Survival of *Yersinia entomophaga* MH96 in a pasture ecosystem and effects on pest and non-target invertebrate populations

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Abstract *Yersinia entomophaga* MH96 (Ye MH96) has shown activity against the pasture pest porina (*Wiseana* spp. larvae) in laboratory bioassays. In this field trial Ye MH96 was applied to pasture as three separate formulations. The presence of viable Ye MH96 was detected in soil for 112 days following application but the number of bacteria decreased rapidly following heavy rainfall 4 days after treatment. Porina numbers were reduced by all formulations of Ye MH96 at 5 and 17 weeks (34-40% and 61-72%) and by fenitrothion, an insecticide used to control porina (93% and 96% respectively). Other non-target Lepidoptera were also reduced by Ye MH96. No effects of Ye MH96 were observed on the earthworms, *Aporrectodea caliginosa* and *Lumbricus rubellus*, the pasture pest *Listronotus bonariensis* or pasture inhabiting Staphylinidae. The survival of Ye MH96 for long enough to affect the target insect, despite the heavy rain, suggests that a microbial alternative to conventional insecticide management of porina may be possible.

Keywords biopesticide, microbial control, porina, *Wiseana* spp., *Yersinia entomophaga* MH96

INTRODUCTION

There is a recognised need for effective bioprotection products in agriculture. This is driven by changing regulations in New Zealand and overseas governing the use of pesticides and their residues in agricultural commodities. In addition market and environmental demands mean that more sustainable production practices have to be devised to ensure access for export products. The enterobacterium *Yersinia entomophaga* MH96 (Ye MH96) has shown activity against a range of insect pests (Hurst et al 2011) and has previously demonstrated potential for porina (*Wiseana* spp. larvae) control (Brownbridge et al. 2009). Porina are perennial pasture pests particularly of young (2-4 year old) pastures in the South Island (Dugdale 1994; Barratt et al 2001) consuming most pasture species but particularly perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*). Control is usually by targeted applications of diflubenzuron against young larvae before damage appears or by application of broad-spectrum insecticides against large larvae after porina feeding becomes apparent. Diflubenzuron is a popular choice due to its low

cost and because it usually leaves sufficient porina in pastures (Ferguson et al 1996) to allow the persistence of naturally occurring viruses leading to the subsequent suppression of pest populations (Crawford & Kalmakoff 1977). The effectiveness of diflubenzuron, however, is influenced by the timing of application as the porina larvae must moult shortly after application for death to occur. Optimal control requires prediction of damage, an awareness of differences between porina species, times of adult flights and egg laying, and of larval development (Ferguson & Crook 2004). For this reason farmers often choose to use organophosphate insecticides that require less knowledge about pest development and for which application time is less critical but that have inherent wide ranging environmental impacts (e.g. Odenkirchen & Eisler 1988; Grue et al. 1997) and are increasingly less tolerated by export markets. Biopesticides are a recognised, environmentally-friendly, plant protection tool, and an effective biopesticide, active against all porina larval stages, would be a significant benefit to farmers trying to utilise sustainable pasture protection techniques. However, widespread adoption of biopesticides has been hindered by lack of efficacy and inconsistent field performance. A significant barrier to successful field use of biopesticides is survival in the environment for long enough to infect the target pest (Glare et al. 2012). This field investigation evaluated the survival of Ye MH96 in a pasture ecosystem and subsequent effects on invertebrate pest and non-pest populations when applied against a porina population beginning to cause noticeable damage and too far developed to be effectively controlled by diflubenzuron.

MATERIALS AND METHODS

Production of *Yersinia entomophaga* MH96

Ye MH96, from stock cultures maintained at AgResearch, Lincoln, was cultured by fermentation and concentrated by centrifugation. Viability of cells was confirmed using serial dilutions in 0.1 M phosphate buffer (10 mM sodium phosphate buffer, pH 7.4; 0.65 mM K_2HPO_4, 0.35 mM KH_2PO_4) spread onto Ye MH96 selective agar (M.R.H. Hurst, unpublished data) plates that were incubated at 30°C for 48 h prior to enumeration of the bacterial numbers. The bacteria were suspended in a 1% biopolymer gel to produce Ye MH96 Gel using patented biopolymer technology (patent WO02/15702A1) and two bait formulations (Baits 1 and 2) that differed in structural integrity. Bait 1 contained 2.06×10^{10} Ye MH96 cells/g and a structurally more rigid Bait 2 contained 1.44×10^{10} Ye MH96 cells/g.

