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# RESEARCH REGARDING INTERACTION OF MON810 BIOTECH CORN ON THE *HELICOVERPA ARMIGERA* IN ROMANIA

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## Abstract

*Corn earworm Helicoverpa or (Helicoverpa armigera Hb.) is considered by some to be the most important insect pest in corn after the stage of seventh collars (V7) together with the European corn borer (Ostrinia nubilalis Hb.). In Romania, the pest is spread all over the country but it seems to be very important in south part of the country, so along the Danube plain, during recent years, the damages were significantly and many farmers were afraid of it. In Europe, the species Helicoverpa armigera Hbn. has the status of "invasive foreign species". In the last years there has been significant damage in numerous crops both in Romania and in its neighboring countries (Hungary, Serbia, Bulgaria, Ukraine, etc.). Research carried out aimed at completing the knowledge of the biology and ecology of the species Helicoverpa armigera Hbn. in the conditions of our country, monitoring the appearance and flight of the adults, the appearance and succession of larvae generations in the maize crop in the year 2009, as well as the duration of parts of the development cycle in laboratory conditions.*

## INTRODUCTION

Corn earworm Helicoverpa or (*Helicoverpa armigera* Hb.) is considered by some to be the most important insect pest in corn after the stage of seventh collars (V7) together with European corn borer (*Ostrinia nubilalis* Hb.) [1].

In Romania, the pest is spread all over the country but it seems to be very important in south part of the country, so along the Danube plain, during recent years, the damages were significantly and many farmers were afraid of it. Corn earworm has a wide host range (more than 120 host species), in addition to corn and tomato, perhaps its most favored vegetable hosts. Among field crops injured by corn earworm are: alfalfa, clover, cotton, flax, oat, millet, rice, sorghum (particularly favored), soybean, sugarcane, sunflower, tobacco, vetch, and wheat. Fruit and ornamental plants may be attacked [2, 3]. Pest is most abundant in the crops during silking of corn, when adults are mate female laid singly egg around the silks. Adults collect nectar or other plant exudates from a large number of plants, trees and shrub species are especially frequented. On corn, its most common host, young larvae tend to feed on silks initially, but eventually they usually gain access to the kernels. There are 6 larval stages, and the complete development

larvae reach 35-40 mm long. Pupa is dark brown, 14-18 mm long, with smooth surface, rounded both anteriorly and posteriorly, with two tapering parallel spines at posterior tip.

In Romania losses are reported throughout the country, and pest cause attacks in some years, on certain sole, in the south of the country. Larvae chew leaves and tunnel down the silk channel of the cob. The presence of larvae and associated frass increase level of mycotoxins [4, 5, 6].

In Europe, the species *Helicoverpa armigera* Hbn. has the status of "invasive foreign species" [7]. Adults can migrate over long distances, borne by wind. In the last years there has been significant damage in numerous crops both in Romania and in its neighboring countries (Hungary, Serbia, Bulgaria, Ukraine, etc.), we thought it proper to choose this research topic referring to importance of pest for corn in southeastern part of Romania. Research carried out aimed at completing the knowledge of the biology and ecology of the species *Helicoverpa armigera* Hbn. in the conditions of our country [3, 8, 9], monitoring the appearance and flight of the adults, the appearance and succession of larvae generations in the maize crop in the year 2009.

## MATERIAL AND METHODS

To establish a level for *Helicoverpa*, we proposed that, in south and east area of Romania, 10 corn field (having check Bt refugees or regular corn hybrid field with the same vegetation group) were inspected, and the presence of larvae and their number/ear was monitorised during the late vegetative stage and the silking stage. At the beginning we searched in different localities, with Bt. corn field, 1. Oltenita, CALARASI-field/farmer Dume; 2. Dalga, CALARASI -field/farmer Liviu Georgescu; 3. Dragos Voda, CALARASI - field/farmer Ildu; 4. Chiciu, CALARASI Soc. Transliberta - field/farmer Ciobanu; 5. Tudor Vladimirescu, BRAILA, Pietrosu S.A.- field/farmer Mihai Popa and regular corn hybrid field with approximately the same vegetation stage 6. Mihailesti, BUZAU demonstration field; 7. Berceni, PRAHOVA 8. Fundulea, CALARASI; 9. Moara Domneasca, ILFOV; 10. Dragos Voda, CALARASI – corn production field.

