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RESEARCH ARTICLE

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Efficacy of invasive alien plants in controlling Arionidae slugs

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Abstract

Aim of study: To develop an alternative slug control method, we explored the use of plant material from seven invasive plant species against *Arion* slugs.

Area of study: The experiments were performed at the University of Ljubljana (Slovenia).

Material and methods: In laboratory (exp. A-C) and semi-field studies (exp. D), we investigated the contact and barrier efficacy of plant material (powder or liquid formulation) of seven invasive plant species (Japanese knotweed, bohemian knotweed, Canadian goldenrod, giant goldenrod, staghorn sumac, tree of heaven, and false indigo) against *Arion* slugs. In order to test a contact efficacy of the substance (exp. A), slugs were rolled in a plant material powder. In exp. B, powder made from a plant material was used as a barrier for slugs. Antifeedant effect of the slugs was tested in exp. C, where lettuce leaves were treated with a liquid formulation of a plant material. In exp. D, all above mentioned techniques were used in a semi-field trial.

Main results: The results of our studies showed that the plant material of staghorn sumac, giant goldenrod, and Japanese knotweed showed the strongest anti-feedant and barrier effects against the slugs. In the semi-field trial, only 7% of the plants treated with giant goldenrod plant material were attacked by slugs.

Research highlights: A contact efficacy of plant powders against *Arion* slugs was not confirmed in our investigation. Furthermore, several plant powders (goldenrods, staghorn sumac) showed good barrier efficacy. A semi-field trial showed that plant material (giant goldenrod) could represent an alternative solution in slug control.

Additional key words: *Arion*; plant extract; plant powder; invasive alien plants; slug control.

Authors' contributions: Conceived, designed and performed the experiments: ZL, TB, KF and IM. Analyzed the data: IM, ZL and ST. Contributed reagents/materials/analysis tools: ST. Wrote the paper: ZL. All authors read and approved the final manuscript.

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Introduction

Slugs of the genus *Arion* (Gastropoda: Arionidae) have been classed as a major agricultural economic pests in Asia, Australia, Europe, and North America (Barker, 2002; Ahmadi, 2004; Douglas & Tooker, 2012; Rowson *et al*., 2014). They cause damage to vegetables, field crops, fruit trees, lawns, and wild plants (Peters *et al*., 2000; Douglas & Tooker, 2012). They may also appear as stored product pests (Henderson & Triebskorn, 2002). Slug feeding can severely damage the plants and thus subject them to stress (Rowson *et al*., 2014). Consequently, such plants are less resistant and more susceptible to diseases and are less likely to survive unfavourable weather conditions (Hammond & Byers, 2002).

The economically important agricultural pest species from the Arionidae family in Europe are *Arion distinctus* Mabille, *A. hortensis* (Férussac), and *A. vulgaris* (Moquin-Tandon) (Rowson *et al*., 2014; Laznik & Trdan, 2016). For the purpose of effective management of any agricultural pest, knowledge of its biology and

ecology is required. The life of the *Arion* slugs is closely connected to its environment, with temperature and humidity directly affecting biological processes (Barker, 2002; Slotsbo *et al*., 2011). Furthermore, body pigmentation may depend on age, diet or environment (Barker, 2002; Kozlowski, 2005; Slotsbo *et al*., 2011). Slugs activity is correlated with different factors, such as air temperature, soil surface temperature, wind speed, humidity and soil moisture content (Young & Port, 1989; Kozlowski, 2007; Slotsbo *et al*., 2013; Rowson *et al*., 2014). Slugs favour heavier soils, since they can survive over the summer in cracks in the soil and under clods (Barker, 2002). Terrestrial molluscs are generally susceptible to desiccation, and several studies have found habitat selection to be strongly correlated with the availability of water (Carne-Cavagnaro *et al*., 2006). The most obvious water regulating behaviour of slugs is their preference for moist habitats (Slotsbo *et al*., 2011). In contrast to snails, slugs lack the physical protection of a shell and must find other ways to reduce water loss. Thompson *et al*. (2005) suggested that water conductance of the skin is an important factor. However, the ability to survive drought cannot be explained only by water conductance alone but is also influenced by body size and the magnitude of water reserves. Active slugs can lose up to 60% of their initial body weight (Thompson *et al*., 2005). Furthermore, dehydrated slugs can rapidly recover from dehydration by absorbing water through their integument; this process is termed contact re-hydration (Prior, 1985; Thompson *et al*., 2005).

Increased mucus secretion is one of the first reactions of slugs to mechanical or chemical irritation (Barker, 2002; Schüder *et al*., 2003; Simms *et al*., 2006). The production of mucus enables the slugs to form a protective barrier preventing direct contact between the toxin and the surface of the epithelial cells. Slugs are mainly controlled with bait pellets, which usually contain either metaldehyde or iron (III) phosphate (Garthwaite & Thomas, 1996; Speiser & Kistler, 2002). The major concern related to slug control is that the chemical compounds are also toxic to non-target organisms and pets (Castle *et al*., 2017). Due to the physico-chemical properties of metaldehyde, it is highly mobile in soil, and hence once applied, it can run off under wet conditions into field drains, gullies and surface waters (Castle *et al*., 2017). Furthermore, farmers and growers often experience difficulty controlling slugs with bait pellets containing molluscicides. Hata *et al*. (1997) reported that in wet conditions, the efficacy of these baits can be very low. Slugs can be difficult to kill by contact molluscicides because they are covered by a layer of slime that prevents chemicals from coming in contact with skin, and at the same time, slime may serve to dilute the toxin (Barker, 2002; Speiser & Kistler, 2002; Gonzáles-Cruz & San Martín, 2013). In addition to environmental problems, human health problems also arise from agricultural pesticide usage (Nicolopoulou-Stamati *et al*., 2016). Many farmers are searching for new alternatives to curb pesticide usage to address the many concerns.