Field trial

A field trial was established at Ratanui, South Otago, in a ryegrass/white clover sheep pasture approaching its 3rd winter since cultivation and containing a potentially damaging density of porina. A randomised block design of 3 × 4 m plots was implemented to suit an uneven distribution of porina over the trial area. To facilitate sampling of Ye MH96 each plot was divided into six 2 × 1 m subplots (labelled A-F). There were five replicates of each treatment to compare the Ye MH96 Gel applied at an average of 2.99×10^7 bacteria per cm^2 (range of 1.9 – 3.9 $\times 10^7$) and the two bait formulations applied at the rate of approximately 6.7 g/m^2, with untreated control plots and a standard insecticide treatment fenitrothion (Caterkil 1000) applied at 900 g ai/ha in 300 litres water. The treatments were applied on 3 May 2011. Sprayed treatments were applied using a pressurised backpack sprayer fitted with TeeJet 8002A nozzles at 207 kPa for fenitrothion and 345 kPa for Ye MH96 Gel. The Ye MH96 Gel was tank mixed with water, 2% (w/v) skim milk powder (added as a UV protectant) and Spreadwet 1000 (0.25 ml/litre) added as a wetting agent. To meet EPA (ERMA) requirements for field testing of formulated Ye MH96 (ERMA application 200647), the trial area was fenced to exclude stock and covered with 25 mm mesh bird netting pinned at the sides and suspended 30 cm above the ground to exclude birds and small mammals (Figure 1a). Climate data were obtained from the nearest NIWA climate station at Catlins Lake approximately 2.5 km NE of the trial site.

Yersinia entomophaga MH96 field persistence

The persistence of Ye MH96 on pasture foliage, in the baits and in soil was assessed, until it could no longer be detected (154 days), to quantify viable

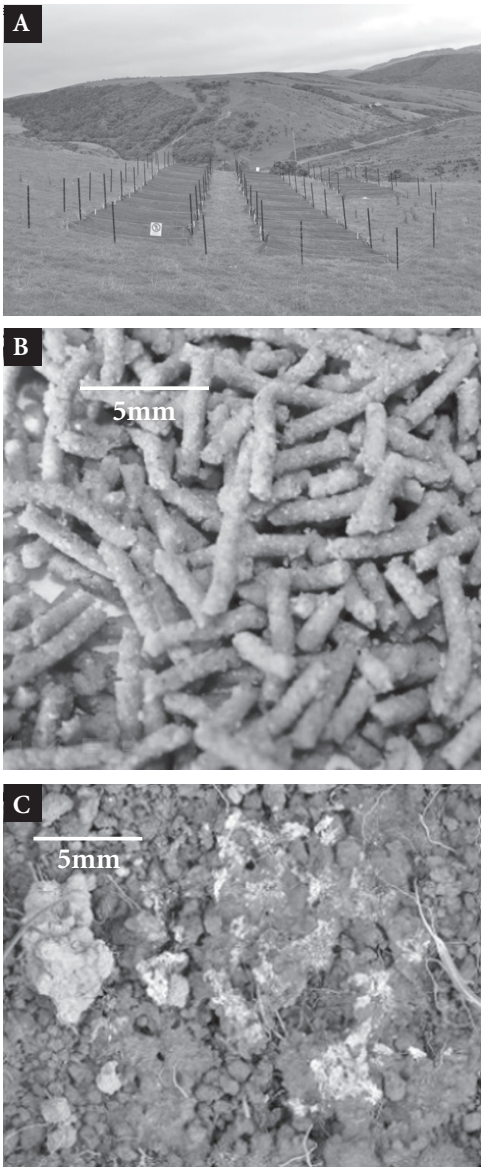

Figure 1 (a) Field trial layout, (b) baits ready for application and (c) disintegrated baits 7 days after application.

