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THE CURRENT ASSESSMENT OF THE STRUCTURE OF *Diabrotica virgifera* (COLEOPTERA: CHRYSOMELIDAE) POPULATIONS AND THE POSSIBLE CORRELATION OF ADULT COLORISTIC WITH THE TYPE AND COMPOSITION OF INGESTED MAIZE PLANTS

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ABSTRACT

***Diabrotica virgifera* is a chrysomelid that destroys maize in green, both in adult and larvae. Adults have a feeding regime focused on several organs of the maize plant. Foods ingested by beetles are an essential factor in the biological development of the individual, but also in the printing of morphological features, such as body coloring. In our studies we have tried to see the causes of the color variation of beetles that feed on maize plant organs different in structure, color and chemical composition. To determine if there is a link food consumed by insects, at a time, and the background color of insects, more precisely if pigmentation has chemical causes, analyzes were made based on samples collected in 2017-2018 period from several lots with different maize hybrids. Firstly, it is worth mentioning that observations began in 2015 in these lots in order to determine population size and monthly dynamics, and by 2017 attention has focused on the link between food composition and insect color. The results showed that the appearance of morphological forms, expressed by body color (elytra, abdomen), is largely due to the pigments in the maize plant. It is not excluded, however, that there are other factors that contribute to the coloring of the insect's body, such as genetic, climatic, or others. We cannot conclude if food is the only determining factor, if it is primary or secondary. But there is certainly a link between the color beetles and the maturity type of maize hybrids, the color of the ingested plant tissue, and the chemical composition of the maize.**

Keywords: Coleoptera, Chrysomelidae, body color, chemical composition of beetles, maize hybrids, leaves, maize silk, pigments in food, western maize rootworm, *Diabrotica virgifera*.

INTRODUCTION

Diabrotica virgifera virgifera LeConte 1868 (Chrysomelidae, subfamily Galerucinae), known as the "western corn rootworm" (Krysan and Smith, 1897) is an important invasive species for maize crops in Europe (Beenen and Roques, 2010; Kriticos et al., 2012; Kiss et al., 2005).

Currently the insect is present on two continents, America (considered to be the place of origin) (Chen et al. 2015) and Europe (OEPP/EPPO, 2017). Current scenarios point to the species' expansion on other continents (Asian, Australian and African) (Xiping et al., 2017). Yonow and Kriticos (2014) models show an extension of the species to the southern part of the African continent (in South Africa).

On the European continent it order to determine population size and monthly dynamics, and by 2017 attention has focused on the link between food composition and insect color. The results showed that the appearance of morphological forms, expressed by body color (elytra, abdomen), is largely due to the pigments in the maize plant. It is not excluded, however, that there are other factors that contribute to the coloring of the insect's body, such as genetic, climatic, or others. We cannot conclude if food is the only determining factor, if it is primary or secondary. But there is certainly a link between the color beetles and the maturity type of maize hybrids, the color of the ingested plant tissue, and the chemical composition of the maize. was mentioned in 1992 (in Belgrade) (Baca et al., 1995) and in

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Romania 4 years later (in 1996) (Vonica, 1996).

Although it was scientifically identified for the first time more than a century ago, the insect continues to expand today into new areas and especially within the continents mentioned above (Xiping et al., 2017). Evolution in Europe has experienced rapid spread times, especially in the early years of installation. It seems that over the last 5 years expansion has been slower. And that, perhaps due to the special attention given by scientifically groups (the Diabrotica subgroup within the International Working Group on Ostrinia-IWGO) and European organizations (European and Mediterranean Plant Protection Organization-EPPO) that effectively deal with species management and finding limitation solutions. In some European countries the species was reported as an important pest, and then it was not found anymore. And the explanation may be the intervention in limiting the expansion. For example, in the United Kingdom, Diabrotica (framed in EPPO A2 List) occurred from 2003 to 2007; after several years of absence, a small number of specimens were caught in a maize field in 2013 (EPO RS 2013/240). As subsequent surveys did not detect the pest, the EPPO considers that pest has been eradicated (EPPO, 2018). Recently, a re-evaluation of the current situation and management strategies of the species has been carried out which highlights the possibilities of limiting the extension to other areas (Reddy, 2018).

Similar situation was observed in Romania, where until 2010 the insect was a real danger for maize plants in the western half of the country, the speed with which it expanded in the first 10 years was astounding. Then it continued to expand but slower, probably due to the Carpathian Mountains that prevented the flight of adult forms (Grozea, 2010). Even though adults have not migrated to other parts of the country, they have grown to size in the western populations (where they first appeared).

For maize crops on the American continent, the insect is considered to be “corn Colorado”, producing considerable annual production of grain maize (Branson and Krysan, 1981). Both in Europe and America adults mainly feed on flowering maize pollen, silks, leaves and young developing kernels, but can also feed on a wide range of other plants that flower in the summer and offer alternative sources of pollen (Moeser and Vidal, 2005).

Diabrotica affect both field and sweet maize crops, the latter containing more sugar and less starch. Several varieties of sweet maize color are described (by Branderberger): yellow, red, white, bicolor, poly-color. These were correlated with the number of days until maturity, and it was found that bi-colors needed several days to maturity (Tracey, 1994).

This species has been and is in present an important subject of research, one part on the dissemination, development, damage assessment, population reduction and morphology too (Florian et al., 2011).

There are few references to feeding behavior and exploration of attractiveness to a certain type of plant tissue, even at international level, most of the research addressing the issue of harm and the impact on the economy (Fisher et al., 1986; Vidal et al., 2005; Wesseler and Fall, 2010; EPPO 2018).

