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Helicoverpa zea selected on Bt corn have wing shapes better suited to long distance flight

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Evolution of resistance within insects to pest control has resulted in changes to the organism's morphotype, including changes in wing shape. Both geometric morphometric and finite element method (FEM) were used to examine wing changes in *Helicoverpa zea* sampled from 4 different Bt corn treatments in North and South Carolina, United States. The 4 treatments were pure-stand non-Bt corn (treatment 1); pure-stand Bt corn with 2 toxins (Cry1Ab and Cry1F; treatment 2); pure-stand Bt corn with 3 toxins (Cry1Ab, Cry1F, and Vip3A; treatment 3); and seed blended Bt corn with 80% containing 3 toxins (Cry1AB, Cry1F, and Vip3A) and 20% having no toxins (treatment 5). Geometric morphometric analyses revealed significant wing shape differences in both female and male moths were driven by moderately selected moths (treatments 2 and 5). Male and female moths, especially from treatment 5, had longer and more slender forewing shape conducive for longer distance flight. FEM modeling of the flight potential in both male and female *H. zea* revealed that the highest wing elastic deformation values for wind speed, indicating the most impact on wing structure, occurred for treatment 2> treatment 1> treatment 3> treatment 5. Wing elastic deformation was significantly more pronounced in female than male moths. In conclusion, we found that one generation of selection on Bt corn in the field could induce *H. zea* wing phenotypes more conducive for potential long-distance dispersal and should be further investigated by directly testing the impact on migratory flight. Our study contributes to the growing body of evidence that selection of *H. zea* on Bt crops may influence adult dispersal behavior.

Keywords: geometric morphometrics, finite element method, forewing shape, flight, Vip3A

Introduction

The corn earworm, *Helicoverpa zea* (Boddie) is recognized as one of the most economically damaging insect pests of crops in southeastern United States. *Helicoverpa zea* has a long history as a pest of cotton and feeds on almost all agricultural crops, although corn (*Zea mays*) is its preferred host (Lincoln and Isely 1947). Currently in the United States, over 90% of corn, cotton, and soybean crops are genetically engineered varieties; 80% of corn hybrids grown in the United States express Bt (Dodson 2025).

The increase in planting of Bt crops throughout the United States has led to *H. zea* evolving practical resistance to some Bt toxins. *Helicoverpa zea* has had 3 cases of field-evolved practical resistance to Bt crops being detected from 1996 to 2005, and 19 cases detected in 2018 (Tabashnik and Carrière

2017). Corn expresses 3 families of Bt toxins, of which southeastern United States corn earworm is resistant to 2 (Cry1 and Cry2) (Tabashnik and Carrière 2017, Bilbo et al. 2019, Gassmann and Reisig 2023, Pezzini et al. 2023). Multiple generations of *H. zea* are likely to be exposed to a wide variety of Bt crops. This provides suitable conditions for quick evolution of resistance to Bt proteins, buoyed by the movement behavior of *H. zea*, of which it and other Noctuids are well known for their capacity for a variety of dispersal strategies involving short- and long-distance flights (Farrow and Daly 1987, Fitt 1989, Wolf et al. 1990, Beerwinkle et al. 1994). The propensity for short- and long-distance flight is a hall mark of the movement behavior of Noctuid moths, some of which are known to migrate in the hundreds of thousands many hundreds of kilometers a night: *Helicoverpa armigera* (Hübner) in Australia

(Farrow and Daly 1987) and *H. zea* in North America (Wolf et al. 1990, Beerwinkle et al. 1994, Westbrook et al. 1997). When dealing with a pest insect from the Noctuid family, which are known for their remarkable flight behavior, it is important to understand how Bt crops can influence their flight behavior and the resulting ability of resistant moths spreading their alleles throughout the agricultural landscape.

Evolution of Bt resistance in *H. zea* has become a key threat for the efficacy of Bt corn crops. There is concern about how this may affect future integrated resistance management strategies. Bt-resistant *H. zea* populations are exceptionally hard to control with foliar insecticides once they have established in cotton (Reisig et al. 2019, Reisig and Goldsworthy 2023). Moreover, there is fear that Bt resistance will hinder possible future pest management strategies, which include pyramided Bt crops (Brevault et al. 2013). Pyramiding is described as transgenic plants that express 2 or more dissimilar Bt proteins. It is believed that they will be more effective as there are a variety of Bt proteins available to kill insect pests if one protein was to fail (Zhao et al. 2003). Proteins that are adopted in Bt corn typically include the Cry1 and Cry2 families. Currently, planted hybrids also include the Vip3A (Vip3Aa20) protein, which is highly effective for *H. zea* (Burkness et al. 2010, Yang et al. 2019). However, there is concern that the efficacy of further pyramided Bt corn will be reduced because Vip3A is not produced at a high enough dose to delay *H. zea* resistance given the current resistance management practices in the United States, including inadequate levels of non-Bt refuge (Brevault et al. 2013, Tabashnik and Carrière 2017, Reisig and Kurtz 2018). Predictions suggest that *H. zea* will follow a similar pattern of resistance evolution for Vip3A as the Cry toxins (Gassmann and Reisig 2023). Recent studies have reported that *H. zea* is demonstrating early warning signs of resistance to this toxin (Yang et al. 2020, Dively et al. 2021, Yang et al. 2021a, 2021b, 2024).

The continued development of resistance within *H. zea* is a dynamic process. To understand this development, the evolutionary changes related to dispersal (eg wing shape change) require ongoing monitoring. This will guarantee that new circumstances and knowledge are considered, guaranteeing that management recommendations are still valid. To ensure that the damage done by pests remains contained, early detection of possible resistant populations to Bt corn is needed and strategies to combat these populations need to be present.

Understanding the movement potential and flight patterns of *H. zea* provides crucial information about their current and potential distribution and therefore how resistance to Bt toxins can be spread through the landscape. It highlights which geographic areas may be at risk of an invasion and allows for the creation of an effective management plan. For this pest, mass migrations can occur over long distances in a single night (Wolf et al. 1990, Beerwinkle et al. 1994). Investigations into the pollen found on migrating *H. zea* moths provided evidence that these migrations are occurring over hundreds of kilometers (Westbrook et al. 1997). Although the sample size was small, roughly half of the *H. zea* moths collected in Florida, South Carolina, and North Carolina during the summer were migrants (Paula-Moraes et al. 2024). When migrating, these insects use powered flight to disperse within the boundary layer, reaching altitudes of up to 900 m (Beerwinkle et al. 1994). They then rely on upward convection and prevailing winds to reduce expenditure during migration, which is gliding flight, eg

Monarch butterflies (*Danaus plexippus* L.) (Gibo and Pallett 1979). In most insects, including *H. zea*, forewings generate the main power for the flight compared to hindwings that possess weaker muscles. In general, insect forewings provide the main aerodynamic force, acting as stable lift-generating structures, whereas the hindwings contribute to agility and maneuverability, especially during sudden changes in flight direction (Howland 1974). Despite the important roles that hindwings play within insects, including *H. zea*, it is the forewings that dictate the ability to fly longer distances for migration purposes. Wing shapes have long been studied to determine the dispersal capabilities of flying insects (Denno et al. 2001, Guerra 2011, Sanzana et al. 2013). Generally, long and elongated wing shapes are better suited to flying with high altitude air currents and longer distance dispersal (eg migration), while shorter and broader wing shapes are more efficient for flapping low-level flights that require more precision (Altizer and Davis 2010, DeVries et al. 2010).

