PALATABILITY AND CONSUMPTION OF 95 SPECIES OF HERBACEOUS PLANTS AND OILSEED RAPE FOR *ARION LUSITANICUS* MABILLE 1868

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Abstract Arion lusitanicus *Mabille (=* A. vulgaris *Moquin-Tandon) causes significant crop damage in some parts of Poland. In the course of research into alternative methods of limiting the damage caused by* A. lusitanicus *to crop plants, no-choice tests were carried out on the palatability and consumption of 95 species of herbaceous plants and oilseed rape. In controlled laboratory conditions, over six consecutive days, the damage to the plants caused by the slug was observed (in the 2–3 leaf phase). For each tested plant species, a palatability index (P.I.) and a consumption index (C.I.) were determined. As a result of the observations the slugs' feeding behaviour was described, palatable and unpalatable plants were distinguished, and the degree of acceptance of those plants by the slug was determined.* A. lusitanicus *showed differentiated preferences in respect of various plant species. Approximately 23% were unpalatable plants, and 48% low-palatability plants. Unpalatable plants included such species as* Impatiens roylei, Geranium robertianum, Saponaria officinalis, Glechoma hederacea, Epilobium palustre *and* Geum urbanum. *26% of the tested species are palatable to the slug. These include* Artemisia dracunculus, Tripleurospermum inodorum, Datura stramonium, Lamium amplexicaule, Myosotis arvensis, Satureja hortensis *and* Conium maculatum. *The remaining two species* (Ocimum basilicum, Trifolium repens) *have palatability equal to that of the control plant* Brassica napus. *It was noticed that the level of acceptance of particular plant species by* A. lusitanicus *was not affected by the plants' life cycle and their family membership. It was found that there was highly significant differentiation in slugs' feeding behaviour depending on the plant species.*

Key words Arion lusitanicus, Arion vulgaris, *oilseed rape, herbaceous plants, palatability index, consumption index*

Introduction

In certain countries of central and western Europe, slugs (Gastropoda: Pulmonata: Stylommatophora) are among the chief pests for winter rape, winter wheat, and certain vegetables and ornamental plants (Martin & Kelly, 1986; Glen *et al.*, 1993; Mesh, 1996; Frank, 1998a, 1998b; Kozłowski & Kozłowska, 2002; Moens & Glen, 2002). Apart from cultivated plants, they also cause damage to many species of weeds and wild herbs. In spite of their wide nutritional range, slugs demonstrate specific preferences in relation to vegetable feeding matter. This is proven by the results of research into the palatability of plants and the degree to which they are accepted by slugs. Numerous experiments have been carried out on the palatability of various species of plants, using their seeds (Cook *et al.*, 1996, 1997), seedlings (Cook *et al.*, 1996; Frank & Friedli, 1999; Keller *et al.*, 1999; Kozłowski & Kałuski, 2004; Kozłowski & Kozłowska, 2000, 2003, 2004; Schädler *et al.*, 2005), leaves (Duval, 1971; Otte, 1975; Mølgaard, 1986; Cook *et al.*, 1996, 1997) or leaf parts (Cates & Orians, 1975; Dirzo, 1980; Cook *et al.*, 1997; Briner & Frank, 1998). Research has been done into the food preferences of slugs and snails, for example *Deroceras panormitanum* (Dirzo, 1980; Whelan, 1982), *D. reticulatum* (Duval, 1971; Pallant, 1972; Jennings & Barkham, 1975; Rathcke, 1985; Clark *et al.*, 1997; Cook *et al.*, 1996, 1997; Frank & Friedli, 1999; Kozłowski & Kałuski, 2004; Kozłowski & Kozłowska, 2003, 2004), *Arion ater* (Cates & Orians, 1975; Jennings & Barkham, 1975; Mølgaard, 1986), *A. fasciatus* (Rathcke, 1985), *A. hortensis* (Duval, 1973), *A. lusitanicus* (Briner & Frank, 1998; Barone & Frank, 1999; Keller *et al.*, 1999; Kozłowski & Kałuski, 2004; Kozłowski & Kozłowska, 2000, 2003, 2004; Schädler *et al.*, 2005), *A. rufus* (Mølgaard, 1986; Kozłowski & Kałuski, 2004; Kozłowski & Kozłowska, 2003, 2004), *A. subfuscus* (Whelan, 1982; Rathcke, 1985), *Ariolimax columbianus* (Cates & Orians, 1975), *Cepaea nemoralis* (Grime *et al.*, 1968) and *Helix pomatia* (Mølgaard, 1986). The range of host plants for particular species of slugs has not been determined, but the research carried out has shown that these animals have

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specific nutritional requirements. According to some authors, slugs' co-evolutionary nutritional specialization is still at an early stage of development (Dirzo, 1980). This means that the process by which slugs acquire and select new sources of food is still ongoing, and therefore we should expect that new threats to crops will arise due to these pests.

For practical purposes, research is under way to establish the properties of plants which exert an influence on slugs. The main goal of this research is to identify the mechanisms which affect the choice of plant food. It has been shown that slugs' selection or rejection of particular plant species may be influenced by such features as physical leaf structure (leaf hardness) (Dirzo, 1980), nutritional component content (Port & Port, 1986; Spaull & Eldon, 1990), and the quantity and quality of secondary plant metabolites. Of greatest importance are chemical properties, which affect both the choice of plant and the slugs' feeding behaviour. Among these, secondary plant metabolites are among the main factors in plants' defence mechanisms against herbivorous slugs (Kloos & McCullough, 1982; Webbe & Lambert, 1983; Mølgaard, 1986; Stahl, 1988; Desbuquois & Daguzan, 1995; Hanley *et al.*, 1995; Clark *et al.*, 1997).

