Breeding *Deroceras reticulatum* under controlled conditions, a prerequisite for controlled efficacy tests of molluscicides

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ABSTRACT

For standardised tests with slugs for developing new slug control formulations, Lonza Ltd. established a breeding unit for *Deroceras reticulatum* at Visp (Switzerland). The animals are held under simulated conditions with an 8h day (photoperiod) and at a temperature of 15° C and 16 h in darkness (scotoperiod) at 10°C. They are fed every second working day (3 times a week) with cereal flour enriched with vitamins and mineral compounds. The time to reach adult stage takes about 90 to 110 days and egg laying starts at the age of 120 to 140 days under these breeding conditions.

INTRODUCTION

Developing specific and high-quality slug control formulations requires appropriate test and evaluation equipment and facilities. In this procedure, a standardised reliable slug mortality test is a key element. Tests performed with wild catches in most cases lead to high variability in mortality, making the results difficult to interpret. For *Deroceras reticulatum* (Müller), animal body size or live weight are shown to be uncertain indicators of physiological age, nutritional status or fitness. South (1982) found considerable differences in body weight of *D. reticulatum* reaching the egg-laying stage of animals reared at different temperatures. Rollo and Shibata (1991) observed a considerably reduced development time of *Deroceras leave* kept on a short nutrition regimen. These observations and the simple fact that it is impossible to track back the life history of animals in the wild show that tests based on such animals will have a high level of uncertainty.

To overcome this problem, Lonza Ltd the manufacturer of Meta® metaldehyde established a fully controlled breeding station for *D. reticulatum* at Visp (Switzerland). It is part of a standardised test procedure in developing slug control formulations. In this presentation, data about the development and the reproduction of this species are presented.

MATERIAL AND METHODS

Rearing of *D. reticulatum* is carried out in fully controlled climatic chambers. The animals were held under simulated conditions with an 8 h day (photoperiod) and at a temperature of 15° C and 16 h in darkness (scotoperiod) at 10° C. This breeding regime corresponds to a physiological time of 7.7 day degrees. The animals are fed every second working day (3 times a week) with an optimum dose of cereal flour enriched with vitamins and mineral compounds. The trays in which the different age stages of *D. reticulatum* are held are checked and cleaned daily.

To find the optimum feeding regime slugs were fed daily (intensive feeding) and every second day only with the same standard daily dose.

RESULTS

Halving the amount of food approximately also leads to a doubling of the development time. As depicted in Figure 1 the animals fed every second day reached 600 mg live weight within 77 days, whereas the daily fed animals have reached a live weight of 330 mg only.

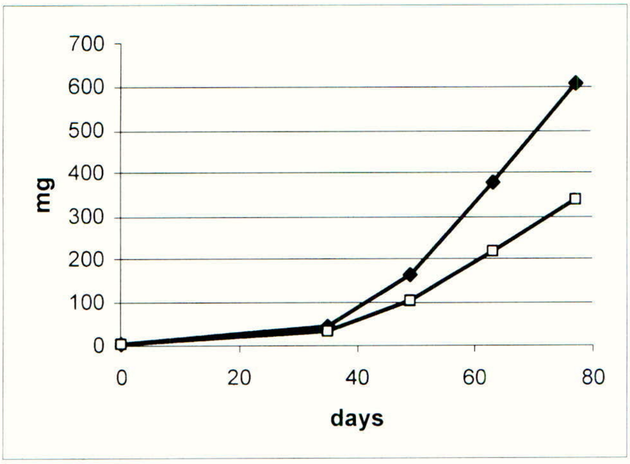

Figure 1. Weight gain depending of the nutritional regimen of *Deroceras reticulatum* (mean of 275 animals per treatment; upper line: Standard feeding, lower line: half rate feeding).

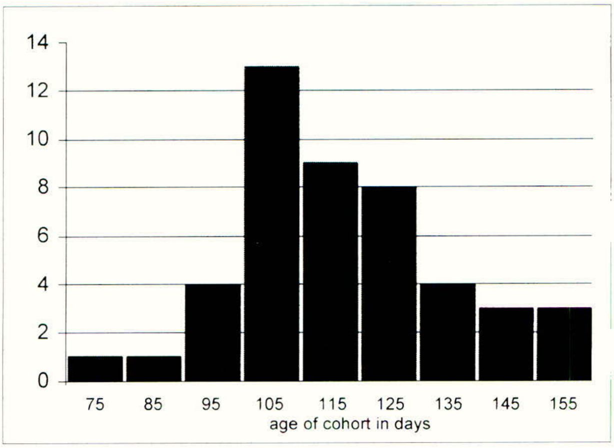

Figure 2. Number of egg batches per cohort of *Deroceras reticulatum* depending on age (Mean of 136 cohorts of 25 to 30 animals, egg production: 1468).

