

## DYNAMICS OF INCIDENCE AND FREQUENCY OF POPULATIONS OF *Fusarium* SPECIES ON STORED MAIZE GRAIN\*\*

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**Abstract:** Production and providing of high quality maize grain are of primary importance for livestock production since maize is the main component of livestock feed. Contamination with fungi not only diminishes the quality of grain, but some fungi species can produce highly toxic compounds known as mycotoxins. Considering that maize is economically the most important grown plant in Serbia, content and intensity of frequency of these pathogen fungi species are investigated in maize stored in the storage facilities.

Based on studies of the fungi incidence in stored maize intended for nutrition of livestock, it was established that during the period from February 2005 to March 2006, some fungi species were present successively (*Fusarium* spp., *Penicillium* spp.), other with interruptions of one (*Nigrospora* spp., *Aspergillus* spp., *Acremonium* spp., *Alternaria* spp), three (*Mucor* spp., *Chaetomium* spp., *Rhizopus* spp.) to four months (*Cladosporium* spp.), and also sporadically during three (*Epicoccum* spp., *Rhizopus* spp., *Trichothecium* spp.), two (*Gliocladium* spp.) or one month (*Papulaspora* spp., *Phialophora* spp.). Period June-October is the most critical for preservation of the quality of stored maize since the number of fungi species present (8–14 of total of 17 identified fungi genera) and their frequency during this period are the highest, especially of toxigenic species of *Fusarium* (43.5–62.5%) and *Penicillium* (10.0–33.5%) genera. Positive correlation ( $r = 0.5979^{**}$ ) between dynamics of incidence and frequency of isolated fungi species indicates that fungi with higher frequency of incidence remain longer on the grain during storage.

*F. verticillioides* and *F. subglutinans*, of total of 6 identified *Fusarium* species were present on the grain during entire year and with the highest frequency (24.7% and 5.9%, respectively). During single year (2005) *F. verticillioides* was present in the highest percentage from February to October (22.0–39.5%), and *F. subglutinans* from April to October (8.0–12.5%), whereas the both species were isolated the least during winter period December-January (4.0–8.0% and 0.5–1.0%, respectively). *F. graminearum* is the third toxigenic *Fusarium* species which from the mycotoxicological aspect can be important for period June-September when it is mostly present (5.0–11.0%). Incidence of other *Fusarium* species is sporadic (1.3% *F. proliferatum*, 1.0% *F. sambucinum* and 0.5% *F. poae*).

**Key words:** maize, grain, stored mycopopulation, *Fusarium* species, dynamics

## Introduction

Maize (*Zea mays* L.) is one of the most important cultivated crops in Serbia grown on approximately 1,2 million ha (*Statistical Yearbook of Serbia*, 2001). This crop is mainly used as feed or as a component of livestock feed especially for pigs and poultry (*Lazarević et al.*, 1998). The production and supply of high quality maize grain remains of prime importance in Serbia. Cereals must thus be protected in the field against disease and in storages after harvest against fungal attack. Invasion by fungi not only reduces the quality of the grain but some species of moulds can produce highly toxic compounds known as mycotoxins (*Marasas et al.*, 1984). Due to it and to the fact that maize is economically the most important cultivated plant in Serbia, a composition and the intensity of the incidence of these pathogenic fungal species are observed in stored maize grain.

Determining ear mould problems prior to harvest is critical to managing and minimizing the impact of these diseases through timely harvest and proper drying/storage conditions. It is also important to identify which ear moulds are present since not all of these fungi produce toxins. *Fusarium* species are the most economically important causal agents reported worldwide as responsible for ear rot (*White*, 2000; *Tenuta*, 2006).

In Brazil, the species of the genus *Fusarium* were mostly distributed (83.8%) in maize grain sampled both, after harvest and in storages (*Pozzi et*

*al.*, 1995). These species were followed by *Penicillium* spp. (55.3%), *Aspergillus* spp. (40.7%), *Cladosporium* spp. (32.3%), then by yeasts (19.2%), *Mycelia sterilia* (10.7%), *Rhizopus* spp. (9.2%), *Mucor* spp. (7.7%), *Absidia* spp. (2.3%), *Wallemia* spp. (2.3%), *Trichoderma* spp. (0.7%), *Paecilomyces* spp. (0.7%), *Neurospora* spp. (0.7%), *Alternaria* (0.7%) and *Aureobasidium* spp. (0.7%).

