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EVALUATION OF DAMAGE TO SEEDS AND SEEDLINGS OF DIFFERENT WINTER WHEAT CULTIVARS CAUSED BY *DEROCERAS RETICULATUM* (O.F. MÜLLER, 1774) AND *ARION LUSITANICUS* MABILLE, 1868 IN LABORATORY CONDITIONS

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ABSTRACT: Susceptibility of 18 winter wheat (*Triticum aestivum* L.) cultivars to damage by *Deroceras reticulatum* (O. F. Müll.) and *Arion lusitanicus* Mabille was estimated in laboratory conditions. For each slug species six experiments, two with seeds, two with seedlings at stage 07–12 BBCH and two with seedlings at stage 10–13 BBCH, were performed. The susceptibility to feeding of the two slug species differed between the cultivars; the two slugs differed in their preferences to some of the cultivars. The Nadobna cultivar was the most severely damaged by both species. The cultivars Jawa and Nutka were the least damaged by *D. reticulatum* and *A. lusitanicus,* respectively.

KEY WORDS: slug, pest, *Arion lusitanicus, Deroceras reticulatum*, winter wheat, cultivar

INTRODUCTION

Among all cereals grown as agricultural crops in western and central Europe, the winter wheat (*Triticum aestivum* L.) is the one most seriously and most often damaged by slugs. The increase in the significance of damage caused by these pests to winter wheat within the last 30 years is illustrated, for example, by a 70-fold increase in molluscicide usage in the UK (SPEISER & KISTLER 2002). The most important gastropod pests are slugs of the families Agriolimacidae and Arionidae, and especially the grey field slug *Deroceras reticulatum* (O. F. Müller, 1774) (COOK et al. 1996; GLEN & MOENS 2002). In the last decade, the slug populations and the damage caused by them to wheat and rape in Poland have increased considerably. In plantations of winter wheat in south-western Poland, besides *D. reticulatum*, slugs of the family Arionidae occur. *Arion lusitanicus* Mabille, where it appears in masses, causes up to 100% damage to crops in the margins of fields (KOZŁOWSKI 1999, KOZŁOWSKI & KOZŁOWSKA 2002).

Winter wheat is damaged in early growth stages, mainly soon after sowing. The slugs eat embryos and a part or the whole endosperm, preventing germination and sprouting (PORT & PORT 1986, MARTIN & KELLY 1986, MOENS 1989, GLEN 1994, GLEN et al. 1989, GLEN & MOENS 2002). The second kind of damage involves destruction of whole seedlings after emergence. The leaf sheath and the first developing leaf may be consumed by slugs (GAIR et al. 1987, GLEN & MOENS 2002, KOZŁOWSKI & KOZŁOWSKA 2002). In the following development phases, when the leaves are harder, the intensity of damage is limited. At that stage, slugs injure the tops of leaves and scratch leaf tissue, causing characteristic shredded leaves. Flag leaves of some plants are also eaten, reasulting in decrease in assimilative surface and inhibition of growth, which can lead to decreased harvests (KEMP & NEWELL 1987). However, the most significant damage to winter wheat occurs directly after sowing and during sprouting.

In Poland and other European countries, winter wheat is protected against slugs mainly by the use of molluscicide bait pellets during or immediately after sowing. Moreover, the number of slugs is reduced by numerous cultivation and agricultural measures (GLEN et al. 1990, 1992, GLEN & MOENS 2002, KOZŁOWSKI 2003). Besides these universally applied methods, different possible ways of reducing slug-caused damage to plants are sought. One is the use of cultivars with a limited sensitivity to slug feeding. Potato cultivars differ in their sensitivity to damage caused by *D. reticulatum* (JOHNSTON et al. 1989, SOUTH 1992, JOHNSTON & PEARCE 1994), and winter oilseed rape cultivars – to damage caused by *D. reticulatum* (GLEN et al. 1990, MOENS et al. 1992) or by *A. lusitanicus* (KOZŁOWSKI & KAŁUSKI 2004). Previous attempts at testing the sensitivity of winter wheat cultivars to slug feeding (PORT & PORT 1986, SPAULL & ELDON 1990, COOK et al. 1996, EVANS & SPAULL 1996) gave contradictory results. This was the reason for the investigations described here.

