Project Title:	Hardy nursery stock: Integrated control of snails and slugs
Project Number:	HNS 105
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Location of Project:	One commercial nursery ADAS, Boxworth, Cambridge, CB3 8NN Department of Agricultural and Environmental Science, Ridley Building, University of Newcastle upon Tyne, NE1 7RU
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Key Words:	*Oxyloma pfeifferi*, *Deroceras panormitanum*, parasitic nematode, *Phasmarhabditis hermaphrodita*, copper ammonium carbonate, Croptex Fungex, repellent barrier, Tex-R (Spin Out) matting, Snail-Ban, mulch, cinnamamide, copper foil, aluminium foil, garlic.

The results and conclusions in this report are based on an investigation conducted over one year. The conditions under which the experiments were carried out and the results obtained have been reported with detail and accuracy. However because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results especially if they are used as the basis for commercial product recommendations.

PRINCIPAL WORKERS

J A Bennison, Senior Research Entomologist, ADAS Boxworth (Project Leader and author of report)

H M Maher, Senior Scientific Officer, ADAS Boxworth

Dr G R Port, Senior Lecturer, University of Newcastle

I Schüder, PhD Student, University of Newcastle

AUTHENTICATION

I declare that this work was done under my supervision according to the procedures described herein and that this report represents a true and accurate record of the results obtained.

..... J A BENNISON
Project Leader

Date.....

Report authorised by: .....

Dr M Heath
Team Manager, ADAS Boxworth

Date.....

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CONTENTS

GROWER SUMMARY

Headline

- In laboratory tests, the parasitic nematodes 'Nemaslug' at both recommended and lower rates were effective against the main species of slug and snail occurring on HNS nurseries and could offer a more effective and environmentally-friendly alternative to slug pellets. Trials comparing the efficacy of different rates of 'Nemaslug' and slug pellets on commercial nurseries are planned for year 3 of the project.

- Tex-R matting is repellent to both slugs and snails. Using Tex-R as a ground cover could reduce infestation of new plants by preventing immigration of slugs and snails from surrounding infested areas.

Background and expected deliverables

Slugs and snails damage a wide range of container-grown HNS species and are difficult to control with slug pellets. Feeding damage is very obvious and causes plant losses or downgrading. This project aims to develop improved, sustainable integrated control strategies for slugs and snails on HNS, to reduce plant losses, improve plant quality and satisfy increasing customer demands for ornamental produce grown with minimal use of pesticides. Expected deliverables include:

- The identification of the main species of slugs and snails damaging HNS.
- The identification of plant species damaged.
- An improved knowledge of the biology of the main slug and snail species so that the optimum timing and components of control strategies can be planned.
- Recommendations for an integrated control strategy, including cultural control methods, biological control of existing infestations with parasitic nematodes ('Nemaslug') and use of barriers or repellents to prevent infestations of new plants.

Summary of the project and main conclusions

Identification of slug and snail species
During year 1 of the project, the main slug species found on ten selected HNS nurseries was *Deroceras panormitanum* and the main snail species was the small semi-aquatic snail, *Oxyloma pfeifferi*. Photographs to aid recognition of these species are included in Factsheet 07/02, issued to HNS growers during 2002.

Identification of plant species damaged

During year 1, a wide range of plants were identified as being damaged by slugs and snails, including herbaceous and perennial species, alpines, shrubs, grasses and herbs, in plugs, liners and containers, both under protection and outdoors. *O. pfeifferi* were also observed to be associated with algae on the substrate, compost or polythene tunnel coverings. During year 2, feeding studies with both *D. panormitanum* and *O. pfeifferi* were carried out, to confirm the plant species damaged and the damage symptoms. Hosta and Choisya were selected as representative herbaceous and perennial host plants respectively.

Replicated laboratory tests where individual slugs or snails were offered Hosta or Choisya leaves showed that *O. pfeifferi* grazed the surface of Hosta leaves and stems but did not feed on Choisya. *D. panormitanum* caused leaf holes and petiole severing on Hosta and leaf holes and shredding on Choisya. Damage to Hosta depended on the variety, with more slug damage occurring on var. 'Elegans' than on 'August Moon'. Both *O. pfeifferi* and *D. panormitanum* fed on algae but could not survive on a diet of algae alone. *O. pfeifferi* was also confirmed to feed on liverwort and decaying leaves. It was concluded that *D. panormitanum* damages both herbaceous and tougher-leaved perennial plants. *O. pfeifferi* causes primary damage to soft-leaved herbaceous plants, but experimental evidence to date indicates that it does not damage some perennial plants including Choisya. In some situtations, *O. pfeifferi* may survive on a mixed diet of algae, damaged or decaying leaves (including those from perennial plants) and liverwort. As the snails are active and visible during the day and slugs are mainly nocturnal and tend to hide during the day, the snails may be mistakenly held responsible for slug damage on some HNS species e.g. Choisya. If present in large numbers on plants or pots, the snails may lead to quality problems as contaminants.

Slug and snail biology

During year 2, relevant gaps in knowledge of the biology of *O. pfeifferi* and *D. panormitanum* were addressed by experimental work at ADAS Boxworth, the University of Newcastle and on a commercial HNS nursery. *O. pfeifferi* hibernated between September/October and February/March on the sides of pots, on the structure of polythene tunnels and on plants. Snail activity started again during February and most were active by late March. *O. pfeifferi* laid eggs between late March and August, with mixed ages and overlapping generations occurring during this period. Wet conditions stimulated activity and egg-laying and favoured survival. The life cycle from egg to egg was 14 weeks at 20-22ºC. *O. pfeifferi* is more active during the day than at night and can easily be found on HNS plants and pots, particularly if present in large numbers.

D. panormitanum was active all year round, with peaks of activity during spring and autumn. Large adult slugs died in late winter/early spring. Egg-laying started in

March and large numbers of juveniles were present in April. Slug growth rates and onset of egg-laying was very variable, so generations are likely to overlap. Under optimum conditions the life cycle takes 10 weeks. *D. panormitanum* is well-adapted to the high temperatures and damp conditions present in glasshouses and polythene tunnels, where two or three slug generations may develop each year. *D. panormitanum* tends to be mainly active at night, hiding beneath pots and trays and at the base of plant stems during the day. This tendency for nocturnal activity means that slug numbers are often under-estimated.

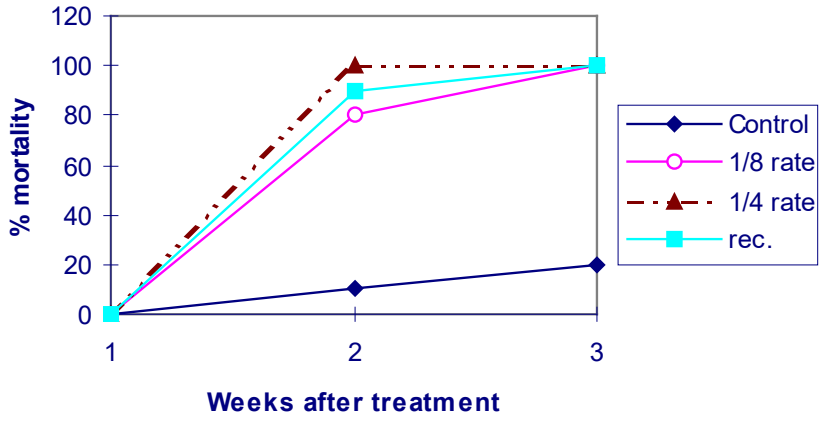
The optimum times for control of both *O. pfeifferi* and *D. panormitanum* are likely to be spring and early autumn.

Cultural control

Experimental work and observations on commercial HNS nurseries have shown that wet conditions favour slug and snail activity and that algae are a source of food, therefore avoiding over-watering will help to reduce infestation levels and damage. Slugs and snails can feed and shelter amongst weeds, liverworts, unmarketable plants and trimmed off plant material, therefore close attention to nursery hygiene is an important cultural component of an integrated control strategy.

Biological control with 'Nemaslug'

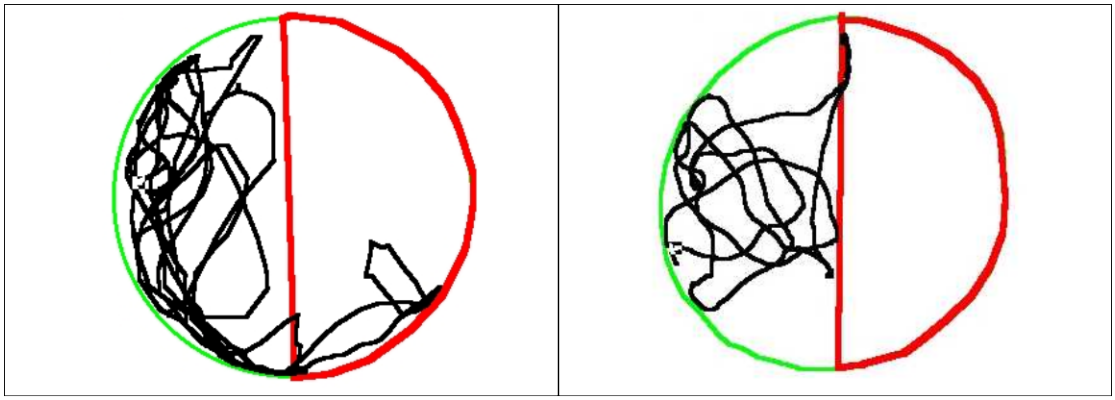
In laboratory tests, 'Nemaslug' was effective against both *D. panormitanum* and *O. pfeifferi*. During year 2, tests with recommended and half-rates of nematodes done in year 1 were repeated with *D. panormitanum*, using younger, summer-collected slugs, as results in year 1 had been inconclusive due to high natural mortalities in older, winter-collected slugs. The year 2 results showed that half-rate 'Nemaslug' was equally as effective as full-rate, killing 80% slugs within four weeks of treatment. 'Nemaslug' at recommended, 1/4 or 1/8- rates were all equally effective against *O. pfeifferi*, killing 100% snails within three weeks of treatment. Trials on commercial HNS nurseries are planned for year 3, comparing control of both slugs and snails by 'Nemaslug' at various rates and by slug pellets.



Mean % mortalities over three weeks in *O. pfeifferi* treated with recommended, 1/8 and 1/4-rates of 'Nemaslug'

Barriers or repellent techniques

During year 2, 14 potential barrier or repellent products were further tested in the laboratory against both *D. panormitanum* and *O. pfeifferi*. Several of these products, including cinnamamide, Croptex Fungex, garlic, Tex-R and similar 'Spin Out' ® products and ureaformaldehyde showed potential for further evaluation of their various effects on the slugs and snails. These effects include repellency, barrier effect, irritation, antifeedant, slug/snail and/or egg mortality and reduction in egg-laying. Tex-R matting was selected for further testing as a repellent barrier in large-scale field trials during year 2, due to its strong repellent effect against both the slugs and snails in laboratory tests, its current availability and practicality. In laboratory tests, when given the choice of either Tex-R matting or Mypex, video behavioural experiments showed that over 90% slug and snail tracks were on the Mypex. In a field trial with *D. panormitanum*, there were 89% fewer slugs on plants in Tex-R plots than on those in the Mypex plots. In a trial with *O. pfeifferi* on a commercial HNS nursery, results with Tex-R were inconclusive due to experimental problems.



A. B.

Representative examples of track images from (A) *D. panormitanum* and (B) *O. pfeifferi* when offered either Mypex (left half of circular dish) or Tex-R (right half of dish).

Financial benefits

- During year 1, case studies of slug and snail damage on ten selected HNS nurseries showed that plant losses and downgrading of individual plant species was variable, with estimated plant losses ranging from less than 1 to 100%. The annual value of the UK HNS industry is currently estimated at £355 million. At a conservative estimate of a minimum of 1% plant losses, current difficulties in controlling slugs and snails are causing annual losses of at least £3.5 million to the HNS industry. In addition, considerable time is lost in selecting undamaged plants for lifting, or in trimming off damaged leaves before market.

- Until the third year of the project has been completed, the cost/benefits of implementing the improved, integrated control strategy developed in this project cannot be confirmed. However, the results in the project to date indicate that the development of the integrated control strategy is likely to reduce the current use of chemical molluscicides, improve plant quality and marketability, and satisfy increasing demands for high quality plants grown with environmentally-friendly production methods and minimal use of pesticides.

Action points for growers

- Until the third year of the project has been completed, only preliminary action points can be recommended on the use of parasitic nematodes and potential barriers / repellents. If wishing to try 'Nemaslug' or Tex-R matting, use on a small scale first for susceptible plant species in areas of the nursery prone to slug or snail damage. The optimum time for treatment with 'Nemaslug' is likely to be spring or early autumn, when minimum compost temperatures are above 5ºC and when slugs and snails are active. Care must be taken to follow label directions for 'Nemaslug' application, in particular to apply to moist compost and to keep the compost damp for at least two weeks after application. Tex-R matting should be used as a ground cover for new plants uninfested with slugs or snails. If used in very wet conditions, algal growth on the Tex-R will reduce its efficacy, particularly with Landscape Pro which is a thick, water-retentive matting designed for use on sand beds. Tex-R and Supercover Plus are alternative, more free-draining Tex-R products and options should be discussed with the supplier.

- Use slug pellets at the recommended rate if / when necessary.

- Maintain good nursery hygiene to reduce shelter and food sources for slugs and snails. Control algae, mosses, weeds and liverworts as thoroughly and frequently as possible and dispose of old unmarketable plants and trimmed off plant material promptly.

- Avoid over-watering as wet conditions favour both slug and snail activity and also the growth of algae and liverworts.

- Check and clean up bought-in plants or liners produced elsewhere on the nursery for slug and snail infestation and damage. Check under pots and trays where slugs often hide during the day.

- Encourage natural predation of slugs and snails by providing habitats for birds, hedgehogs and beetles and by minimising the use of pesticides by adopting IPM.

SCIENCE SECTION

INTRODUCTION

Snails and slugs damage a wide range of container-grown HNS plant species and are difficult to control with conventional molluscicide pellets. Populations have become established on many nurseries throughout the country, and are damaging plants in plugs, liners and containers, both under protection and outdoors. Plant species affected include alpines, herbaceous plants, perennial and deciduous shrubs, grasses and herbs. Feeding damage is very obvious and causes plant losses or downgrading.

Preliminary investigations prior to the project confirmed the snail species causing problems on one HNS nursery as *Oxyloma pfeifferi*, a small semi-aquatic species. Several slug species have been found on HNS nurseries, but the relative importance of the different species of both slugs and snails as pests of HNS was not known. Methiocarb and metaldehyde pellets are used extensively on susceptible plants on most HNS nurseries, but seem to give only partial control of slugs and little control if any, of the snail species.

The development of alternative, integrated, more effective control measures for snails and slugs would reduce plant losses and use of chemical molluscicides, improve plant quality and offer a sustainable strategy for controlling the pests on HNS. As more growers of HNS are now adopting Integrated Pest Management (IPM) techniques, the development of non-chemical snail and slug control methods will be compatible with IPM strategies and satisfy increasing market demands for plants grown with environmentally-responsible production methods.

The parasitic nematode, *Phasmarhabditis hermaphrodita* (Nemaslug®) has been available for the control of slugs since 1997, but has been promoted mainly for the home/garden market, due to cost implications for large-scale commercial use. Research funded by DEFRA (previously MAFF) and MicroBio Ltd (now Becker Underwood) has been done on the efficacy of the nematodes against both slugs and snails (Glen *et al*, 1996). The nematodes have been shown to be effective against the field slug, *Deroceras reticulatum* and other pest species in wheat and lettuce. The snail species tested were mainly those found in hedgerows around arable fields and these did not include *O. pfeifferi* (Wilson, 2001). However, preliminary laboratory tests with the nematodes before this project began, indicated that 'Nemaslug' had potential against *O. pfeifferi* (Bennison & Maulden, unpublished data).

Copper is known to both kill and repel slugs and snails. Various copper formulations are approved for use on HNS for bacterial disease control and these may have potential against slugs and snails. Copper ammonium carbonate (Croptex Fungex) is widely used in the HNS industry. 'SpinOut' products containing copper are now commercially available in the UK for use as a root retardant and weed suppressant. Products include Tex-R matting for use as a sandbed cover or as an alternative to

Mypex, a liquid for application to Mypex, a 'pot-topper' for weed suppression in container plants and an impregnated paper pot. Tex-R matting may have potential for use as a barrier against snails and slugs (Sopp, personal communication).

A grit-like mineral product, 'Snail-Ban'® is available on the amateur market for use as an environmentally-friendly physical barrier to deter snails and slugs. When dry, the product is claimed to prevent snails and slugs moving across the material by absorbing their mucus and when wet, the product acts as an irritant. The product does not dissolve during wet conditions and is claimed to act as a persistent barrier.

Research has been done in the UK on various novel slug and snail repellents. A LINK project part-funded by the HDC Field Vegetables Panel and led by the collaborator project HNS 105, is investigating improved methods for controlling slugs on field-grown brassicas and lettuce, including the potential role of novel slug repellents.

The main objective of project HNS 105 is to identify the snail and slug species currently causing damage to HNS on commercial nurseries, and to develop an integrated management strategy, including both control of established populations and prevention of infestation of new plants brought onto the nursery.

Detailed objectives of the project are:-

1. To identify the species of snails and slugs currently causing damage and difficulties in control on HNS nurseries.

2. To identify the plant species damaged on a range of nurseries, the time of year infestation and damage occurs, and to estimate the extent of damage both under protection and outdoors.

3. To investigate the biology and likely sources of the predominant snail and slug species in order to plan the optimum timing and components of potential control strategies.

4. To evaluate the efficacy of 'Nemaslug' at various dose rates against the selected predominant snail and slug species, compared with that of chemical molluscicides, both in the laboratory and under commercial conditions.

5. To evaluate the efficacy of physical barrier or repellent techniques against the predominant snail and slug species, to prevent infestation of new plants brought onto the nursery. Techniques to be evaluated will include a copper fungicide spray, Tex-R

matting, ‘Snail-Ban’ and novel repellents such as cinnamamide or those based on wood mulches.

6. To produce a factsheet for growers, with details of the snail and slug species and the results of the research.

OBJECTIVES 1 AND 2

Work to meet these objectives was completed during year 1.

OBJECTIVE 3: BIOLOGY OF THE PREDOMINANT SLUG AND SNAIL SPECIES

During year 1, the most common slug and snail species found on HNS nurseries were *Deroceras panormitanum* and *Oxyloma pfeifferi* respectively. During year 2, relevant gaps in knowledge of the biology and behaviour of these two species were addressed by experimental work at both ADAS Boxworth and the University of Newcastle, and by observations on a commercial HNS nursery.

Materials and Methods

Biology of O. pfeifferi

O. pfeifferi hibernation period - study on a commercial HNS nursery
Three adjacent polythene tunnels on a commercial nursery were selected for monitoring the hibernation behaviour of *O. pfeifferi* between September 2001 and March 2002. The tunnels were heavily infested with *O. pfeifferi* and contained Hedera plants in 2-litre pots stood on gravel. Monthly assessments were done as follows:

- Numbers of snails per plant (i.e. on plant or surface of compost), on 45 plants per tunnel in September and October, and on 30 plants per tunnel on the remaining assessments.
- Numbers of snails per pot (i.e. on sides/under pot), on 45 plants per tunnel in September and October, and on 30 plants per tunnel on the remaining assessments.
- Numbers of snails on the wooden door frame of each tunnel, and on 1/4 of the outer wooden door, which was left open throughout the monitoring period.
- Length (mm) of 20 snails on each door frame per tunnel in September and October, and on ten snails per door frame on the remaining assessments.
- Temperatures were recorded throughout the monitoring period with a ‘Tiny talk’ datalogger, placed in an upturned plant pot amongst the plants in one tunnel.

