Research regarding the relationship among the pests *Ostrinia nubilalis, Helicoverpa armigera* and the fungi *Fusarium verticillioides, Aspergillus flavus* in corn in the climatic conditions from Lovrin (Timiș County)

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Abstract

Maize is sensitive to the infection with myco-toxigenous fungi from the genera Fusarium and Aspergillus when the climatic conditions are favourable. The specific maize pests play an important role in the spread of the spores of the myco-toxigenous fungi among the climate conditions. The main Fusarium species are able to produce infections are the following: Fusarium verticillioides, Fusarium proliferatum and Fusarium graminearum. The first two species are producing mycotoxins from the fumonisines group (considered as carcinogenous) and the third produces mycotoxins by the types trichothecene and zearalenone. The most frequent species from the genus Aspergillus in crops is Aspergillus flavus. Near this species there can be found Aspergillus parasiticus. Both fungi are major aflatoxins producers strongly carcinogenous for humans and animals. The infections of these fungi are associated with the dry years, warm climate and the damages produced by insects. The insects play an important role in the epidemiology of those pathogens. This research was developed in a selection field formed from 21 lines of corn placed in the perimeter of the Station of Agricultural Research and Development Lovrin (Timiș County). The purpose of this study is to set the interrelation between pathogens and pests and the implication of the last ones in the fungal epidemiology. The observations regarding the attack of the myco-toxigenous fungi Fusarium verticillioides and Aspergillus flavus were developed in conditions of natural infection. In parallel was noted the frequency of the attack of the pests Helicoverpa armigera and Ostrinia nubilalison cobs and the density of the larvae on cobs. The incidence of the cobs infested with mycelia of Fusarium verticillioides was comprised in the interval 33% – 100% and the severity between 3% and 17.5%. In the case of the fungus Aspegillus flavus the incidence of the cobs with mycelium was comprised between 0 and 27% and the severity doesn't overpass 15%. There was noticed the high incidence of the cobs damaged by Helicoverpa armigera between 40% and 100% and that coincides with the frequency of the cobs infested by Fusarium. The density of the Helicoverpa larvae on cob was one, rarely two and three. Comparative with Helicoverpa armigera, the incidence of the cobs attacked by Ostrinia nubilalis was lower than 40%. The density of the larvae on cobs was similar as in the case of Helicoverpa. The obtained results show the indirect implication of those two pests in the dissemination of the above mentioned myco-toxigenous fungi. The infections level with Fusarium verticillioides was higher on the cobs damaged by larvae, those being entirely covered with white, pink or salmon colour mycelia, characteristic for this fungus.

Key words: *Fusarium verticillioides, Aspergillus flavus, corn, mycotoxins, Helicoverpa armigera, Ostrinia nubilalis.*

INTRODUCTION

In Romania in the year 2019 the surface cultivated with corn was about 2.6 million of hectares (data from the National Institute of Statistics), greater than 2018 when the corn cultivated surface was 2.37 million of hectares. The increase of the land surfaces cultivated with maize in 2019 was due in a great part to the reseeding of some rape and wheat damaged

crops that haven't developed due to the unfavourable climatic conditions from some certain areas from Romania.

Climate changes from the last years have favoured increases of the populations of the harmful organisms from corn crops, mainly the pests *Helicoverpa armigera* and *Ostrinia nubilalis.* Thus, the temperatures and humidity during summer of the year 2019 were favourable to the infections with *Fusarium verticillioides* and *Aspergillus flavus* on cob. Those two pathogens are producing mycotoxins dangerous for the human and animal health. The most common infestation way of the corn with *Fusarium verticillioides* is through the tassel. The spores from air are sweeping down on the corn tassel where germinate. The infection is produced in the moment when the corn tassel is brown-greenish and brown coloured. There was noticed that the green tassel is relatively resistant to infection, when the brown and brown-greenish is proper for colonisation with this fungus. The fungus can enter via the cracks from the rain surface and also with the pathogenic insects. The insects are able to spread the spores to tassel or directly to grains during the feeding process. The rotting caused by *F. verticillioides* and the contamination of the grains with fumonisin following the infection is usually favoured by the dry weather prior and during the tassel formation. Corn grain rotting caused by *F. verticillioides* (*G. fujikuroi*) is associated with the dry and warm years and with injured produced by insects [M. C. SHURTLEFF, 1980]. Temperature is one of the important factors for development. *F. verticillioides* grows very well when temperatures are over 26^0C [L. M. REID *et al.*, 1999]. Beside temperatures there are several factors that interferes with *F. verticillioides*, respectively other corn pathogens, corn genotype and the stress determined by drought [J. D. MILLER, 2001]. The mycotoxins produce by this fungus were first identified in 1988 by a group of researchers from South Africa and were named fumonisins (FB_1, FB_2 and FB_3). They are associated with malfunctions of brain in horses and lung deficiencies in pigs. Fumonisin is often produced in field, but it is formed in the stored corn grains too [J. D. BU'LOCK, 1975; J. D. MILLER *et al*., 1994]. Researches show that the toxins are produced by the penultimate cells of every mycelium. That can be assumed that the toxins production appears at the microlevel of every individual filamentous structure [J. D. MILLER, 1988].

