

## Western Corn Rootworm (WCR), *Diabrotica Virgifera Virgifera* Le Conte – Several Years of Research in Western Part of Romania

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**Abstract.** *Diabrotica virgifera virgifera* Le Conte (Coleoptera: Chrysomelidae), known as western corn rootworm (WCR), is an important pest for maize crops from Romania, after corn borer larvae. Fourteen years ago, this pest has entered in our country. Since its introduction *Diabrotica v.* has been spreading in western part of Romania and many investigations were started by us to understand very well the morphology, eco-biology and control of the species.

**First occurrence and activity period.** The immature developmental stages of WCR (larvae, pupae, eggs) have been observed for the first time in Romania, in soil sample during 2000-2002. Adults' activity developed from during June to September. The males have appeared earlier than females with approximate 4-6 days. The females have begun to lay eggs in middle of July. In soil, the larvae were observed from May to August and pupae were observed from June until August, too.

**Colour variability.** In monitoring activities, the most of specialists use the color criterion for establishing males and females. However, this is not so precise method, because the great variability of color what is frequently in many populations. Therefore, we tried to eliminate any doubt regarding sex ratio.

**Influence of weather and geographical areas.** Based on our data during 2003-2005, we can emphasize a very serious influence of air temperature and rainfall on WCR flight dynamics in adults. Regarding the altitude, we observed a decreasing number of WCR adults at once the altitude increase.

**Control strategies.** In preventive control avoiding monoculture play a main role, because the larvae survived only maize roots. Some researches realized in western part pointed efficacy of seed treatments (using imidacloprid, clothianidin, thiametoxam) against larval populations. Very important is using a chemical product (thiametoxam) against adults (like an additionally product). From an-polluting method may mention follows: pheromone traps, color traps, autochthonous natural enemies. The spider species *Argiope bruennichi* (Araneae:Araneidae) and *Theridion impressum* (Araneae:Theridiidae) are able to diminish significantly population of adults, especially in appearance of maize silk.

**Keywords:** *Diabrotica virgifera virgifera* Le Conte, morphology, eco-biology, control, strategies.

### INTRODUCTION

Romania is part of the southeast European expansion zone of the invasive rootworm species *Diabrotica v. virgifera* Le Conte (Col.:Chrysomelidae) (WCR). The pest originated in North America and reached Belgrade airport with US foreign aid air shipments prior to 1992. Today, about 20 years after its introduction, WCR is a major maize pest which regionally causes economic damage to maize (Hummel *et al.*, 2009). For this reason the pest western corn rootworm (*Diabrotica virgifera virgifera* Le Conte) present a danger for Romanian corn production and its monitoring was carried out since the first occurrence (1996) until now (Cean, 2005, Grozea 2009, Vonica, 1998). The *Diabrotica* populations have increased

strongly year by year up to 2001 (Hancu *et al.*, 2003). In 2000 the first areas with larval attack in a maize field of the Jimbolia zone was found. In this year the attack is stronger than before (Grozea *et al.* 2006, 2007, 2009; Palagesiu *et al.*, 2001). For monitoring of the adults were used Hungarian pheromone traps (type of Csalomon ® Diabrotica v.v. tip panou/2). The traps were installed from June to September. Together, the climatic conditions, altitude and their influence on species behavior and distribution have become more apparent. Their habitat and survival strategies are strongly dependent on local weather patterns and altitude. Based on our observation and other, we can emphasize a very serious influence of air temperature, rainfall and wind in WCR spreading (Grozea and Unipan, 2007, Grozea *et al.*, 2009, Hatala-Zseller and Szell, 2000).