O. pfeifferi hibernation period - study in mini-tunnels at ADAS Boxworth
Three 'mini' tunnels (1.6 m long, 83 cm wide and 60 cm high) were built to monitor the hibernation behaviour of *O. pfeifferi* between October 2001 and April 2002. The tunnels had a wooden frame, a wooden-framed mesh door and were covered with polythene. The tunnels were placed inside a standard-sized polythene tunnel with a Mypex substrate. Algae was collected from the floor of a tunnel at the commercial monitoring site, and spread over the Mypex in the mini-tunnels, to simulate commercial conditions. Eight plants each of Choisya, Hosta and Phormium in 1-litre pots were placed in each tunnel. In addition, one 1-litre pot of liverwort-covered compost and four liner pots with moss/algae-covered compost were placed in each tunnel. *O. pfeifferi* were collected from the commercial nursery and 1,750 snails were added to each mini-tunnel. Approximately half the released snails were 6-10 mm long and and half were under 6mm long. The snails were released to the plants, compost and substrate in each tunnel. Monthly assessments were done as on the commercial nursery (see above), but on all pots and all parts of the wooden structure of each mini-tunnel. During November, ten snails on each of the Choisyas and Phormiums were marked to assess snail activity i.e. whether they changed their positions between assessments.

O. pfeifferi hibernation period - study at University of Newcastle field station
Laboratory-reared snails weighing 60 – 80 mg were used for this experiment. Twenty snails were placed in a small cage (50x50x50 cm), made of perspex , mesh and wood. The snails were provided with one large Chinese cabbage plant and four large Chinese cabbage leaves, which were replaced each week. The bottom of the cage was covered with 1.5 cm of damp soil. The cage was placed in a polythene tunnel at the University field station. The tunnel had two large openings to avoid excessive temperatures in autumn and spring during the day and allowing low temperatures at night during the winter. The experiment started in September 2001. The position of the snails and their activity state (not moving/ sealed operculum) was recorded, if appropriate marking their position on the cage. Damage to the plant and leaves were recorded once a week in the summer and once a fortnight during the winter. The experiment ended in May 2002.

Seasonal life cycle and breeding periods of O. pfeifferi
O. pfeifferi were collected from the commercial HNS nursery in late October 2001 and late March 2002, to investigate whether those snails still active in the autumn were still breeding and to identify the time egg-laying commenced after the winter hibernation period. The largest snails found (8-10 mm long) were placed into perspex sandwich boxes containing damp compost as a substrate, pieces of Chinese cabbage leaf as a food source, a piece of cuttlefish bone as a source of calcium and pieces of liverwort (previously observed as egg-laying sites). The boxes were placed in a mini-

tunnel at ADAS Boxworth and checked three times a week for eggs. Any egg batches were transferred to fresh boxes to monitor hatching, growth and survival of juvenile snails. The observations continued until late May 2002. Observations were also made on the range of snail sizes in the polythene tunnels on the commercial nursery, during visits throughout the year to collect snails for experimental work and to carry out the field trial on barriers and repellents in Objective 5.

Development of O. pfeifferi at different temperatures

Snails were collected from HNS nurseries and kept in incubators at 15° C or 20° C and a 16:8 light:dark cycle and fed with Chinese cabbage. Egg batches with 15 –30 eggs were removed on a daily basis (a total of 20 egg batches per temperature), transferred into Petri dishes with damp filter paper, transferred into incubators with temperatures of 12, 15, 20 and 22° C. Egg development time and hatching rate were recorded. Once the snails had hatched, they were transferred into 500 ml transparent sandwich boxes, containing the snails from one egg batch each and provided with damp filter paper, Chinese cabbage, and a piece of cuttle fish bone. Cultures were cleaned once a week. Mortality and egg production was recorded weekly and the snails were weighed every 14 days for 30 weeks.

Feeding studies with O. pfeifferi

Snails measuring 6-8 mm long were collected from the commercial monitoring site in mid-April, i.e. once activity had resumed after hibernation and before the snails had reached full size. Twenty replicate perspex boxes were set up, each containing a whole Choisya leaf with the base of the petiole placed in a cube of 'Oasis' which was kept wet throughout the study. Twenty similar boxes were set up with individual Hosta (var. 'August Moon') leaves. The leaves were misted with water and one snail was added to each leaf. The boxes were incubated at 20ºC at a 12:12 hrs light:dark cycle for one and four weeks for the Choisya and Hosta respectively. The boxes were checked weekly, the leaves misted with water and any leaf damage and snail mortality was recorded.

As *O. pfeifferi* are often associated with algae on the surface of compost, gravel, Mypex or polythene coverings of tunnels on commercial HNS nurseries, an experiment was set up to identify whether immature snails could grow and complete their development on algae. Food sources tested were:

- Chinese cabbage leaf portion (control)
- Algae-covered compost, collected from the commercial monitoring site
- Algae-covered gravel, collected from the commercial monitoring site

Each food source was added to 20 replicate liner pots containing damp compost. *O. pfeifferi* measuring 6-8 mm long were collected from the commercial monitoring site in mid-May. One snail was added to the surface of each pot and the pot was covered

with a square of mesh and a square of perforated polythene, secured with an elastic band. The pots were incubated at 20ºC at a 12:12 hrs light:dark cycle for four weeks. The pots were checked weekly, misted with water and the length of each snail was measured. Snails were recorded as live, dead or 'attached', i.e. when the snail sealed its operculum with a layer of mucus and became inactive, often prior to death unless the snail is entering hibernation.

Biology of D. panormitanum

Development of D. panormitanum at different temperatures
Slugs were collected in the field and kept in incubators at 15º C and a 16:8 light:dark cycle and fed with Chinese cabbage and carrots. Egg batches with 15 –30 eggs were removed on a daily basis, transferred into Petri dishes with damp filter paper and placed into incubators with temperatures of 12, 15 and 20º C (a total of 20 egg batches per temperature). Egg development time and hatching rate were recorded. Once the slugs hatched, slugs from one egg batch each were transferred into 500ml transparent sandwich boxes and provided with damp filter paper, Chinese cabbage, chicken food "Layers Mash" (mainly wheat seeds) and a piece of cuttle fish bone (calcium source). Cultures were cleaned once a week. Mortality and egg production was recorded weekly; the slugs were weighed every 14 days for 30 weeks.

Performance of field-collected D. panormitanum at different temperatures
Slugs were collected in the field and kept separately in 3 litre transparent sandwich boxes in incubators at 12, 15, 20 and 22º C and a 16:8 light:dark cycle. There were two boxes with 20 slugs each per temperature. Slugs were fed with Chinese cabbage and carrots and provided with a piece of cuttle fish bone. The boxes were cleaned once a week and slug mortality and egg production were recorded. The slugs were weighed every 14 days. The experiment continued until all slugs were dead.

Population dynamics of D. panormitanum in the field
Thirty slugs were collected from the field (clover patch at the University of Newcastle Close House field station near Heddon-on-the–wall, Northumberland) each month. Wooden boards, approximately 40x40 cm were used as "traps", using the slugs' tendency to seek shelter under material at the end of the night. Slugs were weighed and then kept separately in Petri dishes in a shaded area of an unheated greenhouse (long-term temperature range 4-40º C). Slugs were fed with Chinese cabbage and carrots and provided with a piece of cuttle fish bone. The Petri dishes were cleaned once a week and slug mortality and egg production were recorded for a period of two weeks. This experiment started in November 2001 and will run until February 2003.

Population dynamics of D. panormitanum in the glasshouse
One hundred and fifty slugs were collected from the field at the University of Newcastle field station. The slugs were weighed and then transferred into plant propagators (40x60x20 cm), 30 slugs each in five propagators. The propagators were filled with approximately 1cm of coarse sand, covered with Mypex matting and contained four Chinese cabbage plants each. The Chinese cabbage plants were replaced twice a month. Each month six slugs were randomly sampled from each of the five propagators, weighed and then transferred into Petri dishes and treated as described above under "population dynamics in the field". After 14 days all surviving slugs and all the eggs laid during this period were returned to their respective propagator.

One set of five propagators was started in late October 2001 and kept in a polythene tunnel at the University field station until January 2002, when two of the five propagator populations were lost due to severe frost and the remaining three propagators were transferred to an unheated glasshouse. A second set of five propagators was set up in late February 2002 and was kept in the glasshouse all of the time. This experiment will continue until March 2003.

Feeding studies with D. panormitanum
Slugs weighing 150-300mg were collected in the field and then kept separately in 650ml transparent sandwich boxes in incubators at 15^{o} C and a 16:8 light:dark cycle. They were fed with one of the following food sources:

- Chinese cabbage
- *Choisya ternata*
- *Hosta*, variety "August Moon"
- A suspension of algae, collected from a local nursery and kept on an algal growth medium

Plants were offered as whole leaves, which were kept fresh by keeping their base immersed in water in 5ml glass tubes. The boxes were cleaned once a week, and plant damage, slug mortality and egg production were recorded. The slugs were weighed every 14 days. The experiment was continued until all slugs were dead.

Diurnal behaviour of D. panormitanum and O. pfeifferi

Slugs were collected in the field and acclimatised to the conditions in the laboratory for two weeks. Snails were reared in the lab under a 16:8 light:dark cycle. The diurnal behaviour of the snails and slugs was identified using low-light, time-lapse video.

One slug or snail was placed in each of 20 replicate, 16 cm-diameter white plastic containers containing damp compost. Dishes contained a 9cm^2 piece of Chinese cabbage leaf and a 9 cm^2 shelter, made of '2H' wool-based black horticultural matting (see Fig. 1). The compost was kept damp at all times. Animals were kept in the arena 24h prior to the start of the video to allow them to explore the new environment and were then recorded for 24 hours. Individuals of *D. panormitanum* weighed between 300 and 400 mg and those of *O. pfeifferi* weighed between 100 and 160 mg. The arenas were filmed at 15^o C and a 16:8 light:dark cycle. The video was then digitised and analysed using the software package EthoVision ®.

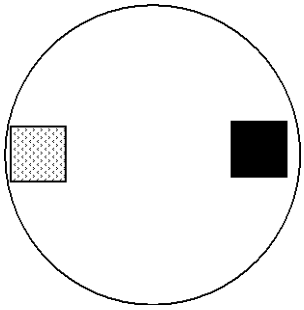


Fig.1. Design of the arena for the video experiments on diurnal behaviour (dotted square is Chinese cabbage leaf portion and black square is matting provided as shelter).

Results and Discussion

Biology of O. pfeifferi

O. pfeifferi hibernation period - study on a commercial HNS nursery

In early August 2001, high numbers of large *O. pfeifferi* were observed on the plants, compost, sides of pots, gravel floor and on algae growing in and alongside the concrete drainage channels in the tunnels. At the next visit in September, when the monitoring of the hibernation period began, none of the large snails were visible, but many very small snails (1-4 mm long) were present. It is likely that the large snails seen in August had died after laying eggs, and the small snails seen in September were the next generation of juveniles. Some of these juvenile snails began to move onto the structure of the polythene tunnels during September and October. By mid-

November, all snails had settled into their hibernation positions on the structure, sides of the pots or on the plants. The snails clamped themselves down onto the chosen surface and sealed their operculums (shell openings) with a layer of mucus. Between September 2001 and mid-February 2002, a mean of 40% of the snails recorded in the sampled areas were found on the structure, 49% were found on the sides of the pots and 11% were found on the plants (see Fig.2). Most of the snails on the structure of the tunnels were found on the underside of the upper horizontal cross bar of the door frame and the mean length of these snails was 4-5 mm. Many of the snails overwintering on the sides of the pots were very small; it is possible that these snails were too small to migrate onto the tunnel structure. Numbers of snails on the structure and sides of pots decreased by approximately 50% between November and February. This decrease is likely to be due to natural mortalities over the winter, possibly due to minimum temperatures dropping to -4ºC in January and sub-zero temperatures occurring on 18 dates between December and February.

On the February assessment, most snails were still inactive, but a few had begun to move around on the damp compost and pots. It was not possible to record snail positions and activity in the assessment tunnels in March, as two of the tunnels had blown down in a severe gale and all the plants had been trimmed and moved in the third tunnel. However, observations were made in late March in another adjacent tunnel and all snails were seen to be actively moving on the wet compost and on the sides of the pots, and feeding had commenced as the mean body length had increased to 7.6 mm. It is thought that surface moisture is one factor stimulating activity in the spring, as a sample of snails remaining on the pot sides and door structure in April became active when removed and placed onto wet tissue paper.

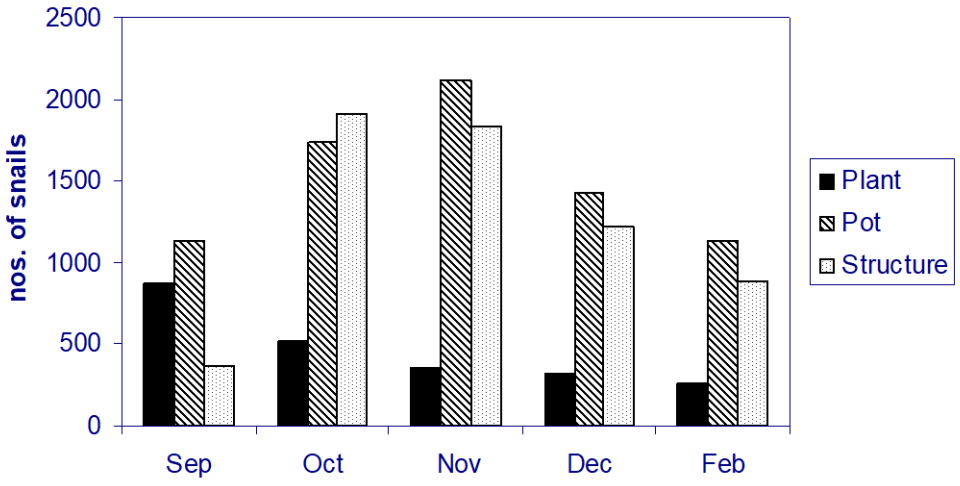


Fig.2. Total numbers of *O. pfeifferi* recorded on 30 plants and pots and the door frames in three polythene tunnels on a commercial HNS nursery between September 2001 and February 2002.

O. pfeifferi hibernation period - study in mini-tunnels at ADAS Boxworth
As at the commercial site, most of the snails had settled into their hibernation positions by November 2001. Although some snails spent the winter on the wooden structure of the mini-tunnels, far fewer (a mean of 11%) chose this position to overwinter than the snails at the commercial site (see Fig.3), possibly due to the smaller available area of structure in the mini-tunnels. However, the numbers overwintering in different positions at the two sites should only be used as a guideline and cannot be compared quantitatively, as only a sample of the total area was assessed at the commercial site, whereas all the area and pots were assessed at Boxworth. Similar proportions (50%) were found on the sides of the pots between October and February as at the commercial site during the same period. A higher proportion (43%) were found on the plants between October and February than at the commercial site.

Although the snails seemed to favour the Hostas during October and November, after the Hostas had senesced at the end of November, most of the snails overwintered on the Choisyas rather than the Phormiums (see Fig.4). The Choisya foliage was denser than either the Phormiums at Boxworth or the Hederas at the commercial site, and its plant architecture may have provided a more protected hibernation site for the snails than the other plant species. The mean body length of the snails overwintering in the mini-tunnels (6-7 mm) was higher than on the commercial nursery, possibly due to the extra protection and thus higher mean temperatures allowing a higher proportion of time spent feeding before inactivity commenced. Although there were approximately 20% decreases in numbers of snails recorded on the pots and structure between November and February, snail mortalities over the winter were not as high as at the commercial site. The factors affecting snail mortality are not fully understood, but both temperature and moisture levels are likely to be involved. Although maximum temperatures in the mini-tunnels were at least 5ºC higher in each month than at the commercial site, minimum temperatures were similar, with sub-zero temperatures occurring on 16 dates between November and February.

The positions of marked snails used to indicate activity showed that although very few snails were active during January, some were still active during December and activity began to resume in February. As at the commercial site, by late March most of the snails were active and by early April the mean body length had increased to 8 mm. At both sites, distribution of the snails was related to surface moisture, with most active snails being found on the damp compost or substrate, whereas no snails

were found on dry compost. Some snails in the mini-tunnels were found on the pots covered with liverwort, moss or algae, but these pots did not seem to be preferred to than those planted with the HNS species.

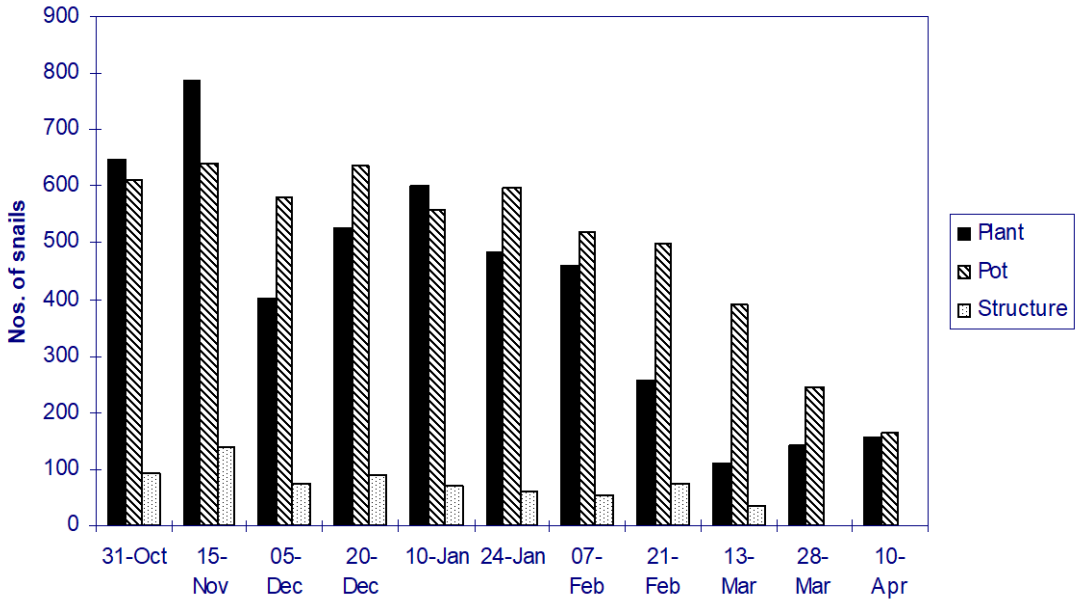


Fig.3. Total numbers of *O. pfeifferi* recorded on 24 plants and pots and the wooden structure of three mini-tunnels at ADAS Boxworth between October 2001 and April 2002.

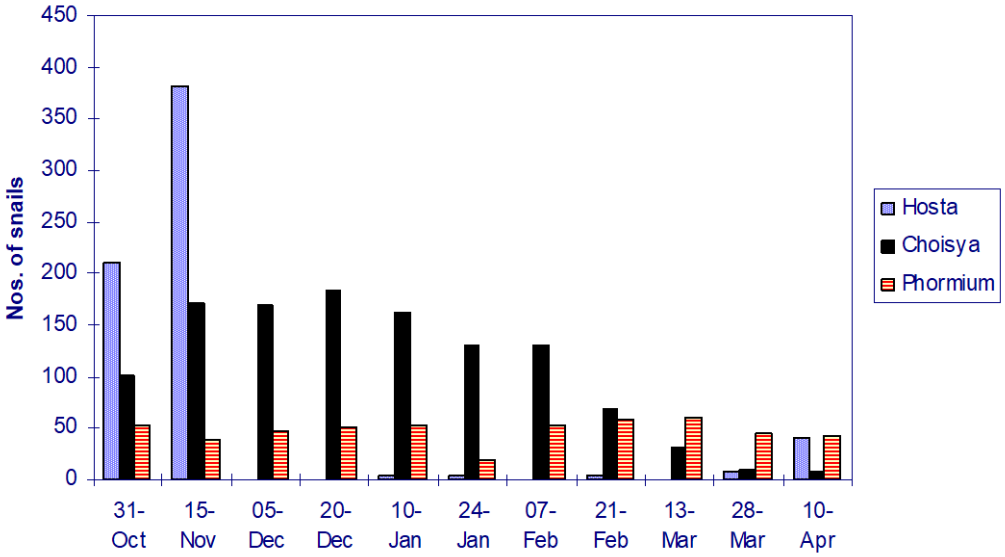


Fig.4. Numbers of snails on Hosta, Choisya and Phormium between October 2001 and April 2002 in mini-tunnels at ADAS Boxworth.

