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DAMAGE TO THE LEGUME (FABACEAE) AND RAPESEED (BRASSICACEAE) PLANTS CAUSED BY *ARION VULGARIS* MOQUIN-TANDON, 1855, *A. RUFUS* (LINNAEUS, 1758) AND *DEROCERAS RETICULATUM* (O. F. MÜLLER, 1774)

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ABSTRACT: In Poland, the most common pest slugs in agricultural crops are *Arion vulgaris* Moquin-Tandon, *Deroceras reticulatum* (O. F. Müller), and locally occurring *Arion rufus* (Linnaeus). They cause significant damage to winter oilseed rape and winter wheat. In recent years, they have also occurred in cultivations of various legume species (Fabaceae). However, information on the extent of damage caused by these species is still insufficient. To assess the extent of damage, young plants of common sainfoin, bird's-foot trefoil, red clover, alfalfa, white melilot, and oilseed rape were exposed to grazing by the slug species investigated under laboratory conditions. It was found that oilseed rape plants were more susceptible to damage by the slugs studied compared to bird's-foot trefoil plants. The susceptibility of the other plants studied varied depending on the slug species.

KEY WORDS: slugs, Fabaceae, damage

INTRODUCTION

Legume plants contribute to proper nitrogen management in the soil by nitrogen fixation and are a valuable component of nutritive fodders due to the high nutritional value of their proteins. However, they are also attractive food for numerous pests, including slugs (Gastropoda: Arionidae, Agriolimacidae). *Deroceras* and *Arion* are the most important genera of pest slugs which damage legume crops and may affect their productivity and durability (RUNHAM & HUNTER 1970, SOUTH 1992, HANLEY 1998, BYERS 2002, BROOKS et al. 2003, KOZŁOWSKI & JASKULSKA 2014, KOZŁOWSKI et al. 2017a, b). The plants frequently attacked by these slugs are red clover (*Trifolium pratense* L.), alfalfa (*Medicago sativa* L.), narrow-leaved lupin (*Lupinus angustifolius* L.), yellow lupin (*L. luteus* L.) and field beans (*Vicia faba* L.). The slugs damage seed embryos and destroy seedlings after the emergence of leaves, leading to significant plant damage and crop loss.

The degree of plant damage caused by slugs tends to vary. This is related to the content of nutritional components and, above all, to secondary plant metabolites which are specific to each plant species or cultivar. They have great influence on slug feeding behaviour and, consequently, the extent of plant damage (DIRZO & HARPER 1982, AGUIAR & WINK 1999, ESTER & TRUL 2000, MOENS & GLEN 2002, KOZŁOWSKI et al. 2016, 2017a). Due to the presence of metabolites each plant species has its own characteristic smell and taste which in turn makes it attractive or repellent to slugs. The fact that slugs feed on plants which they find most palatable results in their selective grazing, and influences the susceptibility of plants to damage (HANLEY

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et al. 1995, Cook et al. 1996, Frank & Friedli 1999). Identifying the degree of susceptibility of different plant species is an important element of developing protection strategies against slugs.

The aim of the present research was to evaluate the extent of damage caused by *Arion vulgaris* Moquin-Tandon, 1855, *Arion rufus* (Linnaeus, 1758) and *Deroceras reticulatum* (O. F. Müller, 1774) to five plant species of the legume family as compared with the damage to oilseed rape. The three slug species are among the most harmful ones in Europe. *A. vulgaris* is an invasive species, growing to a length of 12 cm. It lives approximately one year and produces a single generation. In the last 50 years it has spread across many countries of Western, Central and Northern Europe (Frank 1998, Anderson 2005, Kozłowski 2012, Welter-Schultes 2012). It occurs in various habitats, usually as a synanthropic species. As well as being a pest of vegetables and decorative plants cultivated in gardens, it also causes damage to crops, especially on field edges. *A. rufus* reaches 15 cm in length (Wiktor 1973). It has a one-year life cycle and produces a single generation. It is native to Western Europe and parts of Central Europe (Wiktor 1973). It lives in the British Isles and Scandinavia (Anderson 2005). As an alien invasive species, it also occurs in the USA and Canada (Forsyth 2004). The slug prefers damp habitats: lake and pond shores, river banks, forests, thickets and meadows. It causes damage to vegetables and some crops (Kozłowski 2012). *D. reticulatum* reaches 4.5 cm in length and produces one or two generations in a year. It is common in Europe, particularly in its central part (Wiktor 2000). It has also been widely introduced to Caucasian countries, Central Asia, North and South America, South Africa, Australia, Tasmania and New Zealand (Forsyth 2004). The species is commonly found in open habitats, much less frequently in forests and thickets. Its massive populations often occur in crops and it is regarded as one of the most dangerous pests of horticultural and agricultural crops (South 1992). Although the three slug species are increasingly found in legume cultivations, information on the extent of damage to different species of these plants is still insufficient. This study is an attempt to fill this gap in the knowledge.

