A BIOASSAY TECHNIQUE TO ASSESS THE MOLLUSCICIDAL EFFECTS OF MICROBES

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ABSTRACT

Terrestrial slugs (Order Stylommatophora) can cause significant damage in cropping and pastoral systems. Pathogenic microbes have long been shown to have potential as biocontrol agents for insect pests and a number of retail products are available, but there is no equivalent product for molluscs. A research program is underway to investigate the potential of micro-organisms as slug biocontrol agents and this paper describes a bioassay methodology that can be used to assess the molluscicidal properties of bacterial isolates. Its feasibility has been demonstrated in laboratory tests against pest slugs of the genus *Deroceras*. Fourteen bacterial strains were screened by feeding them to the slugs in a mix based on oat bran, a food that is highly palatable to these molluscs. Mortality was assessed 3 and 4 days after introduction of the treated food. Two of the bacterial strains tested resulted in significant mortality, killing 100% slugs within 4 days.

Keywords: biomolluscicide, mollusc, slug, *Deroceras*, microbial control, bacteria.

INTRODUCTION

Slug damage in cropping and pastoral systems is a common problem in temperate climates, especially at establishment when a severe infestation can completely destroy seeds and young seedlings (Port & Port 1986). The increased use of no-tillage cultivation has exacerbated the problem as slugs can shelter in the residue of a previous crop and attack direct drilled seeds as soon as they are sown. This is particularly problematic when conditions prevent closure of the drill slot (Hughes & Gaynor 1984; Voss et al. 1998). Damage to emerging seedlings is often mistakenly attributed to other pests. For example, slugs can severely damage shoots below the soil surface causing seedlings to wilt, although such damage is more often associated with sap-sucking invertebrates or microbial infection of the plant.

The range of control options currently available is dominated by chemical baits, but there are objections to these including variable efficacy, increased pesticide burden and non-target effects on domestic animals, native birds and other wildlife (Glen et al. 1991; Cook et al. 1997). Certain cultivations, such as deep ploughing and consolidation, can help (Glen et al. 1989), but are not universally applicable, or indeed desirable in sustainable systems. Biological control with the nematode, *Phasmarhabditis hermaphrodita* (Schneider) is effective (Wilson et al. 1994, 2004), but it is currently too costly for use on a field scale and is prohibited in countries such as New Zealand where this nematode species has not been identified. A microbial approach to slug control represents a biological alternative that could be used alone or in integrated pest management systems to augment existing control measures.

A research program is underway to investigate the potential for microbial control of pest slugs of the genus *Deroceras*. The approach takes account of important facets of slug biology. For example, slugs produce copious quantities of mucus to prevent desiccation and assist with locomotion, hence applying direct contact poisons is often ineffective because they are sloughed off before penetrating the integument (Iglesias et al. 2002).

Slugs are also relatively sensitive to olfactory stimuli and therefore controls designed to be ingested must be presented on a non-repellent food source (Pickett & Stephenson 1980; Ali et al. 2003). This paper presents a bioassay methodology that incorporates such criteria in a test of bacterial isolates against slugs.

MATERIALS AND METHODS

Test organisms

Adult *D. reticulatum* (Müller) and *D. panormitanum* (Lessona and Pollonera) were field collected from rough grassland at Ruakura Research Centre, Hamilton, using upturned polystyrene seed trays as refuge traps. They were maintained at 15°C in ventilated plastic boxes lined with moist paper towel, and fed on a diet of carrot (*Daucus carota* L.) and Chinese cabbage (*Brassica chinensis* L.) *ad libitum*. Food was withheld for 24 h prior to the bioassays. As the two species are phenotypically similar and of comparable pest status, they were treated as one mixed culture.

Bioassay methodology

Fourteen bacterial strains indigenous to New Zealand (labelled A-N, Table 1) were selected from AgResearch culture collections and used in the bioassay. Each test bacterium was grown on tryptic soy agar (TSA) in a 9 cm diameter Petri dish at 24°C for 48 h. Bacterial growth was then aseptically suspended in 9 ml of sterile distilled water (SDW). The spread-plate method was used to inoculate each of a further ten TSA plates per bacterial strain with 0.2 µl of this suspension. These were also incubated at 24°C for 48 h. Bacterial growth was then removed from the surface of these plates using a rounded spatula, before being suspended in 12 ml SDW.

To prepare the slug food, 11 ml of the bacterial suspension, or SDW for controls, was mixed thoroughly with 11 g oat bran in a sterile beaker. The bacterial concentrations were in excess of 10⁸ bacteria per gram of oat bran (supermarket 'Homebrand'). For each treatment, 10 shallow plastic trays (3 × 3 × 0.5 cm) were each filled with 1 g of the treated oat bran and one tray was added to each of 10 Petri dishes (9 cm diameter) lined with a sheet of filter paper moistened with 2 ml SDW. One slug was placed into each Petri dish using soft forceps. The species of *Deroceras* within the mixed culture were used at random and not separated for statistical analysis.

