

## CHARACTERISTICS OF POLYMORPHISM FOUND IN ADULTS FROM POPULATIONS OF *DIABROTICA VIRGIFERA VIRGIFERA* IN DIFFERENT MAIZE HYBRIDS

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**Abstract.** The species *Diabrotica virgifera virgifera* (Le Conte) is part of the Chrysomelide family of the Coleoptera order. Overall, the *Diabrotica* genus includes insects that prefer cucurbitaceae. In particular, beetles are adapted to differentiated feeding diet, so that *Diabrotica virgifera* feeds and survives predominantly in corn crops. In its living environment, the beetles are often seen on plants or flying, looking for food or in moments of copulation and feeding. However, at the level of morphology and taxonomy of adult form, the species of the *Diabrotica* genus are still confused, with a pronounced polymorphism. It is difficult to establish some genetic standards in identifying interspecific features. It is known that geographical locations can influence the ratio of individuals of a population, especially among individuals of the same species (males and females). Going on this point, in this paper we want to show whether there are or not morphological variations between adult insect populations, that have previously been collected from different places and diverse corn hybrids. Studies have been conducted on beetle populations from 7 different corn cultures. Crops were located in different locations (different localities from Timis and Arad County) in the western area of Romania. The collecting of the beetles was done manually, directly from plants (leaves and silk) in July and August, during of 2007 year. Detailed studies have been conducted at microscope. Later on, the beetles were divided into morphological categories. The targeted morphological characters were body length, antenna length, elytra coloration, and background color of the body. The observed results concluded that there are morphological differences between beetles from various populations but also between beetles belonging to the same population. Additionally, the variation of coloration has strengthened the fact that food color can imprint a similar nuance to the background color of the beetle and this is because feeding intensely on the same plants.

**Key words:** *Diabrotica virgifera*, polymorphism, insect populations, corn hybrids.

### INTRODUCTION

Populations of living organisms have the ability to change over time, depending on variability. These changes can also be characterized by the many forms an organism can have in various conditions. By definition, polymorphism is the ability of entities to take different forms or the ownership of some species to exist under several morphological aspects (according to Dex-online). As such, in the present work we have proposed to highlight this aspect of a species of Coleoptera, very little studied from this point of view. Broadly, research by MUSTAFA ET AL (2014) has shown that there are obvious structural variations between insect populations collected from different geographic locations. Thus, geographical locations can influence the ratio of individuals of a population, and especially among individuals of the same species (males and females).

Strictly speaking, the genus *Diabrotica* comprises insects that prefer cucurbitaceae, also called leaf beetles and sums up numerous species (KHRYSAN AND SMITH, 1987). There are 338 species (OEPP/EPPPO, 2004), some mention 335 species (KHRYSAN AND SMITH, 1986).

At the level of morphology, biology and ecology of adult form, the species of the *Diabrotica* genus can easily be confused. The most common confusions are between the species *Diabrotica virgifera virgifera*, *Diabrotica barberi*, *Diabrotica undecimpunctata howardi* (KHRYSAN AND SMITH, 1986). Another very similar species is *Acalymna vittatum*. It

has the most morphological characteristics identical to *Diabrotica virgifera virgifera* Le Conte, especially the females of this species. Most are found on the American continent, the continent of origin. Only *Diabrotica virgifera virgifera* is present in Europe, appearing much later on this continent in 1992 (VIDAL ET AL., 2005).

**MATERIAL AND METHODS**

**Experimental lots.** The research was carried out during 2017 and consisted of 7 experimental lots. Each lot was located in another locality, on the grounds of avoiding the mixing of individuals and individualizing a population of a culture at a given time.

The lots were included in two counties in western Romania, respectively Timis and Arad. At the establishment of the lots/locality the corn crop research techniques were considered. The number of rows varied between 8 and 16; most of the plots were evaluated for 16 rows with the exception of hybrids SY ZEPHIR and SY IRRIDIUM, with 8 rows evaluated. Each hybrid/locality was repeated four times (4 rehearsals).The identification of each lot was based on GPS coordinates (Table 3).

**The type of corn hybrids.** Corn hybrids, which constituted the base material from which beetles were sampled, were largely of the early (EE-extra-early and E-early); except for the hybrid DKC 5276, which is part of the semi-late SL (Table 3) hybrid category. Their choice was made on the basis of availability on the commercial areas.