There are suspicions that fumonisin could be implied in the oesophagus cancer, but wasn't set a direct connection yet [ARVID L. HAWK, 2008]. Association of the oesophagus cancer with the mycotoxins from the fumonisines group was reported in the black population from Transkei [IARC, 1993]. The population from there areas consumes mash made from mouldy corn flour. Thus, the bear ingested by adults is produced from mouldy corn too, with a high content in fumonisins of till to 118 mg/kg. fumonisins concentration in this beer is 30 mg/l [J. P. RHEEDER *et al*, 1992; P. M. SCOTT *et al*., 1995].

Aspergillus flavus is a fungus that prefers tropical conditions, but with all these is reported all over the world with the mention that dominates the tropical soils. Together with *Aspergillus parasiticus* is considered major producers of aflatoxins that are very carcinogenic for animals. *Aspergilus flavus* predominates and can create problems in corn, cotton, nuts and peanuts crops [MAREN A. KLICH, 2007]. *Aspergillus flavus* is manifesting in corn crops by the appearance of a yellow-greenish mycelium or yellow-brownish on the surface of the corn grains or among them. The fungus is setting on the corn tassel when its colour is yellow-brownish and is moist, but also on the grains from the top of the cob attacked by insects and birds [ALISON ROBERTSON, 2005]. The spread of the conidia is by wind (anemochore dissemination) or by insects (zoochore dissemination) [DIENER *et al*., 1987]. The resistance organs are represented by sclerots with the diameter comprised between 400 – 700 μm, spherical as shape and coloured from dark red to black. The insects play an important role in the epidemiology of this fungus. The primary infections are realised by the conidia produced by the mycelium but also

by the sclerots from soil. The sclerot could represent the main source of inoculum during winter in the cereal crops agro-ecosystems [D. T. WICKLOW, B. W. HORN, R. J. COLE, 1982]. Secondary infections are produced by conidia when the environmental conditions are favourable for the development of the disease [SCHEIDEGGER and PAYNE, 2003].

If in the past was known that the fungi from the genus *Aspergillus* attack corn during storage now is known that the infection appears in the corn fields. The appearance of the fungus in the corn fields leads to the formation of the aflatoxins before harvesting. The favouring factors are: temperature comprised between 26 – 37^{0}C, air relative moisture of 85%, hotness and drought during the development of the corn (mainly if it they intervene during pollination and grain maturation), the deficit of nitrogen and the cobs attacked by insects. Hail, storms and early frost produce grain cracking and prepossesses the corn to the infection with this fungus. The corn from field can be contaminated with aflatoxins if the night temperature is 7^{0}C and the day temperatures are over 32^{0}C. This thing happens during the drought periods when the insects are bringing the fungal spores beneath the husks. During the feeding with grains they are introducing the spores inside the grain. Thus, the conclusion is that the aflatoxins concentrations are greater in the corn grown in stress conditions and the stress factors are drought, hotness, fertilizers and insects attack [S. KOENNING and G. PAYNE, 1999].

Contamination with aflatoxins can be produced in the storage period too [DEAN MALVICK, 2007]. In storages the fungus prefers air moisture of 18 – 18.5%. If air moisture is lower than 13% the fungus doesn't appear indifferent by temperature. For growth it needs high temperatures. The growth will be slowed at temperatures of 4 – 10^{0}C and fast at 26 – 32^{0}C. Is important to know that the diseased corn stored will deteriorate rapidly even the moisture and temperature are low comparative with the healthy corn free of *Aspergillus flavus* [J. A. WRATHER, LAURA E. SWEETS, 2008]. Aflatoxin B was confirmed as being as very carcinogenic for humans. In high concentrations can cause liver cancer. It is assumed that this toxin is responsible by the intestines and kidney [ARVID L. HAWK, 2008]. Thus, it diminishes the immunity system, the appetite and has effects on the nutrition of the children (interferes with the vitamins A and D, with iron, selenium and zinc).

