***Serratia entomophila*-coated seed to improve ryegrass establishment in the presence of grass grubs**

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Abstract The entomopathogenic bacterium, *Serratia entomophila*, is an alternative to chemical control of grass grub (*Costelytra zealandica*) and is applied in a granule formulation to established pastures. Treatment of seed with microbial inoculants is an ideal mechanism for delivery and establishment of microbial control agents into the plant root zone where soil dwelling pests, such as grass grub, are located. Seed treatment with *S. entomophila* was tested in three glasshouse pot trials, for its ability to protect germinating ryegrass seedlings from grass grub damage. A range of larval densities was used and microbial seed treatment was compared with the insecticide imidacloprid. At medium larval densities (equivalent to 70 larvae/m²), use of *S. entomophila*-coated seed resulted in 85% seedling establishment in comparison with 82% emergence from imidacloprid-treated seed. At a high larval density of 300/m², where there was no establishment of untreated seed, 35–51% of seedlings established from *S. entomophila*-treated seed. Results suggest there is potential for seed coating to aid ryegrass establishment in autumn-sown pastures.

Keywords *Serratia entomophila*, grass grub, ryegrass, coated seed.

INTRODUCTION

The New Zealand grass grub (*Costelytra zealandica*) is a serious pest of pasture. The larvae feed on the roots of established grasses and on emerging seedlings in autumn-sown pastures (Chapman 1984). Chemical control measures to protect ryegrass seedlings from grass grub include the neonicotinoids imidacloprid (Gaucho®, Bayer) and clothianidin (Poncho®, Bayer) applied as seed dressings.

Microbial control agents can be used as alternatives for, or in addition to, chemical insecticides. On organic farms, microbial control agents may be the only suitable option. The

pathogenic bacterium *Serratia entomophila*, formulated as a granule, is sold in New Zealand (under the trade name bioshield™, Ballance Agri-Nutrients, Tauranga) for control of grass grub larvae in established pasture. The granules are applied beneath the surface of pasture where larvae are feeding using a seed drill. Infected grubs cease feeding within 3–4 days of infection and die within 3 weeks.

Seeds are an ideal delivery mechanism for the introduction of entomopathogenic microbes into the plant rhizosphere, as they allow targeted contact with and infection of insect pests and

can introduce high populations of viable cells. Seed coating has been previously investigated as a means of introducing *S. entomophila* into the root zone of wheat (Young et al. 2009) and carrot (Wright et al. 2005) for the control of grass grub larvae.

This study examines the use of seed coating to deliver *S. entomophila* directly into the grass grub feeding zone. The efficacy of *S. entomophila* as a seed coating on ryegrass for control of grass grub larvae was compared with a commonly applied chemical alternative, imidacloprid, and with the use of the microbial control agent bioshield™.

METHODS

Seed treatment

Inoculum for preparation of seeds coated with *S. entomophila* was prepared as described previously (Young et al. 2009). Bacterial cells were concentrated by centrifugation and applied to seed using patented biopolymer technology (Swaminathan & Jackson 2008). Imidacloprid was dressed onto seed in the laboratory at the recommended field rate of 5.8 ml/kg seed.

Enumeration of bacteria on seed and in soil

Bacteria present on seed were enumerated by mixing 1 g of seed with 10 ml of Tween buffer (1.0 ml Tween 80 and 1.0 g $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$) and shaking at maximum speed for 10 min on a wrist action shaker. The coating suspension was then dilution plated onto Luria Bertani agar and the plates incubated at 30°C for 24 h before enumeration of colonies. Bacterial counts were in the range $1.4\text{--}6.5 \times 10^9$ cfu/g seed prior to planting.

Serratia entomophila was enumerated from soil by dilution plating onto selective media, using the method described by O'Callaghan & Jackson (1993).

Pot trials

Three glasshouse pot trials were set up using 2-litre plastic pots (15 cm × 8 cm) filled with sieved Templeton silt loam (approximately 1800 g dry weight). Three rows of 10 ryegrass seeds (Colosseum®, PGG Wrightsons, Christchurch,

New Zealand) were planted to a depth of 1–2 cm, approximating a 22 kg/ha seeding rate. In trial 1 the treatments were: bare seed, *S. entomophila*-coated seed and seed coated but without bacteria as the control. All treatments were challenged at three larval densities: 0, 3 and 9 larvae per pot, equivalent to 0, 70 and 300 larvae/m² (zero, moderate and severe damage-causing populations). In the second trial treatments were: bare seed and *S. entomophila*-coated seed at the same larval rates as trial 1. In the third trial treatments were: bare seed, *S. entomophila*-coated seed, imidacloprid-coated seed, *S. entomophila* and imidacloprid combined coated seed, and bioshield™ granules sown together with bare seed. In all trials there were five replicates per treatment with the pots laid out in a randomised block design.

