

The prospect of using a necrotic protective barrier in the creation of potato varieties resistant to the Colorado potato beetle

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Abstract. The purpose of the research was to investigate the effectiveness of the necrotic protective barrier in creating resistance to the Colorado beetle in potatoes. **Methods.** The research was carried out in a field experiment according to the accepted methodology for 3 years. The research involved 30 different potato varieties grown in conditions of the Cis-Ural forest-steppe of the Bashkortostan Republic. The analysis was focused on the correlation between the leaf blade hypersensitive response intensity on the Colorado potato beetle egg clutch on the one hand, and the level of plant resistance to the pest and loss of tuber yield from damage by parasite on the other side. **Results.** There was observed a strong association between the resistance of potato tops and the plant yield (the correlation coefficient is 0.763–0.804) when potatoes are grown without the control of the phytophage number. When growing potato varieties with insecticide application, the productivity of plants practically did not depend on their resistance level to phytophage. There has been found a strong positive correlation between the hypersensitive reaction of the leaf blades to Colorado beetle egg disposition and the resistance of different potato varieties to the pest (correlation coefficient 0.568–0.671). On the contrary, the relationship between the hypersensitive response of the leaf blades to the egg clutch and the decrease in yields was negative (correlation coefficient –0.646...–0.763). Based on the analysis of the obtained data, it is concluded that the stability of potatoes and the reduced loss of tuber yields from pest damage are closely related to the potato leaf response against the Colorado beetle clutch. The use of a necrotic protective barrier is a promising direction in breeding potato varieties resistant to the Colorado potato beetle. **The scientific novelty** lies in the study of the possible application of a new type of resistance and creating on this basis potato tolerance donors against the Colorado beetle.

Keywords: potato breeding, Colorado potato beetle, hypersensitive response.

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Introduction

The Colorado potato beetle (*Leptinotarsa decemlineata* Say) has been a very dangerous pest for potato almost 45 years and is an acute problem for the country's potato industry [1, p. 67]. Insecticides control the number of pest larvae on the crop. However, the parasite quickly adapts to widely used pest control chemicals [2, p. 54]. Without protective measures unstable potato cultivars can lose 25–50 %, in some cases, 80–100 % of tuber resulted from a high number of insects [3, p. 65]. Currently the Colorado beetle has a high level of resistance to most insecticides. Thus, the only economically feasible way to stabilize potato agrocenoses is to develop highly tolerant varieties that can provide a tuber yield of good quality with a minimum level of chemical protection [4, p. 50], [5, p.14].

To successfully create potato cultivars resistant to the Colorado beetle, it is necessary to modify the elements of its interaction with the plant that are critical for the insect's life [6, p. 165]. Breeding potato tolerant to the Colorado beetle is

complicated by the fact that this insect is a changeable, genetically and adaptively polymorphic, plastic species. In this regard, when creating resistant varieties, protective genes should act equally on all genotypic forms of the pest in the general population and not cause violations of the population structure [7, p. 85]. The traditional approach to breeding potato cultivars tolerant to the Colorado beetle involves the use of wild potato species *S. demissum*, *S. chacoense*, *S. commersonii*, etc. in crosses. At the same time, the stability of potato hybrids in these combinations is mainly due to the presence of glycoalcoloids in the leaves [8, p. 27]. Zarevo, Peresvet, Bryanskiy nadezhnyy, Nikulinskiy and a number of other cultivars developed on this basis are characterized by the relative resistance against pests at 6–8 points. However, this type of resistance is overcome by the pest, so it is necessary to use insecticides to control the number of insects even on these varieties [9, p. 39].

In this regard, to create potato varieties tolerant to the Colorado beetle, there is a need to find new types of resistance

and develop new approaches to creating potato tolerance donors against this type of insects. One of the possible directions of breeding work in this direction is to develop stable genotypes based on the use of a necrotic protective barrier in the form of hypersensitive response of plant leaves to the insect egg clutch. Possible control of the phytophage number based on a necrotic protective barrier has been reported for the first time relatively recently. It was based on the phenomenon observed when individual plants of black mustard (*Brassica nigra*) in climatic conditions of California valley (significant lack of air humidity) caused the death of eggs laid on the leaves by the cabbage white butterfly (*Pieris rapae*) and green-veined white butterfly (*P. napi*). The researchers claimed that the death of the insect clutch occurred as the result of the leaf blade necrosis at the place of the egg attachment followed with leaf drying [10, p. 631]. Insect eggs can't control their temperature and completely depend on the host plant microenvironment. Thus, necrosis at the clutch place can isolate eggs from the thermoregulation process of the host plant leaf and lead to death [11, p. 3451].

