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The comparative study of Bt corn and conventional corn regarding the *Ostrinia nubilalis* attack and the *Fusarium* spp. infestation in the central part of Oltenia

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Abstract

Ostrinia nubilalis or the European corn borer is a dangerous corn pest, especially in warmer Romanian regions, including Oltenia. To compare the attack produced by O. nubilalis on the transgenic Bt corn (MON 810) vs. the conventional corn (Deliciul verii), there were performed studies on unirrigated soil, in conditions of natural infestation. The experiments were made at ARDS Şimnic (in the central part of Oltenia) in 2012 and 2013. It was proven that there is a functional direct relation between the O. nubilalis attack and Fusarium spp, in that an intensive Ostrinia attack favors the installation of Fusarium-type pathogens. Our results have shown that the use of transgenic hybrid Bt-MON 810, which contains the gene CrylAb, reduced the O. nubilalis attack by 99.55% in 2012 and by 100.0% in 2013, and the infestation with Fusarium spp. by 95.54% in 2012 and by 100.0% in 2013. Therefore, Bt-MON 810 corn can reduce the damages caused to corn harvest by Ostrinia and implicitly by Fusarium, both quantitatively and qualitatively. Also, it can reduce the number of necessary pesticides by reducing the number of treatments.

Keywords: attack frequency, Bt-MON 810 corn, efficacy, European corn borer

1. Introduction

With the help of modern biotechnology there were made many Bt corn events, but only one is cultivated in Romania, namely hybrid Bt-MON 810 (BADEA & al. [1]). GM corn event MON810 was genetically modified by introducing a gene from a soil bacterium called *Bacillus thuringiensis* (Bt), the resulting hybrid producing an insecticide protein Cry1Ab (δ-endotoxin)which controls certain pest insects, such as *Ostrinia nubilalis* (Hbn.) or the European Corn Borer (POPESCU & al. [2]).

The European corn borer - ECB (*Ostrinia nubilalis*) is apolyphagous pest which attacks many species of both cultivated plants (corn, sorghum, hemp, hops, etc.) and spontaneous plants (*Echinochloa crus galli*, *Artemisia vulgaris*, etc.), but the greatest damage is caused to corn (*Zea mays* L.) (GHIZDAVU & al. [3]).

With corn, the early larvae feed with leaves or male inflorescences, whereas later-stage larvae dig galleries in the stem or in the cob peduncles, a fact which can cause serious damage, affecting up to 50% of the harvest (PAULIAN &al. [4]).

In Romania, the corn production loss due to *O. nubilalis* attacks reaches 1.3% in Dobrogea, 8.5% in Transilvania, 10.5% in the south of Moldova, 11.7% in Bărăgan and 17.7% in The West Plain (POPOV & ROŞCA [5]).

The damage may be direct, by reducing production potential, but also indirect, by breaking the stems and installing of some pathogenic insects such as *Fusarium* spp. or *Ustilago maydis*. Fusarium-type fungi cause the reduction of corn production, affecting negatively

both production quality and human and animal health, due to the mycotoxines produced (KMOCH *&* al. [6]).
The toxins produced by *Fusarium* spp. were identified in corn grains and in the derived products used in human alimentation and domestic animals' nutrition, these mycotoxines causing severe diseases both to man and domestic animals (REGNAULT-ROGER *&* al. [7]).
Many studies show that *O. nubilalis* attacks facilitate *Fusarium* spp. infestation (IACOB [8]; PAPST *&* al. [9]; JURCĂ *&* POPESCU [10]; OSTRY *&* al. [11]).
A cause for concern is the fact that recent climate changes, especially the increase of the average air temperature, result in the extension of ECB damage area in Romania, too, especially in warmer regions. In the south part of Oltenia, DRĂGHICI *&* al [12]; DRĂGHICI [13] reported an attack frequency of up to 60.5%, depending on climate conditions.
Consequently, it is necessary to identify and develop strategies of reducing the damage caused by these pests. This study compares the *O. nubilalis* attack on Bt corn vs. conventional corn and the involvement of this pest in expressing the level of *Fusarium* ssp. infestation, in the pedoclimatic conditions specific to the central part of Oltenia.