With the disposal of toxic pesticides, commonly used in the control of larvae, one of the alternatives has remained the treatment against adults. For this it is necessary to know the morphology, biology and food preferences. An important aspect is also the causes that determine the color, considered as the criterion of intraspecific differentiation. A single approach of this kind was initiated in 2005 and then in 2018 by Horgoş and Grozea (2018). The approach remained at the level of quantification and testing of the demonstration of the link between adult coloring and the color of feed, as a publication of data without statistical interpretation.

The color of the insects may be pigmentary or structural, direct or indirect, or determined by the pigments in the consumed food. It can also be directly caused by epidermal or cuticle pigments through chemical composition, cuticle structures or light interference and diffraction. In insects with transparent skin, without color, the waxy layer is extremely fine, the light penetrating directly into the cavity. In the epidermis, more precisely in the epidermal cells, there are pigments that determine the visible color of the insect body surface, as specified by Schroeder et al. (2018).

They have a wide range of colors, both warm and cold, separate or combined. The warm colors are: red, orange, yellow. The cool colors are: blue, green, purple (Shamim et al, 2014).

At the beetle wings, surface structures lead to interference or diffraction scattering (dispersion), resulting in the appearance of white, blue and iridescent colors. These structures are known as schemocromes (Shamim et al., 2014).

Generally, polymorphism based on color was studied by Khormizi et al. (2016), not in Diabrotica, but in another species of Coleoptera (*C. flavipes*) and the phenotypic classes of elytra color were demonstrated by variance analysis. The frequency of phenotypic classes varied significantly throughout the collection and the copulation time did not affect the color of the females and males. This is important in order to form an opinion on the influence of colorism on attracting the opposite sex as supposed by Machado and Mellender de Araújo (1980) and on eliminating it in its possible role in insect color printing.

The evolution of the organic forms of a species, known as phylogeny, is little detailed in the species of the Diabrotica genus. It is believed that due to their homogeneity it is very difficult to establish their filiation and development over time, as specified by Khrysan (1986).

Both plant and animal kingdom color are determined by biosynthesized substances in secondary metabolism. Compounds belonging to classes are associated with anthocyanins, phenols and polyphenols, anthraquinones, steroids etc. (Tsao, 2010; Agati et al., 2012).

The basis of the study was the general considerations of the coleoptera, including that the insects may have a pigmentary color determined by the pigments in the consumed food. For example, carotenoids (also called tetraterpenoids) are, organic pigments produced by plants or other organisms (Kinoshita, 2008). Plant carotenoids are yellow and red pigments, or may have dark yellow to dark red tones (Bartley and Scolnik, 1995). These are found in plants (Chourkova, 2012), but also in some insects, even those of Coleoptera type.

All the results of research on *Diabrotica virgifera*, the monitoring and prediction of spreading, control and management, but also other aspects of feeding and attraction to a particular plant type are extremely important. Through these studies, we intended to bring new information about the feeding behavior of adults of *Diabrotica virgifera* and to identify the link between the color of the insect with the color of the vegetal organ and chemical composition of the plant tissue consumed by them.

MATERIAL AND METHODS

Organizing experimental lots in the field

In the field, 7 experimental maize lots were organized in different localities, keeping the distance of at least 5 km between them so that Diabrotica populations do not combine and the beetles belonged onlyto a single lot. These were repeated annually between 2015-2017, in 2 counties (Timiş and Arad) along the border with Hungary and Serbia (Figure 1).

The localities in Timiş County with established research lots were Jimbolia, Sânnicolau Mare, Grabaţ, Nerau and Variaş. The choice of these locations was based mainly on the Jimbolia area and near the border with Serbia. In this part of the county, since the first signal (1997), the most numerous exemplars of *Diabrotica virgifera* have been reported so far (Hancu et al., 2003).

For example, the places of Jimbolia and Grabaţ are located very close to the border, Grabaţ being 2 km away from the border with Serbia (the first signal in Europe, in 1992) (Baca et al., 1995).

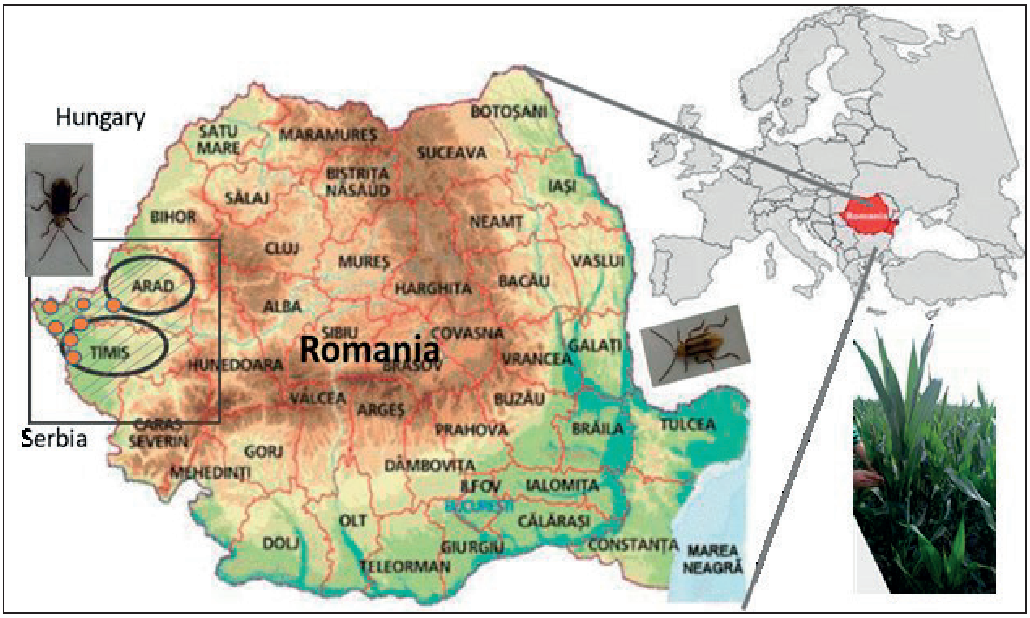


Figure 1. The area in which the 7 experimental maize hybrids of different maturity groups were located. It covered 2 of the western counties of Romania, Arad and Timiş, located near the border with Hungary and Serbia respectively

We took into consideration the same criteria in the choice of the localities of the county Arad, namely Felnac and Zabrani (Vonica, 1998).

