Automated analysis of slug and snail behaviour

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ABSTRACT

A new method for a fully automated tracking of slugs and snails is presented. Applications include methodological, academic and applied aspects. Firstly, arena size and shape was shown to significantly influence the level of activity and the track shape of the slug *Deroceras reticulatum*. Secondly, the diurnal behaviour of *D. panormitanum* was shown to be mainly nocturnal, presenting 24-hour time budgets and a detailed analysis of locomotor activity in 30-minute intervals over 24 hours. Thirdly, in a choice test the strong repellent effect of cinnamamide to the snail *Oxyloma pfeifferi* was demonstrated. Fourthly, the behavioural response of *D. panormitanum* to ureaformaldehyde was shown to increase significantly with concentration. The new software allows the automatic analysis (presence and locomotor activity) for any time and any zone in combination with semi-automatic analysis through a key-stroke activated event recorder. The presented techniques may be transferable to many invertebrates.

INTRODUCTION

Analysis of behaviour can be an important tool for developing an integrated pest management strategy for slugs and snails in agriculture or horticulture. Whilst early work on mollusc behaviour relied on labour-intensive observations (Karlin, 1961) the introduction of video analysis together with time lapse recording allowed the constant observation of animals (Cook, 1989). The analysis was further advanced by software packages such as EthoVision ®, which allow fully automated tracking of animals (Spink & Tegelenbosch, 2001). The software allows behavioural analysis for small specimens, which show little background contrast. Applications include methodological aspects, such as choice of appropriate arena size, academic interests such as diurnal behaviour patterns and applied approaches such as response of animals to pesticides. The experiments reported here were done with the slug *Deroceras reticulatum*, a major pest in agriculture (Godan, 1983) and the slug *D. panormitanum* and the snail *Oxyloma pfeifferi*, the major mollusc pests in Hardy Nursery Stock, a valuable sector of the horticulture industry (Schüder, *et al.*, 2002).

The basic steps of the analysis include:
1) Recording behaviour with a video camera
2) Digitisation of video material
3) Definition of key-strokes for behaviour event recorder (optional)
4) Definition of size and shape of arena(s) and (hidden) zone(s)
5) Automated acquisition of tracks and manual acquisition of pre-defined behaviours
6) Separate analysis of tracks by zone, time interval or behaviour

The basic technical requirements of the software include a contrast between animal and background (under infra-red light), a visually homogenous background (i.e. no bright objects of similar size to the animal), a video recorder (with time lapse mode), a (infra-red sensitive) video camera and a computer with 64 MB ram.

METHODS

Slugs and snails were collected in the field and kept under experimental conditions for at least one week to acclimatise them. Slugs weighed between 300 and 400 mg, snails between 120 and 160 mg. All experiments were done at 15 °C and with a 16:8 light:dark cycle (dark from 22:00 to 06:00). During the dark cycle, the experimental arenas were illuminated with infra-red light. Arenas were filled with damp compost and the inner sides of the walls were painted with Fluon® repellent paint. Behaviour was recorded with a time lapse mode of 1:60 (diurnal behaviour) or 1:80 (other experiments).

Does Arena size and shape matter?

Slugs (*D. reticulatum*) were placed in the centre of individual arenas, which were either circular (16 cm in diameter) or rectangular (40 x 60 cm). Arenas were divided into two zones of equal size, "centre" and edge" (100 cm^2 for circular arenas and 1200 cm^2 for rectangular arenas respectively). There were 16 replicate dishes for the small and four for the large arena.

Diurnal behaviour

Slugs (*D. panormitanum*) were placed into circular arenas (16 cm diameter) 24 hours prior to start of the recording to allow more natural behaviour after exploration of the arena. Arenas contained a shelter (horticultural matting; 3 x 3 cm) and a piece of Chinese cabbage (3 x 3 cm). Pre-defined behaviours "resting", "moving" "feeding" and "sheltering" (under leaf or shelter) were analysed with the event recorder. There were 20 replicate dishes.

Repellent effect of novel molluscicides

Snails (*O. pfeifferi*) were placed in the centre of circular arenas (16 cm diameter), of which one semi-circle was treated with a 1 % dispersion of cinnamamide. The snail behaviour was analysed separately for the treated and untreated semi-circles. There were 10 replicate dishes.

Dose-dependent behavioural response of slugs to molluscicides

Slugs (*D. panormitanum*) were placed in the centre of circular arenas (16 cm diameter), which were either untreated or treated with 0.1 %, 0.5 % or 1 % ureaformaldehyde solution. There were 10 replicate dishes per treatment.

RESULTS

Does Arena size and shape matter?

