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

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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).

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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.

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Chapter 1 Introduction

Overview

In order to attain and maintain sustainable land use of arable and horticultural soils farmers will be required to scientifically monitor changes in soil quality. Sustainable use of crop soils will require farmer commitment to minimise soil tillage-related risks, such as degraded soil structure, accelerated rates of soil erosion, and changes in soil biota diversity and/or activity, (Altieri, 1987; Doran & Jones *et al*, 1996; Spedding, 1996).

The current study examines soil tillage effects on a wide range of physical, chemical and biological soil quality parameters, which are compared with several others, less commonly used ecological soil quality parameters.

The study primarily examines the effects of soil disturbance on arbuscular mycorrhizas (AM aka VAM), commonly occurring soil-fungi in symbioses with roots of many crops and weeds. Maize seedling roots were grown in field-soil samples from two adjacent pastures, permanent pasture (PP) and new pasture (NP) and compared to maize roots grown in control conditions, in non-mycological (NM) low nutrient pasteurised soil.

In addition, microscopy instrument research tested a new 'energy-efficient' light source, in order to attain high illumination, low heat, and low cost transmitted light microscopy of intact cleared and stained root segments in a Petri dish.

Further instrument research was conducted into microscope glass slide root 'chambers' made of grooved glass, to examine intact (un-squashed) and unstained *in vivo* root segments by UV to blue light epifluorescence microscopy. Similar root-chamber preparation methods were also used to examine intact root segments by confocal laser scanning microscopy (CLSM).

A further study examined the effects of soil disturbance treatments on water-stability of soil structure, and soil aggregation processes, mediated by diverse populations of active soil organisms.

It may be possible that land-use sustainability lessons for the future of soil tillage usage can be found by examining past soil disturbance effects. The tillage and cultivation history of a particular field site may affect specific economic and ecological limitations or requirements on subsequent tillage operations and crop management, (Miller, 2000; Mäder *et al,* 2000; Drijber *et al*, 2000; Karasawa *et al*, 2000a&b, and 2001; Boddington & Dodd, 2000a&b; Kabir *et al,* 1999; Höflich *et al*, 1999).

Mycorrhizal Symbioses Descriptions and Terminologies.

Symbiosis is the term that describes when two or more interactive organisms '*live together*'. The range of outcomes (+, -, 0), resulting from interactions between two populations, have previously been characterised by ecologists, (Odum, 1959 1983, Boughey, 1973), (see Appendix M1).

"*The symbiosis between VA mycorrhizal fungi and autotrophic plants is generally regarded as mutualistic, with the basis of mutualism assumed to be the bi-directional transfer of nutrients*" …, (Smith & Read, 1997, emphasis added).

However, and in contrast, some autotrophic plants grown under adequate mineral and light nutrition conditions may be facultative (non-obligate) fungal-root symbionts. In contrast, AM fungi are obligate biotrophic endo-symbionts of roots, (Smith & Read, 1997).

The current study uses the following definition of fungal "*endo-symbiosis*" of plant roots: all fungi that '*inhabit inner-spaces*' of plant roots, … not only active mutually beneficial plant intra-cellular and inter-cellular mycorrhizal fungal structures inhabiting

plant roots, (Odum, 1983; van Demark & Batzing, 1987; Brown, 1993; Smith & Read, 1997).

It is necessary to avoid potential for reader and or writer confusion about referring to symbioses of mycorrhizae in more strictly ecological terms such as 'mutualism', compared to mycorrhiza fungal taxonomic classification terms, (Odum, 1983; Smith & Read, 1997).

Both the *Basidiomycete* and *Ascomycete* classes of root symbiotic fungi are together described as "ecto-mycorrhizae" (ECM). This term depicts a characteristic mycorrhizal fungal structure, a loosely woven cloak of fungal hyphae, a so-called 'mantle' or 'sheath' fungal structure that may cover the root surface of distinctively shortened root tips of mostly woody plants. Some ectomycorrhizal fungal symbionts may have a capacity as organisms with non-obligate (facultative) saprotrophic nutrient adsorption.

Soil fungal-plant ecto-mycorrhiza (ECM) and associated rhizosphere soil micro-organisms may adsorb soil organic-matter (OM) associated mineral nutrients extracted from soil-Carbon such as decaying soil-biota detritus and more chemically stable soil-humus pools.

In comparison, the *Glomales* genera of the *Zygomycete* soil fungus class, aka *arbuscular mycorrhizas* (AM) are also commonly called "*endo-mycorrhizas*". The taxonomic term "*endo-mycorrhiza*" is morphologically descriptive of intracellular *Arum* type arbuscules, and reflects biological taxonomic sciences from a historic scientific era prior to current sciences of cell biology, physiology, molecular biology, ecology, and current terminology and descriptions (Allen, 1991;Allen 1992).

The taxonomic term *endo-mycorrhiza* used to describe arbuscular mycorrhizas (AM) still remains useful to distinguish between two commonly observed mycorrhiza morphology types: root-intracellular endo-mycorrhizas or arbuscular mycorrhizal fungi

(AMF) or *Zygomycete* fungi; contrasted to *Basidiomycete* and *Ascomycete* fungi that form a characteristic morphology of a root-surface fungal hyphal 'mantle' (cloak) of ecto-mycorrhizae (ECM) fungi, (Smith & Read, 1997; Brundrett *et al*, 1996).

However, both AM and ECM types of mycorrhizal fungi contain root intercellular hyphae, thus, by the previous definition of *endo-symbiosis*, both AM and ECM mycorrhiza groups may be described as fungal endo-symbionts (*endophytes*) of plant roots, (Odum, 1983).

The co-evolution of terrestrial soil colonisation by plants, was probably mediated by biotrophic ancestors of extant arbuscular mycorrhizal AM soil-fungal endo-symbionts of many roots, i.e. fungi capable of forming mutually beneficial root symbioses now known as mycorrhizas, meaning 'fungus-root', Smith & Read, (1997).

Until recently the earliest ancestral evidence came within archaeological fossils from the Devonian period. These plant and fungal fossils show that as early as 360-410 million years ago, early proto-root endo-symbiotic fungi had characteristic 'branched', or 'arbuscule-like' fungal structures that once grew inside cells of primitive terrestrial flora, the *Aglaeophyton*, (Remy *et al.,* 1994).

The early terrestrial inhabitation by plants is hypothesised to have occurred with plant root-like structures that contained intra-cellular fungal structures with characteristic '*small-tree-shaped*' ('arbuscule-shaped') branched fungal hyphae, similar to arbuscules of extant *Glomus* genus AM fungi, (Smith & Read, 1997). More recently, molecular data together with the fossil record in Wisconsin have shown that the co-evolutionary history of arbuscular mycorrhizal fungi (*Glomales*, *Zygomycetes*) and Bryophyte plants goes back at least to of the Ordovician period about 460 million years ago (Redecker, 2000).

Most below ground structures of the gametophytes of many bryophytes, the sporophytes and gametophytes of pteridophytes, as well as the roots of seed plants have co-evolved in soils containing fungal hyphae with functions in both mineral adsorption and transportation networks.

The soil-fungal hyphal filamentous networks are connected back to plant root endo-symbiotic associations of mycorrhizal fungal structures, intercellular hyphae and vesicles, and intracellular vesicles and / or arbuscules, (Smith & Read, 1997).

We only have rudimentary understandings of the evolutionary histories and ecological functioning of today's surviving (extant) mycorrhizal fungal species. Less still is known about the evolution of ancestral or extinct photosynthetic organisms in the colonisation of terrestrial soils by primordial fungal-root symbionts in a wide variety of terrestrial plants, soils, geological, and hydrological localised conditions. Nonetheless, fungal endo-symbioses of root-like structures may have contributed to the survival of symbiotic attributes or processes in modern arbuscular mycorrhizal (AM) plant and fungal species interactions, (Smith & Read, 1997).

In most minimally disturbed soil ecosystems, approximately 90% of existing plant species examined to date may form mycorrhizal symbioses. Of these, approximately two-thirds form arbuscular mycorrhizal AM fungal symbioses. Today, most domesticated crop plants may benefit from effective AM fungal symbioses. However, there are two important economic crop plant families that are non-mycorrhizal, the Brassica family (*Cruciferae*, *Brassicaceae*), and the Beet family (*Chenopodiaceae*), (Smith & Read, 1997).

The arbuscule forming mycorrhizal fungi are classified as belonging to the fungal class *Zygomycetes* that contains only one order *Glomales* comprised of five families (*Glomus, Gigaspora, Scutellospora, Acaulospora,* and *Entrophospora*). The *Glomales* order

fungi contain 149 AM fungal species identified so far, (Harrison, 1999). Molecular phylogenetic analyses of an AM fungal small subunit rRNA has placed the estimated origin of ancestral AM fungi between 353 and 462 million years ago, (Harrison, 1999; Redecker, 2000).

Arbuscular mycorrhizal (AM) fungi asexually reproduce by spores and sporocarps, and may also be propagated from viable (endo-symbiotic) AM hyphal fragments inhabiting *in-vivo* plant roots or root segments. AM fungi have aseptate hyphae, and thus may potentially have large populations of coenocytic nuclei per fungal organism, (Smith & Read, 1997). It has previously been proposed that reassortment of genetically different nuclei may provide a mechanism by which AM fungi maintain genetic diversity, (Zézé *et al*, 1997; Harrison, 1999).

Thus, it may be hypothesised that in aseptate multi-nucleate coenocytic fungi like the arbuscular mycorrhizal fungi (AMF), the occurrence of genetic variations in nuclear DNA within a single organism AM fungal isolate, may then in turn potentially alter genetic effects on population interactions between AM fungal and plant symbionts.

Until more recent molecular phylogenic methods were developed, historical taxonomic classifications of the *Glomales* order, previously known as the *Endogonales* order. Previously the identification of soil fungi has largely relied upon microscopy observations of fungal characteristics or morphological identification of AMF spores. Microscopy examinations include identification and classification of spore wall-layer structures, spore surface-textures, sizes, shapes and colours, (Brundrett et al, 1996).

The characteristic 'branching' or *'small tree-like'* shape of *arbuscule* fungal structures of mature stages some AM fungal species may also be accompanied by *"vesicles"*. AMF *vesicles* are fungal 'storage' structures that are spherical-shaped *'seed-like'* endo-symbiont intracellular and intercellular inhabitants of root-cortex tissues.

Early biologists and taxonomists named the *Glomales* fungal order *Endogonales* (or "*inner-seed*") possibly in recognition of the potential plant-beneficial physiological importance of (AM aka VAM) or (vesicular) and (arbuscular) mycorrhizal characteristic fungal structures that may inhabit root cortex tissues.

Plant mycorrhizal hosts may receive benefits from improved soil-fungal and arbuscule mediated mineral nutrition, eg. phosphate (P), zinc (Zn). Mycorrhizal fungal inhabited plant roots effectively occupy larger soil volumes. In adequate light conditions mycorrhizal-inhabited roots may result in more total photosynthetic fixed carbon, and or more effective water-potentials between plants and mycorrhizal soil-fungi, (Smith & Read, 1997; Augé, 2001)

Mycorrhizal fungal symbionts (mycobionts), together with associated symbiont heterotrophic soil micro-organisms in the area adjacent to mycorrhizal hyphae, the 'mycorrhizosphere', may receive improved access to (C) as energy-rich plant photosynthates. In general, non-mycorrhizal plant growth limits are generally dependant on plant uptake of on relatively immobile soil phosphate (P) minerals.

In contrast, in mycorrhizal inhabited roots, in adequately watered soils, the biological productivity or growth limits may be more dependent on sources of the carbon (C) economy, plant symbiont autotrophy, carbon-dioxide (CO_2) and received irradiance, light quantity and quality (Fitter AH, 1991; Smith & Read, 1997).