The development of alternative slugs control methods compatible with integrated pest management (IPM) strategies used to control other pests would help satisfy increasing market demands and environmental safety issues. In the early 1990s, a biocontrol method for slugs based on the parasitic nematode *Phasmarhabditis hermaphrodita* was developed (Wilson *et al*., 1993). The biocontrol method with parasitic nematodes proved to be a promising alternative to chemical molluscicides (Iglesias *et al*., 2003). Many countries have strict regulations for using biopesticides that allow only indigenous species to be used (Laznik & Trdan, 2016). Such regulations have slowed the uptake of use of *P. hermaphrodita* in several countries. Physical barriers, such as continuous lines of sawdust or ash, provide a dry surface which slugs avoid (Barker, 2002; Laznik & Trdan, 2016); however, the effectiveness of these barriers is reduced once they become wet. Copper barriers present an effective mechanical and physiochemical barrier for slugs (Schüder *et al*., 2003; Laznik *et al.*, 2011). As shown in previous studies, mollusks take up copper through ingestion (Berger & Dallinger, 1989), and directly through the foot, at least in the form of copper compounds (Ryder & Bowen, 1977), causing internal damage and irritation (Schüder *et al*., 2003).

Due to the negative environmental impacts of pesticides, their non-target effect and increasingly stringent environmental policies, researchers are searching for new, more environmentally acceptable methods of plant protection against pests. One of these measures is the study of plant extracts in the control of economically important harmful organisms (Pavela *et al*., 2008). To develop an alternative slug control method, we explored the use of plant material from seven invasive plant species: knotweeds (*Fallopia japonica* [Houtt.] Ronse Decr.*, F. × bohemica* [Chrtek & Chrtková] Bailey), goldenrods (*Solidago canadensis* L., *S. gigantea* Aiton), staghorn sumac (*Rhus typhina* L.), tree of heaven (*Ailanthus altissima* [Mill.] Swingle), and false indigo (*Amorpha fruticosa* L.). It was a selection of plants that are widespread in urban areas and whose control is practically impossible in practice. The main idea was to use these plants in order to see if they have any potential in plant protection programmes (Laznik *et al*., 2018). The aims of our laboratory and semi-field

studies were to test (1) the contact control efficacy of an individual use of the selected substances, (2) the barrier effect of the tested substances, and (3) the effect on the slug eating ability.

Material and methods

Slugs

The experiments were performed at the Laboratory of Entomology and the laboratory field of the Biotechnical Faculty (Dept. of Agronomy, University of Ljubljana, Slovenia). *Arion* slugs (mainly representatives of *A. vulgaris* and *A. rufus*) were collected at the laboratory field of the Biotechnical Faculty in Ljubljana (46°04'N, 14°31'E, 299 m a.s.l.) during May and August 2018. Slugs were identified to species level with the use of identification charts (Rowson *et al*., 2014). The slugs collected were of various lengths and ages, as we wanted to analyse a comprehensive sample of outdoor slug behaviours (Laznik *et al*., 2011; Laznik & Trdan, 2016). The slugs were starved for 48 hrs prior to the experiment (Schüder *et al*., 2003).

Experimental design

Plant material was collected in the area of the municipality of Ljubljana (Slovenia). For the purpose of the experiments, we used aerial parts of the plants (leaves, flowers). Plant samples were air-dried. They were tied together and hung to expose the plant to air at ambient temperature for 7-10 days until dry. This drying method does not force dried plant materials using high temperature, meaning heat-labile compounds are preserved (Azwanida, 2015). An electric blender was used to reduce the particle size of the samples to increase the surface contact between the samples and extraction solvents (Azwanida, 2015).

To obtain a liquid formulation, we used a maceration technique (Azwanida, 2015). Maceration involved soaking the plant material (previously prepared powder) in tap water. For the purpose of the experiment, we used two concentrations, 2.5 and 10% w/v (2.5 or 10 g of dry powder was added into 100 mL of tap water). We soaked the plant material for 24 hrs. After this period, the mixture was pressed manually through medical gauze (type 12/8, produced by the company Tosama d.d., Vir pri Domžalah).

The experiment involved the following substances: [1,2] knotweeds (*Fallopia japonica, F. x bohemica*), [3,4] goldenrods (*Solidago canadensis*, *S. gigantea*), [5] staghorn sumac (*Rhus typhina*), [6] tree of heaven (*Ailanthus altissima),* and [7] false indigo (*Amorpha fruticosa)*. The substances were studied individually. Each treatment was repeated ten times. The control sample [8] was a slug sprinkled with water. All laboratory experiments were carried out in a growth chamber (type: RK-900 CH, produced by the company Kambič laboratorijska oprema d.o.o., Semič, Slovenia) at 20 °C, 12/12 h photoperiod, and 75% relative air humidity.

The study of the contact efficacy of the substances (experiment A)

The experiment, which lasted 48 hrs, included 80 slugs (8 different treatments which were repeated 10 times). The experiment was carried out in plastic petri dishes (150 × 20 mm). Moistened tampons (35 × 11 mm) and fresh leaves of lettuce (*Lactuca sativa* L.) were placed in plastic petri dishes. Before starting the experiment, the slugs were weighed. The slugs were then rolled in individual substances (dry powder of tested plant material). In control treatment slugs were only sprinkled with water. In the 48 hrs experiment, we checked the survival of slugs once a day, weighed them again, replaced the lettuce leaf (the source of food), added additional moisture to the tampon, and rolled them again in the substances studied (or sprinkled with water in control). The slugs that died during the experiment were not replaced with live slugs (Laznik *et al*., 2011). The aims of experiment A were (i) to test the contact control efficacy of the tested substances and (ii) to test the effect of the substances on slug eating ability.

The substances studied as barriers for slugs (experiment B)

The experiment lasted 48 hrs and involved 80 slugs (8 different treatments, which were repeated 10 times). The experiment was carried out in glass insectaria (width-length-depth 500-350-400 mm). Moistened tampons were placed in glass insectaria with fresh leaves of lettuce. Before starting the experiment, the slugs were weighed. We sprinkled the barrier (40 g of a substance) 3 cm wide and 2 cm thick around the lettuce leaf. In control treatment only lettuce was given to slugs without any barrier. In the two-day experiment, we checked daily whether the slugs had crossed the barrier, whether they had eaten lettuce leaves, how much they weighed, replaced the leaves of lettuce (the source of food), added additional moisture to the tampons and repaired barriers if they were damaged. The slugs that died during the experiment were not replaced

with live slugs (Laznik *et al*., 2011). The aims of experiment B were (i) to test the barrier effect of tested substances and (ii) to test the effect of the substances on slug eating ability.