According to *Pozzi et al.* (1995) *Fusarium verticillioides* was the most dominant species (80.7%), while *F. proliferatum* ranked second (3.0%). According to *Marasas* (1991), *F. verticillioides* occurs worldwide and is one of the most prevalent fungi associated with corn in most of the corn-producing areas. *Marasas et al.* (1979) revealed that the mean percentage of kernel infection by fungi increased during 8 months of commercial storage in temperature, subtropical, and intermediate areas of South Africa.

The *Fusaria* frequently isolated from maize in Mediterranean countries are widespread *F. verticillioides* and *F. graminearum* (*Logrieco et al.*, 1995). *Fusarium* species commonly isolated from Mediterranean cereals produce high amounts of fumonisin FB<sub>1</sub> and zearalenone (ZEN) *in vitro* on different media. A study of FB<sub>1</sub> production by 28 strains of *F. verticillioides* isolated from cereals in Italy, Spain and France revealed fumonisinotoxigenic potential for all strains with maximum produced concentration of 4100 mg kg<sup>-1</sup> (*Visconti*, 1994).

The obtained results in the period 1994–1996 by *Lević et al.* (1997) indicate that *F. verticillioides* (63.0%) is predominant maize ear rot in Serbia, followed by the *F. subglutinans* (50,6%), *F. graminearum* (12.2%), *F. proliferatum* (9.6%) and *F. oxysporum* (5.8%). According to these authors *F. solani* occurred each year, but its frequency was not so high (2.4%), and other *Fusarium* species (*F. equiseti*, *F. sporotrichioides*, *F. chlamydosporum*, *F. crookwellense* and *F. semitectum*) occurred sporadically on a few number of samples.

Some of the fungi associated with grain in the field (often referred to as "field fungi") can form mycotoxins, such as *Fusarium*, *Aspergillus* and *Penicillium*, either immediately before, or just after harvest (*Hocking*, 2003). Fungal contamination in storage can also be related to levels in the field (*Hell et al.*, 2003). Mycotoxin contamination of grain is not always a result of poor storage, as some mycotoxins, particularly *Fusarium* toxins, may already be in the grains when they are brought into storage. However, if moulds do start to grow in stored grains, mycotoxins may be formed if the fungal succession develops to a point where mycotoxigenic species are able to grow. Whether or not mycotoxins are an issue, any mould development in

stored grains is undesirable (Hocking, 2003).

Globally, it is considered that following species of *Fusarium* genus are the most toxigenic *F. sporotrichioides*, *F. graminearum* and *F. verticillioides* since their mycotoxins can cause serious diseases in animals and humans (Marasas, 2000; Marasas et al., 1984). In agro ecological conditions of Serbia *F. graminearum* is mainly studied as toxigenic species (Bočarov-Stančić, 1996), although also *F. verticillioides* and *F. subglutinans* can be considered as species which most frequently cause fusariosis of maize grain (Lević et al., 1997). In Serbia, two epidemics of mycotoxicosis in livestock were registered in the years when fusariosis of the maize ear was progressing in epidemic proportions, such was the case in years 1968 or 1972 and in subsequent years when this maize was used in livestock nutrition (Marić, 2002). Based on expressed disease symptoms in domestic animals (vulvovaginitis, refusal of feed in pigs) in years of maize ear fusariosis epidemics it can be concluded that maize was contaminated with zearalenone and trihothecenes.

Because of extraordinary importance of use of healthy feed in livestock production, in this paper, the dynamics of incidence and intensity of presence of potentially toxigenic fungi species during longer time period were investigated, especially of *Fusarium* genus, and on stored maize grain intended for livestock nutrition.

## Material and methods

A total of 28 samples of stored maize originated from around Belgrade were used in this study. Stored grains of maize (1000 g) were collected from each monthly twice during period of 14 months, i.e. from February 2005 to March 2006. The average grain moisture in collected samples amounted 12.7%.

