmic loss assessment of maize production caused by the fall armyworm *Spodoptera frugiperda* and investigation of control strategies in dehong prefecture of Yunnan province. *Plant Prot* **48**:220–226 (2020) (in Chinese).
21 Qin Y, Yang D, Kang D, Zhao Z, Zhao Z, Yang P *et al*., Potential economic loss assessment of maize industry caused by fall armyworm (*Spodoptera frugiperda*) in China. *Plant Prot* **46**:69–73 (2020) (in Chinese).
22 Mota-Sanchez D and Wise J, *Arthropod Pesticide Resistance Database*. Michigan State University, Michigan (2017). https://www.pesticideresistance.org/ [accessed 20 October 2022].
23 Gutierrez-Moreno RA, *Susceptibility of the Fall Armyworm, Spodoptera frugiperda (JE Smith) (Lepidoptera: Noctuidae) to Bacillus thuringiensis (Bt) Proteins and Synthetic Insecticides from Different Corn Production Systems in Mexico and Puerto Rico*. Michigan State University, Michigan (2017).
24 Yang X, Wyckhuys KAG, Jia X, Nie F and Wu K, Fall armyworm invasion heightens pesticide expenditure among Chinese smallholder farmers. *J Environ Manage* **282**:111949 (2021). https://doi.org/10.1016/j.jenvman.2021.111949.
25 Huang FN, Andow DA and Buschman LL, Success of the high-dose/refuge resistance management strategy after 15 years of Bt crop use in North America. *Entomol Exp Appl* **140**:1–16 (2011). https://doi.org/10.1111/j.1570-7458.2011.01138.x.
26 ISAAA. Global status of commercialized biotech/GM crops in 2019: biotech crops drive socio-economic development and sustainable environment in the new frontier. ISAAA Brief No.55 (2019).
27 Castro BA, Leonard BR and Riley TJ, Management of feeding damage and survival of southwestern corn borer and sugarcane borer (Lepidoptera: Crambidae) with *Bacillus thuringiensis* transgenic field corn. *J Econ Entomol* **97**:2106–2116 (2004). https://doi.org/10.1093/jee/97.6.2106.
28 Graeber JV, Nafziger ED and Mies DW, Evaluation of transgenic, Bt-containing corn hybrids. *J Prod Agric* **12**:659–663 (1999). https://doi.org/10.2134/jpa1999.0659.
29 Siegfried BD and Hellmich RL, Understanding successful resistance management: the European corn borer and Bt corn in the United States. *GM Crops Food* **3**:184–193 (2012). https://doi.org/10.4161/gmcr.20715.
30 Lauer J and Wedberg J, Grain yield of initial Bt corn hybrid introductions to farmers in the northern corn belt. *J Prod Agric* **12**:373–376 (1999). https://doi.org/10.2134/jpa1999.0373.
31 Buntin GD, Corn expressing Cry1Ab endotoxin for management of fall armyworm and corn earworm (Lepidoptera: Noctuidae) in silage production. *J Entomol Sci* **45**:283–295 (2010). https://doi.org/10.18474/0749-8004-45.3.283.
32 Buntin GD, Lee RD, Wilson DM and McPherson RM, Evaluation of yieldgard transgenic resistance for control of fall armyworm and corn earworm (Lepidoptera: Noctuidae) on corn. *Fla Entomol* **84**:37–42 (2001). https://doi.org/10.2307/3496660.
33 Siebert MW, Babock J, Nolting S, Santos A, Adamczyk J Jr, Neese P *et al*., Efficacy of Cry1F insecticidal protein in maize and cotton for control of fall armyworm (Lepidoptera: Noctuidae). *Fla Entomol* **91**:555–565 (2008). https://doi.org/10.1653/0015-4040-91.4.555.
34 Burkness EC, Dively G, Patton T, Morey AC and Hutchison WD, Novel Vip3A *Bacillus thuringiensis* (Bt) maize approaches high-dose efficacy against *Helicoverpa zea* (Lepidoptera: Noctuidae) under field conditions: implications for resistance management. *GM crops* **1**:337–343 (2010). https://doi.org/10.4161/gmcr.1.5.14765.
35 Gampala SS, Fast BJ, Richey KA, Gao ZF, Hill R, Wulfkuhle B *et al*., Single-event transgene product levels predict levels in genetically modified breeding stacks. *J Agric Food Chem* **65**:7885–7892 (2017). https://doi.org/10.1021/acs.jafc.7b03098.
