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THE EFFICACY OF A FUNGICIDE MIXTURE IN CONTROLLING BLACK LEG AND STEM ROT IN WINTER OILSEED RAPE

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Abstract

Lately, winter oilseed rape has become more and more grown by farmers in Romania, being a rewarding and versatile crop. Black leg (Leptosphaeria maculans) and stem rot (Sclerotinia sclerotiorum) continue to cause significant yield losses in Romania in winter oilseed rape, despite widespread use of fungicides. A series of three trials were conducted in Călăraşi County in 2021 to quantify the contribution of a mixture of Azoxystrobin 20% and Difenoconazole 12.5% at three different rates and of cultivar resistance. In the field, assessments were performed to conclude the frequency (F%) and intensity (I%) of the attack, in order to calculate the degree of attack (DA%). In the end, the effectiveness of the treatment scheme was calculated. Significant effectiveness and yield differences were recorded between cultivars and fungicides dose rates in the fields. Fungicides higher rates gave better control of diseases than lower ones and contributed to disease control and to yield responses to a greater extent than cultivar resistance. DA% of black leg infection was lower than of stem rot.

Key words: *effectiveness, Leptosphaeria maculans, Sclerotinia sclerotiorum, winter oilseed rape.*

INTRODUCTION

Due to its use both for nutritional and industrial needs, winter oilseed rape or canola (*Brassica napus* var. *oleifera* M. Delile) continues to increase in popularity among farmers. It is considered the second most important oilseed crop of the world after soybean (*Glycine max* L. Merrill) (Friedt et al., 2018; Zheng et al., 2020). Nowadays it has a current share of approx. 15% of world production (https://apps.fas.usda.gov), which shows the high value of the seeds as a source for oil and animal feed. The importance of winter oilseed rape has increased in recent years among consumers since provide a health market niche, having the less level of saturated fat of all edible oils and a very rich level of mono-unsaturated fat (Butkute et al., 2006). In Romania production of winter oilseed rape has increased very fast over the last 20 years. According to FAO 2022 data, in the last 20 years, the areas cultivated in our country with this crop have expanded at least 5 times, currently it is cultivated on approx. 500,000 ha. Consequently, the average annual production per hectare tripled during this period (https://www.fao.org/faostat/en/#data) due to the modern technology and to the occurrence of new hybrids that have high requirements for production increases.

Winter oilseed rape is a crop with multiple uses, and nowadays his oil is more desirable than ever. The main sense for this are the widespread use of his oil in a lot of fields, which include human nutrition, raw material in the chemical industry, an alternative regenerative fuel but also as ecological lubricating oil used for various purposes.

One of the major reasons that causes great losses to winter oilseed rape crop are pathogenic fungi, so that without phytosanitary protection measures there is a great risk to limit crop production or in the worst case yields will be lost. Winter oilseed rape may be infected by plant pathogens such as phoma stem canker, stem rot, black spot, grey mould, powdery mildew and others.

Black leg or phoma stem canker, *Leptosphaeria maculans* (Tode) Desmazières, is a widespread important disease of winter oilseed rape which causing severe losses on crops in all European countries (Fitt et al., 2006). This disease has gained an increasingly large scale in recent years in cultures from Romania but also from all over Europe. Having a world-wide distribution is probably due to the transmission of pathogen through the seeds of many others Brassica crops (West et al., 2001). According to Fitt et al. (2006), there is concrete evidence of an eastward spread of *L. maculans* from Western Europe. The pathogen infects cotyledons, leaves and pods in the form of brownish spots, further causes canker on stems and root crowns and lesions on upper stems (Mitrović & Marinković, 2007). Blackening of the stem base is dangerous if it settles during flowering, pods formation and seed maturation. Most of the time, spots at the base of the stem may coalesce, after which they break forming dry rots. This type of manifestation is considered the most harmful (Padmathilake & Fernando, 2022). Despite the deployment of resistant variety, the winter oilseed rape growing in Romania continues to suffer serious losses from phoma stem canker.

