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Insect pathogens and Entomoparasitic Nematodes:

"Slugs and Snails"

editor:

David A. Bohan

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Working Group "Insect pathogens and entomoparasitic nematodes"

"Subgroup "Slugs and Snails".

Proceedings of the Working group Meeting

at

Schloss Flehingen, Stuttgart (Germany)

26th to 29th of October 2004.

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Preface

The following papers were presented at a meeting of the IOBC/WPRS Working Group "Insect pathogens and entomoparasitic nematodes", Subgroup "Slugs and Snails", held at Schloss Flehingen, Stuttgart (Germany) from the 26th to 29th of October 2004. The meeting was arranged by a local organiser, Adel El Titi of The State Institute for Plant Protection Stuttgart, and the Subgroup Convenor, Bill Symondson of Cardiff University.

The meeting took place in a relaxed atmosphere. It brought together workers from all over the WPRS and others from as far afield as South Korea, and allowed a number of younger researchers to present their work for the first time to an interested and supportive audience. Broadly organised into 4 paper presentation session and a poster session, the spirit of the meeting was congenial to a broad range of discussion on slugs and snails of pest importance, as reflected by the wide-ranging papers in this Proceedings.

The papers and posters included presentations on slug and snail biology, population dynamics and ecology, the biological control of slug and snail pests using nematodes and natural enemies, molluscicides and novel chemical for slug and snail control, and a synthesis of these areas in the integrated control of slugs and snails. These submissions showed that slug and snail research is a dynamic area of scientific activity across the WPRS.

For myself, I thought the meeting was extremely successful at fostering future slug and snail research by promoting interactions between researchers who might not normally come together. I am sure I was not alone in finding it stimulating, and would like to thank both Adel and Bill for their hard work in organising this well run meeting. I look forward to the next one.

Dave Bohan

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Contents

Proceedings of the meeting at Schloss Flehingen, Stuttgart (Germany) of the IOBC Working Group "Insect pathogens and entomoparasitic nematodes", Subgroup "Slugs and Snails"
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pp.1 - 7

Snails and slugs as non-targets for environmental chemicals

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Abstract: Due to their global distribution and comparatively high resistance to several environmental pollutants, terrestrial slugs and snails can be used as monitor organisms for the detection of sublethal chemical contamination of ecosystems. In this context, the stress status of the animals, which gives information about the severity of environmental pollution can be investigated at different levels of biological organization. In the present paper, we studied cellular alterations and stress protein induction (1) in slugs after exposure to heavy metals and (2) in snails exposed to pesticides in vineyards. The results made evident that (A) lead causes more harmful effects in slugs than cadmium although cadmium is much more intensely accumulated by the animals, and (B) an elevated stress status of snails could be detected in vineyards which have been treated with pesticides. Laboratory experiments made evident that the pesticides *Polyram* and *Sufran* were probably not solely responsible for this effect.

Key words: slug, snail, metal, pesticide, histology, stress proteins

Introduction

In order to evaluate the extend of pollution in aquatic and terrestrial ecosystems and to assess possible consequences for abundant organisms in these environments, in the last decade, ecotoxicologists established biomarker and bioindicator assays which are suitable to inform about adverse effects in vertebrates and invertebrates at different biological levels (Kammenga et al., 2000, Adams, 2002, Köhler & Triebskorn, 2004). In this context, terrestrial slugs and snails have been widely used as monitor organisms due to their global distribution and comparatively high resistance to several environmental pollutants (Berger & Dallinger, 1993, Triebskorn & Köhler, 1996).

In the present paper, we investigated cellular and biochemical effects in the slug *Deroceras reticulatum* and the snails *Helix pomatia* and *Cepaea hortensis* in response to metal (cadmium and lead) and pesticide (*Polyram*, *Sufran*) exposure. The level of the stress protein hsp70 provided information about the proteotoxic potential of these chemicals, histological and ultrastructural investigations allowed us to assess the health status of the animals and to interpret the data obtained by stress protein analyses.

Material and methods

Experimental design

Adult *D. reticulatum* were exposed for three weeks via food and soil to 1, 10, 25 and 100 mg/L Pb^{2+} (as $PbCl_2$) or 0.1, 1, 2.5, 10, 50 and 100 mg/L Cd^{2+} (as $CdCl_2$). Control animals were kept on untreated soil and fed untreated food. Details concerning the exposure

conditions and actual concentrations in food and soil can be obtained from Triebskorn & Köhler (1996).

Adult *H. pomatia* and *Cepaea hortensis* were collected in May and June 2003 at two locations in a Southern German vineyard. In the laboratory, snails were exposed to the fungicides *Polyram* and *Sufran* in concentrations calculated on the basis of the recommended application rates. The experiments were conducted at 15°C and 22°C. Samples for biomarker studies were taken before exposure (controls) as well as 8h and 32 h after exposure to the respective fungicides.

Metal analyses

Metal concentrations in the slugs were determined on a dry weight basis by graphite furnace atomic absorption spectrophotometry (AAS). For details see Triebskorn & Köhler (1996).

Stress protein analyses

For analyses of the stress protein hsp70, samples were frozen in liquid nitrogen and analyzed by a highly reproducible Western blotting assay with subsequent densitometric image analysis according to Köhler *et al.* (2001).

Electron microscope investigations

After perfusion of slugs with 2 % glutardialdehyde dissolved in 0.01 M cacodylate buffer (pH 7.4), samples of the hepatopancreas were dissected and processed for electron microscopy as described by Triebskorn *et al.* (1998).

Histological analyses

After dissection, samples were fixed in 2% glutardialdehyde, dehydrated in a graded series of ethanol, and embedded in paraffin wax. Sections of about 7 µm were cut and routinely stained with hematoxylin/eosin. For each individual, the histology of the hepatopancreas was qualitatively described and semi-quantitatively assessed using a classification scheme comprising 3 categories: cat.1: control status; cat. 2: status of reaction; cat. 3: status of destruction (according to Triebskorn & Köhler, 2003).

Results and discussion

Exposure to metals

The comparison of accumulation data in *D. reticulatum* for similar treatment concentrations of lead and cadmium shows that cadmium was much more intensely accumulated in *D. reticulatum* than lead. Concentrations in control slugs were 2.9 ± 1.4 mg/kg for cadmium and 4.4 ± 7.2 mg/kg for lead.

Stress protein analyses revealed a highly significant increase of the hsp70 level in slugs treated with 1 mg/L Pb^{2+} or Cd^{2+} with a maximum stress response at 1 mg/L Pb^{2+} or 2.5 mg/L Cd^{2+}. At higher concentrations, hsp70 levels were significantly lower than the maximum stress response, but were still significantly higher than in the controls. The electron microscope investigations showed that cytopathological damage was much more intense in lead-treated animals than in cadmium-treated slugs when comparing similar treatment concentrations. The effects of cadmium reflected the reaction status of the animals and were mainly pronounced in mitochondria of the basophilic cells which were heavily enlarged, and in vacuoles of the digestive cells which were enlarged and showed an electron-dense content. After exposure to lead, symptoms of cell death (general disintegration of membranes) became

obvious in digestive and basophilic cells. In many digestive cells, the microvilli were reduced and large vacuoles occurred (Triebskorn & Köhler, 1996).

The chemical analyses of cadmium and lead reveal that, after 3 weeks, accumulation of cadmium in *D. reticulatum* is about 40 times higher than that of lead. The toxic metal cadmium is known to enter cells via calcium channels and to be bound intracellularly by metallothioneins. Therefore, up to a certain limit, effective metal-scavenging storage-excretion mechanisms are available for cadmium to protect cells from toxic action, even though the total cadmium level may be high. In contrast, lead is less effectively detoxified in the cells and therefore, is able to induce more intense cytopathological effects than cadmium, despite its total concentration in the animal. A maximum level of hsp70 was reached with 1mg/L Pb^{2+} and 2.5 mg/L Cd^{2+}. The decreased hsp 70 values at higher concentrations of lead and cadmium are due to a high proteotoxicity correlated with increased histopathological damage in the cells. They can be explained by the specific kinetics of the hsp response, which follows an optimum curve. In contrast, cellular responses show saturation kinetics with increasing exposure (Triebskorn & Köhler, 2003). When comparing the toxicity of the two metals it becomes evident that three weeks after exposure to 1mg/L Pb^{2+} or 2.5 mg/L Cd^{2+}, the defence responses of exposed slugs are at their maximum. After surpassing these thresholds, cellular destruction becomes predominant in wide areas of tissues and cell death symptoms indicate the moribund status of the gastropods.

Exposure to pesticides

In the digestive gland of *H. pomatia* and *Cepaea hortensis*, the structure of excretory and basophilic cells indicated a higher stress level of animals sampled in May than in those sampled in June (Fig. 1). These structural alterations probably reflect an activated metabolism in the digestive gland of these animals.

The structural responses could not directly be related with stress protein levels in the hepatopancreas. However, in the skin of *H. pomatia*, higher stress protein levels were also detected in animals collected in May when compared to those collected in June (Fig. 2). This was the case, although the mean temperature in June was about 3.5°C higher than that in May, which should have resulted in higher stress protein (also called "heat shock protein")levels in June.

Animals sampled in May were exposed to *Polyram* for 32 h at 15°C and 22°C In the digestive gland of *H. pomatia*, decreased hsp70 levels (Fig. 3) as well as decreasing mean assessment values for the histology of basophilic and digestive cells became evident (Fig. 4). The amount and size of excretory vacuoles, however increased with progressed exposure time indicating a process of recovery of the hepatopancreas (Fig. 5).

Animals collected in the field in June were exposed to Sufran for 32 h at 15°C and 22°C. Slightly increased mean assessment values were determined for the hepatopancreas in snails exposed to the pesticide at 22°C (Fig. 6). The major responses were found in excretory and basophilic cells which increased in number and/or size. The recorded mean assessment values, however, do not indicate severe cellular damage but an activated metabolism in this organ. The (non-significant) decrease of stress protein levels in snails after exposure to Sufran (Fig. 7) might be explained by the fact that stress protein levels were always calculated relatively to the whole protein content of the measured organ. As a result of an increased metabolism, decreasing relative stress protein levels can occur although the absolute values of stress proteins remain unchanged.

To summarize these results it can be concluded (1) that the two investigated metals were differently toxic for slugs leading to cellular damage and decreasing stress protein levels due to a high proteotoxic potential especially of lead. (2) The two investigated pesticides, however, did only moderately influence the two snail species tested. They probably led to a general activation

of their metabolism resulting in decreasing stress protein levels relatively to the total protein content of the investigated organ. The different stress levels of snails observed in the field can probably not be explained by an exposure of snails to the two fungicides *Polyram* and *Sufran*.

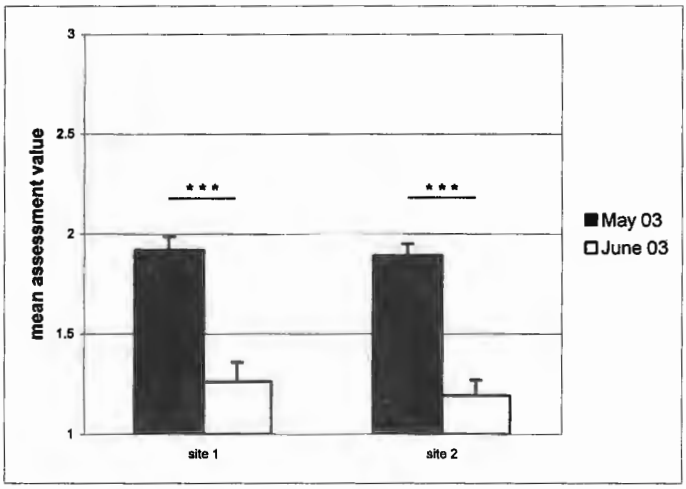

Figure 1. Mean assessment values for the histopathology of basophilic cells in *Cepaea hortensis* sampled at two field sites in a vineyard of Southern Germany prior to exposure to the respective pesticides.

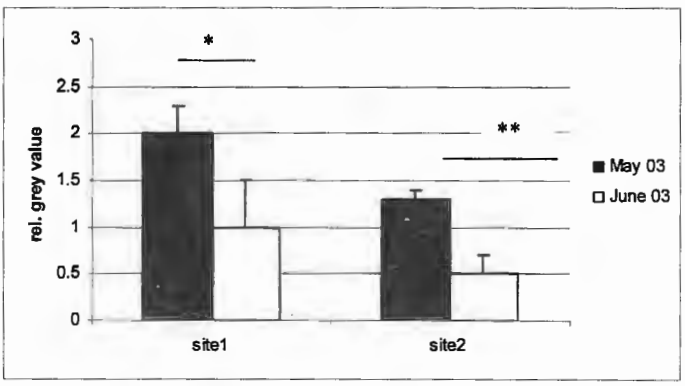

Figure 2. Stress protein levels in the skin of *H. pomatia* sampled in may and june 2003 at two sampling sites in a vineyard in Southern Germany.

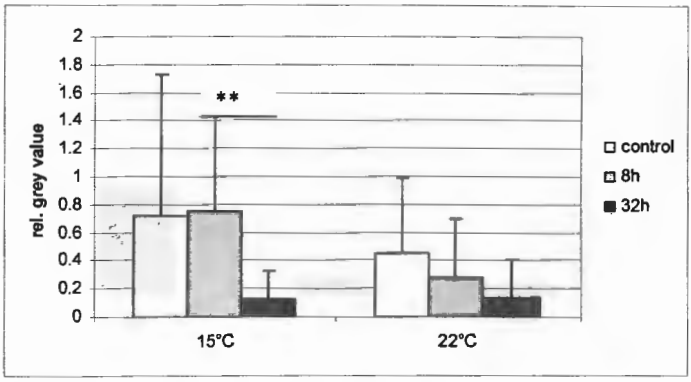

Figure 3. Decreasing stress protein levels in the hepatopancreas of *H. pomatia* exposed to *Polyram* at 15°C and 22°C.

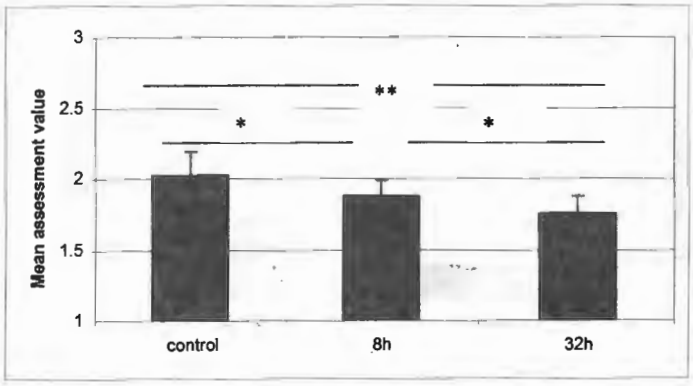

Figure 4. Mean assessment values for the histopathology of digestive cell compartmentation in *H. pomatia* exposed to *Polyram* for 32 h.

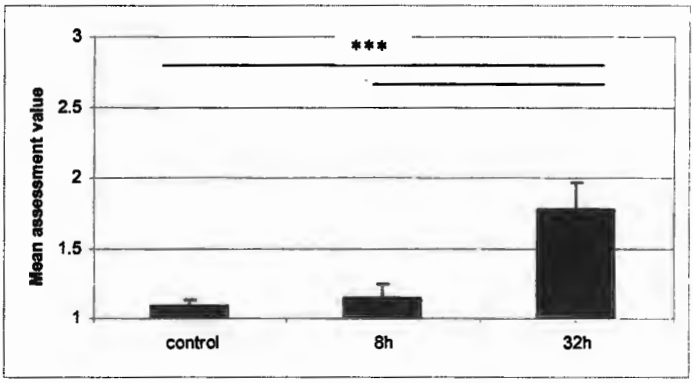

Figure 5. Increasing mean assessment values for the histopathology of excretory cells in *H. pomatia* exposed to *Polyram*

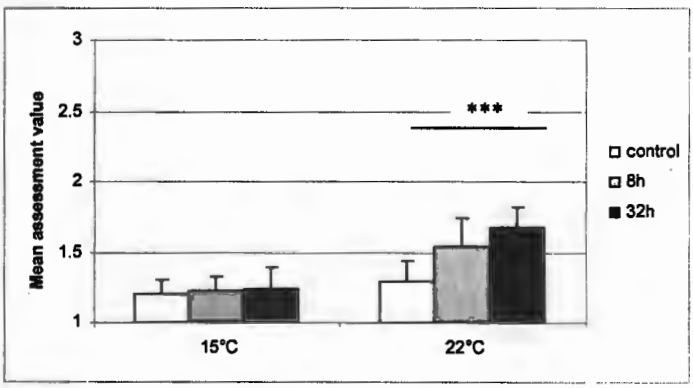

Figure 6. Increasing mean assessment values for histopathological symptoms in the hepatopancreas of *H. pomatia* exposed to *Sufran*, especially at 22°C.

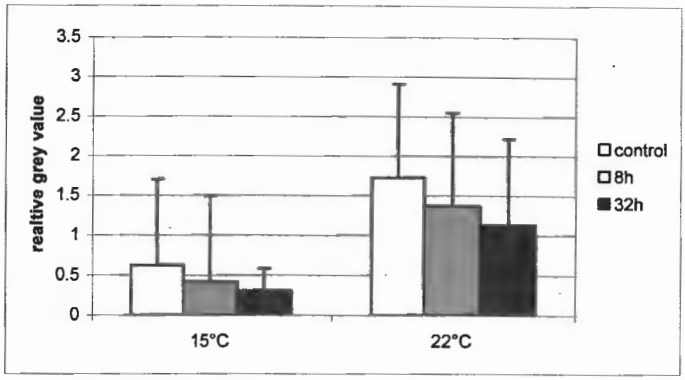

Figure 7. Stress protein levels in the hepatopancreas of *H. pomatia* exposed to *Sufran* for 32 h.

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Insect Pathogens and Entomoparasitic Nematodes
IOBC wprs Bulletin Vol. 28(6) 2005
pp.9 - 13

Antifeedant and molluscicidal activity of scented myrrh applied as a spray

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Abstract: The effect of scented myrrh (*Commiphora guidotti*) essential oil and a chemical component (CDF1), on the feeding behaviour of terrestrial molluscs was investigated. Lettuce leaf discs coated with various extracts were offered to both the field slug (*Deroceras reticulatum*) and the common garden snail (*Helix aspersa*). A range of essential oil concentrations were solubilised in different media and a fixed volume (50 µL) applied to lettuce leaf discs. The amount of lettuce leaf disc consumed by the molluscs and incidences of mortality were recorded.

Slugs were deterred from feeding on lettuce leaf discs by 0.5 - 1% scented myrrh essential oil and snails by 3% of the same oil. Antifeedant efficacy was dependent upon the media used to solubilise the essential oil. Low incidences of mortality were observed for both molluscs when coming into contact with leaf discs coated with essential oil extracts.

A major chemical component of scented myrrh, CDF1, was found to significantly affect the feeding behaviour of both terrestrial molluscs. Slugs were deterred from feeding on lettuce leaf discs when coated with 0.5% CDF1 and snails deterred from feeding by 5%. Incidences of slug mortality were very high for *D. reticulatum* (70-95%) when exposed to aqueous extracts of CDF1, whilst negligible mortalities were observed for *H. aspersa*.

Spray trials confirmed the antifeedant properties of scented myrrh and its component CDF1 resulting in 7% and 0% leaf damage respectively. Scented myrrh (3%) formulated as a spray significantly deterred the slug *D. reticulatum* from feeding on the young lettuce seedlings for seven days. Both scented myrrh and its component CDF1, formulated as a spray, were found to be effective antifeedants and potent molluscicides resulting in very high mortalities (47% and 93% respectively) over the seven day test period. Scented myrrh applied, as an antifeedant spray, significantly deterred snails from feeding on lettuce seedlings for six days whilst CDF1 was an effective antifeedant for four days, both resulting in only 38% leaf damage and no snail mortalities.

This study has shown that sprays based upon scented myrrh and its chemical component CDF1 are potent antifeedants and mollusicides and are recommended to be used as part of an integrated pest management system to control terrestrial molluscs.

Key words: Antifeedant, *Deroceras reticulatum*, *Helix aspersa*, integrated pest management, scented myrrh.

Introduction

As the use of synthetic molluscicides is increasingly becoming unpopular in the agricultural and horticultural industry new means of deterring or repelling slugs and snails are actively being sought. Sales of organic food, in the UK, have been estimated as being worth one billion pounds, illustrating the importance of alternative forms of controlling pests such as slugs and snails in organic farming.

One alternative form of pest control is the use of non-toxic plant extracts and their components as mollusc antifeedants or repellents. There have been many reports, over the years, evaluating the repellent and antifeedant properties of natural compounds but none so far have been exploited commercially (Barker 2002). Airey *et al.* (1989) identified a number of

chemicals of plant origin which prevented slugs from feeding (2-phenylethyl isothiocyanate, and fenchone), however, the former was found to be phytotoxic and the latter too volatile to be persistant in the field. More recently Schüder *et al.* (2003) found garlic to be an effective barrier against slugs (*Deroceras panormitanum*) resulting in up to 95% mortality and 30% mortality for the snail *Oxyloma pfeiffer*. Dodds *et al.*, (1999) used electrophysiological and feeding bioassays to identify *Petroselinum crispum, Conium maculatum,* and *Coriandrum sativum* plant extracts as being both neuroactive and antifeedant against slugs

In our laboratories, we previously reported the repellency and molluscicidal properties of African plants (Ali *et al.*, 2003). This research study is an extension of that work with the aim of testing scented myrrh (*Commiphora guidotti*) extracts, and its components, as an antifeedant / molluscicidal spray against the field slug (*Deroceras reticulatum*) and the common garden snail (*Helix aspersa*).

Material and methods

Test animals

Adult *D. reticulatum* were collected from nearby fields and maintained in plastic trays lined with moist, unbleached, absorbent paper. They were housed in the dark and at a constant temperature of 10±1°C. Adult *H. aspersa* snails, obtained from Blades Biological Limited, were kept in an aquarium, layered with peat soil and maintained at room temperature (18-22°C). Twenty four hours prior to testing, both the snails and slugs were starved for 24 hours and acclimatised to a temperature of 15°C, in an environmentally controlled chamber.

Leaf disc assay

A known volume of plant extract (50 µl) was pipetted onto individual lettuce leaf discs (1.4 cm^2) and any residual solvent left to evaporate. Slugs were introduced to Petri-dishes, whilst the snails were introduced to plastic sandwich containers. Both were placed in an environmentally controlled chamber (15°C: 12 h day, 15°C: 12 h night) for 24 h and the amount of lettuce leaf disc consumed (leaf damage) quantified by comparing digital photographs of treated leaf discs with untreated (control) leaf discs. The experiment was repeated 20 times for each treatment with one slug or snail being introduced per treatment. Treatments comprised of solubilising scented myrrh essential oil (0.5% and 1%) in ethanol, water, aqueous Tween 80 and aqueous DMSO.

Spray trials

Plastic bowls (approximately 30 x 30 cm) were painted with Fluon to prevent snails from leaving the arena. These were layered with about 2 cm depth of peat. Three young lettuces, at the 5-8 leaf stage, were planted per bowl and each plant sprayed with about 5 ml of spray formulation. In addition to this, the soil area adjacent to each lettuce plant was sprayed with 5 ml of spray formulation. Either eight snails or five slugs were added to each arena. The bowls were placed in the same controlled environment conditions as above. The amount of leaf damage was assessed daily and recorded over a seven day period. Three replicates were prepared per treatment. Any mortality was noted and each plant visually assessed for leaf damage. Treatments comprised of 3% scented myrrh essential oil solubilised in 3% aqueous tween 20, 5% CDF1 solubilised in 5% aqueous Tween 20 and a control (water).

Statistical Analysis

As the between-treatment variances were not homogenous (Bartlett's/Levene's tests) and the residuals not normally distributed (Anderson-Darling test) the data were analysed using the

non-parametric Kruskal-Wallis test to show significance of differences between group medians or the Mann-Whitney test for pairwise comparisons of the medians. In each case, the level of significance was established for $p<0.05$.

Results

*Leaf disc assay (***D. reticulatum***)*

The solvents ethanol, aqueous Tween 80 (0.2%) and aqueous DMSO (10%), had not deterred the slugs from feeding on the lettuce leaf discs resulting in 95%, 94% and 97% leaf damage respectively, compared to 95% for the control (water). The Kruskal-Wallis test showed no significant differences between the group medians for the solvent treated leaf discs and the water treated leaf discs ($N = 20$, $P > 0.05$).

Coating the leaf discs with extracts of scented myrrh (0.5-1%) significantly deterred the slugs from consuming the lettuce leaf discs, when compared to the control (water) ($N = 20$, Kruskal-Wallis test, $P < 0.001$) as illustrated in Figure 1. Because of the large variation in standard deviation, Mann-Whitney pair-wise comparisons of the group medians for all the scented myrrh extracts, with the ethanol solubilised extract, showed only the water solubilised extracts (0.5% and 1%) to be on the margin of significance (P=0.058 for both sample groups). Incidences of mortality were not significant for any of the scented myrrh samples tested ($N = 20$, Kruskal-Wallis test, $P > 0.05$).

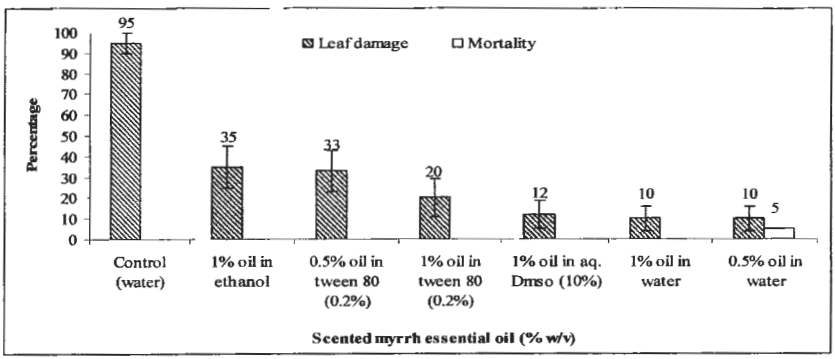

Figure 1: Comparison of the antifeedant and molluscicidal properties of various extracts of scented myrrh essential oil, when tested against *D. reticulatum*.

Comparing between group medians for all the CDF1 treatments showed them to be significantly effective in deterring the slugs from consuming the lettuce leaf discs, as shown in Figure 2 ($N = 20$, Kruskal-Wallis test ($P < 0.001$). The leaf damage data showed significant differences between 0.5% CDF1 solubilised in ethanol and all the other CDF1 treated leaf disc treatments ($N=20$, Mann-Whitney, $P < 0.001$), confirming that ethanol was the poorest solvent to use as a medium for this chemical.

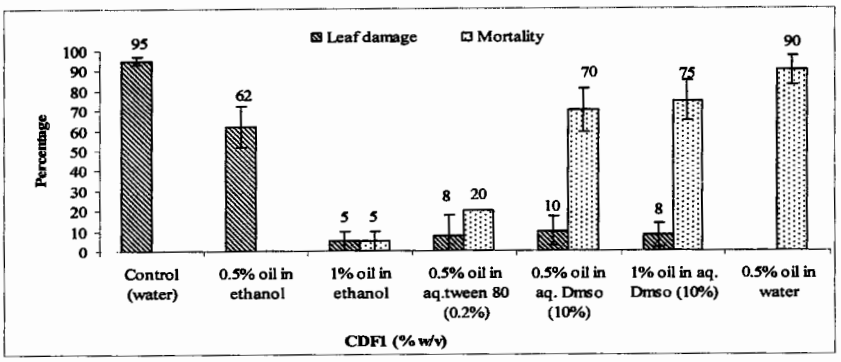

Figure 2: Comparison of the antifeedant and molluscicidal properties of various extracts of CDF1, when tested against *D. reticulatum*.

Incidences of slug mortality, on contact with the CDF1 treated leaf discs, were found to be highly significant ($N = 20$, Kruskal-Wallis test, $P < 0.001$). The molluscicidal properties of the chemical CDF1 (0.5%) was significantly enhanced when solubilised in water or aqueous DMSO (75% and 70% slug death) compared to when solubilised in ethanol (0% slug death) (N=20, Mann-Whitney test, $P < 0.001$). Between treatment comparisons of the group medians showed no significant differences between the high slug mortalities observed for the 0.5 - 1% CDF1 treatments solubilised in aqueous DMSO and water ($N = 20$, Kruskal-Wallis test, $P > 0.05$).

***Leaf disc assay* (H. aspersa)**

The solvents aqueous Tween 20 (3% and 5%) and the control (water) had no significant effects in deterring the snails from feeding on the lettuce leaf discs, all treatments showing 100% leaf damage. The leaf disc assay confirmed the antifeedant properties of scented myrrh essential oil (3%) and its major chemical component CDF1 (5%), solubilised in aqueous Tween 20, against *H. aspersa* snails, resulting in 0% leaf damage for both treatments. No snail mortalities were observed when they were exposed to any of the solvent, scented myrrh or CDF1 treated leaf discs.

***Spray trials: Leaf damage* (D. reticulatum *and* H. aspersa)**

Both scented myrrh essential oil and its chemical component CDF1 both proved to be potent antifeedants, against *D. reticulatum* slugs, when solubilised in aqueous Tween 20. Low levels of leaf damage were observed, for both treatments, over seven days. Scented myrrh essential oil gave rise to only 7% leaf damage whilst CDF1 gave rise to 0% leaf damage compared to 78% leaf damage for the water treated plants (control). Mann-Whitney pair wise comparisons of the medians for scented myrrh and CDF1 treatments compared to the control (water) showed potent antifeedant effects for both treatments ($N = 9$, $P < 0.001$). When the lettuce plants were exposed to *H. aspersa* snails, both scented myrrh and CDF1 were effective antifeedants, for six and four days respectively, with 38% leaf damage being assessed for both treatments, compared to the water treated lettuce plants (100% and 81% respectively). Mann-Whitney pair-wise comparisons of the medians, after six days, showed scented myrrh treatments to be significant in reducing leaf damage compared to the control ($N = 9$, $P < 0.05$). Similarly Mann-Whitney comparisons of group medians, after four days, showed the antifeedant properties of the chemical CDF1 to be marginally significance ($N = 9$, $P = 0.058$) when compared to the control (water).

Spray trials: Mortality (*D. reticulatum *and* H. aspersa*)

Both 3% aqueous scented myrrh and 5% CDF1 sprays, formulated with aqueous Tween 20, resulted in significantly higher incidences of slug death ($N = 15$, Kruskall-Wallis test, $P < 0.001$). Over a seven day period scented myrrh and CDF1 sprays resulted in 47% and 93% slug mortalities, respectively, compared to 7% for the control (water). No snail mortalities were observed over the seven day test period.

Discussion

None of the solvents tested were found to have any antifeedant effects on terrestrial molluscs, when tested using the leaf disc assay. Scented myrrh extracts (0.5–1%) possessed very good antifeedant properties against slugs. A higher scented myrrh concentration (3%) was required to have the same affect against snails. Similarly, 0.5-1% CDF1 was found to deter the slugs from consuming the lettuce leaf discs, whilst again a higher dose (5%) was required to modify the feeding behaviour of the snails. For the slugs, antifeedant activity was stronger when scented myrrh and CDF1 were solubilised in water or aqueous DMSO than when solubilised in ethanol and aqueous Tween 80, indicating that both oils are more active in polar media.

Spray trials, conducted on young lettuce seedlings, confirmed the antifeedant properties of scented myrrh and CDF1 against both molluscs. High incidences of mortality were found to be specific to slugs, when the lettuces were sprayed with these extracts.

This study has shown that sprays based upon scented myrrh and its chemical component CDF1, when applied to lettuce plants, are potent antifeedants and mollusicides and thus may have the potential to be used as part of an integrated pest management system to control terrestrial molluscs.

Acknowledgements

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Insect Pathogens and Entomoparasitic Nematodes
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New findings on metaldehyde as a slug control active ingredient in integrated production systems

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Abstract: According to the Integrated Production Principles and Technical Guidelines of the IOBC (Boller *et al.* 2004) direct plant protection measures should be used when threshold values indicate a need to intervene. But, priority must be given to those measures which have minimal impact on non-target organisms and the environment. In slug control Metaldehyde is the mostly widely used active ingredient for direct intervention. It's specifity and the positive environmental profile is documented in several published investigations and studies (Bieri, 2003). In addition to these findings Langan *et al.* (2003) recently showed that Carabid beetles prefer feeding on slugs having taken Metaldehyde. The surviving rate of these beetles was the same as in the control. In studies carried out at the Central Science Laboratory (York, UK) with 16 house sparrows (*Passer domesticus* L), which were starved for 24 h before being offering slug pellets containing Metaldehyde for 6 hours, no intoxication or even signs of behavioural change was observed. Similar results were found with another grain feeding species the Japanese quail *(Cotumix japonica)* at LPT Hamburg. The IOBC concept of Integrated Production is based on threshold values for intervention. This concept also stipulates that a direct intervention should instantly stop pest activity. Metaldehyde has been shown to be a fast acting ingredient that irreversibly destroys the mucus cells of the intestine, instantly stopping slugs feeding preventing any further substantial damage to the crop stand.

Key words: Metaldehyde, Integrated Production (IP), Seed Feeding Birds, Carabid Beetles

Introduction

According to the Integrated Production Principles and Technical Guidelines of the IOBC (Boller *et al.* 2004) preventive (indirect) measures and observations in the field on pest, disease and weed status must be considered before intervention with direct plant protection measures takes place. In cases where indirect measures are not sufficient to prevent a problem and forecasts and threshold values indicate a need to intervene with direct measures, priority must be given to those which have the minimum impact on human health, non-target organisms and the environment. This implies interventions with highly selective, but also highly efficacious active ingredients to stop plant damages at a level where the grower will not suffer any losses.

