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RESEARCH ARTICLE

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Is a combination of different natural substances suitable for slug (*Arion* spp.) control?

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Abstract

In a laboratory study we investigated the contact and barrier efficacy of different natural substances (wood ash, sawdust, hydrated lime, and diatomaceous earth) against slugs of the genus *Arion,* an important agricultural pest. Natural substances were tested individually and in combination with each other. The experiment was carried out in plastic petri dishes and in glass insectaria. Moistened tampons and fresh leaves of lettuce were placed into both experimental arenas. The slugs were starved for 48 hours prior to the experiment. Six categories of behaviour were identified for slugs in the presence of the natural substances: (1) slug survived the experiment, (2) slug died during the experiment, (3) slug crossed the barrier, (4) slug did not cross the barrier, (5) slug fed on the lettuce, and (6) slug did not feed on the lettuce. The effect of different treatments (natural substances) was significant. The results of our study have shown that hydrated lime had the best contact efficacy on slugs (the mortality of slugs was 100%), both individually and in combination with other substances. The treatments with hydrated lime also proved to be the most efficient barrier preventing slugs from feeding on lettuce. Hydrated lime shows great potential in *Arion* control in our investigation; however, further research is needed to investigate the practical value (how to avoid the problem when the substance becomes wet), safety and economics of hydrated lime used in this way.

Additional key words: slugs; *Lactuca sativa*; barrier; contact efficacy; diatomaceous earth; hydrated lime; sawdust; wood ash.

Authors' contributions. Designed and performed the experiments, and wrote the manuscript: ZL. Analysed the data: ST. Both authors read and approved the manuscript.

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Introduction

Slugs of the genus *Arion* (Gastropoda: Arionidae) have been classified as a major economic pest in Europe (Frank, 1998), North America (Hammond *et al.*, 1999), Asia (Ahmadi, 2004), and Australia (Barker, 2002). Slugs are primarily pests of ground crops, such as vegetable and cereals (Port & Port, 1986; Barker, 1991, 2002). They can damage tuber crops, such as potato and cause losses of seeds, seedlings and fruit. Damage to seedlings usually leads to major losses following the death of plants (Barker, 2002).

For the effective management of any pest, a knowledge of its ecology and biology is required. The life of the *Arion* slug is closely connected to its environment, with temperature and humidity directly affecting biological processes (Barker, 1991). Young & Port (1989) reported that air temperature, soil surface temperature, wind speed, humidity and soil moisture content are all correlated with slug activity. Slugs have a preference for areas of high humidity and the choice of a daytime resting site can be crucial to their survival. Slugs are extremely susceptible to dehydration due to evaporative water loss across their integument and lung surface, and through the deposition of their slime trail (Prior, 1985). Consequently, active slugs can lose up to 40% of their initial body weight in less than 2 hours (Prior & Uglem, 1984; Barker, 2002). Slugs favour heavier soils, being able to survive over summer in cracks in the soil and under clods (Barker, 2002).

Early chemical control has been based on burnt lime, hydrated lime and dehydrated copper sulphate. The chemical control of pest slugs is usually reliant on the application of baits containing a molluscicide. Slugs can be difficult to kill by contact poisons because they are covered by a layer of slime which prevents chemicals from coming in contact with the skin (Hunter & Symonds, 1970; Barker, 2002). Furthermore, growers and farmers often experience difficulty in controlling these pests 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. For example, in wet conditions the efficacy of these pellets can be very low (Hata *et al*., 1997) leading to unsatisfactory control levels. Furthermore, poison baits can be toxic to other non-target soil invertebrates, as well as birds, frogs, and mammals (Martin, 1993; Purvis, 1996). In addition to environmental problems, human health problems also arise from agricultural pesticide usage. Many farmers are seeking new ways to curb pesticide usage to address the many concerns.

The development of alternative slug control methods compatible with integrated pest management (IPM) strategies used to control other pests would help satisfy increasing market demands and environmentally safety issues. In field experiments in organic farming carried out with the nematode biocontrol agent *Phasmarhabditis hermaphrodita*, Wilson *et al*. (1993) concluded that *P. hermaphrodita* is a promising alternative to chemical molluscicides. Reidenbach *et al*. (1989) explored the possibilities of using Sciomyzidae (Diptera) as biological control agents for crop pest molluscs with some success. Symondson (1989) reported that carabid beetles reduced slug population by up to 80%. Physical barriers, such as continuous lines of sawdust or ash, provide a dry surface which slugs avoid (Barker, 2002); however, the effectiveness of these barriers is reduced once they become wet. Copper barriers are believed to create an electrical current when they react to snail or slug secretions (Schüder *et al*., 2003; Laznik *et al*., 2011).