O. pfeifferi hibernation period - study at University of Newcastle field station
The survival rate of the snails over the winter was 75%. Most snails hibernated on the plant (50%), others on the cage structure (44%) or on the pot (6%). Snails were active as late as early December (see Fig.5). However, damage on the Chinese cabbage leaves or the plant stopped in mid-October. Snails started moving around by the end of March, a month later than when first activity was recorded at the other two monitoring sites, and damage occurred shortly afterwards. The maximum temperature in the cage was 37°C, the minimum temperature was –6.1°C. In 27 nights between 10 December and 13 March the temperature dropped below 0 °C.

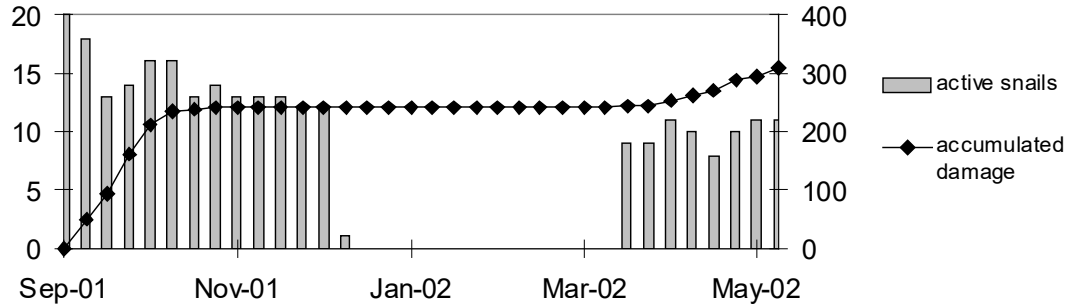


Fig.5. Small-scale hibernation experiment in Northumberland

Activity patterns are likely to depend both on temperature and daylength. As this experiment was carried out in Northumberland, activity and occurrence of damage may be expected to stop later in autumn and to start earlier in spring further south and in glasshouses and polythene tunnels with less heat loss than the one used in this experiment. The two periods in spring and autumn when the snails did not feed on the Chinese cabbage (and thus may represent periods when the snails do not feed on higher plants but move actively around) should be considered when planning the optimal timing of control measures.

Seasonal life cycle and breeding periods of O. pfeifferi
The snails collected from the commercial nursery in October 2001 did not lay any eggs over the next four months and by early February 90% of these snails had died. It was concluded that *O. pfeifferi* do not breed over the winter period when left in similar environmental conditions to those on commercial nurseries. The snails collected on 21 March began to lay eggs within one week and continued to lay eggs during April and May. Many of the eggs were laid under the pieces of liverwort. Some egg batches did not hatch and it was observed that they were highly susceptible

to desiccation. Eggs took between a few days to three weeks to hatch, with most hatching in approximately two weeks. Not all juvenile snails survived the study period, possibly due to high maximum temperatures during April and May reaching 39 and 35ºC respectively . Juveniles surviving in late May when the study was finished had reached 1.5-2mm in length. Minimum and maximum temperatures in the mini-tunnel during the study period between late March and late May were 1 and 39ºC respectively. The study showed that *O. pfeifferi* laid eggs continuously throughout the 2-month study period, leading to a continuous hatching period and a mixed age structure of snails.

Observations on snail sizes during the year at the commercial site confirmed that between late March /April and late August, the snails seemed to be actively breeding and were of mixed size and age, so that overlapping generations were present at any one time. Damp or wet conditions seemed to encourage egg-laying or hatch, with many small snails often being observed on pots adjacent to drainage channels in the commercial tunnels.

Development of O. pfeifferi at different temperatures
Some parameters of the development of lab-reared *O. pfeifferi* are summarized in Table 1.

Table 1. Summary of development parameters of *O. pfeifferi*

Parameter	**Temperature**	
	20º C	**22º C**
Egg development [days]	11.6	10.6
Hatch rate [%]	86	82
Mean weight week 4 [mg]	5	6
Mean weight week 14 [mg]	64	73
Mean weight week 30 [mg]	188	177
Mortality rate [%] week 4	86	96
Mortality rate [%] week 14	98	98
Mortality rate [%] week 30	99.3	98.9
Onset egg production [week]	14	14
Min. life cycle [weeks][1]	16	16

[1] life cycle = egg development + time until onset of egg production of next generation

It was not possible to breed *O. pfeifferi* at temperatures of 15°C or below (100% mortality of juvenile snails occurred within a few weeks). Even at higher temperatures mortality rates were very high in the first four weeks. Once the snails had reached 50-75 mg (7-8mm long and 10-16 weeks old respectively), mortality at 20 and 22°C was much lower and at the age of four months there was hardly any mortality. Growth rates at 20 and 22°C were very similar (see Fig. 6 and Table 1).

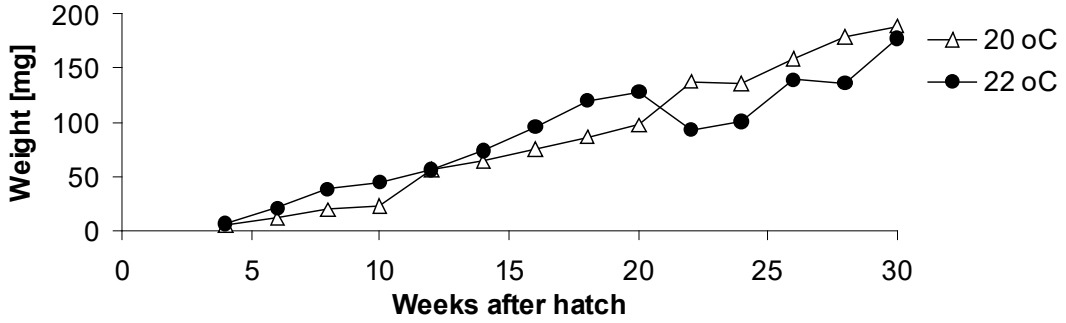


Fig.6. Weight of laboratory-bred *O. pfeifferi* over 30 weeks

It is likely that the results from controlled, constant conditions in the laboratory do not or only partly represent what occurs in the field. Unfortunately the conditions and food sources provided for the snails were in many cases inappropriate to sustain snail development (see results of feeding experiment).

Feeding studies with O. pfeifferi

No snail damage was recorded on the Choisya leaves and all 20 snails had died within one week. Several other attempts had been made prior to this experiment, to identify whether *O. pfeifferi* feed on Choisya leaves and the same result was given. It was concluded that although the snails are often found on Choisya leaves or the compost/sides of pots containing Choisya, they are not the cause of the damage commonly found on this plant species. It is possible that on Choisya the snails are feeding on leaves damaged by slugs, or on decaying leaves/algae on the surface of the compost, and as most slugs hide during the day, the true culprits are often missed by growers. Although only Choisya was fully tested as a perennial host plant, *O. pfeifferi* may not feed on some other thick-leaved perennials; on the commercial site used for the hibernation studies, no damage was seen on the Hedera plants despite large numbers of snails being present. At this site, *O.pfeifferi* were often found in groups on dead or decaying leaves and it is likely that they were using these as a food source.

The snails fed on the Hosta leaves but only surface grazing to the leaves and stems was recorded over the 4-wk period. The majority of snails did not commence feeding on the Hosta until two to three weeks after the experiment was set up (see Fig.7), indicating that as for the slugs, this variety was not a favoured food source. After two and three weeks 20 and 65% snails respectively had died. Observations on *O. pfeifferi* feeding damage to a range of other herbaceous plants are currently underway. Damage similar to that caused by slugs i.e. leaf holes and shredding has been observed on Viola (see Appendix) and the full results will be given in the final report.

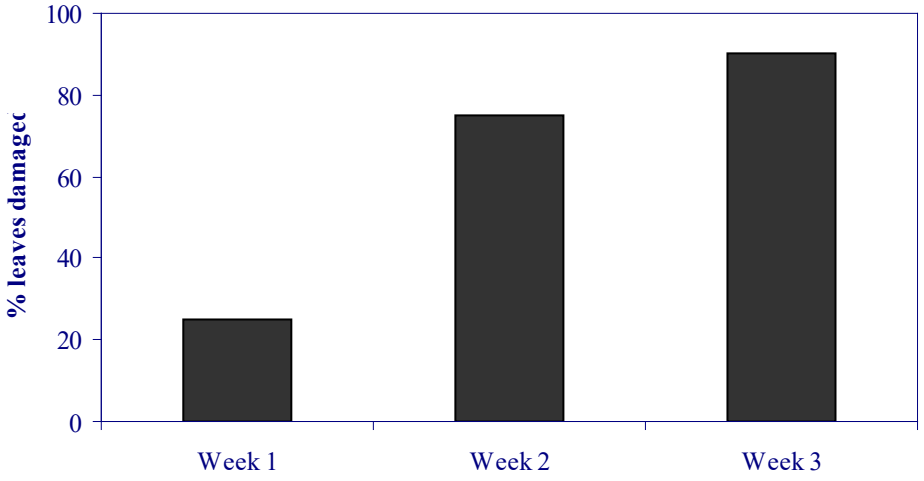


Fig.7. Percentage Hosta var. 'August Moon' leaves damaged by *O. pfeifferi* over a 3-wk period.

When offered either Chinese cabbage, algae on compost or algae on gravel as food sources, the snails grew faster and survived longer on the Chinese cabbage than on the algae. After a 4-wk period, 90% of the snails fed on Chinese cabbage were still alive and the mean body length had increased by 1.3mm i.e. by 19%. Most of the snails were 'attached' after three weeks when offered only algae on either compost or gravel as food, and mean body length had increased only marginally. It was concluded that although the snails can survive for two to three weeks on algae, they need an additional source of nutrients to thrive and complete their development. *O. pfeifferi* were often observed on decaying leaves left on the surface of the compost or substrate after trimming on the commercial monitoring site. An experiment is currently underway to identify whether snails of a similar size used in the study above can complete their development when offered both algae and decaying leaves. The results of this experiment will be given in the final project report.

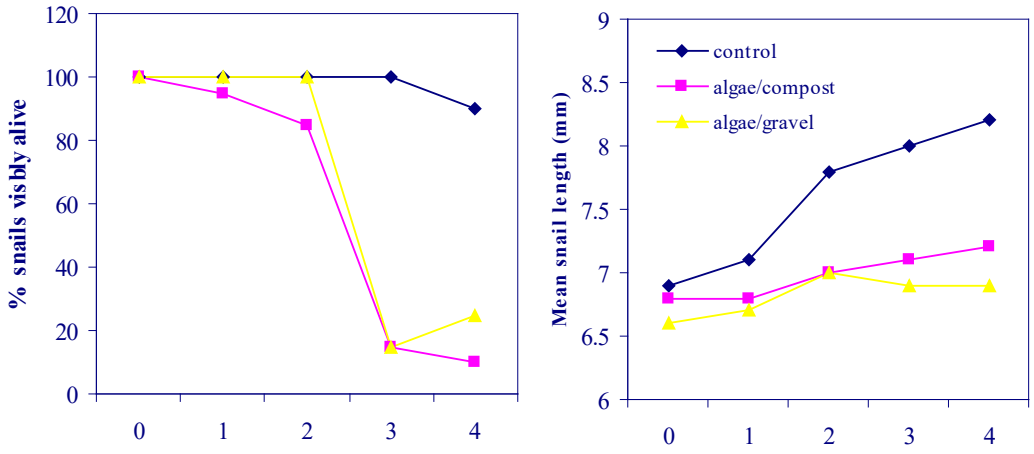


Fig.8 . Mean % live *O. pfeifferi* and mean snail length (mm) over a 4-wk period when offered Chinese cabbage (control), algae on compost or algae on gravel as a food source.

Biology of D. panormitanum

Development of D. panormitanum at different temperatures

The data presented here is preliminary, as the work is still continuing. Some parameters of the development of laboratory-reared *D. panormitanum* are summarized in Table 2.

Table 2. Summary of development parameters of *D. panormitanum*

Parameter	**Temperature**		
	12º C	**15º C**	**20º C**
Egg development [d]	32	22	15
Hatch rate [%]	82	88	87
Mean growth rate [mg/day] [1]	0.43	0.45	1.0
Max. individual weight week 14 [mg][2]	156	310	443
Mean weight week 14 [mg]	42	45	98
Max. individual weight week 16-30 [mg]	197	896	x
Mean weight week 30 [mg]	93	165	x
Max individual weight @ onset egg production [mg]	x	393	289
Mean weight @ onset egg production [mg]	x	57	62
Mortality rate [%][1]	56	61	66
Min. life cycle [weeks][3]	x	40	12

[1] based on week 1-14
[2] the heaviest animals found in the field are 350-400 mg only
[3] life cycle = egg development + time until onset of egg production of next generation

Based on the egg development time at 12, 15 and 20° C, the threshold for egg development can be extrapolated as 5.5 – 6° C (with approximately 210 day degrees needed for full development). This suggests that eggs laid in late autumn could survive in frost-free areas (e.g. in pots), being locked in a temperature-induced winter rest period (similar to the diapause of insects) and could then continue development very early in spring.

Mortality of laboratory-reared juvenile slugs was generally high during the first four to six weeks (Fig. 9. and Table 2). With the larger juveniles i.e after week 16 in the experiment, mortality was very low at the lowest temperature studied (12°C). However, with the data available at present there does not seem to be a clear trend for mortality rates in relation to temperature, e.g. higher mortality rates at higher temperatures.

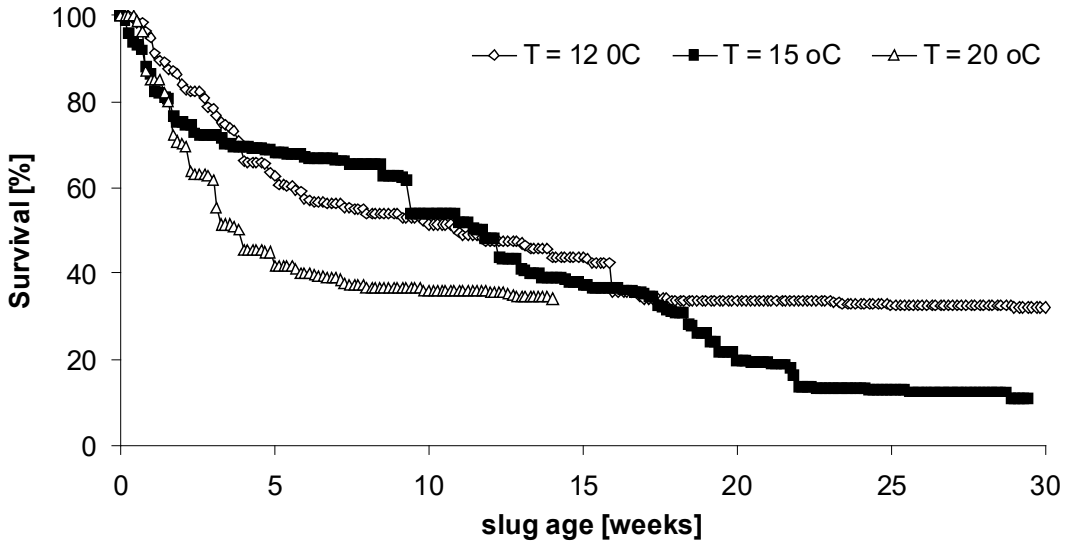


Fig. 9. Survival of *D. panormitanum* juveniles at different temperatures.

Slug growth was very slow in the first four weeks. Thereafter growth was more or less linear, with weight at 12°C levelling out at approximately 100mg after week 24 (see Fig. 10). Weight at 15° C was similar to that at 12°C , except that it continued to increase until the end of the experiment. Weight varied significantly between and within batches of slugs at the same temperature.

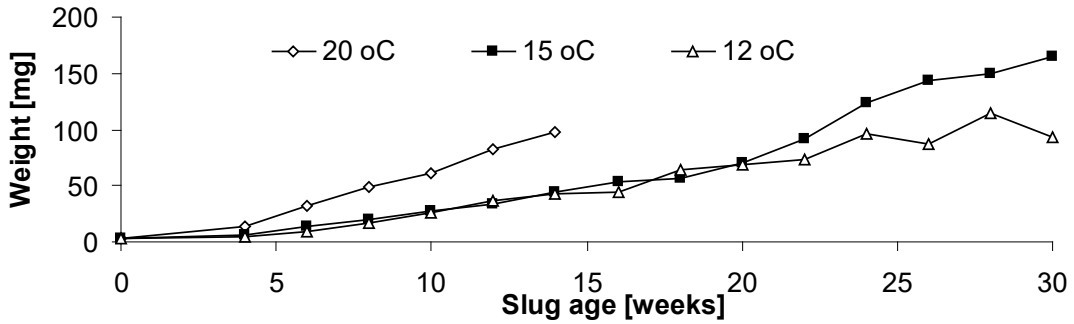


Fig.10. Growth of *D. panormitanum* juveniles at different temperatures.

Growth seemed to be substantially faster at 20ºC. Better performance at this high mean temperature is also represented through an early onset of egg production just before week 10 (Fig.11 and Table 2). At 15ºC only a very few eggs were laid and onset of egg production took until week 18. No eggs were laid at 12 ºC.

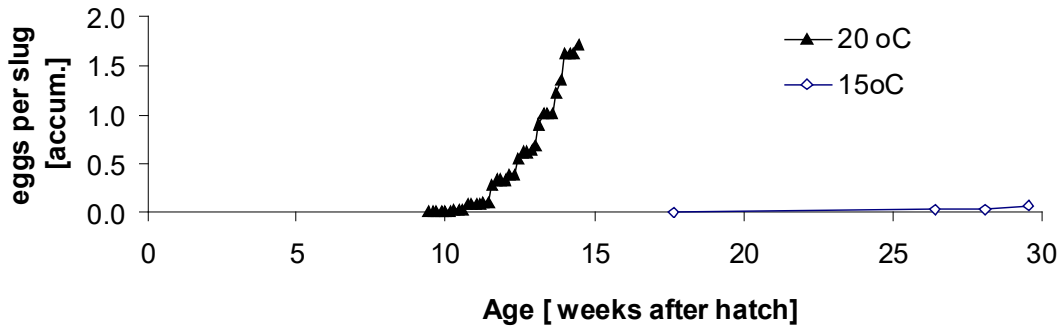


Fig.11. Egg production of young *D. panormitanum* adults (data shows eggs per original number of slugs, i.e. hatched slugs)

Based on the sexual maturation time (onset of egg production) at 15 and 20º C, the threshold for sexual maturation could be extrapolated to be as high as approximately 12.9º C (with approximately 600 day degrees needed for full sexual development). This would explain why slugs reared at 12º C did not lay any eggs, even though some individuals reached weights of nearly 200mg. However, sexual maturity and onset of egg production is probably linked to both individual body weight and temperature. The full mechanism might become clearer once this experiment is completed and results can be compared with data from field-collected and glasshouse slug populations (see following sections).

Especially in protected situations , i.e. polythene tunnels or glasshouses, favourable conditions will prevail for most of the year, with possibly two or even three generations developing each year, allowing a steep increase in population size and infestation pressure. Both slug survival rates and growth performance at a mean

temperature of 20° C were high, which indicates that *D. panormitanum* is well-adapted to the high temperatures present in glasshouses and polythene tunnels.

