SCREENING OF GRASS GRUB POPULATIONS AND SOIL TYPES FOR THEIR INFLUENCE ON PATHOGENICITY OF THE BACTERIUM *SERRATIA ENTOMOPHILA*

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SUMMARY

Grass grub (*Costelytra zealandica*) larvae from 27 sites throughout New Zealand were screened for susceptibility to the pathogenic bacterium, *Serratia entomophila* in an insectary pot trial. Soils from the same sites, representing eight groups, were also tested to determine their influence on pathogenicity. Bacterial application increased disease incidence and larval mortality regardless of grass grub or soil origin. In a second experiment infection levels and mortality in two yellow-brown pumice soils and a Kowhai loam reference soil were similar. Use of *S. entomophila* as a biological control agent for grass grub is unlikely to be limited by intrinsic differences in soil type or grass grub populations.

INTRODUCTION

The naturally occurring bacterium, *Serratia entomophila*, which causes amber disease, is currently under development as an inundative biological agent for control of the grass grub, *Costelytra zealandica* (Jackson 1989). While amber diseased larvae and the bacterium have been found in many areas of New Zealand (O'Callaghan *et al* 1989), the efficacy of the bacteria in a range of situations needs to be examined before its widespread use can be adopted. The purpose of this study was to look for variations in susceptibility to amber disease of larvae collected from major grass grub areas. Soil was also collected from the same sites and the influence of soil type on infectivity examined.

METHOD

Two experiments were carried out. In the first, soil and larvae were collected from 27 sites in Canterbury, Hawkes Bay, Taranaki, Wairarapa and Waikato. Samples were taken when larvae were in the early third instar, with treatments initiated in autumn/winter 1988. The soil from each site was sieved and added to six 0.5 litre pots. Grass and clover seedlings were transplanted into the pots. An additional six pots per site were prepared for comparison with a standard soil (Kowhai loam, Canterbury). Healthy larvae from each site were weighed and added to pots of soil taken from the same site and also to the standard soil. Three larvae were added to each pot. Two pots of soil from each site were left untreated. Bacteria were applied at two rates equivalent to 4×10^{14} and 40×10^{14} bacteria/ha. Each rate was applied to two pots of soil from each site. One pot from each pair was destructively harvested after 3 weeks and the remaining pot after 6 weeks. At harvest, surviving larvae were counted, weighed and assessed for visual symptoms of amber disease (Jackson 1988). The pot trial was carried out in an open sided insectary at Lincoln and pot soil temperatures, measured at 3 pm, fell from 17-6°C during the experiment. Pots were regularly watered throughout the experiment.

Initial analysis was carried out using analysis of variance. Further comparisons were made after grouping according to region or district and soil type. The soils used in the experiment could be placed into eight groups: Balmoral silt loams; Lismore silt loams; Eyre sand and silt loams; Takapau sandy and silt loams; Castlecliff sand, Tirau sandy loam and Waihou sandy loam; Egmont yellow-brown loam, Pahaoa yellow-brown earth; Laharic soil; and the reference Kowhai loam.

As the North Island yellow-brown pumice soils were not screened in the first experiment a second experiment was carried out using these soils collected from two sites on the volcanic plateau and the standard Kowhai loam for comparison. Healthy third instar grass grub larvae collected from Canterbury were used in this experiment.

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There were five replicates of each treatment and bacteria were applied at a rate equivalent to 4×10^{14} /ha. The experiment was initiated in January 1989 and assessed with a single destructive harvest 12 weeks after application. The data were analysed by analysis of variance.

RESULTS

Experiment 1.

Application of bacteria caused a highly significant ($P < 0.001$) increase in disease and mortality (i.e., percent mortality plus percent disease) among larvae in the treated soil (47%) compared to larvae in the untreated soil which had a mean level of 2%. When sites were grouped according to geographical area (Figure 1) a significant ($P < 0.05$) increase in disease and mortality was recorded in the treated larvae from each area. The effect on North Island larvae was significantly ($P < 0.05$) greater than that for the South Island larvae but the response between individual regions could not be differentiated.

Fig. 1: Effect of *S. entomophila* on grass grub larvae from different regions. N = number of sites within each region.

The effects of bacterial application on seven of the major soil groups used in the experiment are shown in Figure 2. The bacteria caused a highly significant ($P < 0.01$) increase in mortality and disease in all soil types. The increase in disease and mortality with bacterial rate differed between soil types with a significantly ($P < 0.05$) lower increase with bacterial rate in the Eyre and Lismore soils compared to the standard Kowhai loam. A high level of disease (58%) and mortality (42%) occurred in the Laharic soil type (Taranaki). As this was from one site only, it is not represented in Figure 2.

Experiment 2

The results from this experiment are presented in Table 1. There was a significant ($P < 0.05$) effect of bacteria on survival of the larvae and a highly significant ($P < 0.01$) effect on health of the larvae in each of the three soils. There was no significant effect of soil type on mortality or disease.

Fig. 2: Effect of *S. entomophila* on grass grub larvae in different soil types. N = number of trials with each soil type.

TABLE 1: The effect of bacterial application on survival of grass grub larvae in yellow-brown pumice soils and a standard Kowhai soil 12 weeks after treatment with *S. entomophila*.

Soil type	% Survival (% healthy)		
	Untreated	<i>S. entomophila</i>	Unt. vs <i>S. ento.</i>
YB pumice 1	64 ¹ (52)	28 (8)	* (**)
YB pumice 2	60 (60)	24 (8)	* (**)
Kowhai	48 (48)	16 (8)	* (**)

¹For comparison of means SEM = $p(1-p)/25$, where $p = \%/100$.

DISCUSSION

The results indicate that disease was induced in larvae from all regions and in all soil types following application of bacteria and that differences in response of larvae from different regions or effects of soils were small in comparison to the main effect of bacterial application. North Island larvae were more affected by the bacteria but the differences were small, 61% disease and mortality among the treated larvae compared with 55% in the South Island larvae.

The effects of soil type also showed some variation. Least effect of the bacteria was recorded in the Eyre and Lismore soils but this may not have been due to soil factors. The Burnham larvae tested in the Eyre soil, which showed only 37% disease and mortality, may have been more mature at treatment than others, affecting their susceptibility (Jackson *et al* 1986). The average weight of the Burnham larvae at treatment was 186 mg cf. the experiment mean of 111 mg. In the Lismore soils mean disease and mortality was 64% but the level of disease and mortality in the untreated soils was also high at 31%. In three of the Lismore soils, field collected larvae developed disease symptoms in the untreated pots as well as the treated despite having been classified as healthy at the initiation of the experiment. The high level of disease in the untreated larvae reduced the apparent effect of the applied bacteria in this soil type. The highest level of disease and mortality was recorded from the Laharic soil, but as only

one site of this soil type was included in the experiment conclusions as to the performance of the bacteria in this soil type cannot be made.

The second experiment ran for a longer period than the first allowing the disease to run its course with the principle assessment made on the basis of mortality rather than disease expression. Similar results were obtained for the two pumice and the Kowhai soil samples. Survival in the treated pots was less than half that in the untreated and more than two thirds of the treated survivors showed disease symptoms.

Results of this preliminary study indicate that there is no major geographical variation in susceptibility of larvae or effect of soil type that is likely to reduce the efficacy of *S. entomophila* when used as a biological control agent for grass grub.

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