Environmentally acceptable substances (wood ash, hydrated lime, diatomaceous earth, sawdust) were tested in a laboratory experiment to investigate their application value for controlling slugs. The aim of our investigation was to study: (1) the contact control efficacy of an individual and combined use of tested substances; (2) the barrier effect of tested substances (individual and combined); and (3) an effect on slug eating ability of tested substances (individual and combined).

Material and methods

Slugs

The experiments were performed at the Laboratory of Entomology (Dept. of Agronomy, Biotechnical Faculty, University of Ljubljana). Two hundred and twenty slugs, mainly representatives of *Arion vulgaris* Moquin-Tandon and *Arion rufus* (L.), were collected at the laboratory field of the Biotechnical Faculty in Ljubljana (46°04'N, 14°31'E, 299 m a.s.l.) in May 2014. The gathered slugs were of various ages and lengths as we wanted to analyse a comprehensive sample of outdoor slug behaviours (Laznik *et al*., 2011). The slugs were starved for 48 hours prior to the experiment (Schüder *et al*., 2003). We weighed slugs using electronic scales.

Experimental design

The experiment involved the following substances: wood ash (ash remaining after burning of beech firewood which was additionally crushed in a mortar), hydrated lime (Cl 90-S; IMG Zagorje d.o.o.; Zagorje ob Savi, Slovenia), diatomaceous earth (from Bela Cerkva at Dolenjska region, Slovenia) (Rojht *et al*., 2010), and sawdust (from beechwood, finely ground). The said substances were studied individually or in combination in the ratio 1:1 (the studied substances were of the same weight and mixed in larger vessels). Each treatment was repeated ten times. The control sample was a slug sprinkled with water. All experiments were carried out in a growth chamber (type: RK-900 CH, produced by the company Kambič laboratorijska oprema d.o.o., Semič) at 22 °C and 75% relative air humidity.

The study of substances' contact efficacy (Experiment A)

The experiment A, which lasted three days, included 110 slugs (11 different treatments which were repeated ten times). The experiment was carried out in plastic petri dishes (150 × 20 mm; produced by the company Kemomed d.o.o., Kranj, Slovenia). Moistened tampons (35 × 11 mm; produced by the company Tosama d.d., Vir pri Domžalah) 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 or their combinations (treatments): wood ash, hydrated lime, diatomaceous earth, sawdust, wood ash + hydrated lime, wood ash + diatomaceous earth, wood ash + sawdust, hydrated lime + diatomaceous earth, hydrated lime + sawdust, diatomaceous earth + sawdust and the control. In the three-day experiment we checked once a day the survival of slugs, weighed them again, replaced the lettuce leaf (the source of food), additionally moistened the tampon, and again rolled them in the studied substances. For the statistical analyses the mass of dead slugs were treated as the mass 0 g. The slugs which died during the experiment were not replaced with live ones (Laznik *et al*., 2011). The aims of the experiment A were (1) to test the contact control efficacy of an individual and combined use of tested substances, and (2) an effect on slug eating ability of tested substances (individual and combined).

The studied substances as barriers for slugs (Experiment B)

The experiment B, which lasted two days, involved 110 slugs (11 different treatments which were repeated ten times). The experiment was carried out in glass insectaria (width-length-depth 500-350-400 mm). Moistened tampons were placed in the glass insectaria (35 × 11 mm; produced by the company Tosama d.d., Vir near Domžale) with fresh leaves of lettuce. Before setting the experiment the slugs were weighed. Around the leaf of lettuce we sprinkled the barrier (20 g of a substance) 3 cm wide and 2 cm thick. We studied the following barriers: wood ash, hydrated lime, diatomaceous earth, sawdust, wood ash + hydrated lime, wood ash + diatomaceous earth, wood ash + sawdust, hydrated lime + diatomaceous earth, hydrated lime + sawdust, diatomaceous earth + sawdust and the control (without barriers). In the two-day experiment we (once a day) checked whether slugs had crossed the barrier or not, whether they had eaten, we weighed them again, replaced the leaves of lettuce (the source of food), additionally moistened tampons and repaired barriers if they were damaged. For the statistical analyses the mass of dead slugs were treated as the mass 0 g. The slugs which died during the experiment were not replaced with live ones (Laznik *et al*., 2011). The aims of the experiment B were (1) to test the barrier effect of tested substances (individual and combined), and (2) an effect on slug eating ability of tested substances (individual and combined).