Today, the protection of crops against slug and snail pests primarily involves the use of molluscicidal bait pellets. Although treatment with such agents is carried out in accordance with established rules and recommendations, its effectiveness, particularly in humid conditions, is often unsatisfactory (Moens *et al.*, 1992). Reasons for this include the short durability of molluscicidal bait pellets, the short length of time for which they attract the slugs (3–4 days following application) and the repellent effect of the active substances (methiocarb and metaldehyde) (Henderson & Parker, 1986; Bailey & Wedgewood, 1991). Both of these substances may be poisonous to vertebrates (cats, dogs, sheep, poultry and wild animals) (Homeida & Cooke 1982), and methiocarb is toxic to beneficial invertebrates, for example to Carabidae, which are important predators of slugs (Purvis & Bannon, 1992). This means that molluscicidal bait pellets cannot be used in areas of organic crop production (Frank & Friedli, 1999). Molluscicidal bait pellets containing iron phosphate may potentially be used on organic farms. This substance is safer for the environment and is an alternative active substance to methiocarb and metaldehyde (Speiser & Kistler 2002).

For these reasons, alternative ways of protecting plants from slugs are sought. One of these involves offering the slugs other food sources in the form of palatable plant species containing attractants or phagostimulators. It has been observed that certain common weed species which grow among crops, such as *Taraxacum officinale*, *Capsella bursa-pastoris* and *Stellaria media*, are very palatable to the slug *Deroceras reticulatum* and may act as an easily available alternative food source (Cook *et al.*, 1996, 1997; Frank & Friedli, 1999). It has been shown that providing slugs with plants of *T. officinale*, grown between rows of winter wheat, may reduce slug damage to the wheat seeds and seedlings (Cook *et al.*, 1997). It has also been found that *S. media* and *C. bursa-pastoris* may protect oilseed rape seedlings against feeding by *D. reticulatum*. Slugs eat these weeds in preference to oilseed rape plants (Frank & Barone, 1999). Attempts are also being made to use plants, plant extracts or plant-derived chemical compounds (deterrents and antifeedants), in order to reduce the palatability of plants and to reduce slug feeding on young crops (Webbe & Lambert, 1983; Mølgaard, 1986; Clark *et al.*, 1997; Briner & Frank, 1998; Barone & Frank, 1999; Kozłowski *et al.*, 2003). Slugs generally reject plants which contain specific secondary plant metabolites, such as glycosides, alkaloids, flavonoids, phenols, saponins, terpenes and others. Some of these are strong deterrents or antifeedants, which prevent or reduce slug feeding on plants and may be potentially used in the protection of plants against slugs (Dirzo, 1980; Kloos & McCullough, 1982; Mølgaard, 1986; Webbe & Lambert, 1983; Sthal, 1988; Airey *et al.*, 1989; Desbuquois & Daguzen, 1995; Schädler *et al.*, 2005). For example, certain antifeedants used on the seedlings of oilseed rape may protect sensitive stadia of that plant against slug feeding (Barone & Frank, 1999).

Research on alternative methods of limiting slug damage using selected plants or plant-derived chemical compounds is focused on learning the feeding behaviour of these animals on plants and determining the attractiveness of various species of plants to slugs. Research in this area has been conducted for several years at the Institute of Plant Protection – National Research

Institute in Poznań. Some of the results, relating to *Arion lusitanicus* Mabille, 1868, are presented in this paper. *A. lusitanicus* was carried to Poland in the early 1990s and quickly became a dangerous pest to plants. It causes significant damage to vegetable crops, ornamental plants and herbs, and damages rape and grain plants in the edge parts of fields (Kozłowski, 2005). As is the case in other European countries, combating this slug is very difficult and involves serious problems.

The experiments carried out were aimed at determining this slug's feeding preferences and evaluating the palatability and acceptance of various plant species. Results are presented from research on the palatability and consumption by slugs of 96 species of plants. Alongside oilseed rape, the plants tested included weeds commonly occurring among agricultural crops, as well as wild and cultivated medicinal herbs.

Materials and Methods

Collection and breeding of slugs used in tests The slugs used in the experiments came from a population of *A. lusitanicus* occurring on garden crops at Łańcut near Rzeszów. They were collected in three consecutive years (2004–2006), the collection taking place several weeks before the tests were due to begin. The collected slug specimens were placed in plastic containers (50 cm×35 cm×40 cm) filled with a 5 cm layer of argillaceous humus soil. The containers had several ventilation holes, protected by gauze. Three times a week the slugs were fed and their food was changed (wheat bran, Chinese cabbage leaves, powdered milk, calcium carbonate). The slugs were raised in a growth chamber at a temperature of 19°C by day and 16°C by night, RH 93% ± 2%, length of day 15 hours. The slugs were starved for 48 hours before the tests began. We carried out three no-choice tests X, Y and Z. In all tests young slugs were used whose average weights at the start of the tests were as follows: for plant first group (X: 58 species) 1.4 g; for plant second group (Y: 20 species) 1.5 g, and for plant third group (Z: 20 species) 1.7 g.

Cultivation and preparation of plants for testing The seeds of 95 species of herbaceous plants (Table 1) were obtained from a collection of cultivated plants at the Herbal Institute in Poznań. The seeds of oilseed rape (*Brassica napus* var. *oleifera*), Kana variety, came from a commercial supplier. The seeds were sown in semi-transparent containers (22 cm×18 cm×13 cm) set up in a greenhouse, filled with a 4 cm layer of garden soil. Each species of plant was sown in ten containers, in order to obtain 10 plants in each container. After the plants reached the 2–3 leaf stage and a height of 5–8 cm, one starved slug was placed in each container. The containers were closed with lids which had two ventilation holes protected with gauze, and were placed in the growth chamber.

No-choice tests The experiments were carried out in the period from March to July in the years 2004, 2005 and 2006, in a growth chamber, at a temperature of 19°C by day and 16°C by night, relative humidity 93% (±2%), length of day 15 hours. The first test involved observation of 57 species of herbaceous plants and oilseed rape, while the second and third tests each involved observations of 19 species of herbaceous plants and oilseed rape. Plant damage was observed daily for a period of six days. The defoliation index was determined, i.e. the percentage damage to the surface of the above-ground organs of each plant. An evaluation was made according to a 5-level scale of damage: 0% (no damage), 25%, 50%, 75% and 100% of plant surface eaten. In total 96 plant species were investigated.