From this 10 field we chose 3 fields (1. Tudor Vladimirescu, BRAILA, Pietrosu S.A.- field/farmer Mihai Popa; 2. Dalga, CALARASI -field/farmer Liviu Georgescu; 3. 1. Oltenita, CALARASI-field/farmer Dume) with heavy attack which were monitored, (five adjacent plants in the row at six widely spaced positions in the crop, a total of 30 plants, and record pest as frequency and attack intensity).

Recording and estimation of the moth flight was registered visually [counting flying or resting moth], when we passed through field after the scale: 0=no moth; 1=1-2 moth at 100 m of corn row; 2=more than 3 moth at 100 m of corn row.

Readings were done from middle of June until the end of September when corn plants were dried.

Monitoring the pest's larvae populations was done by periodically visiting of fields biweekly during middle of June and late vegetative stage and once or twice per week during late vegetative stage (2 weeks before silking/flag leaf development) and silking when pest activity monitoring is critical, every two weeks until 30 September.

At the beginning, to establish a level for *Helicoverpa*, there were inspected, in 10 localities, 100 plants (25 plants in 4 replicateed) and noted the presence of larvae and their number/ear or plant. We count the number of pupae in Tudor Vladimirescu-BRAILA, Dalga-CALARASI and Oltenita, CALARASI by digging an area of 0.5 $m^2$ on the right size of row, in 10 replications/hybrid plot with higher larvae attack. At the same time, at Tudor Vladimirescu, BRAILA, we counted pupae from an area of 0.5 $m^2$ in 5 replications/hybrid plot (Bt and conventional). At the end of September, it was evaluated the *Helicoverpa* attack taking into consideration: percentage of plants attacked by *Helicoverpa armigera*, attack intensity of *Helicoverpa armigera* [Attack intensity of *Helicoverpa armigera* was noted on a scale from 1 to 3, where 1 means larvae attack only on the tip of the ear and on the silk, 2 means *Helicoverpa* spp, attacked ear was destroyed in tip (0.5-1.5 cm), 3 means that *Helicoverpa* spp. larvae have destroyed (by tunneling) till 1/3 from distal area of ear].

Establishing of quantity loses due to *Helicoverpa* attack was done by weighting 15 ears with attack level of 1, 2 and 3 together with unattacked ears. Establishing of quality loses due to *Helicoverpa* attack was done by establishing percentage of ears with pest attack and presence of fungi on attacked area.

## RESULTS AND DISCUSSION

In southern Romania, there are two complete generations/year and a partial third, winter being passed in the pupae stage in the soil.

Monitoring *Helicoverpa armigera* Hbn. adult's (Table 1) supplies useful information we can see that butterflies in May and June are relatively small in number compared to the butterflies observed in July, August, and even in September, which shows that the butterfly generation emerging from hibernating pupae (G1) is smaller, while the larger number of butterflies in July, August, and September, i.e. adults from the generations G2 and G3 develop on maize crops and some of the captured butterflies might come from migration. Migration is, therefore, as mentioned in literature, more intense in summer, when the butterflies fly large distances in search for food.

**Flight of *Helicoverpa* spp.**

***Table 1***

**Flight of *Helicoverpa* spp. observed in different corn fields**

| | **Localities** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| DATA | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** |
| 15-21 VI | ND | ND | ND | ND | ND | ND | 1* | 0* | 0 | 1 |
| 29 VI-05 VII | ND | ND | ND | ND | ND | ND | 1 | 0 | 1 | 1 |
| 06-12 VII | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 2* |
| 13-19 VII | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 2 |
| 20-26 VII | 0 | 1 | 0 | ND | 0 | ND | 0 | 0 | 0 | 0 |
| 27 VII-02 VIII | 2 | 2 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 |
| 03-09 VIII | ND | ND | ND | ND | ND | ND | 0 | 0 | 0 | ND |
| 10-16 VIII | 1 | 2 | 0 | 0 | 2 | 1 | 0 | 1 | 0 | 1 |
| 17-23 VIII | ND | ND | ND | ND | ND | ND | 0 | 1 | 1 | ND |
| 24-30 VIII | ND | ND | ND | ND | ND | ND | 1 | 1 | 1 | ND |
| 31 VIII-06 IX | 1 | 2 | 0 | 0 | 2 | 2 | 0 | 0 | 0 | 1 |
| 07-13 IX | 0 | 1 | ND | ND | 1 | ND | 0 | 0 | 0 | ND |
| 14-20 IX | 0 | 0 | ND | ND | 1 | 0 | 0 | 0 | 0 | ND |
| 21-27 IX | 0 | 0 | ND | ND | 0 | 0 | 0 | 0 | 0 | ND |
| 28 IX-04 X | 0 | 0 | ND | ND | 0 | 0 | 0 | 0 | 0 | ND |

