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## Changes in corn rootworm wing morphology are related to resistance development

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## Changes in corn rootworm wing morphology are related to resistance development

### Abstract

The western corn rootworm, *Diabrotica virgifera virgifera* LeConte, is the most serious pest of maize across the US Corn Belt and now Europe. The beetle has repeatedly demonstrated its ability to adapt to pest management strategies through evolution of resistance to conventional insecticides, the cultural practice of crop rotation and now to the *Bacillus thuringiensis* (Bt) toxin which is produced by genetically modified maize. In this study, the wing morphology of 358 western corn rootworm adults from Iowa, Indiana and Illinois, USA, was investigated using geometric morphometric procedures. The populations investigated comprised resistant (i.e. soybean-maize rotation and Bt-maize variants) and non-resistant beetle populations. Data analysis was divided into two groups: (1) resistant versus non-resistant and (2) rotation-resistant versus Bt-maize rootworm populations. Results showed that morphological differences exist in the hind-wing shape of both rotation and Bt-maize-resistant versus non-resistant populations and rotation-resistant versus Bt-maize-resistant variants. Across all three types of rootworm variants investigated, the movement of landmarks 8, 9 and 14 drove the wing shape differences found. These landmarks relate to the basal radial vein and are a key anatomical character used to distinguish different wing morphotypes in rootworm. This study demonstrates the utility of hind-wing morphology/shape as an inexpensive and accessible population biomarker for rootworm. With simple equipment (camera mounted microscopes or flatbed scanners) and readily available free software to capture and analyse landmark (shape and size) data, it is possible to effectively monitor pest resistance development and associated field-based population-level differences. The biological implications of the differences in wing shape found and how this relates to rootworm flight and consequently its dispersal and invasion capabilities are also explored.

### Publication Details

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1 **Changes in corn rootworm wing morphology are related to resistance development**

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8

9 **Author contributions**

10 KMM, RB and DL conceived and designed the experiments; KMM, DL and HAB performed the experiments; DL and HAB analysed the data;  
11 and KMM, DL, HAB and RB wrote the paper.

12

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15 Agriculture, North Central Agricultural Research Laboratory (NCARL) in Brookings, South Dakota; and Joseph Spencer, Thomas Sappington  
16 and Larry Bledsoe for providing field collected rotation resistant and susceptible beetles from Iowa, Indiana and Illinois.

17

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20 Effective Management Strategies’.

21

22

23 **Abstract**

24 The western corn rootworm, *Diabrotica virgifera virgifera* LeConte, is the most serious pest of maize across the US Corn Belt and now Europe.  
25 The beetle has repeatedly demonstrated its ability to adapt to pest management strategies through evolution of resistance to conventional  
26 insecticides, the cultural practice of crop rotation and now to the *Bacillus thuringiensis* (Bt) toxin which is produced by genetically modified  
27 maize. In this study the wing morphology of 358 western corn rootworm adults from Iowa, Indiana and Illinois, USA, were investigated using  
28 geometric morphometric procedures. The populations investigated comprised resistant (ie. soybean-maize rotation and Bt-maize variants) and  
29 non-resistant beetle populations. Data analysis was divided into two groups: 1) resistant versus non-resistant; and 2) rotation resistant versus Bt-  
30 maize rootworm populations. Results showed that morphological differences exist in the hind-wing shape of both rotation and Bt-maize resistant  
31 versus non-resistant populations and rotation resistant versus Bt-maize resistant variants. Across all three types of rootworm variants  
32 investigated, the movement of landmarks 8, 9 and 14 drove the wing shape differences found. These landmarks relate to the basal radial vein and  
33 are a key anatomical character used to distinguish different wing morphotypes in rootworm. This study demonstrates the utility of hind wing  
34 morphology/shape as an inexpensive and accessible population biomarker for rootworm. With simple equipment (camera mounted microscopes  
35 or flatbed scanners) and readily available free-software to capture and analyse land mark (shape and size) data, it is possible to effectively

36 monitor pest resistance development and associated field based population level differences. The biological implications of the differences in  
37 wing shape found and how this relates to rootworm flight and consequently its dispersal and invasion capabilities is also explored.

