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# YIELDING OF GRAIN MAIZE AND PRESENCE OF FUSARIUM TOXINS IN DEPENCE ON HARVEST TIME*

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Abstract. Later harvest time increased the infection of stems by *Fusarium* spp. and plant lodging. With the delay of harvest, the dry matter content in grain increased, but the crop in the later periods decreased. The optimal harvest time seems to be 20 days after reaching physiological maturity.
In spite of using different genotypes and a wide range of harvest dates, the chemical analysis used to determine the presence of trichothecens B (DON, 3-AcDON, 15-AcDON and NIV) did not reveal their presence above the detection limit 0,001 mg/kg.

Key words: maize, harvest time, grain, mykotoxins

## I. INTRODUCTION

Fungi of the genus *Fusarium* are common, world wide pathogens and are known as causal agents of diseases of many plant species. Species of *Fusarium* can develop on various plant tissues destroying them and leading to accumulation of toxic metabolites. Maize belongs to the plant species relatively sensitive to pathogenic action of the *Fusarium* fungi. It is often attacked at germination but the greatest damages caused by the pathogen occurs at maturing. External symptoms of fusariosis are most often observed on stems or cobs by the end of maturing – at wax and full maturity.

In the countries of warmer climate the main cause of fusarioses is said to be *Fusarium graminearum* Schwabe. It predominates in warm regions of the USA, Australia, southern Europe and China. In cooler regions, such as northern states of the USA, Canada and middle and northern Europe, a dangerous pathogen is *Fusarium culmorum* (W.G.Sm.) Sacc. Mycological studies carried out in Poland revealed that *F. cumulorum* occurs as frequently, if not more, as *F. graminearum* (Czaplińska et al. 1979; Bojarczuk et al. 1983; Chełkowski 1989). Besides also *F. crookwellense* (B.N.& T.), *F. sporotrichioides* Sherb., *F. avenaceum* (Fr) Sacc., *F. tricinctum* (Corda) Sacc. and *F. equiseti* (Corda) Sacc. are found. These fungi cause, so called, "red rot of maize cobs" (Chełkowski 1989) with characteristic clear reddish discoloration of elements of the cobs – grain, cores or covering leaves. oxic metabolites from B group: deoxynivalenol (DON), its two acetyl derivatives 3-AcDON and 15-AcDON, nivalenol (NIV) and zearalenon (ZEA) accumulate mostly in the cobs with the red rot symptoms.

Another cob disease called "pink rot" is caused by: *Fusarium moniliforme* Sheld. *F. subglutinas* (W&R) N and *F. prolieratum* (Matsushima) Nirenberg. Among the metabo-

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lites synthetized by this group of fungi the most important are moniliformin, fusarin C and fumonisines (Chełkowski et al. 1984).

Besides attacking the cobs species of *Fusarium* can result in considerable economic losses destroying maize stems. Visual symptoms of stems attack become evident 5 to 8 weeks after flowering and intensify with the time in which plant remains in the field (Krűger 1978). Under the influence of the pathogen tissues inside the stem become soft and mechan-ically not resistant and the maize plant becomes chlorotic and lodges easily breaking particularly at lower internodes. The attack on stems is often accompanied by damage to cob base due to which the cobs sag or detach and are not collected at threshing. The caused agents of the fusarial stem rot are the same species which attack the cobs. In the central and western Europe they are mainly *F. graminearum, F. moniliforme*, and, particularly, *F. culmorum* (Krüger 1978).

In the silage maize, particularly that produced using a traditional technology, damage caused by fusariosis are usually not serious, because earlier harvest and not full development of the pathogen. However, recently more and more popular technology of corn production for, so called, high energy silage assumes late time of harvest, at wax maturity, when fusariosis can already cause lodging and toxic metabolites can be harmful for animals. But most often negative effects of the attack by *Fusarium* spp. concern maize grown for grain which, after reaching full maturity, remains on the field for a long time to lower the grain water content.

The issue of harmfulness of fusariosis was at first considered only in the aspect of lodging and cob sagging which make harvest difficult or impossible. Intensive work in the 1980s facilitated breeding and introduction into farming practice of a cultivars with higher resistance to fusarial infection and not lodging. However, recently an increased lodging is observed even in the cultivars considered to be the most resistant, as confirmed by a survey of the results of studies carried out by COBORU and PZPK (Siódmiak and Heimann 1998; 1999; Kurczych 1999; 2000), which may indicate breaking of the resistance and increasing threat by *Fusarium* attacs.

