EVALUATION OF METHODS FOR APPLYING *SERRATIA ENTOMOPHILA* TO PASTURE

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SUMMARY

Conventional boom spray, jet stream and turf treatment application methods were evaluated for their success in placement of the bacterium *Serratia entomophila* into pasture. Turf treatment deposited more bacteria into the target band, 20-50 mm, and throughout the soil profile than surface treatments, jet stream and spray. Turf-applied bacteria survived in higher numbers than jet stream and sprayed bacteria for the 30 days of the trial. The establishment of bacteria in soil was enhanced when higher volumes of water were used in jet stream and boom spray application methods.

INTRODUCTION

The method of application of bacterial pathogens for insect pest control is one of the most important, yet frequently neglected, factors involved in their field use (Falcon 1971). Preparations are commonly applied with equipment developed for chemical insecticides often without attention to the special requirements for bacterial establishment on the target site. Most work on application of insect pathogens has centred on foliar applications of bacteria by aerial sprays or dusts. There are few examples of application to soil.

The bacterium *S. entomophila* has good potential for development as a biological insecticide for grass grub *Costelytra zealandica* (White) (Jackson and Stucki 1986). In early field trials, *S. entomophila* was applied in suspension as a soil drench through a watering can (Jackson *et al* 1986) but this method was clearly inappropriate for field scale use because of the high volume of water involved. The bacteria must be deposited into the soil where they can be ingested by second and third instar larvae. Placement beneath the surface should also protect the bacteria from ultraviolet light and desiccation.

Trought (1979) reviewed the use of specialised machinery for application of insecticides for grass grub control. Turf treatment and jet squirt machines were developed to improve the efficacy of relatively less persistent organophosphorus insecticides after the prohibition of DDT. Trought and Wood (1970) found the jet squirt method was less reliable when used on pastures with a relatively dense cover of grasses and clover.

This paper describes preliminary field trials to compare turf treatment, jet stream and spraying as application methods for *S. entomophila*. Their success in depositing bacteria into the soil profile is discussed.

MATERIALS AND METHODS

Culture method

The bacterium used was *S. entomophila* strain A2UC6 originally isolated from a diseased grass grub larva collected from pasture at Fairton, Canterbury. Bacteria required for the field trials were produced by culturing in 10 litre batches of nutrient broth. A fermenter multiple drive assembly (New Brunswick Scientific Co., New Jersey) enabled continuous aeration and stirring during the 24 h incubation at 30 °C. Cultures contained approximately 2 x 10^9 bacteria/ml and were stored at 4 °C until application.

Field application

A field trial was carried out on pasture at the Templeton Agricultural Research Station, in November and December 1986. The soil was a Waimakariri sandy loam and contained no *S. entomophila*. Applications were made on December 5, 10 and 17. On each date bacteria were applied by conventional spray, jet stream and turf treatment. Four rates of water were compared within each plot: 250, 500, 1000 and 2000 litres/ha. Bacteria were applied at a rate of approximately 1.7 x 10^{14}/ha. The trial design was a split plot with two replicates of method x time of application on main plots and rates of water on sub-plots (2.5 x 20 m). Main plots were separated by a 2 m wide strip.

A Duncan 734 triple disc drill with discs spaced at 300 mm centres was modified for bacterial application. For the spray treatment, a boom with eight Teejet 6510 flat fan spray nozzles at 300 mm centres was mounted 500 mm above the ground on the rear of the drill. For the jet stream and turf treatments, solid stream nozzles, 2 mm in diameter, were mounted individually between the rear pairs of discs on the triple disc drill. While applying the jet stream the cutting dics were held above ground level but for the turf treatment they were driven into the pasture to a depth of approximately 40 mm. All systems were supplied by a ground driven "John Blue" variable stroke piston pump operating at approximately 172 kPa.

Drill/treatment rows were marked with wire pegs to enable sampling later. Weather conditions at each time of application were noted. The first and second applications were applied in dry conditions with an average soil moisture of 25%. Overhead irrigation was used before and after the third application to give an average soil moisture of 31%, approximately field capacity.

Sampling and enumeration of *Serratia*

All plots were sampled 7 and 30 days after application. Four intact cores (25 x 150 mm) were taken from along the marked treatment rows in each sub-plot. The zone between 20 and 50 mm deep was considered to be the target stratum, as the majority of larvae are present in the top 50 mm (Stewart and Stockdill 1972). Soil from this stratum was cut from the cores and pooled for each sub-plot. To determine the distribution of bacteria in the soil profile, cores taken from 500 litres/ha treatment were processed in four sections (0-20, 20-50, 50-100, 100-150 mm from surface). Herbage from the spray treatment was sampled for bacteria after 7 and 30 days.

Soil samples were dilution plated on an agar selective for *Serratia* spp. and incubated for 4 days at 30°C. The colonies were counted and identified by further biochemical tests (O'Callaghan, unpublished).