Flight potential in pest insects of corn has been the subject of numerous studies to date (Mikac et al. 2013, Lemic et al. 2014, Mikac et al. 2016, Mikac et al. 2019, Pajač-Živković et al. 2019, Kadoić-Balaško et al. 2021, Mikac et al. 2024). Studies have examined how wing shape and size are related to control, eg soybean-maize crop rotation on western corn rootworm, *Diabrotica virgifera virgifera* (Mikac et al. 2013, 2016). Others have examined how wing shape changes as a function of Bt corn resistance in western corn rootworm (Mikac et al. 2019); however, others still have reported on changes in wing shape of Tortricid moths as a function of resistant to insecticides (Pajač-Živković et al. 2019).

A combined approach was taken in the codling moth (*Cydia pomonella*) where it was possible to demonstrate wing shape differences in moths from resistant and susceptible treatment types using geometric morphometrics, which then through numerical modeling (finite element method, FEM) revealed the influence that the resulting wing shape had on flight potential (Pajač-Živković et al. 2019). Likewise this approach was taken for the western corn rootworm where it was found that the wings of non-resistant beetles were significantly more elongated in shape and narrower in width (chord length) in comparison to beetles that were resistant to Bt corn or crop rotation (Mikac et al. 2019). FEM for the same western corn rootworm showed that Bt corn-resistant and rotation-resistant corn rootworm hindwings could potentially resist higher wind speeds compared to susceptible corn rootworm (Mikac et al. 2024). Such differences may impact upon the dispersal or long-distance movement of resistant and susceptible beetles and warrant investigation in other insects that have reported practical field resistance like *H. zea* on Bt corn in southeastern United States. Investigating wing morphometric changes and modeling the flight potential provides a robust alternative to investigate the movement ecology of insect pests, especially when flight mill experiments provide inconclusive outcomes (Jiang et al. 2013, De Bortoli et al. 2024, Pezzini et al. 2023).

In several laboratory-based studies, when related noctuid-pest species were fed Bt diet, their flight capacity was reduced (Liang et al. 2007, Jiang et al. 2013, De Bortoli et al. 2024). A single study tested this in the field. Pezzini et al. (2023) hypothesized that *H. zea* developing from Bt plants using a structured refuge (non-Bt corn planted in a pure stand) would have reduced flight ability compared to insects developing from non-Bt plants in a structured refuge, but not from Bt and non-Bt plants in seed

blend refuge (non-Bt corn planted within Bt corn). This hypothesis was based on previous studies suggesting that ear-feeding insects that developed from ears contaminated with Bt and non-Bt pollen would evolve Bt resistance quicker than those that developed in a pure stand of Bt corn (Burkness et al. 2011, Yang et al. 2014, Caprio et al. 2016, Carrière et al. 2016, Carrière et al. 2021). The authors tested this using flight mill experiments on *H. zea* collected from the respective treatments. The authors concluded that there was no effect on Bt, from either a pure-stand or a seed blend, for adult parameters (total flight distance and duration, average speed, and longest flying bout) tested using a flight mill. Nevertheless, it was found that across all treatments, *H. zea* flew an average of 12.1 km, with 56% of moths engaging in short distance flight (< 5 km), 10% in long-distance flight (5–10 km), and 34% engaging in migratory flight (> 10 km). The longest flight distance was 82.1 km, over 20 h of flight activity (Pezzini et al. 2023). There are a myriad of biotic and abiotic factors that affect insect flight, and it has been suggested that flight mill studies should be coupled with additional methods, such as FEM, to better understand flight behavior and potential in pest insect species (Mikac et al. 2019).

Despite the negative finding for flight, in this same experiment, the genetic signature for Bt resistance could be detected from *H. zea* individuals that developed in a seed blend for only a single generation (Pezzini et al. 2024). Therefore, Bt could have phenological impacts that were not measured in that experiment.

Here we build on the work of Pezzini et al. (2023), by testing their same hypothesis using insect wings from their flight mill experiment. However, we incorporate a morphometric analysis of wing shape and use numerical modeling (FEM) to understand flight potential in *H. zea* across treatments of non-Bt and Bt corn hybrids, which express 2 and 3 Bt toxins, either in structured or seed blend refuges. We tested the hypothesis that *H. zea* developing from Bt corn using a structured refuge would have different wing shapes and reduced flight ability (capacity) compared to insects developing from non-Bt corn in a structured refuge, but not from Bt and non-Bt corn in seed blend refuge.

Materials and Methods

Sampling Sites and Specimen Collection
Adult *H. zea* from experimental field sites in North and South Carolina were used in geometric morphometric (total n = 143; n-females = 66 and n-males = 77) and finite element modeling (n = 8). Detailed information about the field experiments and treatments from which adults were collected is outlined in Pezzini et al. (2023). Here, a subset of adults from each treatment that were already flown on the flight mill experiments in Pezzini et al. (2023) were then prepared for geometric morphometric analysis, followed by finite element modeling. As per Table 1 from Pezzini et al. (2023), the treatments were as follows: a control containing non-Bt corn in a pure stand (treatment 1, n-females = 15, n-males = 18)); Bt corn with 2 toxins (Cry1Ab and Cry1F) in a pure stand (treatment 2, n-females = 9, n-males = 20); Bt corn with 3 toxins (Cry1Ab, Cry1F, and Vip3A) in a pure stand (treatment 3, n-females = 32, n-males = 28); and a seed blend with 80% containing Bt corn with 3 toxins (Cry1Ab, Cry1F, and Vip3A) and 20% non-Bt corn (treatment 5, n-females = 10, n-males = 11). Briefly, ears with larvae were pulled directly from the field in each treatment.

When the larvae pupated, several phenological parameters were measured, such as pupal depth, pupal weight, and time to eclosion. Upon eclosion, those moths were tethered to a flight mill and flight parameters were measured. After flight, the moths were preserved in ethanol and the wings were removed.

Due to insufficient sample size and quality of specimens, moths from treatment 4—a seed blend with 80% containing Bt corn with 2 toxins (Cry1Ab and Cry1F) and 20% non-Bt corn—were discarded from further analyses in this study. Pezzini et al. (2024) performed whole-genome sequencing on individuals from our study and found that those exposed to corn pollinated with Cry pollen carried resistance-associated signatures of selection (Legan et al. 2024, Taylor et al. 2024). Furthermore, individuals exposed to corn pollinated with Vip3A pollen showed allele frequency divergence in genomic regions adjacent to known Vip3A resistance loci (Singh et al. 2010, Osman et al. 2019). Therefore, based on the detection of genomic signatures for resistance when exposed to Bt (Pezzini et al. 2024), we grouped treatments as non-selected (treatment 1), moderately selected (treatments 2 and 5—Cry), and intensely selected (treatment 3—Vip3A).

