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STUDY OF THE EFFECTS OF N-FERTILIZATION AND PLANT DENSITY ON THE RESISTANCE OF MAIZE HYBRIDS TO FUSARIAL EAR ROT

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Abstract

Eight maize hybrids grown in Hungary were studied in order to investigate the effects of some agrotechnical factors (N-fertilization and plant density) on the resistance to Fusarium moulds. The plants grown on an experimental farm were artificially infected using the tooth pick method with *F. graminearum* and *F. culmorum*. In addition, maize grain meals were also inoculated with isolates of moulds. The surface area of ears covered by moulds 9 weeks after inoculation and the toxin content (Zearalenone and T2 toxin) of infected grain meals were measured. For characterization of resistance, the *toxin-mould index* (*TMI*) was introduced which takes into consideration both the growth rate of moulds and their toxin production.

A slight decrease in the mould-covered surface area of ears was observed with growing N-supply, and surprisingly no significant differences were found in the toxin content with higher plant density.

Keywords: maize, ear rot, Fusaria, N-fertilization resistance, plant density.

Introduction

In Hungary, similarly to other countries of moderate climate, contamination of maize and other cereals with mycotoxin may occur, particularly in rainy years (TANAKA et al., 1988, GAREIS et al., 1989, TÉREN et al., 1990). In Hungary among the fungi infecting maize and other cereals the Fusaria, particularly *F. graminearum* and *F. culmorum* are the most prevalent (MESTERHÁZY, 1984).

In the framework of the efforts to eliminate (or reduce) the losses caused by mycotoxins produced by Fusarium moulds, the breeding of cereal varieties resistant to Fusarium contamination play an important role

(MESTERHÁZY and KOVÁCS, 1986, CHIANG et al., 1987, CULLEN et al., 1983, SCOTT and KING, 1984, PERKOWSKI et al., 1995).

Relatively few data have been published on the role of agrotechnical factors in the resistance of maize to Fusarial ear rot. The aim of our studies presented in this paper was to collect some useful data in this field in order to improve the agrotechnical factors of growing new resistant varieties.

Materials and Methods

Eight maize hybrids grown in Hungary were studied in 1993 and 1994.

The hybrids were provided by IKR Company (Bábolna, Hungary). The field experiments were realized on the Experimental Farm of the same company.

In addition, laboratory experiments were performed with grain maize meals.

Two isolates with different pathogenicities of *F.graminearum* and *F.culmorum* were used in experiments for artificial inoculation of maize plants and maize grain meals.

Methods

On the experimental farm a random block distribution with three replications for every maize hybrid was used for growing maize hybrids, and the generally known agrotechnology was applied. For artificial infection the tooth pick method was used. The inoculum of Fusarium strains was transferred into the middle of the ears 10-12 days after female flowering of the plants (in Hungary July 26–August 6). The evaluation of the fungal damage of ears was performed 9 weeks after artificial infection. The basis of evaluation was the proportion of ear surface covered by moulds.

In laboratory experiments 50 g of grain were ground. The meal was mixed with 50 ml of distilled water and sterilized at 105 °C for 2 h. Next day the sterilization process was repeated under the same conditions. The sterilized mixture, containing about 22–25% water was inoculated with mycelia of F.graminearum or F.oxysporum. The moulds were grown on Czapek agar medium. The final inoculum contained $10^5 - 10^6$ microbes/g.

The inoculated substrate was incubated for 7 days at $20 - 22$ °C, then for further 7 days at a temperature of 4 °C and finally for 7 days again at $20 - 22$ °C in a thermostat. After incubation the material was sterilized at 150 °C for 2 h under the same conditions.

The T-2 toxin and zearalenone content of materials was determined by capillary GC as described by Bata et al. (1983). Concerning agrotechnical conditions, the following parameters were used:

- number of plants/ha: 65000, 75000, 80000
- N-fertilizer: none, 50 kg/ha, 100 kg/ha

Evaluation of the Tolerance

The results obtained by means of the two methods described above revealed poor correlation between the proportion of mould-covered surface of maize ears and the quantities of T-2 and F-2 toxin in the infected maize grain meals. Therefore, in addition to the

- mould covered surface proportion of the maize ear,
- zearalenone content of the infected maize grain meal and
- T-2 toxin content of the infected maize grain meal, a new number was proposed and used, namely the 'toxin mould index' (TMI). This index was calculated according to following formula:

Toxin-Mould Index = TMI $= ZX(T-2)XA$

where: Z = zearalenone content of maize grain meal

$(T-2)$ = T-2 toxin content of maize grain meal

A = proportion of mould-covered surface area of ears in % of the total area

The hybrids were classified as follows

- Highly tolerant hybrids: TMI lower than 200
- moderately tolerant hybrids: TMI = 200–400
- sensitive to Fusarium infections: TMI over 400

Results and Discussion

Effect of Plant Density (Number of Plants/ha)

In *Tables 1,2* and *3* the data relating to the proportion of mould covered area of ears at different plant densities are collected. The results summarized in the tables confirm the views that the plant density influences the growth of moulds on maize plants and probably on ears. It is interesting and to some extent surprising that the mould-covered area has a decreasing tendency which is most pronounced at lower nitrogen supply of growing plants. It seems that this statement is generally valid for all hybrids, representing a wide range of resistance resp. susceptibility to Fusaria.

MESTERHÁZY et al. (1972) found similar behaviour when investigating the resistance to head blight of winter wheats, noting that higher plant density also caused higher rates of stalk rot.

