USE OF THE BACTERIUM *SERRATIA ENTOMOPHILA* FOR CONTROL OF GRASS GRUB IN LAWNS

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ABSTRACT

The bacterium *Serratia entomophila*, cause of amber disease in grass grub (*Costelytra zealandica*), was tested to determine its potential as a biocontrol agent for protection of lawn turf from grass grub attack. Pots containing lawn turf and introduced grass grub were treated with bacteria applied by surface application or by soaking roots in bacterial suspension before planting. The bacterial treatments maintained vigour of the turf, reduced grass grub populations and resulted in amber disease in 79 % of the remaining larvae. Four home lawns, naturally infested with grass grub, were treated with bacteria in a water drench. Sampling one month after treatment confirmed establishment of the applied strain, *S. entomophila* 154, and indicated an average of 46% amber disease among grass grub larvae in the treated lawns. These trials demonstrated that *S. entomophila* can establish, infect grass grub larvae and reduce damage when applied to lawn turf.

Keywords: Grass grub, *Costelytra zealandica*, *Serratia entomophila*, amber disease, lawn turf.

INTRODUCTION

Grass grub (*Costelytra zealandica* White) is one of New Zealand's most important pasture pests (Garnham and Barlow 1993), and is also recognised by homeowners and urban park managers as a cause of damage to amenity and recreational turf. In the past, persistent chemicals were the main defence against grass grub and other garden pests and little thought was given to their residues around the home. With increasing awareness of chemical residues, persistent organochlorine insecticides have been replaced by more transient chemicals, but toxicity and residues remain important issues.

Over the past decade, biological control has been emphasised as the preferred option for pasture pest management and some of the technologies developed may also be appropriate for use in the urban setting. The bacterium *Serratia entomophila*, marketed as the commercial biocontrol product *Invade*, has been used for grass grub control in pastures for almost a decade. For protection of pastures, a bacterial suspension is injected into the soil using a modified seed drill and the applied bacteria establish a cycle of amber disease in the treated grass grub population, reducing both the pest population and level of pasture damage (Jackson *et al.* 1992). Use of a large seed drill, however, is not appropriate for urban lawns and use of a simple drench application was investigated. Two experiments were carried out to determine whether the bacterium could be used to reduce the grass grub problem in lawns. The first was a small outdoor pot trial to investigate whether the application of bacteria could prevent damage to lawn turf grasses. In the second experiment, bacteria were applied to natural populations of grass grub in home lawns.

METHODS

Bacteria

The commercially available product *Invade*, a bacterial suspension containing $>4 \times 10^{13}$ *S. entomophila* (strain 154) per litre was used in the lawn trials. *Invade* was manufactured by Industrial Research Ltd., Lower Hutt on January 21st 1999 and stored at 4°C until use.

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Pot trial

Sections of PVC culvert pipe (300 mm internal diameter, 250 mm in length) were placed vertically on a bed of sand. Tubes were then filled with sieved Templeton silt loam soil to 70-80 mm below the rim. Thirty-eight, healthy, early 3rd instar grass grub larvae (average weight 0.158 g), collected from the AgResearch Lincoln farm, were added to each tube and allowed to burrow into the soil. A circle of 1-year-old lawn turf, composed of a browntop/red fescue mix (*Agrotis tenuis* Sibth./*Festuca rubra* L.) (Prebble's Turf World, Christchurch), approximately 50 mm thick with a 25% moisture content, was cut accurately to fit in the top of the tube. Bacteria in the drench application were applied by watering can with 0.1 ml of bacterial suspension in 1 litre of water (an application rate of approximately 6×10^{10} bacteria/m²) and washed into the surface with a second litre of water. For the soak treatment, turf was placed in a 1% bacterial suspension for five minutes resulting in the absorption of 600 ml of suspension per turf producing an application rate of 2×10^{12} bacteria/m² followed by a surface drench with 1 litre of water. Control sections of turf were drenched with 2 litres of water (equivalent to 28 mm rain). The trial layout was completely randomised with 8 replicates of each treatment. Treatments were applied on March 22nd 1999, in warm conditions, and the tubes were subsequently watered as needed to maintain the soil moisture at approximately 20 %, sufficient for turf establishment. Turf vigour was visually assessed after seven weeks by scoring each plot for growth (scale 1-5, where 1 = poor vigour and 5 = vigorous growth) by eight independent observers. Following this assessment, four replicates from each treatment were destructively sampled for estimation of level of disease and grass grub survival. Larvae showing a clear gut typical of amber disease were classified as diseased (Jackson *et al.* 1993). Disease was reassessed after a 3 day feeding period to provide a more accurate estimate of percentage infected (Townsend 1998). Data were statistically analysed using analysis of variance.