Metaldehyde is known as a specific molluscicide that irreversibly destroys the mucus cells of slugs (Triebskorn *et al.* 1998). In several studies carried out by different authors no reverse effects of Metaldehayde on naturally occurring organisms, such as earthworms, carabid beetles, roof beetles and other arthropods could be found. It's environmental profile also shows it to be advantageous (Bieri, 2003). Integrated Production is an ongoing and progressing process, continuously generating an increasing knowledge and experience in handling complex agro-ecosystems. In this context, the providers of active ingredients for pest control are obliged in widening their knowledge about the properties of their substances to provide correct information on an appropriate and efficacious application to the users.

In this paper some new trial results are presented, to widen knowledge about the properties of Metaldehyde as an active ingredient in slug control.

Materials and Results

Carabid beetles fed with slugs having ingested Metaldehyde pellets (Langan et al. 2004)
Any intervention in cropping systems should not cause drastic perturbation on the beneficial complex. The more the beneficials maintain their original function after a direct intervention the less the natural control potential is reduced. In their study Langan *et al.* (2004) investigated the behaviour of the carabid beetle *Pterostichus melanarius* F., known as an efficient slug predator.

Grey field slugs (*Deroceras reticulatum*), having just being fed with Metaldehyde slug pellets, were presented to unfed *P. melanarius* beetles in Petri dishes. In each dish a single slug per beetle was kept and the feeding behaviour observed and compared with a control group (untreated slugs). The results of these tests are presented in Fig. 1 and 2.

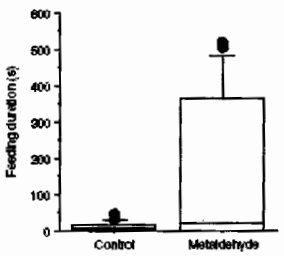

Figure 1: Feeding duration of *P. melanarius* on untreated *D. reticulatum* (Control) and on *D. reticulatum* fed with Metaldehyde slug pellets.

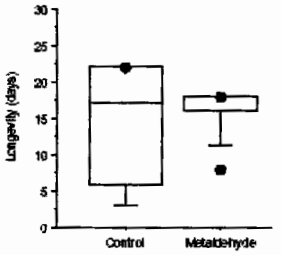

Figure 2: Longevity of *P. melanarius* on untreated *D. reticulatum* (Control) and on *D. reticulatum* fed with Metaldehyde slug pellets.

Slugs fed Metaldehyde pellets were clearly more vulnerable to carabid attack. Generally, the feeding duration on slugs having ingested Metaldehyde lasted longer. On the other hand beetles fed slugs containing Metaldehyde did not show any response concerning longevity. These findings indicate that *P. melanarius* does not suffer any reduction in its function as a natural enemy of the grey field slug after interventions with Metaldehyde slug pellets.

Metaldehyde slug pellet acceptance studies in birds (House Sparrow & Japanese Quail)
In 2003 and 2004, two pellet acceptance studies with two seed feeding bird species were carried out (Anon A, 2003; Anon B, 2004; Lonza internal data).

At the Laboratory of Pharmacology and Toxicology [LPT], Hamburg, Germany a worst case study and a test under realistic conditions with 6 week old Japanese quail *(Cotumix japonica)* were made. Twelve animals (mixed sexes), starved for 16 hours, were allocated to 2 groups of 6 animals were exposed for 8 h to Metaldehyde slug pellets (12 g Metaldehyde pellets + 4 g diet per animal). Afterwards the animals were observed for 14 days.

In a second study performed at the Central Science Laboratory (York, UK), the feeding on Metaldehyde slug pellets of 16 house sparrows (*Passer domesticus* L.) was investigated. The sparrows were held as 8 separate couples (1 male and 1 female). In a first no choice test a

group was starved for 24 h before offering slug pellets containing Metaldehyde for 6 hours. In a second test the sparrows could choose their food out of a mixed diet containing Metaldehyde slug pellets. The general findings of these two studies can be summarised as follows:

- Metaldehyde pellet formulations pose no risk even under worst case deprivation test conditions as well as under real application conditions.
- Metaldehyde pellets were avoided even under no-choice conditions.
- Birds preferred seeds instead of Metaldehyde pellets under Choice conditions.
- Therefore, under real application conditions no risk to birds from Metaldehyde pellet formulations can be expected.

Metaldehyde a fast acting efficacious active ingredient against slugs - a prerequisite in Integrated Pest Management (IPM)

Integrated Pest Management direct plant protection measures have to be used when threshold values indicate a need to intervene. This approach requires that no further plant damage be tolerated when threshold values are reached. The pest has to be stopped immediately to prevent any further feeding to head off losses of the growers.

In several trials made in the Lonza laboratory at Visp (Switzerland), Metaldehyde slug pellets show a fast action, immediately reducing any feeding on carrots close to zero. In Fig. 3 a general pattern in feeding reduction is presented compared with the pattern of another active ingredient.

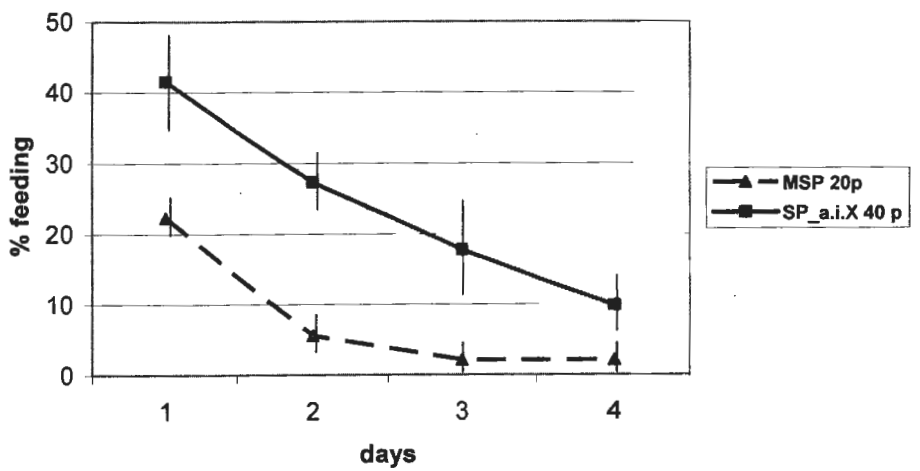

Fig. 3: Slug feeding in % of daily presented food after exposing slugs to slug pellets at day 1. (MSP 20p: 20 Metaldehyde slug pellets per tray; SP_a.i.X 40p: 40 slug pellets per tray containing another active ingredient).

Discussion

As with a previous study (Bieri, 2003), no adverse effects of Metaldehyde on the carabid beetle *P. melanarius* (Langan et al. 2004) and on the two seed feeding bird species the house sparrow, *P. domesticus*, and the Japanese quail, *C. japonica*, could be found. This substantiates once more the advantageous profile of Metaldehyde as molluscicide. It can be concluded that Metaldehyde fulfils all criteria of Integrated Plant Protection for an active ingredient for direct plant protection measures. It is specific without disturbing the behaviour of non target organisms and as a dry alcohol it is degraded by soil micro-organisms into water and CO_2. Metaldehyde also leads to an instant interruption of slug and snail feeding, which is an imperative for a successful implementation of the threshold concept and even after more than 40 years in use, no signs of resistance can be observed.

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Ferramol (Sluggo) – new results of slug and snail control on various crops worldwide

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Abstract: The molluscicide Ferramol (Sluggo) contains a naturally occurring soil component, iron phosphate, as an active ingredient. Ferramol was first introduced to the consumer market in 1998 and is currently marketed throughout Europe and North America. In order to expand the label for agricultural use, trials were done against slugs and snails on various crops worldwide from 2000 to 2004. Some of these trials will be presented here including: Artichokes / *Deroceras reticulatum* USA; Tobacco seedlings / *Helix aspersa* Brazil; Winter wheat / *D. reticulatum* Denmark; Oilseed rape / *D. reticulatum* Germany; Ornamentals/ *Limax marginatus* Japan

Key words: molluscicide, Ferramol, Sluggo, iron phosphate

Introduction

Neudorff's molluscicide is called Ferramol in Europe and Sluggo in North America and the rest of world. Ferramol contains a naturally occurring soil component, iron phosphate, as an active ingredient, combined with unique slug and snail additives in a pellet formulation. The bait contains only 1% active ingredient.

Similar to all molluscicidal baits, the bait must be scattered over the soil around plants. The bait does not kill on contact and must be ingested to exert its effects. Once ingested the slugs and snails will stop feeding, so that no further plant damage occurs. The active ingredient causes pathological changes on the cellular basis in the crop and hepatopancreas of slugs.

Ferramol has an excellent toxicological profile. Because of the extremely low mammalian toxicity (LD50 rats, oral > 5000 mg/kg) it is virtually harmless for higher animals (dog, cat. hedgehog). Also earthworms, bees and other beneficials are not affected and there has been no evidence of aquatic toxicity. In the USA, the Environmental Protection Agency (EPA) has given Ferramol (Sluggo) an exemption from residue tolerance . In Europe, IFOAM recognizes iron phosphate as an acceptable active for slug/snail control in organic farming.

Consequently the bait is ideal for using around cats and dogs and other household pets. For this reason Ferramol was first introduced to the consumer market in 1998 and is currently marketed throughout Europe and North America. For consumer use, a standard rate of about 0.5 kg/100 m^2 or 5 g/m^2 is recommended for good control. The baits are usually scattered in areas where slug damage has been observed or in areas where slugs are known to hide.

In the agricultural areas lower amounts of bait are usually used for control in order to make it cost effective. Several parameters are responsible for successful slug and snail control in field situations. Besides slug/snail density, weather, type of soil, attractiveness of the crop the application rate and way of application is affecting the field efficacy. In order to find out the correct application rate for each crop various tests were conducted in different countries worldwide.

Results and discussion

Artichokes* / Deroceras reticulatum *USA

The grey garden slug has become a serious pest of artichokes in recent years. One reason for this is the sub-surface drip irrigation system that seems to discourage slugs from foraging/crawling on the dry soil surface. The slugs prefer to sit on the plants or the shoots of the artichokes or inside the bud since these parts of the plants remain moist during the day. The slugs feed on the buds thereby lowering their quality and market value.

In order to control the slugs many growers apply the bait manually especially during the crop bud production phase in July/August. The bait is uniformly scattered over the bud top close to the artichoke plant. The following graph gives the results of a field test on Artichoke done in California, USA. Sluggo (the US brand of Ferramol) was applied at 6 or 16 kg/ha at four times during the growing season and was compared to a commercial standard metaldehyde.

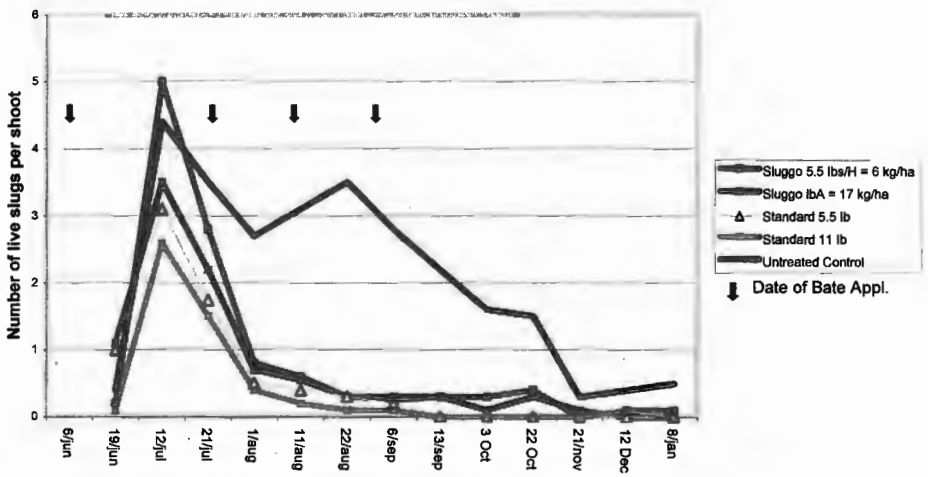

Figure 1. Density of *D. reticulatum* in artichokes following treatment with Sluggo

In July the slug density rose to its seasonal peak in all treatments after the hatch of the summer generation (Figure 1). After the second treatment on July 21 the slug density showed a significant decline in all bait treatments as compared to the control.

Tobacco / Brazil

The purpose of this trial was to assess the efficacy of the product Ferramol in controlling slugs of the genus *Helix aspersa*. The tobacco plants were planted in rows and the bait was scattered over the plants/rows manually (row application).

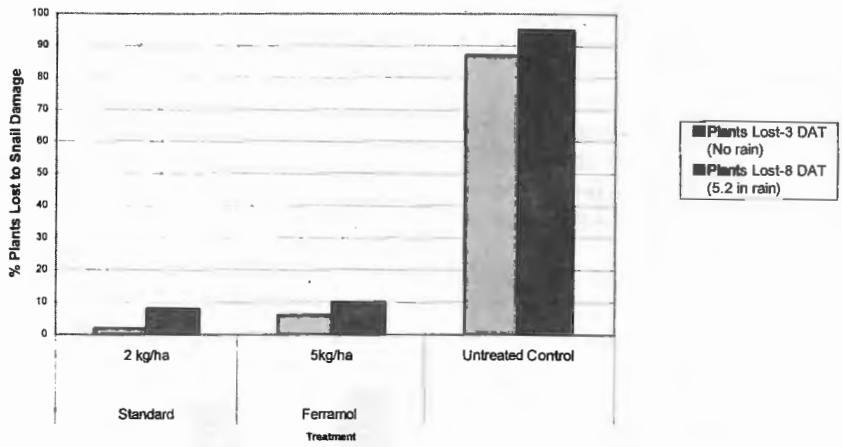

Figure 2. % of tobacco plants lost to *H. aspersa* damage following treatment with Ferramol

Ferramol was effective in controlling *H. aspersa* at the application rate of only 5 kg / ha (Figure 2).

Winter Wheat* / D. reticulatum / *USA

Although slugs feed on wheat plants in every development stage, plants are most susceptible to slugs from seeding until the three leaf stage. Slug attack in later development stages is less of a problem because wheat crops can compensate for the damage through development/growth effects. Figure 3 shows the results from a test done in the US on wheat.

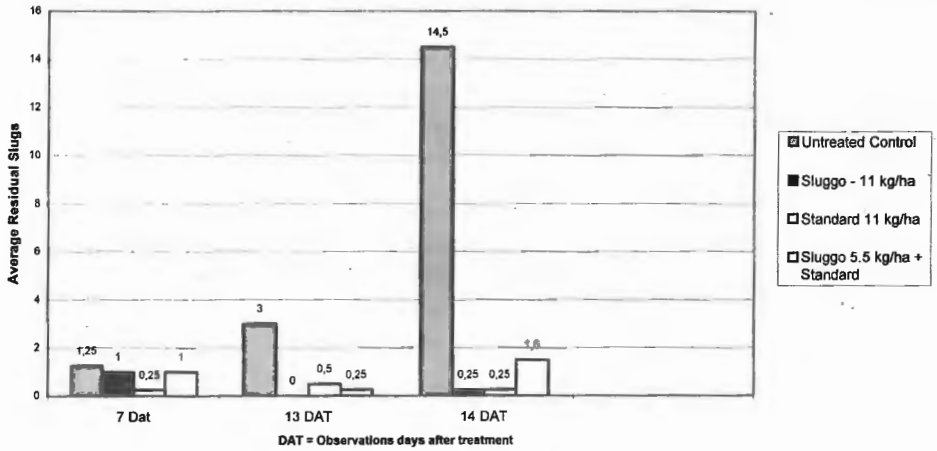

Figure 3. Counts of residual slugs found in plots a number of days after treatment

All slug baits and a combination of Ferramol and standard bait tested were effective in controlling grey garden slug in winter wheat. All the bait treatments were statistically different from the untreated control. No statistical difference was found between the bait types or combinations of bait.

Oilseed rape* / D. reticulatum *Germany

The application occurred shortly after planting. A broadcast application of the slug baits was done. The following graph presents the increase in number of plants / m^2 (in %) of bait treatments compared to untreated plots (control plots = 100%).

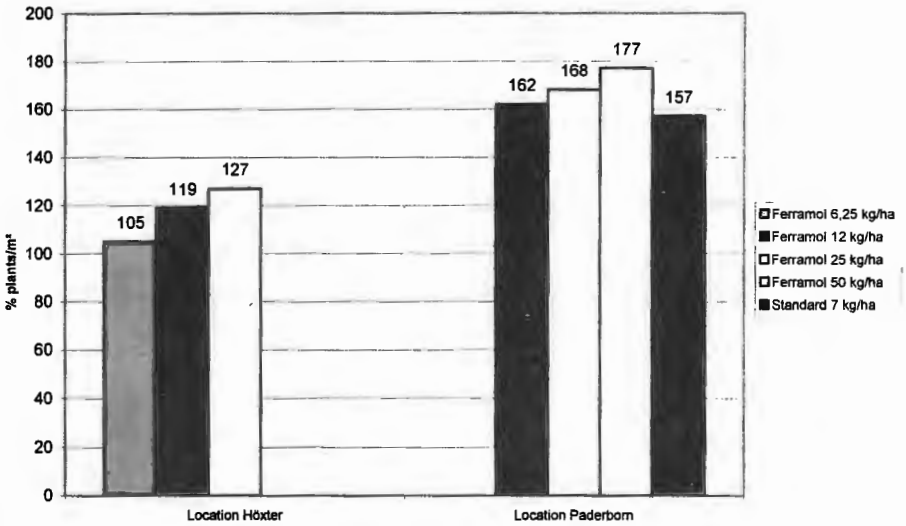

Figure 4. % number of plants, compared to an untreated control, under different treatements of Ferramol at Höxter and Paderborn trial sites

At all tested application rates, the number of plants was increased (Figure 4). At 12 kg Ferramol per hectare provides a significant increase in emerged plants.

***Ornamentals* / Limax marginatus**

The purpose of this trial was to assess the efficacy of Ferramol on ornamentals against the slug species *Limax marginatus*. The slug baits were scattered over the soil around the plants and during the course of the test the dead slugs were counted. The mortality at the end of the trial is shown in the graph below.

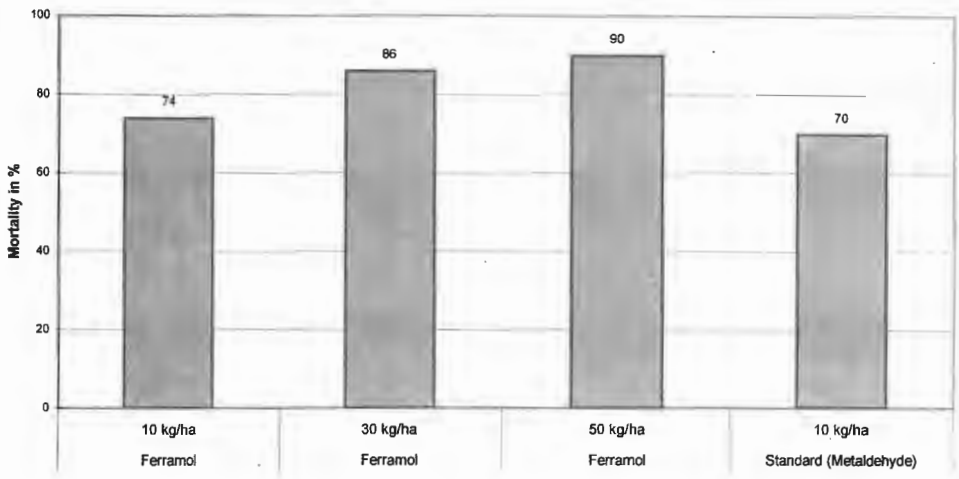

Figure 5. % mortality of *L. marginatus* under different treatment conditions of Ferramol compared to a Metaldehyde control

Even at the low application rate of only 10 kg/ha Ferramol gave high kill on *L. marginatus* (Figure 5).

Summary

The trials conducted clearly showed that in agricultural applications of Ferramol, the correct application rate and method of application must be determined for each crop and that a generalized rate is not suitable for field use. Ferramol (Sluggo) is an effective bait on various crops at low application rates and is cost effective in the commercial agricultural market.

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Evaluating the effects of beetle predators on slug population dynamics

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Abstract: Adults of the generalist, predatory carabid beetle, *Pterostichus melanarius*, have been shown to prey upon slugs in field studies. This interaction between *D. reticulatum* and *P. melanarius* is one not well characterised by generalist, opportunistic predation. In locales where slug numbers are high, *P. melanarius* appears to specialise upon slugs and exert a strong selective pressure. What is not clear is how many slugs are taken and how strong the regulation is in these situations. Over the past four years, the Rothamsted Research slug group has developed a validated simulation model for slug population dynamics (Choi *et al.* 2005). In this paper, we describe the construction and results for an additional model for *P. melanarius* designed to evaluate how important predation by this predator is for *D. reticulatum* population dynamics.

Key words: predation, individual based model, slugs, carabids, *Deroceras reticulatum*, *Pterostichus melanarius*

Introduction

Adults of the generalist, predatory carabid beetle, *Pterostichus melanarius*, have been shown to prey upon slugs in field studies. In a five year study of the dynamics of the slug *Deroceras reticulatum*, Symondson *et al.* (2002) showed that the population dynamics of *P. melenarius* were coupled to those of *D. reticulatum*. Numbers of *P. melanarius* in year $t + 1$ were related to the abundance of slugs in year t, suggesting that slug numbers could be, in part, driving carabid dynamics. Within years, though, Bohan *et al.* (2000) showed that carabids aggregated to locales of high slug abundance and over the course of one month slug numbers declined through predation – where previously there were high slug numbers there were now few slugs. This suggested, in turn, that the carabid could regulate slug numbers, at least during the period of study. Armsworth *et al.* (2005) has recently studied the effects that this predation could have exerted upon the behaviour of slugs. Armsworth *et al.* (2005) found that *D. reticulatum* individuals of both sexes and all size classes will avoid locales previously frequented by carabids, showing for the very first time that slugs have evolved evasion responses to *P. melanarius*. The picture that emerges for the *D. reticulatum* and *P. melanarius* interaction is one not well characterised by generalist, opportunistic predation. In locales where slug numbers are high and make up the predominant prey group, *P. melanarius* appears to specialise upon slugs and exert a strong selective pressure. However, it is not yet clear how many slugs are taken and how strong the regulation is in these situations.

Over the past four years, the Rothamsted Research slug group has developed a simulation model for slug population dynamics (Choi *et al.* 2005). This model simulates the behaviour of individual slugs of a population residing within a field. The model is driven by the daily environmental variables of temperature and rainfall and has been shown to predict, well. the population dynamics of slugs for data from various localities and for runs of up to 3.5 years. Moreover, the model can also predict the magnitude and duration of crashes in slug numbers. However, one intriguing phenomena seen within the model is that it often over-predicts the abundance of slugs during the summer, during the period when adult *P. melanarius* are

present in fields. One explanation for this may be that the model, which includes no predation, is in actuality predicting the dynamics of slugs that would have occurred if no carabids had have been present in the field. The over-prediction would, therefore, represent the slugs that had been 'regulated' by *P. melanarius*.

In this paper, we describe the construction and results for a model of the *P. melanarius*–*D. reticulatum* predation interaction designed to test this explanatory hypothesis.

Materials & Methods

The construction of the individual-based model (IBM) of slug population dynamics is discussed in detail in Choi *et al.* (2005). Briefly, though, the IBM simulates a population of *D. reticulatum* within a "virtual" field. The IBM explicitly models two classes of object, slug individuals and field cells, which interact with each other according to the status of each individual slug. The slug population is divided into four distinctive stages: eggs, neonates, juveniles and adults. Procedures govern egg development, egg hatching, slug weight gain, movement, mortality and egg-laying, and are influenced by individual slug weights, food availability, and daily environmental information on rainfall and temperature (Choi *et al.* 2004). Stochasticity in the procedures permits individual slug status to change so that each grows uniquely. These stochastic procedures are based on statistical distributions estimated from the laboratory and field observations. Two time delays allow seasonality to be modelled. Each field cell represents the geographical information on that fraction of the field, having an explicit position within the field and particular properties relevant to slug habitation. Slug dispersal is determined by 1) whether they will move out from the current location according to crop attraction and slug damage and, if a slug decides to move, 2) the direction of movement as determined by a random process and 3) the distance of movement as determined by the current slug stage, weight, and environmental conditions. Through these processes, the field cells and individuals interact and co-modify the status of the other.

Carabids were introduced using similar individual-based modelling approaches. Because of poor understanding of the population dynamics of *P. melanarius*, particularly as larvae, all carabids were assumed to enter the field simultaneously as adults on the 15th May and leave the virtual field on the 20th August, simulating the presence of adult individuals in the field. During this period of field occupation, the carabids were assumed to have no mortality and all individuals that entered the field did not die. Once a day the carabids dispersed randomly, in both direction and distance to a maximum of 10 cells, to a new cell location and searched for slugs over the 8 adjacent cells and the one they then occupied. The carabids were assumed to target only juvenile slugs (Bohan *et al.* 2000) and successful contact with a slug led to a probability of attack, slug predation and death. Successful carabids then became satiated and rested for 11 days (Bohan *et al.* 2000) within the cell that predation occurred. In some simulations, a carabid avoidance behaviour was implemented to test the importance of predator avoidance by slugs (Armsworth *et al.* 2005).

The carabid model was simulated for a range of values of the distance of carabid dispersal, attack probability and abundance, and for the presence or absence of predator avoidance behaviours by the slugs. The number of carabid attacks, which can be used to simulate the results of ELISA tests for trophism, and abundance of adult and juvenile slugs were noted.

Results and discussion

We found that for some combinations of parameter values, the model described the observed population dynamics of slugs well, suggesting that the presence of carabids could explain the over-prediction of slugs by the IBM. The results of one simulation are presented in Figure 1.

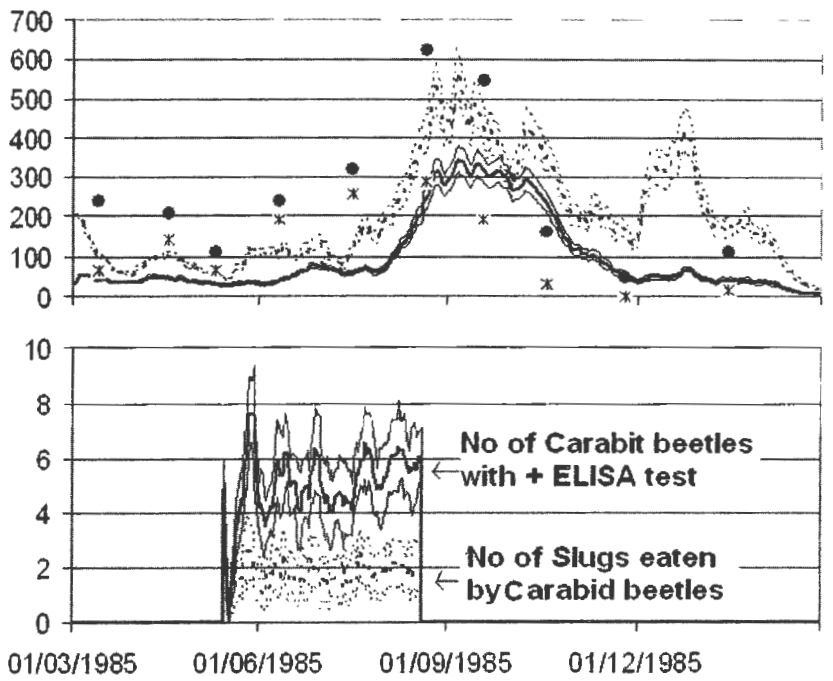

Figure 1. In the upper plot; observed adult (filled circles) and juvenile (stars) slug abundance with adult (thick lines) and juvenile (broken lines) numbers simulated in the IBM with 95% confidence intervals of the simulation. In the lower plot; the simulated number of carabids containing slug protein (thick lines) and the number slugs eaten by carabid beetles (broken lines) with 95% confidence intervals.

We found that the amount of simulated slug damage, over the year, was significantly lower in simulations where carabids were present. In general, the number of slugs eaten by the carabids was low, particularly when compared to the total population of slugs. The carabids appeared to be having effects that were more pronounced than expected from the numbers of carabids they ate. Interestingly, this effect was not due to predator avoidance. In simulations where slugs were allowed to avoid predators, the numbers of slugs taken and the damage caused by slugs was not significantly different from simulations where slugs could not adopt avoidance behaviours.

The simulation also produced output equivalent to ELISA analyses of carabid trophism. Because of the decay of rate of slug protein in carabid guts, which limits the ability to detect a

slug meal in carabid guts to about 2 days (Bohan *et al.* 2000), the estimated ELISA results for carabid predation are much lower than the number of slug attacks. On average, 5.7% of the carabids have consumed slugs each day. Typically, 16.8% of carabids contain slug protein at any one time although some 25.7% have recently been involved in slug attack.

Acknowledgements

We would like to thank Dr Bill Symondson and Prof. David Glen for their general discussions about slug-carabid interactions.

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Insect Pathogens and Entomoparasitic Nematodes
IOBC wprs Bulletin Vol. 28(6) 2005
pp.29 - 33

A comparison of growth trends among separate populations of the slug *Arion ater* (L.) in Biscay (Northern Spain).

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Abstract: Growth trends of four distinct populations of *Arion ater* from Northern Spain were followed over periods of one to two years. Growth patterns recorded in terms of live weight (LW, in mg) have been modelled by means of both logistic and exponential parabolic equations, which provide information about maximal weight attained (MW, in mg), intrinsic growth rate (r= mg/mg/d), starting point for growing processes (t_0 = days) and time when maximal weight is reached (t_{max} = days). As hatching occurs continuously from mid autumn to the end of winter, in each population studied, day one has been standardized to November 1st. Maximal weight appears equally established by both modelling equations revealing wide differences among populations: from 23.5 g LW in Kanala, to 18.5 g in Forua and Andoain, being the lowest in Atxuri: 16.0 g. The moment in which growth starts appears markedly different for the various populations (January 11th for Kanala, mid February for both Atxuri and Andoain, and around March 21st for Forua) which in combination with similar times for t_{max} (from mid September to mid October) results in growing periods from 245 to 292 days. Since intrinsic growth rate tends to common values (0.0244 mg/mg/d) irrespective of the population studied, maximal weight is directly correlated to the length of the growing period and, presumably, to the extension of winter associated restrictions (such as temperature or wind) upon foraging behaviour of slugs. As a consequence, geographically closely located populations as those under study, would be showing the effects of specific variations in orientation, distance from the sea shore, open lands vs. woodlands, leading to large variations (over 70%) in maximal weight attained.

Key words: slug, growth models, population dynamics, Gastropoda Pulmonata

Introduction

Arion ater, the black slug, is a conspicuous and ubiquitous representative of terrestrial molluscs - 64% of combined presence in a variety of habitats in Great Britain (Wareborn, 1969) - and is commonly blamed for horticultural damage in Atlantic Northern Spain. However, and in spite of the fact that it may well account for 19 to 40% of slug biomass (estimations for Great Britain given in Jennings & Barkham, 1976 and Lutman , 1978), information about pulmonate life cycles refers largely to small species (Lutman, 1978; South, 1989) and few studies have undertaken growth modelling in *A. ater* (Txurruka *et al.*, 1996; Txurruka *et al.*, 2003). In those works, growth curves built in terms of organic or dry matter of the various body organs have proved a useful tool to establish the time sequence of the different developmental stages. The aim of this work has been to analyze variability among growth patterns computed as live weight increases in four populations of *A. ater* from similar environments.

Material and methods

Sampling: Site selection and characterization

Four geographically distinct populations, inhabiting the coastal Atlantic grassland, were repeatedly sampled (every 3 wks.) over periods from 14 to 18 months at Kanala, Forua, Atxuri and Andoain in northern Spain. Mean temperature range was similar at all sites, ranging from 8.5 to 22 °C on yearly basis, while height from sea level ranged from 15m (Kanala & Forua) to about 150 m (Atxuri and Andoain).

Field studies based on the inspection of reduced areas (traps, cores, usually of 30 x 30 cm, see South, 1992 for a review of methods) provide scant data when it comes to lower density, large size species such as A. ater, where captures become erratic. If a modelling approach is to be followed, each stage of the life cycle should be adequately represented along the sampling period. We found that surface searching of a defined area (approx. 1250 m^2) at nightime in rainy weather rendered a stable number of captures (from 30 to 75 per sampling episode with a mean value of 45) which gives a reliable idea if not the "true" density of the surface active population. Total numbers of slugs obtained by this procedure range from 459 to 883. When captured, slugs were individually weighed and taken to the laboratory for further analysis.

Growth curves

Experimental live weight data (mg) has been fitted to both a logistic curve and an exponential parabolic curve by means of an iterative algorithm (Levenberg-Marquardt method). The following equations have been employed:

$$\text{Logistic: } W_t = W_{max}/1 + e^{(a-rt)}$$

where W_t = live weight (mg) at age t, W_{max} = asymptote to maximal weight, e= base of natural logarithms, a = a parameter of integration defining the position of the curve relative to the origin, r = intrinsic growth rate (mg/mg/d) and t = age in days;

and an Exponential Parabolic (Txurruka et al., 2003);

$$W_t = (t\text{-}to/tmax\text{-}to)^2 \cdot e^{[1-(t\text{-}to/tmax\text{-}to)2]} \cdot W_{max}$$

where t_o=day of the year when exponential growth initiates, t_{max}= time (days) when maximal growth is attained and W_{max} = maximal weight (mg).

Standardizing a common starting point (day 1) involved determining the period over which egg batches hatch. Considering every population under study, hatching occurs continuously from mid autumn (beginning of November) till the end of winter (february-march) and we have decided on November 1st as the starting point of the growth curve. This procedure does not estimate age of a given individual but growth dynamics of the year class. However, since A. ater is a semelparous species who experiences corporal exhaustion followed by death after egg laying (Chevalier, 1971; Txurruka et al., 1996, 2003) its life cycle is roughly annual.