The paper presents the results of laboratory studies on the susceptibility of seeds and seedlings of different winter wheat cultivars to damage caused by the slugs *D. reticulatum* and *A. lusitanicus*.

MATERIAL AND METHODS

The experiments were carried out on 18 winter wheat cultivars (Tables 1, 2), with *D. reticulatum* and *A. lusitanicus*. Adult slugs were collected in the field a few weeks before the experiments and kept in laboratory conditions in clear plastic boxes (temperature 19±2°C; relative humidity RH 95±3%; natural photoperiod). Slug breeding was carried out on loamy and humus soil, in closed and ventilated plastic boxes. The slugs were fed with Chinese cabbage leaves, wheat seeds, carrots, potatoes and wheat bran with calcium carbonate.

The experiments were carried out in laboratory conditions (temperature 17°C during a day, 14°C at night; RH 95±3%; photoperiod 12D:12N). Separate multiple choice tests were carried out for winter wheat seeds (00–03 BBCH; STAUSS 1994), seedlings soon after germination with up to 2 leaves (07–12 BBCH) and for seedlings with 1–3 leaves (10–13 BBCH). These tests were carried out in a nested block design for 18 cultivars with 6 replications, separately for *D. reticulatum* and *A. lusitanicus*. In all six experiments, the percentage damage to winter seeds or seedlings was recorded once a day. Observations were made for the next few days according to the feeding rate of the slugs. In all tests the same scheme of arrangement of cultivars in the experimental units was adopted (Fig. 1).

EXPERIMENTS WITH SEEDS

Five winter wheat seeds of each cultivar were placed in Petri dishes (7 cm diameter) with damp filter paper. For each cultivar six dishes were prepared. Two open dishes with seeds of each cultivar were placed in 3 closed plastic boxes (54.5 × 76.0 × 13.5 cm) with ventilation holes. Then, 10 individuals of *D. reticulatum* or *A. lusitanicus*, starved for 24 h before the experiment, were placed in each box. The mean weight of the slugs was: *D. reticulatum* 0.49 g, *A. lusitanicus* 0.73 g.

EXPERIMENTS WITH SEEDLINGS

Tests with winter wheat seedlings were made in plastic containers (80 × 40 × 20 cm) with a 4.5 cm soil layer, the surface being divided into 36 small plots. On randomly chosen plots seeds of 18 winter wheat cultivars were sown, five seeds of each cultivar on two plots. Then, 10 individuals of *D. reticulatum* (mean weight 0.45 g) or *A. lusitanicus* (mean weight 1.85 g), starved for 24 h before the experiment, were placed in each box.

In tests with winter wheat seedlings, in the first experiment, slugs were placed in boxes 24 hours after the seeds were sown, and damage was recorded from sprouting until the stage of 2 leaves (07–12 BBCH). In the second experiment, slugs were placed in the containers when the plants had 1 leaf, and damage was recorded up to the phase of 3 leaves (10–13 BBCH).

EVALUATION OF DAMAGE AND STATISTICAL ANALYSIS

In all multiple choice tests visual evaluation of damage to seedlings or seeds was applied. A 5–degree

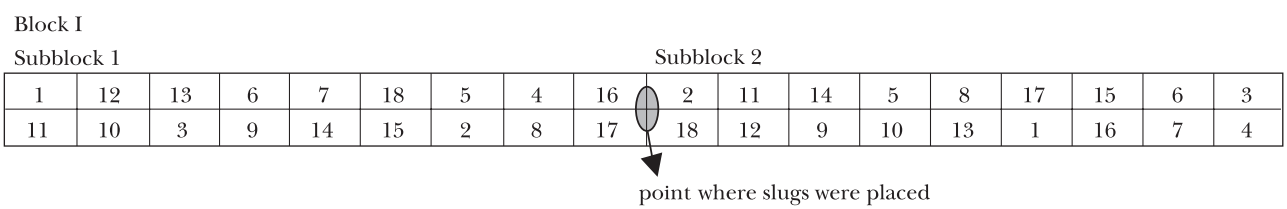

Fig. 1. Example of arrangement of winter wheat cultivars in experimental units (first container: 1–18 particular wheat cultivars)

scale was used: no damage, 25%, 50%, 75%, 100% of plant area consumed. All wheat seeds or plants in the experimental units were subject to visual evaluation. From these observations a weighted mean percentage of damaged surface of wheat seeds or plants was calculated for each experimental unit.

