**EVALUATION OF TWO STRAINS OF *BACILLUS THURINGIENSIS*
VAR. *KUSTAKI* AGAINST EARLY INSTAR GRASS GRUB
(*COSTELYTRA ZEALANDICA*) LARVAE**

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Sustainable agricultural practices in the foreseeable future will rely on integrated pest management systems that contain a soft insecticide option. The availability of these insecticides with negligible environmental and human health hazards is limited for the control of grass grub (*Costelytra zealandica* White). However *Bacillus thuringiensis* (Bt), a naturally occurring bacterium containing a toxic protein crystal that produces no measurable residues and has no mammalian toxicity, is a possible candidate.

The pathogenicity of Bt is complex as the toxicity of the components are different for different bacterial strains and its action may differ depending on the host involved. The rapid environmental degradation of Bt, particularly in soil where microbial competition is strong, is well documented (eg. Pinnock *et al.* 1975). Encapsulation technology, in particular starch encapsulation (Dunkle and Shasha 1988), offers a useful method of protecting Bt against environmental degradation. This technology is applicable as a seed coating which can both regulate Bt dose rate and extend the duration of seedling protection.

Bt may act synergistically with insecticides, in particular stomach poisons. The Bt toxin destroys the midgut epithelium facilitating the uptake of the poison (Ker-Sang Chen *et al.* 1974). For some insecticides this may reduce the lethal dose required for grass grub control.

This study reports on an initial pot trial which screened two Bt preparations (SNZ917 and SNZ907) known to have activity against Coleoptera at a range of rates against first instar grass grub larvae.

Fifty pots (150 x 80 mm diameter) for each of seven treatments were filled with approximately 250 g of sieved soil (Taupo sandy loam). Two first instar grass grub larvae field collected from Kuratau near Taupo were introduced to each pot (100 larvae per treatment) and allowed to burrow. This approximated a larval density of 80/m². Any larvae failing to burrow were replaced. Five ryegrass seeds (cultivar Yatsyn 1, 1991 harvest, germination 91%) were placed equidistant in a 10 mm deep furrow bisecting the soil surface of the pot. The treatments (Table 1) were applied along the furrow using a Gilson P5000 micro pipette at a water rate equivalent to 100 litres/ha and the furrows filled with sieved soil. The pots each received 20 ml of water. The subsequent watering regime of 20 ml/pot was determined by soil moisture conditions in 'monitor' pots that were not part of the trial. Pots were weeded as necessary. The trial was set up on 30 March 1992 and continued for 30 days.

Germination was assessed daily until it was obvious that no further germination would occur. At day 30 post treatment, the surviving seedlings were examined for symptoms of stress or growth deformity then cut at ground level and the cuttings weighed immediately. All surviving larvae were weighed, and their developmental stage recorded.

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TABLE 1: The effects of two isolates of *Bacillus thuringiensis*, applied to ryegrass seedlings against first instar grass grub larvae, on larval survival and perennial ryegrass seedling germination and growth.

Assessments	Treatment rate:	0	SNZ 917					SNZ 907		SED
			8	16 (ppm)	32	64	1000	5000 (g/ha)		
mean days to germination		13.3	13.1	13.2	12.8	12.9	12.4	12.9	0.46	
% germination		84	95	97	96	96	94	96	3***	
ryegrass fresh weight (g)		57.6	85.0	82.2	87.5	88.8	76.6	88.2	5.3***	
% seedling survival		89	96	96	98	96	93	96	2**	
% grass grub mortality		30	38	43	38	49	39	25	5*	
% 3rd instar		91	89	85	89	82	85	85	5 NS	
mean larval weight (mg)		85.4	83.0	90.5	81.2	85.6	85.6	80.6	15.0 NS	

* significant at $P < 0.05$; ** significant at $P < 0.01$; *** significant at $P < 0.001$

There were no delays in germination attributable to the treatments but there was a significant ($P < 0.01$) improvement in the proportion of seeds that germinated in all treatments compared to the untreated control (Table 1). Either these treatments protected seedlings from grass grub feeding between germination and emergence or the treatments enhanced germination. The latter is unlikely because seed germination on filter paper moistened with water or Bt. solution was similar.

Seedling survival was significantly reduced ($P < 0.05$) by 7% in the untreated control. The treated plants showed significantly ($P < 0.01$) more growth, with a 49% increase (range 43%-54%) on the SNZ 917 treatment. Grass grub larval mortality was significantly greater ($P < 0.05$) on all the Bt. treatments with the exception of SNZ 907 at 5000 g/ha. The increase in mortality compared to the untreated control was between 7-19%. The vigorous plant response to the treatments, despite the relatively low larval mortality, may indicate that feeding deterency was operating. Insect physiological responses to Bt include irreversible paralysis of the midgut or complete cellular deterioration of the midgut epithelium, or temporary paralysis of the jaw muscles (Dulmage *et al.* 1977). The latter response may have prevented larvae feeding on the seedlings but allowed recovery when they moved away. There were no differences in subsequent development or survival of surviving grass grub.

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