Assessing the potential of *Yersinia entomophaga* to control plantain moth in a laboratory assay

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Abstract The recent increased use of the high-quality drought-tolerant forage plant plantain (*Plantago lanceolata*) (Plantaginaceae) has been associated with outbreaks of native geometrid caterpillars, such as *Scopula rubraria*. The outbreaks most often occur under warm climatic conditions and within plantain monocrops. Pest populations can reach 11,500 larvae/m². Stands can be severely damaged with 90% of plants destroyed and stand life expectancy reduced by 2–3 years. This study assessed the efficacy of the insecticidal bacterium *Yersinia entomophaga* against *S. rubraria* under laboratory conditions using a leaf dip bioassay. A LD₅₀ of $2.26 \pm 0.33 \times 10^5$ cells per larva was determined with an LT₅₀ defined as 3.1 days when treated with 4.8×10^6 cells per larva. These data combined with the life history of *S. rubraria* and the pathobiology of *Y. entomophaga* suggest the bacterium could be developed as a biopesticide for use against *S. rubraria*.

Keywords plantain, *Plantago lanceolata*, plantain moth, *Scopula rubraria*, *Yersinia entomophaga*, biopesticide, control agent.

INTRODUCTION

In recent years, the forage plant plantain (*Plantago lanceolata*; Plantaginaceae) has been sown by increasing numbers of farmers who perceive advantage can be gained from the plant's drought tolerance and high feed quality that provides high stock live weight gains. Plantain is now commonly grown in both the North and South Islands, typically planted as a plantain/clover mix (Stewart et al. 2014) but also as a monoculture forage crop or as a component of mixed pastures. In the North Island native looper caterpillars, now commonly referred to as plantain moth caterpillars, have emerged as significant pests due to their voracious feeding and ability to rapidly reach very high population densities as a consequence being

able to complete several generations per year (Ferguson & Philip 2014).

“Plantain moths” belong to the family Geometridae, and the group comprises at least two species, *Scopula rubraria* (Doubleday) and *Epyaxa rosearia* (Doubleday), which are widespread and common in both islands. To a layperson these species appear very similar. Both are thought to be New Zealand native species, with *S. rubraria* also present in Australia (Philip & Ferguson 2014). Other closely related species in both genera may also form part of this complex. Neither of the recognised species has been extensively studied within an agricultural context but one study has shown that *S. rubraria*

can reach numbers as high as 11,500 larvae/m² in plantain stands, at times consuming up to 90% of the crop (Ferguson & Philip 2014). This damage typically occurs in areas where plantain is sown as a monoculture, or in conjunction with clover, and under dry summer conditions that favour *S. rubraria* growth and multiplication. Both *S. rubraria* (Philip & Ferguson 2014) and *E. rosearia* (P.J. Gerard, AgResearch, personal communication) appear to have several generations per year, with the number of generations determined by spring and summer temperatures. Consequently both species can become very abundant by late summer but populations decline towards late autumn. Anecdotal evidence suggests that first year crops are relatively untouched by pests but those in the second or third year can be badly hit particularly when adverse drought conditions place stress on plantain slowing its growth. Within pastoral systems, *S. rubraria* damage appears to be confined to plantain, but *E. rosearia* has previously been recorded damaging Caucasian clover (*Trifolium ambiguum*) and white clover (*T. repens*) (Watson et al. 1996a,b).

There are currently no insecticides registered specifically for control of plantain moth caterpillars but insecticides effective against a range of other lepidopteran pests may also provide control of plantain moths. Stock withholding periods associated with insecticide applications may, however, be incompatible with grazing.

The non spore-forming bacterium *Yersinia entomophaga* (Enterobacteriaceae) originally isolated from the cadaver of a *Costelytra zealandica* (Coleoptera: Scarabaeidae) larva, has been shown to be pathogenic by *per os* challenge to a wide range of lepidopteran, coleopteran and orthopteran species (Hurst et al. 2011a). Field efficacy of *Y. entomophaga* has also been demonstrated against larvae of the pasture pest porina (*Wiseana* spp. *Lepidoptera*: Hepialidae) (Ferguson et al. 2012) and adult bronze beetles (*Eucolaspis* sp., *Coleoptera*: Chrysomelidae) in caged field trials in apple orchards (Hurst et al. 2011b).

This study aimed to demonstrate efficacy of *Y. entomophaga* against larvae of *S. rubraria*, one of the plantain moths. Dose response assays were carried out on laboratory-reared larvae as

a prerequisite to determining the potential of *Y. entomophaga* as an efficacious biopesticide of plantain moths.

