Technology of the European Corn Borer (*Ostrinia nubilalis* Hbn) Mass Rearing, Successive Generations, in Controlled Conditions, at NARDI FUNDULEA

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ABSTRACT

Obtain insects egg batches in laboratory conditions, used for artificial infestation of the maize plants, to establish maize lines and hybrids tolerance to pest attack. Because in nature, the attack of *Ostrinia nubilalis* are not at same level, every year, as result of the climatic conditions, especially at egg batch deposition and larva emergence, artificial infestation of the maize plants is one of the best method to evaluate maize lines and hybrids reaction at this pest. European corn borer is reared at Plant Protection Laboratory, from NARDI Fundulea, Calarasi County, Romania. Field experiments were made at experimental fields of both, Plant Protection and Maize Breeding laboratories. In 2011 it has obtained 136043 egg batches, in 2012 it has obtained 121945 egg batches and in 2013 it has obtained 133550 egg batches. At the end of the year 2013, the insect colony created in 1979 arrived at 442nd consecutive generation, the insect colony created in 2008 arrived at 77th consecutive generation, the insect colony created in 2010 arrived at 51st consecutive generation and insect colony created in 2011 arrived at 38th consecutive generation. Even if insects are reared more then 440 generations in laboratory conditions, they don't lose capacity of attacking maize plants. Rearing of the European corn borer (*Ostrinia nubilalis* Hbn) in laboratory conditions, in continuous flux, on same artificial diet, is one of the best methods for mass production of the egg-batches necessary for the field researches concerning maize hybrids and lines tolerance at the attack produced by this pest.

Keywords: diet, insects, laboratory, maize, rearing.

INTRODUCTION

In last years, European corn borer (*Ostrinia nubilalis* Hbn) area has increasing in some countries from west or south-west of the Europe, such as: France, Germany, Spain, Portugal (Brookes, 2007; Brookes, 2008). Same author mentioned that, in some countries from Central Europe, such as Czech Republic or Slovakia, the area of this pest extend to north. According of

some studies made in Germany, ECB area extend in north and east of the country (Gathmann and Rothmeier, 2005). Similar studies in Poland, made by Plant Protection Institute from Warsaw and Poznan, arrive at same conclusion, that *O. nubilalis* area extend from south-east to north and east of the country (Beres and Konefal, 2010; Beres, 2012). Recent research made in Hungary, make in evidence that ECB area extend from south-east

to north-west (Keszthelyi, 2010). A possible cause for area increasing of European corn borer from south to north is climate change such as average air temperature increasing (Kocmánková *et al.*, 2010). Same author mentioned that, as result of the climate changes, this pest present more than one generation/year. Increasing numbers of generations per year in some areas are reported in different countries from Central and West Europe (Brookes, 2008; Derron *et al.*, 2009; Keszthelyi, 2010). *O. nubilalis* were first time signalised in south-west of England, on maize crops (Korycinska and Cannon, 2010).

In Romania, favourable areas for European corn borer are in west part of the country, Transylvania, hilly areas, along meadows of the main rivers and Danube (Barbulescu *et al.*, 2001). In south and south-east of the country is second pest like economical importance, after *Tanymericus dilaticollis* (Cristea *et al.*, 2004). In specialty recent literature it has mentioned that average yield losses in Romania, because of the European corn borer attack arrive at 7,5 %, but in West Plane arrive at 17,7 % and 10,5 % in Transylvania (Popov and Rosca, 2007). In last years, in west part of the Romania and Transylvania it has signalized increasing of the damages produced by European corn borer to maize crops (Alexandri, 2011). The author mentioned that possible causes for ECB attack increasing, climate changes and lack of information about new foreign maize hybrids tolerance to this pest. Malschi *et al.* (2013) report increasing of the *O. nubilalis* average attack frequency in 2012, at ARDS Turda (Transylvania) and a fly peek in second decade of July. Previous this report, in same region, Ivas and Muresan (2011) mentioned that, higher adults of ECB were cached in pheromone traps, which proves that pest population level increasing in maize crops.

