MASS REARING OF THE EUROPEAN CORN BORER (*OSTRINIA NUBILALIS* HBN) IN LABORATORY CONDITIONS, SUCCESSIVE GENERATIONS AT NARDI FUNDULEA, 2010-2012

Emil GEORGESCU[1], Lidia CANA [1], Horia Lucian IORDAN[1], Nicoleta BALABAN[2],

emilgeorgescu@yahoo.com

Abstract

European Corn Borer (*Ostrinia nubilalis* Hbn) is one of the most dangerous pests of the maize crop in Transylvania and Moldova. In south and south-east of the Romania is the second pest like economical importance after maize leaf weevil (*Tanymecus dilaticollis* Gyll). In last three years it has registered higher attacks of ECB at maize plants, especially in west part of country, but in some areas of south-east of the Romania, too. At NARDI Fundulea, this insect is rearing in continuous flux, in laboratory conditions, successive generations for obtaining of the egg batches for artificial infestation of the maize plants in field conditions, for established of the maize hybrids and lines tolerance for this pest. The researches started in 1973 with testing of different types of artificial diet and mass rearing system (spaces, boxes for larvae, cages for moths, egg batches storage period depending of temperature, etc). In year 2010 it has obtained in laboratory conditions 203253 egg batches, in year 2011 it has obtained 136043 egg batches and in year 2012 it has obtained 121945 egg batches witch were used for artificial infestations of the maize plants in field conditions. The average number of the egg batches/female obtained in laboratory conditions was 3,0 in year 2010, 2,7 in year 2011 and 2,4 in year 2012. Almost every year it has started rearing in laboratory conditions of a new insect's colony. At the end of the year 2012 the insect colony created in 1979 arrived at 425th consecutive generation, the insect colony created in 2008 arrived at 59th consecutive generation, the insect colony created in 2010 arrived at 33rd consecutive generation, while the insect colony created in 2011 arrived at 22nd consecutive generation. The insects from colony started in year 1979 and grown for 425 successive generations at the end of the 2012, in laboratory conditions, don't lose capacity of producing damages at maize plants in field conditions, comparative with insects from colonies created in last years.

Key words: laboratory, continuous flux, generations, egg-batches

Maize is one of the most important crops from Romania. In conformity with Has V. et al. (2010), an effective production of this crop is a matter of national interest. Year by year it has registered quantitative and qualitative yield losses because of unfavorable meteorological conditions (draught, high temperatures, storms and hailstone) or because of weeds or attack produced of diseases and pests. Only because of the pest attack, yield losses at maize can arrive at 23 % (Trotus E. et al., 2011). European corn borer (*Ostrinia nubilalis* Hbn) is main pest of the maize crop in western part of the country, Transylvania, in hilly area and along meadows of the main rivers and Danube (Barbulescu A. et al., 2001). In south and south-east of the country is second pest like economical importance, after *Tanymecus dilaticollis* (Cristea M. et. al., 2004). In recent specialty literature it has mentioned that yield losses because of the European Corn Borer attack can arrive at 17,7 % in western part of the Romania or at 10,5 % in north area of the Moldova (Popov C. et Rosca I., 2007). In last two decades it hasn't signalized high problems because of the *Ostrinia nubilalis* attack. But, in period 2010-2012 it has signalized again problems with European corn borer, especially in west part of the country (Alexandri A., 2011; Plants Health, 2012). The possible explications for this situation is climatic changes, that can favored insect in first stages of development or new foreign maize hybrids cultivated in our country with none or less information concerning resistance to European Corn Borer attack. The best method for finding of the answers at this problem is field testing of the maize plants, using *Ostrinia nubilalis* egg batches, obtained from moths grown in laboratory conditions, in continuous flux.

[1] INCDA, Fundulea
[2] Eco Ela Import Export

European corn borer is first pest grown in laboratory conditions, on artificial diet (Barbulescu A., 1980). In Romania, researches concerning mass rearing of the *Ostrinia nubilalis* in laboratory conditions starting in years `70 by Barbulescu A., at ICCPT Fundulea (actual NARDI Fundulea) for south of the Romania and Mustea D., at ARDS Turda for Transylvania. In frame of the Plant Protection Laboratory, from NARDI Fundulea, it has boarded many researches, such as: finding of the better diet for growing of the European Corn Borer, consecutive generations in laboratory conditions (Barbulescu A., 1977, 1978, 1980, 1984), temperature influence concerning storage time of the egg batches (Barbulescu A., 1979), stability of the insects grown, consecutive generations on same artificial diet, in laboratory conditions (Barbulescu A., 1984, 1986, 1993, 1996, 2001). Egg batches obtained from the moths grown in laboratory conditions were used for artificial infestation of the maize plants for establishment of the hybrids tolerance for this pest (Barbulescu A., Cosmin O., 1997; Barbulescu A. et al., 1999, 2001). Now days, only at NARDI Fundulea it has continued rearing of the European corn borer in laboratory conditions, for obtaining of the egg-batches, with an production per year between 121.000 and 200.000 egg-batches, used for different researches in field conditions (plant or insecticide testing). In this paper are presented results obtained in laboratory conditions regarding egg batches productions and data concerning behavior of the insects from different colonies in field conditions.