Analysis of data from the six experiments was performed using a nested block design analysis of variance. Multiple pairwise comparisons corresponding to the specific objectives stated in the Introduction were made using Tukey's test at the $\alpha = 0.05$ level.

Diverse results were obtained from multiple choice tests on seeds, seedlings at stage 07–12 BBCH and seedlings at stage 10–13 BBCH, for each *D. reticulatum* and *A. lusitanicus*. To assess the sensitivity of winter wheat cultivars to feeding by each slug species separately, nonparametric statistical method was used. Ranks were given for the mean percentage from three experiments, but the ranking was made independently for the assumed classification factor – nine dates (b=9). The sums of ranks were calculated and the Friedman test at $\alpha = 0.05$ was used. Tukey's test was used for multiple comparisons of treatments. Then the standard deviation of the mean was calculated from the formula $S_d = \sqrt{12^{-1}\, bv(v+1)}$, where v denotes number of treatments and b denotes the number of levels of the classification factor. This analysis was carried out at a significance level $\alpha = 0.05$.

RESULTS

DAMAGE TO SEEDS AND SEEDLINGS OF WINTER WHEAT CAUSED BY *DEROCERAS RETICULATUM*

In our tests for winter wheat seed damage caused by *D. reticulatum,* seeds of the Sakwa and Nadobna cultivars were stronger damaged (Table 1). There was slight damage to seeds of the Symfonia, Jawa and Kris cultivars. Statistically significant differences between the percentage of seed damage of the examined winter wheat cultivars were noted after the first day of slug feedisng (p=0.045, Table 1). After three days of slug feeding, the percentage of seed damage of the Sakwa cultivar (36.7%) was statistically significantly higher, compared to the Symfonia (7.5%) and Jawa (8.3%) cultivars. After four days, statistically significant differences were found between seed damage of

Table 1. The percentage damage to winter wheat seeds and seedlings caused by *D. reticulatum* in multiple choice tests, significance level p for Fisher-Snedecor test and results of Tukey's test at α=0.05

Cultivar	Seeds at stage 00–03 BBCH						Seedlings at stage 07–12 BBCH				Seedlings at stage 10–13 BBCH			
	Day 1		Day 3		Day 4		Day 5	Day 7	Day 10		Day 1	Day 3	Day 6	
	p=0.045		p=0.007		p=0.010		p=0.444	p=0.073	p=0.027		p=0.642	p=0.744	p=0.002	
Clever	13.3	a	28.5	ab	30.0	ab	28.3	26.7	30.8	a	14.2	27.5	46.7	ab
Elena	5.0	a	14.2	ab	15.0	ab	27.5	34.2	42.5	a	18.3	25.8	52.5	ab
Jawa	0.0	a	8.3	b	14.2	ab	16.7	17.5	20.8	a	3.3	15.0	31.7	b
Kobiera	15.8	a	28.3	ab	27.5	ab	20.0	30.8	32.5	a	15.8	33.3	46.7	ab
Kobra	5.0	a	25.0	ab	24.2	ab	22.5	36.7	38.3	a	15.0	27.5	58.3	ab
Korweta	0.0	a	10.8	ab	20.8	ab	21.7	36.7	40.0	a	19.2	34.2	76.7	a
Kris	3.3	a	13.3	ab	12.5	b	25.8	39.2	49.2	a	5.8	27.5	45.0	ab
Mewa	5.8	a	19.2	ab	22.5	ab	20.0	27.5	35.0	a	8.3	35.0	52.5	ab
Mobela	5.8	a	18.3	ab	22.5	ab	13.3	17.5	29.2	a	21.7	38.3	57.5	ab
Nadobna	19.2	a	25.8	ab	32.5	ab	25.8	36.7	45.0	a	19.2	35.0	50.0	ab
Nutka	5.8	a	20.0	ab	23.3	ab	20.8	25.8	30.8	a	1.7	14.2	33.3	b
Roma	4.2	a	19.2	ab	17.5	ab	20.0	24.2	29.2	a	20.0	34.2	50.0	ab
Rywalka	10.8	a	24.2	ab	23.3	ab	13.3	21.7	26.7	a	12.5	28.3	42.5	ab
Sakwa	14.7	a	36.7	a	40.0	a	30.8	36.7	42.5	a	26.7	35.0	43.3	ab
Sukces	5.0	a	9.2	ab	16.7	ab	12.5	25.0	32.5	a	8.3	19.2	33.3	b
Symfonia	0.0	a	7.5	b	9.2	b	13.3	20.8	25.8	a	13.3	22.5	34.2	b
Tonacja	5.0	a	16.7	ab	15.8	ab	16.7	28.3	40.0	a	7.5	22.5	37.5	b
Zyta	0.8	a	10.0	ab	13.3	ab	11.7	16.7	22.5	a	10.8	32.5	47.5	ab