Statistical analysis

The typical behavioral 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 the experiment A, the value of the event was 2 (see Table 1).

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 crossed the barrier
4	Slug did not cross the barrier
5	Slug fed on lettuce
6	Slug did not feed on lettuce

A two-way analysis of variance (ANOVA) was carried out to evaluate the differences in the response of *Arion* slugs to different treatments. Before analysis, each variable was tested for homogeneity of variance and the values transformed by arcsine square root if necessary. Duncan's multiple range test ($\alpha = 0.05$) was used to analyse the differences between individual treatment means (Hoshmand, 2006). All statistical analyses were performed using Statgraphics Plus for Windows 4.0 (Statistical Graphics Corp., Manugistics, Inc., Rockville, Maryland, USA). The data are presented as untransformed means (Laznik *et al*., 2011)

Results

General analysis

The analysis of the events indicated that the mortality of slugs in the experiment ($F = 94.57$; df = 10, 329; $p < 0.0001$) and their feeding ($F = 81.40$; df = 10, 329; $p < 0.0001$) were influenced by different treatments, while the exposure time (DAT) did not influence the mortality of slugs in the experiment ($F = 1.25$; df = 2, 329; $p = 0.2865$) nor their feeding ($F = 0.29$; df = 2, 329; $p = 0.7476$). Different treatments did influence the slugs' crossing of the barriers ($F = 9.30$; df = 10, 219; $p < 0.0001$), while the exposure did not influence their crossing ($F = 0.06$; df = 1, 219; $p = 0.8131$). The feeding of slugs which crossed the barriers was influenced by both the exposure time ($F = 7.88$; df = 1, 239; $p = 0.0054$) and different treatments ($F = 16.06$; df = 10, 239; $p < 0.0001$).

Individual analysis

The analysis of the events has shown that in the experiment A slugs, which were sprinkled with hydrated lime individually or in combination with other substances, died (average event value: 2.0 ± 0.0)

(Fig. 1a). Satisfactory mortality rates of slugs were confirmed also with individual application of wood ash (average event value: 1.73 ± 0.08). In other treatments mortality rates were below 50%. During the control treatment, the individual application of sawdust and the combined application of diatomaceous earth and sawdust did not produce statistically significant differences in the mortality rates of slugs in our experiment.

The best barriers for slugs in the experiment B were treatments which included hydrated lime. The average values of events ranged between 3.75 ± 0.1 (hydrated lime, and hydrated lime in combination with sawdust) and 3.85 ± 0.08 (hydrated lime in combinations with diatomaceous earth and wood ash) (Fig. 1b). Less than 50% of slugs crossed the barrier composed of wood ash and diatomaceous earth (3.55 ± 0.11). In the remaining treatments the slugs that crossed the barrier exceeded 50% (Fig. 1b).

The analysis of events has shown that slugs in the experiment A did not eat in all those treatments which involved hydrated lime, individually or in combination with other substances (average event value: 6.0 ± 0.0) (Fig. 2a). Satisfactory rates of preventing the feeding was achieved also with the individual application of wood ash (average event value: 5.76 ± 0.08). In the remaining treatments the rates of feeding of slugs exceeded 50% (Fig. 2a).

Treatments with hydrated lime individually or in combination with other substances proved to be the most efficient in preventing the feeding of slugs in our experiment (Fig. 2b). The analysis of the events has shown that satisfactory efficiency in preventing their feeding in the experiment was achieved also with barriers made from wood ash, individually or in combination with other substances except sawdust, in which case the feeding of slugs was more pronounced (average event value: 5.45 ± 0.11) (Fig. 2b).

The analysis of the average mass of slugs in the experiment A is presented in the Table 2. The results have shown that the largest share of mass was lost by those slugs which were treated with hydrated lime individually or in combination with other substances already after the first day (−100 ± 0%). Individual application of wood ash or its combination with diatomaceous earth was among other studied substances which satisfactorily effected loss of mass in slugs (Table 2).

