Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.
BIOLOGICAL CONTROL OF CLOVER CYST NEMATODE \textit{HETERODERA TRIFOLII}

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

\textbf{Doctor of Philosophy}

in Plant Science

(Plant Pathology)

at

\textit{Massey University}

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% Hbitane 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 (Vietnam), 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 (Kagyee) 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

ABSTRACT ........................................................................................................................... III
ACKNOWLEDGEMENTS ...................................................................................................... V
TABLE OF CONTENTS ......................................................................................................... VIII
LIST OF TABLES .................................................................................................................. XIV
LIST OF FIGURES ............................................................................................................. XVI

1  GENERAL INTRODUCTION ............................................................................................... 1
  1.1 WHITE CLOVER .......................................................................................................... 1
     1.1.1 Pests and diseases .............................................................................................. 1
     1.1.2 Nematodes ........................................................................................................ 3
  1.2 LIFE CYCLE OF CLOVER CYST NEMATODE H. TRIFOLII (TYLENCHIDA: HETERODERIDAE) ......................................................... 5
  1.3 POPULATION DYNAMICS OF H. TRIFOLII IN NEW ZEALAND ........................................... 6
  1.4 FUNGI ASSOCIATED WITH CYST NEMATODES ............................................................ 7
  1.5 BIOLOGICAL CONTROL OF CYST NEMATODES ............................................................ 8
  1.6 V. CHLAMYDOSPORIUM (= V. CHLAMYDOSPORIUM VAR. CHLAMYDOSPORIUM GODDARD = Pochonia Chlamydosporia Var. Chlamydosporia Zare et al.) ................................................................. 8
  1.7 MONOXENIC CULTURE OF NEMATODES .................................................................. 10
  1.8 OBSERVATION OF VARIATION AMONG NEMATOPHAGOUS FUNGI BY PCR-BASED TECHNIQUES ......................................................... 10
  1.9 AIMS OF THIS STUDY .................................................................................................. 11
  2.0 REFERENCES .............................................................................................................. 13

2  SEASONAL POPULATION DYNAMICS OF THE CLOVER CYST NEMATODE (HETERODERA TRIFOLII) AND ASSOCIATED FUNGI .................................................................................. 23
  2.1 INTRODUCTION ........................................................................................................... 23
     2.1.1 Clover cyst nematode (Heterodera trifolii) ........................................................... 23
     2.1.2 The role of fungal parasitism of H. trifolii .......................................................... 25
  2.2 OBJECTIVES ............................................................................................................... 26
  2.3 MATERIALS AND METHODS ....................................................................................... 26
     2.3.1 The site and sampling .......................................................................................... 26
     2.3.2 Extraction of second stage juveniles (J2) of H. trifolii .......................................... 27
     2.3.3 Extraction of clover cysts .................................................................................... 29
     2.3.4 Staining of clover roots and pasture dry matter yields ......................................... 30
     2.3.5 Culturing fungi from H. trifolii ............................................................................ 31
        2.3.5.1 Surface-sterilisation of cysts ........................................................................ 31
        2.3.5.2 Isolation of the surface-sterilised cysts ........................................................ 31
2.3.6 Soil moisture, temperature and rainfall ................................................................. 32
2.3.7 Soil temperature .................................................................................................... 32
2.3.8 Statistics ............................................................................................................... 32
2.4 RESULTS .................................................................................................................. 32
2.4.1 Second stage *H. trifolii* and other plant-parasitic nematodes in the soil .............. 33
2.4.2 Second stage juveniles in clover roots ................................................................. 33
2.4.3 White females in soil ............................................................................................ 34
2.4.4 New cysts in soil .................................................................................................. 34
2.4.5 Old cysts in soil .................................................................................................... 35
2.4.6 The wet root weight and white clover dry matter yield ........................................ 35
2.4.7 Soil moisture ....................................................................................................... 35
2.4.8 Soil temperature .................................................................................................. 36
2.4.9 Fungi from clover cysts ...................................................................................... 41
2.5 DISCUSSION ............................................................................................................ 44
2.6 CONCLUSION .......................................................................................................... 47
2.7 REFERENCES .......................................................................................................... 49