Stem rot, *Sclerotinia sclerotiorum* (Libert) de Bary, is one of the main harmfull fungal diseases of winter oilseed rape worldwide, as almost of the crop could die when lesions spread onto the principal stems. It has a wide range of hosts and can infest more than 600 plant species, including member family of the *Brassicaceae*, *Fabaceae*, *Solanaceae*, *Asteraceae*, *Apiaceae* and *Amaranthaceae* causing huge economic losses every year (Ding et al., 2021). Severe yield losses are reported in winter oilseed rape due to the lack of effective control measures. In some regions it causes damage every year starting from 10-20% and where the disease is severe it can reach 80% (Ding et al., 2021). Yield losses caused by stem rot are mainly significant when infestations occur at the early stage of flowering under humidity conditions (Baicu & Șesan, 1996). Ascospore of *S. sclerotiorum* initially infects petals, which fall onto leaf surfaces, resulting in infection of those organs (Jamaux et al., 1995). Early detection of the fungus on petals demands fungicide applications in cases of substantial disease risk.

The management of this stem rot disease is difficult due to a lack of genetic resistance in most commercially grown winter oilseed rape cultivars (Ni & Punja, 2020). The recommended strategies for management are crop rotations and fungicides. One of the most efficient ways to prevent and control crop diseases is the use of chemical fungicides with different chemical structures and modes of action. However, resistant of winter oilseed rape cultivars are unavailable, chemical control is the most important way to control *S. sclerotiorum* (Ma et al., 2016).

The active ingredients Azoxystrobin 20% + Difenoconazole 12.5% SC, a methoxy-acrylate and a triazole respectively, have different modes of action that complement each other. The active substance Azoxystrobin belongs to the chemical class of strobilurin fungicides and is active as a systemic broad-spectrum, translaminar fungicide with protective, curative and eradicate action. Difenoconazole is a broad-spectrum fungicide that controls a wide variety of fungi. It acts as a seed treatment, foliar spray and systemic fungicide. It is taken up through the surface of the infected plant and is translocate to all parts of the plant. It has a curative effect and a preventative effect (Henegar et al., 2019).

The aim of this study was to analize the efficacy of three rates of a fungicide mixture (Azoxystrobin 20% + Difenoconazole 12.5% SC) applied to control stem diseases at the most appropriate stages.

The efficacy of fungicide applications is dependent on being able to predict when fungal ascospores are first present on floral tissue. Failure to apply fungicides at the most effective time can result in an economic cost to the grower.

MATERIALS AND METHODS

Three field trials were carried out in three locations at Ileana, at Marsilieni and at Dâlga (Figure 1) from Southern Romania in Călărași County on chernozem soils, over the 2021-2022 growing seasons. Winter oilseed rape crops were sown in September 2021 according to local practices (Table 1). The trials were organized in a randomized complete block design, in agreement with the EPPO method 1/152(4) (EPPO Standards, 2012). Individual treatment plot size was of 24 m^2 and four replicates per each treatment were conducted.

Table 1. Agrotechnical measures in the field experiments

Location	Ileana	Marsilieni	Dâlga
Preceding crop	Winter wheat	Winter barley	Winter wheat
Sowing date	Sep-1-2021	Sep-15-2021	Sep-15-2021
Emergence date	Sep-23-2021	Oct-10-2021	Oct-7-2021
Variety	SY Iowa	Phoenix CL	SY Floretta
Density (plants/ha)	500000	450000	500000
Fertilisation	Complex NP-200 kg/ha Ammonium Nitrate-200 kg/ha	Ammonium Nitrate-200 Kg/ha Wuxal Sulphur (3 l/ha)	Ammonium Nitrate-200 Kg/ha Wuxal Sulphur (3 l/ha)
Treatment date	Apr-14-2022	Apr-15-2022	Apr-20-2022
Weeds control	Centurion plus (0.8 l/ha)	Pantera 40 EC (1.5 l/ha)	Pantera 40 EC (1.5 l/ha) BRASAN (2 l/ha)
Pest control	Sumi alpha 5 EC (0.2 l/ha) Karate Zeon (0.15 l/ha)	Lamdex Extra (0.3 kg/ha)	Lamdex Extra (0.3 kg/ha) Scatto (0.2 l/ha)

Assessments were conducted in fields looking for *L. maculans* and *S. sclerotiorums* stem infection, checking and comparing the samples taken from the field with the help of the phytopathological atlas (Zală, 2008).

Samples were analysed in laboratory by microscope too.