Ye MH96 bacteria. For the initial assessments two sub-plots from opposite sides of the plot were randomly allocated for sampling. In each subsequent sampling the sub-plot next to that sampled previously was sampled in a clockwise sequence (i.e. sub-plot B followed A, E followed F, etc.). For all sample types collected, fresh and dry weights (weight after drying at 80°C for 24 h) were determined. Serial dilutions were carried out for all non dried samples in specM buffer (0. 1% Triton-X-80; 2 mM tetra-sodium pyrophosphate), with subsequent dilution plating onto Ye MH96 selective agar. Plates were incubated at 30°C for 48 h, and colony counts were recorded as bacteria number/cm^2 or g of soil to estimate mean bacterial deposition rates. To validate the colony counts, a subset of colonies was transferred to CHROMagar™ Orientation agar (Fort Richard Laboratories (PO Box 22172, Otahuhu, Auckland), where Ye MH96 turns purple on the chromogenic agar at 25°C. A sub-selection of these was bioassayed against grass grub (*Costelytra zealandica* (White)), the natural host of Ye MH96, to verify their disease causing ability.

Foliage samples were collected from two 10 × 10 cm quadrats from each random subplot in the Ye MH96 Gel treated plots at days 0, 7, 14, 28, 35, 42 and 56. From each sample approximately 4-5 g foliage was stomached for 1 min with 25 ml of SpecM buffer in an Interscience Bagmixer. This step was repeated after the addition of a further 20 ml of SpecM, to make a combined total of 45 ml. After day 56, soil cores were used to monitor Ye MH96 persistence in this treatment.

To assess bacteria levels in soil, ten 2.5 cm diameter cores were collected to a depth of 7.5 cm from each subplot. For the final sampling, two cores from all sub-plots of each treatment were collected. Soil cores were taken from Ye MH96 Gel subplots at days 56, 112 and 154, and for the Ye MH96 Bait1 and 2 subplots at days 21, 28, 42, 56, 112 and 154. For bacterial enumeration at these times the cores from each plot were combined and then homogenised by hand. Approximately 20 g of the mixed soil was added to 180 g of 0.1% bacto peptone in a 250 ml flask. After hand stirring, each flask was subjected to 3 min of sonication in a Julabo USR-3 sonicating water bath.

Field-collected bait samples were weighed and added to approximately 9 times their weight of SpecM and machine shaken in a Lab-Line multi Wrist shaker for 10 min at maximum speed. The bait treatments were only able to be assessed for day 0 and day 7 after which time the baits had disintegrated (Figure 2c)

Mean values of Ye MH96 in Baits 1 and 2 were calculated using direct bait bacterial enumerations at days 0 and 7 and soil core samples at day 21 through to 154. Values of Ye MH96 in the Gel were calculated using foliage (day 0–42) and soil core samples (day 56–112). It should be noted that that values of 10^3 bacteria/g of soil are at the threshold of detection.

Invertebrate survival

To assess porina numbers, pre-treatment sampling (28 April 2011) was carried out by digging six 20 × 20 × 25 cm deep sample units per plot. Post treatment sampling, 5 and 17 weeks after treatment, comprised five such samples per plot. For the pre-treatment and the 5 week post treatment assessments the turf layer (50 mm deep) of each sample unit was processed using modified Berlese funnels (Crook & Ferguson 2008) to extract and collect porina and some non-target invertebrates (the earthworms *Aporrectodea caliginosa* and *Lumbricus rubellus*, adult Argentine stem weevil (*Listronotus hyperodae*), Staphylinidae and non-target Lepidoptera larvae). No attempt was made to identify Staphylinidae collected beyond family level. The soil from 50-250 mm on these occasions, and the whole of each sample for the final sampling, was hand sorted in the field for porina. Non-target invertebrates were not counted in the 50-250 mm portion of the samples or at the final assessment.

Statistical analysis

For enumeration of Ye MH96 persistence a generalised linear model (GLM) assuming a negative binomial distribution through log link was used. The model included the effects of treatments, post-treatment days and their interaction. These analyses used statistical software SAS version 9.1. Post-treatment mortality of porina at 5 and 17 weeks after application was compared between treatments and blocks using a GLM with binomial distributions and logit link function. This analysis was carried out with statistical software Minitab version 15. Pre- and 5 week post-treatment densities of non-target invertebrates were analysed by GLM with negative binomial distribution and log link function. The model included the effects of pre- and post-treatment times and their interaction to treatments, as well as block effects.

