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MYCOTOXIN CONTENT IN MAIZE IN THE CASE OF *Fusarium graminearum* INOCULATION IS REDUCED AFTER FUNGICIDAL TREATMENT

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Abstract

The present study aims to investigate the effect of two pesticide products against Gibberella ear rot (Fusarium graminearum) on yield quality and quantity of maize (Zea mays L.) and choose the more appropriate application dose and time. Three field experiments were performed in the period 2019-2021 in the Plovdiv region, Bulgaria. Three different varieties of maize were used. The pesticides used were Propulse 250 SE (prothioconazole 125 g/l + fluopyram 125 g/l) - applied in two doses - 0.6 l/ha or 1 l/ha and Prosaro 420 SC (prothioconazole 210 g/l + tebuconazole 210 g/l) - applied in only one dose - 1 l/ha. Seven variants, including untreated control and six different treatments, were analyzed. The treatments were divided into two groups. To understand which application moment was more appropriate, half of the plots were treated before the inoculation, and the others - during the sporulation phase of the pathogen. The results showed a significant reduction in the disease severity and incidence after the application of both products. There were also differences in yield quantity and thousand kernel weight, hectoliter mass, and the content of deoxynivalenol.

Key words: *deoxynivalenol, gibberella ear rot, maize, prothioconazole, yield.*

INTRODUCTION

Human health and nutrition, as well as animal feed, are major priorities of farmers all over the world. Providing high-quality production could be a challenge, especially when the pesticide application should be reduced year after year.

One of the most important plant pathogens that attacks a wide range of plant species, including maize (*Zea mays* L.) (ear and stalk rot), wheat and barley (head blight), is *Fusarium graminearum* (sexual stage *Gibberella zeae*) (Harris et al., 1999). The infection leads to a reduction of both yield and grain quality. The reduction in quality is partially a result of the mycotoxins produced by this fungus. Usually, the ears which were infected with *Gibberella* ear rot contain the trichothecenes deoxynivalenol (DON), 15-acetyldeoxynivalenol (15AcDON), and 3-acetyldeoxynivalenol (3AcDON), as well as zearalenone (Mirocha & Christensen, 1974; Miller et al., 1983; Bennett et al., 1988) and fusarin C (Farber & Sanders, 1986).

Mycotoxins are secondary metabolites synthesized by molds. These substances adversely affect humans, animals, and crops, resulting in illnesses and economic losses (Zain, 2011). Aflatoxins, ochratoxins, trichothecenes, zearalenone, fumonisins, tremorgenic toxins, and ergot alkaloids are the mycotoxins of greatest agro-economic importance. Some molds could produce more than one mycotoxin, and many of the mycotoxins are produced by more than one fungal species.

The contamination of human food can happen at different stages in the food chain (Bennett and Klich, 2003).

The main toxic effect of trichothecene mycotoxins (TCT) is the primary inhibition of protein synthesis. These substances commonly contaminate poultry feed and feedstuffs (Leeson et al., 1995), where they could affect cells, which divide actively like the cells in the gastrointestinal tract, the skin, lymphoid and erythroid cells. After contact with the mycotoxin a necrosis of the oral mucosa and skin appears. An adverse effect on the digestive tract and decreased bone marrow and immune function is also observed (Schwarzer, 2009).

Using fungicides on grain corn or silage aims to control fungal diseases such as *Gibberella* ear rot (*Fusarium graminearum*). Several studies have demonstrated that applying fungicides at the proper stage reduces DON contamination in corn (Edwards et al., 2001; Cardoso, 2020). On the contrary, others suggest that the use of some chemicals could stimulate DON synthesis as a defense mechanism against pesticide-induced stress (Magan et al., 2002).

The maximum limit (ML) for DON in maize grain for further processing by procedures proven to reduce DON levels was agreed upon at 2,000 μg/kg (Schaarschmidt & Fauhl-Hassek, 2021).

Different agricultural practices could be applied to reduce the adverse effect of pathogen infection (Edwards, 2004). Some are focused on developing resistant varieties (Kolb et al., 2001; Rudd et al., 2001; Snijders, 2004). Others recommend crop rotation (Dill-Macky & Jones, 2000). According to many researchers, using pesticides with a fungicidal effect could, to some extent, alleviate the negative effects of the pathogen (Wise & Mueller, 2011; Luna & Wise, 2015).