3 PATHOGENICITY TO WHITE CLOVER PLANTS OF SOME FUNGI ISOLATED FROM CLOVER CYST NEMATODE .......................................................... 53

3.1 INTRODUCTION ....................................................................................................... 53
3.2 OBJECTIVE .............................................................................................................. 54
3.3 MATERIALS AND METHODS ................................................................................ 55
3.3.1 Agar plate test .................................................................................................... 55
3.3.2 Pot test ............................................................................................................... 55
3.4 RESULTS .................................................................................................................. 56
3.4.1 Agar plate test .................................................................................................... 56
3.4.2 Pot test ............................................................................................................... 58
3.5 DISCUSSION ............................................................................................................ 60
3.5.1 Agar plate test .................................................................................................... 60
3.5.2 Pot test ............................................................................................................... 61
3.6 CONCLUSION .......................................................................................................... 62
3.7 REFERENCES .......................................................................................................... 64

4 PATHOGENICITY TO CLOVER CYST NEMATODE OF SOME FUNGI ISOLATED FROM CLOVER CYST NEMATODE: GLASSHOUSE TESTS .................................. 67

4.1 INTRODUCTION ....................................................................................................... 67
4.2 OBJECTIVE .............................................................................................................. 69
4.2.1 *Trial 1* Screening a range of fungal species ...................................................... 69
4.2.2 *Trial 2* Pathogenicity of *V. chlamydosporium* isolate 6 (Vc6) to *H. trifolii* and to clover seedlings ............................................................ 69
4.2.3 *Trial 3* Influence of fungal inoculum medium on pathogenicity tests ............... 69
5 MONOXENIC CULTURE OF CLOVER CYST NEMATODE HETERODERA TRIFOLII ON WHITE CLOVER TRIFOLIUM REPENS ................................................................. 92

5.1 INTRODUCTION ........................................... 92

5.2 TRAIL (5.1) SURFACE-STERILISATION OF H. TRIFOLII J2 WITH HIBITANE AND CULTURE OF H. TRIFOLII IN SAND OR ON AGAR ...................................................... 95
5.3 TRIAL (5.2) EFFECT OF HIBITANE AND ANTIBIOTICS AS SURFACE STERILANTS

5.3.1 Objectives

5.3.2 Materials and Methods

5.3.3 Results

5.3.4 Discussion

5.4 TRIAL (5.3) SURFACE STERILISATION OF J2 WITH VARIOUS CONCENTRATIONS OF HIBITANE

5.4.1 Objectives

5.4.2 Materials and Methods

5.4.3 Results

5.4.4 Discussion

5.5 TRIAL (5.4) SURFACE STERILISATION OF J2 WITH HIBITANE, USING A CENTRIFUGE

5.5.1 Objective

5.5.2 Materials and Methods

5.5.3 Results

5.5.4 Discussion

5.6 TRIAL (5.5) SURFACE STERILISATION OF J2 WITH 0.25% HIBITANE EITHER IN EPPENDORF TUBES/STORED IN SDW OR IN EPPENDORF TUBES

5.6.1 Objective

5.6.2 Materials and Methods

5.6.3 Results

5.6.4 Discussion
6 SCREENING ISOLATES OF VERTICILLIUM CHLAMYDOSPORIUM FROM CLOVER CYST NEMATODE FOR PATHOGENICITY TO CLOVER CYST NEMATODE. 120