RESULTS

***Yersinia entomophaga* MH96 field persistence**

Ye MH96 was detectable for 112 days after application. However, there was a decline from 3×10^7 bacteria to 3.4×10^5 bacteria/g of foliage in the Gel treatment within the first 7 days (Table 1, Figure 2c), which coincided with a major rainfall event (59 mm) on day 4 (Figure 2a). The number of bacteria detected in all treatments decreased over time (Figure 2c) but decreased more rapidly for Ye MH96 Gel than Bait 1 ($P<0.005$) and Bait 2 ($P<0.001$). There was no difference in Ye

Table 1 Mean and SEM of average Ye MH96 (cfu/g) on each day for each of the three formations. From day 21 to the end of the trial soil cores were used for all extractions and enumerationof bacteria.

Day	Gel	Bait 1	Bait 2
0	$2.99 \pm 0.38 \times 10^7$	$2.06 \pm \text{NA}^1 \times 10^{10}$	$1.44 \pm \text{NA} \times 10^{10}$
7	$3.36 \pm 0.78 \times 10^5$	$1.65 \pm 0.32 \times 10^{10}$	$4.49 \pm 3.59 \times 10^{10}$
21	$1.18 \pm 0.37 \times 10^5$	$1.40 \pm 0.72 \times 10^5$	$1.94 \pm 1.66 \times 10^6$
28	$9.51 \pm 4.42 \times 10^3$	$5.12 \pm 4.19 \times 10^5$	$1.31 \pm 0.58 \times 10^5$
42	$4.37 \pm 3.11 \times 10^4$	$1.91 \pm 0.71 \times 10^4$	$3.60 \pm 1.10 \times 10^4$
56	$1.00 \pm 0.93 \times 10^5$	$2.72 \pm 1.38 \times 10^4$	$1.47 \pm 0.87 \times 10^4$
112	$3.56 \pm 0.90 \times 10^4$	$3.27 \pm 3.27 \times 10^3$	$3.94 \pm 3.12 \times 10^4$
154	0 ± 0	0 ± 0	0 ± 0

[1]SEM on Day 0 for Baits 1 and 2 are NA because field-collected bait samples on Day 0 were combined into single sample.