The studied substances in liquid formulation (experiment C)

The experiment, which lasted 48 hrs, included 160 slugs (8 different treatments that were repeated 10 times at 2 different concentrations). The experiment was carried out in plastic petri dishes (150 × 20 mm). Moistened tampons (35 × 11 mm) and fresh leaves of lettuce were placed in plastic petri dishes. The slugs were weighed before starting the experiment. We prepared 2.5% and 10% (w/v) concentration of selected plant material (Azwanida, 2015). A lettuce leaf (the source of food) was soaked into the liquid formulation. In the experiment, we checked the survival of slugs once a day, weighed them again, replaced the lettuce leaf (the source of food and soaked it again in liquid formulation) and added additional moisture to the tampon. The slugs that died during the experiment were not replaced with live slugs (Laznik *et al*., 2011). The aims of experiment C were (i) to test the contact control efficacy of the substances tested and (ii) to test the effect of the substances on slug eating ability.

The studied substances in the semi-field trial (experiment D)

The preparation of the field began in autumn 2017, when stable manure (30 t ha^{-1}) was spread and then the field was ploughed. In spring, the field received the mineral fertilizer NPK (15:15:15). The wooden boxes (frame size: 1 m × 1 m × 0.5 m) with net covers (doors) were made by a local carpenter. In experiment lettuce variety 'Isabel' was used. We dug the box frame into the soil (at least 10 cm to prevent slugs from escaping from the boxes). Lettuce seedlings were transplanted into the experimental field (inside the wooden boxes). We achieved a density of 5 lettuce plants m^{-2}. The lettuce was not treated with other plant protection products. In a semi-field trial, only *Solidago gigantea* plant material was used (in previous laboratory trials, this compound showed the most promising results). Five treatments were used: [1] liquid formulation (lettuce seedlings were sprinkled with selected plant maceration in 10% concentration, [2] barrier (we constructed the barrier around the lettuce seedlings), [3] combination of the barrier and liquid formulation (the seedlings were sprinkled with selected plant maceration in 10% concentration + we constructed the barrier around the lettuce seedlings), [4] positive control (lettuce without slugs), and [5] negative control (lettuce with slugs only). Each treatment was repeated 5 times (25 boxes were used). In every box (except treatment 4), 5 slugs (*Arion vulgaris*) were added. In all the experiments, 100 slugs were used. We placed a tile in 1 corner of the box. This place was called the hiding area for the slugs during the experiment. Boxes were randomly distributed in the experimental field. The experiment lasted 72 hrs. On day 3, we cut all the lettuce and measured different parameters (total weight of the lettuce, weight of the slug damaged leaves, the weight of undamaged leaves, and the weight of leaves that showed phytotoxicity).

Statistical analysis

The typical behavioural responses of the slugs during the experiment were classified in terms of six events, as described in Table 1. The numbers used to index the events were used to quantify the analysis. To perform the data analysis, these index values were used as the values of the response variable "event". For instance, if the slug died in experiment A, the value of the event was 2 (see Table 1).

Two-way analysis of variance (ANOVA) was carried out to evaluate the differences in the response of the *Arion* slugs to different treatments (experiments A-C). Before analysis, each variable was tested for homogeneity of variance. Duncan's multiple range test (α= 0.05) was used to analyse the differences between individual treatment means (Hoshmand, 2006).

A multifactor ANOVA was conducted (semi-field trial, experiment D) to determine the differences in mortality rates (%) between the slugs in different treatments. Prior to the analysis, all the data were corrected for the mortality rate of the control group using Abbott's correction. Student's multiple range

Table 1. Definitions of behavioural events occurring during the experiments.

Index	Event
1	Slug survived the experiment
2	Slug died during the experiment
3	Slug fed on lettuce
4	Slug did not feed on lettuce
5	Slug crossed the barrier
6	Slug did not cross the barrier

test (p<0.05) was used to separate the mean differences among the parameters in all the treatments. All statistical analyses were performed using Statgraphics Plus for Windows 4.0 (Manugistics, Rockville, MD, USA). The data are presented as the untransformed means ± SE.

Results

General analysis of the events at different experiments is presented in Tables 2 and 3.

Individual analysis

Experiment A

The feeding inhibitor effect on the slugs after 24 and 48 hrs is presented in Fig. 1A. Results of our study showed that 40% of the slugs treated with the powder of *S. canadensis* did not feed on the lettuce after 24 hrs. The feeding inhibitor effect was also confirmed after 24 hrs in treatments with *A. fruticosa*, *R. typhina*, and *S. gigantea* (30%, 20%, 10% of the slugs tested did not eat the lettuce, respectively). In all other treatments, the feeding inhibitor effect was not confirmed. After 48 hrs, 30% of the slugs treated with the powder of *R. typhina* did not feed on the lettuce. The feeding inhibitor effect was also confirmed after 48 hrs in treatments with *F. japonica* (20% of the slugs did not eat the lettuce), *S. canadensis* (20% of the slugs tested did not eat the lettuce), *A. altissima* (20% of the slugs tested did not eat the lettuce) and *S. gigantea* (10% of the slugs v did not eat the lettuce). In all other treatments, the feeding inhibitor effect was not confirmed (see Fig. 1A).

Slug mortality after 24 and 48 hrs is presented in Fig. 1B. Results of our study showed that 20% of the slugs treated with powder of *S. canadensis* died after 24 hrs. Slug mortality was confirmed after 24 hrs in *R. typhina* (10%). In all the other treatments, slug mortality was not confirmed (see Fig. 1B). The results of our study showed that 30% of the slugs treated with the powder of *R. typhina* died after 48 hrs. Slug mortality was also confirmed in treatments *A. fruticosa* (10%), *F. japonica* (10%), and *S. canadensis* (20%). In all the other treatments, slug mortality was not confirmed (Fig. 1B).

Experiment B

The feeding inhibitor effect towards the slugs after 24 and 48 hrs is presented in Fig. 2A. Results of our

Table 2. ANOVA results for the experiments A (df for the error term: 159), B (df for the error term: 159), and C (df for the error term: 259).

