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SUSCEPTIBILITY OF FIELD BEAN CULTIVARS TO SLUG DAMAGE

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ABSTRACT: In recent years field bean crops (*Vicia faba*) have been frequently attacked by slugs. As there are no molluscicides registered for protection of field beans against slugs, alternative anti-slug measures are needed. One of them may be using specific cultivar properties of the plant. The aim of the study was to assess the susceptibility of different field bean cultivars to damage by slugs and to identify the effect of tannin content in plants on the extent of damage. The experiments were performed in the laboratory and in experimental plot conditions on seeds and plants at stages of 3–4 and 5–6 true leaves, which were exposed to *Arion vulgaris* (Moquin-Tandon) and *A. rufus* (Linnaeus). The extent of damage was analysed in four cultivars with low seed tannin content and five with high seed tannin content. The extent of damage to the seeds and plants varied depending on the cultivar and the slug species. The plants of the high-tannin Optimal cultivar were less susceptible to damage.

KEY WORDS: field bean, tannins, *Arion rufus*, *Arion vulgaris*, damage

INTRODUCTION

The field bean (*Vicia faba* L.) is a leguminous, high-yield plant of the family Fabaceae grown for seeds in the main crop. It has an important function in soil improvement and due to its high protein content is a valuable component of nutritive fodders. It is successfully used as a forecrop for other arable crops, especially cereals. As the area of cultivation of field bean crops in Poland has grown in recent years, protecting this plant against diseases and pests has become increasingly difficult. The most important pests of the field bean are aphids (Aphididae), pea weevils (Curculionidae) and recently also slugs (Gastropoda: Arionidae, Agriolimacidae). With regard to the Fabaceae, research on risks and protection of plants against gastropod pests has focused primarily on lupin, clover, alfalfa, peas and other species of beans and vetch (SOUTH 1992, BYERS 2002, GEBAUER 2002, PORT & ESTER 2002, BROOKS et al. 2003). No information has been found on the field bean, although our observations indicate considerable damage to the plant in areas with abundant slug populations.

Making use of plant biochemical defence mechanisms may prove essential in protecting plants against slugs. Certain plants produce compounds with deterrent and anti-feedant properties, which may affect the feeding activity of slugs and, as a result, the degree of crop damage. Secondary plant metabolites such as glycosides, terpenoids, flavonoids, alkaloids, saponins, phenols and others may prevent or inhibit feeding of herbivorous gastropods (KLOOS & MCCULLOUGH 1982, WEBBE & LAMBERT 1983, MØLGAARD 1986, STAHL 1988, DESBUQUOIS & DAGUZAN 1995, HANLEY et al. 1995, CLARK et al. 1997). Some potato and lupin cultivars have been shown to contain alkaloids which reduce plant damage caused by *Deroceras reticulatum* (O. F. Müller, 1774), *Tandonia budapestensis* (Hazay, 1881), *Arion hortensis* Férussac, 1819, *A. lusitanicus* Mabille, 1868, *A. distinctus* Mabille, 1868 and *Helix aspersa* (O. F. Müller, 1774) (WINFIELD et al. 1967, AIREY 1989, AGUIAR & WINK 1999, CHEVALIER et al. 2000). A similar effect has been demonstrated for glucosinolates in certain cultivars of the oilseed

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rape against *D. reticulatum* (Glen et al. 1990, Moens et al. 1992) and for cyanogenic glucosinolates in some forms of clover against *D. reticulatum*, *Agriolimax caruanae* Pollonera, 1891, *Arion ater* Linnaeus, 1758 and *H. aspersa* (Dirzo & Harper 1982a, b, Burgess & Ennos 1987). Some of the currently grown cultivars of the field bean contain derivatives of phenolic compounds – tannins, which reduce seed germination. They have also been shown to defend plants against pests and diseases (Kigel 1995). The deterrent effect of tannins has been demonstrated in studies on the preference of *Arion subfuscus* (Draparnaud, 1805) to willow seedlings (*Salix sericea* and *S. eriocephala*) (Fritz et al. 2001, Albrectsen et al. 2004). The attractiveness of the seedlings to slugs is significantly reduced at high levels of tannins in the plants. Tannins in field bean seeds may produce a similar effect. Some reports suggest that when field bean seeds are devoid of tannins their seed coat is damaged more often, which makes the seeds less vigorous and healthy; they may also be more sensitive to environmental stressors (Bond & Duc 1993).