In dimensioning of the individual lots, no account was taken of the characteristics of the literature, but they were set up on other adapted criteria,: the criterion of the location of traps at the required distances, the criterion of account that they are located near the town of Nădlac, where Diabrotica was first reported in Romania (Vonica, 1996) providing a broader harvesting base (the collection of beetles) and the criterion isolation (within a radius of at least 5 km around the plot of the study).

Selected maize hybrids for each lot were comprised in different maturity groups: DKC 3811 (extra-early type), SY Respect and SY Zephyr (semi-early type), P8523, SY Arioso, SY Irridium (early) and DKC 5276 (semi-late type).

Each lot was associated with a hybrid as follows: SY Zephyr/N: 45.976466 E: 20.567432; P8523/N: 46.046170 E: 20.627468; SY Arioso/N: 45.864602 E: 20.733524; SY Irridium/N: 45.999448 E: 21.005185; DKC 5276/N: 45.816152 E: 20.703100; DKC 3811/N: 46.107296 E: 21.152720 and SY Respect /N: 46.062100 E: 21.564735.

The principle of selection of the hybrids constituting the individual lots was based on the maturity nature. We chose those hybrids that offer silk (female inflorescence) and pollen from panicle (male inflorescence), when Diabrotica adults are present in large populations for feeding in crops.

Insect collection

In the 7 lots described above 4 collection activities were carried out:

- catching insects by direct trapping in order to determine the population level and population structure;
- collecting trapped insects for morphological study and phenotypic groups;
- collecting live insects to see the connection between the color of the insect and the color of the plant tissue consumed (Figure 2);
- collecting live insects for chemical analysis and establishing polyphenols and flavonoids, essential pigmentary substances (Figure 3).

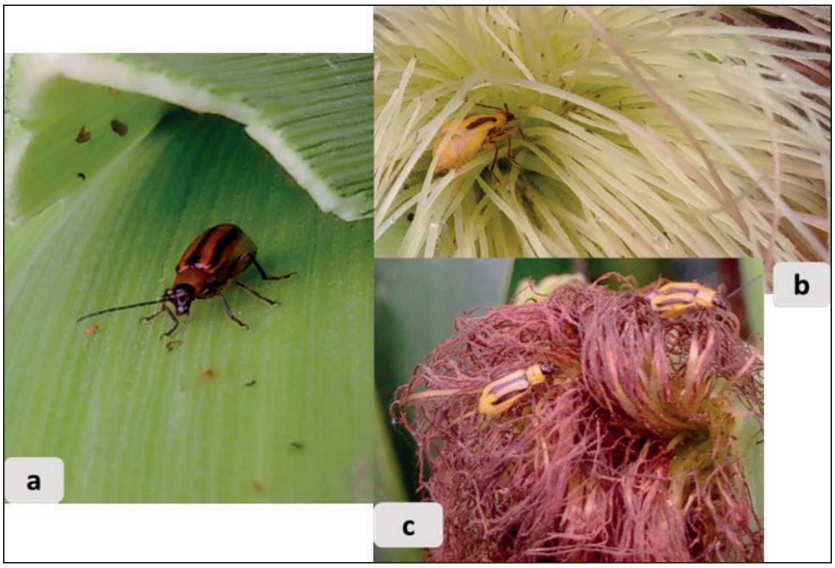


Figure. 2. Insects feeding on different maize organs before collecting them and establishing the link between the color of the beetle and the color of the tissue consumed: a - on the leaf; b - on yellow silk; c - on red silk

Using traps

In order to conduct insect catching activities by direct quantification to determine the population level and population structure and to collect insects for morphological study and phenotypic groups, Csalomon PAL traps from Hungary, Plant Protection Institute, MTA ATK, Budapest were used. These are known as sticky traps or sticky clover traps that have proven effective for Diabrotica (Toth et al., 2007).

Manual collection

To have an intact material (s) without adhesive, we used manual collection directly from the experimental lot. Of each lot/ type of hybrid, 60 adults were harvested, when 80% of the maize plants had silk. The beetles were used as follows: 50 individuals for color studies and 12-20 individuals for chemical analysis.

Figure 3. Adult insect samples collected from individual lots (L) L_1-L_7 before chemical extractions. In each lot there were both male and female beetles with various color combinations As an example, in this image (bottom left) is a female from Gr.3 and a male from Gr.1 of color

Analysis of polyphenols and flavonoids by spectrophotometric methods

To determine whether there is a link between the ingested food by the insects at one time and the background color of the insects, more precisely if the pigmentation has chemical causes, polyphenols and flavonoids were determined on the basis of samples collected during the period 2017-2018, considered to be representative. The 7 samples were extracts from the insects collected from the 7 experimental lots (respectively from the 7 hybrids) in June, July and August, when all the organs of the plant were formed. The total content of polyphenols was determined spectrophotometrically by the Folin-Ciocalteu method. The calibration curve was performed using gallic acid standard (0-30 μg/mL). Samples (diluted as appropriate) (100 μL) were mixed with 3 mL water and then for 3 minutes with Folin Ciocalteu (100 μL). The total flavonoid content was determined using the spectrophotometric method based on the reaction with aluminum chloride. The calibration curve was performed by using rutin (0-30 μg/mL) as a standard. Determinations have been made in accordance with national and international standards in accordance with ISO (International Organization for Standardization) and Official Methods of Analysis of AOAC INTERNATIONAL, 20th Edition (2016).