More many studies are highlighting the implication of the pests *Ostrinia nubilalis* (Hübner) and *Helicoverpa armigera* (Hübner) in the epidemiology of the fungi *Fusarium verticilliodes* and *Aspergillus flavus* Link. The great densities registered in the last years for those pests are justified mainly by the climate changes where can be added the absence of crop-rotation, practice of short crop-rotations, minimal soil works, neglection of the prophylactic measures *etc*. The larvae of the above-mentioned pests can bring the spores of the myco-toxigenous fungi beneath the husks in the area of the grains favouring the pathogeny. Some researches show that the *Ostrinia* and *Helicoverpa* larvae are feeding with mycelia of *Fusarium verticillioides* contributing to the dissemination of the conidia by their excrements [DARVAS BELA *et al.*, 2011]. MASSIMO BLANDINO *et al*. (2015) shows that *Ostrinia nubilalis* has an important role in the dissemination of the spores of *Fusarium verticillioides* favouring the appearance of the infections and lately the contamination with fumonizins in the corn cultivated in the areas with temperate climate.

The purpose of this research was to evaluate the attack of the pests *Ostrinia nubilalis* and *Helicoverpa armigera* on corn cobs in the year 2019 and their implication in the propagation of the infections with *Fusarium verticillioides* and *Aspergillus flavus*. There were monitored in this way 21 consanguineal corn lines from a selection field set at Station of Agricultural Research and Development Lovrin from Timiș County.

MATERIAL AND METHOD

The observations in the selection field were realised during August – September 2019. In

this period were monitored 21 consanguineal corn lines. There was analysed the attack of the pests *Ostrinia nubilalis* and *Helicoverpa armigera* on cobs and in parallel the infections with *Fusarium verticillioides* and *Aspergillus flavus*. The climatic data were registered at the Meteorological Station of SCDA Lovrin (temperature and rainfalls). The analysed 21 consanguineal corn lines were organized in the experimental field after the method of Latin rectangle with three replicates. Every line has occupied 4 m^2 with about 20 plants per plot.

The registered data regarding the incidence and virulence of the pathogens analysed there was realised in natural conditions using the classical formulas from plant pathology domain. The severity of the pathogenic fungi was evaluated on a 0-9 scale. In the case of pests, the data collection was applied in the same way as in the case of fungi regarding the calculation of the density of larvae on cobs and the frequency of the damaged cobs.

Statistical analysis of the research results was realised using the statistical method ANOVA.

RESULTS AND DISCUSSIONS

The increasing trend of the temperatures associated with the modern technologies used in agriculture have leaded to the increase of the populations of *Helicoverpa armigera* and *Ostrinia nubilalis* in the western side of Romania. The present climate changes can influence positively or negatively the development of the pest insects and the myco-toxigenous fungi. The year 2019 had proved to be extremely favourable the development of the corn pests and of the fungi *Fusarium verticillioides* and *Aspergillus flavus* due to the temperatures and rainfalls registered at Lovrin. Those fungi are major producers of mycotoxins extremely toxic for humans and animals, even carcinogenic in some conditions. According with MIRAGLIA *et al.* (2009) food safety and security are neglected aspects in comparison with climate changes. Today, vegetal food safety is in a strong correlation with climatic factors with significant impact on insects and pathogenic agents from the point of view of the contamination with mycotoxins. The main climate parameters are in continuous change in the last decade, respectively temperature, drought, rainfalls and atmospheric carbon dioxide. From this point of view in the susceptible crops exists the risk of mycotoxins formation. The appearance of mycotoxins in the corn grains depends exclusively by temperature, relative air moisture, the stress produced by drought and the attack of the insects on cobs.

The period January – August from the year 2019 at Lovrin was characterised from climatic point of view by exceeding rainfalls (mainly in the spring months), but also by high temperatures during summer there being gathered the optimal conditions for the infection with fungi from *Fusarium* genus in wheat and corn. The higher temperatures from August were favourable to the infections with *Aspergillus flavus* on corn cobs.