36 Niu Y, Guo J, Head GP, Price PA and Huang F, Phenotypic performance of nine genotypes of Cry1A.105/Cry2Ab2 dual-gene resistant fall armyworm on non-Bt and MON89034 maize. *Pest Manage Sci* **75**:2124–2132 (2019). https://doi.org/10.1002/ps.5331.
37 Li Y, Wang Z and Romeis J, Managing the invasive fall armyworm through biotech crops: a Chinese perspective. *Trends Biotechnol* **39**:105–108 (2021). https://doi.org/10.1016/j.tibtech.2020.07.001.
38 Buntin GD, Corn expressing Cry1Ab or Cry1F endotoxin for fall armyworm and corn earworm (Lepidoptera: Noctuidae) management in field corn for grain production. *Fla Entomol* **91**:523–530 (2008). https://doi.org/10.1653/0015-4040-91.4.523.
39 Huang F, Resistance management for Bt maize and above-ground Lepidopteran targets in the USA: from single gene to pyramided traits, in *Bt Resistance: Characterization and Strategies for GM Crops Producing Bacillus thuringiensis toxins*, Vol. **4**. CABI Biotechnol **Ser**, Wallingford, UK, pp. 173–185 (2015). https://doi.org/10.1079/9781780644370.0173.
40 Huang F, Resistance of the fall armyworm, *Spodoptera frugiperda*, to transgenic *Bacillus thuringiensis* Cry1F corn in the Americas: lessons and implications for Bt corn IRM in China. *Insect Sci* **28**:574–589 (2021). https://doi.org/10.1111/1744-7917.12826.
41 Botha AS, Erasmus A, du Plessis H and Van den Berg J, Efficacy of Bt maize for control of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in South Africa. *J Econ Entomol* **112**:1260–1266 (2019). https://doi.org/10.1093/jee/toz048.
42 Li G and Wu K, Commercial strategy of transgenic insect-resistant maize in China. *J Plant Prot* **49**:17–32 (2022) (in Chinese).
43 Abendroth LJ, Elmore RW, Boyer MJ and Marlay SK, *Corn Growth and Development*. Iowa State University Extension, Ames, IA (2011).
44 Zhang D and Wu K, The bioassay of Chinese domestic Bt-Cry1Ab and Bt-(Cry1Ab+Vip3Aa) maize against the fall armyworm, Spodoptera frugiperda. *Plant Prot* **45**:54–60 (2019) (in Chinese).
45 Liang J, Zhang D, Li D, Zhao S, Wang C, Xiao Y *et al*., Expression profiles of Cry1Ab protein and its insecticidal efficacy against the invasive fall armyworm for Chinese domestic GM maize DBN9936. *J Integr Agric* **20**:792–803 (2021). https://doi.org/10.1016/S2095-3119(20)63475-X.
46 Székács A, Lauber É, Juracsek J and Darvas B, Cry1Ab toxin production of MON810 transgenic maize. *Environ Toxicol Chem* **29**:182–190 (2010). https://doi.org/10.1002/etc.5.
47 Nguyen HT and Jehle JA, Expression of Cry3Bb1 in transgenic corn MON88017. *J Agric Food Chem* **57**:9990–9996 (2009). https://doi.org/10.1021/jf901115m.
48 Eghrari K, Oliveira SC, Nascimento AM, Queiroz B, Fatoretto J, De Souza BHS *et al*., The implications of homozygous Vip3Aa20- and Cry1Ab-maize on *Spodoptera frugiperda* control. *J Pest Sci* **95**:115–127 (2022). https://doi.org/10.1007/s10340-021-01362-7.
49 Gutierrez-Moreno R, Mota-Sanchez D, Blanco CA, Chandrasena D, Difonzo C, Conner J *et al*., Susceptibility of fall armyworms (*Spodoptera frugiperda* J.E.) from Mexico and Puerto Rico to Bt proteins. *Insects* **11**:831 (2020). https://doi.org/10.3390/insects11120831.
50 Tellez-Rodriguez P, Raymond B, Moran-Bertot I, Rodriguez-Cabrera L, Wright DJ, Borroto CG *et al*., Strong oviposition preference for Bt over non-Bt in *Spodoptera frugiperda* and its implications for the evolution of resistance. *BMC Biol* **12**:48 (2014). https://doi.org/10.1186/1741-7007-12-48.
