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Optimization of biological (*Phasmarhabditis hermaphrodita*) and chemical (iron phosphate and metaldehyde) slug control

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ABSTRACT

Slugs are serious pests of a range of crops worldwide and are commonly controlled using chemical bait molluscicides. *Phasmarhabditis hermaphrodita* is a slug parasitic nematode that can be an effective alternative to chemical pellets. We conducted a field trial with Chinese cabbage to assess the potential of new nematode application techniques (dipping of root plugs, spraying around plant base and three low doses of *P. hermaphrodita*) compared to standard broadcast spraying of nematodes and chemical molluscicides. We also performed a series of miniplot trials investigating persistence of *P. hermaphrodita*, the efficacy of *P. hermaphrodita* compared to chemical molluscicides (iron phosphate and metaldehyde pellets) and using three repeated low doses of *P. hermaphrodita* compared to one broadcast spraying treatment. In field trials new application strategies of three low doses of *P. hermaphrodita* and dipping the plant roots in *P. hermaphrodita* reduced slug damage but did not reduce slug numbers. In miniplot trials we found that *P. hermaphrodita* persisted in soil and caused significant slug control 38 days after initial application. Also three low dose applications of *P. hermaphrodita* provided slug control comparable to one broadcast spraying. Also, we found that new chemical molluscicides such as iron phosphate can significantly reduce slug damage caused by *Deroceras reticulatum* and *Arion ater*.

1. Introduction

Slugs are major pests of arable crops including oilseed crops and cereals (Glen and Moens, 2002; Moens and Glen, 2002) and horticultural crops including vegetables and ornamentals (Port and Ester, 2002). They cause reductions in yield by killing seeds and seedlings, destroying stems and growing points and reducing plant leaf area (Port and Port, 1986; South, 1992). Damage to germinating seeds can be so severe that whole fields may need to be resown (Willis et al., 2006). Slugs also contaminate harvested plants with their bodies, eggs, faeces or slime, leading to deterioration in product quality and financial loss (Iglesias et al., 2002).

Traditionally, control methods have relied on chemical bait pellets containing metaldehyde or carbamates (methiocarb or thiodicarb). Gastropods encounter them via food uptake or by dermal contact, and the chemicals act either as stomach or contact poisons (Henderson and Triebskorn, 2002). There are application and environmental problems with both types of molluscicide. Both are poisonous to vertebrates (Homeida and Cooke, 1982; Fletcher et al., 1991, 1994), and methiocarb is toxic to beneficial invertebrates including earthworms and carabid beetles (Purves and Bannon, 1992). However, a new slug pellet containing iron phosphate (Ferramol®, Neudorff GmbH, Germany) has recently been registered in several European countries (Speiser et al., 2001). Iron phosphate is commonly used as a human nutritional supplement and as a fertilizer ingredient; it shows little toxicity towards mammals and occurs naturally in the forms of strengite and metastrengite (EPA, 1998; Roberts et al., 1990; Clark, 1993). The potential of iron phosphate to reduce slug damage has been demonstrated under laboratory and glasshouse conditions and in the field (Jäckel, 1999; Koch et al., 2000; Speiser and Kistler, 2002). The pellets are broadcast evenly over the soil surface and upon ingestion, iron phosphate causes cellular pathological changes in the crop and hepatopancreas of the slug. Iron phosphate causes feeding inhibition and mortality in slugs such as *Deroceras reticulatum*, *Arion lusitanicus* and *A. hortensis* (Koch et al., 2000; Iglesias and Speiser, 2001; Speiser and Kistler, 2002).

An alternative to chemical molluscicides is the slug parasitic nematode *Phasmarhabditis hermaphrodita*, which can kill several slug and snail species (Wilson et al., 1993, 2000; Speiser et al., 2001; Iglesias and Speiser, 2001; Grewal et al., 2003; Rae et al., 2008) and is available as a biological control agent called Nemaslug® from

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Becker Underwood, UK (see Rae et al., 2007). The nematode is applied using standard spraying equipment at an application rate of 3×10^9 infective juveniles per hectare. Nematodes search for slugs in soil responding to slug mucus and faeces (Rae et al., 2006, in press), enter through the shell cavity at the back of the mantle (Wilson et al., 1993; Tan and Grewal, 2001) and kill the slug between 4 and 21 days after infection. The nematodes feed on bacteria present on the decaying slug and eventually produce new infective juveniles. *P. hermaphrodita* has been used successfully to protect a range of crops against slug damage, including oilseed rape (Wilson et al., 1995; Speiser and Andermatt, 1996), winter wheat (Wilson et al., 1994b), strawberries (Glen et al., 2000), asparagus (Ester et al., 2003a) and Brussels sprouts (Ester et al., 2003b) and the use in floriculture has been demonstrated in orchids (Ester et al., 2003c) and hostas (Grewal et al., 2001).

Nematodes are much more expensive than chemical molluscicides and this prevents more widespread use. Novel approaches to reducing numbers of nematodes applied (and hence treatment cost) include, repeated applications of low doses (Ester et al., 2003a,b) application of nematodes to artificial slug shelters and (Grewal et al., 2001) application of the recommended rate of nematodes in bands or around individual plants. *D. reticulatum* and *A. ater* actively avoid areas where nematodes are applied at rates of 38 cm^2 and 120 cm^2 (Wilson et al., 1999). This is very close to the recommended rate of *P. hermaphrodita* (30 nematodes per cm^2) and thus, it is possible that slugs will avoid these treated areas.