Sub samples (100 kernels) were rinsed under running tap water for 1 hour. Then, kernels were superficially disinfected in 5% NaOCl and rinsed three times with sterile distilled water for 5 minutes. Kernels were dried on the filter paper and placed on the 2% agar medium in 10-cm Petri dishes. These kernels were incubated in the thermostat at the temperature of 26°C. Six days later, the identification of fungal colonies, especially of those of the genus *Fusarium*, developed in the area around incubated maize kernels was carried out by the microscopic examination.

The identification of *Fusarium* species on the basis of morphological macroscopic and microscopic characteristics on potato-dextrose agar (PDA)

and synthetic nutrient agar (SNA) was done after *Nelson et al.*, (1983) and *Burgess et al.* (1994), while remaining fungal genera were determined after *Ellis* (1971) and *Watanabe et al.* (1994).

Data obtained for dynamics (number of months) and intensity of fungi incidence on stored maize grain during the period from February 2005 to March 2006 (14 months) were used for calculation of correlation coefficient. Significance of interrelations of these two factors was tested at the level of  $P_{0.05}$  and  $P_{0.01}$ .

## Results and discussion

Based on study of dynamics of incidence of certain species of fungi during storage in period from February 2005 to March 2006 (table 1), they can be divided into three groups with traits: (1) successive incidence throughout the year (*Fusarium* spp., *Penicillium* spp.); (2) discontinuous incidence: not identified in one (*Nigrospora* spp., *Aspergillus* spp., *Acremonium* spp., *Alternaria* spp), three (*Mucor* spp., *Chaetomium* spp., *Rhizopus* spp.) or four months (*Cladosporium* spp.); (3) sporadic incidence: determined only during three (*Rhizopus* spp., *Epicoccum* spp., *Trichothecium* spp.), two (*Gliocladium* spp.) to one month (*Papulaspora* spp., *Phialophora* spp.).

Of total 17 fungi genera, determined on stored maize grain from February 2005 to March 2006, 7 (May 2006) to 11 (February, June and December 2005 and January 2006) were always present, and only 14 in September and 12 in October 2006 (table 1).

Species of genera *Fusarium* and *Penicillium* are always present, in average 33.6 and 21.7%, respectively. During 2005, presence of *Fusarium* spp. varied from 4.5% (December) to 62.5% (July) in 2005, and they were the most frequent in period from April (52.0%) to October (45.0%). *Penicillium* species on stored maize corn were most frequent in March (43.0%), April (29.0%), May (28.0%) and October (33.5%), whereas in other months their presence varied from 10.0% (July 2005) to 25.5% (December 2005 and February 2006). Species of *Nigrospora*, *Aspergillus*, *Acremonium* and *Alternaria* were determined in 13 of 14 months in average 11.2%, 7.0%, 6.3% and 4.0%, respectively. *Nigrospora* spp. was approximately equally frequent in April (18.0%), May (19.5%), June (19.0%) and August (19.5%), whereas *Aspergillus* spp. was the most frequent in March (24.0%), *Acremonium* spp. in October (18.5%) and *Alternaria* spp. in August (18.0%).

On stored maize grain, also, frequent is incidence of genera *Mucor*, *Chaetomium* and *Rhizopus* (11 months), as well as *Cladosporium* (10 months), but in average of considerably lower intensity (5.7%, 4.8%, 1.9% and 3.3%, respectively) than previous species. Except species of genus *Trichoderma*, which were determined in discontinuity over 6 months, other fungi genera were determined only in three (*Epicoccum* spp., *Trichotecium* spp. *Dreschlera* spp.), two (*Gliocladium* spp.) or one month (*Papulospora* spp. and *Phialophora* spp.) on stored maize grain. Considerable was also presence of *Chaetomium* (11.5%) and *Cladosporium* (13.0%) species during September 2005 and *Trichoderma* spp. (11.0%) in March 2006.

