International Journal of Agriculture, Environment and Bioresearch

Vol. 08, No. 01; 2023

ISSN: 2456-8643

EVALUATION OF FUSARIUM RESISTANCE TESTS ON MAIZE APPLIED IN THE NATIONAL VARIETY REGISTRATION IN HUNGARY

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https://doi.org/10.35410/IJAEB.2023.5807

ABSTRACT

With respect to maize, as one of the most significant agricultural plant species in the world, resistance to Fusarium is particularly important since chemical control against it is difficult. For food safety, the National Food Chain Safety Office of Hungary (NFCSO) tests the resistance of varieties reported for national registration against Fusarium stalk and ear rot (Fusarium spp.). In this study, we present the results obtained based on the current methodology of resistance test. Based on the comparison of Fusarium infection of the variety trial set up in 12 different production sites (Variety Trial Station) in years under test (2014, 2015 and 2016), we test the effects of the harvest year and trial site based on the rate of infection and the susceptibility/resistance of 19 hybrids tested for 3 years. There is significant difference between harvest years, trial sites, and hybrids based on the infection of stalk and ear rot. Based on our findings, the current methodology can be successfully applied to determining the level of field resistance.

Keywords: Maize, Fusarium Stalk And Ear Rot, Disease-provocation Trials, Natural Infection, Mycotoxins.

1. INTRODUCTION

Sustainability is a key part of the Green Deal and the EU Biodiversity and Farm to Fork Strategy these days. Their objectives include reducing the use of fertilizers and pesticides and increasing the share of organic land. To meet these objectives without (the) decrease in yields, varieties with satisfying nutrient balance and adequate disease resistance are needed. Maize is one of the most important agricultural plant species in Hungary and in the world. According to data from the Food and Agriculture Organization of the United Nations (FAO) for 2019, the United States was the leading producer of maize globally, followed by China and Brazil, Ukraine ranked the 4th place, Romania was the 9th on the list. It is produced nearly on 1 million ha in Hungary every year (Gadóc, 2021).During selection, the number of hybrids constantly produced is rising to achieve ever-increasing yields. In addition to high yields, the role of crop safety is becoming particularly important due to changing climatic factors (Széles and Huzsvai, 2020). Hybrids produced are reported to the Directorate of Agricultural and Genetic Resources of the National Food Chain Safety Office of Hungary (NFCSO) for trial for national variety registration. Of the agricultural plant species reported for national registration, maize is one of the species with the largest number of varieties, with over 100 varieties to be tested each year. Registration may be based on a 3-year testing period, but 2-year consistent positive trial result.

The National Catalogue of Varieties contains 320 (Csapó, 2021), while the European Common Catalogue has a total of 6288 (European Commission, 2021) registered maize hybrids. For crop safety, it is worth choosing a variety that has been tested under Hungarian climatic conditions and is resistant to diseases occurring in Hungary. Some climatic conditions favour the occurrence of different pathogens. One of the major pathogens of maize in Hungary includes various *Fusarium* species. *Fusarium* stalk rot results in a decrease in thousand-grain weight due to a weakened stalk, inducing a yield loss of 10% or even, in severe cases, up to 50% (Yu et al. 2017). The direct damage caused by *Fusarium* ear rot is yield loss, but the more significant indirect damage is mycotoxin production, which poses threat to human and animal health. The most common mycotoxins are deoxynivalenol (DON), followed by zearalenone (ZEA) and fumonisin (FUM). Additionally, mycotoxins do not occur on their own and therefore a multitoxic effect is to be expected. However, tolerable thresholds were determined based on the inherent toxicity of mycotoxins. It would be worth considering how certain toxin interactions may affect tolerable values determined for mycotoxins (Kovács et al. 2018).

Resistance test of diseases of *Fusarium* origin in maize hybrids was already the focus of national variety registration in the late 1960s. At the Variety Trial Station in Baja, resistance of maize hybrids against stalk rot has been studied in monoculture since 1966, and test methodology has been developed. At the Variety Trial Station in Isaszeg, an artificial infection trial was carried out in 1969 to study resistance against ear rot with 4 isolates of *Fusarium* species (Hinfner and Békési, 1969). Variety trial has been set up in monoculture since 1959 at the Variety Trial Station in Röjtökmuzsaj. Initially, the aim of the trial was to study the resistance of maize against *Sorosporium holci-sorghi*. Since 1980, provocation trial has been applied to test *Fusarium* stalk and ear rot. The exclusion factor in the national variety registration is if a variety is in a highly susceptible category (5) to stalk and ear rot or if it belongs to the susceptible category (4) to both forms of disease based on our multi-year, multi-site studies (Ruga-Kovács, 2016).

2. MATERIALS AND METHODS

2.1. Trial Sites

Trial sites were chosen from the Variety Trial Stations of the National Food Chain Safety Office of Hungary (Table 1). The Variety Trial Stations are situated in areas of the country with different climatic conditions, including regions important for maize production in Hungary (Figure 1).

Table 1: List of the Trial Sites ('+' = trial sites '-'= no testing was made) in the years of test (2014-2016)

Trial site	Ear rot			Stalk rot		
	2014	2015	2016	2014	2015	2016
Abaújszántó	+	-	-	+	+	-
Debrecen	+	+	+	+	+	+

Eszterágpuszta	+	+	-	+	+	-
Gyulatanya	+	+	+	+	+	+
Iregszemcse	+	+	+	+	+	+
Jászboldogháza	+	+	-	+	+	-
Kaposvár	+	-	-	+	+	-
Röjtökmuzsaj	+	+	+	+	+	+
Szarvas	-	+	+	-	+	+
Székkutas	+	+	+	+	+	+
Szombathely	+	-	+	+	+	-
Tordas	-	-	+	-	-	+