In another example, *there was* the hypersensitive response of the leaf blade and the necrosis at the place of the Colorado potato beetle egg clutch (*Leptinotarsa decemlineata* Say) in potato species hybrid *Solanum* spp. It caused egg detachment, falling on the soil surface and subsequent death [12, p. 655]. Moreover, the selection evaluation of potato hybrids, showed that relatively stable varieties reacted with the necroses on the leaves under the egg clutch, while unstable varieties did not have this reaction [13, p. 10]. However, a detailed study of the effect of this protective barrier on potato tolerance to the Colorado beetle has not been conducted.

It is undoubtedly relevant for further scientific and breeding programs to assess the effectiveness of the necrotic protective barrier on pest resistance and the prospects for its using to develop tolerance of potato varieties against the Colorado beetle [14, p. 69]. Thereby, the authors of the very paper conducted a comparative study of the way the leaf blade hypersensitive response intensity on pest egg clutch decreases the yield of different potato varieties resulted from damage by the Colorado beetle.

Methods

The research was conducted in the Cis-Ural climate zone (northern forest-steppe) on the lands of the Birsks scientific division of the Bashkir Research Institute of Agriculture. The experiment was performed in 2016, 2017 and 2019. Meteorological conditions in the research area for all years of studies were within the climate norm. Annually the period from the moment the Colorado beetle females lay eggs on potato leaves to the development of larvae of the IV age was from the beginning of the 2nd decade of June to the end of the 3rd decade of July. This period in 2016, the average daily temperatures were 19.6–23.9 °C and the amount of precipitation was 60 mm. At the same time, there was insufficient water due to a lack of rainfall in the first three decades of this period. In 2017, during the embryonic development of phytophage larvae, the average daily temperatures were within 16.8–21.5 °C, the amount of precipitation was 166 mm, which fell evenly. In 2019, the mean daily temperatures in the studied period were 16.1–20.6 °C; there were 95 mm of precipitation that fell relatively evenly.

The experiments were based on 30 different potato cultivars. Samples were planted on two row plots of 10 plants in three-fold repetition. The planting scheme was 70 × 30 cm. Pest dispersal and egg laying by female Colorado beetles on experimental potato plants took place naturally. At the time of mass larvae hatching from eggs, one row of each plot was fenced off with a protective screen and treated with 0.005 % solution of Regent insecticide at the rate of 200 ml, which is a hectare rate of 10 g/ha. The other row of this plot was not treated with insecticide. The biological effectiveness of the insecticide against larvae of age 1–2 was at the level of 95–98 %. The degree of damage to the potato tops on the unprotected half of the plot was assessed visually 15 days after hatching, when the pest larvae reached age IV and fed intensively before going into the soil for pupation. The damage degree on each Bush plot was evaluated visually on a 9-point scale:

- 9 points – high stability, weak damage up to 10 %;
- 7 points – relatively high stability, damage from 10 to 24 %;
- 5 points – average stability, damage from 25 to 49 %;



Fig. 1. Development of leaf blade necrosis on 100 % of the area occupied by the Colorado beetle egg clutch (view from the upper (a) and lower (b) sides of the leaf blade), Bashkirskiy variety

3 points – weak stability, damage from 50 to 79 %;

1 point – lack of stability, severe damage from 80 to 100 %.

Each row of the plot was harvested separately. The yield was recorded in the 1st decade of September. The extent of losses from pest damage was estimated by comparing the yield of potato tubers from the protected row and the yield from the row without the use of insecticide.

The development of a leaf blade hypersensitive response to a Colorado potato beetle clutch was evaluated on 3–5 day after the clutch placement on the following scale:

0 points – no hypersensitive response;

1 point – protrusion of the leaf blade at the place of the clutch attachment;

2 points – necrosis on 25 % of the area occupied by the clutch;

3 points – necrosis on 50 % of the area under the clutch;

4 points – necrosis on 100 % of the area under the clutch (fig. 1);

5 points – perforation of the leaf blade at the place of the clutch attachment.