Rhizosphere micro-organism populations receive plant-derived and easily absorbed low molecular weight carbon compounds and cytoplasm mineral nutrients derived from root exudates and sloughed root cells. Rhizosphere and mycorrhizosphere associated micro-organisms may increase the mobilisation rates of organic and mineral soil nutrients (Perez-Moreno & Read, 2001).

All fungal structures of mycorrhiza endo-symbionts form in root apoplast spaces outside the plant cell symplast membrane and cytoplasm, (Smith & Smith, 1997; Smith & Read, 1997). In plant root cortex and epidermal tissues arbuscular mycorrhizal fungal structures may inhabit the following root apoplast spaces:

(1) AM fungal arbuscules inhabit root cortex intra-cellular spaces, enveloped within a cortex cell peri-arbuscular membrane;

(2) coiling hyphae of '*Paris* -type' morphology AM symbioses may inhabit root intracellular spaces and are enveloped by a plant root cortex cell membrane;

(3) root cortex cell *trans-wall* spaces may contain either intercellular coiling '*Paris* -type' hyphae, or else '*Arum*-type' arbuscule 'trunks';

(4) the inter-cellular spaces between root cortical cells and or between epidermal root cells may be inhabited by AM fungal (intercellular) hyphae.

Mycorrhizal fungal hyphae that grow outside root tissues in soils or else in aqueous or aeroponic nutrient solutions, may be variously named as 'extracellular', 'extra-matrical', 'extra-radicle' mycorrhizal fungal hyphae, or else may also be named 'mycorrhizal soil fungal hyphae', (Smith & Read, 1997).

As obligate endo-symbionts, AM fungi are biotrophic heterotrophs that require plant carbon and aqueous soil nutrient substrates in order for AM spore propagules to live beyond the spore germination-tube development stage, (Smith & Read, 1997). In contrast to obligate symbiosis of arbuscular mycorrhizal fungi, many cultivated plants commonly display facultative mycorrhizal symbioses, as evidenced by common horticultural potting and soil-less nutrient media practices.

Relatively few plants have evolved strategies to become non-mycorrhizal (NM), ie. 'non-hosts', or 'non-symbiotic' or "*roots that are normally without mycorrhizal*

inhabitants'. A common non-mycorrhizal plant example, the *Brassicaceae* family, includes many wild and weedy genetic relatives of cultivated crop plants. Many non-mycorrhizal plants may have evolved in, and are now commonly found as early seral stage weedy colonisers in physically disturbed soils or soils where low levels of mycorrhizal inocula could be expected, (Smith & Read, 1997).

Crop plant rotations that normally form AM symbioses, that are grown following cultural practices such as soil fumigation, fallow or non-mycorrhizal monoculture crops including Brassicas, have subsequently been shown to have reduced soil and plant colonisation (inhabitancy) by arbuscular mycorrhizas, and subsequently reduced concentrations plant cell minerals such as phosphate, (Thompson *et al,* 2001; Miller, 2000; Mäder *et al,* 2000; Karasawa *et al*, 2000a&b, 2001; Drijber *et al*, 2000; Koide 1985; Koide *et al,* 1989; Singer & Cox, 1998; Borie *et al,* 1997; Douds *et al,* 1988; Hamel, 1996; Kurle & Pfleger, 1994; Jawson *et al,* 1993; Ocampo & Hayman, 1981).

AM fungi are capable of forming symbioses with a wide range of plants. However, in any particular combination of symbionts, a fungus and plant population interaction may have more or less costs to each symbiont (partner). Similarly, in any particular combination of symbionts, AM fungus and plant population interactions may have relatively more or less benefits to each symbiont, (Harrison, 1999; Smith & Read, 1997).

For example, a single fungal endo-symbiont may also share a common plant root system with multiple fungal root symbionts, including mycorrhizal or other soil fungi. Additionally, it is possible for a single AM fungus to form multiple symbioses with adjacent plants, of the same or different species (Smith & Read, 1997). Thus, the possible effects of multi-species mycorrhizal links between communities of plants and soil fungi complicates 'conventional' plant population interactions proposed by simple

pot-trial plant growth inter and intra-population 'competition' experiments performed in pot or growing media without mycorrhizal symbionts (Brundrett *et al.*, 1996; Smith & Read, 1997).

In terrestrial soils inhabited by mycorrhizas and other soil fungal hyphae, there may often be temporal changes in seasonal, geographical and phenological factors across a wide hydrological range of dry and wet climates and habitats. In contrast, there is a relatively improved potential for fungal survival in the internal habitats of root apoplast spaces provided to plant biotrophic endo-symbionts, including soil AM fungi, (Margullis, 1970; 1981; 1982; 1998; Smith & Read, 1997).

Biotrophic endo-symbiotic fungi (*mycobionts*) reside in relatively homeostatic growth conditions, in close proximity to access to carbohydrate energy rich nutrient media from 'host' (*phyto-biont*) plant roots, (Smith & Read, 1997)

Soil Aggregation Effects Derived From Soil Fungi and Roots.

Soil particle enmeshment by soil-fungal hyphae networks, including AM soil-fungi, act in combination with a sticky fungal exudate, eg. 'glomalin', to assist physical stabilisation of soil micro- and macro- aggregates, (Miller & Jastrow, 1990; Tisdall, 1991; Oades, 1993; Haynes & Beare, 1996; Wright *et al.* 1999; Bethlenfalvay *et al,* 1999; Jastrow *et al*, 1998; Wright & Anderson, 2000).

The visual examination of carefully dug roots and rhizosphere soils allows rapid differentiation between non-mycorrhizal (NM) versus mycorrhizal seedlings separately grown in low nutrient soils. It is possible to see increased shoot and root plant growth and aggregated soil 'crumbs' clinging to mycorrhizal inhabited roots and soil fungal hyphae. In contrast, non-mycorrhizal (NM) plants are smaller in size, and result in few to nil formation of soil aggregates adjacent to roots (McFall, 1994).

It may be argued that soil habitat and biotic diversity is increased by soil aggregation rhizosphere biotic mediated effects deriving from fungal hyphal temporal enmeshment and soil bacterial faunal waste 'gum' compounds. Biologically active and 'healthy' soils contain many micro-habitats in which soil biota can exhibit a wide range of nutrient uptake capabilities. A single soil-aggregate can support aerobic organisms at the aggregate surface, whilst possessing microhabitats for anaerobic micro-organisms in the centre of the soil aggregate, (Macgregor, 2001).

Quantitative observations of microbial mediated effects on field soil aggregation may thus be a useful gross indicator of soil rhizosphere micro-organism biological activity. Of further interest to land users and crop scientists, physical stability tests of wet saturated *in-vitro* soil aggregates may also assist assessment of the ameliorative potential for soil micro-organisms to promote the physical stability of soil aggregates and hence subsequently also improve root and soil aeration and drainage, (Carter, 1996; Clapp, 1962).

Physical Soil-Disturbance Effects on Soil Organic Matter.

Effective maintenance of levels of soil organic matter (OM) is critical to effective soil-moisture and nutrient management in physically disturbed soil ecosystems, ie. crop and soil 'production' ecosystems. Soil organic matter (OM) improves the soil's water holding capacity, and soil water solution is more biologically available if temporarily sequestered into hydrophilic carbon-based molecules of complex soil-humic substances that comprise the non-living soil OM. In contrast, excessively dried soil OM commonly becomes hydrophobic, impossible to re-hydrate, and thus then rendered unsuitable as plant growing media. Dry soil OM carbon is also more prone to oxidation into gaseous carbon dioxide CO_2, (Carter, 1996).

Carbon from roots and from rhizosphere soil micro-organisms are generally the major sources of abiotic soil carbon forming soil organic matter (OM). Carbon throughput from photosynthetic captured energy may subsequently become available as consumer 'food' energy for heterotrophic organisms.

Photo-period and daily carbon flux are thus key factors determining biological energy limits to ecosystem productivity. In turn, plant growth is partly dependant on limits of plant-available mineral nutrients, which are often derived from soil micro-organism mineralisation processes. Plant growth rates interact with localised soil biota and with mobile terrestrial populations of heterotrophic or 'consumer' organisms that in turn produce plant-available by-products in soil nutrient solutions in upper soil surface strata, (Carter 1996; Werner, 1992;).

Soil Disturbance Effects on Soil Structure and Nutrient Cycles

Soils habitats that are affected by significant and or frequent soil physical or biological disturbances, e.g. cultivation practices or vegetation removal, have a subsequently reduced biological capacity to produce plant and rhizosphere biomass, such as photosynthate carbon nutrients and other biologically incorporated soil-derived minerals. Subsequently, physically disturbed soil ecosystems have reduced rates of carbon C deposition as a source of soil organic matter, (Carter, 1999; Tan *et al*, 1999; Ball *et al,* 1998; Tisdall, 1996; Baker *et al,* 1996;).

Naturally occurring soil disturbance events such as land-slips, alluvial and volcanic deposits, may bury surface biota and 'top-soil' layers to form unique localised soil sequences of geological strata. Many terrestrial fauna cause a wide range of soil disturbance effects, for example ant mounds and rabbit burrow excavations. In comparison, human population derived soil disturbance is derived from a wide range of

crop preparation and maintenance soil tillage practices, as well as physical and chemical soil-disturbances from construction, consumerism wastes and mineral mining operations.

Contemporary methods of so-called "conventional" tillage are also comprised of both primary and secondary tillage operations. Conventional primary tillage methods now use mould-board ploughs or rotary-hoes, to invert, fracture or mix soils. Primary tillage also assists burial of soil-surface vegetation including pasture, weeds or crop residues, to prevent nutrient and water 'weed' competition within crops. In comparison, secondary tillage practices include many soil cultivation methods that mechanically seriously disturb surface soil strata. Secondary tillage assists the further breaking-up of large clumps of soil, and may also aim to physically and / or chemically eliminate or disturb weed regrowth. In preparation for crop establishment, secondary tillage methods may be used to produce level soil surfaces, aiming to deter uneven soil drainage of surface water. Soil levelling secondary tillage may also be aimed to improve the reproducibility and evenness of seed sowing depth, (Baker *et al*,1996, Carter, 1996).

Further soil biological disturbances may occur following chemical or energy intensive horticultural and arable crop or soil treatments applied for crop health or nutrition, eg. fungicides, herbicides, stubble burning, and soluble mineral fertilisers, (Alvarez & Alvarez, 2000; Dick, 1997; Doran & Jones, 1996; Chen *et al.*, 2000).

The seedbed benefits of conventional tillage methods used for crop establishment include the amendment of hardened soil structure (decreased bulk density), temporarily increased soil-aeration, improved soil-moisture evaporation, and raised soil temperatures. Tillage operations are continued until such changes provide optimal qualities of soil structure, or 'tilth', appropriate to a particular seed-size. Tillage may

also be required to provide a suitable tilth for moulding of soil to form ridges or raised seed-beds, (Quirk & Murray, 1991; Baker *et al*,1996, Carter, 1996).

The timing of tillage operations is aimed to occur so that crop establishment coincides with optimal soil temperatures for seed germination and seedling growth. In temperate seasonal and maritime climates such as in the current field studies in the Manawatu district in the lower and eastern coast of North Island in New Zealand, the optimal timing of soil tillage practices usually coincides with soil warming and drying in spring, or else before maximal soil-wetting and cooling in autumn.

Successful crop seedbed preparation, crop seedling establishment, and effective weed management all require optimal soil-moisture levels so that cultivation tools may pass through soils which are not too hard or dry; or else too wet, and subsequently soft, pliable or sticky when worked, (Baker *et al*, 1996, Carter, 1996).

When conventional tillage practices are intensively repeated, or when tillage and traffic practices occur that are inappropriately timed during excessively dry or wet soils, these soils may become prone to soil compaction and / or treading effects from ploughs, rotary hoes, vehicle wheels, and / or animal feet.