Determination of indices and analysis of data For each plant species the amount of damage to above-ground parts was determined using the defoliation index, and on this basis the palatability index (P.I.) and consumption index (C.I.) were found.

Palatability index (P.I.) values were calculated for the first day of slug feeding, as the ratio of the average consumed surface area of the tested plant species (S) to the average consumed surface area of the control plant, namely oilseed rape (K), according to the formula:

$$P.I. = S/K$$

A palatability index of 1 means that the tested plant species is equally palatable as the control plant (oilseed rape). A value of 0.0 given for the palatability index means that the plant species is unpalatable (remark: P.I. = 0.0 means that P.I. is less than 0.05).

Plant species	Family	D	G	P.I.	C.I. (day)						Rank Sum
					1	2	3	4	5	6	
Achillea millefolium L.	Asteraceae	P	Y	0.6	7.0	7.0	5.3	5.4	5.1	4.4	339.5
Aegopodium podagraria L.	Apiaceae	P	X	0.9	7.8	5.3	5.1	4.4	3.8	3.3	285.0
Agrostemma githago L.	Caryphyllaceae	A	X	0.4	3.8	6.6	6.0	5.5	4.6	4.8	323.0
Amarantus retroflexus L.	Amaranthaceae	A	X	1.1	10.7	7.9	6.8	6.0	6.6	6.4	401.5
Anagallis arvensis L.	Primulaceae	A	X	0.4	3.8	3.1	3.2	2.7	2.1	1.9	175.5
Anethum graveolens L.	Apiaceae	A	Z	0.3	3.2	4.9	4.9	4.4	3.9	3.5	248.0
Artemisia absinthium L.	Asteraceae	P	Z	1.1	12.6	9.1	8.1	6.7	5.6	5.4	414.0
Artemisia dracunculus L.	Asteraceae	P	Y	5.5	60.3	31.5	21.4	16.5	13.3	11.1	582.5
Artemisia vulgaris L.	Asteraceae	P	X	0.3	2.2	2.0	1.6	1.4	1.8	2.6	139.5
Bellis perennis L.	Asteraceae	P	X	0.5	5.0	4.0	4.6	4.8	3.4	2.9	246.0
Borago officinalis L.	Boraginaceae	A	Y	0.4	4.3	4.3	4.0	3.3	2.7	3.1	218.5
Brassica napus L. var. *oleifera*	Brassicaceae	A	X	1.0	10.0	7.9	5.7	4.4	3.9	3.8	325.5
Brassica napus L. var. *oleifera*	Brassicaceae	A	Y	1.0	11.0	12.0	12.4	10.4	9.8	8.7	505.5
Brassica napus L. var. *oleifera*	Brassicaceae	A	Z	1.0	11.5	10.7	10.7	10.1	9.5	8.7	497.0
Calamintha vulgaris (L.) Druce	Lamiaceae	P	Y	1.5	16.7	13.2	12.1	11.5	9.5	8.6	521.0
Calendula officinalis L.	Asteraceae	A	Y	0.9	9.7	9.0	6.9	5.7	4.9	4.2	368.5
Capsella bursa-pastoris (L.) Medik.	Brassicaceae	A	X	0.9	8.9	10.4	10.5	9.0	8.2	7.8	463.5
Centaurea cyanus L.	Asteraceae	A	X	0.4	3.1	3.9	4.4	5.1	5.1	5.0	281.5
Chamaenerion angustifolium (L.) Scop.	Onagraceae	P	Y	0.0	0.3	0.7	0.6	0.7	0.9	0.9	77.0
Chelidonium majus L.	Papaveraceae	B	X	0.4	3.6	6.8	6.1	6.8	6.4	5.6	352.5
Chenopodium album L.	Chenopodiaceae	A	X	0.6	6.4	6.3	7.0	6.7	6.6	5.9	377.0
Cichorium intybus L.	Asteraceae	P	X	2.0	17.8	13.6	10.2	8.4	7.7	7.1	491.0
Cirsium arvense (L.) Scop.	Asteraceae	P	X	1.4	12.2	10.2	9.9	9.0	7.4	6.8	458.0
Conium maculatum L.	Apiaceae	B	X	2.0	20.0	18.6	17.4	14.7	12.1	10.4	556.0
Coriandrum sativum L.	Apiaceae	A	Y	2.3	25.7	15.8	14.0	11.5	9.9	8.7	541.5
Datura stramonium L.	Solanaceae	A	X	4.0	35.0	25.6	19.5	15.2	12.4	10.4	570.0
Digitalis grandiflora P. Mill.	Scrophulariaceae	P	X	0.7	7.7	6.2	5.0	4.3	3.6	3.3	279.0
Echinochloa crus-galli (L.) Beauv.	Poaceae	A	Z	0.0	0.3	0.1	0.1	0.2	0.2	0.2	41.0
Epilobium hirsutum L.	Onagraceae	P	X	0.0	0.4	0.8	0.9	0.8	0.8	0.8	82.5
Epilobium palustre L.	Onagraceae	P	Z	0.0	0.0	0.0	0.0	0.2	0.4	0.4	35.5
Erigeron canadensis L	Asteraceae	A	Z	0.6	7.4	5.6	4.9	4.7	4.0	3.6	294.5
Euphorbia helioscopia L.	Euphorbiaceae	A	X	1.1	11.1	12.0	9.6	11.3	9.7	8.2	489.5
Galeopsis tetrahit L.	Lamiaceae	A	Z	0.3	3.2	2.5	2.0	2.0	1.6	1.5	145.5
Galium aparine L.	Rubiaceae	A	Z	0.0	0.0	0.4	1.2	1.7	1.9	1.7	98.0
Geranium pratense L.	Gerianiaceae	P	X	0.0	0.4	0.4	0.4	0.4	0.4	0.4	58.0
Geranium pusillum L.	Gerianiaceae	B	Y	0.5	5.3	5.0	5.1	4.4	4.0	4.3	286.5
Geranium robertianum L.	Geraniaceae	B	X	0.0	0.0	0.0	0.2	0.2	0.3	0.3	35.0
Geranium sanguineum L.	Geraniaceae	P	X	0.1	0.8	0.6	0.4	0.4	0.3	0.3	57.0
Geum urbanum L.	Rosaceae	P	Z	0.0	0.0	0.0	0.0	0.1	0.1	0.1	22.0
Glechoma hederacea L.	Lamiaceae	P	X	0.0	0.0	0.0	0.0	0.0	0.1	0.1	20.0
Helichrysum arenarium (L.) Moench	Asteraceae	A	Y	0.1	1.0	3.2	4.1	5.8	6.9	6.7	293.5
Hieracium pilosella L.	Asteraceae	P	Z	0.2	1.8	2.1	2.0	1.7	1.5	1.3	131.5
Holcus lanatus L.	Poaceae	P	X	0.7	7.7	5.0	4.4	4.2	3.8	3.3	263.0
Hyssopus officinalis L.	Lamiaceae	P	X	1.3	13.5	12.5	10.3	9.1	8.5	8.0	491.0
Impatiens balsamina L.	Balsaminaceae	A	Y	0.7	8.0	7.3	6.3	5.9	4.9	4.5	358.0
Impatiens roylei Walp.	Balsaminaceae	A	Z	0.0	0.0	0.0	0.0	0.1	0.1	0.0	20.0
Lamium amplexicaule L.	Lamiaceae	A	X	2.9	28.6	23.6	17.3	14.9	12.6	10.8	569.5
Lamium purpureum L.	Lamiaceae	A	X	0.4	3.6	2.7	2.4	2.1	1.9	2.5	168.0
Leucanthemum vulgare Lam.	Asteraceae	P	Y	0.5	5.7	5.2	5.1	4.9	4.8	4.0	306.0
Lithospermum arvense L.	Boraginaceae	A	X	0.9	7.5	5.5	4.4	3.9	3.8	3.3	265.5
Lycopsis arvensis L.	Boraginaceae	A	X	0.3	2.2	1.1	0.7	0.7	0.6	0.6	90.0
Malva sylvestris L.	Malvaceae	B	Y	0.8	8.3	9.7	9.1	8.3	7.5	6.4	431.0