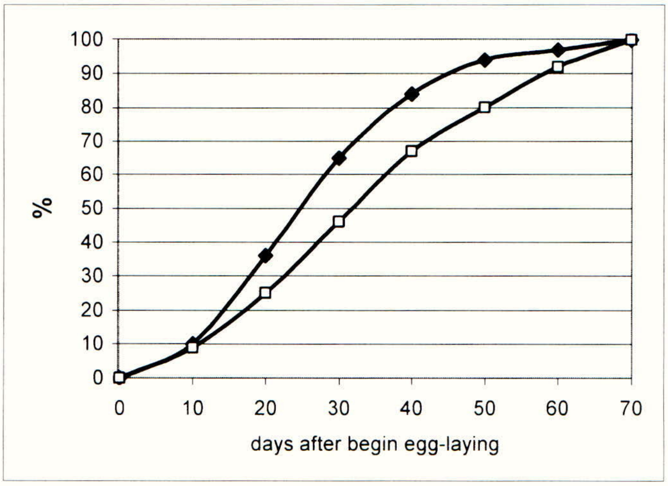

Figure 3. Cumulative number of eggs (upper line) and observed mortality (lower line) of *Deroceras reticulatum* at 8h day (photoperiod) and at a temperature of 15° C and 16 h in darkness (scotoperiod) at 10° C (calculated out of 2900 animals observed)

The turnover of the different slug cohorts is depicted in Figure 4 below.

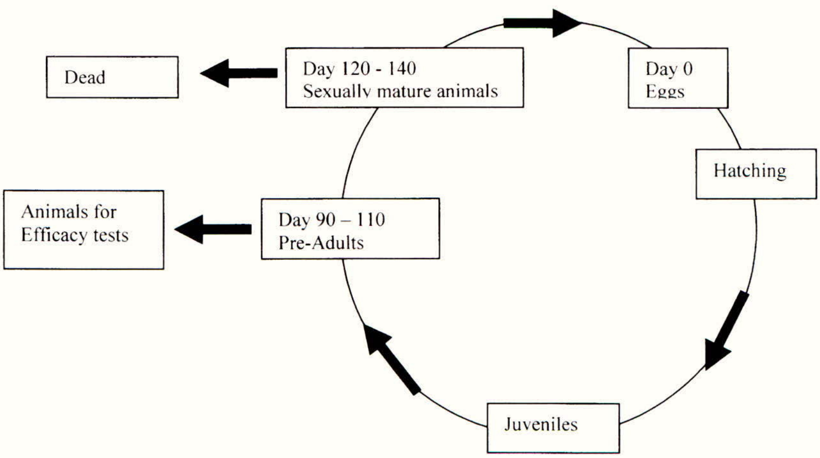

Figure 4. Life cycle and timing of the slug breeding under controlled conditions

DISCUSSION

Optimum feeding conditions are of high importance for *D. reticulatum*. Especially if the live weight of the animals reaches 50 mg, food becomes an essential factor for the further development of the animals as was found by Rollo and Shibata (1991) for *D. leave*. High quality food supply for slugs in nature, however, can vary to a great extent in time and space.

The average age of slugs starting egg laying is about 111 days (850 day degrees). Under the given conditions, egg laying is first observed at the age of 80 days (615 day degrees), lasting about 80 days, ending at about day 160 (1230 day degrees). This matches the observations of South (1982) who found similar development times with animals kept at 18° C or under variable temperature conditions. At Visp the average egg-laying period is about 60 days, i.e. about half the growth time. According to South (1982) the survival time of the adult slugs at 10° C, however, lasts up to 530 days. Under the laboratory conditions at Visp slugs lived for a maximum of 10 further days after ending egg-laying.

Therefore we can assume that the results obtained at Visp are also valuable for slugs in the northern regions of Europe. Moreover, the high potential for variability in this animal (South 1982) also showed that a good and stable breeding unit is a prerequisite for obtaining reliable data.

REFERENCES

Rollo C D; D M Shibata (1991). Resilience, robustness and plasticity in a terrestrial slug, with particular reference to food quality. *Canadian Journal of Zoology* **69**, 978 – 987.

South A (1982). A comparison of the life cycles of *Deroceras reticulatum* (Müller) and *Arion intermedius* Normand (Pulmonata: Stylomatophora) at different temperatures under laboratory conditions. *Journal of Molluscan Studies* **48**, 233 – 244.

Xenobiotic metabolising enzymes of freshwater snails: Implications for molluscicide metabolism

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ABSTRACT

Several species of aquatic snails are vectors of medically important parasitic diseases. Efforts to control the snail species have proven to be difficult and both chemical and biological means have met with limited success. There is now a renewed interest in the identification of newer plant molluscicides. With a greater understanding of molluscan biochemistry and physiology it would be possible to develop molluscicides that are not only more effective but also much safer to the environment. Xenobiotic metabolising enzymes (XMEs) play an important role in the metabolism and excretion of various xenobiotics encountered by organisms and have been identified in four aquatic snail species namely *Lymnaea natalensis, Bulinus tropicus, Physa acuta* and *Helisoma duryi*. We have shown that these snails posses many of the XMEs and that exposure to a number of pesticides resulted in altered XME activity. Our results suggest the need to carry out more detailed studies to determine the interaction of these enzymes with candidate molluscicides, both synthetic and natural, in order to understand molluscicidal action. This would allow for the development of safer and more effective molluscicides in order to control snail-transmitted diseases.