Results and discussion

Prior to modelling growth, size frequency distributions for every sampling episode were analyzed in order to ascertain evolution of population structure. In Figure 1, the Kanala population has been taken to exemplify the clear separation between the parental (female) slugs and the newly hatched slugs.

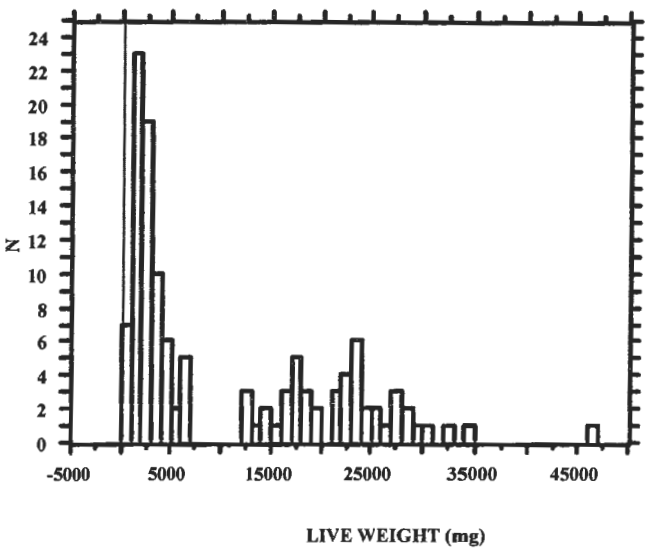

Figure 1. Size frequency distribution of *Arion ater* from Kanala: months of November & December 1995 .

Slugs captured along the month of January adjust to the cohort on the left-hand side (newly hatched) growth along this period appearing negligible. This finding probably reflects low viability of autumn born slugs along with continuous recruitment through hatching. Since embriogenesis appears to be suppressed in slugs below 4°C (South, 1992) and the period over which an egg batch hatches is extremely dependent on temperature (Q_{10}>3 between 5 and 15°C from Carrick, 1942 and Cook & Radford, 1988) variations due to geographical and even microhabitat differences are to be expected. This aspect will be dealt with later when models are discussed.

The main objective of this study was to combine the benefits of the logistic and exponential parabolic equations to portray main features of growth: W_{max}, intrinsic growth rate, t_0 and t_{max}. The results have been summarized in Table 1.

Maximum size is equally predicted by both models appearing as a robust parameter. Mean maximal weights range from 24 g in Kanala to 16 g in Atxuri. These figures largely exceed data for *A. ater* in the British Isles which could be explained in terms of reduced life span: data from Lutman (1978) shows that the adult breeding population in montane grasslands extends its presence no longer than September, while they can still be found in

November or even December in our latitude. Time when active growth is reinitiated shows significant differences among populations (January 11th vs. mid-March; C.V.= 17%) whereas the opposite is true for the moment when maximal size is reached which shows little variation (C.V.= 9%). Interestingly, although we have no knowledge of field studies about the length of the period prior to hatching, predictions derived from the exponential parabolic model for t_o (82-138 days) relate well to the figures reported by Cook & Radford (1988) for several *Limax* species in Northern Ireland whose egg-lying peaks show seasonal correspondence with *A. ater* in our area: between 56 and 183 days between 5°C and 10°C. Attempts to establish a relationship between t_o and temperature in terms of day-degree are not conclusive (South, 1992). Recently, Gillooly et al. (2002) have presented an equation that explains well such temperature dependence for both embrionic and post-embrionic development in a variety of ectotherms. In any case, precise information about cumulative temperature exposure remains uncertain when field studies are undertaken.

Intrinsic growth rate appears similar (mean = 0.024 mg/mg/d) with the exception of Atxuri (0.050). This last figure gives unrealistic predictions in terms of maximum weight for this population. Since the shape parameter of the equation (a) covariates positively with t_o, some distortion appears when a values increase, which happens when the "lag phase" becomes longer. The common 0.024 value predicts a mean weight-doubling time (1/r) of around 40 days.

The length of the growing period (between 8 and 10 months) is largely determined by t_0, and, since intrinsic growth rate is common (0.024), maximum weight is also largely dependent on t_o. Live weight doubles at a mean rate of 40 days which considering the period between t_0 and t_{max}, implies 6-7 doubling episodes over the year. Smallest sizes of infantile slugs range from 68 to 800 mg with a mean value around 250 mg. Starting from that size, maximum weight predictions would range between 24 g for Kanala (6.5 duplications) and 16 g for Atxuri (6.0 duplications), such difference mainly relying on the time extension of winter associated restrictions (such as temperature or wind) upon hatching and foraging behaviour of small slugs.

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Insect Pathogens and Entomoparasitic Nematodes
IOBC wprs Bulletin Vol. 28(6) 2005
pp.35 - 39

On the importance of temperature and moisture to the egg laying activity of a pest slug, *Deroceras reticulatum* (Müller)

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Abstract: Damage caused by slugs has been increasing in the last 30 years, which could be caused in part by changing climate. Egg laying has been shown to be important when describing the dynamics of populations, therefore understanding the effects of temperature and moisture on oviposition will be key when predicting how populations will change with climate. Here we found that most eggs are laid at 53% soil moisture and 18.36^{0}C. The number and proportion of fertile eggs also varies with temperature and moisture as does the number of hatching eggs. However the proportion of hatching eggs is more strongly drive by moisture. Our results show that slugs adjust their egg laying behaviour to the surrounding temperature and moisture conditions.

Key words: slug, egg, oviposition, temperature, moisture, activity, weather

Introduction

Slugs are highly sensitive to changes in environmental conditions; predicted changes in climate will therefore affect the distribution of slug species and the damage that they cause. A good understanding of how slugs adapt to changes in environmental conditions is vital when predicting their future distribution. Egg laying has already been shown to be important in determining population sizes (Schley & Bees 2002) and it is therefore important to know how oviposition behaviour will change with climate.

The eggs of *D.reticulatum* have no structural adaptation to avoid desiccation (Runham & Hunter 1970), although they are laid in batches reducing evaporation. *D.reticulatum* has been found to lay it's eggs down the sides of the grass *Dactylis glomerata* L. (South 1965; Stephenson 1975), in soil composed of fine or medium soil particles and also amongst the large lumps resulting from cultivation, these behaviours help eggs to retain water. The development of eggs is highly dependant on environmental conditions, the risk of predation and attack by fungi increases the longer they take to develop. Newly hatched slugs are also sensitive to desiccation via their permeable integument, small size and low dispersal abilities.

Modification of egg volume and the size of batches also help to maintain the moisture required for egg development. In *Laevicaulis alte* (Férussac), the garden slug, specialised faecal pellets are laid around egg masses, which are thought to maintain a humid environment for the egg and enable proper development to take place. It has been shown that eggs require a high moisture content to enable proper development to take place (Raut & Panigrahi 1988).

The most complete account of oviposition behaviour with varying temperature and moisture was given by Carrick 1942 and states that eggs are laid between 3 and 20 ^{0}C and 25-75% soil moisture, with 10-20^{0}C and 64% being optimal. Few other studies consider fertility and egg laying with temperature and rainfall in *D.reticulatum*. Further, the effects of different environmental conditions on resource allocation in egg batches have not, to our knowledge, been investigated.

Here we consider how slugs adapt their behaviour to the environmental conditions of temperature and soil moisture with respect to egg laying. Eggs per slug per week, total number fertile, proportion fertile, number hatching and proportion hatching are investigated.

Material and methods

Boxes were lined with 1.4 g of cotton wool, 60 g of sterilized loam; 20, 40 60 or 80mls of tap water and labelled. Four replicates of each were placed inside larger foil wrapped boxes in a random order at; 4, 10, 15 and 23^{0}C. Boxes were lit by a fluorescent desk lamp (9.5/14.5) at a height of 28cms; the height was kept uniform using a frame.

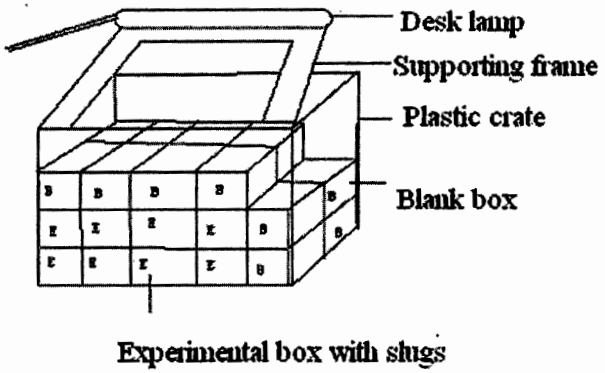


Figure 1. Diagram of experimental boxes.

Boxes containing just soil were placed on top of the experimental boxes and around the exposed sides, including an extra replicate of each moisture. A thermometer was also placed in the bottom of each large box (Figure 1). Chinese cabbage (cv Wong Bock) oil seed rape (both grown in Rothamsted greenhouses) and a filter paper disc with dry 'Ready Brek' breakfast cereal on it were supplied in ample quantities to each box.

Slugs were separated into weight classes, four were placed in each box, the same combination of classes was used in each. A stock of replacement slugs were kept at 15 ^{0}C. Samples were taken three times a week for six weeks. Dead slugs were replaced with one of the average weight in the box whenever available. All slugs were weighed each Monday. Any decaying food was removed and the box searched for eggs. Eggs were counted, five removed, placed in Petri dishes lined with damp cotton wool and measured using an eye piece graticule under 6x magnification. They were then incubated at 15 ^{0}C for two weeks after which fertility was assessed, dishes over two weeks old were searched for newly hatched slugs.

One moisture replicate from each temperature was retained after the sampling period along with the blank replicates. The soil from these boxes was weighed and then oven dried to a constant weight, from this the percentage soil moisture in each box at each temperature was calculated. The results were weighted and analysed using ANOVAs. They were then modelled using surface response curves.

Results and discussion

Number of eggs per slug per week

Eggs per slug per week were found to vary over the course of the experiment ($p<0.001$) and also significantly with both temperature ($p<0.001$) and moisture ($p<0.001$). When modelled using quadratic surface response curves the average optimum temperature was 18.36 ^{0}C and moisture 53.1% (Figure 2).

These results show that slugs adjust egg laying to optimal conditions. Producing eggs is costly and the individual may suffer reduced future fitness as a result of laying large batches. In order to produce the maximum number of offspring which survive to reproduction larger numbers of eggs may be laid in conditions which are optimal for the development, hatching and survival of the newly hatched neonate.

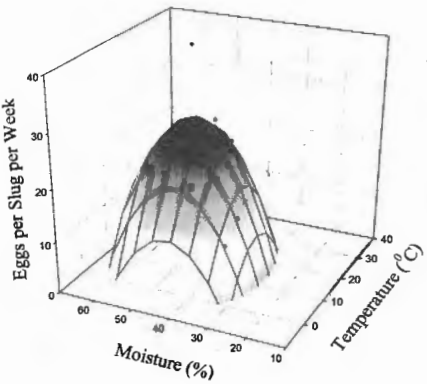
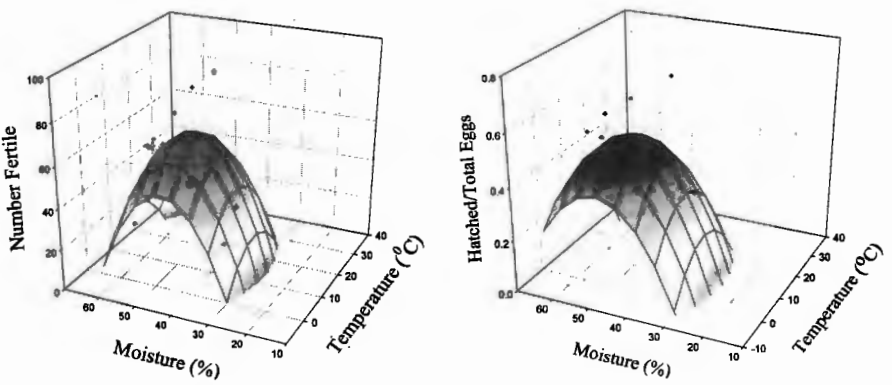


Figure 2. Surface response curve showing the number of eggs produced per slug per week in week 5.

Fertility

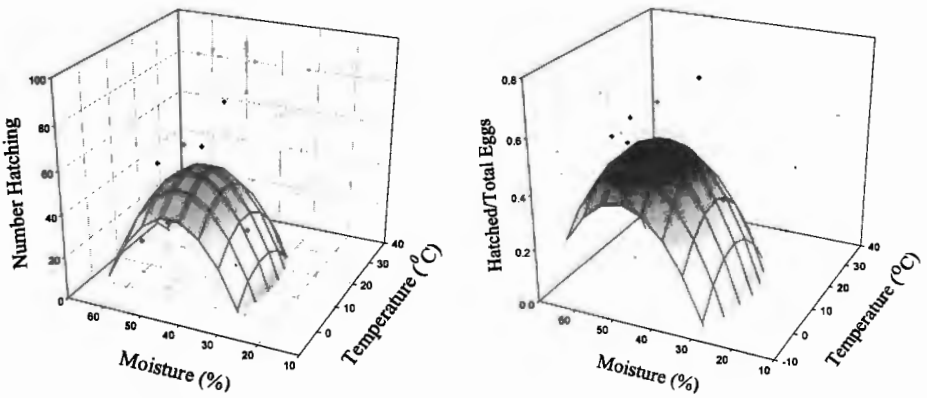
The number and proportion of fertile eggs also vary significantly with respect to temperature and moisture ($p<0.001$ and $p<0.001$ for number and $p=0.002$ and $p<0.001$ for proportion respectively). When modelled with quadratic surfaces the optima were estimated to be 10.28 ^{0}C and 47.7 % soil moisture (Figure 3) for number fertile and 5.35 ^{0}C and 47.9 % soil moisture (Figure 4) for proportion fertile. These results suggest that slugs adjust the number of fertile eggs that they lay according to the temperature and moisture conditions.



Figures 3 & 4. Surface response curves showing the number of fertile eggs laid and the proportion of fertile eggs laid.

Hatching

When the total number and proportion of eggs hatching were analysed using ANOVAs significant relationships were found between both variables and temperature and moisture ($p<0.001$ for all). When modelled as surface response curves the optimum conditions for the total number of hatching eggs was predicted to be 9.46 ^{0}C and 47.9 % soil moisture (Figure 5). The predicted optimum for the proportion of hatching eggs was predicted as 50.6 % soil moisture (Figure 6). Unfortunately, this model does fit well with respect to temperature and therefore we were unable to make an accurate estimate of the temperature optimum. The proportion of hatching eggs doesn't appear to be strongly related to temperature suggesting that the moisture is more important. However the number of hatching eggs is driven by both temperature and moisture.



Figures 5& 6. Surface response curves showing the number of hatching eggs and the proportion of hatching eggs in week 5.

General conclusions

Our results suggest the slugs alter the number of eggs that they lay according to temperature and moisture conditions. The number of realised (hatching) offspring also changes with temperature and moisture increasing the probability of them being born in good survival conditions. Slugs appear to be fertilising a greater proportion of their eggs in certain temperature and moisture conditions. These results show that slugs adjust their egg laying activity to optimise their fitness, investing the most when conditions are optimal.

The optimum moisture levels appear relatively constant at 48-53% soil moisture, the temperature optima, however, varies. The optimum temperature for laying eggs is higher than those predicted for the fertility or hatching measures, this may reflect the higher energetic expenditure in relation to laying eggs.

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Insect Pathogens and Entomoparasitic Nematodes
IOBC wprs Bulletin Vol. 28(6) 2005
pp.41 - 51

Modelling growth of the reproductive tract of slugs (*Arion ater*) from two populations of Urdaibai (Biscay, Northern Spain)

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Abstract: Growth of somatic tissues as a whole and three reproductive structures have been fitted to a bell shaped and an exponential parabolic curve models. In this minireview, using modelled parameters as a framework, published data on the endocrinology, histology, physiology, biochemistry and allometry of slugs have been for the first time integrated in a theoretical framework that may help researchers to precisely time their experiments in key moments of the life cycle of slugs. To know 'when' it is most likely to find a hormone and it is easier to study its controlling role on a specific tissue or organ will aid to deepen our present knowledge on mollusc endocrinology and will help to manage mollusc populations more effectively, from snail farming to pest control.

Key words: slug, growth models, population dynamics, Gastropoda, Pulmonata

Introduction

In temperate regions *A. ater* (L) has an annual life cycle (Abeloos, 1944; Chevallier, 1971; Godan, 1983) that, as has been reported by many authors, may be strongly influenced by climatic factors (South, 1992 and references therein). In fact, unfavorable weather conditions may affect growth rate, delay the onset of reproduction (Laviolette, 1950) or, in extreme cases, even prevent reproduction until next year (Chevalier, 1971).

Growth curves of slugs in the field follow the usual S-shaped, logistic trajectory (Calow, 1981), but weight losses occur frecuently during senescence (Smith, 1966; South, 1992). Growth of *A. ater* in a population at Kanala in northern Spain has been modeled in some detail by our research group (Txurruka et al., 1996; Txurruka et al., 2003). Modelling techniques using equations with parameters of immediate biological interpretation have proved to be powerful tools for extracting order and patterns from an apparently chaotic collection of data points. According to the values of the parameters from the logistic, bell shaped and exponential parabolic models it seems that the growth patterns shown by the different body sections of *A. ater*, belonging to both somatic and reproductive tissues, are the result of a tightly coordinated process. It has been the aim of the present research to study the effect of subtle differences in climatic conditions on key life cycle parameters of slugs in two populations.

Material and methods

The two sampling areas studied in this work at Forua (43°20'20"N 2°40'20"W) and Kanala (43°22'50"N 2°40'20"W), are geographically close and slightly above sea level. The sampling area at Kanala faces WSW whereas that at Forua has a ENE orientation and is more shadowy

leading to an expectation that Forua might be colder than Kanala. Slugs were collected in 1995 every 3 weeks. In total 424 slugs from Kanala and 401 from Forua were dissected, freeze-dried and ashed for 14 h at 450 °C. Weights of the somatic organic matter and of the organic matter in the three germinal tissues (Ovotestis, Accessory Sex Organs (ASO) and Albumen Gland (AG)) were fitted to two three-parameter curves, respectively described by a bell shaped (BS) curve model (Txurruka et al., 1996):

$$W_{(t)} = W_{max} \cdot e^{\left[-\frac{1}{2}\left(\frac{t-t_{max}}{t_{inf}-t_{max}}\right)^2\right]}$$

where, e = base of natural logarithms, t = day of the year, t_{inf} = day of the year at which the left inflexion point is reached, t_{max} = day of the year at which maximal organic matter content is attained, $W_{(t)}$ = Weight (mg) of the organic matter content at day t of the year, and W_{max} = Weight (mg) of the maximal organic matter content, and an exponential parabolic (EP) model (Txurruka et al., 2003):

$$W_{(t)} = \left(\frac{t-t_0}{t_{max}-t_0}\right)^2 \cdot e^{1-\left(\frac{t-t_0}{t_{max}-t_0}\right)^2} \cdot W_{max}$$

where, e, t, t_{max}, $W_{(t)}$ and W_{max} are as in the previous equation, and t_0 = day of the year at which exponential growth starts.

The values of the parameters which best fitted the observations were computed in a three-step process. First, a continuous simulated annealing algorithm was applied (Goffe et al., 1994), in order to properly identify the global maximum, without being trapped in local minima. Then, the results of the simulated annealing method were refined using a Levenberg-Marquardt optimization step. Finally, the average value and standard deviation in the parameters that can be achieved with observational data were estimated by means of a bootstrap method with replacement (Press et al., 1992) of 37% of the elements in the sample, with 5000 random subsamples. The fittted line would describe the growth curve followed by the modelled organ of an ideal slug, usually known as "young-of-the-year".

Results and discussion

Parameter values of the fitted curves are shown in Table 1. Values given for t_{inf} in the EP model and values listed in the column entitled MGR (Maximal Growth Rate) and MIGR (Maximal Intrinsic Growth Rate) are derived quantities, because, not being equation parameters, these values cannot be directly estimated during the iterative process of curve fitting: therefore they were obtained indirectly, after substituting the values of the fitted parameter in the equations.

Somatic tissues

According to values of t_0 parameter in the EP model, exponential increase in weight of organic matter in somatic structures (body wall + digestive gland + empty gut) would start at the very beginning of February (day 32 of the year) in Kanala, whereas this process would be delayed for nearly one month and half in Forua (up to the 72th day of the year, mid March). The t_0 date indicates the end of the long apparent lag phase observed in the increase in mean

weight of slugs populations during winter. The apparent lag phase is probably the result of, at least, the following two phenomena: firstly, the slow weight gain of slugs during winter (Chevallier, 1971); and secondly, the more or less continuous recruitment of newly hatched very small individuals throughout winter, because the length of embrionary development, and therefore hatching, is negatively correlated with temperature (Le Calve, 1989). The northeastward orientation of the sampling site in Forua will probably lead to lower mean temperatures than those of Kanala. These colder temperatures will likely be responsible for the delay in exponential growth at Forua. As far as the time-to-go for the life cycle of *A. ater* in our study area is quite fixed and more or less coincident with winter beginning (Txurruka et al., 1996), other factors being equal, a smaller average size at maturity (Kozlowski & Wiegert, 1986) could be predicted. This is really the case, as can be seen in the W_{max} column in Table 1.

Also in Table 1, it may be observed that the around 40 days delay in the t_0 of Forua in relation to Kanala becomes shortened to an about 30 days lag when t_{inf} of both populations are compared, and shortened again to circa 20 days when t_{max} are likened. Therefore, slugs in Kanala have an about 7 months growing season, which is around one month shorter in Forua. Maximal Growth Rate (MGR) is about 40% higher in Kanala, (≈11 mg of Organic Matter (OM) of somatic tissues /day) than in Forua (≈8 mg OM/day), a fact that, combined with the alredy cited longer growing period, brings about a ≈60% heavier slugs in Kanala than in Forua. Nevertheless, when values of the Maximal Intrinsic Growth Rates (MIGR, calculated dividing a Maximal Growth Rate by its corresponding W_{inf}) are compared, it may be seen (Table 1) that in both sampling sites values are very similar, about 13 µg OM/mg OM/day for the whole fraction of somatic tissues. It seems that this value represents the upper limit of the growing capacity of the somatic structures in our study area.

Looking at the subject from photoperiodic and hormonal viewpoints, it is worthy to note that t_0 in Kanala lies just in the middle point between winter solstice (when daylight is shortest) and spring equinox (the day of the year in which day and night become equal length); that is to say, after continuous lengthening of the daylight may have been photoentraining some of the photosensitive cerebral cells, as the neurosecretory Dark Green Cells (DGC) of the mesocerebrum (Ezzughayyar, 1993; Flari & Edwards, 2003). These DGC synthesize and release somatotropic growth hormones (Wijdenes & Runham, 1977; Ezzughayyar, 1993) that may be homologous to some molluscan insulin-related peptides (MIPs) (Flari & Edwards, 2003). Furthermore, Ezzughayyar (1993) proposed that DGC may control the activity of the dorsal bodies (DB), a type of endocrine cell located in the connective sheath of the circumoesophageal brain (South, 1992; Saleuddin, 1999).

Lengthening daylight might lead to increased production of somatotropic hormone and, as a consequence, higher growth rates. The hormone–growth relationship is not straightforward as, besides hormones, the actual growth rate depends on a number of factors, with food being one of the most important. The same holds true at Forua, where lower mean winter temperatures may constrain both the foraging activity of newly hatched slugs as well as plant growth.

of the bell shaped (B S) and exponential parabolic (E P) curve models (Weight of organic matter in the organs (W) vs day of
G R (Maximal Growth Rate) and M I G R (Maximal Intrinsic Growth Rate) are diagnostic values, i.e., derived quantities.
ssory Sex Organs, OVO=Ovotestis, SOM= Somatic Tissues.

n	r^2	Organ	$t_0 \pm$ Sd (day)	$t_{inf} \pm$ Sd (day)	$t_{max} \pm$ Sd (day)	$W_{inf} \pm$ Sd (mg)	$W_{max} \pm$ Sd (mg)	M G R ± Sd (mg /day)
24	0.523	SOM	-	154.24 ± 0.06	248.95 ± 0.05	982.28 ± 0.22	1619.5 ± 0.36	10.371 ± 0.00
24	0.537	SOM	32.025 ± 0.17	131.61 ± 0.09	244.71 ± 0.01	761.25 ± 0.09	1590.6 ± 0.18	11.937 ± 0.01
24	0.720	OVO	-	221.60 ± 0.03	249.87 ± 0.03	51.407 ± 0.02	84.756 ± 0.04	1.819 ± 0.00
24	0.512	OVO	122.86 ± 1.41	184.11 ± 0.68	253.67 ± 0.19	27.402 ± 0.25	57.246 ± 0.14	0.699 ± 0.02
30	0.624	A S O	-	247.98 ± 0.03	285.40 ± 0.07	170.55 ± 0.07	281.18 ± 0.11	4.558 ± 0.00
30	0.661	A S O	207.87 ± 0.06	242.60 ± 0.02	282.06 ± 0.02	181.23 ± 0.10	286.01 ± 0.16	6.154 ± 0.01
87	0.396	A G	-	276.67 ± 0.01	312.59 ± 0.06	215.68 ± 0.34	355.59 ± 0.56	6.004 ± 0.01
87	0.405	A G	225.89 ± 0.04	265.56 ± 0.02	310.54 ± 0.07	219.24 ± 0.13	346.00 ± 0.20	6.525 ± 0.00
01	0.464	SOM	-	185.77 ± 0.05	266.98 ± 0.07	620.92 ± 0.16	1023.7 ± 0.26	7.646 ± 0.04
01	0.472	SOM	71.914 ± 0.75	165.39 ± 0.30	271.55 ± 1.47	485.25 ± 1.45	1013.9 ± 3.02	8.107 ± 0.04
01	0.664	OVO	-	235.99 ± 0.02	265.18 ± 0.02	31.6105 ± 0.04	52.115 ± 0.07	1.083 ± 0.01
01	0.558	OVO	146.98 ± 0.37	204.00 ± 0.06	268.79 ± 0.32	18.898 ± 0.05	39.482 ± 0.18	0.517 ± 0.00
70	0.353	A S O	-	265.38 ± 0.02	289.06 ± 0.03	124.35 ± 0.27	205.02 ± 0.45	5.252 ± 0.01
70	0.338	A S O	233.47 ± 0.03	257.7 ± 0.02	285.22 ± 0.02	117.37 ± 0.13	185.22 ± 0.20	5.713 ± 0.00

Remarkably, t_{inf} at Kanala happens about six weeks later than a key point of the photoperiod cycle, about one and half months after spring equinox, that in the Northern hemisphere happens on day 21±1 of March (day 81±1 of the year). This day too, lies just in the middle point of two key days of the photoperiodic cycle, the spring equinox and the summer solstice. Present knowledge of molluscan endrocrinology does not allow us to elucidate the probably sequential photoactivation of different parts of the slug brain (and tentacles, see below) (Gomot de Vaufleury, 2001; Flari & Edwards, 2003). However, decelerating growth rate in somatic tissues will be the result of complex interacting neurocrine and endocrine products modulated by signals coming from somatic tissues and information about energentic reserves. Signals of this kind will be ultimately responsible for Forua slugs reaching their maximal weight one month later than those at Kanala (Table 1).

Finally, about six weeks after the summer solstice and, therefore, under a photoperiodic regime of shortening daylight, actual t_{max} (Table 1) in each population will depend on how much food resource can be obtained to fulfill metabolic requirements for sustaining growth once development of all the reproductive tissues has been triggered (see below).

Ovotestis

For ovotestis development, there was a shorter delay between the t_0 of the two populations than observed for the somatic structures: t_0 occurs at day 123 (early May) at Kanala and day 147 (late May) at Forua (Table 1). Therefore, the lag in ovotestis development between the two populations has been reduced about 50% compared with that estimated for somatic tissues. It takes ≈130 days to attain full growth of the ovotestis in Kanala and ≈120 days in Forua (Table 1). As occurred in somatic tissues, $\dot{W}_{max}$ and MGR of the ovotestis are also higher, ≈55%, in Kanala than in Forua. Nevertheless, as was the case in somatic tissues, MIGRs are essentially identical in both populations, about 30 µg OM/mg OM/day. This value is ≈2.2 times higher for ovotestis alone than for the somatic tissues (Table 1). From these data it can be easily deduced that in *A. ater* the structural capacity for growth is remarkably higher in germinal organs than in somatic tissues, a common feature of animals.

Ovotestis development is controlled by photoperiod and temperature (Chevalier, 1971; Gomot de Vaufleury, 2001) as well as by food (Laviolette, 1950; Chevalier, 1971) and putative "organic correlations" among different tissues (Laviolette, 1954). It seems that light exerts its influence through the direct action on the ovotestis of a "tentacular hormone" (TH) produced by the endocrine "collar cells gland" (CCG) (Takeda, 1982; Joose, 1988; Flari & Edwards, 2003), with increasing light inducing and stimulating spermatogenesis (Pelluet, 1964; Takeda, 1982) and inhibiting autodifferentiation of female line cells, i.e., precluding oogenesis (Takeda, 1985; Joose, 1988; Gomot de Vaufleury, 2001). Light also controls ovotestis growth by means of the cerebral ganglia (CG); directly, through the secretion of a male gonadotropic neurohormone (MGN) (Takayanagi & Takeda, 1985; Gomot de Vaufleury, 2001) and, indirectly, inhibiting the secretion of (a) hormone(s) by DB. The hormone(s) secreted by these cells inhibit(s) male and stimulate female cell lines in the ovotestis (Joose, 1988). Moreover, an extraocular photoreceptive pathway may exist in some slugs (Flari & Edwards, 2003).

Looking at t_0 values for ovotestis development (Table 1), it can be realised that the start of exponential growth of the ovotestis takes place about 6 (Kanala) to 9 (Forua) weeks later than the spring equinox. These seem reasonable values for a process involving an initial photoperiodical activation of CCG and CG, and the ensuing synthesis and secretion of, respectively, TH and MGN, at the same time that neuronal inhibitory control of DB by CG is taking place (Gomot de Vaufleury, 2001). The overall resultant of all those interrelated

hormones will be the triggering of ovotestis development, probably in coincidence with the initiation of the "early spermatid stage" described by Smith (1966).

Certainly, as far as the photoperiod regime must essentially be the same in our two sampling sites, the approximate 3 week lag in Forua demands complementary explanations. If temperature affects development of the male cell line in the ovotestis of *A. ater* in a way similar to that pointed out by Gomot de Vaufleury (2001) in the snail *Helix aspersa*, it may be predicted that, compared to a site facing ENE (Forua), the higher mean spring temperature expected in other with WSW orientation (Kanala) will result in an earlier t_0 in this last site. Furthermore, temperature may influence ovotestis growth indirectly, through its effect on plants growth, because, as pointed by Laviolette (1950), genital maturation is directly dependent on nutritional factors. Allometric changes induced by food in the slug's tissues as a consequence of preferential accumulation of different biochemical compounds in the reserve organs (Txurruka et al., 2003) may account for the "organic correlations" influencing genital maturation (Laviolette, 1954).

From some developmental stage on, the ovotestis behaves as an endocrine gland and produce hormones, chemical messengers that are steroid in nature (Takayanagi & Takeda, 1985) and resemble the steroid sex hormones of vertebrates (Joose, 1988), although up to date no one molluscan gonadal hormone has been neither identified nor purified (Flari & Edwards, 2003). Androgens will stimulate the development of the male parts of the ASO and, by means of a negative feedback, will inhibit production of TH by CCG. On the other hand, female parts of ASO, including AG, will be the target of estrogens synthesized by the ovotestis (Joose, 1988).

t_{inf}, and therefore beginning of deacceleration in ovotestis growth (Figure 1), happens in mid-July at Kanala and in early-August at Forua, in close synchrony with t_0 of development of ASO (Table 1). At this time the ovotestis will probably be at the "mid-spermatozoa stage" (Smith, 1966; South, 1992) and several structures of the ASO, specially the male parts, are going out from their "undifferentiated stages" (Smith, 1966).

Day of t_{max} of ovotestis is synchonous with t_{max} of somatic tissues and it is attained when ovotestis is in its "late-spermatozoa stage" (Smith, 1966). Several authors have mentioned the apparent lack of a relationship between age, body weight and reproductive development in *A. ater* (Abeloos, 1944; Laviolette, 1950). There may exist great individual variability in the body weight to genital weight ratio in slugs when their entire life cycles and/or the reproductive systems as a whole are considered. However, if specific and relatively short time periods of the life cycle (specifically, time lapse between somatic and ovotestis t_{inf} and t_{max}) and, furthermore, if only somatic and ovotestis weights are considered, inter-individual variability may be reduced to such a degree that direct correspondence between somatic and gonadal weights may be brought to light (Txurruka & Ortega, 1996). This "growth dynamics agreement" may be clearly seen in the fact that synchronous occurrence of somatic and ovotestis t_{max} is predicted in the modelled young-of-the-year slug (Table 1).

From t_{max} date on, the ovotestis loses weight (Figure 1), probably because some sperm begins to be passed out (Laviolette, 1950) and stored in the still developing hermaphrodite duct, a part of the male ASO (Smith, 1966). Afterwards, the ovotestis enters in its "oocyte stage" (Smith, 1966) and by this time TH has surely become ineffective (Flari & Edwards, 2003). Then, the ovotestis shrinks considerably (see Fig 8 in Abeloos, 1944) and follows a weight trajectory that fits particularly well to the descending part of a bell shaped curve (see r^2 values in Table 1 and Txurruka et al. 1996).