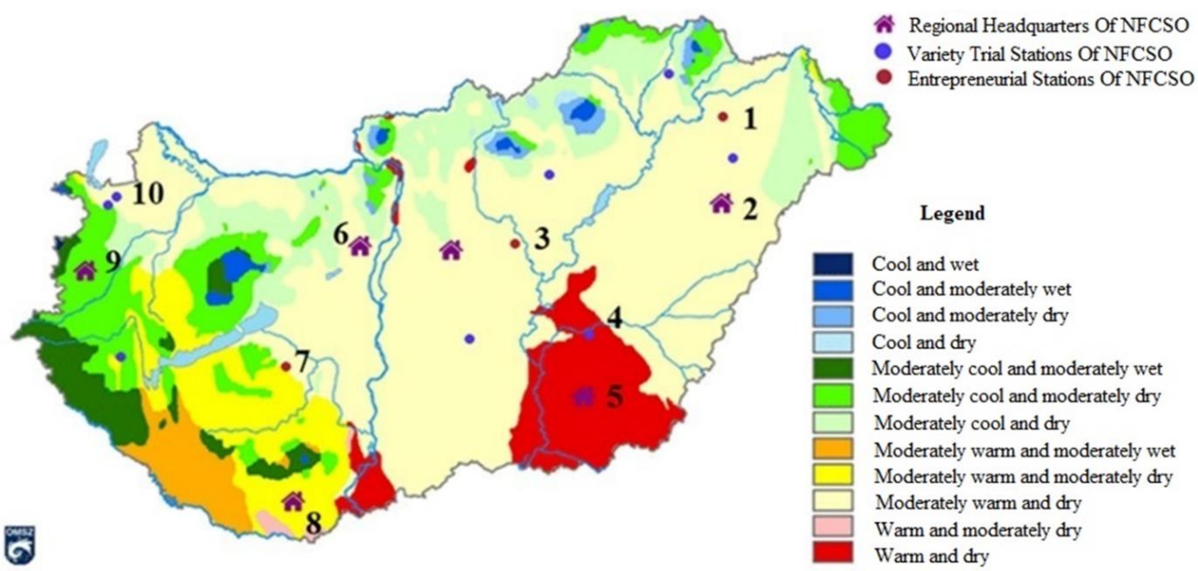

Figure 1: Climatic conditions of the Variety Trial Stations tested by the National Food Chain Safety Office of Hungary: 1: Gyulatanya, 2: Debrecen, 3: Jászboldogháza, 4: Szarvas, 5: Székkutas, 6: Tordas, 7: Iregszemcse, 8: Eszterágpuszta, 9: Szombathely, 10: Röjtökmuzsaj) (Péczely György, 2021)

Soil preparation at trial sites was the same: seedbed preparation with disc in the spring after deep ploughing in the autumn. Maize was sown in crop rotation at each location and year, except for Szarvas and Röjtökmuzsaj (Table 2). Candidate Varieties are tested in a monoculture of 2 and 55 years in Szarvas and in Röjtökmuzsaj, respectively. The ground for nutrient replenishment was a soil test based on a N:P:K ratio of 10 t/ha (120:60:120), adjusted for previous crop effects. Chemical weed control and control against animal pests were carried out with maize herbicides and insecticides from the list of approved chemicals. There was no control against fungal diseases in any of the areas.

Table 2: Previous crops of trials during years under test by trial site (2014-2016) (*= no testing was made)

Trial site	Year		
	2014	2015	2016
Debrecen	Wheat	Wheat	Wheat
Gyulatanya	Wheat	Wheat	Potato
Iregszemcse	Wheat	Wheat	Rape
Röjtökmuzsaj	Maize	Maize	Maize
Székkutas	Wheat	Wheat	Wheat
Szombathely	Wheat	Wheat	Wheat
Abaújszántó	Wheat	Wheat	*
Eszterágpuszta	Mustard	Soya	*
Jászboldogháza	Wheat	Wheat	*
Kaposvár	Rape	Rape	*
Szarvas	*	Wheat	Maize
Tordas	*	*	Wheat

2.2. Meteorological data

Table 3: Precipitation characteristics of the years under test, indicating deviations (Δ) from precipitation data of 2015 by sites (DE=Debrecen, GYTA=Gyulatanya, IR=Iregszemcse, RMU=Röjtökmuzsaj, SZKU=Székkutas, SZH=Szombathely, AÚSZ= Abaújszántó, EPU= Eszterágpuszta, JBH= Jászboldogháza, KA= Kaposvár, Sz= Szarvas, TO= Tordas)

Month/Site	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016
	DEΔ	DE	DEΔ	GYTAΔ	GYTA	GYTAΔ	IRΔ	IR	IRΔ	RMUΔ	RMU	RMUΔ	SZKUΔ	SZKU	SZKUΔ
April	6.0	20.4	-11.7	13.0	14.0	-4.0	46.2	9.3	14.4	42.0	21.0	4.3	36.9	9.5	6.7
May	41.5	38.1	11.6	38.2	54.0	-15.0	-70.5	130.8	-57.9	66.0	122.0	-4.0	8.5	68.1	-29.2
June	-6.5	33.3	106.8	1.2	26.0	30.0	-10.7	56.7	-35.5	8.0	16.5	32.3	29.1	24.3	53.4
July	31.8	35.3	63.7	98.2	41.0	52.0	61.8	36.0	51.8	69.6	38.4	43.0	117.1	59.0	66.4
August	-3.0	70.7	-5.8	36.2	15.0	21.0	67.0	47.4	21.5	41.7	92.3	-39.6	-66.2	84.7	-60.9
September	22.7	39.6	28.4	13.7	40.5	-3.5	140.2	26.0	20.2	4.9	40.1	-11.9	83.8	44.7	-0.9

