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Review Paper

IMPLEMENTATION OF BIOTECHNICAL MEASURES FOR THE CONTROL AND PREVENTION OF AFLATOXIN CONTAMINATION IN MAIZE PRODUCTION

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ABSTRACT

Maize represents one of the most important cereal crops for meeting both human and animal nutritional requirements. However, its production is increasingly threatened by aflatoxins, primarily those produced by Aspergillus flavus, which can cause aflatoxicosis in both humans and animals. High relative humidity (80–90%) and temperatures (25–30 °C) promote the growth of Aspergillus species and the subsequent production of aflatoxins. Under such environmental conditions, the implementation of appropriate biotechnical measures is essential to ensure the production of health-safe maize. This study aims to examine the harmful effects of aflatoxins and the efficacy of various biotechnological strategies for the prevention and reduction of aflatoxin contamination during maize cultivation. As demonstrated herein, the application of diverse biotechnical practices—including the prevention of drought stress, the use of windbreaks, optimal plant density, timely sowing, and the control of insect and weed infestations—can significantly reduce aflatoxin levels, often to the values compliant with the official safety regulations. In particular, biological control using indigenous non-aflatoxigenic A. flavus strains has been shown to reduce aflatoxin contamination in maize fields by 45–100%. Comparable reductions have also been achieved through proper irrigation management, intercropping maize with legumes, and the use of windbreaks to optimize microclimatic conditions. In addition to implementing these measures, raising farmers' awareness of their importance and routinely analyzing maize samples for aflatoxin content are crucial steps toward ensuring the production of safe and high-quality maize.

Key words:
maize, aflatoxins, Aspergillus flavus, biotechnical measures

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INTRODUCTION

Alongside wheat and rice, maize ranks among the most important crops globally. According to FAOSTAT (2022), approximately 203 million hectares of arable land were allocated to maize production in 2022. With about 950,000 ha devoted to maize, the Republic of Serbia (hereafter R. Serbia) accounted for 5% of the total harvested maize area in Europe. Over the past few years, R. Serbia has emerged as one of the leading maize exporters in Europe. As noted by Prasanna et al. (2001), maize is not only a staple food and animal feed but also serves as a key feedstock for a wide range of industrial products, underscoring its considerable economic significance.

In subsistence agricultural systems, maize is primarily cultivated as a staple food crop. In contrast, in developed countries, maize is mainly utilized as animal feed, representing more than 85% of the total maize consumption, although the demand for maize-based flour and animal-derived food products has increased in recent years (FAO, 2018). As the global human population continues to grow, the demand for maize products is expected to increase correspondingly.

The aflatoxin contamination of maize is a widespread concern. The fungus *Aspergillus flavus*, a primary causal agent of maize ear rot, is the most common source of contamination (Klich, 2007). Symptoms typically appear at the ear tip, although in severe infections the damage can extend to the entire ear (Figure 1). The pathogen produces olive- to green-colored powdery spores that disperse readily, resembling dust (Figure 2). The presence of this fungus not only reduces grain quality but also leads to the production of aflatoxins, which pose serious health risks to both humans and animals (Amirahmadi et al., 2017). In response, a range of prevention strategies has been developed, with biotechnological measures receiving particular attention for their effectiveness in controlling aflatoxin contamination.

This study aims to highlight the hazards posed by aflatoxins and to demonstrate the importance of various biological and agrotechnical interventions for their prevention and reduction during maize cultivation. Such measures are essential to mitigate the harmful effects of aflatoxins on human and animal health, as the ingestion of toxic substances can result in severe illness or even death (Barrett, 2005).

AFLATOXINS

Mycotoxins are toxic secondary metabolites that pose significant health risks to both humans and animals and are a common source of agricultural product contamination worldwide (Pleadin et al., 2023). These naturally occurring contaminants frequently accumulate in food and feed, representing a persistent threat to public and animal health. The presence of mycotoxins in agricultural commodities is a globally recognized issue, one that is expected to intensify due to the ongoing effects of climate change. According to the World Health Organization (WHO, 2000), by the end of the 20th century, approximately 25% of the global cereal supply was contaminated with at least one mycotoxin. However, more recent studies report that between 60% and 80% of agricultural products now exhibit mycotoxin contamination, largely attributed to climate-driven shifts in fungal ecology (Eskola et al., 2020).

