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Describing Shapes of the Wings of the Mango Leafhopper, *Idioscopus Clypealis* (Lethierry) Collected from Different Orchards

Mark Ronald S. Manseguiao[1], Cesar G. Demayo[2]*

[1] *Institute of Education, Davao del Norte State College, Panabo City, Philippines*

[2] *Department of Biological Sciences, Iligan Institute of Technology, Mindanao State University, Philippines*

ABSTRACT

Farm management methods have always used pesticides to mitigate the pests and improve the crop yields. This study investigated the population structures of Mango leafhopper, Idioscopus clypealis *in mango orchards unsprayed or sprayed with pesticides. The analysis of the populations was based on the wing shapes used by insects to fly. Geometric and morphometric techniques were used to analyze the shapes of the forewings of the pest. The results of the thin plate spline image showed asymmetry in the left and right wings in the two sexes of the insect pest. The relative warp analysis showed that wing shapes of insects in the unsprayed orchard had a mean shape close to the consensus wing shape unlike those populations where there was a routine application of pesticides. The differences in wing shapes of the populations collected from unsprayed and sprayed orchards were argued to be due to the effects of pesticides.*

Keywords: Thin-plate Spline, asymmetry, pests, mitigate, yield, geometric morphometrics

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Corresponding author: Cesar G Demayo
E-mail ✉ cgdemayo @ gmail.com
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INTRODUCTION

Pesticides have been used on crops to control the populations of insect pests from attacking the plants [1, 2]. These pesticides can be artificially produced or distilled from the biological products with differing modes of action and application [3-6]. However, they have a concurrent effect on the pest populations particularly in the surviving populations especially in monocultures such as mango orchards.

Resistance in insects has been observed leading to the development of new insecticides [3]. Despite an array of chemicals in use, pests continue to develop resistance worldwide [4, 7, 8]. Pests feeding on transgenic crops have also been observed with the increased resistance to the toxins [9, 10]. These surviving populations have had physiological and behavioral adaptations to the chemicals which may then in turn be passed on to the next generation [11, 12]. Avoidance from chemicals has also been possible where the detection might lead to a migration to other compatible plants [13-15]. The mobility of the insect and the application of pesticides can influence the direction and rate of the evolutionary change in the pests [16] thus this study was conducted to examine the changes in wing shapes in the mango leafhopper in mango orchards managed with or without pesticides. The wing as the agent of mobility can measure the changes affected by pesticides during wing development [17, 18]. Geometric and morphometric techniques were employed since this tool can identify the variation in the wing shapes as it relates to the effects of pesticide on the insect population structure in response to the use of pesticides in mango orchards [19, 20]. The study of changes or variations in the wing shape may lead to a clearer understanding of the nature of the pest infestations, thus may help in the formulation of better management strategies to prevent or mitigate the emergence of resistant strains in the pest.

MATERIALS AND METHODS

Sample Collection

Mango orchards were visited in 4 geographical locations in the Visayas and Mindanao (Table 1). A clear plastic bag with approximately 2ml of ethanol was used to capture the mango leafhoppers on the leaves and branches by encasing them quickly with the bag then removing it slowly. Only leafhoppers in the lower fringes of the tree were collected. The leafhopper became entrapped by the liquid. The specimens were then transferred into a small plastic container with ethanol as a preservative.

Table 1. Location of sampling sites.

Location	Code	Remarks	Latitude	Longitude
Brgy. Sapad, Kapatagan, Lanao del Norte	A	20-40-year-old, Chemical pesticides	7°52'23.1"N	123°46'31.1"E
Sibunag, Guimaras	B	Century-old trees, untended unsprayed	10°29'04.8"N	122°38'49.5"E
Guimaras Wonders Farm, San Lorenzo, Guimaras	C	10-20-year-old, organic pesticides	10°37'50.8"N	122°36'42.2"E
Lacida Farm, Brgy. Buru-un, Iligan City	D	20 years old, 2-3 years unsprayed, sprayed neighbor orchards	8°10'54.6"N	124°10'17.7"E

Identification and Dissection

The specimens were identified using the guide by [21]. Male and female specimens were separated (Fig. 1). The forewings were dissected by teasing the wing from the thorax using needles separating left and right forewings. Each wing was then mounted into a glass slide with a small drop of glycerin.