38 **Keywords:** geometric morphometrics, bt-maize, monitoring, resistance, hind wings

39

## 40 **Key message**

- 41 • Morphological differences exist in the hind-wing shape of both rotation and Bt-maize resistant versus non-resistant populations and  
42 rotation resistant versus Bt-maize resistant variants of western corn rootworm from across the US Corn Belt.
- 43 • The rotation resistant wing shape was the shortest in length and greatest in width (chord length), followed by Bt-maize resistant beetles,  
44 meaning that these beetles have a greater propensity for short distance movement and dispersal, while susceptible (non-resistant) beetles  
45 had longer and more narrow wings that are more predisposed to long distance dispersal and sustained flight at higher altitudes.
- 46 • Geometric morphometrics is an affordable and accessible technique that has been used to show that hind-wing shape and size differences  
47 among rootworm populations. Hence, wing size and shape can be used as a biomarker for resistance detection as part of a larger  
48 integrated resistance management strategy for the western corn rootworm.

49

## 50 **Introduction**

51 The western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), is a major pest of maize, *Zea mays* L., in  
52 North America, and over the past two decades has spread into Europe (Miller et al. 2005, Ciosi et al. 2010). The first invasion detected was in  
53 Serbia in the early 1990s, where economic levels of damage to maize occurred (Bača and Berger 1994). Intercontinental introductions of the

54 western corn rootworm from North America into Europe are still occurring (Ciosi et al. 2008; 2010; Lemic et al. 2015). Through such  
55 introductions the spread of resistant alleles to various control methods (chemical, cultural or genetically modified) could occur in Europe and  
56 render once effective control programs ineffective (eg. Levine and Oloumi-Sadeghi, 1991; Gassmann et al. 2011; Narva et al. 2013; Tabashnik et  
57 al. 2013; Jakka et al. 2016), and as such innovative pest resistance monitoring and surveillance tools and technologies must be implemented to  
58 mitigate such risks. Across the US Corn Belt the rootworm has progressively developed resistance to most of the control methods used to once  
59 curb its spread and the extensive economic damage caused. The first case of resistance to insecticides in rootworm was noted in 1959 in  
60 Nebraska to cyclodiene insecticides such as aldrin and heptachlor (Ball and Weekman 1962; 1963). To date the species has become resistant to  
61 organophosphates (methyl-parathion), carbamates (carbaryl) (Mainke et al. 1998; Wright et al. 2000) and pyrethroids (bifenthrin and tefluthrin)  
62 (Periera et al. 2015; 2017). European populations of western corn rootworm have shown resistance to Aldrin (Ciosi et al., 2009). The first case of  
63 resistance to the *Bacillus thuringiensis* (Bt) -toxin Cry3Bb1 was reported by Gasmann et al. (2011) in beetles from Iowa and resistance has since  
64 spread to other US States (Gasmann 2012; Wangila et al. 2015). Cross-resistance between Cry3Bb1 and mCry3A toxins also has since been  
65 established (Gasmann et al. 2014; Wangila et al. 2015). Under laboratory and greenhouse conditions, selected rootworm populations developed  
66 resistance to Cry34/35Ab1, Cry3Bb1 and mCry3A toxins (Lefko et al. 2008; Meihls et al. 2008, 2011; Meihls 2010; Oswald et al. 2011).  
67 Proactive programs of monitoring for resistance to Bt crops involves the screening of field populations via lengthy laboratory based bioassays  
68 (Tabashnik 2015).

69 Currently in Europe corn rootworm beetles still respond favourably to the relatively straight forward cultural control method involving the  
70 annual rotation of corn usually with soybean known simply as ‘crop rotation’. Crop rotation acts to break the lifecycle of beetles as the eggs laid  
71 in a rotated corn field will hatch in fields planted with different crops (usually soybean) where larvae are unable to survive (Kiss et al. 2005).  
72 Crop rotation was successful in some areas of the USA until ca. mid-1990 when beetles developed resistance through a behavioural adaptation  
73 by laying eggs in a non-host crop (Levine and Oloumi-Sadeghi 1996; Sammons et al. 1997; Levine et al. 2002). Knolhoff et al. (2006)  
74 demonstrated that rotation resistance was an inherited trait, while Onstad et al. (2001) suggested that a single locus model with an allele for

75 reduced fidelity to maize was responsible for the evolution of the variant beetle's behaviour. Crop rotation as a method of control is still effective  
76 in Europe because farmers there use a more diverse array of cropping systems (Kiss et al. 2005), rotate more often with a greater variety of  
77 crops, and frequently utilize relatively smaller fields compared with farmers in the US who operate on much larger scales (Gray et al. 2009). The  
78 utilization of relatively small fields rotated with various crops enables crop diversity, thus reducing the selection pressure of soybean-maize crop  
79 rotation to the beetles. In contrast to other rootworm resistant variants, there is currently no simple assay available to identify rotation resistant  
80 rootworm variants. Rather, studies use emergent cages to sample rotation resistant variants that survive on non-host crops or simply sample  
81 beetles from areas that are known to be the epicentre of rotation resistance (eg. Gray et al. 2009; Chu et al. 2013; Mikac et al. 2013).