2. Materials and Methods

2.1. Field trials and evaluation

In 2012, a 1900 sq. m. experimental area was divided into seven experimental plots (each comprising 14 rows) in which Deliciul verii conventional corn (sweet hybrid) was sown according to the cardinal points (North-South) at various distances (10, 20 and 30 m) from the genetically modified corn MON 810 (DKC 5784 YG) at Agricultural Research and Development Station (ARDS) Şimnic (Figure 1).
In 2013 the experiment was repeated as in the previous year, but the orientation in relation to the cardinal points was changed (this time it was East-West).It was attempted to use the same experimental area as in 2012 (just changing the sowing direction) in order to increase the level of *Fusarium* infestation, considering the vegetal remnants in the soil.
During the blooming period, when the plants ended their process of growth and development, having the maximal level of nutrients in the stem (a sure food source for the larvae) it was determined the *O. nubilalis* attack frequency for each separate plot.
The attack frequency was calculated according to the formula:

$$F = n \times 100/N,$$

where n = number of attacked plants and N = total number of evaluated plants.
During the harvest period, the number and length of the stem tunnels was determined by splitting 5 plants/plot.
At the same time, there were made observations regarding the level of cobs' infestation with *Fusarium* spp. using for this purpose a five-score scale (KWAŚNA *&* al. [14]):very small (1–6 grains, 2%), small (7–30 grains, 3–10%), medium (1/3 of cob, 11–30%), large (1/2 of cob, 31–50%); veery large (>1/2 of cob, 51–100%).
The efficacy (E) parameter was calculated according to the formula (REGNAUT-ROGER *&* al. [7]:

$$E\ (\%) = [(\text{Control} - \text{Treatment})/\text{Control}] \times 100,$$

where „control" and „treatment" were attack and infestation levels with *O. nubilalis* and *Fusarium* spp. in respectively conventional corn and Bt corn plots

2.2. Data analysis

Results are presented as mean with their standard error (SD). Significance of the difference between average values was assessed by analysis of variance (ANOVA) with Duncan's multiple range test ($P \leq 0.05$).

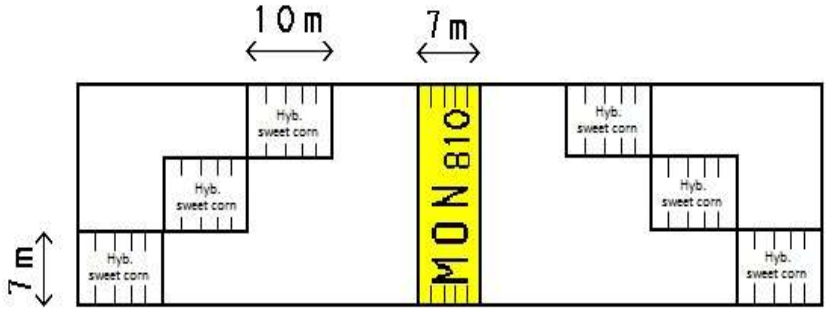

Figure 1. The field design in 2012 and 2013

3. Results and Discussions

*3.1. Climaticaly conditions in relationship to Ostrinia nubilalis attack*and *infestation by Fusarium* spp.

The analysis of the climatic conditions during the research period (2012 and 2013) in ARDS Şimnic (Table 1) reveals the climate differences between the two years of study that have significantly influenced the frequency of attack produced by *O. nubilalis.*

The year 2012 was an extremely dry year, with an excessive pedological drought, to which very high average monthly temperatures were added, a fact which favored the developmental biologic cycle of *O. nubilalis.* During the same year, the first butterflies appeared at the end of May, and the larvae appeared in the first two decades of June, their attack manifesting on the leaves, male inflorescences and especially the stems. The average air temperature in June was higher than the multiannual average by +0.3° C (Table 1).

Table 1. Meteorological data at ARDS Simnic during the research period (2012 and 2013)

Parameter		**IV**	**V**	**VI**	**VII**	**VIII**	**Average**
Rainfall mm	2012	79.3	130.0	28.0	13.5	-	50.16
	2013	56.0	55.0	88.0	20.0	27.0	49.20
	Multiannual average	54.5	70.8	75.1	86.1	48.8	67.06
Temperature ^{0}C	2012	13.7	17.9	21.8	26.6	25.4	21.05
	2013	14.5	19.4	21.3	23.4	24.5	20.60
	Multiannual average	12.1	18.6	21.5	23.8	22.2	19.40

Generally, the year 2013 was a good year for the development of corn, without hot days, with moderate rainfall, even if they were not uniformly spread. Considering the conditions of 2013, at ARDS Simnic the appearance of the first *O. nubilalis* butterflies was noticed at the beginning of June, and the appearance of the larvae took place in the last decade of June and the first decade of July. In June there was a +15.9 mm rainfall exceeding compared to the multiannual average.