The analysis of the average mass of slugs in the experiment B is also presented in the Table 2. The

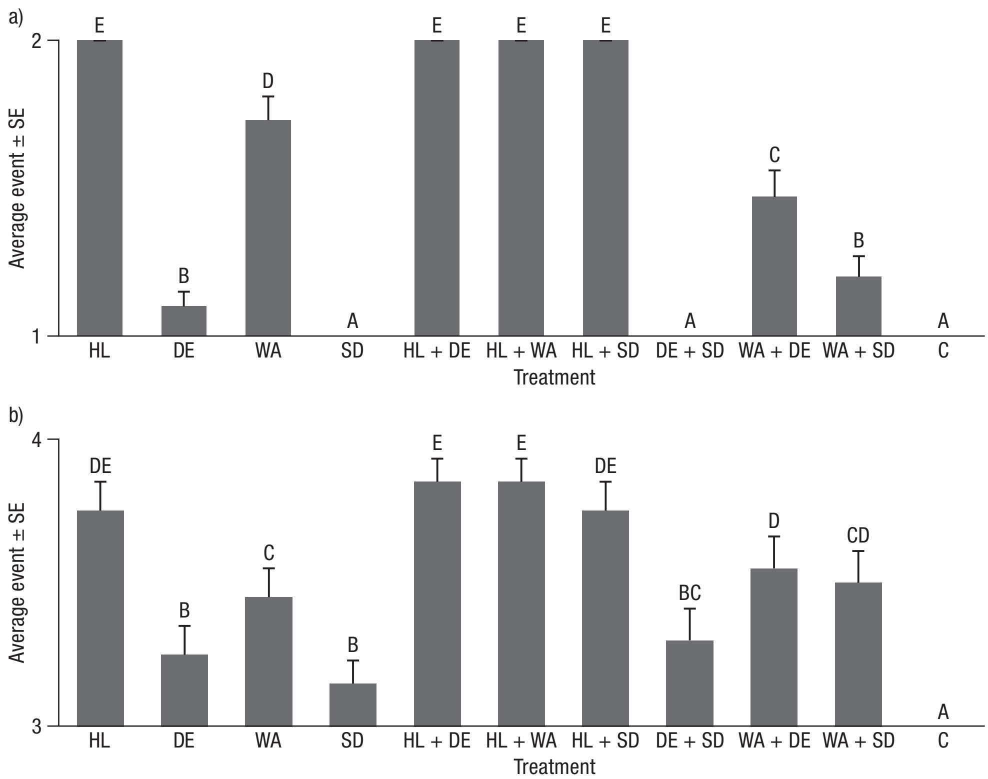

Figure 1. Average values of events (±SE) during the experiment A in which we studied contact efficacy of the studied environmentally acceptable substances (n = 10) (a) or during the experiment B in which we studied the effect of substances as barriers (n = 10) (b). Different letters above the histogram bars indicate significant differences between groups according to Duncan's multiple range test ($p < 0.05$). HL, hydrated lime; DE, diatomaceous earth; WA, wood ash; SD, sawdust; C, control. Event 1, slug survived the experiment. Event 2, slug died during the experiment. Event 3, slug crossed the barrier. Event 4, slug did not cross the barrier.