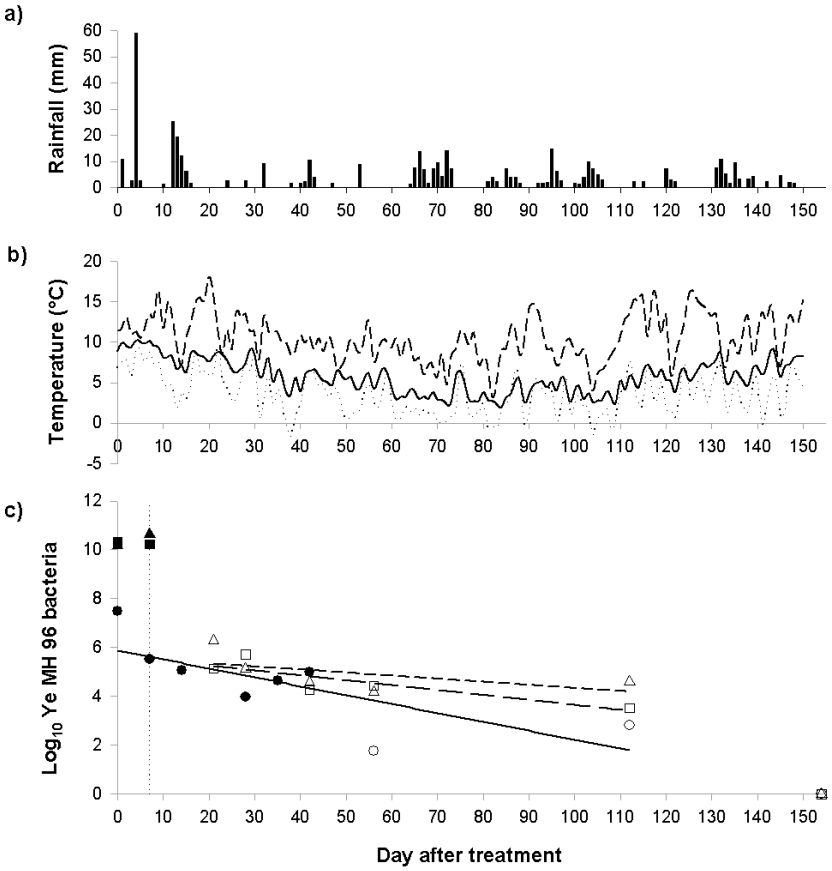

Figure 2 (a) Rainfall (mm) and (b) Temperatures (°C) at Ratanui over the duration of the field trial (solid line for soil temperature, dashed line for maximum air and dotted line for minimum air temperatures). (c) Persistence of Ye MH96 for the three formulations (closed or open circle for observed average bacterial count of Gel from foliage samples or soil samples, closed or open square for Bait 1 from field-collected bait sample or soil samples, and similarly closed or open triangle for Bait 2). Solid line is estimated decreasing rate of Gel, dashed line and dotted line are of Baits 1 and 2 respectively. Vertical dotted line denotes bait disintegration observed at day 7.

MH96 decline rate between the baits ($P>0.05$). Ye MH96 was not recovered from any treatment after 112 days.

Porina

Pre-treatment sampling indicated a mean porina density of 81/m^2 (range 35–170, SEM 8). When assessed 5 weeks after treatment the numbers of porina in the control plots were 20% lower than pre-treatment counts. All the Ye MH96 treatments and fenitrothion had further reduced porina numbers ($P<0.001$) (Table 2). The differences between fenitrothion and the Ye MH96 treatments were also significant ($P<0.001$). At 17 weeks numbers of porina in the control plots had decreased to 42% of pre-treatment counts. Treatment differences evident at 5 weeks remained and further reductions had occurred for both Ye MH96 bait treatments (Table 2). The level of mortality associated with fenitrothion at 5 weeks was still significantly higher than any Ye MH96 treatment ($P<0.001$). Ye MH96 Baits were associated with greater porina mortality than Ye MH96 Gel ($P<0.033$).

Table 2 Percent reduction in porina numbers relative to pre-treatment counts at 5 and 17 weeks after treatment (P-value for the difference compared to untreated control).

	5 weeks	17 weeks
Control	20	42
Ye MH96Gel	38 (<0.001)	61 (<0.001)
Ye MH96 Bait 1	40 (<0.001)	72 (<0.001)
Ye MH96 Bait 2	41 (<0.001)	69 (<0.001)
Fenitrothion	93 (<0.001)	96 (<0.001)

Non-target invertebrates

Neither fenitrothion nor Ye MH96 had observed effects on the abundance of either earthworm species (Table 3). None of the Ye MH96 treatments were associated with any differences in densities estimated for the untreated plots for adult *L. bonariensis* or Staphylinidae (Table 3). Reductions of non-target Lepidoptera (comprised of several unidentified species) occurred in the Ye MH96 Bait plots after 5 weeks relative to the untreated plots and were significantly different from pre-treatment levels ($P<0.01$ and $P<0.05$ for Baits 1 and 2 respectively) (Table 3). Fenitrothion was associated with reductions of both *L. bonariensis* and the non-target Lepidoptera (Table 3).

DISCUSSION

The heavy rainfall 4 days after treatment application very likely washed the Ye MH96 from plant foliage in the Gel treated plots, as indicated by the rapid decline in bacteria numbers from foliage samples when measured on day 7, and into the soil as shown by the level of bacteria detected in soil cores taken from these plots at day 56. This rainfall was also associated with the disintegration of both bait formulations observed on day 7 (Figure 1c) and evidenced by Ye MH96 levels in the soil cores taken from both bait treatments on day 21 by which time baits were not discernable on the pasture or soil surface. The movement of bacteria from the substrates of all Ye MH96 formulations into the soil eliminated the influence of substrate on bacteria survival. In all cases survival remained consistent in the soil until day 112 before falling below the detection limit at day 154. Survival was possibly favoured by regular low level rainfall, which kept the soil moist, and stable soil temperatures. The rainfall at day 4 was not unusually high but clearly compromised the integrity of formulations and is an issue that needs to be addressed.