The effects of excess tillage or traffic cropping practices include the following effects: (1) increased soil bulk-density (>compaction), (2) reduced soil macro-pore sizes (<porosity), thereby also (3) decreasing (<) soil-drainage and (4) decreasing (<) soil-aeration, (Bevan, 1944; Faulkner, 1945, 1948; Smith, 1950; Poincelot, 1988; Angers & Carter, 1996; Carter, 1996; Tisdall, 1996; Baker *et al,* 1996; Angers *et al*., 1997).

Conventional soil tillage practices may also potentially increase the risks and or rates of soil degradation from wind and water erosion processes. Soil and water ecosystems may experience widespread physical, biological, and chemical effects derived from soil

disturbance and vegetation clearance cultural practices associated with cropping systems, (Bezdicek *et al.*, 1996; Doran & Jones, 1996; Doran & Parkin, 1996).

As a result of sub-optimally wet soil plasticity, soil smearing and soil compaction pans may result when soils are ploughed when sub-optimally too wet. When dry, such soils form a hard and water impermeable 'plough pan', thus adversely affecting crop soil and plant water potentials, or else increasing drought effects, (Baker *et al*, 1996, Carter, 1996).

In addition, there are further associated risks of inappropriately timed tillage and / or soil treading cultural practices that may at times result in the following effects:

(1) wet smeared and dryness hardened soil plough pans may reduce the upward 'wicking' capillary movement of soil water from lower soil strata and or water tables, into the uppermost strata in seasonally dry cultivated soils;

(2) restricted downward drainage of excess surface soil water by impermeable pan may subsequently increase soil saturation, resulting in soil erosion effects of surface water 'runoff', degrade the visual quality, and increase nutrient and particulate loads of surface waters;

(3) physically restricted root soil volumes with decreased ability for root penetration through sub-surface plough pans, resulting in reduced plant access to lower soil minerals;

(4) poor rates of seedling shoot emergence through rain-splash hardened soil surface compaction 'crusts';

(5) soil mixing dilution or soil burial reducing root zone population concentrations of viable soil micro-organisms, including AM fungi.

(6) Restricted aerobic activities of plant roots and soil rhizosphere micro-organisms.

In more recent years there are growing numbers of tillage practitioners motivated by aims to monitor ecological soil effects derived from tillage practices, and who aim to decrease accelerated soil erosion by a range of soil conservation methods that include conservation tillage direct drilling of seeds into less physically disturbed 'no-till' seedbeds.

Most conservation tillage practices are usually dependant on herbicide weed control to reliably reduce nutrient competition between crops and weeds. In order to protect of soil surfaces from deterioration by wind and water erosion, conservation tillage methods also aim to conserve root-anchored surface residues of plants from the previous growing season, or otherwise aim to achieve at least 30% ground-cover.

Conservation tillage practices are also aimed to reduce the scale of detrimental effects on soil structure and soil biota, by avoiding unnecessary working of soils during periods that are normal peak seasonal soil-moisture minima or maxima, ie. avoiding either excessively wet or dry soils, (Baker *et al*, 1996, Carter, 1996).

Soil disturbance effects on arbuscular mycorrhizal symbioses.

Previously the consideration of the fragile morphology and physically delicate nature of fine filamentous mycorrhiza soil-hyphal networks have prompted researchers to suggest that disruption of AM soil fungal networks may cause observable detrimental ecological effects on the functional integrity of nutrient transport provided by filamentous fungal hyphal networks in natural soils and or in crop and soil AM fungal symbionts. Physical soil disturbance practices may also alter the spatial distribution of soil fungal spores, (Brundrett et al., 1996; Smith & Read, 1997; Miller, 2000).

A body of AM research has examined effects from physical soil disturbance, comparing the effects of soil fragmentation caused by intensive, repeated or prolonged use of

conventional tillage methods. Mycorrhiza researchers have compared tillage effects on AM fungal infectivity (inhabitation) of roots of 'bait' plants; as well as on the effectiveness on plant nutrient uptake, and ecosystem productivity effects resulting from physically and or biologically disturbed soil mycorrhizal hyphal networks, (Miller, 2000; Mäder *et al,* 2000; Drijber *et al*, 2000; Karasawa *et al*, 2000a&b; Karasawa *et al* 2001; Boddington & Dodd, 2000; Kabir *et al,* 1997; Kabir *et al* 1998; Höflich *et al*, 1999; Boswell *et al,* 1998; Singer & Cox, 1998; Hamel, 1996).

There is now a growing body of research which indicates that one of the effects of mechanical soil disturbances, including tillage, is the disruption of functional integrity of ecologically active AM soil fungal networks involved in uptake and bi-directional transport of water, soil minerals and plant carbon to or from mycorrhizal soil hyphae and root endosymbiotic fungal mycelia.

Consequently, the repeated mechanical disruption of soil fungal hyphal networks, as in cropping soils, may reduce the effectiveness of AM symbiosis population interactions on plant drought tolerance and nutrient uptake, thereby retarding plant and AM fungal development (ontogenesis), and subsequently may reduce crop and soil ecosystem biomass growth (productivity), (Miller, 2000; Smith & Read, 1997).

In contrast, there is also contradictory evidence suggesting that physical soil disturbance by conventional tillage practices has positive propagative effects on AM fungi. (Höflich *et al*, 1999). As a possible explanation of the variability of these results, it has been recently been speculated that differences of AM propagative sensitivity to soil-disturbance effects may be due to varied capabilities to propagate from soil-hyphal fragments, and varied AM species reliance on propagation by spores (Karasawa *et al,* 2000a&b; Karasawa *et al,* 2001).

Conventional tillage practitioners have previously used intensive tillage soil disturbances to disrupt pathogenic soil-fungi, in order to control crop production losses resulting from high fungal 'infectivity' disease levels of roots, (Baker *et al*, 1996, Carter, 1996). Problem or disease (infecting) fungi residing within crop plants may have various nutrient acquisition fungal strategies ranging from parasitic to pathogenic: e.g. biotrophic, saprotrophic, and necrotrophic,

Many conventionally managed agricultural and horticultural soils receive intensive inputs of soluble nutrients for pastoral and crop systems. In other nutrient intensive input systems, including soils used for phyto-remedial waste treatment systems, plant roots may directly uptake soluble mineral nutrients. The AM fungal inhabitation (or fungal infectivity) of roots may often but not always be reduced soil-plant ecosystems that have adequate or abundant luxury nutrient consumption circumstances, in plants which contain adequate cellular cytoplasmic levels of phosphate P, or are hypothetically 'P-sufficient' root cells, (Smith & Read, 1997).

Chapter 2 Methods and Materials

Research Aims:

1. Investigate effects of field-soil disturbance on seedling growth and soil fungal infectivity (inhabitation) in roots.

2. Instrument research to assess an alternative light source in order to achieve low cost, low heat and rapid stereomicroscopy methods for cleared and stained intact (un-squashed) root segment examinations.

3. Instrument research to assess grooved glass microscope slides as 'chambers' to contain *in-vivo* intact root segments for examinations by epifluorescent UV microscopy and confocal laser scanning microscopy CLSM.

The costs of materials described, and the details of suppliers of materials or time are annotated as footnotes.

Field Site Soil Characteristics and Soil Management Histories.

Background information into field site pasture soil characteristics discussed here include soil pedology, soil mineral nutrient tests, and plot histories of soil nutrient and production management. In addition to the study focus on arbuscular mycorrhizas and soil fungi, soils were also compared for microbiologically mediated soil effects, in particular, the wet-stability of artificially made, incubated soil aggregates.

Soil pedology descriptions were not feasible as a result of not digging and photographing a measurement scale with soil profiles at the various field-soil core sampling sites in two pasture soil treatments and a pasteurised sandy silt loam soil.

The physical geography of all field sites examined can be characterised as recent river terrace, mostly almost flat, unshaded and unsheltered, and are thus commonly

somewhat wind exposed sites. The pasture field soils examined in current studies were obtained from an organic dairy and crop farm called "Biofarm".[1]

The study field sites are situated on Koehlers Road at Whakarongo, within the flood plains of the Manawatu River and Stoney Creek, a few kilometres northeast and upstream of the city of Palmerston North in the lower North Island Manawatu region of Aotearoa New Zealand.

Field-soil fungal populations were examined from sites at a farm that has practiced 'organic' production methods since 1986. This was primarily because literature indicates that mycorrhizal abundance is often increased under soil conditions which use nil or low external inputs of soluble plant-available nutrients such as super-phosphate. Organic production methods used by Biofarm have excluded the use of fungicides and agrochemical biocides, (Smith & Read, 1997; Brundrett *et al* 1996; Macgregor, 2001; Tait-Jameson, 1986).

Biofarm provides around-the-year pasture for dairy cattle grazing or for making silage. A few sheep are also grazed to assist with pasture weed control. Biofarm also produces arable crops including cereal grains and potatoes, and small quantities of various horticultural vegetable crops.

At paired field study sites, field-soil samples were taken from land that had been managed as permanent pasture (PP) for approximately twenty-five years. Until recently, the adjacent un-grazed new pasture (NP) soils were also previously managed as permanent pastures for approximately twenty-five years. As a result of being located closer to the existing riverbed bend, the new pasture (NP) field site has occasionally previously been flooded and has subsequently drained quickly (Figure 1 and Figure 2).

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[1] Biofarm pasture field-soils were a koha (gift) for farmer-based assessment of soil quality assessment (Sarrantonio *et al*, 1996; Romig, 1996). Many thanks and kia ora korua Jamie & Cathy Tait-Jameson, & katoa & nga whanau, friends and co-workers associated with this soil-disturbance mycorrhizal fungal assessment project.

In October 1999 (spring), the new pasture (NP) field site was conventionally ploughed under and then followed by a summer-fallow soil management with intermittent physical soil disturbances by discing and harrowing secondary tillage, and earth levelling operations. During the summer, fallow and physical disturbance field-site treatments of 1999-2000, new pasture (NP) soils were not sown in pasture seed but instead were allowed to regenerate as mixed and patchy clusters of a mixed herbal ley, ruderal weed plants, pasture grasses, and legumes, red clover and lupin seedlings.

Figure 1 Permanent pasture field site (left) and new pasture field-site (right)

Figure 2 New pasture (left) and permanent pasture (right) field -sites

Field Soil Core Sampling.

All field-soil core sample methods used a 52mm diameter soil corer to obtain 32mm deep soil samples, see Figure 3. Small-sized soil core samples were relatively easy and quick to collect, especially during summer and prototype soil-fungal-root bioassay trials

when pasture soils were dry and hard from wind drying and animal compaction effects. The top 50mm of a pasture soil profile is where approximately 90% of pasture roots are usually present, and hence where most AM spores and soil hyphae are also likely to be found Hall, (1996); Macgregor (2001).

Figure 3 Soil corer and intact soil core.

In order to ensure safe transport in and from the field sites, each of the twelve sets of about twenty field-soil core samples obtained was separately placed into clean plastic bags and packed into 4-Litre sealable and stackable plastic rectangular containers.

The field-soil core sampling method allowed later microscopy assessment of the effects on AM fungal infectivity (inhabitation) variability that may result from field-soil sample sub-sites. As illustrated in Figure 4 and Appendix AP, on June 15th 2000, approximately twenty field-soil core samples were obtained at each of four sample sub-sites (NP1, NP2, NP3, NP4) within the new pasture (NP) field site. Similarly, within the permanent pasture (PP) field-site soil disturbance treatment, approximately twenty field-soil cores were taken at each of four sample sub-sites (PP1, PP2, PP3, PP4).

