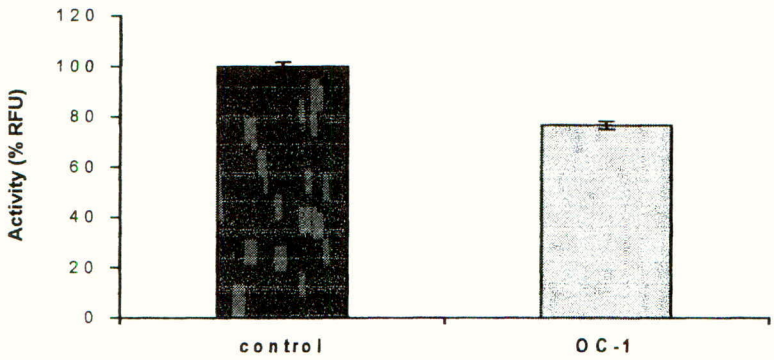

Figure 4. The relative proteolytic activity of *D. reticulatum* digestive gland after treatment in the bioassay.

PI; or an alteration in the form of proteolytic enzymes produced by the digestive gland leading to an alteration of its natural form.

These results raise many questions on the ability of PIs to affect slug populations. For example, how does the presence of OC-1 cause a change in the digestive gland morphology? Does this observation, together with the fact that endogenous proteolytic activity is significantly reduced, suggest that over the long term OC-1 may be effective in controlling slugs, at least to a limited extent ?

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Activity patterns of *Arion lusitanicus* using shelter traps

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ABSTRACT

The daily activity of the slug *Arion lusitanicus* was studied using time-lapse video analysis in the laboratory. Under constant temperature (18°C) and 16-hours photoperiod, the activity of slugs was measured in half-hourly periods as locomotor activity, feeding or resting. Track lengths were determined using image analysis. Locomotion of *A. lusitanicus* was greatest at 5:30, 1.5 hours after sunrise, and at 20:30, 1 hour after sunset; least locomotor activity occurred between 13:00 and 14:00. The mean distance travelled by *A. lusitanicus* in 24 hours was 10.8 m. The largest slug was the most active and the smallest the least. Slugs spent 68 % of 24 hours resting, mainly under the artificial shelter traps, 27 % in locomotion and 4 % feeding. Feeding occurred mainly during the hours of the scotophase (76 %). All categories of behaviour investigated varied greatly between individuals and also between times of day. These behavioural patterns agreed with those found in a previous field investigation.

INTRODUCTION

Whereas activity in *Deroceras reticulatum* (Müller), the most important pest slug in western Europe, has been examined extensively (e.g. Bailey, 1989; Hommay, *et al.* 1998), little is known about the activity of an equally important pest species in northern and central Europe, *Arion lusitanicus* Mabille. Only its daytime activity (Grimm, *et al.*, 2000) and the influence of weather on its activity (Crawford-Sidebotham, 1972) have previously been investigated.
One of the facts of basic interest for farmers and gardeners dealing with a pest slug population is to estimate the population density. Shelter traps have proved to be an easy method, and therefore one of the most frequently used means, of evaluating slug populations. Additionally, shelter traps can be used for controlling pest populations when combined with additional measures such as collecting or baiting. However, the effectiveness of these artificial shelters is highly dependent upon the activity of the slugs on the soil surface (Hommay, *et al.*, 1998). In the case of a pest species like *A. lusitanicus*, activity can be considered a key factor in causing crop damage, and is even more important than the actual size of the population (Grimm, 2001).

Therefore, the aim of the present study was to establish the complete 24-hour activity pattern for *A. lusitanicus* in order to determine the preferred times of day for locomotor activity, feeding, resting, and for the acceptance of shelter traps. This information can subsequently be used to determine the timing and application of specific pest-management measures in the control of *A. lusitanicus*.

MATERIALS AND METHODS

The activity of slugs was examined using infra-red, time-lapse video recording, applying the protocols of Bailey & Wedgwood (1991) and Hommay, *et al.* (1998). Track lengths were measured and summed using image analysis.