One of the best method for control *Ostrinia nubilalis* attack is using of maize hybrids with tolerance at this pest (Popov and Barbulescu, 2007). Because in nature, the attack of European corn borer are not at the same level, every year, as result of the climatic conditions variability, especially at egg batch deposition and larva emergence, artificial infestation of the maize plants is one of the best method to evaluate maize lines and hybrids reaction at this pest. *O. nubilalis* is first pest grown in laboratory conditions, on artificial diet (Barbulescu, 1980). In Romania

researches concerning mass rearing of the *O. nubilalis* in laboratory conditions starting in years '70 at ICCPT Fundulea (actual NARDI Fundulea) for south of the Romania and ARDS Turda for Transylvania. At NARDI Fundulea it has researched many themes, such as: finding of the better diet for growing of the European corn borer, consecutive generations in laboratory conditions (Barbulescu, 1977, 1980, 1984), influence of the temperature concerning storage time of the egg batches (Barbulescu, 1979), stability of the insects grown consecutive generations on same artificial diet, in laboratory conditions (Barbulescu, 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 inbreed lines and hybrids tolerance for this pest (Barbulescu and Cosmin, 1997; Barbulescu *et al.*, 1999). At ARDS Turda it has researched chemical control of European corn borer (Mustea, 1977; Muresan and Mustea, 1995) or maize hybrid resistance at this pest, in artificial and natural infestation conditions (Mustea, 1981, 1990).

Now days, only at NARDI Fundulea it has continued growing of the European corn borer in laboratory conditions, for obtaining of the egg-batches, with a production per year between 121000 and 200000 egg-batches, used for different researches in field conditions, plants or insecticide testing. In this paper, authors collective present results obtained in laboratory conditions regarding egg batches productions, data concerning behaviour of the insects from different colonies in field conditions and data concerning behaviour of some maize hybrids to *O. nubilalis* attack, in field condition.

MATERIALS AND METHODS

O. nubilalis is grow in laboratory conditions, in continuous flux, on same artificial diet, at Plant Protection Collective in frame of the National Agricultural Research and Development Institute Fundulea, Calarasi County, Romania. For larvae stage, the growing conditions in laboratory are: air temperature 27-28 °C and air relative humidity between 60 and 90 % (Fig. 1). 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 °C, 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 °C and 6 for hours, air temperature 20 °C (simulation of the day-night alternation); air relative humidity between 82-85 % and permanent ventilation (Fig. 2). 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 (Tab. 1). At each generation in frame of each colony it has registered average number pupae/rearing box,

Tab. 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

Fig. 1. Rearing chamber of *Ostrinia nubilalis* Hbn larva's, at NARDI Fundulea

total number of the moths, female number and egg batches number.

Almost every year it has started a new insect's colony. At the end of maize vegetation period, larva's are harvested from the maize stalks. Until the spring, harvested larva's are stored in a place where air temperature is similar with environment temperature. In the spring, larva's are moved in laboratory where there will be transformed in pupae. In this way it has starting multiplication of a new insect colony, successive generations, in laboratory conditions. It has kept colonies created in last years, well as a reference colony, created in year 1979. For testing of the European corn borer insect colonies, starting in different years, for evaluation of the capacity of these insects for damaging maize plants, it has made artificial infestation in field conditions.

It has used one maize hybrid, considered sensitive of *O. nubilalis* attack. Also, in field conditions, at Plant Protection Collective it has tested maize hybrids, produced at NARDI Fundulea, both in natural and artificial infestation conditions. Egg batches produced in laboratory conditions in frame of Protection Collective it has used for testing maize inbred lines tolerance at *O. nubilalis* attack, in frame of Maize and Sorghum Breeding Laboratory,

Maize plants were sowing at the last decade of the April. Plots length was of 10 m, plots width was of 4,2 m (6 rows per plot). Distance between rows was of 70 cm. Equivalent plant densities were of 50000 per hectare. Plots were arranged according randomized blocks scheme. With,

Fig. 2. Chamber of egg-batches deposition, at NARDI Fundulea

appreciative 10 days before panicle emergence (end of the June or beginning of July), 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. 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 (cm) and number of the alive larva/plant. The results were statistical analyzed through variance analysis, using ANOVA software.