Wing Preparation
Wings of individual moths were prepared for slide mounting by removing their scales. This was done by soaking wings in a solution of bleach (10%) for 3 minutes, followed by an ethanol solution (70% v/v) for another 3 minutes, and a final rinse in a distilled water bath (Pajač-Živković et al. 2019). When necessary, the chemically treated wings were lightly brushed with a fine paint brush to ensure scale removal and clear exposure of wing vein anatomy. Left and right forewings of each specimen were then slide mounted on glass slides (75 mm × 26 mm), using coverslips (22 mm × 22 mm), and the fixing agent Euparal (Australian Entomological Supplies, Brisbane) based on standard methods (Upton and Mantle 2010).

Geometric Morphometric Analysis
Wing Landmarks. We chose 15 type 1 landmarks established in previous studies (Table 1 and Fig. 1), which were defined by vein junctions or vein terminations to improve accuracy and repeatability of landmarking (Bookstein 1991, Cañas-Hoyos et al. 2016, Nagoshi et al. 2020).

Slide mounted wings were photographed using a Nikon D 7500 camera (20.9M Pixel) on a trinocular mount with a Micro Nikkor 40 mL lens. The camera was placed so that each specimen occupied approximately the same area in the viewing field, to minimize distortion. Damaged and folded wings caused by the slide-mounting process were discarded and excluded from further analysis. Images were saved in a NEF file, before being converted into a TIFF file using Nikon Transfer 2 (v2.15). The program tpsUtil v1.82 was used to convert files from TIFF to TPS format. tpsDig v2.32 (Rohlf 2008) was then used to landmark, record the scale of and digitize each specimen. This produced cartesian coordinates (x, y) of the pairwise distances between the landmarks. The landmark data were saved as a TPS file and imported into MorphoJ v1 (Klingenberg 2011) for geometric morphometric statistical analysis.

Systematic measurement error was assessed for each specimen by photographing and landmarking twice. This resulted in a total of 4 sets of cartesian coordinates for each specimen. A Procrustes ANOVA was applied to this data to compare the measurement

Table 1. Location and description for "type 1" landmarks used for geometric morphometric analysis for *Helicoverpa zea*

| Landmark number	Anatomical location and description
1	Intersection of origin of Radial R1 Vein from the Primary Radial Vein
2	Second division on Posterior Radial Vein
3	Intersection of Radial R4 and Radial R5 Vein
4	Intersection of origin of Medial Anterior MA Vein from Radial Vein
5	Termination of Radial R4 Vein at the distal wing border
6	Termination of Radial R5 Vein at the distal wing border
7	Termination of Media Anterior MA Vein at the distal wing border
8	Termination of Medial Posterior MP1 Vein at the distal wing border
9	Termination of Medial Posterior MP2 Vein at the distal wing border
10	Termination of Cubitus Anterior CuA1 Vein at the distal wing border
11	Termination of Cubitus Anterior CuA2 Vein at the distal wing border
12	Termination of combined Anal A1+A2 Vein at the distal wing border
13	Branching of Cubitus Anterior CuA2 Vein from Primary Cubitus Anterior Vein
14	Branching of Cubitus Anterior CuA2 Vein from Primary Cubitus Anterior Vein
15	Intersection of origin of Media Posterior R4 Vein from Primary Cubitus Posterior Vein
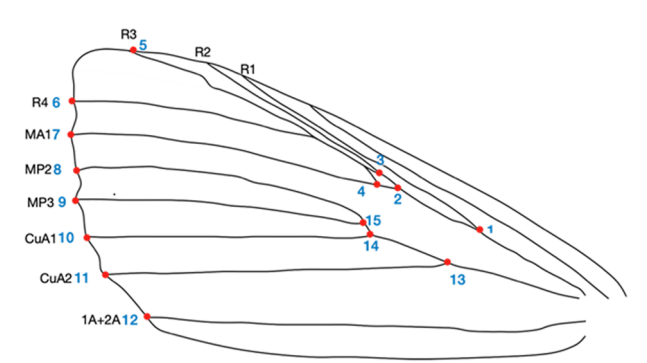
Fig. 1. Venation pattern of the forewing of *Helicoverpa zea*. Vein types are represented by Radius (R1-4), Medius (MA, MP1, and MP2), Cubitus Anterior (CuA1-2), and Anal (A1-2) and shown in black text. Landmark placement shown as red point and blue number (1-15).

error (Klingenberg and McIntyre 1998). The means squares of the Procrustes ANOVA between the individual and the error values indicated that measurement error (MS = 0.00000444) was less than individual error (MS = 0.0002401), which was a negligible influence on the data set.

Bilateral asymmetry was tested to determine if there were significant differences between left and right wings. A Procrustes ANOVA was used to test for asymmetry for both wing shape and centroid size (Palmer and Strobeck 1986). No significant shape difference was detected between the left and right wings of specimens tested; therefore, no bilateral asymmetry was observed ($F = 1.32$; $P = 0.134$). Therefore, one wing per specimen was used and wings were reflected as needed.

Allometry was tested for to ensure that size did not have any influence on shape changes, and that differences detected because of the treatments of Bt corn were not confounded by size. To test for the presence of allometry within the *H. zea* samples, a multivariate regression was conducted on shape versus size (Loy et al. 1996, Monteiro 1999, Drake and Klingenberg 2008). Centroid size was used to describe size while Procrustes coordinate data described shape; 10,000 iterations of permutation was not significant ($P = 0.24$) suggesting that size and shape were not associated. Therefore, the effects of allometry did not need to be considered when interpreting results.

Sexual dimorphism was tested using discriminant function analysis (DFA). There was a significant difference found between males and females for the centroid size and shape of the wing (Table S1). Therefore, sexes were separated for all further analysis.

Notably, Pezzini et al. (2023) found no effect on flight parameters by location and the impacts of Bt on pupae are consistent between these 2 locations in North and South Carolina (Bryant et al. 2024). Despite this, geographic differences were tested (North Carolina vs South Carolina) using DFA. There were no significant differences found between males and females by region for wing shape (Table S2). Therefore, wings from samples from North and South Carolina were combined by sex.

Statistical Analysis

To identify if there was a separation among the treatments a canonical variate analysis (CVA) was used. To determine if wing shape can act as an indicator for different treatments of Bt corn a DFA was used (Zelditch et al. 2012). Pairwise analysis of treatments was undertaken along with 10,000 permutations to distinguish among the different treatments. These determined if there was a discriminating function present that would allow moths from unknown treatments to be characterized into certain treatments with high certainty. The difference in Procrustes and Mahalanobis distance between the means of the 2 groups was measured again as part of the DFA. A parametric P-value test was also calculated, as well as the permutation P-value for Procrustes distance and Hotelling's T^2. These statistical analyses were performed using MorphoJ v1.07a (Klingenberg 2011).