XENOBIOTIC METABOLISING ENZYMES

Aquatic snail species possess cellular metabolic pathways that resemble those of mammals. Xenobiotic metabolising enzymes (XMEs) are ubiquitous and play an important role in the metabolism and removal of xenobiotics (Jakoby & Ziegler, 1990). There is now a large amount of information on the role and characteristics of these enzymes in mammalian systems where they have been shown to play a pivotal role in the removal of pharmacological agents from the system (Oscarson, 2003). They also play a protective role against the toxic effects of xenobiotics and at other times have been shown to be responsible for the adverse effects of xenobiotics (Knudsen, *et al*., 2001). Resistance to certain drugs (in humans) and pesticides (in insects and plants) has also at times been attributed to the XMEs.

The XMEs include cytochrome P-450 (P450), glutathione S-transferase (GST), esterases, and the antioxidant enzymes (AOEs). The cytochrome P-450 and Flavin Monooxygenases play a crucial role in the metabolism of drugs and other endobioitcs by converting lipohillic compounds to hydrophilic compounds that can be easily excreted form the body. The GSTs play a major role in the removal of electrophilic compounds that are usually toxic or mutagenic. The AOEs include enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and DT-Diaphorase (DTD). Collectively they play a role in protecting against oxidative stress whether exerted directly through reactive oxygen species or by the generation of reactive oxygen species through redox cycling compounds such as quinones. Esterases have been best studied in insect species where they are the targets of insecticidal action (Hassal, 1990). There are several different esterases although the best studied are the cholinesterase that are responsible for nerve inhibition (Walker, 1993). While esterases are not able to metabolise xenobiotics *per se* they are able to act as temporary reservoirs for certain pesticides before elimination.

MOLLUSCICIDES

Only a limited number of synthetic molluscicides are currently available resulting in few available options for snail control. Several species of aquatic snails are vectors of parasitic diseases such as schistosomiasis and fascioliasis but only Niclosamide has been used in snail control programmes (Sturrock, 2001). A number of terrestrial snail species are responsible for the destruction of wide variety of agricultural produce including fruit and vegetables. More options are available with the control of terrestrial snail pests but metaldehyde and copper based molluscicides are usually the preferred molluscicides for control (Hassal, 1990).

Although synthetic molluscicides were widely used previously (WHO document, 1992) there has recently been a trend towards the identification of natural plant products with molluscicidal activity (Mott, 1987). Newer potential plant molluscicides are reported regularly in the literature and the list continues to grow steadily (Markouk, *et al.* 2000; Melendez & Capriles, 2002). The literature also indicates that the candidate plant molluscicides have been identified variety of different species and have active ingredients that includes terpenes, flavones etc. (Henderson, *et al.*,1987).

The mechanism of action of synthetic molluscicides is still poorly understood. The widely used Niclosamide is an effective molluscicide but little is known of its mechanism of action besides that it affects osmoregulation, carbohydrate metabolism and components of the respiratory chain (Andrews, *et al.*, 1983). The chemical structures of various candidate plant molluscicides is also becoming available but there is again little information on their mechanism of action (Duncan, 1987). There is a need to have a better understanding of the mode of action of candidate molluscicides at the biochemical and physiological levels in order to make them more effective and safe. Characterisation of molluscan enzymes and macromolecules would allow for a better understanding of the nature of their interaction with molluscicides.

XME'S OF MOLLUSCS AND XENOBIOTIC METABOLISM

Besides the P-450 of *Mytulis edulis* (Livingstone, *et al.* 1989) only limited, if any, information is available on the other XMEs of marine molluscs. In freshwater snails P450 like activity has been shown in *Lymnaea stagnalis* (Wilbrink, *et al.*,1991) and implicated in *Lymnaea natalensis* (Naik, *et al.*, in press) but again information is lacking on other XMEs except esterases. Esterases, but not other XMEs, have been reported in terrestrial molluscs and their interactions with certain pesticides has also been studied (Coeurdassier, *et al.*, 2002). At least four species of freshwater aquatic snail species namely *Lymnaea natalensis, Bulinus tropicus*, *Physa acuta* and *Helisoma duryi* have been shown to possess a number of XMEs. Esterase activity was detected using three different substrates as have the AOEs (Maredza & Naik, 1996) and GST (Naik, *et al.*,1995).