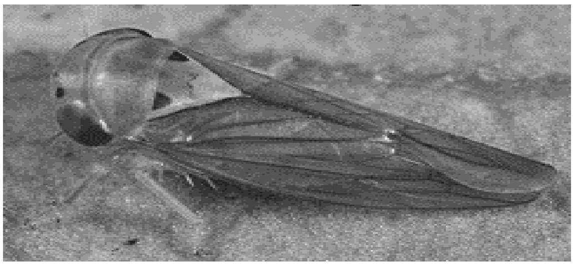

Fig. 1. *Idioscopus clypealis*

Image Acquisition and Landmarking

Images of each wing was acquired using a digital camera attached to a stereomicroscope. The images were then replicated three times. Eighteen (18) landmarks were selected using the venation of the wing (Fig 2; Tab. 2). These landmarks were selected to indicate vein junctions in the wing. The tps Utilility program v1.44 software [22] was used to create an image directory where the landmarks could be superimposed using tpsDig v2.12 [23].

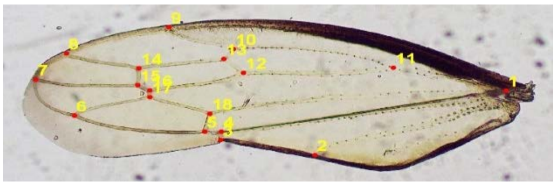

Fig. 2. Landmarks used on the wing of *Idioscopus clypealis*.

Table 2. Descriptions of anatomical landmark points on the wing of *Idioscopus clypealis*.

Landmark no. (LM)	Description	Landmark no. (LM)	Description
1	Proximal end of Cubitus and Median vein	10	Proximal Radial vein 1
2	Distal Anal vein 1	11	Proximal end of anterior and posterior Radial vein
3	Posterior end of Cu vein	12	Posterior end of Radial crossvein
4	Cross vein from LM3 to Cubitus vein	13	Anterior end of Radial cross vein
5	Distal end of Cubitus vein	14	Proximal Radial vein 2
6	Distal Radial vein 4	15	Proximal Radial vein 3
7	Distal Radial vein 3	16	Distal end of posterior Radial vein
8	Distal Radial vein 2	17	Distal end of Radial and Median cross vein 2
9	Distal Radial vein 1	18	Distal end of Median vein

Statistical Analysis

Raw landmark coordinates were extracted and Procrustes-transformed using PaST v2.15 [24] to standardize the dataset [25]. Procrustes transformation removed the variation of the landmarks due to the digitizing location, orientation and scale projecting the image into a common coordinate system [26]. Thin plate splines were then generated to observe the mean shape of the wing within the populations.

The relative warp analysis was done to determine the within-group variability [26, 27]. [28] posited that this method can identify the major trends of variations among the specimens within a sample as the deformations of shape, by creating a space where the landmark coordinates are superimposed, and it can create a standardized shape compared to the samples. Relative warp program v1.46 [29] was used to provide the analysis and visualization of the landmarks. The analysis of the variance of the relative warps was computed to verify the significant differences in the populations visualized through the canonical variate analysis. The distribution of the populations was visualized using histograms and boxplots generated through PaST v2.15 [24].

RESULTS AND DISCUSSION

The nature of variations in the wing shapes have been graphically shown as the thin plate splines where the contraction and expansion of the wing landmarks to each other were located. The contractions were denoted as blue while, the expansion was denoted by red in the deformation grids. The shapes of the insect wings from four locations were observed that the location of wing landmarks across the populations was not uniform (Fig. 3). These observations were done on the left and right wings in all populations of the insect pest. No similarity between the male and female wings was also observed indicating the dimorphism in the shapes of the wings.