The development of the leaf blade hypersensitive response was analyzed under ten clutches found in a row on the plot. Then the average value of the necrosis development extent was deduced in points.

Mathematical processing of the obtained data was performed by calculating the arithmetic mean of 10 observations and the standard error of the average. Correlation analysis of the obtained data was done according to the accepted methodology [15. p. 269].

Results

The results of three years of field experiments indicate that the Colorado beetle is a particular danger as a pest of potato culture. Annual losses from the insect, depending on the variety, reached up to 93 % of the yield (tables 1, 2, 3, 4). At the same time, varietal characteristics were a decisive factor in the level of yield reduction when the crop was damaged by the Colorado beetle. Thus, the data on the reduced yield of potato tubers without pest control by insecticide, in comparison with the conditions of using protection means, prove that the most resistant cultivars had 8–18 times lower yield reduction than the least resistant varieties.

Table 1

The effect of the hypersensitive response of potato leaves to the Colorado beetle egg clutch on potato tolerance to the pest damage, 2016

Item No.	Cultivars	Hypersensitive response development, points	Potato top stability, points	Potato yield capacity, g/bush		Yield reduction, %	Rating in tolerance to yield reduction
				Treated with insecticide	Untreated with insecticide		
1	Gibrid 53	3.7 ± 0.31	5.5 ± 0.88	477	424	11.2	1
2	Freska	3.5 ± 0.28	5.1 ± 0.58	410	362	11.6	2
3	Bashkirskiy	3.2 ± 0.34	5.5 ± 0.66	225	197	12.5	3
4	Burnovskiy	3.3 ± 0.21	5.8 ± 0.29	398	339	14.8	4
5	Safo	1.0 ± 0.25	6.8 ± 0.74	205	173	15.6	5
6	Belosnezhka	1.5 ± 0.39	6.5 ± 0.87	387	313	19.0	6
7	Kondor	2.5 ± 0.21	5.5 ± 0.55	351	280	20.0	7
8	Udacha	2.7 ± 0.22	4.5 ± 0.54	595	469	21.1	8
9	Soldatik	2.2 ± 0.13	4.6 ± 0.88	375	290	22.6	9
10	Resurs	0	5.8 ± 0.89	446	329	27.7	10
11	Svetanok kievskiy	2.3 ± 0.28	4.8 ± 0.87	158	114	28.0	11
12	Institutskiy	1.5 ± 0.29	4.6 ± 0.38	387	271	30.1	12
13	Skarb	1.0 ± 0.27	4.5 ± 0.87	560	362	35.4	13
14	Zhigulevskiy	1.2 ± 0.11	4.0 ± 0.88	291	184	36.6	14
15	Liga	1.0 ± 0.09	3.1 ± 0.95	371	222	40.1	15
16	Alegro	1.3 ± 0.14	3.5 ± 0.54	332	181	45.5	16
17	Disko	0	3.0 ± 0.65	295	148	49.8	17
18	Amaliya	0	2.1 ± 0.87	382	181	52.6	18
19	Sentyabr'	0	2.4 ± 0.71	420	168	60.1	19
20	Nevskiy	1.0 ± 0.15	2.3 ± 0.18	197	66	66.6	20
21	Vesna	0	2.5 ± 0.87	384	116	69.9	21
22	Lugovskoy	0	2.4 ± 0.29	379	111	70.7	22
23	Raya	0	1.5 ± 0.87	375	79	78.9	23
24	Roksana	0	1.9 ± 0.17	310	63	79.8	24
25	Nayada	0	1.5 ± 0.12	405	81	80.1	25
26	Andro	0	2.0 ± 0.54	340	58	82.8	26
27	Rannyaya roza	0	2.3 ± 0.28	417	56	89.0	27
28	Bronitskiy	0	2.1 ± 0.29	314	35	90.1	28
29	Antoshka	0	1.2 ± 0.37	459	45	92.2	29

Table 2
The effect of the hypersensitive response of potato leaves to the Colorado beetle egg clutch on potato tolerance to the pest damage, 2017