Performance of field collected D. panormitanum at different temperatures
Survival rates of slugs were similar at 15 -22°C (Fig.12). The slugs lived significantly longer in the laboratory at 12° C than at the other temperatures. It is likely that conditions in the laboratory are not ideal (e.g. available food resources, space, cross-infection) and that slugs would have lived longer at the same temperatures in the field.

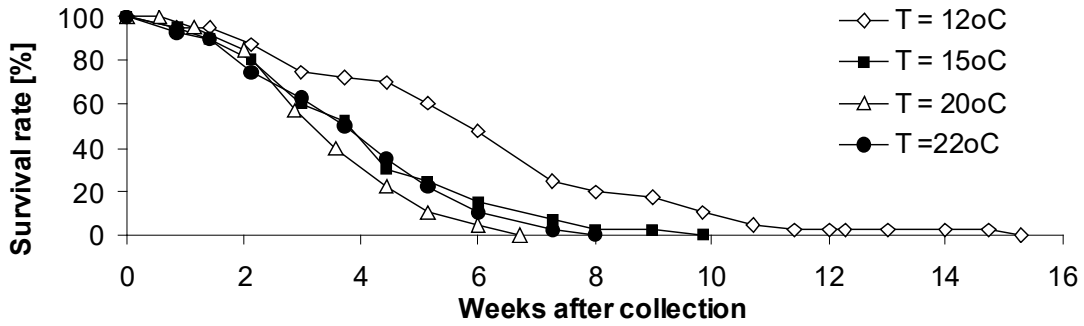


Fig.12. Survival of field-collected *D. panormitanum* in the laboratory

At all temperatures the slugs gained a lot of weight in the first two weeks (Fig.13). At higher temperatures, slug weight dropped either immediately or two weeks after the first two weeks. The slugs were able to maintain the high weight for a period of several weeks only at 12° C.

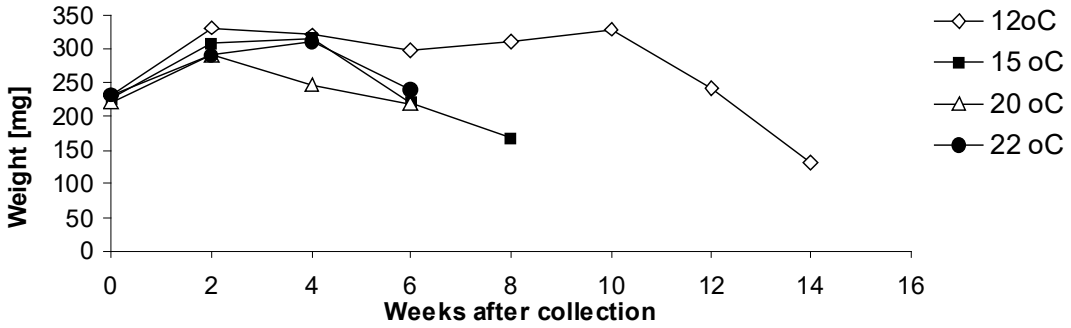


Fig.13. Weight change of field-collected *D. panormitanum* in the laboratory (curves end when all slugs are dead)

After field collection, it took the slugs at least one week before they started laying eggs (Fig.14). At 15, 20 and 22°C egg production grew logistically, peaking after eight, six and three weeks respectively. Egg production at 12° C had a late onset, was very low and increased in a more linear function.

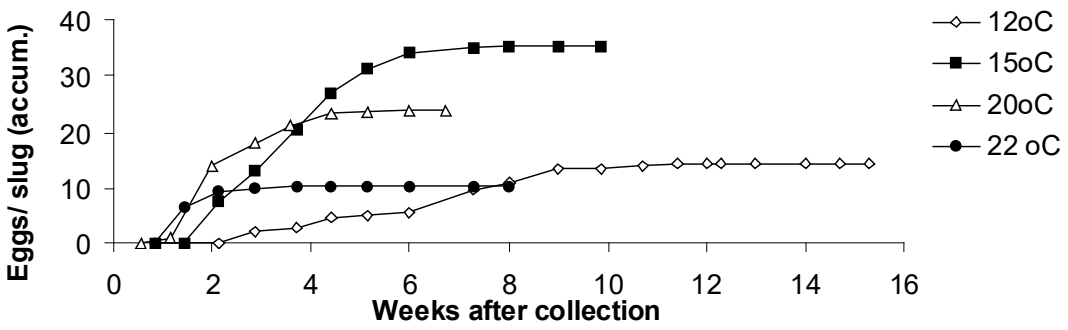


Fig.14. Accumulated egg production of field-collected *D. panormitanum* in the laboratory (curves end when all slugs are dead)

Population dynamics of D. panormitanum in the field

The size-class distribution of field-collected slugs did not vary much over time. During any month the majority of slugs weighed 150- 300mg (Fig.15). Size classes were slightly shifted towards heavier individuals at the end of winter. With the onset of egg production in the field, slightly more small adults/ large juveniles were caught in the traps.

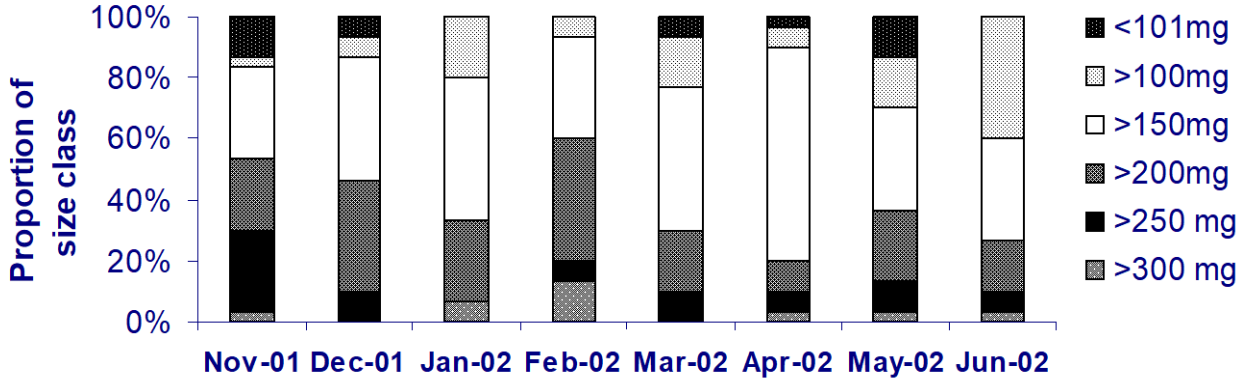


Fig. 15. Size class distribution of field-collected *D. panormitanum*

No egg production was observed during late autumn and winter (Fig.16). Egg production started in March and peaked in May, with a mean of six eggs per slug per week.

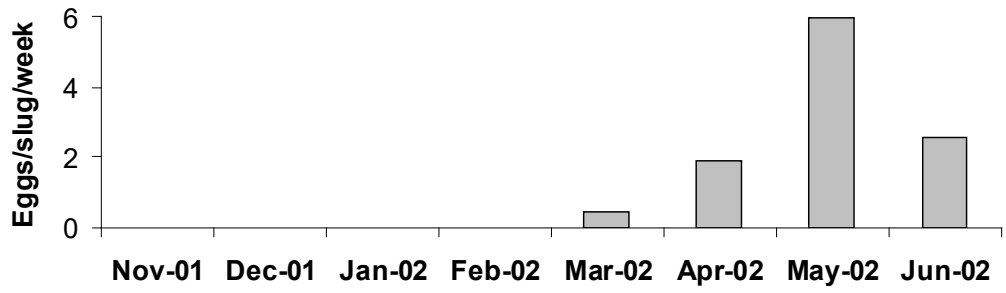


Fig.16. Egg production of field-collected *D. panormitanum* after transfer into an unheated glasshouse.

Population dynamics of D. panormitanum in the glasshouse

The plant propagator populations continuously shifted towards heavier size classes from the beginning of the experiment in October 2001 until March 2002 (Fig.17.). After sampling in March 2002 all individuals from the original population must have died, because only juveniles and adults of the second generation (distinguishable by their paler colour due to diet) were found. In March the size class distribution shifted dramatically towards the two smallest size classes (slugs below 100mg). During spring these small juveniles increased in weight and the population structure shifted towards the heavier size classes again.

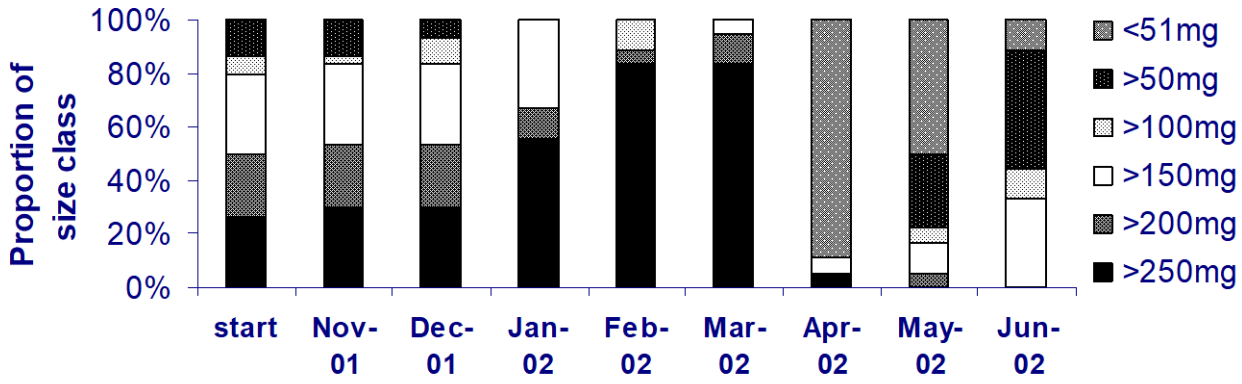


Fig.17. Size class distribution of *D. panormitanum* plant propagator populations

It is likely that in the field or glasshouse, slugs will manage to avoid too hot conditions during the day, but will benefit from mild night temperatures in the summer.

The data on population dynamics is indicating the following trends:

- At/below 12° C longevity is much higher, but does not allow sexual maturation and only little egg production of already mature slugs (see temperature threshold for sexual maturation above)
- *D. panormitanum* is adapted to average temperatures as high as 20 °C

Feeding studies with D. panormitanum

For all food sources the egg production peaked in week 2 (Fig.18). When feeding on Chinese cabbage the egg production in the first three weeks was higher than with all other food sources and also higher than at 15°C in the study on "performance of field-collected *D. panormitanum*".

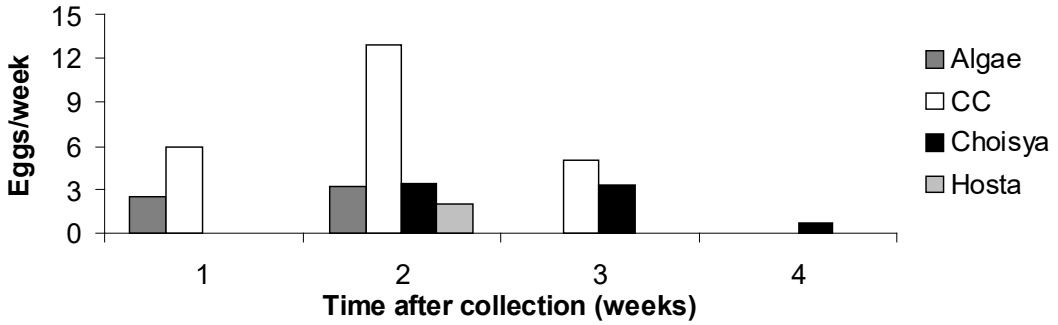


Fig.18. Egg production of *D. panormitanum* during feeding trial

Only Chinese Cabbage supported an increase in body weight (Fig.19). With algae and Choisya leaves slugs were able to maintain their body weight for one week, then their weight started to drop as recorded for Hosta from the very start of the experiment.

Some of the slugs fed on the damp paper tissue and blue faeces (the colour of the paper) were found. As with the snails, the damage on Hosta var. "August Moon" was very low, indicating that this variety is not very susceptible to slug or snail damage. Some examples of feeding damage to Choisysa and Hosta var. "elegans" leaves are shown in the Appendix.

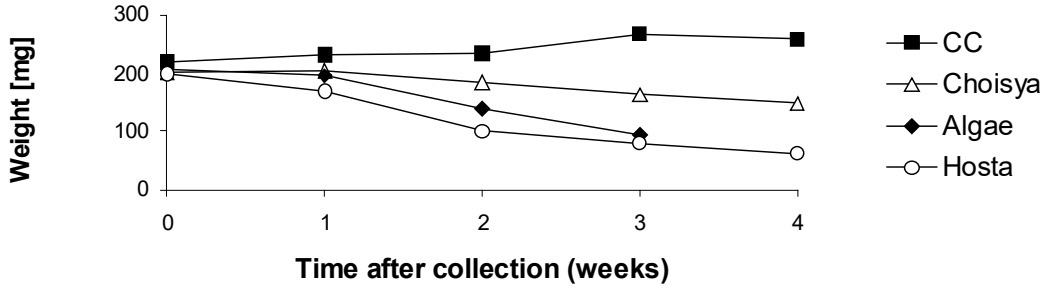


Fig. 19. Weight change of *D. panormitanum* during feeding experiment

The survival rate was highest on Choisya, followed by Chinese cabbage (Fig.20). With Hosta and algae the survival rate was much lower, and all slugs feeding on algae were dead after four weeks.

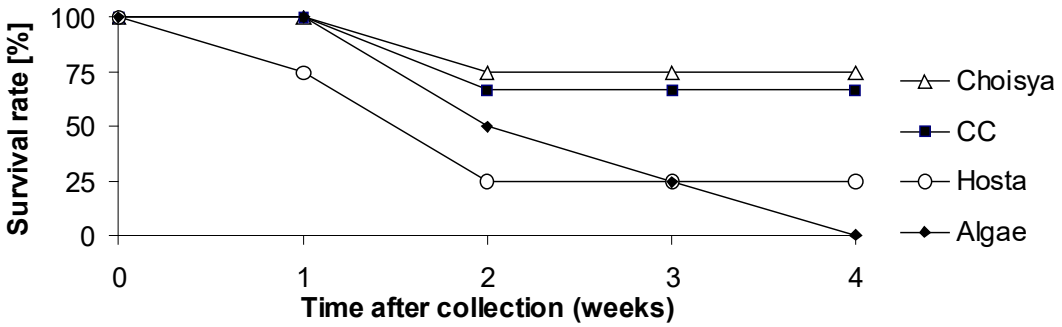


Fig.20. Survival rate of *D. panormitanum* during feeding trial

Diurnal behaviour of D. panormitanum and O. pfeifferi

The data presented here is preliminary, with 12 and eight replicates analysed to date, for slugs and snails respectively.

D. panormitanum

During the light period the slugs hid under the shelter or the leaf, for 66% of the time. (Fig. 21A). Slugs had an equal tendency to hide near the food source (under the leaf) or further away (under the matting). The slugs rested for 20% of the time on the damp compost when exposed to the light. They were active, either feeding or moving around, for 14% of the time.

During the dark period slugs were under shelter for only 50% of the time(Fig 21B). They spent a larger proportion of their time resting on the compost and were active for 24% of the time.

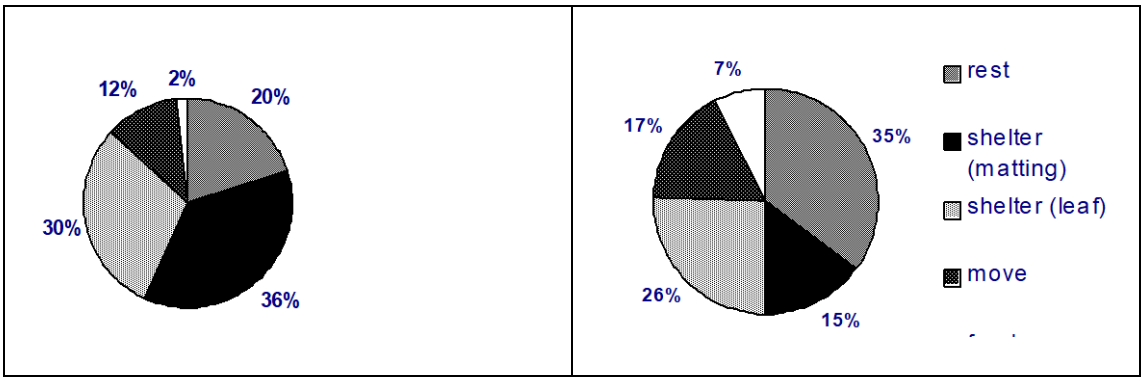


Fig.21. Activity pattern of *D. panormitanum* during light (left) and dark (right) period

Slugs are usually described as being nocturnal. Whilst laboratory results do not fully reflect the natural behaviour, this nocturnal activity pattern is confirmed by these experiments. Individual observations that slugs are sometimes active during the day if conditions are favourable (cool and moist), were also confirmed with the video experiments, and with observations on commercial nurseries during years 1 and 2.

O. pfeifferi

The activity pattern of the snails was quite different from that of the slugs. During the light period the snails were active for 22% of the time, i.e. similar to that of the slugs during the dark period (Fig.22A). However, most of the snail activity was spent moving around not actually feeding. The snails seemed to ignore the shelter made of horticultural matting. If seeking shelter it was under the leaf, but they remained exposed to the light for two thirds of their time.

During the dark period the snails were much less active (Fig.22B). Most of the time (94%) they were resting, either on the compost or under the shelter (with equal proportions of 47% in each position). As snails carry their shell with them, they may not be as dependent as slugs on finding shelter. Hiding under a leaf might be preferred to seeking other shelter, in order to stay close to the food source.

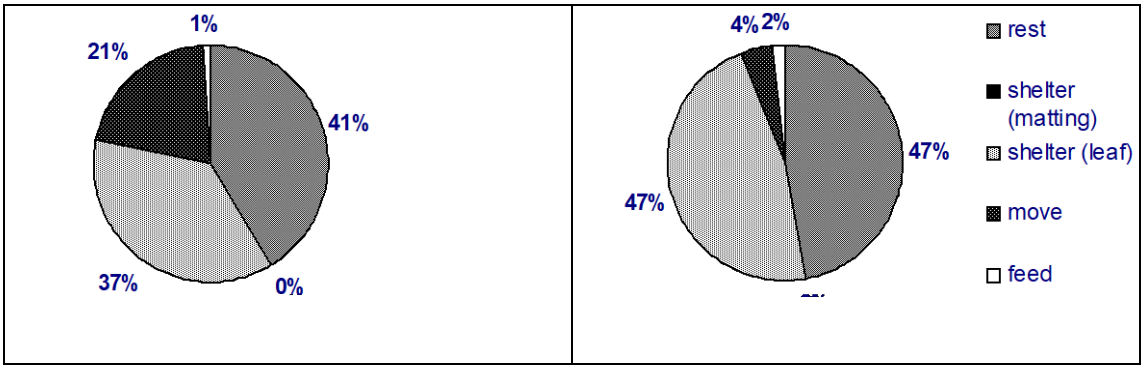


Fig.22. Activity pattern of *O. pfeifferi* during light (left) and dark (right) period

As slugs spend most of their time during the day hiding, it is difficult to monitor the level of infestation when sampling during day-time. However, the snails were exposed and visible for 63% of the time during the light period. Thus slug infestations may often be under-estimated in relation to snail infestations.