FEM was used to examine flight capacity in *H. zea*. The elastic deformation of forewings was based on a range of wind speeds experienced in the field, which were numerically modeled from the 4 treatments (detailed above). Eight simplified finite element models of the *H. zea* forewings (one individual, from each sex and treatment 1, 2, 3, and 5) were tested using ANSYS Workbench v 2025 R2 (ANSYS® Academic Research Mechanical, Release 2025 R2, ANSYS Inc., Canonsburg, PA, USA). A single specimen from each sex and treatment was modeled for flight potential, as this number of samples has been demonstrated by others to be adequate for robust output using FEM (Combes and Daniel 2003b, Pajač-Živković et al. 2019, Mikac et al. 2024). The *H. zea* forewing model was represented as a 3D skeleton model of a round solid bar as a simulation of the real model, based on the actual 2D images of *H. zea* forewings from each sex and treatment (generated in Scan2CAD v Pro, Avia Systems Limited, UK).

For all vein elements represented in the 3D skeleton model, the material properties were characterized as being isotropic

linear elastic materials with a density of 1200 kg m − 3, thickness of 45 µm, Poisson's ratio of 0.3, and Young's modulus of 150 MPa (Combes and Daniel 2003b). The mesh analysis (FEM model) of the vein structure (by treatment) included quadratic elements that ensured asymptotic performance of the model—as previously established in Pajač-Živković et al. (2019) and applied in Mikac et al. (2024). To represent the connection of the forewing to the thorax of the *H. zea* adult, a fixed support (no displacement or rotation) was applied to the base of the forewing where it connects to the pterothorax (Fig. 1).

Incremental loads were applied along the length of the 3D skeleton model from the dorsal side (z axis) to represent the wind force applied to *H. zea* forewings under field conditions. It has been documented that *H. zea* and other Noctuid migrants ascend into the lower troposphere at night (1-2 km in altitude) and encounter winds of up to 15 m/s $^{-1}$ in southeastern United States (Beerwinkle et al. 1994). To examine the extremes of wind speeds experienced in the field by *H. zea*, the incremental loads modeled ranged from 2.8 m/s $^{-1}$ up to 27.8 m/s $^{-1}$ (Beerwinkle et al. 1994). To assess the effects of treatment and sex on wing elastic deformation while accounting for variation in wind speed, a 1-way analysis of covariance (ANCOVA) was conducted in JMP® v2025 (JMP Statistical Discovery LLC, Cary, NC). Treatment and sex were fixed factors, and wind speed was modeled as the covariate. Interaction terms were included to determine whether the rate of elastic deformation varied by treatments or between sexes. This approach allowed for the assessment of treatment and sex effects and controlled for the variance associated with wind speed.

Results

Geometric Morphometrics: Overall Wing Shape Changes in Males and Females

For males and females, CVA showed 4 clusters among the treatments examined (Figs 2 and 3). These differences were significant for males (Goodall's $F = 1.61$, $P < 0.05$) and females (Goodall's $F = 1.71$, $P < 0.05$).

Wing Shape Changes Among Bt Corn Treatment Groups in Males and Females

Male *H. zea* pairwise comparisons among treatment groups revealed varying degrees of shape differentiation based on the interpretation of Procrustes and Hotelling's T^2 statistics (Table 2). The Procrustes permutation tests indicated statistically significant shape differences for males from moderately (treatment 2) versus intensely selected treatments (treatment 3) (Table 2).

The Hotelling's T^2-based permutation tests statistics agreed in some cases but diverged in others. For males from moderately selected treatments (ie treatment 2 vs treatment 5) and
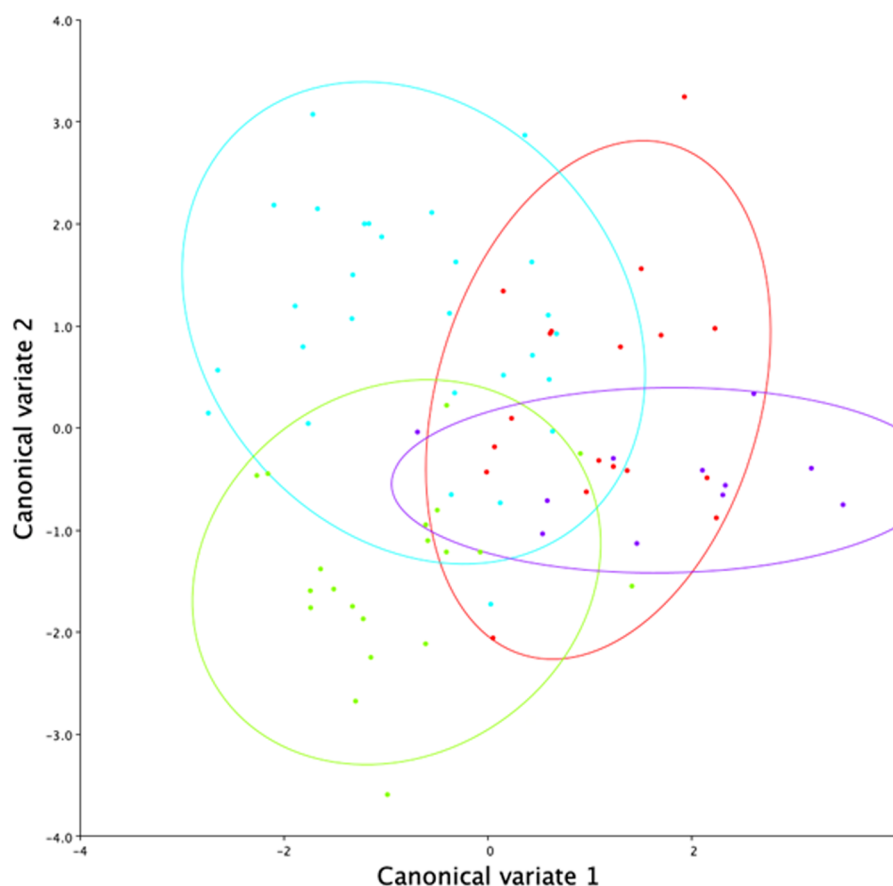
Fig. 2. A canonical variate analysis (CVA) conducted on the forewings of male *Helicoverpa zea* (n = 77). Points are color coordinated dependent on which treatment of corn the moths were extracted from, treatments of corn are as follows: Red: treatment 1, non-Bt corn in a pure stand; Green: treatment 2, Bt corn with 2 toxins in a pure stand; Pink: treatment 3, Bt corn with 3 toxins in a pure stand; and Purple: treatment 5, a seed blend with 80% containing Bt corn with 3 toxins and 20% non-Bt corn. Ellipses are 95% confidence intervals.
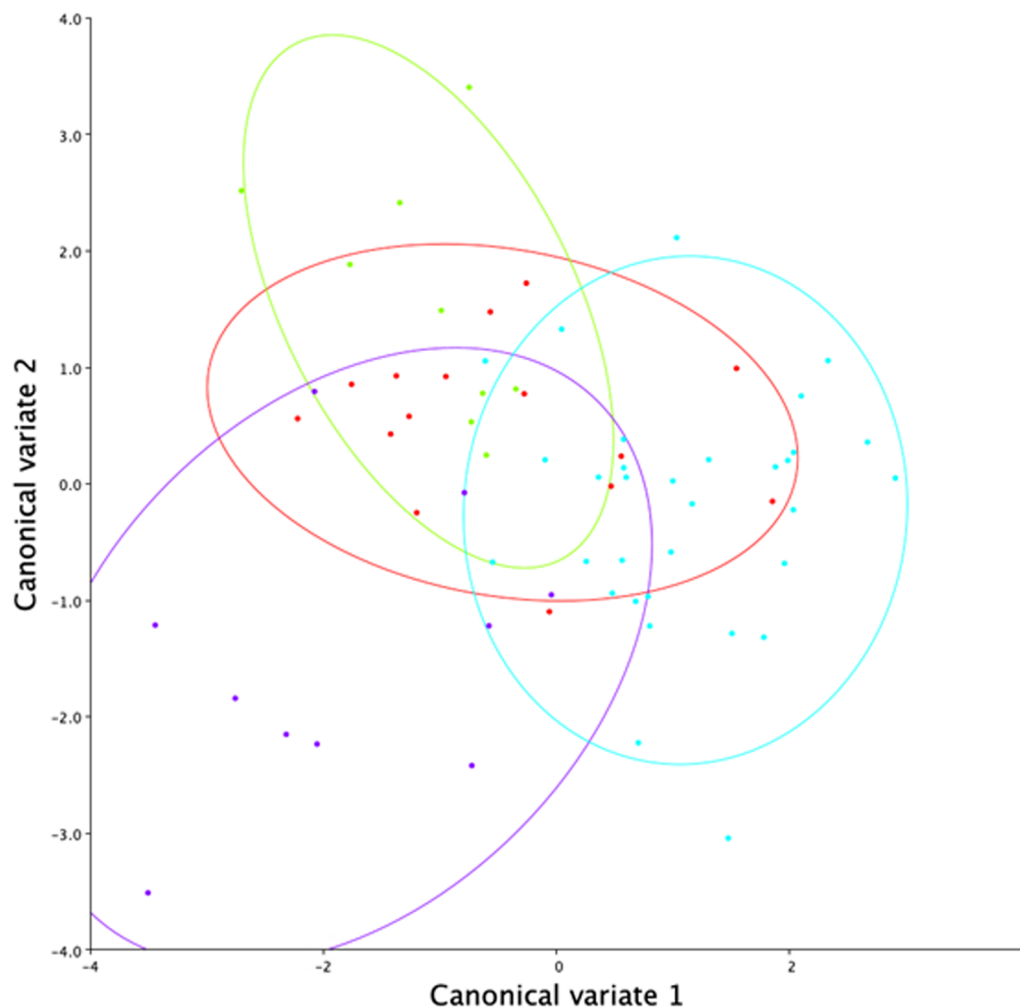
Fig. 3. A canonical variate analysis (CVA) conducted on the forewings of female *Helicoverpa zea* (n = 66). Points are color coordinated dependent on which treatment of corn the moths were extracted from, treatments of corn are as follows: Red: treatment 1, non-Bt corn in a pure stand; Green: treatment 2, Bt corn with 2 toxins in a pure stand; Pink: treatment 3, Bt corn with 3 toxins in a pure stand; and Purple: treatment 5, a seed blend with 80% containing Bt corn with 3 toxins and 20% non-Bt corn. Ellipses are 95% confidence intervals.