Month/Site	2014	2015	2016	2014	2015	2014	2015	2014	2015	2014	2015	2015	2016	2016
	SZHΔ	SZH	SZHΔ	AÚSZΔ	AÚSZ	EPUΔ	EPU	JBHΔ	JBH	KAΔ	KA	SZ	SZΔ	TO
April	41.1	18.9	10.6	89.0	17.0	56.5	26.1	26.4	3.6	43.8	9.4	16.2	-7.3	30.9
May	-2.1	98.4	10.6	-2.0	73.0	-38.3	141.4	43.2	30.4	-44.5	129.0	53.2	-38.8	93.5
June	11,1	29.9	39.4	-12.0	32.0	25.4	31.0	-19.8	39.4	-12.7	64.2	20.8	116.8	63.9
July	67.7	71.5	48.6	101.0	21.0	57.2	56.1	135.7	18.1	29.8	42.1	24.4	85.0	119.7
August	143.0	23.4	48.5	-31.5	74.5	66.7	34.5	25.5	37.3	167.3	17.6	38.5	10.0	46.7
September	86.7	42.3	-14.2	73.0	15.0	59.5	53.8	26.9	64.6	45.6	54.8	50.0	-42.3	38.5

In 2014, spring was warm at a national average. Summer was initially dry, but there was a large amount of precipitation during the period of fertilization and incorporation.

The vegetation period in 2015 was characterized by extreme heat and drought (Figure 2). The summer in 2015 was the 4th hottest summer since 1901. A severe drought developed due to heat and lack of precipitation. The amount of summer precipitation was only two-thirds of long-term average, with frequent heat waves. 41 heat days (tmax> 30 ° C) and 13 hot days (tmax> 35 ° C) were registered in the country. Heat during silking resulted in fertility defects. In addition, we experienced grain saturation problems due to drought. Due to the warm, droughty weather, the drying of maize started early. In 2016, a warmer and drier spring than average was followed by a cooler and wetter summer than usual. Summer was followed by warm and rainy weather. In 2015, precipitation in July was lower at each trial site than in 2014 and 2016 (Table 3).

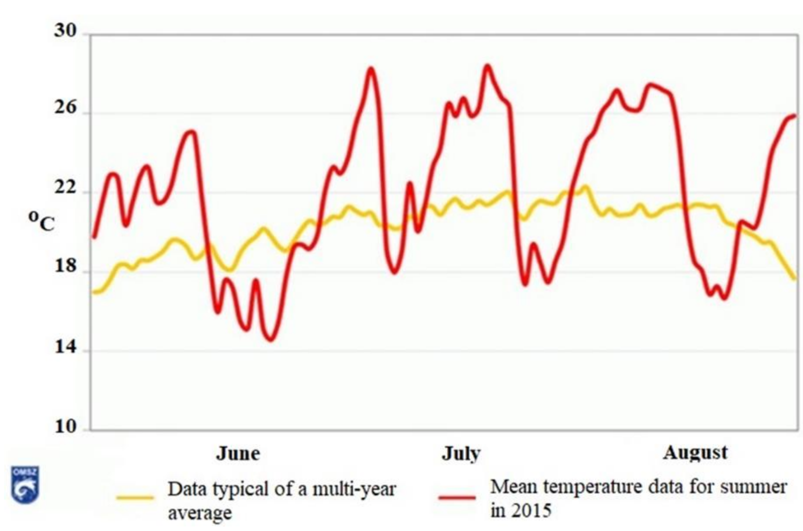

Figure 2: Mean temperature data for summer in 2015 and data typical of a multi-year average (Source: OMSZ, 2021)

2.3. Hybrids under test

During the evaluation we used the results of hybrids and standards applicated for national registration in 2014-2016. Statistical analyses were carried out based on the infection rates of all the hybrids tested to study the effect of year, trial site, and maturity group. The number of hybrids in the maize variety trial differs in years under tests, because new reports, registrations and withdrawals took place year by year (Table 4). The number of samples tested is therefore different.

Table 4: In the years of tests, the number of hybrids in the trial per maturity group (2014-2016)

FAO maturity group/year	2014	2015	2016
FAO 180-239	11	10	10
FAO 240-199	35	23	36
FAO 300-399	112	107	91
FAO 400-499	42	45	24
FAO 500-599	10	13	11
Total	210	198	172

During the test of the differences between hybrids, we narrowed the analysis to the standards and varieties to be registered included in the trial in all three years between 2014 and 2016. We tested 19 hybrids that were applicated by different plant breeding companies. The hybrids are divided into five maturity groups: super early 3 pc, very early 5 pc, early 4 pc, mid-maturing 4 pc, late 3 pc. Their resistance to *Fusarium* differs in the average of 3 years tested (Table 5).

Table 5: Distribution of 19 hybrids per maturity group and their resistance to *Fusarium* stalk and ear rot (2014-2016)

FAO maturity group (pc)		Resistance category	Stalk rot (pc)	Ear rot (pc)
FAO 180-239	3	resistant (1)	1	-
FAO 240-199	5	moderately resistant (2)	5	5
FAO 300-399	4	mid- susceptible (3)	11	11
FAO 400-499	4	susceptible (4)	2	3
FAO 500-599	3	highly susceptible (5)	-	-

2.4. Trial setting, sowing period

The trial plots include 4 rows. Each plot is of 9.20 m x 2.80 m. The territory of the plot is 25.76 m^2. In case of a row distance of 70 cm, the planting distance is 19 cm in the super early maturing group, 21 cm in the very early maturing group, and 24 cm for the mid and late maturing groups. **Sowing of the trial took place on 15-25 April in each year and site.**

2.5. Methods of resistance testing

Assessment of ear rot was carried out per maturity group when the grain moisture of the hybrids in the group at harvest was below 20%. The tests were performed in 2 replicates in the 4^{th} row of the 4-row plots. In the 4^{th} row, cobs were evaluated on a scale of 0-4 (0=no cob infection, 1=cob end has an infection of roughly 1-10%, or 1-5 grains on the cob, 2=cob infection of 4-25 %, 3=cob infection of 26-50 %, 4=cob infection of 51-100 %). With respect to all the cobs and infected cobs, we determined the frequency of infection, which is given in infected plant % according to Hinfner and Békési (1969). Based on the estimated rate of infection, an infection index was calculated with the formula by McKinney (1923), which indicates the severity of diseases as well.