MATERIALS AND METHODS

Insects

Scopula rubraria adults and larvae were field-collected from a mixed plantain/grass sward at Halcombe, Manawatu, in February 2014. The identity of these was subsequently confirmed by Brian Patrick, Wildland Consultants, Christchurch (Ferguson & Philip 2014). These individuals were used to start a laboratory colony. Adults were kept in 180 × 160 × 70 mm clear acrylic containers with mesh lids during mating and oviposition. Plantain foliage and paper towels were provided as substrates for egg-laying and cotton wool dental wicks moistened with a 10% honey/water solution were supplied as food. Larvae were mass-reared in containers described above and were fed plantain leaves on a layer of paper towels. Leaf material and paper towels were replaced as necessary. Pupae were removed to 60 ml specimen containers for moth emergence. All rearing stages were kept at 17 ± 1°C and 16:8 h light:dark. Fourth instar and early 5th instar larvae (5–10 mm length) from the 3rd laboratory generation of caterpillars were used in the experiment.

Bioassay

Yersinia entomophaga was grown in Luria-Bertani (LB) broth at 25°C for 18 h as described previously (Hurst et al. 2014). For assessment in the bioassay, five rates of *Y. entomophaga* cells were prepared by performing ten-fold serial dilutions to give doses ranging between 5 × 10³ and 5 × 10⁷ cfu / dose. Dilutions were made in water with the addition of 0.04% DuWett (Elliot Chemicals Ltd), which assisted in application of the bacterial suspension to the plantain leaf discs in the bioassay.

Immediately prior to the assay, 2.5 cm leaf discs were excised from freshly cut plantain leaves, collected from plants that had been maintained in pots in the glasshouse. The leaf discs were dipped into dilutions of the culture described above, and were air dried for 1 h at ambient room temperature. Individual discs were then placed on a piece of Whatman® filter paper (grade no. 5)

located at the base of a 70 ml specimen screw cap container and five fourth instar *S. rubraria* larvae were added to each container. The five replicate containers per treatment, including a 0.04% DuWett in water only control, were arranged in a randomised block design within a covered plastic container kept at 18°C. The larvae were observed daily over 6 days for mortality. Feeding activity was determined by visually assessing the percentage of leaf disc that had been eaten. On day 3 of the assay any uneaten leaf was removed from the containers and replaced with a fresh uninoculated leaf disc. This was to prevent larval mortality due to lack of food and cannibalism.

To validate that *Y. entomophaga* was the causative agent of larval mortality, 48 h after commencement of the assay, two dead larvae from each treatment were randomly selected. The cadavers were surface sterilised by submerging in 30 ml of 70% ethanol for 60 s, washed in sterile distilled water and surface moisture removed with tissue paper. The individual larvae were homogenised in 1.5 ml of sterile 0.1M phosphate buffer (pH 7.4) in a micro-centrifuge tube. The homogenates were streaked onto LB agar plates and *Y. entomophaga* colonies confirmed by patching 10 random colonies onto Orientation ChromAgar (Fort Richard Laboratories, Auckland, New Zealand) and incubating for 24 h at 25°C. *Yersinia entomophaga* colonies appear purple following incubation.

Statistical methods

Minitab version 16 was used to assess larval mortality and feeding activity data to calculate means and standard errors. For the live *Y. entomophaga* treatment groups, LD₅₀ on day 6 was estimated using probit analysis. The dose level of *Y. entomophaga* live cells of each treatment group was calculated per caterpillar, by multiplying the applied cell count per leaf disc by average percentage of the disc consumed until day 3 across five replicates, then, dividing by five caterpillars. The time to achieve 50% mortality (LT₅₀) value for each treatment group was estimated in parametric survival (or time-to-event) analysis. In the analysis, standard statistical curved functions were fitted to

cumulative mortalities of the group during the 6-day post treatment period and the function that most closely reflected mortality was chosen to estimate the LT₅₀ value.

RESULTS

Yersinia entomophaga cells applied at the two highest rates of 4.8×10^7 cells and 4.8×10^6 cells /leaf disc caused 100% mortality of *S. rubraria* larvae within 6 days and there was a highly significant effect ($P < 0.001$) on larval mortality on day 6 where an application rate of 4.8×10^5 cells/leaf disc was used (Figure 1a) in comparison to the untreated control. Mortality levels (<15%) at lower concentrations were not significantly different to the control group mortality of 10%.