Petri dishes grouped by treatment were maintained at 15°C in a controlled environment room (16:8 h light:dark) for 4 days, and mortality was assessed at days 3 and 4. Numbers of dead slugs were compared between the bacterial treatments and control using Fisher's exact test.

RESULTS

Slugs readily ate the control and treated oat bran and many individuals were observed to feed soon after introduction to the Petri dishes. Filter paper remained moist during the 4 day assessment period and the plastic feeding trays were effective in preventing food absorbing moisture from the filter paper lining the dish. Their dimensions relative to the Petri dishes also meant that they could not be tipped by slugs whilst feeding. The treated oat bran became more glutinous after initial mixing with the bacterial solutions, but the humidity in the closed Petri dishes meant it did not harden and remained palatable to slugs.

Two bacteria clearly caused significantly greater mortality than controls (Table 1). These were bacteria B ($P < 0.001$) and G ($P < 0.001$), both of which killed 100% slugs within 4 days post-treatment, with a minimum of 90% slugs killed within 3 days. This was in marked contrast to all other microbes, none of which differed significantly from the control, with little or no mortality observed. Frequently, the mouthparts of dead slugs were characteristically elongated, and microscopic examination revealed the radula to be everted.

TABLE 1: Mean % mortality ($\pm$ upper and lower 90% binomial confidence intervals) of *Deroceras* spp. 3 and 4 days after exposure to food treated with bacteria in the molluscicidal bioassay. There were 10 slugs per treatment.

Code	Test Bacterium	AgResearch Collection	Mean % mortality			
			Day 3		Day 4	
A	(AgR3433)	Lincoln	0	(0–25.9)	0	(0–25.9)
B	(AgR3434)	Lincoln	90	(60.6–99.5)	100	(74.1–100)
C	(AgR0735)	Ruakura	20	(3.6–50.7)	20	(3.6–50.7)
D	(AgR1218)	Ruakura	0	(0–25.9)	10	(0.5–39.4)
E	(AgR1219)	Ruakura	0	(0–25.9)	0	(0–25.9)
F	(AgR1221)	Ruakura	10	(0.5–39.4)	10	(0.5–39.4)
G	(AgR1973)	Ruakura	100	(74.1–100)	100	(74.1–100)
H	(AgR1974)	Ruakura	0	(0–25.9)	0	(0–25.9)
I	(AgR2283)	Ruakura	0	(0–25.9)	10	(0.5–39.4)
J	(AgR2290)	Ruakura	30	(8.7–60.7)	30	(8.7–60.7)
K	(AgR2297)	Ruakura	0	(0–25.9)	0	(0–25.9)
L	(AgR2294)	Ruakura	0	(0–25.9)	0	(0–25.9)
M	(AgR1979)	Ruakura	0	(0–25.9)	10	(0.5–39.4)
N	(AgR1976)	Ruakura	0	(0–25.9)	0	(0–25.9)
Control	–		0	(0–25.9)	0	(0–25.9)

DISCUSSION

The bioassay described in this paper was developed to test the molluscicidal effects of bacteria on common species of pest slug. There have been very few studies of this nature published in the literature because exploring the use of microbes as a slug biocontrol is a relatively novel concept. A notable exception is the paper of Kienlen et al. (1996), which investigated the effect of a well-known insect pathogen, *Bacillus thuringiensis* (Berliner), on three pest species of slug. In their work, none of the bacterial strains tested showed toxicity to slugs. The methodology, however, used treated fresh food (lettuce), which is likely to have varied in water content and quality between replicates with possible impacts on palatability and quantities ingested.

In the current bioassay, dry oat bran is used, which has been shown to be a highly attractive food to slugs (Young 1990). A fixed weight of the oat bran is moistened with an equal volume of bacterial suspension for each treatment to give a consistently replicable method. Slugs readily accepted the microbe-treated food orally, therefore avoiding problems associated with contact-based control substances, the absorption of which may be impeded by the mucus barrier covering the body surface (Iglesias et al. 2002).

A range of bacteria was assessed for effectiveness in killing slugs. These were all sourced in New Zealand, and labelled A–K for reasons of commercial sensitivity. The two strains showing promise (B and G) were both Gram negative. Strain B was motile, oxidase negative and catalase positive while strain G was non-motile, oxidase and catalase positive. There is no other published work using similar microbes against which to compare our results. It has been shown, however, that the nematode *P. hermaphrodita* acts as a vector for the slug-pathogenic bacterium, *Moraxella osloensis* (Wilson 1995). Like strains B and G in the present study, *M. osloensis* is also Gram negative. This organism causes mortality through the production of an endotoxin, a possibility for further investigation to elucidate the mechanism by which bacteria B and G exert their effects.