Species of *Fusarium* genus were most present in July (62.5%) 2005, and the least in December (4.5%) 2005. Of six identified species of *Fusarium* genus in all investigated months, *F. verticillioides* and *F. subglutinans* were determined with intensity of 4.0–39.5% and 0.5–12.5% or in average 24.7% and 5.9%, respectively (table 2). In investigation period, *F. verticillioides* was very highly present in April (39.5%), May (34.5%), June (33.0%), July (39.0%), August (32.0%) and October (34.5%) of 2005, and the lowest presence was in December (4.0%) of 2005. Presence of *F. subglutinans* in most of the months of investigation was low (0.5–4.5%) and relatively high (5.0–8.5%), except in April, May and July 2005, when it's presence was very high (10.0–12.5%). During 14 months, discontinuous presence of *F. graminearum* during 7 months, 0.5% (November 2005) to 11% (September 2005) or in average 5.0% was established. Other species (*F. proliferatum*, *F. sambucinum* and *F. poae*) were determined sporadically (4, 2 or 1 months) and in poor intensity (0.5–1.3%).

Analysis of results obtained for contamination with fungi of maize grain stored in warehouses, which was intended for livestock nutrition, shows that in the period February – March there were differences between investigation years in regard to incidence of certain species as well as their intensity (Tables 1, 2).

Based on PPU parameters (table 1) positive correlation ( $r = 0.5979^{**}$ ) between dynamics of incidence (number of months during which the presence of species was determined) and frequency of fungi (%) on stored maize grain during storage from February 2005 to March 2006 was established.

**Table 1. Dynamics of fungi occurrence and frequency (%) on maize storage in the period of February 2005 to March 2006**

Fungal species	Month of investigation <sup>a</sup>														PPU <sup>b</sup>
	2005											2006			
	F	M	A	M	J	J	A	S	O	N	D	J	F	M	
<i>Fusarium</i> spp.	39.0	27.5	52.0	44.5	48.0	62.5	43.5	48.0	45.0	13.0	4.5	9.0	13.0	17.5	14/33.6
<i>Penicillium</i> spp.	10.5	43.0	29.0	28.0	14.5	10.0	16.0	15.5	33.5	17.0	25.5	16.5	25.5	19.0	14/21.7
<i>Nigrospora</i> spp.	5.5	14.5	18.0	19.5	19.0	7.5	19.5	11.0	0.0	1.0	8.5	7.0	11.0	4.0	13/11.2
<i>Aspergillus</i> spp.	3.5	24.0	9.0	2.0	5.0	0.0	2.5	1.5	13.5	9.0	1.0	3.0	7.5	9.0	13/7.0
<i>Acremonium</i> spp.	12.5	5.5	8.5	12.5	4.0	9.0	2.5	1.0	18.5	2.5	0.0	3.5	1.5	1.0	13/6.3
<i>Mucor</i> spp.	0.5	4.0	8.5	0.0	0.0	0.0	2.0	4.5	0.5	10.5	13.0	12.5	0.5	6.0	11/5.7
<i>Alternaria</i> spp.	2.5	1.0	2.0	0.0	3.5	7.0	18.0	4.5	1.5	4.0	5.0	1.0	1.0	1.5	13/4.0
<i>Chaetomium</i> spp.	0.0	0.0	0.0	2.0	2.0	2.0	3.5	11.5	2.0	2.0	4.0	10.5	4.0	10.0	11/4.8
<i>Cladosporium</i> spp.	1.5	0.5	0.0	0.0	0.0	3.5	8.0	13.0	0.5	2.5	1.5	1.0	0.5	0.0	10/3.3
<i>Trichoderma</i> spp.	0.0	0.0	2.5	0.0	7.5	0.0	0.0	0.5	4.5	0.0	0.0	0.0	3.0	11.0	6/4.8
<i>Rhizopus</i> spp.	4.0	1.5	2.0	0.5	0.5	0.5	1.0	3.0	3.0	0.0	2.5	2.5	0.0	0.0	11/1.9
<i>Epicoccum</i> spp.	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5	1.5	0.5	0.0	0.0	0.0	3/1.2
<i>Trichotecium</i> spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5	0.5	0.0	0.0	1.0	0.0	0.0	3/1.0
<i>Dreschlera</i> spp.	0.0	0.0	0.5	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	3/0.7
<i>Gliocladium</i> spp.	1.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2/1.0
<i>Papulaspora</i> spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	1/2.0
<i>Phialophora</i> spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	1/2.0

<sup>a</sup>First letter of month when the phytopathological analysis of maize sample stored in the period of February 2005 to March 2006 was carried out.