The rainfall amount fallen at Lovrin in the interval January – August 2019 was 375 mm, with 24.2 mm higher comparative with the multiannual average of the area that is 350.8 mm. on entire year the multiannual average of rainfall amount at Lovrin is 520 mm. Rainfall regime was characterised by negative and positive deviations comparative with the monthly multiannual averages. Thus, negative deviations were registered in the months February (-14.6 mm), March (-17.3 mm), April (-9.7 mm), July (-0.8 mm) and August (-14.3 mm). The exceeding rainfalls amount for the entire period was 24.2 mm and for the summer months 4.8 mm. In the case of thermal regime, the deviations were positive almost on the entire period analysed, less in the months May and July when they were negative. The positive deviations comparative with the multiannual monthly average were comprised between 0.7^0C (January) and 3.8^0C (February). In the summer months the positive deviations were registered in June ($+2,5^0$C) and August ($+2,2^0$C) (Figure 1.)

In this work were analysed 21 consanguineal corn lines there being approached the analysis of the influence of the attack of the pests *Helicoverpa armigera* and *Ostrinia nubilalis* on the infections with *Fusarium verticillioides* and *Aspergillus flavus.* The lines were coded as it follows: from 6200 to 6202 and from 6205 to 6222.

Climatic conditions from Lovrin in the year 2019 have supported the infection with *F. verticillioides* on cobs, the registered frequencies being comprised between 33 – 100%. Even the frequency of the attacked cobs was high the infections severity didn't surpass over 25% (line 6208). The infections with *Aspergillus flavus* were lower, the incidence of the cobs with mycelia being maximum 27% (line 6221) and the severity of 15% (lines 6208 and 6210). The analysis of every cob shows that the injuries produced by feeding the larvae of *Helicoverpa* and *Ostrinia* were covered in a great rate by mycelia of *Fusarium verticillioides* and less by *Aspergillus flavus* (Figures 2, 3, 4, 5 and 6).

Incidence of the attack of *Ostrinia nubilalis* on cobs was generally low in comparison with *Helicoverpa armigera.* Only in 13 lines the pest was present on cobs (on top or at the base) with a frequency comprised between 7 - 50% (Figure 7). Density of the larvae was 1/cob. The low incidence of the attack of *Ostrinia* on cobs can be assumed to the fact that the larvae prefer to feed in stems and less on cobs.

Analysing the relationship between the cobs attacked by *Fusarium moniliforme* and the frequency of the cobs with attack of *Helicoverpa* there can be noticed that there are situations identical in 7 lines (P6207, 6212, 6213, 6214, 6218, 6219 and 6220), respectively 33% from the analysed cases (Figure 8). In the other 14 lines from the analysed plots (67%) the attack frequencies aren't coinciding, the incidence of the cobs with symptoms of *Fusarium moniliforme* being greater compared with the cobs with damages produced by *Helicoverpa*. There is the possibility that the larvae of *Helicoverpa armigera* to leave the cobs with *Fusarium* mycelia. According with DARVAS BELLA *et al.* (2011) the larvae of *Helicoverpa armigera* doesn't tolerate mycelia of *F. verticillioides*, having the trend to leave the cobs in the area covered with mycelium. Thus, the short time feeding of the larvae with mycelia and their passing over the infected areas of the cob contribute to the spread of the fungus *F. verticillioides* by the microconidia that remain viable in excrements and mycelium fragments that remain attached to the body of the larvae.

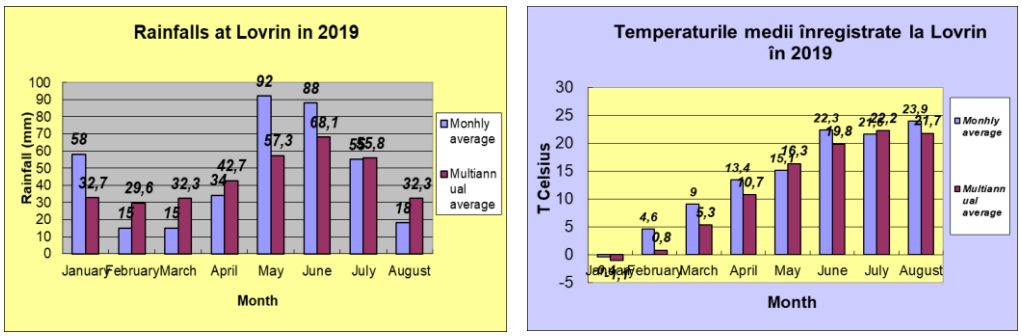

Figure 1. Average rainfalls and temperatures registered at Lovrin during January – August 2019 period - comparison with multiannual averages

Figure 2. *Fusarium verticillioides* on corn cob [Cotuna Otilia, 2019]