51 Ingber DA, McDonald JH, Mason CE and Flexner L, Oviposition preferences, Bt susceptibilities, and tissue feeding of fall armyworm (Lepidoptera: Noctuidae) host strains. *Pest Manage Sci* **77**:4091–4099 (2021). https://doi.org/10.1002/ps.6434.
52 He L, Zhao S, Gao X and Wu K, Ovipositional responses of *Spodoptera frugiperda* on host plants provide a basis for using Bt-transgenic maize as trap crop in China. *J Integr Agric* **20**:804–814 (2021). https://doi.org/10.1016/S2095-3119(20)63334-2.
53 Goncalves J, Rodrigues JVC, Santos-Amaya OF, Paula-Moraes SV and Pereira EJG, The oviposition behavior of fall armyworm moths is unlikely to compromise the refuge strategy in genetically modified Bt crops. *J Pest Sci* **93**:965–977 (2020). https://doi.org/10.1007/s10340-020-01219-5.
54 Wang W, Zhang D, Zhao S and Wu K, Susceptibilities of the invasive fall armyworm (*Spodoptera frugiperda*) to the insecticidal proteins of Bt maize in China. *Toxins* **14**:507 (2022). https://doi.org/10.3390/toxins14080507.
55 Kurtz RW, A review of Vip3A mode of action and effects on Bt cry protein-resistant colonies of Lepidopteran larvae. *Southwest Entomol* **35**:391–394 (2010). https://doi.org/10.3958/059.035.0321.
56 Lee MK, Walters FS, Hart H, Palekar N and Chen JS, The mode of action of the *Bacillus thuringiensis* vegetative insecticidal protein Vip3A differs from that of Cry1Ab delta-endotoxin. *Appl Environ Microbiol* **69**:4648–4657 (2003). https://doi.org/10.1128/aem.69.8.4648-4657.2003.
57 Estruch JJ, Warren GW, Mullins MA, Nye GJ, Craig JA and Koziel MG, Vip3A, a novel *Bacillus thuringiensis* vegetative insecticidal protein with a wide spectrum of activities against lepidopteran insects. *Proc Natl Acad Sci U S A* **93**:5389–5394 (1996). https://doi.org/10.1073/pnas.93.11.5389.
58 Trtikova M, Wikmark OG, Zemp N, Widmer A and Hilbeck A, Transgene expression and Bt protein content in transgenic Bt maize (MON810)

under optimal and stressful environmental conditions. *PLoS One* **10**: e0123011 (2015). https://doi.org/10.1371/journal.pone.0123011.
59 Girón-Calva PS, Twyman RM, Albajes R, Gatehouse AMR and Christou P, The impact of environmental stress on Bt crop performance. *Trends Plant Sci* **25**:264–278 (2020). https://doi.org/10.1016/j.tplants.2019.12.019.
60 Dively GP, Venugopal PD, Bean D, Whalen J, Holmstrom K, Kuhar TP *et al.*, Regional pest suppression associated with widespread Bt maize adoption benefits vegetable growers. *Proc Natl Acad Sci U S A* **115**:3320–3325 (2018). https://doi.org/10.1073/pnas.1720692115.
61 Perry ED, Ciliberto F, Hennessy DA and Moschini G, Genetically engineered crops and pesticide use in U.S. corn and soybeans. *Sci Adv* **2**:e1600850 (2016). https://doi.org/10.1126/sciadv.1600850.
62 Romeis J, Meissle M and Bigler F, Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. *Nat Biotechnol* **24**:63–71 (2006). https://doi.org/10.1038/nbt1180.
63 Xie W, Ali T, Cui Q and Huang J, Economic impacts of commercializing insect-resistant GM maize in China. *China Agri Econ Rev* **9**:340–354 (2017). https://doi.org/10.1108/CAER-06-2017-0126.
64 Uslu T, Advantages, risks and legal perspectives of GMOs in 2020 s. *Plant Biotechnol Rep* **15**:741–751 (2021).
65 Storer NP, Kubiszak ME, King JE, Thompson GD and Santos AC, Status of resistance to Bt maize in *Spodoptera frugiperda*: lessons from Puerto Rico. *J Invertebr Pathol* **110**:294–300 (2012). https://doi.org/10.1016/j.jip.2012.04.007.