*0=no moth; 1=1-2 moth at 100 m of corn row; 2=more than 3 moth at 100 m of corn row.
ND=not done

**Localities:** 1. Oltenita, CALARASI-field/farmer Dume; 2. Dalga, CALARASI -field/farmer Liviu Georgescu; 3. Dragos Voda, CALARASI - field/farmer Ildu; 4. Chiciu, CALARASI Soc. Transliberta - field/farmer Ciobanu; 5. Tudor Vladimirescu, BRAILA, Pietrosu S.A.- field/farmer Mihai Popa and regular corn hybrid field with approximately the same vegetation stage 6. Mihailesti, BUZAU demonstration field; 7. Berceni, PRAHOVA 8. Fundulea, CALARASI; 9. Moara Domneasca, ILFOV; 10. Dragos Voda, CALARASI – corn production field.

Analyzing results obtained in 2009, when we monitored the flight of the adults of *Helicoverpa armigera*, we can conclude that the emergence of the butterflies from pupae in spring takes place in May-June.

In corn, the appearance of larval populations of *Helicoverpa armigera* Hbn. from the second generation during the studied period of time took place starting with the second decade of June, with a peak between the second and third decades of July, the last appearances being in the last decade of July. In the third larval generation, the first signs were starting with the second decade of August, with a peak in the third decade of September, the last signs being in the first decade of September. We can consider that the periods in which larval populations appeared were

determined largely by the climate conditions of each year, which could favor or not the early appearance of the butterflies and the development of the larval populations earlier or later on maize crops.

The second and third generations were the generations that cause damage on maize crops, the second generation being more important since it appeared the moment maize was in the milk stage. The climate conditions had a big impact not only on the pest's biology, but also on plant growth and development, both cultivated and spontaneous that represented a food source for the butterflies of the first generation and, thus, acted indirectly on the pest population growth and multiplication.

**Monitoring the pest's larvae populations**

Monitoring the pest's larvae populations was done by periodically visiting of fields. At the beginning, to establish a level for *Helicoverpa*, it was inspected, in 10 localities, 100 plants (25 plants in 4 replicates) and noted the presence of larvae and their number/ear or plant (Table 2), after 13 June, 3 fields (1. Tudor Vladimirescu, BRAILA; 2. Dalga, CALARASI; 3. Oltenita, CALARASI) with evident *Helicoverpa* attack have been monitorised, (five adjacent plants in six replicates widely spaced positions in the crop, a total of 30 plants, were noted for evolution of total alive larvae number/corn ears (no. larvae/ear or plant) (Fig. 1).