$$I = \Sigma \frac{(n * k)}{N} * K$$

K – all the cobs in the parcel

k – cobs for each infection category

n – infection category (0-4)

N – maximum value of the scale (4)

Hybrids were classified into resistance categories based on their infection index. Assessment is performed in each set of performance trials, however, classification into the resistance category is performed only on the basis of data from trials with adequate infection pressure (at least one hybrid tested has a minimum infection of 20 %). in each maturity group. If the trial mean is less than 50 %, classification into resistance category is based on the deviation between the infection rate of each genotype and the actual trial mean, which considered being 100% (Hinfner and Homonnay, 1966) as follows:

If the actual trial mean is less than 50 %, a given candidate variety is: resistant (1) if its rate of infection does not exceed 25 % of the trial mean, moderately resistant (2) if its rate of infection is 26 - 75 % of the trial mean, mid-susceptible (3) if its rate of infection is 76 - 125 % of the trial mean, susceptible (4) if its rate of infection is 126 - 175 % of the trial mean, and highly susceptible (5) if its rate of infection exceeds 175 % of the trial mean.

If the trial mean of infection is over 50 %, classification is based on the percentage of the actual infection: resistant (1): 0-20 %, moderately resistant (2): 21-40 %, mid-susceptible (3): 41-60 %, susceptible (4): 61-80 %, highly susceptible (5): 81-100 %.

The evaluation of stalk rot was determined by the pressure test of the 2nd internode of the stalk of the plants. Soft stalks were visually evaluated and the number of plants showing *Fusarium* disease was recorded. Stalk rot is expressed as a percentage of the total number of plants. Subsequently, *Fusarium* stalk rot resistance was classified into the appropriate resistance category, similar to ear rot.

2.7. Statistical processing of data

The difference was due to the fact that the number of hybrids tested varied in years, and to the fact that the number of registered trial locations varied year by year per disease type. The reason is that *Fusarium* natural infection did not occur to a significant extent in the trial locations every year. Due to the variable number of elements, a non-parametric statistical method was required. The Kruskal-Wallis test is a non-parametric procedure used to compare more than two independent samples along a variable, which is suitable for comparing non-normally distributed, different number-independent samples. It is an effective alternative to one-way analysis of variance. In the Kruskal-Wallis H test, data from each group were ranked and H values were calculated.

$$H = \frac{12}{N(N+1)} + \left(\left(\frac{R2}{n}\right) - 3(N+1)\right)$$

R is the sum of the ranks, N is the sum of the total scores, n: is the sum of the scores of a given group. The magnitude of the P value shows the relationship between the studied groups: if P=<0.05 we reject the null hypothesis and state that the groups are statistically different from each other (Ostertagová et al. 2014).

For pairwise comparisons of groups, a Dunn post-test was applied, where Bonferroni correction was used to exclude the probability of errors resulting from multiple comparisons, which gives a less sensitive result for different number of elements in the groups (Sinkovits and Prohászka, 2021). The figures for the results (Figure 3-8) were based on the analysis of the Kruskal-Wallis test. In the tables (Tables 6-11), significantly different data pairs from the Dunn-Bonferroni post-tests are presented. The Kruskal-Wallis test and the Dunn-Bonferroni post-test were carried out with SPSS statistical program.

3. RESULTS

3.1. The effect of year on the evaluation of stalk and ear rot

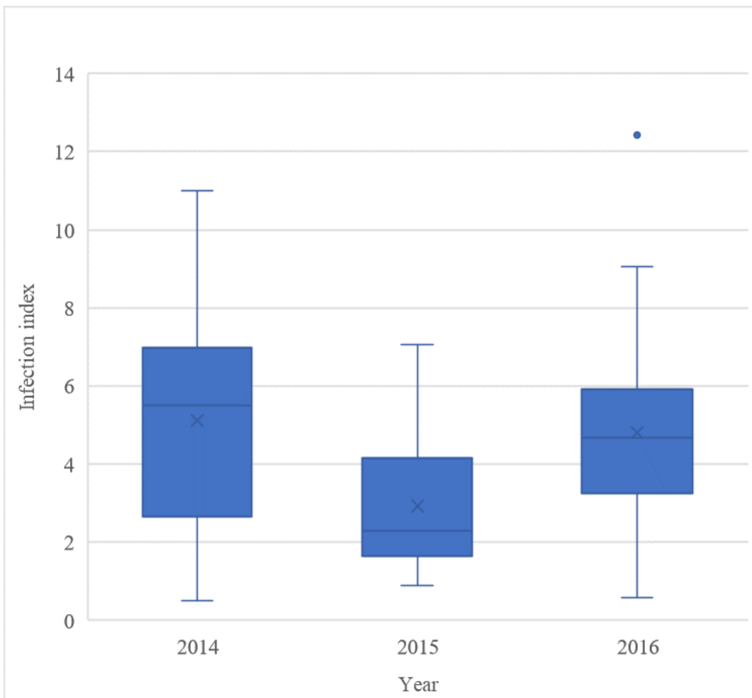

Figure 3: Annual change in ear rot infection index of the trial set up at Variety Trial Stations (2014-2016)

Table 6: Pairwise comparison of *Fusarium* stalk rot in the years tested with Dunn-Bonferroni post-test at p=5% significance level (Budapest, 2023)

Years tested	P
2015-2016	0.000
2015-2014	0.000

Figure 3 and Table 6 show that the 3-year period tested had a significant effect (H (2) =28.920, P=0.000) on the degree of ear rot infection. Based on the post-test, 2015 differs significantly from the infection data of 2014 and 2016, the level of infection is significantly lower compared to data of the other two years. As a conclusion, 2015 was characterized by a smaller degree of ear rot compared to 2014 and 2016.