Cytosolic GST of *L. natalensis* is inhibited by niclosamide (Naik, *et al*., in press). The GST as well as the AOE's were altered differentially by pyrethroids (deltamethrin), phenoxyacetic acids (24D), organochlorines (Endosulfan) and organophosphates (pirimiphos/ malathione) (Maredza & Naik, 1996; Naik, *et al.*, 1995). The esterases were, however, only inhibited consistently by the organophosphates. Pesticide induced alterations in esterase activity has also been reported in other aquatic (*Pila globosa* and *Lymnaea acuminata*) (Singh, *et al.*, 1982) and terrestrial (*Helix aspersa*) molluscan species (Coeurdassier, *et al.*, 2002). Finally, pesticide induced adverse effects have been noted and suggest that a variety of pesticides including insecticides and herbicides are able to adversely affect molluscs.

XME'S AND MOLLUSCICIDES

There is a vast amount of information on the targets, routes of metabolism and toxicity of drugs for human diseases and insecticides (Hassal, 1990). Available data suggest that molluscs possess many of the XMEs already described in mammalian systems and, more importantly, that these enzyme levels can be modulated by exposure to xenobiotics. The XMEs of snails are therefore likely to play a role in the metabolism and removal of molluscicides and also possibly confer resistance to molluscicides. Just as XMEs are targets for the development of drugs in medicine, molluscan XMEs are also potential targets for the development of molluscicides. A more detailed understanding of the characteristics of molluscan XMEs would allow for the identification and/or modification of candidate molluscicides in a manner similar to that used for drug design and discovery using ADME studies (Norris, *et al.*, 2000). Development of inhibitors of esterases using existing knowledge of various pesticides would be a good start. Using available information in the scientific literature, inhibitors could be identified that are more potent and less detrimental to the environment. For example, the active ingredients in *Phytolacca dodecandra* have been identified and information on the metabolism within snails would help generate safer and more potent derivatives. Studies on the interaction between XMEs and molluscicides would also shed much light on the mechanisms, metabolism and possible resistance mechanisms of existing and candidate molluscicides. Finally, other molecular targets, other than XME's, such as transaminases that are affected by metals (Masola, *et al.*, 2003), should also be pursued in developing molluscicides. Collaborative studies between phytochemists and malacological biochemists on the mode of action of newer molluscicides are likely to provide some useful data and leads.

ACKNOWLEDGEMENTS

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REFERENCES

Andrews P; Thysen J; Lorke D (1983). The biology and toxicology of molluscicides, Bayluscide. *Pharmacology and Therapeutics* **19,** 245-295.

Coeurdassier M; Gomot-De Vaufleury A; Saint-Denis M; Ribera D, Narbonne JF; Badot P M (2002). Effects of dimethoate on snail B-esterase and growth as a function of dose, time and exposure route in a laboratory bioassay *Biomarkers*. **7**,138-50.

Duncan J (1987). Mode of action of molluscicides. In: *Plant Molluscicides,* ed. K E Mott, pp 27-44. John Wiley and Sons Ltd: Chichester.

Hassal K A (1990). *The Biochemistry and use of Pesticides*. 2nd Ed, Macmillan: UK.

Henderson T; Farnsworth N; Myers T (1987). Biochemistry of recognised molluscicidal compounds of plant origin. In: *Plant Molluscicides*, ed. K E Mott, pp. 109-130. John Wiley and Sons Ltd: Chichester.

Jakoby W B; Ziegler D M (1990). The enzymes of detoxication. *Journal of Biological Chemistry* **265,** 20715- 21718.

Knudsen L E; Loft S H; Autrup H (2001). Risk assessment: the importance of genetic polymorphisms in man. *Mutatation Research* **482,** 83-88.

Livingstone D R; Kirchin, M; Wiseman A (1989). Cytochrome P-450 and oxidative metabolism in molluscs. *Xenobiotica* **19,** 1041-1062.

Markouk M; Bekkouche K; Larhsini M; Bousaid M; Lazrek H B; Jana M (2000). Evaluation of some Moroccan medicinal plant extracts for larvicidal activity. *Journal of Ehnopharmacology* **73,**293-7.

Maredza A; Naik Y S (1996). Altered esterase activity due to pesticide exposure in the aquatic snail *Physa acuta*. *Proceedings of the First Pan-African Conference on Biochemistry and Molecular Biology. Nairobi, Kenya 1996,* 300- 304.

Masola B; Chibi M; Naik Y S; Kandere E; Zaranyika M F (2003). Activities of glutatmate dehydrogenase, alanine and aspartate aminotransferases in freshwater snails *Helisoma duryi* and *Lymnaea natalensis* exposed to copper. *Biomarkers* **8**, 33-42.

Melendez P A; Capriles V A (2002). Molluscicidal activity of plants from Puerto Rico. *Annals of Tropical Medicine and Parasitology* **96,** 209-18.

Mott K E (ed). (1987). *Plant Molluscicides*. John Wiley and Sons Ltd.: Chichester.

Naik Y S; Magwere T; Matiyenga M; Hasler J A. (in press). Glutathione S- transferase of the freshwater snails *Planorbella duryi* and *Lymnaea (Radix) natalensis*: Partial characterization and interaction with niclosamide. *Journal of Medical and Applied Malacology*.