Table 1

The proportion of mould-covered are of ears (%) at different plant densities (number/ha) (No additional N - fertilizer)

Hybrid	Proportion of mould-covered area (%) 65000 plants/ha	75000 plants/ha	80000 plants/ha
1.	17.18	21.20	17.58
2.	4.45	6.33	3.20
3.	28.43	14.48	17.10
4.	17.95	15.05	13.33
5.	24.40	20.28	23.28
6.	9.15	9.33	2.98
7.	22.93	8.75	6.93
8.	4.48	5.23	5.48
Average	16.17	12.66	11.23

Table 2

The proportion of mould-covered area of ears (%) at different plant densities (number/ha) (+ 50 kg/ha N - fertilizer)

Hybrid	Proportion of mould-covered area (%) 65000 plants/ha	75000 plants/ha	80000 plants/ha
1.	18.68	16.65	9.70
2.	5.38	6.40	3.15
3.	24.55	10.43	6.20
4.	16.63	10.13	14.75
5.	29.20	6.85	22.83
6.	12.63	3.43	9.35
7.	9.65	5.10	12.88
8.	6.58	3.71	7.50
Average	17.84	7.84	10.79

KÜKEDI (1988) did not find significant differences. In a recent research report RAFAI (1995) also reported a decrease in mould-covered ear surface area and an increase in stalk rot of maize with increasing plant density.

The toxin contents of artificially infected maize grain meals are summarized in *Tables 4* and *5*. In *Table 4* the data of all hybrids are shown (at the basic level of N-supply). It can be seen that generally the hybrids being more susceptible to Fusaria (e.g. hybrids Nr. 5,1,3) have a higher toxin

Table 3

The proportion of mould-covered area of ears (%) at different plant densities (number/ha) (+ 100 kg/ha N - fertilizer)

Hybrid	Proportion of mould-covered area (%)		
	65000 plants/ha	75000 plants/ha	80000 plants/ha
1.	11.25	12.05	10.63
2.	5.18	5.75	4.35
3.	16.05	16.00	16.13
4.	14.38	11.90	11.38
5.	19.83	12.28	21.50
6.	7.85	6.23	9.08
7.	12.58	8.08	10.48
8.	6.63	4.40	4.50
Average	11.72	9.58	11.00

content, nevertheless there are also exceptions (e.g. hybrid No.8) showing that the growth rate and toxin production of moulds may be different even in the case of the same strain.

Table 4

Toxin content of infected maize grain meals (mg/kg) (No additional N-fertilizer used)

Hybrid	Toxin content					
	65000 plants/ha		75000 plants/ha		80000 plants/ha	
	T-2	Zea	T-2	Zea	T-2	Zea
1.	42.40	43.20	48.80	3.70	90.80	1.90
2.	2.90	48.20	10.30	15.20	15.70	62.40
3.	28.50	15.40	26.50	1.60	102.00	6.20
4.	27.30	32.20	24.60	12.70	85.70	97.70
5.	57.20	87.40	3.30	87.50	63.70	5.70
6.	18.20	17.50	23.40	11.30	30.40	38.60
7.	13.70	27.50	60.10	39.00	83.60	67.00
8.	60.10	42.20	50.50	43.70	92.50	12.70
Average	31.32	39.20	30.94	26.83	70.59	36.50

The differences at different plant densities are not too high except for the T-2 toxin content at 80000 plant/ha. If the total toxin content is taken into account it seems that a 75000 plant/ha density is optimal.

Studying the average values of 8 hybrids (*Table 5*) the same tendency may be observed at all three levels of N-supply.

In *Table 6* the TMI values are collected. These values are most interesting from the point of view of feeding because they provide information

Table 5
Toxin content of infected maize grain meals (mg/kg)
as influenced by plant density (plants/ha) and N-fertilizer (kg/ha)
(Averages of 8 hybrids)

Additional N-fertilizer	Toxin content 65000 plants/ha T-2	65000 plants/ha Zea	75000 plants/ha T-2	75000 plants/ha Zea	80000 plants/ha T-2	80000 plants/ha Zea
None	31.32	39.20	30.94	26.83	70.59	36.50
+50 kg/ha	35.63	31.37	21.10	13.26	48.87	30.09
+100 kg/ha	30.45	24.90	54.28	23.16	72.15	41.67

Table 6
Toxin-mould index (TMI) values for the investigated hybrids
(No additional N-supply)

Hybrid	TMI Plant number/ha 65000	75000	80000
1.	1470.6	1113.0	1629.7
2.	227.4	161.4	249.9
3.	1248.1	406.9	1850.2
4.	1068.0	561.4	2444.7
5.	3600.5	1841.4	1615.6
6.	326.6	333.1	205.6
7.	944.7	867.1	1045.7
8.	499.2	481.7	576.5
Average	1173.1	720.8	1202.3

about the quantity of mycotoxins which may be present in the maize grains included in the mixed feeds of animals. So for animal husbandry the TMI values are valuable information.

Naturally, from the point of view of breeders other factors, e.g. yield, inheritance of resistance, etc. must also be considered.

The Effect of N-Supply

Comparing the data in the *Tables 1,2* and *3* a slight decrease may be observed in the mould covered surface area of ears at a plant density of 75000 plants/ha with increasing N-supply. In other cases no significant changes may be observed.

According to the data in *Table 5*, it seems that on the average a 50 kg/ha additional N-supply is optimal for the hybrids investigated. This tendency is most pronounced at a plant density of 75000 plants/ha.

The TMI values (*Table 6*) show the same tendency if the average data of 8 hybrids are calculated.

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