82 It has become apparent that ongoing introductions of rootworm into Europe will eventually result in the introduction of individuals and their  
83 associated alleles that are resistant either to insecticides, crop rotation or Bt toxins. Onstad et al. (2003) predicted that from ca. 2017 onwards,  
84 rootworm could independently evolve a loss of ovipositional fidelity to maize in Europe, even in the absence of accidental inter-continental  
85 introductions of resistant alleles. At present no such alleles or resistant beetles have been detected in Europe, though methods of monitoring and  
86 detection must be improved to facilitate the ongoing and rapid surveillance (and reporting) of resistance.

87 Consequently, for contingency planning and associated risk management there is an ongoing imperative to seek out resistance monitoring  
88 techniques that are cost effective, do not require expert equipment or analysis and that can assist scientists and pest managers alike in the  
89 surveillance and bio-monitoring of resistance. Mikac et al. (2013 and 2016) have reiterated the importance of this need and investigated how  
90 changes in corn rootworm wing shape and size, using geometric morphometrics, can be used *in lieu* of more expensive and time consuming  
91 genetic techniques as a population biomarker. Thus far it has been repeatedly demonstrated that hind-wing shape and size differences can act as  
92 biomarkers (Benitez et al. 2014, 2014a; Lemic et al. 2014; 2016; Mikac et al. 2016) and can be used to discriminate between and among crop  
93 rotation resistant populations sampled from the epicentre of rotation resistance in the USA (Mikac et al. 2013).

94 Here we extend the use of hind-wing size and shape differences to examine changes in corn rootworm as related to the development of  
95 resistance, specifically investigating possible differences among rotation resistant, Bt-maize resistant and non-resistant (or susceptible)  
96 populations in the USA. The consequences of changes in beetle wing shape and size are discussed in relation to their long distance flight and  
97 dispersal capabilities (ie. possible spread of resistant alleles). Also, discussed is how hind-wing morphology can be used as an inexpensive and  
98 accessible population biomarker for corn rootworm resistance detection globally.

## 99 **Materials and Methods**

100 The hind-wing morphology of 358 individual rootworm from various populations across the US Corn Belt (Fig. 1) was measured. Bt-maize  
101 resistant (Gassmann et al. 2011) and susceptible insects were obtained from two laboratory colonies maintained at the United States Department  
102 of Agriculture, North Central Agricultural Research Laboratory (NCARL) in Brookings, South Dakota. Rotation resistant western corn  
103 rootworms were collected in fields with documented resistance (J. Spencer, Univ. Illinois, pers. comm.).

104 The individuals sampled were subjected to geometric morphometric procedures and analyses based on hind-wing venation as described below.  
105 The populations investigated comprised resistant (ie. crop rotation and Bt-maize) and non-resistant populations with subsequent shape data  
106 analyses divided into: 1) resistant versus non-resistant populations; and 2) rotation resistant versus Bt-maize resistant (Fig. 1).

107 Left and right hind wings were removed from each individual and slide mounted using the fixing agent Euparal (Australian Entomological  
108 Supplies, Melbourne, Australia) based on standard methods (Upton and Mantel 2010). Slide-mounted wings were photographed using a Leica  
109 DFC295 digital camera (3 megapixel) on a trinocular mount of a Leica MZ16a stereo-microscope and saved in JPEG format using the LEICA  
110 APPLICATION SUITE, version 3.8.0 (Leica Microsystems Ltd). Fourteen type 1 landmarks defined by vein junctions or vein terminations were  
111 used (Bookstein, 1991). The type 1 landmarks used in this study represent the ideal characteristics of type 1 landmarks in that they are  
112 anatomical loci that are homologous and are reliable and repeatable in their position on the specimen of interest, ie. vein intersections on  
113 rootworm hind wings (see also Fig. 1: Mikac et al. 2013).



## 114 **Shape Analysis**

115 Fourteen landmarks previously established for the corn rootworm (Benitez et al. 2014) were digitized using TPSDIG v 2.16 (Rohlf 2016). The  
116 landmark coordinates were obtained and the shape information extracted using a full Procrustes fit (Rohlf and Slice 1990; Dryden and Mardia  
117 1998; Klingenberg 2011). To test for and thus avoid measurement error that may occur via inaccurate digitizing, a Procrustes ANOVA of 30 left  
118 or right hind-wings was undertaken (asymmetry was not shown for this species: Mikac et al. 2013). Hind-wings were digitized twice and values  
119 of the mean squares (MS) from the ANOVA between the error 1 component of variation and individuals compared (Klingenberg and McIntyre  
120 1998).