Table 1 Palatability index P.I. and consumption index C.I. of the investigated herbaceous plants for *A. lusitanicus.* D = duration: A = annual, B = biennial, P = perennial; G – Group of plants: X = first set of 58 plant species; Y = second set of 20 plant species; Z = third set of 20 plant species. Rank sum: Friedman's test, Fisher LSD (0.05) = 193.1*.

Plant species	Family	D	G	P.I.	C.I. (day)						Rank Sum
					1	2	3	4	5	6	
Matricaria chamomilla L.	Asteraceae	A	Y	1.2	13.3	9.5	8.7	8.1	7.2	6.8	448.0
Melandrium album (P. Mill.) Garcke	Caryophyllaceae	B	X	1.6	16.4	13.6	11.9	9.2	7.7	6.9	497.0
Melissa officinalis L.	Lamiaceae	P	X	0.5	5.4	3.8	3.2	2.7	2.3	1.9	193.0
Mentha piperita L.	Lamiaceae	P	Y	0.6	6.3	4.8	4.7	4.5	4.1	3.8	279.0
Myosotis arvensis (L.) Hill	Boraginaceae	A	X	2.4	24.3	22.1	16.7	14.0	11.6	10.5	556.5
Ocimum basilicum L.	Lamiaceae	A	Y	1.0	10.7	10.5	9.9	8.8	8.5	7.4	466.5
Origanum vulgare L.	Lamiaceae	P	X	0.5	5.0	2.5	1.9	2.2	2.5	2.6	179.5
Papaver argemone L.	Papaveraceae	A	Z	0.7	7.6	8.8	7.8	6.7	6.2	5.8	390.5
Papaver rhoeas L.	Papaveraceae	A	X	1.3	13.8	15.0	13.7	13.3	12.5	11.1	544.5
Plantago indica L.	Plantaginaceae	A	X	0.0	0.0	0.6	1.8	1.6	1.5	1.6	98.0
Plantago lanceolata L.	Plantaginaceae	P	X	0.3	2.5	5.0	5.5	5.0	5.1	4.5	299.5
Plantago major L.	Plantaginaceae	P	X	0.9	8.6	7.5	7.0	6.4	6.4	6.0	391.5
Plantago media L.	Plantaginaceae	P	Z	0.0	0.3	1.0	1.6	1.2	1.0	0.9	95.5
Poa annua L.	Poaceae	A	X	0.1	0.9	0.8	0.5	0.5	0.4	0.6	74.0
Polygonum aviculare L.	Polygonaceae	A	Y	0.1	1.3	2.0	3.9	3.8	3.8	4.2	209.0
Polygonum convolvulus L.	Polygonaceae	A	X	0.8	7.2	6.1	4.7	4.8	4.8	4.4	315.5
Polygonum nodosum Pers.	Polygonaceae	A	X	0.9	8.6	6.8	5.7	4.6	4.2	3.7	323.0
Polygonum persicaria L.	Polygonaceae	A	Z	0.9	10.6	10.6	9.8	10.1	9.2	8.5	477.5
Potentilla anserina L.	Rosaceae	P	Y	0.0	0.0	0.7	0.4	0.7	0.9	1.1	70.5
Ranunculus repens L.	Ranunculaceae	P	X	0.6	6.5	4.4	3.6	3.2	2.7	2.6	225.5
Rosmarinus officinalis L.	Lamiaceae	P	Z	0.5	5.6	3.2	2.9	3.0	2.9	2.5	204.0
Rumex acetosa L.	Polygonaceae	P	X	1.3	13.2	12.7	9.5	7.8	6.6	5.8	448.0
Rumex acetosella L.	Polygonaceae	P	X	1.4	14.3	8.8	8.2	6.6	6.6	6.8	432.0
Ruta graveolens L.	Rutaceae	P	X	0.1	1.2	1.3	1.8	1.5	1.2	1.0	113.5
Salvia officinalis L.	Lamiaceae	P	Y	0.8	8.7	5.3	5.1	4.8	4.5	4.3	324.5
Saponaria officinalis L.	Caryophyllaceae	P	X	0.0	0.0	0.0	0.0	0.0	0.0	0.0	16.0
Satureja hortensis L.	Lamiaceae	P	Y	2.2	24.7	21.3	17.6	14.2	12.0	10.2	558.0
Senecio vulgaris L.	Asteraceae	A	Z	0.3	3.8	3.1	3.6	2.9	2.6	2.2	188.5
Setaria glauca (L.) P.B.	Poaceae	A	X	0.1	0.9	1.6	1.1	1.3	1.3	1.5	109.0
Sinapis arvensis L.	Brassicaceae	A	X	1.3	12.5	8.9	8.5	7.6	7.5	6.9	445.0
Sisymbrium officinale (L.) Scop.	Brassicaceae	A	Z	0.4	5.0	3.4	2.7	2.6	2.1	2.2	184.0
Solanum nigrum L.	Solanaceae	A	X	0.4	3.1	3.3	2.4	2.0	2.3	2.2	166.0
Solidago canadensis L.	Asteraceae	P	Z	0.4	4.4	5.0	4.5	4.3	3.8	3.6	252.0
Stellaria media (L.) Vill.	Caryophyllaceae	A	X	1.6	16.1	12.3	11.2	9.8	9.3	8.5	504.5