The abundant rain and the temperatures slightly below the multiannual average (-0.2 ^{0}C in June and -0.4 ^{0}C in July) limited the flight of *O. nubilalis* butterflies, their oviposition and incubation, consequently, the attack frequency was significantly reduced compared to 2012. It can be said that the *O. nubilalis* attack frequency was influenced by climate conditions, especially by the air temperature. Similar results were obtained by KOCMÁNKOVÁ *&* al. [15] and WALIGÓRA *&* al. [16], who reported that the air temperature is the most important factor in the development of this pest.

The comparative study of Bt corn *vs.* conventional corn showed that the *O. nubilalis* attack frequency and the level of *Fusarium* spp. infestation were significantly lower with Bt corn than with conventional corn in both reference years (Table 2 and 3).

From data obtained regarding the *O. nubilalis* attack frequency in 2012, the sweet corn hybrid (Deliciul verii) was preferred by the larvae, the percentage being very high compared to MON 810 hybrid, respectively between 18.57% (N 20 m) and 27.07% (S 10 m) (Table 2).

Under favorable development conditions for *O. nubilalis*, DRĂGHICI [13] reported an attack frequency between 31.2% and 52.1%, depending on the hybrid, in the RDCFCSS Dăbuleni area, GEORGESCU *&* al. [17] reported an attack frequency of 79.4% for NARDI Fundulea area and JURCĂ *&* POPESCU [10] reported an average attack frequency of 51.27% at Horia, Arad County.

On the MON 810 plot there was a single plant exhibiting a low leave attack, which, in terms of percentage, meant an attack frequency of only 0.1% (Table 2).These results suggest the fact the frequency of the plants attacked by *O. nubilalis* during their flowering period was not influenced by the wind direction or the distance from the genetically modified hybrid. It is certain, though, that the ARDS Şimnic experimental area is under the attack of this pest and MON 810 hybrid is very resistant to it.

Regarding the *Fusarium* spp. cob infestation in 2012, it was noticed that MON 810 corn had a very small infestation rate of 2.1% (Table 2). The significantly reduced level of *Fusarium* spp. infestation in the case of MON 810 corn can be explained by a reduced level of *O. nubilalis* larvae attack (KMOCH *&* al. [6]).

Table 2. The attack frequency by *Ostrinia nubilalis* and infestation levels by *Fusarium* spp. (average ± SE in plot) at Bt corn *vs*. conventional corn in 2012

Genotype	Wind direction and distance	No. evaluated rows	No. evaluated plants	Attack frequency *O. nubilalis* (%)	Levels infestation *Fusarium* spp. (%)
DELICIUL VERII	N30m	14	210	22.35 ±2.25 bc	63.21±0.62 e
DELICIUL VERII	N 20m	14	200	18.57±1.94 b	48.42±0.58 d
DELICIUL VERII	N 10m	14	183	21.35±2.62 bc	67.0±0.86 f
MON 810		10	578	0.1±0.09 a	2.1±0.34 a
DELICIUL VERII	S 10m	14	213	27.07±4.18 c	19.42±1.45 b
DELICIUL VERII	S 20 m	14	189	22.21±2.34 bc	48.78±0.61 d
DELICIUL VERII	S 30 m	14	215	22.71±2.47 bc	36.0±1.99 c

a, b, c, d, e, f - values with different letters denote statistically significant difference at $P \leq 0.05$

Table 3. The attack frequency by *Ostrinia nubilalis* and infestation levels by *Fusarium* spp. (average ± SE in plot) at Bt corn *vs*. conventional corn in 2013