RESULTS AND DISCUSSION

From 1975, at NARDI Fundulea it has rearing, *O. nubilalis* in continuous flux, on same artificial diet for obtaining of the egg batches used for artificial infestation of the maize plants, in field conditions. This is a better method to test maize lines and hybrids reaction to pest attack or testing effectiveness of both, chemical and biological methods to control this pest. The rearing activity in laboratory conditions has two distinct periods. First period, from July to March, when we maintain biological material from one month to another and second period, from March to July when biological material it has mass rearing, for obtain more then 100000 egg-batches used in different research themes, in period last decade of June-first decade of July. Data from table 2 demonstrate that between 2009 and 2013, in period March-July it has obtained a variable number of egg batches, between 109843 and 203253. Along the time, egg-batches number, obtained at the beginning of July, used for artificial infestations of the maize plants,

ranged between 120000 and 135000. But in some years, like in 2009, it has obtained less number of the egg batches. In some year, like 2010 it has obtained more then 200000 egg batches.

Total number of moths, obtained in laboratory conditions, in period March-July, ranged between 115112 and 150758 moths. Female percent has close values in all years taken in study, of 45 %. This data is in accordance with those obtained by Barbulescu (1993, 1996, 2001). Average number of the egg batches/female varied from one year to another. In 2010 it has registered highest value of this parameter (3,0 egg batches/female). One year early it has registered lowest alue of this parameter, from period taken in study. Generally, average number of the egg-batches/female, in period March-July ranged between 2,0 and 2,5. Number of rearing boxes used for mass rearing of the *O. nubilalis*, in period March-July, depends of the research plan. The highest number of rearing boxes used in last 5 years it has registered in 2010 (329 boxes). Generally, rearing box number, ranged between 240 and 300. In last years, average number of the pupae/rearing box ranged between 466 and 565.

Data from tables 3-5 show behavior of the insect colonies, created in different years, in field conditions. One maize hybrid was artificial infested with egg-batches, produced from different insect colonies, in laboratory conditions. Regard as average gallery length per plant, it hasn't registered higher difference of this parameter between recent insects colonies (2008, 2010 and 2011) and insect colony started from 1979. Highest values of gallery length/plant it has registered in 2011, comparative with 2012 and 2013. A possible explication for this is because of favorable climatic conditions for insect development in 2011, especially in period of

Tab. 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 2009-2013

Specification	2009	2010	2011	2012	2013
Number of the rearing boxes	248	329	260	286	243
Average number of the pupae/box	565	505	486	466	524
Total number of the moths	131041	150758	116342	134085	115112
Total number of the females	59058	67922	52354	60338	51799
% female	45,07	45,05	45,00	45,00	45,01
Total number of the egg-batches	109843	203253	136043	121945	133550
Average nr. egg-batches/female	1,9	3,0	2,7	2,4	2,5

larva eclosion and larva first stage. In 2011 gallery length/plant ranged between 30,0 and 36,1 cm. In 2012, gallery length/plant ranged between 13,3 and 14,7 cm and in 2013 gallery length/plant ranged between 15,9 and 16,4 cm.

Regard as average number of alive larva/plant, in 2011, data from table 3 show 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 ($P < 0,0001$). In climatic conditions of

years 2012 and 2013, average number of alive larvae/plant have lower values, comparative with 2011, and ranged between 1,3 and 1,8 (2012) and 1,9 and 2,2 (2013). In 2012, only in case of insect colony created in 2010, alive larvae/plant value is statistically assigned ($P < 0,001$). In 2013, the differences between insect colonies are not statistically assigned. All the data from period 2011-2013 demonstrate that even insects is rearing more then 440 generations in laboratory conditions, they don't lose capacity of producing damage at maize plants in field conditions and

Tab. 3. Behavior of the *O. 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	30,0o	5,7**
2010	22	100	33,4	6,3***
2011	9	100	36,1	6,0***
			LSD _{5%} =3,04	LSD _{5%} =0,40
			LSD _{1%} =4,26	LSD _{1%} =0,55
			LSD _{0,1%} =6,17	LSD _{0,1%} =0,80

Tab. 4. Behavior of the *O. 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
			LSD _{5%} =0,46	LSD _{5%} =0,31
			LSD _{1%} =0,64	LSD _{1%} =0,43
			LSD _{0,1%} =0,93	LSD _{0,1%} =0,63

Tab. 5. Behavior of the *O. nubilalis* Hbn colonies, starting in different years, in field conditions, year 2013

Colony	Generation number	Attack frequency (%)	Gallery length (cm/plant)	Alive larvae (nr./plant)
1979 (mt)	442	100	15,9	1,9
2008	77	100	16,2	2,0
2010	51	100	16,1	2,2
2011	38	100	16,4	2,2
			LSD _{5%} =0,91	LSD _{5%} =0,41
			LSD _{1%} =1,28	LSD _{1%} =0,57
			LSD _{0,1%} =1,85	LSD _{0,1%} =0,83

they can be used for artificial infestation of the maize plants, in frame of the different research themes.