MATERIAL AND METHOD

The experiences were made at Plant Protection Laboratory in frame of the National Agricultural Research and Development Institute Fundulea, Calarasi County. Growing conditions for European corn borer are different and depending of the development stages of the insect. For larvae stage, the growing conditions in laboratory are: air temperature 27-28 ºC and air relative humidity between 60 and 90 %. The ventilation and light must be assigned all day. At the pupae stage, the growing conditions in laboratory are: air temperature between 21 and 25 ºC, air relative humidity between 60 and 90 %, permanent ventilation and continuous dark. At the moth stage, for favoring egg batches deposition, growing laboratory conditions must be similar with natural conditions from the field. In egg-batches deposition room, growing conditions are: for 18 hours air temperature 27-28 ºC and for 6 hours, air temperature 20 ºC (simulation of the day-night alternation); air relative humidity between 82-85 % and permanent ventilation. For mass rearing insects in laboratory conditions it has used diet with main ingredient, “bean flour” (Barbulescu A., 1980). Comparative with original formulation, now days, diet used for insects mass rearing are without “dried milk for calf’s” ingredient (table 1). At each generation in frame of each colony it has registered average number pupae/rearing box, total number of the moths, female number and egg batches number. For testing of the European corn borer insect colonies, starting in different years, for evaluation of the capacity of the larvae for damaging maize plants, it has made artificial infestation in field conditions. It has used one maize hybrid, considered sensitive at the *Ostrinia nubilalis* attack (Rapsodia). With, appreciative 10 days before panicle emergence (second decade of the June), maize plants were infested with 10 egg batches/plant, twice, 5-6 days, between infestations. Egg-batches used for artificial infestation are in “black-head” stage, when larva head become visible. Attack level of the European corn borer at the maize plants were analyzed in autumn (September), after the end of the maize vegetation period, before harvesting. From each plot it has taken 20 maize plants, the stalks was cooped in twice and it has determined three parameters: attack frequency, gallery length/plant (cm) and number of the alive larvae/plant. The results were statistical analyzed through variance analysis, using Microsoft Excel.

RESULTS AND DISCUSSIONS

For maize hybrids and lines testing at the *Ostrinia nubilalis* attack, every year at Maize and Sorghum Breeding Laboratory and Plant Protection Laboratory experimental fields, it has made artificial infestation of the maize plants with egg-batches obtained from the moths reared in laboratory conditions. Data from table 2 show that in 2010, it has obtained 203253 egg-batches; in 2011 it has obtained 136043 egg-batches, while in 2012 it has obtained 121945 egg-batches. Average number of pupae/rearing box was 505 in 2010, 486 in 2011 and 466 in 2012. Total number of moth obtained in laboratory conditions in 2010, at NARDI Fundulea was 150758, the highest value from all years taken in study. In 2011 it has obtained 116342 moths, while in 2012 it has obtained 134085 moths. It is important to mention that even in 2012, it has obtained more females (60338) comparative with 2011 (52354), average number of egg-batches/female was higher in 2011, with a value of 2,7. In 2012 it has obtained 2,4 egg-batches/female. Higher value of this parameter was in 2010 (3,0 egg-batches/female). Generally, female percent was 45 % in all years taken in study. Almost every year it has started a new colony of *Ostrinia nubilalis* for insect rearing in

laboratory conditions in continuous flux. Larva's are harvested in autumn period from the field, then are stored until spring at temperature of the environment. When spring arrive, larva's are taken in laboratory where there are transformed in pupae and then starting multiplication of the insect in laboratory conditions. It has kept colonies created in last years, well as a reference colony, created in

Table 1

Ingredients used for diet preparation for *Ostrinia nubilalis* Hbn insects mass rearing, quantity for 8 rearing boxes

Ingredient	Quantity
Bean flour	572 g
Wheat bran	160 g
Beer yeast	156 g
Premix	40 g
Sugar	155 g
Ascorbic acid	15,6 g
Sorbic acid	10 g
Glacial acetic acid	14,8 ml
Formaldehyde	8,4 ml
Agar	40 g
Water	3500 ml