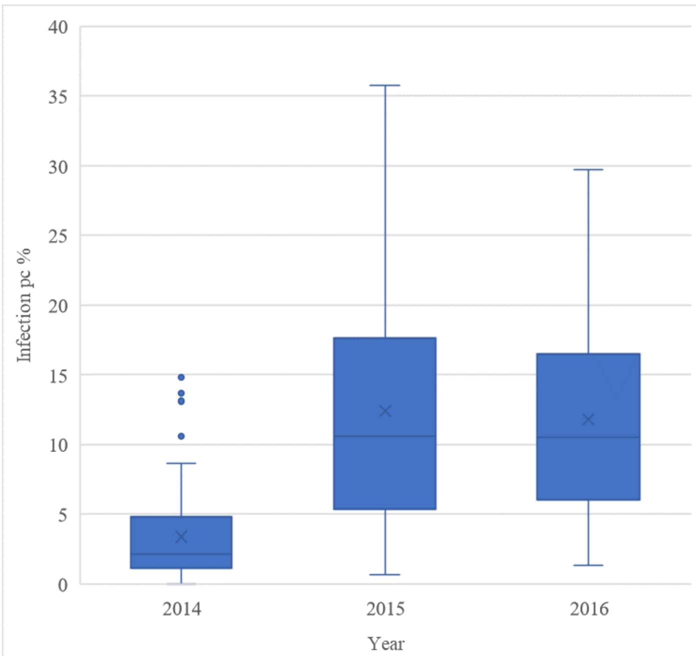

Figure 4: Annual pieces percentage (pc %) change in stalk rot infection of trial set up at Variety Trial Stations (2014-2016)

Table 7: Pairwise comparison of *Fusarium* stalk rot in years with Dunn-Bonferroni post-test at p=5% significance level (Budapest, 2023)

Years tested	P
2014-2015	0.000
2014-2016	0.000

It can be concluded based on Figure 4 and Table 7 that year had a significant effect on stalk rot. Based on the Kruskal-Wallis test (H (2) =71.640, P=0.000) and its post-test, the infection rate in 2014 was significantly ($p < 0.05$) lower than in both other years. However, there is no significant difference between the values of 2015 and 2016. As a conclusion, there was a lower degree of stalk rot in 2014 than in 2015 and 2016 (Figure 4).

3.2. Effect of trial site on the evaluation of stalk rot and ear rot

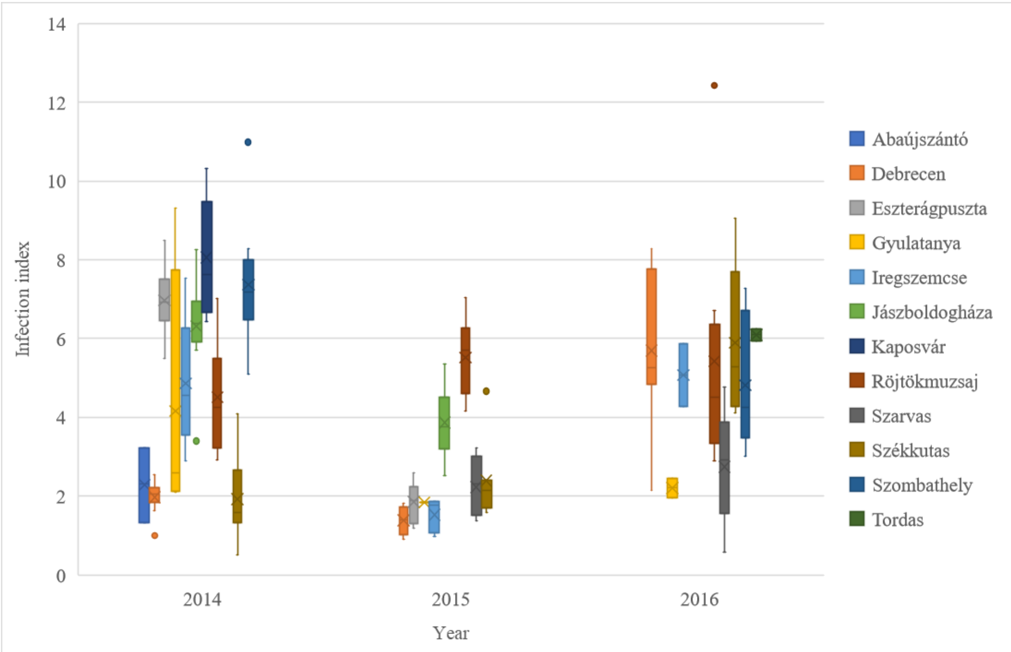

Figure 5: Changes in the index of ear rot infection in the trial set up at Variety Trial Stations of the years tested (2014-2016)

Table 8: Significant pairs and locations obtained during the comparison of the extent of Fusarium ear rot at the trial sites, evaluated by the Dunn-Bonferroni post-test at the p=5% significance level (Budapest, 2023)

Year	Pairs of trial sites	P
2014	Székkutas- Jászboldogháza	0.011
	Székkutas- Eszterágpuszta	0.001
	Székkutas- Szombathely	0.000
	Székkutas- Kaposvár	0.000
	Debrecen- Jászboldogháza	0.009
	Debrecen- Eszterágpuszta	0.001

ISSN: 2456-8643

	Debrecen- Szombathely	0.000
	Debrecen- Kaposvár	0.000
2015	Debrecen- Jászboldogháza	0.030
	Debrecen- Röjtökmuzsaj	0.001
	Iregszemcse-Röjtökmuzsaj	0.001
	Eszterágpuszta-Röjtökmuzsaj	0.002
	Szarvas- Röjtökmuzsaj	0.011

Based on the Kruskal-Wallis test, it can be stated that within a given year there was significant difference between the distribution of infection values for sites in 2014 (H (9) = 59.427, P = 0.000) and 2015 (H (7) = 36.427, P = 0.000). In 2014, infection of *Fusarium* ear rot in Szeged and Debrecen was significantly different from most places (Table 8). In 2015, the values of Debrecen and Röjtökmuzsaj differed significantly from most places in the pairwise comparisons of the post-test. Infection data in Debrecen were low and data in Röjtökmuzsaj were high (Figure 5). Data from Röjtökmuzsaj, although not significantly different from the data from the other sites in 2014 and 2016, can be said to be overall that Röjtökmuzsaj provided reliably high infection pressure for the evaluation in all 3 years tested. In 2014, Jászboldogháza, Eszterágpuszta, Szombathely, Kaposvár were trial sites with a higher risk of ear rot infection. In 2015, in addition to Röjtökmuzsaj, as in 2014, the ear rot infection of Jászboldogháza was outstanding (Figure 5).