On winter oilseed rape, the mixtures of fungicides Azoxystrobin 20% + Difenoconazole 12.5% SC was sprayed at the rate of 0.6, 0.8 and 1.0 l/ha. So, at Ileana, treatments were applied at BBCH 50 (stage of flowers buds present, still enclosed by leaves), at Marsilieni on BBCH 51 (flowers buds visible from above, "green bud" stage) and at Dâlga during BBCH 53 (flowers buds raised above the youngest leaves).

The fungicides were applied by a knapsack sprayer (Solo 425, Germany) to imitate practical application by farmers (water volume: 400 litters/ha). Three assessments were conducted at BBCH 81 (10% of pods ripe, seeds dark and hard), at BBCH 83 (30% of pods ripe, seeds dark and hard) and at BBCH 85 (50% of pods ripe, seeds dark and hard) according to EPPO method no. PP 1/78(3) (EPPO Standards, 2021). A number of 50 randomly chosen stems per plot were analysed, assessed as percentage infected plants. Statistical data - processing of the assessments was made on the analysis of ARM-9 software (P = .05, Student-Newman-Keuls).

The values of the frequency and intensity of the diseases were determined according to the formulas: Frequency (F%) = n x 100/N, where N means number of plants observed (%) and n means number of plants specific symptoms (%). The intensity was evaluated in percentages and calculated according to the formula: Intensity (I%) = Σ (ixf)/n (%), where I means percentage given, f means number of plants/organs with the respective percentage and n is total number of attacked plants/organs.

Based on the data obtained by evaluating the frequency and intensity, the degree of attack was calculated: DA = F x I/100 (%), where DA means attack degree (%), F means frequency (%) and I means intensity (%).

The effectiveness of the treatments was determined according to the formula: E = [Gam-Gav/Gam] x 100 (%) (Abbott 'formulas), in which: Gam- degree of attack on the control sample, Gav- degree of attack on the treated check (Iosub et al., 2022).

Figure 1. Trial location of Dâlga

RESULTS AND DISCUSSIONS

A high amount of rainfall (litters on square meters) occurred at trail fields in the first half of April as follows: 50.3 at Ileana, 28.8 at Marsilieni and 20.1 at Dâlga. This fact created specific conditions for the outbreak of winter oilseed rape diseases so that *L. maculans* and *S. sclerotiorum* infection damaged the plants. In untreated checks due to weather conditions and lack of treatments, frequency and intensity had an upward evolution and increased at every assessments (Tables 2 and 3). Consequently, the degree of attack reached high values.

Thus, for *L. maculans* at winter oilseed rape, the DA% on stem increased in the untreated plots from 6 to 14.3% at Ileana, from 8 to 16.8% at Marsilieni and from 3.6 to 8.4% at Dâlga (Table 2).

Table 2. Evolution of *L. maculans* in the trials

Location/ Hybrid	Samples	BBCH 81			BBCH 83			BBCH 85		
		F%	I%	DA%	F%	I%	DA%	F%	I%	DA%
Ileana/ SY Iowa	Untreated	24a	25a	6a	29a	30a	8.7a	36a	40a	14.3a
	Azoxystrobin + Difenoconazole 0.6 l/ha	12b	15b	1.8b	15b	17.5b	2.6b	18b	25b	4.5b
	Azoxystrobin + Difenoconazole 0.8 l/ha	8c	12.5c	1c	10c	15b	1.5c	14c	17.5b	2.4c
	Azoxystrobin + Difenoconazole 1.0 l/ha	5d	5d	0.25d	8c	5c	0.4d	9d	7.5c	0.67d
	LSD (P=.05)	2.416	2.870	0.762	4.502	4.114	0.977	4.882	8.940	3.172
Marsilieni/ Phoenix CL	**Samples**	**BBCH 81**			**BBCH 83**			**BBCH 85**		
		F%	**I%**	**DA%**	**F%**	**I%**	**DA%**	**F%**	**I%**	**DA%**
	Untreated	27a	30a	8a	33a	35a	11.6a	42a	40a	16.8a
	Azoxystrobin + Difenoconazole 0.6 l/ha	18b	15b	2.7b	20b	20b	4b	24b	25b	6b
	Azoxystrobin + Difenoconazole 0.8 l/ha	12c	10c	1.20c	14c	15b	2c	17c	20b	3.4c
	Azoxystrobin + Difenoconazole 1.0 l/ha	6d	5d	0.3d	8d	10c	0.8d	12d	10c	1.2d
	LSD (P=.05)	5.211	6.216	2.171	4.208	6.765	2.645	3.343	9.130	3.898
Dâlga/ SY Floretta	**Samples**	**BBCH 81**			**BBCH 83**			**BBCH 85**		
		F%	**I%**	**DA%**	**F%**	**I%**	**DA%**	**F%**	**I%**	**DA%**
	Untreated	18a	20a	3.6a	23a	25a	5.75a	28a	30a	8.4a
	Azoxystrobin + Difenoconazole 0.6 l/ha	12ab	10b	1.2b	16b	12.5b	2b	20b	15b	3b
	Azoxystrobin + Difenoconazole 0.8 l/ha	9b	7.4b	0.67b	12c	7.5c	0.9c	16b	10b	1.6c
	Azoxystrobin + Difenoconazole 1.0 l/ha	3c	1.83c	0.1c	7d	2.85d	0.2d	9c	5c	0.45d
	LSD (P=.05)	6.941	6.802	1.250	4.593	5.661	1.740	6.568	7.382	2.349

