ADULT GRASS GRUB DISPERSAL OF *SERRATIA ENTOMOPHILA*

M. O'CALLAGHAN and T.A. JACKSON

AgResearch, PO Box 60, Lincoln, Canterbury

Keywords: *Costelytra zealandica*, amber disease, dispersal, biological control

Grass grub (*Costelytra zealandica* White) larvae infected with amber disease, caused by the bacterial pathogens *Serratia entomophila* and *S. proteamaculans*, (Enterobacteriaceae) are frequently found in established pastures but are seldom found in young pastures (O'Callaghan *et al.* 1989). Cultivation causes death of the larvae and presumably a consequent decline in bacterial numbers leading to low disease levels. The source of inoculum by which the disease re-establishes and the manner in which the disease builds up and spreads in new pastures is unknown. At sites where *S. entomophila* had been applied for grass grub control, the results of Jackson *et al.* (1988) suggested that there was some spread of disease from the treated to the untreated plots following the year of application. Further work has shown that there is little spread of the bacteria from treated plots within the season of application (O'Callaghan unpubl.). In the study reported here, the possible role of adult grass grub in the spread and transmission of amber disease was investigated.

In November 1992, samples of soil and larval, pupal and adult grass grubs were collected from a field trial site in Canterbury where *S. entomophila* had been applied the previous March. Samples were taken from five replicate plots and pooled into treated and untreated samples prior to extraction and enumeration of *S. entomophila*. Groups of 10 larvae, pupae or adults were macerated in sterile phosphate buffer before dilution plating on a *Serratia* specific medium, caprylate thallous agar (Starr *et al.* 1976). Bacterial colonies were identified by transfer to a series of differential agars (O'Callaghan and Jackson 1993a). Numbers of the applied strain were differentiated from total *S. entomophila* by phage typing (O'Callaghan and Jackson 1993b). Bacteria in soil were enumerated as described previously (O'Callaghan and Jackson 1993a).

To determine the proportion of adults carrying *S. entomophila*, bacteria from 18 individual adults collected from treated plots were enumerated and identified as described above.

To simulate the death and decay of beetles in the field, 24 adults collected from treated plots were placed in pairs in twelve small plastic tubes (25 cc) containing *Serratia* spp. free soil and were incubated at 15°C. Twelve tubes containing pairs of adults from untreated plots were also set up. Adult cadavers and soils were sampled separately after 4 months incubation and *S. entomophila* enumerated.

Serratia entomophila was found in the soil from the treated plots (5.0×10^4 bacteria/g soil) but not in untreated plots. Larvae, pupae and adults from the treated plots contained 1.2×10^7 , 3.9×10^7 and 2.2×10^8 *S. entomophila*/insect respectively. *S. entomophila* was not detected in insects from the untreated plots. *S. entomophila* could be detected in only 3/18 adults when beetles were macerated individually but high numbers of bacteria were found in each of these adults (1.3 , 9.7 and 4.0×10^7 *S. entomophila*/adult). Phage typing showed that the extracted bacteria corresponded to the applied strain. *S. entomophila* was recovered more frequently when adults had been incubated in soil for 4 months. Where beetles had been collected from treated plots, 11/12 samples contained *S. entomophila*. Numbers of bacteria ranged from 1×10^4 to 3×10^6 *S. entomophila*/adult (mean of 9.0×10^5). *Serratia entomophila* were also found in 10/12 of the soil samples in which the adults from treated plots had been incubated (mean of 8.5×10^3 /g soil). Isolates recovered from beetles and soil were shown, by phage typing, to be the strain applied. *Serratia entomophila* was also

Proc. 46th N.Z. Plant Protection Conf. 1993: 235-236

detected in 6/12 of the adults originally collected from untreated plots (1×10^4 to 6×10^5 /adult; mean of 1.9×10^5). Five out of 12 soils containing adults from untreated plots contained *S. entomophila* (mean of 4.4×10^3 /g soil). Phage typing of isolates from soil and untreated adults showed they were not the strain applied, indicating a low level of background *S. entomophila* at the site.

The results indicate that, with high levels of bacteria in the soil, *S. entomophila* bacteria can be present in late instar larvae without causing disease symptoms and are carried through from larvae to the adult, probably in the midgut of the insect which is retained through metamorphosis.

Enumeration of bacteria in the live adults indicated that only a small proportion of the adults were carrying high numbers of the bacteria. However after death and disintegration, high numbers of the applied strain of bacteria were associated with most of the cadavers of beetles collected from the treated plots. This indicates that at least some bacteria were present in nearly all of the adults but were at levels too low to be detected. These appear to have multiplied following death of the insect, possibly using their ability to degrade chitin in the insect cuticle.

The results indicate that adult beetles can carry significant numbers of bacteria which can multiply on the cadaver and which may act as foci for infection of larvae in the following season. Such a mechanism would assist bacterial survival over the early summer period, when no host larvae are available, and also provide a method by which the bacteria could be introduced into disease-free areas.

REFERENCES

- Jackson, T.A., Pearson, J.F., Broad, T.M., O'Callaghan, M. and Mahanty, H.K., 1988. Subsurface application of the bacterium *Serratia entomophila* for the control of grass grub in Canterbury. *Proc. 41st N.Z. Weed and Pest Control Conf.*: 108-112.
- O'Callaghan, M. and Jackson, T.A., 1993a. Isolation and enumeration of *Serratia entomophila* - a bacterial pathogen of the New Zealand grass grub, *Costelytra zealandica*. *J. Appl. Bacteriol.* (in press).
- O'Callaghan, M. and Jackson, T.A., 1993b. Bacteriophages of *Serratia entomophila* - a bacterial pathogen of the New Zealand grass grub. *Proc. 6th Australasian Conf. Grassl. Invert. Ecol.*: (in press).
- O'Callaghan, M., Jackson, T.A. and Noonan, M.J., 1989. Ecology of *Serratia entomophila* in soil. *Proc. 5th Australasian Conf. Grassl. Invert. Ecol.*: 69-75.
- Starr, M.P., Grimont, P.A.D., Grimont, F. and Starr, P.B., 1976. Caprylate thallous agar medium for selectively isolating *Serratia* and its utility in the clinical laboratory. *J. Clin. Micro.* 4: 270-276.