Conclusions

- *O. pfeifferi* hibernated between September/October and February/March on the sides of pots, on the structure of polythene tunnels and on plants. All hibernating snails were juveniles (less than 8 mm long). Over the winter period, 20-50% natural mortalities occurred. Snail activity began again during February and most were active by late March.
- *O. pfeifferi* laid eggs between late March and August, with mixed ages and overlapping generations occurring at any one time during this period. Damp or wet conditions stimulated activity and egg-laying and favoured survival. Most eggs took two weeks to hatch but development time was dependent on temperature. The life cycle from egg to egg was 14 weeks at 20-22ºC.
- *O. pfeifferi* fed on soft-leaved herbaceous plants including Hosta and Viola but did not feed on certain tougher-leaved perennial plants such as Choisya and Hedera. The snails also fed on algae, liverwort and decaying leaves but could not survive on a diet of algae alone. As the snails are active and visible during the day, they could be mistakenly held responsible for slug damage on some HNS species e.g. Choisya. However, even if not causing primary damage, if present in large numbers they may lead to quality problems as contaminants.
- The optimum times for control of *O. pfeifferi* could be late March/April and again in late August/early September, i.e. when snails are fully active, just after and just before the hibernation period. However, if using parasitic nematodes for control, minimum compost temperatures need to be above 5ºC and this will affect timing of application.

- *D. panormitanum* was active all year round, with peaks of activity occurring in spring and autumn. Large adult slugs of this species die in late winter / early spring.
- *D. panormitanum* egg production started in March and large numbers of juveniles were present in April. Under optimum conditions the life cycle can be as short as 10 weeks. Laboratory-reared young slugs showed a great variation in growth rates and onset of egg production, which indicates that generations will probably overlap.
- *D. panormitanum* survival, growth and egg-laying rates were high at 20ºC, and this species seems to be well-adapted to the high temperatures present in glasshouses and polythene tunnels. In protected HNS where favourable mild, damp conditions are present between spring and autumn, two or even three generations may develop each year, leading to heavy infestations. Outdoors, in very dry summers and cold winters, slug activity may be very low.
- *D. panormitanum* fed on Choysia and Hosta, but the damage on Hosta depended on variety, with more damage on var. 'Elegans' than on 'August Moon'. The slugs also fed on algae but survival rates on both Hosta and algae were low.
- *D. panormitanum* showed a nocturnal behaviour pattern typical of slugs, although in favourable conditions they can also show some activity during the day. The tendency for nocturnal activity means that the abundance of *D. panormitanum* can be underestimated by growers.
- The optimum times for control of *D. panormitanum* are likely to be similar to those for *O. pfeifferi* i.e. in spring (from late March onwards) and in early autumn (September and October).

OBJECTIVE 4: EVALUATION OF PARASITIC NEMATODES ('NEMASLUG') AGAINST THE PREDOMINANT SNAIL AND SLUG SPECIES

Materials and Methods

Efficacy bioassays

Further tests on the efficacy of the parasitic nematode *Phasmarhabditis hermaphrodita* ('Nemaslug'®) against both *D. panormitanum* and *O. pfeifferi* were done, using the same replicated 'semi-field' laboratory bioassays as used in year 1. The tests in year 1 with recommended and half-rates of nematodes against *D. panormitanum* were repeated, but using younger, summer-collected slugs in May/June 02. (Tests in year 1 during winter 01 gave inconsistent results due to high natural mortalities in older, winter-collected slugs). The tests in year 1 with *O. pfeifferi* were repeated, but using lower rates of nematodes.

Treatments - D. panormitanum

1. Water control
2. 'Nemaslug' at a double rate (600,000 nematodes per m^2)
3. 'Nemaslug' at the recommended rate (300,000 nematodes per m^2)
4. 'Nemaslug' at half-rate (150,000 nematodes per m^2)

Treatments – O. pfeifferi

1. Water control
2. 'Nemaslug' at the recommended rate (300,000 nematodes per m^2)
3. 'Nemaslug' at half-rate (150,000 nematodes per m^2)
4. 'Nemaslug' at quarter rate (75,000 nematodes per m^2)
5. 'Nemaslug at eighth rate (37,500 nematodes per m^2)

All treatments were applied in a water volume of 2 litres per m^2.

Bioassays

Replicate one-litre plant pots filled with a commercial compost suitable for HNS were used for each treatment. The base of each pot was lined with muslin to prevent potential escape of slugs or snails, or migration between pots. Compost was added to each pot to within four cm of the top. The pots were watered well and allowed to drain. Using a calibrated pipette, the required amount of nematode suspension was applied to the surface of the damp compost in the pots. The nematode suspension was agitated gently before and during pipetting to ensure that the nematodes did not settle out. A 4-cm^2 piece of Chinese cabbage leaf was treated on each side with the appropriate treatment and placed on the compost in the middle of each pot, as a food source for the slugs or snails. The square of Chinese cabbage leaf was replaced weekly. One slug or snail was added to each of ten replicate pots per treatment. Slugs weighing 110-260 mg (mean 170 mg) and adult snails measuring 1 cm long were used.

The individual pots were covered with muslin, overlaid with polythene pierced with five ventilation slits, and secured around the rim of the pot with an elastic band. The pot coverings acted as 'lids' to prevent slug or snail escape, and simulated the environmental conditions in a glasshouse or polythene tunnel. The pots for each treatment were placed on sheets of polythene overlaid with paper towel and incubated at 10°C, 12:12 hours light:dark for 3-5 weeks, until all the snails or slugs respectively were dead.

Assessments

After 24 hours and daily for six days, the position of the slugs or snails were recorded i.e. on the compost or leaf, or avoiding contact with the compost or leaf, i.e. on the side of the pot or on the muslin covering the pot. On each daily assessment, mortality and percentage leaf feeding were recorded. Following the 6-day period, the assessments were made weekly until all the slugs or snails were dead. The compost was kept moist by misting with water three times a week.

Statistical analysis

Percentage reduction in feeding compared with the controls, and percentage live slugs or snails in contact with the compost for the first six days, were analysed by ANOVA (analysis of variance). Percentage mortality of slugs or snails was analysed by GLIM (generalised linear model).

Time-lapse video experiments - irritant effect of nematode-treated compost

The behavioural response of the slugs and snails to treatment of compost with the standard recommended rate of 'Nemaslug' was studied using low-light, time-lapse video in a no-choice experiment. One slug or snail was placed in each of 16 replicate, 16 cm-diameter white plastic containers containing damp compost, onto which the nematodes were added in 30 ml of water, and slug or snail behaviour was recorded for one night (14 hours). Individuals of *D. panormitanum* weighed between 300 and 400 mg and those of *O. pfeifferi* weighed between 100 and 160 mg. The video was then digitised and analysed using the software package EthoVision ®. The temperature was 15ºC and the 14- hr period included eight hours darkness.

Time-lapse video experiments - repellent effect of nematode-treated compost

The same methods as in the no-choice experiments above were used in choice experiments. One half of each container was treated with a 15ml suspension of the 'Nemaslug' formulation, applying the recommended rate onto the surface and the other half remained as untreated compost. Only the 8-hr dark period was used for the analysis. These experiments were carried out to collect some quantitative information on the possible sub-lethal behavioural effects of the nematodes on the slugs and snails.

Results and Discussion

Efficacy bioassays

Effect of 'Nemaslug' on D. panormitanum mortality

All three rates of 'Nemaslug' significantly increased % slug mortality when compared with the controls, at three and four weeks after treatment. Mean % mortalities in pots treated with recommended, double and half-rates were 70, 70 and 80% respectively three weeks after treatment and 80, 90 and 80% respectively four weeks after treatment (see Table 3 and Fig. 23). There were no significant differences between the nematode treatments i.e. half-rate killed as many slugs as recommended or double rates. These high levels of mortality occurred sooner than in year 1 when older, winter-collected slugs were used, and were significantly different from those in control pots due to the lower natural mortalities in the controls than in year 1.

Table 3. Mean % *D. panormitanum* mortalities after treatment with three rates of 'Nemaslug'

Weeks after treatment	**% mortality Untreated control**	**% mortality Half-rate 'Nemaslug'**	**% mortality Rec. rate 'Nemaslug'**	**% mortality Double rate 'Nemaslug'**
1	0	0	0	0
2	30	40	30	20
3	30	80*	70*	70*
4	30	80*	80*	90*
5	50	90	80	100

* significantly different from controls, $P<0.05$

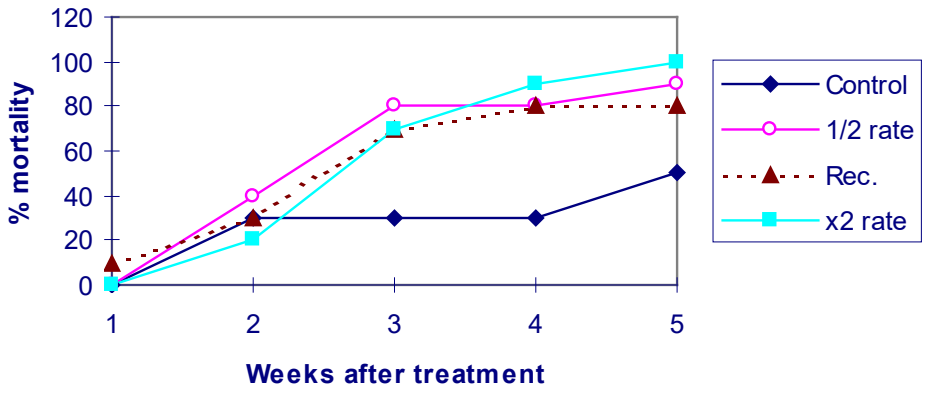


Fig.23. Mean % *D. panormitanum* mortalities after treatment with recommended, double and half-rates of 'Nemaslug'.

Effect of 'Nemaslug' on O. pfeifferi mortality

All rates of 'Nemaslug' killed 100% *O. pfeifferi* within three weeks of treatment and there were only low mortalities in the controls (see Table 4 and Fig.24). All the snails were killed within three weeks after treatment and there were no significant differences between the nematode rates. The results with recommended and half-rates were similar to those in year 1, when all the snails were killed within three and four weeks respectively. The laboratory results in year 2 indicate that rates as low as 1/8-rate kill all *O. pfeifferi* within three weeks of treatment.

Table 4. Mean % mortality in *O. pfeifferi* treated with three rates of 'Nemaslug'

Weeks after treatment	**Control**	**% mortality 1/8 rate 'Nemaslug'**	**% mortality 1/4 rate 'Nemaslug'**	**% mortality Rec. rate 'Nemaslug'**
1	0	0	0	10*
2	10	80*	100*	90*
3	20	100*	100*	100*

* significantly different from the controls, $P<0.05$

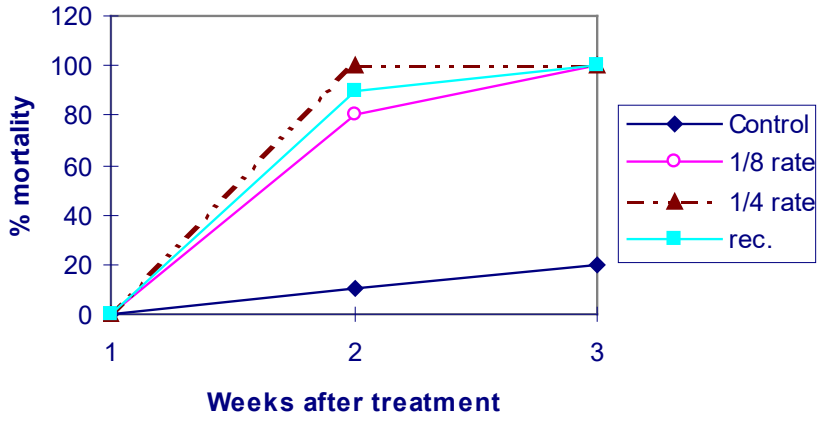


Fig.24. Mean % mortalities over three weeks in *O. pfeifferi* treated with recommended, 1/8 and 1/4 rates of 'Nemaslug'

Reduction in feeding by slugs and snails treated with 'Nemaslug'

Over the 5-wk period of the experiment, all rates of 'Nemaslug' significantly reduced mean % leaf feeding by the slugs when compared with the water control ($P<0.001$, see Fig. 25). The double rate of nematodes reduced mean % feeding by significantly more (70%) than the recommended and half-rates (45 and 43% respectively). The reductions in mean % leaf feeding by recommended and half-rates were very similar to those given in year 1, when 45 and 48% reductions were given respectively. No significant reduction in % leaf feeding by the snails were given by any of the nematode treatments, as the individual snails grazed only a very small amount of the surface of the Chinese cabbage leaf portions and this was very difficult to measure.

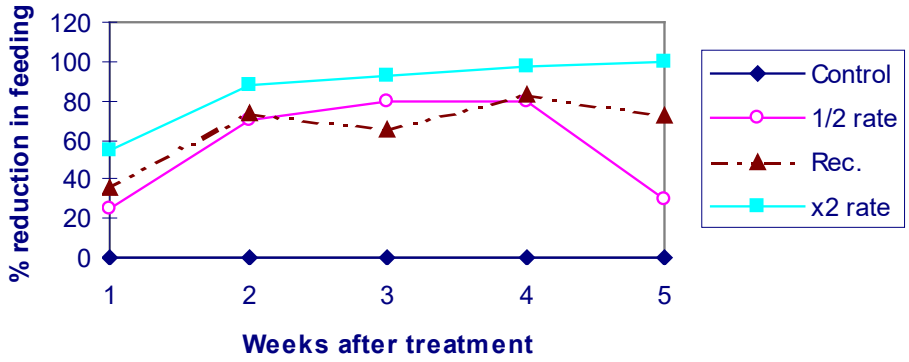


Fig.25. Mean % reduction in feeding by *D. panormitanum* over a 5-wk period after treatment with recommended, half and double rates of 'Nemaslug'

Repellent effect of 'Nemaslug' on the slugs and snails

In the 'Nemaslug' efficacy bioassays, all rates of nematodes i.e. recommended, double and half rates seemed to repel the slugs from remaining in contact with the treated compost or leaf, during the first week after treatment (see Table 4 and Fig.26). The mean % slugs remaining in contact with the compost or leaf treated with recommended, double and half rates were significantly lower (33, 17 and 38% respectively) than those treated with water (83%). In similar tests in year 1, mean % slugs remaining in contact with treated compost were significantly reduced by the recommended rate but not by the half-rate. This result is consistent with recent work in the Netherlands (de Werd *et al*., 2001) which was referred to in the year 1 report. The Dutch work reported that smaller, juvenile *Deroceras reticulatum* (the field slug, a related species to *D. panormitanum*) are more susceptible to the nematodes than larger slugs, with feeding reduction being greater with the juvenile slugs. From one week after treatment, increasing slug mortality made any continued repellent effects of the nematodes difficult to assess.

Table 4. Mean % live *D. panormitanum* remaining in contact with the compost during the 1-wk period after treatment with 'Nemaslug'.

Treatment	**mean % live slugs in contact with compost during 1 wk after treatment**
Control	83.3
Double-rate 'Nemaslug'	16.7***
Recommended rate 'Nemaslug'	32.5***
Half-rate 'Nemaslug'	38.3***

*** significantly different from control, P<0.001

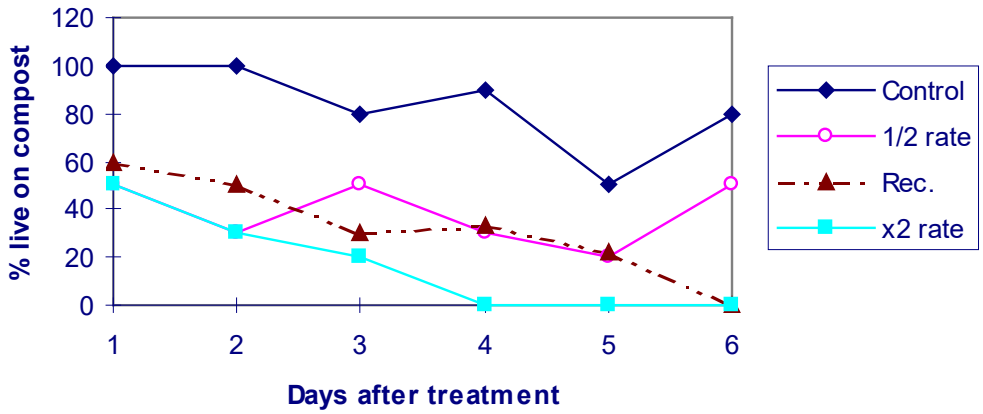


Fig.26. Mean % live *D. panormitanum* remaining in contact with compost during the 1-wk period after treatment with 'Nemaslug' .

None of the nematode rates significantly reduced the % snails remaining in contact with the compost during the first week after application. As in similar tests in year 1, all rates led to high snail mortalities from one week after treatment, so any repellent effect could not be assessed.

Time-lapse video experiments – irritant/repellent effects of 'Nemaslug' on slugs and snails

When given the choice between an untreated and nematode treated area, *D. panormitanum* significantly discriminated against the treated compost (N= 16, paired T-Test: $P < 0.01$, Fig. 27). The slugs spent 44% of their track on the treated surface. This result is consistent with that in the efficacy bioassays above, where the slugs avoided contact with compost treated with three rates of nematodes, for one week after treatment, i.e. a repellent effect was demonstrated.

As in the efficacy bioassays above, *O. pfeifferi* did not discriminate against the treated compost (N= 16, paired T-Test: $P > 0.05$, fig. 27). Although they spent relatively less time (41%) on the treated compost than *D. panormitanum,* due to a greater variation within the treatments this effect was not statistically significant. No mortality occurred during these short-term experiments.

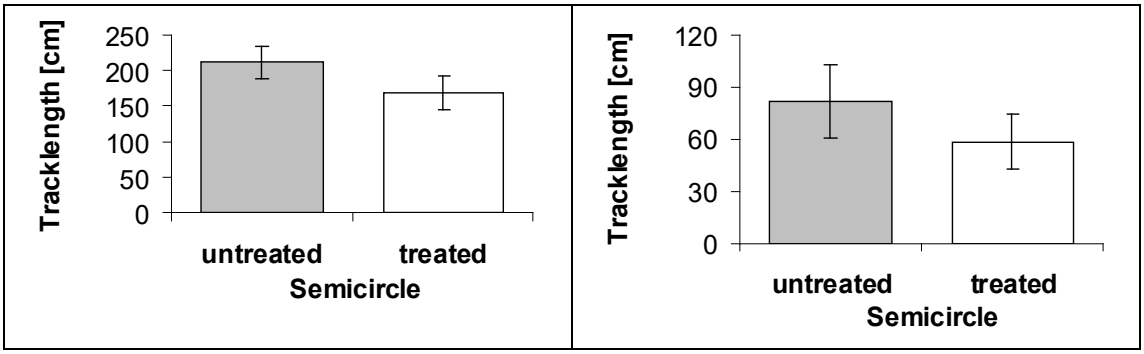


Fig. 27. Track length of *D. panormitanum* (left) and *O.pfeifferi* (right) during an 8-hr period with a choice between untreated compost and nematode-treated compost. Bars represent standard error.

In the no-choice time-lapse video experiments, the nematodes had no short-term effect on the activity of *D. panormitanum* (N=16, U-Test, P > 0.05, Fig. 28A). The track length over the 14-hr period was statistically similar. In the first four hours of the experiment, the slugs were more active on the nematode-treated surface, possibly due to them seeking escape from the nematodes.