The multivariate analysis of variance of the relative warp scores generated from the different landmarks from the wings of insects collected across the different mango orchards, showed that there were significant differences (Tab. 3). The differences have been graphically shown by the distribution of individuals in the scatter plot generated from CVA where it could be shown that the variations in wing shapes between the populations were attributed to the presence of individuals outside the overlap (Fig. 4).

Relative warp analysis showed the shape and distribution of individual shapes in all the populations of the insects from the different orchards (Figs. 5 and 6). Three significant relative warps explained that the variations in wing shapes were considered as the basis for the comparison of the different populations of the insects. Of the three warps, the first relative warp explained about 60% of the variance.

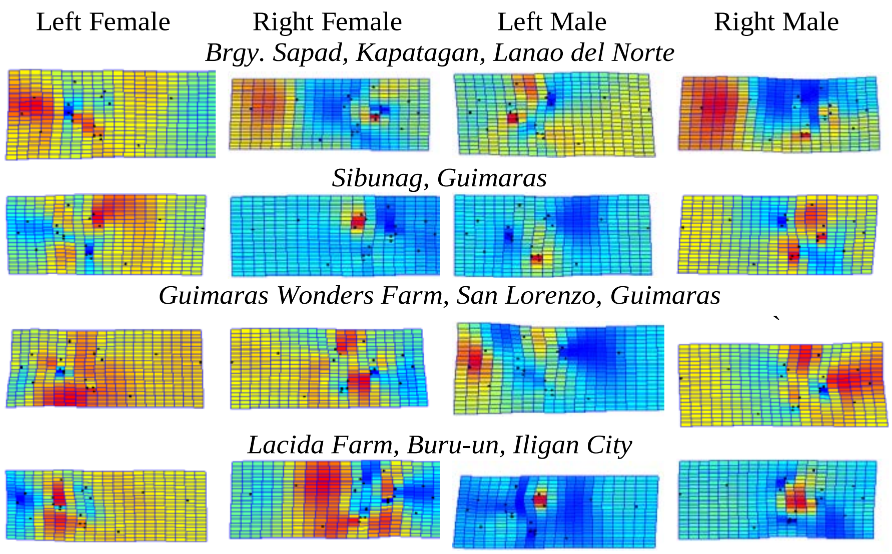

Fig. 3. Thin plate splines of the wing landmarks across the sample locations. Blue areas denote the contraction between landmarks and Red areas denote the expansion between the landmarks

Table 3. Multivariate analysis of relative warp scores across four different modes of pesticide use.

Source of Variation	Wilks λ	F	p(same)
Left Wing Female	03684	17.53	5.094×10^{-237}
Left Wing Male	0.2996	21.17	3.582×10^{-285}
Right Wing Female	0.3771	16.62	2.707×10^{-223}
Right Wing Male	0.3028	21.54	1.158×10^{-291}

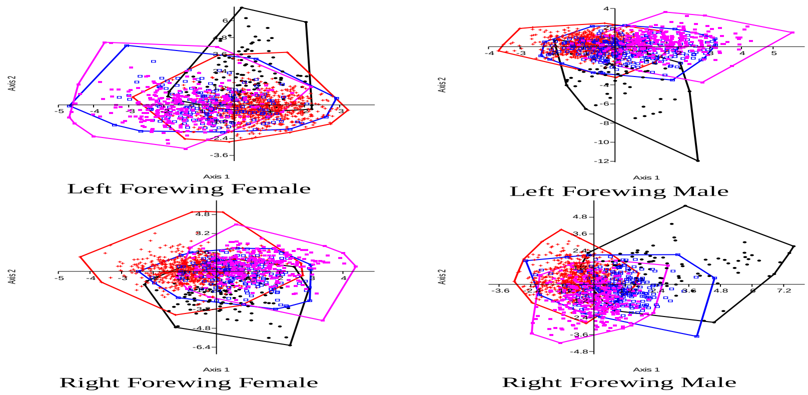

Fig. 4. Morphological spaces of the first two canonical variables (CV)1 and 2 originated from the comparison of the forewing shape across all the four populations of of *Idioscopus clypealis*.