In the small circular arenas, some of the slugs spent a large proportion of their time going around the edge of the arena close to its wall. The centre of the arena was only crossed occasionally (Figure 2a). The presence in the centre zone, the time spent moving and the distance travelled there were significantly lower than in the edge zone (N = 16, paired t-test: $P < 0.001$, Table 1 and Fig. 1). The slugs moved slightly faster when in the centre of the arena. However, this trend was not significant. When the slugs were allowed to move in a much larger rectangular arena, their behaviour changed (Figure 2a). There was no significant difference between the centre and edge of the arena regarding presence, time spent moving and the track length (N = 4, paired t-test: $P > 0.05$, Table 1 and Fig. 1). The slugs moved significantly faster in the centre of the arena than at the edge (N = 4, paired t-test: $P < 0.01$, Table 1). When comparing the two arenas types, it was obvious that slugs moved about twice as much in the large rectangular arena than in the small circular arena, but at a lower speed.

Table 1. Presence and activity (± SE) of *D. reticulatum* in "centre" and "edge" zones during a 14-hour period.

	Small circular arena				**Large rectangular arena**			
	arena	**centre**	**edge**	***P***	**arena**	**centre**	**edge**	***P***
Area	**100%**	**50%**	**50%**		**100%**	**50%**	**50%**	
Presence [min]	**840**	**85 ± 25**	**755 ± 25**	**< 0.001**	**840**	**373 ± 109**	**467 ± 116**	**> 0.05**
Moving [min]	**227 ± 15**	**35 ± 6**	**192 ± 12**	**< 0.001**	**577 ± 56**	**284 ± 47**	**293 ± 74**	**> 0.05**
Velocity [cm/min]	**3.4 ± 0.3**	**3.5 ± 0.2**	**3.4 ± 0.3**	**> 0.05**	**2.5 ± 0.1**	**2.8 ± 0.1**	**2.2 ± 0.1**	**< 0.01**

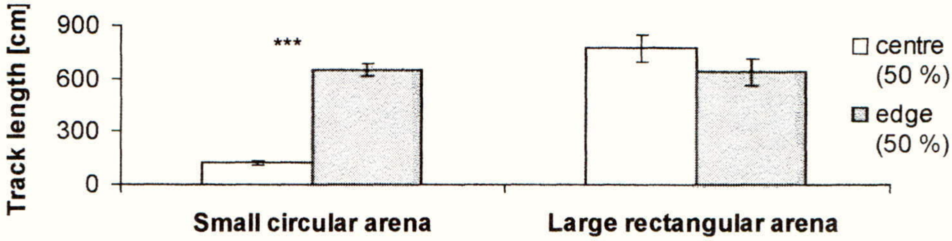

Figure 1. Distance (± SE) *D. reticulatum* moved in 14-hour period in "centre" or "edge" zone.

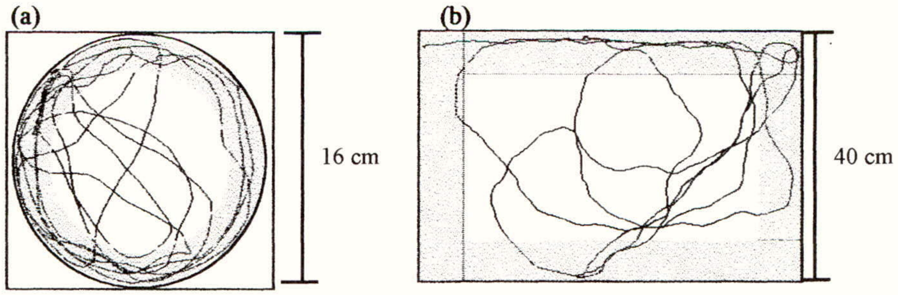

Figure 2. Representative tracks of *D. reticulatum* during an eight-hour dark period
(a) Small circular arena. (b) Large rectangular arena.

Diurnal behaviour

The slugs spent a large proportion of their time under the shelter or under a leaf (Figure 3a). The total active time was approximately 20 %, of which 16 % was spent moving and 4 % was spent feeding. The movements of the slugs could be followed precisely. Several times slugs were observed to move straight from the shelter to the Chinese cabbage leaf after a long period of sheltering (Figure 3b). Slugs was mainly nocturnal. However there were three peaks of activity during the light period, two in the morning and one around the time when the experiment was started at 17:00 hours (Figure 4).