Naik Y S; Chirombe L; Hasler J A (1995).Effect of pesticides on the glutathione S-transferases of aquatic snails. *Proceedings of the 3rd Congress of Toxicology in Developing Countrie, Cairo, Egypt*. **II**, 51-57.

Norris D A; Leesman G D; Sinko P J; Grass G M (2000). Development of predictive pharmacokinetic simulation models for drug discovery. *Journal of Control Release* **65,** 55-62.

Oscarson M (2003). Pharmacogenetics of drug metabolising enzymes: importance for personalised medicine. *Clinical Chemistry in Laboratory Medicine* **41,** 573-80.

Singh D; Singh O; Agarwal R A (1982). Comparative study of cholinesterase in two snails *Pila globosa* and *Lymnaea acuminata*. *Journal of Physiology* **78**, 467-72.

Sturrock R F (2001). Schistosomiasis epidemiology and control: how did we get here and where should we go? *Memoria Insituto Oswaldo Cruz* **96**, 17-27.

Walker C H (1993). The classification of esterases which hydrolyse organophosphates: recent developments. *Chemical Biological Interactions* **87,** 17-24.

Wilbrink M; Groot, E J; Jansen R; De Vries, Y; Vermuelen N P E (1991). Occurrence of a cytochrome P-450 containing mixed-function oxidase system in the pond snail *Lymnaea stagnalis. Xenobiotica* **21**, 223-233.

WHO document. WHO/SCHISTO/92.107. The role of mollusciciding in schistosomiasis control. (1992). World Health Organisation, Geneva.

The potential use of an alternative food source (legumes) as a pest management strategy for the field slug, *Deroceras reticulatum* (Müller), in winter wheat

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ABSTRACT

A series of laboratory-based studies determined the most suitable species of legume to act as an alternative food for *D. reticulatum*, thereby reducing the amount of damage caused by the slug species to winter wheat. Of all legumes screened, red clover was favoured because it was the most palatable species as well as affecting wheat survival the least. Slugs also continued to consume red clover, even when wheat seeds were fed as a novel food. Furthermore, red clover was as effective at reducing damage to wheat seedlings as the commonly used molluscicide, metaldehyde.

INTRODUCTION

Deroceras reticulatum (Müller) is the most important slug pest of winter wheat crops in temperate climates. Slugs can damage the crop in one of two ways, firstly below ground where the grain is hollowed soon after sowing, and secondly at the base of stems and young leaves (Duthoit, 1964).

Slug control continues to be a problem as molluscicides, conventionally applied, are often unreliable. The provision of an alternative source of food for slug pests could further reduce the level of damage to a wheat crop. Many species of slug, including *D. reticulatum*, spend most of their time foraging for green plant material on or just below the soil surface, penetrating deeper only when large enough spaces exist between clods of soil. Cook *et al.* (1996) demonstrated in the laboratory that, while some agricultural weeds are highly palatable, species differ in their acceptability to *D. reticulatum*. In further work Cook *et al.* (1997) highlighted the importance of weed palatability to slugs and suggested that weeds could provide a degree of protection to the crop until it has developed beyond the vulnerable seedling stage. However, because weeds, by definition, are a problem in cereal production, and require their early removal, this would seem unfeasible. In contrast, legumes, could be a viable alternative, not only because they can be controlled within the crop but because they have the added advantage of potentially increasing soil organic matter and enhancing biodiversity.

Presented here, therefore, is an overview of key themes central to this study, namely to:
1. Assess the palatability of a wide range of legume species to *D. reticulatum*, so that the potentially most suitable alternative food sources could be identified.
2. Quantify the effects that highly palatable legume species grown within the wheat crop have on wheat development.

3. Investigate the extent to which the addition of wheat to previously sown legumes affects slug feeding preferences. The proposed scheme of providing an alternative leguminous food source would require that the legume be sown before the wheat seed. Earlier work has indicated that slugs tend to eat more of a new food item than when it is familiar (Wareing, 1993), suggesting that slugs might show a temporary increase in preference for wheat seeds immediately after they are sown.
4. The ability of the most suited legume to reduce the amount of damage caused to winter wheat by *D. reticulatum*.

MATERIALS AND METHODS

Evaluation of the palatability of legumes to *D. reticulatum*

Experimental slugs were given a choice between equal amounts of 1 month old leaves of ten legume species (Table 1) and wheat with one leaf emerged (GS11). A detailed methodology of this experiment can be found in Brooks *et al.* (2003).

The relative preferences of each legume species were calculated using an established method of Acceptability Indices (AI) to express the degree of preference for the legume species. The method has been adopted from previous work (Cook *et al.*, 1996):

$$AI = \frac{\text{area of legume leaf eaten (cm}^2\text{)}}{[\text{Area of legume leaf eaten (cm}^2\text{)} + \text{area of wheat leaf eaten (cm}^2\text{)}]}$$

The index therefore ranges from 0 when no legume was consumed, to 1, when only legume was eaten.