Application to lawns

Areas of lawn showing evidence of grass grub attack in the suburb of Halswell, Christchurch, were selected for application of Invade in 1998 and 1999. Two lawns were treated in each year. The lawns showed typical patchy damage with larval density in the damage patches estimated at $>200/\text{m}^2$. Larvae were collected from the damaged lawns at each site prior to application and examined for visual symptoms of disease. *S. entomophila* was applied (Table 2) as a soil drench through a watering can to pre-watered damaged lawns ranging in size from 50 to 275m². The application rate (4×10^{10} bacteria/m²) was equivalent to 10 times the normal commercial application rate to pasture. A light watering of 5 mm followed application. At each site, a second similar area of lawn was selected as an untreated comparison. Approximately one month after treatment, each site was sampled and 20-30 larvae were collected and taken to the laboratory for disease assessment. At least five soil samples of approximately 100 g were taken from treated and untreated areas to a depth of 70-80 mm and mixed together as a pooled site sample. *Serratia* spp. bacteria from these samples were quantified and identified using the method of O'Callaghan and Jackson (1993a) and further phage typed to enable identification of the applied strain (O'Callaghan and Jackson 1993b).

RESULTS

Pot trial

After seven weeks, clear visual differences were evident between treatments. Both bacterial treatments were producing vigorous turf with assessment scores significantly ($P < 0.05$) higher than the untreated turf (Table 1) which showed less growth and yellowing of the tillers. Turf in the untreated plots had poor root growth compared to turf treated with bacteria, which had extensive nodal roots extending down to the sand bed at 220 mm depth. Grass grub numbers were significantly ($P < 0.05$) reduced following bacterial treatment (Table 1) and a mean of 79 % grass grub in the *S. entomophila* treated plots showed symptoms of disease compared with 17 % apparent disease in larvae from the untreated plots. While the soak treatment resulted in a significant ($P < 0.05$) increase in turf vigour above the drench treatment, higher levels of disease were recorded from the drench treatment.

TABLE 1: Effect of *S. entomophila* applied by soak and drench treatments on turf vigour, grass grub numbers and disease levels in a pot trial 7 weeks from application.

Treatment	Mean turf vigour score ¹	Grass grub larvae/ m ²	% disease in grass grub larvae	Healthy grass grub/m ²
<i>S. entomophila</i> – soak	4.0	283	72	80
<i>S. entomophila</i> – drench	2.9	274	85	43
Untreated	1.8	375	17	316
LSD (5 %)	0.6	69	13	48

¹ Vigour score 1 = poor vigour; 5 = vigorous growth

Application to lawns

At each site the drench treatment resulted in colonisation of the soil with a mean density of 2×10^4 bacteria/g soil of the applied strain *S. entomophila* 154 recovered one month after application (Table 2). *S. entomophila* (7.4×10^3 /g) was recovered from the untreated lawn area of Site 3, while no *S. entomophila* was detected at other sites. A mean of 46 % of larvae showing symptoms of amber disease was recovered from the treated sites. The lowest percentage disease was recorded at site 4 where there was a high proportion (33 %) of less susceptible early second instar larvae (3rd instars 39 % diseased). No amber disease had been observed among larvae from the treated sites prior to application and none of the untreated sites showed amber disease although protozoan diseases and milky disease were evident in both treated and untreated areas at site 4. The visual signs of damage, thin turf and yellowing grass tillers, were rapidly reduced on the treated sites, while on the untreated check sites, grass grub damage remained evident.

TABLE 2: Bacterial numbers per gram dry soil and percentage diseased grass grub larvae, one month after application of *S. entomophila* to four lawns in Christchurch.

Lawn	Date of application	<i>S. entomophila</i> /g soil	% diseased grass grub recovered
1	28 April 1998	1.5×10^4	70
2	28 April 1998	9.3×10^3	41
3	16 February 1999	5.3×10^4	48
4	28 March 1999	2.5×10^3	26

DISCUSSION

Product safety, reliability and ease of use are priorities for pest control in the home environment. Biological controls, which are specific to the target pest, are ideal for meeting the first of these criteria but have not always provided consistent control following simple application procedures (Grewal 1998). In these experiments, we have applied bacteria to the soil using a simple soil drench and obtained high levels of disease among the treated grass grub larvae in all trials. Amber disease in grass grub larvae produces a rapid cessation of feeding followed by a long, chronic amber stage eventually leading to death (Jackson 1994). This cessation of feeding explains the increase in turf vigour after application and the decline in visible damage in the treated turf.

The high levels of disease obtained in these experiments suggest that the bacteria were well distributed through the soil. When bacteria are applied in drill rows, as in commercial pasture applications, disease levels are usually lower over the first month as infection is confined to larvae coming into contact with bacteria in the drill row. The

drench treatment appeared adequate for establishment of the bacteria in the turf as bacterial density in the lawn soil one month after treatment was within 1 log of the theoretical maximum density at application ($4 \times 10^6/\text{g}$) (O'Callaghan 1998). The increased vigour of turf after soaking in the bacterial suspension was interesting and may have an application in preparation of new lawns with pre-cut turf.

Infection by amber disease is common among grass grub in New Zealand pastures. It is surprising, therefore, that no amber diseased grass grub larvae were recovered from the untreated lawns in this trial and the bacterium *S. entomophila* was only recovered from one site. Where naturally occurring grass grub pathogenic bacteria are lacking, we have demonstrated that fermenter-produced *S. entomophila* can be applied and will establish in lawn turf to produce amber disease infection and prevent damage by grass grub. These experiments indicate that *S. entomophila* has a good potential as a tool for grass grub management in lawn turf.

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