Field bean and other leguminous plants grown in Poland are mainly attacked by *Arion vulgaris* (Moquin-Tandon, 1885), *A. rufus* (Linnaeus, 1758) and *D. reticulatum*. Other slug species are also found in crops of this plant, though larger populations are rare. The attractiveness of seeds and legumes as a source of food for slugs has been well known for decades (Runham & Hunter 1970, Gebauer 2002). Slugs exhibit a dietary preference for more palatable plants without naturally-occurring molluscicidal toxins (Hanley et al. 1995, Cook et al. 1996, Byers 2002, Frank 2003, Barlow et al. 2013). Consequently, there is considerable variation in the degree of slug-induced crop damage. This fact may be potentially used in integrated plant protection against these pests.

This study was motivated by the lack of information on dietary relationships between field bean cultivars and *A. vulgaris* and *A. rufus* which damage field bean crops. The aim of the study was to assess the susceptibility of field bean cultivars to slugs and determine the impact of tannin compounds on the extent of damage to seeds and plants.

MATERIAL AND METHODS

The experiments were performed in the laboratory and in experimental plot conditions on seeds and plants of the field bean exposed to two slug species. Young slugs were collected in Poland, from horticultural crops in the environs of Poznań (*A. vulgaris*) and Wronki (*A. rufus*) in the spring of 2015. The slugs were kept in plastic containers filled with soil at 16°C and fed with cabbage leaves, potato tubers and wheat bran with addition of calcium carbonate. Food was changed twice a week. Prior to each experiment the slugs were weighed after being starved for 48 hours and individuals of the most similar weight were selected. Seeds used in the experiments were obtained from commercial growers. Four high-tannin field bean cultivars: Granit, Bobas, Neptun and Optimal (0.466–0.563 mg/g DW) and five low-tannin cultivars: Albus, Amulet, Kasztelan, Leo and Olga (0.032–0.035 mg tannins/g DW) (Domański & Osiecka 2014) were used.

LABORATORY TESTS

Experiments were performed on germinated seeds and plants at the 3–4 leaf stage. In the first experiment, seeds were stored for two days in high-humidity conditions to swell. Subsequently, they were placed on moistened filter paper in plastic containers (20 × 16 × 13 cm), 12 seeds of each cultivar per container. Finally, one *A. vulgaris* or *A. rufus* was placed in each container. The average slug weight was 1.33 g and 1.28 g, respectively.

In the second experiment, plants that had been grown in raised beds up to the 3–4 leaf stage were planted in a 5 cm thick layer of soil in plastic containers (26 × 26 × 14 cm), five plants per container. After two days one *A. vulgaris* or *A. rufus* was placed in each container. The average slug weight was 1.26 g and 1.44 g, respectively. All containers had openings to provide air circulation. Both experiments were conducted in an environmental chamber with air temperature of 17°C, RH 70±3% and 12-hour photoperiod. Damage to organs of germinating seeds and plants was assessed once a day on a 5-point scale (0; 25%; 50%; 75% and 100% plant surface damaged). Six replicates were performed for plants and seeds of each cultivar and for each slug species.

EXPERIMENTAL PLOT RESEARCH

The field experiment was conducted in July 2015 in the Institute of Plant Protection – National Research Institute, Poznań. Four field bean cultivars (two low-tannin cultivars: Albus, Amulet and two high-tannin cultivars: Neptun, Optimal) were sown into each of the sixteen randomly allocated field plots, each 0.16 m^2 in area. The plots were separated by 0.5 m wide strips without vegetation. After two weeks there were 15 plants in each plot at the 5–6 true leaf stage. Eight slug shelters made of plant pot saucers were positioned between the plots. The saucers were 35 cm in diameter and covered with a 1 cm layer of felt and aluminium foil which reflected sunlight. Prior to the ex-

periment, six *A. vulgaris* with an average weight of 2.87 g were placed in each shelter. The extent of damage to plants was determined every two days according to the 5-point scale described above. Four replicates were performed for each cultivar.

The results of all experiments were analysed with ANOVA, and Fisher's test was used at a significance level of a = 0.05 (STATISTICA software v. 10).

