FORMULATION OF *SERRATIA ENTOMOPHILA* FOR BIOLOGICAL CONTROL OF GRASS GRUB

V.W. JOHNSON, J.F. PEARSON and T.A. JACKSON

AgResearch, PO Box 60, Lincoln, New Zealand

Corresponding author: von.johnson@agresearch.co.nz

ABSTRACT

Cultures of the bacterium *Serratia entomophila* (Enterobacteriaceae) have been applied as the biological control product Invade® for the control of grass grub for more than a decade. However, the use of the bacterium is limited by the specific requirements of the live microbial cultures for distribution and delivery. The cultures must be maintained under refrigeration and applied through a modified seed drill. To overcome these problems we have developed a system for stabilising the bacterium in a biopolymer matrix, which can then be incorporated into clay-based granules. The resulting formulation can be stored at ambient temperatures for extended periods and applied to the soil through conventional farm machinery. Such thermostable formulations of sensitive micro-organisms are likely to have a wide application in the biological control of pests and diseases.

Keywords: formulation, *Serratia entomophila*, biocontrol, stability, delivery.

INTRODUCTION

Grass grub (*Costelytra zealandica* (White)) (Coleoptera: Scarabaeidae) is among the most important pests of pasture in New Zealand, since feeding by larvae on roots can cause serious damage resulting in pasture deterioration. A biopesticide, containing a high density culture of the bacterium *Serratia entomophila* (Invade®), has been developed for control of grass grub. Use of Invade® requires the delivery to the soil of live bacteria that are capable of competing with other soil micro-organisms and maintaining viability long enough to initiate a cycle of disease in the target pest population. Hence delivery of the maximum possible number of high quality micro-organisms is crucial, and maintaining their viability from the laboratory to the field is a major challenge.

High density cultures of *S. entomophila* are produced *in vitro* by batch fermentation, but the liquid Invade® product must be maintained under refrigeration to avoid cell death during storage (Jackson et al. 1992). Application must be made through a modified seed drill to ensure that the bacteria do not die on the soil surface (Jackson et al. 1989). While these methods have successfully provided a means of effective delivery of bacteria to the soil, they pose a severe constraint to the use of the product, through limiting outlets and flexibility in application. To overcome these constraints we have investigated formulation of *S. entomophila* into clay-based granules. We discuss the implications of this technology for other biocontrol products where formulation continues to be a challenging and often success-limiting step (Paau 1998).

METHODS

A granular formulation was developed by incorporating *S. entomophila* cells into a biopolymer gel. This was mixed with clays and extruded to form clay pellets, and then air dried to a desired moisture level (NZ Patent Applications No. 506484 to No. 506488). To test viability during storage, the formulation was produced in six 300 g batches and stored in plastic containers in the laboratory at ambient conditions. Bacterial viability within the formulation was evaluated throughout storage by taking triplicate 1 g sub-samples which were each dissolved in 9 ml phosphate buffer. Colony forming units (cfu) were counted

New Zealand Plant Protection 54:125-127 (2001)

after dilution plating onto selective caprylate thallos agar (CTA) (O'Callaghan & Jackson 1993). Samples were taken from the containers at regular intervals for six months. The size of clay granules was varied by using different diameter granule extruding dies and the 100-granule weight of different batches was recorded. The theoretical relationship between granule size and distribution of granules within the row was calculated using an application rate of 15 kg/ha. Storage requirements and application systems required for the new granular formulation of *S. entomophila* were compared with those required for liquid Invade®.

RESULTS

Viability in storage

Bacterial viability was maintained within the granules throughout storage. After six months, cell viability was $1.8 (\pm 0.6 \text{ S.E.}) \times 10^9$ cfu/g granules; 89% of the initial viability of $2.2 (\pm 0.3 \text{ S.E.}) \times 10^9$ cfu/g granule.