121 Principal component analysis (PCA) was used to visualize hind-wing shape variation related to the development of resistance (Jolliffe 2002).  
122 The PCA was based on the covariance matrix of individual hind-wing shape. In order to visualize the average change of the crop rotation versus  
123 Bt-maize resistant strains, a covariance matrix of the average data (for all specimens irrespective of sex) was created. A PCA was used for the  
124 better visualization of shape morphospace (Klingenberg 2013). To compare the morphological relationships among resistant (Bt-maize and field  
125 versus laboratory rotation resistant populations) and non-resistant populations a Canonical Variate Analysis (CVA) was performed in order to  
126 extract morphological distances.

127 To determine if there were significant differences among the three beetle population variants, a Procrustes ANOVA was performed. Finally, a  
128 multivariate regression of shape versus centroid size was undertaken to confirm if size had an allometric effect (Monteiro 1999). All the  
129 aforementioned statistical analyses were performed using MorphoJ v1.06d (Klingenberg 2011).

## 130 **Results**

131 The MS of Procrustes ANOVA between the individual and the error values indicated that measurement error (MS=0.000007) was less than  
132 individual error (MS=0.000074); overall this had a negligible influence on the results.

133

134 The PCA of the variation of hind-wing shape between the two resistance variants showed that more than half of the variation was explained by  
135 the first three PCs (PC1 = 27.6 %; PC2 = 18.3 %; PC3 = 11.6 %). After averaging the data by rootworm variant, clear differences were found  
136 between Bt-maize resistant and rotation resistant beetle wings (Fig. 2). Across all three beetle variants these differences were mostly driven by  
137 movement of landmarks 8, 9 and 14 resulting in shorter and wider wings, whereas in comparison non-resistant beetles had a more elongated  
138 wing shape (Fig. 3). Rotation resistant beetles had the shortest and widest wing shape of all three types of beetles, resulting from a larger  
139 expansion of landmark 14. Similar variation of this section of the wing was also found for Bt-maize resistant beetles; nevertheless these  
140 individuals displayed more changes related to the structural portion of the wing for landmarks 4, 10 and 11 (Fig. 3). Overall, two different  
141 morphotypes (and wing shapes) were found for the beetles variants investigated. The CVA showed minimal differences in relation to field  
142 versus laboratory reared rotation resistant populations (Fig. 4) justifying further analyses where these populations were treated collectively as a  
143 single rotation resistant treatment.

144 The multivariate regression showed that shape variation was not influenced by allometry, in that only 1.75% of shape differences were predicted  
145 by size. Consistent with previous results presented here, the Procrustes ANOVA for centroid size and shape variation further confirmed  
146 statistical shape differences found between resistant and non-resistant beetles (Table 1).

147

## 148 **Discussion**

149 A reliable pattern of difference in hind-wing wing shape related to resistance type was shown in this study. Morphological differences exist in  
150 the hind-wing shape of both rotation and Bt-maize resistant versus non-resistant populations and rotation resistant versus Bt-maize resistant  
151 strains of western corn rootworm from the USA. Difference in wing shape as related to rearing method (laboratory versus field collected rotation  
152 resistant samples) was explored as it can effect wing morphology principally when the properties of the rearing system are related directly to the  
153 developmental stability of the organism (Gerard et al. 2018). However, the minimal differences found between rotation resistant laboratory

154 versus field collected populations suggests that rearing system was not the main driver of the small differences observed; rotation resistant  
155 samples were thus further investigated by treatment type. Overall, the largest difference in wing shape was noted between non-resistant beetles  
156 and the two resistant beetle variants examined. In general, the hind wings of non-resistant beetles were significantly more elongated in shape and  
157 narrower in width (chord length) in comparison to beetles that were resistant to Bt-maize or crop rotation. Such differences may impact upon the  
158 dispersal or long distance movement of resistant and susceptible beetles considering the fact that wing morphology is a critical element of an  
159 insect's dispersal capacity (DeVries et al. 2010). Understanding which beetle morphotype is the superior flier and disperser (with the best chance  
160 of spreading resistant alleles long distance) has implications for the way in which beetles are managed via integrated resistance strategies.