Table 2

Data concerning mass rearing of the European corn borer (*Ostrinia nubilalis* Hbn), in controlled laboratory conditions, în continuous flux, on the same artificial diet, period 2010-2012

Specification	2010	2011	2012
Number of the growing boxes	329	260	286
Average number of the pupae/box	505	486	466
Total number of the moths	150758	116342	134085
Total number of the females	67922	52354	60338
% female	45,05	45,00	45,00
Total number of the egg-batches	203253	136043	121945
Average nr. egg-batches/female	3,0	2,7	2,4

Table 3

Behavior of the *Ostrinia nubilalis* Hbn colonies, starting in different years, in field conditions, year 2010

Colony	Generation number	Attack frequency (%)	Gallery length (cm/plant)	Alive larvae (nr./plant)
1979 (mt)	402	75,0	7,1	0,7
2008	37	70,0	7,3	0,7
2010	11	70,0	7,2	1,0*
			DL5 %=0,45 DL1 %=0,68 DL0,1 %=1,09	DL5 %=0,26 DL1 %=0,40 DL0,1 %=0,64

Table 4

Behavior of the *Ostrinia nubilalis* Hbn colonies, starting in different years, in field conditions, year 2011

Colony	Generation number	Attack frequency (%)	Gallery length (cm/plant)	Alive larvae (nr./plant)
1979 (mt)	413	100	33,8	5,0
2008	48	100	30,0°	5,7**
2010	22	100	33,4	6,3***
2011	9	100	36,1	6,0***
			DL5 %=3,04 DL1 %=4,26 DL0,1 %=6,17	DL5 %=0,40 DL1 %=0,55 DL0,1 %=0,80

Table 5

Behavior of the *Ostrinia nubilalis* Hbn colonies, starting in different years, in field conditions, year 2012

Colony	Generation number	Attack frequency (%)	Gallery length (cm/plant)	Alive larvae (nr./plant)
1979 (mt)	425	100	13,3	1,3
2008	59	100	14,1*	1,5
2010	33	100	14,7***	1,8**
2011	20	100	13,7	1,3
			DL5 %=0,46 DL1 %=0,64 DL0,1 %=0,93	DL5 %=0,31 DL1 %=0,43 DL0,1 %=0,6

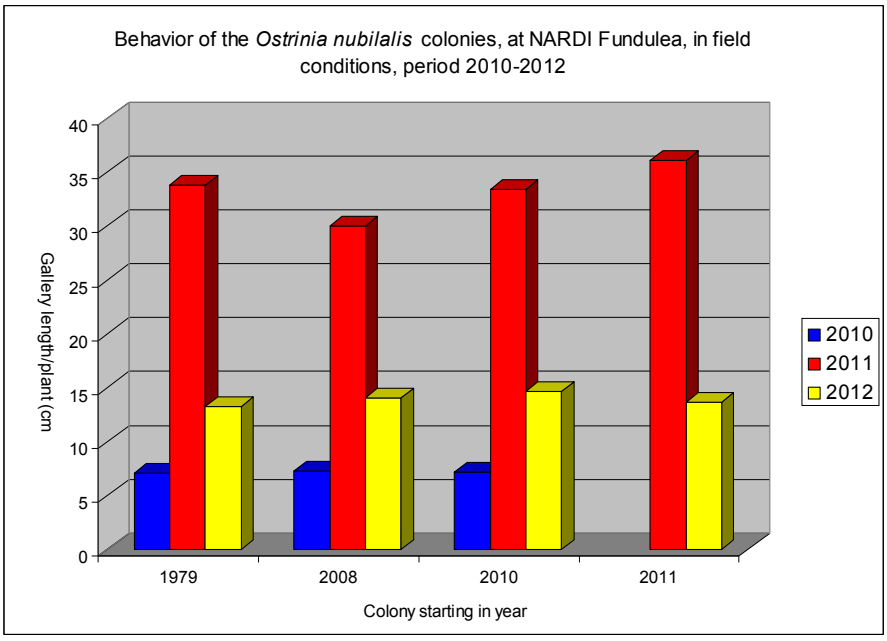

Figure 1 **Behavior of the Ostrinia nubilalis colonies at NARDI Fundulea, in field conditions, period 2010-2012**