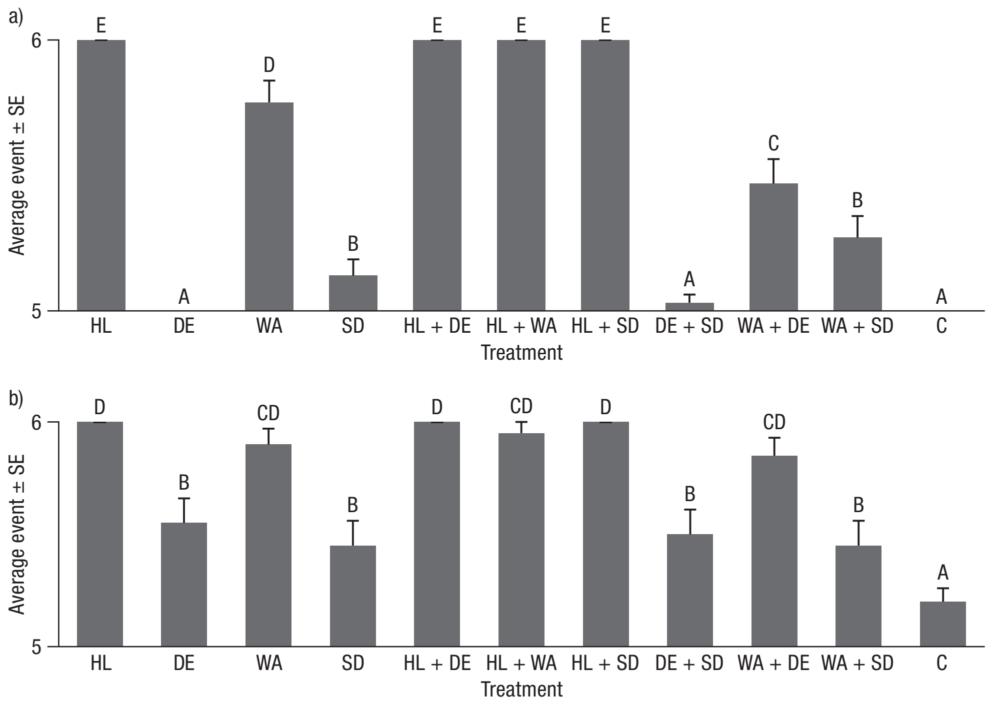

Figure 2. An effect on slug eating ability of tested substances during experiment A (n = 10) (a) or during experiment B (n = 10) (b). Different letters above the histogram bars indicate significant differences between groups according to Duncan's multiple range test ($p < 0.05$). Legend: HL, hydrated lime; DE, diatomaceous earth; WA, wood ash; SD, sawdust; C, control. Event 5, slug fed on lettuce; event 6, slug did not feed on lettuce.

Table 2. Average Δ percentage (% ± SE) of mass of slugs in the experiment with contact and barrier efficacy of the applied substance or the combination of substances (n = 10).

Substance[1]	Contact efficacy			Barrier efficacy		
	Mass of slugs 0 hrs	Δ Mass of slugs after 24 hrs	Δ Mass of slugs after 48 hrs	Mass of slugs 0 hrs	Δ Mass of slugs after 24 hrs	Δ Mass of slugs after 48 hrs
HL	100 ± 0 Aa	-100 ± 0 Fa	-100 ± 0 Fa	100 ± 0 Aa	-16 ± 2 Db	-32 ± 3 Fc
WA	100 ± 0 Aa	-68 ± 2 Eb	-94 ± 2 Ec	100 ± 0 Aa	-22 ± 3 Eb	-20 ± 3 Eb
DE	100 ± 0 Ab	-2 ± 2 Cb	+18 ± 3 Aa	100 ± 0 Aa	-2 ± 2 Ca	-17 ± 3 DEb
SD	100 ± 0 Ac	+11 ± 3 ABb	+18 ± 3 Aa	100 ± 0 Aa	+2 ± 2 Ca	+2 ± 2 Ba
HL + WA	100 ± 0 Aa	-100 ± 0 Fa	-100 ± 0 Fa	100 ± 0 Aa	-36 ± 4 Fb	-53 ± 4 Hc
HL + SD	100 ± 0 Aa	-100 ± 0 Fa	-100 ± 0 Fa	100 ± 0 Aa	-13 ± 3 Db	-45 ± 4 Gc
HL + DE	100 ± 0 Aa	-100 ± 0 Fa	-100 ± 0 Fa	100 ± 0 Aa	-15 ± 3 Db	-28 ± 3 Fc
DE + SD	100 ± 0 Ab	-1 ± 1 Cb	+11 ± 2 Ba	100 ± 0 Aa	+1 ± 1 Ca	-7 ± 2 Cb
WA + SD	100 ± 0 Ab	+8 ± 2 Ba	+6 ± 1 Ca	100 ± 0 Aa	-11 ± 3 Db	- 13 ± 3 Db
WA + DE	100 ± 0 Ac	-26 ± 3 Db	-34 ± 2 Da	100 ± 0 Ab	+24 ± 4 Aa	+17 ± 3 Aa
C	100 ± 0 Ab	+13 ± 2 Aa	+14 ± 1 ABa	100 ± 0 Ab	+13 ± 3 Ba	+14 ± 3 Aa

[1]HL, hydrated lime; DE, diatomaceous earth; WA, wood ash; SD, sawdust; C, control. Mean values followed by different uppercase or lowercase letters indicate significant differences in Δ percentage (±SE) of slug mass among different treatments or different day of exposure, respectively ($p<0.05$, Duncan's test).

results have shown that the largest share of mass was lost by those slugs which were treated with hydrated lime individually or in combination with other substances. The most efficient barrier which caused the most substantial loss of mass in slugs was made from hydrated lime and wood ash (after 48 hours they lost 53 ± 4% of their body weight) (Table 2).