Experimental design

30 specimens of *A. lusitanicus* of different size (4.19 to 7.62 cm length at full body extension) were collected in Austria in April 1999 and transferred to Manchester University (UK) three weeks before the investigations started for acclimatisation. 10 slugs were transferred into a rectangular arena to be kept there for the whole period of the experiment which lasted 13 days. Eight slugs remained traceable for almost the whole duration of the experiment and were used for all the calculations. The arena (94 x 124 cm) was filled with soil (standard garden compost with 10 % sand), which was "top-dressed" with a thin layer of silver sand to facilitate video analysis, and edged by a barrier of dried, salt-impregnated blotting paper serving as a fence to prevent the slugs from escaping. Two artificial shelters (lids of transparent plastic dishes, 12 x 18.3 cm, to allow visibility of the slugs) were placed in the SW and NE corner of the arena, 10 cm from the edges. A petri-dish was placed in the centre of the arena to serve as the feeding site. Ready Brek® (oat-based cereal, available in the UK) was used as food and replaced daily. The arena was situated in a controlled-environment room, at constant temperature (18°C ± 0.1°C), a relative humidity of 96 %, and an artificial photoperiod of 16 hours (LD: 16:8) with "sunrise" at 4:00 a.m. (GMT) and "sunset" at 8:00 p.m. (GMT), simulating middle-European conditions at that time of the year. Soil moisture was maintained by spraying with water once or twice daily during different times at the diurnal rest period of the slugs.

Durations of the different phases in the slugs' behaviour (locomotor activity, feeding, resting) were recorded in intervals of 30 min. Locomotor activity was defined as any displacement. Feeding was determined as the time spent at the feeding place because it was not possible to distinguish actual feeding from resting and staying in contact with the food. In any part of the arena other than the feeding place, slugs not moving for more than 10 min were considered to be resting. The different positions of resting (shelter traps, corners, edges, other places) were also recorded.

Statistics

Statistical analysis was performed using GenStat 4.2 on logarithmically ($\log_{10} + c$) transformed data. The ANOVA was expressed with a slug/day/times-of-day stratification.

RESULTS

Locomotor activity

Locomotor activity of *A. lusitanicus* was essentially nocturnal with its highest peak in the early morning at 5:30, 1.5 h after the artificial sunrise (Figure 1). Locomotor activity slowed down in mid-scotophase when the animals either fed or rested. On average, 27 % of 24 hours was spent in locomotor activity (Figure 2).

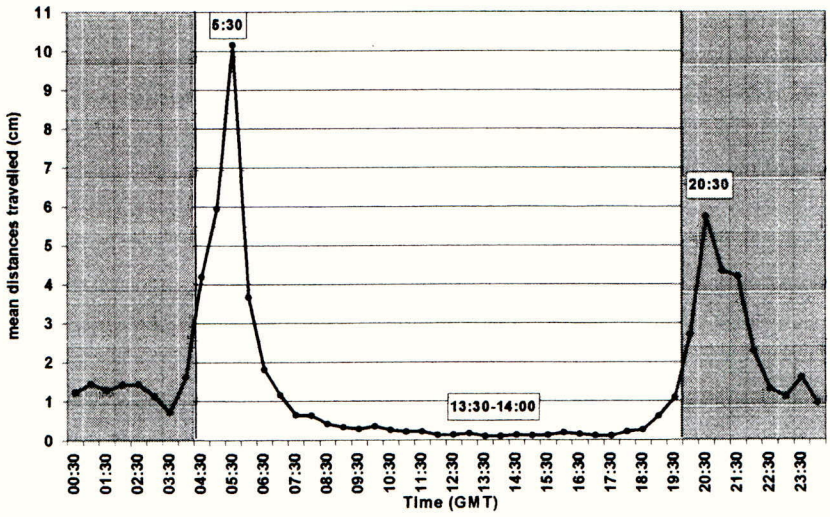

Figure 1. Distances travelled (in cm) of *Arion lusitanicus* during different times of day. The scotoperiod is shaded.

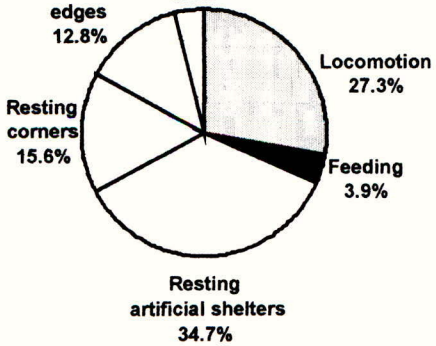

Figure 2. Percentage time per 24 hours *Arion lusitanicus* was found in the different categories of behaviour.

The mean distance travelled by *A. lusitanicus* in 24 h was determined as 1080.8 cm (df = 7, SE = 120.9). The mean time spent in locomotion amounted to 393.4 min per 24hrs (df = 7, SE = 9.6) (Figure 2). In a comparison between the smallest and the largest slug, the former (4.2cm long while moving) covered only about half the distance (65 %) of the largest one (7.6 cm long while moving). Additionally, the largest slug was found to be the most active (29.2 % per day) and the smallest the least (23.4 % per day). Slugs sometimes started to move when they were touched by conspecifics. In one case, the smallest slug left its roost site when touched by the largest one, whereas it remained at the same place when touched by a smaller one. Initiation of locomotor activity was usually spontaneous, but could also be observed in non-sheltering slugs when suddenly exposed to full light.

