PASTEURIA PENETRANS AS A POTENTIAL CONTROL AGENT FOR ROOT KNOT NEMATODE IN KIWIFRUIT

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Root knot nematodes (RKN, *Meloidogyne* spp.) are potentially the most significant nematode pathogens of kiwifruit (*Actinidia deliciosa*) (Grandison 1990). *Meloidogyne hapla* is the most prevalent species in New Zealand. It can cause heavy root deformation throughout the root system to greater than 1 m depth. Successful control using chemicals is unlikely. Kiwifruit cultivars with resistance to RKN have so far not been developed. As a long term perennial crop, with a single dominant pathogenic nematode, kiwifruit is suited to the buildup and activity of biological controls for RKN. *Pasteuria penetrans* is a bacterial disease which has shown promise in reducing RKN populations in crops including vineyards (Bird and Brisbane 1988). It may reduce the nematode burden on kiwifruit if strains effective on *M. hapla* are found. Such effective strains will have wider applications in control of RKN in New Zealand. *Pasteuria penetrans* has not yet been found to occur naturally in New Zealand. It is the first example of a biological control agent introduced for nematode control in this country.

Pasteuria penetrans

When second stage RKN juveniles move through soil they contact *Pasteuria* spores which adhere to the cuticle. Spore encumbered larvae become less mobile to the extent that they may be unable to invade roots (Stirling 1984). Less encumbered larvae enter roots and start to feed normally. At the same time germination of the spores occurs with penetration of the nematode cuticle by a germ tube. A vegetative microcolony forms and proliferates inside the body of the growing nematode. Eventually most of the internal tissues disintegrate, egg production is inhibited and the female becomes a container for around 2×10^6 bacterial spores which are subsequently released into the soil (Sayre and Starr 1985).

As yet *P. penetrans* has not been cultured outside its nematode hosts. Strains of the disease have varying although restricted host ranges. Some have intrageneric specificity while others only infect certain populations within a nematode species (Stirling 1985). In general *M. hapla* are more difficult to infect than RKN species adapted to warmer climates so careful selection of candidate *Pasteuria* strains is necessary. Inoculation of larvae is crucial to successful control. Excessive spores adhering to RKN juveniles means too few nematodes survive to mature further *Pasteuria* spores while insufficient attachment means too many nematodes escape infection to effectively reduce the population (Stirling 1984).

Research objectives

1. Multiplication of *Pasteuria* cultures. Small quantities of spore cultures have been received from overseas sources. This spore material was inoculated on *M. javanica*, the most widely used *Meloidogyne* host. Infected nematodes were grown on tomato roots at 25 °C and after 8-10 weeks females containing mature spores were dissected from roots and frozen. These provide a uniform inoculation source for future comparative testing.

2. Inoculation tests. In order to optimise infection of nematodes two inoculation methods were evaluated: (i) agitation of an aqueous larvae/spore suspension using a flat bed shaker or aeration pump (Stirling and Wachtel 1980); (ii) a passive method using 1% water agar plates which allow larvae to migrate over spores previously spread over the plates (Verdejo and Jaffe 1988). Water agitation increased the frequency of spore/larval contact and allowed more rapid infection of larvae. Water agar plates required longer to give the same infection level but larvae had a lower mortality and were in better condition for later plant inoculation.

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Mature spores have a parasporal protective covering which is normally broken down by natural processes in soil. Infectivity of unweathered spores can be improved by sonicating the spore suspension prior to inoculation to artificially disrupt this protective cover (Stirling *et al* 1986). Our tests showed that sonication improved inoculations using the water agitation method.

3. Strain tests. Each culture needs to be evaluated to find the best strain for control of *M. hapla*. Strains most suitable for other purposes will also be identified, eg. for control of *M. javanica* in the glasshouse.

4. Safety tests. Before *Pasteuria* is released for field evaluation it is necessary to show that these strains attack target nematodes, and not species in the soil which are either beneficial or innocuous. Nematode fauna from various environments will be exposed to *Pasteuria* and examined for spores adhering to the cuticle.

5. Preliminary efficacy studies. The efficacy of selected strains to reduce RKN on plant hosts such as kiwifruit will be conducted in quarantine.

Increasing environmental concerns and other major limitations to the use of chemical nematicides necessitate the development of alternative controls which are environmentally "friendly". *Pasteuria penetrans* provides a promising start in this direction.

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