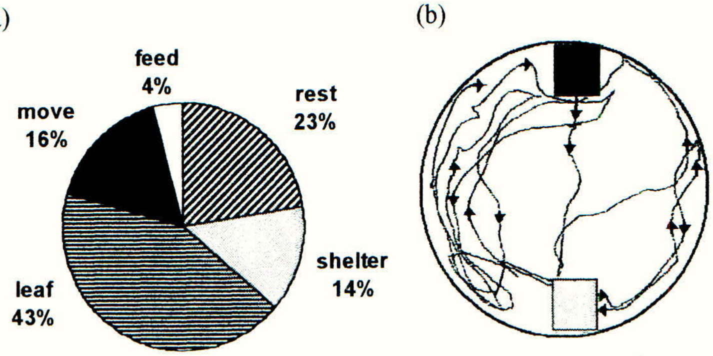

Figure 3. Diurnal behaviour of *D. panormitanum*: (a) Time budget over 24 hours as analysed with an event recorder. (b) Details of the visual output (shelter black, leaf grey).

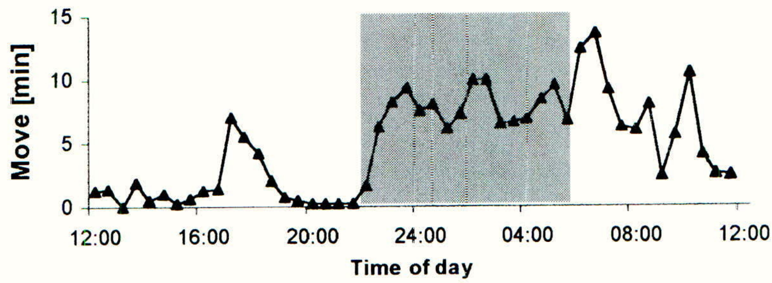

Figure 4. Activity of *D. panormitanum* (N = 20) over 24 hours analysed in 30-minute intervals.

Repellent effect of novel molluscicides

Cinnamamide had a strong repellent effect on the snail *O. pfeifferi*. They were present in the treated semi-circle only 12 % of the total time and the proportion spent moving was even lower at 3 %, which was significantly lower than in the untreated semi-circle. (N = 10, paired t-test: $P < 0.01$, Table 2 and Figure 5b). The actual distance moved on cinnamamide was significantly lower than on untreated compost, a mere 1 % of the track length (N = 10, paired t-test: $P < 0.01$, Table 2 & Figure 5a). The speed of the snails was also reduced on the cinnamamide-treated surfaces. However, this effect was not significant (N = 10, paired t-test: $P > 0.05$, Table 2).

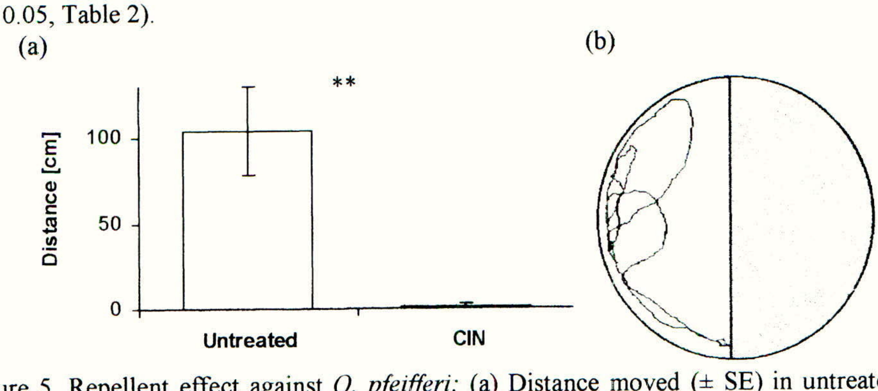

Figure 5. Repellent effect against *O. pfeifferi:* (a) Distance moved (± SE) in untreated and treated (CIN) semi-circle during a 10-hour period. (b) Track for an eight-hour period.

Table 2. Presence and activity (± SE) of *O. pfeifferi* in choice test over a 10-hour period.

	Untreated	CIN	*P*
Area	50%	50%	
Presence [min]	526 ± 60	74 ± 60	< 0.01
Moving [min]	195 ± 35	5 ± 5	< 0.01
Velocity [cm/min]	0.5 ± 0.1	0.3 ± 0.1	> 0.05

Dose-dependent behavioural response of slugs to molluscicides

Treating surfaces with ureaformaldehyde had a significant effect on the behaviour of *D. panormitanum* at all three concentrations. In comparison with the untreated compost, both the time spent moving and the distance travelled were significantly reduced over a 14-hour period (N = 10, Anova: $P < 0.001$ Table 3, Figures 6 and 7). The medium and high concentration also showed a significant reduction of activity in comparison with the low concentration. For the highest concentration of ureaformaldehyde, the velocity of the slugs was also significantly reduced in comparison with the untreated compost (N = 10, Anova: $P < 0.01$ Table 3).