Several active ingredients were examined in the last years. Prothioconazole is a demethylation-inhibiting fungicide (DMI) that blocks or prevents the fungus from producing essential sterol compounds, such as ergosterol, that are important for fungal membrane and structure (Latin, 2011; Mueller et al., 2013). Tebuconazole is also a DMI fungicide and acts the same way as prothioconazole.

Fluopyram is a pyridyl ethyl benzamide broad-spectrum fungicide and nematicide. It belongs to the group of Succinate dehydrogenase inhibitors (SDHIs) – a class of fungicides that act on succinate dehydrogenase and inhibit the respiration of pathogenic fungi (Xu et al., 2019). Fluopyram is effective against parasitic nematodes and soil-borne fungi (Nadeethara Lohithaswan et al., 2022).

MATERIALS AND METHODS

Experimental design

Three field experiments were performed in the period 2019-2021. The trials were set up in the Region of Plovdiv using the block plot method with a plot size of 30 m^2. Seven variants including untreated control, Propulse 250 SE in two dose rates (0.6 l/ha and 1 l/ha), and Prosaro (1 l/ha) were tested. Propulse 250 SE (Bayer Crop Science) contains prothioconazole 125 g/l and fluopyram 125 g/l. Prosaro 420 SC (Bayer Crop Science) contains prothioconazole 210 g/l and tebuconazole 210 g/l. The details about the applications are presented in Table 1.

Table 1. Application data

Variants	Application	Product
1 (Control)	-	-
2	Application A (foliar application before inoculation)	Propulse 250 SE 0.6 l/ha
3	Application A (foliar application before inoculation)	Propulse 250 SE 1 l/ha
4	Application A (foliar application before inoculation)	Prosaro 420 SC – 1l/ha
5	Application B (foliar application during pathogen sporulation)	Propulse 250 SE 0.6 l/ha
6	Application B (foliar application during pathogen sporulation)	Propulse 250 SE 1 l/ha
7	Application B (foliar application during pathogen sporulation)	Prosaro 420 SC – 1 l/ha

Experimental design 2019

In 2019 the trials were set up in Purvenets (GPS 42.095237 and 24.677521). The experiment was performed with maize cv. DKS5075 after oats (*Avena sativa* L.) as a previous crop. The seeds were sown on 28 March 2019, with a sowing rate of 68,000 plants/ha with a row spacing of 70 cm and space within row of 18.6 cm. Every plot included four rows. The plants emerged on 9 April 2019. The first application (Application A) with the test products was performed on 19 June 2019. The air temperature during the application was 18.3°C, the relative humidity - 59%, the wind velocity - 0.3 m/s, the cloud cover was 0%, and the soil temperature was 24.7°C. The developmental stage of the plans during the application was BBCH 61 (Majority), and the plant height at that time was 215 cm. The second application (Application B) was performed 11 days after the first one, on 1 July, during the sporulation phase of the pathogen. The air temperature was 23.2°C, the relative humidity - 70%, the cloud cover - 10%, the wind velocity - 0.3 m/s, and the soil temperature was 26.6°C. The plants were treated in BBCH stage 67 (Majority) and the plant height at that time was 220 cm.

Experimental design 2020

In 2020, the experiment was performed in the village of Katunica (GPS 42.077679 and 24.676725) with maize cv. SY Phenomen after sunflower (*Helianthus annuus* L.) as a predecessor. The seeds were sown on 3 April and the young plants emerged on 18 April. The row spacing was 70 cm, and the space within row was 18.1 cm (four rows per plot). The sowing rate was 70,000 plants/ha. The first application of the test products (Application A) was performed on 25 June 2020 before the inoculation. The air temperature during the application was 19.1°C, the relative humidity - 57%, the cloud cover - 20%, the wind velocity - 1.7 m/s, and the soil temperature was 25°C. The developmental stage of the plants was BBCH 61 (Majority), and the plant height was 173 cm. The second application (Application B) was performed 12 days after the first one, on 7 July 2020, during the sporulation phase. The air temperature during the application was 22.1°C, the relative humidity - 59%, the cloud cover - 10%, the wind velocity - 0.3 m/s, and the soil temperature was 26.4°C. The developmental stage of the plants during the application was BBCH 69 (Majority), and the plant height was 215 cm.

