EFFECT OF INFECTION BY THE BACTERIUM *SERRATIA ENTOMOPHILA* ON FEEDING AND PASTURE DAMAGE BY GRASS GRUB LARVAE

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Serratia entomophila is a naturally occurring bacterium currently being developed as a biological control agent for grass grub (*Costelytra zealandica*) (Grimont *et al* 1988). The bacteria, once ingested, cause the larvae to cease feeding and clear the gut resulting in a translucent, amber colouration. Once infected, larvae may remain active in the soil for more than a month prior to death, although during this period they are not feeding. The nil feeding response to infection and the effect of infected larvae on plant growth are described in this research note.

To examine the feeding response of the larvae to infection by the bacteria, individual larvae were confined in 13 x 13mm compartments in trays held at 20 °C. Early third instar larvae were fed every 2 days with a small piece of carrot, approx. 20 mg fresh wt. Once acclimatised to the trays and feeding, the carrot for the test group of 30 larvae was treated by impregnation with 1 x 10^6 bacteria. The dosed carrot was consumed at a similar rate to untreated carrot. After 48 hours larvae in the treated group stopped feeding while the untreated larvae continued to feed on fresh carrot. After 5-7 days the larvae developed symptoms of amber disease while untreated larvae remained healthy and continued to feed normally.

To determine the effect of infection on larvae and plant growth, 20 white clover (*Trifolium repens*) seedlings were pricked out into 2 litre pots of soil . The plants were grown in a glasshouse for 4 weeks until the pot was covered with a thick clover sward. The plants were then trimmed to a height of 2 cm and 20 healthy early third instars were added to each pot. Five pots were treated with a 20ml drench of *S. entomophila* at the rate of 4 x $10^{10}/m^2$. The remaining five pots were treated with a nutrient drench of the equivalent volume of bacterial culture medium. The pots were destructively harvested after 4 weeks and plant shoots, insect measurements and levels of disease assessed. A feeding test was carried out on larvae as described above. The results are presented in Table 1.

TABLE 1: Effect of application of *S. entomophila* on grass grub larvae and growth of white clover in grass grub infested pots.

	Treated	Untreated	SED
Number of live plants	17.8	12.4	2.7 ns
Clover fresh weight (g)	5.1	2.5	0.7 **
Number larvae surviving	12.4	15.2	1.9 ns
Mean larval weight (mg)	90	113	6 **
No. diseased	9.0 (73%)	1.4 (9%)	1.9 **
No. feeding	0.6 (4%)	13.2 (80%)	1.9 **

The results show that, despite little difference in total numbers of larvae in the treated and untreated groups, there was a considerable effect on both plant survival and regrowth. Fresh weight of the plants in treated pots was double that in the untreated. The treated larvae had a lower mean weight and 73% of the survivors showed symptoms

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of the disease. In the treated population only 4% of the surviving larvae were feeding while in the untreated population 80% of the larvae were actively feeding.

Pasture growth measurements and visual damage assessments were made on one of a series of 1987 field trials (Jackson *et al* 1988). Bacteria were applied in March 1987 by subsurface application at a rate of 2 x $10^{10}/m^2$ to 3 year old pasture plots near Windwhistle, Canterbury. Plot size was 72 m^2 with five replicates of each treatment. The grass grub population density at the time of application was over $1000/m^2$ with most larvae in the early third instar. After 8 weeks population estimates were made by taking 20 (62 x 100 mm) cores per plot. A visual estimate of proportion of pasture damaged in each plot was made by six independent observers using an aerial photograph of the site taken in May. Dry matter assessments were not possible at this time as cattle had grazed the trial site. A further assessment was made 25 weeks after application, when both grass grub populations and pasture production were estimated. The results are presented in Table 2.

TABLE 2: Effect of application of *S. entomophila* on grass grub larvae and pasture growth in field plots at Windwhistle, Canterbury.

	Treated	Untreated	SED
May assessment			
Larval density/m^2	24.3 (596)[1]	23.3 (550)	1.9 ns
% Amber disease	15	1	4.6 *
% Feeding	24	80	12.5 **
Estimated pasture damage %	19	51	7 *
September assessment			
Larval density/m^2	16 (266)	17.2 (310)	1.9 ns
Pupae + prepupae/m^2	7.9 (73)	13.7 (199)	1.7 **
% Amber disease	21	0	3.5 **
Pasture production May-Sept (kg D.M./ha)	1000	730	130 *

[1] square root transformed (Raw data mean in brackets).

The May results showed a clear difference in the percentage of damaged pasture between treated and untreated plots despite the high numbers of larvae present in both. Of the larvae in the treated plots, 15% of the population showed symptoms of amber disease, yet 76% were not feeding compared with 20% in the untreated population. A similar pattern emerged in September. The reduction of feeding was reflected in 37% more dry matter being produced in the treated plots over the May-September period compared with the untreated, while total populations were again similar. Disease levels in the treated plots remained at 21%. Similar reductions in pasture damage have been noted in other field trials. There were significantly fewer prepupae and pupae in the treated plots compared to the untreated.

The results of both the pot and field trials show that the amount of damage is not simply the result of the number of larve present but is related to the number that are feeding. Although visual symptoms of amber disease are a clear indication that feeding has ceased, many larvae may have latent effects of the disease. These larvae are, presumably, in the early stages of disease development or responding to low doses of bacteria. In both pot and field trials a high proportion of apparently healthy larvae had ceased feeding. Thus the effects of bacterial application in the short term may be underestimated by visual evaluation of larvae alone.

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