	Source	Slug mortality			Feeding ability		
		F	df	*p*	F	df	*p*
Expt. A	Treatment (T)	2.76	7	0.0100	2.22	7	0.0355
	Exposure time (DAT)	1.80	1	0.1818	0.00	1	1.0000
	Source	**Barrier crossing**			**Feeding ability**		
		F	df	*p*	F	df	*p*
Expt. B	Treatment (T)	15.83	7	<0.0001	21.06	7	<0.0001
	Exposure time (DAT)	11.84	1	0.0007	20.29	1	<0.0001
	Source	**Slug mortality**			**Feeding ability**		
		F	df	*p*	F	df	*p*
Expt. C	Treatment (T)	32.64	7	<0.0001	21.45	7	<0.0001
	Exposure time (DAT)	20.08	1	<0.0001	18.69	1	<0.0001
	Concentration of the compounds	0.0	1	1.0000	1.52	1	0.2190

Table 3. ANOVA results for the experiment D (df for the error term: 238).

	Source	Slug mortality			% damaged plants			% damaged leaves		
		F	df	*p*	F	df	*p*	F	df	*p*
Expt. D	Treatment (T)	6.22	4	<0.0001	6.39	4	<0.0001	4.92	4	<0.0001
	Exposure time (DAT)	11.68	2	<0.0001	24.09	2	<0.0001	16.50	2	<0.0001

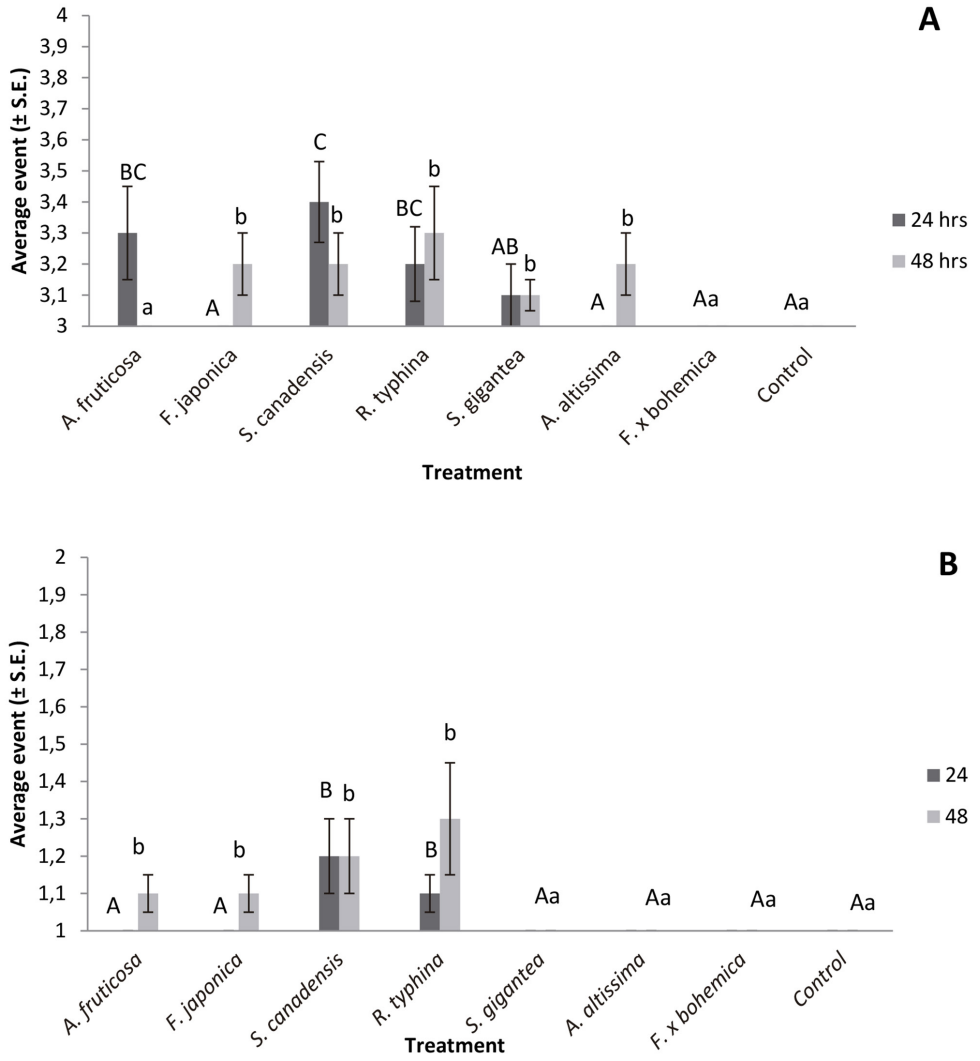

Figure 1. Antifeedant effect of slugs (A) and slug mortality (B) among different treatments within 24/48 hrs, respectively. Average values of events (± S.E.) during experiment A, in which we studied the contact efficacy of the studied plant powders (n = 10). Means with the same capital/small letter above the histrogram bars are not significantly different at $p = 0.05$ (Duncan's multiple range test) among different treatments within 24/48 hrs, respectively. Event 1: slug survived the experiment; event 2: slug died during the experiment; event 3: slug fed on lettuce; event 4: slug did not feed on lettuce.

study showed that after 24 hrs, 100% of the slugs did not feed on the lettuce when the barriers were made from the powders of *S. canadensis*, *R. typhina* and *S. gigantea*. Positive results were also observed in the treatments *A. altissima* and *F. x bohemica* (90% of the slugs tested did not eat the lettuce). For more details, see Fig. 2A. The results of our study showed that after 48 hrs, when barriers were made from the powders of *R. typhina* and *S. gigantea*, 100 % of slugs did not feed on the lettuce. Positive results were also observed in the *A. altissima* treatment (80% of the slugs tested did not eat the lettuce).

The efficacy of the barrier after 24 and 48 hrs is presented in Fig. 2B. Results of our study showed that after 24 hrs, the efficacy of powders (*R. typhina*, and *S. gigantea*) as slug barriers was 100%. Positive results were also observed in the *A. altissima* and *F.* × *bohemica* treatments (90% of the slugs tested did not cross the barrier). The results of our study showed that after 48 hrs, the efficacy of powder *R. typhina* as a slug barrier was 100%. Positive results were also observed in the *S. gigantea* treatment (90% of the slugs tested did not cross the barrier). For more details, see Fig. 2B.