Experimental design 2021

In 2021, the experiment was performed in the village of Trud (GPS 42.191797 and 24.722284) with maize cv. ES Faradi. The seeds were sown on 13 April after winter barley (*Hordeum vulgare* L.) as a predecessor, and the young plants emerged on 4 May 2021. The row spacing was 70 cm, and the space within row was 18.1 cm. Every plot had four rows. The sowing rate was 80,000 plants/ha. The first application with the test products (Application A) was performed on 13 July 2020. The air temperature during the application was 23.1°C, the relative humidity was 63%, the cloud cover was 50%, the wind velocity - 1.7 m/s, and the soil temperature was 25.5°C. The developmental stage of the plants during the application was BBCH 63 (Majority), and the height of the plants was 200 cm. The second application was performed six days after the first one, on 19 July 2021, during the sporulation phase. The air temperature during the application was 21.9°C, the relative humidity was 53%, the cloud cover - 20%, the wind velocity - 1.1 m/s, and the soil temperature was 25.3°C. The developmental stage of the plants during the application was BBCH 67 (Majority) and the plant height was 220 cm.

Fusarium graminearum inoculation

The artificial inoculation with *Fusarium graminearum* was performed at BBCH Stage 65 with a spray concentration of 10^3 conidia/ml. The leaves, tassels, and the stems of the plants on every experimental plot were uniformly sprayed with a volume spray of 800 l/ha three days after the first fungicide treatment.

Mycotoxin estimation

The content of deoxynivalenol (DON) was measured using high-performance liquid chromatography (HPLC) with tandem mass spectrometry (MS/MS) meeting VLM 92 2010.

Statistical data analysis

The data are presented as the mean of four replicates. The experimental results were statistically processed with the SPSS program using a one-way ANOVA dispersion analysis and Duncan's comparative method, with the validity of the differences determined at a 95% significance level. The different letters (a, b, c, d) after the mean value show statistically significant differences between the variants.

RESULTS AND DISCUSSIONS

During the first experimental year, the disease severity (% of the infected area on corn cobs) and disease incidence (% of the plants with disease symptoms) were evaluated on 6 August 2019. The analysis of the disease severity and disease incidence in 2019 is presented in Figure 1. The results show that the disease severity and incidence were the highest in the untreated plots. The disease severity in variant 2 (Propulse 250 SE 0.6 l/ha, applied before the inoculation) was 27% lower than the control. In variant 5, where Propulse 250 SE was applied at the same dose of 0.6 l/ha, but in the sporulation phase, the disease severity was reduced by 34% compared to the control. The severity was the lowest in variant 3 (Propulse 250 SE 1 l/ha before inoculation), variant 4 (Prosaro 420 SC before inoculation), variant 6 (Propulse 250 SE 0.6 l/ha during sporulation phase), and variant 7 (Prosaro 420 SC during sporulation phase). The reduction was by 62%, 63%, 59%, and 65%, espectively. The effect of pesticides on disease incidence was similar. The disease incidence was reduced by 27% in variant 2 and by 25% in variant 5, respectively. The reduction of the disease incidence in variants 3, 4, 6, and 7 was by 56%, 69%, 63%, and 66%, respectively.

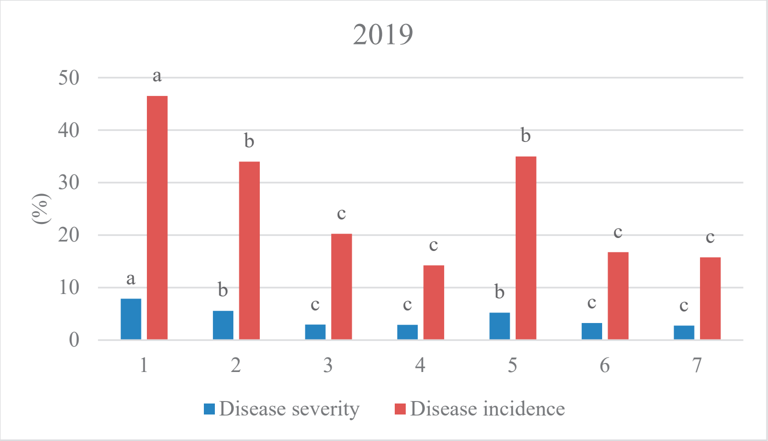

Figure 1. Disease severity and disease incidence of *Fusarium graminearum* in maize, cv. DKS5075 in 2019