Table 3. Dose dependent response of *D. panormitanum* to ureaformaldehyde during a 14-hour period (mean ± SE). Different letters represent sig. differences between treatments.

	Treatment				
	Untreated	UF 0.1 %	UF 0.5 %	UF 1 %	*P*
velocity [cm/min]	2.6 ± 0.1 a	2.5 ± 0.1 a	1.8 ± 0.2 ab	2.1 ± 0.2 b	< 0.01
move [min]	484 ± 23 a	313 ± 36 b	161 ± 20 c	123 ± 11 c	< 0.001

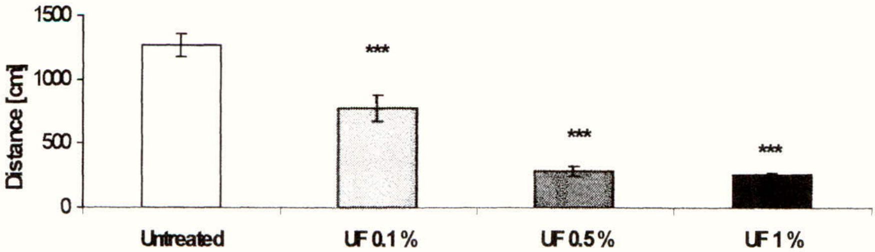

Figure 6. Distance moved (± SE) by *D. panormitanum* in a 14-hour period on untreated compost and ureaformaldehyde (UF).

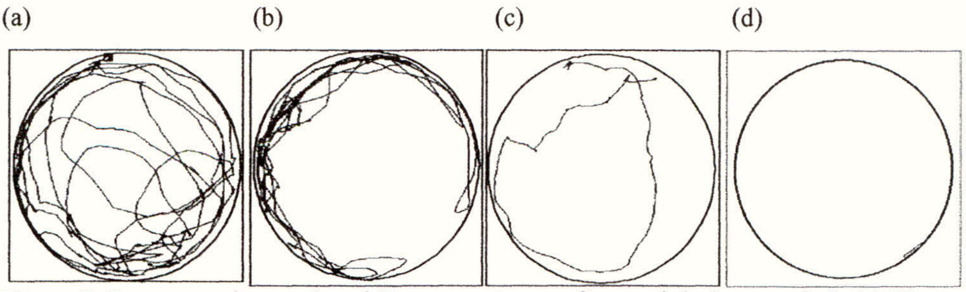

Figure 7. **Representative tracks of *D. panormitanum* for an eight-hour period. (a) Untreated** compost (b) – (d) Ureaformaldehyde: (b) 0.1 % (c) 0.5 % (d) 1 %.

DISCUSSION

D. reticulatum clearly behaved differently depending on the arena shape and size (Figures 2a and 2b). The choice of arena size will have to be a compromise between effective use of time and effort and the desire to observe a more "natural" behaviour such as slugs searching for pellets. The small arena allowed up to six animals to be recorded each night on the same video recording, which was time-effective, as shown for experiments analysing a dose-dependent physiological response (Figure 7a-d). As seen in the diurnal behaviour experiments with *D. panormitanum*, the introduction of shelter(s) and a food source - and their position in the arena - can make the animal spend more time in the centre of the arena (Figure 6 and 7). When a small circular arena is divided into two semi-circles (treated and untreated), there is a relatively large border between the treated and untreated areas in relation to area. Slugs and snails may tend to carry on moving along the arena wall instead of being repelled by a surface treatment. This would mean that the repellent properties of a product would have to be stronger to cause an actual repellent effect.

Overall, the possibilities of analysing video recordings with this software are numerous. The combination of fully automated analysis (track, presence in zones, moving, velocity, turning angles, frequency, total and mean duration of parameters) for any zone and any time interval with parameters recorded with the help of the event-recorder (any behaviour) allow the acquisition and analysis of large quantities of data. Constraints include the number of replicates per night (depending on arena size), the strict requirements regarding illumination and background and the restriction to one animal per arena (a second animal is only possible if different in size, e.g. predator and prey).

This fully automated analysis offers a significant sophistication and improvement in comparison with software packages which only allow manual tracking or the technique of tracking animals in certain intervals on acetates attached to video screens. The techniques described in this paper could be transferred to many groups of vertebrates and invertebrates.