Aflatoxins are among the most extensively studied and widely distributed mycotoxins identified to date. These toxic and highly carcinogenic secondary metabolites have been conclusively linked to adverse health effects in both humans and animals, making them one of the most critical contaminants in global food and feed safety. Owing to their frequent occurrence, high toxicity, and significant impact on human health and international trade, aflatoxins present a persistent challenge for maize producers and processors (Wilson et al., 2002).

Aflatoxins B1 (AFB1), B2 (AFB2), G1 (AFG1), and G2 (AFG2) are produced primarily by fungi of the *Aspergillus* genus, most notably *A. flavus* and *A. parasiticus* (Klich, 2007; Yabe et al., 1999). Among these, AFB1 is considered the most prevalent and the most toxic (Oliveira and Germano, 1997). Toxigenic strains of *A. flavus* predominantly synthesize AFB1 and AFB2 (Ehrlich et al., 2004), whereas *A. parasiticus* is capable of producing all four major aflatoxins (Diener et al., 1987). In maize, the primary aflatoxin-producing species include *A. flavus*, *A. fumigatus*, and *A. terreus*, although *A. niger* has also been implicated in some cases (Zulkifli & Zakaria, 2017). Moreover, aflatoxins M1 (AFM1) and M2 (AFM2)—hydroxylated derivatives of AFB1 and AFB2, respectively— are found in the milk of animals that have consumed feed contaminated with AFB1. Following ingestion, AFB1 is metabolized in the liver to AFM1, which is subsequently excreted in milk and other biological fluids (Murphy et al., 2006). It has been estimated that approximately 0.3–6.2% of the AFB1 ingested through contaminated feed is converted into AFM1 in milk (Creppy, 2002).


Figure 1. Symptoms of maize ear rot caused by *Aspergillus flavus* (Photo credit: Ferenc Bagi)

Aflatoxicosis

Mycotoxins are recognized as one of the most significant chronic, non-infectious foodborne risk factors, as they can induce both acute and chronic intoxication—collectively known as mycotoxicosis—in humans and animals (Yu et al., 2007). Among them, aflatoxins are a prominent cause of aflatoxicosis, the severity of which is influenced by the dose and frequency of exposure to contaminated food, as well as individual factors including age, sex, immune status, and overall health condition (IARC, 2002). It is estimated that, as of 2015, approximately five billion people worldwide were at risk of dietary exposure to aflatoxins (Wu et al., 2015).

To date, more than 16 distinct types of aflatoxins have been identified, with aflatoxin B1 (AFB1) recognized as the most toxic naturally occurring compound due to its carcinogenic, immunosuppressive, and teratogenic effects in both humans and animals (IARC, 2002). In humans, aflatoxin exposure can result in a wide range of health consequences, from acute hepatic toxicity to chronic diseases such as liver cancer, hemorrhages, edema, and, in severe cases, sudden death. Chronic, low-level exposure to aflatoxins has been associated with immune suppression, malnutrition, and growth impairment in children, potentially leading to a long-term disability or fatal outcomes (Barrett, 2005). A notable example is provided by the study of Gong et al. (2002), which reported a strong correlation between aflatoxin exposure and both stunting (an indicator of chronic malnutrition) and underweight status (an indicator of acute malnutrition) among children in West Africa. Further evidence of the harmful effects of aflatoxins is demonstrated by the 2004 outbreak of acute aflatoxicosis in Kenya, which was linked to the consumption of contaminated maize and resulted in 125 fatalities (Probst et al., 2007). Occupational exposure is also a concern, particularly for food industry workers who may inhale aflatoxin-contaminated dust, especially AFB1. Prolonged exposure in such settings has been associated with an increased incidence of upper respiratory tract and lung cancers, particularly among workers involved in grain shelling and processing (Coulombe, 1994). The International Agency for Research on Cancer (IARC) has classified both AFB1 and its hydroxylated metabolite, AFM1, as Group 1 human carcinogens, although AFM1 is considered approximately ten times less carcinogenic than AFB1 (IARC, 2002; IARC, 2012).