Symphytum officinale L.	Boraginaceae	P	X	0.5	5.4	5.0	5.0	4.6	4.0	4.2	286.0
Tanacetum vulgare L.	Asteraceae	P	X	0.0	0.0	0.8	2.2	3.6	4.5	4.3	188.0
Taraxacum officinale Web.	Asteraceae	P	X	1.2	10.6	8.9	7.8	6.8	6.4	6.6	416.0
Thlaspi arvense L.	Brassicaceae	A	X	1.8	17.5	14.8	13.2	12.1	10.3	9.2	537.0
Thymus vulgaris L.	Lamiaceae	P	X	0.6	6.5	7.5	6.8	6.3	5.8	5.2	365.5
Trifolium repens L.	Fabaceae	P	X	1.0	10.4	7.7	6.9	6.2	5.4	4.7	380.0
Tripleurospermum inodorum (L.) S.-B.	Asteraceae	A	X	4.1	40.7	30.7	22.5	17.4	14.0	11.7	586.0
Urtica dioica L.	Urticaceae	P	X	0.3	3.5	3.8	3.6	3.8	4.0	3.6	229.0
Urtica urens L.	Urticaceae	A	Z	0.4	5.0	4.0	3.5	3.2	2.5	2.3	204.0
Verbascum phlomoides L.	Scrophulariaceae	B	X	1.5	16.5	11.5	10.6	9.1	7.3	6.9	481.5
Verbascum thapsus L.	Scrophulariaceae	B	X	0.4	3.4	1.7	1.3	1.8	3.0	3.6	171.5
Viola arvensis Murr.	Violaceae	A	Z	0.1	1.5	0.7	1.0	0.9	0.9	0.9	93.0

* – species of plants for which the difference of the rank sums is greater than 193.1 differ significantly in terms of the amount of consumption

Values of the consumption index (C.I.) were calculated for successive days of slug feeding, as an average percentage of consumed surface area of the tested plant species (S), divided by the initial slug weight (M) and by the number of days of feeding (T). The following formula was used:

$$C.I. = S/(MxT).$$

For the results of the three no-choice tests, a joint analysis was performed. The analysis included the control species *B. napus* from the first, second and third no-choice tests. For comparison of annual (A), biennial (B) and perennial (P) plant species, and for comparison of plant families in terms of P.I., the rank sum test of Kruskal & Wallis (1952) was used. For comparison of plant species in terms of consumption in the first six days of slug feeding, Friedman's test (Friedman, 1937) was used, and Fisher's LSD procedure was used at a significance level of 0.05. For investigation of the relationship between P.I. and C.I. in the first six days of feeding, a correlation and polynomial regression analysis was applied.

Results

Palatability It was shown that considered plant species have different levels of palatability (P.I.) to *A. lusitanicus* (Table 1). Plants more palatable than oilseed rape (*B. napus*), with P.I. > 1.0, include 25 species, approximately 26% of all of the examined plant species. A lower palatability than oilseed rape (P.I. < 1.0) was found for 68 plant species (70.8%). For the plants *Ocimum basilicum* and *Trifolium repens* the P.I. is equal to 1.0, meaning that these species show the same palatability as *B. napus*.

The plant species most palatable to slugs are *Artemisia dracunculus* (P.I. = 5.5), *Datura stramonium* (P.I. = 4.0) and *Tripleurospermum inodorum* (P.I. = 4.1). These plants were very intensely damaged throughout the slug feeding period. Other palatable plants include nine species with P.I. between 1.6 and 2.9, and thirteen species with the index in the range 1.1 to 1.5.