Item No.	Cultivars	Hypersensitive response development, points	Potato top stability, points	Potato yield capacity, g/bush		Yield reduction, %	Rating in tolerance to yield reduction
				Treated with insecticide	Untreated with insecticide		
1	Bashkirskiy	3.2 ± 0.44	6.5 ± 0.55	475	439	7.5	1
2	Burnovskiy	3.9 ± 0.25	7.0 ± 0.45	647	589	8.9	2
3	Freska	2.5 ± 0.65	7.1 ± 0.66	327	297	9.1	3
4	Kondor	3.0 ± 0.33	6.5 ± 0.26	205	183	10.5	4
5	Resurs	0	7.0 ± 0.25	277	243	12.2	5
6	Udacha	2.3 ± 0.58	5.4 ± 0.22	547	476	12.9	6
7	Safo	1.2 ± 0.33	4.4 ± 0.14	229	198	13.6	7
8	Gibrid 53	3.0 ± 0.11	7.5 ± 0.88	666	570	14.4	8
9	Liga	1.5 ± 0.24	6.1 ± 0.58	191	161	15.9	9
10	Svitanok kievskiy	3.0 ± 0.28	5.4 ± 0.55	329	276	16.1	10
11	Alegro	1.3 ± 0.24	5.5 ± 0.78	297	187	36.9	11
12	Institutskiy	0.5 ± 0.25	4.8 ± 0.87	440	262	40.4	12
13	Belosnezhka	2.8 ± 0.12	4.7 ± 0.19	335	184	45.2	13
14	Zhigulevskiy	0.3 ± 0.25	4.0 ± 0.22	417	228	45.3	14
15	Soldatik	3.3 ± 0.32	3.6 ± 0.29	653	334	48.8	15
16	Skarb	1.0 ± 0.22	3.5 ± 0.25	291	147	49.5	16
17	Disko	0	3.1 ± 0.23	294	146	50.4	17
18	Raya	0	2.5 ± 0.21	422	187	55.6	18
19	Nayada	1.5 ± 0.11	1.5 ± 0.23	369	123	66.8	19
20	Roksana	1	2.5 ± 0.55	267	81	69.6	20
21	Vesna	0	2.4 ± 0.23	273	81	70.2	21
22	Lugovskoy	0	2.5 ± 0.21	350	97	72.3	22
23	Antoshka	0	2.2 ± 0.66	264	65	75.5	23
24	Sentyabr'	0	2.8 ± 0.35	511	120	76.6	24
25	Nevskiy	2	2.0 ± 0.47	474	103	78.2	25
26	Rannyaya roza	0	2.5 ± 0.46	154	32	78.9	26
27	Sadovyy	0	2.0 ± 0.41	355	68	80.8	27
28	Andro	1	2.4 ± 0.44	239	35	85.5	28
29	Amaliya	0	2.1 ± 0.53	416	42	89.9	29
30	Bronitskiy	0	1.1 ± 0.25	427	34	92.1	30

Table 3
The effect of the hypersensitive response of potato leaves to the Colorado beetle egg clutch on potato tolerance to the pest damage, 2019