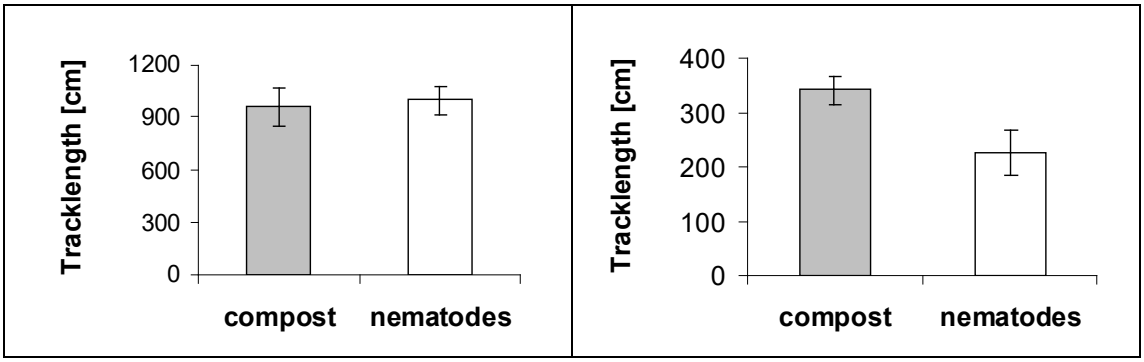


Fig. 28. Track length of *D. panormitanum* (left) and *O.pfeifferi* (right) during a 14-hr period on compost and nematode-treated surface (no choice given). Bars represent standard error.

In the no-choice test with *O. pfeifferi*, the nematodes had a significant effect on reducing the activity of the snails (N=16, T-Test, P < 0.01, Fig. 28). The snail track was 34 % shorter on the nematode-treated surface than on the untreated compost, possibly due to the snails clamping themselves down on the compost in an attempt to escape nematode invasion. This result suggested that the nematodes have an irritant effect on the snails, although this effect was very low in comparison with the novel molluscicides investigated in Objective 5. No mortality occurred during these short-term experiments.

Conclusions

- In laboratory tests, 'Nemaslug' at half-rate was as effective as recommended and double rates in killing *D. panormitanum* , with a mean of 80% mortality four weeks after treatment.
- In laboratory tests, 'Nemaslug at 1/8 rate was as effective as recommended or 1/4 rates in killing *O. pfeifferi*, with 100% mortality three weeks after treatment.
- In laboratory tests, half, recommended and double rates of 'Nemaslug' all significantly reduced mean % leaf feeding by *D. panormitanum* over a 5-wk period after treatment, with double rate giving significantly better reduction (70%) than recommended (45%) and half-rates (48%).
- In laboratory tests, half, recommended and double rates of 'Nemaslug' all significantly reduced the mean % *D. panormitanum* remaining in contact with treated compost during the first week after treatment, i.e. the nematodes had a repellent effect on the slugs.
- Time-lapse video recording confirmed that when given the choice, the slugs preferred untreated compost rather to that treated with 'Nemaslug'.
- Time-lapse video recording showed that activity of *O. pfeifferi* was significantly reduced on compost treated with 'Nemaslug' when no choice of untreated compost was given, ie. an irritant effect was demonstrated.

OBJECTIVE 5: EVALUATION OF BARRIER OR REPELLENT TECHNIQUES AGAINST THE PREDOMINANT SNAIL AND SLUG SPECIES

Barriers or repellents could prevent infestation of new plants brought onto the nursery and also reduce the abundance of resident snail and slug populations.

Materials and Methods

In year 1, the potential repellent, irritant, antifeedant or barrier effects of nine products were investigated against *D. panormitanum* and *O. pfeifferi*, using replicated laboratory bioassays. During year 2, an additional five products were tested, using similar bioassays to those in year 1, in conditions conducive to slug and snail activity i.e. 15˚C, high RH% and 16:8 Light:Dark.

Full list of Treatments

1. Compost (Control)

2. Snail-Ban'® (incinerated kaolin minerals)
3. Croptex Fungex'® (copper ammonium carbonate, 0.1 -0.625% solution)
4. Cinnamamide dispersion (0.1-1% w/w, with 0.1 -1% non-ionic surfactant)
5. Mulch (based on chipwood waste, old and new batches)
6. Garlic concentrate (2.5 and 5% dispersion)
7. Ureaformaldehyde (0.1 -10 % of resin, containing ca. 60-75 % ureaformaldehyde and ca. 1-3% formaldehyde)

Metal foils:

8. Aluminium
9. Copper (new and oxidised - "old")

Mattings:

10. Tex-R ® non-woven matting treated with 'Spin Out'® (copper impregnated)
11. Tex-R Landscape Pro ® (sandbed cover with a 'Spin Out' ® coating)
12. Supercover Plus ® (Mypex matting with a 'Spin Out' ® coating)
13. Mypex ® (both as a treatment and as a control versus Tex-R matting)
14. Geobond ® (sandbed cover)
15. Florimat 3 ® (sandbed cover)

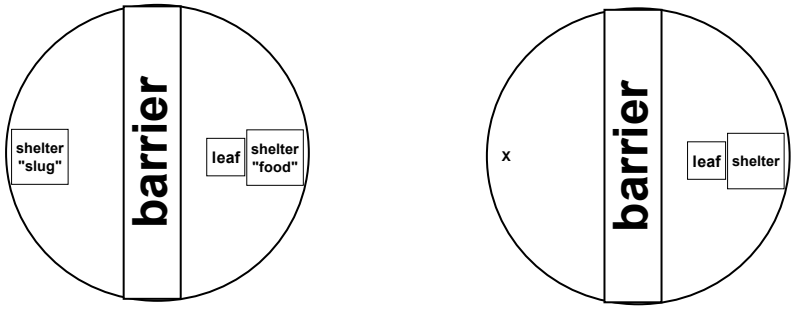
Products 6, 7 & 11-15 were added to the list of products after the preliminary tests in year 1.

Barriers against horizontal movement

The methods were the same as those used in year 1:

D. panormitanum were field-collected and *O. pfeifferi* were collected from glasshouses and polythene tunnels on HNS nurseries. Both species were starved for 48 hours prior to the experiments. Experiments were carried out using white circular plastic dishes 16 cm in diameter and filled with approximately 2 cm of damp compost. A 3 cm-wide strip in the middle of each dish was treated with one of the treatments (30 ml solution for liquid treatments, 1mm-thick foils or discs of aluminium, copper, Tex-R matting or Mypex, or approximately 1 cm-depth (mulch or Snail-Ban). Soluble products were applied at their maximum concentration (garlic 2.5 %). The treatment strip divided the dish into two semicircles (see Fig.29). A 4 cm^2 square of Chinese cabbage was offered in one semicircle and two slugs or one snail were placed in the other semicircle (snail availability limited the number used per experiment). Slugs weighing 150-400 mg and snails weighing 60-120 mg were used. Square pieces of cardboard (3x3 cm) were provided as shelters as shown in Fig. 5.1. There were 20 replicate dishes per treatment, for both *D. panormitanum* and *O.*

pfeifferi. Leaf damage (cm^2) and position of the slugs or snails were recorded every 24 hours for two and seven days respectively.



A B

Fig. 29. Experimental design for horizontal barrier experiments with (A) *D. panormitanum* and (B) *O. pfeifferi* (x = snail placed here). All components of the experimental arena are shown in accurate proportion to each other.

Barriers against horizontal movements (low concentrations)
This experiment evaluated the effect of cinnamamide, and ureaformaldehyde at concentrations between 0.1 and 1 % and for Croptex-Fungex at concentrations between 0.1 and 0.625 % (the current maximum concentration when applied as fungicide). If proved effective, these low concentrations would be more cost-effective than higher dose rates.

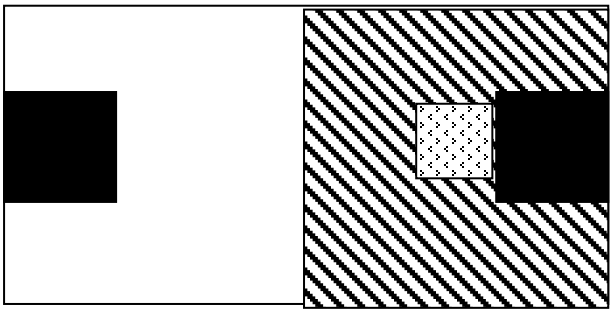


Fig.30. Experimental design of barrier experiment with low concentrations

Both species were starved for 24 hours prior to the experiments. Experiments were carried out using transparent 650 ml boxes (approx. 16x8x4 cm) and filled with approximately 1 cm of damp compost. One half of each box was treated with 10 ml of the solutions. Slugs weighing 150-300 mg and snails weighing 60-120 mg were used. Square pieces of cardboard (9x9 cm) were provided as shelters as shown in Fig.

30. There were 20 replicate dishes per treatment. Leaf damage (cm^2) was recorded every 24 hours for seven days.

Barriers against vertical movement

Preliminary data with nine products was presented in the year 1 report. In year 2, an additional treatment, ureaformaldehyde was tested and the results of the final analyses are presented in this report.

The experiment was carried out only with *D. panormitanum*, which has been observed to hide in the soil during the day. Slugs were starved for 24 hours prior to the start of experiment. One slug was buried in each of 20 individual transparent plastic beakers (300 ml, 6 cm diameter) under a 2 cm layer of damp compost (see fig. 31). The treatments were applied to the surface of the compost as a solid disc, 6 mm-deep layer of material or 8 ml solution respectively. The Tex-R matting was used with the treated surface uppermost. The position of slugs (if visible) and damage caused to a 4 cm^2 piece of Chinese cabbage leaf were recorded every 24 hours for seven days.

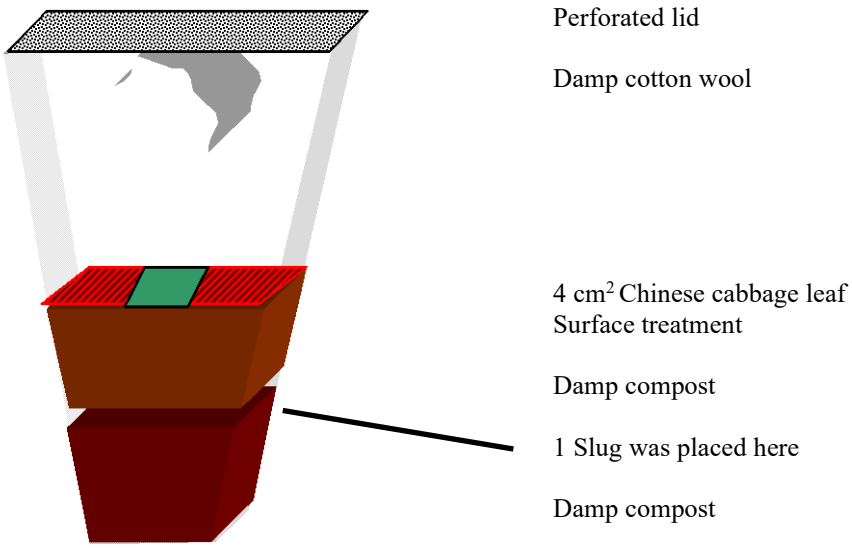


Fig.31. Experimental design of barrier experiment (vertical movement)

Behavioural response of slugs and snails (no-choice experiments)

The behavioural response of the snails and slugs to the treatments were identified using low-light, time-lapse video. One slug or snail was placed in each of 10 replicate, 16 cm-diameter white plastic containers containing damp compost, onto which the treatment was added, and recorded for one night (14h). Individuals of *D. panormitanum* weighed between 300 and 400 mg and those of *O. pfeifferi* weighed between 100 and 160 mg. The video was then digitised and analysed using the software package EthoVision ®. For technical reasons only soluble products, mulch, and black mattings could be analysed with EthoVision.

Behavioural response of slugs and snails (choice experiments)

Initial data with four treatments was given in the first year report. In this report only the results of an additional two treatments are presented:

- Compost (control) vs. ureaformaldehyde (10%)
- Mypex (control) vs. Tex-R matting

The same methods as in the no-choice experiments above were used in choice experiments. One half of each container was treated and the other half remained as untreated compost or Mypex matting

Suppression of D. panormitanum egg development

Cinnamamide, Croptex-Fungex ® and ureaformaldehyde were evaluated at 0.1 -1%. Egg batches with 15-30 eggs were collected from *D. panormitanum* laboratory cultures on a daily basis. Egg batches were transferred into Petri dishes containing filter paper slightly dampened with 1 ml water per dish. Four ml of each test solution were poured onto the filter paper around the egg batch. Hatching rate and egg development was recorded every day for four weeks after egg laying commenced. Future work in the project will extend this experiment to concentrations as low as 0.001%.

Small-scale field trial - Newcastle

This experiment took place in a polythene tunnel at the University of Newcastle field station. Each circular plot (40 cm diameter) had four small potted Chinese cabbage plants in the centre (see Fig. 3a, Appendix I) and was infested with five adult slugs or snails at the edge of the plot. The plots were covered with Mypex matting and surrounded by a vertical barrier painted with Fluon to prevent slugs escaping. The products were applied to the surface of pots (cinnamamide, Croptex-Fungex , garlic and ureaformaldehyde at maximum concentration) or the whole plot surface was covered with Tex-R instead of Mypex matting. There were 10 replicates of each treatment. Plants were watered by sub-irrigation. The number of damaged leaves was recorded over a 4-wk period.

Large-scale field trial with mattings and slugs -Newcastle

An area at the University field station planted with clover was used for the trial. This area had a very large slug population and is regularly used for slug collection. Square pieces of horticultural matting (1.4 m^2 of Mypex ® and Tex-R ® Landscape Pro ®) were placed on the ground. Thirty-six potted plants, six plants each of Hosta, Choisya, Impatiens, Geranium, Marigold and Chinese cabbage were placed on the matting. All plants were re-potted immediately before the trial and were presumed to be "slug free". Plants were arranged in six rows with a random row arrangement (see Fig. 4a, Appendix I). A 15-20 cm-wide strip at the edge of the matting was left free of pots to act as a barrier against slugs. The number of slugs on the plot was assessed by sampling all pots (presence under pots). The plant damage was assessed by counting damaged leaves on 12 specific plants on each plot. The assessments took place every 14 days for eight weeks. There were 10 replicates.

Large-scale trial with mattings and snails – commercial nursery

One polythene tunnel on a commercial nursery was used for this field trial. The floor of the tunnel was covered with gravel. Square pieces of horticultural matting (1.4 m^2 of Mypex and Tex-R Landscape Pro ®) were placed on the ground. Sixteen potted plants, four plants each of *Choisya* and *Euonymus* and eight plants of *Elaeagnus* were placed on the matting in four rows (4x4 square). A 15-20 cm wide strip at the edge of the matting was left free of pots to act as a barrier against slugs and snails. Each plot was surrounded by 60-80 *Elaeagnus* plants with an infestation of *O. pfeifferi* of approximately 15-30 snails per plant. The number of slugs and snails on the plot and plants and the damage caused to the plants were assessed for eight specific plants per plot after four and eight weeks. There were six replicates of each treatment.

Results and Discussion – laboratory experiments

Barriers against horizontal movement - D. panormitanum

The barrier efficacy of different products varied significantly, with some products having no effect and garlic and ureaformaldehyde repelling more than 60% of all slugs. Four products had a significant barrier effect (N=20, multiple comparison of proportions, $P < 0.001$, Table 5). Three products significantly raised the mortality rate within the two-day experiment, with mortalities being highest with copper foil and Tex-R ® matting (30%). Six of the tested products gave a significant reduction in damage when compared with the control (N = 20, Kruskal-Wallis-Test: $P < 0.001$,

Wilcoxon & Wilcox statistics for posthoc), ranging from between 41% reduction with cinnamamide and mulch and 95% with garlic (Table 5 and Fig. 32).

Table 5. Barrier efficacy of treatments against horizontal movement of *D. panormitanum* and reduction in leaf damage over 48 hours.

Treatment	Barrier efficacy[1] (%)	Mortality rate (%)	Leaf damage (cm^2 ±SE)	Reduction in leaf damage (%)
Control	0	5	6.6 (±0.4)	n.a.
Aluminium	0	15	6.5 (±0.3)	1
Cinnamamide	10***	5	3.9 (±0.6)***	41
Copper foil	25***	30***	2.7 (±0.5)***	60
Croptex-Fungex	0	10	5.2 (±0.4)	21
Garlic	65***	0	0.3 (±0.1)***	95
Mulch	13***	25***	3.9 (±0.6)***	41
Mypex	0	0	5.5 (±0.4)	16
SnailBan	0	0	6.9 (±0.3)	-5
Tex-R	0	30***	2.8 (±0.3)***	58
Ureaformaldehyde	70***	5	0.4 (±0.3)***	94

[1] Barrier efficacy is defined as the number of animals that do not manage to cross the barrier until the end of the experiment

*** significantly different from control, P<0.001

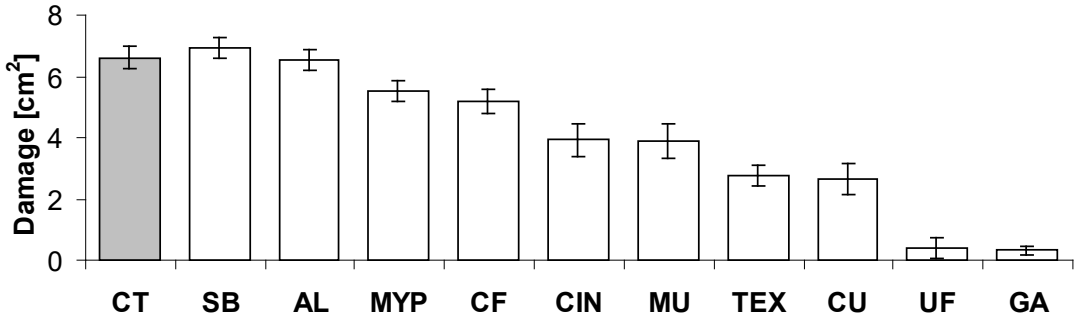


Fig. 32. Accumulated leaf damage caused by *D. panormitanum* after 48 hrs when treated with barriers against horizontal movement. Bars represent standard error.

Abbreviations of treatment names:
CT = control (compost), MYP = Mypex ® matting, AL = aluminium foil, TEX = Tex-R ® matting, MU = mulch, SB = SnailBan ®, CF = Croptex-Fungex ®, GA = garlic, CIN = Cinnamamide, CU = copper foil, UF = ureaformaldehyde

For many products the horizontal barrier width was obviously not wide enough in relation to the body length of the slugs. Once the slugs had crossed the barrier there was no or little reduction in their activity. Thus it is thought that if commercially acceptable and practicable, the treatments should be applied to the entire pot surface, and/or substrate, to stop migration from pot to pot.

Barriers against horizontal movement – O. pfeifferi

The products showed variable efficacy against horizontal movement of *O. pfeifferi* (see Table 6 and Fig. 33). The barrier efficacy was generally higher than in the experiment with *D. panormitanum*. Seven products had a significant barrier effect ranging from between 25% (garlic) and 90% (ureaformaldehyde) (N=20, multiple comparison of proportions, $P < 0.001$, Table 5.2). The experiment was done over a longer period than the one with *D. panormitanum* as snail mortality rates were generally lower than those of the slugs. However, five products increased snail mortality significantly, with a maximum rate of 30% (garlic) (N=20, multiple comparison of proportions, $P < 0.001$, Table 5.2). Leaf damage was significantly reduced by all treatments except aluminium foil and Mypex matting (Table 5.2 and Fig. 5.8, N =20, Kruskal-Wallis-Test : $P<0.001$, Wilcoxon & Wilcox posthoc multiple comparisons). The reduction in damage when compared with the control ranged from 60% (Tex-R) to nearly 100% (ureaformaldehyde).

Overall, horizontal barriers were more effective against *O. pfeifferi* than *D. panormitanum* . This was probably due to the ratio of barrier width to body length, which is in favour of *D. panormitanum*. Mortality rate was probably lower in the snails, because they can retreat into their shells if they experience adverse conditions.

Table 6. Barrier efficacy of treatments against horizontal movement of *O.pfeifferi* and reduction in leaf damage over seven days.