The results show that in 2019 the effect of the applied products depended on the application dose and was not related to the moment of application. The higher application dose resulted in a better ability to control the disease severity and incidence. The lower dose of the test product Propulse 250 SE (0.6 l/ha) reduced the disease severity and disease incidence compared to the untreated plots, but the higher dose of application (1 l/ha) of both products led to a bigger reduction of the disease severity and incidence.

Regarding disease incidence, the tendency was different. The untreated plants were still the ones that were most affected by the pathogen. In this case, the rate of damage on the pesticide-treated plots was affected not only by the application dose but also by the application time. It was observed that the disease incidence was lower on the test plots, which were treated before inoculation (Application A), compared to the variants which were treated during the sporulation phase (Application B). The preventive treatment resulted in a better protective effect of the tested products. The pretreatment with Propulse 250 SE 0.6 l/ha (variant 2) led to a decrease in the disease incidence by 56%, and the treatment in the sporulation phase with the same application dose (variant 5) resulted in a 33% reduction. The application of Propulse 250 SE in the dose of 1 l/ha (variant 3) reduced the disease incidence by 89% vs. 75% when the same dose was applied during the sporulation phase. The treatment with Prosaro 420 SC reduced the disease incidence by 77% when applied before the inoculation and by 54%, respectively, when the plots were treated during the sporulation phase. The same tendency was observed also in the third experimental year. The disease severity and incidence during 2021 were measured on 18 August 2021. The data are presented in Figure 3. The results show that the highest disease severity and incidence were measured on the untreated test plots. The disease severity on plots that were treated with Propulse 250 SE 0.6 l/ha before the inoculation was 62% lower than the control. The rate of 1 l/ha Propulse 250 SE led to a 94% decrease. The application of Prosaro 420 SC decreased the severity by 90%. When Propulse 250 SE 0.6 l/ha was applied during sporulation, the reduction was by 63%, and by 93%, when the higher dose of 1 l/ha was applied, respectively. The treatment with Prosaro 420 SC during sporulation led to a decrease in the disease incidence by 83% compared to the untreated control.

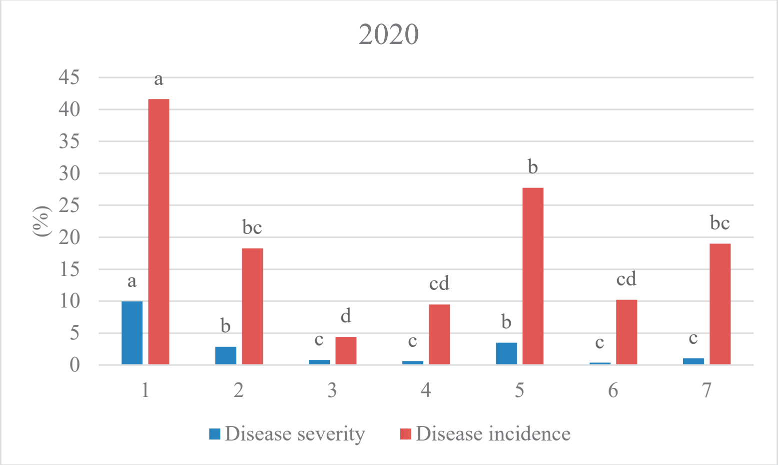

Figure 2. Disease severity and disease incidence of *Fusarium graminearum* in maize, cv. SY Phenomen in 2020

In 2020 the disease severity and disease incidence were measured on 11 August 2020. The results are presented in Figure 2. It is seen that the disease severity and incidence were the highest in the untreated control. The disease severity was reduced by 71% in variant 2 and by 65% in variant 5 respectively, compared to the control. The damage was the lowest in variants 3, 4, 6, and 7. The reduction was by 92%, 94%, 96%, and 89% respectively. Both of the test products were able to reduce the disease severity. The effect was dose-dependent and it was not related to the moment of application of the fungicides.