Field-collected, third instar grass grub larvae were pre-fed carrot cubes in trays over 3 days to select healthy, feeding individuals. Larvae were placed on the soil surface post-sowing and allowed to bury themselves in the soil. Any grubs remaining on the surface after 20 min were removed and replaced with healthy specimens to avoid using any larvae damaged by handling. Watering was carried out as required to maintain a soil moisture content of at least 15%, equivalent to approximately 200 ml per pot per week.

Sampling

The trial was conducted in an unheated glasshouse. After 7 weeks seedlings were counted and the total plant dry matter and numbers of surviving grubs recorded. Surviving grubs were characterised as either diseased or healthy by assessment of visual disease symptoms (Jackson et al. 1993). Numbers of *S. entomophila* bacteria in the soil (colony forming units/g dry soil) were calculated by dilution plating of soil onto selective media.

Statistical analysis

Seedling establishment numbers were analysed using Fisher's exact tests for trials 1 and 2, and logistic regression for trial 3 to compare interactions between treatment effects and numbers of larvae. Plant weights in trials 1 and 2

were analysed using the 1-sample *t* test with the null hypothesis of mean = 0 where larvae were present, and in the absence of larvae by analysis of variance (ANOVA). Plant dry weights from trial 3 were analysed by ANOVA. Surviving grass grub numbers were analysed by Fisher's exact tests. *Serratia entomophila* cfu/g values were compared using either a negative binomial model or a quasi-Poisson model for treatment groups with at least one non-zero value and by Fisher's exact tests for comparison of treatment groups having all zero values with another group.

RESULTS

In trials 1 and 2 there were no significant differences between treatments in seedling establishment or final plant dry weights in the absence of grass grub larvae (Table 1). No seedlings survived in the bare seed or coated control treatments at larval densities of 70 or 300/m². In the *S. entomophila*-coated seed treatments, around 72% of seedlings were established in both trials at medium larval density (70 larvae/m²). At high larval densities (300 larvae/m²), seedling establishment was reduced to 35 and 51% for trials 1 and 2 respectively.

In trials 1 and 2, plant dry matter weights from *S. entomophila*-coated seed were not significantly different between larval densities of 0 and 70/m².

However, high larval densities resulted in significantly lower dry matter production than the medium larval densities in both trials (Table 1). Trial 1 was conducted in winter and trial 2 in early spring when average temperatures were higher, which may account for the difference in plant dry matter weights recorded between the two trials.

In the third trial, in the absence of larvae, the mean seedling establishment was 88.7% of seeds sown and the mean final dry matter weight 1.43 g/pot with no significant differences between treatments. Where the treatments were challenged by larvae, the best performing treatment, in terms of seedling establishment, was *S. entomophila*-coated seed, imidacloprid-dressed seed or a combination of both (82–85%). These three seed treatments performed significantly better than either bioshield™ granules incorporated with bare seed (42.7%) or bare seed (20.7%). Bioshield™ performed significantly better than bare seed (Table 2).

In trial 3, in the presence of grass grub larvae, the highest dry matter weights resulted from the seeds coated with *S. entomophila*, imidacloprid alone and in combination. The pots inoculated with bioshield™ granules yielded significantly lower dry matter weights than the three seed coating treatments (Table 2).

Table 1 Mean ryegrass seedling establishment (%) and mean plant dry weights (g/pot) 49 days after sowing bare, coated (without *Serratia entomophila*) or *Serratia entomophila*-coated seed at three larval densities of *Costelytra zealandica* in trials 1 and 2.