Incidence of this pest in Europe and Romania attract the specialist's attention and European organisms regarding substantial changes which save the yield. Usually, the most control used is chemical products. After Edward *et al.*, 2002, the most efficient insecticides were Gaucho 600 (imidacloprid) and Poncho (chlorfenthrin) that have reduced significantly the attack at roots. The researchers from Purdue University have suggested a high damages decreases by using: tefluthrin (Force) and fipronil (Regent). Current trends in control regard the using natural enemies' because non-pollutants effects. In this way it follows protection of useful scale from agroecosystems and their exploitation in control of invasive population (Grozea *et al.*, 2008). As part of a sustainable pest management, knowledge pertaining to the community of indigenous natural enemies of *Diabrotica virgifera virgifera* was required from its areas of introduction in Europe. Natural enemy surveys were conducted on different life stages of *D. v. virgifera* in Hungary, Romania, Serbia, Croatia, Slovenia and Italy between 2000 and 2006. (Toepfer *et al.*, 2009).

## MATERIALS AND METHODS

**First occurrence and activity period.** This kind of research has been carried out made in monoculture cornfields from Timis county (Grabat locality), in 2000-2002 period. Because the first appearances of adults (1996) were registered in western part of country the researches regarding some aspects of biology of WCR it could be presented only in these places. The larval and pupae populations were checked weekly, by taking of soil samples. The samples were moved to laboratory for details study. For eggs estimation soil samples were passed through a special filter (method by Krysan and Miller, 1986). The same of researches were made in laboratory conditions, needed for comparative study.

**Colour variability.** Researches what have made in a maize crop from Didactic Station of USAMVB Timisoara, Timis District (2004-2006) praised more many phenotypic (color) groups. Adults were selected after the shape of the last abdominal segment. By the total number of researched adults (614 ad.), just 256 (252-matures, 4-immatures) adults were males and 358 adults were females (352-matures, 6-immatures). The beetles were divided in several categories. It was establish the normal characteristics (described in literature) for males' color and females and other characteristics (original method).

**Influence of weather and geographical areas.** The locations of researches regarding the influence of temperature and rainfall in development of adults were made in Timis county (in this place, in 1997 was the first appearance of pest). For establish the number of adults compared with altitude we made observations in 11 localities (in 2008) and 17 localities (in 2009), beginning to 110 respectively 115 m until 557 m altitude. For movement we used the

car of Entomology and Agricultural Zoology department and for determination of altitude, we used a GPS apparatus. For each location, we have put two pheromone traps (Csalomon<sup>®</sup>). The date for installing the traps was within the June, 20 and July, 1. The traps were placed in middle part of the field, to a distance of 50 or 100 m. Traps were attached to maize stalk and placed approximately at ear height. Their checking (numbering of adults/trap) was made daily and its have been removed each 2 week.

**Control strategies. Using traps.** One of the an-pollutant capturing methods is installing of traps on maize plants. We used follows kind of trap: pheromone trap (Csalomon) and colour traps (Cluj Napoca and Multigard). These were placed in maize crops, at 100 m distance; readings were made each 2 weeks.

**Chemical products.** We are made seed treatments (using imidacloprid, clothianidin, thiametoxam) against larval populations a chemical product (thiametoxam) against adults (like an additionally product). The product based on thiametoxam splashing in the form of vegetation, more exactly in the generative phase of developing maize.

**Using natural enemies.** In each experimental variant (I, II) were placed 2 emergence cages, 4 maize plants/variant. The height of cage was by 2.20 m, so this size to let the normal development and growing of the plants. The soil (into the cage) was covered with synthetically netting very finely for a good ventilation but to stop the adults to exit in outside. In the stage of cob development and silk appearance, we started the observation about the working of this kind of biological control. In each cage it was introduce by 50 *Diabrotica* adults (males) and 5 *Argiope bruennichi* spiders. These were taken from nearly of cages place, for to keep their in the same development conditions. After 10 days, the *Diabrotica* adults from into the cages were counted and degree attack was rated (by detailed study and pluck up by the root). There were made daily observations (readings, photos, videos).

