ORIGINAL ARTICLE

Selective response of *Ricinus communis* seedlings to soil borne *Rhizoctonia* infection

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Abstract – Seedlings of Ricinus communis *tolerated soil-borne* Rhizoctonia *infection in strain dependent manner. There was no connection revealed between pathogenicity of strains and their origin or taxonomic position, however, the castor plant proved to be susceptible to most strains highly pathogenic to other host plants as well.* Rhizoctonia zeae *(teleomorph:* Waitea circinata*), a species new for European flora, was less aggressive to* R. communis *as the most potent* R. solani *strains. The effect of* Rhizoctonia *infection on mass accumulation of hypocotyls was more prominent than that on cotyledons. The protein content and glutathione S-transferase (GST) activity increased in parallel with evolution of disease syndrome. Metalaxyl, an acetanilide type systemic anti-oomycete fungicide induced locally the GST activity in* R. communis *cotyledons with 24 hours lag phase, and this induction was altered in the seedlings grown in* Rhizoctonia *infested soil by strain dependent manner. It might be concluded, that the stress response related detoxication mechanisms of plants in tolerant host/parasite pairs take effect at higher level than in highly susceptible relationships.*

Introduction

Castor bean (*Ricinus communis* L.; *Euphorbiaceae*) is an oleaginous plant with mean cycle of 150 days for the majority of annual cultivars and 120 to 130 days for annual early cultivars [1]. This plant is one of the oldest cultivated crops, but currently it represents only 0.15% of the vegetable oil produced in the world. Nevertheless, having near 1000 industrial uses castor oil is important to industry because it is the only commercial source of a hydroxylated fatty acid. This plant has future potential as an industrial oilseed crop because of high seed oil content (45-50% w/w), potentially high oil yields (1250-2500 L ha^{-1}), and ability to be grown under drought and saline conditions [2]. In Hungary decorative value of purple varieties has been exploited for centuries and locally produced castor oil has been used for pharmacological purposes. Topically applied ricinoleic acid, the main component of castor oil, exerts analgesic and anti-inflammatory effects [3]. Globally, around 1 million tons of castor seeds are produced every year, the leading producing areas being India, PR China, and Brazil [4]. *R. communis* is a primary food plant for rearing of cultivated eri silkworm, *Samia ricini* (Donovan). Eri silk production in India during 2007/8 was 1,530 tons [5]. *Ricinus communis* intoxications in humans and animals have been known for centuries. The lectin ricin is among the most toxic natural substances [6-8]. Ricin is a by-product in castor oil production. On the basis of its availability, toxicity, ease of preparation and the current lack of medical countermeasures, ricin has gained attention as potential biological warfare agent [9-11] as well as attempts have been made for using these by-products in biocontrol of agricultural pests [12-15]. Rotations with American jointvetch (*Aeschynomene americana*), castor (*Ricinus communis*), hairy indigo (*Indigofera hirsuta*), partridge pea (*Cassia fasciculata*), sesame (*Sesamum indicum*), and velvetbean (*Mucuna deeringiana*) have resulted in good nematode control and increased yields of peanut and soybean [16]. The oil seed press cakes also reduced significantly the population of plant parasitic nematode and some phytophathogenic fungi [17]. The residual effects of oil-seed cakes were also noted in the subsequent crop, chickpea, in the next growing season. It was possible to detect the presence of ricin in a soil after harvest and two years post-harvest [18]. Results on the use of castor oil against antracnose are contradicting as in some cases the pathogen was stimulated [14].

The *R. communis* cultivation is troubled by several diseases [19] and the yield is constrained by insects as well. Serious losses are made by *Botryotinia ricini* (G.H. Godfrey) Whetzel [20] *Rhizoctonia solani* Kühn and *Alternaria ricini* (Yoshii) Hansf. were transmitted by seed and caused severe seedling damping-off [21]. Sometimes severe problems arise of seed and soil borne pathogens. Among them *Fusarium oxysporum* (Wollenw.) W.L. Gordon, *Sclerotium rolfsii* Sacc. and *R. solani* can interfere with castor been production in main areas of cultivation. The *Thanatephorus* anamorphs are known producers of several mycotoxins that are involved into the pathogenesis (Fig. 1), where the deteriorative capacity of plant and associated microbes is related to the tolerance of host plant to the pathogen [22, 23]. Thus, the selection of tolerant plants to these pathogenic fungi is important both for quality food production and crop rotation. The new for Europe fungal pathogen, *R. zeae* Voorhees (teleomorph *Waitea circinata* Warcup & P.H.B. Talbot1) also occurred together with a *R. solani* strain (teleomorph *Thanatephorus cucumeris* (A.B. Frank) Donk; AG3) both species of Ceratobasidiaceae (Cantharellales, Basidiomycota). We aimed to study the tolerance of castor bean plant to this new pathogen. Also, we have measured the capacity of