non-selected (treatment 1) and moderately selected treatments (treatment 2) significant shape differences were found (Table 2).

In female *H. zea*, only 2 moderately selected (treatment 5) comparisons were significant for Procrustes statistics (Table 2). That is, moderately selected versus non-selected moths (treatment 1) and moderately selected (treatment 5) versus intensely selected moths (treatment 3) (Table 2). Overall, in both male and female comparisons, the moderately selected treatments were driving the significant difference found (Table 2).

By visually assessing a wireframe construction superimposed over an *H. zea* wing for females from significant DFA treatment comparisons (treatment 3 vs treatment 5 and treatment 1 vs treatment 5; Table 2), it was evident that the mean wing shape of females from moderately selected moths (treatment 5) was longer and narrower compared with females from both non-selected (treatments 1) and intensely selected moths (treatment 3) (Fig. 4). Wing length in moderately selected moths (treatment 5) appeared longer overall than wings from treatments 3 and 1. Wing width in treatment 5 was narrower in the middle wing base area compared to intensely selected (treatments 3) and non-selected moths (treatment 1), which showed more breadth across the midsection, resulting in a more oval

or rounded wing. The wing shape differences in moderately selected (treatment 5) wings were characterized by a narrow and tapered form, with sharper angles, especially at the posterior tip of the wing. For intensely selected (treatments 3) and non-selected moths (treatment 1), the shape of the wing was broader and more rounded, particularly in the middle and at the wing base. In short, moderately selected moth wings from treatment 5 were longer, narrower, more tapered and angular, while intensely selected (treatments 3) and non-selected moth (treatment 1) wings were shorter, broader, more rounded, and oval (Fig. 4).

Similarly for males, comparisons were visually assessed via a wireframe construction superimposed over an *H. zea* wing for males from significant DFA treatment comparisons (moderately selected (treatments 2) vs intensely selected (treatment 3), moderately selected (treatments 2 (pure stand) vs 5 (seed blend)), Table 2). For the treatment 2 vs treatment 3 comparison, the wireframe for treatment 3 appeared longer than that of treatment 2, as the posterior tip extended further back (Fig. 5). The wireframe for moderately selected moth wings (treatment 2) was wider in the midsection and basal areas, while the intensely selected (treatment 3) moth wing wireframe

Table 2. Discriminant function analysis of *Helicoverpa zea* forewings in females (n = 66) and males (n = 77), and comparison of Bt corn treatments

Bt corn treatment comparisons		P-values	
		Procrustes P	Hotelling's T²
Males			
1	2	ns	ns
1	3	ns	ns
1	5	ns	ns
2	3	*	ns
2	5	ns	**
3	5	ns	ns
Females			
1	2	ns	ns
1	3	ns	ns
1	5	**	ns
2	3	Ns	ns
2	5	Ns	ns
3	5	**	ns

Treatment 1, non-Bt corn in a pure stand; treatment 2, Bt corn with 2 toxins in a pure stand; treatment 3, Bt corn with 3 toxins in a pure stand; treatment 5, a seed blend with 80% containing Bt corn with 3 toxins and 20% non-Bt corn.
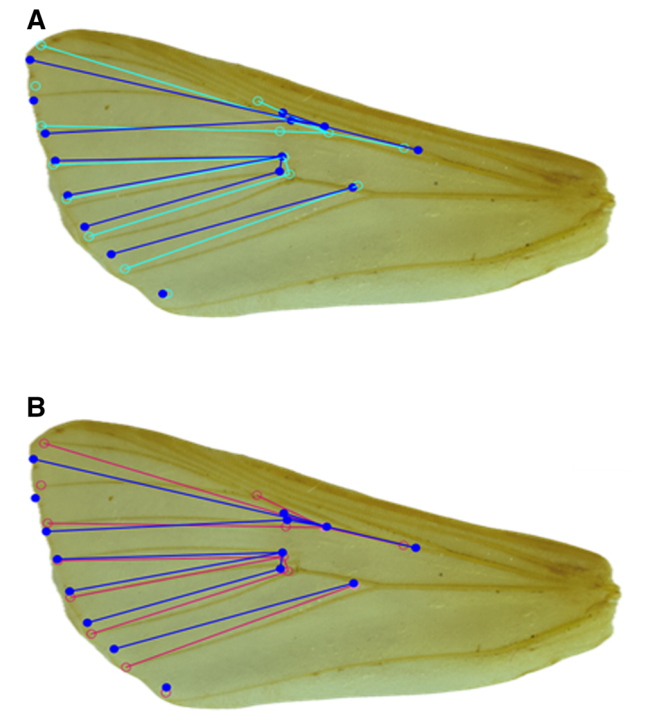*P < 0.05, **P < 0.01, ns > 0.05.