6.1 INTRODUCTION. 120
6.2 OBJECTIVE. 121
6.3 MATERIALS AND METHODS. 121
6.3.1 Screening of fungi for pathogenicity to H. trifolii eggs on agar. 121
6.3.2 Screening of V. chlamydosporium fungal isolates in sand-tips. 122
6.3.3 Screening of V. chlamydosporium fungal isolates against H. trifolii on white clover grown on Hoagland & Knop's agar plates. 122
6.4 RESULTS. 123
6.4.1 Screening of V. chlamydosporium in water agar plates. 123
6.4.2 Screening of V. chlamydosporium in sand-tips. 123
6.4.3 Screening of V. chlamydosporium in Hoagland & Knop's agar plates. 125
6.5 DISCUSSION. 128
6.6 CONCLUSION. 130
6.7 REFERENCES. 131

7 OBSERVATIONS ON MOLECULAR VARIATION OF VERTICILLIUM CHLAMYDOSPORIUM POPULATIONS ISOLATED FROM HETERODREA TRIFOLII. 133

7.1 INTRODUCTION. 133
7.2 OBJECTIVES. 135
7.3 MATERIALS AND METHODS. 135
7.3.1 Culturing V. chlamydosporium. 136
7.3.2 Extraction of DNA. 136
7.3.3 Measuring DNA concentration by the fluorometer method. 137
APPENDIX I. NUMBERS OF *H. TRIFOLII* (CHAPTER 2) ......................................................... 181
APPENDIX II. SYSTEMATIC POSITIONS OF NEMATODES (CHAPTER 2) ........................................... 182
APPENDIX III. BUFFERS, SOLUTIONS AND MEDIA (CHAPTER 7) ...................................................... 183
List of tables

Table 2-1 Nematode counts from fortnightly sampling in a white clover/ryegrass pasture at AgResearch, Palmerston North, over a 2-year period (March 1999 – March 2001) .................. 42
Table 2-2 Percentage of microbial taxa isolated from young cysts of *H. trifolii* on each of 51 samples over the study period (March 1999 – March 2001) ............................................... 43
Table 3-1 Mean dry weights of white clover seedlings on agar harvested 6 weeks after inoculation with a range of fungi .................................................. 57
Table 3-2 Lesion formation on white clover seedlings on agar 6 weeks after inoculation with fungi .................................................. 57
Table 3-3 Mean dry weights of roots (g/plant) harvested from white clover plants 8 weeks after inoculation by dipping the roots in a suspension of fungal conidia (1 x 10^4, 1 x 10^6 or 1 x 10^8/ml) with or without wounding .................................................. 59
Table 3-4 Mean dry weights of shoots (g/plant) harvested from white clover plants 8 weeks after inoculation by dipping the roots in a suspension of fungal conidia (1 x 10^4, 1 x 10^6 or 1 x 10^8/ml) with or without wounding .................................................. 60
Table 4-1 The effect of a range of fungal isolates on reproduction of *H. trifolii* and on yield of white clover in the presence or absence of *H. trifolii* (no. of nematodes/pot) .................. 76
Table 4-1a The effect of a range of fungal isolates on reproduction of *H. trifolii* (no. of nematodes/g of root wt) .................................................. 77
Table 4-2 The effect of selected fungal isolate (Vc6) on reproduction of *H. trifolii* and on yield of white clover in the presence or absence of *H. trifolii* (no. of nematodes/pot) .................. 78
Table 4-2a The effect of selected fungal isolate (Vc6) on reproduction of *H. trifolii* (no. of nematodes/g of root wt) .................................................. 78
Table 4-3 The effect of *V. chlamydosporium* (Vc6) grown on a range of media on reproduction of *H. trifolii* and on yield of white clover in the presence or absence of *H. trifolii* (no. of nematodes/pot) .................. 81
Table 4-3a The effect of *V. chlamydosporium* (Vc6) grown on a range of media on reproduction of *H. trifolii* (no. of nematodes/g of root wt) .................................................. 82
Table 5-1 Percentage survival of J2 after treatment with Hibitane, using filter holder .................................................. 100
Table 5-2 Percentage survival of J2 after treatment with antibiotics, using Eppendorf tubes ....... 101
Table 5-3 Percentage survival of J2 after treatment with Hibitane and Streptomycin, using Eppendorf tubes .................................................. 101
Table 5-4 Percentage survival of J2 after treatment with Hibitane in Eppendorf tubes ............... 104
Table 5-5 Percentage survival of J2 in SDW, using Eppendorf tubes or filter holders ............... 105
Table 5-6 Percentage survival of J2 after treatment with Hibitane, using Eppendorf tubes with and without centrifugation .................................................. 107
Table 5-7 Percentage survival of J2 after treatment with Hibitane either in Eppendorf tubes/stored in SDW or in Eppendorf tubes .................................................. 109
Table 5-8 Percentage survival of J2 after treatment with Hibitane in glass test tubes (25 x 5 mm) 112
Table 5-9 Percentage survival of J2 after treatment with Hibitane in glass test tubes (53 x 5 mm)
Table 5-10 Percentage survival of J2 after treatment with Hibitane in watch glasses