Item No.	Cultivars	Hypersensitive response development, points	Potato top stability, points	Potato yield capacity, g/bush		Yield reduction, %	Rating in tolerance to yield reduction
				Treated with insecticide	Untreated with insecticide		
1	Freska	4.0 ± 0.41	7.1 ± 0.89	791.0	758.0	4.2	1
2	Kondor	3.3 ± 0.32	6.5 ± 0.81	511.1	488.9	4.3	2
3	Bashkirskiy	3.0 ± 0.21	7.5 ± 0.88	716.7	660.0	7.9	3
4	Safo	3.5 ± 0.23	6.9 ± 0.54	533.0	478.0	10.3	4
5	Resurs	0	8.0 ± 0.44	673.0	595.0	11.6	5
6	Udacha	0.8 ± 0.41	5.5 ± 0.48	715.0	623.0	12.9	6
7	Liga	1.0 ± 0.54	7.1 ± 0.44	733.3	612.2	16.5	7
8	Belosnezhka	3.3 ± 0.32	6.5 ± 0.64	944.4	743.3	21.3	8
9	Gibrid 53	3.5 ± 0.25	6.5 ± 0.75	728.0	570.0	21.7	9
10	Zhigulevskiy	0.3 ± 0.23	4.2 ± 0.87	997.5	770.0	22.8	10
11	Burnovskiy	2.0 ± 0.21	5.8 ± 0.48	924.3	703.0	23.9	11
12	Svetanok kievskiy	0.3 ± 0.87	4.8 ± 0.46	817.0	615.6	24.7	12
13	Disko	1.0 ± 0.48	4.0 ± 0.34	744.0	534.0	28.2	13
14	Alegro	1.3 ± 0.74	4.5 ± 0.47	843.8	590.9	30.0	14
15	Institutskiy	0.5 ± 0.56	4.1 ± 0.87	833.0	557.0	33.1	15
16	Soldatik	1.3 ± 0.25	4.6 ± 0.88	796.7	528.9	33.6	16
17	Skarb	1.0 ± 0.22	6.5 ± 0.81	738.9	482.0	34.8	17
18	Vesna	0	3.5 ± 0.17	844.0	476.0	43.6	18
19	Lugovskoy	0	3.4 ± 0.16	767.0	430.0	43.9	19
20	Amaliya	0	2.1 ± 0.25	499.1	255.0	48.9	20
21	Sentyabr'	0	2.4 ± 0.32	836.7	411.1	50.9	21
22	Rannyaya roza	0	2.3 ± 0.15	519.0	247.8	52.3	22
23	Raya	0	3.5 ± 0.10	613.3	280.0	54.3	23
24	Roksana	1.0 ± 0.15	2.5 ± 0.44	509.1	225.0	55.8	24
25	Nayada	1.5 ± 0.12	1.5 ± 0.32	594.0	223.0	62.5	25
26	Andro	2.0 ± 0.11	3.9 ± 0.24	794.0	206.0	63.9	26
27	Bronitskiy	0	1.0 ± 0.12	503.3	159.1	68.4	27
28	Antoshka	1.8 ± 0.17	2.0 ± 0.32	585.0	148.0	74.7	28
29	Nevskiy	1.2 ± 0.15	2.4 ± 0.51	688.0	172.0	75.0	29
30	Sadovyy	0	1.5 ± 0.33	718.0	172.7	75.9	30

Table 4
Inheriting hypersensitive response of leaf blades to the Colorado beetle egg clutch by the offspring of the Bashkirskiy potato cultivar

Item No.	Crossing combination	The number of studied hybrids, pcs	Distinguishing between hybrids in manifesting the hypersensitive response in points after 5 days from egg laying by the pest					
			0	1 point	2 points	3 points	4 points	5 points
1	Bashkirskiy × Dubrava	80	51	14	9	5	1	0
2	Phenotype distribution, %	100	63.75	17.5	11.25	6.25	1.25	0
3	Bashkirskiy × Aurora	57	38	10	5	3	1	0
4	Phenotype distribution, %	100	66.7	17.5	8.8	5.3	1.8	0.0
5	Self-pollination of the Bashkirskiy cultivar	107	77	17	4	4	3	2
6	Phenotype distribution, %	100	72.0	15.9	3.7	3.7	2.8	1.9

The hypersensitive response of the potato leaf blade to the Colorado beetle clutch has been found to be a genetically determined trait. The varieties with these genes manifest this characteristics every year. The cultivars without these genes did not display this trait during the entire observation period. At the same time, the trait manifestation rate has been found to vary depending on the specific conditions of the growing season, which fall on the period of egg laying by insects. This is quite natural, since each variety is known to have a certain rate of reaction to different growing conditions, and this property is the basis for varietal zoning. Three-year field data on the hypersensitive response of potato leaves to the Colorado beetle clutches provide evidence and confirm the previously made conclusion that a warmer temperature background inhibits the process of necrosis formation [13. p. 10]. Thus, the highest point for the hypersensitive reaction of potato leaves to the clutches of the Colorado beetle was 3.7 (Gibrid 53) in the warmest 2016. In a more moderate 2017, it was 3.9 (Burnovskiy cultivar). In the coolest year of 2019, the maximum point was 4.0 (Fresca cultivar). The temperature influence on the hypersensitive reaction development of potato leaves on the Colorado beetle clutches, apparently, lies in the biochemical features of this reaction.

To assess the effectiveness of the necrotic protective barrier on pest resistance and the prospects of using this protective barrier in creating resistance of potato varieties to the Colorado beetle, a correlation analysis of the studied factors was conducted.