The comparison of the wings of male insects in the populations collected from different mango orchards showed three significant warps that would account for the variations observed (Fig. 5). The first relative warp explained 59.66% of the variance. This variance was exhibited by the landmarks LM10-13 and LM15-17 among the others. Wing shapes of female insects from the population A was towards the negative first relative warp shape. The rest of the samples were near the consensus shape. The B and D populations had relatively the same mean shape.

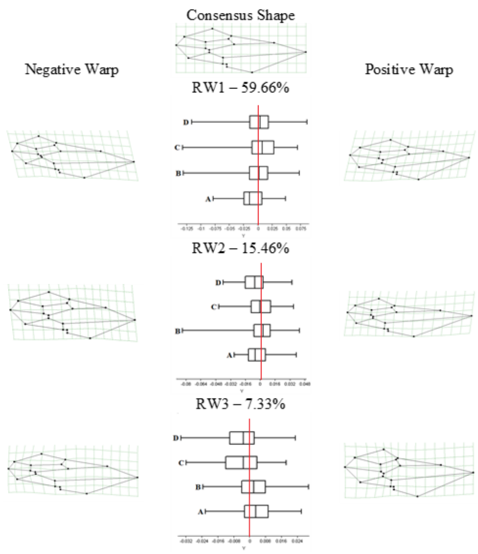

Fig. 5. Relative warp scores of the female left forewing and the distribution of the wing shape from 4 orchards with different modes of pesticide use. (A – Brgy. Sapad, Kapatagan, Lanao del Norte, B – Sibunag, Guimaras, C – Guimaras Wonders Farm, San Lorenzo, Guimaras, D - Lacida Farm, Brgy. Buru-un, Iligan City)

Considering the shapes of the male forewing, three significant warps were also observed (6) where the first relative warp explained 61.32% of the variance observed among the male populations. The variations were observed in the distances between the medial points from the perimeter points. Population A had its landmark points moving towards the positive warp of the first relative warp. Population B and D were near the consensus shapes, while population C was slightly towards the negative warp (Fig. 6).

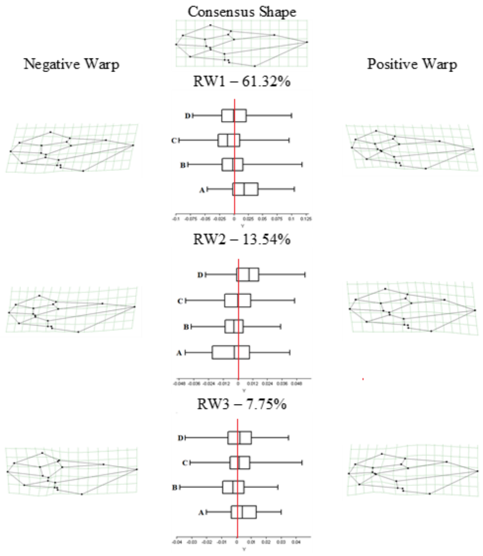

Fig. 6. Relative warps analysis of the males' left forewing and the distribution of the wing shape in 4 orchards with different modes of pesticide use. (A – Brgy. Sapad, Kapatagan, Lanao del Norte, B – Sibunag, Guimaras, C – Guimaras Wonders Farm, San Lorenzo, Guimaras, D - Lacida Farm, Brgy. Buru-un, Iligan City)

Relative warp analysis of the shapes of the right female forewings also showed three significant warps (Fig. 7) where the first warp had a value of 56.89% explaining that the variation was observed in landmarks LM9-10 and the LM15-17 among the other points. The mean shape of population B was towards the negative relative warp shape, whereas the other three population leant toward the positive relative warp shape (Fig. 7).