MATERIAL AND METHODS

Experiments assessing the rate and extent of damage to five legume species exposed to three slug species were performed in laboratory conditions. The following species were studied: common sainfoin (*Onobrychis vicifolia* Scop.) – 'Taja' cultivar, bird's-foot trefoil (*Lotus corniculatus* L.) – 'Skrzeszowska' cultivar, red clover (*Trifolium pratense* L.) – 'Rozeta' cultivar, alfalfa (*Medicago sativa* L.) – 'Perfecta' cultivar, white melilot (*Melilotus albus* L.) – 'Adela' cultivar and, as a comparative plant, oilseed rape (*Brassica napus* var. *napus* L.) – 'Sy Saveo' cultivar. The plants were exposed to grazing by *A. vulgaris*, *A. rufus* and *D. reticulatum*. The slugs came from populations found in horticultural crops in the environs of Poznań (*A. vulgaris*, *D. reticulatum*) and Wronki (*A. rufus*). Young slugs collected in the spring 2017 were kept in containers filled with 5 cm of soil, at 17 °C and a photoperiod of 12 hours. They were fed on pieces of vegetables (Chinese cabbage leaves, potato tubers) and wheat bran, changed twice a week. Before each experiment, the slugs were starved for 48 hours and then weighed. Individuals with the most similar weight were selected. The plants at 3–4 leaf stage were grown in raised beds in the greenhouse of the Institute of Plant Protection, National Research Institute in Poznań.

1	4	2	5	2	6
5	3	6	3	1	4

3	6	4	6	3	1
1	5	2	4	2	5

4	1	5	2	5	3
2	6	3	6	4	1

Fig. 1. Nested block design – diagram of arrangement of treatments (plant species) on experimental units (plots), where numbers denote: 1 – common sainfoin ('Taja'); 2 – bird's-foot trefoil ('Skrzeszowska'); 3 – white melilot ('Adela'); 4 – alfalfa ('Perfecta'); 5 – red clover ('Rozeta'); 6 – oilseed rape ('Sy Saveo'). Three plants per plot

Three experiments were performed using a nested block design (Fig. 1). In each experiment, 108 plants were placed in three containers (3 superblocks) divided into two parts (2 blocks) such that there were three plants of one species on each plot. Eighteen plants of each species on six plots were planted in containers 72 × 35 × 15 cm in size in a 5 cm-thick layer of soil. After two days, six slugs of one species were placed in the central part of each containers. The average weight of the slugs was: *A. vulgaris* – 1.47 g, *A. rufus* – 1.53 g, and *D. reticulatum* – 0.41 g. The experiments were conducted in an environmental chamber with the air temperature of 17 °C, RH 90%±3% and the photoperiod of 12 hours. Damage to plants was assessed once a day for seven days on a 5-point scale (0; 25; 50; 75 and 100% of plant surface damaged).

Six replicates were performed for all the plant and slug species. The results were analysed with ANOVA, and the differences were assessed with the F-test at the level of significance $\alpha = 0.05$ (STATISTICA software v. 12).

RESULTS

First significant differences in the extent of plant damage occurred after the first day of grazing for all slug species. *A. vulgaris* caused significantly more damage to oilseed rape in comparison with other plants, except for alfalfa (Table 1). Bird's-foot trefoil and red clover were the least damaged after 24 h. After two days, oilseed rape, alfalfa and common sainfoin were more severely damaged, with no significant differences in the extent of damage. From the second day until the end of the observation, the plants of bird's-foot trefoil were significantly less damaged compared to the other plant species. After four days, oilseed rape plants sustained 100% damage, while bird's-foot trefoil plants – only 33%.