At the end of the year 2013 the insect colony created in 1979 arrived at 442th consecutive generation, the insect colony created in 2008 arrived at 77th consecutive generation, insect colony created in 2010 arrived at 51rd consecutive generation, while insect colony created in 2011 arrived at 38nd consecutive generation.

Every year, at experimental field of the Plant Protection Collective, there wre tested maize hybrids produced at NARDI Fundulea, in conditions of artificial infestation with European corn borer (*O. nubilalis*) egg-batches, produced in laboratory conditions. In this paper are present some result from field testing trials, between 2011 and 2013. Data from Fig. 3 show that, in climatic conditions of the year 2011, attack frequency was of 95 % in most of the cases, except hybrid Crisana with attack frequency of 80 % and Iezer with attack frequency of 100 %. In condition of the year 2012, at plants artificial infested with ECB egg batches, attack frequency present lower values comparative with 2011, except variants Danubian and Crisana. Attack frequency at the maize hybrids taken in study, ranged between 85 and 100 %, in climatic conditions of the year 2013,

except hybrids F 475 M (F=65 %) and Iezer (80 %).

Data from table 6 show that attack intensity of the *O. nubilalis* at maize plants, in conditions of artificial infestation where higher in 2011 comparative with years 2012 and 2013, especially because of the climatic conditions from the end of June, favorable for larva development, from emergence until they enter in plant stalks. Regard average gallery length/plant, in 2011, higher values of this parameter registered at hybrids Rapsodia, Danubian and Generos while lowest values registered at hybrids Olt and Mostistea. At new hybrids, Iezer and Crisana, average gallery length/plant, as result of the larva attack, has lower values, 10,3 and 10,0 cm. Regard as alive number of larvae/plant, in climatic conditions of the year 2011, higher values of this parameter were meet at hybrids Generos and Danubian. At hybrids Rapsodia, Paltin and F 376 it has registered more then 3 larvae/plant. Lowest value of this parameter, in conditions of artificial infestation of the maize plants has registered in case of Mostistea hybrid (2,0 alive larvae/plant). In climatic conditions of the year 2012, average gallery length/plant has lower values comparative with 2011. However at hybrids Generos and Danubian it has registered higher values of this parameter, close to values

Fig. 3. Behaviour of the maize hybrids to *Ostrinia nubilalis* Hbn attack, at NARDI Fundulea, period 2011-2013

Tab. 6. Behavior of maize hybrids assortment at attack of the *O. nubilalis*, at NARDI Fundulea (2011-2013), in conditions of artificial infestation

Hybrid	2011		2012		2013	
	Gallery length (cm/plant)	Alive larvae (nr./plant)	Gallery length (cm/plant)	Alive larvae (nr./plant)	Gallery length (cm/plant)	Alive larvae (nr./plant)
Rapsodia (mt)	19,9	3,2	13,0	2,6	10,1	1,9
Paltin	14,9 ^{ooo}	3,2	10,2 ^{ooo}	2,5	10,7	2,1
Olt	9,3 ^{ooo}	2,0 ^{ooo}	2,6 ^{ooo}	0,6 ^{ooo}	6,9 ^{ooo}	1,4
F 376	13,0 ^{ooo}	3,1	8,6 ^{ooo}	2,8	9,1	1,5
F 475 M	11,6 ^{ooo}	2,1 ^{ooo}	7,3 ^{ooo}	0,7 ^{ooo}	3,8 ^{ooo}	0,4 ^{ooo}
Genneros	20,2	4,1 ^{**}	16,2 ^{**}	3,6 ^{***}	10,0	2,3
Danubian	19,7	4,2 ^{**}	15,2 ^{**}	2,5	8,8	1,4
Mostistea	9,3 ^{ooo}	2,0 ^{ooo}	6,1 ^{ooo}	1,6 ^{ooo}	9,7	1,7
Crisana	10,3 ^{ooo}	2,3 ^{oo}	5,6 ^{ooo}	1,0 ^{ooo}	6,5 ^{ooo}	0,9 ^{ooo}
Iezer	10,0 ^{ooo}	2,8	3,4 ^{ooo}	0,5 ^{ooo}	4,7 ^{ooo}	0,9 ^{ooo}
	LSD _{5%} =1,34	LSD _{5%} =0,63	LSD _{5%} =0,80	LSD _{5%} =0,43	LSD _{5%} =1,23	LSD _{5%} =0,51
	LSD _{1%} =1,80	LSD _{1%} =0,84	LSD _{1%} =1,07	LSD _{1%} =0,58	LSD _{1%} =1,65	LSD _{1%} =0,68
	LSD _{0,1%} =2,35	LSD _{0,1%} =1,10	LSD _{0,1%} =1,40	LSD _{0,1%} =0,76	LSD _{0,1%} =2,16	LSD _{0,1%} =0,89