RESULTS

DAMAGE TO SEEDS

The effects of slug grazing on the germinating seeds of the field bean cultivars studied were similar for *A. vulgaris* and *A. rufus*. The slugs mostly fed on radicles, endosperm and seed embryos. Hypocotyls, cotyledons and leaf primordia were eaten to a much lesser extent.

After one day of *A. vulgaris* grazing, seeds of the Amulet, Kasztelan and Leo cultivars were significantly more damaged compared to Granit seeds (Table 1). From the second day onwards, seeds of the Optimal cultivar were slightly damaged, whereas those of the Kasztelan and Bobas sustained more damage. This trend continued until the last day of slug grazing. After seven days, Kasztelan and Bobas seeds were significantly more damaged by *A. vulgaris* than Optimal, Olga, Leo and Granit seeds.

After one day of grazing Leo seeds were most damaged and Kasztelan seeds were least damaged by *A. rufus* (Table 2). On the second and third day more damage was observed in seeds of the Granit cultivar. Neptun seeds were more damaged on the fourth and fifth day. Significantly less damage between days 2 and 5 was observed in the Olga cultivar. During the seven days of *A. rufus* grazing, Granit, Leo and Neptun seeds were more damaged compared to those of Olga and Kasztelan. Importantly, these differences were not significant on the seventh day.

CROP DAMAGE AT THE 3–4 LEAF STAGE

The first significant differences in the extent of damage caused by *A. vulgaris* to different field bean cultivars were found after two days of grazing (Table 3). Amulet plants were more damaged

Table 1. Average damage to seeds (%) in different field bean cultivars (*Vicia faba* L.) caused by *A. vulgaris* and Fisher's test results at significance level α = 0.05. Values in columns with at least one letter the same do not differ significantly

Cultivar	Days of slug feeding													
	1		2		3		4		5		6		7	
Albus	4.9	bc	9.0	bcd	12.8	b	16.3	cd	19.1	de	19.8	bcd	21.2	bcd
Amulet	5.9	c	10.4	cd	13.2	bc	15.6	cd	18.0	cde	19.1	abcd	21.2	bcd
Bobas	4.5	bc	10.1	cd	12.5	b	16.7	de	19.1	de	20.5	cd	23.6	cd
Granit	2.1	a	4.9	a	8.7	ab	10.4	ab	11.4	ab	13.5	a	17.7	ab
Kasztelan	5.9	c	11.8	d	17.7	c	21.5	e	23.3	e	24.3	d	24.3	d
Leo	5.9	c	7.3	abc	10.1	ab	12.8	bcd	15.6	bcd	17.0	abc	17.4	ab
Neptun	3.5	ab	6.9	abc	11.1	ab	13.5	bcd	17.0	cd	18.0	abc	19.1	abc
Olga	5.2	bc	7.6	abc	9.7	ab	11.4	abc	13.5	abc	14.6	ab	14.7	a
Optimal	4.2	abc	5.5	ab	6.9	a	7.6	a	9.4	a	13.5	a	14.6	a

Table 2. Average damage to seeds (%) in different field bean cultivars (*Vicia faba* L.) caused by *A. rufus* and Fisher's test results at significance level α = 0.05. Values in columns with at least one letter the same do not differ significantly

Cultivar	Days of slug feeding													
	1		2		3		4		5		6		7	
Albus	2.8	bc	4.5	abc	4.9	ab	5.2	ab	5.5	ab	5.9	a	6.6	a
Amulet	2.8	bc	4.9	bc	6.2	ab	6.9	ab	6.9	ab	8.3	ab	8.7	a
Bobas	1.4	ab	2.4	ab	4.2	ab	5.5	ab	5.9	ab	6.6	ab	6.9	a
Granit	2.8	bc	7.3	c	8.3	b	8.3	ab	8.7	ab	9.7	b	10.1	a
Kasztelan	0.3	a	3.1	ab	4.9	ab	5.2	ab	5.2	a	5.2	a	6.9	a
Leo	3.8	c	6.9	c	7.6	ab	7.6	ab	8.0	ab	9.0	b	9.4	a
Neptun	2.4	abc	4.9	bc	8.0	ab	9.4	b	9.7	b	10.1	b	10.4	a
Olga	1.4	ab	1.4	a	3.8	a	4.5	a	5.2	a	5.2	a	6.2	a
Optimal	2.4	abc	3.5	ab	4.9	ab	5.9	ab	6.2	ab	6.6	ab	6.9	a