Accessory Sex Organs

It may be observed that, compared with ASO t_0 for Kanala (day ≈208, last week of July), the ASO t_0 for Forua (day ≈ 233, 3th week of August) shows more or less the same delay estimated for the ovotestis; at about 25 days. Nevertheless, a trend to synchronization between the two populations is clearly more evident in the ASO than in somatic tissues and ovotestis, because when the ASO of slugs in the two populations reach their inflexion points, timing difference between Kanala (t_{inf} ≈ day 245, early September) and Forua (t_{inf} ≈ 261, mid September) has alredy become shortened to half, i.e., to about 13 days. Further synchronization continues up to the ASO attain their maximal weight, during the second week of October, just when timing coincidence between the two populations is nearly perfect, with a delay at Forua of only 3 days (Table 1).

Onset of exponential growth of the ASO, revealed by the t_0 value in the exponential parabolic model (Figure 1), will probably be the gravimetric consequence of a morphogenetic process that comprises the genital atria, epiphalus and spermatheca (GAES) entering their "enlarging stage" as well as the common duct (CD) leaving its "early male stage" (Smith, 1966).

When the subject is analysed from a photoperiodic viewpoint, it is clear that exponential growth of the ASO starts 4 to 7 weeks after summer solstice (Figure 1), and, therefore, it may be assumed that production of TH by CCG is occurring at its highest rate. It is reasonable to think that at such time lapse after daylight started to shorten, on the one hand, photoperiodically controlled TH secretion will be showing lowered rates and, consequently, feminization of the gonad is being permitted (Flari & Edwards, 2003). On the other hand, and in a similar way, from summer solstice on, photoperiod-sensitive CG have probably being releasing less and less neurosecretory inhibitory products and, as a result, DBCs have increased the synthesis and secretion of DBH, a hormone involved in the control of multiple female-related reproductive processes, from organogenesis to mating behaviour (Joose, 1988; Saleuddin, 1999; Flari & Edwards, 2003). Moreover, it may be noted that t_{inf} is happening just a short time before autumm equinox (21st±1 of September) (Figure 1),. By this time, GAES have probably gone into their "copulation stage" and CD may be in its "early female stage" (Smith, 1966).

Change to a decelerated growth rate of the ASO development, as it is revealed by t_{inf}, may indirectly indicate when mating can occur. Likewise, copulation happens in a remarkably tight synchrony with weight of somatic structures attaining their highest value (Table 1). It seems that, in both study sites, up to this moment of the life cycle of *A. ater*, external resources derived mainly from food have been enough to sustain the development of all the growing structures in the slug. From early-to-mid September, though, declining plant production, as a result of shortened daylight length, in combination with increasing energetic and plastic requirements from rapidly growing reproductive tissues (Table 1), lead to the necessity of beginning the withdrawal of materials from internal sources (i.e., from reserves accumulated in the somatic tissues) (Txurruka et al., 2003) as a means of supplementing the increasingly scarce external supplies.

Finally, as has been pointed out earlier, our models predict that the maximal weight (t_{max}) will be reached just at the end of those "copulation" and "early female" stages, about 3 weeks later than the day of the year in which night begins to last longer than day (Figure 1),. Therefore, as was the case for ovotestis, key points (t_0, t_{inf} and t_{max}) of the growth curve of the ASO appear correlated with main shifts in daylight length at the equinoxes and summer solstice.

In order to fully understand the physiological and hormonal processes underlying growth patterns described by our models, it would be worthwhile to consider the precise timing of photoperiodical switching for several extra-gonadal (neuro-) endocrine tissues whose products interplay in several activation/inactivation cascades dealing with the development of the ASO. Furthermore, as far as it has been established in terrestrial pulmonates the ovotestis exerts a hormonal control of the ASO development (Joose, 1988; Gomot de Vaufleury, 2001; Flari & Edwards, 2003), it should be a compelling task to start the study of the time-course production of the gonadal hormones, so that it might be known which are the hormones that in each moment are actually envolved in the control of the development of the different parts of the ASO.

The overall result of all those intertwined processes is that at about 2 and half months at Kanala and in slightly less than 2 months at Forua the ASO has fully grown. The ASO in slugs of Kanala (W_{max} ≈283 mg OM) are about 45 % larger than the ASO in slugs of Forua (W_{max} ≈195 mg OM). Nevertheless, as a consequence of the already mentioned synchronization, on the one hand, MGR is nearly identical in both populations (slightly more than 5 mg OM/day) but, quite unexpectedly, MIGR is about 50 % higher in slugs of Forua (≈45 µg OM/mg OM/day) compared with slugs of Kanala (≈30 µg OM/mg OM/day) (Table 1). A key point to understand this phenomenon may be to take into consideration the fact that the structural demands of completely functional ASO with adequate working capacity (for example for mating, egg-laying, etc.) may require a minimal size. According to our models (Table 1), it seems that in the two study areas this minimal size must be attained before a photoperiodically fixed but physiologically modulated date, regardless of when exponential growth of the ASO has actually started. To attain this functionally adequated minimal size, with a delayed t_0 in Forua with respect to Kanala, and coupled to synchronous t_{max} in both populations, will require higher intrinsic growth rates in Forua (Table 1).

Albumen Gland

When compared with t_0 of AG development in Kanala (day ≈226, mid August), slugs of Forua showed a t_0 (day ≈258, mid September) with the usual delay of about 1 month observed in the previously described two reproductive structures. It was a date so late in the season that in the studied year (1995) female slugs were found in Forua only in two samplings after that day, 3 and 6 weeks later, this last one just in the end of November. Therefore, with only two samples providing slugs with non-zero-weight albumen glands, models of AG yielded unreliable parameter values in Forua; for example, t_{max} in mid February of the next year, maximal weight close to 2000 mg, etc. In consequence, parameter values of AG models of Forua have not been used for comparison with Kanala.

Anyhow, the reliable t_0 values of both populations may give insight into the link between resource availability and changes in growth dynamics, as has alredy been pointed out, concretely, when synchronism between t_{max} of somatic structures and t_{inf} of ASO development has been discussed. As a matter of fact, it looks like, after triggering the exponential growth of the AG, at Kanala, the accelerated weight increase of the AG may be compatible during 3 weeks or so with a decelerated accretion of materials in the somatic structures as well as with the still exponential development of the ovotestis and the ASO. Up to this moment (in very late August), this growth may have been sustained all at the same time by means of resources coming from food. Just in the middle point between t_0 and t_{inf} of AG development, resource demands derived from exponentially growing ASO and AG (Table 1) become higher than the amount of resources that can be delivered by the digestive system. To compensate for the increasing imbalance, reserves (especially carbohydrates and proteins)

must be withdrawed from somatic tissues, probably from body wall (Txurruka et al. 2003). Furthermore, this growth is so 'explosive' that from this date on its competing demands will alter, lowering, ASO growth rate (Figure 1), which just in this moment will reach the inflexion point (Table 1). At this point AG will be entering the "secreting stage" in which "eosinophilic secretion is being stored in the cytoplasm" (Smith, 1966). The highly significative negative correlation that in the female stage of *A. ater* exists between the dry weight of genitalia and the dry weight of the body wall, and more specifically between the genital index and the carbohydrate content of the body wall (Txurruka & Ortega, 1996), would likely be indicating which is the main source of the materials that are being stored in the AG. A glucagon-like hormone will probably facilitate breakdown and export of polysaccharides in the "source" tissues (specially body wall) and, meanwhile, some other insuline-like hormone will likely favour polysaccharide synthesis in the "sink" tissues ASO and AG.

Under the influx of both the growth-promoting female steroid hormones secreted by the ovotestis (Flari & Edwards, 2003) as well as the vitellogenic hormones released by DB, freed under short daylight photoperiodic regimes from the inhibition imposed by the CG (Joose, 1988), AG will grow very rapidly (Figure 1), up to reaching a MGR of about 6.3 mg/day or a MIGR ≈29 µg/mg/day, values that are very similar to those showed by the ASO (Table 1). By t_{inf} of AG (end of September), DBCs are probably secreting their products at its highest rate, galactogen synthesis in the AG is at its peak (Flari & Edwards, 2003), ovotestis is in its "oocyte stage" (see above) (Smith, 1966) and it is producing (posibly) steroid hormones at a high rate (Gomot de Vaufleury, 2001). As regards the histology, AG is entering its "mature stage" in which the cells are "full of secretion" (Smith, 1966).

When t_{max} is reached, ≈310th day of the year (Table 1), in the first week of November, AG is fully mature, and probably an egg-laying hormone is being produced in the brain (Gomot de Vaufleury, 2001; Flari & Edwards, 2003). As far as the AG is the major supplier of biochemical resources for the fabrication of eggs, with some contribution of the ASO (Txurruka et al. 2003), oviposition will drain materials from these two parts of the reproductive tract, especially from the AG. At this time of the year resources derived from food will most likely not suffice to replenish organic and inorganic materials removed from reproductive tissues and channeled to the eggs, and the AG will begin to lose weight (Figure 1). Therefore, t_{max} of the models may be indicating the onset of egg-laying in the "young-of-the-year". After this date on, actual number of clutches and total weight of laid eggs will depend on the weather severity and the amount of reserves accumulated during the growing season. Consequently, and taking into account that *A. ater* is a semelparous species with an annual life cycle, it may be expected that fecundity in this animal will be a feature that will show both high individual and interannual variability.

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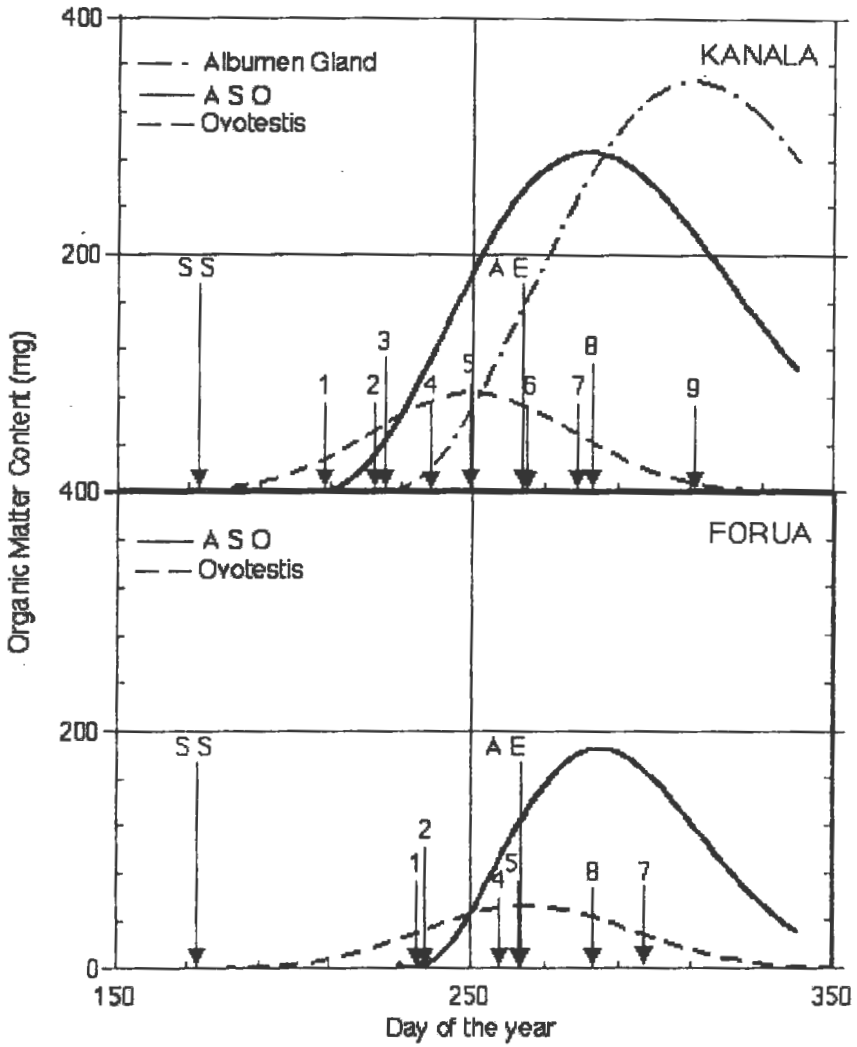

Figure 1 - Modelled growth of the reproductive structures in the two sampling sites, Kanala and Forua. SS: Day of the summer solstice; AE: Day of the autumn equinox; 1: t_0 of ASO; 2: t_{inf} in the ascending part of the bell shaped curve of ovotestis growth model; 3: t_0 of albumen gland; 4: t_{inf} of ASO; 5: t_{max} of ovotestis; 6: t_{inf} of albumen gland; 7: t_{inf} in the descending part of the bell shaped curve of ovotestis growth model; 8: : t_{max} of ASO; 9: t_{max} of albumen gland.

Insect Pathogens and Entomoparasitic Nematodes
IOBC wprs Bulletin Vol. 28(6) 2005
pp.53 - 57

Estimation of surface active slug populations using refuge traps

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Abstract: It has been suggested that refuge traps underestimate the number of small slugs that are active on the soil surface, whereas counts of large slugs are thought to be more reliable. This was investigated using time-lapse video techniques to record individual movement of *Deroceras reticulatum* (Müller) of different sizes underneath refuge traps made of infra-red transparent material. Aspects of slug activity studied included characteristics of trap entry (e.g. numbers of each size entering, the timing of entry and the number of re-entries during the night), the numbers present at dawn and activity patterns after sunrise. No significant differences were found between large (>500mg) and small (<100mg) slugs for any of the activities studied. Our results show that there is no difference in the efficiency with which traps estimate the activity-density of large and small slugs. For both large and small slugs refuge traps underestimate the total numbers of slugs and this is due to slugs leaving traps before dawn.

Key words: *D. reticulatum*, population, refuge trap

Introduction

Refuge traps are used to estimate the level of slug activity on the soil surface in order to aid the decision of whether to treat an area with molluscicides. The traps consist of a shelter material, e.g. an upturned plant pot saucer or sacking, which may cover bait (Young *et al.*, 1996). The shelter is laid on the soil surface and provides a moist environment attractive to slugs. It is left in the field overnight when conditions are favourable for slug activity and slug numbers are counted early the following morning. If the average catch exceeds a threshold it is recommended that molluscicides are applied.

Numerous studies comparing refuge traps with other more labour intensive methods of population assessment, for example defined area traps, have reported that refuge traps tend to underestimate the numbers of small slugs present, although they are thought to be more reliable for large slugs e.g.(Clements & Murray, 1991; Glen & Wiltshire, 1986). Reasons for this could be that fewer small slugs enter refuge traps or do so, but leave before the catch is assessed. Alternatively small slugs may be present, but simply missed in counting. The study presented in this paper investigated these possibilities. The species used was *Deroceras reticulatum* (Müller 1774). Movement of small and large individuals underneath saucer traps transparent to infra red light was recorded using time-lapse video techniques.

Materials and methods

Recording technique

A high resolution, infra-red sensitive camera (Sanyo VCB 3572IRP) was connected to a time-lapse video recorder (Panasonic AG-6720A) set to record 180 h of slug activity on a standard 3 h VHS cassette. Daytime lighting was provided by two 400W halogen bulbs which were switched on and off at prevailing sunrise and sunset times by a timer switch.

Refuge trap
A refuge trap made of infra-red transparent plastic was designed. This was manufactured to the same dimensions as an 18cm diameter 'standard' plastic plant pot saucer. It was opaque to the naked eye, but was transparent to infra-red radiation.

Indoor experiments
Experiments were conducted at 13±2°C in a controlled temperature room. Arenas (57 x 36 x 16 cm) were filled with loamy soil dug from an agricultural plot. Any large organic particles, stones and soil invertebrates were removed. The soil surface was raked to a fine tilth and watered with approximately 750ml tap water. Three large (>500mg) and three small (<100mg) *D. reticulatum* were introduced to the arena. They were allowed to acclimatise for 24 hours prior to recording during which period they were starved. A fluon barrier painted on the upper rim of the arenas prevented escape. After the acclimatisation period 5ml of Chicken Layers' Mash was placed in the centre of the arena. This was then covered with an upturned infra-red transparent saucer and recording began. There were 8 replicates in total.

Outdoor experiments
Experiments were repeated outdoors in semi-field conditions. The set up was similar to that described for the indoor experiments, but the camera was encased in waterproof housing and additional daytime lighting was not required. The arena was sunk into the ground so that it was flush with the soil surface. The infra-red transparent saucer traps were lightly sprayed with an anti-mist coating to prevent condensation from obscuring the image. Again, there were 8 replicates in total.

Statistical analysis
Numbers of slugs of each size entering or leaving the refuge trap at various points throughout the night were analysed using Chi-square or Fisher's Exact test as appropriate. One-way analysis of variance (ANOVA) was carried out on transformed data on the timing of events. The influence of slug size on whether individuals were present under the trap at dawn was assessed using the Scheirer-Ray-Hare test.

Results and discussion

Preliminary experiments showed that the anti-mist coating on saucers used outdoors had no significant effect on slugs compared with uncoated saucers.

Trap entry
Results from indoor experiments were confirmed by outdoor experiments. The number of large and small slugs entering the refuge trap did not differ significantly. For both size classes, the majority of slugs entered the trap at least once during the night (Fig. 1).

Although, on average, the elapsed time between onset of activity and the first occasion slugs entered the trap was shorter for large slugs than small slugs in indoor experiments and the converse in outdoor experiments there was considerable variation in entry times and these differences were not significant in either case (Table 1).

When traps are baited with a non-toxic food, e.g. chicken layer's mash, it is possible for slugs to leave again. In this study many slugs were observed to enter and leave the traps several times per night, rather than enter and remain underneath for the duration. There was, however, no difference in the number of slugs of each size class that re-entered the trap once or two-plus times.

These results show that, not only do small slugs enter the traps in comparable numbers to large slugs, but that the timing of this is also similar between size classes. Furthermore, slug size does not appear to influence the frequency of re-entries. It would seem unlikely, therefore, that reported underestimates of small slugs in refuge trap catches are due to some difference in the behaviour of small and large slugs when they encounter the trap or in their response to the trap environment once underneath.

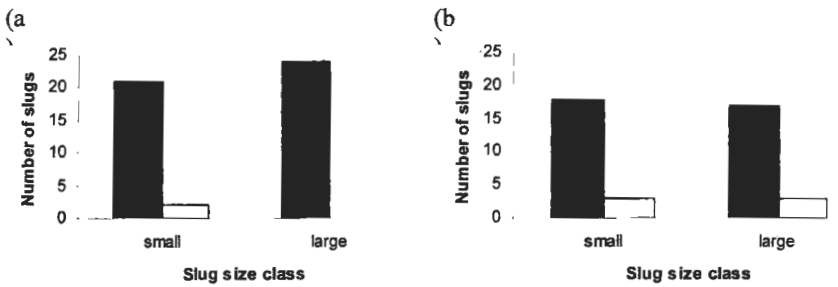

Figure 1. Number of small and large slugs entering the refuge trap at least once during the night (a) indoors and (b) outdoors. (Black bars: slugs entering trap; White bars: slugs not entering trap)

Table 1. Mean time before the first trap entry for small and large *D. reticulatum* in indoor and outdoor experiments.

	Mean time to first trap entry (± SE)	
Slug size	Indoors	Outdoors
Small	107.84 ± 38.37	50.36 ± 26.41
Large	75.24 ± 20.48	129.31 ± 91.84

Number of slugs present at dawn

More slugs of both sizes entered the trap during the night than were present at dawn (indoor and outdoor experiments: $P<0.05$) (Fig 2). In indoor experiments slightly more large slugs entered the trap than small slugs whereas this was reversed in outdoor experiments. In both conditions, however, the reduction in numbers observed at dawn did not differ significantly according to size. For both small and large slugs there were a third or fewer under the trap at dawn than had entered during the night.

These results, suggest, therefore, that estimates of surface active slugs provided by refuge traps are comparable for small and large individuals; similar proportions of the total active population are trapped for each size.

Activity after dawn

Under the constant temperature conditions of the indoor experiments there was no significant difference in the number of small and large slugs leaving traps between dawn and midday; only one individual of each size class was observed to do so. Under normal field conditions, however, it might be expected that the environment under a refuge trap would become increasingly inhospitable for slugs as air temperaturs rise after dawn and heat trap surfaces. This could result in slugs leaving the trap before the catch has been counted. In the outdoor experiments presented here, however, this was found not to be the case. Again, only one slug of each size left the trap between dawn and midday.

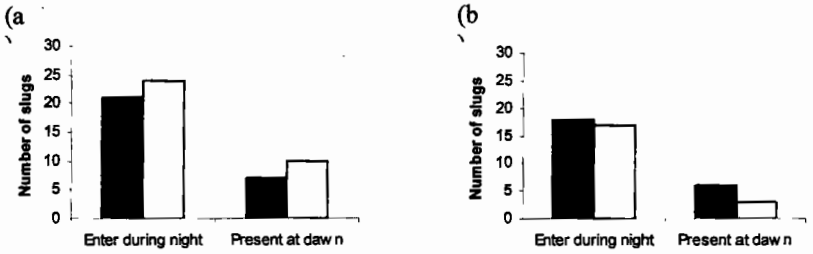

Figure 2. Number of small and large slugs entering refuge traps during the night and number of each size present at dawn (a) indoors and (b) outdoors. (Black bars represent small slugs and white bars represent large slugs).

The experiments were conducted in April 2004. Whilst the weather was mild (mean maximum teamperature 11.6°C; range 8°C–21°C) with some sunny days, Tinytalk™ dataloggers showed that there was only 1°C difference in the mean soil temperature and mean temperature under the trap suggesting, at least under the conditions of the experiment, any heating effect was minimal. It is possible that in the summer when there are wider extremes of temperature the results may have been different, but in spring we found no evidence that underestimates of small slugs by refuge traps could be due to them leaving the traps after sunrise.

Summary

In summary, these experiments indicate that there is little difference in activity patterns of small and large *D. reticulatum* under refuge traps. Catches represent a sample of the total surface active population that is comparable for small and large slugs. The difference in counts of slugs between the sample and total active individuals in an area is most likely due to slugs leaving the trap during the night and not returning. Reported underestimates of small slugs relative to large slugs does not appear to be due to any differences in their behaviour towards traps. The simplest explanation is that it is caused by observer error.

Acknowledgements

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Insect Pathogens and Entomoparasitic Nematodes
IOBC wprs Bulletin Vol. 28(6) 2005
pp.59 - 63

Responses of *Deroceras reticulatum* to the annual effects during the last three years

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Abstract: The population density of the pest slug *Deroceras reticulatum* was assessed between 1999 and 2004 in two adjacent 1.25 ha sections of a commercial arable field at Berolzheim-Ahorn, Boxberg, Germany. One of the two field sections had been shallow ploughed annually since 1996 **(Pl-section),** whereas the other was tine cultivated (non-inversion soil tillage) **(Cu-Section).** These two regimes were replicated twice on each field. All other husbandry measures, including crop varieties, were identical during the study period. The number of eggs laid per litre of soil was used as a density parameter. Three mixed samples at each of three different depths (0-5 cm, 5-10 and 10 - 15 cm) were taken at weekly intervals from 2002, excluding periods when the topsoil was frozen. The eggs were washed out in a washing tower with six different mesh sizes starting with 0.6 cm^2 and ending with 0.1 cm^2 at the bottom. Weather records were obtained from a full automatic weather station nearby, providing standard measurements.

The results indicate significant annual changes in the slug densities in the two field sections. In contrast to 2002, the egg number fell drastically from spring 2003 until mid-2004. The impacts of the extreme high temperatures in both 2002 and 2003, along with the associated drought, exerted a 'knock-down' effect on slug populations. Significant differences were observed between the tillage regimes, with highest densities in non-inversion tillage soils. The distribution pattern of *Deroceras reticulatum* eggs in the soil profile showed the highest abundance in the 0-5 cm top layer. This declined in the 5-10 cm layer and in the 10-15 cm layer there were almost no slug eggs to extract.

Key words: *Deroceras reticulatum,* soil tillage, population dynamics, impacts of abiotic factors.

Introduction

A reliable parameter for population assessment of pest slugs is an essential prerequisite for population dynamics studies of molluscs as well as for forecasting damage risk (South 1964; Armsworth et al 2003). A number of techniques are commonly used to assess slug densities. The refuge bait trap is said to be easy to handle (Glen & Wiltshire 1986; Frank 1998; Voss et al 1998) but the catches are rather difficult to interpret. Traps currently in use are either baited with slug pellets (Glen et al., 1993) or non-poisonous bait (e.g. chicken layers mash) (Young et al, 1996). None of them, however, seem to be sophisticated enough to fulfil practical requirements, ease of use and accuracy. In contrast, the barrier trap is reported to be extremely laborious and as difficult to implement in standing crops as the widely recommended and most accurate floatation method in regaining mobile slugs (Glen et al 2004, this volume). Sieving soil samples under flowing water enables recovery of slugs from different development stages, but is also reported as laborious and destructive. Sieving under gently flowing water, however, provides considerable information about the slug population present and its structure, assuming that the methodological bias of sampling and washing is acceptable. In the present studies slug eggs were used as a population size indicator, generating basic data for population models of the pest slug species (Shirley & Bees 2001; 2003). Egg density analysis allowed comparisons of the impact of soil tillage management

regimes, descriptions of the vertical distribution pattern of the slug population and an illustration of the impacts of the extreme climatic conditions in the preceding years. The aim is to obtain a practical means to trace population development at a given site sown with common arable crops.

Material and methods

The field site

Two field strips each measuring 50 x 250 m (1.25 ha) were used in these studies. The first strip had been ploughed since 1996 using a shallow mouldboard plough whereas the adjacent strip was annually cultivated by a broadshare tine cultivator (Dutzi).

A soil sample of 5-7 kg was taken at weekly intervals. This was composed of three subsamples dug at random from each of three depths (0 -5, 5-10 and 10-15 cm), in both field sections (PL and CU). The mixed samples were transported in PVC-containers into the laboratory, where they were washed out separately. Samples of each soil layer were examined between 2002 and 2004. The extraction procedure began by pre-soaking the soil of each sample separately before distribution over the surface of the top sieve (largest mesh size). A gentle stream of water was applied until all clods were broken. The remaining sediments on each of the six sieves were examined to detect the presence of eggs (and slugs), when necessary using a magnifying lens (Fig. 1).

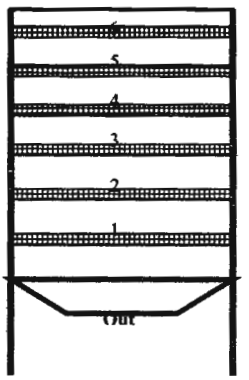

Figure 1. The structural layout of a heavy duty metal tower with a 6-sieve-set for washing out slugs and slug eggs from soil samples as used at Berolzheim, Germany

Eggs, juveniles and adult slugs were picked out of the soil sediments and identified as far as possible to species. The egg numbers obtained were extrapolated to calculate slug number per litre of soil.

Results and discussion

The extraction technique

A total of 300 soil samples were analysed until mid-July 2004. These provide the base-line data for the following interpretation. The extraction sieve tower was shown to be very effective. A large amount of soil can be processed in a reasonable time span and immediate results can be obtained. The dark brown colour of wet soil on the sieve surface served as a high-contrast background and improved the detection of the hyaline white coloured eggs. A disadvantage of this method, however, is that both soil sampling and the operation of washing out slug eggs are time consuming procedures, requiring a considerable input of labour.

Changes in the population density in response to weather conditions

Average slug density varied significantly between 2002 and 2004 (Fig. 2). The largest egg densities were generally recorded in spring and/or autumn, whereas extremely low numbers or no eggs at all were recovered during the summer months. The number of eggs extracted was highest in 2002 and dropped drastically through 2003. The low egg recovery rate lasted into Spring and Summer 2004. The temperature records of summer 2002 may provide an explanation for the reduced activity during this period. Until May 2002 the egg density was high with the average number of extracted eggs ranging from 1-7 per litre of soil during spring of this year. The egg density, however, fell to zero from June 2002 onwards.

The low population size indicated by slug egg numbers in autumn 2002 lasted into the Spring and Summer of 2003. The peak temperature in Summer was greater than 35° C with a daily average exceeding 25°C. This temperature range is far beyond the optimum temperature for *D. reticulatum*. The extreme temperatures are likely to have a lethal desiccation effect on this species leading to diminution of egg laying rate. *D. reticulatum* is reported to respond to temperatures over 17 °C by weight loss and reduced growth rate. The highest temperature of 2004, along with the persistent drought of that year have obviously restricted the reproduction of the slugs causing a further decline in the population.

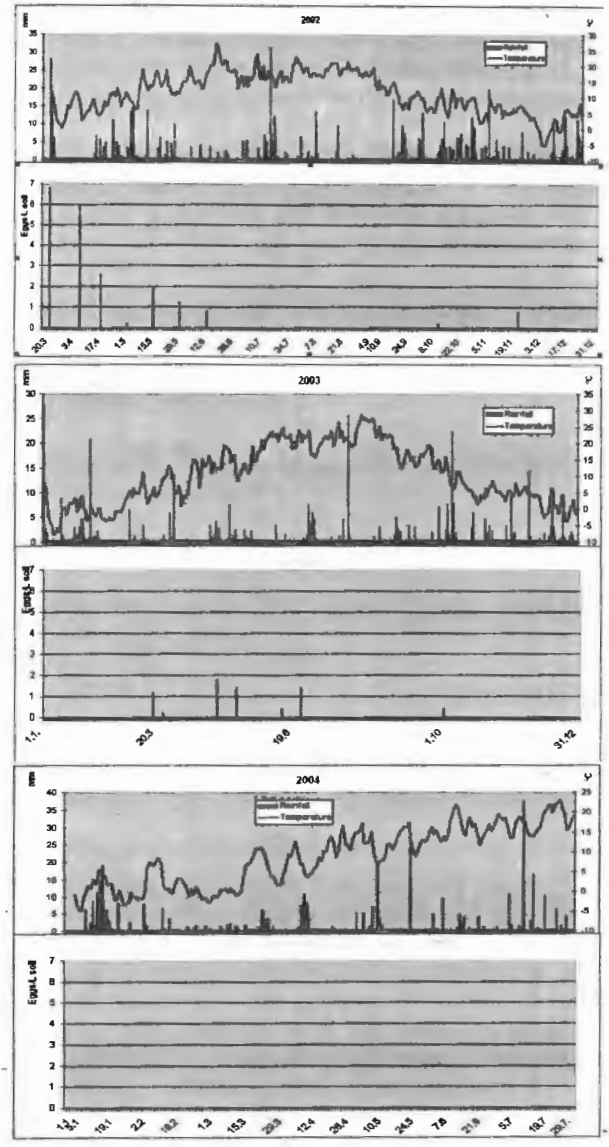

Figure 2. Fluctuation of egg density of *D. reticulatum* between 2002 - 2004 under the prevailing weather conditions of these years

No eggs were extracted over the 23 sampling occasions from 2nd February until July 2004. The absence of eggs indicates a severe scarcity of oviparous slugs. Both the prevailing drought and the extremely high temperature of the preceding optimal reproductive period seem to have caused a dramatic reduction in the local slug population. There is no indication of a recovery in the populations up until the present time (August 2004).

A few slugs were, however, trapped after a rainy period, suggesting that the population is capable of recovery given favourable conditions.

Impacts of tillage regime on slug egg density

The average number of eggs in the cultivator managed soils was generally higher than in ploughed soils. However, the differences were not consistent over the study period. The vertical distribution of slug eggs within the top 15 cm soil layer showed a distinct pattern (Fig. 3).

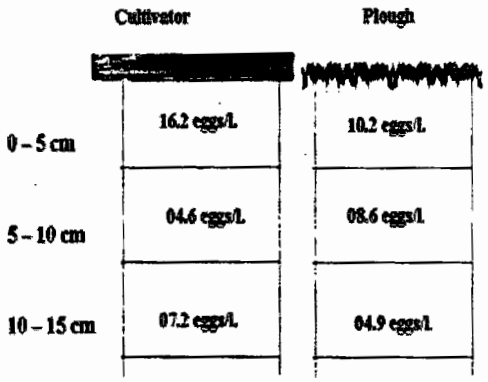

Figure 3: Annual average number of slug eggs obtained over period of 139 weeks as distributed in the three topsoil layers (0-5, 5-10 and 10-15 cm) at Berolzheim, Germany.

In the ploughed soils there was a gradual decline in the number of slug eggs with increasing soil depth. The surface top 5 cm contained the highest average number of eggs per litre of soil, whereas the deepest layer of 10-15 cm contained just under half this amount. In the cultivator treatment there was a higher number of eggs laid in topsoil but the distribution did not show the same pattern. Soil cracks, burrows and numerous holes resulting from decomposed roots may have provided good routes for slugs to move up and down. This hypothesis is supported by the higher soil humidity in deeper layers.

In summary, the prevailing weather conditions during 2003 and 2004 had an adverse effect on slug populations. According to the literature *D. reticulatum* is likely to reduce its activity as temperature exceeds 20°C. When average daily temperature exceeds the optimum of 17 °C slugs are expected to loose weight with negative consequences for oviposition and the following generation (South 1982. The long–term drought (soil moisture < 4%) induced by the severe shortfalls in precipitation during 2003 and 2004 may have exacerbated this impact on the slug population. Lethal effects on slug eggs, juveniles and adults are expected to occur. When soil water content falls below 10% of the field capacity, body water losses are expected (Shirley & Bees 2003). This was indeed the case during the observation period. The

synergistic adverse effects of both high temperature and drought seem to have caused a 'knock-down' effect on slug population in the study area.

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Insect Pathogens and Entomoparasitic Nematodes
IOBC wprs Bulletin Vol. 28(6) 2005
pp.65 - 73

A change in *Monacha cantiana* distribution following a change in the use of arable farmland.