Some natural reduction of porina numbers not associated with any of the treatments occurred during the trial. Despite this the application of fenitrothion had an acute impact resulting in almost complete destruction of the porina in those plots. This is an expected response and one most

Table 3 Densities (number/m^2) (P-value for the difference relative to untreated control) of non-target invertebrates at 5 weeks after treatment application. Pre-treatment levels are presented for comparison.

	L. rubellus	*A. caliginosa*	*L. bonariensis*	Staphylinidae	Non-target Lepidoptera
pre-treatment	111	113	30	25	25
post-treatment					
control	90	206	47	51	27
Ye MH96 Gel	135 (ns)	154 (ns)	25 (ns)	20 (ns)	13 (ns)
Ye MH96 Bait 1	166 (ns)	210 (ns)	26 (ns)	33 (ns)	12 (0.04)
Ye MH96 Bait 2	163 (ns)	173 (ns)	27 (ns)	14 (ns)	15 (0.10)
fenitrothion	175 (ns)	141 (ns)	17 (0.05)	16 (ns)	8 (0.03)

farmers would welcome. However, a prime reason for porina causing damage to young pastures is that cultivation disrupts an association between porina and a range of natural pathogens. When the association is undisturbed it provides some long term regulation of porina numbers (Crawford & Kalmakoff 1977). The level of reduction achieved by fenitrothion limits the number of hosts available for these natural pathogens and may consequently increase the length of time required for equilibrium to re-establish. By comparison, the level of control provided by Ye MH96 is modest and, especially after 5 weeks, unlikely to appeal to farmers. However, the control at 17 weeks associated with both bait formulations is acceptable because surviving porina would not cause noticeable damage and their presence would allow the persistence of natural pathogens. The timeframe of 17 weeks to achieve this level of control is longer than desirable but the results obtained suggest that, with refinements to improve the integrity of formulation, Ye MH96 may have a future role to play in limiting the impacts of porina. Temperatures measured over the duration of the trial, and especially over the 5 weeks following treatment application (Figure 2b), were such that porina would have been active and had ample opportunity to ingest Ye MH96. However, this may have been affected after the rain on day 4 by the rapid loss of the applied bacterium from the pasture foliage, the disintegration of the baits and associated loss of formulated porina attractants, and release of bacterium into the soil profile. These factors may have lessened the amount of bacteria consumed by porina and/or the number of porina that may otherwise have ingested Ye MH96. The observed reductions in porina numbers 5 weeks after treatment may largely reflect ingestion of Ye MH96 in the 3-4 days following application. However, the increased levels of mortality observed at 17 weeks indicate that Ye MH96 was still active and ingestion of bacteria may have been occurring from the "soil surface litter" layer. The result of cumulative ingestion of sub-lethal doses of Ye MH96 has not been assessed but it is possible that continuous ingestion of low numbers of bacteria may allow the bacterium to establish in the insect and multiply to cause lethality.

Ye MH96 and fenitrothion were not observed to have any effect on the abundance of either earthworm species. Densities of both *L. rubellus* and *A. caliginosa* (Table 3) estimated in this investigation relate only to the top 50 mm of soil and as both species are also found deeper, densities for the plots would be higher than those presented. However, *L. rubellus* is a surface/dung feeding earthworm generally found in the top 50 mm of the soil profile and the numbers measured are typical of pastures. *Aporrectodea caliginosa* is a shallow (to ca 150 mm) burrowing earthworm but feeds predominantly in the top 50 mm of soil and is numerically most common in that zone. The densities estimated for the latter species are lower than optimal in pasture but typical of the environment the investigation was carried out in. Soil moisture and temperatures over the 5 week period after treatment were very suitable for earthworm activity and the likelihood of the earthworms coming in contact with Ye MH96 would have been high as there were at least 3×10^4 bacteria/g soil until 112 days after application. Adult *L. bonariensis* numbers were low but not untypical for south Otago pastures in late autumn. At this time of year they generally have undeveloped flight muscles and are likely to have been largely restricted to living within a single plot over the 5 weeks since treatment. As grass foliage feeders it could be expected they would most likely come into contact with the gel formulation of Ye MH96. However, none of the Ye MH96 treatments were associated with any differences from untreated plots. Staphylinids are carrion feeders and would most likely be exposed to Ye MH96 after they consumed other invertebrates killed or infected by the bacteria. If this was the case it does not seem to have had any effect on Staphylinidae abundance. Hurst et al. (2011) have previously shown that Ye MH96 affects a range of lepidopteran larvae so the possible effect on non-target species observed in this study is not unexpected. This result indicates this is an area that should be given more attention in future investigations.