Treatment	**Barrier efficacy (%)**	**Mortality rate (%)**	**Leaf damage (cm^2 ±SE)**	**Reduction in leaf damage (%)**

Control	0	0	5.5 (± 0.5)	n.a.
Aluminium	0	5	2.9 (± 0.3)	29
Cinnamamide	35***	20***	0.5(± 0.2)***	88
Copper foil	80***	5	0.2(± 0.1)***	77
Croptex-Fungex	30***	0	1.1 (± 0.3)***	87
Garlic	25***	30***	0.7 (± 0.2)***	88
Mulch	55***	10***	1.7 (± 0.5)***	69
Mypex	0	5	3.0 (± 0.4)	46
SnailBan	35***	15***	1.5 (± 0.4)***	77
Tex-R	5	5	1.9 (± 0.4)***	60
Ureaformaldehyde	90***	20***	< 0.1***	> 99

*** significantly different from control, P<0.001

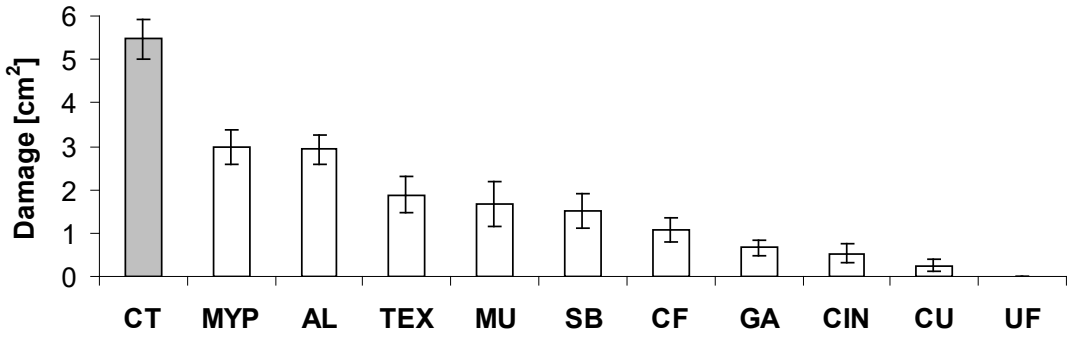


Fig.33. Accumulated leaf damage by *O. pfeifferi* after seven days. Bars represent standard error.
Abbreviations as in Fig. 32.

Barriers against horizontal movement – low concentrations - D. panormitanum
As in the previous barrier experiment the maximum concentrations of cinnamamide (0.625%), Croptex-Fungex and ureaformaldehyde (1% each) significantly reduced the damage (N=20, Kruskal-Wallis-Test: P < 0.001, Wilcoxon & Wilcox posthoc multiple comparisons, Fig. 34) when applied to half of the arena. For cinnamamide and ureaformaldehyde, the medium concentration (0.5%) also had a significant effect. All three products had little effect at their lowest concentration (0.1%). Ureaformaldehyde performed best at all concentrations, achieving a reduction of damage of 93% at 0.5%.

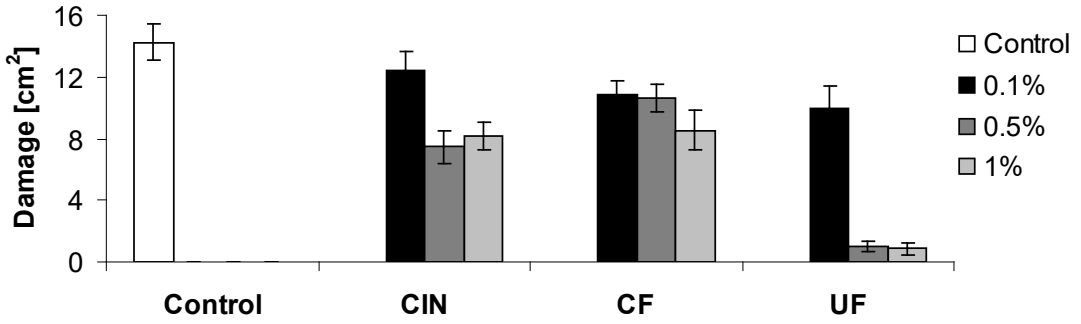


Fig. 34. Accumulated leaf damage by *D. panormitanum* after seven days. Bars represent standard error.
Abbreviations as in Fig. 32.

Data and statistics on mortality rates and barrier efficacy are not yet available and will be presented in the final report.

Barriers against horizontal movement – low concentrations – O. pfeifferi
The trends for *O. pfeifferi* were similar to those for *D. panormitanum*, with products achieving significant reductions in damage with exactly the same products and concentrations (N=20, Kruskal-Wallis-Test: $P < 0.001$, Wilcoxon & Wilcox posthoc multiple comparisons, Fig. 35). However, the reduction in damage was generally greater with the snails than with the slugs. Maximum concentrations of cinnamamide and ureaformaldehyde, for example, reduced the damage by 95 and 100% respectively. At the lowest concentration (0.1%) cinnamamide performed the best (damage reduced by 46%).

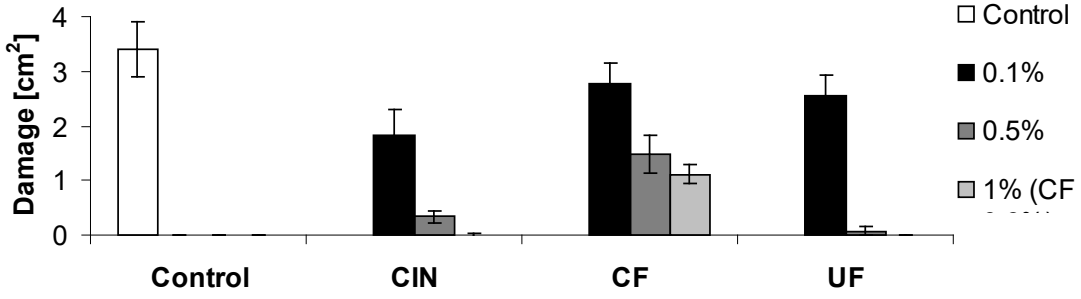


Fig. 35. Accumulated leaf damage by *O. pfeifferi* after seven days. Bars represent standard error.
Abbreviations as in Fig. 32.

Data and statistics on mortality rates and barrier efficacy are not yet available and will be presented in the final report.

Barriers against vertical movement - D. panormitanum

All products had a significant effect as barriers against vertical movements (Table 5.3, N=20, multiple comparison of proportions, $P < 0.001$), i.e. in sealing off the pots in order to stop buried slugs coming to the surface. The most effective product was garlic (100%), followed by SnailBan (75%). Most products increased the mortality rate significantly (Table 7, N=20, multiple comparison of proportions, $P < 0.001$). A 5% solution of garlic concentrate killed 95% of all slugs before they had the chance to come to the surface. Egg production within the one-week experiment was generally lower than in the control. Due to a very great variation within the treatments there were no significant effects on egg production (Table 7, N=20, Kruskal-Wallis-Test $P > 0.05$). However, egg production was reduced by over 50% by eight of the ten products.

All products gave a significant reduction in leaf damage (N=20, Kruskal-Wallis-Test: $P < 0.001$, Wilcoxon & Wilcox posthoc multiple comparisons, Table 7 and Fig. 36), ranging from 61 % with Tex-R and 100% with garlic and ureaformaldehyde.

Table 7. Barrier efficacy, mortality rate, egg production and reduction in leaf damage caused by *D. panormitanum* over seven days.

Treatment	**Barrier efficacy (%)**	**Mortality rate (%)**	**Eggs (N)**	**Leaf damage (cm^2±SE)**	**Reduction leaf damage (%)**
Control	0	0	8.2	13.6 (±1.3)	x
Aluminium	35***	5	6.0	5.1 (±1.3)***	63
Cinnamamide	40***	35***	1.0	2.2 (±0.6)***	84
Croptex-Fungex	10***	5	8.4	4.2 (±1.0)***	69
Copper foil	20***	15***	3.4	3.1 (±0.7)***	77
Garlic	100***	95***	0.1	0.0 (±0.0)***	100
Mulch	45***	35***	2.1	1.4 (±0.6)***	89

Mypex	50***	0	3.6	2.3 (±0.9)***	83
SnailBan	75***	10***	1.8	1.1 (±0.6)***	92
Tex-R	25***	10***	2.7	5.3 (±1.1)***	61
Ureaformaldehyde	65**	25***	1.9	0.0 (±0.0)***	100

*** significantly different from control, P<0.001

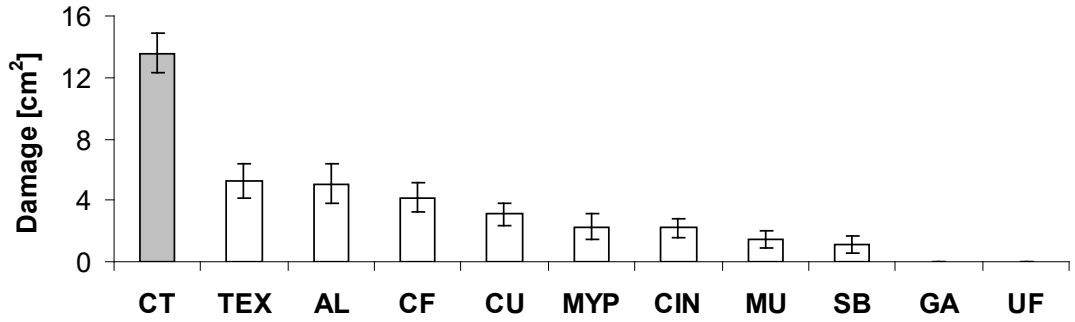


Fig. 36. Accumulated leaf damage by *D. panormitanum* after seven days when treated with barriers against vertical movement. Bars represent standard error. Abbreviations as in Fig. 32.

Suppression of development of D. panormitanum eggs

Cinnamamide, Croptex-Fungex and ureaformaldehyde caused 100% egg mortality at concentrations of 0.1%, 0.5% and 1%. The egg development time in the control was 15 days with a mean hatching rate of 92%. Future work in the project will evaluate the effect of these three products at concentrations as low as 0.001%.

Behavioural responses of slugs and snails (no-choice experiments)

The data will be presented in three sections:

- Mulch and soluble products (maximum concentration)
- Soluble products (low concentrations i.e. 0.1-1%, preliminary data, for *D. panormitanum* only).
- Mattings (preliminary data for *O. pfeifferi*)

Behavioural response (no-choice video) – mulch and soluble products (maximum concentration) - D. panormitanum

When slugs were forced to move onto surfaces treated with each product, all products significantly reduced the activity of the slugs (N= 10, ANOVA, Tukey posthoc

multiple comparisons, P<0.001, Table 8 and Fig. 37). The most effective products were cinnamamide and Tex-R matting, which reduced activity by 95 and 86% respectively. A high production of mucus was observed in some cases, especially in the Tex-R matting and cinnamamide treatments, followed by death within the experimental period. High mortalities were recorded for cinnamamide and Tex-R (80 and 90% respectively, Table 8. Mortality within the experimental period is one of the factors that strongly contributed to the significant reduction of activity of the slugs.

Table 8. Reduction of activity and mortality of *D. panormitanum* by no-choice surface treatment with mulches and soluble products (max. concentration).

Treatment	Track length (cm ±SE)	Reduction of activity (%)	Mortality rate (%)
Control	910 (±146)	n.a.	0
Cinnamamide	47 (±15)***	95	80
Croptex-Fungex	290 (±71)***	68	10
Mulch	590 (±64)***	35	10
Tex-R	129 (±20)***	86	90
Ureaformaldehyde	390 (±55)***	57	0

*** significantly different from control, P < 0.001

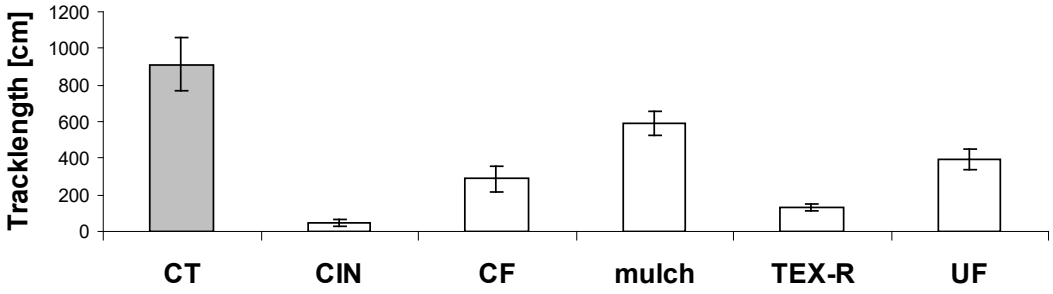


Fig. 37. Effect of no-choice surface treatment with mulches and soluble products (max. concentration) on the activity of *D. panormitanum* during a 14-hr period. Bars represent standard error.
Abbreviations as in Fig. 32.

Behavioural response (no-choice video) – mulches and soluble products (maximum concentration) - O. pfeifferi

The results with *O. pfeifferi* were similar to those with *D. panormitanum*. Again the effect was generally stronger with the snails than with the slugs. All treatments

significantly reduced the activity of the snails (N=10, ANOVA: P < 0.001, Tukey posthoc for multiple comparisons, Table 9 and Fig. 38). The most effective treatment was cinnamamide which reduced activity by 98 % and performed significantly better than mulch and Croptex-Fungex. No mortality occurred within the experimental period. However, 24 hours after the end of the experiment 60% mortality was observed with the cinnamamide treatment.

Table 9. Reduction of activity of *O. pfeifferi* by no-choice surface treatment with mulches and soluble products (max. concentration).

Surface treatment	**Track length (cm ±SE)**	**Reduction in activity (%)**
Control	315 (±9)	N.A.
Cinnamamide	7 (±1)***	98
Croptex-Fungex	50 (±13)***	84
Mulch	49 (±12)***	84
Tex-R	36 (±5)***	89
Ureaformaldehyde	40 (±5)***	87

*** significantly different from control, P<0.001

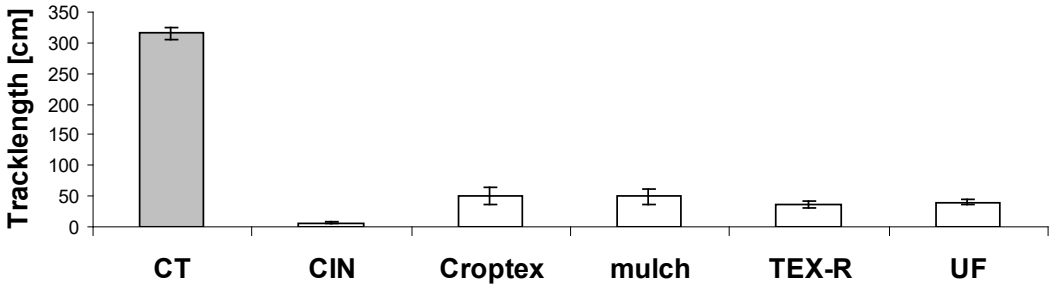


Fig. 38. Effect of no-choice surface treatment with mulches and soluble products (max. concentration) on the activity of *O. pfeifferi* during a 14-hr period. Bars represent standard error.
Abbreviations as in Fig. 32.

Behavioural response (low concentrations of soluble products & no-choice video) – D. panormitanum

This data is preliminary with four out of ten replicates analysed. The trends show that the irritating/ poisoning effect which reduces activity usually increases with the concentration of the product (Fig. 39). As in the experiments with maximum concentrations, cinnamamide performed best and Croptex-Fungex had the smallest effect on slug activity.

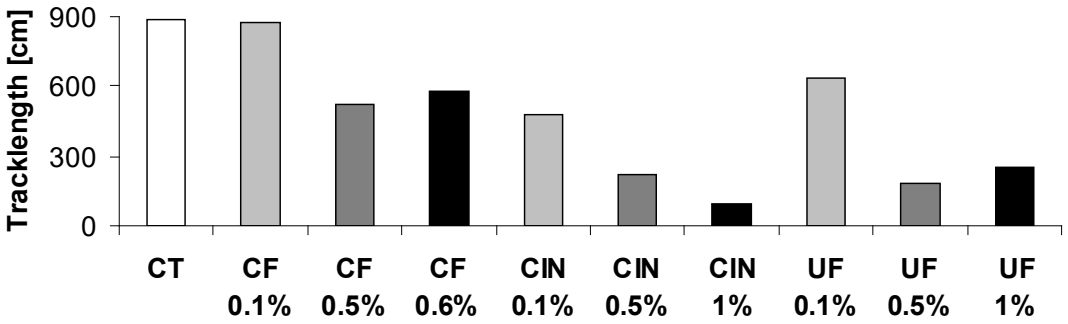


Fig. 39. Effect of high, medium and low concentration surface treatment on the activity of *D. panormitanum.*
Abbreviations as in Fig. 32.

Behavioural response (mattings no-choice video) - D. panormitanum

When slugs were forced to move onto surfaces of different horticultural mattings, only those impregnated with copper formulations (Supercover Plus, Tex-R, Tex-R Landscape Pro) significantly reduced the activity in comparison to the Mypex control (N= 10, ANOVA, Tukey posthoc multiple comparisons, $P<0.001$, Table 10 and Fig. 40). There was no significant difference between the three copper-impregnated mattings. Mortality only occurred with copper-impregnated mattings, and mortality rates were very high (90 or 100%). Weight loss over one night was only monitored for Supercover Plus and Tex-R Landscape Pro and was 73 and 67% respectively.

Table 10. Performance of *D. panormitanum* on different horticultural mattings.

Matting	Track length (cm)	Reduction in activity (%)	Mortality (%)	Weight loss (%)
Mypex	1381	n.a.	0	N/A
FM3	1012	27	0	N/A
GB	1381	0	0	N/A
SCP	87	94	100	73
Tex-R	129	91	90	N/A
Tex-R LP	73	95	100	67

Abbreviations:
FM3 = Florimat 3, GB = Geobond, SCP = Supercover Plus, Tex-R = standard Tex-R matting, Tex-R LP = Tex-R Landscape Pro.

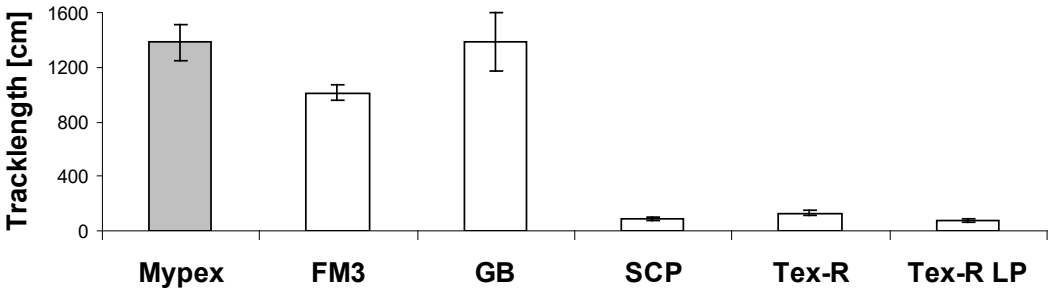


Fig. 40. Effect of different mattings on the activity of *D. panormitanum* during a 14-hr period. Bars represent standard error.
Abbreviations as in table 10.

Behavioural response (mattings no-choice video) - O. pfeifferi
This data is preliminary with six out of ten replicates analysed. The trends are similar to those described for the slugs. On Florimat 3 there was even more activity than on the Mypex (Fig. 41). Geobond seemed to dry out on the surface quicker than other products and showed a small reduction in the track length in comparison with the Mypex.