Feeding

Most feeding occurred during the hours of darkness (between 1:00 and 2:30) but some foraging was observed during daytime (Figure 3).

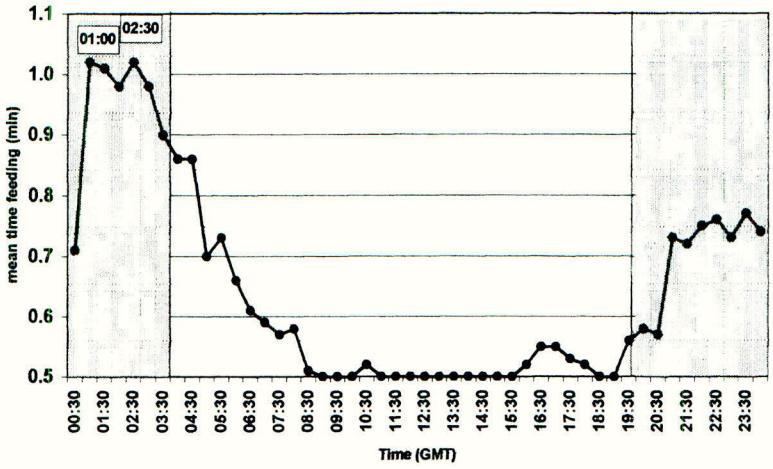

Figure 3. Time spent feeding (in min) of *Arion lusitanicus* (geometric mean) during different times of day. The scotoperiod is shaded.

The overall daily mean duration of time spent at the foraging site for all the slugs observed feeding was 56.6 min (SE = 12.3) (Figure 2).

Resting and Homing

The animals were found to spend 68 % of the time (990.0 min, SE = 7.8) resting (Figure 2). Slugs mainly rested during the photophase. From 6:00 to 14:00 the number of resting animals increased continuously; after 15:00 an almost continuous decline (except for 17:00) was recorded until 21:00. The artificial shelters were the preferred roost sites (Figure 2).
All the individuals used several roost sites and frequently shared them with others. Large slugs were found to be more active, and so spent less time resting than smaller slugs.

DISCUSSION

Locomotor activity

The activity of *A. lusitanicus* under laboratory conditions was essentially nocturnal, as previously shown for many other slug species with an additional rise of activity after the light was switched on, and the highest peak at 1.5 h after this artificial sunrise. This supported previous field observations by Grimm *et al.* (2000) using opaque shelters. In the laboratory, as in field conditions, activity then declined steadily until it almost ceased around noon. Field data showed initial movements after the resting period in the late afternoon, peaking at 20:00. This again is in close agreement with the present observations where the second phase of activity peaked during the dark phase at 21:00. Thus, the difference in methodology

(transparent versus opaque shelter traps) seems not to have a fundamental effect on the basic activity pattern of *A. lusitanicus*.

Like *A. lusitanicus*, *D. reticulatum* also shows two main peaks, the major one after dusk (e.g. Hommay, *et al.*, 1998) and a second, lesser peak of activity around dawn (Bailey, 1989). Our observations clearly suggest that the pattern of activity of *A. lusitanicus* is closer to that of the limacid pest-species *D. reticulatum*, than to most of the other arionids investigated. Moreover, the pest snail *Helix aspersa* Müller shows similar rhythms of nocturnal behaviour to pest slugs (Blanc, *et al.*, 1989). These appear to be examples of ecological traits overriding taxonomic relationships. Apart from the endogenous rhythms which predominantly trigger slug activity exogenous factors such as temperature and light cycle are also known to be influential. However, amongst exogenous factors, photoperiod proved to be the more important for *A. lusitanicus*, as similar patterns of activity were found both in the field under fluctuating temperatures and in the present laboratory study where temperature was constant.

Feeding

Patterns of feeding activity have been found to differ greatly between the different species of pest slug investigated. Apart from differences in abiotic conditions (temperature and humidity), the variablility in the timing of the peaks of foraging activity may also result from differences in the types of food offered. Because of restrictions in the video analysis, the exact proportion of time spent feeding and resting at the feeding place could not be determined, but it is highly likely that slugs spent some time at the feeding place without feeding. The duration of feeding in *A. lusitanicus* (63 min) is closer to *A. distinctus* Mabille (64 min) than to the equivalent west-European limacid pest species *D. reticulatum* (102 min; Hommay, *et al.*, 1998).