a, b – values followed by the same letter within columns do not differ statistically in Tukey's test

Table 2. The percentage damage to winter wheat seeds and seedlings caused by *A. lusitanicus* in multiple choice tests, significance level p for Fisher-Snedecor test and results of Tukey's test at α=0.05

Cultivar	Seeds at stage 00–03 BBCH						Seedlings at stage 07–12 BBCH			Seedlings at stage 10–13 BBCH					
	Day 1		Day 3		Day 4		Day 5	Day 7	Day 10	Day 1		Day 3	Day 6		
	p=0.027		p=0.011		p=0.005		p=0.183	p=0.095	p=0.089	p<0.001		p=0.128	p=0.072		
Clever	9.2	a	34.2	a	38.3	ab	4.2	12.5	20.0	4.2	b	34.2	46.7		
Elena	1.7	a	15.8	a	20.8	ab	4.2	10.8	22.5	11.7	b	28.3	41.7		
Jawa	1.7	a	11.7	a	15.0	ab	2.5	11.7	18.3	6.7	b	16.7	35.0		
Kobiera	15.8	a	37.5	a	38.3	ab	15.5	25.0	34.2	15.8	ab	37.5	39.2		
Kobra	8.3	a	25.0	a	30.0	ab	5.8	13.3	25.0	4.2	b	26.7	35.8		
Korweta	5.8	a	28.3	a	30.8	ab	9.2	15.8	30.0	19.2	ab	34.2	45.8		
Kris	17.5	a	35.0	a	35.8	ab	8.3	15.0	21.7	2.5	b	19.2	38.3		
Mewa	2.5	a	10.0	a	10.8	ab	8.3	14.2	23.3	5.0	b	33.3	36.7		
Mobela	20.0	a	44.2	a	45.0	ab	12.5	24.2	28.3	41.7	a	44.2	51.7		
Nadobna	28.3	a	46.7	a	53.3	a	20.8	31.7	40.0	20.0	ab	29.2	49.2		
Nutka	0.8	a	11.7	a	12.5	ab	1.7	5.8	15.0	0.8	b	25.0	42.5		
Roma	14.2	a	32.5	a	38.3	ab	11.7	18.3	35.8	18.3	ab	35.8	50.0		
Rywalka	5.8	a	32.5	a	35.0	ab	0.8	6.7	15.0	17.5	ab	40.0	53.3		
Sakwa	0.8	a	1.7	a	4.2	b	5.8	15.0	22.5	10.8	b	28.3	30.8		
Sukces	6.7	a	18.3	a	21.7	ab	6.7	13.3	24.2	10.0	b	32.5	46.7		
Symfonia	2.5	a	9.2	a	9.2	ab	3.3	8.3	20.8	20.0	ab	40.0	47.5		
Tonacja	14.2	a	29.2	a	31.7	ab	8.3	15.0	24.2	11.7	b	29.2	42.5		
Zyta	5.0	a	13.3	a	16.7	ab	6.7	8.3	15.8	2.5	b	23.3	33.3		

a, b – values followed by the same letter within columns do not differ statistically in Tukey's test

the Sakwa cultivar (40%) and the Symfonia and Kris cultivars (9.2% and 12.5%, respectively).