Table 6-1 Percentages of *V. chlamydosporium* infected *H. trifolii* eggs on water agar after 1-week incubation at 21°C
Table 6-2 The effect of *V. chlamydosporium* isolates on mean numbers of females, new cysts and mean clover shoot dry weights in sand in pipette tips
Table 6-3 The effect of *V. chlamydosporium* isolates on mean numbers of females, new cysts, and old cysts in Hoagland & Knop's agar plates
Table 6-4 The effect of *V. chlamydosporium* isolates on mean numbers of root tips, trifoliated leaves, and clover shoot lengths in Hoagland & Knop's agar plates

Table 7-1 PCR and sequencing primers
Table 7-2 Amplification by 27 RAPD primers with 10 *V. chlamydosporium* isolates
Table 7-3 Groups of *V. chlamydosporium* isolates by size of polymorphic amplification products from RAPD primers
Table 7-4 The sizes and scores of reproducible RAPD PCR polymorphic amplification products for RAPD cluster analysis
Table 7-5 Mean fungal growths, numbers of viable spores and sizes of chlamydospires of 10 *V. chlamydosporium* isolates
List of figures

Figure 1-1 Life cycle of *H.* trifolii on sterile *T.* repens roots ................................................................. 5
Figure 2-1 Soil sampling process .......................................................................................................................... 27
Figure 2-2 Extraction process for vermiform nematodes from soil ................................................................. 28
Figure 2-3 The extraction of *H.* trifolii cysts and their observation ................................................................. 30
Figure 2-4 Staining of white clover roots ............................................................................................................ 31
Figure 2-5 Numbers of *H.* trifolii in roots and soil based on fortnightly samples from a permanent white clover/ryegrass pasture at Palmerston North from March 1999-March 2001 expressed as a four-fortnight running averages ................................................................. 37
Figure 2-6 (a) Soil temperature (°C, 10 cm soil depth) from the Meteorological records at AgResearch Palmerston North and mean soil moisture levels (% dry weight), (b) Mean wet root weights (g) / 100 ml soil in fortnightly samples from a permanent white clover/ryegrass pasture at Palmerston North from March 1999-March 2001 expressed as a four-fortnight running averages. Bars represent ± SE. (n=4) ................................................................................................................................. 39
Figure 2-7 Rainfall (mm) from the Meteorological records at AgResearch Palmerston North expressed as a four-fortnight running averages ................................................................................................................................. 40
Figure 2-8 White clover yield (dry matter g/m²) .................................................................................................... 40
Figure 2-9 Mean percentage of young cysts *H.* trifolii infected with *Fusarium* spp., *Gliocladium roseum*, and *Verticillium chlamydosporium* in a permanent white clover/ryegrass pasture at AgResearch, Palmerston North, New Zealand, over a 2-year period (March 1999-March 2001) .................................................................................................................................................. 41
Figure 5-1 Variations in the procedure for surface-sterilisation of *H.* trifolii J2 ........................................................................................................................................................................................................ 94
Figure 5-2 Procedure for surface-sterilisation of *H.* trifolii J2 in trial (5.1) ........................................................................................................................................................................................................ 95
Figure 5-3 Procedure for surface-sterilisation of *H.* trifolii J2 in trial (5.2) ........................................................................................................................................................................................................ 99
Figure 5-4 Procedure for surface-sterilisation of *H.* trifolii J2 with Hbitane in trial (5.3) .......................... 103
Figure 5-5 Procedure for surface-sterilisation of *H.* trifolii J2 with Hbitane using centrifuge in trial (5.4) ........................................................................................................................................................................................................ 106
Figure 5-6 Procedure for surface-sterilisation of *H.* trifolii J2 with Hbitane using Eppendorf stored in SDW in trial (5.5) ........................................................................................................................................................................................................ 108
Figure 5-7 Procedure for surface-sterilisation of *H.* trifolii J2 with Hbitane using glass test tubes in trial (5.6) ........................................................................................................................................................................................................ 111
Figure 5-8 Procedure for surface-sterilisation of *H.* trifolii J2 with Hbitane in watch glasses in trial (5.7) ........................................................................................................................................................................................................ 113
Figure 5-9 *H.* trifolii J2, females, and cyst monoxenically cultured from surface-sterilised J2 with 0.25% Hbitane in a watch glass on Hoagland & Knop’s agar ...................................................................................... 115
Figure 6-1 A comparison of CCN female from control check with that from *V. chlamydosporium* treatment on Hoagland & Knop’s agar ........................................................................................................................................................................................................ 127
Figure 7-1 Gel electrophoretic analyses of genomic DNA isolated from three *V. chlamydosporium* isolates (duplicates A and B of each isolate).................................................................143