Discussion

In recent times, the use of environmentally acceptable substances for pest control in agriculture has gained unprecedented impetus all over the world (Radwan & El-Zemity, 2007; Rojht *et al*., 2010; Singh *et al*., 2010). 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 (Marston & Hostettmann, 1985; Rojht *et al*., 2010; Singh *et al*., 2010). In our research we studied both contact efficacy and the so-called barrier efficacy of different environmentally acceptable substances (hydrated lime, wood ash, sawdust, diatomaceous earth) on slugs. A novelty in our research was the study of combinations of different environmentally acceptable substances on mortality rates, feeding rates and effects of barriers on slugs.

The results of our research have shown that hydrated lime had the best contact efficacy on slugs both individually and in combination with other substances (wood ash, diatomaceous earth, sawdust). Slugs were observed producing large amounts of mucus when the animals came into contact with the hydrated lime. Satisfactory contact efficacy was also achieved by applying wood ash. The effect of wood ash is similar to that of hydrated lime, as both substances cause dehydration of cuticle and blocking of airways (Boiteau *et al*., 2012). Prior (1985) reported that slugs are extremely susceptible to dehydration due to evaporative water loss across their integument and lung surface, and through deposition of their slime trail. It is surprising that diatomaceous earth in our experiment did not prove to be a substance which causes dehydration of slugs. Even more, when we rolled a slug in diatomaceous earth, the slug got rid of the substance with the help of its slime and carried on feeding. Diatomaceous earth, nonetheless, has proved to be an efficient agent by damaging the cuticle and airways of insects (Athanassiou *et al*., 2005; Rojht *et al*., 2010; Bohinc *et al*., 2013). Hydrated lime individually, as well as in combination with other studied substances, proved to be the best barrier against slugs in this study. Ryder & Bowen (1977) reported that the slug foot is an important route for the uptake of different compounds that can cause an irritant effect. This irritation can cause significant dehydration and loss of weight (Prior & Uglem, 1984; Gebauer, 2002). Hydrated lime (individually and in combination with other substances) and wood ash (individually and in combination with diatomaceous earth) were the two studied substances which had the biggest effect on the feeding of slugs. On the basis of our observations we conclude that the tested substances efficiently prevented slugs from crossing the barrier to reach the source of food. The tested substances in the experiment with contact efficacy also caused the highest mortality rate of the studied slugs, which meant that the slugs did not eat.

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). It is important not to attempt to eradicate slugs completely but simply aim to limit damage to economically acceptable levels. This is particularly difficult for growers of crops such as salads, where the tolerance level is effectively zero. A key point in integrated control is that the best control of slug damage in many crops is achieved only when slug control measures are implemented shortly before slug damage is seen (Schüder *et al*., 2003). Slug control in organic systems presents particular problems because the use of chemicals is greatly restricted. Various methods of slug control are recommended for organic growers, but most are untested and unproven. However, recent research has shown that the use of hydrated lime can be recommended, at least for special situations with high-value crops. Physical barriers such as hydrated lime or wood ash provide a dry surface which slugs avoid (Barker, 2002). The effectiveness of these barriers could be reduced once they become wet. For example, in wet conditions the efficacy of the molluscicide pellets can be very low (Hata *et al*., 1997). In areas with dry summers (Mediterranean region) the efficacy of such barriers could be more effective. However, there are no published data to support our concern and further investigation in needed.

The relatively small size of the market for molluscicides, combined with stringent testing that new compounds must undergo before approval, means that new molluscicides are only likely to be introduced into the market if a pesticide developed for another purpose shows molluscicidal properties or if low toxicity compounds can be used (Henderson & Triebskorn, 2002). Hydrated lime shows great potential in *Arion* control in our investigation. Possible field application of tested substances could be reached with the use of hand rotary duster/hand dusting around the plants. However, further research is needed to investigate the practical value (how to avoid the problem when the substance becomes wet), safety and economics of hydrated lime used in this way. Nonetheless, the use of hydrated lime could be suitable for private gardens as well as for large crop growing areas.

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