Genotype	Wind direction and distance	No. evaluated rows	No. evaluated plants	Attack frequency *O. nubilalis*(%)	Levels infestation *Fusarium* spp. (%)
DELICIUL VERII	W 30m	13	249	1.38±0.73 ab	20.23±1.26 d
DELICIUL VERII	W 20m	14	298	4.85±1.64 d	13.5±1.11 c
DELICIUL VERII	W 10m	14	301	1.42±0.82 ab	11.14±1.09 bc
MON 810		10	472	0 a	0 a
DELICIUL VERII	E 10m	14	282	2.57±1.01 bc	10.64±1.11 bc
DELICIUL VERII	E 20 m	14	346	4.0±1.36 cd	9.07±1.31 b
DELICIUL VERII	E 30 m	9	198	3.77±3.93 cd	9.33±1.42 b

a, b, c, d - values with different letters denote statistically significant difference at $P \leq 0.05$

For the sweet hybrid (Deliciul verii) there was observed a range of infestation levels from medium to very large, between 19.42 % (S 10m) and 67.0 % (N 10 m) (Table 2). The high infestation level with this hybrid is probably due to the fact that, being a very early hybrid, it reached its maturation earlier, and *Fusarium* spp. attached to the grains almost at the stage of the latter's physiological maturity, since it had favorable weather conditions. This was the result of the high sugar content of the grain and of the *O. nubilalis* larvae attack.

The year 2013, a moderate year from a rainfall point of view, can't be said to have been favorable to *O. nubilalis* attacks in this ecological area. Therefore, the infestation levels for conventional corn were lower than in 2012, ranging between 1.38 % (W 30m) and 4.85 % (W 20m) (Table 3). Under these unfavorable development conditions, DRĂGHICI [13] reported an attack frequency between 11.7% and 18.3%, depending on the hybrid, for the RDCFCSS Dăbuleni area, and GEORGESCU *&* al. [17] reported an average attack frequency of 43.3% for the NARDI Fundulea area.

There was no *O. nubilalis* attack on the plot cultivated with MON 810 corn (0%), as expected (Table 3).

Comparing the plots, we noticed that there are significant differences between them, but it was noticed again, for the second year in a row, that there is no connection between the frequency of attacked plants and the orientation in relation to the cardinal points or the distance from the genetically modified hybrid.

As far as *Fusarium* spp. infestation is concerned, it is obvious in the second year of the study (2013), too, but only in the case of conventional corn and the level of infestation ranges from small to medium, the percentage being lower than in 2012, between 9.07 % (E 20 m) and 20.23 % (W 30 m), respectively Bt-MON 810 corn was not affected by *Fusarium* spp. (0%) (Table 3).Therefore, the functional relation between *O. nubilalis* attacks and *Fusarium* spp. mentioned in literature is proven to be valid.

MUNKVOLD *&* al. [18] and MUNKVOLD [19] reported that Bt corn, genetically modified to control *Lepidoptera*, caused a significant decrease both in *Fusarium* spp. infestation and the content of mycotoxines.

CZEMBOR *&* al. [20] consider that, as a result of the lesions caused in the stem and the cob, *O. nubilalis* larvae can spread *Fusarium* spp. infections in two ways: firstly, they can carry

the spores of the various types of Fusarium on the surface of the plants, grains and into the stems; secondly, when the larvae feed, they cause lesions and the spores laid onto the injured tissue germinate more easily and infest the plant.

3.2. The number and dimensions of the tunnels produced by O. nubilalis into conventional corn stems

The conventional corn, on the exterior part of the stems attacked by *O. nubilalis* we could notice one or more orifices, starting from the superior internodes towards the inferior ones, the orifices having corresponding irregular tunnels of various dimensions into the stem.

Consequently to the transversal sectioning of the corn stems under study, it was noticed that the majority of the orifices had been placed below the insertion point of the main ear, becoming more numerous towards the stem base. In very few cases did the tunnels made by the larvae reach the area near the top of the male inflorescence.

In 2012 there were plants which had, on the average, more tunnels, the highest number being on the plot S 20 m (1.88). Concerning the tunnel length, it ranged between 5.11 on the plot S 20 m and 8.50 cm on the plot S 20 m (Table 4).

In 2013, all the plants under study had a single tunnel and the average length of the tunnels ranged between 7.40 cm on the plot S 30 m and 13.20 cm on the plot V 20 m (Table 5). There was no statistical significance established between the studied characters and the orientation in relation to the cardinal points or the distance from the genetically modified hybrid.