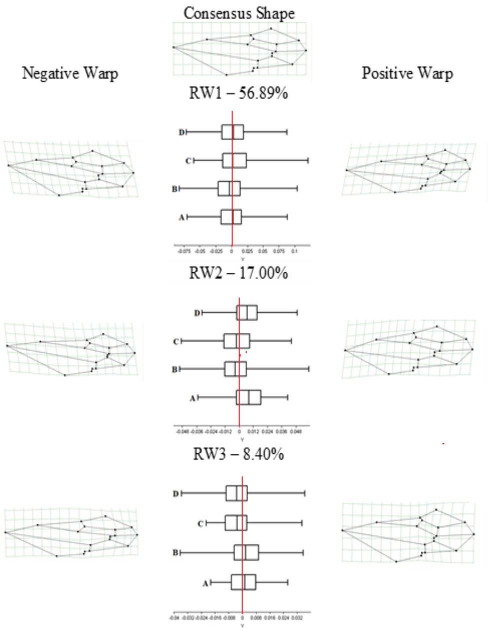

Fig. 7. Relative warps analysis of the right forewing of females and the distribution of the wing shape in 4 orchards with different modes of pesticide use. (A – Brgy. Sapad, Kapatagan, Lanao del Norte, B – Sibunag, Guimaras, C – Guimaras Wonders Farm, San Lorenzo, Guimaras, D - Lacida Farm, Brgy. Buru-un, Iligan City)

Considering the shapes of the right forewing of the male insects, three significant relative warps were observed with the first relative warp accounting for 59.60% of the observed variations at landmarks LM 9-10 and LM 15-17 among the other points. The mean shape of the right wings of insects in populations A and D was towards the positive relative warp, whereas in the population B and C, it was towards the negative relative warp.

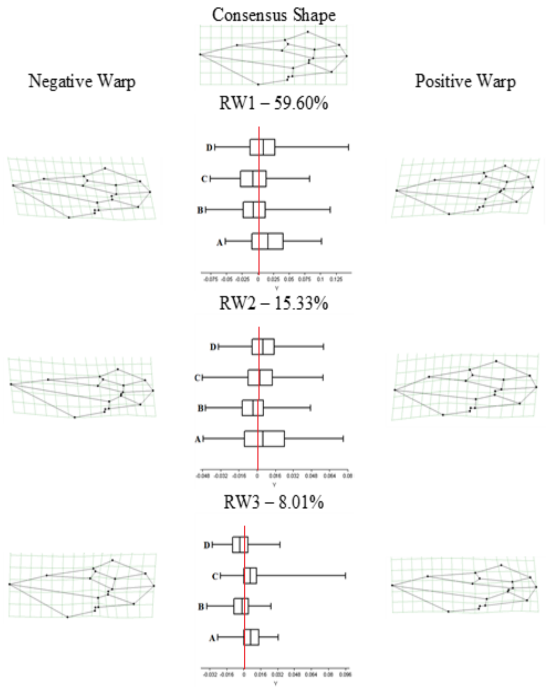

Fig. 8. Relative warps analysis of the right male forewing and the distribution of the wing shape in 4 orchards with different modes of pesticide use. (A – Brgy. Sapad, Kapatagan, Lanao del Norte, B – Sibunag, Guimaras, C – Guimaras Wonders Farm, San Lorenzo, Guimaras, D - Lacida Farm, Brgy. Buru-un, Iligan City)

The relative warp analysis of the different wings considering different sexes in different populations collected from mango orchards from different locations in Visayas and Mindanao, Philippines, was done. Mango leafhoppers showed significant differences in the shapes of the wings. It was interesting to note from the results that Population B which has been untreated with pesticides, had its mean wing shape close to the calculated wing shape. Organic pesticides might have a direct contribution to the variation between the wing shapes of the insects from pesticide-treated orchards when compared to the untreated ones. [30] explained that the shape variations as shown by the differences in the directions of the movement of landmarks can be attributed to the differences in the wingbeat, and the force exerted on them may have been affected and actively selected by the pesticide use.

CONCLUSION

This study was conducted to describe the variability in the shapes of the wings within sexes, within and between the populations of the mango leafhopper, *Idioscopus clypealis* collected from different mango orchards located in different places in Philippines. The mango leafhoppers in orchards untreated with pesticides were shown to have wing shapes that were similar as the consensus wing shapes unlike those populations where there was a routine application of the pesticides. The variations within and between the sexes and populations in both treated and untreated orchards can be attributed to the developmental instability of the asymmetric shapes between the left and right wings.

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