## RESULTS AND DISCUSSION

**First occurrence and activity period.** Analysing the situation through a review a researches carried in field condition the adult of *Diabrotica virgifera virgifera* Le Conte stage during from second decade of June until third decade of September. The pupal stage can be observed from the end May until the middle of August and larval stage can be found from beginning of May until beginning of August. The eggs oviposition carried from the middle of July until middle of September. In laboratory, the adults were observed beginning with last decade of April until second decade of August. The pupal stage is from second decade of April until last decade of June and larval stage can be found from first decade of April until third decade of June. Females lay eggs beginning from second decade of June until first decade of August. Is important to mention that the first adults of western corn rootworm in Romania were discovered in a cornfield from Arad district (Nadlac) in 1996. In Timis district the first beetles were observed in 1997 (Deta) and the first damage occurred in 1999 in a monoculture cornfield. The other developmental stages of WCR (larvae, pupae, eggs) have been observed in next two years.

**Colour variability.** It was observed a lower variability of males' color (4 color groups) than of females (6 color groups), maybe because the less number of males. The most

of adults have presented normal characteristics described in specialty literature (group 1), but there is a significantly number of them with another characteristics.

Color groups. Males. Gr.2- black elytra with diffuse longitudinal light stripes; similarity with females; Gr 3- black elytra and reddish brown in posterior part; Gr. 4- black elytra with diffuse longitudinal reddish brown stripes; similarity with females. Females. Gr.2- yellow-green elytra with diffuse black stripes; similarity with males; Gr.3- dark elytra with yellow-green posterior and lateral side, similarity with males; Gr.4- reddish brown elytra and visible black longitudinal stripes; Gr.5- yellow-brown elytra with diffuse black stripes; similarity with males; Gr.6- dark elytra with reddish brown posterior and lateral side, similarity with males (Tab 1).

Tab. 1

Percent of individuals from different color groups in studied population

Adults	Color groups (%)					
	Gr.1	Gr. 2	Gr. 3	Gr. 4	Gr. 5	Gr. 6
Males	66.66	20.63	7.93	4.76.	-	-
Females	31.25	28.97	19.88	7.38	6.25	6.25

**Influence of weather and geographical areas.** Based on our data from the years 2003, 2004 and 2005, we can emphasize a very serious influence of air temperature and rainfall on WCR flight dynamics in adults.

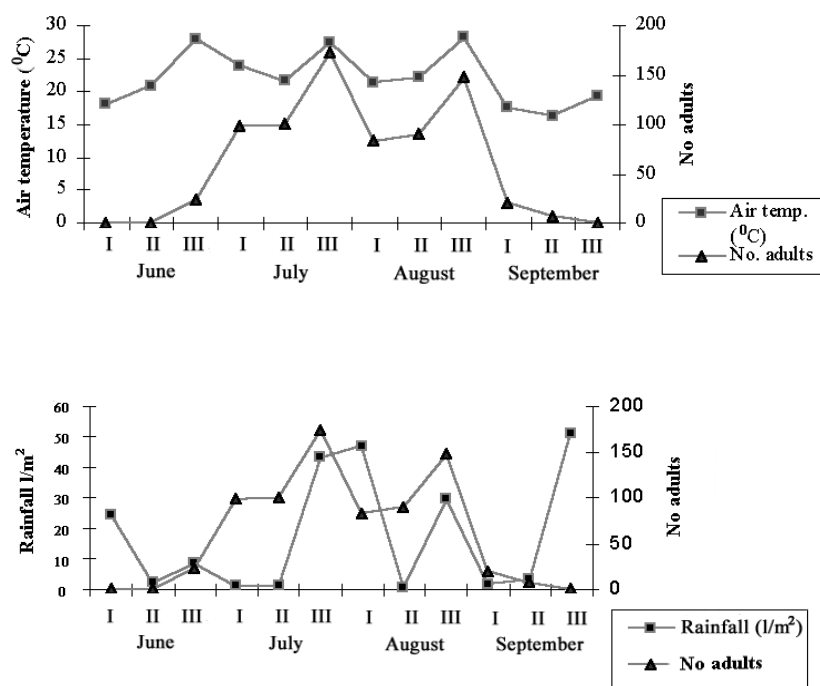


Fig. 1 The influence of air temperature and rainfall on flight dynamic of *Diabrotica v.v. L.C*

Regarding the altitude, we observed a decreasing number of WCR adults at once the altitude increase. For capture of adults, we used pheromone traps, in three replications (T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>) at difference altitude where maize was grown.