Table 3. Average damage to plants (%) at the 3–4 leaf stage across different field bean cultivars (*Vicia faba* L.) caused by *A. vulgaris* and Fisher's test results at significance level $\alpha = 0.05$. Values in columns with at least one letter the same do not differ significantly

Cultivar	Days of slug feeding													
	1		2		3		4		5		6		7	
Albus	10.0	a	15.8	ab	20.0	ab	25.0	ab	28.3	ab	30.8	ab	34.2	ab
Amulet	10.8	a	21.7	b	27.5	b	33.3	b	39.2	b	43.3	b	48.3	b
Bobas	8.3	a	13.3	a	17.5	a	24.2	ab	30.0	ab	32.5	ab	35.8	ab
Granit	10.0	a	12.5	a	15.8	a	24.2	ab	27.5	ab	30.0	ab	31.2	a
Kasztelan	9.2	a	14.2	ab	18.3	ab	20.8	a	23.3	a	25.8	a	26.7	a
Leo	6.7	a	15.0	ab	20.8	ab	25.0	ab	27.5	ab	31.7	ab	35.8	ab
Neptun	6.7	a	9.2	a	15.8	a	21.7	a	25.8	a	32.5	ab	35.0	ab
Olga	9.2	a	15.0	ab	20.0	ab	22.5	ab	30.0	ab	30.0	ab	32.5	a
Optimal	7.5	a	13.3	a	15.8	a	20.8	a	23.3	a	26.7	a	30.8	a

Table 4. Average damage to plants (%) at the 3–4 leaf stage across different field bean cultivars (*Vicia faba* L.) caused by *A. rufus* and Fisher's test results at significance level $\alpha = 0.05$. Values in columns with at least one letter the same do not differ significantly

Cultivar	Days of slug feeding													
	1		2		3		4		5		6		7	
Albus	9.2	b	17.5	c	20.8	c	23.3	b	26.7	b	26.7	b	27.5	ab
Amulet	5.0	ab	8.3	ab	11.7	abc	15.0	ab	19.2	ab	21.7	ab	22.5	ab
Bobas	10.8	b	14.2	bc	20.8	c	24.2	b	26.7	b	30.0	b	33.3	b
Granit	7.5	ab	10.0	abc	15.8	bc	17.5	b	21.7	ab	24.2	ab	25.8	ab
Kasztelan	6.7	ab	12.5	abc	14.2	abc	17.5	b	20.0	ab	21.7	ab	23.3	ab
Leo	7.5	ab	7.5	ab	10.8	ab	14.2	ab	17.5	ab	22.5	ab	23.3	ab
Neptun	7.5	ab	10.8	abc	15.8	bc	18.3	b	23.3	b	25.0	ab	25.8	ab
Olga	7.5	ab	15.0	bc	18.3	bc	20.8	b	25.8	b	27.5	b	28.3	ab
Optimal	1.7	a	4.2	a	5.8	a	6.7	a	9.2	a	12.5	a	15.0	a

compared to Granit, Bobas, Optimal and Neptun. Between the fourth and seventh days of observation, Amulet beans were more damaged than Kasztelan and Optimal cultivars. Throughout the seven days of *A. vulgaris* grazing, the Amulet cultivar was more susceptible to damage than Optimal, Neptun and Kasztelan.

Significant differences in the extent of damage to field bean cultivars caused by *A. rufus* were found after one day of slug grazing (Table 4). Compared to the Optimal cultivar, Albus and Bobas suffered more damage. Similar differences in the extent of damage to the cultivars mentioned above persisted until the last, seventh day of *A. rufus* grazing.

CROP DAMAGE AT THE 5–6 LEAF STAGE

During the experiment the daily air temperature ranged from 2.8 to 24.43°C (mean 18.7°C), humidity from 69.6 to 90.8% (mean 79.4%), while the total precipitation was between 0 and 12.7 mm (mean 3.7 mm). Due to the low precipitation the plots were watered every three days. The number of *A. vulgaris* observed during the day on the plots and in the shelters varied between 33 and 49. The plant damage increased gradually in consecutive days (Fig. 1).