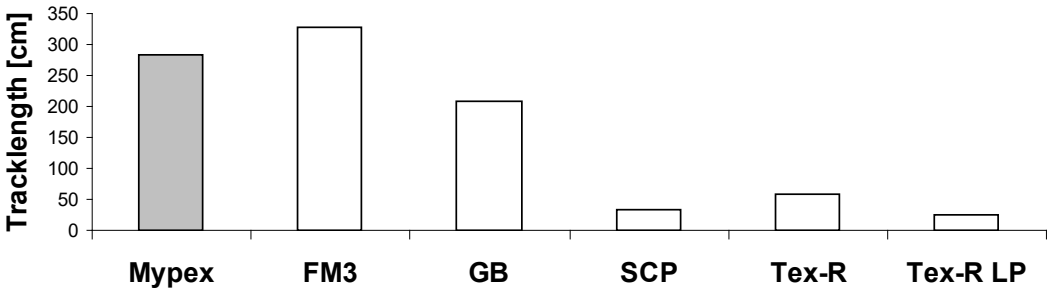


Fig. 41. Effect of different mattings on the activity of *O. pfeifferi* during a 14-hr period. Abbreviations as in Table 10.

Behavioural response (choice experiments) - D. panormitanum
When given a choice over an 8-hr dark period, the slugs showed a significant preference for the control area than areas treated with ureaformaldehyde and Tex-R

respectively, where the mean track length was only 9% and 6% of the total track length (N = 10, Wilcoxon's signed ranks test, P < 0.01, Fig.42 and 43).
No mortality occurred during these short-term experiments.

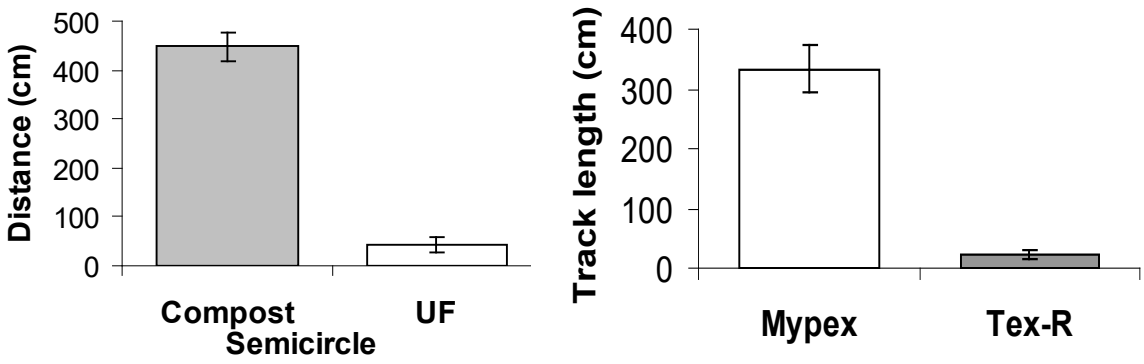
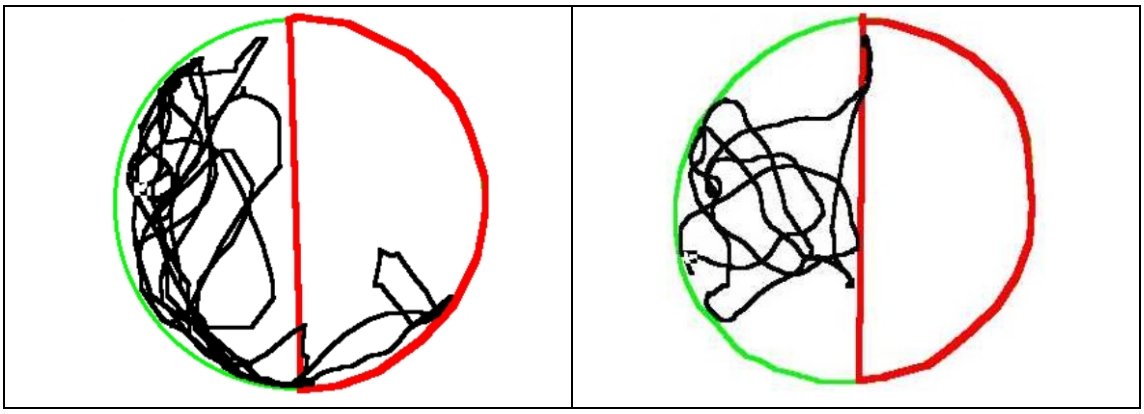


Fig. 42. Track length of *D. panormitanum* in choice tests. Bars represent standard error.



A B

Fig 43. Representative examples of track images from (A) *D. panormitanum* and (B) *O. pfeifferi* when offered either Mypex or Tex-R. 88% and 93% of the slug and snail tracks respectively were on Mypex (left half of circular dish) rather than on Tex-R (right half of dish).

Mode of action of treatments (choice experiments) - O. pfeifferi

When given a choice over an 8-hr-dark period, the snails showed no significant preference for the control area or the area treated with ureaformaldehyde. (N = 10, Wilcoxon's signed ranks test, P > 0.05, Fig.44). However, a mean of only 20 % of the track was spent on the ureaformaldehyde. In some cases the snails moved onto the treated surface at an early stage and then stopped moving due to irritation. This behaviour caused a large variation within the experiment. When given a choice

between Mypex and Tex-R matting, the snails showed a strong preference for the Mypex, where they spent 93% of their total track (N = 10, Wilcoxon's signed ranks test, $P < 0.05$, Fig.43 and 44). No mortality occurred during these short-term experiments.

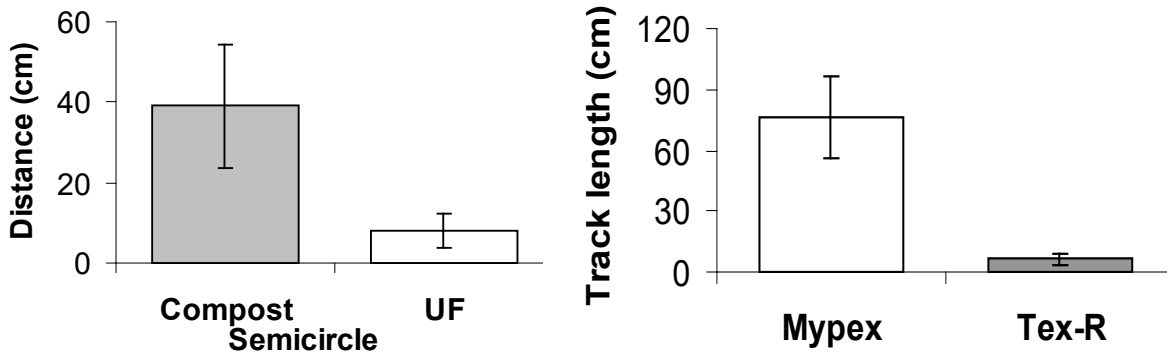


Fig. 44. Track length of *O. pfeifferi* in choice tests. Bars represent standard error.

General Discussion of all laboratory data in Objective 5

From the laboratory bioassays it can be concluded that a combination of effects leads to the reduction in damage, including:

- Repellency, causing a chemical barrier effect
- Physical barrier effect (e.g. foils and mattings as barriers against vertical movement)
- Irritation
- Mortality
- Antifeedant effect
- Any combination of the above five effects

The effectiveness of repellents depends on the width of the barrier, i.e. on the barrier width:body size ratio. For smaller species, such as *O. pfeifferi,* treating a pot surface and thereby creating a barrier only a few cm wide may be sufficient. For larger species, such as *D. panormitanum* this might not be adequate and treating larger areas or using products with antifeedant or irritating effects or might be more effective.

The video behavioural no-choice experiments showed that even the best product, Tex-R Landscape Pro allowed slugs and snails to move 73 and 26 cm respectively, before they died or finally stopped moving. However, the animals' tracks tended to be in small circles rather than unidirectional, e.g. on the copper-impregnated mattings some of the snails never reached the edge of the arena (i.e. eight cm from the centre). It can be concluded that the repellent effect of a product at the border of treated/untreated

areas is more important than the irritant or killing effect once the animals are on the treated surface. The data presented for the Mypex vs. Tex-R choice test showed the strongest repellent effect of all the products, with over 90% of the tracks of both slugs and snails being on the Mypex control). This result, together with Tex-R's commercial availability and practicality led to Tex-R matting being selected for evaluation in the large-scale field trial.

Irritation can cause a reduction in activity, for example by intense mucus production which will lead to dehydration and finally death. The general effect of copper (the active ingredient of Croptex-Fungex and the Tex-R mattings) on slugs and snails is well known. The effect of the copper in the Tex-R range of mattings seems to be very specific and direct. However, the mechanism is not fully understood at present. It can be assumed that substances in the slug and snail mucus cause the release of small amounts of copper from the matrix of the matting and that the copper then enters the body via the foot (i.e underside of the slug or snail), which has already been described as the site of copper uptake (Ryder & Bowen 1977).

An ideal product would be effective both as a vertical and horizontal barrier and would act in more than one of the five described ways. The results show that soluble products could be effective when applied at high or medium concentration, giving a short-term effect on the adults (repellent, anti-feedant, irritant, mortality, reduction in egg laying). After dilution of products through irrigation it is likely that they would still have a long-term effect on the mortality of eggs already laid.

Results and Discusion – field trials

Small-scale field trial –D. panormitanum
The plots treated with the five products had significantly less damage than the control ($N = 10$, Kruskal-Wallis-Test: $P < 0.001$, Wilcoxon & Wilcox multiple comparisons Fig. 45). Although Tex-R matting seemed to be the most successful product with 94% fewer damaged leaves than in the control, there was no significant difference between the five products. The results indicate that treating larger areas might be more effective than treating small areas such as just the surface of pots. This is particularly true where sub-irrigation is not possible and overhead irrigation would leach out any of the treatment solution from the pots. In this trial the soluble products were used at very high concentrations, which might not be commercially viable or environmentally sustainable.

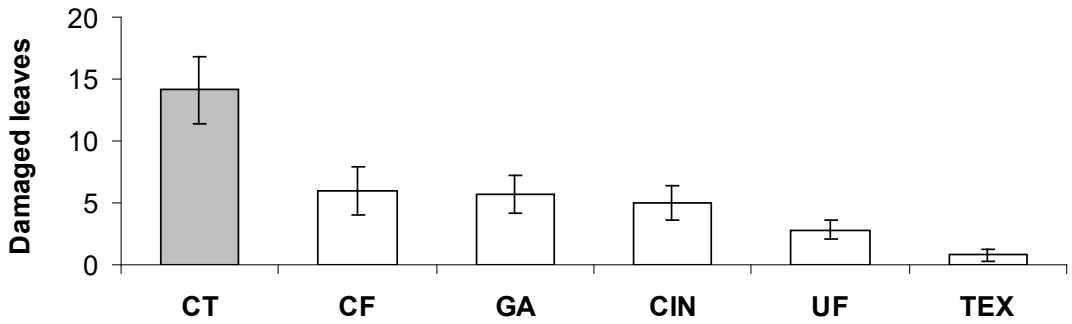


Fig. 45. Accumulated leaf damage caused by five *D. panormitanum* to four Chinese Cabbage plants over a 4-wk period. Bars represent standard error.
Abbreviations as in Fig. 32.

Small-scale field trial –O. pfeifferi

The level of damage was much lower than in the trial with the slugs. The plots treated with all the five products had significantly less damage than the control (N =10, ANOVA: $P < 0.001$, Tukey posthoc multiple comparisons, see Fig. 46). Tex-R matting seemed to be the most successful product with 92% fewer damaged leaves than the control, although it was not significantly better than the other four products.

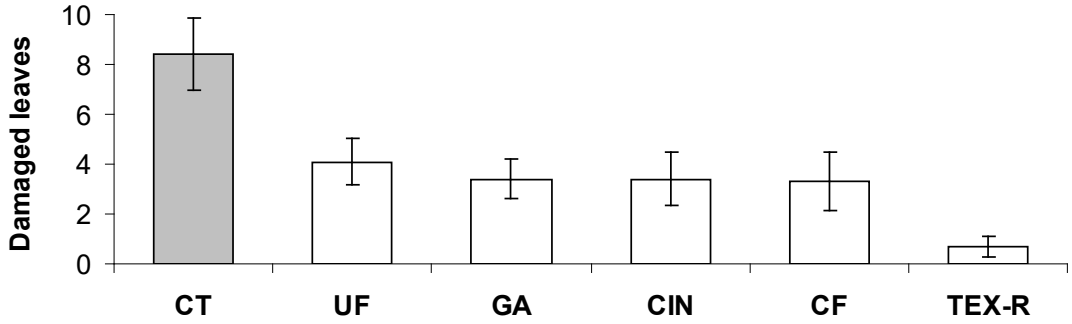


Fig. 46. Accumulated leaf damage caused by five *O. pfeifferi* to four Chinese Cabbage plants in 4 weeks. Bars represent standard error.
Abbreviations as in Fig. 32.

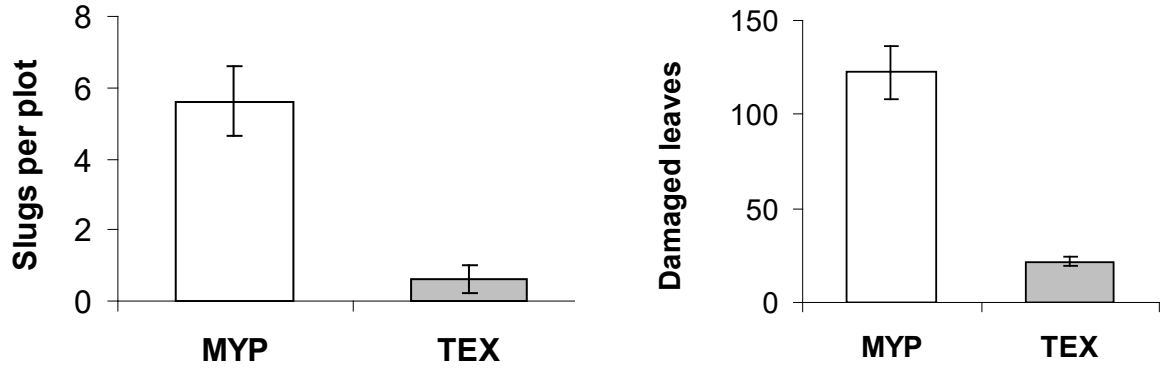
Large-scale field trial with mattings and slugs – Newcastle

The data presented in this report is preliminary, with results of the four-week intermediate assessment. This experiment evaluated the effect of Tex-R matting on *D. panormitanum*, which were known to be highly abundant in the clover patch at the University field station. *O. pfeifferi* is not present at the field station and very few other snail species were found. Slug species found included *D. panormitanum, Deroceras reticulatum, Tandonia budapestensis* and several *Arion* species. Both slug numbers and damage were significantly reduced by Tex-R matting. In comparison with the conventional Mypex matting the slug abundance on the plots with Tex-R

matting was reduced significantly (N =10, T-Test P< 0.01, Fig 47A.). Slug numbers were reduced by 89 %.

As slugs are nocturnal, the numbers found by searching during the day were relatively low. However, the sampling technique provided a fair comparison between the two products. It is very likely that at night the slugs migrated onto the plots from the surrounding clover. Another indication of slug abundance is the number of eggs, which were mostly found under pots on the Mypex matting.

Plant damage was also significantly reduced on the Tex-R matting (N =10, U-Test: P< 0.001, Fig 47B). The number of damaged leaves on Tex-R was reduced by 82 % compared with that on the Mypex.



A B

Fig. 47. Number of slugs (A) and damaged leaves per plot (B) on Mypex (MYP) and Tex-R Landscape Pro (TEX) matting after four weeks. Bars represent standard error.

Large-scale trial with mattings and snails – commercial nursery
The data presented in this report is preliminary, with results of the four-week intermediate assessment. This trial evaluated the effect of Tex-R matting on *O. pfeifferi* ,which were very abundant in the polythene tunnels at this nursery. In addition, a high number of slugs were found, including *D. panormitanum, Deroceras reticulatum* and *Arion ater.*

After four weeks the slug and snail numbers and the number of damaged leaves on the Tex-R plots showed only marginal reductions in comparison to the Mypex plots (Fig. 48).

Fig. 48. A. Number of slugs and snails and damaged leaves found per plot on Mypex (MYP) and Tex-R Landscape Pro (TEX-R) matting after four weeks.

The trial had the following limitations:

- Plants were already partly damaged when the trial started.
- Plants were already infested with snails when the trial started (adults were removed when found, but some adults and probably nearly all eggs were overlooked)
- Plants grew more quickly than anticipated and the surrounding plants grew to touch the plants on the matting and functioned as "bridges" for the slugs and snails to move onto the plants on the plots.
- Tex-R Landscape Pro was used, which is a thick matting intended for use on sandbeds with capillary irrigation. The plants in the trial were watered by overhead irrigation and the gravel floor was always wet and the relative humidity very high. Thus the mattings were probably always damp and algae growth developed on the Tex-R matting, reducing its efficacy.

The above factors reducing the performance of the matting in this trial will provide pointers for practical use of Tex-R matting.

Conclusions

- The products tested in Objective 5 can act in seven different ways:
  - Repellency, causing a chemical barrier effect
  - Physical barrier effect (e.g. foils and mattings as barriers against vertical movement)
  - Irritation
  - Mortality of adults and juveniles
  - Antifeedant effect
  - Suppression of egg development (egg mortality)

- Reduction in egg production

- In laboratory tests, *O. pfeifferi* proved to be generally more susceptible to the treatments than *D. panormitanum*.

- Although several of the products tested, including cinnamamide, Croptex Fungex, garlic, Tex-R products and ureaformaldehyde have potential for further evaluation against slugs and snails, Tex-R matting was selected for testing in the large-scale field trials in this project. Tex-R was selected due to its current commercial availabilty, practicality, and the results of the laboratory video behavioural experiments showing a strong repellent effect, with over 90% tracks of both slugs and snails being on the Mypex control rather than on the Tex-R.

- In a field trial with *D. panormitanum*, there were 89% fewer slugs on plants stood on 1.4 m^2 plots of Tex-R than on plants stood on the Mypex plots. In a trial with *O. pfeifferi* on a commercial nursery, results with Tex-R were inconclusive due to experimental problems.

- For controlling infestations of slugs already present, laboratory tests indicated that medium to high (0.5 –1%) concentrations of cinnamamide and ureaformaldehyde seem to be the most promising of the products tested in Objective 5 against adult slugs and especially against eggs. Any further development work with these products should take into account the peak activity of relevant life stages (see section on Objective 3 - Biology).

OBJECTIVE 6: PRODUCTION OF FACTSHEET FOR GROWERS

The text and photographs for Factsheet 07/02 were provided by the research partners, summarising the year 1 results of the project, and the Factsheet was distributed to all HNS levy payers by HDC.

TECHNOLOGY TRANSFER

- Jude Bennison presented the results of the project to date at the HDC / Efford Open Day on 25 September 2001.

- Jude Bennison and Ingo Schüder wrote an article for 'HDC News' No. 77, October 2001, summarising the results of the project to date.

- During 2002, Jude Bennison discussed the results of the project to date and the practicality of using parasitic nematodes and Tex-R matting with selected growers who were visited during year 1 of the research.

- Jude Bennison discussed laboratory results with the parasitic nematodes 'Nemaslug' with the suppliers, Becker Underwood.

- Jude Bennison and Ingo Schüder provided information on the laboratory results with Tex-R matting, to the suppliers, Fargro Ltd, to include in their technical newsletter.

ACKNOWLEDGEMENTS

Thanks to Becker Underwood for supplying the Nemaslug, Fargro for the Tex-R mattings and commercial HNS growers for providing experimental sites and plants for the research.

REFERENCES

Ryder, T.A. and Bowen, I.D. (1977). The slug foot as a site of uptake of copper molluscicide. *Journal of Invertebrate Pathology* **30,** 381-386.

Fig. 1a. Damage to Viola by *O. pfeifferi*: leaf holes (left) and shredding (right)

Fig. 2a. *D. panormitanum* damage to Hosta 'Elegans' (left) and Choisya (right).

Fig. 3a. One plot of small-scale field trial with *D. panormitanum* and barriers/repellents

Fig. 4a. One plot of large-scale field trial with *D. panormitanum*, Mypex and Tex-R (this plot is stood on Mypex).