Experiment C

The antifeedant effect of the slugs after 24 and 48 hrs at 2.5% concentration is presented in Fig. 3A.

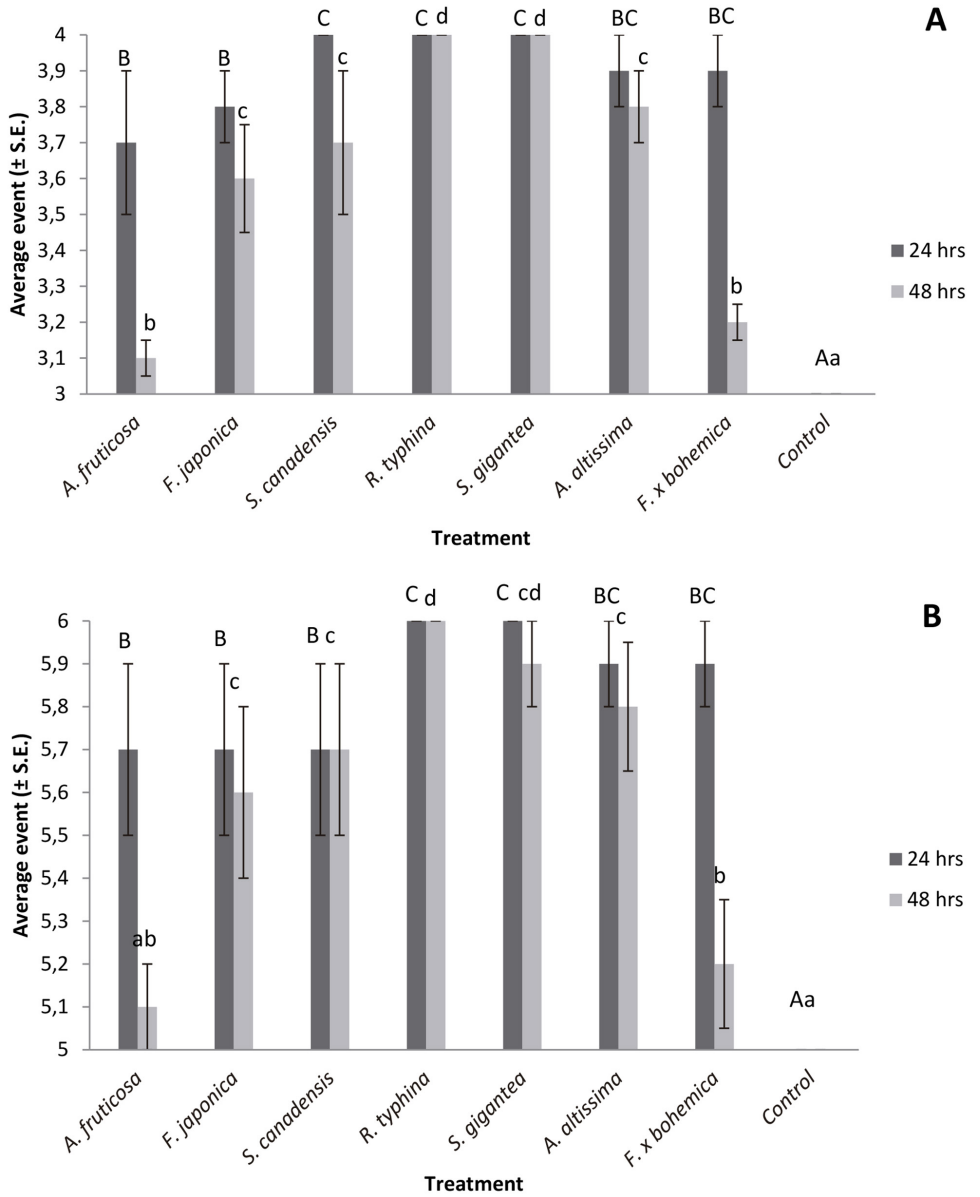

Figure 2. Antifeedant effect of slugs (A) and efficacy of barrier (B) among different treatments within 24/48 hrs, respectively. Average values of events (± S.E.) during experiment B, in which we studied substances as barriers for slugs (n = 10). Means with the same capital/small letter above the histrogram bars are not significantly different at $p = 0.05$ (Duncan's multiple range test) among different treatments within 24/48 hrs, respectively. Event 3: slug fed on lettuce; event 4: slug did not feed on lettuce; event 5: slug crossed the barrier; event 6: slug did not cross the barrier.

Results of our study showed that after 24 hrs, 30% of the slugs did not feed on the lettuce when the leaves were treated with a liquid preparation of *S. gigantea* and *F. japonica*. Antifeedant activity was also confirmed in treatments with *S. canadensis* (20% of the slugs tested did not eat the lettuce) and *R. typhina* (10% of the slugs tested did not eat the lettuce). In all other treatments, the antifeedant effect was not confirmed. The results of our study showed that after 48 hrs, when the lettuce leaves were treated with liquid preparation of *R. typhina*, 20% of the slugs did not feed on the lettuce. Antifeedant activity was also confirmed in treatments with *S. canadensis* (10% of the slugs tested did not eat the lettuce), and *F. japonica* (10% of the slugs tested did not eat the lettuce). In all other treatments, the antifeedant effect was not confirmed (see Fig. 3A).

The antifeedant effect towards the slugs after 24 and 48 hrs at 10.0% concentration is presented in Fig. 3B. Overall, the non-tested plant powders showed satisfactory results. Moreover, the results of our study showed that after 24 hrs, 60% of the slugs did not feed on the

lettuce when the leaves were treated with a liquid preparation of *F. japonica*. Antifeedant activity was also confirmed in the treatments with *S. canadensis* (10% of the slugs tested did not eat the lettuce), *S. gigantea* (20% of the slugs tested did not eat the lettuce), and *A. fruticosa* (20% of the slugs tested did not eat the lettuce). The results of our study showed that after 48 hrs, 40% of the slugs did not feed on the lettuce when the leaves were treated with a liquid preparation of *F. japonica*. Antifeedant activity was also confirmed in the treatments with *S. canadensis* (10% of the slugs tested did not eat the lettuce), *F.* × *bohemica* (10% of the slugs tested did not eat the lettuce), and *A. fruticosa* (20% of the slugs tested did not eat the lettuce). In all other treatments, the antifeedant effect was not confirmed (see Fig. 3B).