Effect of competition of legumes on winter wheat survival

The experiment took place in a polytunnel at Harper Adams University College. On two separate occasions, two months before the wheat (sowing date 1) and one month before the wheat (sowing date 2), red clover (cv. Britta), lucerne (cv. Vela) and white clover (cv. Alice) (varieties used previously) were sown at the highest rate which would be sown in the field into 20 cm pots using John Innes No. 2 compost. The control pots consisted of wheat seeds only (cv. Cadenza at 325 seeds/m^2) The pots were arranged on the ground in six blocks of 8 treatment combinations. Presented here are the numbers of wheat plants that survived to GS30 (start of stem elongation), as a percentage of the number of seeds sown.

Effect of recent dietary history on the palatability of legumes to *D. reticulatum*

In order for the chosen legume species to be established in the field so that it can act as an alternative food source, it must have established sufficiently before the wheat crop. During this time, slugs would become familiar with the legume species as a source of food. Once the wheat is sown it would become a novel food, to the slugs. Experiments were designed to assess whether the wheat would be treated as a new food and therefore be consumed in preference to the legume. Three pre-test diets, red clover leaves only, red clover leaves and wheat seeds and lettuce leaves, were fed for a period of one week to slugs, prior to a test diet of legume leaves and wheat seeds. The test diet was fed for three days, with the amounts of the two foods consumed assessed daily, using a six point damage score. A Preference Index was used to express the relative consumption of wheat to red clover:

$$PI = \frac{\text{damage score of wheat seed eaten}}{[\text{damage score of wheat seed} + \text{damage score of red clover leaf}]}$$

The Index ranges from 0 when no wheat was eaten to 1, when only wheat was eaten. A score of 0.5 indicated that equal amounts of wheat seeds and red clover leaves were consumed.

The potential for red clover and metaldehyde to reduce slug damage to winter wheat

To assess the potential for an alternative food source to reduce slug damage to winter wheat, three treatments, wheat seedlings only, wheat seedlings and red clover plants and wheat seedlings and metaldehyde (6% w/w), were tested using 10 litre plastic food boxes. Boxes were half filled with a sandy clay loam soil and allocated boxes planted with red clover seeds at the standard seed rate. Once the spade leaf of the red clover plants had emerged, wheat seeds, at a standard autumn seed rate, were sown in three rows between the clover plants, so that that by the time the first set of trifoliate leaves were fully emerged, the wheat was at GS11 (one leaf emerged). At this point the boxes were well watered and metaldehyde pellets were applied to allocated boxes at (15 kg/ha). A high population of slugs (40/m^2) were added to all boxes and the lids lightly fastened before being placed in an unheated glasshouse cubicle. No artificial lights were used. After seven days, the slugs were removed and damage to both wheat seedlings and red clover leaves were assessed using a six point damage score.

RESULTS

Evaluation of the palatability of legumes to *D. reticulatum*

Table 1. Ten legume treatments and their Acceptability Indices (untransformed means), in order of acceptability to *D. reticulatum*. (Adapted from Brooks *et al.*, 2003)

Legume	Latin name	AI	SE
Red clover	*Trifolium pratense*	0.810	0.038
Lucerne	*Medicago sativa*	0.792	0.032
White clover	*Trifolium repens*	0.770	0.040
Lupin	*Lupinus perennis*	0.746	0.033
Tufted vetch	*Vicia cracca*	0.604	0.066
Trefoil	*Medicago lupulina*	0.532	0.054
Birdsfoot trefoil	*Lotus corniculatus*	0.530	0.079
Sainfoin	*Onobrychis sativa*	0.502	0.071
Common vetch	*Vicia sativa*	0.444	0.077
Hairy tare	*Vicia hirsuta*	0.419	0.064

Table 1 shows the Acceptability Index for each of the ten legume species averaged over the 72 hr test period. There was a significant difference in AI between legume species over the whole 72 h period ($P<0.05$) and a clear hierarchy of acceptability of legumes to *D. reticulatum* can be seen. Red clover, lucerne, white clover and lupin showed significantly higher AIs compared to the other six species tested (LSD tests at $P<0.05$), with the exception of tufted vetch, possibly because they exhibit little cyanogenesis. However, there was no significant difference in legume acceptability between these four species (LSD tests at $P>0.05$).

Acceptability Indices (AIs) provide a useful overall view on the relative palatabilities of different items of food; however, they do not give an indication of the absolute amount of the foods consumed in this case, winter wheat and ten different legume species. Nevertheless, the four legumes with the highest AIs consumed large amounts of legume and the smallest amounts of wheat, of the ten species screened. The three most palatable legumes were taken forward to the next stage of the screening process.

Effect of competition between legumes and wheat

When sown two months before the wheat, all legume treatments had a highly significant negative effect ($P<0.001$) on the survival of winter wheat (Figure 1). This is unsurprising because when the wheat sown the legumes were large, well established plants. In comparison, red clover sown one month before the wheat appeared to have much less of an effect on wheat survival (LSD test at $P<0.05$), compared to lucerne and white clover sown at the same time, indicating that red clover was the least competitive of the three. This may be, in part, due to less shading resulting from a smaller red clover leaf canopy and that white clover and lucerne tended to produce a more erect growth habit. Observations suggest that at this stage the legumes would need to be removed from the wheat crop, as GS30 is considered the latest time to remove the legumes.