There was little variation in the results for a given bacterial strain; it tended to work well or not at all, which might be expected given that slugs co-exist with a wide range of bacteria in their natural environment, the majority of which are harmless. Any that are pathogenic are likely to be relatively virulent in order to overcome innate slug defence mechanisms. Further development of the work described in this paper will be undertaken to establish minimum threshold concentrations of bacteria B and G required to kill slugs so that an LD50 value may be quantified.

Preliminary tests indicated that mortality tended to occur after an initial time lag of up to 2 days following ingestion of the microbe-treated food and hence the first assessment was made on the third day. The duration of this lag period will be influenced by a number of factors, such as the amount of microbe ingested, its speed of action and the general health of the slug at the time of ingestion. After day 4, there was little change in the numbers of dead slugs, and it was deemed that since the aim is to kill slugs as quickly as possible following the application of molluscicide there would be no further assessment beyond this time.

In conclusion, the bioassay described in this paper is a replicable, inexpensive way to rapidly screen a range of microorganisms in order to identify a subset with molluscicidal activity for detailed investigation. It gave consistent results at 15°C, a temperature commonly encountered by slugs under New Zealand field conditions.

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REFERENCES

- Ali AY, Muller CT, Randerson P, Bowen ID 2003. Screening African plants for mollusc repellency. In: Slugs & Snails: Agricultural, Veterinary and Environmental perspectives. Dussart GBJ ed. British Crop Protection Council, Norwich, UK. Pp. 319-324.
- Cook RT, Bailey SER, McCrohan CR 1997. The potential for common weeds to reduce slug damage to winter wheat: Laboratory and field studies. *Journal of Applied Ecology* 34: 79-87.
- Glen DM, Milsom NF, Wiltshire CW 1989. Effects of seed-bed conditions on slug numbers and damage to winter wheat in a clay soil. *Annals of Applied Biology* 115: 177-190.
- Glen DM, Wiltshire CW, Butler RC 1991. Slug population changes following molluscicide treatment in relation to distance from edge of treated area. *Crop Protection* 10: 408-412.
- Hughes KA, Gaynor DL 1984. Comparison of Argentine stem weevil and slug damage in maize direct-drilled into pasture or following winter oats. *New Zealand Journal of Experimental Agriculture* 12: 47-53.
- Iglesias J, Castillejo J, Ester A 2002. Laboratory evaluation of potential molluscicides for the control of eggs of the pest slug *Deroceras reticulatum* (Müller) (Pulmonata: Limacidae). *International Journal of Pest Management* 48: 19-23.
- Kienlen J, Gertz C, Briard P, Hommay G, Chaufaux J 1996. Recherche de la toxicité de diverses souches de *Bacillus thuringiensis* Berliner vis-à-vis de trois espèces de limaces. *Agronomie* 16: 347 - 353.
- Pickett JA, Stephenson JW 1980. Plant volatiles and components influencing behaviour of the field slug. *Journal of Chemical Ecology* 6: 435-444.
- Port CM, Port GR 1986. The biology and behaviour of slugs in relation to crop damage and control. *Agricultural Zoology Reviews* 1: 253-299.

- Rae R, Verdun C, Grewal PS, Robertson JF, Wilson MJ 2007. Biological control of terrestrial molluscs using *Phasmarhabditis hermaphrodita* - progress and prospects. *Pest Management Science* 63: 1153-1164.
- Voss MC, Ulber B, Hoppe HH 1998. Impact of reduced and zero tillage on activity and abundance of slugs in winter oilseed rape. *Zeitschrift fuer Pflanzenkrankheiten und Pflanzenschutz* 105: 632-640.
- Wilson MJ, Glen DM, Hamacher GM, Smith JU 2004. A model to optimise biological control of slugs using nematode parasites. *Applied Soil Ecology* 26: 179-191.
- Wilson MJ, Glen DM, George SK, Pearce JD 1995. Selection of a bacterium for the mass production of *Phasmarhabditis hermaphrodita* (Nematoda, Rhabditidae) as a biocontrol agent for slugs. *Fundamental and Applied Nematology* 18: 419-425.
- Wilson MJ, Glen DM, Hughes LA, Pearce JD, Rodgers PB 1994. Laboratory tests of the potential of entomopathogenic nematodes for the control of field slugs (*Deroceras reticulatum*). *Journal of Invertebrate Pathology* 64: 182-187.
- Young AG 1990. Assessment of slug activity using bran-baited traps. *Crop Protection* 9: 355-358.