161 Flight studies of winged animals (bats, birds and insects) have demonstrated that selection pressure from migration distance, sexual selection,  
162 foraging strategies and geographic clines can drive changes in wing shape (eg. common starling, *Sturnus vulgaris*: Bitton and Graham 2015). It  
163 is known that dispersal ability in a winged animal is related to body size, wing loading (function of body mass ratio to wing area) and wing  
164 shape as shown, for example, for monarch butterflies, *Danaus plexippus* (Altizer and Davis 2010). Of functional importance to flight is the  
165 aspect ratio which is the length of the wing relative to its width (chord length). The general principle of aspect ratio is that high aspect ratio  
166 wings are characterised as longer and narrower (thinner) wings, whereas low aspect ratio wings are characterised as being shorter and wider in  
167 shape. Bird species that undertake long distance migration are known to have a higher wing aspect ratio with a longer and more slender wing  
168 shape compared to non-migrating species, such a wing shape results in a faster and more energetically efficient flight (Mönkkönen 1995). This  
169 pattern appears to be reversed in some insect species, where by low aspect ratio wings are more important for long distance and dispersal related  
170 flight. In the speckled wood butterfly, *Pararge aegeria* recently founded populations have lower aspect ratios (Hill et al. 1999) and this is also  
171 true for some *Drosophila* species (Azevedo et al. 1998; Bhan et al. 2014). In our study Bt-maize and rotation resistant beetles both had shorter  
172 and wider wings suggesting a lower aspect ratio, while susceptible beetles (non-resistant) had much longer and narrower wings and thus possibly  
173 a higher aspect ratio. Given that such obvious intraspecific variation in wing shape was found from the corn rootworm collected in this study  
174 within a relatively short time period (within 3 generations) and from similar latitudes as one another, it is unlikely that the significant difference

175 found is being driven by a large scale difference in latitude (eg. *Calopteryx* damselflies: Outomuro and Johansson 2011), pronounced temporal  
176 differences or as a result of a geographic cline (eg. tsetse fly, *Glossina palpalis gambiensis*: Bouyer et al. 2007). Understanding which wing  
177 shape is most efficient in assisting long(er) distance movement and dispersal in rootworm beetles is important because it is likely that the  
178 associated genotype will spread over a larger distance in a shorter period of time. Given these important results, a more detailed understanding of  
179 the aspect ratio including the wing geometry of the rootworm investigated here is warranted and should be the focus of future work.

180 Mikac et al. (2013) was the first study to investigate wing shape and size differences in rotation resistant and susceptible beetles and  
181 demonstrated clear phenotypic differences between the beetle types. Similar to our current findings, Mikac et al. (2013) showed that rotation  
182 resistant beetles also had broader wings (cf. susceptible beetle). In Mikac et al. (2013) it was hypothesized that the unsuitability of soybean as a  
183 nutritional source necessitated the longer distance flight related to in-season long inter-field flights back and forth between soybean and maize  
184 fields, compared to susceptible beetles that usually fly shorter distances within continuous maize fields. Though again this warrants further  
185 research to understand the importance that wing geometry has on the rootworm's ability to disperse short and long distances.

186 In this study we have demonstrated evidence of distinct wing shapes related to resistance development and in future studies we will concentrate  
187 on testing European populations for resistance using wing shape as a biomarker. Future work, as well, will investigate corn rootworm  
188 intraspecific flight morphology, in addition to modelling wing structure and flight efficiency of wing shape differences based on crop resistance  
189 using finite element analysis eg. Jin et al. (2010). A deeper understanding of rootworm wing shape and flight morphology, wing geometry,  
190 aspect ratio and flight efficiencies will assist with discerning which resistant phenotypes are most likely to invade geographic areas where they  
191 not yet present (ie. rotation resistant beetles entering Europe where such variants are absent). Such information is crucial to biosecurity measures  
192 and resistance management strategies for the western corn rootworm globally.

193 Findings from this study support our previous work (Mikac et al. 2013 and 2016) and highlight the importance of wing shape as a reliable, cheap  
194 and yet effective biomarker for resistance monitoring in corn rootworm. The wing shape analyses undertaken here should be extended and

195 applied to other well-known pest species to confirm whether discernible wing shape patterns can also be found for resistance (eg. Colorado  
196 potato beetle: Grapputo et al. 2005; Codling moth: Franck and Timm 2010). The results of this study have important implications for ongoing  
197 rootworm monitoring and management, particularly where genetic capabilities and monetary investment in such techniques may not be feasible  
198 (eg. in developing countries). Here we have demonstrated an affordable and accessible technique that reliably demonstrates that hind-wing shape  
199 and size differences can be used to reveal differences among rootworm populations, and thus can be used as a biomarker for resistance detection  
200 as part of a larger integrated resistance management strategy for the species.