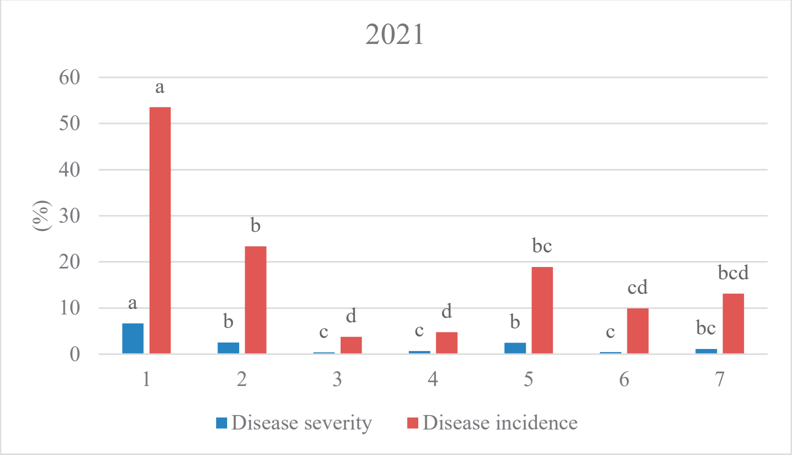

Figure 3. Disease severity and disease incidence of *Fusarium graminearum* in maize, cv. ES Faradi in 2021

The disease incidence was 56% lower than the control when Propulse 250 SE 0.6 l/ha (variant 2) was applied before the inoculation. The reduction in variant 3 was by 93%. The treatment with Prosaro at the same phase (variant 4) led to a 91% decrease in disease incidence. When Propulse 250 SE was applied in a dose of 0.6 l/ha during sporulation (variant 5) the reduction was by 65%. After the application of Propulse 250 SE at a dose rate of

1 l/ha during sporulation (variant 6), the disease incidence was reduced by 81%. The treatment with Prosaro 420 SC at the same phase (variant 7) reduced the disease incidence by 75%.

To explore the effect of the different treatments on the yield, some of its parameters and the content of mycotoxins in grain, namely deoxynivalenol (DON), were analyzed. The yield was harvested on 4 September 2019 during the first experimental year. The analysis of the yield for 2019 is presented in Table 2. The lowest yield was measured in the untreated control. In variant 2, the yield was 1.5% higher; in variant 3-32% higher; in variant 4-3.3% higher, and in variant 5-2% higher than the control. The highest yield was measured in variant 6, where Propulse 250 SE was applied in a dose of 1 l/ha during sporulation (33% higher than the control). The enhancement in variant 7 was by 19% compared to the untreated control. In Table 2, there are also data about moisture content, thousand kernel weight (TKW), hectoliter mass (HLM), and the content of deoxynivalenol (DON) for 2019. It is seen that there are no statistically significant differences in the moisture content of the variants. Regarding the thousand kernel weight, the highest value was measured in variant 3 and variant 4, where Propulse in a dose of 1 l/ha and Prosaro, respectively, before the inoculation, were applied. The lowest values were measured when Propulse was applied during sporulation, regardless of the application dose. The hectoliter mass was the highest in variants 2 and 7 and the lowest - in variant 5. Regarding deoxynivalenol, the highest content was observed in the control plots. In variant 2 (Propulse 250 SE 0.6 l/ha before the inoculation), the reduction of DON was by 73% compared to the control. In variant 5 (Propulse 250 SE 0.6 l/ha during sporulation), the reduction was 65%. In all of the other variants, the content of DON was lower than 100 µg/kg. The accredited laboratory analyses could not detect DON contents below 100 µg/kg.

Table 2. Parameters of the yield and content of DON in maize, cv. DKS5075 in 2019

Variants	Yield (kg/ha)	Moisture content (%)	TKW (g)	HLM (kg)	DON (µg/kg)
1 (Control)	9,015b	10.1	290.1b	77.7c	416
2	9,157ab	9.8	279.1c	78.4a	112
3	11,893ab	9.8	305.6a	77.4e	<100
4	9,320ab	10.3	305.7a	78b	<100
5	9,198ab	9.4	265.3d	75d	147
6	12,029a	9.7	262d	77.6c	<100
7	10,762ab	10.3	290b	78.4a	<100

During the second experimental year, the yield was harvested on 25 September 2020. The data are presented in Table 3. There are no statistically significant differences in the yield quantity and moisture content. Regarding the weight of thousand kernels, the highest value was measured in variant 3 and variant 7, and the lowest was observed in variant 6. There were no statistically significant differences in the hectoliter mass of the variants. The content of DON was the highest in control. In variant 2, DON was reduced by 40% compared to the control. In variant 5, the reduction was by 49%. In all the other treatments, the content of DON was lower than 100 µg/kg.