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Abstract: Despite the weather, arable farming practises and predators, *Monacha cantiana* is apparently both widespread and abundant in the field margins of arable farmland in the Debden Valley of North West Essex. As agricultural practises change, *M. cantiana* could become a pest in this area. In July 1996 no live snails were found during hand sampling within a Debden Valley field of wheat. However in 1997, one year after an area of this wheat field was re-seeded to permanent grassland, seven snail species were recorded in the new grassland; *M. cantiana* was the most abundant but juvenile growth was slower than expected from the literature. Data obtained from sampling in an area of the wheat field that had been re-seeded with mixed vegetation, suggest that both snail abundance and population structure were affected by the vegetational differences. Although the majority of juvenile *M. cantiana* survived from May to August 1998, most failed to grow to a size capable of reproduction, even though this species is thought to have an annual life cycle.

Key words: *Monacha cantiana,* distributional change, growth, gastropod, agriculture

Introduction

Monacha cantiana is a largely nocturnal gastropod snail principally found in the Mediterranean area but spread by man in England (Kerney, 1999). Currently *M. cantiana* is common, and increasing over much of eastern Britain (Killeen, 1992) where it occurs among tall grasses and herbs, in roadside verges, field edges and railway embankments but not in woodland (Paul, 1975). During the last century, the distribution of this species increased in England, despite the temperate climate and intensive farming. For example, according to Kerney (1999), *M. cantiana* was first noted in Shropshire (1909), Fife (1920), Staffordshire (1943), Cornwall (1947), Pembrokeshire (1957), Derbyshire (1969), Anglesey (1974), Sutherland (1975), Aberdeenshire (1980), Cumberland (1980), Cardiganshire (1983), Carmarthenshire (1985) and Lancashire (1994). The common name for *M. cantiana* is the Kentish snail, which perhaps reflects the fact that, in Britain, it is most abundant in the southeast of England (Kerney & Cameron, 1979).

In the East Anglian region of England, a high proportion of the land is devoted to arable farming and molluscs within the crops are controlled by the use of molluscicides. However, seasonal sampling, at 18 field margin sites, recorded populations of snails. Snail abundance was greatest in ditch field margins alongside cropped fields on chalk or sandy soil; the most abundant species was *M. cantiana* which accounted for 37.4% of a total of 14,091 live snails and shells recorded across five consecutive seasons of the year in the Debden Valley of East Anglia (Ward-Booth, Paglia & Dussart, 1996). *M. cantiana* has attacked clover, navel oranges and lettuce in Egypt (El-Khodary, Helal, Sharshir & Shahawy, 2000) and, similarly, if agricultural practises change, could become a pest in England

Agricultural practises are changing in England. Thus oil seed rape is drilled into harrowed but not ploughed wheat fields; because of expense, molluscicide use is being

restricted to periods of high crop prices (Brooks & Crook, 2004); there is an increase in organic farming; alternative crops may provide an improved habitat for invertebrates and vertebrates, for example during biomass production e.g. *Miscanthus giganteus* and, coming shortly, arable farmers applying for the Single Payment Scheme will need to implement a two-metre buffer zone around hedges and watercourses by July 2005. Such a buffer zone may be increased in width to as much as ten metres if set-aside strips are put along the field margins (Department for Environment, Food and Rural Affairs, July 2004).

No information on the colonising ability of *M. cantiana* was found in the literature so an experiment was carried out to ascertain the colonisation of land by *M. cantiana* after a change of land use from arable crop to permanent grassland and also a change of land use from arable crop to mixed vegetation.

Material and methods

The colonisation of land by* M. cantiana *following a change in monoculture. (arable crop to permanent grassland)

In the spring of 1996, a piece of an arable field alongside a ditch field margin on clay soil in the Debden valley, was sold (Ordnance Survey reference TL 552 342). Timed hand searches in 1996 in the area of site TL 552 342 had recorded live snails in the field margin but no live snails had been found within the field of wheat. The previously ploughed and harrowed land was left fallow over the summer. Over the summer of 1996, stones were removed by hand and in the autumn the land was hand-seeded with fodder mix and rolled by the new owners. No live snails were found when the area was surveyed before the ground was seeded. The Fodder mix for permanent grassland contained a mixture of grass seeds plus red clover seed (Trifolium pratense). The earth was dry and friable. Many of the seeds failed to germinate until the following spring.

On 2nd August 1997 the grassland was cut for hay. The experimental design was for a survey of snails within 8 adjacent strips each 76.2 cm. wide and 58.0 m. long. The last strip was 3 metres from a ditch field margin. The grass was cut using an Allen scythe with a 76.2 cm. cutter bar. Table 2 shows the arrangement of the area sampled. Each strip was divided into four sections. Each section of the strip was cut and sampled in turn. The cutter bar was set high to cut the grass approximately 8cm. above ground level in order to cut the grass above the level of occupants such as toads, mice and snails. Snails on grass stalks dropped to the ground, as did the cut hay. All live *M. cantiana* and shells were recorded and where the shell had an internal rib as well as a reddish colour near the aperture the individual was classed as a fully grown adult. The abundance of other snail species in the samples was also recorded. Finally, in order to compare species occurrence in the grassland with species occurrence in the nearby ditch field margin, six 15-minute hand searches were carried out. Each of the six searches was within a 3 x 1 metre strip laid transversely across the ditch field margin.

The colonisation of land by* M. cantiana *following a change from monoculture to mixed vegetation.

When the new grassland site was seeded with fodder mix in 1996, one plot of 9.02 m^2 was left unseeded. This area, 4.3 metres from a ditch field margin containing an established population of snails, was planted with mixed vegetation in 1997. Given that the average distance moved by 70 *M. cantiana* in a flat grassland situation had been measured at 37.5 cm in 24 hours (Ward-Booth, 2003), the plot of mixed vegetation was potentially accessible by *M. cantiana* from the ditch within 12 days. The vegetation included vegetables that had been planted

intentionally to represent alternative crops but, as this site was available for the investigation of land colonisation by *M. cantiana,* the area was left undisturbed between sampling sessions and other plants also grew within the 9.02 m^2 area.

Vegetables planted:- Carrot, turnip, cabbage, Jerusalem artichoke.
Other plants which also grew within the 9.02 m^2 area :-

Species of Gramineae,	Grass,
Urtica dioica	Stinging nettle,
Cirsium arvense	Creeping thistle,
Papaver somniferum	Opium poppy,

Both grass and stinging nettles are known to be food for *M. cantiana* (Chatfield, 1976).

In October 1997, the mixed vegetation area was carefully searched by hand for four hours and 30 adults, but no juvenile, *M. cantiana* colonists were found. The shells of these 30 individuals were numbered near the aperture using paint and a fine pen nib. The site was monitored for a year to follow the success, if any, of this colonisation. As before, the surrounding grass was cut for hay in August using an Allen scythe. The mixed vegetation was left undisturbed between monitoring sessions.

From the end of October 1997 to September 1998, snails were collected by hand each month from the 9.02 m^2 of taller vegetation in the seeded plot and the surrounding 7.4 m^2 of grassland border, working carefully over the site. Thus the total area sampled was 16.42 m^2 (9.02+7.4). Snail species and abundance were noted. In the first survey, all *M. cantiana* were weighed and individuals without a painted number were marked with a paint dot in the suture. In subsequent monthly surveys, any unmarked individuals were marked using a different colour for each month. The paint mark was added to the suture near the apex, since paint marks on the shell surface become indistinct within a month or two and may be covered by a whorl as the snail grows. Paint in the suture remained visible, under a microscope for small juveniles, throughout the year of the investigation. In September 1998 the ditch field margin was hand-searched for a period of four hours, to see if snails marked in the area of mixed vegetation had moved to the ditch field margin.

The weight of each *M. cantiana* in monthly samples was recorded in order to give an indication of size. The usual method of recording size is by shell measurement. However, in this case, one person had to process a large number of young snails in the laboratory, for example 489 juveniles <0.2 g. at the end of January 1998. Therefore a quicker but reliable method was sought so that snails could be returned to their habitat before the change in their environment affected their normal pattern of behaviour. Weighing the snails was an alternative method available. In order to see if snail weight was a reliable indication of snail size, the relationship between shell breadth, in cm. measured with Vernier callipers, and mass, in grams, was investigated for *M. cantiana.* Shell breadth for 366 *M. cantiana* was log.2.5 transformed to straighten the curve of the graph. As may be seen from Fig. 1, there is close correlation between shell breadth and mass up to the 1 cm. sized snail (r_s=0.943 n=138 $P >$ 0.2). This size coincides with full development of the reproductive system (Chatfield, 1968). Thereafter the correlation is not quite so strong, presumably due to reproductive activities (r_s=0.898 n=117 $P > 0.2$).

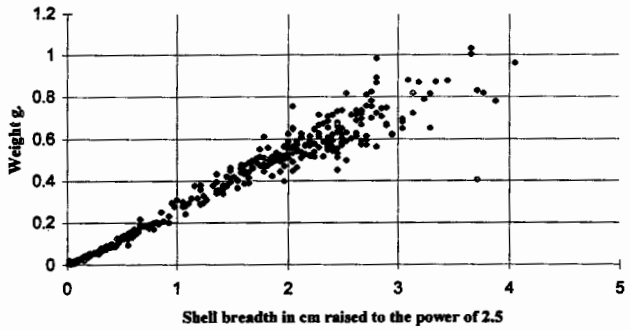

Fig.1. The relationship between weight and shell breadth of 366 *M. cantiana.*

Weighing was therefore thought to be a sufficiently accurate and reliable method of indicating snail size especially as it was the growth of offspring to reproductive age that was of particular interest. This decision allowed the juvenile snails to be grouped, under the microscope, according to size. Batches of 6-8 similar sized snails were weighed together before being marked and released.

Results and discussion

The colonisation of land by* M. cantiana *following a change in monoculture. (arable crop to permanent grassland)

Changing the use of land from arable cropping to grassland affected the distribution of snails. No live snails had been found when hand sampling was carried out within a wheat crop in this field or, later, on the soil before grass seed was sown. Table 1 shows that the change in monoculture, from wheat crop to grassland, resulted in the immigration of seven snail species into the cultivated area.

All but one of the immigrating snail species had also been present in the ditch field margin. However, *Candidula gigaxii* was an exception. It was not found in the ditch despite repeated sampling across the seasons of the year but now appeared to be a successful coloniser with 13 live specimens recorded. Four snail species were present in the ditch but not in the grassland. These four snail species, *O. helveticus*, *C. lubrica*, *E. obscura* & *D. rotundatus*, are small in size compared to *M. cantiana* but they are also known to occur in moist or shady places (Killeen, 1992). Ecology and not size might be the relevant factor affecting their ability to colonise grassland. This point was later investigated.

Although *C. gigaxii* and *C. intersecta* were the most successful colonisers of the new habitat, in terms of an increase in abundance, by far the most abundant snail recorded in the newly available grassland was *M. cantiana*

M. cantiana individuals were not evenly spread throughout the grassland sampled. Table 2 shows the distribution of M. cantiana within the eight adjacent strips of grassland that were cut and sampled for snails by hand searching on 2nd August 1997.

Table 1. Abundance of terrestrial snail species recorded in a ditch field margin and an adjacent grassland area after a change of use from arable crops to grassland

Field		Species		Ditch	
Live animal	Dead shell	Ordered by abundance Field	Ditch	Live animal	Dead shell
472	184	*M. cantiana*	*M. cantiana*	233	55
22	3	*Candidula intersecta*	*Cepaea hortensis*	11	9
14	6	*Cepaea hortensis*	*Oxychilus helveticus*	4	9
13	4	*Candidula gigaxii*	*Trichia striolata*	4	5
4	0	*Trichia striolata*	*Trichia hispida*	3	2
3	0	*Cernuella virgata*	*Discus rotundatus*	1	3
0	2	*Trichia hispida*	*Ena obscura*	2	0
			Candidula. intersecta	1	1
			Aegopinella nitidula	0	9
			Cernuella virgata	0	1
			Cochlicopa lubrica	0	1

Table 2. Abundance of *M. cantiana* distributed in permanent grassland, 16 months after a change from arable cropping to permanent pasture. Adjacent strips were 76.2 cm. wide. Key: - emboldened numbers = total live adult abundance; un-emboldened numbers = total live juvenile abundance; and italicised numbers = total dead shell abundance.

Sections S1 – S4	Strip 1	Strip 2	Strip 3	Strip 4	Strip 5	Strip 6	Strip 7	Strip 8
14.5 m	**2**	**2**	**1**	**0**	**1**	**4**	**5**	**5**
Section 1	0	1	1	0	1	2	2	2
	3	0	1	1	0	1	6	9
14.5 m	**8**	**5**	7	**6**	**10**	**12**	**12**	**10**
Section 2	4	4	3	1	6	2	1	6
	21	0	0	0	4	6	10	30
14.5 m	**17**	**11**	**10**	**7**	**9**	7	**13**	**12**
Section 3	6	7	6	3	4	1	4	1
	16	1	0	0	5	6	11	4
14.5 m	**19**	**16**	**14**	**10**	**20**	**5**	**32**	**41**
Section 4	9	7	5	2	8	4	15	21
	14	2	1	0	2	6	10	12

The 472 live *M. cantiana* (333 adults + 139 juveniles) were contagiously dispersed as shown in Fig. 2, (Dispersal index 7.18, n=31, $\chi^2 = 222.6$, $P < 0.05$ Green's index 0.013) Three peaks of abundance are evident in the following sections, S4 strip8, S4 stripl and S4 strip 5.

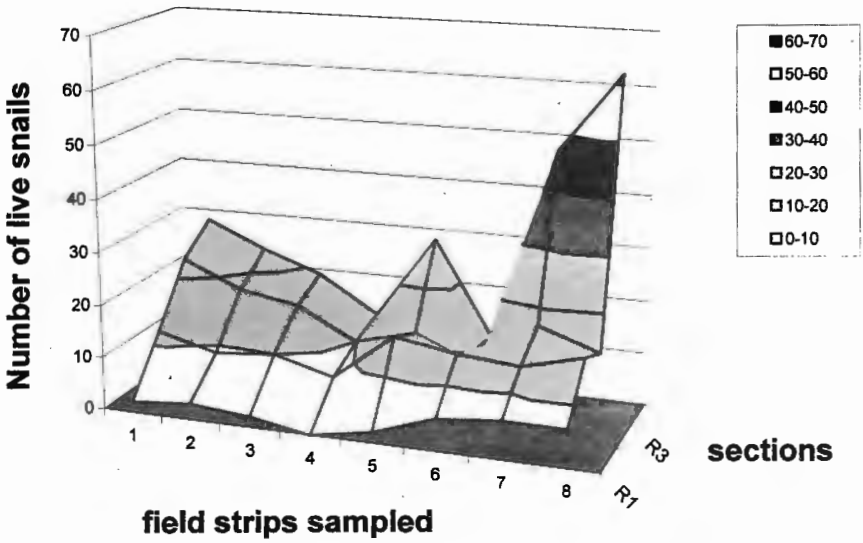

Fig.2. Contagious distribution of live *M. cantiana* (total adults and juveniles) in adjacent grassland strips 16 months after a change from arable cropping.

The *M. cantiana* shells were also contagiously dispersed. Fig. 3 shows two peaks of abundance, sections S2 strip1 and S2 strip8, but very few shells in the intervening strips. However the 182 *M. cantiana* shells in this newly colonised site were more contagiously dispersed than the 472 live individuals (Dispersal index 8.66, n=31, χ^2 268.46 $P < 0.05$ Green's index 0.042). This differs from the distribution of live *M. cantiana* and shells in an established population in a ditch field margin where the shells were both more abundant and more evenly spread than the live individuals (Ward-Booth, 2003).

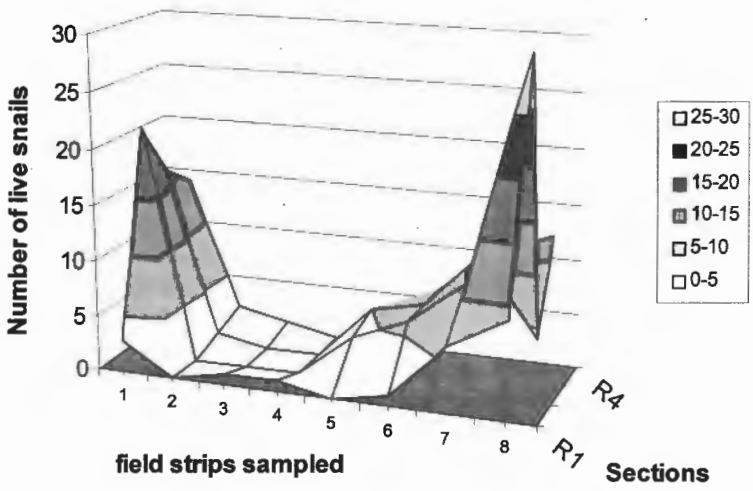

Fig.3. Contagious distribution of *M. cantiana* shells in grassland 16 months after a change from arable cropping.

When the following evidence is considered, it becomes apparent that it is possible that the two peaks of abundance of *M. cantiana* shells in Fig.3 represent adult colonists that bred in the autumn of 1996. Adult *M. cantiana* have an internal rib and a reddish colour near the shell aperture. Out of the 182 dead *M. cantiana* shells recorded, 123 had both an internal rib and a reddish brown colour near the aperture as shown in fig.4.

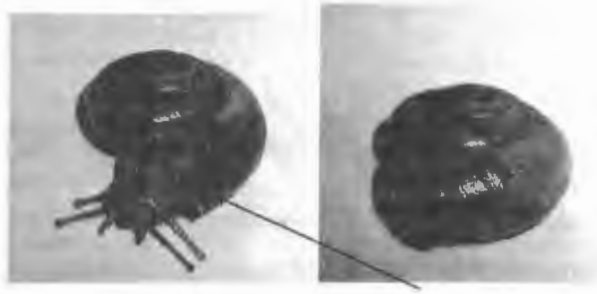

Fig. 4. A photograph of an adult *M. cantiana* showing an internal rib and reddish brown colour near the aperture.

This configuration indicates arrest of mantle growth in adults of 15-17 mm shell breadth and there is a large mortality of adults with these characteristics of shell morphology during

September to December after the breeding season (Chatfield, 1968). It is therefore possible that the adult shells found represent *M. cantiana* that colonised the field soon after a change of land use in 1996 and died, after breeding, in the autumn of 1996. By August 1997, 472 live individuals were recorded.

The colonisation of land by* M. cantiana *following a change from monoculture to mixed vegetation.

By May 1998 the four species *O. helveticus*, *C. lubrica*, *E. obscura* & *D. rotundatus*, that had failed to colonise the area of grassland, were present in the new mixed vegetation habitat. Of these species, *O. helveticus* was particularly successful since with 29 live animals it was, apart from *M. cantiana*, the most abundant colonist. Thus it may be concluded that the macro-snail species encountered in the Debden valley are competent colonisers of suitable new habitats, although their requirements vary. Some species, for example *M. cantiana*, are ubiquitous in this area but abundance varies with soil type, land use and typography (Ward-Booth, Paglia & Dussart, 1996). Within a year of a new habitat becoming available, an August 1998 count of live *M .cantiana* found considerably more live snails where a small area of taller mixed vegetation in an experimental plot was incorporated into the grassland (61.3 /m ^{2}, of which 22.7 / m^2 were adults) as compared to abundance of *M. cantiana* in grassland without the taller herbs (between 0 and 6 /m^2) and abundance in a field margin (between 18 and 44 /m ^{2}). Thus, as Boycott (1934) predicted, the type of vegetation influences snail abundance. In this case both abundance and population structures were affected by these vegetation differences.

Fig. 5 shows that 30 fully-grown *M. cantiana*, which colonised the area containing mixed vegetation, had died and all their shells were found in the sampling area in the winter of 1997, though reproduction had been successful.

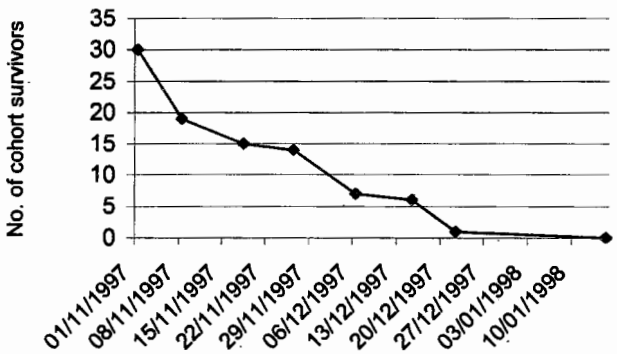

Fig.5. Survival of a cohort of 30 *M. cantiana* in an area of mixed vegetation from 1/11/1997 to 10/01/1998

Counting adulthood as the existence, in an individual, of a fully developed reproductive system and thus approximately1cm shell breadth (Chatfield, 1968), 959 juveniles and 242 adults were recorded within the mixed vegetation sampling area in May 1998, followed by 634 juveniles and 373 adults in August . This was contrary to Boycott's experience in 1934 that: 'we seldom see young snails much more abundant than the adults will be later on, and

the main loss falls I believe on the eggs and the infant young.' Thus, in this newly colonised site, young *M. cantiana* were much more abundant than were the adults later on. There is, therefore, a question concerning the growth rates of *M. cantiana*. Although this species has, essentially, an annual life cycle (Taylor, 1917 & Chatfield, 1968), and the majority of marked snails survived from May to August, the majority of juveniles failed to grow to a size capable of reproduction; in July 1998 there was an influx of unmarked adult snails suggesting re-colonisation of the site (Ward-Booth & Dussart, 2003) and 19% of the 548 *M. Cantiana* recorded in the ditch field margin 4.3m away in September 1998 had shell paint marks indicating that they had been captured, marked and released in the mixed vegetation site in June and July.

Currently *M. cantiana* is common, and increasing over much of eastern Britain (Killeen, 1992). The distribution of *Monacha cantiana* increased in the Debden Valley following a change in the use of arable farmland. If wheat crops in East Anglia were to be replaced by alternative crops providing an improved habitat for snails, this snail species appears to have the potential to increase its distribution to become a pest. However, this research raises questions about the growth of *M. cantiana* and the importance of ditch field margins to its growth and survival.

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Assessing the risk of slug damage to oilseed rape and the need for control measures

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Abstract: A method of rapid soil sampling for slugs was developed, involving digging soil samples with a spade and flooding them over three days, with a further one day for collecting slugs. This technique provided data on all stages in the slug life-cycle except eggs. It is suitable for widespread use to assess slug populations in soil in the period leading up to establishment of oilseed rape crops. The method was compared with slug trapping at field sites in Germany and England from 2002-2004 to investigate relationship between slug population density and the severity of slug damage to oilseed rape at establishment. However, because of dry weather in 2002 and 2003, slug populations were mostly low and there was little or no damage at the majority of sites. Further investigations are needed under a range of weather, soil and agronomic conditions. Opportunities for slug trapping were greatly constrained by dry soil conditions and trapping provided variable results.

Key words: soil sampling, trapping, slugs, *Deroceras reticulatum*, *Arion* spp., Milacidae, oilseed rape.

Introduction

Slugs are important pests of oilseed rape at establishment (Voss *et al.*, 1998; Glen, 2002; Moens & Glen, 2002) and it is important to identify in advance which fields are likely to suffer from severe slug attack, so that control measures can be taken before seedling emergence. In a collaborative project from 2002 to 2004, we developed and tested a simple, rapid technique for estimating the population density of slugs in the top 10 cm of soil in fields to be sown with oilseed rape. The technique is based on soil flooding as devised by South (1964) and as modified and used in arable crops by Glen *et al.* (1989, 1992). In the rapid technique described here, soil samples are dug with a spade then placed in water-tight and slug-proof containers in a cool place. Slices of kohlrabi are placed on top of the soil (to attract slugs) and the soil is gradually flooded (to force slugs to the surface), with the water level raised twice each day, over a period of three days, with a further 1-day period of observation to collect slugs. Tests of the technique started before harvest of cereal crops and continued through the inter-crop period and the establishment of winter oilseed rape crops.

Materials and methods

Study sites

The research was done at locations throughout Germany and at one location in England (Somerset). At each location, except Gettorf, at least two fields were selected each year, to be

sown with oilseed rape. Within each field, 8-10 plots were marked out, each plot a minimum of 12 m x 12 m. At Gettorf, this was done in fields going from oilseed rape into winter wheat.

Soil sampling

From the middle 8 m x 8 m area of each plot, one soil sample (18 cm x 18 cm or 20 cm x 20cm) was dug with a spade to a depth of 10cm. Each soil sample was placed in a watertight container with a lid, so that the samples were kept in the dark to encourage slugs to come to and remain at the soil surface. The containers with the samples were kept out of direct sunshine and transported to a cool shaded place for flooding.

Slices of kohlrabi 0.5 – 1.0 cm thick were placed on the surface of the soil in each container to attract slugs. Slugs were also encouraged to come to the surface by flooding the sample from below. Water (2-3 cm deep) was introduced to the base of the container as soon as possible and 2 cm depth of water was added each morning and evening until the water level was about 2 cm below the level of the soil surface. For the final stage, water was added to reach just below the surface of the soil sample. Before the water was added on each occasion, each container was examined and all slugs that had come to the soil surface were identified and weighed individually while they were fully hydrated. The flooded samples were left for a further seven days and examined daily for slugs. In order to verify whether any slugs remained in soil after completion of extraction, the soil samples were washed through a set of graduated sieves at one location in Germany.

On each sampling occasion, soil samples were also taken at two depths in the soil (0-2 cm and 2-10 cm) to determine soil moisture content gravimetrically. Daily weather records of temperature, and rainfall were obtained from nearby weather stations.

A series of samples was taken from each study field: (1) in the preceding cereal crop before harvest, (2) in the stubble after harvest and before cultivation, (3) after emergence of oilseed rape seedlings. Additional samples were taken at some sites.

Investigation of the rate of soil flooding

At one field site in each location in 2003, extra sets of samples were taken in order to compare flooding over 1 day and 2 days with the 3-day flooding period described above.

Depth of soil sampling

Because slugs may move below the 10 cm depth fixed for soil sampling, it was important to investigate if there were any slugs to extract below this level. Accordingly, soil samples were taken to greater depth (20 cm) on at least one sampling occasion at each location in 2003.

Slug trapping

Mat traps as developed by Hommay & Briard (1988) were used. They are composed of three layers with the top one being metallic silver for maximum light reflection and the bottom one consisting of black perforated plastic. Between these layers an insulating fabric is enclosed to hold moisture within the mat. The mats are *ca.* 50 cm x 50 cm and are placed on the soil surface, with the corners held in place by stones or tent pegs. Metaldehyde pellets (Metarex, 10g/trap) were used as bait, distributed evenly over the 15 x 15 cm central area underneath each mat, to poison the slugs and thus reduce the likelihood of escape. In 2003, slug catches in these traps were compared with similar traps surrounded by slug-proof barriers.

Provided that the soil surface was moist at the time when soil samples were taken, one mat trap was placed in each plot and examined the following morning. If the soil surface was dry when soil samples were taken, traps were put out on the first suitable opportunity afterwards. A sample of up to 20 trapped slugs of each species was removed from each trap

type and weighed individually. Immediately after examination, the traps were removed and the pellets covered with soil. In order to improve the attractiveness of the traps under dry soil conditions in August and September at Göttingen, the mats and the soil surface underneath the mat traps were moistened with 2 litres (2002) or 5 litres (2003) of water per mat.

Upturned plant-pot saucers (25 cm diameter) baited with chicken layers' mash (20 ml) were also used at the sites in Somerset, as described by Glen *et al.* (2003). These traps were left overnight and examined the following morning in the same way as the mat traps.

Assessing slug damage to oilseed rape

Slug damage to oilseed rape at establishment was assessed by dividing each experimental plot into two sub-plots (each at least 6m x 6m). One subplot in each plot was treated with metaldehyde pellets broadcast at drilling and after crop emergence. The number of plants and the % plants damaged by slugs were assessed at intervals to the 4-true-leaf stage. Significant differences between treated and untreated sub-plots were taken as evidence of slug damage.

Results and discussion

The simple soil sampling technique provided valuable data on the populations of slugs, from hatchlings to adults, in soil. It requires no specialised equipment and is suitable for wide use as a rapid and accurate method for estimating slug populations. 82-98% of all slugs were extracted after four days. The population densities of slugs declined markedly at almost all sites in August and September 2002 and especially 2003, with the exception of one direct-drilled field. It is likely that dry weather conditions, especially in 2003, contributed to the relatively low levels of slug infestation and the marked declines. Soil moisture declined substantially after cereal harvest, especially in the sites in Germany in 2002 and 2003. In wet weather in August 2004, slug populations increased at sites in northern Germany and south west England, but numbers remained low at most sites in Germany.

Comparisons of sampling to 10 and 20 cm depth showed, in all but one case, no evidence of substantial numbers of slugs in the lower 10-20 cm layer. The exception was in oilseed rape stubble in mid September 2003, where the density of slugs in the 10-20 cm layer was about half that in the upper, 0-10 cm layer. This may have been a consequence of the dead roots of oilseed rape plants providing channels to enable slugs to move deeper into the soil than they would normally do in cereal crops or cereal stubbles before drilling oilseed rape.

Slug activity on the soil surface was also estimated using: (1) mat traps, (2) enclosed mat traps (both baited with metaldehyde pellets) and (3) plant-pot saucer traps baited with chicken layers' mash. Traps recorded mainly the larger individuals of each species and did not provide reliable estimates of the numbers of smaller, immature slugs. The use of traps was greatly constrained by dry soil conditions in 2002 and 2003 and trapping provided variable results, probably depending largely on soil surface moisture together with weather conditions on the day of examination. At Göttingen, an attempt was made to overcome dry soil conditions by wetting the soil surface under each trap with water (2 litres/trap in 2002, 5 litres/trap in 2003). However, no improvement in trap catch was recorded.

For the purpose of practical risk assessment, there appeared to be no advantage in using enclosed traps, surrounded by barriers (similar to the defined area traps described by Ferguson *et al.*, 1989), which could only record slugs from the defined area of the trap. These traps were time-consuming to set up in the field, especially in stony soils where it was difficult to insert the barriers into the soil. (Such traps can, however, have advantages for research purposes). The large mat traps, each 0.5 m x 0.5 m, used as the standard method of trapping, appeared to have no consistent advantage over smaller traps when the traps were left out

overnight for monitoring *D. reticulatum* and *Arion* spp. Only for keeled slugs (Milacidae) was there evidence of any advantage in using the larger mat traps baited with metaldehyde pellets. However, it is not possible to say whether this difference was due to the trap or the bait.

Smaller traps of dimensions 0.25m x 0.25 m square or 0.25 m in diameter have considerable advantages in ease of use over the larger traps: they are easier to transport, especially when the traps have been exposed to rain overnight and the heavy wet traps are removed in the morning. One further important advantage of the smaller traps is the ability to place them in standing cereals before harvest without having to cut down a patch of the crop.

The data from the field sites in this study provide preliminary information on the relationship between slug population density and the severity of slug damage to oilseed rape at establishment. However, the weather was dry and slug populations were low at most sites so that no significant damage was recorded at 30 of 37 sites from 2002 to 2004. Significant damage was recorded at one direct drilled site each year, emphasising that direct-drilling is associated with high damage risk, as described by Glen & Moens (2002). Significant damage was also recorded at four sites with reduced tillage. However, it is not yet possible to specify threshold slug numbers in soil samples or traps indicating different levels of risk of slug damage to oilseed rape. Further investigations are needed under a wide range of weather, soil and agronomic conditions.

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Insect Pathogens and Entomoparasitic Nematodes
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Integrated control of slug damage in winter wheat

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Abstract: A systematic approach for integrated control of slug damage in winter wheat is described. Firstly, damage risk is assessed, based on the slug catch in traps baited with chicken layers' mash, left out in the field overnight before cultivation, together with other factors known to influence the ability of slugs to inflict severe damage. Damage risk is minimised by the use of cultural control measures. However, if the risk is high, slug pellets should be applied at around the time of drilling, before significant damage has occurred. All crops should be monitored closely from emergence until tillering, then at intervals throughout the winter and slug pellets broadcast if the crop is slow to emerge or to grow through the early vulnerable stages and does not appear to be outgrowing slug damage.

Key words: integrated control, risk assessment, trapping, slugs, winter wheat.

Introduction

Winter wheat is highly vulnerable to slug attack during establishment (Glen & Moens, 2002). Wheat seeds are especially at risk and severe damage can result in complete crop failure or such severe loss of stand that the crop will not recover. This paper describes a system for integrated control of slug damage in winter wheat that has been developed in the UK by the partners in a Sustainable Arable LINK Project.

Risk assessment and cultural control

Risk assessment and cultural control measures are very closely interlinked.
There are four main elements, as described below:-

1. Trap to assess slug activity during period before cultivation and possibly after drilling.
2. Use trap catches together with other information to assess the risk of slug damage
3. Reduce the risk of slug attack by cultivations and adjustment of drilling depth
4. Monitor crops throughout the early susceptible growth stages

1. Trapping to assess slug activity

Slug activity on the soil surface is dependent on moist and mild conditions. Traps act as refuges for slugs active on the soil surface in the surrounding area. Traps predominantly record slugs >100 mg, whereas smaller slugs are under-recorded compared to their densities in soil, even though behavioural studies have shown no differences in the rates of entry and leaving traps (Howlett *et al.*, 2005). If conditions are suitable for surface activity, the catch will give an indication of the number of slugs >100 mg in the area. Placing bait beneath traps increases the numbers of slugs trapped.

Although traps baited with slug pellets have been used routinely for monitoring slug activity (e.g. Gratwick, 1992), we do not now recommend slug pellets as trap bait because recent research shows that chicken layers' mash is a safe and effective alternative (Young *et al.* 1996; Glen *et al.*, 2003). Traps with this bait need only be left out for one night to record slug numbers similar to traps baited with slug pellets left out for three nights (Glen *et al.*, 2003). Traps may consist of inverted plant saucers, mats (for example, as described by Hommay & Briard, 1988) or pieces of hardboard etc. Traps should be of about 25 cm diameter or width. A heap of around 20 ml (two heaped teaspoonfuls) of chicken layers' mash is placed under each trap.