Regarding the infection of *S. sclerotiorum* evolved in the untreated plots from 8.4 to 17.6% at Ileana, from 11.2 to 22% at Marsilieni and from 5.25 to 14.4% at Dâlga (Table 3). During the assessments from BBCH 81 to 85 diseased plants showed symptoms of yellowing and wilting. Wet weather formed a white surface, the mycelium of the fungus, in which sclerotia were later formed. When the disease progressed, sclerotia of the fungus were observed inside the stem in the medullary tissue. After completing the lifecycle, the fungus produced sclerotia or long-lived resting bodies which remained active, emphasizing even more the problem of this disease. In these infestation conditions in experimental fields, the fungicidal mixture of Azoxystrobin 20% + Difenoconazole 12.5% SC had a good efficacy in controlling of diseases at all doses tested, the best results being obtained at higher rates.

On consequence, at Ileana, the efficacy on BBCH 85 for black leg at rate of 0.6 l/ha was 68.11, at 0.8 l/ha was 82.17 and at 1.0 l/ha was 95.23% (Figure 2).

In Marsilieni trial the efficacy value at 0.6 l/ha was 61.54, at 0.8 l/ha was 79.3 and at 1.0 l/ha was 92.63% (Figure 3).

As far as concerns Dâlga experiment, efficacy was at 0.6 l/ha was 62.38, at 0.8 l/ha was 79.42 and at 1.0 l/ha was 93.58% (Figure 4).

Table 3. Evolution of *S. sclerotiorum* in the trials

Location/ Hybrid	Samples	BBCH 81			BBCH 83			BBCH 85		
		F%	I%	DA%	F%	I%	DA%	F%	I%	DA%
Ileana/ SY Iowa	Untreated	28a	30a	8.4a	39a	35a	13.7a	44a	40a	17.6a
	Azoxystrobin + Difenoconazole 0.6 l/ha	16b	20b	3.2b	24b	22.5b	5.4b	27b	25b	6.7b
	Azoxystrobin + Difenoconazole 0.8 l/ha	12c	15c	1.8c	17c	17.5b	3c	19c	20c	3.8c
	Azoxystrobin + Difenoconazole 1.0 l/ha	7d	5d	0.35d	12d	10c	1.2d	13d	12.5d	1.6d
	LSD (P=.05)	4.57	4.29	1.993	4.688	6.412	3.131	6.53	5.336	3.962
Marsilieni /Phoenix CL	Samples	BBCH 81			BBCH 83			BBCH 85		
		F%	I%	DA%	F%	I%	DA%	F%	I%	DA%
	Untreated	32a	35a	11.2a	36a	40a	14.4a	44a	50a	22a
	Azoxystrobin + Difenoconazole 0.6 l/ha	21b	22.5b	4.72b	23b	25b	5.75b	30b	30b	9b
	Azoxystrobin + Difenoconazole 0.8 l/ha	16c	17.5c	2.8c	17c	20c	3.4c	22c	22.5c	4.95c
	Azoxystrobin + Difenoconazole 1.0 l/ha	9d	10d	0.9d	12d	10d	1.2d	13d	12.5d	1.63d
	LSD (P=.05)	4.39	4.624	2.126	3.499	4.815	2.186	4.76	7.849	3.933
Dâlga/ SY Floretta	Samples	BBCH 81			BBCH 83			BBCH 85		
		F%	I%	DA%	F%	I%	DA%	F%	I%	DA%
	Untreated	21a	25a	5.25a	29a	35a	10.2a	36a	40a	14.4a
	Azoxystrobin + Difenoconazole 0.6 l/ha	15b	15b	2.25b	20b	20b	4b	24b	25b	6b
	Azoxystrobin + Difenoconazole 0.8 l/ha	10c	10bc	1c	16b	15b	2.4b	18c	17.5c	3.2c
	Azoxystrobin + Difenoconazole 1.0 l/ha	7c	7.3c	0.51c	9c	10c	0.9c	11d	10d	1.1d
	LSD (P=.05)	4.69	6.850	1.769	5.651	6.730	2.90	6.62	7.21	3.604