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IPM approach to control of the slug *Arion lusitanicus* Mabille – a new pest species in Serbia and Montenegro

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ABSTRACT

The molluscicidal activity of an ecologically friendly and biodegradable molluscicide based on Fe-pyrophosphate-hydrate was investigated against slugs. The product effectivity was tested on *Arion lusitanicus*, a noxious slug species recently introduced to Serbia and Montenegro and now developing into a major local pest. The trials were conducted in both a cabbage field and a horticultural nursery in Belgrade. The tested product was applied in granular formulation as baits, and compared with conventional methiocarb-based products. In both trials Fe-pyrophosphate-hydrate treatments demonstrated significant efficacy (89.4-91%) on *A.lusitanicus*, similar to methiocarb-based products (89-91.6%). Therefore the product has potential in the management of this introduced slug species as a part of an integrated pest management programme.

INTRODUCTION

The extensive economic damage caused to vegetable and horticultural crops by *A.lusitanicus* (Mabille), an invasive arionid species, was first recorded in Serbia and Montenegro in 2002. This slug had previously only been reported from nearby Bulgaria (Wiktor, 1983). It is known to be a pest throughout Europe, where it has developed into a serious danger to cultivated plants (e.g. Godan, 1999).

Data on its new distribution in Serbia and Montenegro are still scarce. Reliable information concerning its presence is limited to a few isolated highland localities in Southwest Serbia and Eastern Montenegro with prominent outbreaks and severe agricultural damage. There is also anecdotal evidence of this slug having been found in the lowland region of Vojvodina, where it promises to cause economic damage in the near future.

From an economic point of view, slugs constitute the most important pest group amongst molluscs in Serbia and Montenegro. Over the past decades, molluscicides have been used mainly by vegetable and horticultural growers. Arable and fodder crops have not usually been endangered.

The latest trends in the management of slugs include the use of Integrated Pest Management (IPM) programmes which have not yet successfully been tried in Serbia and Montenegro. Slug control so far only consists of conventional chemical treatments with standard molluscicidal baits, mostly methiocarb-based. To make a first step towards an effective IPM programme, we have focused our efforts on introducing a natural and ecologically friendly molluscicide based on Fe-pyrophosphate-hydrate.

The iron compounds, mainly chelated iron salts, are both stomach and contact poisons for a range of terrestrial molluscs. Henderson, *et al.* (1989) focused much of their investigations on slug sensitivity towards these compounds. Furthermore, some iron chelates used in slug pellets offer considerable advantages over metaldehyde and methiocarb in that they pose no threat to the environment. Indeed, they can be sources of trace elements in agriculture (Young, 1996).

The active component of Fe-pyrophosphate-hydrate is iron in the form of ferric pyrophosphate. It is biodegradable into Fe and phosphate, i.e. natural products that are nutrient elements to plants, soil microorganisms and animals. In these trials on the recently introduced pest slug *A. lusitanicus*, the efficacy of a Fe-pyrophosphate-hydrate-based bait was compared with a conventionally-used and highly effective methiocarb-based bait.

MATERIALS AND METHODS

Trials were conducted in September 2002. Despite a warmer and drier autumn than usual for the time of year, slug densities were found to be high at two locations in the Belgrade area. These were:

a) a cabbage field at Krnjaca (cabbage cultivar Futog, stage of development 45,47 and 49; soil - chernozem); percentages of pest slugs and snails found on the plot : *A.lusitanicus* - 97%, *Deroceras agreste* (L.) and *Helix pomatia* (L.) - 3%.

b) a horticultural nursery at New Belgrade, Public Greenery Company (*Thymus*, *Campanula*, *Cineraria* and *Iberis* spp.; soil humus); percentages of pest slugs and snails found on the plot: *A. lusitanicus* - 95% and *H. pomatia* - 5%.

Standard test methods were used (Anonymous 1999; Anonymous 2002). The test Fe pyrophosphate hydrate-based product used in our trials was ARION (treatment F1), Unichem, Vrhnika, Slovenia, ready-to-use granulated bait (0.4% Fe). Since no Fe pyrophosphate hydrate-based product has so far been registered in Serbia and Montenegro, we used as a reference product the same product from a different manufacturer - FERAMOL (treatment F2), Neudorf GmbH KG, Emmerthal, Germany (0.4% Fe). Another reference product used was Mesurol GR (treatment M), Bayer-Leverkusen, Chemical Agrosava, Beograd, (4% methiocarb).

The type of field experiment was a randomised block design, with 4 replicates per treatment. The application rates of pellets were 5 g/m^2 band sprayed (2 replicates) or 30 pellets (5 g) applied in heaps (2 replicates). The plot size was 8 x 3 m. Slugs were counted one day prior to each treatment; the following day, baits were deposited, and plots were investigated after 1, 3, 5 and 7 days.

The molluscicidal efficacy was evaluated by the Henderson–Tilton formula of percentage efficacy (Anonymous, 1975). Data from the different treatment efficacy were compared and analyzed by analysis of variance (ANOVA).