Table 4. The number and dimensions of the tunnels (average ± SE in plot) produced by *O. nubilalis* into conventional corn stems in 2012

Wind direction and distance	Total length of sectioned plant (cm)	No. tunnel/plant (cm)	Tunnel length (cm)
N 30 m	96.40±3.66	1.5±0.26	7.62±1.57
N 20 m	99.00±4.04	1.5±0.26	7.50±2.37
N 10 m	102.40±5.63	1.16±0.16	7.50±1.26
S 10 m	96.80±3.65	1.00±0	5.20±0.70
S 20 m	104.80±3.41	1.88±0.28	5.11±0.45
S 30 m	92.00±6.13	1.5±0.26	8.50±2.19

Measurements and observations made on five plants/plot

Table 5. The number and dimensions of the tunnels (average ± SE in plot) produced by *O. nubilalis* into conventional corn stems in 2013

Wind direction and distance	Total length of sectioned plant (cm)	No. tunnel/plant (cm)	Tunnel length (cm)
W30 m	108.20±5.45	1.00±0	8.40±1.02
W 20 m	121.20±1.68	1.00±0	13.20±3.53
W 10 m	113.80±4.63	1.00±0	10.20±1.79
E 10 m	108.80±5.45	1.00±0	8.40±1.02
E 20 m	122.80±5.50	1.00±0	7.60±0.88
E 30 m	121.00±5.13	1.00±0	7.40±0.85

Measurements and observations made on five plants/plot

3.3. The efficacy of Bt corn

The transgenic corn MON 810 is promoted to be used against *O. nubilalis* larvae, which usually feed on the leave base after incubation (first stages) and afterwards dig tunnels through the stems (DARVAS & al. [21]).

Our results show that MON 810 corn controlled the *O. nubilalis* attack with an efficiency rate of 99.55 % in 2012 and 100% in 2013. In fact, in 2012, the 0.1% attack addressed the leaves, not the stems, therefore we can say that the stem infection efficiency was 100 % in 2012, too (Table 6). Similar results regarding the Bt corn efficiency in reducing the *O. nubilalis* attack on corn stems have been obtained by JURCĂ & POPESCU [10], DARVAS & al. [21], KOCOUREK & STARÁ [22].

Table 6. Efficacy of MON 810 corn (DKC 5784 YG)

Species	Deliciul verii %		MON 810 %		Efficacy %	
	2012	2013	2012	2013	2012	2013
Ostrinia nubilalis	22.37±1.12	2.99±0.58	0.1	0	99.55	100
Fusarium ssp.	47.13±7.21	12.31±1.71	2.1	0	95.54	100

The *Fusarium* spp. cob infestation was controlled by MON 810, the efficiency rate being 95.54% in 2012 and 100% in 2013 (Table 6). The results obtained by JURCĂ & POPESCU [10] show an efficiency rate of 81.78% in 2008 and 75.67% in 2009, respectively, regarding the control of *Fusarium roseum* f. *cerealis* attacks.

Bt corn hybrids are efficient in reducing the lesions caused by the insects, by exerting an indirect control on the plant sensitivity to fungus infections and mycotoxine contamination (MUNKVOLD & al. [18]; OSTRÝ & al. [11]; BOWERS & al. [23].

MEISSLE & al. [24], considers that Bt corn is one of the tools in the integrated pest management, a highly specific and efficient pest control measure that allows the farmes to produce high-quality grain.

By choosing to grow Bt corn hybrid, the use of agricultural pesticides is reduced, especially in those areas where the pressure of the lepidopters is extremely high and where the pest insect control requires more treatments. This type of Bt corn (MON 810), which biosynthesizes an insecticide protein continuously, reduces the necessity for chemical treatments (FOLCHER & al. [25]).

4. Conclusions

In the central part of Oltenia, the *O. nubilalis* attack exists in corn conventional crops, frequency reaching an average 22.37%, whereas the *Fusarium* spp. infestation rate reaches up to 47.13%, depending on climate conditions.

There is a positive functional relation between the attack frequency of *O.nubilalis* and the infestation level with *Fusarium* spp. thus, in 2012, when *O. nubilalis* had favorable growth conditions, the level of *Fusarium* spp. infestation was also higher.

The attack frequency and the level of conventional corn infestation do not vary according to the cardinal point orientation or the distance in relation to the genetically modified hybrid.

Bt corn (MON 810) controls efficiently both *O. nubilalis* attacks and *Fusarium* spp. infestation of corn cobs, proving to be one of the best control strategies.

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