In animals, aflatoxins primarily induce liver damage, along with a reduction in milk yield and reproductive efficiency. Clinical manifestations are similar to those observed in humans, including vomiting and pulmonary edema, which may progress to coma and death in severe cases. In dairy and beef cattle, acute aflatoxicosis is typically characterized by anorexia, depression, weight loss, gastrointestinal disturbances (e.g., abdominal pain, bloody diarrhea), reduced feed intake and feed efficiency, jaundice, blindness, abnormal behaviors such as walking in circles, ear twitching, frothing at the mouth, internal bleeding, and, ultimately, death (Agag, 2004).

Figure 2. *Aspergillus flavus* – the fungal growth on PDA medium (Photo credit: Nemanja Pavković)

EFFECTS OF ENVIRONMENTAL FACTORS ON AFLATOXIN CONTAMINATION

Various agricultural products may become contaminated with aflatoxins either during field production or storage. The most frequently affected and economically significant crops include maize, wheat, peanuts, cottonseed, rice, pistachio nuts, and oilseeds such as sunflower and coconut. Sorghum, soybean, beans, figs, and almonds have also been identified as potential carriers of aflatoxins (Rustom, 1997). Furthermore, as noted earlier, when animal feed is contaminated with high concentrations of aflatoxins, the toxins can be transferred into animal-derived products, including milk (Nleya et al., 2018).

Extreme weather conditions caused by climate change and global warming have contributed to the progressive expansion of the global distribution of mycotoxins, including aflatoxins. According to Nleya et al. (2018), high temperatures and humidity levels are expected to sustain aflatoxin contamination across Sub-Saharan Africa and parts of Southern Asia. In these tropical and subtropical regions, maize offers an especially favorable substrate for the growth of aflatoxigenic fungi and subsequent aflatoxin synthesis, compared to regions with temperate climates (Rustom, 1997). However, as a result of climate change, aflatoxin contamination is expected to emerge as a major concern even in these temperate zones in the near future.

Studies from different geographic regions confirm that *Aspergillus* species are capable of colonizing diverse substrates under a wide range of environmental conditions. In many cases, their proliferation is also driven by poor post-harvest storage practices (Park and Liang, 1993). The optimal conditions for *Aspergillus* growth and aflatoxin production typically involve high relative humidity (80–90%) (Sinovec et al., 2006) and temperatures ranging from 25 °C to 30 °C (Abbas, 2005; Bernaldez et al., 2017). Therefore, regulating these factors during both the cultivation and storage stages represents a crucial preventive strategy.

Aflatoxin contamination in maize is directly promoted by plant stress, especially stress inducted by drought and heat (Farfan et al., 2015; Kebede et al., 2012), as well as insect damage and improper irrigation timing (Tola and Kebede, 2016). Kos et al. (2018) reported that extreme drought conditions significantly contributed to the elevated aflatoxin concentrations in the maize samples collected during 2012. In contrast, although 2013 was similarly hot and dry, higher soil moisture reserves during the summer growing season resulted in a nearly 48% reduction in the aflatoxin contamination compared to the previous year. Moreover, no aflatoxin contamination was detected in the maize samples from 2014, a year characterized by favorable agroecological conditions in the Republic of Serbia.

A study by Pleadin et al. (2023) further highlighted the impact of extreme weather, noting that the precipitation levels in 2021 were the lowest among the examined years, while the number of days with maximum temperatures exceeding 30 °C and 35 °C was unusually high. These conditions led to the detection of aflatoxin B1 (AFB1) in 84%

of the maize samples, and 61% of the tested samples exceeded the maximum permissible levels for human consumption.

Obradović et al. (2018) reported that AFB1 was detected in 97.3% of the maize kernel samples during the drought-affected 2012 season, whereas the contamination rate dropped to 18.3% in 2013. The year-dependent variations in contamination rates were also confirmed by Lević et al. (2013), who recorded the presence of aflatoxigenic *A. flavus* in 13.3% to 95.3% of the maize samples, with the highest prevalence observed in 2012. Parallel findings from Croatia and North Macedonia confirmed the widespread occurrence of aflatoxins during the same period, with contamination detected in 38.1% and 29.2% of the maize samples, respectively (Dimitrieska-Stojković et al., 2016; Pleadin et al., 2014). These studies consistently attributed the surge in aflatoxin contamination during the 2012 growing season to the combination of high temperatures and prolonged drought stress. The reports from the Rapid Alert System for Food and Feed of the European Union (RASFF, 2012-2015) for the years 2012 through 2015 confirmed the presence of aflatoxin-contaminated maize in multiple European countries, including the Republic of Serbia, Croatia, Greece, Italy, Poland, Romania, Slovakia, Bulgaria, and Hungary.