This investigation demonstrated the need to improve the delivery of Ye MH96 particularly with respect to maintaining formulation integrity during heavy rain. Survival of the bacteria in the soil for 112 days and possible ingestion of sub-lethal doses of Ye MH96 with subsequent natural multiplication illustrate the importance of conducting environmental persistence studies to gain an understanding of the behaviour and ecology of Ye MH96 when introduced to agricultural environments. In terms of biopesticide efficacy Ye MH96 in this investigation provided encouraging levels of porina control with minimal non-target effects observed. With further development to increase its efficacy farmers may soon have an effective biological solution to an intransigent pasture pest.

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REFERENCES

Barratt BIP, van Toor RF, Ferguson CM, Stewart KM 1990. Grass grub and porina in Otago and Southland. A guide to management and control. MAF, Mosgiel, New Zealand. 104 p.

Brownbridge M, Ferguson C, Saville DJ, Swaminayhan J, Hurst MRH, Jackson TA 2008. Potential for biological control of porina (*Wiseana* spp.) with a novel insecticidal bacterium, *Yersinia* n. sp. (MH96) EN65 strain. New Zealand Plant Protection 61: 229-235.

Crawford AM, Kalmakoff J 1977. A host-virus interaction in a pasture habitat: *Wiseana* spp. (Lepidoptera: Hepialidae) and its baculoviruses. Journal of Invertebrate Pathology 29: 81-87.

Crook KE, Ferguson CM, Barratt BIP 2004. Heat extraction of invertebrates from grassland turf samples. In: Winder LM, Goldson SL ed. Proceedings of the 8th Australasian Grasslands Invertebrate Ecology Conference. Pp. 102-106.

Dugdale JS 1994. Fauna of New Zealand No. 30, Hepialidae (Insecta: Lepidoptera). Manaaki Whenua Press, Lincoln, New Zealand. 164 p.

Ferguson CM, Crook KE 2004. The development of two *Wiseana* species and the implications for their management as pastoral pests. In: Winder LM, Goldson SL ed. Proceedings of the 8th Australasian Grasslands Invertebrate Ecology Conference. Pp. 87-93.

Ferguson CM, Logan RAS, Barratt BIP 1999. *Wiseana* species flight patterns in Otago and Southland. Proceedings of the 52nd New Zealand Plant Protection Conference: 275.

Glare T, Caradus J, Gelernter W, Jackson T, Keyhani N, Köhl J, Marrone P, Morin L, Stewart A 2012. Have biopesticides come of age? Trends in Biotechnology 30(5): 250-258.

Grue CE, Gibert PL, Seeley ME 1997. Neurophysical and behavioural changes in non-target wildlife exposed to organophosphate and carbamates pesticides: Thermoregulation, food consumption, and reproduction. American Zoologist 37: 369-388.

Herigstad B, Hamilton M, Heersink J 2001. How to optimize the drop plate method for enumerating bacteria. Journal of Microbiological Methods 44: 121-129.

Hurst MRH, Becher SA, Young SD, Nelson TL, Glare TR 2011. *Yersinia entomophaga* sp. nov. isolated from the New Zealand grass grub *Costelytra zealandica*. International Journal of Systematic and Evolutionary Microbiology 61: 844-849.

Jäkel A, Roth M 1998. Short-term effects of selected insecticides on non-target soil invertebrates of a forest ecosystem. In: Pižl V, Tajovský K ed. Soil zoological problems in central Europe. Proceedings of the 4th Central European Workshop on Soil Biology, České Budějovice, Czech Republic. 23-24 April 1997. Pp. 65-70.

Odenkirchen EW, Eisler R 1988. Chlorpyrifos hazards to fish, wildlife, and invertebrates: A synoptic review. Fish and Wildlife Service, U.S. Department of the Interior. Biological Report 85 (1.13). Contaminant Hazard Reviews Report No. 13. 37 p.