Thus, the analysis of the relationship between the potato top stability rate in points and the yields of different potato cultivars made based on the data obtained showed that these two indicators are closely related only when the studied crop is grown without the use of insecticides to control the pest population. In addition, the correlation coefficient between the stability in points and the level of plant productivity against the background of free development of the pest was 0.763 in 2016, 0.779 in 2017 and 0.804 in 2019, meaning that the relationship between these indicators is strong. When growing potato varieties under the control of the Colorado beetle population by insecticide, the potato yields practically did not depend on the level of resistance to phytophage (the correlation coefficient for years ranged within 0.066–0.348). The level of yield in this case depended on other features of potato varieties. There is the rationale for this: in conditions of free phytophage nutrition, the level of potato stability acts as a limiting factor for yields, so the relationship between them is strong.

Analysis of correlation dependence between the degree of hypersensitive response to the Colorado potato beetle clutch and potato top resistance to the pest, as well as a yield reduction of different potato cultivars shows that this relationship is strong enough. Thus, the correlation coefficients of the dependence between the degree of development of the hypersensitive reaction to the Colorado beetle clutch and the level of stability of the potato tops, which shows the share of the preserved leaf surface after the larvae left the plants, were 0.671 in 2016, 0.631 in 2017 and 0.568 in 2019. These values correspond to the average degree of dependence between these two characteristics. This is entirely due to the fact that potato cultivars have also other immunogenetic barriers of stability. A similar conclusion can be drawn between the hypersensitive response development to the Colorado beetle clutches and the decrease in yields of different potato varieties. The negative correlation coefficient between these factors was – 0,763 in 2016, – 0,646 in 2017, – 0,571 in 2019.

A clear proof that the hypersensitive response of potato leaves to Colorado beetle clutches and the level of resistance of potato varieties to the pest are closely related is that in most cases the top rating cultivars tolerant to yield losses have this protective barrier, while the varieties that close this rating do not have it. It is possible that the hypersensitive reaction is also a trigger for other immunogenetic barriers of resistance to the Colorado beetle [16, p. 794].

It is known that most of the potato cultivars selected for the study were developed on the basis of productivity and other economically valuable traits. The manifestation rate of the hypersensitive response of potato leaves to the Colorado beetle egg clutches was not taken into account in the selection. At least, there are no scientific reports about this. Therefore, in our experience, the level of stability of most potato varieties was at an average and weak level. Only some cultivars showed relatively high stability in some years, which is clearly visible in the field experiment (fig. 2).

The data obtained provide evidence that targeted selection of genotypes with a well-expressed hypersensitive response of potato leaves to the Colorado beetle egg clutches and a high level of productivity make it possible to create qualitatively new potato cultivars. The use of a phenotypic trait for evaluating hybrid material to effectively reject Colorado beetle clutches by means of a pronounced hypersensitive response of potato leaves to them will significantly increase the effectiveness of the selection process for resistance to this pest. The combination of the necrotic resistance barrier with other



Fig. 2. The state of potato after the IV-aged larvae go into the soil for pupation.

On the left, there are rows of unstable varieties treated and not treated with insecticide. On the right, rows of stable varieties

immunogenetic factors of tolerance by pyramiding protective genes will further create potato genotypes that can be grown with a significant reduction in the pesticide load on the crop.

Discussion and Conclusion

The conducted research has revealed that the necrotic immunological barrier of potato resistance to the Colorado beetle is available in different manifestation rates in most of the varieties studied in the experiment. However, the level of the hypersensitive reaction of potato leaves to the egg clutches of the Colorado beetle in most varieties that have this trait is not high enough to radically reduce the damage of the insect. This seems to be the reason why there is no purposeful selection based on this trait.

Based on the data obtained and their correlation analysis, it was found that the potato resistance to the Colorado beetle and the decrease in the loss of tuber yields resulted from damage by the pest are closely related to the manifestation rate of the hypersensitive reaction of leaves to the Colorado beetle egg clutches. The use of a necrotic protective barrier and the creation of genotypes with intensive formation of necrosis in the clutch attachment zone up to the rejection of eggs from the leaf blade is a promising direction in the creation of potato varieties resistant to the Colorado beetle.

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