Plants with lower palatability than *B. napus* (P.I. < 1.0) include fifteen species with P.I. = 0.0 and seven species with P.I. = 0.1 (Table 1). Slugs did not feed on *Impatiens roylei*, *Geranium robertianum*, *Saponaria officinalis*, *Glechoma hederacea*, *Epilobium palustre* or *Geum urbanum*. Moreover on the plants of another 13 of these species slight loss of leaf tissue was observed, meaning that the slugs had tried the plants but had not come to feed on them permanently. Included in the set of plants with low palatability were *Helichrysum arenarium*, *Polygonum aviculare* and *Tanacetum vulgare*, although permanent feeding on these plants on subsequent days was observed (rank sums for consumption are high, 293.5, 209 and 188 respectively). Therefore except for the last three species, it can be concluded that the plants of these 19 species were unpalatable to slugs. These plants contain the minor secondary plant compounds which they probably limit or make impossible feeding by *A. lusitanicus* (Table 2).

There were 46 species of plants with P.I. in the range 0.2 to 0.9. The slugs had fed on these plants and caused damage.

The palatability of plants from 27 families to *A. lusitanicus* was differentiated, although significant differences were not found (Table 3, p = 0.056). The value P.I. = 0.0 was obtained for all plant species from the families *Onagraceae* (3 species), *Rosaceae* (2 species) and *Rubiaceae* (1 species), while P.I. = 0.1 was found for plants from the families *Rutaceae* and *Violaceae*, each represented by one species. A very low palatability was also shown by plants from the families *Geraniaceae* (4 species), *Poaceae* (4 species) and *Plantaginaceae* (4 species).

Considerable differentiation was observed between the palatability of plant species within families such as *Apiaceae* (4 species), *Asteraceae* (19 species), *Boraginaceae* (5 species), *Lamiaceae* (14 species) and *Solanaceae* (2 species). For example, among the best represented family – *Asteraceae* – there were species with low palatability to slugs, such as *T. vulgare* (P.I. = 0.0) and *H. arenarium* (P.I. = 0.1), as well as species with very high palatability index, such as *A. dracunculus* (P.I. = 5.5) and *T. inodorum* (P.I. = 4.1). Similarly among plants from the family *Lamiaceae* (14 species) we can find both unpalatable species such as *G. hederacea* (P.I. = 0.0) and palatable ones such as *L. amplexicaule* (P.I. = 2.9).

A consistently high palatability was found for plants from the *Brassicaceae*. Moreover a high palatability was found for plants from three other families: *Amaranthaceae*, *Euphorbiaceae* and *Fabaceae*, with one species tested from each.

Table 2 The examined plant species unpalatable for *A. lusitanicus* and the best-known important secondary plant compounds contained in these plants (taken from Kohlmünzer, 2000).

Plant species	Family	Secondary compounds
Chamaenerion angustifolium (L.) Scop.	Onagraceae	Flavonoids, glycosides
Echinochloa crus-galli (L.) Beauv.	Poaceae	Flavonoids, phenols
Epilobium hirsutum L.	Onagraceae	Ellagic and caffeic acid
Epilobium palustre L.	Onagraceae	Flavonoids, glycosides
Galium aparine L.	Rubiaceae	Iridoid glycosides
Geranium pratense L.	Gerianiaceae	Flavonoids
Geranium robertianum L.	Geraniaceae	Flavonoids (cempherol, quercetin), ethereal oils
Geranium sanguineum L.	Geraniaceae	Flavonoids
Geum urbanum L.	Rosaceae	Phenols (eugenol), tannins
Glechoma hederacea L.	Lamiaceae	Ethereal oils, tannins, bitter principles
Impatiens roylei Walp.	Balsaminaceae	Ethereal ols
Plantago indica L.	Plantaginaceae	Tannins, aucubin, ols
Plantago media L.	Plantaginaceae	Glycosides, flavonoids, caffeic acid
Poa annua L.	Poaceae	Silica bodies (phytoliths)
Polygonum aviculare L.	Polygonaceae	Flavonoids, phenols acids, caffeic acid, tannins
Potentilla anserina L.	Rosaceae	Flavonoids, catechinic tannins, saponins, sterols
Ruta graveolens L.	Rutaceae	Alcaloids, flavonoids (rutin), ethereal oils
Saponaria officinalis L.	Caryophyllaceae	Saponins, triterpenes, sterols
Setaria glauca (L.) P.B.	Poaceae	Silica bodies (phytoliths)

The tested plants included 45 species of annual plants, 43 of perennials and 8 species of biennials (Table 4). Analysing the palatability (P.I.) of plant species to *A. lusitanicus* in relation to maturity (age), it was found that perennial plants were less palatable than annuals and biennials. It was found, however, that the observed differences between the palatability of plants from the compared age groups were statistically insignificant ($p = 0.096$).

Consumption For the analysis of consumption of particular species of plants over the first six days of slug feeding, highly significant differences were identified between species (Table 1, $p < 0.001$). Values of the consumption index (C.I.) determined for six consecutive days of feeding were assigned ranks, and the rank sums were calculated. Species of plants for which the difference of the rank sums is greater than 193.1 differ significantly in terms of the mass consumed.

The highest consumption was found for *T. inodorum* (rank sum = 586). Among the investigated species, the consumption of 29 species was found not to differ significantly from that of *T. inodorum.* Apart from *T. inodorum*, high consumption was found for *A. dracunculus, D. stramonium, Lamium amplexicaule, Satureja hortensis, Myosotis arvensis* and *Conium maculatum*. These species are examples of plants with high palatability index, i.e. on which the slugs fed intensively only during the first 24 hours, after which a large fall in consumption took place (Fig. 1). High consumption was found also on the control plants of *B. napus.* The intensity of slug feeding on these plants was fairly equal on successive days of observation. Most of the 29 species discussed here are species with a palatability index greater than 1 (P.I. > 1.0).

The lowest consumption was found for plants of *Saponaria officinalis*. Similarly low consumption (no significant difference from *S. officinalis*) was found for 34 of the investigated plant species. These include 15 species with P.I. = 0.0. Apart from *S. officinalis*, they include such species as *G. hederacea*, *G. urbanum* and *I. roylei,* for which almost up to the last day of slug feeding no plant damage was observed. Low slug feeding activity and minimum plant damage was observed for most of the 15 species having P.I. = 0.0. An exception was *T. vulgare*. On plants of that species no feeding was observed on the first day, then on following days up to the fifth day the C.I. value successively rose, remaining at a similar level on the sixth day.