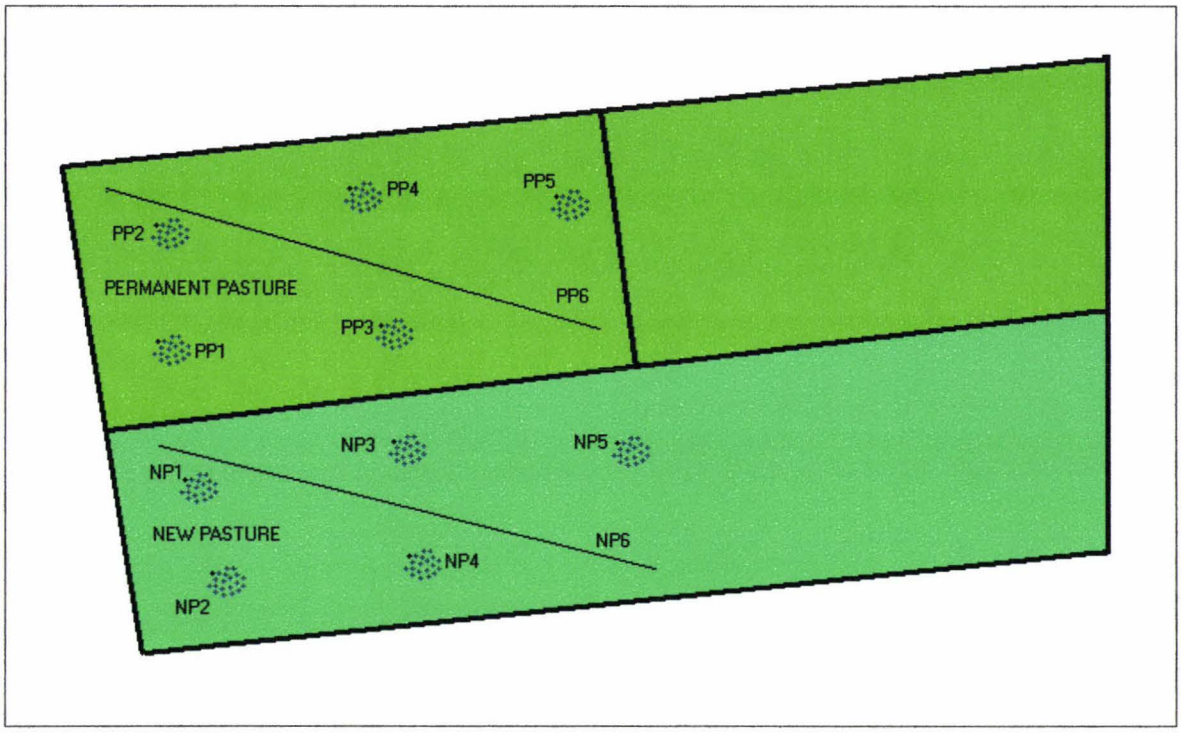

Figure 4 Diagram of field-soil sample sites within adjacent soil-disturbance treatments, new pasture (NP), and permanent pasture (PP)[2].

Water-stability of soil aggregates

As illustrated in Figure 4, soil-cores were obtained from paired field-soil sample sites at permanent pasture and new pasture sub-sites (PP5 & NP5). Each field-site (NP5 & PP5), provided approximately twenty field-soil core samples that were air dried for several days to enable wet-stability tests of artificially made soil aggregates, Clapp *et al.,* (1962), Tisdall (1991), Wright *et al* (1999).

Air-dried field-soils were ground into dust by mortar & pestle. The pulverised field soils were mixed with small quantities of powdered cellulose or glucose, or else had nil added nutrients (control).

The dry dusty soil was rewet by water droplets to artificially make soil aggregates that were placed into Spot Plates[3], sealed and incubated at 28^0C in high humidity atmospheres for three weeks. A high-humidity incubation atmosphere was provided by

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[2] see also appendix AP

[3] Spot Plates (12) INR Soil Science Lab, no cost for use

the placement of four spot plates onto a wet capillary mat[4] cut to fit a plastic tray base. The plastic tray containing four spot plates and artificial soil aggregates were enclosed in aluminium foil.

After three weeks incubation, artificial soil aggregates were soaked in water and or ethanol. Soaked soil-aggregates were examined for differences in water stability or soil physical degradation effects. Biologically mediated soil aggregation and stabilisation were assessed for the effects of glucose and cellulose nutrient availability, compared with non-nutrient amended control soil aggregates, (see Appendix A).

Soil chemistry tests

As illustrated in Figure 4, field-soil samples for later soil chemistry tests were obtained by myself and separately two Massey soil laboratory and field technicians; Furkert I, and an unrecorded other technician. Field-soil sample cores were obtained from both new and permanent pasture field-soil sites (PP6 & NP6) that each contained approximately twenty soil cores obtained along diagonal transects of each pasture [5] (see also appendix C).

Control Non-mycological bioassay plants

In order to provide a non-mycological (NM) control soil media for assessment of *Z. mays* root fungal infectivity (inhabitation), sandy silt-loam soils were excavated from below pasture root zones of sandy silt loam under a sand dune in a nearby riverside field. The sandy silt-loam soil samples collected were steam pasteurised for bioassay plant propagation soil media as described by Brundrett *et al* (1996),[6] (Appendix V).

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[4] 2 X capillary mats (370mm X 250mm) cost $5.00

[5] Soil chemistry tests , Massey University, Institute of Natural Resources. cost $560.00 & $250.00

[6] Crop & Food Research Palmerston North nursery stationary electric steam generator, & mobile trailer soil pasteuriser & mixer. cost $ u/k ? calculate heat units/ cost / hourly staff labour equivalents ... voluntary student labour exchange)

Bioassay Pot-Culture Methods for Greenhouse Propagation

The following points summarise a few reasons why we chose to use bioassay and microscopy methods to examine for the presence of AM fungi inhabiting maize plant roots:

- AM fungi are obligate biotrophic plant symbiotic organisms that must be cultured together with *in-vivo* (living) compatible plant root symbiotic cortex and epidermis tissues.

  The arbuscular mycorrhiza 'AM' fungi, aka 'endomycorrhizas', cannot be grown *in-vitro* as axenic container cultures of fungal micro organisms like it is possible to with ectomycorrhizas and other 'decomposer' fungi that may utilise abiotic (non-living) nutrient growth media.
- Field-soil mixing may cause "irregular" "patchy" or "clumped" dispersal of viable and non-viable fungal spore inocula throughout the field-soil samples collected or examined.
- There are potential problems of stock and pasture management associated with using large sized field-soil samples. These include live-stock and or pasture disruption resulting in increased work and resources required to back-fill pasture soil holes left by a large soil-corer diameter, or else by spade dug field-soil samples.

The current root-fungal bioassay studies propagated and examined young maize seedling roots and shoots. Seedling roots were used for microscopy examinations in preference to more mature larger root tissues with difficult logistics of exponential increases in materials & time costs for experiment preparation, maintenance, harvests and processing required for microscopy examinations. More mature roots are usually

thicker, harder and darker pigmented and hence consequently require further chemical bleaching and dissection treatments.

Nylon mesh fabric has previously been used to provide pouches or semi-permeable mesh barriers to contain various soil-volumes of field-soil in-vivo AM fungal inoculum for the evaluation of physical soil disturbance effects, (Evans & Miller, 1990; Addy *et al*, 1994; Brundrett *et al*, 1996; McGonigle & Miller, 1999; Kabir *et al*, 1999).

I considered that the use many metres of surgical quality non-stretch nylon mesh in order to make field-soil pouches had a prohibitive financial cost. As a result of a research aim to use low-cost methods it was decided to use new stretch-nylon 'stocking' mesh [7], sold in bulk 2Kg bags from paint-trade suppliers, usually used to strain paint impurities.

Nylon stretch stockings were cut into approximately 210mm lengths. Two knots were tied close together in the middle of the open-ended stretch-nylon tube. These were then cut into two 'pouches' or 'socks', each about 100mm long. The nylon mesh soil pouches were sterilised by soaking in 5% hyper-chlorite solution for ten minutes, and then thoroughly rinsed clean several times in distilled water.

The relatively wet soil-core samples of winter required air-drying for three days before field-soils became friable and non-sticky. Air-dried soils from individual pasture sampling sites were combined and thoroughly mixed together using clean rubber-gloved hands. Air-drying of soils assisted the manual crumbling soil cores, and also assisted removal of soils from around roots whilst minimising hand-rubbing derived soil-smearing effects.

Pasture vegetation was removed by hand from soil samples that had previously been combined, dried and mixed. Using a kitchen knife, air-dried pasture vegetation was cut

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[7] Nylon stretch stockings cost = NZ$30.70 per 2kg bag (X2)

into 1cm lengths. A small and approximately equal volume of diced pasture vegetation was then placed into the base of each pre-sterilised nylon pouch. Field soil sub-samples were then added to the pouches containing vegetation fragments. Each pasture field-soil sample site of approximately twenty soil-core samples was assessed by sixteen 120g field-soil replicates contained in nylon mesh pouches.

A nylon mesh pouch containing an approximately 120g field-soil sample was uniformly recompressed into soil 'pellets' by using an adapted second-hand bottle-top press[8], (see Figure 5) to obtain an even bulk-density (un-measured!) of field-soil stretch nylon mesh 'pouches', looking a bit like a compressed peat & mesh-pots trade named 'Jiffy'™ pots.

Placement of Field-Soil Pouches in Bioassay Pots

With the use of a purpose built Perspex 'jig'[9], a suitable sized core of pasteurised sandy silt-loam growing medium was removed from the bioassay pot to accommodate the field soil pouch, (Figure 6)

Re-compressed field soil pellets were placed into one-litre pots containing pasteurised low-nutrient sandy silt-loam soil, (Figure 7). To reduce risks of potential fungal contamination derived from using wooden tools, the soil surfaces of the bioassay pots containing soil pellets were levelled using a purpose-built glass tamper[10].

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[8] apparatus comprised of a second-hand manually operated bottle-cap press fitted with a wooden plunger. This apparatus was also comprised of two stainless steel tubes and two wooden dowel pistons. $10.00 ... hand press $15.00... / wooden dowel cost $10.00 / 4 X stainless steel tubes $35.00.

[9] Perspex 'jig' materials provided for free by Jens Jorgensen, Ecology Engineer, INR Massey, Perspex $ nil recycled materials Perspex glue $20.00

[10] Glass tamper, constructed by IFS glassblower cost $25.00

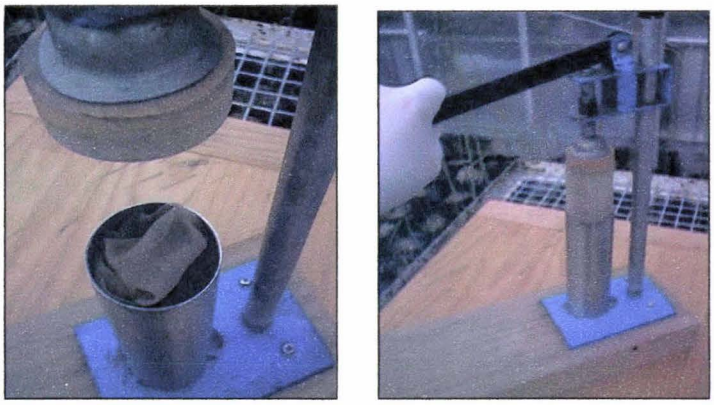

Figure 5 Hand press with stainless steel tube containing nylon mesh pouch and field-soil.

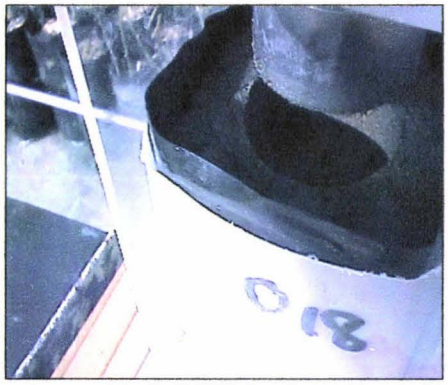

Figure 6 Sandy silt-loam core removed from bioassay pot container

Figure 7 Nylon mesh pouch containing field-soil inserted into pasteurised sandy silt-loam soil bioassay growing media

Bioassay Plant Seeds

Bioassay methods to examine fungal infectivity (inhabitation) of roots requires that seed is free of fungicide treatments commonly found coating commercially available seeds. Early bioassay prototypes used organically sourced untreated *Z. mays* seeds[11], cv. "Early Gem". However, these open pollinating dwarf corn seeds did not have

[11] Certified 'organic' Zea mays , an open-pollinating dwarf sweet-corn cv. 'Early Gem', was obtained from Dave Treadwell at "Organically Yours" , Wellington. cost $ 25.00.

regular plant growth habit phenotypes necessary to compare between bioassay infectivity (inhabitation) and growth replicates, thus making void any potential quantitative AM bioassay analysis. Fortunately it was possible to obtain sufficient quantities of untreated *Z. mays* cv. "CF1" hybrid maize seed[12].