Shelters

A. lusitanicus largely remained under the shelters during the photophase. The same behaviour was observed in both the other slug species (*A. distinctus*, *D. reticulatum*) investigated by Hommay *et al.* (1998). In both investigations, the rapid commencement of locomotor activity was related to the fast transition between the artificial light and dark phases.

Distances travelled

In general, arionids are known to be less active than limacids. However, a comparison of *A. lusitanicus* and *D. reticulatum* shows the former to be the more active pest slug. With total distances of 10.8 m travelled in 24 h, *A. lusitanicus* covers more than twice the distance of *D. reticulatum* (4.03 m) under similar conditions (Hommay, *et al.*, 1998). This result is further supported by the mean duration of movements per day. These two factors can be considered to be extremely important in contributing to the high dispersive ability of *A. lusitanicus*.

Pest control

A knowledge of the variation in daytime activity and the use of artificial shelters in a pest-slug species is of considerable importance from the applied point of view. As explained above, the results of the present laboratory study are generally applicable to field situations. Although this was a study under controlled conditions using different shelter traps in size and material, the agreement of these results with a previous field investigation on the same species (Grimm, *et al.*, 2000), allows some general conclusions to be drawn.

Our study clearly confirms the high general level of acceptability of shelter traps. In the field, artificial shelter traps should provide more stable microclimatic conditions, so as to compete efficiently with any natural shelters (Young, *et al.*, 1996; Grimm, 2001). Therefore, well-insulated traps of the type used by Grimm *et al.* (2000) should be favoured. To make the best use of these shelter traps for reducing a population of *A. lusitanicus*, the preferred time for collecting slugs from these shelters should be between 9:00 and 18:00, ideally in the early afternoon, when most slugs are resting or barely active. One of the most effective ways of reducing a pest-slug population is to use traps baited with molluscicide pellets, as these are known to be highly frequented by the slugs, not just for their climatically favourable conditions, but also because of the olfactory attractiveness of the bait. The traps also provide shelter for the molluscicide from abiotic factors, and protect it from being consumed or dragged away by other animals. Thus, used in this way, the traps can provide an easy, safe and molluscicide-saving way of killing slugs.

A further important practical conclusion to be drawn from the present results is that any watering of agricultural plots or gardens should not be carried out in the evenings, prior to one of the two main peaks of slug activity, but rather should be undertaken in the late morning when activity is at a minimum. Although this is less beneficial from the water-conservation point of view, this simple measure will help to minimise the benefit of any watering to the slugs.

ACKNOWLEDGEMENTS

The authors want to thank Dr S E R Bailey, University of Manchester, and Mr C Moncrieff, Head of Biometrics, The Natural History Museum, London, for their help.

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Distribution of metaldehyde in the different organs of *Deroceras reticulatum* after oral application of the molluscicide

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ABSTRACT

Deroceras reticulatum was fed with ^{14}C labelled Metaldehyde, whereas 1.5 µCi per animal were orally administered per animal. After 5 min, 1 h, 3 h, 6 h and 12 h, samples were taken for autoradiographic studies. Already 5 minutes after oral application of the radio labelled material, radioactivity was found in almost every organ in the slug body. Metaldehyde then accumulated in some essential organs of the body. It is evident that the action of the molluscicide instantly starts after ingestion and that the animal cannot recover from the damage caused even in cold and wet conditions.

INTRODUCTION

Metaldehyde, the tetramer of acetaldehyde, is a highly specific slug control agent in all weather conditions e.g. when it is cold and wet (Triebskorn, 1998). To get a deeper insight into the mode of action of metaldehyde several studies were undertaken by Triebskorn *et al.* (Triebskorn, 1989; Triebskorn & Ebert, 1989; Triebskorn & Schweizer; 1990; Triebskorn *et al.* 1998). These investigations led to a more detailed knowledge of the impact of metaldehyde on the microstructures of the mucus cells in skin and digestive tract which were irreversibly destroyed by the active ingredient. Information concerning the distribution and the accumulation of metaldehyde within the animals after ingestion, however, was still lacking. In this paper, the distribution of ^{14}C labelled metaldehyde in slugs will be explained and described.

MATERIALS AND METHODS

Deroceras reticulatum (Müller) was fed with ^{14}C-labelled metaldehyde, so that 1.5 µCi were orally administered per animal. After 5 min, 1 h, 3 h, 6 h and 12 h, samples were taken for autoradiographic studies. In order to assess the distribution of the ^{14}C Metaldehyde and its metabolites, histological cryosections and paraffin sections were exposed for 5 weeks with light-sensitive film in the dark. The regions where ^{14}C-marked molecules accumulated occurred as dark zones on the film.