Trapping should be done during the period before cultivation. After cultivation, trapping may under-estimate the true slug population as surface activity is reduced. However, trapping between drilling and emergence is valuable if wet weather persists, because under these conditions increasing slug populations may pose a threat to emerging wheat. It is essential to take advantage of suitable weather for trapping. Traps are put in place only when the soil surface is moist and temperatures are favourable for slug activity (minimum night temperature greater than 5°C, maximum daytime temperature less than 25 °C).

Nine traps should be laid out in a 'W' pattern in each field (13 traps if the field is larger than 20 ha). If certain areas of the field are known to suffer from slug damage (e.g. areas of heavy clay or silt soil), traps should be concentrated in these areas. Traps are left overnight and examined the following morning. Slugs will remain in traps while the sky remains overcast, but will leave traps if they heat up when exposed to sunshine. In sunny weather, traps should be examined as early as possible, before direct exposure to sunshine.

For winter wheat, a catch of an average of four or more slugs per trap will justify slug pellet treatment, provided that favourable conditions for slug activity (and control) continue and provided that other risk factors (described below) are positive.

Trapping will provide a useful guide to levels of slug activity, when carried out under favourable conditions while following the guidelines given above. Monitoring should be considered well in advance of drilling to maximise flexibility in subsequent operations.

2. Use trap catches together with other information to assess the risk of slug damage

When trap catch exceeds the threshold, slug pellet treatment is advised when one or more of the following criteria are met:

- the field is drilled during a period of generally wet weather
- wet weather delays sowing in a prepared seedbed
- the seedbed tilth is coarse and cloddy, and further consolidation is not possible following sowing
- wet weather continues after drilling and further trapping shows evidence of high slug activity on the seedbed
- the crop is slow to emerge or to grow through the early vulnerable stages and symptoms of slug damage are seen.

3. Reduce the risk of slug attack by cultivations and adjustment of drilling depth

Damage to seeds and seedlings of winter wheat before emergence directly affects yield, but is the hardest to predict as it may not be linked to surface activity. The best approach to prevent early damage is sowing at sufficient depth (3 cm) in a fine, consolidated seedbed to deny access to the seeds by slugs and provide conditions for rapid germination. In cloddy seedbeds, seeds should be sown a little deeper than normal (4-5 cm).

The more cultivations and the more intensive the cultivation method, the greater the likelihood that slug numbers will be reduced, especially if the weather is dry. However, whatever method of cultivations are used, it is important that that the seedbed is fine and firm to protect seeds and young seedlings. Thus, although reduced tillage methods can allow more slugs to survive compared with ploughing, they can have advantages for slug control if the farmer is able to produce finer seedbeds compared with ploughing. Moreover, surviving slugs are not buried to some depth by reduced tillage, as happens after ploughing, so slugs are more likely to be surface-active following reduced tillage and therefore vulnerable to slug pellets. Reduced tillage methods also retain seedbed moisture for germination under dry conditions, which helps the crop to grow rapidly through the early vulnerable stages.

4. Monitor crops throughout the early susceptible growth stages

Crops should be examined regularly for slug damage. Slug trapping is not normally necessary at this stage, but trapping should be done if there is any doubt about whether the damage is caused by slugs. Cereal crops are most susceptible to damage from sowing to first tillering (GS 21). After this growth stage is reached, further damage is unlikely to result in additional loss of plants. However it is important to continue to monitor crops throughout the winter and be ready to treat if there is evidence of fresh damage to young leaves and plants show signs of being set back by slug damage.

Control using slug pellets

Timing of slug pellet treatment

An application of slug pellets at the recommended rate will generally depress the slug population and feeding activity for several weeks following treatment. The population will recover more rapidly if conditions are especially favourable (mild and wet), or more slowly if the intervening period is dry. The proportion of the population killed will depend on surface activity in the week following application. Heavy splashy rain soon after treatment could result in reduced efficacy. If there is heavy rain within three days of application, treated fields should be examined to check whether pellets are still visible. The highest probability of an economic response to treatment occurs during the first month of crop growth, so that an application immediately following drilling and rolling is most likely to produce a useful effect.

Application shortly before drilling may be effective if the conditions at the time are suitable for surface activity and if the soil can be left undisturbed for three days after treatment. However, because of the importance of timely sowing in good conditions for control of slug damage, it is not worth delaying sowing to allow a treatment to be applied (Gratwick, 1992).

In the dry autumns of 2002 and 2003, pellets applied to stubble up to 6 weeks before drilling winter wheat were as effective as pellets applied after drilling. In contrast, in the wet autumn of 2004 pellets applied to stubble had lost their efficacy by the time that wheat was at risk; pellets applied after drilling were significantly more effective. Thus, the earlier before

sowing that treatments are applied the more likely it is that slug populations will have recovered by the time the crop is exposed to damage.

When risk assessment shows that an application of pellets is not justified around the time of drilling, a pre-emergence treatment may be justified if wet weather has continued since drilling and traps placed on the seedbed show high slug activity.

Treatments are often applied after crop emergence in response to fresh leaf shredding damage. They will normally only be worthwhile in the period before the crop has reached the less susceptible 3-4 true-leaf/tillering growth stage and, as always, if the conditions following treatment are suitable for surface activity. However treatment after tillering may be justified if there is evidence of fresh damage to young leaves and plants show signs of being set back by slug damage.

Slug pellet application

Pellets may be applied broadcast to the soil surface or admixed with seed and applied at drilling. Mixing should be carried out immediately before use, calculating the quantity needed to ensure that there is negligible surplus of the mixture. Storage of grain/slug pellet mixtures is not good practice and should be avoided.

Broadcasting is the method of application that will give the most consistent slug control, especially when combined with the preparation of fine, firm seedbeds that protect seeds and young seedlings from attack before emergence.

Pellet admixtures with wheat seeds may be effective when winter wheat is direct-drilled or drilled into open cloddy seedbeds. However when seeds are sown, as recommended, into fine seedbeds, admixed pellets will be ineffective because, like the seeds, they will be unavailable to slugs. Slugs will survive to attack the emerging seedlings.

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Influence of slug populations on green manure crops

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Abstract: Slugs can cause severe losses in many arable crops. Slug control has become increasingly important in recent years. Reasons for this are: reduction in soil cultivation; increasing acreages of land under set-aside; and consumers have elevated their quality standard, resulting in a lower tolerance for slug damage. One of the main reasons for increases in slug damage are larger slug populations, produced as a consequence of growing green manure crops such as forage rape *(Brasslca napus)* and fodder radish *(Raphanus sativus)* in crop rotations.
The aim of our research is to determine the susceptibility of 22 green manure crops to the grey field slugs. In semi-field trials with and without slugs, green manure crops were compared with each other on the number of emerged plants and slug damage.

Key words: Green manure crops, *Deroceras reticulatum*, semi-field trials

Introduction

In Western Europe, as a consequence of the decrease of the input of soil pesticides in combination with the reduction of intensive soil cultivation (Bolton, *et al.* 1996), soil pests have increased over the last decade (Glen, 1989). Meanwhile it has been necessary to achieve high levels of yield and quality of the crops. This leads to intensive production of high value crops such as potatoes, Brussels sprouts and sugar beets, which often are susceptible to slugs (Beer, 1989; Ester & Geelen, 1996; Ester *et al.* 2003). These arable crops often are grown on a heavy clay soil that, after ploughing in autumn, has a high shelter-capacity for slugs (Glen *et al.* 1989).

In these intensive cropping systems no damage by slugs is tolerated. Slugs are likely to increase in importance as pests in integrated crop management systems. In these systems a population can build up quickly where green manure crops such as oil radish, white mustard and Italian and perennial ryegrass or bulky old crop residues are used as a part of a soil management programme. This paper reports on the results of a project which aims to improve the control of slugs in an arable crop rotation system, which is focussed on the tolerance of green manure crops for slug damage in semi-field trials.

Material and methods

The trials were laid out in 1 x 1 m and 30 cm high boxes of iron fence with a copper barrier around the top. The experiments were randomised blocks with four replicates. Each crop was sown into two boxes; in the first box slugs were added and in the second box no slugs were added, as a standard. The soil was a marine loam with 22 % silt and a pH = 7.3. The seed rate of each crop was comparable to Dutch practice (Table 1). Assessment was focussed on two phases of plant development, namely the damage from germination up to plant emergence and secondly damage to the plants of the green materials (stem and leaves). Statistical analysis was performed with the statistical package Genstat 7.2.

Semi-field trial 1

All green manure crops (Table 1) were sown on the 8th July 2004, irrespective of the recommended sowing date for each crop. Twenty slugs were added to each box. The damage to the crops by slugs was assessed 10 days after sowing by counting the number of plants per plot in the boxes with and without slugs. From these numbers the relative percentages of emerged plants were calculated. Also the numbers of attacked plants by slugs were counted, in the boxes with slugs only.

Semi-field trial 2

All crops (Table 1) were sown on 21 August 2004. Thirty slugs were added to each box. Assessment took place 26 days after sowing the seeds by counting the number of emerged plants in both boxes of each crop (box with and without slugs). Also the numbers of plants affected by slugs were counted.

Table 1. Numbers of seeds counted for different green manure crops.

Crops	Latin name	Cultivar	G. seeds/m²	Number of seeds
Italian ryegrass	*Lolium multiflorum*	Montblac	2.5	666
Buckwheat	*Fagopyrum esculentum*	-	1	42
Common vetch	*Vicia sativa*	Hifa	21	143
White mustard	*Sinapis alba*	Concerta	1.3	158
Lacy phacelia	*Phacelia tanacetifolia*	Angelica	0.9	479
Sticky nightshade	*Solanum sisymbriifolium*	Sharp	0.3	108
Oil radish	*Raphanus sativus*	Commodore	1.6	150
Oilseed rape	*Brassica napus*	Jet neuf (0)	0.8	154
Oilseed rape	*Brassica napus*	Express (00)	0.8	178
Sudangrass	*Sorghum sudanense*	Piper	3.5	358
Red clover	*Trifolium pratense*	Rotra	1.4	511
Black medick	*Medicago lupulina*	Virgo	1.5	932
Rye	*Secale cereale*	Sorom	13	373
Perennial ryegrass	*Lolium perenne*	Elgon	2	1228
Red fescue	*Festuca rubra*	Bargreen	2.4	2464
White clover	*Trifolium repens*	Retor	0.7	827
Lucerne	*Medicago sativa*	Sanditi	1.5	776
French marigold	*Tagetes patula*	Singel gold	0.3	130
Coriander	*Coriandrum sativum*	Caribe	2	172
Blue lupin	*Lupinus angustifolius*	Rosalin	16	113
Yellow lupin	*Lupinus luteus*	Juno	16	111
Forage rape	*Brassica napus*	Stego	1	240

Results and discussion

It appears that buckwheat, white mustard, lacy phacelia, oil radish, oil seed rape (0), Sudangrass and rye result in the same percentage of emerged plants in comparison with the same crops without slugs (Table 2). The remaining crops had a significantly lower relative percentage of emerged plants. This is the consequence of the damage caused by slugs in the

period between germination of the seeds and the plant emergence. Especially the susceptibility of the double low cultivar in comparison with the single low cultivar of the oil seed rape is remarkable, because the significant differences between the single- and double zero cultivars, which have respectively a high and a low content of glucosinolates. Common vetch, white mustard, phacelia, sticky nightshade, Sudan grass, red clover and black medick have no significant slug damage in comparison with the same crops in the boxes without slugs. What this means is that the activity of the slugs on the soil surface is much lower in plots with a low percentage of attacked plants compared with the remaining crops. The surface-active slugs are much lower in number in the plots with an increase in damage. The remaining crops have a significant slug damage $p<0.001$ (Table 2). The trial in July showed after 10 days after sowing, that the legumes white mustard, lacy phacelia, Sudangrass and black medick have a high tolerance against the grey field slug.

Table 2. Relative percentage of emerged plants and percentage of attacked plants, 18 July 2004.

Crops	Emerged plants	Attacked plants
Italian ryegrass	67	23
Buckwheat	93	49
Common vetch	83	2
White mustard	93	1
Lacy phacelia	85	3
Sticky nightshade	48	0
Oil radish	99	17
Oilseed rape (0)	86	20
Oilseed rape (00)	56	16
Sudangrass	98	9
Red clover	70	3
Black medick	80	1
Rye	96	54
LSD ($\alpha = 0.05$)	15.3	14
F-prob.	< 0.001	< 0.001

Nearly four weeks after sowing, oil radish, blue lupin, yellow lupin and forage rape resulted in the same percentage of plants as the same crops in boxes without slugs (Table 3). In the remaining crop, slugs decrease the percentage of emerged plants enormously. Barratt *et al.* 1989, reported that lucerne seedling density in autumn with a high population density eight days after sowing was reduced by 51 %, this is corresponding with the second trial in Lelystad. On September 16, white clover, lucerne, French marigold, coriander, blue lupin and yellow lupin showed no significant differences in the percentages of attacked plants compared to the same crops without slugs. Bolton *et al.*, 1996, found on most occasions the perennial ryegrass plots contained the most surface-active slugs and in mustard plots the slug numbers were significantly lower. These slug-activities are related to the percentages of emerged plants and the damaged plants in trials 1 and 2 respectively. From trial of September it was concluded 26 days after sowing, that the crops blue lupin as well as yellow lupin have a high level of tolerance against slugs.

Table 3. Relative percentage of emerged plants and percentage of attacked plants, 16 September 2004

Crops	Emerged plants	Attacked plants
Perennial ryegrass	36	98
Red fescue	71	72
White clover	3	24
Alfalfa	51	27
Oil radish	90	49
French marigold	3	8
Coriander	58	17
Blue lupin	103	18
Yellow lupin	85	27
Forage rape	96	41
LSD ($\alpha = 0.05$)	16	38
F-prob.	< 0.001	< 0.001

Summarizing the green manure crops white mustard, lacy phacelia, Sudangrass and black medick have a high tolerance to slugs in mid-summer, and the crops blue and yellow lupin have a high tolerance against slugs in the early autumn.

With the positive results of these trials new research will be conducted to establish whether crops can have a controlling effect on slug populations over winter. The most slug-tolerant and therefore the most promising crops will be screened for their sensitivity to nematodes as well as their yield biomass, to ensure successful practical use.

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Decision Support Systems for management of slugs

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Abstract: Slugs and snails are persistent problems in horticultural and agricultural crops. Farmers and growers need a wide range of information and support to help them manage these pests effectively. The development of Decision Support Systems for selected horticultural crops in the UK has involved extensive consultation with growers. The requirements of the growers and how they can be met by researchers is discussed.

Key words Decision Support System, slugs, integrated management

Introduction

Slug and snail pests cause considerable problems for farmers and growers in Europe. Given the close link between weather and mollusc activity, the extent and duration of crop damage can vary dramatically from year to year and place to place. For arable crops, such as wheat and oilseed rape, the crop can withstand a certain amount of damage before a loss occurs, but linking losses to mollusc numbers is difficult as feeding activity can vary so much. For horticultural crops, such as lettuce and Brussels sprouts, which have a higher market value, the damage caused by feeding is compounded by the presence of molluscs, and/or contamination with mucus and faeces and the pest threshold is low or effectively zero for many crops.

There has been research published on many aspects of slug and snail biology that may have some relevance to managing the pest problem. However, a major constraint on employing these techniques is whether they are practically feasible in commercial cropping systems and whether they economically worthwhile. What is required is a system which evaluates the research and informs the user of those techniques that are both practical and worthwhile.

To address this problem in a structured fashion we have surveyed growers and crop protection advisors to assess what information they require from the researchers. We have then evaluated the best ways of meeting their requirements using a range of tools to provide support for their pest management decisions.

Material and methods

As part of a project developing Decision Support Systems for slug pests in horticultural crops we held a series of focus groups with 19 growers and crop protection advisors. Once the outcomes of these meetings were analysed we sent a postal questionnaire to a larger group to seek their views on the initial findings. Finally, when the specific Decision Support Systems had been planned, a further series of focus group meetings were held to get feedback on the

proposed product. The results reported here are based on the replies to the questionnaire survey.

Results and discussion

Questionnaire respondents

Data from 73 completed questionnaires were collated: Of these there are replies from 53 growers, 19 consultants, 5 distributors and 3 others (a few do two things). Of the growers, 23 produced salads (3514 ha in total) and 44 produced brassicas (5334 ha). Of the consultants, 10 advised salad growers (800 ha) and 19 advised brassica growers (3680 ha). Slug control programmes were based mainly on crop and soil characteristics (58-60 respondents) and assessments of slug numbers by eye (58 respondents). Traps were also used as guide by 34 respondents. Table 1 shows the issues regarded by these respondents as important for improving the management of slug pests

Table 1. Information required by growers and consultants to improve the management of slug pests with an indication of how these requirements might be met.

Information required	Demand	Way in which demand might be met		
		Encyclopaedia or database	Action model	Projection model
Slug Biology: Slug species and the differences between them; Lifecycle; Habitat and distribution within a field; Food types and preferences; Slug movement (how far/fast); Population dynamics; Impact of weather on lifecycle and movement; Resistance to treatments.	56-90%	✓		
Slug Identification: Taxonomic key	85%	✓		
Chemical control: Formulations, standard and alternative; Impact of weather on the product; Effectiveness over time; Environmental impact; Non-molluscicide products with slug management properties; Repellents.	78-95%	✓		
Non-chemical control: Predators; Out of crop treatments; Sterilisation; Cultivation; Managing organic matter; Varietal susceptibility.	75-84%	✓		
Support for determining **whether treatment is necessary**	88%	✓	✓	
Support for determining the **optimum timing and application rate** for pellet application	97%	✓	✓	
Warnings of imminent damage	94%		✓	
Indication of **risk of using a particular field**	86%			✓

The Decision Support System

Some of the information required is already available, but is information the user needs to have close to hand. Furthermore the information is found in a wide range of resources (Port et al., 2002). The obvious way of addressing this demand for encyclopaedic or database information is to bring the data together into a single resource such as a booklet or computer based information system.

A large number of the respondents wanted information on whether treatment (with molluscicides) is necessary and what the optimum timing and application rate would be. These requirements are best met by a mixture of approaches. Some of the information, such as recommended methods for assessing slug populations and application rates for molluscicides can be conveyed by a booklet or computer based information system. However, molluscicide applications are only required in some weather conditions when the slugs are likely to be active. These weather conditions are difficult to predict and we have used a simple threshold model, nicknamed *Trap or Treat,* which takes account of temperature and soil surface moisture in the crop. This threshold model is also useful for providing a warning of imminent damage.

Longer term risks, for example of planting in particular fields, require predictive modelling. To tackle the problem of long term forecasting we have developed models which simulate changes in populations of slugs taking into account growth rates, fecundity and mortality together with meteorological data (Shirley *et al.*, 2001).

This group of materials and models is being developed as a Decision Support System for horticultural growers in the UK. It is possible that similar systems will be useful for farmers producing arable crops, although the different thresholds for damage in the crops may mean that the farmers have different priorities for the management of slug pests. The iterative approach, of consulting the user groups at several stages during the development of the support system, means that effort is expended where it will be most effective.

Acknowledgements

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Insect Pathogens and Entomoparasitic Nematodes
IOBC wprs Bulletin Vol. 28(6) 2005
pp.91 - 94

Influence of the farming system and specific cultivation methods on the slug damage level in Swiss potato production

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Abstract: Swiss potato production is increasingly faced with quality problems. A three year project (2001-2003) aimed at identifying the most important quality deficiencies in Swiss potato production studied how these are influenced by farm management and production systems. On approximately 95 farms (278 plots) of different production systems (organic, integrated label production and conventional production) all relevant data on crop rotation, farm management and site parameters was collected and the quality of the harvested potatoes was assessed for a sample of 800 tubers from 270 plants collected according to a specific sampling plan.

The most important cause of outer quality deficiencies of potatoes in the years 2001 to 2003 were slugs, wire worm, Rhizoctonia solani *(Drycore) and powdery scab. About 20% of the plots included in the project did not meet the potato trade quality standards. Slug damage was more frequent on organic farms and on plots with grassland or vegetables in the crop rotation. A high soil cover achieved by catch crops in the years preceding the potatoes significantly increased the slug damage risk. The use of molluscicide pellets can reduce the damage, but a treatment is preventive in absence of a reliable system of forcasting slug damage.*

Key words: potato, *Solanum tuberosum*, quality, slugs

Introduction

During the past few years a decline of potato quality is increasingly evident in Switzerland. It is frequently attributed to increasing importance of ecological criteria that farmers have to fulfil. Nevertheless, the actual cause is unclear and there is a lack of scientific evidence to support the above hypothesis. A three year project (2001-2003) aimed at identifying the most important quality deficiencies in Swiss potato production studied how these are influenced by farm management and production systems.

Material and methods

Experimental design and data collection

All relevant data concerning crop rotation, management and site parameters was recorded for 278 plots (organic production 58, integrated label production 69, conventional 151) of approximately 95 farms distributed across the major potato growing regions of Switzerland. Farms were only included in the project if they had practiced the same production system for several years. Immediately before the harvest a sample consisting of 800 tubers from 270 plants was collected on each plot according to a specific sampling plan. The assessment of the quality was done with specifically designed evaluation system which contained both scientific criteria and requirements set by the trade. Besides the detailed assessment of different quality criteria using grades we recorded for each tuber and each quality criteria if the official quality standard (Swiss potato commission, 1989) was met.

Influence of crop rotation and soil cover on slug damage
To assess the influence of crop rotation and soil cover on slug damage of potatoes, all the plots included in the project were grouped into five risk groups according to the soil cover during previous years. For this each main crop and each catch crop was assigned a specific number of risk points and the sum of the risk points was determined for each plot for a period the three years preceding the potatoes. Based on the sum of the risk points each plot was assigned to one of the five risk groups (5 = highest risk of slug damage).

Statistical analysis
To determine the influence of various factors on different quality parameters, the project plots were grouped according to management practice (e.g. production system, crop rotation). Measurement results were statistically checked for normal distribution and comparable variability. Depending on the result, averages were compared with the following methods:

- 2- and higher way analyses of variance
- t-test for two independent samples of normally distributed but heterogeneous data.
- Wilcoxon rank-sum test for not normally distributed data.
- In order to have a global α-level of 0.05 for the family of the tests performed, the tukey-Kramer Test resp. the Bonferroni-Holm procedure was applied.

All statistical analysis was done with NCSS (Number cruncher statistical software).

Results and discussion

Overall assessment of quality deficiencies
According to the Swiss potato quality standard, the maximum tolerance for outer quality deficiencies is 18% of weight. On average for the three years this requirement was achieved for 30% of the plots without sorting out deficient tubers. For further 48% of the plots the requirements could be reached if it is assumed that half the deficient tubers are sorted out. For 22% of the plots the quality was insufficient to meet the standard.

Looking at the results of the variety Agria only, the quality was comparable for the production systems IP-Suisse (integrated label production) and conventional integrated production (22% insufficient quality for each). In contrast, the proportion of plots with insufficient quality was 47% for organic production.

Slugs: more important than expected
Slug damage was more frequent than expected and was the most important outer quality deficiency besides wire worm damage in all production systems. Over 95% of the slugs collected on the project sites were Deroceras reticulatum, while Arion ssp. was found only relatively infrequently. For organic production the proportion of plots with serious slug damage (2001 75%, 2002 55%, 2003 40%) was significantly higher than for other production systems (figure 1). The most serious slug damage were observed in 2001 after a mild winter and a wet spring. The least damage was observed in the exceptionally dry year of 2003. With increasing soil cover (catch crops, grassland) in the years preceding potatoes slug damage increased (figure 2). The clearly higher slug damage on plots with regular vegetable production (spinach, carrots) was especially remarkable. If the plot was not used for grassland or vegetable for three years preceding potatoes, slug damage was significantly reduced. On plots where molluscicide pellets were used, slug damage was also significantly reduced. If potatoes were preceded by wheat, major slug damage was only observed in the conventional and the integrated label production systems if a catch crop was grown after the wheat.

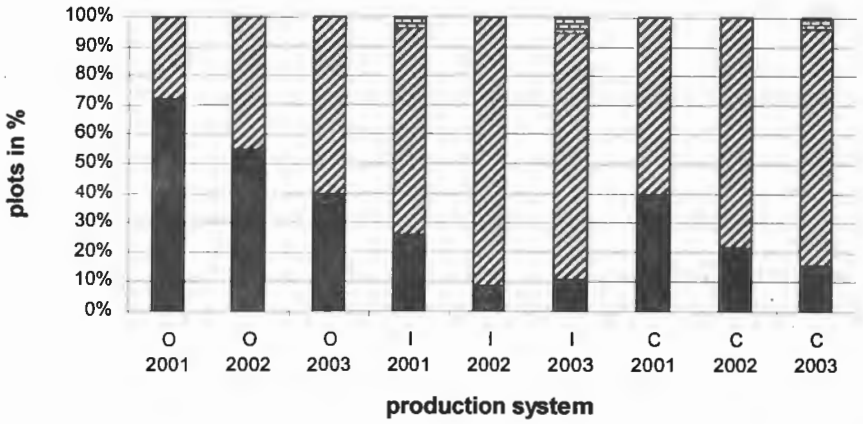
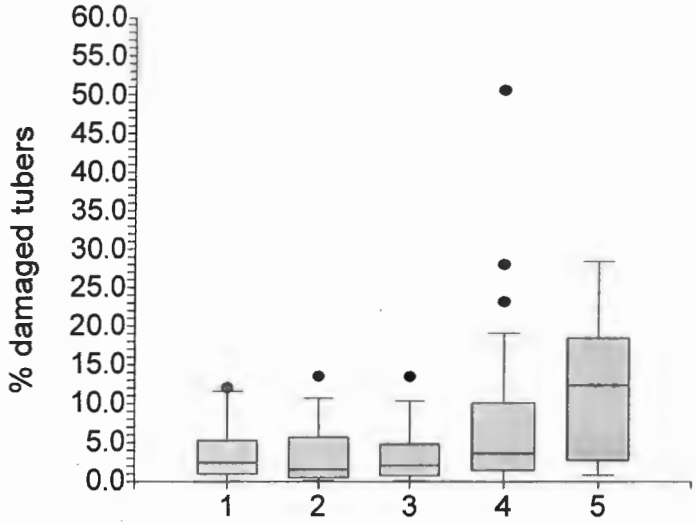


Figure 1. Slug damage depending on production system and year. ■ More than 5% of the tubers with slug damage, ▨ less than 5% of the tubers with slug damage, ▦ no slug damage, O= organic, I= Integrated Label production, C= Conventional.



N	*27*	*22*	*50*	*63*	*11*
Statistical group	*ab*	*ab*	*a*	*bc*	*c*

Figure 2. Slug damage in the years 2001 to 2003 depending on crop rotation (only plots without use of molluscicide pellets). A sequentially rejective multiple test was performed according to the Bonferroni-Holm procedure (Holm 1979).

1 = Crop rotation with cereals, maize, sugar beets, no catch crops
2-4 = As 1 but with increasing importance of catch crops
5 = High proportion of sown grassland and vegetables and high importance of catch crops

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Simultaneous detection of the remains of multiple species of mollusc and other prey in carabids using multiplex PCR

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Abstract: DNA-based techniques are providing valuable new approaches to tracking predator-prey interactions. The gut contents of invertebrate predators can be analysed using species-specific primers to amplify prey DNA to confirm trophic links. The problem is that generalist predators need to be analysed with primers for the tens of potential prey available at a field site, even though the mean number of species detected in each gut may be as few as one or two. Conducting all these PCRs is a lengthy process, and effectively precludes the analysis of the hundreds of predators that might be required for a meaningful ecological study. We report a new, rapid, more sensitive and practical approach. Multiplex PCRs, incorporating fluorescent markers, were used to amplify mitochondrial DNA fragments from 10+ species simultaneously.

New primers to detect the remains of aphids, earthworms, weevils and molluscs in the guts of the carabid beetle *Pterostichus melanarius* were developed and characterised. The system was then applied to field-caught beetles in a project studying the response of generalist beetle predators in a low-input arable farming system to changes in number and density of various prey species. Results are presented on the relationship between number of molluscs found in two years of field survey and the proportion of 'PCR-positive' beetles.

Key words: *Pterostichus melanarius*, mollusc, multiplex, mitochondrial DNA

Introduction

Without direct observation (which is impractical), it is difficult to obtain quantitative data on rates of predation on molluscs by invertebrate predators in the field. This is less of a problem with vertebrates where examination of indigestible remains in regurgitated pellets or faeces may provide valuable clues to the range of prey taken by a predator (Love *et al.* 2000). Several problems are encountered when studying invertebrate predators, however, as these are often small and cryptic (Chen *et al.* 2000) and the nocturnal habits of many species, such as carabid beetles, make direct observation of predation difficult (Symondson 2002). Also, most invertebrate predators are fluid feeders, resulting in little or no identifiable remains in gut samples or faeces (Symondson 2002).

To overcome the problems of prey identification in general, and in insect predators in particular, we have developed DNA-based techniques for prey identification. Briefly, this approach involves the sequencing of short stretches of DNA from both predator and prey species and designing PCR primers specific to particular prey species or groups. The wide

availability of molecular biology facilities, and the generally low cost of development, makes DNA-based methods a practical means of studying predator foodwebs.

The majority of studies have focused on various regions of mitochondrial DNA (mtDNA). Several hundred or thousands of copies of the mitochondrial genome may be present within each cell (Hoy 1994) greatly increasing the probability that prey DNA can be amplified from a predator's gut. There are also several sets of 'universal' primers available for the amplification of mtDNA genes (Folmer *et al.* 1994; Simon *et al.* 1994) facilitating the rapid screening of suitable regions from both predator and prey species from which prey-specific primers can be designed.

To date all DNA based predator-prey studies have applied specific PCRs and agarose gel electrophoresis to identify single prey items from predators. In order to apply this to generalist predators, where the aim is to detect the multiple prey species that could be present, a lengthy process of multiple PCRs and agarose gel electrophoresis would be required. If the field is to develop, techniques must be developed that will reduce both the time and effort needed to screen generalist predators for the wide range of prey species that may potentially be encountered.

To overcome this problem, we have developed an adaptation of a multiplex approach used in population genetics to detect microsatellites. Utilising fluorescently labelled PCR primers, all targets can be detected simultaneously using a highly sensitive DNA sequencer-based detection system. This allowed us to screen the gut contents of the generalist carabid beetle predator *Pterostichus melanarius* simultaneously for the presence of multiple groups and species of prey. The beetles were collected from a field experiment looking at the effects of different forms of cultivation on invertebrates generally and on the responses of *P. melanarius* to different prey and to prey diversity.

Material and methods

Primers were developed to detect the major mollusc species present at our field site. These included the slugs *Deroceras reticulatum* and three *Arion* spp., *Arion distinctus, Arion hortensis* and *Arion intermedius.* These slug primers were developed by Dodd (2004). The *D. reticulatum* primers were species-specific, while a general *Arion* primer pair amplified different sized DNA fragments for each of the three target species. Species-specific primers were also created to detect two common snails, *Candidula intersecta* and *Vallonia pulchella.* We were also interested in measuring predation of other components of the beetles' diet at the same time. The density and type of alternative prey available, for example, may well affect predation on molluscs, at any time. General group-specific primers were developed for earthworms and aphids, while species-specific primers were designed for *Sitona* weevils and the aphids *Myzus persicae, Aphis fabae* and *Megoura viciae.* Previously published primers were used for the aphids *Rhopalosiphum padi* and *Sitobion avenae* (Chen *et al.* 2000). All PCR products were below 250 bp (base pairs) in length as many studies have reported a negative relationship between length of prey amplicon and the detection period in the predators' guts (Chen *et al.* 2000; Hoogendoorn & Heimpel 2001; Zaidi *et al.* 1999).

A single multiplex PCR was optimised to amplify ten of the twelve invertebrate amplicons (general earthworm; general aphid, *M. persicae, A. fabae, M. dirhodum, R. padi,* general *Arion, C. intersecta, V. pulchella, and D. reticulatum* and *Sitona* sp.). Amplifications were performed as described elsewhere (Harper *et al.* submitted) and separated on 5% denaturing polyacrylamide gels, using 36 cm well-to-read gel plates, on an ABI Prism® 377 DNA sequencer (Applied Biosystems) running Genescan software. Data collected from the

DNA sequencer was stored in an electronic format. Electropherograms were analysed and scored using GENOTYPER v 2.5 (Applied Biosystems).

Calibratory feeding trials were carried out using the ground beetle *Pterostichus melanarius* (Illiger) and four different prey species (*S. avenae*, *Allolobophora chlorotica*, *A. intermedius* and *D. reticulatum*). These were designed to measure the rate at which the DNA decayed in the guts of the predators during digestion.

As well as controlled feeding experiments, the multiplex system was used to analyse the gut contents of fifty *P. melanarius*, caught by pit-fall trapping from a crop of spring beans at Rothamsted Research at Long Ashton, Bristol on the 9th of May and the 7th June 2001. The beetles were analysed to estimate the efficiency of detecting predation on the targeted invertebrates within the field. Molecular analysis of several hundred such beetles from the same site, plus statistical analysis of the relationship between prey within the predators and prey availability in the field, will be reported elsewhere.

Results and Discussion

All of the primers were also tested for cross-reactivity against DNA from other potential prey species. In all cases, primers were found to be specific to the species or group for which they were designed. The robustness of the multiplex system was also tested to assess if, when numerous targets were co-amplified, any amplicon 'dropout' occured. We needed to be sure that there was no preferential amplification of particular DNA targets. When the multiplex was simultaneously tested against multiple species, the results show no preferential amplification, or amplicon dropout, and the system was capable of identifying all targets.