<sup>b</sup>Number of positive samples of total 14/Average for positive samples

**Table 2. Dynamics of *Fusarium* species occurrence and frequency (%) on maize storage in the period of February 2005 to March 2006**

<i>Fusarium</i> species	Month of investigation <sup>a</sup>														PPU <sup>b</sup>
	2005											2006			
	F	M	A	M	J	J	A	S	O	N	D	J	F	M	
<i>Fusarium verticillioides</i>	33.5	22.0	39.5	34.5	33.0	39.0	32.0	27.0	34.5	10.5	4.0	8.0	11.0	17.0	14/24.7
<i>Fusarium subglutinans</i>	4.5	4.5	12.5	10.0	8.5	12.5	6.5	7.5	8.0	5.0	0.5	1.0	0.5	0.5	14/5.9
<i>Fusarium graminearum</i>	0.0	0.0	0.0	0.0	5.0	11.0	5.0	11.0	1.0	0.5	0.0	0.0	1.5	0.0	7/5.0
<i>Fusarium proliferatum</i>	0.0	0.0	0.0	0.0	1.0	0.0	0.0	1.0	1.5	1.5	0.0	0.0	0.0	0.0	4/1.3
<i>Fusarium sambucinum</i>	1.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2/1.0
<i>Fusarium poae</i>	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1/0.5

<sup>a</sup>First letter of month when phytopathological analysis of maize sample stored in the period of February 2005 to March 2006 was carried out.

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During the last 20 years, there were no significant changes in the distribution of genera *Aspergillus* (5.0%), *Fusarium* (41.0%) and *Penicillium* (46.0%) in maize grain in Serbia (Lević *et al.*, 2004), except in the species composition and frequency. Noory (1983) reported the dominance of *F. oxysporum* and a two-fold lower occurrence of *F. verticillioides* (syn. *F. moniliforme*) and *F. graminearum*, while Lević *et al.* (2003) detected the dominance of *F. subglutinans* and *F. verticillioides*, the unchanged distribution of *F. graminearum* and the tendency of *F. proliferatum* to spread. In general, these four species can be considered the most important pathogens of maize under agro ecological conditions of Serbia, but with different frequencies over years (Bočarov-Stančić *et al.*, 1997; Lević *et al.*, 1997).

Comparing with Lević *et al.* (1997), results obtained on stored maize kernels in the period February 2005 to March 2006 show some similarity in dominance of certain species of *Fusarium*, and also changes in number of isolated species and frequency of particular *Fusarium* spp. Obtained differences can be interpreted by small number of analyzed samples, different method of sample storing and climatic conditions in which maize was produced and stored.

## Conclusions

Based on investigation of fungi incidence on stored maize grain intended for livestock nutrition, it was determined that during period from February 2005 to March 2006 certain fungal species were present successively (*Fusarium* spp., *Penicillium* spp.), others with interruptions of one (*Nigrospora* spp., *Aspergillus* spp., *Acremonium* spp., *Alternaria* spp), three (*Mucor* spp., *Chaetomium* spp., *Rhizopus* spp.) or four months (*Cladosporium* spp.), and remaining species sporadically during three (*Epicoccum* spp., *Rhizopus* spp., *Trichothecium* spp.), two (*Gliocladium* spp.) or one month (*Papulaspora* spp., *Phialophora* spp.).

Period June - October was the most critical for preservation of stored maize since the number of present species (8–14 of total 17 identified fungal genera) and frequency of fungi during this period were the highest, especially of toxigenic species of *Fusarium* (43.5–62.5%) and *Penicillium* (10.0–33.5%) genera.