Treatment	Larvae (no./m ²)	Seedling establishment		Plant dry weights	
		Trial 1	Trial 2	Trial 1	Trial 2
Bare seed	0	92 a ¹	87 a	0.706 a	3.018 a
	70	0	0	0.000	0.000
	300	0	0	0.000	0.000
Coated control	0	91 a	nt ²	0.687 ab	nt
	70	0	nt	0.000	nt
	300	0	nt	0.000	nt
<i>S. entomophila</i>	0	92 a	86 a	0.596 ab	3.274 a
	70	72 b	73 b	0.459 b	2.845 a
	300	35 c	51 c	0.186 c	1.590 b

¹Values followed by the same letter within a column are not significantly different at *P* < 0.01.

²nt – not tested.

Table 2 Mean ryegrass seedling establishment (%) and dry matter weights (g/pot) 49 days after sowing bare seed with and without bioshield™, or seed with either *Serratia entomophila* or imidacloprid or both in the presence of *Costelytra zealandica* larvae (70 larvae/m²) in trial 3.

Treatment	Rate	Establishment (%)	Dry matter
Bare seed		20.7 a ¹	0.19 a
bioshield™	30 kg/ha	42.7 b	0.50 b
<i>S. entomophila</i>	6.50 x 10 ⁹ cfu/g	85.3 c	1.08 c
Imidacloprid	5.80 ml/kg	82.0 c	1.12 c
<i>S. entomophila</i> +imidacloprid	as above	82.7 c	1.41 c

¹Values followed by the same letter within a column are not significantly different at P<0.001.

Grass grub survival

In trial 1 there were no significant treatment effects on numbers of surviving healthy grubs at the lower rate of 70 larvae/m² (Table 3). At the high larval density, there was a significant reduction in the number of larvae recovered from the pots with *S. entomophila*-coated seeds (Table 3). In trial 2, there were no significant differences in the number of surviving larvae at either rate.

In trial 3, no surviving healthy larvae were recovered from the *S. entomophila*, *S. entomophila* plus imidacloprid or imidacloprid seed treatments. In the bare seed treatment there were seven surviving larvae and there were two in the bioshield™ treatment.

Serratia entomophila in soil

In trials 1 and 2, at the larval rate of 70/m², significantly more bacteria were recovered from the soil in the *S. entomophila*-coated treatments than from the non *S. entomophila*-coated treatment. At the higher rate of 300 larvae/m², *S. entomophila* were recovered from the bare seed treatment; these bacteria were possibly introduced to the pots by the field-collected larvae but were evidently non-pathogenic strains as there was no inhibition of grass grubs in this treatment, resulting in zero plant establishment.

Serratia entomophila survival on seed

Samples of the *S. entomophila*-coated seed from trial 3 were stored at 20°C, and sampled at 2 and 4 weeks after coating. From initial counts of 5.2–6.5 x 10⁹ colony forming units/g, the bacterial

counts decreased to 2.0 – 0.88 x 10⁷ cfu/g over 4 weeks (Table 5).

DISCUSSION

Previous work on application of *S. entomophila* as a seed coating to control grass grub in carrot (Wright et al. 2005) and wheat (Young et al. 2009) has demonstrated improved seedling establishment in the presence of grass grub larvae. In the pot trials reported here, coating of ryegrass seed with *S. entomophila* significantly improved establishment of ryegrass seedlings in the presence of grass grub larvae at densities that would typically result in moderate to severe pasture damage in the field. *Serratia entomophila*-coated seed performed as well as seed dressed with imidacloprid, and in these short term pot trials there was no significant advantage in combining *S. entomophila* with imidacloprid.

The high density grub treatment used in the pot trials (equivalent to 300 larvae/m²) far exceeds the population density considered safe for sowing new pasture. At this larval density, the recommendation would normally be to carry out two or three surface workings prior to re-sowing, which would kill 40–70% of the larvae (van Toor 1996). Thus it is unlikely that *S. entomophila*-treated seed would be used under this intense level of feeding pressure in the field.

Serratia entomophila used as a seed coating provided better protection of young seedlings than application of bioshield™ granules in combination with bare seed. This result is not unexpected as within the confined space in pots

Table 3 Number of surviving healthy *Costelytra zealandica* larvae per treatment with bare, coated (without *Serratia entomophila*) or *Serratia entomophila*-coated seed at equivalent field rates of 70 or 300 larvae/m².

larvae/m ²	Trial 1		Trial 2	
	70	300	70	300
Bare seed	10 a ¹	21 a	4 a	7 a
Coated control	13 a	22 a	nt ²	nt
<i>S. entomophila</i>	4 a	6 b	0 a	1 a

¹Values followed by the same letter within a column are not significantly different at P<0.01.