Dry and warm conditions promote growth of insects' number. Therefore, in 2003, July (III) the temperature raised to 26.5°C, led to increasing of adult's number (189). For 2004, the data shown a positive correlation between temperature and adults captured on pheromone traps (Fig. 1). Similar aspects were recorded in 2005. Excessive or insufficient rainfall is an important variable that affected adults' dynamics in maize fields. The most observations, in all studied years, shown a decreasing number to rainfalls increasing (Fig. 1).

In 2008, at altitude of 115 m we counted 2611 beetles on traps while at altitude of 557 m were counted 807 beetles (Fig. 2). Similar situation was recorded in 2009 by values of 18462 beetles/traps at altitude of 115 m and only 1148 beetles at 557 m altitude (Fig. 3).

The total values registered in 2009 were higher than in 2008; the maximum of captures was reached values of 21323 beetles (2009) comparative with 3969 beetles (2008).

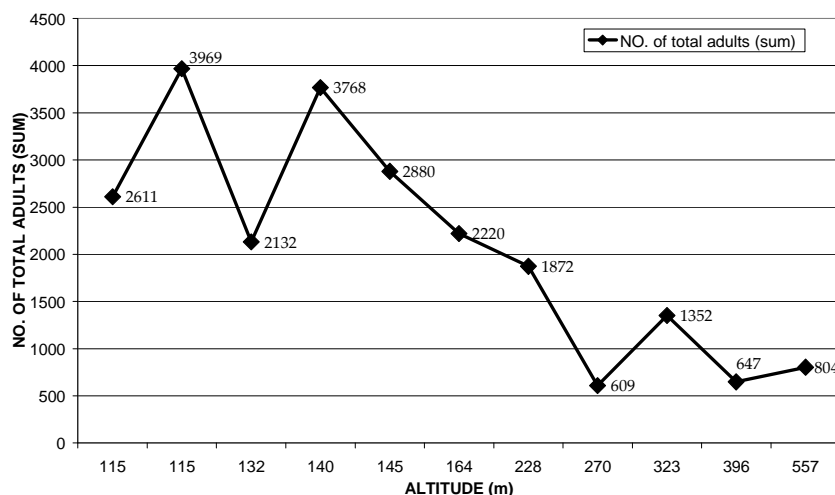


Fig. 2. Total number of WCR adults caught per traps (June 20 and August 31, 2008) in maize fields from different localities (altitudes), in western part of Romania

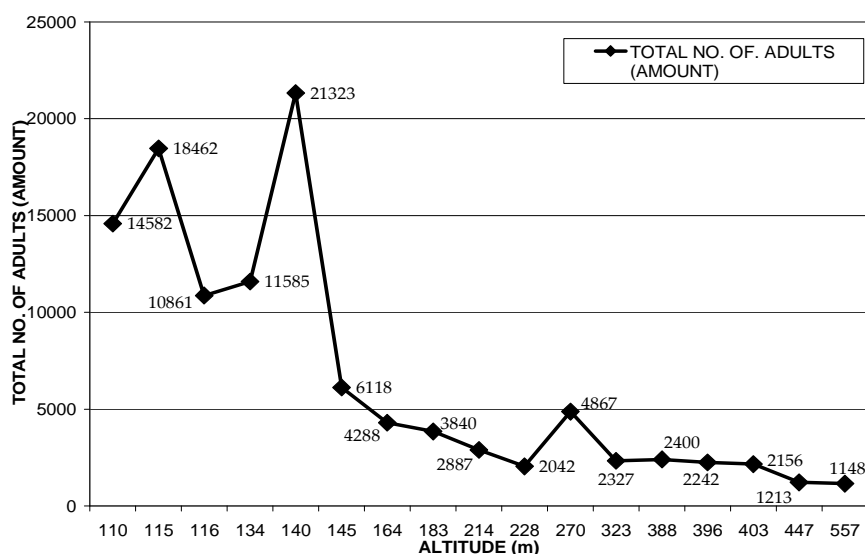


Fig. 3. Total number of WCR adults caught per traps ((June 20 and August 31, 2009) in maize fields from different localities (altitudes), in western part of Romania

