COMPATIBILITY OF MICROBIAL CONTROL AGENTS *SERRATIA ENTOMOPHILA* AND *BEAUVERIA BASSIANA* WITH SELECTED FERTILISERS

R.J. TOWNSEND, M. O'CALLAGHAN, V.W. JOHNSON and T.A. JACKSON

Biocontrol and Biosecurity Group, AgResearch, PO Box 60, Lincoln
Corresponding author: richard.townsend@agresearch.co.nz

ABSTRACT

Microbial control agents targeting soil-dwelling organisms need to be compatible with commonly used fertilisers. The bacterium *Serratia entomophila* is used as a microbial control agent for control of the New Zealand grass grub, *Costelytra zealandica*, and *Beauveria bassiana* is an entomopathogenic fungus used to control a range of insect pests. These biocontrol agents were formulated into granules and applied to pots together with five fertilisers commonly used on pastures throughout New Zealand. Compatibility with *S. entomophila* was also assessed in a field trial where treatments were applied by direct drilling and surface application. There appeared to be no deleterious effect from the application of the fertiliser treatments on the establishment and survival of either *S. entomophila* or *B. bassiana*. On the contrary, there was a suggestion that some nitrogenous fertilisers may lead to an increase in numbers of the bacterial biocontrol agent.

Keywords: microbial control agent, fertiliser, compatibility, *Serratia entomophila*, *Beauveria bassiana.*

INTRODUCTION

A liquid formulation of the bacterium *Serratia entomophila* (marketed as the product Invade®) has been applied to New Zealand pastures for biological control of the New Zealand grass grub, *Costelytra zealandica*, for more than a decade (Jackson et al. 1992). This product is applied using specialised application equipment. Recently, solid formulations of *S. entomophila* that allow improved distribution and application of this insect pathogen to pasture using conventional seed drills have been developed (Johnson et al. 2001). Field trials with these granular formulations are currently underway, as a prelude to registration. Another insect pathogen is also currently being tested in the field in New Zealand. Granular formulations of the insect pathogenic fungus *Beauveria bassiana* have been prepared and applied to pasture for control of the introduced pest *Sitona lepidus* (T.R. Glare, pers. comm.).

For control of soil dwelling pests, such as *Costelytra zealandica*, it is necessary that the biocontrol inoculum be placed beneath the soil surface where it can be ingested by feeding larvae. Ideally, subsurface inoculation of microbial control agents can be integrated with many routine agricultural practices, including application of fertilisers and pesticides or cultivation. Such practices may involve a risk of damage to the pathogen (Burges & Jones 1998). For example, agricultural chemicals could be toxic to the microbes and lead to a reduction in efficacy. Precautions to minimise risk include encapsulation of either the pathogen or the chemical and separation of application of the two in time.

Information on the compatibility of micro-organisms with materials such as fertilisers and chemical pesticides is scarce and where it has been studied, the tested materials have often been applied to micro-organisms during their growth in broth and on agar, or to bioassays with target insects. These forms of laboratory tests have consistently shown

little correlation with limited field results and can be misleading. Burges & Jones (1998) considered compatibility between chemicals and micro-organisms could be reliably assessed only in field tests.

For *S. entomophila* and *B. bassiana* to be used under current agricultural practises, it is advantageous if they are compatible with commonly used fertilisers. The establishment of these biocontrol agents in soil was measured in the presence of five fertilisers commonly used on pastures in New Zealand.

MATERIALS AND METHODS

Production and formulation of microbial control agents

Serratia entomophila strain 626 was originally isolated from pasture soil in Canterbury and is pathogenic to the New Zealand grass grub. This strain is held in the Microbial Control/Insect Pathogen Culture Collection, AgResearch, Lincoln. *Serratia entomophila* cells for formulation were produced as described previously (O'Callaghan et al. 2002). *Serratia entomophila* was formulated into granules using methods developed at AgResearch, Lincoln, for the stabilisation of non-spore forming bacteria (NZ Patent 506487). Briefly, broth cultures of *S. entomophila* were concentrated by centrifugation and then stabilized in a biopolymer matrix. The formulation was designed to have 25% moisture content with 0.989 water activity and appropriate loading of bacteria to achieve field application rate.

Beauveria bassiana strain B17 was isolated at AgResesearch, Lincoln, from a *Sitona lepidus* cadaver collected in Romania (29-08-00), and is stored in the Insect Pathogen Collection at Lincoln. *Beauveria bassiana* was produced by Seed & Grain Biotechnology, Wodonga, Victoria, Australia, as a fine spore powder having a spore count of $1\text{-}3x10^{10}$ spores/g. The spores were then formulated in to a granular formulation using patented technology as above. The formulation was designed to have 5-8% moisture content to avoid spore germination and carried the required spore loading to achieve field application rate.