201

## 202 **Compliance with ethical standards**

## 203 **Conflict of interest**

204 The authors declare that they have no conflict of interest.

## 205 **Ethical approval**

206 Western corn rootworm is an established pest of maize in USA and Southern Europe. No special permission was needed for its collection in this  
207 study.

208

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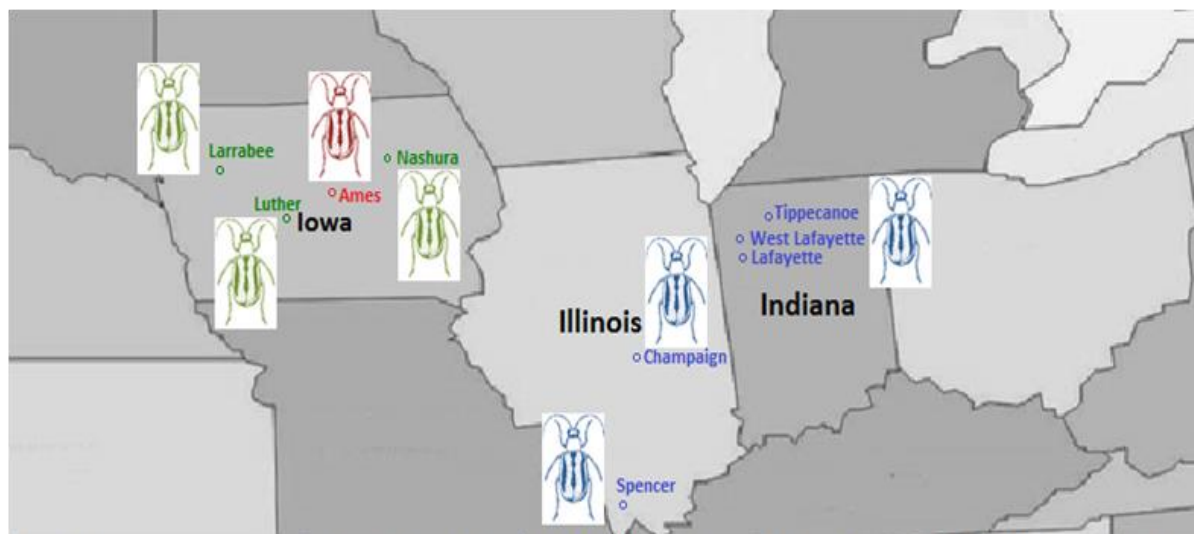
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323

324 **Figures**



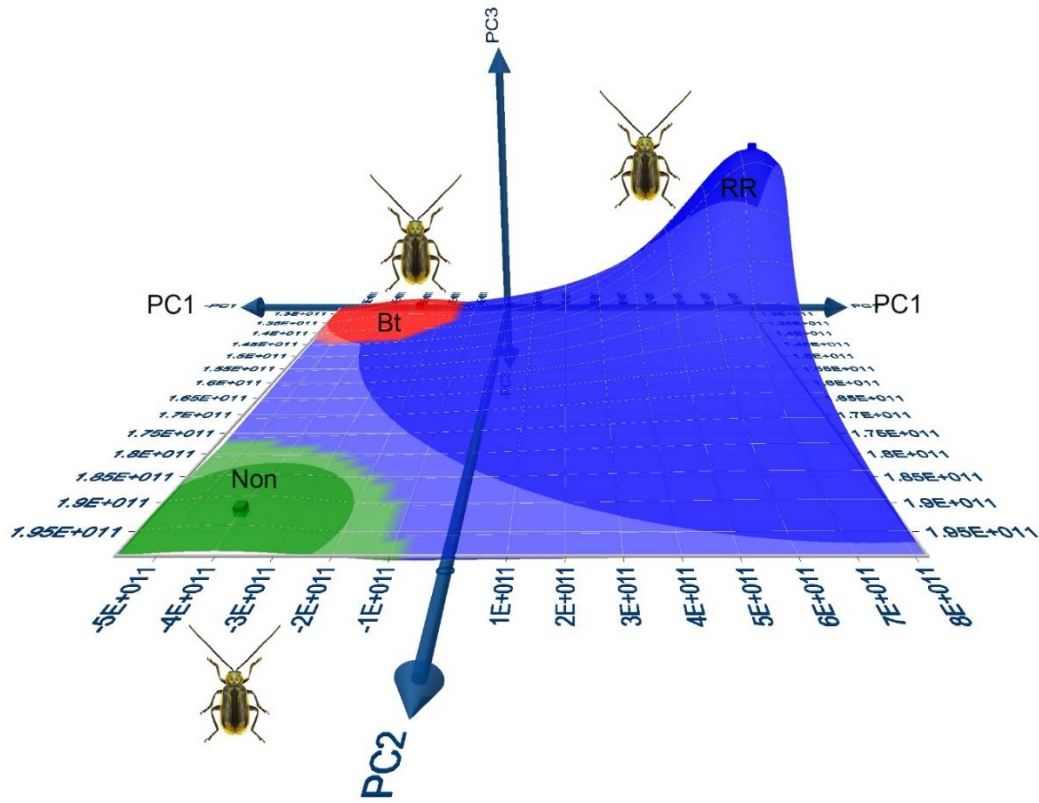
Location	No. of samples	Males/Females	Year of sampling	Resistance status
Luther, Iowa	120	30/30	2011	NON
Nashua, Iowa	120	30/30	2011	NON
Larrabee, Iowa	82	11/30	2011	NON
Ames, Iowa	86	13/30	2012	Bt resistant - field
Lafayette, Indiana	36	4/14	2012	RR - field
West Lafayette, Indiana	60	15/15	2014	RR - lab
Tippecanoe, Indiana	60	15/15	2014	RR - lab
Champaign, Illinois	60	15/15	2014	RR - lab
Spencer, Illinois	60	15/15	2014	RR - lab