Fig. 4. Mean wing shape differences visualized using a wireframe construction superimposed over a *Helicoverpa zea* wing for females from (a) Bt corn with 3 toxins in a pure stand (treatment 3) (light blue) and a seed blend with 80% containing Bt corn with 3 toxins and 20% non-Bt corn (treatment 5) (dark blue), and (b) non-Bt corn in a pure stand (treatment 1) (red) and a seed blend with 80% containing Bt corn with 3 toxins and 20% non-Bt corn (treatment 5, seed blend) (dark blue).

was narrower overall, giving it a slender appearance. For wing shape, treatment 3 displayed a more tapered and angular form, particularly at the apex and posterior end, whereas for moderately selected moths (treatment 2), the wings had a broader and rounder shape with smoother transitions from base to tip.
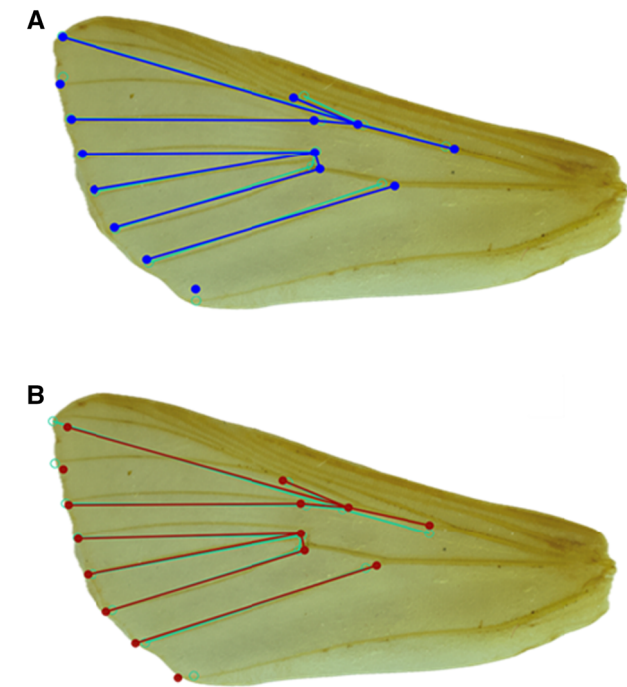

Fig. 5. Mean wing shape differences visualized using a wireframe construction superimposed over a *Helicoverpa zea* wing for males from (a) Bt corn with 2 toxins in a pure stand (treatment 2, pure stand) (green) and a seed blend with 80% containing Bt corn with 3 toxins and 20% non-Bt corn (treatment 5) (dark blue); (b) Bt corn with 2 toxins in a pure stand (treatment 2) (green) and Bt corn with 3 toxins in a pure stand (treatment 3) (red).

The wireframe for moderately selected moths from treatment 5 (seed blend) was longer than that of moderately selected moths from treatment 2 (pure stand), with the wing tip extending further, particularly in the posterior region. The wireframe for treatment 2 (moderately selected, pure stand) was wider across the central and basal regions, while the treatment 5 (moderately selected, seed blend) wireframe was noticeably narrower, giving it a more streamlined appearance. For shape, the treatment 5 wireframe showed a more tapered and angular form, especially at the posterior and apex, whereas the treatment 2 wireframe was broader and rounder in comparison (Fig. 5).

Finite Element Method

The elastic deformation measurements, recorded in millimeters, revealed distinct patterns across treatments and sexes, indicating varied levels of wing frame stiffness under increasing wind pressures (Fig. 6). For elastic deformation magnitude, for moderately selected moths (treatment 2) exhibited the highest values across both sexes, reaching 0.57 mm in males and 0.38 mm in females at the maximum wind speed of 27.8 m·s⁻¹. The sharpest increase in elastic deformation across wind speeds was observed in this treatment, indicating lower flexural stiffness in the wing vein structure.

For non-selected moths (treatment 1) showed the next highest elastic deformation, with values of 0.54 mm in males and 0.36 mm in females, which suggested moderate structural compliance. Although slightly less deformable than treatment 2 (moderately selected moths), this group still exhibited considerable elastic deformation under the modeled aerodynamic loading (Fig. 6).
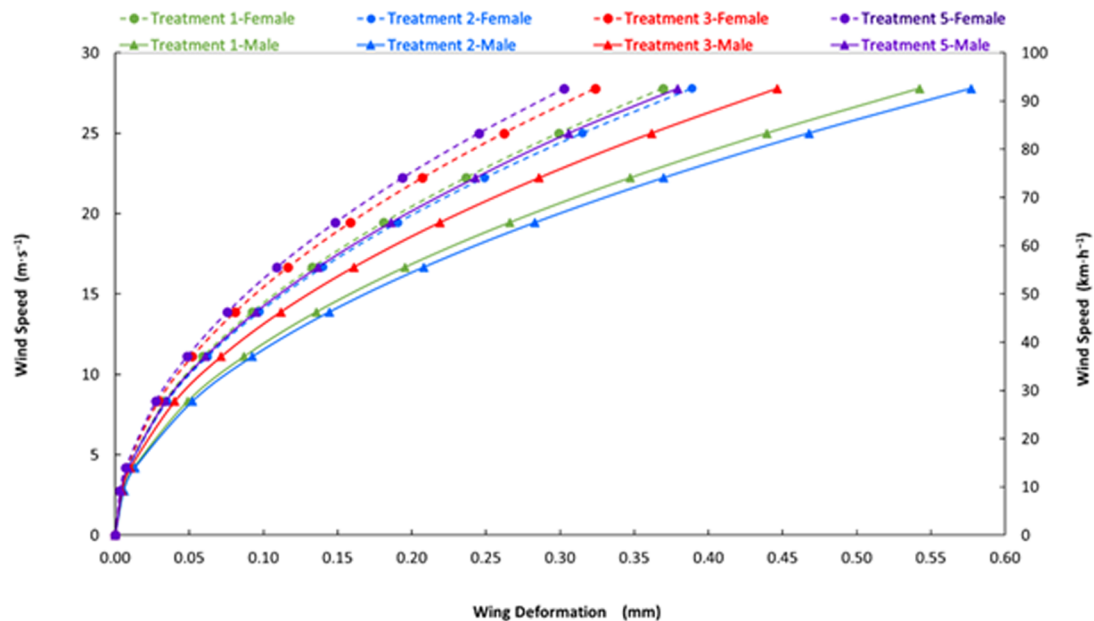
Fig. 6. Finite element method analysis of *Helicoverpa zea* wing deformation for males and females under the 4 treatments to evaluate potential flight capacity. Treatment details: treatment 1, non-Bt corn in a pure stand; treatment 2, Bt corn with 2 toxins in a pure stand; treatment 3, Bt corn with 3 toxins in a pure stand; treatment 5, a seed blend with 80% containing Bt corn with 3 toxins and 20% non-Bt corn.