In experiments with wheat seedlings with up to 2 leaves (07–12 BBCH), statistically significant differences in damage to particular cultivars appeared after 10 days of observations (p=0.027, Table 1). Seedlings of the Kris, Nadobna, Elena and Sakwa cultivars were rather seriously damaged, while those of Jawa, Zyta and Symfonia were only slightly damaged.

At the stage of 1–3 leaves (10–13 BBCH), *D. reticulatum* caused the most severe damage to plants of the Korweta cultivar (Table 1). Statistically significant differences between damage to seedlings of different winter wheat cultivars were noted on the 6th day of slug feeding. The percentage of plant injury for the Korweta cultivar after six days of feeding was 76.7%, and was statistically significantly different from the damage to the plants of other five cultivars: Jawa (31.7%), Nutka and Sukces (33.3%), Symfonia (34.2%) and Tonacja (37.5%) (Table 1). The Kobra and Mobela cultivars were also strongly damaged, but the observed differences were not statistically significant.

The cumulative analysis (Table 3) of damage to seeds and seedlings (07–12 and 10–13 BBCH) caused by *D. reticulatum* showed that the most seriously damaged cultivars were Nadobna and Sakwa. Also strongly damaged were Clever, Kobra and Kobiera. Jawa was the cultivar the least damaged by *D. reticulatum*. The Symfonia cultivar was also only slightly damaged.

DAMAGE TO SEEDS AND SEEDLINGS OF WINTER WHEAT CAUSED BY *ARION LUSITANICUS*

Observations of damage to wheat seeds caused by *A. lusitanicus* showed statistically significant differences between some of the examined cultivars after the first day of slug feeding (p=0.027, Table 2). After four days of feeding, the percentage of seed damage for the Nadobna cultivar (53.3%) was statistically significantly higher than that for the Sakwa cultivar (4.2%). Apart from the Nadobna cultivar, seeds of the Mobela cultivar (45.0%) were also strongly injured. The least damaged cultivars at the seed phase were Sakwa (4.2%) and Symfonia (9.2%).

Damage to winter wheat seedlings with up to 2 leaves (07–12 BBCH) did not show statistically significant differences between the cultivars (Table 2). Seedlings of the Nadobna cultivar were fairly much dam-

Table 3. Combined comparison of susceptibility of different winter wheat cultivars to damage caused by *D. reticulatum* or *A. lusitanicus* by sums of ranks, results of Friedman test and Tukey's test at α=0.05

Cultivar	Ranks sums			
	Deroceras reticulatum		*Arion lusitanicus*	
Clever	112.5	ab	84.5	abcd
Elena	97.5	abc	62.5	bcd
Jawa	23.0	c	37.0	cd
Kobiera	111.0	ab	130.0	ab
Kobra	111.5	ab	73.0	abcd
Korweta	98.5	abc	109.0	abc
Kris	82.5	abc	85.5	abcd
Mewa	98.5	abc	65.5	bcd
Mobela	97.5	abc	150.0	a
Nadobna	141.5	a	148.0	a
Nutka	65.0	abc	26.5	d
Roma	84.5	abc	133.5	ab
Rywalka	78.0	abc	85.5	abcd
Sakwa	141.5	a	48.0	cd
Sukces	49.0	bc	85.0	abcd
Symfonia	35.5	bc	72.5	abcd
Tonacja	67.5	abc	100.5	abcd
Zyta	44.0	bc	42.5	cd

a, b – values followed by the same letter within columns do not differ statistically in Tukey's test

aged, while only a slight damage was suffered by Nutka, Rywalka and Zyta.

At the stage of 1–3 leaves (10–13 BBCH), *A. lusitanicus* caused the strongest damage to plants of the Mobela cultivar (Table 2). The percentage (41.7%) of damage to plants of this cultivar after the first day of slug feeding was statistically significantly higher than that recorded for other eleven examined cultivars. On successive days, the strongly damaged cultivars, besides Mobela, were Rywalka, Roma, Nadobna and Symfonia, whereas Sakwa and Zyta were only slightly damaged, but the differences were not statistically significant.