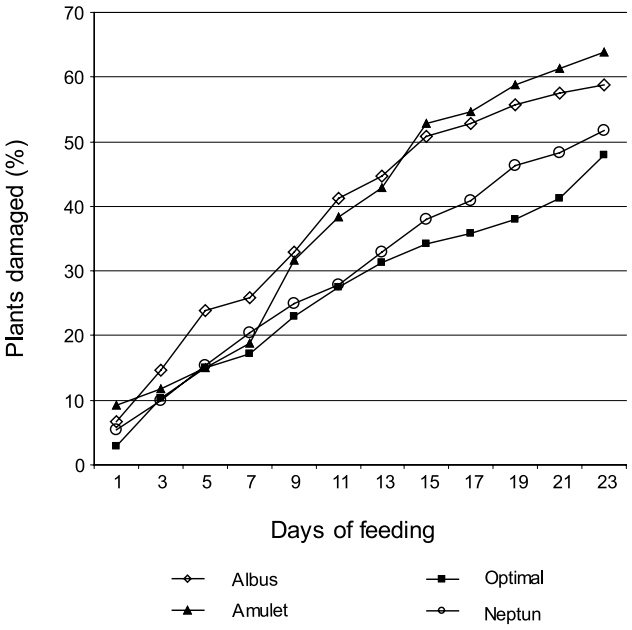

Fig. 1. Average damage to plants (%) at the 5–6 leaf stage across different field bean cultivars (*Vicia faba* L.) caused by *A. vulgaris* and Fisher's test results at significance level $\alpha = 0.05$ (significant differences: Amulet and Optimal from day 15 to 23 of slug grazing)

After one day of *A. vulgaris* grazing, significant differences in the extent of damage were noted between the Amulet and Optimal cultivars. On day 13, the damage to plants of the four cultivars tested was similar. Clear differences in the damage appeared between days 15 and 23 of slug grazing. The Amulet cultivar sustained significantly more damage than the Optimal. As for the extent of damage to plants, similar results for the cultivars tested were obtained in the laboratory experiment (plants at 3–4 leaf stage).

COMPARISON OF DAMAGE TO SEEDS AND PLANTS CAUSED BY *A. VULGARIS* AND *A. RUFUS*

Pooling the results of seed damage revealed significant differences between the two slug species (Tables 1, 2). Granit seeds sustained minor damage from *A. vulgaris* but were severely damaged by *A. rufus*. Conversely, Kasztelan seeds were only slightly damaged by *A. rufus* and severely damaged by *A. vulgaris*. Substantial differences were also found in the extent of damage to seeds across high- and low-tannin cultivars. *A. vulgaris* caused minor damage to seeds of the high-tannin Optimal and Granit cultivars and severe damage to seeds of the high-tannin Bobas and low-tannin Kasztelan. As for *A. rufus*, greater seed damage was recorded for the high-tannin Granit and Neptun cultivars and for the low-tannin Leo.

In the experiments on plants, the high-tannin Optimal cultivar suffered minor damage from both slug species. The extent of damage to the other cultivars was different for the two slug species. *A. vulgaris* caused the greatest damage to the Amulet cultivar, while *A. rufus* – to Bobas.

DISCUSSION

The diverse feeding preferences of slugs and the influence of plant substances result in different susceptibility of plant cultivars to slug grazing and damage. There are documented examples of cultivars and plant forms whose secondary metabolites or specific physical traits may provide defence against slugs (Dirzo & Harper 1982a, b, Kloos & McCullough 1982, Webbe & Lambert 1983, Mølgaard 1986, Stahl 1988, Airey 1989, Moens et al. 1992, Desbuquois & Daguzan 1995, Hanley et al. 1995, Clark et al. 1997, Chevalier et al. 2000, Albrectsen et al. 2004). Such plants are usually less susceptible to slug grazing.