RESULTS

The findings are summarised in Table 1. Already 5 minutes after oral application of the radio-labelled material, radioactivity was found in almost every organ in the slug body. Most probably, it rapidly passed the tissue of the crop and the oesophagus and got distributed by the haemolymph throughout the whole body afterwards. Metaldehyde then accumulated in some essential organs of the body. After 60 min. it distinctly accumulated in the salivary gland, the epithelium of the stomach and the intestine as well as in the kidney and the heart. After 3 h, high concentrations were found in addition in the upper pharynx, the genital tract and in the

fallopian tube. The overall presence of the radio-labelled material in the slug body over a long time period is an indication that the effect on the mucus cells lasts long enough for a complete destruction of essential cell formations such as the mucus cells. Moreover the observed accumulation in kidney and heart most probably indicate a disturbance of the ionic and water balance of the body.

Table 1. Distribution of ^{14}C labelled metaldehyde in *Deroceras reticulatum* at different times after administration. The intensity of staining is assessed by ranging 0 (weak staining) to 4 (highly intensive staining).

Body region	Check	5 min	1 h	3 h	6 h	12 h
Mouth cavity	0	2	3		3	**4**
Oesophagus	0	2	3		3	3
Upper pharynx	0	2	3	3	**4**	3
Pharynx ganglion	0	1	2			
Salivary gland	0	1	3	**4**		3
Crop cavity	0	2	3		3	3
Crop epithelium	0	2	2		2	2
Stomach/Intestine cavity	0	2	3	3	**4**	2
Stomach/Intestine epithelium	0	3	**4**	2	3	3
Pancreas	0	2	2	2	3	2
Genital tract	0	1		**4**	**4**	**4**
Fallopian tube	0	3	3	**4**	**4**	**4**
Eyes & tentacles	0	3	3		3	3
Kidney, heart	0	1	**4**	**4**	**4**	**4**
Skin of the head	0	2		2	3	3
Skin of the back	0	2	2	2	3	2
Foot sole	0	1	2	2	2	3
Mucus	0	1				

CONCLUSIONS

The present study shows the rapid distribution of Metaldehyde and/or its metabolites in the body of the slug and its accumulation in essential organs of *Deroceras reticulatum*. It also made evident that the action of the molluscicide instantly starts after ingestion and that the animal cannot recover from the damage caused, even in cold and wet conditions.

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Molluscicidal and repellent properties of African plants

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ABSTRACT

Screening of Nigerian plants, for aquatic molluscicides has been previously reported, however their use as terrestrial mollusc repellents is examined here for the first time.

The plants evaluated were *Detarium microcarpum* (bark), *Ximenia americana* (bark and leaves) and *Polygonum limbatum* (shoot). The plant raw materials as well as their aqueous and alcoholic extracts were tested, against the field slug *Deroceras reticulatum*, using a variety of assays including; terraria trials, split substrate, contact toxicity, and caged field trials. Split substrate studies demonstrated the repellent nature of all the plant extracts. Laboratory terraria trials showed that all the plant raw materials exhibited significant mollusc repellency properties, when applied as a barrier. Metaldehyde pellets (4%) and *Detarium microcarpum* demonstrated potent molluscicidal properties in the first week. Mixing the plant materials with sawdust (50:50) as well as coating the alcoholic extracts on to sawdust provided an alternative option for application as a barrier. The barks from *Detarium microcarpum* and *Ximenia americana* showed significant mollusc repellency properties. Contact toxicity tests for all of the plants showed high slug mortality over a 24 hour period. Promising results obtained, from caged field trials, validate the use of these indigenous Nigerian plants as a natural barrier for slug control.

INTRODUCTION

Many laboratories are today screening plants as a potential source of cheaper, safer and more effective molluscides. African plants, in particular, have been widely reported as possessing molluscicidal activity (Adewunmi, 1991). The molluscicidal activity of Nigerian plants was reported to be very effective against 12 week old *Lymnaea natalensis* (Kela *et al.*, 1989) the aquatic intermediate host of *Fasciola gigantica*, the parasite responsible for transferring the disease schistosomiasis. Previous reports have shown the molluscicidal activity of Nigerian plants, (*Detarium microcarpum*, *Ximenia americana* and *Polygonum limbatum*) against *Pomacea canaliculata* (Lamarck), in an aquatic environment (Arthur *et al.*, 1996) and tested their toxicity to non-target species.