glutathione conjugation system (GCS) as this biochemical function is of primary importance for plant's responses to unfavorable conditions [24-26]. The influence of *Rhizoctionia* infection on the induction of GCS in castor bean seedlings was also investigated. The metalaxyl was used for the induction of the glutathione S-transferase [GST, EC 2.5.1.18]. This acetanilide fungicide *per se* does not react with GCS. However, it is a capable inducer of GST at subtoxic doses as demonstrated in diverse objects [27-29].
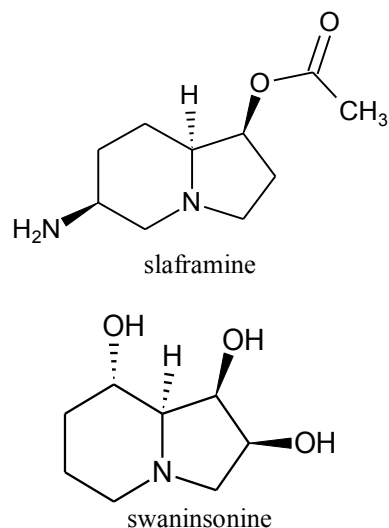

Figure 1. Structure of mycotoxins produced by *Rhizoctonia leguminicola* Gough & E.S. Elliott.

Materials and Methods

The susceptibility of *R. communis* to *R. zeae* was compared to eight strains of *R. solani* isolated from various sources. The plants suggested for crop rotation in cultivation of *R. communis* were involved in these tests (Table 1).

The soil was infested with *Rhizoctonia* in the following manner: The sterile soil prepared as above was admixed with barley seeds previously infected with the pathogen [29, 30]. After sowing, pots were maintained at 16–32 °C in a glasshouse. Disease symptoms were observed after complete opening of the cotyledons or development of coleoptyles in control plants grown in pots with uninoculated soil. Damage assessment was done using a fourfold scale, where 0=healthy, 1=depressed growth and sporadic lesions on root neck, no damping off; 2=typical disease symptom and as minimum as one plant survived; 3=all plants died.

Measurement of enzyme activity: cytosol was extracted after dissecting the seedlings. Tissues were homogenized in 0.2 M TRIS·HCl buffer (pH 8.3) at the ratio of 1:6 w/v and centrifuged at 12000 rpm for 10 min. Aliquots of supernatant were filtered via Sephadex 10 column equilibrated with Dulbecco's phosphate buffer (pH 7.4) to change the buffer and eliminate fractions interfering with enzyme assays. The GST [EC 2.5.1.18] activity was measured spectrophotometrically by following the protocol of SIGMA GST assay kit using CDNB and glutathione (GSH) as substrates. Protein content in the extracts was measured by Coomassie Blue dye binding method [31].

Induction of GST activity: One of the fully opened cotyledons of each seedling was treated with metalaxyl (5 μL of 100 nM solution per leaf). The fresh weight, protein content and GST activity of roots, hypocotyl, treated and untreated cotyledons were measured after 48 hours.

The experimental data were analyzed by employing Fisher's test to disclose differences either in susceptibility of crops or pathogenicity of *Rhizoctonia* strains. Results are presented in the form of graphs, where the respective LSD values are shown at p=0.05. Microsoft Office Excel statistical functions (Microsoft, Redmond, USA) were used for calculations and Statistica 5 program (StatSoft, Tulsa, USA) was applied for multidimensional scaling (non-linear mapping). The graphical representation of the results of data analysis was uniformly edited in Microsoft Office Power Point.