Bioassay Plant Seedling Propagation

In order to reduce potential risks of non soil-fungal contaminants, bioassay maize seeds were surface sterilised for 15 minutes in a 10% solution of sodium hypochlorite, (Brundrett, 1996). After a number of prototype root-fungal bioassay methods, the current research used two maize seeds per bioassay pot.

Each pair of seeds was uniformly sown into a centrally located field-soil pellet. The two surface-sterilised seeds were placed immediately adjacent to the inner wall of the nylon mesh soil pouch, aligned with opposite corners of a bioassay pot container. The regularised placement of seed spacing and depth was achieved using a small diameter steel corer, together with the Perspex jig illustrated in Figure 8.

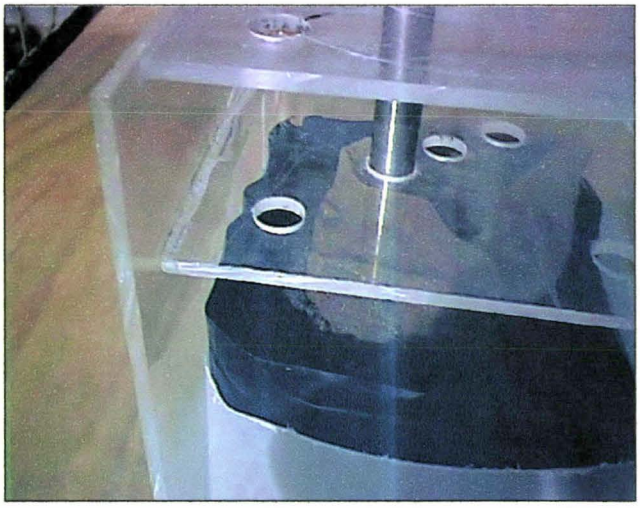

Figure 8 An oblique view of stainless steel seed-drill corer, Perspex jig, bioassay pot and nylon-mesh pouch containing sample of field-soil and fungal inocula.

[12] CF1hybrid Zea mays seeds (quantity) were supplied gratis by Dr Allan Hardacre, Crop & Food Research, Palmerston North, NZ. No cost $ - gratis

The surface cleaned seeds were placed into the prepared holes in the sandy silt loam using tweezers. Oven-dried pre-pasteurised sandy silt-loam was poured into the holes and evenly covered the seeds.

Bioassay maize seeds were sown in mid-winter on June 21st 2000. The seedling maize plants were propagated in a greenhouse at Massey University Plant Growth Unit[13], see Figure 9. Accordingly, supplementary overhead lighting was supplied to each of three benches containing a total of 160 bioassay pots organised in a randomised block design. Supplementary lighting was supplied between the daylight hours of 7.30am and 5pm. Over the duration of propagating bioassay maize plants, the greenhouse minimum recorded overnight temperature was 8.5^0C, whilst the maximum recorded daytime temperature was 25^0C.

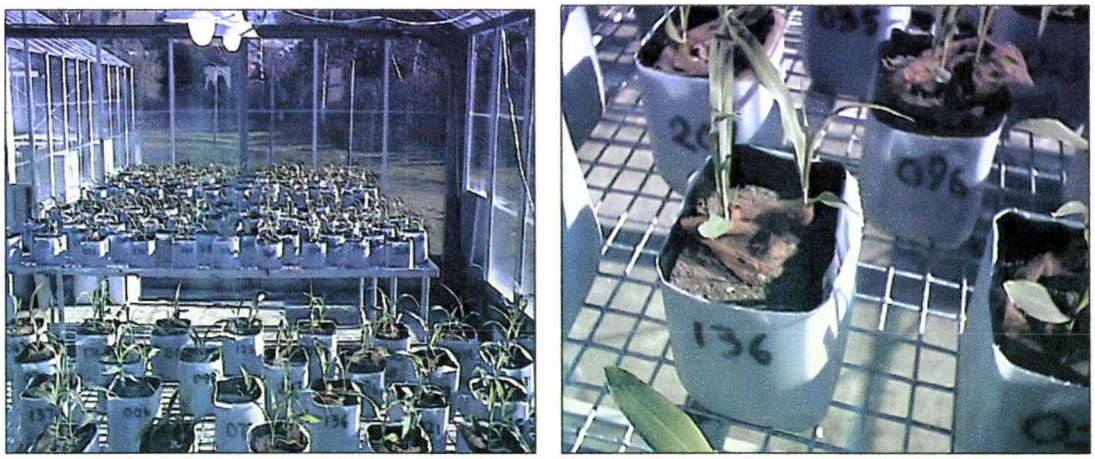

Figure 9 Greenhouse propagation of maize seedlings growing in field-soil fungal inocula placed within pasteurised sandy silt-loam growing media

Bioassay maize plants were watered with distilled water applied in average volumes determined gravimetrically as recommended by mycorrhizal manuals (Brundrett *et al*, 1996). No additional nutrients were supplied to seedling maize plants. In order to ensure regularisation of water use across all bioassay pots during the propagation of bioassay plants, it was also necessary to remove weed seedlings by hand.

[13] PGU greenhouse costs (not recorded)

Bioassay Plant Harvests

The experience derived from labour intensive and time consuming earlier prototype bioassay methods led to the present research methods where bioassay pot plants were not watered for two days prior to root harvesting. Bioassay pots were inverted to remove the PB2 planter bag containing the soil and plants. A number of roots emerging from drain holes of the planter bag were removed and discarded. The planter bag was held upside down by the soil surface, thus enabling careful intact removal of the plants and soil media.

To speed up the harvest method, the outermost bioassay roots were not immediately washed, instead the dry sandy silt loam was gently shaken off the roots, (Figure 10).

Figure 10 Harvested nylon mesh pouch containing field-soil fungal inocula and unwashed innermost and outermost roots of maize seedlings.

Once the majority of the sandy silt loam had been removed, these roots were replaced into the bioassay pots for soaking in distilled water. The soil and plants were then placed onto a large kitchen sieve sitting atop a large volume bucket situated within a bench sink. The field-soil was washed off the bioassay plant roots using a garden hose water jet. As illustrated in Figure 11, the maize plant roots were then returned to the bioassay pots for 10 minutes soaking to loosen and separate the remaining soil from them.

Figure 11 Soaking and washed nylon mesh pouch containing pair-planted maize seedlings.

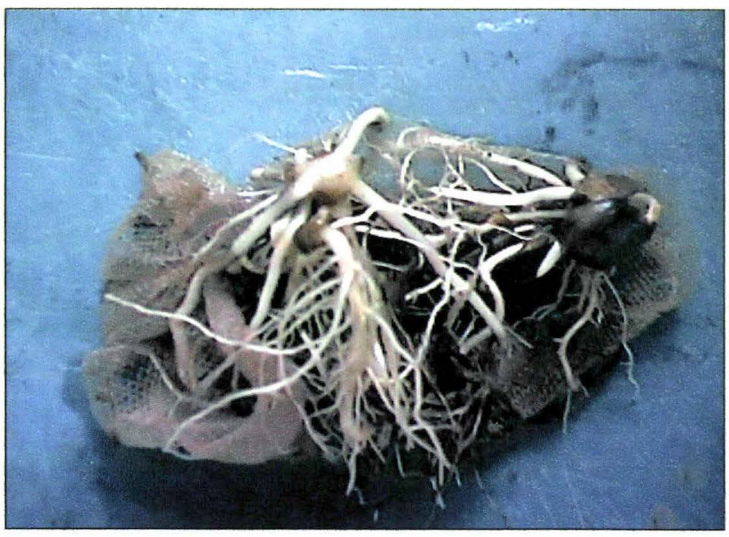

Figure 12. Washed maize seedling roots contained in a nylon mesh pouch

The maize root segments were excised from three regions; (a) upper-outermost roots, (b) lower-outermost roots, and (c) inner-most roots contained in the nylon mesh pouch. Segmented bioassay roots were placed in labelled Universal bottles or Schott 50ml bottles containing distilled water. Soil residual pasture derived plant material was discarded. At the completion of washing each root harvest, the Schott bottles containing roots were drained of water and replaced with 10% KOH[14] to clear cytoplasm from these root segment samples.

Bioassay plant shoot descriptions were recorded for each plant in each bioassay pot. Records were made of the number of fully emerged leaves, as well as the presence of any emergent leaf. As illustrated by Figure 12 maize shoots were excised directly above the uppermost root, the distance between the excision point and the 4th ligule was then recorded for each plant. Oven dried maize bioassay seedlings were oven dried and weighed.

Preparation of maize root-segments for microscopy examinations.

Freshly harvested *Z.mays* maize seedling roots were prepared for transmitted light microscopy examinations by adding to a 10% KOH solution and autoclaved @121°C for 30 minutes[15]. The KOH cytoplasm 'cleared' roots were drained of KOH, and rinsed three (3) times in distilled or reverse-osmosis 'RO' water[16]. Cleared and rinsed roots were stained in 0.1% CBE[17] in lactoglycerol solution[18] autoclaved @121°C for 30minutes.

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[14] KOH - Potassium Hydroxide - BDH Chemicals , 500g @ $ 31.08 (X2)

[15] Autoclaving of bioassay root samples for KOH clearing & for CBE staining, IMBS 'kitchen' services free to IMBS post-grads & staff. much thanks to Pat, cost $ u/k? paid by IMBS enrolment fees & labs

[16] RO-water easy to record, but as not in this study measured then difficult to calculate ? total volumes of water, compared to costs of possible alternative use of rainwater vs. tap water.

[17] Chlorazol Black E stain, cost $50.00 per 100g

[18] Lactoglycerol solution consists of three (3) equal parts (1:1:1); Lactic acid/ Glycerol/ Water, 2.5 Li Glycerol analar $ 121.95 (X2), 2.5 Li Lactic Acid $189.96 (X2) .

Stained root segments were drained of CBE stain and stored in clear lactoglycerol mountant solution. CBE black-stained fungal structures include dichotomously branched arbuscule structures that may occupy intracellular spaces of root cortical cells. Arbuscules are characteristic phenotypic intracellular fungal structures that assist plant mineral beneficial endo mycorrhizal associations.

It was necessary to wear gloves and safety glasses when handling 10% KOH-cleared bioassay roots. It also took patience, time and experience to develop methodological skills in order to avoid potential risks of accidental squashing or breaking soft and delicate root samples. A pair of "bent-nose" tweezers[19] proved to be an indispensable tool for handling KOH cleared root segments. Individual roots were also handled and lifted gently using a plastic pipette[20] with a widened tip to suck up roots till the lowermost end of the root was just inside the pipette tip meniscus.

Bulk samples of root segments were manually transferred into stain or lactoglyerol Maize root segments were stored in, or poured into and from Universal bottles[21], Schott bottles[22], and Petri dishes[23]. Residual roots were rinsed out of containers with recycled lactoglycerol solution.

Microscopy examinations of squashed maize seedling root segments.