Fusariosis of cobs and grain did not at first draw much attention but increasing number of reports about diseases of animals fed with attacked maize caused greater interest in this issue. With increasing demand for maize grain for industrial processing, including human feed products, the problem of improving its healthiness and estimation of the presence and frequency of mycotoxins became of a particular importance.

The goal of the studies carried out since 1998 is to assess the usefulness of maize varieties for industrial processing and the effect of environmental and agrotechnical conditions on their economic and technological value. One of the main factors deciding about this value is the degree of *Fusarium* attack and its negative effects such as lodging before harvest and remains of toxic metabolites in the grain.

## II. MATERIAL AND METHODS

Two factor field experiment with maize grown for grain in split-plot design was conducted in four replications in the years 1998-1999 in the Experimental Station of the Poznań Agriculural University Swadzim. The prime factor were 4 dates of harvest of corn maize carried out every 10 days starting from physiological maturity (black spot stage) reached by each cultivar. The secondary factor were cultivars with different structure of caryopsis, earliness and origin: Janna – semi flint type grain, FAO 190; Dragon – semi flint type grain, FAO 210; Marietta – dent type grain, FAO 240 and LG 2310 – dent type grain, FAO 280.

Maize was sown with a single-seed drill on 26.4 m$^2$ plots in the amount assuring density of 8 plants per 1 m$^2$. Agrotechnical treatments were typical for grain maize experiments. The plot was weeded with Azoprim + Dual preparations (2 + 2 l) once, before sowing, mixed with soil. In 1998 harvest was carried out with a plot harvester, while in 1999 the cobs were picked by hand. The grain (or cobs) were dried in a floor drier.

Moisture content and yield of grains, number of plants after germination and before harvest, and the infection of stems by *Fusarium* spp. was assessed. Occurrence of stem base gangrene and lodging was given in % of the total number of plants per plot, while statistical analysis was carried out following transformation of per cent into °Bliss. Hence, in the tables, for a given per cent value significance of differences are indicated by reference letters.

Grain samples from all objects (4 harvest dates x 4 cultivars x 2 years) were analysed for fusarium toxins content. The analysis was carried out in three replications using gas chromatograph with mass spectrometer (Hewlett Packard GC/MS HP 6890 Series with capillary column HP-5MS) following extraction, separation of the substances and transforming them into silil derivatives.

Determination of toxin content was performed on full basis selected ions: for DON the 236 ion was chosen, for NIV ion 585 while for acetyl DON derivatives ion 392. The level of described detectability was 0.001 mg/kg.

## III. RESULTS AND DISCUSSION

Calendar dates of harvest in both years differed considerably. In the cool year 1998 the harvest started on September 28 and finished on November 11 (Tab. 1). Differences in maturing of earlier varieties – Janna, Dragon and Marietta were slight whereas LG 2310 matured considerably later. In the warmer, more favourable for maize year 1999 the Janna variety first term of harvest was September 10. At similar time Dragon matured while dent shaped varieties Marietta and LG 2310 matured definitely later. Different reaction of the varieties to weather conditions caused that significance of differences in the investigated traits concerned only single factors and no interaction was found (Tabs. 2-5).

With the delay of harvest time in both years grain moisture content decreased significantly while the yield increased slightly at the beginning to fall significantly later (Tabs. 2, 3). It is worthwhile to notice that in the specific conditions of 1999 the yield was high and grain moisture content at the first date of harvest (after reaching physiological maturity) was on average 30.4%, hence close to that obtained in 1998 only in the last date of harvest. The investigated hybrids differed significantly with respect to grain moisture content and the results confirmed the earliness scale assumed for these varieties. In the cool year 1998 the hybrids of medium earliness (Dragon, Marietta) yielded better while in conditions of the warmer 1999 season later hybrids proved better. It should be stressed that the mean level of moisture

