

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.

**An analysis of the effects of field-soil disturbance treatments
on arbuscular mycorrhizal fungi.**

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

Master of Sciences in Plant Biology

**Massey University
Palmerston North, New Zealand**

**Donald Wayne Graves
2002**

Abstract

In soil and root ecosystems the partitioning of carbon is ubiquitously affected by interactions with heterotrophic rhizosphere micro organisms, including the potentially mutually beneficial (+,+) arbuscular mycorrhizal (AM) fungi. However, the existence and sustainable management of AM fungi is threatened by prolonged and or intensive disturbances of soil. Therefore this study set out to explore the relationships between plants, soil fungi and soil disturbance treatments. A containerised bioassay of maize seedlings was used to assess root inhabitation of arbuscular mycorrhizal fungi from samples of Manawatu silt loam pasture field soils, methods were adapted from Brundrett *et al* (1996).

Development of a rapid method to visualise the AM fungal inhabited maize seedling roots was enhanced by an alternative light source on an Olympus SZIII dissection microscope. A 100W-equivalent fluorescent light tube produced less heat, but provided approximately five-fold more illumination than the original 20W Olympus incandescent light bulb.

It was found that propagation of maize seedlings during mid to late winter and greenhouse environments with relatively limited light day-length and irradiance levels may have resulted in 'parasitic' (+,-) soil-fungal interactions, or reduced growth of maize seedling plant biomass. Soil fungal parasitism of plant growth was attributed to mutual competition (-,-) for carbon photosynthate resources shared between soil fungi and plant host symbionts.

In addition, a Venn-diagram model is proposed with three entities depicting fungal and plant population interactions that include mutual costs and benefits derived from bi-directional exchange of mineral and carbon nutrients as follows; *mutualism* and *proto-cooperation* (+,+); *neutralism* (0,0); and *competition* (-,-). Intersecting sets of these entities depict a three-way continuum of population interactions; *parasitism* or *predation* (+,-), and prey or host *escape* (-,+); *amensalism* (0,- or -,0); and *commensalism* (0,+ or +,0).

Acknowledgements

Kia ora katoa ... and thank you all many times over to those many people who have helped to fulfil some of my sometimes enthusiastic aims during these mycorrhiza field-soil analyses and microscopy instrument research projects.

There is too short a space here and too many peoples' names to do justice to directly recalling their friendships, kinship, land, workspace and other help and resources that I may have directly or indirectly benefited from during my employment, communications and my part-time studies during the last five years and earlier.

Table of Contents

Chapter 1 Introduction	7
Overview.....	7
Mycorrhizal Symbioses Descriptions and Terminologies.....	8
Soil Aggregation Effects Derived From Soil Fungi and Roots.....	16
Physical Soil-Disturbance Effects on Soil Organic Matter.....	17
Soil Disturbance Effects on Soil Structure and Nutrient Cycles.....	18
Soil disturbance effects on arbuscular mycorrhizal symbioses.....	22
Chapter 2 Methods and Materials	25
Research Aims:.....	25
Field Site Soil Characteristics and Soil Management Histories.....	25
Water-stability of soil aggregates.....	29
Soil chemistry tests.....	30
Control Non-mycological bioassay plants.....	30
Bioassay Pot-Culture Methods for Greenhouse Propagation.....	31
Preparation of maize root-segments for microscopy examinations.....	39
Chapter 3 Results	46
Microscopy Instrument Research.....	46
Soil Nutrient Status Chemical Analyses.....	49
Soil Aggregate Wet-Stability Examinations.....	49
Soil-Disturbance Effects on Percentages of Fungal-Inhabited Root Segments.....	50
Soil Disturbance Effects on Mean Numbers of Fungal-Inhabited Root Segments.....	54
Soil Disturbance Treatment Effects on Root-Fungal Inhabitation ('Infectivity') ratios: RATIOA, RATIOB, and RATIOC.....	59
Soil-disturbance effects on root-fungal inhabitation (infectivity) ratios: RATIOB and RATIOC.....	61
Soil Disturbance Effects on Bioassay Maize Seedling Shoot Lengths (mm).....	64
Soil Disturbance Effects on Maize Seedling Shoot Mean Dry Mass (g).....	69
Chapter 4 Discussion	72
Ecological models and interpretations of soil-disturbance treatment effects on maize seedling AM fungal symbiosis microscopy and biomass data:.....	72
Shoot biomass of maize seedlings and effects of soil disturbance treatments.....	77
Initial assessments of fungal ' <i>parasitism</i> ' of host plant maize seedlings, (+, -).....	77
Comparisons of results with previous mycorrhiza research into a competitive carbon economy induced by low solar irradiance effects.....	79
Discussion of new and existing models and descriptions of mycorrhizal symbiosis plant and fungal population interactions.....	79
A social context of researchers and practitioners of plant, fungal and soil health.....	84
Models and descriptions of symbiosis and arbuscular mycorrhizas.....	85
A proposed Venn diagram model of symbiont population interactions between arbuscular mycorrhizal fungi and plant host roots.....	86
Microscopy Instrument Research.....	99
Chapter 5 Conclusions	102
The observed effects of field-soil disturbance treatments.....	102
Ecological models of interactions between two populations.....	103
Microscopy instrument research.....	103
SPSS.....	104
Appendices	104
APPENDIX F: Pasture Field-soil Sample Sites.....	105
APPENDIX AP: Aerial Photographs of Pasture Field-soil Sample Sites.....	107
APPENDIX P: Pasteurisation of Low-Nutrient Soils as Bioassay Growing Media.....	108
APPENDIX A: Wet-stability soil aggregate tests.....	111
APPENDIX C: Chemical laboratory tests of permanent pasture, new pasture and sandy-silt-loam field-soil samples.....	112
APPENDIX S: Microscopy examinations of squashed maize root segments.....	113
APPENDIX R: Intact Root Segment Microscopy Examinations.....	119
APPENDIX M: Models of Two-Population Interactions.....	129
APPENDIX T: SPSS Tables of Statistical Analysis of Results.....	132
Bibliography	159