Yersinia entomophaga live cells applied at the highest rate had a highly significant effect on amount of leaf material consumed by day 3, with approximately 8% of leaf material consumed by larvae in comparison with 89% consumption in the control (Figure 1b). Larvae in the highest dose treatment began feeding but had consumed less than 10% of the leaf disc before dying. There was no significant reduction in feeding at lower dose rates compared to the control group by day 3, and larvae in these groups continued to feed when provided with a fresh uninoculated leaf disc. At day 6 the reduced levels of leaf consumed in the 4.8×10^7 , 4.8×10^6 and 4.8×10^5 treatments most likely reflected the high level of larval mortality (> 62%) in those groups.

Through probit analysis the predicted LD₅₀ of *Y. entomophaga* cells per *S. rubraria* larva was estimated to be $2.26 \pm 0.33 \times 10^5$ (Mean $\pm$ SEM) (Figure 2).

The time taken for larvae to die was proportional to the dose rate of *Y. entomophaga*. Rapid death (LT₅₀ 2.5 ± 0.1 days) (Mean $\pm$ SEM) occurred at the highest rate (4.8×10^7 /leaf disc) but was much slower (LT₅₀ 4.9 ± 0.3 days) (Mean $\pm$ SEM) with 100-fold less cells (4.8×10^5 /leaf disc) (Figure 3).

Yersinia entomophaga was detected in all cadavers sampled at 48 h, with numbers of *Y. entomophaga* varying between 1×10^3 and 5×10^4 cells per larvae (total of 4 cadavers assessed).

Figure 1 (a) Mortality (%) of *Scopula rubraria* larvae after treatment with different concentrations of *Yersinia entomophaga* live cells/leaf disc over time. (b) Mean proportion (%) of treated leaf disc consumed by day 3, and fresh leaf disc consumed from day 3 until day 6 of the bioassay period. Error bars denote the 95% confidence intervals

Figure 2 Fitted relationship using the probit model between log dose of *Yersinia entomophaga* and mortality of *Scopula rubraria* after 6 days showing LD_{50} of 2.26×10^5 cells/larva.

Figure 3 Mean cumulative mortality curves estimated from the best-fit survival functions derived from the observed mortality of *Scopula rubraria* larvae after treatment with *Yersinia entomophaga* at 4.8×10^7 cells, 4.8×10^6 cells or 4.8×10^5 cells/leaf disc.

DISCUSSION

The results of this laboratory study have shown that *Y. entomophaga* caused rapid death of 4th instar *S. rubraria* larvae, with an LD_{50} of $2.26 \pm 0.33 \times 10^5$ *Y. entomophaga* cells/larva. At a leaf dip rate of greater than 4.8×10^7 cells/leaf disc, 100% mortality was observed within 6 days, while at a leaf dip rate of 4.8×10^5 cells/leaf disc, a LT_{50} of approximately 5 days was defined.

These bacterial foliar concentrations are within the range of cell concentrations typically found after foliar application of *Y. entomophaga*. For example, approximately 5×10^5 – 5×10^6 cells/cm² were recovered from apple leaf surfaces or grass following field application (Hurst et al. 2011b; Ferguson et al. 2012).

Yersinia entomophaga survives for a relatively short time on foliage with no significant field-

based foliar persistence of the bacterium from 3 days post spray application (Hurst et al. 2011b; Ferguson et al. 2012). As a non spore-forming bacterium it is sensitive to environmental stresses such as desiccation and exposure to UV light. To be effective in control of plantain moths, it must achieve rapid knockdown of these pests and the laboratory tests reported here suggest this can be achieved. In the field, plantain moth larvae can reach densities in excess of 11,500 larvae/m² (Philip & Ferguson 2014). These high densities, combined with the voracious appetite of plantain moth larvae, make them an ideal target for a *Y. entomophaga*-based foliar application. Further to this, the population build-up of *Y. entomophaga* in the plantain larvae cadavers, coupled with observed cannibalistic habits of the pest may assist in spread of infection and prolong persistence of *Y. entomophaga* in the field.

The limited foliar persistence combined with the speed of kill means that the bacterium *Y. entomophaga* should be applied using a strategy similar to that of an insecticide, where the bacterium is sprayed to rapidly knock down an infestation. Due to the multiple generations of plantain moth that can occur within a season, an early application in December should prevent the build-up of caterpillar numbers in late summer leading to less pressure for autumn pastures.

In future studies, the ability of *Y. entomophaga* to kill other plantain pests, e.g. *Epyaxa rosearia* and leafroller caterpillars (Family Tortricidae), *Merophyas leucaniana* (New Zealand native) and *M. divulsana* (Australian species), will also be determined. Improved foliar sprays and other delivery techniques for *Y. entomophaga* are also being investigated in the MBIE funded programme, Next Generation Biopesticides and will benefit efficacy testing in future field trials targeting plantain pests.

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