Statistical analyzes

The gross data from observations related to the size and population dynamics, the correlation between the color of individuals and the food consumed as well as chemical analyzes for the determination of pigment substances in insects were statistically interpreted by the Biostatistics program. Basic descriptive statistics were: average, minimum, maximum, inferior quartz, upper quartet, variance, standard deviation and asymmetry coefficient. For each variable, descriptive statistics/year were made. Also, for assessing the approximate probabilities, the Duncan Test for the variables studied was used.

RESULTS

Evolution and dynamics of adult populations

The results of the observations made in the each lots showed that the invasive species (*Diabrotica virgifera*) is still present in large numbers, with population differences between maize hybrids tested. During the adult flight, the largest number of catches was recorded at the end of July and the beginning of August. The large presence of adults in the experimental plots was somehow associated with the appearance and maintenance of silk and pollen.

In 2015, the DKC 5276 hybrid (semi-late type) was the most attractive for adults of the *Diabrotica* species, where the highest value was recorded (321.00 individuals). The fewest adults were registered in the Sy Zephyr hybrid, which is of the extra-early type (22.33 ind.) (Figure 4).

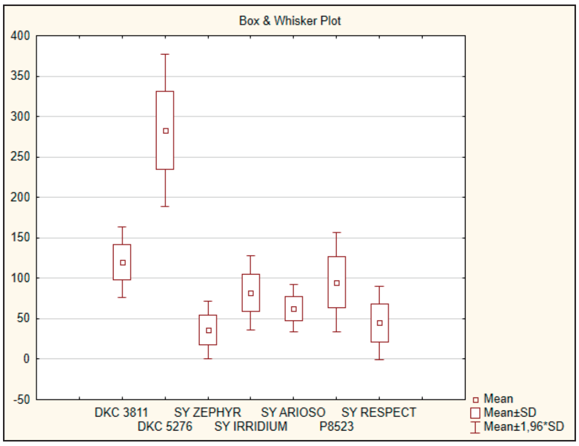


Figure 4. Diabrotica beetles captured on traps (number/Trap 1, Trap 2, Trap 3) placed in the 7 lots with different maize hybrids, in 2015

A detailed analysis of the approximate probabilities by comparing the variants between them in terms of the catches of individuals of Diabrotica shows that there are statistically significant differences between the experimental variant DKC 381 and the following variants: DKC 5276 (p=0.000179)/($p<0.05$), Sy Zephyr (p=0.004900), Sy Arioso (p=0.035339) and Sy Respect (p=0.009108).

In 2016, the situation was similar. The semi-late hybrid, DKC 5276 being highly attractive to insects, compared to the extra-early hybrid Sy Zephyr that attracted the fewest beetles (Figure 5). Duncan test values show that there may or may not be statistically significant differences between lots of individuals in individual varieties /lots (with different maize hybrids). Thus, there are significant differences ($p<0.05$) between the hybrids DKC 3811 and Sy Zephyr ($p=0.002131$), Sy Arioso ($p=0.023525$) and Sy Respect.

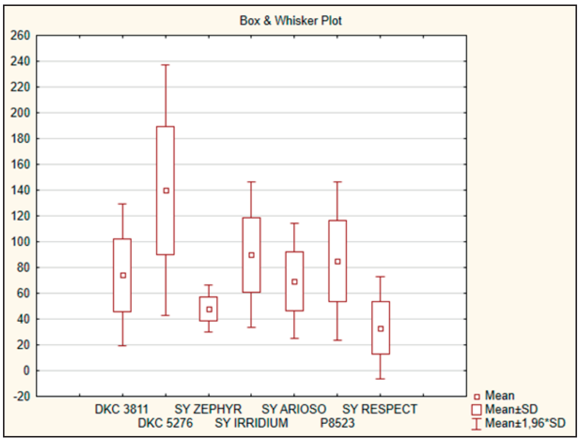


Figure 5. Diabrotica beetles captured on traps (number/Trap 1, Trap 2, Trap 3) placed in the 7 lots with different maize hybrids, in 2016

In 2017, the hybrid with the highest number of beetle catches was DKC 5276 (218 ind.) and the hybrid Sy Zephyr with the lowest (64 ind.) (Figure 6).

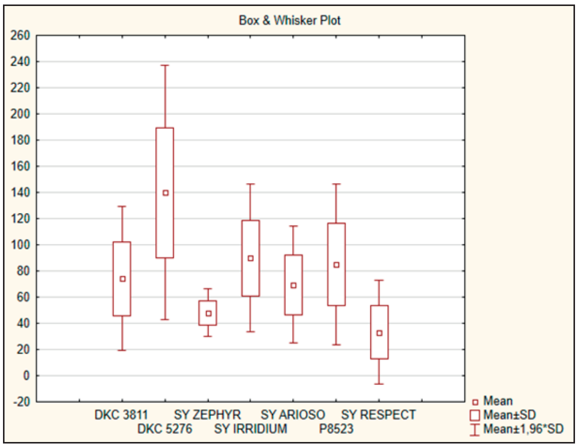


Figure 6. Diabrotica beetles captured on traps (number/Trap 1, Trap 2, Trap 3) placed in the 7 lots with different maize hybrids, in 2017

The approximate probabilities for the variables studied and the number of individuals trapped in the individual lots in 2017, highlights that there are statistically significant differences between experimental variant DKC 3811 and DKC variant 5276 ($p=0.00254$ where $p<0.05$). In the plot of DKC 5276 hybrid, the number of catches showed differences from all other hybrids where values were below the significance threshold ($p<0.05$).