Results of the feeding trial experiments showed that DNA from each of the four species of prey could be reliably detected in the guts of *P. melanarius* for 18 h to 24 h. After this time, the ability to detect the prey DNA dropped quickly, such that after 48 h less than 30% of beetles in the slug and aphid trials tested positive. For the earthworm trial, 40% of beetles still tested positive at 96 h post-feeding.

The multiplex detection system was also tested on 50 field caught *P. melanarius*. Figure 1 shows the results for a single beetle, with peaks for four prey speicies including a slug and a snail. One or more prey species were identified in 80% of the predators. Overall, 48% contained the remains of a single prey species but up to four separate prey items were identified within some individual gut samples. Seven of the twelve prey DNA targets were identified. The most important prey item was earthworms, being found in 40% of beetles, confirming results by Symondson *et al.* (2000). Next to earthworms molluscs proved to be the main prey, especially *D. reticulatum* and *V. pulchella*. The remaining prey detected were aphids and weevils. The former are eaten by *P. melanarius* when they fall from the crop to the ground (e.g. Sunderland & Vickerman 1980, Sunderland *et al.* 1987), although this carabid has been observed to climb up to aphid colonies in the laboratory (Snyder & Ives 2001). *Sitonia* weevils were consumed by 16% of beetles, suggesting that they too were a relatively important prey item in bean crops.

The multiplex system we have developed will provide detailed information on prey choice by mollusc-eating predators. Instead of simply obtaining data on the proportion of predators containing mollusc remains we can now see how availability of alternative food resources affects prey choice. Such food resources can also play a vital role in helping to maintain predator populations in arable fields when mollusc densities are low.

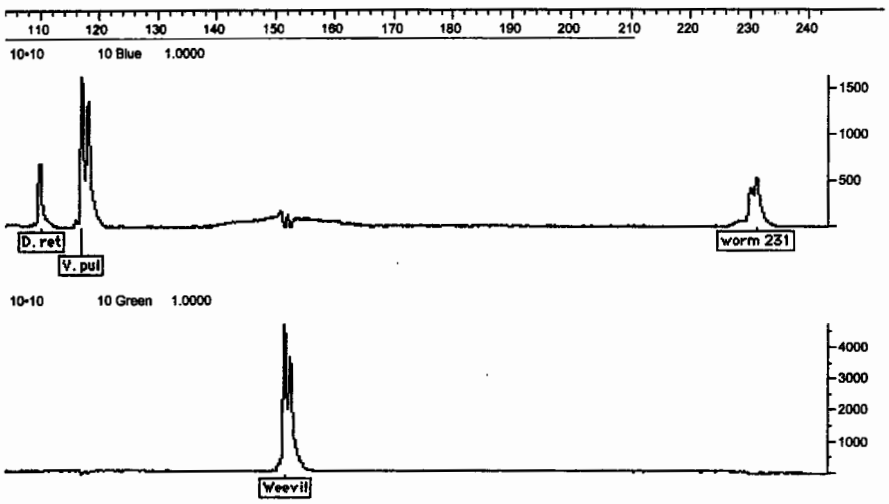

Figure 1. Scan of a single *P. melanarius* gut using an ABI 377 sequencer. The primers were labelled with either blue or green fluorescent markers. Along the top is the DNA fragment length in base pairs. Software was pre-programmed to recognise each DNA fragment size as a particular target prey species. This individual beetle contained the remains of a *Sitonia* weevil, two molluscs (the slug *Deroceras reticulatum* and the snail *Vallonia pulchella*) plus earthworm

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Insect Pathogens and Entomoparasitic Nematodes
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Impact of some agricultural practices on carabidae beetles

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Abstract: In Courseulles, Normandy (France), an experiment has been running for 10 years to compare integrated and conventional farming systems. A technico-economic study was carried out on the site during 1989-1995. Subsequently (1996-1998), further research was conducted on biological indicators, comparing four modes of production: Integrated (I), without ploughing and with optimised treatment; Integrated without treatment (IT); Conventional (C), plots being ploughed and using the treatments normally employed in the area; and Conventional without treatment (CT). Crop rotation on the plots was beetroot-wheat-peas-wheat.

Between 30 and 44 species of carabidae were detected. As in a previous study in the U.K., the number of species and the biodiversity of the captures were strongly correlated. The 5 dominant species accounted for 85% of captures, and 10 species for 95%. The results of the present study showed similar trends. The number of arthropods captured during the various analyses was quite high for intensively farmed plots. Throughout the study, spiders proved most sensitive to insecticides and to changes in cultivation. The beetles were rather less sensitive to such changes, but were nevertheless affected by these 2 factors. Staphylinids, on the other hand, were systematically more numerous in ploughed plots.

Integrated farming and absence of treatment thus seem to increase the number of carabidae and spiders, even if there was not always a significant increase in diversity or abundance over the long term. While their great capacity for colonisation meant that no medium-to-long term effect of production systems could be observed, s hort-term effects of the various technical procedures, on the other hand, were regularly detected. Generally speaking, monitoring the populations of these epigeal arthropods during the study found them to be sensitive to changes in cultivation procedure. The capture rate underscores the size of carabid, staphylinid and spider populations on cultivated land. Factors leading to population reductions were identified.

Key words: agricultural pratices, arable crops, carabidae, spiders, staphylinids

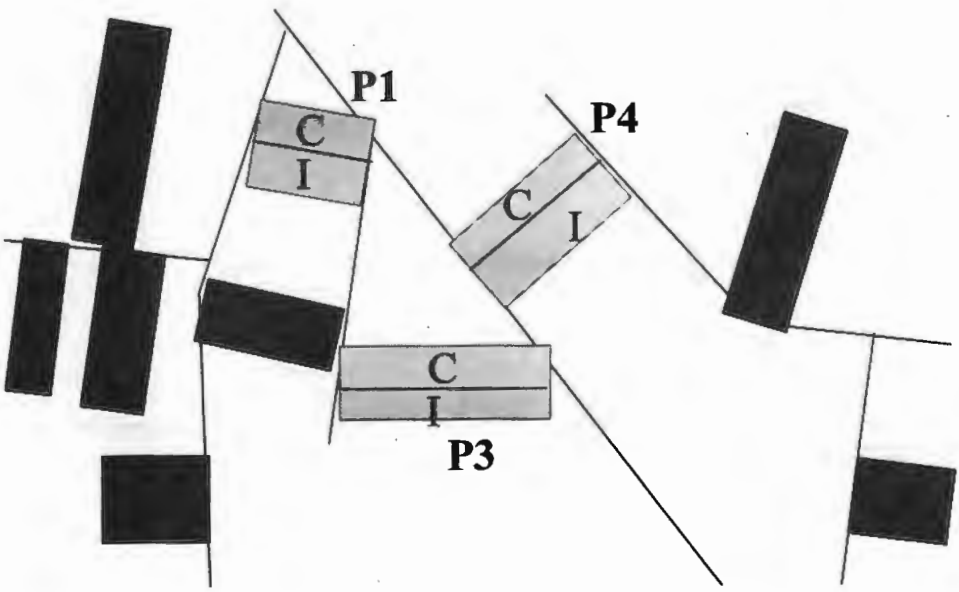
Introduction

In France there are 12 million hectares given over to large-scale farming, 14 million hectares of prairie and 1.5 million hectares of vines. The scale of these cultivated areas explains the importance of developing agricultural systems that respect the environment while still being economically viable and productive, and of doing all possible to preserve their biodiversity.

The arthropod population living on cultivated land comprises several hundred different species, only a few of which are phytophagous. The others are either beneficial organisms (parasites or predators) or else detritivores. The main polyphagous predators on cultivated land belong to the carabidae, staphylinidae and spider families. Aphid remains are frequently found in their digestive tracts. These predators may be found at high density, and become active quite early on in the season, although spiders tend to appear later. Certain carabid species seem to be effective predators when plant-louse populations are expanding in springtime.

Material and methods

The experimental site was in Courseulles-Sur-Mer, in the Calvados region of Normandy (France), near to the Channel coast. The climate there is oceanic, with an annual rainfall of 717 mm, fairly evenly spread over the year.



C: Conventional
I : Integred system
Plots size : 1.5 to 2 ha

Figure 1. Presentation of plots P1, P3, and P4 in Courseulles.

The experiment has been running for 10 years, comparing integrated and traditional farming systems. A technico-economic study was carried out on the site during 1989-1995. Subsequently (1996-1998), further research was conducted on biological indicators.

Of the six study plots, the programme mainly involved three cultivated ones – P1, P3 and P4 – of some 5 ha each (Figure 1).

Integrated farming lies midway between the Conventional system that seeks maximum yield, and organic farming using no synthetic chemicals at all. For present purposes, conventional farming may be defined in terms of the systematic application of treatments and maximisation of production. In this it generally corresponds to the perspective of the agricultural cooperatives and wholesalers. Artificial chemicals may also be used in integrated farming, but in most cases their utilisation is controlled. In large-scale farming, the aim is to reduce applications by 30% or 35% and implementing simplified ploughing. This may well

lead to lower yields, but the fall in gross profit is made up for by savings on field visits and applications.

Savings on applications mainly concern phytosanitary products and fertilisers. In environmental terms, integrated large-scale farming should be aiming at sustainability: "Development meeting the present needs of humanity as a whole, while leaving future generations the chance to survive and prosper."

Since 1990, each plot has been divided between two systems:

- a conventional system (C), with ploughing and abundant use of chemicals;
- an integrated system (I), without ploughing and with limited use of chemicals: herbicides and fungicides, but no insecticides.

Since March 1998, an area of some 1,200m^2 has been set aside in each plot where treatment is kept to an absolute minimum, in both conventional and integrated systems; these areas are known as IT in the integrated system (no pesticides on plots P1 and P4, and only herbicidal treatment on P3 in May 2000), and TT in the conventional system (no treatments in P1, and herbicides in P4 and P3 only during April-May 2000).

Thus, four experimental treatments were studied per plot:

- Integrated (I), without ploughing and with optimised treatment,
- Integrated without treatment (IT),
- Conventional (C), plots being ploughed and using the treatments normally employed in the area,
- and Conventional without treatment (CT).

The crop rotation per plot is shown in Table 1.

Table 1. Crop rotation per study plot in the programme

	1998	1999	2000	2001
P1	Beetroot	Wheat	Beetroot	Wheat
P2	Wheat	Peas	-	-
P3	Peas	Wheat	Flax	Wheat
P4	Beetroot	Wheat	Peas	Wheat
P6	Wheat	Peas	Wheat	-

The next Table presents the insect monitoring data over the entire Courseulles study period.

Table 2. Arthropod populations monitoring by ground-level trapping

Year: 1999	
Plots:	5 plots: P1 (wheat), P2 (peas), P3 (wheat), P4 (wheat), P6 (peas).
Sampling technique	Pitfall traps
Number of samples per plot	7 pitfall traps per plot (C, CT, I, IT): 28 traps raised weekly from 4 May to 29 June
Year: 2000	
Plots:	3 plots: P2, P4, P6
Sampling technique	pitfall traps
Number of samples per plot	7 pitfall traps per plot (C, CT, I, IT): 28 traps raised weekly from 26 May to 30 June
Year: 2001	
Plots:	3 plots: P1, P3, P4
Sampling technique	Pitfall traps
Number of samples per plot	7 pitfall traps per plot (C, CT, I, IT): 28 traps raised weekly from 16 May to 27 June

In this study, only carabidae were counted by species; spider and staphylinid species were all counted together.

Results and discussion

Despite the intensiveness of the farming practised at Courseulles, certain rare species such as the large *Abax* and *Eucarabus* beetles were found. The golden carabid *Carabus auratus* was detected regularly on plot P6.

The study data were analysed by correlating capture rates and cultivation procedures. This enabled the relative importance of ploughing and of phytosanitary treatment to be estimated. Such detailed data analysis can highlight temporal phenomena that other, more general statistical analyses would not do.

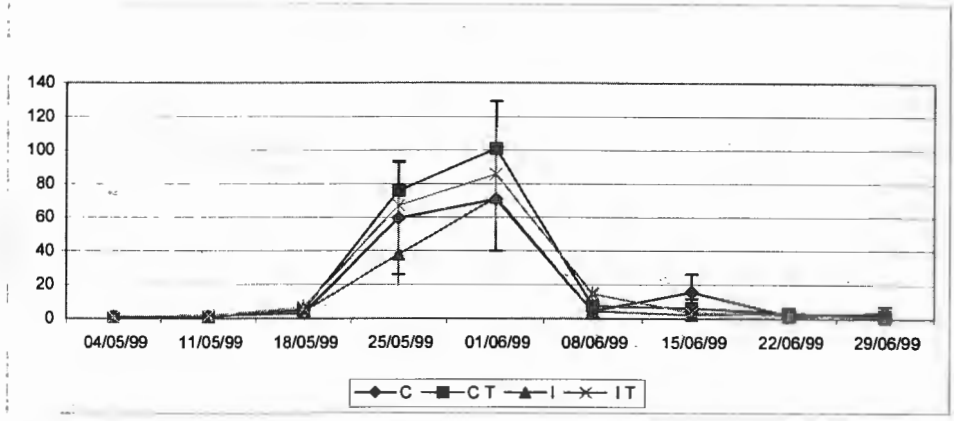


Figure 2: Carabid capture for plot P2 (Peas) in spring 1999

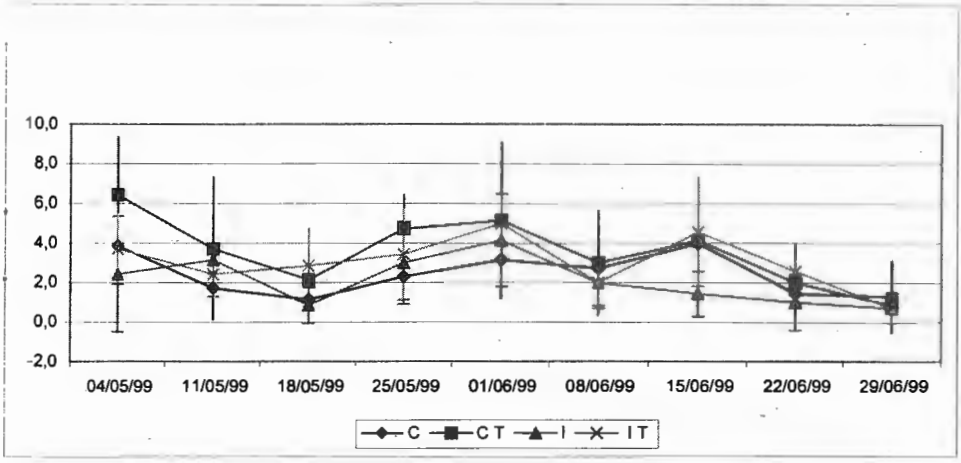


Figure 3: Carabid capture rates for plot P1(Wheat) in spring 1999

Figure 2 and 3 highlight the importance of trapping over a sufficiently long period. Depending on the plot and on the crop, peak capture times may differ. In the pea filed, capture peaked in the second half of May, but was more evenly spread in the wheat fields. In pea fields, the untreated plots (CT and IT) showed much higher capture rates than the treated ones (C and I), whereas this difference was much less pronounced in the wheat fields.

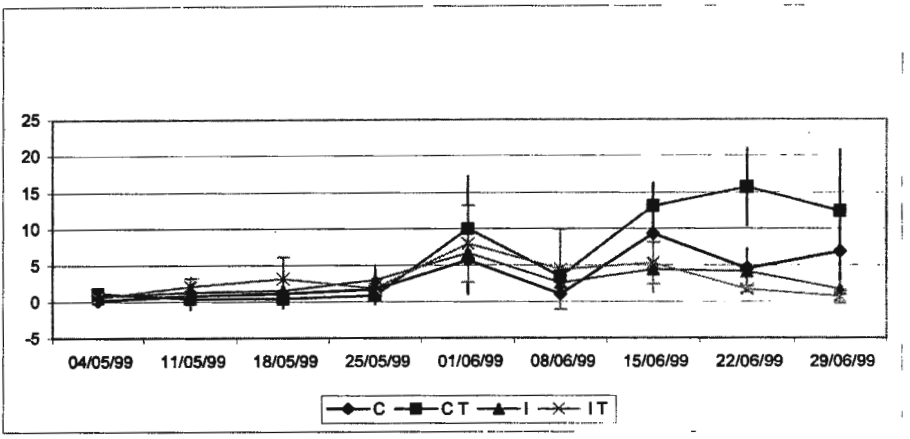


Figure 4 : Staphylins captures in P2 (peas) in 1999

In Figure 4, staphylinid captures during Spring 1999 tended to be higher in conventional pea and wheat fields. In the pea field, the difference between the C and CT curves shows the impact of insecticide application. In the case of wheat, no such effect was found.

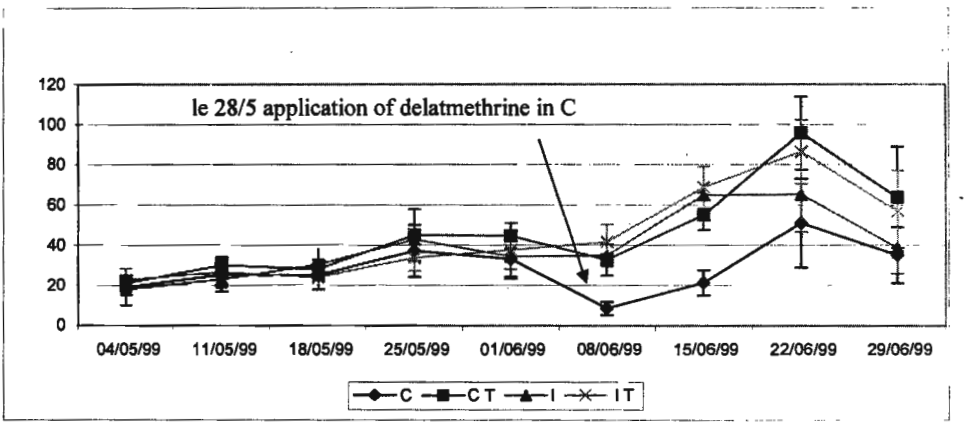


Figure 5 : Spider captures in P1 (Wheat) 1999

Prior to early June, spider capture rates were broadly the same in under the different study conditions (Figure 5). Following deltamethrin treatment in the T condition, the capture rate fell sharply. Afterwards, the differences between conditions grew weaker.

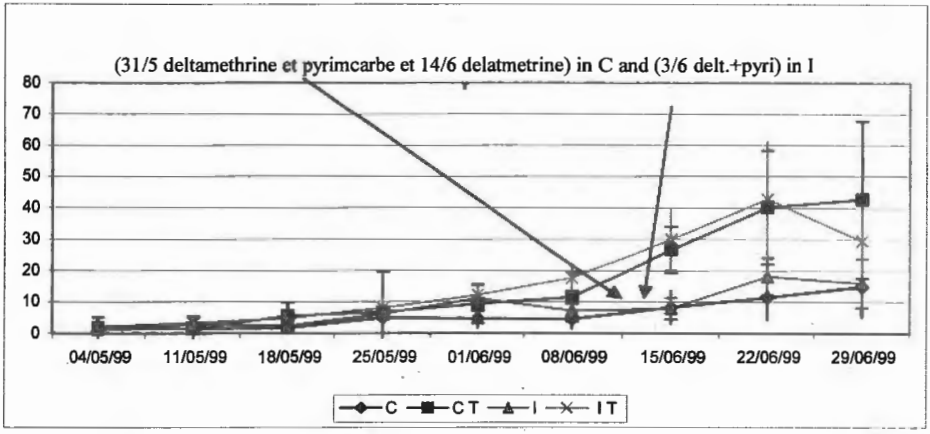


Figure 6 : Spider captures in P2 (peas) in 1999

In wheat, insecticide treatment reduced the spider capture rate in the pitfall traps. Spiders are fairly polyphagous predators, so this fall in numbers is unlikely to be due to prey being killed off by the insecticide. Rather the intrinsic toxicity of the insecticide being used was likely to have been the cause of the decrease in capture.

Statistical analysis

A non-parametric Kolmogorow-Smirnov test was run on the total capture data per plot and per year, comparing the effect of ploughing (C and CT vs I and IT) and of treatment (C and I vs CT and IT).

Table 4. Number of statistically significant (*) or non-significant (NS) differences found in the effect of ploughing (C/CT vs I/IT) or of treatment (C/I vs CT/IT).

		NS	*
carabidae	**C/CT vs I/IT**	**7**	**4**
	C/I vs CT/IT	**6**	**5**
	TOTALS	*13*	*9*
Staphylinids	**C/CT vs I/IT**	**4**	**7**
	C/I vs CT/IT	**9**	**2**
	TOTALS	*13*	*9*
Spiders	**C/CT vs I/IT**	**4**	**7**
	C/I vs CT/IT	**5**	**6**
	TOTALS	*9*	*13*

For carabidae and staphylinids, 9 significant differences emerged from the 22 (13+9) tests; for spiders, the number was 13 out of 22 (Table 4). This difference suggests that spiders are more sensitive as biological indicators than carabidae and staphylinids.

Overall, then, the indicators proved sensitive to the differences in experimental treatment studied here. Carabidae also were sensitive to treatment and ploughing. Staphylinids were

notably more sensitive to ploughing effects than to phytosanitary substances. In this case, the conventional system was more favourable to populations than the integrated system. Quantitatively, spiders, like carabidae, proved as sensitive to insecticides as to ploughing.

Analysing the total capture figures for the three years of the study confirmed the trends found above (Figure 7).

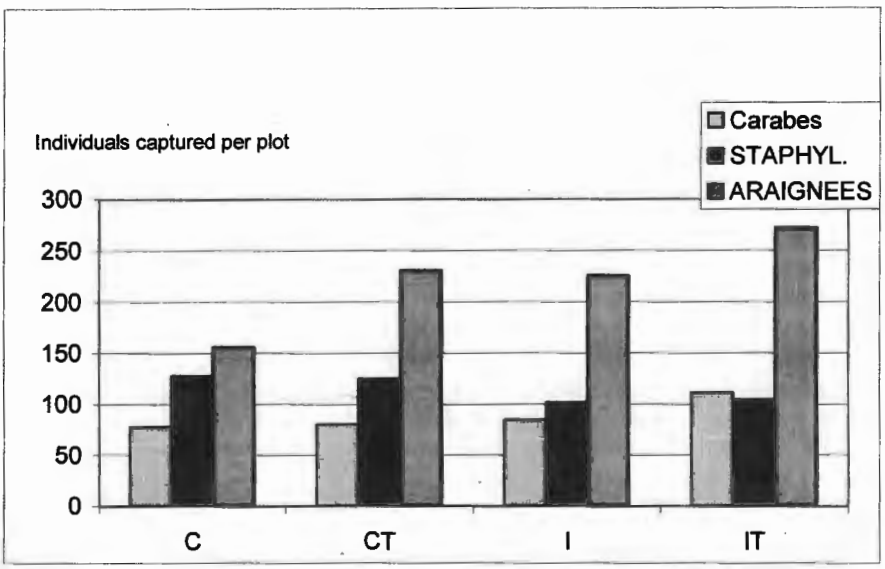


Figure 7 : Total carabid, staphylinid and spider captures: mean values of plots and years.

Carabid populations were on average markedly greater in IT plots, combining the advantages of less intense ploughing and absence of insecticide. These findings confirm the sensitivity of this biological indicator to these two factors.

The number of staphylinids was much greater in the two conventional plots. The mixed impact pattern for spiders can be seen again here. Mean incidence was less in conventional than in integrated plots. Furthermore, in either system the untreated plots showed markedly higher capture rates than those treated, especially with insecticide.

Conclusion

The number of arthropods captured during the various analyses was high for intensively farmed plots. In Courseulles, between 30 and 44 species of carabidae were detected. This matches findings from a U.K. study, which found about 30 carabid species in cultivated land. The numbers of species and the biodiversity of the captures correlated strongly. The 5 dominant species accounted for 85% of captures, and 10 species for 95%. The results of the present study showed similar trends. Monitoring epigeal arthropod populations in the Courseulles study showed these organisms to be sensitive to changes in agricultural technique.

Throughout the study, spiders proved most sensitive to insecticides and to changes in cultivation. The beetles were rather less sensitive to such changes, but were nevertheless affected by these 2 factors. Staphylinids, in contrast, were systematically more numerous in ploughed plots. Generally speaking, these arthropods' capacity for colonisation meant that no medium-to-long term effect of production systems could be observed. Short-term effects of the various technical procedures, though, were regularly detected. Integrated farming and absence of treatment thus seem to increase the number of carabidae and spiders, even if they do not always greatly increase their diversity or numbers over the long term.

This studied has highlighted the role carabidae, staphylinids and spiders play in cultivation. Their sensitivity to production techniques was demonstrated at Courseulles. Factors leading to population reduction were able to be identified. Systems need to be designed that optimise beneficial practices. Identifying practices that favour the populations of beneficial organisms that limit the growth of pests such as plant-lice and slugs on cultivated land is an example of this. More fundamentally, this kind of study sheds light on the role of arthropod beneficials in agriculture. The size of the populations found here underscores their influence on the equilibrium of the biocenosis of a cultivated field.

It would have been desirable to analyse the data in greater depth. The literature contains ecological analyses that are not widely used in agronomy, where environmental variables need to be taken into full account. More elaborate data analysis could be envisaged to reveal quantitative relations with environmental variables such as site, plot, crop, year and treatment.

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Impact of some insecticides on Carabidae and consequences for slug populations

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Abstract: The present study sought to measure the medium-term toxicity of wheatear-louse insecticides (esfenvalerate associated with pyrimicarb, esfenvalerate alone, and tau- fluvalinate) for the main beneficial organisms able to help regulate pest populations.

Each study plot measured 48 by 70 metres. Fauna in the various treated plots was assessed by regular ground-level trapping. Data were compared with those of an untreated control plot and a carbaryl-treated plot. Carbaryl is toxic for the various aphidiphagous beneficials, and led to a strong growth in plant-louse populations.

Carbaryl and esfenvalerate-plus-pyrimicarb proved toxic for Carabidae. In the two plots treated with these substances, the number of slugs captured was much greater than in the other plots. The study thus underscores the importance of Carabidae for the control of slug populations.

Key words: arable crops, Carabidae, Araneae, Staphylinidae, slugs, insecticides

Introduction

The present study sought to assess the medium-term impact of test substances on wheatear-louse predators and parasitoids, comparing it to data for an untreated control plot and to a toxin acting on beneficials but not on plant-lice. The toxin condition was intended to demonstrate plant-louse population growth untrammelled by natural enemies. Plant-louse and beneficial populations were estimated just prior to and during the month following treatment.

Material and methods

The size of the individual plots (70 by 40 metres) was large enough to limit beneficial recolonisation. The following treatment conditions were applied:

1. untreated control
2. KABUTO (6 g/l esfenvalerate plus 100 g/l pyrimicarb) at 1 l /ha
3. SEVIN L85 (85% carbaryl) atl kg/ha
4. SUMI ALPHA (25 g/l esfenvalerate) at 0.3 l/ha
5. MAVRIK FLO (240 g/l Tau fluvalinate) at 0.15 l/ha.

A single treatment was applied on 26 May 1999 at the start of flowering (BBCH 61). It was delivered by the farmer's own sprayer at a rate of 300 l/ha, from a 12 metre boom. The quantity of mixture sprayed onto each plot was controlled.

Traps comprised pitfalls of 11 cm diameter and 10 cm height, ¾ filled with water. A few drops of TEEPOL spreader were added. The pitfalls were buried with their neck at ground level. This technique captures spiders, carabids and staphylinids. The traps were raised on the following days post-treatment (T): T+5, T+12, T+19, and T+25.

Table 1. The numbers of various carabid species trapped and their percentage of the total number of carabids trapped. The two main species found were *Platysma vulgare* and *Poecilus cupreus.*

	Number	Percentage
Platysma vulgare	684	47.30
Poecilus cupreus	447	30.91
Agonum dorsale	138	9.54
Bembidion sp	36	2.49
Ophonus pubescens	34	2.35
Harpalus sp	13	0.90
Nebria sp	12	0.83
Loricera pilicornis	52	3.60
Other	30	2.07
TOTAL	**1,446**	**100**

Table 2. The mean number of Carabidae per pitfall trap by sampling date.

DATES	31 May	07 June	14 June	21 June
Control	17.8	19.8	32.6	21
Carbaryl	5	1.2	8	6
esfenvalerate + pyrimicarb	4	2.6	2.6	3
esfenvalerate	17.2	20.6	16.4	11.8
Tau fluvalinate	15.8	24.6	28.6	13.6

The mean numbers per pitfall trap taken from the control plot lay between 17 and 32. The numbers for the esfenvalerate and Tau fluvalinate treated plots were broadly similar to these figures. Carabid numbers were very low (1 to 8) in the carbaryl and esfenvalerate + pyrimicarb treated plots in the four post-treatment samples. In the carbaryl-treated plot, numbers rose slightly on 14 and 21 June: Carabidae are highly mobile, and the large number of plant-lice in this particular plot may have attracted some.

Carbaryl and esfenvalerate + pyrimicarb proved toxic to the beetles throughout the study.

Table 3. The mean number of slugs collected per pitfall in each of the 5 plots by sampling date

DATES	31 May	07 June	14 June	21 June
Control	0	0.6	0.2	0.4
Carbaryl	10.6	11.6	4	5.4
esfenvalerate + pyrimicarbe	1	4.8	4.2	1.8
esfenvalerate	0.4	0.4	0.8	0.4
Tau fluvalinate	0.2	0.6	0	0.6

There were more slugs in the carbaryl and esfenvalerate + pyrimicarb treated plots than elsewhere. Slug numbers were inversely proportional to carabid numbers.

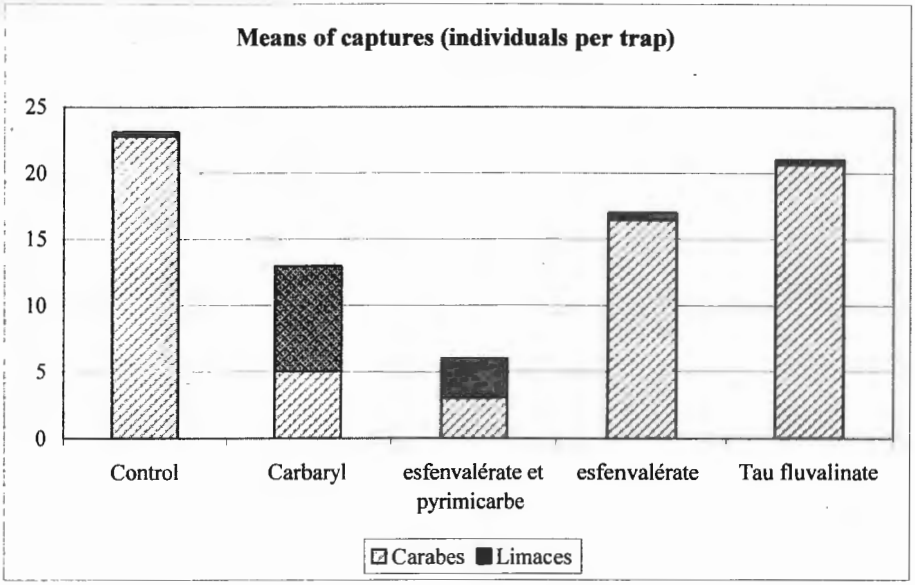

Figure 1. Mean numbers of Carabidae and slugs captured over four days.

There were more slugs in the carbaryl and esfenvalerate + pyrimicarb treated plots than elsewhere. Slug numbers were thus inversely proportional to carabid numbers.

The correlation coefficient between carabid number and the logarithm of the slug number was r = -0.89, significant at the 5% level (Table 4).

Table 4. Relation between carabid and slug capture rates over the whole capture period.

	Carabidae	Log(slugs+1)		
CONTROL	22.8	0.26	R	-0.90
Carbaryl	5.05	2.18	R^2	0.80
esfenvalerate + pyrimicarbe	3.05	1.37		
esfenvalerate	16.5	0.40		
Tau fluvalinate	20.65	0.30		

The correlation coefficients for spiders and staphylinids were respectively 0.57 and 0.82. As there were very few staphylinids, this finding was not investigated further.

Discussion

In this study of wheat crops, two treatments showed highly toxic effects on Carabidae. In plots where such a fall in carabid numbers was found, the number of slugs captured rose sharply.

This study was performed under realistic agricultural conditions, and shows Carabidae to play an important role in controlling slug populations. It is therefore worth implementing agricultural practices favourable to these beneficial organisms.

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Facultative scavenging by *Pterostichus melanarius* on slug carrion: detectability of decayed prey in the predator's guts using PCR

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Abstract: Gut content analyses are widely used for estimating predator-prey interaction in the field. Facultative scavenging by predators could therefore be a potential source of error. We demonstrated that the generalist predator *Pterostichus melanarius* would readily feed on dead *Deroceras reticulatum* under both field and laboratory conditions. Dead slugs placed in the field were rapidly removed by scavenging *P. melanarius*. The degradation rates of the DNA of dead slugs, and the detectability of this decaying material in the predator's guts, were estimated using PCR-based detection techniques. The results revealed that PCR-based gut content analyses are unable to distinguish between true predation and scavenging.

Key words: carrion, decay rates, gut content analysis, PCR, *Pterostichus melanarius*, scavenging, slug.