66 Huang F, Qureshi JA, Meagher RL Jr, Reisig DD, Head GP, Andow DA *et al.*, Cry1F resistance in fall armyworm *Spodoptera frugiperda*: single gene versus pyramided Bt maize. *PloS One* **9**:e112958 (2014). https://doi.org/10.1371/journal.pone.0112958.
67 Omoto C, Bernardi O, Salmeron E, Sorgatto RJ, Dourado PM, Crivellari A *et al.*, Field-evolved resistance to Cry1Ab maize by *Spodoptera frugiperda* in Brazil. *Pest Manage Sci* **72**:1727–1736 (2016). https://doi.org/10.1002/ps.4201.
68 Bernardi O, Bernardi D, Horikoshi RJ, Okuma DM, Miraldo LL, Fatoretto J *et al.*, Selection and characterization of resistance to the Vip3Aa20 protein from Bacillus thuringiensis in *Spodoptera frugiperda*. *Pest Manage Sci* **72**:1794–1802 (2016). https://doi.org/10.1002/ps.4223.
69 Bernardi O, Bernardi D, Amado D, Sousa RS, Fatoretto J, Medeiros FC *et al.*, Resistance risk assessment of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and *Diatraea saccharalis* (Lepidoptera: Crambidae) to Vip3Aa20 insecticidal protein expressed in corn. *J Econ Entomol* **108**:2711–2719 (2015).
70 Fernando SA, Guidolin AS, Salmeron E, Kanno RH, Padovez FEO, Fatorettob JC *et al.*, Geographical distribution of Vip3Aa20 resistance allele frequencies in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) populations in Brazil. *Pest Manage Sci* **76**:169–178 (2020). https://doi.org/10.1002/ps.5490.
71 Fatoretto JC, Michel AP, Silva Filho MC and Silva N, Adaptive potential of fall armyworm (Lepidoptera: Noctuidae) limits Bt trait durability in Brazil. *J Integr Pest Manage* **8**:1–10 (2017). https://doi.org/10.1093/jipm/pmx011.
72 Li G, Feng H, Ji T, Huang J and Tian C, What type of Bt corn is suitable for a region with diverse lepidopteran pests: a laboratory evaluation. *GM Crops Food* **12**:115–124 (2021). https://doi.org/10.1080/21645698.2020.1831728.
73 Zhang L, Liu B, Zheng W, Liu C, Zhang D, Zhao S *et al.*, Genetic structure and insecticide resistance characteristics of fall armyworm populations invading China. *Mol Ecol Resour* **20**:1682–1696 (2020). https://doi.org/10.1111/1755-0998.13219.
74 US EPA (2020) Current and Previously Registered Section 3 Plant-Incorporated Protectant (PIP) Registrations. https://www3.epa.gov/pesticides/chem_search/reg_actions/registration/decision_PC-006599_3-Apr-09.pdf
75 Roush RT, Bt-transgenic crops: just another pretty insecticide or a chance for a new start in resistance management? *Pestic Sci* **51**: 328–334 (1997).
76 Roush RT, Two-toxin strategies for management of insecticidal transgenic crops: can pyramiding succeed where pesticide mixtures have not? *Philos Trans R Soc Lond B Biol Sci* **353**:1777–1786 (1998).
77 Xiao Y and Wu K, Recent progress on the interaction between insects and *Bacillus thuringiensis* crops. *Philos Trans R Soc Lond B Biol Sci* **374**:20180316 (2019). https://doi.org/10.1098/rstb.2018.0316.
78 Tabashnik BE, Evolution of resistance to Bacillus thuringiensis. *Annu Rev Entomol* **39**:47–79 (1994).
79 Tabashnik BE, Gassmann AJ, Crowder DW and Carrière Y, Insect resistance to Bt crops: evidence versus theory. *Nat Biotechnol* **26**:99–202 (2008).
80 Tabashnik BE and Croft BA, Managing pesticide resistance in crop-arthropod complexes: interactions between biological and operational factors. *Environ Entomol* **11**:1137–1144 (1982).
81 Jiang Y, Liu J, Wu Q, Ciren Z and Zeng J, Investigation on winter breeding and overwintering areas of *Spodoptera frugiperda* in China. *Plant Prot* **47**:212–217 (2021) (in Chinese).
82 Xie D, Tang J, Zhang L, Cheng Y and Jiang X, Annual generation numbers prediction and division of fall armyworm, *Spodoptera frugiperda* in China. *Plant Prot* **47**:61–67 (2021) (in Chinese).