The comparative analysis of the values recorded in each lots/hybrid between the three study years showed that between 2015 and 2017 in the DKC 3811 hybrid there were no significant differences. In the DKC 5276 lot between 2015 and 2016, the difference was significant because $p<0.05$ ($p=0.000062$). In the hybrids Sy Zephyr, Sy Irridium, Sy Arioso P8523 and Sy Respect hybrids, there were no significant differences between 2015 and 2017 ($p=0.601601$, $p=0.724868$, $p=0.756535$, $p=0.653582$ and $p=0.603643$).

Comparing the population level of the species *Diabrotica virgifera* recorded between the months of year 2016 and 2017 were made using Duncan Test. In August, most catches were recorded, so in 2016 the maximum was 256 ind. and in 2017 it was 589 ind. (Figures 7 and 8).

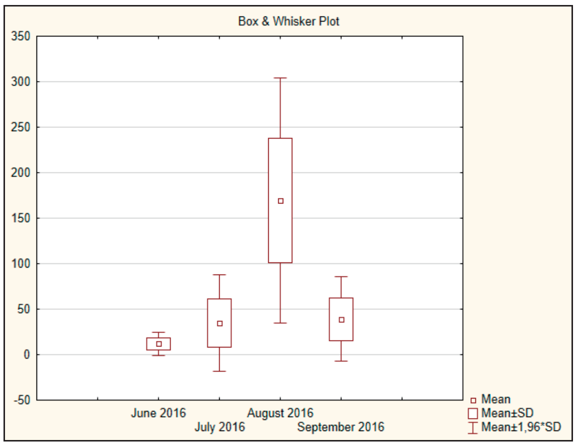


Figure 7. Monthly dynamics of *Diabrotica* adult populations in experimental maize hybrids during June-September, 2016

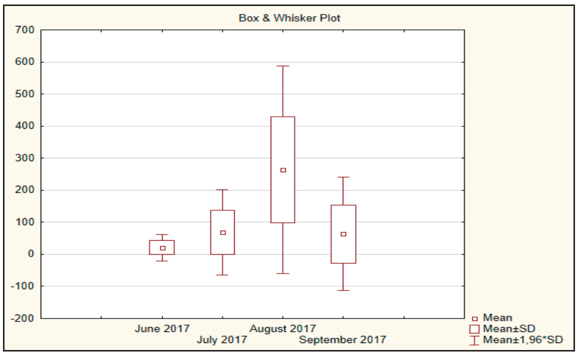


Figure 8. Monthly dynamics of *Diabrotica* adult populations in experimental maize hybrids during June-September, 2017

In 2016, there were significant differences between June and August (p=0.000367), July and August (p=0.001875), August and September (p=0.002351). There were no significant differences between June and July (p=0.607003). The dynamics of the population level is evident also in the year 2017, due to the significant differences between the studied months. Significant differences were reported between June and August (p=0.000018) July and August (p=0.000035) and August and September (p=0.000028).

Gender structure and color categories of adults in populations

The ratio of females to males in these experimental variants with different maize hybrids was about 2:1 (F: M)/2016 and 1:1 (F: M)/2017; the ratio was observed in all lots representing the seven hybrids under study (Figure 9).

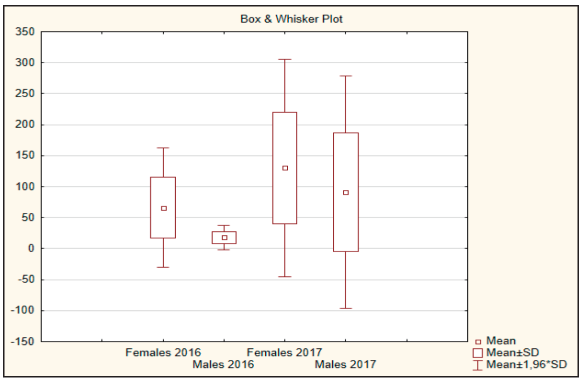


Figure 9. The ratio of females to males (F: M) in experimental variants with different maize hybrids in the period 2016-2017

Most females were observed in the experimental lot with the hybrid DKC 5276, followed at a considerable distance by the hybrids DKC 3811, P8523 and SY Irridium.

In order to better understand the phenotypic categories (or, in the present case, the color categories), it is necessary to know the standard morphological characteristics in detail. Thus, standard females range from 4.2 to 6.8 mm, and standard males from 4.4 to 6.6 mm. Elytra and the pronot are light yellow (Khrysan and Smith, 1987).The femur of the legs is pale yellow with the outer black border. Elytra have longitudinal grooves. Elytra are black on the humeral angle but also in the suture area. In many cases, the elytra cover most of the body. Males are generally darker than females. The male antenna is longer than female (Grozea, 2010).

The results showed that the females had a larger color palette than males. Thus, the females were divided into 5 groups of males and the males in 4 groups (Figure 10).