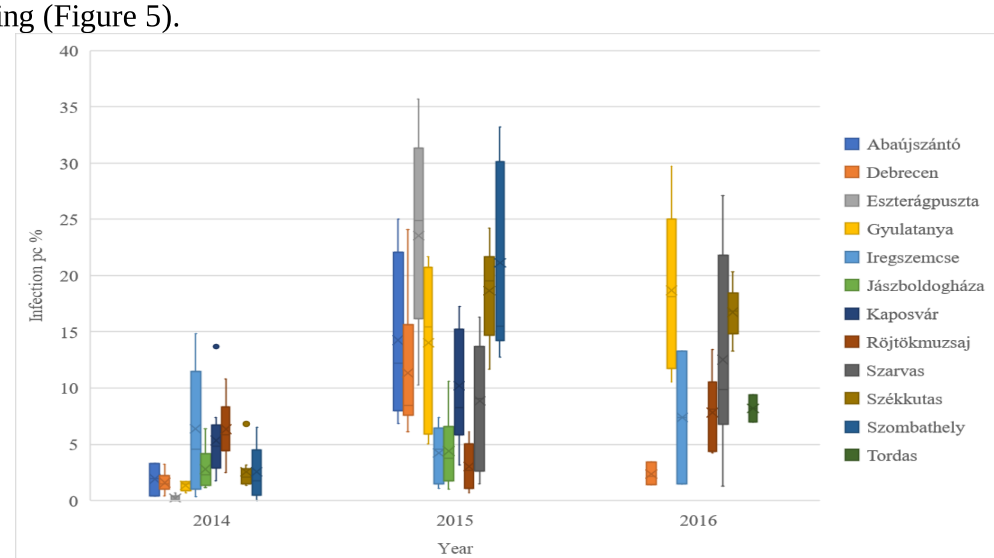

Figure 6: Pieces percentage (pc %) change in stalk rot of the trial set up at the Variety Trial Stations of the years tested (2014-2016)

Table 9: Significant pairs of sites obtained during the comparison of the extent of Fusarium stalk rot at trial sites, evaluated by the Dunn-Bonferroni post-test at the p=5% significance level (Budapest, 2023)

Year	Pairs of trial sites	P
2014	Eszterágpuszta-Iregszemcse	0.001
	Eszterágpuszta-Kaposvár	0.000
	Eszterágpuszta-Röjtökmuzsaj	0.000
	Debrecen-Röjtökmuzsaj	0.022
2015	Röjtökmuzsaj-Székkutas	0.002
	Röjtökmuzsaj-Szombathely	0.000
	Röjtökmuzsaj-Eszterágpuszta	0.000
	Jászboldogháza-Székkutas	0.029
	Jászboldogháza-Szombathely	0.012
	Jászboldogháza-Eszterágpuszta	0.004
	Iregszemcse-Székkutas	0.011
	Iregszemcse-Szombathely	0.004
	Iregszemcse-Eszterágpuszta	0.001
2016	Debrecen-Székkutas	0.029
	Debrecen-Gyulatanya	0.025

Data of Figure 6 and Table 9 show – based on the comparison of the data of stalk rot – there was significant difference between the distribution of data of the sites within the years (H(9)=43,642 in 2014, P=0.000; H(10) in 2015).)=52,160, P=0.000 and in 2016 H(6) = 18,487). In 2014, Eszterágpuszta can be significantly distinguished from Iregszemcse, Kaposvár and Röjtökmuzsaj. Data of Röjtökmuzsaj differed statistically from data of Debrecen (Table 9). In 2015, data of Röjtökmuzsaj, Jászboldogháza and Iregszemcse differed significantly from most places. During the comparisons of the post-test pairs in 2016, data of Debrecen were significantly different from infection data of Gyulatanya and Székkutas. Regarding data of the provocation trial in Röjtökmuzsaj, it can be stated that although there was no significant stalk rot infection in all three years (Figure 6). In 2014, trials of Iregszemcse, Kaposvár, Röjtökmuzsaj, in 2015 in Székkutas, Szombathely, Eszterágpuszta, in 2016 in Székkutas and Gyulatanya were characterized by large stalk rot (Figure 6).