Analysis

It was not possible to directly compare numbers of bacteria from cores in sprayed plots with the other two treatments because spraying distributed the bacteria uniformly while jet stream and turf treatment deposited bacteria in concentrated bands approximately 2.5 cm wide and 30 cm apart. Therefore numbers of bacteria recovered from turf and jet treatments were multiplied by 2.5/30, a conservative adjustment in the light of the results. All counts were log transformed to assist homogeneity of variance and one outlying value was removed before analysis of variance.

TABLE 1: Bacterial numbers (log_{10}/g oven dry soil) remaining in soil 7 and 30 days after application by three methods, meaned over date of application.

Days after application	Application Method			
	Turf	Jet	Spray	LSD (5%)
7	3.99	1.39	1.40	0.88
30	2.44	0.91	0.85	0.66

RESULTS

Mean log numbers of bacteria remaining in the soil 7 and 30 days after application by the three methods are shown in Table 1. The number of *S. entomophila* recovered from turf treated soil was significantly greater ($P<0.01$) than the numbers recovered from jet stream and spray treatments. Despite the variation in soil moisture levels

(25-31%) the date of application had no significant effect on the number of bacteria persisting in the soil.

Increased water volume at application significantly ($P<0.01$) improved the deposition of bacteria into the soil, particularly in the jet stream and spray treatments (Table 2). Turf treatment gave more consistent results at all rates. The effect of water rate on establishment of the bacteria in soil was most obvious when the soil was dry (Dec. 5 and 10).

TABLE 2: Effect of interaction of date or method of application with water rate on bacterial numbers (log_{10}/g oven dry soil) in the soil 30 days after application.

	Water rate (litres/ha)			
	250	500	1000	2000
Date of Application				
Dec 5	0.80	1.48	1.79	1.96
Dec 10	0.86	0.34	1.44	2.07
Dec 17	1.28	1.09	1.85	1.84
Method of Application				
Turf	2.23	1.92	2.75	2.84
Jet	0.39	0.46	1.05	1.77
Spray	0.32	0.53	1.29	1.26
S.E. of differences within row 0.35				
Mean	0.98	0.97	1.69	1.96
S.E. of difference 0.20				

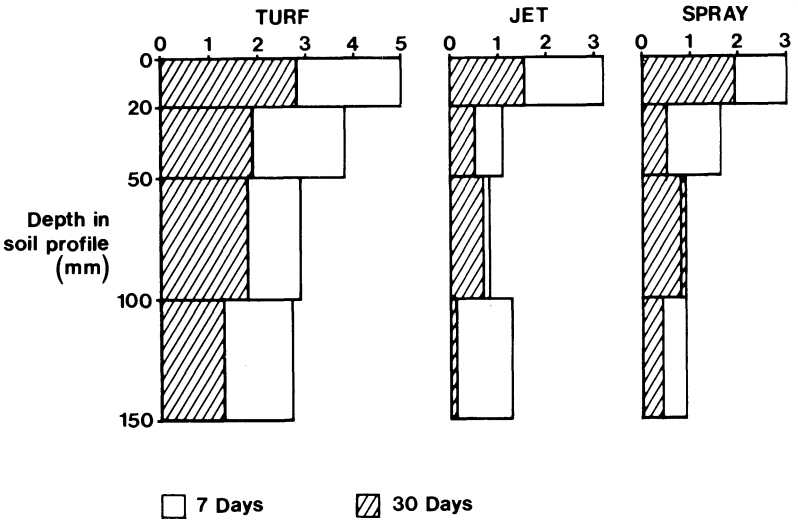

Fig. 1: Bacterial numbers (log_{10}/g oven dry soil) remaining at various depths in the soil profile after application by three methods.

Figure 1 shows the numbers of *S. entomophila* at various depths in the soil profile 7 and 30 days after turf, jet or spray application at a water rate of 500 litres/ha. Turf treatment deposited more bacteria into the soil profile ($P<0.01$) than the other treatments.

DISCUSSION

Penetration of the bacteria into the turf mat is conducive to survival of the bacteria. The spray and jet stream treatments left most bacteria on the herbage and in the 0-20 mm layer. Bacteria remaining above the surface are subject to desiccation and breakdown by ultra violet light. In this trial only a trace of bacteria remained alive on the herbage 7 days after the spray treatment.

The best bacterial deposition and survival followed the turf treatment as the bacteria were introduced into the soil through the surface turf. The bacteria survived in the soil at high levels for at least 1 month which would allow sufficient time for ingestion and infection of actively feeding grass grub larvae. The position of larvae in the soil profile varies with stage of development, availability of food and environmental factors, such as soil moisture. These factors may require consideration when selecting depth and spacing of disc cuts. Applications made in late summer and autumn could be less deep, perhaps 10 mm, since larvae are likely to be feeding close to the surface.

The deposition of bacteria in the turf treatment was affected less by low water rate or dry soil conditions than that in the spray and jet stream treatments. Thus, it seems likely that turf treatment would allow application over a wider range of conditions than the other two methods. The advantages of the turf treatment suggest that construction of a specialised turf treatment bacterial applicator would be of value if the bacteria are to be used for grass grub control.

Field trials on grass grub infested pasture are currently being conducted to further compare the three application methods and to determine the effects of timing and spacing of application on disease incidence.

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