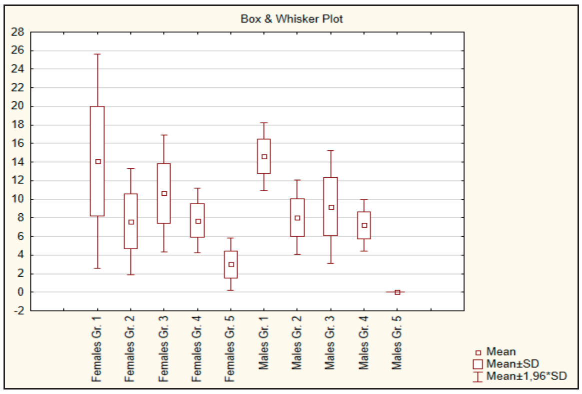


Figure 10. Distribution of *Diabrotica* females and males (from the 7 experimental lots) in color groups

The background color difference can be seen between the base colors, respectively, between the yellow color category and the red color category. In fact, the two standard colors of adults of *Diabrotica virgifera*, are yellow with black, yellow being the background color. The only color changed from one group to the other was the yellow one (which sometimes turned greenish or yellowish-reddish and occasionally marooned).

Females and males in all experimental lots with the 7 different hybrids were predominantly included in the standard category. This is normal if we take into account their predominance in the populations present in the variants.

Possible correlation between feed color and adult color

The results obtained showed that food color, can influence the background color of adults of *Diabrotica virgifera*. It can imprint a hue specific to the insect that feeds intensely on plant organs of the same hue. Chemical compounds usually print the color of a species. Our studies showed that the appearance of morphological forms expressed by the color of the elytra is largely due to pigments in the maize plant.

Maize silk, which is the most consumed organ of the plant by adults of Diabrotica, contains a considerable number of vitamins, proteins and carotenoids.

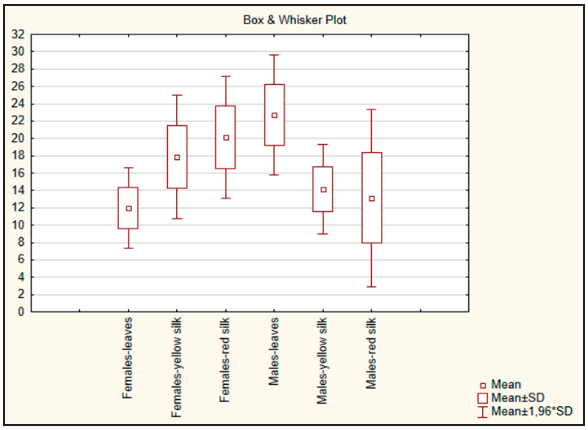


Figure 11. The attraction of *Diabrotica* males and males for various organs of maize plants from lots with different hybrids

Females were observed consuming more silk. Most were present on reddish silk (16-26 individuals/50 collected individuals), with a peak observed in the hybrid Sy Irridium group (Figure 11).

The attraction of females to reddish-colored silk was also reflected in the red color of body color and elytra.

Regarding the linkage between the color of the ingested plant tissue and the coloring of the males, it can be mentioned that they were observed consuming more leaves. Few males were seen on reddish silk compared to females. In the Sy Zephyr hybrid lot, a large number of males was observed on the leaves (25 individuals/50 collected insects) (Figure 11).

Chemical composition of insects after ingestion of food

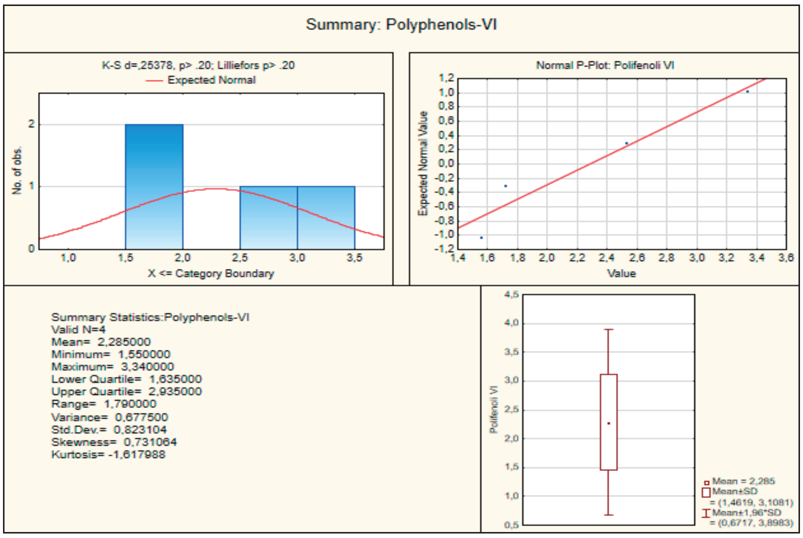
Following laboratory analyzes, the target chemical compounds (polyphenols and flavonoids) were identified in all samples (L1-L7).

Going on the fact that polyphenols print the tissue (vegetal or animal) red color and the flavonoids yellow, we looked to see the amount of these compounds in the beetle samples. So, we made an indirect connection with the maize phenophase, depending on the maturity of the hybrid from which the beetles consumed the plant tissue (organ).