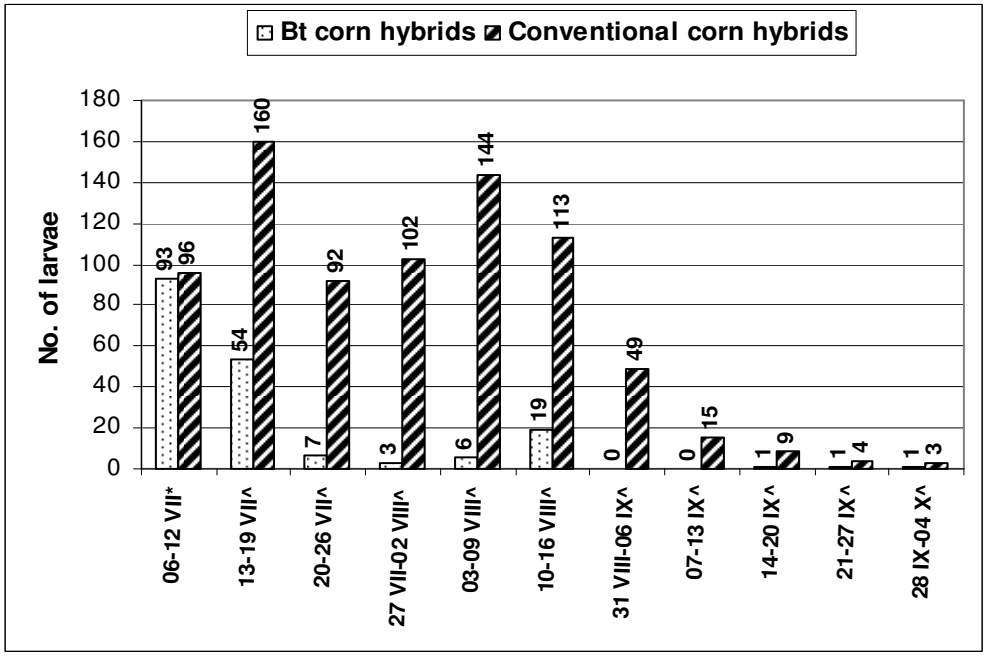


**Fig. 1. Total *Helicoverpa* larvae number**

*Table 2*

**Presence of *Helicoverpa* spp. larvae***

| | Localities | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| DATA | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** |
| 15-21 VI | ND | ND | ND | ND | ND | ND | 10* | 5* | 3* | 14* |
| 29 VI-05 VII | ND | ND | ND | ND | ND | ND | 17* | 5* | 4* | 21* |
| 06-12 VII | 9* | 19* | 5* | 7* | 25* | 8* | 6* | 5* | 4* | 16* |
| 13-19 VII | 25^ | 30^ | 6 | 2^ | 46^ | 7^ | 3^ | 1^ | 0^ | 9^ |
| 20-26 VII | 7^ | 6^ | 3^ | 0^ | 13^ | ND | ND | ND | ND | 8^ |
| 27 VII-02 VIII | 11^ | 17^ | ND | ND | 26^ | 25^ | ND | ND | ND | 5^ |
| 03-09 VIII | 12 | 17^ | ND | ND | 57^ | ND | ND | ND | ND | ND |
| 10-16 VIII | 11 | 16^ | ND | ND | 33^ | 15^ | 10^ | ND | ND | 8^ |
| 17-23 VIII | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| 24-30 VIII | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| 31 VIII-06 IX | 6^ | 7^ | ND | ND | 15^ | 10^ | ND | ND | ND | 2^ |
| 07-13 IX | 5^ | 6^ | ND | ND | 7^ | ND | ND | ND | ND | ND |
| 14-20 IX | 5^ | 5^ | ND | ND | 5^ | ND | ND | ND | ND | ND |
| 21-27 IX | ND | 3^ | ND | ND | 2^ | ND | 1^ | ND | ND | ND |
| 28 IX-04 X | ND | 2^ | ND | ND | 1^ | ND | ND | ND | ND | ND |

*=Number of larve/100 ears or plants (1-6, on most susceptible hybrids); ^= Number of larve/30 ears or plants (1-6, on most susceptible hybrids)
ND=not done
**Localities:** 1. Oltenita, CALARASI; 2. Dalga, CALARASI; 3. Dragos Voda, CALARASI; 4. Chiciu, CALARASI; 5. Tudor Vladimirescu, BRAILA and regular corn hybrid field with approximately the same vegetation stage 6. Mihailesti, BUZAU; 7. Berceni, PRAHOVA 8. Fundulea, CALARASI; 9. Moara Domneasca, ILFOV; 10. Dragos Voda, CALARASI.

## Monitoring the *Helicoverpa* pupae populations

During the last week of July, we counted the number of pupae in Tudor Vladimirescu-BRAILA, Dalga-CALARASI and Oltenita, CALARASI by digging an area of 0.5 $m^2$ on the right size of row, in 10 replications/hybrid plot with higher larvae attack. At Oltenita, CALARASI it was registered, on hybrid DKC 5143, 0.4 pupae/$m^2$ (total 2 pupae), at Dalga-CALARASI it was registered, on hybrid DKC 4626, 0.6 pupae/$m^2$ (total 3 pupae), at Tudor Vladimirescu-BRAILA it was registered, on hybrid DKC 5276, 1.4 pupae/$m^2$ (total 7 pupae). At the beginning of August (the next week) at Tudor Vladimirescu, BRAILA, it was counted pupae from an area of 0.5 $m^2$ in 5 replications/hybrid plot. Of course there is a difference between number of pupae in soil due to larvae population on corn plants, DK 315, 0,4; DKC 3511, 0,8 ; DK 440, 0,4; DKC 4626, 0,4; DKC 4490, 0,8; DKC 5143,

1,2; DKC 5276, 2,4; DKC 5783, 1,6; **average on total conventional corn hybrids 1.1 pupae/m$^2$**.

**Level and importance of NPV which contribute to decreasing population of *Helicoverpa*.**

In Table 3, it is presented the situation at T. Vladimirescu, which shows that from 262 died larvae, 171 (65.27%).