Experiment D

Analysis of the percentage of the plants attacked after 24 hrs showed that there were no significant differences (F=0.70; p=0.6022) among the different treatments. Damage on the plants was recorded in all treatments except in the positive control (without slugs) (see Fig. 4A). Analysis of the percentage of attacked plants after 48 hrs showed that among the different treatments, there were significant differences (F=3.09; p=0.0391). When the lettuce was sprinkled with a liquid formulation of the compound tested, an average of 36% of the plants were attacked by slugs. When powder was used (as the slug barrier), 28% of plants were attacked. In combination with both treatments, 4% of the plants were attacked,

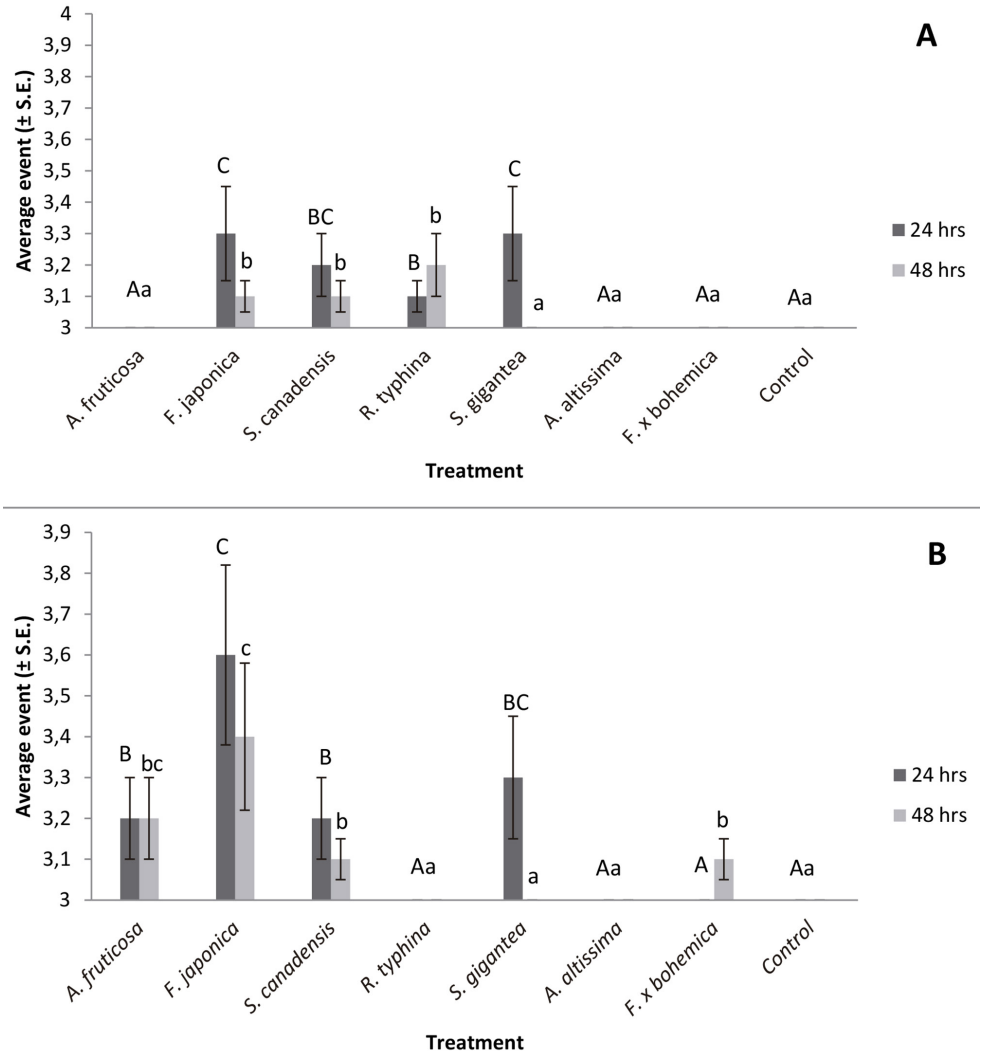

Figure 3. Antifeedant effect of slugs after 24 and 48 hrs at two concentrations: 2.5% (A) and 10.0%. Average values of events (± S.E.) during experiment C, in which we studied substances in liquid formulation against slugs (n = 10).). Means with the same capital/small letter above the histrogram bars are not significantly different at p = 0.05 (Duncan's multiple range test) among different treatments within 24/48 hrs, respectively. Event 3: slug fed on lettuce; event 4: slug did not feed on lettuce.

while in the negative control, 56% of plants were attacked. For more details, see Fig. 4A. Analysis of the percentage of plants attacked after 72 hrs showed that among the different treatments, there were significant differences (F=8.04; p=0.0005). When the lettuce was sprinkled with a liquid formulation of the compound tested, an average of 44% of the plants were attacked by slugs. When powder was used (as the slug barrier), 60% of the plants were attacked. In combination with both treatments, 8% of plants were attacked, while in the negative control, 72% of the plants were attacked. For more details, see Fig. 4A.

Analysis of the percent damage after 24 hrs showed that among the different treatments, there were no significant differences (F=0.89; p=0.4864). When the lettuce was sprinkled with a liquid formulation of the tested compound, an average of 10% of the plants were damaged by slugs. When powder was used (as the slug barrier), 7% of the plants were damaged. In the negative control, 1% of the plants were damaged. Analysis of the percent damage after 48 hrs showed that among the different treatments, there were no significant differences (F=0.87; p=0.4991). When the lettuce was sprinkled with a liquid formulation of the compound tested, an average of 22% of the plants were damaged by slugs. When powder was used (as the slug barrier), 18% of the plants were damaged. In the negative control, 24% of the plants were damaged. Analysis of the percent of damage after 72 hrs showed that among the different treatments, there were no significant differences (F=2.51; p=0.0743). When the lettuce was sprinkled with a liquid formulation of the compound tested, an average of 27% of the plants were damaged by slugs. When powder was used (as the slug barrier), 40% of the plants were damaged. In the negative control, 47% of the plants were damaged. For more details, see Fig. 4B.