Table 3 Rank sum and Kruskal-Wallis test for comparison of plant families in terms of palatability.

Family	Number of species	Rank sum
Amaranthaceae	1	75.0
Apiaceae	4	275.5
Asteraceae	19	1033.0
Balsaminaceae	2	64.5
Boraginaceae	5	267.0
Brassicaceae	7	483.5
Caryophyllaceae	4	219.0
Chenopodiaceae	1	51.5
Euphorbiaceae	1	75.0
Fabaceae	1	71.0
Gerianiaceae	4	80.0
Lamiaceae	14	793.0
Malvaceae	1	60.0
Onagraceae	3	24.0
Papaveraceae	3	173.0
Plantaginaceae	4	108.0
Poaceae	4	102.5
Polygonaceae	6	373.0
Primulaceae	1	36.0
Ranunculaceae	1	51.5
Rosaceae	2	16.0
Rubiaceae	1	8.0
Rutaceae	1	19.0
Scrophulariaceae	3	178.0
Solanaceae	2	132.0
Urticaceae	2	63.0
Violaceae	1	19.0
Statistic	Chi-square	38.4
Significance level	p – value	0.056

Table 4 Rank sum and Kruskal-Wallis test for comparison of groups of plants in terms of palatability. A = annual, B = biennial and P = perennial.

Group of plants	Number of species	Rank sum
A	47	2448.0
B	8	448.5
P	43	1954.5
Statistic	Chi-square	4.686
Significance level	p – value	0.096

The remaining 31 plant species showed a similar average rate of damage caused by slugs, significantly different from the value of consumption of *T. inodorum* and from the lowest consumption (*S. officinalis*).

Among the examined plant species there are some on which, following intensive feeding over the first 24 hours, there followed a large fall in consumption, for example on *A. dracunculus*. There were also plants on which a rise in consumption was observed on the second or third day of slug feeding, followed by a fall in consumption on subsequent days, e.g. *Agrostemma githago*. For most species significant fluctuation in consumption was observed day to day, for example in the case of *Euphorbia helioscopia*.

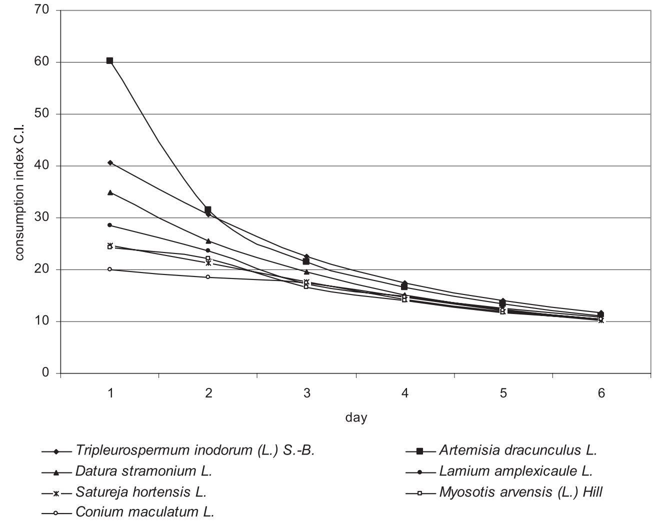

Figure 1 Consumption of plants of selected species on the first six days of feeding by *A. lusitanicus.*

A relation was found between the palatability of the considered species of plants expressed by the palatability index P.I. after the first day of slug feeding, and consumption expressed by the rank sum obtained from the consumption index C.I. calculated for the first six days of slug feeding (Fig. 2). The correlation coefficient for second-degree relation is equal to 0.92 ($p < 0.001$, $n = 98$).

Discussion

The preferences of *A. lusitanicus* varied with respect to plant species. Of the 96 tested species, about 23% were unpalatable and 48% were of low palatability i.e. categorised as of lower palatability than *B. napus*. On plant species (P.I. = 0.0) the slugs consumed some plant tissue on a trial basis, but were not observed to feed actively. In the unpalatable species, trial feeding took place very rarely and occurred only after a period of several days during which the slugs were starved. These included *I. roylei*, *G. robertianum*, *S. officinalis*, *G. hederacea*, *E. palustre* and *G. urbanum*

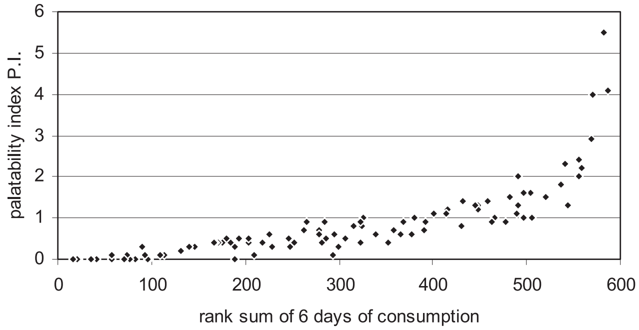

Figure 2 Palatability on the first day and consumption on the first six days of feeding by *A. lusitanicus.*