Table 1

**Time of maize harvest**

| Harvest time | Cultivars | Years | |
|---|---|---|---|
| | | 1998 | 1999 |
| I – physiological maturity | Janna | 09-28 | 09-10 |
| | Dragon | 09-30 | 09-10 |
| | Marietta | 09-30 | 09-21 |
| | LG 2310 | 10-12 | 09-24 |
| II – 10 days after | Janna | 10-08 | 09-20 |
| | Dragon | 10-10 | 09-20 |
| | Marietta | 10-10 | 10-01 |
| | LG 2310 | 10-22 | 10-04 |
| III – 20 days after | Janna | 10-18 | 09-30 |
| | Dragon | 10-20 | 09-30 |
| | Marietta | 10-20 | 10-11 |
| | LG 2310 | 11-02 | 10-14 |
| IV – 30 days after | Janna | 10-28 | 10-09 |
| | Dragon | 10-30 | 10-09 |
| | Marietta | 10-30 | 10-21 |
| | LG 2310 | 11-11 | 10-24 |

content and grain in the experiment as well as the differences brought about by the course of the weather in both years were similar to those obtained in the cultivar experiments carried out by the Polish Association of Maize Producers – PZPK (Kurczych 1999; 2000).

One of the more important factors deciding about maize yield is lodging and the attack maize stems by *Fusarium* spp. Comparing the varieties tested in the COBORU network (Michalski and Siódmak 1993) it was found that there is a strong positive correlation between

Table 2

**Grain moisture by harvest in %**

| Factor | Level | Years | | Average |
|---|---|---|---|---|
| | | 1998 | 1999 | |
| Harvest time | I | 37.8 d | 30.4 c | 34.1 d |
| | II | 34.5 c | 26.8 b | 30.7 c |
| | III | 31.9 b | 25.7 ab | 28.8 b |
| | IV | 30.5 a | 24.6 a | 27.5 a |
| | LSD | 0.35 | 1.98 | 1.04 |
| Cultivars | Janna | 30.4 a | 23.8 a | 27.2 a |
| | Dragon | 32.0 b | 27.1 b | 29.5 ab |
| | Marietta | 35.0 c | 27.0 b | 31.0 b |
| | LG 2310 | 37.2 d | 29.6 c | 33.4 bc |
| | LSD | 0.33 | 1.01 | 3.10 |

Table 3

**Grain yield by 15% of water**

| Factor | Level | Years | | Average |
|---|---|---|---|---|
| | | 1998 | 1999 | |
| Harvest time | I | 75.8 ab | 90.3 | 81.9 |
| | II | 76.8 a | 93.3 | 83.4 |
| | III | 75.1 b | 91.3 | 81.6 |
| | IV | 70.8 c | 91.3 | 79.9 |
| | LSD | 1.13 | d.u. | d.u. |
| Cultivars | Janna | 71.2 b | 80.5 b | 75.9 |
| | Dragon | 77.3 a | 81.8 b | 79.6 |
| | Marietta | 77.8 a | 95.0 a | 86.4 |
| | LG 2310 | 72.2 b | 98.0 a | 85.1 |
| | LSD | 0.88 | 5.64 | d.u. |

d.u. – difference unsignificant

fusariosis and lodging (r = 0.56 to 0.91) while between fusariosis and yield the correlation is negative ranging from r = –0.22 to r = –0.70 in each year. Our own stu-dies indicated that lodging increased with the delay in harvest (Tab. 4). This was particularly clear in 1998 at calendar late terms of harvest. Higher lodging at late harvest was also observed by Waligóra (1990). Varietal differences appeared only in 1998 when higher yielding Dragon and Marietta cultivars were characterised by lower lodging which confirms earlier mentioned correlation relationships. To sum up it can be stated that in view of the obtained results the optimal harvest date was the third one – 20 days after reaching physiological maturity, when lodging was still relatively small, yield did not decrease and moisture content and *Fusarium* mycotoxins were considerably lower.