After 72 hrs, all the lettuce plants were cut, and the total weight of the leaves, mass of damaged/undamaged leaves, and mass of leaves that showed phytotoxicity were measured. Analyses of the total yield of lettuce showed that among the different treatments, there were no significant differences (F=2.13;

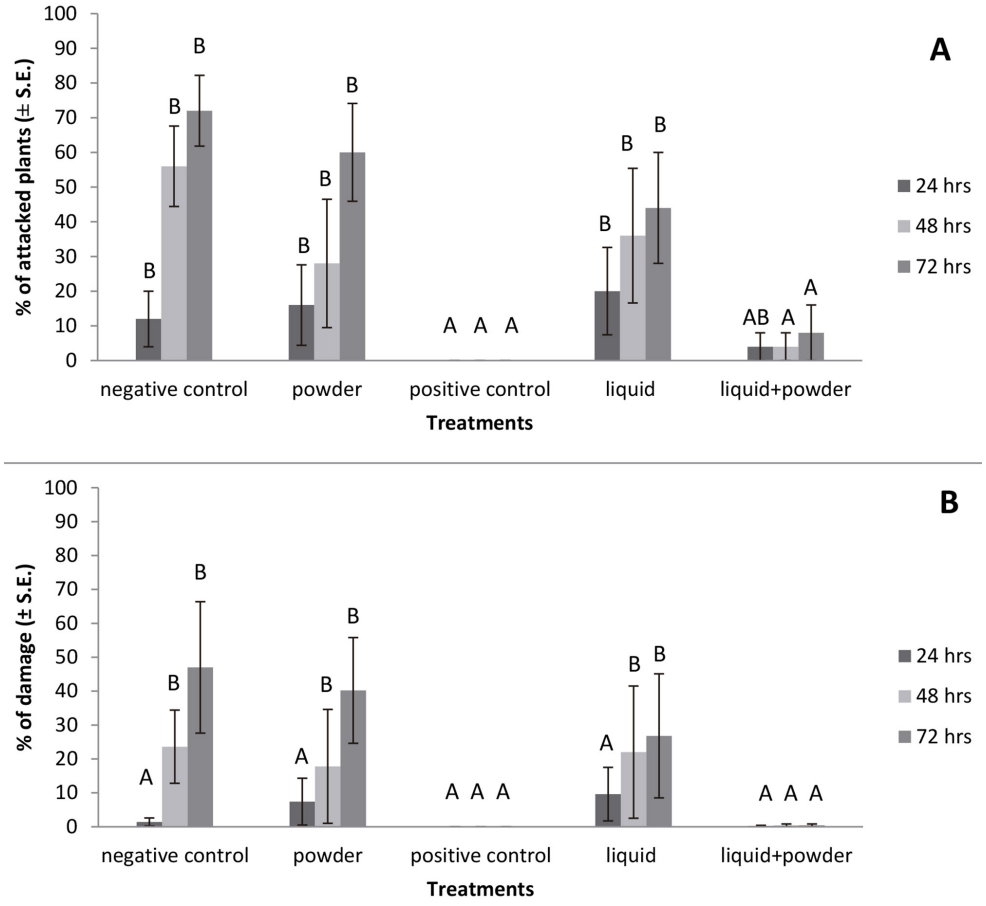

Figure 4. Average% (± S.E.) of attacked plants (A) and damage (B) in experiment D (semi-field trial). Means with the same capital/small letter above the histrogram bars are not significantly different at p = 0.05 (Student's multiple range test) among different treatments within 24/48/72 hrs, respectively.

p=0.1143). The highest yield was obtained in the positive control (on average 29 g of lettuce), the lowest in the powder formulation as a slug barrier (on average 16 g of lettuce). For more details, see Fig. 5. Analyses of the yield of undamaged leaves of the lettuce showed that among different treatments, there were significant differences (F=3.64; *p*=0.0220). The highest yield was obtained in the positive control (27 g of lettuce on average), and the lowest was obtained in the powder formulation as a slug barrier (10 g of lettuce on average). For more details, see Fig. 5. Analyses of the yield of the slug-damaged leaves of lettuce showed that among the different treatments, there were significant differences (F=5.28; *p*=0.0046). The highest yield of the damaged leaves was obtained in the negative control (3 g of lettuce on average), and the lowest was obtained in the positive control (0 g of damaged lettuce on average). For more details, see Fig. 5. Analyses of the yield of leaves with phytotoxic symptoms showed that among the different treatments, there were significant differences (F=11.02; *p*=0.0001). The highest yield of phytotoxic leaves was obtained in combination with powder and liquid formulations (4 g of lettuce on average), and the lowest was obtained in the positive and negative controls (0 g of damaged lettuce on average). For more details, see Fig. 5.

Discussion

Due to the price and other benefits, molluscicides represent the most common strategy in terrestrial gastropod control programmes. Molluscicides are considered emerging pollutants and are frequently detected in surface water bodies above the EU statutory drinking water limit (Castle *et al*., 2017). There are also reports of the negative impact of molluscicides on non-target organisms (Bailey, 2002). Furthermore, growers and farmers often experience difficulty controlling terrestrial gastropods with conventional bait pellets containing molluscicides such as methiocarb (this molluscicide is already being withdrawn from use following the recent ban by the European Commission) and metaldehyde. Due to these problems, researchers are looking for new, more environmentally acceptable ways of protecting plants against harmful organisms, including slugs (Laznik & Trdan, 2016). Recently, the use of environmentally acceptable substances for slug control in agriculture and horticulture has gained unprecedented impetus all over the world (El-Sherbini *et al*., 2009; Laznik *et al*., 2011; González-Cruz & San Martín, 2013; Laznik & Trdan, 2016). Different natural substances are promoted due to their wide range of ideal properties, such as high target toxicity, low mammalian toxicity, low cost, and easy bio-degradability (Laznik *&* Trdan, 2016). One of these measures is also the use of plant extracts against slugs and snails (Barone *&* Frank, 1999; El-Sherbini *et al*., 2009; González-Cruz & San Martín, 2013). In our research (laboratory and semi-field), we studied both the contact efficacy, antifeedant and barrier efficacy with the use of plant material of seven invasive plant species, including knotweeds, goldenrods, staghorn sumac, tree of heaven*,* and false indigo. A novelty in our research was the idea of how invasive plants could be used to control arionid slugs.