AFLATOXIN PREVENTION THROUGH THE IMPLEMENTATION OF BIOTECHNICAL MEASURES

Indirect aflatoxin prevention measures

1. Regulations

Due to their high toxicity to humans and animals, almost all countries have established regulations specifying the permissible levels of aflatoxins in food and feed. The United States Food and Drug Administration (FDA) has set a maximum limit of 20 µg/kg for total aflatoxins in the interstate commerce involving food and feed (Brown et al., 2013). In the Republic of Serbia, the "Pravilnik o maksimalnim koncentracijama određenih kontaminenata u hrani" [Regulation on Maximum Levels of Certain Contaminants in Food] (Official Gazette of the Republic of Serbia, No. 81/2019, 126/2020, 90/2021, 118/2021, and 127/2022) prescribes the maximum permissible levels of 5.0 µg/kg for aflatoxin B1, 10.0 µg/kg for the total aflatoxins B1, B2, G1, and G2, and 0.050 µg/kg for aflatoxin M1 in raw milk, in line with European Commission Regulation 2023/915 (EC, 2023). In both the European Union and Serbia, the maximum permissible concentration of AFB1 in maize intended for animal feed is set at 20.0–30.0 µg/kg (EC, 2003; Official Gazette of the Republic of Serbia, Regulation on the Quality of Animal Feed, No. 4/2010, 27/2012, 113/2012, 27/2014).

2. Agrotechnical measures

Several agricultural practices—including crop rotation, tillage, optimized irrigation and fertilization, and the use of resistant maize hybrids—have been shown to mitigate infection and aflatoxin accumulation in maize (Champeil et al., 2004).

Water management* – *irrigation. Drought-induced stress is a major predisposing factor for aflatoxin contamination, which can be mitigated through proper irrigation management during the maize growth cycle (Chauhan et al., 2008; Guo et al., 2008; Munkvold, 2014). Several studies have demonstrated that adequate irrigation lowers aflatoxin levels in maize (Jones et al., 1981; Payne et al., 1986; Kebede et al., 2012). However, other studies have reported no significant differences between irrigated and non-irrigated maize with respect to aflatoxin contamination (Bruns, 2003; Abbas et al., 2012; Savić et al., 2020). According to Khlangwiset and Wu (2010), the efficacy of aflatoxin reduction can be improved by combining irrigation with insecticide application. Moreover, the effectiveness of irrigation depends not only on the volume of water applied but also on the timing, which should correspond to crop needs, soil moisture capacity, and prevailing weather conditions (Damianidis et al., 2018b; Singh et al., 2019).

Good agricultural practices, such as irrigation, represent the first line of protection against aflatoxin contamination (Lević et al., 2013; Murphy et al., 2006), as proper irrigation impedes the growth of *Aspergillus* fungi (Afzal et al., 2023) and reduces the susceptibility of maize to *A. flavus* infection and subsequent aflatoxin production (Klich, 2007). However, according to Wilson et al. (2002), irrigation has not proven effective in lowering aflatoxin contamination across all regions of the United States.

Crop rotation. Continuous monoculture increases aflatoxin contamination risk, as residues from previous maize crops provide a substrate for toxigenic fungi. Fields devoted to maize for consecutive seasons tend to harbor larger populations of *Aspergillus flavus*, which elevates the risk of contamination in subsequent harvests (Jaime-Garcia and Cotty, 2004, 2010).

Tillage. No-tillage systems encourage the buildup of aflatoxin-producing fungi, and crop residues also act as a source of increased fungal inoculum. However, extensive data suggest that comparable soil conservation benefits could be attained if these residues are incorporated during tillage. Nonetheless, in areas vulnerable to erosion, the no-tillage practice might be necessary for safeguarding the soil, and in this case, a crop which is less susceptible to aflatoxin contamination would be preferable to maize (Ayalew et al., 2017).