A thin layer of cleared and stained squashed root tissues can provide a good media for transmitted-light microscopy (Robson *et al.*, 1994; Brundrett *et al*, 1996; Clapp *et al*, 1996; Varma *et al*, 1998). Squashed-root microscopy methods minimise non-focal-plane light interactions from foreground and background fungal or plant tissues. However, images obtained from squashed-root slide preparations come at a cost of

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[19] A pair of "bent-nose" tweezers, @ $5.50 each (X3)
[20] plastic pipette cost (not recorded)
[21] Universal bottles cost $ nil
[22] Schott bottles cost $ nil
[23] Petri dishes cost $ nil

positional information about roots and fungi. Prototype root-fungal bioassay methods examined squashed maize roots, but were not adopted for the current study primarily because of the additional time and materials required for these methods. Further descriptions of squashed-root preparation and examination methods are provided in Appendix S.

Microscopy examinations of intact (un-squashed) maize seedling root segments

During the development of the current microscopy methods of root-fungal assessment using Petri dishes, intact *in-vivo* roots were also briefly and non-quantitatively examined using purpose-built grooved-glass microscopy slides as water-tight chambers to hold maize root segments, (see appendix R for details).

A numbered sample bottle containing KOH cleared and CBE stained bulk roots was emptied into a Petri dish base. Volumes of lactoglycerol solution were removed by tilting a Petri dish base to allow a small tipped pipette to remove the solution. Care was taken to minimise potential removal of small diameter roots. To obtain an approximately even distribution of intact roots across the Petri dish base, it was necessary to use a combination of tilting and swirling wrist movements and careful pipette use.

A grid-line intersect method illustrated by Brundrett et al (1996) was modified by using an eight by eight grid, (8x X 8y coordinates). The grid was derived from photocopying 5mm X 5mm graph paper onto an overhead transparency (OHT). The diameter of a Petri dish base was drawn onto the photocopied OHT gridline. As illustrated in Figure 13, eight OHT permanent marker coloured pens were used to mark (1 cm X 1 cm) grid lines of eight vertical (x-coordinate) lines, and eight horizontal (y-coordinate) lines.

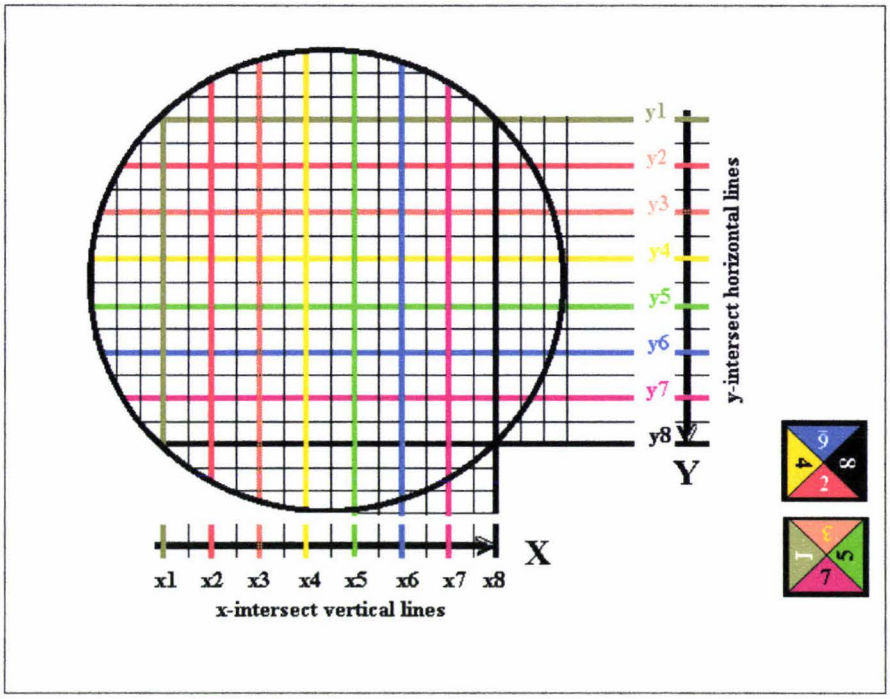


Figure 13 Diagram of transparent OHT gridline placed underneath Petri dishes of intact roots.

As illustrated in Figure 13, a coloured overhead transparency (OHT) plastic sheet was cut out for right-handed use in the shape of a back-to-front capital "D". The OHT was laminated to protect against abrasion and spillages it was placed underneath a Petri dish containing roots during examinations on the SZIII dissection microscope stage.

In order to ensure the continuity of the alignment of root segments in a Petri dish, the Petri dish sample identity label was oriented so that the last alphanumeric root sample position identifier (U or L or I), was always aligned with the vertical (X-coordinate) green line of the laminated gridline sheet.

As depicted in Figure 13, an eight-sided dice was used to randomly select half the total possible transects for scoring root fungal infectivity (inhabitation). The eight-sided dice was used to randomly choose four gridline intersect lines from eight available x-coordinates; and to choose four of eight y-coordinate gridlines.

The microscopy methods for root-fungal bioassays used an Olympus SZIII dissection stereomicroscope, mounted on a standard Olympus 'trans-illuminator' base fitted with a modified transmitted light source. The original light transmission sources for the SZIII

'trans-illuminator' include an "Edison-screw" light-fitting Olympus 20W incandescent bulb. The modified light source was a 21W, 'power-saving', small fluorescent light tube. The alternative fluorescent light source provided the equivalent illumination of a 100W incandescent light bulb[24] (Figure 14).

As a means to assist root segment grid-line intersect measurements, the Olympus SZIII dissection stereomicroscope was re-configured with the pillar at the back of the stage, and the microscope head and eyepiece positioned away from the pillar, 180^0 away from the microscope's normal operational configuration. This microscope configuration assisted the movement of the Petri dish across the transparent stage fitted atop a trans-illuminator base. A cardboard gasket set was used to raise the level of the SZIII microscope stage up to level with the top of the transilluminator base.

The brightness of the 'power-saving' fluorescent light source meant that there was a potential to be an eye hazard risk. This potential eye risk was avoided by using a flexible reflective screen and weighted paper-copy holder[25], (Figure 15).

Figure 14 Olympus 20W light bulb and replacement 'power-saving' fluorescent light tube

[24] Osram 20 watt "Energy Saver" fluorescent light $19.50
[25] Copy holder $9.95

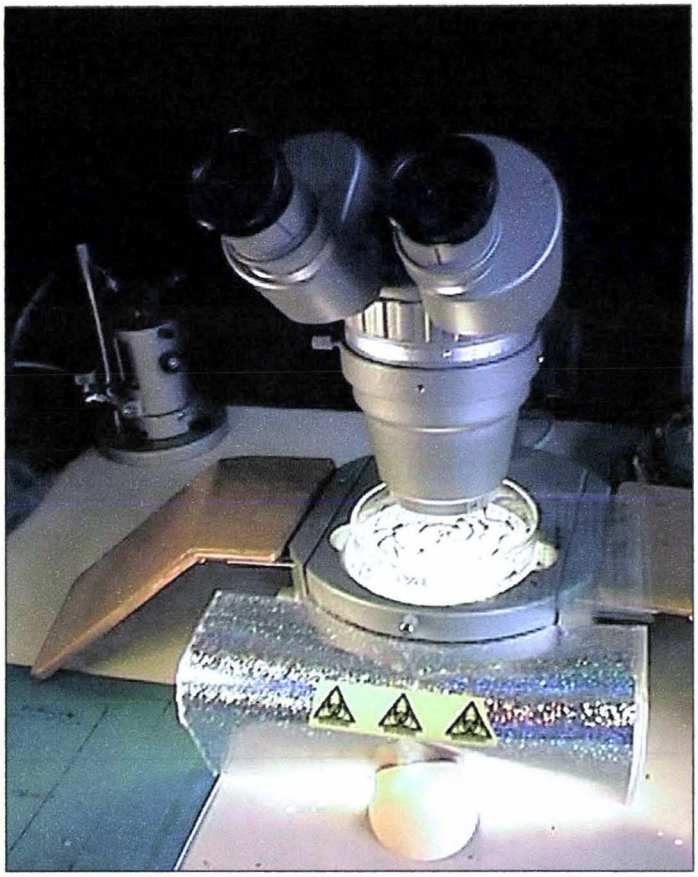


Figure 15 Olympus SZIII dissection stereomicroscope and trans-illuminator base fitted with a flexible sheet to provide eye-safety protection

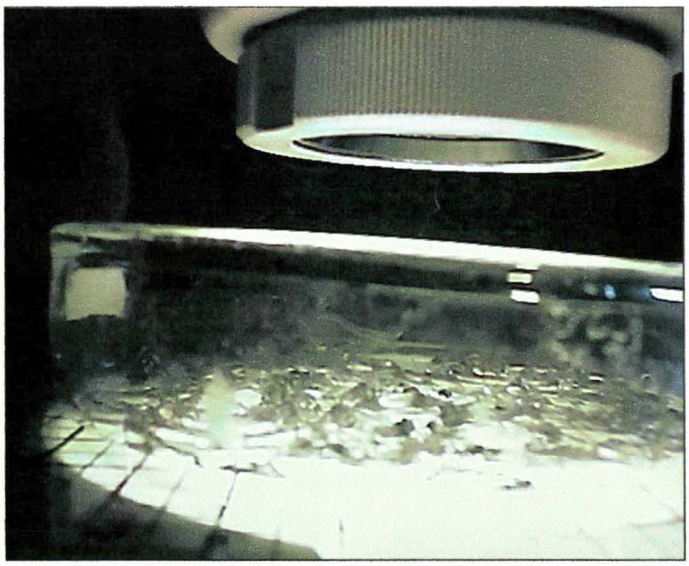


Figure 16 Petri dish containing intact maize root segments examined by transmitted light microscopy by an Olympus SZIII dissection stereomicroscope.

The current 'grid-line root intersect' methods used for counting root-fungal arbuscule populations that intersect gridlines were derived from Brundrett et al, (1996). Cleared and CBE stained maize root segments were examined in Petri dishes in order to reduce

method time requirements, and to reduce material costs for quantitative microscopy to assess AM root-fungal 'infectivity' (inhabitation) , (see Figure 16).

At relatively low magnifications, between 28X to 160X, light transmission microscopy can provide acceptable quality images of intact roots containing fungal structures. These images are suitable for quick visual screening of fungal inhabited (infected) roots, (see Figure 17).

As recommended in literature regarding 'grid-line (root) intersect' methods, three separate counts were made of each of the eight transects counted per Petri dish, [ie. a) root numbers (ROOTS); b) roots containing intracellular arbuscules (ARBUSCUL); and roots containing intercellular hyphae (HYPHAE)], (see Brundrett *et al,* 1996).

Earlier studies of intact root segments also counted fungal vesicles, and appressoria (fungal root infection and potential entry-points). However, these were no longer counted after consultation about similar events in root-hair curling pre-infection events in nodulation of legume roots being colonised or inhabited by *Rhizobia* soil bacteria populations. Fungal appressoria structures and intercellular hyphae are not mycorrhiza specific fungal structures that inhabit plant roots.

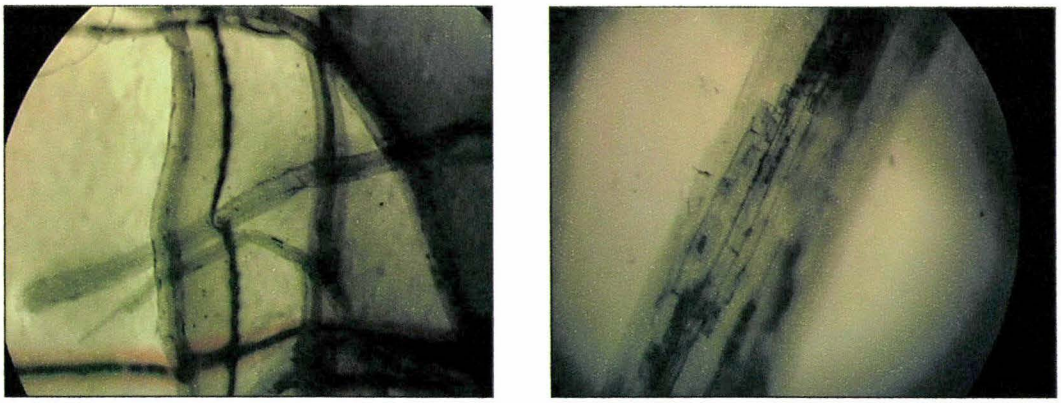


Figure 17 Olympus SZIII dissection stereomicroscope views of intact maize root segments (28X & 160X).

