

Case Report

An Epizootic of *Rickettsiella* Infection Emerges from an Invasive Scarab Pest Outbreak Following Land Use Change in New Zealand

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Abstract

Rickettsiella spp. are tiny obligate intracellular bacteria frequently pathogenic to a range of arthropods. As a consequence of being difficult to diagnose, little is known about their biology and ecology, and the importance of *Rickettsiella* diseases in insect population regulation has been underestimated. Land use change to increase agricultural productivity has produced unintended consequences by generating wide scale pest outbreaks that threaten economic viability of the development initiatives. On the West Coast of the South Island in New Zealand, land improvement through 'flipping' and 'hump & hollow' earth movement has created productive pasture land, but produced a widespread outbreak of manuka beetles (*Pyronota* spp.) reaching unprecedented densities and causing severe damage to pasture. Over time a reduction of manuka beetle densities back to 'normal' levels was observed, and it was determined to be the result of an epizootic of bacterial disease caused by the *Rickettsiella popilliae* pathotype '*Rickettsiella pyronotae*', which spread through the outbreak pest group.

INTRODUCTION

Bacteria of the genus *Rickettsiella* have been long known as insect pathogens infecting a wide range of insect hosts [1]. The genus *Rickettsiella* has been assigned to the taxonomic family Coxiellaceae in the order Legionellales of the Gammaproteobacteria [2]. The *Rickettsiella popilliae* type species consists of a number of synonymized pathotypes that have been associated with specific insect diseases, and seem particularly related to soil dwelling species, but surprisingly from different insect orders (Coleoptera, Lepidoptera, Diptera). *Rickettsiella* spp. are also associated with crickets and ticks; *R. gryllii* causes behavioural fever in crickets and *Rickettsiella* spp. form mutual associations with ixodid ticks and can be transmitted to mammals [3,4]. It is also notable that strains of *Rickettsiella* are also being identified as maternally-inherited endosymbionts [5]. *Rickettsiella popilliae* is known as a pathogen of soil dwelling Scarabaeidae in New Zealand [6]. It is a fastidious intracellular pathogen that typically target the fat body and hemolymph cells of the host [1]. The infective cells are small, dense rods (approximately 0.2 by 0.6 µm) that are ingested during feeding

and subsequently pass through the midgut epithelium to the hemocoel where they enter host cells (e.g. fat bodies) through endocytosis. Once within the cell, pleomorphic forms develop within the cytoplasmic vacuoles, varying from bacteria-like secondary cells to large, round stroma. As the disease develops, characteristic protein crystals form and the bacterial cells revert to small rods that fill the host cells, which appear as rapid Brownian motion under light microscopy observation. Eventually, the infected insect cells lyse, releasing the multitude of *Rickettsiella* cells and crystals into the hemolymph, producing the white to blue coloration of infected larvae. Diseases of insects caused by *R. popilliae* are frequently chronic. The infected host loses vigour over time, becomes whitened, and eventually dies.

In this case report we document an epizootic of *R. popilliae* disease that occurred during an outbreak of manuka beetles (*Pyronota setosa* and *P. festiva*) causing beetle numbers to decline and the pasture to recover from damage.

CASE PRESENTATION

The outbreak of manuka beetles occurred in an area of

large-scale land development from swamp to pasture on the West Coast of the New Zealand South Island [8]. Pastures were monitored to assess pest numbers and *R. popilliae* (pathotype '*R. pyronotae*') was isolated from manuka beetle larvae [7,8]. Five pasture paddocks were systematically sampled from 2009-2012 to monitor insect numbers and possible infections with approximately 12,500 manuka beetle larvae collected over this period. Pest enumeration assessments were made by taking 20 soil spade samples (15 x 15 x 20 cm) per paddock along transects, and insect larvae extracted and counted to provide an estimate of manuka beetle density. Larvae were identified to species level in the laboratory, while estimates of disease rates were determined from sub-samples of collected insects by a combination of observation for characteristic symptoms of infection and microscopic examination. Confirmation of the '*R. pyronotae*' identification was carried out via PCR amplification and DNA sequence analysis of partial 16S rRNA and *ftsY* gene sequences as described in [8].

The pastures sampled had been established on renovated land in 2002 with the manuka beetles reaching damaging levels by 2005/2006 [8], and after several seasons of very high densities of beetles had passed that the first manuka beetle larvae infected with *Rickettsiella* (Figure 1) were recognised in 2009. *R. popilliae* pathotype '*R. pyronotae*' infection was first observed in larvae from a small patch of pasture with a high density of manuka beetles and developed from an initial average infection rate of 0.5% in 2009 to 28.6% of larvae in 2012 showing advanced stages of *Rickettsiella* infection (Table 1). Consequently the numbers of manuka beetles dropped significantly ($p < 0.001$) from an average of 465 larvae/m² in 2009 to 223 larvae/m² by 2011 (Table 1). The density of manuka beetles remained low in subsequent years with widespread '*R. pyronotae*' infection and the pasture had recovered from insect damage.