Resistance breeding, adapted maize hybrids, and plant density. As the aflatoxin presence in maize depends on the genotype, extensive research has been conducted on producing new maize hybrids with tolerance to drought, pests, and toxigenic fungi (Guevara-González et al., 2011; Widstrom et al., 2003), as this could reduce aflatoxin concentration in the open field production. Water loss dynamics during maize ear maturity also appear to be determined by the genotype. Accordingly, breeding is a viable strategy for controlling grain water activity, which is a key parameter in modeling the colonization and infection of *A. flavus* and other fungi (Dövényi-Nagy et al., 2020).
Bacillus thuringiensis maize (Bt maize) is one of the most commonly planted transgenic crops worldwide, as it has significantly lower aflatoxin levels than non-Bt isolines. Therefore, Yu et al. (2020) recommended Bt maize use as an important strategy in reducing aflatoxin risk, with the corresponding economic benefits. If the same principles hold true in other world regions, Bt maize hybrids adapted to diverse agronomic conditions may play an important role in reducing aflatoxin presence in areas prone to high contamination.
Plant density is also one of the important factors for aflatoxin production, necessitating the adoption of recommended and optimal sowing densities. As noted by Krnjaja et al. (2019), a greater plant density (75,000 plants ha^{-1}) results in a higher aflatoxin concentration (55,000 plants ha^{-1}). However, several authors have failed to establish a link between plant density and aflatoxin concentration (Abbas et al., 2012; Damianidis et al., 2018a).

Cover crops. The amount of aflatoxin has been found to decline when maize is planted alongside legumes, such as cowpea and beans (Joutsjoki and Korhonen, 2021). Conversely, Njeru et al. (2019) noted that intercropping maize with beans increased the probability of aflatoxin contamination. According to Mutiga et al. (2015) and Gachara et al. (2022), growing maize in monoculture resulted in a 3-fold increase in aflatoxin contamination relative to intercropping. Bruns (2003) attributed the benefits of maize intercropping to several factors, including increased soil nitrogen levels and lower insect-related harm, due to which *Aspergillus* infections and contamination tend to decline. Mutiga et al. (2014) similarly noted a lower proportion of aflatoxin contamination in the samples taken from farmers who grew their maize within an intercropping system.

Fertilization. Nitrogen stress can significantly increase aflatoxin contamination in maize, especially when crops are exposed to drought stress and high temperatures during pollination and seed maturation. Therefore, the adequate application of NPK fertilizers is likely to counter drought stress and subsequently deter fungal attacks and aflatoxin contamination (Ayalew et al., 2017).

Windbreaks. Windbreaks improve microclimate conditions in open fields through temperature regulation and moisture preservation, thus mitigating plant stress and preventing soil drying. Wind erosion represents a significant risk to agricultural sustainability by decreasing the soil's ability to retain water (Chang et al., 2021). Accordingly, Ucar and Hall (2001) recommended windbreaks as an effective means of wind power reduction, which in turn reduces the likelihood of soil drying out.
Windbreaks also enable greater retention of snow and moisture in the soil, resulting in greater reserves of winter moisture, which is particularly important in the absence of precipitation (Kort, 1988). Windbreaks can ensure enough soil moisture for the next year's crop, as they affect the movement of wind and spread snow evenly across the field (Scholten, 1988).

Direct aflatoxin prevention measures

1. Chemical control

Insect pests, particularly *Ostrinia nubilalis* (European corn borer), cause mechanical damage to maize kernels, creating entry points for fungal infection (Dowd, 2003). Therefore, the presence of *Ostrinia nubilalis* is a significant predisposing factor for mycotoxin contamination (Gnonlonfin et al., 2013; Widstrom, 1996). A significant positive correlation has been established between *O. nubilalis* infestation and *A. flavus* ear rot (Lević et al., 2013), suggesting that regular monitoring of the insect infestation status in maize fields is necessary. If pest infestation is observed, the recommended measures include the application of synthetic sex pheromones, inter-cropping and habitat management, management of crop residues, manipulation of sowing dates and densities, fertilizer use, enhancement in the host plant resistance, and the reliance on chemical and biological pest control. The use of fungicides is not a widespread strategy against *A. flavus* (Logrieco et al., 2021). However, Lagogianni and Tsitsigiannis (2018) reported that cyprodinil + fludioxinil decreased *A. flavus* concentration in maize grain by 83%.