RESULTS

Table 1 shows the results from initial sampling before the treatments. All the tested products showed high efficacy, especially on days 5 and 7 days after treatment. No significant differences were found in the efficacy between different treatments (Table 2), nor between band-spraying or application in heaps. It is noteworthy that the tested molluscicide and both of the standard products had similar effects on the other species found on the investigation sites.

a) Cabbage field trial

Table 1. Numbers of *A. lusitanicus* per m^2 prior to treatment (Sept. 6, 2002)

Treatment	Replicates				Mean	Sd
	I	II	III	IV		
F1	18	27	24	26	23.75	4.03
F2	19	22	17	21	19.75	2.22
M	22	27	21	25	23.75	2.75
Control	16	19	11	13	14.75	3.50

Table 2. Efficacy of different molluscicides (%) on *A.lusitanicus* over a seven-day period after treatment

Treatment	Efficacy (%)			
	1^{st} day	3^{rd} day	5^{th} day	7^{th} day
F1	25.3a	63.1b	88.4c	89.4d
F2	20.2a	55.7b	83.5c	86.1d
M	24.2a	57.9b	87.4c	91.6d

Note: Treatment means, for the same date (across columns) followed by the same letter are not significantly different from each other as established by ANOVA, with means separated by Fisher's least significant difference test ($P < 0.05$); a ($P<0.30$), b ($P <0.45$), c ($P<0.15$), d ($P <0.09$).

b) Horticultural nursery trial

The results from the horticultural nursery were similar to those from the cabbage field (a) described above (Tables 3 and 4). The results of both trials show good effects of the Fe pyrophosphate hydrate-based product in the control of *A.lusitanicus* compared with the conventional methiocarb-based product. The former achieved a reduction of individuals of up to 89.4% in cabbage, and 91% in the horticultural nursery, compared with 91.6% and 89% efficacy for methiocarb respectively.

Table 3. Numbers of *A. lusitanicus* per m^2 prior to treatment (Sept. 2, 2002)

Treatment	Replicates				Mean	SD
	I	II	III	IV		
F1	47	56	21	32	39.00	15.56
F2	39	42	28	25	35.50	8.27
M	41	42	35	27	36.25	6.90
Control	57	37	32	29	38.75	12.61

Table 4. Efficacy of different molluscicides (%) on *A. lusitanicus* over a seven-day period after treatment

Treatment	Efficacy (%)			
	1^{st} day	3^{rd} day	5^{th} day	7^{th} day
F1	25.27a	60.97b	87.18c	91.03d
F2	29.58a	64.09b	85.22c	89.44d
M	32.42a	65.52b	85.20c	88.97d

Note: Treatment means, for the same date (across columns) followed by the same letter are not significantly different from each other as established by ANOVA, with means separated by Fisher's least significant difference test ($P< 0.05$); a ($P<0.06$), b ($P<0.50$), c ($P<0.85$), d ($P<0.83$).

DISCUSSION

For a long period in Serbia and Montenegro, methiocarb was the only available molluscicide. Metaldehyde and aluminium sulphate–based products have not yet been registered. Unfortunately, together with methiocarb-based pellets, a methiocarb spraying-formulation legally registered as a bird repellent is also accessible to some potentially irresponsible growers (Mitic, 2002).

For a number of years, but especially more recently, growers in Serbia and Montenegro appeared willing to try any "home remedy" because of the severe problems they had with *A. lusitanicus*. Consequently, uncontrolled spraying increased potential hazards for many susceptible yet beneficial groups of organisms.

From an economic point of view, the majority of growers cannot afford the commercial use of *Phasmarhabditis hermaphrodita*, which is an efficient biocontrol agent against slugs in general. Moreover, *A. lusitanicus* may have become less sensitive to this nematode (Speiser & Andermatt, 1996).

In such circumstances, Fe-pyrophosphate hydrate-based molluscicides are promising. Concerning its bait formulation, the present results suggest that it is as effective as methiocarb (c.90 %), at least on *A. lusitanicus* adults. We are aware that a period of increased sensitivity of slugs occurs at the peak of their reproductive activity (Godan, 1983). This coincided with the time of our trials and would therefore be expected to have improved the efficacy of the treatments.

Although bait formulation usage has serious limitations in relation to high populations of slugs, the introduction of this ecologically mild molluscicide to our country could be useful. Together with a spectrum of preventive methods which should be encouraged, growers could, in Fe-pyrophosphate-hydrate, have a relatively harmless agent at hand that can help them to minimize the impact of *A. lusitanicus* and other terrestrial pest molluscs.