***Table 3***

**Importance of NPV to decreasing population of *Helicoverpa***

| Hybrid | Number of observed died larvae | Number which seems to be died due to NPV |
|---|---|---|
| DK 315 | 26 | 20 |
| DKC 3511 | 40 | 25 |
| DK 440 | 24 | 17 |
| DKC 4626 | 27 | 18 |
| DKC 4490 | 29 | 17 |
| DKC 5143 | 29 | 16 |
| DKC 5276 | 41 | 28 |
| DKC 5783 | 46 | 30 |
| TOTAL Conventional corn hybrids | **262** | **171** |

Mortality of *Helicoverpa* larvae was from total larvae observed (972) and from total died larvae observed (358), 79.6% seems to be died due to NPV infection.

There are more numerous died larvae which seems that were killed by NPV in conventional hybrids (83.4%) than in Bt. corn hybrids (16.6%).

***Establishing the Helicoverpa importance for corn crop***

At the end of September, it was evaluated *Helicoverpa* attack taking into consideration percentage and attack intensity of plants attacked by *Helicoverpa armigera*. The data presented in Table 4 underline that there was a difference between attack of *Helicoverpa* taking in consideration percentage an intensity of attack in different locality (attack is higher in T. Vladimirescu, followed by Dalga and Oltenita), Bt hybrids were significantly less attacked by pest.

Establishing of quantity loses due to *Helicoverpa* attack was done by weighting 15 ears (in kg.) with attack level of 1, 2 and 3 together with unattacked ears only at T. Vladimirescu taking into consideration conventional corn hybrids DK 315, DKC 4626 and DKC 5783. The results presented in Table 5 show that pest is possible to cause, at the level of 2009 in T. Vladimirescu, losses between 4.4 to 6.6%, depending on the hybrid and the level of attack.

*Table 4*

**Importance of *Helicoverpa* attack on corn**

| | T. VLADIMIRESCU | | DALGA | | OLTENITA | |
|---|---|---|---|---|---|---|
| **Hybrid** | **% of ears attacked** | **Intensity of attack** | **% of ears attacked** | **Intensity of attack** | **% of ears attacked** | **Intensity of attack** |
| DK 315 | 18 | 2.3 | 12 | 1.6 | 7 | 3.0 |
| DKC 3511 | 20 | 2.2 | 15 | 2.0 | 9 | 2.6 |
| DK 440 | 30 | 2.3 | 21 | 2.6 | 14 | 2.3 |
| DKC 4626 | 34 | 2.4 | 22 | 2.4 | 15 | 2.4 |
| DKC 4490 | 32 | 2.3 | 19 | 2.7 | 11 | 2.0 |
| DKC 5143 | 26 | 2.2 | 12 | 2.4 | 9 | 1.6 |
| DKC 5276 | 21 | 2.0 | - | - | - | - |
| DKC 5783 | 28 | 2.0 | 14 | 2.2 | 10 | 1.5 |

*Table 5*

**Loses due to *Helicoverpa* attack**

| **Hybrid** | **Production/ 15 corn ears** | | | | **Percentage of losses** | | |
|---|---|---|---|---|---|---|---|
| | Attack level | | | Check | | | |
| | Note 1 | Note 2 | Note 3 | Unattacked | Note 1 | Note 2 | Note 3 |
| DK 315 | 2.229 | 2.200 | 2.130 | 2.228 | +0.04 | -1.26 | -4.40 |
| DKC 4626 | 2.335 | 2.280 | 2.195 | 2.351 | -0.68 | -3.02 | -6.633 |
| DKC 5783 | 2.540 | 2.485 | 2.395 | 2.545 | -0.196 | -2.35 | -5.894 |

Establishing of quality loses due to *Helicoverpa* attack was done by establishing percentage of ears with pest attack and presence of fungi on attacked area. From this point of view almost 100% of the attacked area was covered with mold.

## CONCLUSIONS

1. The results presented show that pest is possible to cause, at the level of 2009, percentage of ears attacked between 0-34% and losses between 4.4 to 6.6 %, depending on the hybrid and the level of attack.
2. The mortality of *Helicoverpa* larvae was from total larvae observed, 79.6% seems to be died due to NPV infection.
3. There is a significant difference between Bt. corn hybrids and conventional corn hybrids; the first category is not suitable for development of pest taking into consideration.

4. In southern Romania, there are two complete generations a year and a partial third, winter being passed in the pupae stage in the soil, but an important role seems to have immigrant moth population at the end of July, beginning of August observing that there is no a clear gap between the second and third moth flight or larval population level on corn ears.

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