2. Biological control with indigenous organisms

Extant studies on the biological methods aimed at reducing the aflatoxigenic *A. flavus* infection in maize have mostly focused on the use of non-aflatoxigenic strains of *A. flavus* which act by competitively displacing toxigenic strains (Abbas et al., 2011; Atehnkeng et al., 2008; Pekar et al., 2022). This brand is already used in the US as a new technology, and in Nigeria it showed extremely positive results, with the 80−90% reduction of aflatoxin contamination reported for maize and peanut (Bandyopadhyay and Cotty, 2013). Depending on the atoxigenic isolates, Maxwell et al. (2021) reported the 45−79% reduction in aflatoxin contamination in USA, while in Argentina, using the atoxigenic strains of *A. flavus* was found by Camiletti et al. (2018) to reduce aflatoxin contamination by 54−84%. Similar results were obtained by Bandyopadhyay et al. (2016) for sub-Saharan Africa.

As noted by Nešić et al. (2021), the application of the Aflasafe product in open field solved the problem of aflatoxin in Africa. In Nigeria, the application of atoxigenic strains reduced the aflatoxin content by 67% to 95% (Atehnkeng et al., 2014). In Ghana, after the use of biocontrol product Aflasafe GH02, more than 95% of the samples were free from aflatoxin, and in the remaining 5% of the samples, its concentration was reduced by 80–100% (Agbetiameh et al., 2020). In Italy, the application of biopreparation AFIT-01 led to the 83.7−94.8% aflatoxin reduction in 2012 (Mauro et al., 2018). According to Pekar et al. (2022), the use of atoxigenic strains reduced the aflatoxin content by 38% in the USA. In Serbia, the use of biocontrol product Mytoolbox Af01 led to the 51−83% reduction in aflatoxin concentration depending on the year, but at 73% the overall mean reduction was still high (Savić et al., 2020).

Abbas et al. (2006) noted that the atoxigenic strains K49 and CT3 decreased aflatoxin concentration by 58−86% compared to the non-inoculated controls. Moreover, Mauro et al. (2015) found that Italian isolates reduced the aflatoxin concentration by at least 80%, whereas the other isolates achieved a reduction in the 61−80% range. Atehnkeng et al. (2008) showed that the atoxigenic strains of *A. flavus* reduce the concentration of aflatoxin in laboratory and field conditions by 70% to 99%. Abbas et al. (2011) found that the aflatoxin content could be decreased by 69−99% through inoculation. More recently, Agbetiameh et al. (2019) reported 92−98% reductions in aflatoxin concentrations. Atehnkeng et al. (2016) observed that atoxigenic isolates reduced aflatoxin concentrations in maize grain by more than 96%.

CONCLUSION

In light of the continuous growth of the global population, ensuring an adequate food supply—particularly of cereals—remains a fundamental priority. This objective is increasingly challenged by the adverse effects of global climate change on agricultural production systems. Among the most pressing issues is the rising occurrence and geographical expansion of aflatoxin contamination, especially in maize, which poses significant risks to both human and animal health. Effective mitigation of this problem can be achieved through the implementation of good agricultural practices, including a range of biotechnical measures such as crop rotation, the use of optimal plant densities, timely sowing, the introduction of cover crops and windbreaks, the application of adequate irrigation schedules, and the utilization of atoxigenic strains of *Aspergillus flavus*. The adoption of such strategies has been demonstrated to significantly reduce aflatoxin concentrations in maize, thus lowering contamination risks across the food and feed supply chains. To ensure food safety, harvested maize should be regularly monitored and analyzed to verify compliance with the maximum aflatoxin concentrations prescribed by national and international regulatory frameworks. Furthermore, raising awareness among farmers regarding the health risks associated with aflatoxins is essential. In this regard, the organization of open-field demonstration days by the relevant governmental institutions—particularly in developing countries—could facilitate the knowledge transfer on safe maize cultivation practices and aflatoxin prevention. Considering the expected increase in aflatoxin prevalence due to climate change, it is vital to expand the global awareness of this issue. Aflatoxin contamination will likely become an increasingly significant threat to food safety across all regions, underscoring the need for proactive and coordinated preventive measures.

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