3.3. Assessment of the resistance of 19 hybrids tested in 2014-2016 to *Fusarium* stalk and ear rot based on the average of the years and trial sites

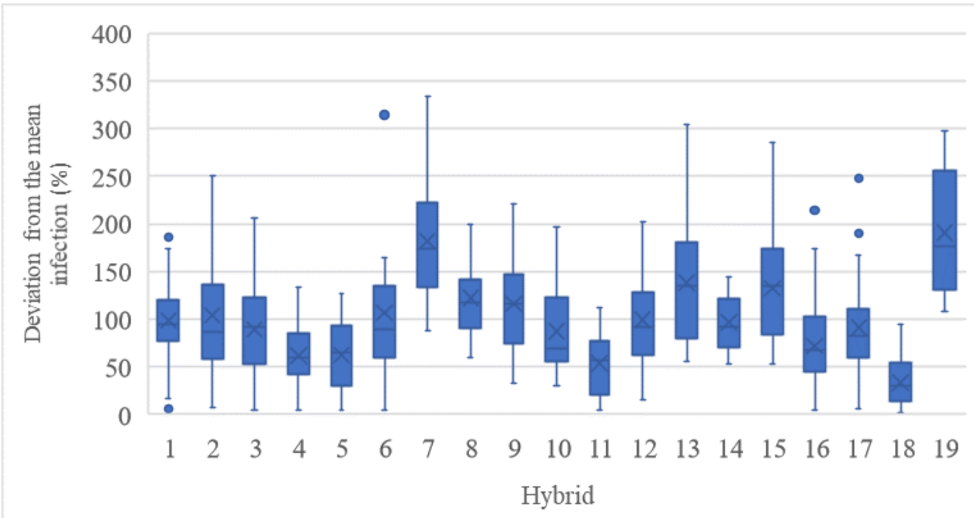

Figure 7: Percentage deviation of *Fusarium* ear rot infection from the mean infection (100 %) in context of 19 hybrids tested based on data of trial years and sites

Table 10: Significant differences in the susceptibility of 19 hybrids to Fusarium ear rot when tested by Dunn-Bonferroni post-test after Kruskal-Wallis test at p=5% significance level (Budapest, 2023)

Hybrid pairs	P
18-16	0.011
18- 10	0.032
18-17	0.001
18-3	0.000
18-2	0.000
18-12	0.000
18-6	0.001

18-14	0.001
18-1	0.000
18-9	0.000
18-8	0.000
18-15	0.000
18-13	0.000
18-7	0.000
18-19	0.000
11-15	0.036
11-13	0.000
11-7	0.000
11-19	0.000
4-9	0.044
4-8	0.014
4-15	0.006
4-13	0.010
4-7	0.000
4-19	0.000
5-7	0.000
5-19	0.000
16-15	0.022
16-13	0.040
16-7	0.000
16-19	0.000
10-7	0.015

10-19	0.044

Evaluating index of infection of 19 hybrids included in the analysis, significant differences in the susceptibility of hybrids to ear rot were observed based on the Kruskal-Wallis test (H (18) =151,593, P=0.000). According to post-tests, mathematically verifiable hybrid 18 was less infected than 15 hybrids (1,2,3,6,7,8,9,10,12,13,14,15,16,17,19) with *Fusarium* ear rot. Hybrid 4 is significantly less infected than 6 hybrids (7,8,9,13,15,19). Infection of hybrid 11 is significantly different from 4 hybrids (7,13,15,19), hybrid 16 is different from 4 hybrids (15,13,7,19) and hybrid 5 is different from 2 hybrids (7,19). Hybrid 10 is significantly less infected than 2 hybrids (7, 19) (Table 10). The infection values of hybrids 4, 5, 11, and 18 are always below the trial mean (100 %). Infections of hybrids 7 and 19 often reached twice the trial mean. Based on our tests, hybrids 7, 13, and 19 proved to be susceptible (4) genotypes. Hybrids 4, 5, 11, 16, and 18 fall into the moderately resistant (2) category (Figure 7).

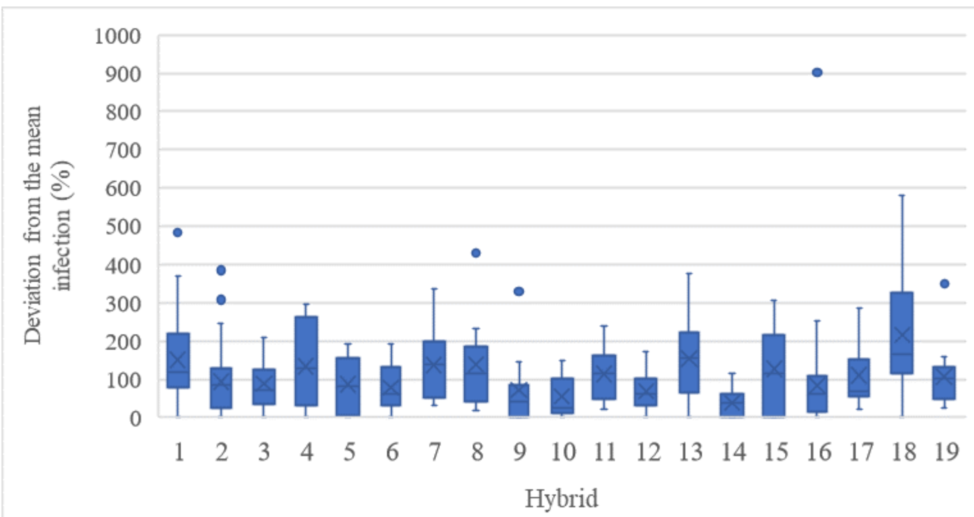

Figure 8: Percentage deviation of *Fusarium* stalk rot infection in 19 hybrids tested from the mean infection (100 %) based on data from trial years and sites

Table 11: Significant differences in susceptibility of 19 hybrids tested to *Fusarium* stalk rot by Dunn-Bonferroni post-test after the Kruskal-Wallis test at p=5 % significance level (Budapest, 2023)

Hybrid pairs	P
14-13	0.033
14-1	0.001

14-18	0.000
10-18	0.011
9-18	0.140
16-1	0.007
16-18	0.000
12-18	0.000

The resistance of 19 hybrids to stalk rot differs (H (18) =75.456, P=0.000). Based on the post-test, hybrid 18 was significantly more infected with stalk rot than 5 other hybrids (9,10,12,14,16). Hybrid 14 proved to be statistically less infected than 3 other hybrids (1, 13, 18). Furthermore, hybrids 1 and 16 differ mathematically (Table 11). In addition to hybrid 14, the results of hybrid 9 were always below the trial mean. Hybrids 1, 4, 7, 13, and 15 were up to twice the trial mean for the rate of infection, and hybrid 18. was three times higher. Based on our tests, hybrids 8 and 18 performed as susceptible (4) genotypes to stalk rot. Hybrids 6, 9, 12, and 16 are moderately resistant (2) and hybrid 14 is resistant (1) (Figure 8).

