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)

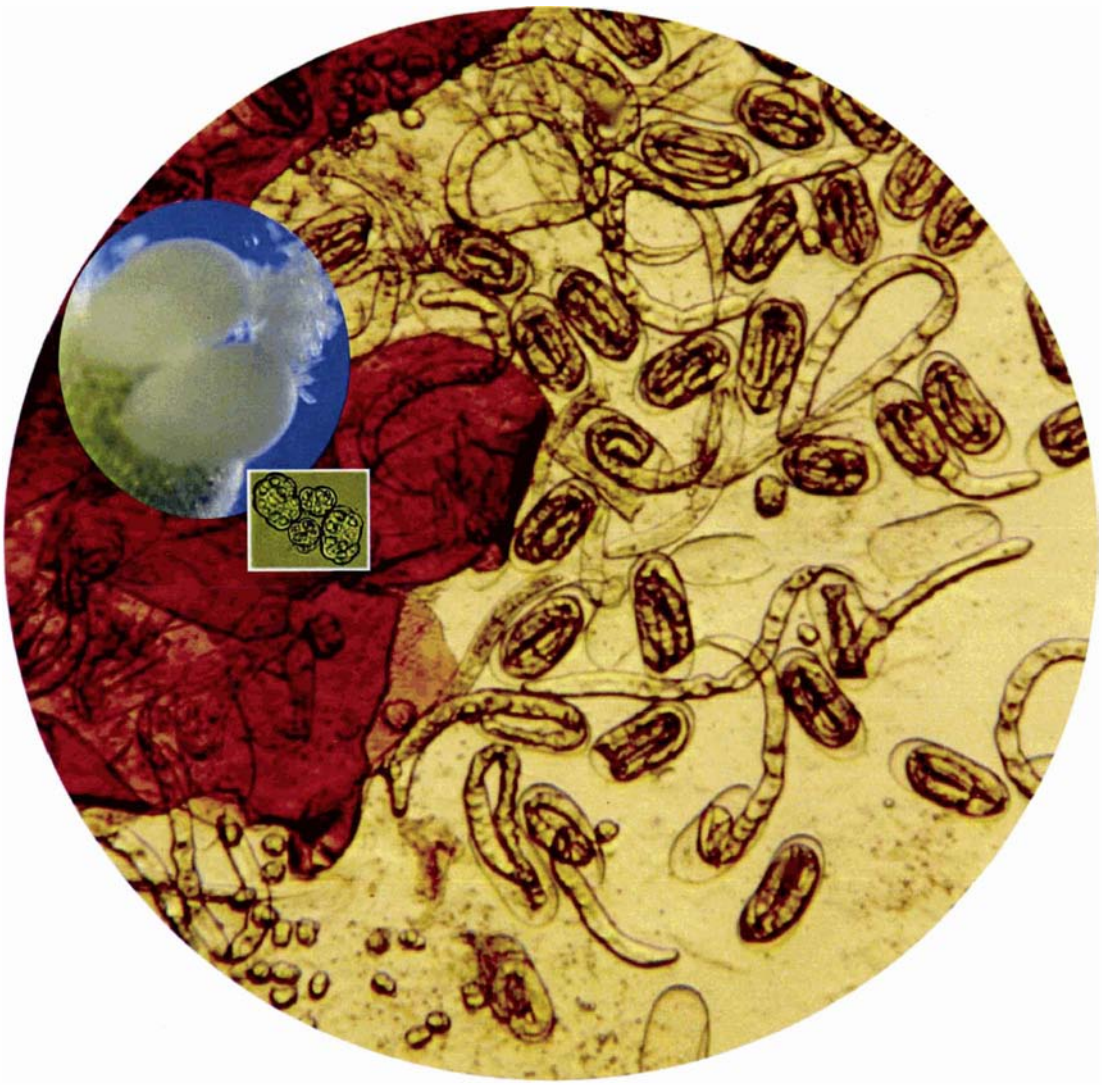
at

Palmerston North

New Zealand

Pyone Pyone Kyi

June 2003



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.

ACKNOWLEDGEMENTS

I wish to express my appreciation to my chief supervisor Dr Peter Long for his advice, encouragement, guidance, patience, his time spent on reading and critically evaluating this manuscript and his moral support throughout my life in New Zealand. Without his support I would not have been able to attend National Conferences and the International Congress of Plant Pathology.