The use of raw plant material, raw plant material/sawdust mixes and plant extracts coated on to sawdust was evaluated against terrestrial molluscs in laboratory terraria experiments. The main aim of the study was to develop an environmentally friendly barrier against terrestrial molluscs, which has little or no toxic effects against non-target organisms. In this form the material could find beneficial use as a home and garden product.

MATERIALS AND METHODS

All plants were kindly provided and identified by Professor S L Kela from the Bauchi area of Nigeria. Spruce sawdust was obtained from RS Biotech, Northants, UK. Sand (lime free agricultural grade) was obtained from Wickes, Cardiff, UK. Absolute ethanol and methanol (hplc grade) was purchased from Fisher Scientific, Loughborough, UK.

Test animals

Adult *Deroceras reticulatum* were collected from nearby fields and maintained in plastic trays lined with moist, unbleached, absorbent paper. They were housed in the dark and at a constant temperature of 10° ± 1°C. Slugs were regularly fed on a mixture of iceberg lettuce and carrots. Slugs were pre-starved for 24 hours prior to testing. The earthworm *Lumbricus terrestris* were also collected from the field for non-target testing.

Preparation of test materials

Plant raw materials were ground to a powder using a Multiquick hand blender (Braun).
Plant material/sawdust mixtures (50%) were prepared by adding 100g of powdered plant material to 100g of sawdust. Alcoholic plant extracts (30%) were prepared by transferring 60g of the powdered barks of *D. microcarpum* and *X. americana* to separate containers, diluting to 200ml with either absolute ethanol or methanol and left overnight. The plant extracts were filtered, under vacuum, using a Buchner flask. Sawdust (30g) was covered with 100ml of alcoholic plant extract, mixed homogenously with a spatula and the solvent allowed to evaporate to air.

Terraria trials

Experiments were performed as described by Ali & Bowen (2003) and involved five replicates using 0.07 m^2 plastic trays lined with moist, unbleached, absorbent paper.
Four pre-starved slugs were placed in each tray, equivalent to 570,000 slugs/ha, which is regarded as representing a heavy infestation. The following test materials (1-2g) were tested as barriers, against *D. reticulatum*: 100% plant material, 50:50 plant material/sawdust, and sawdust coated with alcoholic plant extracts. Controls were employed consisting of untreated trays, sawdust and alcoholic sawdust. Experiments were conducted over fourteen days.

Split substrate test

Experiments were performed as described by Bowen & Antoine (1995) in which treated filter paper sectors are tested in petri dishes to measure slug trail coverage. Once dry the filter papers were photographed to measure slug trail coverage, with a digital camera and manipulated using photographic software (Adobe Photoshop®). The area of the slug slime trail was calculated using an image analyser (Sigma Scan®). The repellency/attractancy properties of the following aqueous extracts (10%) were tested against the field slug *D. reticulatum*; *Detarium microcarpum* bark, *Ximenia americana* bark, *Ximenia americana* leaf, *Polygonum limbatum* shoot.

The repellency index (R.I) was determined using the following equation:

$$\text{R. I (\%)} = 100 \times \frac{\text{\% slug trail area (control)} - \text{\% slug trail area (test)}}{\text{\% slug trail area (control + test)}}$$

Contact toxicity test

A glass tube (75mm x 25mm) was filled with one third plant raw material, i.e. approximately 3.5g, and moistened with about 4ml of de-ionised water. One slug was added to each tube followed by a piece of moistened cotton wool, thus forcing the slug to be in contact with the moistened plant material. The tube was stoppered with a cork and the test replicated a total of ten times. The test was performed under environmentally controlled conditions ((12 hour light; 15°C: 12 hour dark; 15°C) over a 24 hour period. Slugs were recorded as dead if they did not respond to a 9 volt electrical stimulus.

The 100% raw plant materials were tested against *D. reticulatum using s*and filled tubes as controls. The method of Bieri *et al.*, (1989) was used to test the impact of the plant materials on the non-target species *Lumbricus terrestris*. The earthworm was allowed to move about within a soil filled glass filter funnel and did not have enforced contact with the test materials. Mortalities were recorded over seven days.

Caged field trials

Caged field trials were conducted in October 2001, in $1m^2$ hardwood arenas, with rigid pvc foam core side panels (200mm high) using the method described by Ali & Bowen (2003). A known population of slugs (10) was introduced into each arena to represent a population equivalent to 100,000 slugs per hectare. The plant raw materials (100%) were tested in the field against *D. reticulatum* using untreated plots as a control. The percentage area of leaf damage was assessed for each plant over a fourteen day period.