Chapter 3 Results

Microscopy Instrument Research

Transmitted-Light Dissection Microscope

As illustrated in Chapter 2 methods and images in Appendix R1, the aims of instrument research to attain low-cost, low-heat stereomicroscopy methods were met by observations that used an alternative light source, a 'power-saving' 21W fluorescent light tube. The adapted Olympus SZIII dissection stereomicroscope was successfully practically tested by 'gridline-intersect' root-fungal bioassay examinations of un-squashed (intact) maize seedling root segments.

The 1960's model Olympus SZIII dissection stereomicroscope also provided images at higher maximum magnifications (parfocal zoom up to 160X) compared to a maximum (100X) magnification from locally available modern research dissection microscopes. Although in current studies digital images were not used to make measurements, a tripod mounted Casio LCD 100 digital camera provided lower resolution images but relatively good visualisation of CBE stained fungal structures in root segments, with low costs for camera hardware and software. (See Appendix R2)

Ultra-Violet Microscope

In further instrument microscopy research into examinations of mycorrhizas and plant roots, grooved glass microscope slides were tested as sealed chambers to contain un-squashed (intact) *in-vivo* root segments of *Allium porrum* and *Trifolium repens* seedlings that were examined by ultra-violet (UV) microscopy.

There were only relatively low-resolution root and fungal images obtained from ultra-violet (UV) reflected-light autofluorescence of unstained fungal structures inhabiting *in-vivo* root segments, (see Appendix R3).

However, despite low-resolution images, the UV autofluorescence root-fungal microscopy examination methods provided rapid and low-cost examinations of intact *in-vivo* root segments prior to confocal laser scanning microscopy (CLSM) autofluorescence examinations.

Confocal Laser Scanning Microscope

In comparison to UV autofluorescence microscopy results, the grooved glass slide 'root-chambers' used to contain *in-vivo* intact *Allium porrum* root segments provided relatively high-resolution digital images visualised by CLSM blue light (488nm) autofluorescence of fungal and root structures. Multiple 'optical section' CLSM images were overlayed by software to generate 3-D visualisations of autofluorescent arbuscule fungal structures inhabiting fresh, *in vivo*, unstained and uncleared seedling root segments, (see Appendix R4).

Fungal and plant tissue positional information was retained in microscopy observations of fungi inhabiting root cortical and epidermal tissues of un-squashed (intact) root segments. See Appendix R5 for photographs illustrating two CLSM microscopy z-series of multiple optical sections, and composite 3-D images.

However, there were relatively high operational and maintenance costs associated with root sample CLSM examinations, due mostly to an expensive Argon/Krypton gas mixture ($Ar_{(g)}$ /$Kr_{(g)}$) as the laser source in a Leica Confocal laser scanning microscope. [26]

Transmitted-Light Compound Microscopes

Non-polarised transmitted light microscopy observations of glass slides with a thin layer of squashed roots and fungi, provided very good high-resolution images of individual or

[26] The Leica CLSM service user charge-out rate was NZ$20.00 per hour for organizations or individual researchers within the Leica CLSM purchase group of research institutes. The charge for CLSM use by other organizations or individuals was NZ$120.00 per hour.

groups of fungal arbuscules. In contrast to CLSM examinations of intact root segments, there was relatively little tissue positional information available from 'squashed-root' microscopy observations of fungal structures occupying plant root epidermal and cortical tissues.

From my relatively inexperienced 'eye', the squashed-root microscopy observations were relatively time-consuming and material-intensive methods to accurately assess and differentiate between squashed tissues of root and fungal symbionts. Images of Chlorazol Black E (CBE) black-stained root-fungal structures were viewed located within backgrounds and foregrounds of plant tissues ranging from clear and light, to opaque and dark.

Phase Contrast Microscopy

Excellent visualisation of KOH cleared and CBE stained squashed root-borne AM fungi can be achieved by Phase-Contrast microscopy, see Appendix S to compare photomicrographs of squashed-root and fungal samples. The Phase Contrast microscopy photographs illustrate 'small-tree-shaped' fungal arbuscule structures growing within rectangular–shaped maize seedling root cortical cells.

Phase Contrast microscopy images of squashed fungal and root tissues sometimes had relatively low contrast to each other, at least in colour, if not in morphological shapes when viewed close-up. The phase-contrast CBE black-stained AM fungal "arbuscule" structures illustrated in Appendix S have a relatively poor contrast to surrounding plant root tissues which are dark and light tones of green, brown or grey.

Differential Interference Microscopy, or Normarski Microscopy

As shown in Appendix S Normarski microscopy produces polarised filtered light that may cause double refraction or "bi-refringence" colouring effects in certain plant

tissues. On a magenta background, plant cell wall micro-fibre materials like cellulose appear purple, blue and yellow. It was relatively easy to rapidly identify between arbuscules, CBE black stained fungal structures that occupied rectangular-shaped root cortical cells, and elongated rectangular-shaped physically adjacent "bi-refringence" coloured plant root cells such as helical-shaped xylem vessel walls.

In contrast to the preceding 'squashed-root' microscopy methods, much less time and material resources were required in the current root-fungal bioassay microscopy methods that examined intact root segments temporarily mounted in drained Petri dishes.

In comparison, it required more time to randomly select squashed root segments, permanently mount these onto glass slides, and to engrave slides for identification prior to viewing by a transmitted light compound microscope. Root samples that were permanently mounted between glass slides and cover slips also required adequate storage containers.

Soil Nutrient Status Chemical Analyses

As illustrated in tables in Appendix C, the laboratory results found a similar trend in decreased plant-available phosphate, a lower Olsen-P resulting from soil disturbance effects an both an early experimental sites (np1,np2, np3, np4), and later from (NP6) field-soil samples at the current paired pasture transect sites.

Soil Aggregate Wet-Stability Examinations

Inconclusive results were found from wet-stability examinations of artificially made soil aggregates as described by methods in chapter 2, see Appendix A. for photos of soil aggregates and soil-borne fungal hyphae.

Soil-Disturbance Effects on Percentages of Fungal-Inhabited Root Segments

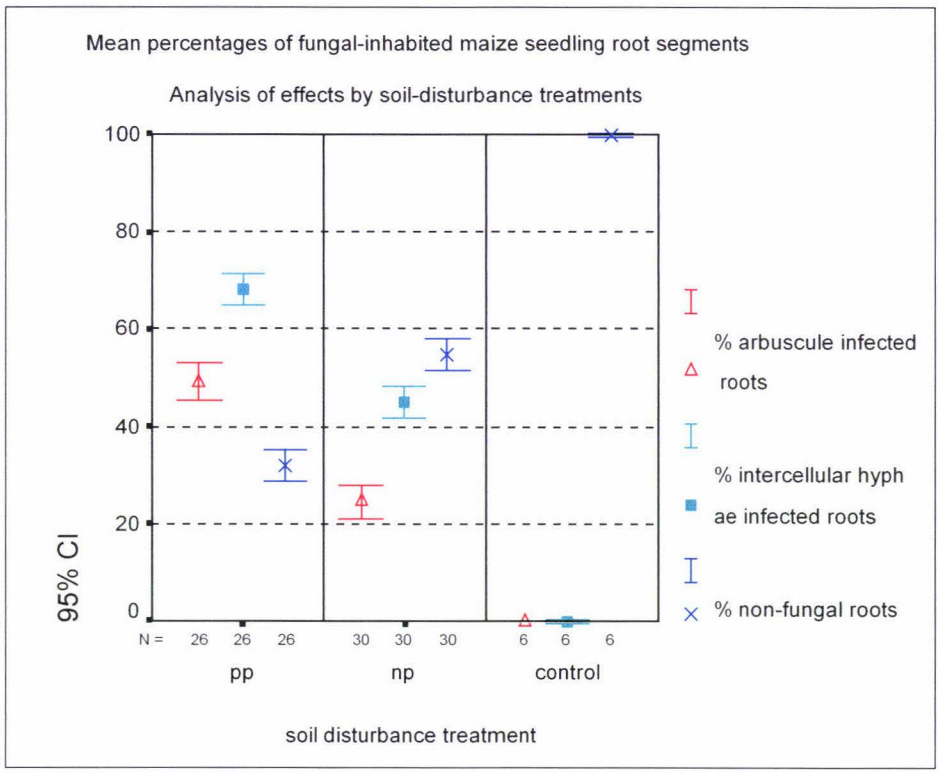


Figure 18 Mean percentages of fungal-inhabited maize root segments observed per bioassay pot at the first bioassay harvest date 20.07.2000.

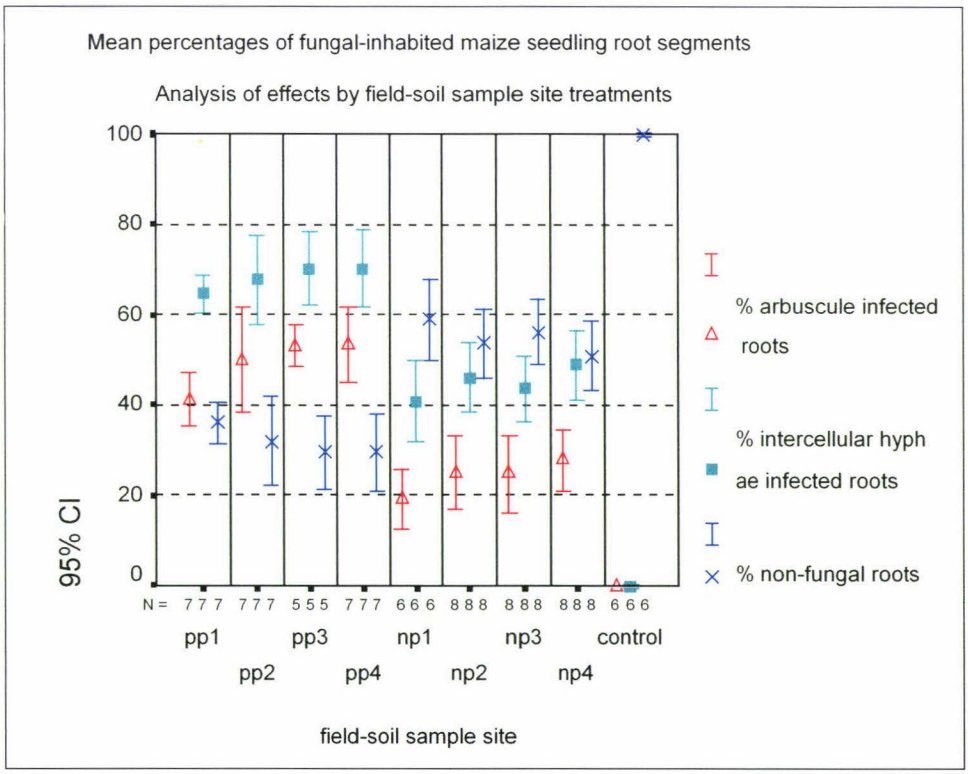


Figure 19 Mean percentages of fungal inhabited maize seedling root segments per bioassay pot at the first bioassay harvest date 20.07.2000.

The effects of soil-disturbance treatments on root-fungal inhabitation were examined using root-fungal microscopy and shoot biomass mid-winter to late-winter bioassays of greenhouse grown *Z. mays* seedlings (Crop & Food Research CF1 maize hybrid). Bioassay maize seedlings were grown in stretch-nylon mesh pouches that contained fixed soil-volume 120g weights of two pasture field soil samples, permanent pasture (PP), new pasture (NP), and in a control soil treatment, non-mycobiont (NM) (ie. non-fungal) pasteurised sandy-silt loam.