4. CONCLUSIONS

4.1. Effect of year on the evaluation of stalk and ear rot

Campa et al. (2005) suggest that 4 critical periods can be distinguished in relation to weather and silking. In their opinion, the amount of precipitation and temperature that fall between 10 days before and 14 days after silking of 50 % determines the amount of toxin at harvest. In the resistance test of maize variety trial against diseases of *Fusarium* origin, data were obtained from trials with natural infection and from provocation trials based on monoculture. Of 3 years tested, higher ear rot was observed in 2014 and 2016 compared to 2015. In July 2014 and 2016 (at the time of silking), there was more precipitation compared to 2015 at all the trial locations. Drought in 2015 favoured the development of stalk rot. Stress caused by drought and warm weather is very favourable to the development of the disease (Balázs, 1990). Several *Fusarium* species are responsible for the development of diseases of *Fusarium* origin in maize. Logrieco et al. (2002) highlight 19 *Fusarium* species, but in Hungary *Fusarium graminearum* and *Fusarium verticillioides* have paramount importance (Mesterházy et al. 1977). The genetic background of resistance to different species causing *Fusarium* ear rot is not the same. The mechanisms of resistance to *Fusarium verticillioides* and *Fusarium graminearum* are different (Löffler et al., 2011; Mesterházy et al., 2012; Szőke et al. 2013), although Presello et al. (2006) identified sources of resistance that showed good resistance to *Fusarium graminearum* and *Fusarium verticillioides*. Szőke et al. (2014) found that the prevalence of *Fusarium verticillioides* increased in stalk samples, and that our warming climate helps the spread of more heat-demanding species. In a resistance test based on natural infection, the species composition of *Fusarium* in a given year should be determined. By trial with artificial infection of two prevalent species, we can ensure the significance of the dominant species in the test location each year. No species identification is performed during visual assessment in the field. In 2015, we collected maize stalk (tissue) samples from the trials with strong Fusarium stalk rot in Szombathely and Kaposvár. According to the results of molecular studies, *Fusarium temperatum* species were also isolated from the samples (Molnár et al. 2017). The emergence and spread of new species as a result of climate change is to be expected, therefore, in addition to trials with artificial infections, it is important to test them under natural infection as well and to supplement them with species identification.

4.2. Effect of trial site on the evaluation of stalk and ear rot

Infection of *Fusarium* species is determined by other local environmental factors, such as previous crop. At the Variety Trial Sites, maize was most commonly found after winter wheat in the years tested. In this case, the amount of precipitation in the previous year during the earing, silking, and harvesting of winter wheat is decisive, as *Fusarium* infects winter wheat, as well. In addition to winter wheat, mustard, soybean, rape, and potato previous crops occurred. Schaafsma et al. (2007) have shown that weather (48 %), variety (27 %) and previous crop (14–18 %) play an important role in predicting DON toxin production in wheat. In the case of maize, hybrids (25 %), wheather conditions (12 %) and the combination of the two (42 %) are responsible for the formation of DON and fumonisin toxins, and in the case of maize, insect damage has an important role. Testing the effect of previous crop, it can be stated that maize as previous crop (monoculture) resulted in high infection every year. There has been a monoculture in Röjtökmuzsaj for 55 years, therefore, accumulation of antagonists of *Fusarium* species is expected in the soil. Such an antagonist in soil is *Bacillus subtilis*, the effect of which was tested on *Fusarium verticillioides* by Cavaglieri et al. (2005). Röjtökmuzsaj had a stalk rot infection in all the years tested, but the rate of infection was not remarkably high. The potential presence of antagonists may cause the development of stalk rot to be generally lower than the degree of ear rot. The humid microclimate, which is characteristic of Röjtökmuzsaj, favours ear rot. In addition to the monoculture in Röjtökmuzsaj, higher infection pressure is expected from the monoculture in Szarvas. In Szarvas in 2016, there was a higher rate of stalk and ear rot infection than in 2015. In context of resistance test, it is important to make founded decisions about locations with natural infection based on knowledge on *Fusarium* species composition.

4.3. Assessment of the resistance of hybrids tested in 2014-2016 to Fusarium stalk and ear rot based on years and trial sites tested

The extent of Fusarium stalk and ear rot is influenced by, among other factors, weather conditions, agrotechnics, insect damage and the resistance of the selected hybrid. While according to the study by Schaafsma et al. (2007), the hybrid has a role of 25%, Campa et al (2005) suggests that 47% is affected by weather, 17% by insect damage, and 14% by the hybrid for fumonisin content. Although the mechanism of resistance to Fusarium is not yet fully clarified, there is a shortage of *Fusarium*-resistant maize genotypes, but our studies have shown several hybrids with high-level field resistance. The susceptibility of 19 hybrids tested can be distinguished based on the combined results of 3 years and different sites. There are hybrids that have good resistance to ear rot but are susceptible to stalk rot (hybrids 18 and 4), while some hybrids are susceptible to both forms of the disease (7 and 13), or resistant to both (16). Unfortunately, there are asymptomatic but toxin-producing hybrids among the hybrids in commercial cultivation that cannot be detected by visual evaluation. To prevent this, in addition to visual assessment, toxin measurement is also required.

In summary, disease resistance tests play an important role in the national variety registration of maize. Great emphasis is placed on testing for resistance during the registration process. To be registered, a hybrid must meet resistance criteria. The disease resistance of hybrids can also be successfully tested on the basis of the currently valid test methodology. Regardless of the year, there are trial sites with natural infection that can be evaluated regarding stalk and ear rot. The methodology covers the fact that we only take into account infections that have developed as a result of the appropriate infection pressure.

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