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Growth measurement in the land snail *Helix aspersa* (Müller) fed artificial diets

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ABSTRACT

Five laboratory feeding assays were performed on the Common Garden Snail *(Helix aspersa*) collected from the field. Individual measurements of growth over 7 days were conducted simultaneously by daily gravimetric determinations of live weight and measurements of parameters of energy balance. Individual daily data of live weight was processed according to an exponential model in the following form (Live weight $_t = a \times e^{r \times time}$) giving a mean growth rate (mg/mg/day) of 0.0275 for lettuce and chicken food, 0.060 for a high moisture content (M.C.) food, and 0.108 and 0.090 for two low M.C. foods. Scope for growth (SFG) investigation gave a mean potential biomass increase of 26.95 (± 9.45) mg/day with the exception of one of the low M.C. diets: Wla = 68.14 (± 26.43) mg/day. Weight increase calculated from gravimetric measurements accounted for 75.6% (± 35.1) of SFG recordings, lettuce showing lower values. Food conversion indexes were lowest for lettuce (14.46 ± 6.03) with moderate values for high M.C. and chicken foods (0.835 ± 0.37) and highest values for low M.C. diets (0.3105 ± 0.107).

INTRODUCTION

Although little information is available on the nutritional requirements of terrestrial gastropods needed to sustain active growth, observations on feeding in natural environments reveals a polyphagous, largely vegetarian diet (for a review see Speiser, 2001). When cultured, the edible snail *Helix aspersa* (Müller) is currently fed artificial, low moisture-content foodstuffs, whereas plant materials available in nature are highly enriched with water. The aim of our study was therefore to relate growth rate to physiological parameters by describing the feeding and digestive behaviour of snails supplied with foods of different water content and similar biochemical composition.

MATERIALS AND METHODS

Individuals of *H. aspersa* (Müller) were collected from the field in Leioa (Biscay: Lat. 43° 24′N; Long. 2° 59′W). The snails were carefully cleaned, weighed and distributed in homogenous groups according to live body weight (b.w.). Size distribution (1.5-2.5 g live wt) corresponds to the so-called second rearing phase in snail farming. Experiments took place between the months of May and August (1998). Snails (10 per diet) were individually maintained in plastic containers with a regular water supply, stable temperature and humidity conditions (20°C ± 2, 85%-90% r.h.) and under a natural photoperiod regime. Animals were fed *ad libitum* and chambers were cleaned, faeces collected and food replaced daily. Two reference diets were assayed (a "natural" diet based on lyophilized lettuce in an agar gel (L : moisture content. 94.2%), and a commercial food (G: moisture content 4.2%) for chicken). These were compared with three diets which had common proportions of biochemical and mineral compounds and variable moisture content (M.C.): Wh (M.C. high, 70.42%), Wl_a and WL_b (M.C. low, 5.3%).

The following aspects were measured daily over a 7 day period: (a) live weight, (b) gravimetric ingestion and egestion rates (both in organic and inorganic terms) (c) metabolic expenditure, as oxygen consumption, measured manometrically. Energy equivalents for foodstuffs were derived from biochemical composition and rates of metabolism, using an oxycaloric coefficient of 20.08 J ml^{-1} O_2^{-1}. After the experiments, snails were carefully dissected and both soft tissues and shell were dried at 100°C for 48 h and weighed, after which they were ashed (450°C for 12 h.) and reweighed.

Live weight is likely to experience hydration variation under experimental manipulation, and this possibility was therefore evaluated in two additional experiments covering every diet. Results indicate that the proportion of both total dry matter (d.m.) and of soft tissue d.m. to live weight remain constant for up to 15 days from the second day of experimentation (Table 1), the initial change being explained by filling of the alimentary canal. Consequently, individual daily data of live weight was processed according to an exponential model which was used as (Live weight$_t$ = a x $e^{r \times time}$) and growth rates (GR = mg/mg/day) were computed.

An effort was made to select uniformly sized snails, and the initial mean for live weight was 1992.07 mg with a variation coefficient of 22%. Nevertheless, differences in physiological rates resulting from size variation are evident throughout the experiment and data were standardized for a common live weight of 2000 mg according to the formula Ystd = Yexp $(2000/Wexp)^{b}$, where Ystd = standard value for the standard size animal; Yexp = direct physiological measurement; Wexp = actual live weight of a given animal and b the specific weight power for each physiological parameter: b = 0.6929 for ingestion and egestion rates and 1.05 for oxygen consumption (unpublished data). Similarly,

GRstd (mg/mg/d) = GR exp x $e^{-(2000/Wexp) \times 0.186}$, where GR stands for growth rate.