Results and Discussions

Figure 2. **Symptoms of soil-borne *Rhizoctonia* infection *on R. communis* seedlings**. Left to right: Pseudosclerotia on the surface of roots (*R. solani* strain isolated of potatota cv. Ella), Lesion on hypocotyl (*R. solani* strain isolated of *Malus*), Cotyledon decay (*R. solani* strain isolated of *Festuca*).

Table 1. Susceptibility of *Ricinus communis* and plants recommended for remediation to *Rhizoctonias*.

No.	Species	*R. zeae*	*Rhizoctonia solani* strains									
			ONI	DES	MAL	HIB	CLEO	ROS	FES	ELL	NI*	K*
1	*Ricinus communis*	1	2	3	2	1	1	1	2	0	1	2
2	*Linum usitatissimum*	0	0	2	2	3	2	2	2	0	2	1
3	*Viola tricolor*	3	2	3	1	3	3	1	3	0	2	4
4	*Carthamus tinctorius*	1	3	2	1	2	1	2	2	0	1	1
5	*Daucus carota*	3	3	1	0	2	3	0	1	1	2	2
6	*Arachis hypogea*	0	3	3	3	2	0	1	2	1	1	3
7	*Glycine max*	3	2	3	2	2	0	1	2	0	2	1
8	*Sesamum indicum*	3	3	3	3	3	0	0	3	0	3	5
9	*Ipomoea purpurea*	1	1	1	0	0	0	0	0	0	4	3
10	*Solanum melongena*	3	3	0	1	3	3	0	0	0	4	3
11	*Eleusine coracana*	0	1	0	0	1	0	0	0	0	6	0
12	*F. arundinacea* Schreb.**	2	2	1	1	1	0	1	1	0	2	0
13	*Pennisetum glaucum*	2	2	2	2	3	0	0	0	0	4	2
14	*Orzya sativa*	1	2	0	0	3	0	0	0	0	6	1
15	*Allium cepa*	1	3	3	1	3	1	0	0	0	3	2
	Rhizoctonia morphotype		A	A	B	B	C	C	D	D		
	No infection (NI)***	3	1	6	4	1	8	8	8	12		
	Killed (K)	5	6	2	2	7	3	0	0	0		

The abbreviations of strains are as follows: *R. solani* strains ELL, CLE, DES, ROS of potato, FES of grass-land, HIB of *Hibiscus rosa-sinensis* L., MAL of *Malus domestica* Bork., ONI of *Allium cepa* L. NI: no infection, K: killed

Taxonomic position (ordo) of plant species: 1-3=Malpigiales, 4=Asterales, 5=Apiales, , 6-7=Fabales, 8=Lamiales, 9-10=Solanales, 11-14=Poales, 15=Asparagales.

* The susceptibility was calculated as a sum for *R. solani* strains. **- *Festuca arundinacea* Schreb. was the dominant species on the field of first appearance of *R. zeae* in Europe. *** - The pathogenicity of *Rhizoctonia* strains calculated as a sum for species 2-15. The susceptibility of plants to soil-borne infection was evaluated by four fold scale as follows: 0=healthy, 1=depressed growth and sporadic lesions on root neck, no damping off; 2=typical disease symptom and as minimum as one plant survived; 3=all plants died.

Response of plants to soil-borne *Rhizoctonia* infection

The susceptibility of *R. communis* to *Rhizoctonia* was strain dependent (Table 1), and the symptomatic picture of disease syndrome varied within large limits. All *Rhizoctonia* strains colonized the roots of castor bean seedlings (Fig. 2). The most frequent symptom observed was the root neck rot which led to either destruction of germlings or damping off of the seedlings (Fig. 2). The size of the root system in seedlings was reduced even in the case of *R. solani* strain that did not cause any other visible symptom (Table 2). Root length reduced and frequency of branching decreased evidently in each case as compared to the seedlings grown up in *Rhizoctonia* free soil. The most pathogenic strain (DES) destroyed the germinating beans within three days after sowing. The radicles of most germinating beans were destroyed by aggressive strains (FES, MAL and ONI), but in few cases adventive roots were initiated and the seedlings survive, although the all symptoms of disease syndrome could be observed. Small brown spots could be sporadically observed on root surface of other variants, but these did not result in the death of young castor plants. There was no connection between pathogenicity of strains and their origin, moreover, their morphotypic characters did not related to their host range (Table 1).