DISCUSSION

The spectacular outbreak of *Rickettsiella* and ensuing local population collapse may be related to specific site conditions [9]. The pest outbreak occurred where a new soil profile was created with an abundance of freshly established, fertilised, grass roots for the insect larvae. Peak densities of manuka beetle larvae (*P. setosa* and *P. festiva*) were reached in 2009, but no significant diseases were recorded in the pest group for the first five years following sowing. Consistent rainfall provides ideal conditions for pasture growth and insect population development. In these conditions, a small inoculum of '*R. pyronotae*' could initiate infections, lead to insect death, and thereby build up propagules in the soil. The subsequent generations of manuka beetle larvae were exposed to this inoculum and became infected by horizontal transmission from the soil. Inoculum would be spread by the surviving beetles as they emerged and flew for mate searching to adjacent local populations.

The combination of a spectacular pest outbreak, the ability of the project team to recognise *Rickettsiella* infections, and the concern of the farmers to support a monitoring project, has meant that this episode demonstrating the actions of *Rickettsiella* disease in stabilising a pest population could be documented. A similar impact of *Rickettsiella* disease was noted by Niklas on May

Table 1: Annual mean larval density and estimated level of '*R. pyronotae*' infection.

Year	Density (/m ²) ^a		Infection rate ^b	
	Mean	±SE	%	±SE
2009	465.4	35.4	0.53	0.3
2010	352.4	25.4	3.25	0.9
2011	223.2	15.9	9.2	1.7
2012	226.5	20.4	28.6	3.3

^aThe manuka beetle larval densities were compared between the years using a generalised estimating equation analysis and demonstrated a significant difference between all year combinations ($p < 0.005$) except between 2011 and 2012 ($p = 0.946$). Analyses utilised statistical software SAS version 9.3. SE, standard error.

^bThe manuka beetle infection rate was compared between the years using a generalised linear model analysis and demonstrated a significant difference between all year combinations ($p < 0.002$). SE, standard error. Analyses utilised statistical software Minitab version 16.

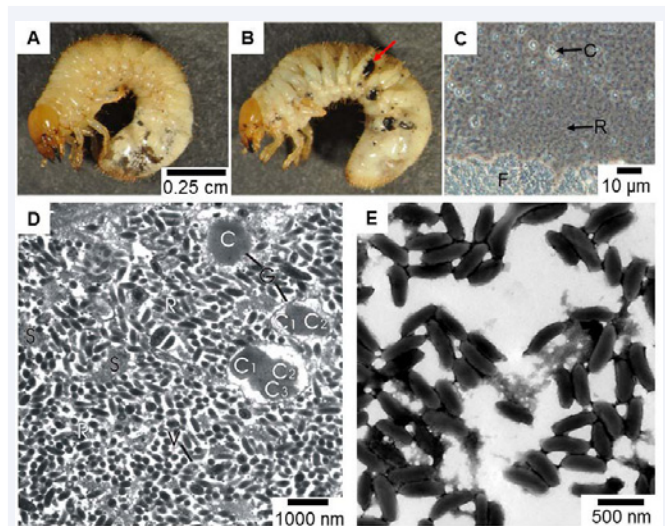


Figure 1 Diagnosis of *Rickettsiella* infection in *Pyronota* spp. A) Healthy *P. setosa*. Cuticle has a yellow-white hue and is free of blemishes. Note that the physical appearance of *P. festiva* is highly similar to that of *P. setosa*. B) *Rickettsiella* infected *P. setosa* (*Rickettsiella popilliae* '*R. pyronotae*' pathotype). Cuticle has a blue-white hue and with several distinct dark melanization patches (red arrow) observable. C) Aqueous wet mount of *P. setosa* fatbody tissue (F) infected with *Rickettsiella* displaying several crystals (C) and a multitude of infected bacterial cells (R) easily observed. D) Transmission electron micrograph of an ultrathin section of infected fat body of *P. setosa*. Multiplication of *Rickettsiella* bacteria (R) is in a progressing stage. *Rickettsiella* bacteria are often observed in vesicles (V) and finally are also replacing areas of relatively dense stroma material (S). Giant bodies (G) transforming into association crystals (C) that can be single or in clusters of smaller crystals (C1, C2, C3). E) Transmission electron micrograph of infective rod-shaped '*R. pyronotae*' bacteria isolated from *P. setosa* fat body tissue.

beetles (*Melolontha* spp.) in Germany in the 1960's [10], but the difficulty of diagnosis and the association with soil insects means that the importance of *Rickettsiella* diseases in insect population regulation is vastly underestimated.

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