The best result were recorded at Ileana trial with an efficacy of 95.23 % at 1.0 l/ha rate and the worst was 61.54 at Marsilieni when 50% of pods ripped and seeds were dark and hard.

No phytotoxicity symptoms have been shown in the experimental plots. No symptoms of chlorosis, necrosis, leaf deformation, height reduction, distortion and delay at flowering in plots treated with fungicidal mixture of Azoxystrobin + Difenoconazole were seen. In experimental plots of the three trials symptoms of stem root were detected at every assessments time. As a result of treatment applied, the efficacy on BBCH 85 for *S. sclerotiorum* at rate of 0.6 l/ha was 59.42, at 0.8 l/ha was 77.36 and at 1.0 l/ha was 90.56%, at Ileana (Figure 5). In plots from Marsilieni, the efficacy of 0.6 l/ha mixture was 58.89, of 0.8 l/ha was 77.22 and of 1.0 l/ha was 92.29 % (Figure 6). Regarding Dâlga trial, efficacy for 0.6 l/ha was 58.42, for 0.8 l/ha was 76.88 and for 1.0 l/ha was 91.41% (Figure 7).

The highest performance was registered at Marsilieni trial with an efficacy of 92.29 % at 1.0 l/ha rate and the lowest was 59.42% at 0.6 l/ha in Dâlga trial when 50% of pods ripped and seeds were dark and hard.

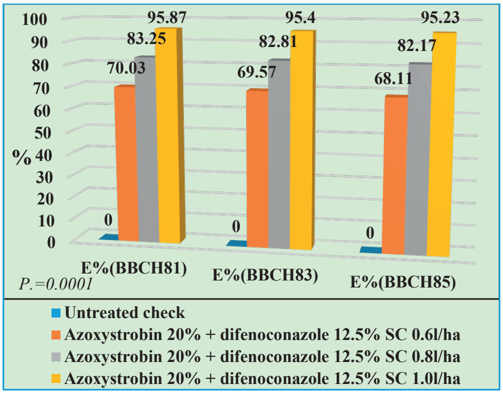

Figure 2. Efficacy of treatments for *L. maculans* at Ileana

The mixture of two active substance with protective and curative action applied at the first stage of infection are a possible and efficient way to control *S. sclerotiorum* and *L. maculans*.