from 2011. Lowest values of gallery length/plant, from the experiment, have registered in case of Olt, Iezer and Crisana hybrids. Also, at hybrid Genneros, in 2012, it has registered higher number of alive larvae/plant, from the experiment, while at hybrids Olt, F 475 M and Iezer this values were subunit. In climatic conditions of the year 2013, average gallery length/plant has slightly higher values comparative with 2012, at Iezer, Crisana, Mostistea, F 376 or Paltin hybrids and lower values comparative with previous year at Rapsodia, F 475 M, Genneros and Danubian hybrids. Regard as alive larvae/plant, lowest value of this parameter it has registered in case of F 475 M (0,4 larvae/plant). Also at hybrids Iezer and Crisana it has registered less then one larva/plant. At hybrids Paltin and Genneros, in climatic conditions of the year 2013, it has registered higher attack intensity of *O. nubilalis*, at maize plat artificial infested.

At experimental field of the Maize and Sorghum breeding laboratory, between 2010 and 2012 it has tested 250 inbreed maize lines, both in conditions of natural and artificial infestation of the maize plants, with *O. nubilalis* egg-batches, produced in laboratory conditions.

From 250 inbreed lines, 67 were partially or total resistant to ECB attack and were keep in maize breeding process. The cooperation between Plant Protection Collective and Maize and Sorghum

Fig. 4. Gallery of *O. nubilalis* larva in maize stalk breeding laboratory materialized in maize hybrids breeding with different degree of resistance to *O. nubilalis* attack.

CONCLUSION

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 insect's egg-batches, necessary for the field research concerning maize hybrids and lines tolerance at pest attack or researches concerning biological or chemical control of this pest.

Between 2009 and 2013, in period March-July it has obtained a variable number of egg-batches, between 109843 and 203253 used for artificial infestations of the maize plants, in field conditions.

The average number of the egg-batches/female obtained in laboratory conditions was 1,9 in year 2009, 3,0 in year 2010, 2,7 in year 2011, 2,4 in year 2012 and 2,5 in year 2013.

At the end of the year 2013 the insect colony created in 1979 arrived at 442nd consecutive generation, the insect colony created in 2008 arrived at 77th consecutive generation, the insect colony created in 2010 arrived at 51st consecutive generation, while the insect colony created in 2011 arrived at 38th consecutive generation.

Even insects is rearing more then 440 generations in laboratory conditions, they don't lose capacity of producing damage at maize plants in field conditions.

Climatic conditions from summer period, at NARDI Fundulea, between 2011 and 2013 were variable. Climatic conditions of year 2011 were most favorable for European corn borer attack and the maize hybrids reaction to this pest, both in natural and artificial infestation conditions were better differentiated.

Between 2011 and 2013, in conditions of artificial infestation of the maize plants with *O. nubilalis* egg-batches, higher attack of the pest it has registered at hybrids Danubian, Generos, Rapsodia while lower attack of the pest it has registered in case of the Olt hybrid.

Good results concerning tolerance at *O. nubilalis* attack, it has registered in case of new maize hybrids, produced at NARDI Fundulea.

From 250 inbreed lines tested in experimental field of the Maize and Sorghum breeding laboratory, 67 were partially or total resistant to European corn borer attack and were keep in maize breeding process.

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