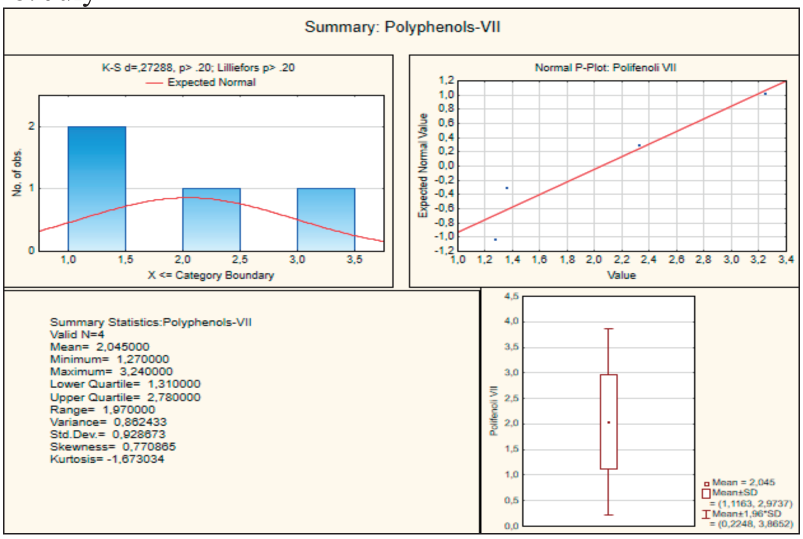
The results record between June and August showed that the amount of polyphenols and flavonoids had an increasing trend, with a slight decrease in values of samples collected in July. Thus, polyphenols were present in all samples starting with June (with an average of 2.29 μg/μL) and finishing with August when the mean value increased to 3.11 μg/μL (Figure 12).

The amount of flavonoids was found in an average value of 0.39 μg/μL in the June samples; then declined slightly in July to 0.34 μg/μL and then increased considerably to 0.50 μg/μL in August (Figure 13).

a. June



b. July



c. August

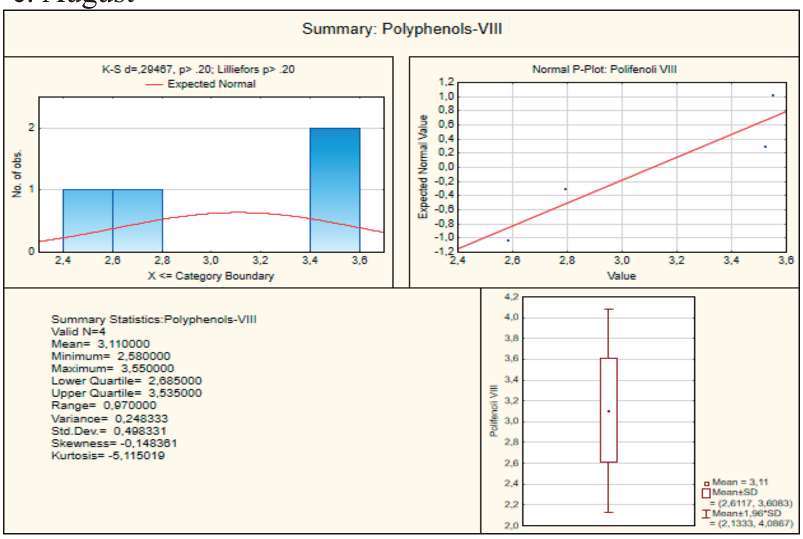
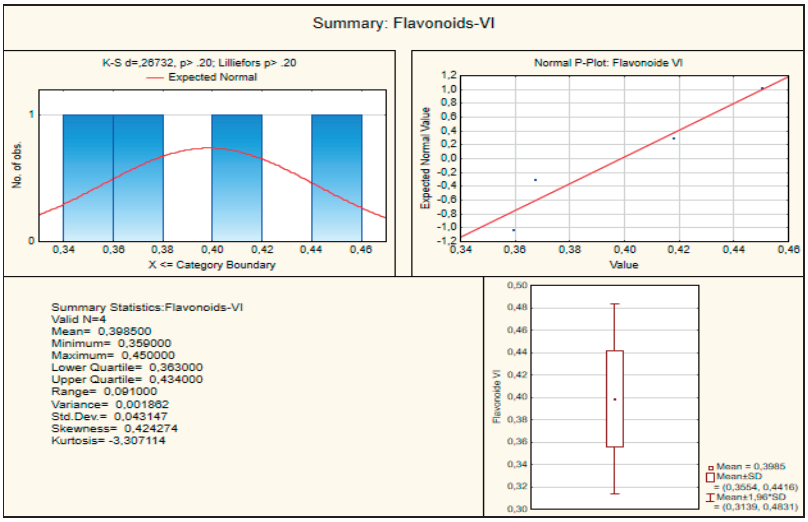
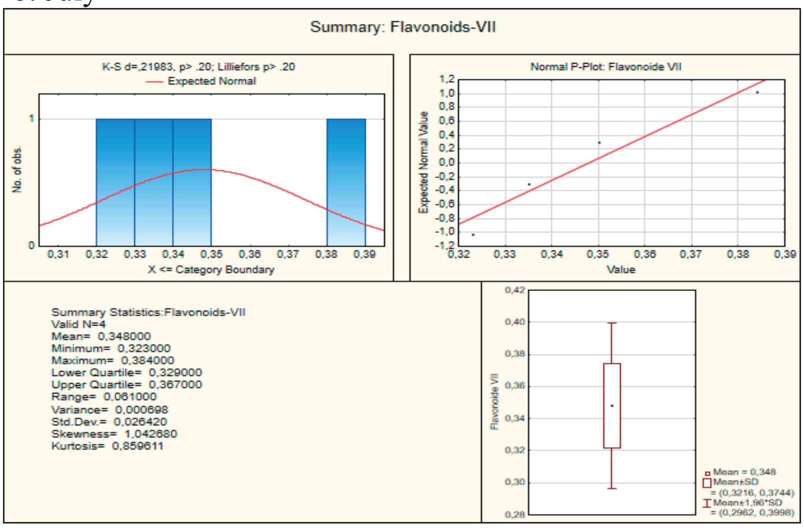