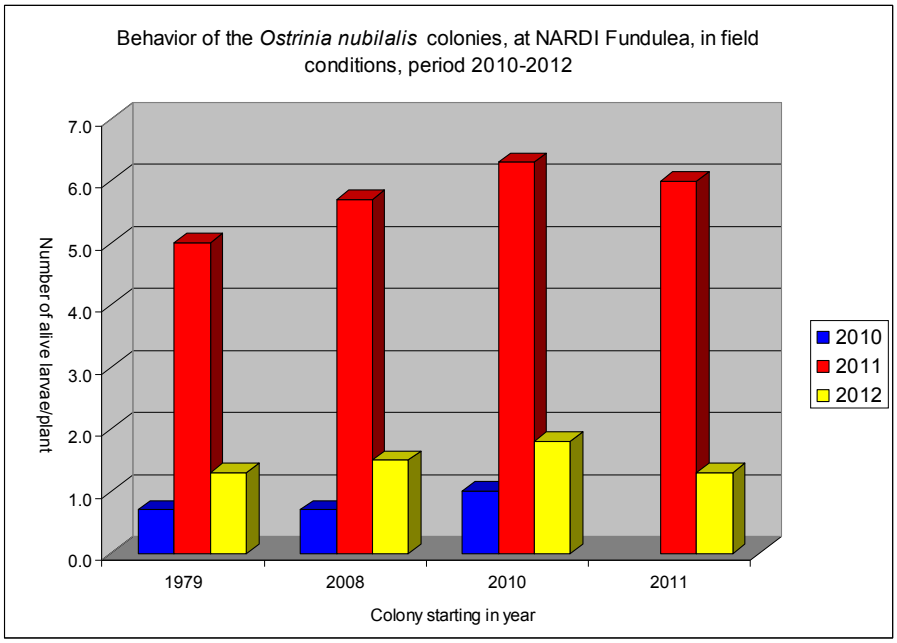

Figura 2 **Behavior of the Ostrinia nubilalis colonies at NARDI Fundulea, in field conditions, period 2010-2012**

year 1979. At the end of the year 2012, the insect colony created in 1979 arrived at 425th consecutive generation, the insect colony created in 2008 arrived at 59th consecutive generation, the

insect colony created in 2010 arrived at 33rd consecutive generation, while the insect colony created in 2011 arrived at 22nd consecutive generation. Every year, in experimental field of the Plant Protection Laboratory, are tested insects from colonies created in different years (*tab*. 3-5). Analyzing data from table 3, it has ascertained that, in field conditions of the year 2010 it hasn't registered high differences regard as attack frequency, average value of the gallery length/plant or number of the larvae/plant. The highest value of larvae/plant was registered at colony created in 2010 (1,0 larvae/plant). Average length of the gallery/plant ranged between 7,1 (colony started in 1979) and 7,3 (colony started in 2008). In climatic conditions of the year 2011 it has tested insects from four colonies (1979, 2008, 2010 and 2011). Data from table 4 show that attack frequency at maize plants was equal at all experimental variants (F=100 %). Regard as average value of the gallery length/plants, from table 4 it has ascertained that attack of the European corn borer at maize plants was higher comparative with previous year. At colony started from 1979, gallery length/plant was of 33,8 cm. This value is higher then attack produced by insects from colony created in 2008 (gallery length/plant=30.0 cm) and almost similar with insects from colony created in 2010 (gallery length/plant=33.4 cm). The highest value of this parameter was registered at insects from colony created at the beginning of the 2011. Regard as average number of alive larvae/plant, from table 4 it has ascertained that this parameter ranged between 5,0 larvae/plant, in case of colony created in 1979 and 6,3 in case of the colony created in 2010. Differences between control variant and last two variants are statistically assigned. In 2012, in field conditions of the Plant Protection Laboratory it has tested insects from four colonies (1979, 2008, 2010, and 2011). Data from table 5 show that, at all experimental variants, attack frequency were 100 %. That means all maize plants from experimental plots were attacked by European corn borer (*Ostrinia nubilalis* Hbn). Average gallery length/plants values were lower comparative with 2011 but higher comparative with 2010. Attack is similar at insects from colony created in 1979 and colony created in 2011 (GA=13, 3 cm and 13,7 cm). The highest value of this parameter was at insects from colony created in 2010 (GA=14,7 cm). This value is statistical assigned. Regard as average number of alive larvae/plants, data from table 5 show that are not high differences between experimental variants, except variant with insect from colony created in 2010, where it has find 1,8 larvae/plant. This value is statistical assigned. All this data, from the field, period 2010-2012 demonstatrate that even insect is rearing more then 400 generations in laboratory conditions, they don't lose capacity of producing damage at maize plants in field conditions.

CONCLUSIONS

Rearing of the European corn borer (*Ostrinia nubilalis* Hbn) in laboratory conditions, in continuous flux, on same artificial diet, is a good method for mass production of the insects egg-batches necessary for the field researches concerning maize hybrids and lines tolerance at the attack produced by this pest or researches concerning biological effectiveness of the insecticides used like vegetation treatment.