The cumulative analysis (Table 3) of damage to seeds and seedlings (07–12 and 10–13 BBCH) of the examined cultivars caused by *A. lusitanicus* showed that the most seriously damaged were the Mobela and Nadobna cultivars; Nutka was the least damaged.

DISCUSSION

The observations on the extent and rate of damage to winter wheat seeds and seedlings caused by slugs *D. reticulatum* and *A. lusitanicus* showed statistically significant differences in the susceptibility to slug feeding between some cultivars. Among the 18 winter wheat cultivars examined in laboratory conditions, the most seriously damaged by both slug species was the Nadobna cultivar. The susceptibility of other winter wheat cultivars to damage was dependent on the slug species. Apart from the Nadobna cultivar, *D. reticulatum* damaged plants of the Sakwa cultivar the most seriously, whereas *A. lusitanicus* caused the most serious damage to the Mobela cultivar. The least damaged by *D. reticulatum* was the Jawa cultivar, while *A. lusitanicus* caused the least damage to the Nutka cultivar. We conclude that some wheat cultivars differ in their tastiness for particular slug species. This is a result of the specific food preferences of slugs and their different reactions to the chemical ingredients in plant food. The Sakwa cultivar is a good example, its seeds being preferred by *D. reticulatum* but not readily accepted by *A. lusitanicus* (Tables 1–3).

The reasons for the observed differences in susceptibility of seeds and seedlings to slug feeding have not been analysed. They are probably associated with specific biochemical properties of particular winter wheat cultivars, but this needs additional detailed experiments. Studies by other authors (PORT & PORT 1986, SPAULL & ELDON 1990, EVANS & SPAULL 1966) show different susceptibility of winter wheat cultivars

to slug feeding, which may arise from the different content of basic food ingredients in the seeds. PORT & PORT (1986) have found that the different susceptibility of winter wheat cultivars to damage caused by *D. reticulatum* is correlated with the total nitrogen content in seeds. SPAULL & ELDON (1990) have shown that differences in susceptibility of winter wheat cultivars to slug feeding are determined by the amount of nutrients extracted from the seeds. In their laboratory experiments, *D. reticulatum* preferred seeds of the Avalon cultivar to those of the Parade cultivar, and the differences were statistically significant. Although the reasons for this preference have not been explained, SPAULL & ELDON (1990) suggest a higher sugar content and a higher quantity of nutrients in the seeds of the Avalon cultivar, which may act as local attractants. The different susceptibility of winter wheat cultivars to *D. reticulatum* feeding has not been confirmed in laboratory experiments carried out by COOK et al. (1996), thought the tests used similar wheat cultivars as those in SPAULL & ELDON's (1990) study. According to COOK et al. (1996) "the cultivars possess no inherent differences in palatability to *D. reticulatum*".

Differences in susceptibility of cultivars to *D. reticulatum* feeding, among plants other than wheat, have been observed for potatoes (JOHNSTON et al. 1989, SOUTH 1992, JOHNSTON & PEARCE 1994) and rape (GLEN et al. 1990, MOENS et al. 1992). Differences relating to *A. lusitanicus* feeding have been found for rape (KOZŁOWSKI & KAŁUSKI 2004). The genotypes of cultivated plants which are more resistant to slug feeding may provide important sources of protection against these pests. The different susceptibilities, observed in the experiments described here, of winter wheat cultivars to damage caused by slugs may be of consequence in practice. Selection of cultivars less susceptible to slug feeding on plantations threatened by slugs may be a useful factor in plant protection.

CONCLUSIONS

1. Susceptibility of seeds and seedlings of some winter wheat cultivars to damage caused by *D. reticulatum* and *A. lusitanicus* is very different.
2. Among the tested winter wheat cultivars the most susceptible to damage caused by *D. reticulatum* were Nadobna and Sakwa cultivars, while seeds and seedlings of the Jawa cultivar were the least damaged.
3. The Mobela and Nadobna cultivars were the most susceptible to damage caused by *A. lusitanicus*; the Nutka cultivar was the least damaged by this slug species.

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