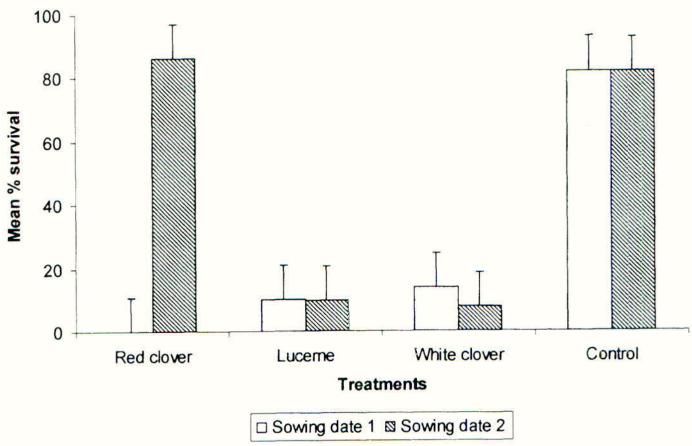

Figure 1. Effect of legume species and sowing date on the survival of wheat plants to GS30 (Bars = +1 SE, n=10)

Effect of recent dietary history on the palatability of legumes to *D. reticulatum*

The general trend illustrated by these results is for *D. reticulatum* to consume more wheat from Day 2 onwards (Figure 2). With the exception of Day 1, the PIs of slugs given the clover only pre-test diet, where wheat is novel, are similar to those given the clover and wheat seed pre-test diet, where the wheat seed is not novel. Interestingly, when slugs were fed the lettuce pre-test diet (where red clover and wheat are both novel) they consumed significantly less wheat than the other two diets (LSD test at $P<0.05$), indicating that lettuce reduced the slugs' consumption of wheat, whilst still consuming the same amount of red clover, within this time period. Irrespective of pre-test diet, though, the mean PIs are still below 0.5, indicating that the slugs still consume more red clover than wheat. This result is encouraging with regard to a potential slug control strategy.

In all treatments, when presented with the test diet, red clover and wheat, slugs consumed red clover in similar amounts. However, the amount of wheat eaten differed between groups, with nearly 50% less wheat consumed after the pre-test diet of lettuce compared with the two other treatments where similar quantities of wheat were eaten.

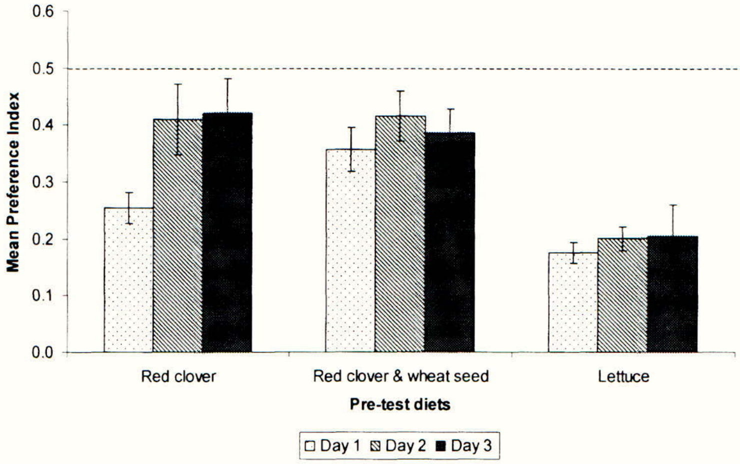

Figure 2. Mean Preference Indices for wheat of slugs fed a test diet of wheat seeds and red clover leaves over a three day period, after being maintained on three pre-test diets (Bars = ± 1SE, n=15). Dotted line = PI of 0.5, no preference for wheat or red clover

The potential for red clover and metaldehyde to reduce damage to winter wheat

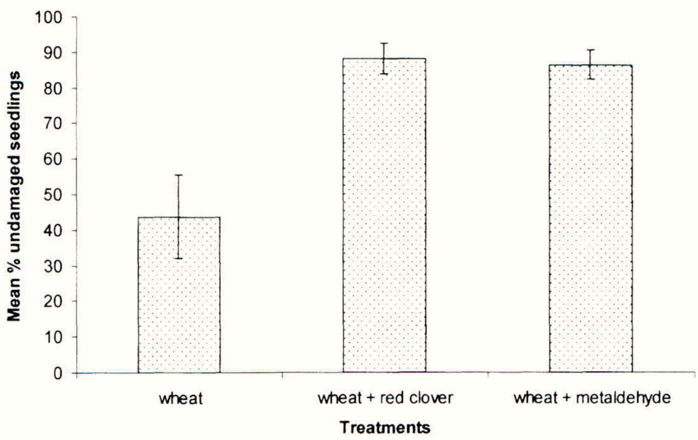

Figure 3. Mean number of wheat seedlings damaged by slugs as a percentage of the total seedlings present, growing in boxes and subjected to three different treatments (Bars = ±1 SE, n=5)

The effect of treatment on the mean number of undamaged seedlings, as a percentage of the total present, was significant ($P<0.05$). Figure 3 shows that red clover was equally effective as metaldehyde in reducing slug damage to winter wheat. In all cases, the damage to the developing crop occurred almost exclusively to the stem base, which usually results in plant death. Both clover and metaldehyde were seen to significantly reduce damage compared with no slug control ($P<0.05$), by some 40%.