Table of Figures

Figure 1 Permanent pasture field site (left) and new pasture field-site (right).....	27
Figure 2 New pasture (left) and permanent pasture (right) field -sites	27
Figure 3 Soil corer and intact soil core.....	28
Figure 4 Diagram of field-soil sample sites within adjacent soil-disturbance treatments, new pasture (NP), and permanent pasture (PP).....	29
Figure 5 Hand press with stainless steel tube containing nylon mesh pouch and field- soil.	34
Figure 6 Sandy silt-loam core removed from bioassay pot container	34
Figure 7 Nylon mesh pouch containing field-soil inserted into pasteurised sandy silt- loam soil bioassay growing media	34
Figure 8 An oblique view of stainless steel seed-drill corer, Perspex jig, bioassay pot and nylon-mesh pouch containing sample of field-soil and fungal inocula.....	35
Figure 9 Greenhouse propagation of maize seedlings growing in field-soil fungal inocula placed within pasteurised sandy silt-loam growing media.....	36
Figure 10 Harvested nylon mesh pouch containing field-soil fungal inocula and unwashed innermost and outermost roots of maize seedlings.	37
Figure 11 Soaking and washed nylon mesh pouch containing pair-planted maize seedlings.	38
Figure 12. Washed maize seedling roots contained in a nylon mesh pouch.....	38
Figure 13 Diagram of transparent OHT gridline placed underneath Petri dishes of intact roots.....	42
Figure 14 Olympus 20W light bulb and replacement 'power-saving' fluorescent light tube	43
Figure 15 Olympus SZIII dissection stereomicroscope and trans-illuminator base fitted with a flexible sheet to provide eye-safety protection.....	44
Figure 16 Petri dish containing intact maize root segments examined by transmitted light microscopy by an Olympus SZIII dissection stereomicroscope.....	44
Figure 17 Olympus SZIII dissection stereomicroscope views of intact maize root segments	45
Figure 18 Mean percentages of fungal-inhabited maize root segments observed per bioassay pot at the first bioassay harvest date 20.07.2000.....	50
Figure 19 Mean percentages of fungal inhabited maize seedling root segments per bioassay pot at the first bioassay harvest date 20.07.2000.....	50
Figure 20 Mean numbers of fungal-inhabited root segments per bioassay pot at bioassay harvest date 20.07.2000.....	54
Figure 21 Analysis of effects by soil disturbance treatments.....	59
Figure 22 Analysis of effects by sample site and soil disturbance treatments.	59
Figure 23 Analysis of effects of soil disturbance treatments at two harvest dates.....	64
Figure 24 Analysis of effects of soil sample-site treatments at two harvest dates.....	64
Figure 25 Analysis of soil disturbance effects and field-soil sample site on two harvest dates, on maize seedling shoot dry mass (g).	69
Figure 26 Analysis of two-species population interactions, type of population interaction and general nature of interactions.	81
Figure 27 Analysis of two-species or population interactions and non-interactions; Effects of relationship on growth and survival of two populations.	82
Figure 28 Venn diagram of three mycorrhizal components or entities : plant, fungus, and soils,.....	86