Statistical methods

Between treatment effects were determined using the non-parametric Kruskal-Wallis (K-W) test to show the significance of differences between group medians. In the majority of cases, between group variances were not homogeneous and residuals were not normally distributed, hence parametric comparisons of group means were not appropriate.

RESULTS

Terraria trials

Table 1 shows the crop protection obtained after a fourteen day exposure of winter wheat seeds to *D. reticulatum*, when Nigerian plant raw materials, their sawdust mixtures and their alcoholic plant extracts are applied as barriers. Over the first seven day period all the plant materials gave significant crop protection against the molluscs, compared to the control. *D. microcarpum* was equally as effective as the commercial molluscicide metaldehyde both showing negligible seed hollowing. On comparison of *X. americana* bark and leaf, the former showed good barrier properties resulting in low seed damage. *X. americana* leaf and *P. limbatum* shoot gave only moderate crop protection over the seven day period. Continuation of the experiment for a further seven days showed *D. microcarpum* and *X.*

americana to have good barrier properties, whereas the total protection with metaldehyde resulted from slug mortality. The leaves and shoot from *X. americana* and *P. limbatum* gave relatively poor crop protection. In terms of mortalities, metaldehyde gave the highest slug death over the first seven day period followed by *D. microcarpum*. The other plant materials gave relatively low slug mortalities. After a further seven days metaldehyde gave 100% mortality and of the plant materials only *X. americana* bark showed moderate molluscicidal properties.

Mixing the raw plant materials 50% with sawdust reduced the efficacy of *D. microcarpum* bark, yielding very poor crop protection properties over the seven day period, whilst mixing *X. americana* with the same amount of sawdust maintained the same high level of crop protection, as before. The level of crop protection afforded by the two plants over the next seven days was only moderate. The highest slug mortality for *X. americana* was obtained in the first seven days. In terms of molluscicidal properties *X. americana* was more effective than *D. microcarpum* over the fourteen day period.

Table 1 Mean (±SEM) and median percentage seed protection and percentage slug mortality with barriers of plant origin

Treatment	Day 7				Day 14			
	Hollowed Seeds		Mortality		Hollowed Seeds		Mortality	
(n = 20 slugs)	Mean	Median	Mean	Median	Mean	Median	Mean	Median
100 % Plant raw material								
Control (No barrier)	98 ± 1	100	5 ± 5	0	87 ± 11	100	15 ± 10	0
Detarium microcarpum bark	1 ± 1	0	60 ± 13	50	6 ± 2	8	10 ± 10	0
Ximenia americana bark	8 ± 1	8	10 ± 6	0	12 ± 4	8	30 ± 15	25
Ximenia americana leaf	19 ± 7	13	10 ± 6	0	48 ± 5	46	5 ± 5	0
Polygonum limbatum shoot	27 ± 6	29	25 ± 8	25	50 ± 4	48	15 ± 6	25
Metaldehyde (4%)	1 ± 1	0	90 ± 10	100	0 ± 0	0	100 ± 0	100
K-W test		P<0.001		P<0.001		P<0.001		P=0.007
50%Plant raw mat./sawdust								
Control (No barrier)	93 ± 2	92	15 ± 10	0	93 ± 6	100	0± 0	0
50%*D. microcarpum*/sawdust	51 ± 3	13	5 ± 5	0	38 ± 10	42	5±5	0
50% *X. americana*/sawdust	10 ± 4	50	40 ± 6	50	28 ± 9	21	15 ± 10	0
K-W test		P=0.002		P=0.028		P=0.009		P=0.291
Sawdust + alcoholic extracts								
Control (No barrier)	98 ± 1	100	10 ± 6	0	79 ± 5	75	25 ± 8	25
Sawdust	93 ± 2	92	0 ± 0	0	77 ± 8	83	30 ± 12	25
Ethanolic sawdust	94 ± 3	92	15 ± 6	25	88 ± 3	92	30 ± 9	25
Methanolic sawdust	99 ± 1	100	5 ± 5	0	88 ± 2	92	30 ± 5	25
D.microcarpum (Ethanolic)	36 ± 9	38	5 ± 5	0	72 ± 6	63	15 ± 6	25
D.microcarpum (Methanolic)	49 ± 14	46	10 ± 6	0	39 ± 8	38	40 ± 6	50
X. americana (Ethanolic)	12 ± 6	8	15 ± 6	25	32 ± 11	17	45 ± 15	50
X. americana (Methanolic)	3 ± 3	0	15 ± 15	0	52 ± 6	50	15 ± 15	0
K-W test		P<0.001		P=0.504		P<0.001		P=0.285

Sawdust treated with plant extracts of *D. microcarpum* showed relatively modest levels of crop protection and molluscicidal activity. However the ethanolic and methanolic extracts of *X. americana* showed superior crop protection, with little seed damage occurring over the first seven days. Crop protection decreased in the second week but the molluscicidal activity of ethanolic *X. americana* increased. The repellent nature of all the plant extracts was confirmed by the high repellency indices obtained in the split substrate test (Table 2).