In contrast, treatments 3 (intensely selected) and 5 (moderately selected) exhibited significantly lower wing elastic deformation. Treatment 3 wing elastic deformation reached 0.44 mm in males and 0.32 mm in females, while treatment 5 (moderately selected) consistently showed the lowest wing elastic deformation, with values of 0.37 mm (male) and 0.30 mm (female) (Fig. 6). These patterns indicate increased vein stiffness and suggest that selection may influence mechanical resistance to bending.

ANCOVA revealed a strong main effect of wind speed on elastic deformation ($F(1,75) = 898.7$, $P < 0.001$), indicating a consistent increase in elastic deformation with aerodynamic load. A significant interaction between wind speed and treatment ($F(3,75) = 5.5$, $P < 0.01$) confirmed that elastic deformation increased at different rates across treatment groups. Additionally, a significant interaction between wind speed and sex ($F(1,75) = 25.5$, $P < 0.001$) indicated sex-specific mechanical responses to increasing aerodynamic pressure.

Wind speed explained a large proportion of variance in elastic deformation (partial $\eta^2 = 0.911$), while the interactions with treatment (partial $\eta^2 = 0.133$) and sex (partial $\eta^2 = 0.218$) also contributed to a lesser extent. Overall, female wings exhibited greater resistance to aerodynamic elastic deformation than male wings.

Discussion

We used a flight-mill-alternative approach to test the hypothesis of Pezzini et al. (2023) that *H. zea* developing from pure-stand Bt corn (treatments 2 and 3, moderately and intensely selected, respectively) would have different wing shapes and reduced flight ability (capacity) compared to moths developing from pure-stand non-Bt corn (treatment 1, non-selected), but wing shape should not differ from moths developed on Bt and non-Bt corn in seed blend refuges (treatment 5, moderately selected). We found that moderately selected treatments drove the comparison differences in males (treatment 2, moderately selected) and females (treatment 5, moderately selected) for wing shape. In males, wing shape in treatment 2 (moderately selected) was characterized as being rounder and broader in shape compared to moths from treatment 5 (moderately selected) that had longer and more slender wings. This pattern was mirrored in females, however, was more pronounced, especially for treatment 5 (moderately selected), where wings were more elongated, narrower, and more tapered and angular compared to the wing shape noted from the other treatments. Thus, we partially accept the original hypothesis: moths from pure-stand Bt treatments did exhibit wing shape differences, particularly in comparison to seed blend refuge moths, but not entirely in the predicted direction. This study demonstrates that differences in forewing elastic deformation among *H. zea* treatments and sexes are attributable to variation in structural stiffness, primarily governed by the architecture of wing veins. Finite element modeling based on cantilever beam theory provided a practical framework for evaluating flexural behavior under mechanical loading conditions, consistent with established approaches in insect wing biomechanics (Combes and Daniel 2003a, 2003b).

Among all groups, moths from treatment 2 (pure stand, moderately selected) exhibited the greatest wing elastic deformations, particularly in males, suggesting lower structural rigidity within the vein network. In contrast, the lowest elastic deformation was observed in moths from treatment 5 (seed blend, moderately selected), which may reflect increased resistance to bending forces. Consistently lower elastic deformation in females compared to males across all treatments points to a sex-based difference in wing stiffness (Combes and Daniel 2003b), potentially linked to flight performance (Doyle et al. 2025) or biological roles (ie mated females with greater payload needs for carrying eggs; Willmott and Ellington 1997).

For the non-selected wing shape (treatment 1), a shorter and rounder shape was found for both male and female moths. This

shape was similarly characteristic of moths from treatments 2 (moderately selected) and 3 (intensely selected). Such a wing shape may confer to short distance or intra-field movement (Mikac et al. 2024). Modeled potential flight capacity for these wing shapes in female moths had higher wind resistance than their male conspecific when approaching wind speeds known to assist in long distance movement of Noctuids (eg 20-50 km; Beerwinkle et al. 1994).

Excessive wing elastic deformation, as observed in moths from treatment 2 (moderately selected), may compromise flight efficiency by diminishing the transmission of aerodynamic forces along the wing and increase the energetic cost of maintaining stable flight. This interpretation aligns with prior work on flexural mechanics in insect wings, where localized compliance has been shown to influence energy transfer and aerodynamic control (Willmott and Ellington 1997, Combes and Daniel 2003a, 2003b).

For the moderately selected wing shape (seed blend, treatment 5), the narrower and elongated wing shape found in males, and that was more pronounced in female moths, ensures that both short- and long-distance flight can be achieved. Modeling of flight capacity showed that this wing shape (narrow/slender, elongated) was the best performer particularly in female moths across all the wind speeds modeled and deformed the least irrespective of wind speed. Female potential flight outperformed males in most treatments (the exception was male moths from treatment 5). Of particular concern are female *H. zea* from treatments selected on pure-stand Bt corn (treatment 2, moderately selected, Cry1Ab + Cry1F) and seed blend Bt corn (treatment 5, moderately selected, Cry1Ab + Cry1F + Vip3A), with significant genomic shifts detectable in regions known to be associated with Cry-resistance (Pezzini et al. 2024).

For the intensely selected wing shape (treatment 3), the wing shape was more rounded on the distal edge, however overall had a rectangular shape. This wing shape performed the second strongest under all wind speeds when modeling potential flight capacity. *Helicoverpa zea* from pure-stand Bt corn with 3 toxins (treatment 3, intensely selected, Cry1Ab + Cry1F + Vip3A) showed this pattern consistently in both sexes, although female moths were able to withstand higher modeled wind speeds than males. As with the moderately selected individuals, genomic regions associated with Bt-resistance could be detected in the intensely selected individuals (Pezzini et al. 2024).

Important to note is the moderately selected wing shape (seed blend, treatment 5), characterized as the narrower and more elongated wing shape, which ensures that both short- and long-distance flight can be achieved. It is this wing shape that is most suited to migration and dispersal (Lockwood et al. 1998, Mönkkönen 1995) and likely the most problematic for integrated resistance management strategies. This is because the long-distance flights that individuals with this wing shape can undertake under a range of wind speeds can facilitate long-distance dispersal. Wing shapes of this nature would render other species unable to fly (cf western corn rootworm FEM; Mikac et al. 2024). A study conducted by Altizer and Davis (2010) compared 2 different populations of monarch butterflies. They determined that the populations that underwent migratory flight had longer wings, compared to their non-migratory conspecifics. In general, longer forewings may create more lift and increase the efficiency of flight allowing for longer flight distances. Longer wings have a larger surface area, which produces more lift and allows the insect to fly with

greater ease and efficiency. The decreased weight associated with the narrowing of *H. zea* wings makes it easier for adult moths to take off and fly. Elongated and slender wings are also much faster and more energetically efficient in flight as shown in birds (Mönkkönen 1995). By minimizing the induced power requirements, this wing shape allows for flight to be maintained for long distances.