(Table 1). These species of plants were not accepted at all by *A. lusitanicus.* Plants not accepted by *A. lusitanicus* also include a number of other species, with palatability P.I. < 0.2, on which slug feeding activity was very low, as was shown by the small amount of damage to the plants (Table 1). The low palatability of a number of the plant species tested by us had already been referred to by other authors in relation to such slugs as *A. lusitanicus* (Briner & Frank, 1998), *Arion ater*, *A. rufus, Deroceras panormitanum* and *D. reticulatum* (Cates & Orians, 1975; Dirzo, 1980; Kozłowski & Kałuski, 2004; Kozłowski & Kozłowska, 2003, 2004). Data from the literature show that plants which are not accepted by slugs have defence mechanisms which strongly inhibit slug feeding, thus protecting the plants from damage. Some research results indicate that the degree of acceptance of plants by slugs is most influenced by specific plant metabolites. These are chiefly secondary metabolites which are species specific (Duval, 1971, 1973; Cates & Orians, 1975; Dirzo, 1980; Kloos & McCullough, 1982; Webbe & Lambert, 1983; Mølgaard, 1986; Stahl, 1988; Desbuquois & Daguzan, 1995; Hanley *et al.*, 1995; Cook *et al.*, 1996; Clark *et al.*, 1997; Briner & Frank, 1998). Secondary metabolites act as antifeedants and strongly influence slugs, preventing or discouraging them from feeding. Table 2 shows the most important secondary metabolites of 19 plant species which we classified as unpalatable to *A. lusitanicus.* These compounds are in the main family or species specific (Kohlmünzer, 2000). The mechanism by which the compounds work is poorly known, but are clearly effective against *A. lusitanicus.*

A smaller number of plant species (26% of those investigated) were palatable to *A. lusitanicus* (Table 1). Although these were plants of various degrees of palatability, all of them can be said to be species which are accepted by the slug. Almost half of the plants in this category were species for which both palatability and consumption indices attained their highest values on the first day of slug feeding. They included *A. dracunculus*, *T. inodorum*, *D. stramonium* and *L. amplexicaule*.

In common with the results of Briner and Frank (1998), plants such as *Papaver rhoeas*, *Sinapsis arvensis, M. arvensis* and *Stelaria media* had relatively high palatability to *A. lusitanicus*. However there were significant differences in relation to *Tanacetum vulgare* and *Viola arvensis*, where low palatability to *A. lusitanicus* was demonstrated. The palatability index values determined in research by different authors for the same plant species may show significant differences. This has already been mentioned by Dirzo (1980), in work concerning the acceptance of 30 species of plants by *Deroceras panormitanum*. The reason for these discrepancies may be the different origins of the experimental plants and slugs. For example Buschmann *et al.* (2005, 2006) found that invasive species of Brassicaceae show greater ability to compensate for slug damage caused by *A. lusitanicus* than native species, which means that they have greater tolerance to damage. Regional climatic conditions may have a large effect on the chemical composition of plants and on slugs' food requirements (Keller *et al.*, 1999). Moreover the palatability of plants may be dependent on the selection of compared species or on the varieties used, the age of the plants, the plant parts tested and the experimental methods used. In our research the control plant was oilseed rape (*B. napus*), which has been noted by other authors as a plant very acceptable to slugs (Frank, 1998a, 1998b; Briner & Frank, 1998). In the present research *B. napus* did not have the highest consumption of investigated species, and 71% of the total were more palatable. High palatability of rape plants has been obtained by the authors compared with 58 other plant species in tests with choice, during research on the food preferences of *A. lusitanicus, A. rufus* and *D. reticulatum* (Kozłowski & Kałuski, 2004; Kozłowski & Kozłowska, 2003, 2004).

In view of the differences in the numbers of plant species from particular families which were tested by us, it is difficult to distinguish categorically families which are more or less palatable to slugs. It was found only that low palatability was demonstrated by plants from the families Onagraceae, Rosaceae, Rubiaceae, Gerianiaceae, Rutaceae and Violaceae, while those of the families Brassicaceae, Amaranthaceae, Euphorbiaceae and Fabaceae were significantly more palatable to *A. lusitanicus*. These results require confirmation in future research on the palatability of other species of plants from the families mentioned. It was also found that species of the Asteraceae and Lamiaceae showed variability in terms of palatability to slugs. Similar differentiation has been observed by other authors (Mølgaard, 1986; Briner & Frank, 1998). Briner & Frank (1998), who found plants of low palatability to *A. lusitanicus* to include Ranunculaceae, Scrophulariaceae, Poaceae, with plants of high palatability including Brassicaceae, Apiaceae, Boraginaceae, explain this fact in terms of the effects of secondary plant substances which are specific to the plants in those families.

An important factor having an effect on the palatability of particular plant species to slugs may be their life cycle. Some authors have shown that annuals are more palatable than perennials (Cates & Orians, 1975; Dirzo, 1980; Briner & Frank, 1998). Similar results were obtained in the present research, but at the non-significance level p = 0.096 (Table 4).

One essential conclusion from this research is the identification of differentiated slug feeding behaviour on plants of various species. There was observed to be differentiation in the pattern of consumption rate. The following consumption patterns were distinguished: (1) intensive consumption on the first day, and a marked fall on subsequent days of feeding; (2) increase of consumption on the second or third day and fall on subsequent days; (3) significant fluctuation in consumption on successive days of feeding; (4) minimum consumption over the whole period of feeding.

The certain species of plants may be potentially used to protect crop plants from slugs. This is indicated by the results of experiments so far carried out with various species of plants, plant extracts and plant-derived substances, used against various species of slugs (Webbe & Lambert, 1983; Mølgaard, 1986; Clark *et al.*, 1997; Cook *et al.*, 1996, 1997; Briner & Frank, 1998; Barone & Frank, 1999; Frank & Barone, 1999; Frank & Friedli, 1999; Speiser, 2001; Frank *et al*., 2002; Port & Ester, 2002; Simms *et al*. 2002; Kozłowski *et al*., 2003; Schüder *et al*., 2004, Schädler *et al*., 2005). Although the results of those experiments have not yet been applied in practice in field conditions, they are sufficiently promising that research in this area is constantly being conducted. This applies both to evaluation of the palatability to slugs of previously untested plant species, and to the search for plant-derived chemical compounds with slug-killing properties, deterrents and/or antifeedants. The observations presented here make it possible to identify plant species which may be useful in reducing damage to rape plants caused by the slug *A. lusitanicus*. However further research is necessary in order to establish which of the chemical compounds contained in those plants influence the feeding behaviour of slugs and by what mechanism they act.

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