DISCUSSION

These laboratory-based studies have clearly identified that there is a potential for a legume crop to help control slug damage to the developing wheat crop, in a pest management strategy. Red clover was the most palatable, least competitive at key growth stages of wheat and was as effective as conventional molluscicides in reducing slug damage to wheat seedlings. Additionally, slugs did not alter their feeding behaviour when wheat became available after a period of a clover only diet.

Extensive field trials are obviously necessary to determine how effective this strategy could ultimately be, both agronomically and economically. One significant difficulty may be implementation under commercial conditions, given that the agronomy of a dual crop could be made difficult by inclement weather conditions preventing correct sowing and herbicide application timings. The latter would be essential to ensure successful clover eradication.

However, it must be noted that whilst using an alternative food source may reduce the use of molluscicides it could actually increase herbicide usage – since clover would need to be controlled after the wheat crop has passed its most vulnerable stages.

REFERENCES

Brooks A S; Crook M J; Wilcox A; Cook R T (2003). A laboratory evaluation of the palatability of legumes to the field slug, *Deroceras reticulatum* (Müller). *Pest Management Science* **59**, 245-251.

Cook R T; Bailey S E R; McCrohan C R (1996). Slug preferences for winter wheat cultivars and common agricultural weeds. *Journal of Applied Ecology* **33,** 866-872.

Cook R T; Bailey S E R; McCrohan C R (1997). The potential for common weeds to reduce slug damage to winter wheat: laboratory and field studies. *Journal of Applied Ecology* **34,** 79-87.

Duthoit G M G (1964). Slug and food preferences. *Plant Pathology* **13,** 73-78.

Hunter P J (1968). Studies on slugs of arable ground. III. Feeding habits. *Malacologia* **6,** 391-399.

Wareing D R (1993). Feeding history - a factor in determining food preference in slugs. *Journal of Molluscan Studies* **59**, 366-368.

Onset of immobilization in the slug *Deroceras reticulatum* (Müller) parasitized by the nematode *Phasmarhabditis hermaprodita* Schneider

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ABSTRACT

The onset of immobilization in *Deroceras reticulatum* after drenching with inoculum of the nematode *Phasmarhabditis hermaphrodita*, at the recommended application rate was observed by infra-red time-lapse videorecording. Small slugs died within two days of inoculation, but larger individuals were still observed active up to 10 days later, although their activity was markedly reduced after three days, and feeding and copulation ceased two days after treatment. In addition, parasitized slugs moved more slowly, with shorter and possibly more circuitous tracks. Parasitized slugs no longer confined the bulk of their activity to the night phase. There was a slight initial increase in activity in the first two days. The rapid immobilization suggests that the possibility of infected slugs transmitting the nematode to new sites is poor.

INTRODUCTION

The nematode *Phasmarhabditis hermaphrodita* has been developed as a biological control agent for the control of many species of pest slugs, including the field slug *Deroceras reticulatum* (Wilson, *et al*., 1993). Wilson, *et al*. (1994) suggested that feeding inhibition of parasitized slugs was the main factor in crop protection. Although the dauer larva of the nematode migrates a negligible distance in soil, it is possible that infected slugs could transport the nematode to new areas before dying and releasing a new generation of dauer larvae.
The aims of the experiments were (1) to see if slugs would be effective vectors of the nematode, carrying the infection to new areas before they become immobilised, and (2) to record any changes in behaviour which might affect the spread of the parasite.

MATERIALS AND METHODS

Slugs, *D. reticulatum*, were collected from a garden which had had no biocontrol and only limited application of molluscicidal baits, and used in trials within two days of collection. Six sets of trials were conducted, over summer, autumn and winter, in a controlled environment room at 15°C ± 0.5°C in winter, and 19°C ± 0.5°C in summer and autumn, using a pair of square wooden boxes 30 cm x 30 cm, and 15 cm high, filled with 5 cm of garden loam. Filter paper impregnated with sodium chloride folded over a supporting acetate sheet was pinned to the outside of the boxes to a height of 6 cm above the rim to prevent slugs escaping. Between 3 and 6 slugs were introduced to each box 24 h before the inoculum of parasites was added. Transparent shelters (short glass tubes or 8 cm Petri dish lids) were placed in the corners of the box, and pelleted food (maize flour with 2% gelatine binder) was placed near the centre. One box served as a control, the other was inoculated with the nematodes. 200 ml of deionised