Figure 7-2 Agarose gel (1.2%) with purified-PCR amplification products of a *V. chlamydosporium* isolate 6B, using universal primers ITS4 and ITSS .............................................................144

Figure 7-3 Location on nuclear rDNA of PCR primers designed to PCR-amplify the ITS regions. The arrowheads represent the 3’ end of each primer .................................................................145

Figure 7-4 Sequence alignments of ITS1+5.8S+ITS2 regions of AJ292397 (*Verticillium chlamydosporium* var. *chlamydosporium* (Zare et al. 2000) and four *V. chlamydosporium* isolates (Vc 1A, Vc 6B, Vc 7B, Vc 12B)).................................................................147

Figure 7-5 The effect of annealing temperature (36-40°C) on reproducibility of anchored primer AnCT and RAPD primer PA 10 amplified with *V. chlamydosporium* isolate 5B.............148

Figure 7-6 Agarose gel (1.2%) electrophoretic analysis of PCR amplification products of *V. chlamydosporium* isolates in TAE (A) or TBE buffer (B)........................................................................149

Figure 7-7 Agarose gel (1.2%) electrophoretic analysis of RAPD PCR products of *V. chlamydosporium* isolates using Q-solution (+) and without Q-solution (-)..............................150

Figure 7-8 Anchored CT RAPD primer analysis of 10 *Verticillium chlamydosporium* isolates.....152

Figure 7-9 P. E03 RAPD primer analysis of 10 *Verticillium chlamydosporium* isolates........153

Figure 7-10 P. E15 RAPD primer analysis of 10 *Verticillium chlamydosporium* isolates..........154

Figure 7-11 Gen 3-60-10 RAPD primer analysis of 10 *Verticillium chlamydosporium* isolates......155

Figure 7-12 P. P05 RAPD primer analysis of 10 *Verticillium chlamydosporium* isolates .........156

Figure 7-13 Gen 3-60-3 RAPD primer analysis of 10 *Verticillium chlamydosporium* isolates ......157

Figure 7-14 A dendrogram from cluster analysis of 10 *V. chlamydosporium* isolates [duplicates (A and B) in each isolate] based on RAPD data using six RAPD primers .........................160

Figure 7-15 The growth of *V. chlamydosporium* on PDA (2 week-old culture)..........................161

Figure 7-16 Chlamydomospores (x 400) of 10 *V. chlamydosporium* isolates on WA (1 month old culture)..........................................................................................................................162