Figure 29 A proposed Venn diagram model of symbiosis and ecological outcomes derived from two-species or population interactions.	87
Figure 30 Un-grazed new pasture vegetation, facing S.W. towards sandy dunes and Manawatu River.	105
Figure 31 Close-up of un-grazed new pasture vegetation.	105
Figure 32 Un-grazed new pasture vegetation.	105
Figure 33 Legumes and other un-grazed vegetation in new pasture soil disturbance treatments.	106
Figure 34 Permanent pasture vegetation, facing NW towards the Manawatu River and Tararua Ranges.	106
Figure 35 Close-up of permanent pasture vegetation.	106
Figure 36 Steam pasteuriser used to pasteurise low-nutrient sandy silt-loam soil media for propagation of root-fungal bioassays of two pasture soils studied.	109
Figure 37 Wet-stable soil aggregate and soil fungal hyphae.	111
Figure 38 Soil fungal hyphae extending from wet-stable soil aggregate.	111
Figure 39 New pasture artificial soil aggregate - control no added nutrients, soil structure is slaking with soaking in 50% ethanol solution.	111
Figure 40 Permanent pasture artificial soil aggregate – no added nutrients, soil structure is slaking in 25% ethanol solution.	111
Figure 41 A phase-contrast microscope image of a CBE Black-stained dendritic ‘branch’ or ‘small-tree’ shaped fungal <i>arbuscule</i> structure inhabiting a rectangular-shaped maize root cortical cell.	113
Figure 42 ‘Paris-type’ intracellular arbuscules and thicker intra-cellular coiled hyphae.	114
Figure 43 ‘ <i>Arum</i> -type’ fungal arbuscules inhabiting rectangular-shaped root cortical cells, and adjacent to distinctive xylem vessels.	114
Figure 44 A Phase-Contrast microscope image of a densely fungal arbuscule inhabited ‘squashed-root’ prepared segment of a maize seedling.	114
Figure 45 ‘Squashed-root’ method microscopy examination of CBE black-stained fungal arbuscules and intercellular hyphae that inhabited squashed root cortical cells.	115
Figure 46 A group or colony of three fungal arbuscules inhabiting root cortical cells.	115
Figure 47 A single ‘ <i>Arum</i> -type’ fungal arbuscule structure, characteristic of the ‘small-tree-shaped’ dendritic branching morphologies associated with transfer of soil Phosphate (P) from AM fungi to plant root cortical cells and whole plants.	115
Figure 48 A close-up view of an ‘ <i>Arum</i> -type’ fungal arbuscule contained in a rectangular-shaped root cortical cell and adjacent to a xylem vessel.	116
Figure 49 An ‘ <i>Arum</i> -type’ arbuscule and a vesicle occupying adjacent rectangular-shaped root cortical cells.	116
Figure 50 A fungal sporocarp, several spores and hyphae adjacent to a ‘squashed-root’ microscopy method sample.	116
Figure 51 ‘Normarski’ or ‘Differential-interference’ microscope image of colourised plant tissues and CBE black-stained fungal arbuscules inhabiting a squashed maize root segment.	118
Figure 52 Side views of Olympus SZIII microscope fitted with 2X objective lens for viewing intact maize root segments contained in a Petri-dish.	119
Figure 53 Transmitted-light microscopy images of KOH cleared and CBE stained intact maize root segments, (160 X magn.)	120
Figure 54 Low magnification image (28X magn.) of maize roots observed using gridline-intersect method described by Brundrett <i>et al</i> (1996)	121

Figure 55 A higher-magnification image of maize root segments observed by in gridline-intersect method as described by Brundrett <i>et al</i> (1996).....	121
Figure 56 Oblique view of grooved-glass ‘root chamber’ glass microscope slide containing maize root segments	122
Figure 57 UV-induced yellow autofluorescence of unstained living fungi inhabiting fresh untreated maize root segments mounted in root-chambers containing optical quality glycerol.....	124
Figure 58 Image software brightness and contrast intensified root-fungal image	125
Figure 59 CLSM 3-D image of a living fungal arbuscule structure inhabiting a white clover root.....	127
Figure 60. Analysis of two-specie population interactions, type of population interaction and general nature of interactions.	129
Figure 61 Analysis of two species population interactions; effects of symbiosis relationship on growth and survival of two populations, eg. plant roots and soil-fungi.....	130
Figure 62 Venn-Diagram of fungal and plant (<i>biotic</i>), and soil (<i>abiotic</i> and <i>biotic</i>) mycorrhizal associations as illustrated by Brundrett <i>et al.</i> (1996).....	131
Figure 63 Proposed Venn-diagram model of symbiosis and twos-species population interactions.	131