The technology used to produce *P. hermaphrodita* is based on that developed for the more widely used entomopathogenic nematode (EPN) biopesticides (Kaya, 1990). It is quite likely that some of the novel application techniques developed for EPN will work for *P. hermaphrodita* too. Such application methods include application of infected hosts, 'nemabags' and root dips (Grewal et al., 2005). Infected cadavers can be applied to target specific sites and have been used effectively in the field (Jansson et al., 1993; Parkman et al., 1993). Nematodes can also be applied in "tea bags" containing a super absorbent gel or mixed with carboxymethylcellulose (CMC) so nematodes adhere to root plugs (Menzler-Hokkanen and Hokkanen, 2003; Peters et al., 2002). Novel methods of applications that are easier to use, may decrease application costs and increase the attractiveness of nematode products to consumers (Grewal et al., 2005).

In a series of field experiments and mini-plot experiments, we aimed to devise new application strategies for *P. hermaphrodita* that would reduce the number of nematodes applied, and hence cost, but would still provide significant protection against slugs comparable with chemical molluscicides. We had four main objectives: (1) to test the efficacy of new application strategies using *P. hermaphrodita* (dipping root plugs, spraying around plant bases and three repeated low doses); (2) to examine the efficiency of three repeated low dose applications of *P. hermaphrodita* versus broadcast spraying miniplot trials; (3) to investigate the persistence of *P. hermaphrodita* applied at the recommended rate in miniplot trials; and (4) to compare the efficacy of iron phosphate pellets, metaldehyde pellets and *P. hermaphrodita* exposed to two very different pestiferous slug species.

2. Materials and methods

2.1. Source of invertebrates and equipment

Slugs (*D. reticulatum* and *A. ater*) were collected from the Cruickshank gardens (Aberdeen University) stored in non-airtight boxes with moist cotton wool and fed on a mixture of Chinese cabbage (*Brassica rapa* var. pekinensis) and lettuce. Any slugs exhibiting signs of infection by *P. hermaphrodita* were removed. *P. hermaphrodita* was supplied by Becker Underwood, UK. Chinese cabbage was grown from seed 4 week prior to use. Fluon® and copper fencing was supplied by Blades Biological, UK. Lawnedge was purchased from B and Q, UK. Metarex green® (5% metaldehyde active ingredient) was purchased from De Sangosse Ltd, UK. Slug Killer® containing 1% iron (ferric) phosphate was purchased from Growing Success, UK. Mean precipitation and temperature of the Aberdeen area during field and miniplot trials was accessed from www.met.office.gov.uk.

2.2. Field trial evaluating efficacy of different application methods of *P. hermaphrodita* applied in Chinese cabbage

The field trial began on 10/4/2006 and was performed on a commercial lettuce farm near Methlick, Aberdeenshire (NJ825403GB). Two rows (30 × 1 m) were treated with glyphosate to kill any weeds present pretrial. Thirty plots (2 × 1 m) were constructed of Lawn edge (30 m × 16.5 cm) and were fixed into the soil to a depth of 5 cm. The top 5 cm were coated with Fluon® to stop emigrating and immigrating slugs (Symondson, 1993). Each plot was separated with a 2 m buffer zone whereby the recommended rate of metaldehyde pellets was applied. There were six treatments: (1) untreated control; (2) metaldehyde pellets (0.8 g/m^2); (3) *P. hermaphrodita* (30 nematodes per cm^2); (4) three applications of one sixth of the recommended rate of *P. hermaphrodita* (5 cm^2) applied every 14 days; (5) dipping of the root plug into a *P. hermaphrodita* and CMC adhesive mix; (6) spraying around the base of the plants with *P. hermaphrodita* (3000 *P. hermaphrodita* per plant). Nematodes (30 per cm^2 and 5 per cm^2) were suspended in 2 l of water and applied evenly over the soil surface using a watering can. Untreated plots received 2 l of tap water. Pellets were broadcast by hand after 2 l of water was added. Three applications of *P. hermaphrodita* (5 per cm^2) were applied to five of the plots; this took place on days 0, 14 and 28 and was the equivalent of 50% of the recommended rate of *P. hermaphrodita*. Chinese cabbage root plugs were placed in a 0.5% CMC nematode solution and were planted immediately. Approximately 7000 nematodes adhered to each cabbage root plug before planting. CMC was added to prevent the nematodes from settling and to adhere to root plugs of Chinese cabbage (Peters et al., 2002). Chinese cabbage plugs were weighed before and after dipping and absorbed approximately 4–5 ml of the solution which contained approximately 7000 nematodes. Nematodes were also sprayed around the base of plants to investigate repellency. This was done on an area basis, i.e. 30 nematodes per cm^2. Bamboo squares (10 × 10 cm) were constructed and placed around each plant and 3000 nematodes were added in 100 ml of water. Twenty-four cabbage plants were planted in six rows of four. There were five replicates of each treatment arranged in a randomised block design. Fleece was then placed over each plot for insulation. Damage was monitored visually by estimating the percentage eaten per leaf every week for 6 weeks. At the end of 6 weeks the slug population in each plot was determined by using refuge traps. Briefly, upside down saucer plots were placed in each plot and filled with a handful of bran placed in the centre of the treated area. Traps were monitored at 3-day intervals for 9 days and any slugs present were removed and taken back to the laboratory and identified to species.

2.3. Miniplot trial investigating potential of three low dose applications versus one broadcast spray of *P. hermaphrodita*

There were two replicate miniplot trials conducted in Aberdeen University gardens that began on 9/10/05 and 20/5/06, respectively. Twenty-four boxes (0.7 × 0.5 × 0.2 m) were half filled with fresh top soil as described by Wilson (2007) and