Table 2 Mean (±SEM) slug trail area and the repellency index calculated from the split substrate test evaluating aqueous Nigerian Plant extracts

Treatment (n = 20 replicates)	Percent slug trail in control sector	Percent slug trail in plant extract sector	Repellency Index (%)
Detarium microcarpum bark	3 ± 1	30 ± 4	89 ± 4
Ximenia americana leaf	10 ± 2	44 ± 2	65 ± 4
Ximenia americana bark	9 ± 1	43 ± 3	66 ± 5
Polygonum limbatum shoot	8 ± 2	50 ± 4	78 ± 4

Enforced contact with all the raw plant materials, over 24 hrs, induced high slug mortalities (80-100%) compared to the control (20%). *D. microcarpum* induced relatively low mortalities (10%) against *L. terrestris* as the animal was allowed to withdraw away from the test source. Even so, *X. americana* leaf and bark were found to be more toxic to earthworms, 40% and 30% respectively, over the seven days.

Table 3. Mean (± SEM) and median percentage leaf damage to lettuce with barriers of plant caged field trials

Treatment (n = 16 lettuces)	Leaf damage (%)			
	Day7		Day 14	
	Mean	Median	Mean	Median
Control (No barrier)	40 ± 3	38	47 ± 4	47
Detarium microcarpum bark	5 ± 1	7	6 ± 1	7
Ximenia americana bark	11 ± 3	9	19 ± 2	17
K-W test		P=0.022		P=0.013

As shown in Table 3, both *X. americana* and *D. microcarpum* conferred good crop protection over seven days, when tested in the field. This protection was extended over fourteen days.

DISCUSSION

All plant materials and their extracts showed some measure of mollusc repellency. Using *D. microcarpum* plant raw material on its own was particularly effective in both laboratory and caged field trials, matching the performance of metaldehyde in crop protection.

D. microcarpum in addition shows very strong molluscicidal activity especially during the first week but declining in the second week, whilst *X. americana* takes longer to exert an effect as a molluscicide. When the raw material is mixed 50:50 with sawdust the best performance is achieved by samples containing *X. americana*, whilst the activity of *D. microcarpum* appears to decline with time. Only the alcoholic extract of *X. americana* bark, applied to sawdust, demonstrated good crop protection properties for the first seven days. The repellent nature of the Nigerian plant extracts were confirmed by the split substrate assay resulting in high repellency indices for all the aqueous plant extracts. In aquatic studies, Arthur *et al.*, (1996) also concluded that *X. americana* was more potent than *D. microcarpum*. In this respect *X. americana* extracts compare well with a range of naturally occurring compounds tested as seed treatments by Powell & Bowen (1996).

Bourne *et al.*, (1988) showed that methiocarb demonstrated better seed protection than metaldehyde and subsequently demonstrated the relative efficacies of the two commercial molluscicides. The efficacies achieved by metaldehyde in terms of crop protection are

Bourne *et al.*, (1988) showed that methiocarb demonstrated better seed protection than metaldehyde and subsequently demonstrated the relative efficacies of the two commercial molluscicides. The efficacies achieved by metaldehyde in terms of crop protection are matched here by *D. microcarpum* (presented as 100% raw material) and approach the same level of molluscicidal activity over a seven day period. The molluscicidal activity of *D. microcarpum,* however, is more labile and declines rapidly over fourteen days. Enforced contact between *D. reticulatum* and the plant raw materials resulted in slug death, confirming that in a terrestrial context all the plants tested are molluscicidal. In terms of impact on non-target species enforced contact is not normally experienced. Earthworms will normally move away from molluscicidal slug pellets (Bieri *et al.*, 1989). In this study where the earthworms *L. terrestris* have some contact with the test materials some mortalities were observed, although these were minimal with *D. microcarpum* over a seven day period. Caged field trials represent a more natural application and resulted in very low slug mortalities (3%) for both plant materials as well as good crop protection especially in the case of *D. microcarpum* where there was negligible leaf damage over fourteen days. Overall the data presented here support the use of particular Nigerian plant materials as successful slug repellents and would have minimal impact on ecological biodiversity.

ACKNOWLEDGMENTS

We wish to thank Professor S K Kela for identifying and supplying the Nigerian plants and the Compton Group for sponsoring this PhD research project and their continued support.

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