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BIOLOGICAL CONTROL OF CLOVER CYST NEMATODE *HETERODERA TRIFOLII*

A thesis presented in partial fulfillment of
the requirements for the degree of

Doctor of Philosophy

in Plant Science

(Plant Pathology)

at



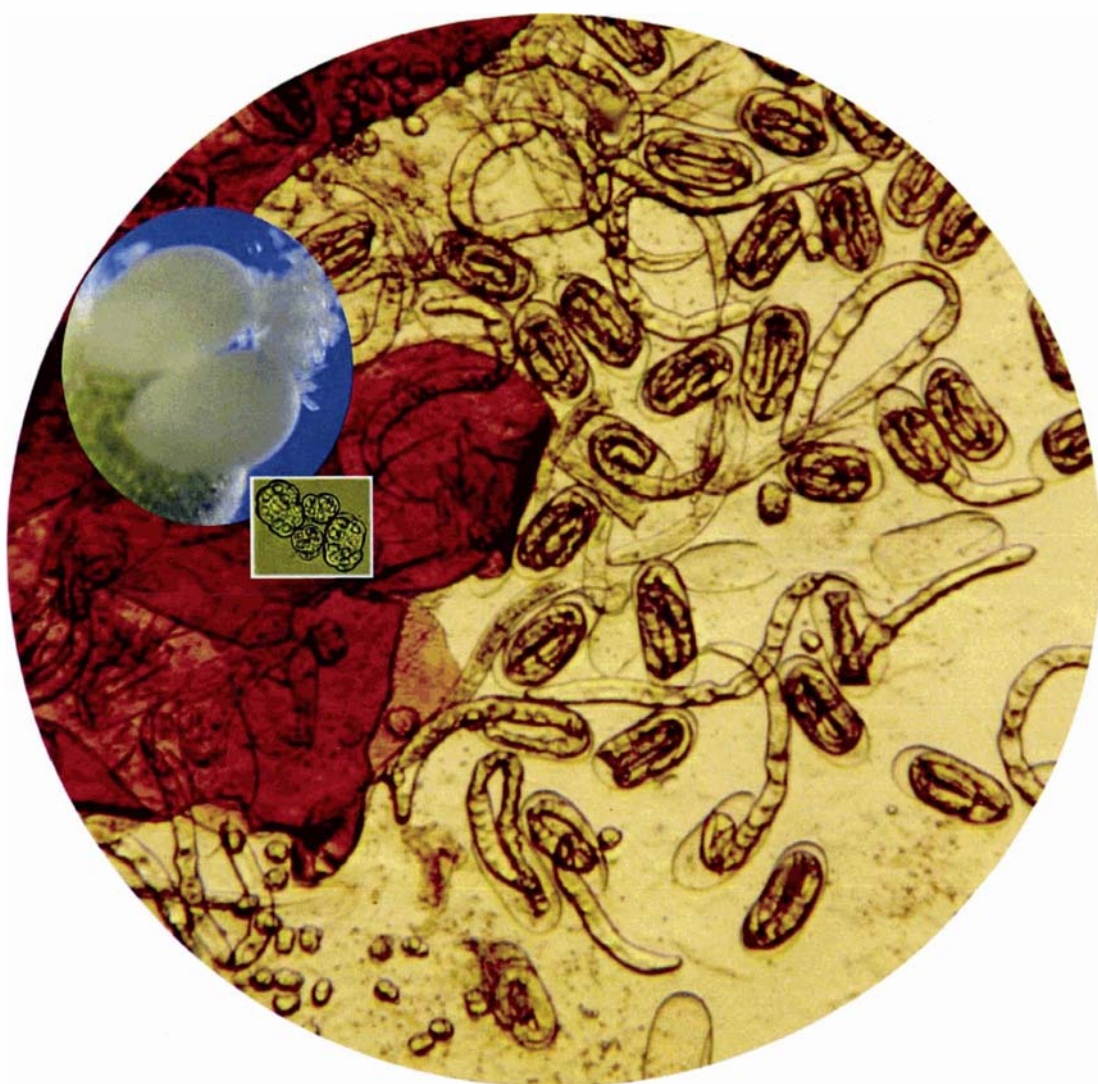
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ABSTRACT

Heterodera trifolii is one of the most damaging pests in New Zealand pastures and the aim of this work was to study the potential use of 'biological control' as an alternative strategy to the use of chemicals. Natural enemies, especially fungi, isolated from *H. trifolii* were the main organisms investigated for control of *H. trifolii* in this study. As a first step, more information was required on population dynamics of this nematode species in pasture soil and the possible causes of its population peaks and troughs. Soil cores were taken at fortnightly intervals from a permanent white clover/ryegrass pasture at AgResearch, Palmerston North, New Zealand, from March 1999 to March 2001 and numbers of second stage juveniles (J2), adult females and cyst stages of *H. trifolii* were recorded together with those of other soil nematodes. Emergence of J2 peaked in winter in both 1999 and 2000, and appeared to follow root growth, which in turn reflected soil moisture levels.

Young cysts and mature females were assessed for parasitism by fungi and such fungi were isolated into pure culture as a first stage in assessing their biological control potential. Fungal genera such as *Verticillium*, *Fusarium*, *Gliocladium*, *Paecilomyces*, and *Trichoderma* were assessed for their pathogenicity to white clover seedlings *Trifolium repens* on 1.0% water agar *in vitro* then in sand in pots. As these fungal isolates were not pathogenic to the *T. repens* plants in pots, some, such as species of *Fusarium*, *Gliocladium*, and *Verticillium* were tested for their potential parasitism on *H. trifolii* in pots of sand with a view to assessing their use as biological control agents. Oatmeal was one substrate on which fungi were grown but it caused poor growth of *T. repens*.

On the basis of these experiments, only isolate Vc6 (a *Verticillium chlamydosporium* isolate) consistently reduced the numbers of *H. trifolii* and was selected for further experiment. Vc6 was grown on a range of media such as alginate beads, bran culture alginate beads, potato dextrose broth culture alginate beads, dry soil inoculum and wheat flour/sand inoculum and it was assessed for its potential pathogenicity to *H. trifolii* females and cysts. Vc6 grown on alginate beads containing wheat bran significantly reduced the numbers of females and of cysts and it increased plant growth of *T. repens*.

There were more than 100 *V. chlamydosporium* isolates from the two-year study so there was a need to screen the isolates for biocontrol activity as they varied in the production of chlamydospores from which eggs of adult females are parasitised. For screening experiments, clover cyst nematode was successfully cultured on 0.5% Hoagland & Knop's agar monoxenically using J2 surface-sterilised with 0.5% Hibitane in a watch glass. A number of *V. chlamydosporium* isolates obtained from young cysts and females of *H. trifolii* in the two-year field study were screened for pathogenicity to *H. trifolii* in monoxenic culture using *T. repens* seedlings on 0.5% Hoagland & Knop's agar, and in sand in pipette tips *in vitro*. Females of *H. trifolii* developed in some of the *V. chlamydosporium* isolate treatments and it was concluded that there were some variations in their pathogenicity to *H. trifolii* in the *in vitro* tests.

In addition to screening the isolates for their parasitism to *H. trifolii in vitro*, variation among the isolates was investigated at the molecular level using the RAPD PCR-based technique. Cluster analysis of 10 *V. chlamydosporium* isolates using RAPD PCR data showed that isolate Vc6 consistently differed from other *V. chlamydosporium* isolates tested.

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