Figure 12. Polyphenols as insect extracts from the L1-L7 experimental lots and collected in June (a), July (b) and August (c). In the graph are presented the basic statistical indicators, the positioning of the values and the normal distribution curve.

a. June



b. July



c. August

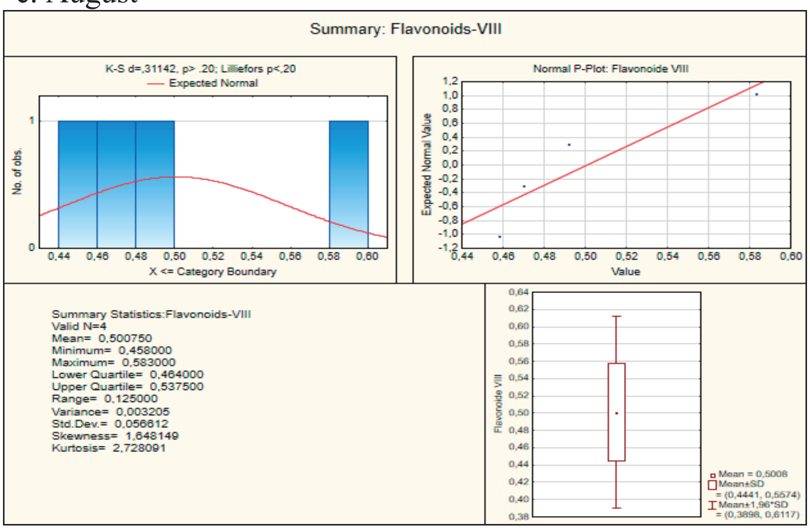


Figure 13. Flavonoids as insect extracts from the L1-L7 experimental lots and collected in June (a), July (b) and August (c). In the graph are presented the basic statistical indicators, the positioning of the values and the normal distribution curve.

DISCUSSION

Our results of the *Diabrotica virgifera* adult population showed that the DKC 5276 hybrid, which is part of the semi-late maturity group (FAO-470), attracted most of the Diabrotica beetles, and at the opposite end are the semi-early hybrids be Sy Zephir (FAO-390) and Sy Respect (FAO-170). It is obvious that beetles are attracted to late maize hybrids rather than the early ones, so in order to avoid the over-development of Diabrotica populations, it would be advisable to plant hybrids that mature earlier. Thus, there will be no concordance between the silk formation and the maximum feeding time of the beetles. This is confirmed by the results of agricultural practice, which argue that maize fields with early flowering maize hybrids may become donor fields when flowering in these fields finishes. Conversely, later flowering maize fields are attractive to beetles and thus may become trap crops (Miller et al., 2005). Meinke (2008) says that they are capable of causing economic losses to field maize, if there are high beet densities, especially when silk occurs, excessive feeding of silk can occur, which can lead to reduced pollination.

Although they can also feed on other plants that bloom in the summer and offer alternative sources of pollen, as Moeser and Vidal (2005) mentioned, in our observations we only noticed that the main attraction is maize plants.

Previous studies of gender structure and internal variability of the population have shown that they are mainly based on color and have interspecific and intra-specific applicability (between the same species known as sexual dimorphism) (Harisson, 1980). The existence of gender structure through sexual differentiation and coloristic variability among individuals was also demonstrated by our studies on populations that had habitat and fed in lots with diverse hybrids as maturity.

Females versus males were predominant in all maize plots in the experiment, and sometimes they even showed double numerical ratios (2:1). And the color range of females was larger, they could be classified into 5 color categories while males only in 4 categories. Our previous research had already revealed these color groups (Horgoş and Grozea, 2017), so that in their present work it was easier to quantify and catalog directly the specimens collected. They also preferred the silk as the basis of feeding while the males were noticed more on the leaves and other organ plant.

No other references are known, or at least we have not found in the literature any other references to the possible correlation between the color of the food and the background color of the body and adult elytra of the Diabrotica, but also of other coleopterans. That is why we tried to address this issue through an adapted methodology and to bring to light other factors that could be taken into account in printing the color of insects, not just the genetic factor.

Our approaches have shown that the emergence of diversity in elytra-colored populations is largely due to the pigments in the maize plant. Maize silk, which is the most consumed organ of the plant by feeding adults of Diabrotica, contains a neglected amount of vitamins, proteins and carotenoids. Pigments targeted and analyzed through this work (flavonoids and polyphenols) were definitely present in the body of specimens of beetles/sample analyzed, which confirms the ones mentioned by Kinoshita (2008) that these organic pigments are plant-specific but are also found in the insect body of Coleoptera type (probably following the ingestion of these plants). The basic colors of plant carotenoids are usually yellow and red, with color tones variations ranging from light to dark, as Bartley and Scolnik (1995) mentioned, which was also observed and demonstrated in our studies. Chemically analyzed beetles from the 7 samples had yellow and red base colors, with more or less prominent design (especially longitudinal stripes), which confirms variability among individuals.

However, the results of chemical determinations in adult insect samples are uncertain and it could be said that the color variation of this species is probably determined by the chemical composition of the plants consumed.

CONCLUSIONS

It is certain that the invasive pest *Diabrotica virgifera* is present in maize culture in high populations and the color variation is also great. Although it is complicated to understand the mechanism behind the coloristic variability trend of this species, from the determinations and observations made for 2 years, it can be stated that the dispersion in the experimental lots was based on the availability of the preferred organs (leaves, silk and pollen) at a time. A number of factors, such as genetic factors, could contribute to color clarification, and the color of ingested food could be considered only secondary, but this may be a new approach and direction of research in studying coloristic variability.

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