R. communis was slightly susceptible to *R. zeae*, the newly introduced pathogen to Europe, and the symptoms were similar to those caused by *R. solani* strains. The germlings of other plants used for comparative tests of susceptibility, but *T. spelta* and *E. coracana*, were susceptible to either *R. zeae* or to most of the strains of *R. solani*. The *Allium* species exhibited surprisingly high susceptibility to soil-borne *Rhizoctonia* infection. Thus only few plants (*E. coracana* and *T. spelta*) exhibiting tolerance simultaneously to both *R. zea* and *R. solani* can be integrated into the crop rotation in castor bean plant cultivation. The *R. communis* form a separate group with dicots when the plants listed in *Table 1* were clustered according to their susceptibility responses to *Rhizoctonia* strains. This indicates that, some traits used for botanical classification might be associated with the properties determining the character of plant/*Rhizoctonia* interaction. The pathogenicity of *R. zea* strain is comparable to that of the most potent *R. solani* strains, but their host spectrum is

Table 2. Mass accumulation in castor bean seedlings.

Rhizoctonia	Fresh weight (mg/plant)			
			Cotyledons*	
	Root	**Hypocotyl**	**Control**	**+Metalaxyl**
Untreated	1556	349	588	518
R. zeae	959	501	464	390
R. solani strains				
Ella**	860	509	535	479
Cleopatra**	576	424	352	514
Rosa**	730	520	449	430
Hibiscus	592	498	257	172
Malus	558	607	194	232
Festuca	652	450	216	298
Desirèe**	0	0	0	0
*Allium***	0	0	0	0

The castor beans germinated in soil infested pre-sowing with *Rhizoctonia* strains of various origin and the mass was measured after full opening of cotyledons. $LSD_{0.05}=107$ (F=21.65, p<0.05)
* One of cotyledons of each seedling was treated with metalaxyl (5 nmol per leaf) and the mass of seedlings was measured after 48 hours. **Strains isolated from potato varieties. ***The damage was too heavy: this variant was omitted of further analysis.

seemingly different. Although on the non-linear map, strains isolated from monocot host (FES and ONI) were the closest to *R. zeae*, there was no clear cluster formation among the tested strains in the study as evaluated either by origin or by pathogenicity. This indicates the role of multiple factors involved in the pathogenic interaction between *Rhizoctonia* and host plants including *R. communis*.

Influence of soil-borne *Rhizoctonia* infection on responses of plants to metalaxyl treatment

The hypocotyls of surviving castor bean seedlings increased in diameter and in mass (Table 2) even in those cases, where no symptoms could be observed visually, and the individual variations were surprisingly low (coefficient of variation < 8%). Contrarily, the mass of cotyledons of seedlings grown up in *Rhizoctonia* infested soil varied, and individual differences were more prominent in cases of severe infections. In the case of strongly pathogenic *R. solani* strains (FES, HIB and MAL) the decrease in mass was evident. Metalaxyl treatment did not significantly alter the accumulation of mass in cotyledons either grown in *Rhizoctonia* infested soil or not (Table 2).

The soluble protein content (SPC) of seedlings that survived the infection varied in a strain dependent manner (Fig. 3). The roots were colonized in each case, although in various degree, and this fact reflects on SPC measured as it was not possible to separate root tissues of *Rhizoctonia* mycelium. Thus the SPC of roots was extremely high in the roots of seedlings densely colonized by ELL strain of *R. solani*. In hypocotyls the alterations of SPC were insignificant. In cotyledons, however, the SPC was similar
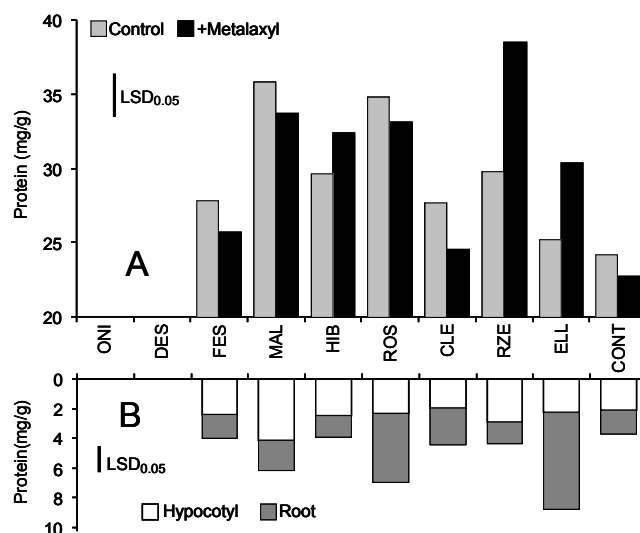