Inoculum loading on the granules was estimated by dilution plating on Luria Bertani agar (Sambrook et al. 1989) for *S. entomophila*, or on Potato Dextrose Agar (PDA) for *B. bassiana*. Pot trials were inoculated with *S. entomophila* (batch FT402: $1.05x10^9$ colony forming units (cfu)/g granules) or *B. bassiana* (batch FT390: $4.22x10^8$ spores/g granules), while the field trial was conducted using only *S. entomophila* (batch FT393: $2.00x10^9$ cfu/g granules).

Recovery and enumeration of microbial control agents from soil

Serratia entomophila populations in soil from the pot and field trial were enumerated by soil dilution plating onto a selective medium, caprylate thallous agar (Starr et al. 1976). After incubation for 6 days at 30°C, the identity of each colony was verified as described previously (O'Callaghan & Jackson 1993). *Beauveria bassiana* populations in soil from the pot trial were enumerated by spreading dilution plating onto PDA containing cycloheximide (BDH, UK; 125 mg/l), streptomycin sulphate (Sigma; 350 mg/l) and tetracycline (Sigma; 50 mg/l) after incubation for 5 days at 23–25ºC.

Fertilisers

The fertilisers and rates used in this study are listed in Table 1. All the fertilisers were supplied by Ballance AgriNutrients Ltd., Mt.Maunganui. Fertilisers were used as per the handling instructions.

Pot trial

Wakanui silt loam free of *S. entomophila* was sieved (5 mm) and half the soil was allowed to air dry until the soil moisture content was 25% soil moisture (w/v) while the other portion was further dried to 15% moisture content. Soil was then weighed into pots; 400 g was placed in 600 ml plastic pots (approximately 10 cm diameter by 10 cm deep) and evenly compacted. Granules containing *S. entomophila* or *B. bassiana* were applied, at rates shown in Table 1, in a line across each pot simulating application along a drill row. The fertilisers listed in Table 1 were then applied along the same line across each pot. Fertiliser application rates per pot were calculated using the surface area of the pot to equate to the field rates in Table 1. A further 200 g of soil was added to the pots

and slightly compacted so that the treatments were 2.5–3 cm below the soil surface. For each biocontrol agent there were five replicates of the biocontrol agent plus fertiliser combinations, ten replicates of biocontrol agent alone and 10 replicates containing neither biocontrol agent or fertiliser; all repeated at the two soil moistures. The trial was laid out in a randomised block design. Pots were placed in a 15°C environment room in randomised replicates for one month. Pots were checked every 4–5 days and watered as required, by weight, to keep the soil moisture levels within 1–2% of the designated levels. After one month, populations of *S. entomophila* or *B. bassiana* were enumerated as described in the previous section.

TABLE 1: Treatments and application rates (kg/ha) of fertilisers and biocontrol granules used in pot and field trials.

Treatment	Rate	Characteristics
Fertiliser		
DAP	125	formulated compound fertiliser N + P
n-rich	100	concentrated nitrogen fertiliser N
hydro™ green	125	formulated compound fertiliser N + P + K
Superphosphate	250	unformulated fertiliser, P + S
Serpentine superphosphate	250	unformulated, low acidic, P + S + Mg
Biocontrol granule		
S. entomophila	30	non-spore forming bacteria on granules
B. bassiana	15	deuteromycete fungal spores on granules

Field trial

The trial was conducted at the AgResearch Research Farm at Lincoln. The pasture was two-year-old Horizon tetraploid ryegrass/Collenso red clover mix on Templeton silt loam. *Serratia entomophila* was applied to all plots, except control plots, using a Duncan 734 triple disc 13 coulter drill. The five incorporated (drilled into the soil) fertiliser treatments were applied through the same drill at the same time as *S. entomophila*. Surface fertiliser treatments were applied by hand shaker (half quantities applied at right angles) after the *S. entomophila* was drilled. There were three replicates of each treatment, arranged in a randomised block design, with a plot size of 15 m x 6 m. Treatments were applied on 7 May 2002, in fine weather. The site was overhead irrigated once with approximately 40–50 mm water 1 week after application of treatments, and an additional 25 mm rain fell before the first soil samples were taken. A further 92 mm rain fell prior to the second soil sampling. Soil samples were collected 5 and 9 weeks after application, by collecting 40 random soil cores (to a depth of 8 cm) per plot.

Statistical analysis

Numbers of colony forming units (cfu)/g soil were compared by analysis of variance.