Table 1

The type of maize hybrids and identification of research sites

Type of corn hybrids	Place/ County	Plot/ locality	FAO	Maturity groups	Producer	GPS coordinates
SY ZEPHYR	Timis	Nerau	390	EE	Syngenta	N: 45.976466 E: 20.567432
SY IRRIDIUM	Timis	Varias	380	E	Syngenta	N: 45.999448 E: 21.005185
SY ARIOSO	Timis	Grabat	300	E	Syngenta	N: 45.864602 E: 20.733524
P8523	Timis	Sannicolau Mare	270	E	DuPont Pioneer	N: 46.046170 E: 20.627468
DKC 5276	Timis	Jimbolia	470	SL	DEKALB	N: 45.816152 E: 20.703100
DKC 3811	Arad	Felnac	280	EE	DEKALB	N: 46.107296 E: 21.152720
SY RESPECT	Arad	Zabrani	170	EE	Syngenta	N: 46.062100 E: 21.564735

EE- extra-early; E-early; SL- semi late

**Taking samples.** The collection of the beetles was done manually, directly from plants (leaves and silk) in July and August, in sunny days. From each experimental plot, 50 beetles were collected. Specific containers and utensils have been used (figure 1).



Figure 1 Searching for beetles from the experimental lot of Nerau (left); containers with beetles from the hybrid lot of DKC from Jimbolia (middle); moment of collecting of beetles on the corn silk (right)

**Morphological identification.** At present there is no methodology for identification and determination. The only way to recognize and differentiate of beetles is related to the main characters that are related to the distinction between males and females.

There are some studies that highlight sexual dimorphism in adults-beetles. This can be done by comparing the size of the antenna segments or by observing the morphological differences of the terminal segments of the abdomen (BRANSON ET AL., 1975). In this respect, it is found that the female abdomen is bigger and sharper, and that of the male is smaller and rounded (GROZEA, 2002). Based on these aspects we started in the morphological identification (figure 2) of the individuals collected from the experimental fields (figure 1). Of course we also focused on secondary characteristics such as body length, elytra coloration and body background color.

Detailed studies were performed on a microscope (figure 2).

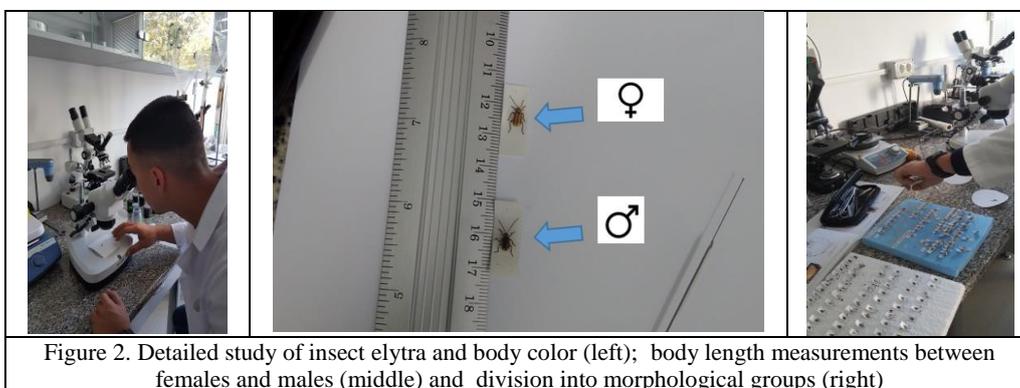


Figure 2. Detailed study of insect elytra and body color (left); body length measurements between females and males (middle) and division into morphological groups (right)

## RESULTS AND DISCUSSIONS

The results refer strictly to the populations of *Diabrotica virgifera virgifera* in the 7 experimental lots and not to the general aspect (Table 2). That is why it is necessary to pay attention to our own results as well as to the information in the literature.

Table 2

The numerical and sex ratio of beetles of *Diabrotica virgifera* collected from the experimental lots

No of lot	Type of hybrids	Total number of beetles collected	Total number of beetles analyzed*	Females analyzed	Males analyzed	Sex ratio
Lot 1	SY ZEPHYR	50	33	18	15	5:4
Lot 2	SY IRRIDIUM	50	35	16	19	4:5
Lot 3	SY ARIOSO	50	33	19	14	6:4
Lot 4	P8523	50	33	19	14	6:4
Lot 5	DKC 5276	50	39	22	17	6:4
Lot 6	DKC 3811	50	39	20	13	5:3
Lot 7	SY RESPECT	50	30	15	15	5:5

\* The females with eggs were eliminated on sorting

*Insect description in the literature.* The adult is a small coleopteran beetle, about half-cm, between 4 and 6 mm, usually with a yellow-green basis color. The female is bigger than the male and differs in color and shape (GROZEA, 2003). On its body, on elytra, darker longitudinal stripes are observed (HILL, 1975). The ventral part of the body is yellow with a

black spot. The pronot is well developed and has a rectangular shape, with larger width and length in female than in males. The mezonot scutelle has a triangular form in both sexes (FLORIAN ET AL., 2011).