²nt – not tested.

Table 4 Mean *Serratia entomophila* (cfu/g dry soil) recovered from soil in *S. entomophila* or imidacloprid treatments under *Costelytra zealandica* larval densities of 70 or 300/m² in Trials 1, 2 and 3.

larvae/m ²	Trial 1		Trial 2		Trial 3
	70	300	70	300	70
Bare seed	0 b	12.9 b	0 b	8339 a	37 ab
Coated control	0 b	0 b	nt	nt	nt
<i>S. entomophila</i> -coated seed	8208 a ¹	3151a	1366 a	16204 a	1120 ab
bioshield™	nt ²	nt	nt	nt	2311 ab
Imidacloprid	nt	nt	nt	nt	0 a
<i>S. entomophila</i> +imidacloprid	nt	nt	nt	nt	583 b

¹Values followed by the same letter within a column are not significantly different at P<0.01.

²nt – not tested.

Table 5 *Serratia entomophila* (cfu/g seed) survival at 20°C.

Treatment ¹	Initial	2 weeks	4 weeks
<i>S. entomophila</i>	6.5×10^9	5.3×10^8	8.8×10^6
<i>S. entomophila</i> +imidacloprid	5.2×10^9	2.0×10^8	2.0×10^7

¹These results were not analysed statistically as they were based on a single sample.

with no food source other than young seedlings, grass grubs would have been attracted to the germinating seed and young plant roots and brought into contact with *S. entomophila* very quickly, possibly before sufficient *S. entomophila* had been released from granules to cause grass grub infection. Enumeration of *S. entomophila* populations established in soil indicated that inoculation by seed or granule resulted in similar overall loadings of the inoculum per gram of soil, suggesting that the differing effectiveness of these two treatments in terms of seedling protection must be related to spatial distribution

of inoculum within the pots. There could be potential for development of a bait product containing *S. entomophila*, which could attract the feeding larvae away from germinating seeds.

Field trials over a longer period are needed to ascertain the efficacy of these treatments with respect to pasture production in subsequent seasons. There is potential that inoculation of the soil with *S. entomophila* by either method is likely to result in more enduring grass grub control than use of chemical seed treatments, as the disease recycles in the grass grub population providing several seasons control (van Toor 1996). The

compatibility of *S. entomophila* with imidacloprid on stored seed indicates there is potential to develop seed treatments with combinations of agents for enduring pasture protection.

There is an obvious market for *S. entomophila*-coated seed in the organic farming industry where grass grubs are as much of a threat to seedling emergence and establishment as in conventional farming. If further work confirms the results reported here in field scale trials, this application could also be utilised on non organic farms when direct drilling grass seed into sprayed out pasture or when over-sowing established pasture with top up seed in late summer and autumn into grass grub prone sites.

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REFERENCES

- Chapman RB 1984. Pasture pests. In: Scott RR ed. New Zealand pest and beneficial insects. Lincoln University College of Agriculture, Canterbury, New Zealand. Pp. 129-142.
- Jackson TA, Huger AM, Glare TR 1993. Pathology of amber disease in the New Zealand grass grub, *Costelytra zealandica* (Coleoptera: Scarabaeidae). *Journal of Invertebrate Pathology* 61: 123-130.
- O'Callaghan M, Jackson TA 1993. Isolation and enumeration of *Serratia entomophila* – a bacterial pathogen of the New Zealand grass grub, *Costelytra zealandica*. *Journal of Applied Bacteriology* 75: 307-314.
- Swaminathan J, Jackson TA 2008. Agent stabilisation and delivery process and product. Patent No. NZ 560574, PCT/NZ2008/000299, WO2009061221A2. Encoate Limited. c/- AgResearch Ltd, East Street, Ruakura Campus, Hamilton, New Zealand.
- van Toor R 1996. Grass grub. Biology, detection and integrated control in pasture. AgReference No 3. AgResearch, Hamilton, New Zealand [ISSN 1173-2768].
- Wright DA, Swaminathan J, Blaser M, Jackson TA 2005. Carrot seed coating with bacteria for seedling protection from grass grub damage. *New Zealand Plant Protection* 58: 229-233.
- Young SD, Townsend RJ, O'Callaghan M 2009. Bacterial entomopathogens improve cereal establishment in the presence of grass grub larvae. *New Zealand Plant Protection* 62: 1-6.