RESULTS

Pot trial

The numbers of *S. entomophila* recovered from soil four weeks after application to the pots when applied alone averaged 3.51 and 2.62 x 10^4 cfu/g soil for the 15% and 25% soil moistures respectively. There was no significant ($P<0.05$) deleterious effect caused by the application of any of the fertiliser treatments on the establishment and survival of *S. entomophil*a and the one low figure for recovery, when the bacteria were applied with superphosphate, appears to be an aberration (Table 2). Interestingly, there was a suggestion of an increase in numbers of bacteria ($P<0.10$) in the high soil moisture treatment when the bacteria were applied with DAP or urea (n-rich). There were also no deleterious effects detectable from application of fertilisers with *B. bassiana*. Fungal spore numbers appeared more variable in the high moisture treatment. Heterogeneity in microbe populations and extraction procedures led to high LSD values. The statistical

analysis was repeated using $\log_{10}$ transformed data and also using square root data without affecting the overall outcome of the analysis.

TABLE 2: Numbers of *Serratia entomophila* 626 (no. x 10^4 cfu/g soil) and *Beauveria bassiana* B17 (no. cfu/g soil) at 15 and 25% soil moisture (w/w), four weeks after inoculation of pots with biocontrol granules and fertilisers.

	S. entomophila		*B. bassiana*	
Treatment	15%	25%	15%	25%
DAP	5.05	7.68	375	778
n-rich	3.22	7.96	176	539
hydro™ green	2.93	2.42	273	49
Superphosphate	0.06	4.79	273	618
Serpentine superphosphate	2.03	4.89	364	1014
Biocontrol granules alone	3.51	2.62	244	772
Control	n.d.[1]	n.d.	n.d.	n.d.
LSD (P<0.05)[2]	4.30	6.01	516	1051

[1]n.d.=not detected.
[2]For comparison of biocontrol granules alone with fertiliser + biocontrol treatments.

Field trial

While there was some variability in numbers (possibly due to heterogeneous soil factors), *S. entomophila* was recovered from the soil at the expected rates in all treatments. There appeared to be no deleterious effect from the application of the fertiliser treatments (applied by surface application or drilling) on the establishment and survival of *S. entomophila* in the field (Table 3). Again, there was a suggestion that DAP may have stimulated bacterial growth by the five week sampling time

TABLE 3: Numbers of *Serratia entomophila* 626 (x 10^4 cfu/g soil) five and nine weeks after application of biocontrol granules in combination with fertilisers to pasture.

	Incorporated		Surface	
Treatment	5 weeks	9 weeks	5 weeks	9 weeks
DAP	1.00	0.26	1.97	0.20
hydro™green	0.32	0.07	0.86	0.12
n-rich	0.90	0.39	0.69	0.47
Serpentine superphosphate	0.62	0.22	0.60	0.22
Superphosphate	0.70	0.33	0.71	0.18
S. entomophila alone	0.67	0.32	0.96	0.11
Control	n.d.[1]	n.d.	n.d.	n.d.
LSD (P<0.05)	1.18	0.32	2.56	0.24

[1]n.d.=not detected.

DISCUSSION

To date, very few studies have examined the compatibility of microbial control agents with chemicals or fertilisers. A search of the literature failed to reveal any studies examining compatibility of microbial control agents with fertilisers, despite the recognised need for this research (Burges & Jones 1998). During (1972) discussed the sensitivity of *Rhizobium* seed inoculants to water soluble fertilisers and extremes in acidity and alkalinity. The acidic nature of superphosphate has been shown to be lethal to *Rhizobium* in culture. This sensitivity led to recommendations that inoculated seed should not be mixed with any fertiliser before sowing.

The results of this study indicate that there was no obvious detrimental effect of fertilisers on survival and establishment of *S. entomophila* and *B. bassiana*, at least when applied separately to soil. There are indications that some of the nitrogenous fertilisers may have stimulated bacterial growth in the first month after application and this aspect warrants further study. Assessing microbial numbers within the heterogeneous soil environment is challenging, and improved extraction methods need to be developed for more precise estimates of microbial numbers and determining the balance between microbial death and growth in the soil. These preliminary tests suggest that the microbial control agents could be applied simultaneously with fertilisers, thus reducing application costs to the farmers. There is the potential for microbial control agents to be used and even formulated with chemicals to obtain a synergistic or additive action. They may be included with chemicals as components of integrated control packages, applied together if compatible, or for application convenience. The potential to develop granules containing both the microbial control agent and fertiliser has yet to be explored, and will require specialised formulation techniques which maintain the viability of the microbial inoculum in the presence of the chemical components of the fertiliser.

ACKNOWLEDGEMENTS

We are grateful to Ballance-Agri Nutrients, Mt. Maunganui for the supply of fertilisers and financial support for this study. Also, Jayanthi Swaminathan and Charlie Dillimore for preparation of bacterial inoculum, Emily Gerard and Tracy Nelson for plating out soil samples and Dave Saville for statistical analysis.

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