Table 4

**Plant lodging in %**

| Factor | Level | Years | | Average |
|---|---|---|---|---|
| | | 1998 | 1999 | |
| Factor | I | 0.0 a | 0.8 a | 0.4 |
| | II | 1.0 b | 0.8 a | 0.9 |
| | III | 4.3 c | 1.4 ab | 2.8 |
| | IV | 18.9 d | 2.4 b | 10.7 |
| | LSD | d.s. | d.s. | d.u. |
| Cultivars | Janna | 7.8 c | 1.1 | 4.5 |
| | Dragon | 4.7 b | 1.4 | 3.1 |
| | Marietta | 3.5 a | 1.6 | 2.6 |
| | LG 2310 | 8.2 c | 1.3 | 4.7 |
| | LSD | d.s. | d.u. | d.u. |

d.s. – difference significant

Percentage of plants attacked by species of *Fusarium* was relatively high, on average, 23% in 1999 and about 8% in 1998 (Tab. 5). The strong attack in 1998 was a function of atmospheric conditions favourable for the spread of the disease as well as of late harvest. With the delay in harvest the attack increased significantly, almost doubling within 30 days. Such a strong attack was probably a cause of lower yields, especially in the fourth harvest date. In both years the studied varieties were attacked at different degree. This concerns mainly Dragon hybrid which in 1998 was definitely characterised by the highest level of

Table 5

**Per cent of plants with symptoms fusariosis**

| Factor | Level | Years | | Average |
|---|---|---|---|---|
| | | 1998 | 1999 | |
| Harvest time | I | 15.0 a | 6.1 a | 10.6 |
| | II | 18.6 b | 8.0 b | 13.3 |
| | III | 27.1 c | 8.6 bc | 17.9 |
| | IV | 30.9 d | 10.5 cd | 20.7 |
| | LSD | d.s. | d.s. | d.u. |
| Cultivars | Janna | 24.9 b | 5.8 a | 15.3 |
| | Dragon | 11.4 a | 12.4 c | 11.9 |
| | Marietta | 25.4 c | 7.4 b | 16.4 |
| | LG 2310 | 29.8 d | 7.6 b | 18.7 |
| | LSD | d.s. | d.s. | d.u. |

healthiness while its infection in 1999 it was the strongest. However, no relationship was found between hybrid earliness and fusariosis, as indicated in the work of Michalski and Siódmiak (1993). In the quoted work it was found that among the early varieties the attack was 2-4 times higher than among medium late ones. It was also proven that the occurrence of stem fusariosis favours the increase in dry mass content in grain which may explain considerable decrease of grain moisture content in the third and fourth harvest dates in 1998 despite late autumn weather and conditions unfavourable for drying. Generally looking, at the obtained results concerning the level of fusariosis it can be supposed that this problem increases it was found as in the 1970s and 1980s. It turns out that the genes of specific varietal resistance found at that time, today cannot ensure full healthiness under a range of conditions. Indirectly this hypothesis is confirmed by a relatively high percentage of lodging of various maize varieties in the PZPK experiments.

Maize lodging and, particularly, high proportion of plants with the symptoms of fusariosis in the lower part of stems indicated a possibility of occurrence of mycotoxins in the harvested maize grain. According to literature survey results that fusarium toxic metabolites are quite often found in maize grain. Among the detected toxins DON was most often encountered. It was found in 69% of 420 tested European maize samples, in the amounts of 0.01 – 67.0 mg/kg (Gareis et al. 1989). Tanaka et al. (1988) studying maize from different parts of the world detected DON in 20% of the samples and determined its mean concentration as 0.402 mg/kg. Besides DON also NIV was found in 16% of the samples (on average 0.766 mg/kg) and ZEA in 58% of the samples (on average 0.165 mg/kg).

Most works dealing with maize cob and grain fusariosis have been published in the USA and Canada where this disease is considered to be one of the most important. Wood and Carter (1989) state that among 123 maize samples from 13 states of the USA and tested in 1984 and 1985, 66 and 30%, respectively, contained DON and the amount of this toxin ranged from 0.10 to 2.47 mg/kg. Confirmation of these results are data given by Olsen et al. (1986) where in the tested 164 samples of maize imported to Sweden from the USA, ZEA was found in 14% (0.08-0.62 mg/kg) and DON in 28% (0.04-0.56 mg/kg).

Despite such common occurrence of fusarium toxins, in the tested maize grain from our own experiment in both years, no fusarium toxins of the group B were found at the detectability level. Considering relatively high level of attack on maize stems, particularly in 1998, this is a very positive result indicating that in Polish conditions, despite of infection of the vegetative parts, attack on the grain may not take place, or occurs relatively late when the maize is being harvested. Both in the studies by Perkowski et al. (1991) and Bennett et al. (1988) in the grain without etiological signs trace amounts of toxins, while high concentrations were found in the attacked grains. Most studies in Poland focus on evaluation of mycotoxin content in the naturally attacked or artificially infected material. While analysing grain from the plants attacked by *F. culmorum* or *F. graminearum*, DON, 3-AcDON, 15-AcDON and ZEA were detected in the concentrations from several to 130 mg/kg in grain and 2-4 times higher in cores (Chełkowski et al. 1984, Perkowski et al. 1991).