325

326 **Fig. 1** The collection sites and resistance variants of adult western corn rootworm collected from the US Corn Belt in areas of known Bt-maize  
 327 and crop rotation resistance (Gray et al. 2009). Types of resistance were: NON (susceptible variants); RR (rotation resistant variants); Bt (Bt-  
 328 maize resistant variants).

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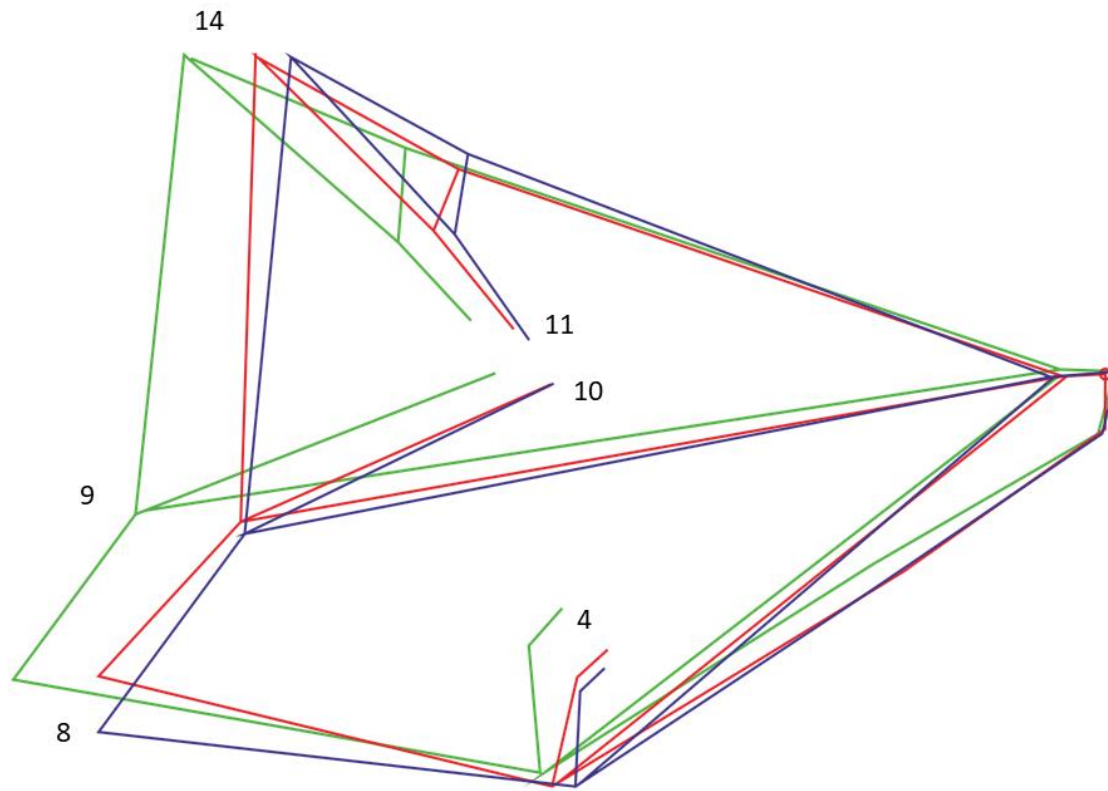


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333 **Fig. 2** Three-dimensional volume dispersion graph of the average shape for the three western corn rootworm groups based on resistant variant.  
334 The volumes simulate the shape space of the wing shape variation found. Red: Bt-maize resistant; Blue: rotation resistant; Green: non-  
335 resistant/susceptible.