Long distance and/or migratory flying is important for the dispersal of the species and colonization of new areas. Wing morphology is a critical element of an insect's dispersal capacity (DeVries et al. 2010). Understanding which morphotype is the potentially superior flier and disperser has implications for the ways in which *H. zea* will be managed with integrated resistance strategies. This is because the superior flier and disperser has the best chance of spreading its alleles long distance, and thus, the associated genotype will spread over a larger distance in a shorter amount of time; a feat that may occur in a short period of time for *H. zea* that are known to fly hundreds of kilometers in a single night (Wolf et al. 1990, Beerwinkle et al. 1994, Westbrook et al. 1997). Slender and elongated wings allow for this rapid colonization of a large area and hence the population at the front of this expansion wave would compose of individuals that possess these traits in wings (Phillips et al. 2010). Wing morphology is heritable in insects (Desender 1989); therefore, the progeny of these colonizers would display increasing wing length and a slender shape. This was confirmed in the wing shape and frequency of wing shape types of cricket populations that were at the forefront of a range expansion (Simmons and Thomas 2004).

The effects of Bt selection on flight have been previously studied. Prior to our investigation on wing shape, these same moths had been tested in a flight mill. Adult flight distance was not different across moths that were selected on non-Bt corn (non-selected), 2-toxin Bt corn (moderately selected), or 3-toxin Bt corn (intensely selected; Pezzini et al. 2023). In contrast, *Spodoptera exigua* (Hübner) and *Spodoptera frugiperda* (JE Smith) fed Cry1Ac protein in diet flew longer distances on a flight mill than those that did not feed on the protein (Jiang et al. 2013, De Bortoli et al. 2024). Both *Spodoptera spp.* findings align with our study's findings on the impact of Bt protein on wing shape. The fact that our findings do not match the flight mill studies of Pezzini et al. (2023) using the same insects could be unique to corn earworm or a difference in study methodology (eg field insects reared on corn in our study vs lab *Spodoptera spp.* reared on diet). Another lab study with a Cry1Ac-resistant strain of *H. armigera*, for example, found a reduction in flight distance after feeding on Cry1Ac diet (Liang et al. 2007). However, comparisons among *Helicoverpa* spp. reveal differences in dispersal within the family (Jones et al. 2019), making comparisons among species difficult. Moreover, while noctuid flight mill studies are excellent for estimating dispersal ability (Jones et al. 2016), their sensitivity to parse potentially small differences among or within species is not known. Hence, it has been recommend combining results from flight mill studies with experimental evidence, like we have done with this study (Minter et al. 2018). Regardless, for measuring the impacts of Bt protein on potential flight of *H. zea*, wing morphometrics and FEM were more sensitive than using a flight mill.

One caveat of our approach is that it does not directly measure flight. *Helicoverpa zea* pupae that develop on Bt corn have lighter pupae than those that develop on non-Bt corn (Reisig

and Reay-Jones 2015, Bilbo et al. 2019, Pezzini et al. 2023, Bryant et al. 2024, Pezzini et al. 2024). Pupal weight has a positive correlation with flight capacity in several related lepidopteran species, such as *Pectinophora gossypiella* (Saunders) (Wu et al. 2006), *H. armigera* (Liang et al. 2007), and *S. exigua* (Han et al. 2009). However, even though *H. zea* selected on Bt corn have a wing morphology better suited for long-term dispersal, there is no effect of pupal weight on flight distance for this species (Pezzini et al. 2023). Therefore, the result is likely more nuanced. For example, *H. zea* moths with a smaller body size and wing-to-thorax ratio fly longer and moths with a smaller wing-to-thorax ratio fly farther than their counterparts (Calixto et al. 2024). Hence, *H. zea* flight capacity is likely a combination of multiple factors, perhaps not limited to body size and wing shape, influenced by the environment during larval development. Also, it is important to acknowledge that a single morphotype or wing shape does not determine dispersal solely. It is a complex trait influenced by interacting morphological, physiological, and behavioral adaptations (Jones et al. 2019, Doyle et al. 2025).

Flight mills have not shown impacts of Bt on flight behavior in this species (Pezzini et al. 2023); therefore, future studies to tease out the impact of sublethal effects that might decrease flight on Bt-selected individuals would be useful. This could include mark-recapture studies or even genomic approaches that could measure selection on Bt (Pezzini et al. 2024) combined with isotopic analyses that could measure whether an individual was a migrant (Paula-Moraes et al. 2024). The flight mill studies of Pezzini et al. (2023) could also be repeated using 3- to 6-day-old moths. Pezzini et al. (2023) tested moths 24 hours after eclosion, but 3- to 6-day-old moths are more likely to engage in migratory behavior (Calixto et al. 2024).

On a final note, we acknowledge that sample sizes for some treatments were relatively small, reflecting the inherent difficulty of collecting sufficient individuals from fields with particular traits. While these constraints are understandable, explicitly recognizing them is important for contextualizing the strength of the inferences drawn.

In conclusion, we found that one generation of selection on Bt corn in the field could induce *H. zea* wing phenotypes more conducive for long-distance dispersal. This matches previous studies in the laboratory on related species (Jiang et al. 2013, De Bortoli et al. 2024) and should be further investigated by directly testing the impact on migratory flight. One way this could be accomplished is by evaluating the wing phenotypes of a large sample size of individuals deemed to be migratory and local dispersals on a flight mill. While the impact of Bt crops on local populations of *H. zea* can be strong (eg Arends et al. 2021, Dorman et al. 2021a, 2021b, Arends et al. 2022), ~40% to 60% of male *H. zea* moths in southeastern United States are migratory individuals (Paula-Moraes et al. 2024). Our study contributes to the growing body of evidence that selection of *H. zea* on Bt crops may influence adult dispersal behavior (Dorman et al. 2021a, 2021b, Arends et al. 2022).

Author Contributions

Katarina M. Mikac (Conceptualization [equal], Data curation [equal], Formal analysis [equal], Funding acquisition [equal], Investigation [equal], Methodology [equal], Project administration [equal], Resources [equal], Software [equal], Supervision [lead], Writing—original draft [lead], Writing—review & editing [lead]), Jose H. Dominguez Davila (Conceptualization [equal], Data curation [equal], Formal analysis [lead], Methodology [lead], Visualization [equal], Writing—original draft [equal], Writing—review & editing [equal]), Meagan J. Powley (Conceptualization [equal], Data curation [equal], Formal analysis [equal], Methodology [equal], Software [equal], Supervision [equal], Writing—original draft [equal], Writing—review & editing [equal]), Sarah Barclay (Formal analysis [supporting], Investigation [supporting], Writing—original draft [supporting]), Daniela Pezzini (Conceptualization [equal], Data curation [equal], Funding acquisition [equal], Writing—review & editing [equal]), and Dominic D. Reisig (Conceptualization [equal], Data curation [equal], Funding acquisition [lead], Investigation [equal], Methodology [equal], Project administration [equal], Resources [equal], Supervision [equal], Validation [equal], Writing—original draft [equal], Writing—review & editing [equal])

Supplementary Material

Supplementary material is available at *Environmental Entomology Journal* online.

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Conflicts of Interest.

None declared.

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