DISTRIBUTION OF THE BACTERIUM *SERRATIA ENTOMOPHILA* IN THE SOIL WHEN APPLIED IN HIGH WATER VOLUMES

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The bacterium *Serratia entomophila* has been developed as a biological control agent for the control of grass grub (*Costelytra zealandica*) and a subsurface application method has been developed to enhance its survival (Jackson *et al.* 1988). However, the cost of subsurface application has presented a barrier to more widespread use of this bacterium and application through overhead irrigation systems has the potential to overcome this. Jackson *et al.* (1986) reported infection of grass grub after application of *S. entomophila* through watering cans at a water rate of 0.8 litres/m² followed by overhead irrigation equivalent to 2.5 mm of rainfall. More recent trials (Garnham, unpublished) applying bacteria through overhead irrigation have had variable results and low recovery of bacteria from the soil. This trial was designed to determine the effect of ground cover and high volume application on the placement and recovery of bacteria.

Sixteen plots of pasture (4 m²) at Templeton Research Farm were treated with *S. entomophila* (strain 154) applied at the equivalent of 4×10^{15} bacteria/ha (100 times commercial rate) by watering can. The bacterium was applied at two water rates of 15 and 30 litres/m², equivalent to 15 mm and 30 mm of irrigation. The paddock was border dyked on a Templeton deep silt loam and had been in pasture for more than 8 years. Prior to application the vegetation was removed at ground level with hand held clippers on eight plots (four at each water rate), taking care not to disturb the soil surface. The pasture on the remaining plots was cut to 3–4 cm height. Soil moisture was determined gravimetrically from samples taken in 5 cm increments through the profile to a depth of 15 cm.

Within one hour of application a litter sample was taken from the surface of the unclipped plots. Twenty four hours after application all plots were sampled to determine the depth of both water and bacterial movement in the soil. Four samples were taken to determine bacterial movement; 0–1, 1–5, 5–10 cm and 10–15 cm. A 10.2 cm diameter corer was used to mark the ground from which both a litter and soil crust sample were taken. The 0–5 cm sample was taken with the 10.2 cm diameter corer and the 5–10 and 10–15 cm sample was taken from within this core using a 2.4 cm diameter corer to avoid contamination of individual cores. Ten samples were taken per plot and the depth increments bulked. Soil and litter samples were dilution plated on caprylate thallous agar, selective for *Serratia* spp. and incubated for 4 days at 30°C. Representative colonies from each sample were transferred onto test agars to confirm identification (O'Callaghan and Jackson 1993). Data for bacterial counts were log transformed.

Vegetation did not affect distribution of bacteria and there were no significant interactions between vegetation and water rate. Soil bacterial numbers from the two vegetation treatments have therefore been pooled. When bacterial numbers were added for each sector of the profile, recovery of applied bacteria was 54% and 41% respectively for the 15 mm and 30 mm water rates. The distribution of recovered bacteria through the profile is shown in Table 1. In both cases a high proportion (>40%) of the bacteria were recovered from the top 5 cm.

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TABLE 1: Distribution of *Serratia entomophila* through the soil profile under two irrigation regimes as a percent of the total number of bacteria recovered.

Depth (cm)	Bacteria as a % of total <i>S. entomophila</i> recovered	
	Water rate	
	15 mm	30 mm
Litter	7.4	3.3
0 - 1	13.5	17.6
1 - 5	29.8	33.7
5 - 10	26.7	28.4
10 - 15	22.6	17.0

Figure 1 shows the number of *S. entomophila* at various depths in the soil profile 24 hours after application at both water rates. At both water rates bacterial numbers declined with depth ($P < 0.01$). Bacterial numbers at each depth for the 30 mm rate were consistently less than the 15 mm rate by an average of 1.5×10^5 bacteria.

Figure 1: Effect of two water rates on the distribution of bacteria in the soil profile

Application in high water volumes placed more than 40% of the recovered bacteria in the top 5 cm of the soil where the majority of the grass grub population are feeding (Jackson *et al.* 1988). While the bacteria were applied at a rate well in excess of that used commercially, the distribution in the profile should not be altered by application at a lower rate. The recovery of between 40 - 55% of applied bacteria 24 h after application is within the range shown by O'Callaghan and Jackson (1993) for bacteria applied by direct drilling. However, there were significant losses from the system and these losses were greater at the higher water rate.

High initial soil moisture may also have contributed to the loss of bacteria. The field capacity of Templeton silt loam is 33.8 (% v/v) (McLaren and Cameron, 1990) i.e. the point at which there is rapid drainage as the macropores are full of water. At the time of application the moisture content of the top 5 cm was about field capacity. The distribution of the bacteria suggests that biphasic flow occurred whereby water moved both through the soil matrix and through the macropores (Smith *et al.* 1985).

Of the bacteria which were applied to the soil surface most were held in the top 0 - 5 centimetres and not moved significantly by subsequent irrigation. A proportion of bacteria entered the macropores and moved rapidly through the profile to below 15 cm. The extent of bacterial movement is dependant on the soil moisture at time of application so that with increasing soil moisture and water rates more bacteria will tend to be moved through the profile in the macropores.

The period of time over which a bacterial suspension is applied to the soil has also been shown to have a large effect on bacterial transport through soil columns (Smith *et al.* 1985). Where low water rates are applied over long periods macropore flow is presumed to be minimal. However, the rate of application in this experiment was approximately 30 or 60 mm/h resulting in significant macropore flow and subsequent loss of bacteria.

Application of bacteria at water rates used in this experiment placed a high number of bacteria in the target zone for grass grub control. However, placement will be influenced by soil moisture at the time of application and rate of application and more knowledge of these factors is required before this method can be recommended.

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