Figure 3. Influence of soil-borne *Rhizoctonia* infection on the protein content of castor seedlings.
The castor beans germinated in soil infested pre-sowing with *Rhizoctonia* strains of various origin (*R. solani* strains ELL, CLE, DES, ROS of potato; FES of grass-land, HIB of *Hibiscus rosa-sinensis* L.; MAL of *Malus domestica* Bork.; ONI of *Allium cepa* L. and RZE = *R. zeae* of grass-land). One of cotyledons of each seedling was treated with metalaxyl (5 nmol per leaf) and the protein content of tissues was measured after 48 hours. The $LSD_{0.05}$ is shown for evaluating differences between effects of *Rhizoctonia* strains.

to the control in case of *R. solani* strain of low infection potential (ELL), where the survivors did not differ from the control. Contrarily, various symptoms of disease syndrome manifested in survivors of strains of higher infection potential, and SPC was significantly higher in these variants when compared to the control. The metalaxyl treatment influenced significantly this parameter only in two cases (ELL and RZE).

The subtoxic dose of metalaxyl locally induced an increase in GST activity (Fig. 4) after 24 hours lag phase, and this level remained high for further 72 hours. This
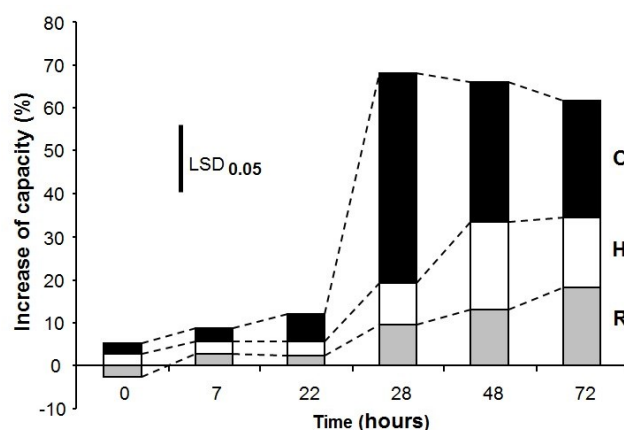

Figure 4. Inducibility of GST activity in castor bean seedlings.
The fully opened cotyledons of seedling were treated with metalaxyl (5 nmol per leaf), while the untreated ones serve as control. The increase of enzyme activity in tissues (C: cotyledon, H: hypocotyl, R: root) of metalaxyl treated seedlings was expressed in percents as related to control. Deviation is shown with bar.
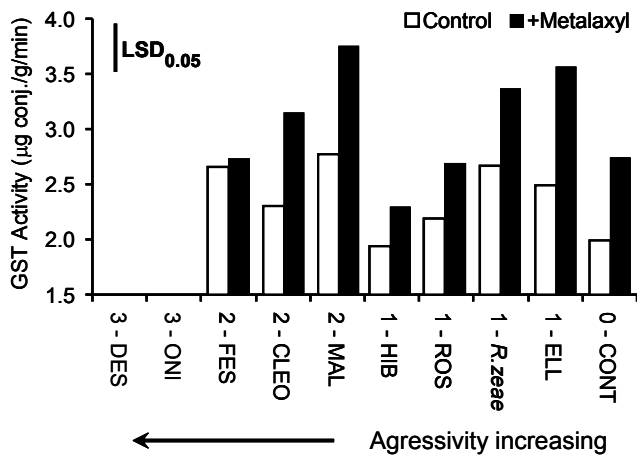
Figure 5. Influence of soil-borne *Rhizoctonia* infection on the inducibility of Glutathione S-transferase in castor cotyledons.
The seeds germinated in soil infested pre-sowing with *Rhizoctonia* strains of various origin (*R. solani* strains ELL, CLE, DES, ROS of potato; FES of grass-land, HIB of *Hibiscus rosa-sinensis* L.; MAL of *Malus domestica* Bork.; ONI of *Allium cepa* L. and RZE = *R. zeae* of grass-land). One of cotyledons of each seedling was treated with metalaxyl (5 nmol per leaf) and the GST activity of tissues was measured after 28 hours. The LSD$_{0.05}$ is shown for evaluating differences between effects of *Rhizoctonia* strains.

increase was not related to the changes in protein content of tissues (Fig. 4). Similar phenomenon was demonstrated in cotyledons of sweet pepper [28] and okra [29].