Granule size

Granules were initially produced through a 1.3 mm die, which resulted in 250 granules/g and a calculated distribution of one granule per 18 mm of row. To improve distribution within the row, die size was reduced to 0.9 mm resulting in 500 granules/g and a theoretical distribution of 112 granules/row metre or one granule per 9 mm of row.

Characteristics of the new formulation

The characteristics of the traditional liquid formulation (Invade®) and the new granular formulation of *S. entomophila* are presented in Table 1. The granule formulation delivers the same number of bacteria to the soil as the liquid formulation but can be transported and stored at ambient temperatures. It does not require water for dispersal and application, although an application rate of 15 kg granules/ha is required to ensure adequate distribution through the soil.

TABLE 1: Characteristics of liquid (Invade®) and granular formulations of *Serratia entomophila* (strain 626).

Product formulation	Invade	Granular
Form	Liquid	Granule
Active ingredient	<i>S. entomophila</i> (626)	<i>S. entomophila</i> (626)
Storage/transport temperature	4°C	20°C
Concentration	4×10^{10} cfu/ml	2.6×10^9 cfu/g
Application rate/ha	1 litre	15 kg
Total bacteria/ha	4×10^{13}	4×10^{13}
Application additives/ha	100 litres water	-
Application machine	Modified seed drill	Seed drill
Drill spacing	150 mm	150 mm
Total cfu/m row	6.0×10^8	6.0×10^8
Quantity/m row	1.5 ml	106 - 112 granules
Placement	solid jet	8-9 mm apart
Granules/g	-	475 - 500
Shelf life at 20°C	LT ₅₀ <7 days	LT ₅₀ >180 days

DISCUSSION

The new granular formulation of *S. entomophila* overcomes the constraints imposed on the liquid Invade® formulation by reducing the need for refrigerated storage and transport, and specialist application machinery. As it is a dry formulation it also avoids the need for obtaining clean, non-chlorinated water for application, which is a problem at some field sites. The new formulation also allows other options for application to be

explored. The granules can be easily stored on the farm and applied through conventional seed drills without modification. This will allow application of the granules exactly when and where the farmer considers best results can be obtained. The bacteria are protected within the granule, but their release rate can be manipulated. By modifying the composition of the granule, formulations for fast and slow release of micro-organisms can be designed for various applications. For example, fast release formulations can be used for curative applications while slow release formulations could be applied at sowing to provide control throughout the season. Controlled release granular formulations are currently being tested against grass grub. This technology, however, also has scope for delivery of a wide range of biocontrol agents to the soil.

ACKNOWLEDGEMENTS

Our thanks to members of the Microbial Control Group at AgResearch, Lincoln for comment and assistance, especially C. Dillimore for technical support.

REFERENCES

- Jackson, T.A.; Broad, T.M.; O'Callaghan, M.; Martin, G. 1989: Development of a subsurface application system for *Serratia entomophila* for control of grass grub in pasture. *Proc. 5th Australasian Conf. Grassland Invert. Ecol.*: 63-68.
- Jackson, T.A.; Pearson, J.F.; O'Callaghan, M.; Mahanty, H.K.; Willocks, M. 1992: Pathogen to product - development of *Serratia entomophila* (Enterobacteriaceae) as a commercial biological control agent for the New Zealand grass grub (*Costelytra zealandica*). *In*: Jackson, T.A.; Glare, T.R. *ed.* Use of Pathogens in Scarab Pest Management. Intercept, Andover, UK. Pp. 191-198.
- O'Callaghan, M., Jackson, T.A. 1993. Isolation and enumeration of *Serratia entomophila* - a bacterial pathogen of the New Zealand grass grub, *Costelytra zealandica*. *J. Appl. Bacteriol.* 75: 307-314.
- Paau, A.S. 1998: Formulation of beneficial organisms applied to soil. *In*: Burges, H.D. *ed.* Formulation of microbial biopesticides. Kluwer Academic Publishers, Dordrecht. Pp. 235-254.