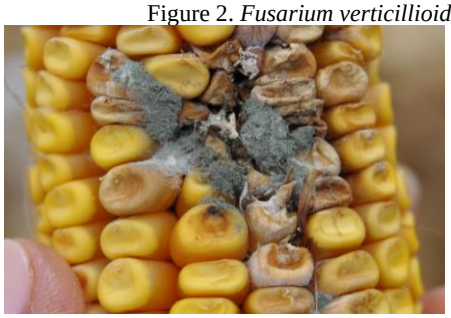

Figure 3. *Aspergillus flavus* on corn in field [Cotuna Otilia, 2019]

Figure 4. *Helicoverpa armigera* – attack on cob [Cotuna Otilia, 2019]

Figure 5. *Ostrinia nubilalis* – attack on cob [Cotuna Otilia, 2019]

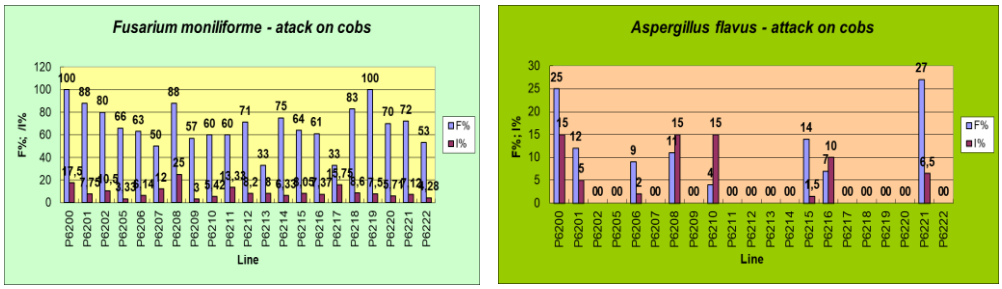

Figure 6. Incidence and severity of the fungi *F. verticillioides* and *Aspergillus flavus* attack on maize in 2019

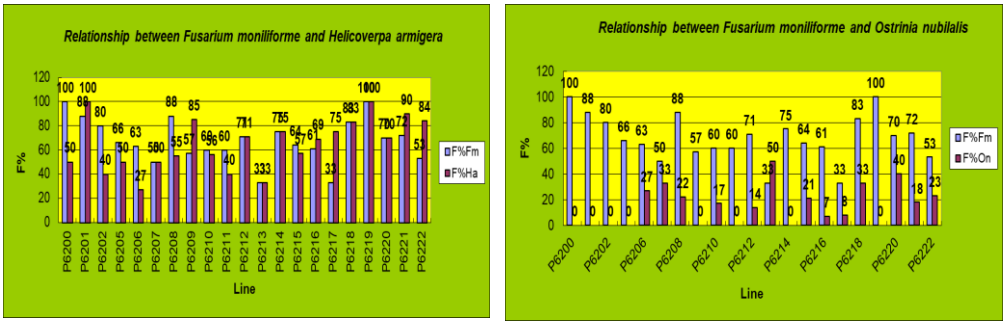

Figure 7. Incidence and severity of the pests *H. armigera* and *O. nubilalis* attack in comparison with the attack of the fungi *F. moniliforme* and *A. flavus* in 2019

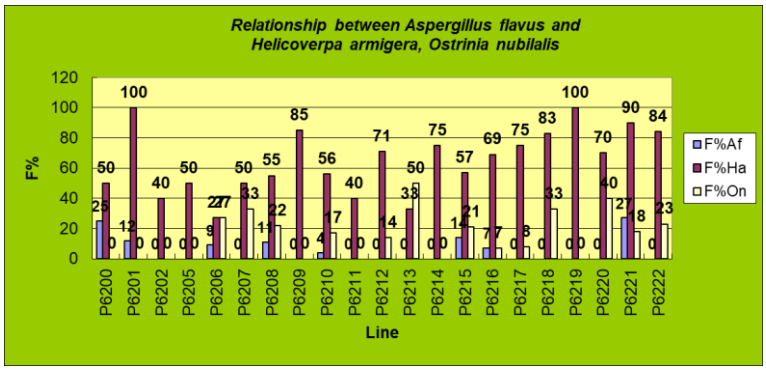

Figure 8. Frequency of the attack of the fungus *Aspergillus flavus* comparative with the attack frequency of the pests *H. armigera* and *O. nubilalis* in 2019 at Lovrin

Table 1

Variance analysis (ANOVA) for the incidence and severity of the attack of *Fusarium verticillioides* and *Aspergillus flavus*