When we rolled the slugs in tested plant powders, the highest mortality of the individuals tested was

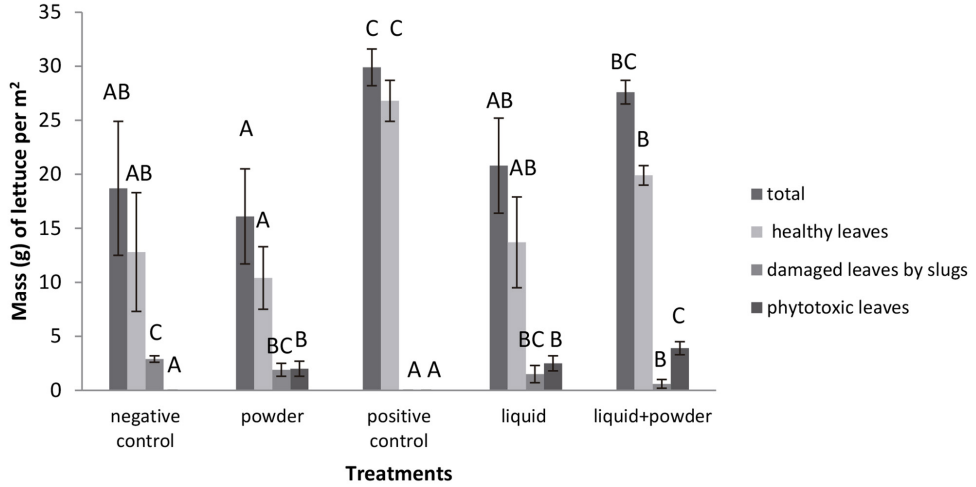

Figure 5. Average mass (± S.E.) of lettuce (g) in a semi-field trial at different treatments. Means with the same capital/small letter above the histogram bars are not significantly different at $p = 0.05$ (Student's multiple range test) among different treatments within 24/48/72 hrs, respectively.

recorded in *R. typhina* (30%). These results also led to very low feeding inhibitor effects in experiment A. Klingauf *et al*. (1988) reported that plant extracts from *R. typhina* have insecticidal efficacy against aphids (up to 70%). Furthermore, laboratory studies have shown that *R. typhina* plant extracts also have bactericidal and fungicidal efficacy (Mosch *et al*., 1989; Rayne & Mazza, 2007). Until now, there had been no reports on the molluscicidal efficacy of *R. typhina*.

In the second experiment (B), the plant powder of selected plants was used as a barrier for the slugs. After 48 hrs, none of tested slugs fed on lettuce when the barrier was made from the plant powder of *R. typhina* and *S. gigantea*. None of the slugs crossed the barrier made of *R. typhina*; however, 10% of the slugs that crossed the barrier made of *S. gigantea* did not feed on the lettuce. In all the treatments, the slugs observed produced a large amount of mucus. The effect of the plant powders is similar to that of wood ash and hydrated lime, as all substances cause dehydration of the cuticle and blockage of the airways (Laznik & Trdan, 2016). Prior (1985) reported that slugs are susceptible to dehydration due to evaporative water loss across their body and lung surface and through the deposition of their slime trail. Mucus production is an energy-wasting process that could also impact the feeding behaviour of slugs. More detailed experiments are merited to confirm this thesis in the future.

In experiment C, our goal was to determine whether the foliar application of the tested plant material in a liquid formulation has any effect on reducing the feeding ability of the individuals tested. In our investigation, the concentration of plant water extracts (2.5% or 10% w/v had no influence on slug feeding behaviour. At both concentrations tested, the best anti-feedant effect was confirmed with the *F. japonica* plant water extract, with slug feeding reduced by up to 40%. The use of knotweed plant extracts had only been cited to control plant diseases, such as powdery mildew (Herger *et al*., 1988; Neuhaus & Pallut, 1992; Konstantinidou-Doltsinis *et al*., 2006), or mites (*Tetranychus urticae*) (Tomczyk, 2006).

In experiment D, our goal was to investigate different techniques of using plant material (as a barrier or foliar liquid formulation) under semi-field conditions. For this matter, only *S. gigantea* plant material was used. The best results were obtained when we used the combination of both techniques (only 7% of plants were attacked by slugs). In the control treatment, over 70% of plants were attacked by slugs. The most important biologically active compounds in goldenrod plants are flavonoids, saponins and terpenes (Starks *et al.*, 2010). The literature concerning the use of plant extracts with a high content of saponins for the control of terrestrial gastropods is limited. The published reports are mainly related to egg control, feed deterrence, seed treatment and the formulation of baits (Winder & Friedrich, 1996; Barone & Frank, 1999; Iglesias *et al*., 2002; González-Cruz & San Martín, 2013). González-Cruz & San Martín (2013) concluded in their investigation that the application of plant extracts with a high content of saponins could be a good means for controlling slugs because saponin residues have been demonstrated to be safe in foods and agronomic products in the USA, EU and Japan. However, the results of our investigation confirmed a high level of phytotoxicity when the lettuce was treated with a combination of powder and liquid formulation. More than 10% of lettuce leaves showed phytotoxic symptoms. Phytotoxicity could be related to the use of a high concentration (10%) of plant extract in our investigation. To support our assumption, further investigation is needed.

The underlying principle of integrated slug control is to reduce the risk of slug damage by means of cultural practices, if possible, and apply molluscicides if necessary. Biological control (*P. hermaphrodita*) has a role to play, together with other techniques that are especially relevant to organic growers (Wilson *et al*., 1993; Schüder *et al*., 2003; Laznik *et al*., 2011; Laznik & Trdan, 2016). It is important not to attempt to eradicate slugs completely but simply aim to limit their damage to economically acceptable levels. Slug control in organic systems presents particular problems because the use of chemicals is substantially restricted. Various methods of slug control are recommended for organic growers, but most are untested and unproven. Our study showed that plant material (dry or in liquid formulation) could represent an alternative solution for private gardens in plant protection against slugs. However, there are still many challenges to overcome (phytotoxicity and economics) in further studies. In addition, the potential challenges are the risk of using products derived from invasive pants, as many of the plant species tested in our paper are controlled by legislation throughout the EU.

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