Introduction

Among arthropod predators occurring in agroecosystems, carabid beetles a common and abundant group. Because of their predominantly carnivorous and polyphagous feeding ecology (Thiele, 1977, Larochelle, 1990), they are considered to be potentially important natural pest control agents (Hengeveld, 1980a,b; Luff, 1987; Kromp 1999; Symondson et al. 2002a). In studies that investigate their feeding habits, pest control efficiency or predation rates, gut content analyses are an invaluable tool. Since many invertebrate predators (including carabids) regularly scavenge (Sunderland, 1996) and gut content analyses are not able to distinguish between scavenging and true predation, scavenging could potentially lead to overestimation of biocontrol impact or even to identification of false predator-prey interactions. Facultative scavenging by carabids under field conditions, and availability/disappearance rates of invertebrate carrion in the field, have been little studied. There has been only one study looking at the detectability of decayed prey in predators by Calder et al. (2005), who used a monoclonal antibody to study consumption of decaying slugs. No-one has yet used this approach to examine detectability using PCR or combined this with an estimation of cadaver removal rates in the field. Further research in this area is needed to improve our understanding of predator-prey interaction between carabid beetles and their pest prey.

We measured the rates at which DNA became undetectable in decaying *Deroceras reticulatum* (Müller) slugs (Mollusca: Pulmonata) and detectability of DNA from this carrion type in the guts of the generalist predator *Pterostchus melanarius* (Illiger) (Coleoptera: Carabidae). The effects of carrion age, weight and beetle sex on detection periods was described. Laboratory trials were used to estimate prey-preference by the beetles between prey that had decayed for different lengths of time and live prey in the laboratory. Further experiments measured the acceptability of slug carrion to *P. melanarius* in the field.

Disappearance rates for slug carrion in wheat fields and grassland was estimated and *P. melanarius* was identified as the major scavenger. Possible implications of facultative scavenging by invertebrate predators for biocontrol and food-web research are discussed.

Material and methods

Deroceras reticulatum slugs were collected by hand in the vicinity of Cardiff, South Wales, UK and killed by freezing at -80°C. The weight of each slug was recorded before freezing. The *P. melanarius* beetles were collected by pitfall trapping from a wheat field and kept individually in a controlled environment. Prior to the experiments, the beetles were fed on a single *Calliphora vomitoria* (L.) larvae and then starved for 7 days.

Sample preparation and processing

Freshly killed slugs were placed on topsoil and were allowed to decay for sixteen different time periods from 0-264 h under controlled conditions. They were then frozen in batches of eight slugs for the each time period, thawed, homegenised and used for the DNA extraction. Batches of thirty slugs were frozen after 0, 3, 6, 12, 24, 48, 72, 120 and 168 h of their decomposition on characterised topsoil from a field. The decayed material was fed to the beetles. Batches of five beetles were killed by freezing at 0, 1, 3, 6, 12, 24 and 48 h after feeding, for each decay period of the prey. Beetle guts were dissected, their gut contents removed and DNA extracted using a QIAamp® DNA stool Mini Kit (Qiagen GMBH, Hilden, Germany). Species-specific primers that amplified a 109 bp sequence of D. reticulatum DNA (Dodd, 2004) were used for PCR. The PCR products were visualised by ultraviolet transillumination following electrophoresis on an ethidium bromide stained agarose gel.

Field experiments

Field experiments were carried out in two wheat fields (2.7 and 3.5 ha) and two pastures (5.4 and 2.2 ha). Fifteen freshly killed *D. reticulatum* were placed directly on the ground in the middle of each of the four experimental sites in 10 m square grids to avoid interaction among them. Presence, absence or partial consumption of the slugs was checked every 3 h for 24 h. The experiment was repeated after 12 h. Potential scavengers were identified visually.

In a parallel experiment, pitfall traps baited with dead slugs or nothing (controls) (N = 10) were set up in 10 m square grids in each of the four experimental sites (120 traps in total). The trap treatments were randomised. The numbers of *P. melanarius* beetles (the dominant predator species in all of the experimental sites) in the traps was recorded and traps were empted every 3 h over 36 h. Regular emptying of the traps avoided the possibility that the beetles would be attracted by conspecifics or other insects that had fallen into the traps.

Choice experiment

Batches of eight freshly killed slugs were placed on the soil in 8.5 cm diameter by 4.5 cm deep uncovered plastic containers (one per container), allowed to decay under controlled conditions for sixteen different time intervals from 0-264 h and then frozen. At the start of the experiment a single thawed slug together with a single live slug was placed on a Petri dish lined with a moisten filter paper. Single *P. melanarius* beetles (sexes in equal proportions) were placed in each container and observed continuously for 1.5 h. The first attack by the beetle on the prey, and feeding on live or dead prey, was recorded. The beetle was considered to have attacked the prey when it took a bite. It was considered have fed on the prey when feeding was continuous for more than 2 min on one prey or if it consumed the whole prey. Data from non-feeding beetles was excluded.

Results and discussion

Degradation rate of the DNA in slug carrion

The relationship between the probability of detection and slug weight was analysed using binomial multiple regression, where detection was regressed against decomposition time and slug weight. The regressions were fitted as generalised linear models and their significance tested by comparing the Akaike information criterion and F values with the null model. Whereas decay time had a highly significant effect on probability of detection, slug weight did not. Fitted functions for the relationship between detectability and time of decomposition were created. This experiment was used to estimate a theoretical threshold for detection of carrion in the predator's guts and described DNA degradation in decaying invertebrate tissues.

Detectability of slug carrion in predators' guts

Multiple GLM regressions with binary response variables (detected vs. undetected) were used to analyse the data and revealed that the decay time for the dead slugs and the time that elapsed before the predator was killed had highly significant negative effects upon the detectability of slug DNA. Slug weight was also significant (the larger the slug the longer their remains could be detected within the beetle) but this effect was weaker than the two time-related variables. Beetle sex had no effect. The multiple regressions showed that the time that the slugs decayed and the time before the carabid was killed (digestion time) had independent additive effects on decreasing the detectability. The strongest factor was digestion time within the beetle.

Response of* P. melanarius *to dead slugs in the field

To analyse the experiment we used analysis of covariance with (wheat vs. pasture) and 'bait' (slug carrion vs. control) as independent variables, and time (linear term) as a covariate. The effect of time was assessed via within-cell regression. Both 'crop' and 'bait' significantly affected numbers of captured carabids, whereas neither their interaction nor the covariable time had significant effects. More carabids were captured in wheat than in the pasture. Regarding bait, traps with dead slugs captured more carabids than controls traps. It follows that (i) the carabids were attracted to traps containing dead slugs, and (ii) the pattern of the attraction did not differ between crops, which presumably differed in abundance (or activity-density) of the beetles, and (iii) the attraction neither declined nor increased during time that the traps were exposed.

Disappearance rate of slug carrion in the field

Cox's proportional hazards regression modeling approach was used to evaluate the effect of carrion weight, experimental site and crop type (pasture/wheat) on the removal rates of dead slugs in the field. This approach revealed that the 'risk' that a dead slug would be consumed significantly decreased with decreasing slug weight. Such a response would be expected in response to decreasing apparency, whether visual or olfactory. Both crop type and experimental site also had highly significant effects and explained more variation in the model than the carrion weight. Carrion disappearance was significantly faster in wheat fields than in pastures. However significant differences were found also between sites within crop type. Interestingly, in sites where there was high activity-density of *P. melanarius* there were high disappearance rates for dead slugs. The order of the sites was identical for both variables. Overall, *P. melanarius* represented more than 80% of identified scavengers recorded *in-situ* in the field and was the only predator found in significant numbers in the pitfall traps. We

conclude that this carabid was the major invertebrate scavenger of dead slugs at our experimental sites.

Choice experiment

No significant difference was found between live and dead slugs in terms of first attacks by the beetles. However, dead slugs were consumed significantly more often than living ones. Furthermore, the interaction dead/living*time of decomposition was highly significant and performed as the best-fitting combination of explanatory variables for attacking and consuming slugs (GLM regressions were used again). Time of decomposition thus systematically and nonlinearly influenced the preference: dead prey was preferentially attacked/consumed in early stages of decay, but later preference shifted towards live prey as the dead prey on offer became increasingly putrid.

Conclusions

The carabid *P. melanarius* has been shown in many studies now to be capable of killing slugs in the laboratory (e.g. McKemey *et al.*, 2001), and of controlling slug populations under semi-field conditions, either as adults (McKemey *et al.*, 2003) or larvae (Thomas 2002). In the field they can significantly affect the spatial (Bohan *et al.*, 2000) and temporal (Symondson *et al.* 2002b) dynamics of slug populations. Other species of carabids have been shown to eat dead slugs in preference to live ones in the laboratory (Mair & Port, 2001; Langan *et al.*, 2001). Here, however, we show in both field and laboratory experiments that *P. melanarius* readily and preferentially feed on dead prey if available, such preferences only changing as the slugs become increasingly decayed. Comparison of the mean decay time for dead slugs in the field with the detection period for slug material (that had previously been allowed to decay for various periods) in the predator's guts showed that PCR-based techniques are not able to distinguish between predated and scavenged food items. Assuming that material in predator's guts was the product of predation could lead to overestimation of the impact of predation on slugs by carabids. Equally, scavenging may have been underestimated as an important ecological process, helping to maintain high populations of known slug predators such as *P. melanarius*. Estimation of carrion availability in the field is essential if we are to improve our understanding of predator-prey interactions in the field.

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Insect Pathogens and Entomoparasitic Nematodes
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The use of *Phasmarhabditis hermaphrodita* (Nemaslug®) for the control of slugs, an update of the most recent results

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Abstract : The last decade has seen Nemaslug® use expand rapidly and it is now used throughout Europe. Up to now, it has been used mostly in the home and garden market and in high value crops. The focus is now to develop this product for use in large scale crops like vegetables and salads. This paper will present the results of some commercial trials done by Becker Underwood and Brinkman Agro in the Netherlands and the United Kingdom in Brussels sprouts and Roman lettuce. Application method to large scale crops and positive results from 2004 will be discussed.

Key words : Brussels sprouts, field trials, lettuce, Nemaslug, *Phasmarhabditis hermaphrodita*, Slugs.

Introduction

Nemaslug® is mostly commercialized in the Home and Garden market and high value crops. However, this product has a great potential in many large scale crops. Indeed, many academic trials done in the last 15 years give hope for commercial success in crops like winter wheat, salad, asparagus and Brussels sprouts.

Therefore, Becker Underwood's aim is now to develop the use of this product in some of these large scale crops in Europe. In this paper the protocols and results for 3 commercial trials, conducted in Brussels sprouts and Romaine lettuce in the United Kingdom and Netherlands during 2004, will be presented and discussed.

Material and methods

Experimental designs and assessments

Brussels sprouts – United Kingdom: The experimental design used for this trial had two replicates in order to compare Nemaslug with Optimol, Sumiagro (4% metaldehyde) applied at the manufacturer's recommended rate. The treatment plots were placed close to the field margin in order to have a higher slug population, making it unnecessary to infest the field artificially. The standard plot size (48 m x 48 m) was large enough to minimize the effects of slugs moving from one plot to an other.

15 plants were assessed per replicate (30 plants per treatment), randomly chosen in the middle of each plot. The number of damaged buttons and slug presence on / around the plant were noted.

Brussels sprouts – The Netherlands: Three plots can be distinguished in this trial site (Figure 1). This particular design resulted from an initial application mistake by the grower. However, this trial site was kept for its dramatic results. 30 plants are assessed per half plot, randomly

chosen in the middle of the area (to avoid slug migration from plot to plot). The number of damaged buttons and slug presence on / around the plant are noted.

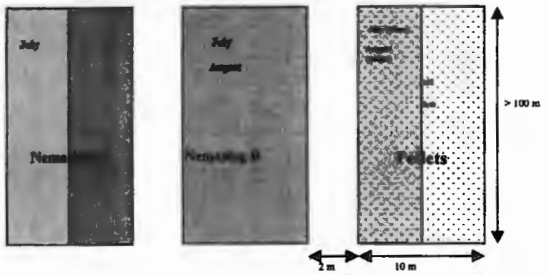

Figure 1. Large field scale Experimental design, Brussels sprouts, The Netherlands

Roman Lettuce – United Kingdom: The assessment areas are 2m x 2m (Figure 2). They have been chosen as far as possible from the margin, in order not to disadvantage one treatment from the other. 15 plants were randomly chosen per 2m x 2m assessment area; 45 plants were assessed per treatment. The number of damaged leaves and slug presence on or around the plant were noted.

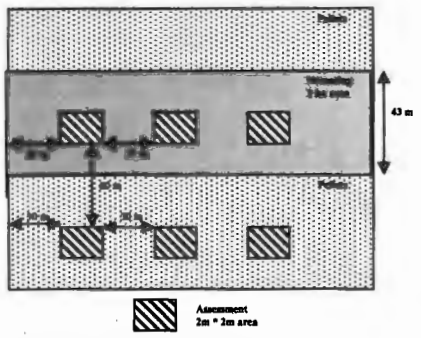

Figure 2. Large field scale Experimental design, Roman lettuce, UK 2004

Nematode Application

In Brussels sprouts and lettuce crops, the applications were made by standard boom sprayers. The rate used is the one developed by the Dutch research institute PPO in Lelystad: 50 000 nematodes per m^2, 3 applications with 1000 L / ha of water (Ester, *et al.* 2003). We chose to apply Nemaslug every two weeks.

Due to the high density of the Brussels sprouts canopy, the recommendation is to apply Nemaslug when it was raining. The idea is to allow the highest number of nematodes to reach the soil and prevent the nematodes dessicating and dying on the leaves.

With regarding to the lettuce, which is generally a summer-grown crop, the recommendation was to apply Nemaslug with 1000L per ha and then wash off the leaves with

water (1500 L per ha) in order to allow the nematodes to go into the soil (if the soil surface is too dry).

The chemical pellets have been applied by the grower in every site at the manufacturer's recommended rate.

Results and discussion

Brussels sprouts – United Kingdom

Significant differences between the two treatments (Nemaslug and Optimol) were seen between the first and the second treatment (Figure 3). The plants in Nemaslug plots show significantly less damage than the ones in the Optimol plots (Mann-Whitney test). However, the damage was very low in both treatments at the time of the last assessment. A last assessment will be done in December 2004, at harvest, to confirm these results.

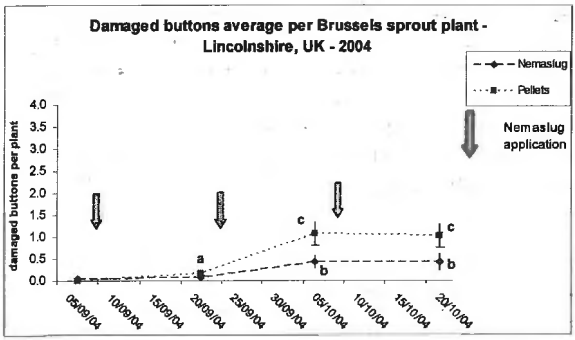

Figure 3. Mean ± SE damaged buttons per plant – UK, 2004

Brussels sprouts – The Netherlands

The graph shows that Nemaslug applied twice has reduced significantly the damage on the sprouts compared to the metaldehyde treated area, where damage is particularly high (Figure 4). The plot where Nemaslug has been applied only once on the half (Nemaslug A) gives intermediary results between Nemaslug B and Pellets.

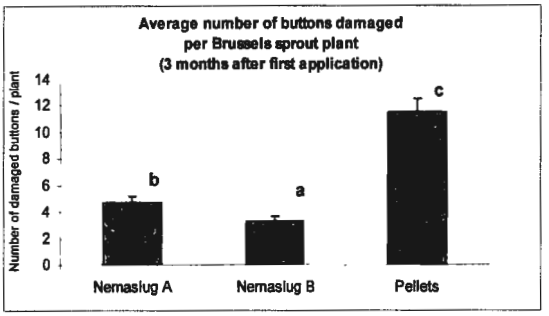

Figure 4. Comparison of Slug damage between metaldehyde and Nemaslug (Mean ± SE, ANOVA, Arcsine transformation)

Although the plots are smaller than in previous trials (slug migration risk from plot to plot), these results indicate that Nemaslug applied twice, has reduced the average damaged buttons by 74 % compared to the half of the Pellets plot, where they have been applied twice during the trial period (Figure 5). In the half plot where Nemaslug has been applied only once, the damage is intermediate between the pellets plot and Nemaslug twice. These particularly good results might be explained by the less dense canopy of the crop which has allowed some more nematodes to reach the soil surface.

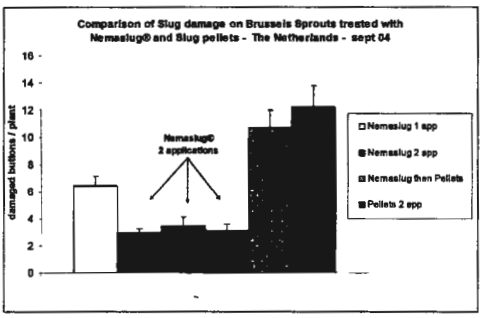

Figure 5. Comparison of Slug damage between metaldehyde and Nemaslug – different plots details (Mean ± SE damaged buttons per plant)

Romaine Lettuce – United Kingdom

Figure 6 presents the results from an assessment done between the 2nd and 3rd nematode application (26 days after planting – 15 days after the first nematodes application). With merging the 3 assessments area data per treatment, it shows that the Nemaslug area has significantly less leaves damaged than the chemical treated area (Mann-Whitney). Globally, the damage were low at harvest.

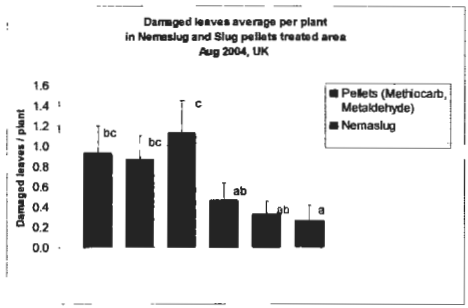

Figure 6. Comparison of Slug damage between Nemaslug and slug pellets (Methiocarb and Metaldehyde). Mean ± SE, Mann-Whitney test.

Conclusion

These trial's results show that Nemaslug application on a large scale is a success and confirms the PPO trials results (Ester *et al.*, 2003).

In broad acre crop, some parameters can be issues as soil moisture or weather during the application. This is especially true in big farms where irrigation or spraying time are restricted. However, the nematodes application remains suitable with the classic sprayer system and some adaptations can be found to solve these problems.

This experience is now expanding the use of Nemaslug into vegetable crops as a viable alternative to chemicals.

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Molecular detection of the nematode *Phasmarhabditis hermaphrodita* within slugs and predators of slugs

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Abstract *Phasmarhabditis hermaphrodita* is a slug parasitic rhabditid nematode that is used for the biological control of the field slug *Deroceras reticulatum*. Two species-specific primer pairs were developed for the molecular detection by PCR of DNA originating from *P. hermaphrodita*. The primers amplified two DNA fragments of mitochondrial Cytochrome Oxidase subunit I, a 353-bp fragment and a 155-bp fragment.

Two experiments were carried out to investigate the potential of these primer pairs to detect nematode DNA within the slug host and within the gut of a generalist predator (the carabid beetle, *Pterostichus melanarius*) that had eaten an infected slug host. Detection of nematode DNA within tissue of the rear of the mantles of infected *D. reticulatum* was possible from some samples as early as 1 h after infection. After 120 h of infection all samples tested positive for nematode DNA. In a second experiment, *D. reticulatum* at varying stages of nematode-infection, were fed to starved carabid beetles (*P. melanarius*). Molecular detection of nematode DNA within the gut of the beetle predator was achieved for a maximum of 24 h after eating the most infected slug host.

This work was carried out to investigate the feasibility of using molecular detection of nematode DNA to track the presence and level of infection of the slug parasitic nematode *P. hermaphrodita* through soil ecosystems.

Key words: *Deroceras reticulatum*, molecular detection, PCR, *Phasmarhabditis hermaphrodita*, soil food webs.

Introduction

Nematodes of the genera Heterorhabditis and Steinernema are commonly used as biological control agents to control a range of insect pests (Gaugler *et al*, 1997). More recently a mollusc-parasitic nematode, *Phasmarhabditis hermaphrodita* Schneider, has been exploited to control pest molluscs (Wilson *et al*, 1993), and in particular the damaging and widespread pest slug *Deroceras reticulatum* Müller. These nematodes are marketed as a biological control agent for slugs in the UK (Nemaslug®, Becker Underwood), and are used mainly in the home garden market. The nematodes are bacterial feeders, and have a non-feeding, free living stage which inhabits the soil called an infective juvenile (IJ). The nematode infects slugs in the area beneath the mantle surrounding the shell, causing characteristic swelling of the mantle and death within 7-21 days of initial infection (Wilson *et al*, 1993).

Rhabditid nematodes, that are used as biological control agents, have been shown to infect and kill a wide range of non-target invertebrates under laboratory conditions (Poinar, 1989). The effects of *P. hermaphrodita* on soil ecosystems and food webs are less well understood. One method of investigating these interactions is by the analysis of host tissue and the guts of soil organism for the presence of parasitic nematode DNA. The use of species-specific oligonucleotide primers in PCR reactions enables the detection of minute amounts of target DNA, even when swamped by non-target DNA (Symondson, 2002). This method has been used successfully in the analysis of predator guts in predator-prey studies (e.g. Agusti *et*

al, 2003) but this is the first time that this technique has been applied to the detection of the consumption of dead and dying nematode-infected prey. Here our aim was test the sensitivity of a molecular approach to determining nematode infection rates in the field. We are also interested in possible negative interactions between nematodes and predators, where predators such as the carabid beetle *Pterostichus melanarius* Illiger may be preferentially attacking nematode-infected slugs. We therefore tested the ability of the nematode primers to amplify nematode DNA from beetles that had fed on nematode-infected slugs.

Material and methods

Slugs and nematodes

The slugs (*D. reticulatum*) weighing 0.075-0.383 g were collected from grass lawns in Wiltshire and Cardiff in July 2004. They were stored in groups of 15 in non-airtight plastic containers at 6°C, and fed *ad libitum* with carrot and cabbage. Nematodes (*P. hermaphrodita*) were supplied by BeckerUnderwood in the form of partially desiccated infective juveniles in a clay aggregate (Nemaslug). The aggregate was diluted in tap water and viable nematodes were counted using a Sedgewick Rafter counting cell.

Primers

The *P. hermaphrodita*-specific primer pairs were designed using sequence data from a 510-bp fragment of the mitochondrial CO1 DNA. Two *P. hermaphrodita* species-specific primer pairs were designed that amplified a 353 bp fragment and a 155 bp fragment.

Detection of nematode DNA within host and predator

The first experiment involved the tracking of nematode infection in the host organism. One hundred and ten *D. reticulatum* were placed individually in 20 ml plastic tubes with a layer of 90 mm whatman filter paper on the inside. Nematodes were diluted in tap water and applied at the field concentration of approximately 30 individuals per cm^2 to the inside of the tube. The tubes were stored at 16°C with a light cycle of 12:12 L:D. Ten tubes were sampled daily and the slugs killed by freezing at -80°C. Slugs were washed in distilled water and a section of the posterior mantle weighting approx 20-25 mg was removed from each slug using a clean scalpel and the DNA was extracted using the Qiagen tissue extraction kit procedure. The extracted DNA was used as template in PCR reactions with the *P. hermaphrodita* species-specific primer pair that amplified a 353-bp fragment. Successful DNA amplifications were visualised using electrophoresis on an ethidium bromide stained agarose gel. The results were plotted on graphs of percentage of samples positive versus time of sampling and linear regression was carried out on the data.

Detection of feeding on nematode-infected slugs was carried out using 350 freshly-collected *D. reticulatum*, infected with *P. hermaphrodita* in 20 ml tubes as above. At one, 24, 96, 144, and 216 hours after the point of infection, 70 tubes were removed and the slugs frozen at -80° C. Once all slugs were killed they were thawed and placed in feeding arenas (90 mm Petri dishes). For each level of infection, 35 male and 35 female *P. melanarius* that had been starved for seven days were placed individually in the feeding arenas with the infected slug cadaver and allowed to feed for 1 h. Slug remains were removed and five male and five female beetles were killed 0, 1, 3, 6, 12, and 24 h after feeding for each infection group. The foreguts of the beetles were removed and the contents of the gut placed in a 1.5 ml Eppendorf tube. The DNA was again extracted using the Qiagen tissue extraction kit. Extracted DNA from each gut sample was used as template in a PCR reaction with the *P. hermaphrodita*-specific primer pair that amplified a 155-bp fragment. Presence of nematode DNA in the

samples was visualised using electrophoresis on a ethidium bromide-stained agarose gel. The results were plotted as percentage positive samples against time of sampling.

Results

Detection of nematode DNA within host

The primers amplifying a 353-bp fragment were capable of detecting *P. hermaphrodita* DNA in slugs from as early as 1 h after exposure to the nematodes (Table 1). By 96 h 100 % nematodes DNA could be amplified from 100 % of the slugs. Regression analysis showed a strong relationship between $\log_e$ percent of samples positive for nematode DNA and $\log_e$ time ($y = 0.1935x + 1.5944$, $R^2 = 0.9903$, $P < 0.001$).

Table 1. Percent of slugs positive for nematode DNA at each sampling time

Time	Percent positive
1	40
24	70
48	80
72	90
96	100
120	100

Detection of nematode DNA within predator guts

The results showed a clear trend of consumption of the most infected slugs resulting in longer detection of nematode DNA within the guts of carabid beetles (Table 2.). Detection times for consumption of the most infected slug tissue was a maximum of 24 h. Regression analysis on the T_{216h} data set showed a strong correlation between $\log_e$ percentage of beetles positive and time ($y = -0.0305x + 0.7557$, $R^2 = 0.9512$, $P < 0.005$).

The data from all the infection categories were pooled to give detection of nematode DNA within the guts of carabid beetles at a range of different nematode infection levels on *D. reticulatum*, emulating conditions in the field. Regression analysis on these data showed a strong correlation between $\log_e$ percentage of beetles positive and time ($y = -0.1133x + 3.6759$, $R^2 = 0.9855$, $P < 0.001$).

Table 2. Percent of *P. melanarius* gut samples positive for *P. hermaphrodita* DNA

Digestion period (h)	Beetle guts positive (%) for nematode DNA after eating slugs infected for different time periods (T)				
	T_{24}	T_{72}	T_{144}	T_{216}	$T_{24}+T_{72}+T_{144}+T_{216}$
0	20	40	40	60	40
1	10	30	30	50	30
3	10	20	30	50	27.5
6	10	20	40	30	25
12	0	0	10	30	10
24	0	0	0	10	2.5

Discussion

The use of a molecular approach to the detection of nematodes within hosts can allow the monitoring of parasite infection in biological control systems in the early stages of infection. Signs of nematode infection are not clear until days 4-6 of *P. hermaphrodita* infection of *D. reticulatum* (personal observation), but the probes were able to detect nematode penetration into the slug mantle far earlier than this (1 h after exposure). The rapid increase in numbers of samples from which nematode DNA could be amplified within the first 24 h was probably a result of nematode accumulation in the mantle rather than reproduction. By 96 h the next generation of nematodes could be observed in and around the slug mantle, resulting in all samples recorded as positive.

The ability to investigate food web links, such as predation or scavenging, by carabid beetles on moribund or dead hosts allows for a better understanding of the ecological effects of innundative application of nematode biopesticides on soil ecosystems. The ability of the primers to detect nematode DNA within the guts of predators that have predated on infected hosts allows the investigation of advanced food chain dynamics.

Acknowledgements

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Insect Pathogens and Entomoparasitic Nematodes
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Molecular detection of slug DNA within carabid predators.

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Abstract: Carabid beetles are important predators of slugs in arable crops and can have a significant impact on the size and distribution of pest populations in the field. These factors make carabids, such as *Pterostichus melanarius*, potential candidates for use as *in situ* biocontrol agent within agroecosystems. In this study PCR-based molecular techniques were used to investigate predation by the beetle, *P. melanarius*, on the slugs *Deroceras reticulatum* and *Arion hortensis*. Species- and genus-specific primers enabled the amplification of small fragments of slug mitochondrial DNA from within the foregut of the beetles. Detection limits for the primers were determined in timed laboratory-based DNA decay rate experiments. The application of the technique to beetles trapped in the field resulted in the detection of slug positive beetles and indicated that this is a suitable technique for detecting predation in field based studies.

Key words: carabid beetle, mitochondrial DNA, predation, slug

Introduction

Slugs are one of the most important pests in European agriculture, responsible for millions of pounds worth of damage to arable crops annually. Previous studies have identified the role of carabids as predators of slugs within agroecosystems (Symondson & Liddell, 1993a; Symondson *et al.*, 1996; Bohan *et al.*, 2000; McKemey *et al.*, 2001). Indeed, *P. melanarius* has been shown to significantly reduce slug populations in the field, has great importance for slug population dynamics and the activity density of *P. melanarius* has a dynamic association with slug biomass (Symondson *et al.*, 1996; Bohan *et al.*, 2000; McKemey, 2000; Symondson *et al.*, 2002).However, in order to be able to investigate such complex trophic interactions, it is necessary to develop sensitive assays that will allow the determination of prey choice by a predator *post-mortem*. Recent development and refinement of DNA-based methods for predator gut content analysis have demonstrated that such techniques are now a viable option for this area of research. Predation on a target species can be detected for at least the 24 hour period prior to the capture of the predator using DNA-based detection methods (Zaidi *et al.*, 1999; Agustí *et al.*, 2003a,b; Dodd, 2004), which is a sufficient period of time to detect predation in predators captured during the previous night (Symondson *et al.*, 1996). The principal objectives of this study were: a) to develop a range of mitochondrial primers that specifically amplify DNA from species of slug which are important pests in agriculture in the UK; b) to determine the rate at which slug DNA degrades in the guts of carabid beetles, detected using these primers and; c) to use the primers to identify predation on slugs by carabid beetles in the field.

Material and methods

Primer design and amplification

Specific primers were designed to detect *Deroceras reticulatum* or *Arion* spp. DNA, targeting fragments of the third domain of the mitochondrial 12s rRNA gene, after the secondary structure for predator and prey DNA sequences had been resolved. Primer pairs were tested for cross reactivity with non-target species to determine their specificity. The forward primer of each pair was fluorolabelled with HEX or FAM and amplified via PCR to produce amplicons that ranged in size from 204-221 bp. These were run on polyacrylamide gel and analysed on ABI377 Semi-automated sequencer using ABI Prism Genescan and Genotyper software.

Decay-rate experiment

A laboratory-based DNA decay rate experiment was conducted for each prey species to determine the detection period of slug DNA following digestion by the beetle for up to 48 h. Starved beetles were allowed to feed ad lib on the target prey for 2 h, after which time it was removed. Beetles that had not been observed to feed during this time were discarded. The beetles that had fed were maintained under controlled conditions until they were killed by freezing at a range of post-feeding time intervals. The detection periods for different PCR amplicons of digested DNA were determined, following polyacrylaminde gel electrophoresis, as mean intensity of fluorescence.

Field caught beetles

The guts of carabid predators that had been caught from the field in pitfall traps, which had been left in position overnight, were removed and the DNA extracted from the contents. Slug DNA was amplified using the above primers to determine upon which species of slug the beetles had been feeding.

Results and discussion

Cross reactivity tests showed that the primers designed for *Deroceras reticulatum* were specific to *D. reticulatum* and that the primers designed for *Arion* spp. amplified all members of the genus that were tested, but did not amplify species from other genera.

Rate of DNA decay

Mean intensity of fluorescence decreased with time since ingestion of the target DNA (Figure 1) with a maximum limit of detection occurring at 49 hours following ingestion of the prey with both sets of primers.

The mean period of detection was calculated from the regression equation for the rate of decay using each primer set. This was reached after 24.37 hours and 26.17 hours for the general *Arion* and *Deroceras* specific primers respectively, which suggested that these primers were suitable for detecting predation on slugs in the field.

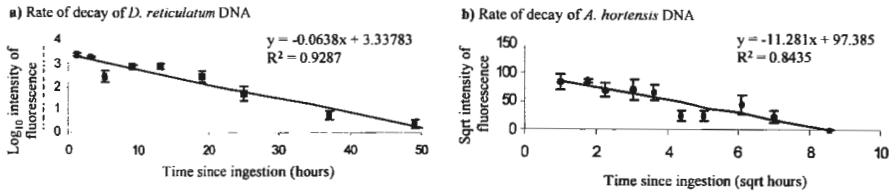

Figure 1. Rate of decay of a) *Deroceras reticulatum* and b) *Arion hortensis* DNA within the foregut of the beetle *Pterostichus melanarius*, determined by mean intensity of fluorescence.

Detection of predation on slugs in the field

Remains of *D. reticulatum* and *Arion* spp. were detected, using the primers described above, in the foregut extracts of beetles that had been caught in the field. In total, 600 individual beetles were analysed, of which 23 (3.8 %) were positive for *D. reticulatum* remains and 0.7 % (6 individuals) were positive in the expected size range (204-218 bp) for *Arion* species. Based on the size of the PCR products obtained, two beetles had consumed *Arion hortensis* Férrusac, two *Arion intermedius* Normand and two *Arion distinctus* Mabille. This was in a year when slug monitoring, by gradual flooding of soil samples, detected few if any slugs in some months when the beetles were active.

The results of the decay rate study clearly show that the *Deroceras* and *Arion* primers tested were capable of reliably amplifying the DNA from the target species from within the predators gut for a sufficient period of time following ingestion to allow the detection of predation in the field. Using these methods, we have demonstrated that it is possible to detect predation by carabids on slugs in the field.

By using a fluorolabelled system with acrylamide gel in an automated sequencer it is possible to accurately separate bands that are only 1 bp apart and by using different coloured fluorolabels, PCR products can be multiloaded in the same gel lane. In addition, the development of genus level primers (i.e. general *Arion* primers) that amplify a fragment of DNA varying in length between species, it was possible to identify prey remains to the species level based on amplicon size. This methodology has now been further developed into PCR multiplexing (King *et al.*, this volume) to enable the detection of multiple prey species within the same PCR reaction.

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