Positive correlation ( $r = 0.5979^{**}$ ) between dynamics of incidence and frequency of isolated fungi indicates that fungi with higher frequency of incidence remain are maintained longer on maize grain during storage.

*F. verticillioides* and *F. subglutinans*, of total of six identified *Fusarium* species were present on grain throughout the year and with the highest frequency (24.7% and 5.9%, respectively). During one year (2005) *F. verticillioides* was present in the highest percentage from February to October (22.0–39.5%), and *F. subglutinans* from April to October (6.5–12.5%), whereas both species were least isolated in winter period December-January (4.0–8.0% and 0.5–1.0%, respectively). *F. graminearum* is the third toxigenic species of *Fusarium* genus which from mycotoxicological aspect can be important for period June-September when it is present in the highest percentage (5.0–11.0%). Incidence of other species of *Fusarium* genus is sporadic (1.3% *F. proliferatum*, 1.0% *F. sambucinum* and 0.5% *F. poae*).

## DINAMIKA POJAVE I UČESTALOSTI POPULACIJA *Fusarium* VRSTA NA USKLADIŠTENOM ZRNU KUKURUZA

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### Rezime

Proizvodnja i obezbeđivanje visokog kvaliteta zrna kukuruza su od primarnog značaja za stočarsku proizvodnju jer je kukuruz osnovna komponenta stočne hrane. Zaraza gljivama ne samo da smanjuje kvalitet zrna, već neke vrste gljiva mogu proizvoditi visoko toksična jedinjenja, poznata kao mikotoksini. S obzirom da je kukuruz ekonomski najvažnija gajena biljka u Srbiji, sadržaj i intenzitet učestalosti ovih patogenih vrsta gljiva se ispituje u kukuruzu u skladištu.

Na osnovu proučavanja pojave gljiva na uskladištenom kukuruzu, koji je bio namenjen za ishranu životinja, utvrđeno je da su u periodu od februara 2005. do marta 2006. godine neke gljive prisutne sukcesivno (*Fusarium* spp., *Penicillium* spp.), druge diskontinuirano sa prekidom od jednog (*Nigrospora* spp., *Aspergillus* spp., *Acremonium* spp., *Alternaria* spp), tri (*Mucor* spp., *Chaetomium* spp., *Rhizopus* spp.) ili četiri meseca (*Cladosporium* spp.), a treće sporadično u toku tri (*Epicoccum* spp., *Rhizopus* spp., *Trichothecium* spp.), dva (*Gliocladium* spp.) ili jednog meseca (*Papulaspora* spp., *Phialophora* spp.). Period jun-oktobar je

najkritičniji za očuvanje kvaliteta uskladištenog kukuruza jer je brojnost (prisutno 8–14 od ukupno 17 identifikovanih rodova gljiva) i frekvencija gljiva u tom periodu najveća, posebno toksigenih vrsta iz rodova *Fusarium* (43.5–62.5%) i *Penicillium* (10.0–33.5%). Pozitivna korelacija ( $r = 0.5979^{**}$ ) između dinamike pojave i frekvencije izolovanih vrsta gljiva ukazuje da se gljive sa većom frekvencijom pojave duže održavaju na zrnu kukuruza tokom skladištenja.

*F. verticillioides* i *F. subglutinans* su od ukupno šest identifikovanih vrsta roda *Fusarium* prisutne na zrnu tokom cele godine i sa najvećom frekvencijom (24.7% i 5.9%). U toku jedne godine (2005) *F. verticillioides* je u najvećem procentu prisutna od februara do oktobra (22.0–39.5%), a *F. subglutinans* od aprila do oktobra (8.0–12.5%), dok su obe vrste najmanje izolovane u zimskom periodu decembar-januar (4.0–8.0% i 0.5–1.0%). *F. graminearum* je treća toksigena vrsta roda *Fusarium* koja sa mikotoksikološkog aspekta može biti značajna za period jun-septembar kada se javlja u najvećem procentu (5.0–11.0%). Pojava ostalih vrsta roda *Fusarium* je sporadična (1.3% *F. proliferatum*, 1.0% *F. sambucinum* and 0.5% *F. poae*).

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