The GST activity was at a higher level in hypocotyl of all plants grown in *Rhizoctonia* infested soil (Fig. 5) when compared to the control except of HIB strain. Considerable
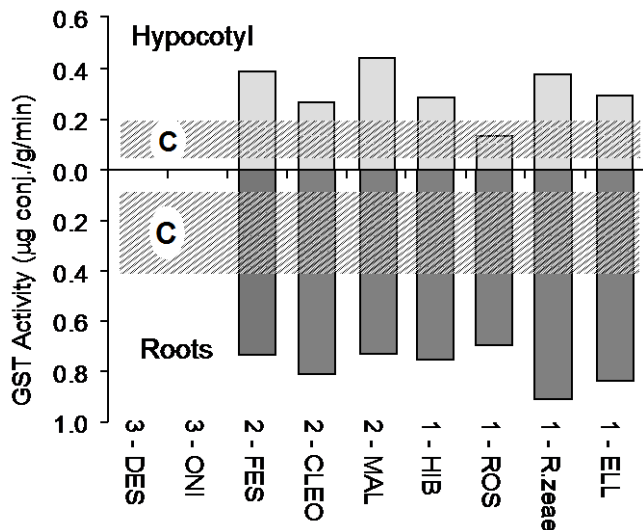

Figure 6. The effect of soil-borne *Rhizoctonia* infection on the Glutathione S-transferase activity in tissues of okra seedlings.
The seeds germinated in soil infested pre-sowing with *Rhizoctonia* strains of various origin (*R. solani* strains ELL, CLE, DES, ROS of potato, FES of grass-land, HIB of *Hibiscus rosa-sinensis* L., MAL of *Malus domestica* Bork., ONI of *Allium cepa* L. and RZE = *R. zeae* of grass-land).
The lines strips show the activity in tissues of untreated healthy (control) plants for evaluating differences between effects of *Rhizoctonia* strains.

differences between variants were not observed. In cotyledons of symptomless plants (potato strains), the GST activity did not differ significantly from the control.

However, it was more than thrice in the plants with manifested symptoms (moderately aggressive strains of *Festuca* and *Malus*) while twice that in heavily diseased plants (strains *R. zeae* and *R. solani* of *Hibiscus*). The metalaxyl treatment induced high level of GST in all cases. The increase was strain dependent and dramatic in symptomless survivors of potato strains.

In cotyledons of symptomless plants (ELL strain), the GST activity was significantly higher as compared to the control (Fig. 5). In the cotyledons of plants with manifested symptoms it varied greatly, however, in heavily diseased plants (CLEO, FES and MAL) the GST activity significantly increased. Metalaxyl treatment induced high level of GST in all cases in strain dependent manner. The GST activity was at a higher level in hypocotyls of plants grown in *Rhizoctonia* infested soil (Fig. 6) when compared to the control except of ROS strain. Considerable differences between variants were not observed. Increased GST activity could be measured in root tissues, but in this case distinctions could not be made between enzyme activities originated of roots or mycelia.

Conclusions

Rhizoctonia strains infected the germlings of castor beans primarily *via* root neck in strain dependent manner, and the hypocotyls were shortened and thickened in most of cases even if any other symptoms were absent. Significant individual differences manifested in the evolution of disease syndrome of fungus/host indicating the complex interaction of factors influencing on the type of plant response.

The glutathion conjugation system was systemically activated in infected castor seedlings, and its inducibility depended on the progress of disease.

No relationship was found between taxonomic position and origin of *Rhizoctonia* strains, indicating that traits used for their classification are not closely related to expression of their pathogenicity against castor plant.

We have got empirical evidence from plant/pathogen system verifying our approach of using simplified scale for disease assessment.

Further research requested to elucidate the role of *Rhizoctonia* toxins in pathogenesis as well as factors regulating development of survivors in presence of *Rhizoctonia* should be identified.

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