RESULTS AND DISCUSSION

Hydration levels remained constant under regular feeding and water supply regimes (Table 1), supporting the observations of Klein-Rollais (1986). Weight data for individual animals relative to moisture content (M.C.), organic matter and ash of both meat and shell were processed according to allometric models (Table 2). Results indicate that, given an homogeneous size class (1.5-2.5 g live weight corresponding to the so-called second rearing phase in snail farming), relationships appeared to be isometric with the exception of the positive allometry found for mineral content of shell vs dry shell weight: a weight exponent >1 could relate to the need to support static loads (Schmidt-Nielsen, 1984). Lack of allometry implies that the various body components were fixed proportions of live weight, (LW) or dry matter (d.m.): i.e. for the mean size snail (LW = 2 g) d.m. represented 22.04% of LW evenly accounted for organics and inorganics (82.32% of total organics would be in the soft tissues whereas 90.87% of minerals would appear in the shell).

Table 1. Summary of analysis of covariance for testing significance among slopes and elevations within the groups of regression equations established for different maintenance times in the laboratory: initial day, 3 days, 6-9 days and 12-15 days. d. f.: degrees of freedom; ns = not significant ($P > 0.05$). SBDW = Soft Body Dry Wt; LW = Live Wt; ASB = Ashed Soft Body; ASW = Ashed Shell Wt; SDW = Shell Dry Wt; SBWW = Soft Body Wet Wt; TWW = Total Wet Wt and SWW = Shell Wet Wt .

	Slope			Elevation		
Equation	Fs	d. f.	Significance	Fs	d. f.	Significance
SBDW vs LW	0.178	3, 112	ns	2.295	3, 106	ns
ASB vs SBDW	0.450	3, 64	ns	1.692	3, 106	ns
ASW vs SDW	0.790	3, 67	ns	3.000	3, 67	ns
SBWW vs TWW	1.145	3, 111	ns	2.339	3, 106	ns
SWW vs TWW	1.000	3, 111	ns	2.740	3, 106	ns

Table 2. Summary of linear regression equations relating the various body parameters of snails. Weights were expressed in mg and subject to log-log transformation. Live Wt (LW), Total Wet Wt (TWW), Soft Body Wet Wt (SBWW), Shell Wet Wt (SWW), Soft Body Dry Wt (SBDW), Shell Dry Wt (SDW), Ashed Soft Body Wt (ASBW) and Ashed Shell Wt (ASW).

Equations	n	Elevation	slope	r^2	*P*	%*
Soft Body Dry Weight vs Live Weight	110	-1.030	1.015 ± 0.081	0.852	0.0001	0.105
Total Wet Weight vs Live Weight	110	-0.118	1.008 ± 0.035	0.969	0.0001	0.811
Soft Body Wet Weight vs Total Wet Weight	110	-0.139	1.013 ± 0.023	0.989	0.0001	0.802
Shell Wet Weight vs Total Wet Weight	110	-0.657	0.980 ± 0.091	0.807	0.0001	0.189
Soft Body Dry Weight vs Soft Body Wet Weight	110	-0.745	0.986 ± 0.072	0.873	0.0001	0.161
Shell Dry Weight vs Shell Wet Weight	110	-0.251	1.055 ± 0.046	0.950	0.0001	0.777
Ashed Soft Body weight vs Soft Body Dry Weight	55	-0.838	0.970 ± 0.198	0.644	0.0001	0.124
Ashed Shell Weight vs Shell Dry Weight	65	-0.078	1.020 ± 0.015	0.996	0.0001	

*Antilog recalculated a for slope = 1 x 100

Analysis of food-intake data (Table 3) gave different results when expressed in fresh or dry terms. Lettuce consumption was significantly higher on a wet weight basis (ANOVA F = 183.22 $P<0.0001$), with common lower rates for W_h and Wl_a (201.42 mg ± 58,89) and Wl_b and G (94.44 mg ± 24.14). Water dilution led to reduced IR for both Wh diets (57.53 mg ± 12.50) while Wl_a registered the highest rates of dry food processing among low M.C. diets (common value of 88.75 mg ± 21.96 for Wl_b and G; ANOVA F = 26.23, $P<0.0001$). Such findings agree with Robertson & Moorhead (1999) who found similar ingestion rates on a d.m. basis for snails fed common garden plants of variable moisture content (84-95%). Data for low M.C. Wl_b and G foodstuffs conform with Daguzan (1985) and Fonolla, *et al.*,(1980) for commercial diets and corn flour (basic compound of G: chicken food), while rates obtained for Wl_a (low M.C.) relate better to results for *H. aspersa.* var. *maxima* (Jess & Marks, 1995).