

The Social Ecology of *Serratia entomophila*: Pathogen of *Costelytra giveni*

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Abstract : The endemic New Zealand grass grub (*Costelytra giveni*, Coleoptera: Scarabaeidae) is an economically significant grassland pest in New Zealand. Due to their impacts on production within the agricultural sector, one of New Zealand's primary industries, several methods are being used to either control or prevent the establishment of new grass grub populations in the pasture. One such method involves the use of a biopesticide based on the bacterium *Serratia entomophila*. This species is one of the causative agents of amber disease, a chronic disease of the larvae which results in death via septicaemia after approximately 2 to 3 months. The ability of *S. entomophila* to cause amber disease is dependant upon the presence of the amber disease associated plasmid (pADAP), which encodes for the key virulence determinants required for the establishment and maintenance of the disease. Following the collapse of grass grub populations within the soil, resulting from either natural population build-up or application of the bacteria, non-pathogenic plasmid-free *Serratia* strains begin to predominate within the soil. Whilst the interactions between *S. entomophila* and grass grub larvae are well studied, less information is known on the interactions between plasmid-bearing and plasmid-free strains, particularly the potential impact of these interactions upon the efficacy of an applied biopesticide. Using a range of constructed strains with antibiotic tags, in vitro (broth culture) and in vivo (soil and larvae) experiments were conducted using inoculants comprised of differing ratios of isogenic pathogenic and non-pathogenic *Serratia* strains, enabling the relative growth of pADAP+ and pADAP- strains under competition conditions to be assessed. In nutrient-rich, the non-pathogenic pADAP- strain outgrew the pathogenic pADAP+ strain by day 3 when inoculated in equal quantities, and by day 5 when applied as the minority inoculant, however, there was an overall gradual decline in the number of viable bacteria for both strains over a 7-day period. Similar results were obtained in additional experiments using the same strains and continuous broth cultures re-inoculated at 24-hour intervals, although in these cultures, the viable cell count did not diminish over the 7-day period. When the same ratios were assessed in soil microcosms with limited available nutrients, the strains remained relatively stable over a 2-month period. Additionally, in vivo grass grub co-infections assays using the same ratios of tagged *Serratia* strains revealed similar results to those observed in the soil, but there was also evidence of horizontal transfer of pADAP from the pathogenic to the non-pathogenic strain within the larval gut after a period of 4 days. Whilst the influence of competition is more apparent in broth cultures than within the soil or larvae, further testing is required to determine whether this competition between pathogenic and non-pathogenic *Serratia* strains has any influence on efficacy and disease progression, and how this may impact on the ability of *S. entomophila* to cause amber disease within grass grub larvae when applied as a biopesticide.

Keywords : biological control, entomopathogen, microbial ecology, New Zealand

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