Table 3. Parameters of the yield and content of DON in maize, cv. SY Phenomen in 2020

Variants	Yield (kg/ha)	Moisture content (%)	TKW (g)	HLM (kg)	DON (µg/kg)
1 (Control)	8,393	8.7	294.8a	74.4	333
2	8,038	8.9	282bc	75.3	201
3	8,460	8.7	299.9a	75	<100
4	8,130	8.5	292.6ab	74.1	<100
5	7,973	9.2	281.8bc	75	169
6	8,488	8.8	277.3c	74.6	<100
7	8,250	8.6	294.5a	75.5	<100

During the third experimental year, the yield was analyzed on 12 September 2021. The data are presented in Table 4. There are no statistically proven differences in relation to the yield and moisture content after the different applications. The TKW was the highest where Propulse 250 SE was applied during sporulation in a dose of 0.6 l/ha (variant 5) and the lowest - in variant 2 and variant 7, where Propulse 250 SE was applied before inoculation in a dose of 0.6 l/ha, and Prosaro during sporulation was applied, respectively. Some differences were also observed in relation to the hectoliter mass. The value was the highest in variant 6 (Propulse 1 l/ha during sporulation) and the lowest - in variant 5 (Propulse 250 SE 0.6 l/ha before inoculation). The DON content was the highest in control, and using Propulse 250 SE in a dose of 0.6 l/ha led to an 85% reduction (variant 2). The same application rate of Propulse 250 SE applied during sporulation led to a decrease of 75% compared to the control. In all of the other variants, the content of DON was lower than 100 µg/kg.

Table 4. Parameters of the yield and content of DON in maize, cv. ES Faradi in 2021

Variants	Yield (kg/ha)	Moisture content (%)	TKW (g)	HLM kg)	DON (µg/kg)
1 (Control)	2,390	9.6	205.9c	62f	834
2	2,115	9.1	201e	62.6e	118
3	2,520	9	211.4b	63.7c	<100
4	2,860	9.8	207.5	63.6d	<100
5	2,755	9.3	212.3a	61.4g	209
6	2,838	8.7	203.7d	64.7a	<100
7	2,465	9.1	201.7e	64.3b	<100

According to Andersen et al. (2017), Prothioconazole was able to reduce the *Gibberella* ear rot severity but did not reduce the content of DON in maize. Regarding the disease severity, these announcements align with our findings. On the other hand, our results are the opposite concerning the DON content. The research of Limay-Rios & Schaafsma (2018) announced that the early application of prothioconazole reduced the content of DON in maize. The authors observed no reduction in *Fusarium graminearum* toxins when the fungicide was applied after the silk senescence stage. Our study confirms that the fungicidal treatment was able to reduce the mycotoxin content. It was established that all the test products reduced the content of deoxynivalenol, and the effect was dose-related.

CONCLUSIONS

The results of the present study showed that using chemical pesticides could reduce the severity and incidence of *Gibberella* ear rot (*Fusarium graminearum)* on maize and the content of deoxynivalenol in the yield. Most of the results in the three experimental years were characterized by a similar tendency. The effect of the applications was dose and, in some cases, time-depended. Both of the experimental doses of Propulse 250 SE reduced the disease incidence and severity, but better results were achieved when the higher dose (1 l/ha) of Propulse 250 SE was applied. The content of mycotoxins was dramatically reduced when Propulse 250 SE in a dose of 1 l/ha and Prosaro 420 were applied. Regarding the moment of application, the treatments before inoculation had a better effect on mycotoxin reduction in 2019 and 2021. In 2020, the DON content was lower when Propulse 250 SE was applied during sporulation. Regarding the yield, applying Propulse 250 SE in a dose of 1 l/ha during sporulation in the first experimental year led to a statistically significant increase of 33% compared to the untreated control. For the second and third experimental years, the differences were not statistically proven.

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