After one day of grazing, *A. rufus* caused significantly more damage to oilseed rape and common sainfoin than to the other species (Table 2). From the second day of observation, the plants of oilseed rape and sainfoin were significantly more damaged than the plants of red clover, bird's-foot trefoil, alfalfa and white melilot. After seven days, 100% of oilseed rape plants were damaged, while the least damaged plant species was red clover (40%). Altogether, during the entire period of slug grazing, *A. rufus* caused the most damage to oilseed rape and common sainfoin, and the least damage to red clover and bird's-foot trefoil.

After the first day of grazing, *D. reticulatum* caused significantly more damage to oilseed rape, red clover and white melilot than to bird's-foot trefoil (Table 3). From the second day of grazing until the end of the experiment, the slug caused severe damage also to alfalfa. Common sainfoin sustained little damage. After seven days, the most damaged plants were white melilot (94%) and oilseed rape (89%), whereas the least damaged ones were bird's-foot trefoil (14%) and common sainfoin (33%). During the seven days of grazing, *D. reticulatum* caused more damage to white melilot, rape and alfalfa than to bird's-foot trefoil and common sainfoin.

The experiments performed showed that all the investigated slug species preferred oilseed rape plants, while bird's-foot trefoil was the least accepted species. Plant susceptibility to the damage caused by *A. vulgaris* was similar across all the species except for bird's-foot trefoil, while in the case of *A. rufus* and *D. reticulatum* it varied for most of the plants investigated.

Table 1. Damage caused by *Arion vulgaris* to different plant species [%] and results of Fisher's test; significance level $\alpha = 0.05$

Plant species	Days of grazing						
	1	2	3	4	5	6	7
Common sainfoin (*Onobrychis vicifolia*)	26.4 ab	69.4 c	81.9 cd	88.9 bc	90.3 bc	100.0 b	100.0
Bird's-foot trefoil (*Lotus corniculatus*)	11.1 a	19.4 a	25.0 a	33.3 a	40.3 a	54.2 a	73.6
Red clover (*Trifolium pratense*)	13.9 a	36.1 b	59.7 b	77.8 b	84.7 b	98.6 b	100.0
Alfalfa (*Medicago sativa*)	43.1 bc	66.7 c	77.8 c	84.7 bc	87.5 bc	95.8 b	100.0
White melilot (*Meliotus albus*)	33.3 b	43.1 b	68.1 bc	83.3 b	87.5 bc	95.8 b	100.0
Oilseed rape (*Brassica napus*)	59.7 c	80.6 c	98.6 d	100.0 c	100.0 c	100.0 b	100.0

Values in columns marked with at least one same letter do not differ significantly.

Table 2. Damage caused by *Arion rufus* to different plant species [%] and results of Fisher's test; significance level $\alpha = 0.05$

Plant species	Days of slug feeding						
	1	2	3	4	5	6	7
Common sainfoin (*Onobrychis vicifolia*)	44.4 c	83.3 c	87.5 c	91.7 c	91.7 c	91.7 c	94.4 cd
Bird's-foot trefoil (*Lotus corniculatus*)	13.9 ab	23.6 ab	27.8 a	34.7 a	36.1 a	44.4 a	50.0 a
Red clover (*Trifolium pratense*)	6.9 a	13.9 a	20.8 a	30.6 a	31.9 a	34.7 a	40.3 a
Alfalfa (*Medicago sativa*)	26.4 b	34.7 b	43.1 b	56.9 b	65.3 b	69.4 b	73.6 b
White melilot (*Meliotus albus*)	18.1 ab	30.6 b	43.1 b	52.8 b	65.3 b	68.1 b	77.8 bc
Oilseed rape (*Brassica napus*)	58.3 c	80.6 c	91.7 c	95.8 c	97.2 c	97.2 c	100.0 d

Values in columns marked with at least one same letter do not differ significantly.

Table 3. Damage caused by *Deroceras reticulatum* to different plant species [%] and results of Fisher's test; significance level $\alpha = 0.05$

Plant species	Days of slug feeding						
	1	2	3	4	5	6	7
Common sainfoin (*Onobrychis vicifolia*)	9.7 ab	12.5 a	15.3 a	16.7 a	25.0 a	29.2 b	33.3 b
Bird's-foot trefoil (*Lotus corniculatus*)	2.8 a	2.8 a	5.6 a	6.9 a	9.7 a	11.1 a	13.9 a
Red clover (*Trifolium pratense*)	20.8 b	34.7 b	45.8 b	48.6 b	56.9 b	62.5 c	73.6 c
Alfalfa (*Medicago sativa*)	11.1 ab	31.9 b	48.6 b	65.3 c	70.8 b	75.0 cd	86.1 cd
White melilot (*Meliotus albus*)	20.8 b	33.3 b	50.0 b	63.9 c	70.8 b	84.7 d	94.4 d
Oilseed rape (*Brassica napus*)	18.1 b	36.1 b	41.7 b	51.4 bc	62.5 b	72.2 cd	88.9 d

Values in columns marked with at least one same letter do not differ significantly.