I offer my sincere gratitude to my supervisors Mr Chris Mercer, Dr Bob Skipp (AgResearch Grassland Research Centre, Palmerston North), Dr Gregor Yeates (Landcare Research, Palmerston North) and Dr Rosie Bradshaw (Massey University, Institute of Molecular BioSciences, Palmerston North) for their valuable advice, supporting references, their help what or when ever I needed it for my thesis. I especially acknowledge Mr Chris Mercer for his keen interest and supervision that enabled me to complete my research works in his glasshouse and his moral support throughout my four year-study here in New Zealand. Without them this thesis could not been. I owe much for all these people and I will never forget.

I am also indebted to Mr Hugh Neilson (Technician, Plant Protection, Massey University, Palmerston North) for his assistance, and advice for my laboratory works. Whenever I needed help, he never hesitated to assist me. I would like to acknowledge the help given by all staff members of Plant Protection, Massey University, Palmerston North, especially Pam Howell, Lorraine Davis, and Ai Lih, Adam Mackres (INR, Information Technology Unit, Massey University, Palmerston North) for their kind help and friendly.

I am deeply grateful to Mrs Li-Yuan Chen and Mr Mike Christensen (Pathology AgResearch Grassland Research Centre) for their kind help for my research, Mr John Brock for weather data and pasture trial related references, Mrs Kylie Miller and Mr Kenyon Moore (Glasshouse AgResearch Grassland Research Centre, Palmerston North) for their help in glasshouse experiments, Mr Greig Cousins for pasture yield data, Dr David Horne (Soil & Earth Sciences, Massey University, Palmerston North) for his help with soil moisture levels which saved me a lot of my time. Dr. Terry Stewart (Plant Protection, Massey University, Palmerston North) for his input in the thesis final version, Dr Janne Lager (Sweden) for kindly sending his valuable thesis and Dr Keith Davies (UK) for his references, to them I am very deeply grateful.

My special appreciations are also extended to Vingnani, Entin (statistical analysis), Christine (with Bryn), Cait (nice bands), Helen, Jianyu, Hatsue, Karma, Xiong Zhao, Alfredo, Jorge, Edgardo, and to my flat mates; Roni & Devi (Indonesia), John, Ponchie, Meggi, Helena (Philippines), Wan Theng (Malaysia), Embe and Hoa (Vietnum), Natilene, Linda, Donata (Kiwi), Vijay (Sri Lanka) for their friendship.

My special thanks goes to Mona (Philippines) for being a close friend, her humour make my life never be boring. I still remember the time we spent together and the sharing of feeling and thoughts.

I will never forget you as we have opposite characters! I have learned so much from you that I have not done/known before in my life. Thanks for being with me all my struggling times in computer room till early morning, not only one night, several nights. You drive for me wherever I want to go (of course just in Palmy) and you cook for me yummy foods (without chillies ☺) almost every dinner. You 'Tuing' from Thailand, I really thank you more than I can write.

I would particularly like to thank some of my fellow countryman: especially Ma Ma Mya in New Zealand and Saya Dr Aung Kyi & Yi Yi in Myanmar for their kind help that was given without my needing to ask for it.

Government of Myanmar for allowing me study in New Zealand, The New Zealand Ministry of Foreign Affairs and Trade for scholarship to study and live in New Zealand and New Zealand Plant Protection Society for providing funds for the 8th International Plant Pathology Congress, Christchurch, New Zealand, for which I would like to express my sincere gratitude. I also thank to the staff of International Students' office, especially to Mr Charles Chua for kindly help and Mella (Residential office) for giving me a chance to live in a nice room of 'Tararua flat 4' in Massey campus.

I am very deeply grateful for moral support which kept me on my toes especially during difficult times, given by Sayadaw U Jo Tika (Burmese monk).

I owe what I have achieved to a great number of people, who teach me from the beginning (Ka gyee) and all of my life, I will not name individually.

Special thanks are due to my uncles and aunties, brother Win Naing Oo, family of sisters Lwin Lwin, Cho Cho who have been a constant source of encouragement, and to lovely nieces and nephews who make Grandma happy.

Finally, I owe so much thanks to my Grandma, my parents who give me wonderful support and love that can't be compared with anything else, but unfortunately I can not share in this personal achievement with my Grandma and my father. Dear Phae Phae, writing these words has meant so much to me because today is anniversary of you leaving us. I am so sad but happy to think of 'how proud you on me'…

To May May Gyi, Phae Phae and May May I dedicate this thesis.

Table of contents

List of tables

List of figures