In the studies carried out in Austria (Lew et al. 1997) it was found that the concentrations of the analysed metabolites increased with the length of plant vegetation. In our experiment harvest dates covered almost all possible time of harvesting for grain (about 40 days). In the countries of warmer or continental climate, after reaching physiological maturity, maize is kept in the field for 50, and often even 100 days which probably favours higher degree of attack.

Genetic resistance of varieties is the basic method for controlling *Fusarium* spp. Introduction of resistant maize varieties by the end of the 1970s considerably limited the threat posed by these diseases but among the registered hybrids there are significant differences with this respect. This was confirmed by the studies in conditions of artificial infection – the least toxins were accumulated by Smolimag variety while the most by Zenit and RAH BE 86101 (Perkowski et al. 1997). High susceptibility for toxin formation in Zenit hybrid, and high resistance in Smolimag and Ruten were also found by Chełkowski et al. (1993) and Kostecki et al. (1994). Therefore, in our own studies we expected the same reaction. The differences were found in stem infection while infection of grain was so small that in the mean sample no mycotoxin presence was detected.

## IV. CONCLUSIONS

1. With the delay in harvest time the dry mass content in the grain increased, while at the beginning the yield slightly rose to fall considerably later.
2. The delay in harvest time was related to increasing infection of stems by *Fusarium* spp. and increasing risk of lodging. This was particularly clear in 1998 in the conditions of late maturing.
3. Considering increasing lodging, increasing stem fusariosis and losses in grain, an optimal harvest date was the third date, 20 days after reaching physiological maturity.
4. Despite differentiation of genotypes and a wide range of harvesting dates, analysis of the grain for the content of fusarium mycotoxins of the group B (DON, 3-AcDON, 15-AcDON and NIV) did not reveal their presence at the detectability level (0.001 mg/kg).

## V. LITERATURE


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# PLONOWANIE KUKURYDZY ZIARNOWEJ I OBECNOŚĆ TOKSYN FUZARYJNYCH W ZALEŻNOŚCI OD TERMINU ZBIORU

## STRESZCZENIE

W latach 1998-1999 przeprowadzono doświadczenie polowe z 4 odmianami kukurydzy: Janna, Dragon, Marietta i LG 2310. Roślin tych odmian zbierano w czterech terminach: po osiągnięciu dojrzałości fizjologicznej ziarna (stadium czarnej plamki) oraz 10, 20 i 30 dni później.

Analizowano przebieg wegetacji, zdrowotność roślin, poziom plonu i jego strukturę oraz przydatność ziarna do przerobu przemysłowego. Zawartość w ziarnie trichotecenów grupy B, tworzonych przez grzyby z rodzaju *Fusarium* oznaczano metodą GC/MS, po wykonaniu prób w pochodne sililowe.

Wraz z opóźnianiem terminu zbioru wzrastała zawartość suchej masy w ziarnie. Plony początkowo nieco wzrastały, natomiast później ulegały wyraźnemu obniżeniu. W kolejnych terminach zbioru rosło porażenie łodyg fuzariozą i zwiększało się wyleganie. Było to widoczne szczególnie w roku 1998, w warunkach chłodnej pogody i opóźnionych zbiorów.

Biorąc pod uwagę rosnące wyleganie, postępujące porażenie łodyg fuzariozą oraz straty masy ziarna, optymalnym terminem zbioru kukurydzy na ziarno okazał się termin III, po 20 dniach od osiągnięcia dojrzałości fizjologicznej. Analizowane odmiany różniły się istotnie: wilgotnością i plonami ziarna oraz odpornością na fuzariozę źdźbła.

Mimo szerokiego zakresu terminów zbioru kukurydzy i zróżnicowanych genotypów, nie wykazano obecności na ziarnie trichotecenów grupy B (DON, 3-ACDON, 15-AcDON i NIV) – na poziomie oznaczalności 0,001 mg/kg.