**Results of intra-population and inter-population morphology.** Individuals collected were involved first under morphological studies related to body length and antenna length (figure 3, 4). Second, the elytra coloration, and background color of the body were analyzed.

For a correct interpretation, females that had their abdomen swollen and full of eggs (observation visible only on a microscope) were removed from the analysis and measurements.

In most experimental lots, the female showed higher body lengths than the male. The difference, however, is not visible macroscopically, only when they have the abdomen through the egg. The values of the evaluated individuals revealed a difference (at the minimum limit) of 0.015 mm. Females ranged between 4.385 and 6.80 mm and males between 4.20 and 6.60 mm. The explanation of lower values than those already mentioned in literature would be that only females without eggs were analyzed. The antenna measurements only targeted the right antenna of each individual, usually on the set of males and set of females analyzed separately. Thus, it was observed that all individuals presented 11 segments at each antenna, except when accidentally broken.

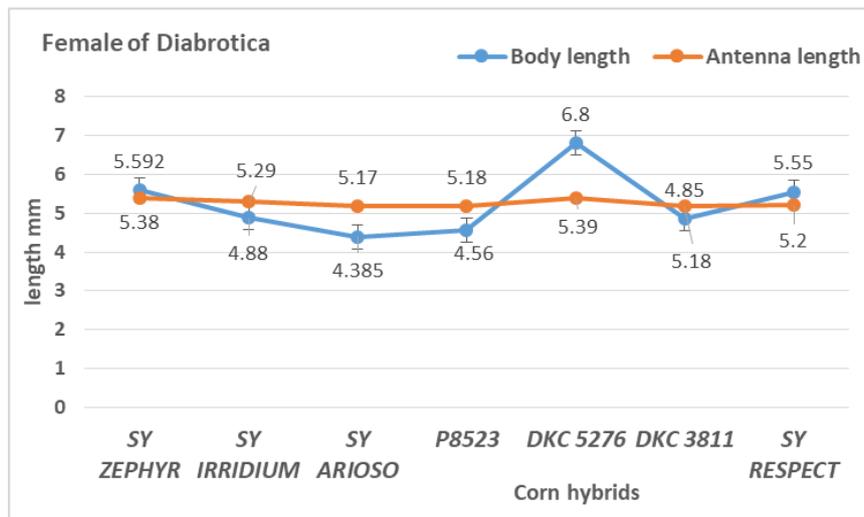


Figure 3 Average females body and antenna lengths of Diabrotica populations collected from lots of different maize hybrids

From the partial results it was found that there were differences between the length of the body and the antenna length of the analyzed individuals but at insignificant level, in the majority of analyzed populations. In female (figure 3), although the differences are visible, the values related to the length of the antennas relative to the body were insignificant in both the DKC 5276 (5.39/6.8 mm  $\pm$  0.19) hybrid and the Sy Arioso hybrid (4.385/5.17mm  $\pm$  0.08).

As for male populations (figure 4), the situation was similar with female registrations, so insignificant results were observed. The only beetles-Diabrotica population that noticed the difference was the Sy Arrioso hybrid (4.20/4.87mm  $\pm$  0.021).

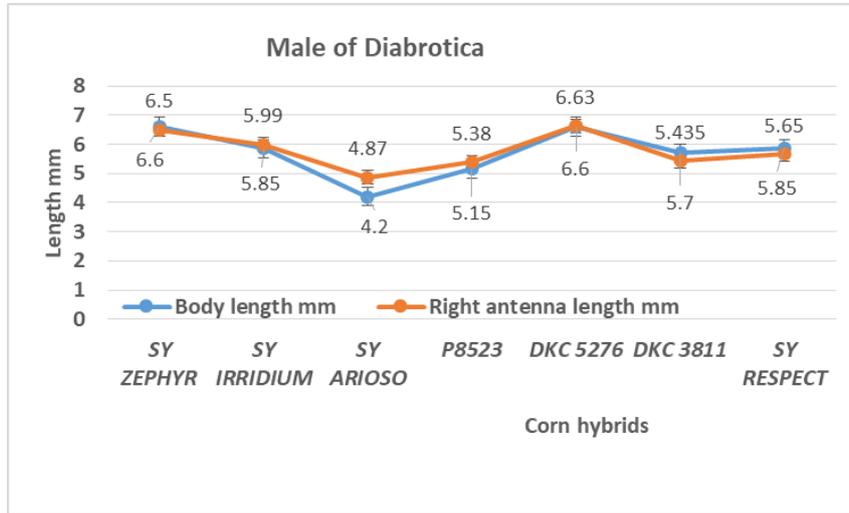


Figure 4 Average males body and antenna lengths of Diabrotica populations collected from lots of different maize hybrids