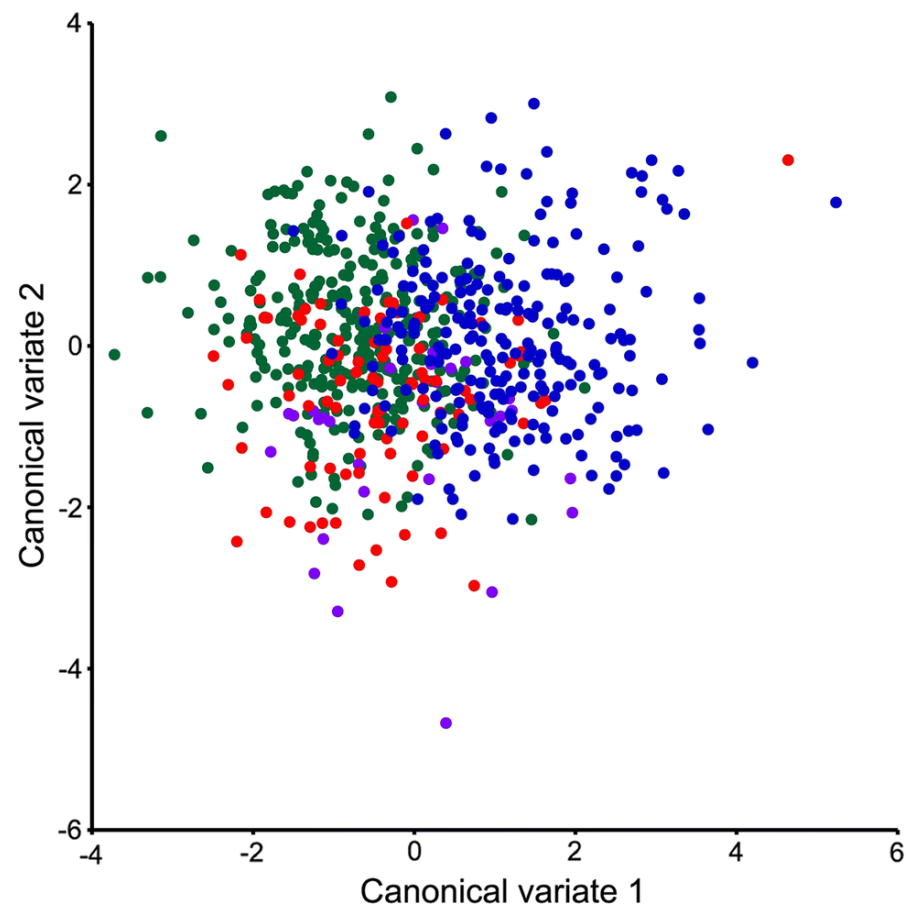
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338 **Fig. 3** Wireframe visualization of the average shape for different resistance type of western corn rootworm: Bt-maize resistant (red); rotation  
339 resistant (blue); non-resistant (green).

340



341

342

343 **Fig. 4** CVA visualization of hind-wing shape differences for western corn rootworm: Bt-maize resistant (red); rotation resistant-laboratory reared  
344 (blue); rotation resistant-field collected (purple); non-resistant (green).



345

346 **Table 1.** Procrustes ANOVA for both centroid size and shape using resistant type as a factor for western corn rootworm (dimensionless).

347 Characterized by matching symmetry. Sums of squares (SS) and mean squares (MS) are in units of Procrustes distances

348

<b>Centroid size</b>							
Effect	SS	MS	df	F	P	Pillai tr.	P(param) <sup>349</sup>
Resistant Type	2.334542	1.167271	2	5.34	0.0052		
Individual	70.821146	0.218584	324	6.27	<0.0001		
Side	0.000016	0.000016	1	0.00	0.9830		
Ind * Side	11.288637	0.034841	324	2171.70	0.0171		
Error 1	0.000016	0.000016	1				
<b>Shape</b>							
Effect	SS	MS	df	F	P	Pillai tr.	P(param)
Resistant Type	0.035879	0.00075	48	9.7	<0.0001	0.59	<0.0001
Individual	0.59937450	0.0000770801	7776	0.89	10.000	17.44	<0.0001
Side	0.00111131	0.0000463047	24	0.54	0.9681	0.16	0.0004
Ind * Side	0.67131634	0.0000863318	7776	1.73	0.0506		
Error 1	0.00119719	0.0000498829	24				