In year 2010 it has obtained in laboratory conditions 203253 egg batches, in year 2011 it has obtained 136043 egg batches and in year 2012 it has obtained 121945 egg batches witch were used for artificial infestations of the maize plants in field conditions.

The average number of the egg batches/female obtained in laboratory conditions was 3,0 in year 2010, 2,7 in year 2011 and 2,4 in year 2012.

At the end of the year 2012 the insect colony created in 1979 arrived at 425th consecutive generation, the insect colony created in 2008 arrived at 59th consecutive generation, insect colony created in 2010 arrived at 33rd consecutive generation, while insect colony created in 2011 arrived at 22nd consecutive generation.

Even insect is rearing more then 400 generations in laboratory conditions, they don't lose capacity of producing damage at maize plants in field conditions.

REFERENCES

Alexandri A., 2011 - *Ostrinia nubilalis-Diabrotica virgifera virgifera*, Plants Health, nr. 159 (8/2011), p. 12.

Barbulescu A., 1977 – *Rearing of the European corn borer Ostrinia nubilalis Hbn. on different artificial diets*, Problems of Plant Protection, vol. V, nr. 2, p. 141-150.

Barbulescu A., 1978 – *Insects rearing on artificial diets*, Problems of Plant Protection, vol VI, nr. 4, p. 375-400.

Barbulescu A., 1979 – *Influence of the temperature concerning European corn borer egg incubation Ostrinia nubilalis Hb.*, Problems of Plant Protection, vol VII, nr. IV, p. 377-384.

Barbulescu A., 1980 – *Techniques of mass rearing on artificial diet of the European corn borer (Ostrinia nubilalis Hbn.)*, Problems of Plant Protection, vol. VIII, nr, 1, p. 1-12.

Barbulescu A., 1984 – *Behavior of the European corn borer (Ostrinia nubilalis) reared a different number of succesive generations on same*

artificial diet, Problems of Plant Protection, vol XII, nr. 4, p. 285-290.

Barbulescu A., 1986 - *Results concerning mass rearing of the European corn borer Ostrinia nubilalis on same diet successive generations*, Annals of NARDI Fundulea, vol. 53, pg. 373-381.

Barbulescu A., 1993 – *Data obtained in period 1985-1989 concerning rearing of the specie Ostrinia nubilalis on artificial diet many successive generations,* Problems of Plant Protection, vol XXI, nr. 1, p. 1-11.

Barbulescu A., 1996 - *Data obtained in period 1990-1992 concerning rearing of the specie Ostrinia nubilalis on artificial diet many successive generations,* Problems of Plant Protection, vol XXIV, nr. 1, p. 1-12.

Barbulescu A., Cosmin O., 1997 – *Maize inbreed lines obtained at Fundulea, characterized by a certain resistance degree to Ostrinia nubilalis Hb*, Problems of Plant Protection, vol XXV, nr. 1, p. 1-8.

Barbulescu A., Cosmin O., Sabau I., 1999 - *Data obtained at Fundulea, concerning maize resistance at European corn borer Ostrinia nubilalis* attack, Problems of Plant Protection, vol XXVII, nr. 2, p. 173-180.

Barbulescu A., 2001, *Data obtained in period 1996-1998 concerning rearing of the specie Ostrinia nubilalis on artificial diet many successive generations,* Problems of Plant Protection, vol XXIX, nr. 1, p. 33-40.

Barbulescu A., Popov C., Sabău I., 2001 - *The behavior of a Monsanto maize hybrid-Dekalb 512 bt to the attack by the European corn borer in Romania*, Romanian Agricultural Research, vol. 15, p. 65-73.

Cristea M, Cabulea I., Sarca T., **2004** - *Maize. Monographic study*, Volume 1, Romanian Academy Publishing house, Cap. 14: 589-626.

Has V., Has I., Antohe I., Copandean A., Nagy E., **2010** - *The variability in production capacity and quality of grain maize hybrids from different FAO maturity groups*, Annals of NARDI Fundulea, vol. 78, nr. 1, p. 37-47.

Popov C., Rosca I., 2007 - *Technology of European corn borer (Ostrinia nubilalis Hbn.) mass rearing, in continuous system and successive generations*, Entomological Research, vol. 37, nr. 1, pg. 126.

Trotus E., Buburuz A.A., Zaharia P., 2011 - *Researches on the protection of maize crops against soil pests*, Agronomical Researches in Moldavia, vol. 4, p. 45-51.