Our studies on the damage caused by *A. vulgaris* and *A. rufus* to the seeds and young plants of the field bean at 3–6 leaf stage with high and low tannin content show that plant cultivars display varying susceptibility to slugs. This study included plants in early development stages, as they are least tolerant to damage inflicted by slugs (Byers 2002). The susceptibility of germinating seeds to damage varied significantly according to both the field bean cultivar and the slug species. The seeds of the high-tannin Optimal and Granit cultivars were less susceptible to damage by *A. vulgaris*, whereas those of the low-tannin Olga were less susceptible to damage by *A. rufus*. In contrast, the seeds of the low-tannin Kasztelan cultivar and high-tannin Bobas were more susceptible to *A. vulgaris*, while seeds of the high-tannin Granit and Neptun and low-tannin Leo were more vulnerable to *A. rufus*. To conclude, severe and minor damage resulting from slug grazing occurred in the seeds of both high- and low-tannin cultivars. Similar results were obtained in the studies on *D. reticulatum* (Kozłowski et al. 2016). This suggests that tannins were not a decisive factor with regard to the extent of field bean damage. On the other hand, the fact that seeds and plants of the high-tannin Optimal cultivar (0.563 mg tannins/g DW) sustained significantly less damage from *A. vulgaris* and *A. rufus* indicates that such an effect of tannins is possible.

Our results do not provide an unambiguous answer to the question whether tannins in the field bean cultivars tested protect the plants against slugs. According to some authors, phenolic compounds and their derivatives, including tannins, may deter slugs from grazing on plants (Mølgaard 1986, Airey 1989, Fritz et al. 2001, Albrectsen et al. 2004). Experiments on leaf discs of willow (*S. eriocephala*) by demonstrated that seedling palatability to *A. subfuscus* was considerably reduced as tannin concentration in the plants increased (Albrectsen et al. 2004). In earlier experiments on leaf discs of *S. sericea* and *S. eriocephala* willows, *A. subfuscus* were repelled at the tannin concentration ranging from 50 to 100 mg/g DW (Fritz et al. 2001). Lower tannin concentration (<5%) did not reduce the palatability of willow leaves for this slug. The data presented suggest that the tannin concentration is important. In the plants we tested, the tannin concentration did not exceed 0.563 mg/g DW. This might explain why slugs did not exhibit a noticeable reaction to these compounds.

As in the case of seeds, the susceptibility of plants to slug damage varied across the cultivars of the field bean, both in the laboratory and in experimental plot studies. The Optimal cultivar was less susceptible to damage by the two slug species. The Amulet cultivar showed higher susceptibility to *A. vulgaris*, while Albus and Bobas were more susceptible to *A. rufus*. Besides, in some cultivars the extent of damage to plants was found to be markedly different from the extent of damage to seeds. Such differences were

observed in Kasztelan in relation to *A. vulgaris* and in Olga in relation to *A. rufus*. It can be suspected that this is associated with different concentrations of plant substances, which change over the course of plant growth.

Our results indicate that the feeding preferences for seeds and plants of the nine field bean cultivars differed for the two examined slug species. This means that each species has a specific range of host plants and is more willing to eat the plants it finds palatable and which, as a result, sustain more damage. This conclusion confirms the results of earlier studies on feeding preferences of slugs and snails in relation to different species of crop and non-crop plants (Cates & Orians 1975, Jennings & Barkham 1975, Dirzo 1980, Webbe & Lambert 1983, Mølgaard 1986, Speiser et al. 1992, Hanley et al. 1995, Cook et al. 1996, Briner & Frank 1998, Clark et al. 1999, Frank & Friedli 1999, Keller et al. 1999, Frank 2003). The reasons behind the varying extent of damage are better understood only for some slug species and plant cultivars. With regard to the field bean, further research is needed to determine the factors and mechanisms which mediate the grazing activity of slugs on particular cultivars of the plant.

The main objective of the experiments reported here was to assess the susceptibility of the field bean cultivars to damage by *A. vulgaris* and *A. rufus*. The results enable us to initially determine which cultivars are highly susceptible to slug damage and which are highly resistant. Once tested in field conditions, the results may prove useful in selecting cultivars of the plant to be grown in areas exposed to the pests in question. Together with various agrotechnical treatments that limit damage caused by slugs and with different combinations of biological control (Wilson et al. 1993, Glen & Moens 2002, Meredith 2003, Douglas & Tooker 2012), limiting the cultivation of crop cultivars more susceptible to slugs will be an important element of integrated plant protection programmes.

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