Groups	*Count*	*Sum*	*Average*	*Variance*		
F% *F. verticillioides* (atack on cob)	21	1427	67.95238	331.8476		
I% *F. verticillioides* (atack on cob)	21	190.88	9.089524	27.28628		
F% *A. flavus* (atack on cob)	21	109.13	5.196667	69.69445		
I% *A. flavus* (atack on cob)	21	70.13	3.339524	30.71502		
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	61055.41	3	20351.8	177.1481	3.14E-35	2.718785
Within Groups	9190.868	80	114.8858			
Total	70246.28	83				

Table 2

Variance analysis (ANOVA) for the incidence and density of the larvae of *Ostrinia nubilalis* and *Helicoverpa armigera*

Groups	*Count*	*Sum*	*Average*	*Variance*		
F% *Ostrinia nubilalis*	21	313,08	14,90857	235,7713		
Density larvae/cob *O. nubilalis*	21	13,08	0,622857	0,242691		
F% *Helicoverpa armigera*	21	1360	64,7619	453,6905		
Density larvae/ cob *H. armigera*	21	21	1	0		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	58077,73	3	19359,24	112,2756	1,37E-28	2,718785
Within Groups	13794,09	80	172,4261			
Total	71871,82	83				

Even *Ostrinia nubilalis* implies in the dissemination of the conidia of the fungus *Fusarium moniliforme* on cobs, the analysis of the field data shows that there isn't correlated attack frequency of the pest with attack frequency of the fungus. In 8 lines from the analysed genotypes (38%) the pest wasn't present on the cobs, but *Fusarium* was. *Ostrinia* tolerates better the *Fusarium verticillioides* mycelia comparatively with *Helicoverpa* [DARVAS BELLA *et al.*, 2011].

Frequency of the cobs with mycelia of *Aspergillus flavus* was maximum 27% comparatively with the incidence of the fungus *Fusarium verticillioides* attack. In 13 lines from the analysed corn genotypes the cobs haven't presented symptoms at the attack of the fungus *Fusarium verticillioides* (Figure 8). Implication of the two pests in the spread of the pathogens is a certitude according with the previous researches. With all of these the climate conditions are determinant because the fungus *Aspergillus flavus* needs higher temperatures for development, respectively over 26^0C. Average temperatures registered at Lovrin during July – August 2019 were comprised between 21.6^0C (July) and 23.9^0C (August).

Statistical analysis ANOVA shows that there exists significance in the group of the processed data, the calculated *F* being greater than *F crit.*. In this situation the null hypothesis is rejected and is accepted the alternative hypothesis. The significance level ($\alpha = 0.05$) show that there exists variability within the group and between the analysed variables, the differences being significant (Table 1 and 2).

The results obtained in this research are in concordance with other similar researches from national and international level. The increasing temperatures favours the development of the insect populations from the corn crops, their spreading in areas where they weren't able to develop or even increasing the number of generations per year. The analysed pests *Helicoverpa* and *Ostrinia* contributes to the setting of the myco-toxigenous fungi by creating lessions to the cob tissues, in this way contributing to the dissemination of the conidia.

The increase of the temperatures with 1 - 3^0C over the annual averages has already a devastating impact in agriculture. In future, the pests and myco-toxigenous fungi of corn will find proper conditions for multiplication, increasing the risk of corn contamination with fumonisines and aflatoxins

CONCLUSIONS

The attack of the pests *Helicoverpa armigera* and *Ostrinia nubilalis* had produced lesions on cobs creating entering gates for the pathogens *Fusarium verticillioides* and *Aspergillus flavus*. Not in all the situations, the lesions produced by the pest insects were followed in all the situations by the infection with *Fusarium verticillioides* and *Aspergillus flavus*. *Helicoverpa*

armigera attack frequency was very high in 2019 reaching to 100%, compared with it *Ostrinia*'s attack that was lower than 50%. Pests implication in the epidemiology of the pathogens cannot be ignored and is studied continuously.

Climate changes concretised as increases of the monthly temperatures with 1 - 3^0C at Lovrin during the analysed period have leaded to the increase of the populations of the harmful organisms in the corn crops, increasing the risk of the formation of the fumonisins and aflatoxins mycotoxins harmful for humans and animals.

Statistical analysis (ANOVA) shows that the analysed variables are interdependent, there being statistical significance within and in the data groups.

In future the pests and the pathogens of corn will be more difficult to control with serious consequences on food security due to the climate changes.

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