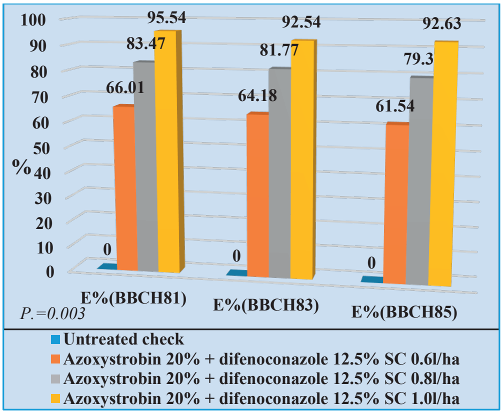

Figure 3. Efficacy of treatments for *L. maculans* at Marsilieni

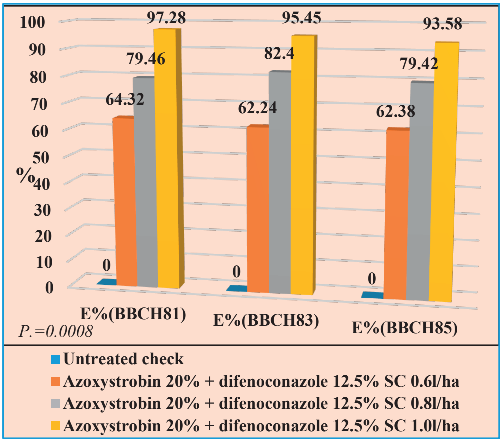

Figure 4. Efficacy of treatments for *L. maculans* at Dâlga

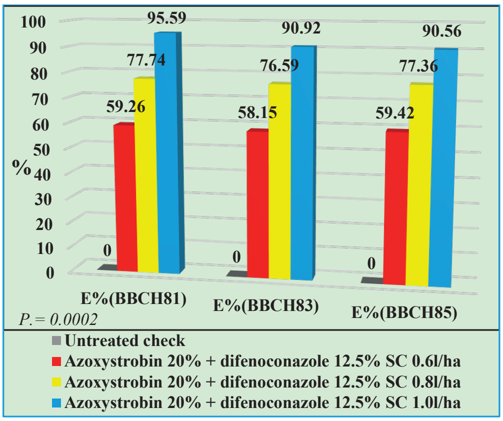

Figure 5. Efficacy of treatments for *S. sclerotiorum* at Ileana

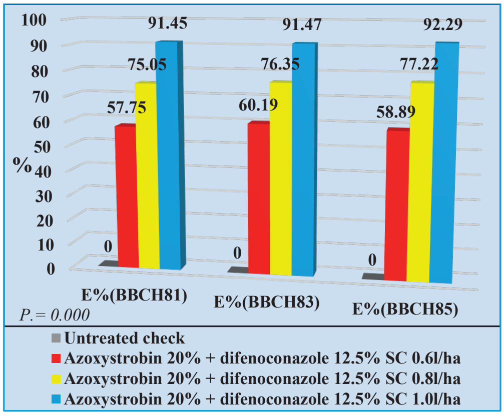

Figure 6. Efficacy of treatments for *S. sclerotiorum* at Marsilieni

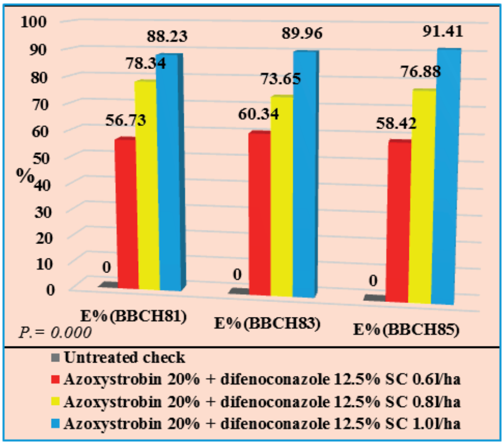

Figure 7. Efficacy of treatments for *S. sclerotiorum* at Dâlga

CONCLUSIONS

There was a gradual increase of the degree of attack of the two diseases studied from late spring to the beggining of summer in every location. It was noted that due to low rainfall the degree of the attack of the two diseases was weaker in the location of Dâlga.

For the control of the *L. maculans* disease, by applying the treatments at the stage of flowers buds present, a good effectiveness was ensured which reached 95.23% in Ileana location.

Also, the fungicide mixture applied proved very useful in the control of *S. sclerotiorum* so that the effectiveness was 92.29% in Marsilieni location, at the final observations.

The application of the treatment with Azoxystrobin 20% + Difenoconazole 12.5%

SC at reduced doses is not economically justified because at 0.6 l/ha it only reached 61.54% for black leg and 58.42% for stem root when 50% of pods ripped and seeds were dark and hard.

The most effective was the combination Azoxystrobin 20% + Difenoconazole 12.5% SC applied at the rate of 1.0 l/ha at winter oilseed rape, which reached over 90.0% efficacy.

L. maculans and *S. sclerotiorum* control assays by fungicides on *B. napus* could be limited to a single treatment with a mixture of Azoxystrobin + Difenoconazole applied at 1.0 l/ha dose rate when flowers buds are present.

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