DISCUSSION

The primary aim of the study was to assess the susceptibility of the selected legumes and oilseed rape to grazing and damage caused by *A. vulgaris*, *A. rufus* and *D. reticulatum*. The tests were performed on plants in their early stage of growth (3–4 true leaf stage), i.e. when they are most vulnerable to slug grazing (Byers 2002, Barlow et al. 2013). The method used in the experiments was choice tests which allow for assessing which plants are the most attractive food for slugs. Our results showed that oilseed rape was preferred by all the investigated slug species. *A. vulgaris* and *A. rufus*, caused, respectively, 100% and 96% damage to the plants after four days of grazing. In the case of *D. reticulatum*, whose weight was three times lower compared to the other species, the rate of damage was lower. However, after seven days of grazing, the damage caused by this species was almost 90%. The high palatability of oilseed rape has been known since the emergence of cultivars with lower glucosinolate content (6–12 mg g^{-1} in seeds). Glucosinolates are known to have a deterrent effect on slugs (Port & Port 1986, South 1992, Byrne & Jones 1996, Frank 1998, Moens & Glen 2002). In the past, rape cultivars used to contain high amounts of these substances (70–90 mg g^{-1} in seeds), which made them very unattractive to slugs. As a result, the extent of damage was negligible from the economic point of view (Moens & Glen 2002). The present study showed that, apart from oilseed rape, *A. rufus* and *D. reticulatum* also caused severe damage to alfalfa, while *A. rufus* – to common sainfoin, which was little damaged by *D. reticulatum*. *D. reticulatum* also preferred white melilot, while *A. rufus* showed little preference towards red clover and bird's-foot trefoil. High preference for white melilot shown by *D. reticulatum* and little acceptance of red clover observed in *A. rufus* was also found in a previous study (Kozłowski et al. 2017b) in which the plants were compared with white mustard, serradella and vetch. The results of the present study indicate that food preferences of *A. rufus* and *D. reticulatum* are more varied with respect to the plant species investigated than those demonstrated by *A. vulgaris*. A similar conclusion was formulated in an earlier study (Kozłowski et al. 2017b).

An interesting observation is the small susceptibility of bird's-foot trefoil (*L. corniculatus*) to damage caused by *A. vulgaris*, *A. rufus* and *D. reticulatum*. Similar results were obtained with regard to other slug species. Based on their laboratory studies, Byers & Bierelein (1982) concluded that *D. reticulatum*, *D. laeve* and *A. fasciatus* preferred seedlings of red clover and alfalfa to those of bird's-foot trefoil. These findings generally corroborated the data from field experiments which demonstrated a low susceptibility of bird's-foot trefoil in comparison with alfalfa (Byers 2002). It is known that some forms of bird's-foot trefoil (*L. corniculatus*) and white clover (*Trifolium repens*) may contain cyanogenic glycosides which have a deterrent effect on many herbivores, including slugs (Jones 1966, Dirzo & Harper 1982). Once damaged by a herbivore, these compounds release hydrogen cyanide (HCN). It is toxic to animals, primarily due to the fact that it inhibits the activity of cytochrome oxidase in the mitochondrial respiratory chain. Using choice tests, Jones (1966) showed that *D. reticulatum* first fed on non-cyanogenic forms of *L. corniculatus* and began to eat the cyanogenic ones only when the others were not available. This shows that slugs tend to avoid plants containing these substances. Plants of bird's-foot trefoil used in our research may have contained cyanogenic glycosides which inhibited slug grazing. This possibility will be explored in further studies on the extent of damage to cyanogenic and non-cyanogenic forms of bird's-foot trefoil and white clover caused by slugs.

The results of the present study show that the susceptibility of legume plants to damage caused by slugs varies. Determination of the extent of plants' susceptibility to damage caused by slugs has an important application in practice. After verification in field conditions, these data will be used to predict the extent of damage to legumes, and implemented in programmes of integrated protection of these plants in areas inhabited by *A. vulgaris*, *A. rufus* and *D. reticulatum*.

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