In order to establish the connection between Diabrotica adults' coloring and their printing of a nuance similar to the color of the ingested feed, we followed the feeding site of the beetles collected from the samples. Females were observed consuming more silk. Most were present on the reddish silk (16-26 ind/50 collected), the maximum observed in the SY IRRIDIUM hybrid (figure 5). The attraction of females to colorful silk in reddish attracted a reddish color of the body color of the body and the elytra.

Regarding the connection between the color of the plant (leaves, silk in green, yellow and reddish) and male coloring, it can be mentioned that they have been observed consuming more leaves. Few males have been observed on reddish silk (figure 6). In the SY ZEPHYR hybrid, a great number of males were observed on leaves (25 ind/50 collected).

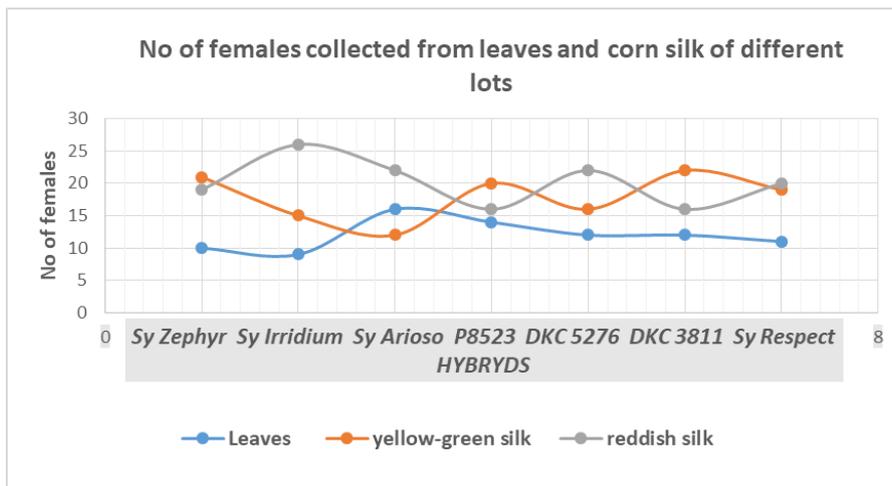


Figure 5 Females of Diabrotica populations and their connection with feeding on different maize hybrids

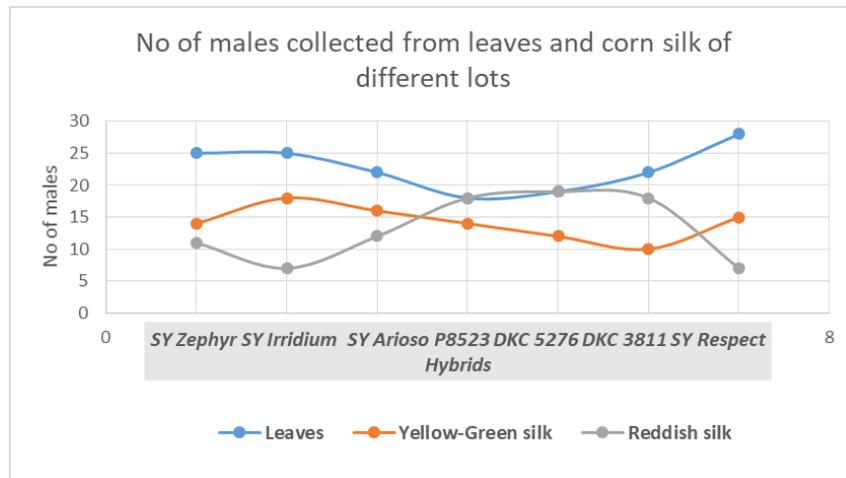


Figure 6. The males of *Diabrotica* populations and their connection with feeding on different maize hybrids

### CONCLUSIONS

In conclusion, there are morphological variations between adult insect populations, previously collected from different places and diverse corn hybrids.

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