**Control strategies.** Previous researches realized in western part pointed efficacy of seed treatments (using imidacloprid, clothianidin, thiametoxam) against larval populations. Very important is using a chemical product (thiametoxam) against adults (like an additionally product). From non-pollutant methods, it can be mentioned: pheromone traps and autochthonous natural enemies. The most efficient traps were pheromone traps, Hungarian provenance (type of Csalomon ® *Diabrotica* v. v, panel/2), used in the same time in monitoring activities. Trying to find solutions to the issues created by invasive species

*Diabrotica virgifera virgifera* we discovered an ecological alternative of the chemical methods, as an-polluting biological methods. In a period when easily apply to chemical substances we consider that is absolutely necessary the introduction of these biological methods.



Fig. 4. The pheromone traps, Hungarian provenance (type of Csalomon ® *Diabrotica* v.v, panel/2), in a crop field from west part of Romania, in 2009 (original photo)

The maximum appearance period of invasive species (July, August) is very important in establishing the analogy with appearance of predator's species. From natural enemies of *Diabrotica* v. can be notice follow species: *Speira diademata*, *Argiope bruennichi*, *Theridion impressum* (Arachnida:Araneae), *Coccinella* sp., *Pseudophomus rufipes* (Insecta: Coleoptera). The most frequent encountered species in maize agro ecosystems was the species *Argiope bruennichi* (60-70% from utile entomofauna who was studied). From our findings, we can observe the high present and aggression upon invasive species especially when the silk is build. Since the beneficially species observed in trials, the spiders of genus *Argiope* (*Argiope bruennichi*) have determinate the highest mortality degree in *Diabrotica* adults populations. Follow the methodology described in previous text at "material and method" we founded that each spider has feed 7.4 – 8.5 *Diabrotica* males (Fig. 5).

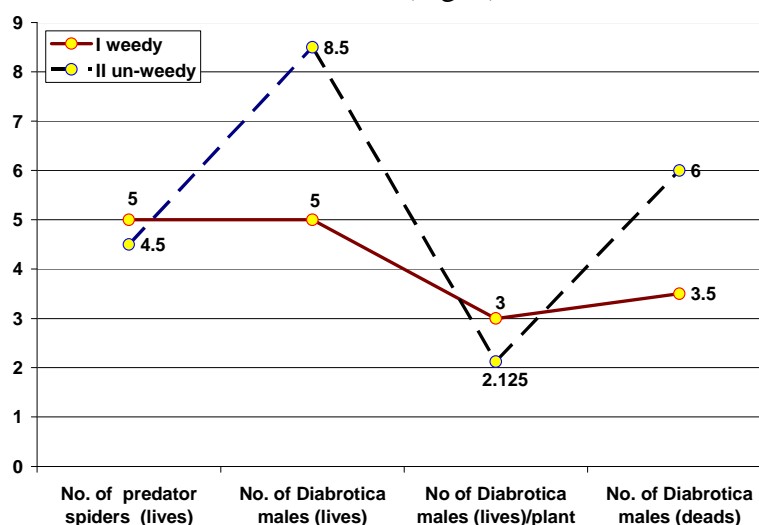


Fig. 5. The differences between those two trials regarding the number of predator live spiders and *Diabrotica* adults live and dead

## CONCLUSIONS

Entire vegetation period of maize (from April until September, in soil or plant) can meet different stages of *Diabrotica virgifera* species.

Among populations of insects there is appreciable variability in colour.

The air temperature, rainfall and altitude can serious influence of on WCR flight dynamics in adults.

The most efficient traps were pheromone traps, Hungarian provenance (type of Csalomon® *Diabrotica* v.v, panel/2)

The spider species *Argiope bruennichi* (Araneae:Araneidae) and *Theridion impressum* (Araneae:Theridiidae) are able to diminish significantly population of adults, especially in appearance of maize silk

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