As illustrated in Figure 18 and Figure 19, maize seedling roots grown in field-soil samples from the permanent pasture (PP) treatment had a majority (68.16%), or approximately two-thirds (≈2/3) of examined maize root segments inhabited by intercellular hyphae (mycelia). In comparison, maize seedling roots grown in field-soil samples from a new pasture (NP) treatment contained less fungi (49.32%), or approximately half (≈1/2) the examined roots were inhabited by intercellular fungal hyphae.

Maize seedling roots grown in field-soil samples from permanent pasture (PP) soil-disturbance treatment had (45.16%), slightly less than or approximately half (≈1/2) of the roots examined inhabited by fungal arbuscule branching structures. In comparison, maize seedling roots grown in field-soil samples from the new pasture (NP) treatment had a minority (24.65 %), or approximately one quarter (≈1/4) of examined roots inhabited by intracellular fungal arbuscules.

The gridline intersect microscopy examination methods previously described were used to compare maize seedling roots grown in field-soil samples from both a permanent pasture (PP) and a new pasture (NP).

These observations show that in the most disturbed soil treatment, field-soil samples from new pasture (NP) bioassay maize root segments showed a decline of percentage

fungal 'infectivity' (inhabitation) effects by both intercellular hyphae (mycelia) and intracellular fungal arbuscule structures.

In order to calculate from microscopy data, numbers and percentages of grid-line intersects with non-fungal (non-inhabited) maize root segment intersects, (NONFUNGA) data were derived from observed total numbers of maize roots intersecting gridlines (ROOTS), minus observed gridline intersect counts of maize roots inhabited by CBE-stained root intercellular fungal hyphae (HYPHAE).

The percentage of gridline intersects examined with non-fungal (non-inhabited) maize root segments (NONINF) was (31.83%) or about one third (≈1/3) in maize seedling roots grown in field-soil samples from permanent pasture (PP) treatment. In maize seedlings grown in new pasture (NP) field soil samples, the fungal-root non-inhabitation value increased to (54.84%), a little more than half (≈1/2) maize seedling roots examined were non-fungal root segments.

Non-mycobiont (NM) (non-fungal) control replicates of maize seedling roots were grown in clean milk bottle pots containing 1 litre of pasteurised sandy silt loam soil. Microscopy observations showed nil (0.0%) intracellular fungal inhabitation of roots, and only one single maize root segment inhabited by an intercellular fungal hypha.

As illustrated in Figure 19, graphs of the percentage (%) root-fungal inhabitation indicates relatively good data replication between four new pasture (NP) field-soil sample sites (NP1, NP2, NP3, NP4). Likewise there was also good data replication between four permanent pasture (PP) field-soil sample sites, (PP1, PP2, PP3, & PP4). For further field site information see Biofarm aerial photograph & diagram of field-soil sample sites, Appendix AP.

A General Linear Model (GLM) Repeated Measures Analysis was used to compare percentages of maize seedling roots inhabited with arbuscules (INFARBUS) and

intercellular hyphae (INFHYPHA) (Within-subject factors); and type of field-soil disturbance treatment (PP or NP) and individual sample sites (Between-subject factors).

As illustrated in Appendix T1, SPSS statistical software outputs from the GLM Repeated Measures Analysis *Multivariate Tests*, and *Tests of Within-Subject Effects* on two (FUNGUS) factors: [percentage root-fungal inhabitation levels, ie. 'percentage (%) root-fungal inhabitation (INFARBUS) and (INFHYPHA)]. Both statistical tests calculated root-fungal percentage inhabitation levels with a very high value (F= 421.345), and with a high statistical significance (sig. = 0.000).[27]

As illustrated by Appendix T1, SPSS outputs from the GLM Repeated Measures Analysis *Tests of Between-subjects effects* by two (DISTURBANCE) soil disturbance treatment factors: [new pasture (NP); and permanent pasture (PP)], were calculated to have a high F value (F= 125.296), with a high statistical significance (sig. = 0.000).

As also illustrated by Appendix T1, SPSS outputs from the GLM Repeated Measures Analysis *Tests of Between-subjects effects,* two (SOILSITE) factors, field-soil sample sites: [new pasture sites (NP1, NP2, NP3, NP4); and permanent pasture sites (PP1, PP2, PP3, PP4), were calculated to have a low F value (F= 1.563), with a low statistical significance (sig. = 0.178).

[27] Multivariate tests, Greenhouse-Geisser & Schericity Assumed tests are all equivalent when a within-subject factor has two levels.

Soil Disturbance Effects on Mean Numbers of Fungal-Inhabited Root Segments

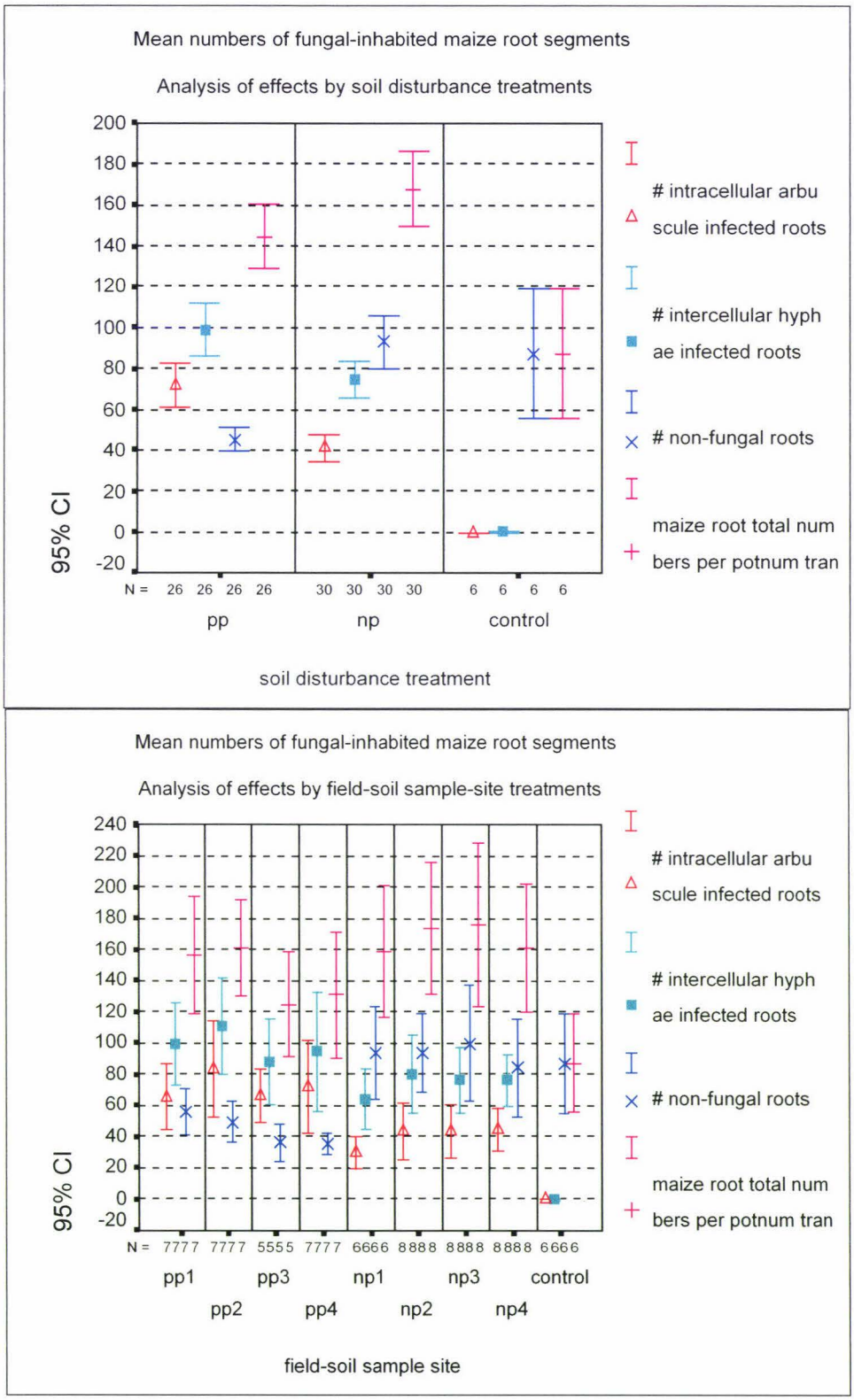


Figure 20 Mean numbers of fungal-inhabited root segments per bioassay pot at bioassay harvest date 20.07.2000.

As illustrated in Figure 20 and statistically analysed in Appendix T2, microscopy examination data relating to numbers of maize roots intersecting gridlines provided an adequate statistical analysis of soil disturbance effects, without requirement to calculate percentages of root-fungal inhabitation, as previously described.

Soil-disturbance Effects on Mean Total Numbers of Maize Root Segments per Sample Soil-Volume.

Each bioassay pot and stretch-nylon mesh pouch contained a uniform volume and weight of field-soil and/or non-fungal control soil. Thus, as illustrated in Figure 20, analyses of the total numbers of maize roots intersecting gridlines per bioassay pot replicate, indicates that there may be statistically significant differences in mean total root numbers between field-soil treatments from permanent pasture (PP = 144.85, sd. = 49.64 roots); new pasture (NP = 168.20, sd. = 38.45 roots); and non-mycological (NM = 87.67, sd. = 30.30 roots) in pasteurised control soils.

As described in Figure 20, replicates of field-soil sample sites of permanent pasture (PP), or new pasture (NP) soil disturbance treatments, two field–soil sample sites (PP3) and (PP4), appear to have relatively lower total numbers of root segments per replicate bioassay pot/pouch soil sample.

In contrast, there is little graphically discernable difference between data of total root segment numbers from the remainder of field-soil sample site replicates (pp1, pp2, np1, np2, np3 & np4). The lowest total numbers of maize roots observed were from control (NM) non-mycobiont (asymbiotic) pasteurised bioassay soils.

Soil Disturbance Effects on Mean Numbers of Fungal Inhabited Root Segments per Soil-Volume

As illustrated by Appendix T3, an SPSS General Linear Model (GLM) Repeated Measures Analysis was used to compare effects on three (FUNGUS) factors, total numbers of observed gridline intersects with maize root segments (ROOTS), mean numbers of roots inhabited with arbuscules (ARBUSCUL), and roots inhabited with intercellular hyphae (HYPHAE), (Within-subject factors).

The observed microscopy data of root-fungal inhabitation levels were tested for effects derived from field-soil disturbance treatments, permanent pasture (PP) and new pasture (NP), control (NM) pasteurised soils; and field-soil sample site effects (Between-subject factors).

As illustrated in Appendix T2, SPSS General Linear Model (GLM) Repeated Measures Analysis *Multivariate Tests*, and *Tests of Within-Subjects Effects* assessed two (FUNGUS) factors; observed numbers of root segments inhabited by fungal arbuscules (ARBUSCUL); and observed numbers of roots inhabited with intercellular hyphae (HYPHAE). These two (FUNGUS) data sets (ARBUSCUL) and (HYPHAE) were calculated to have a high F value (F= 250.957), with a high statistical significance (sig. = 0.000).[28]

As also illustrated in Appendix T2, SPSS outputs of General Linear Model GLM Repeated Measures Analysis *Multivariate Tests*, and *Tests of Within-Subject Effects* assessed two (FUNGUS) factors: (ARBUSCUL) and (HYPHAE); two field-soil disturbance treatment factors (DISTURBA), permanent pasture (PP) and new pasture (NP), and control non-mycological (NM) pasteurised soils. These (FUNGUS) and

[28] Multivariate tests, Greenhouse-Geisser & Schericity Assumed tests are all equivalent when a within-subject factor has two levels.

(DISTURBA) data were calculated to have a low F value (F = 2.942), with a medium high statistical significance (sig. = 0.093).

As illustrated by Appendix T2, SPSS GLM Repeated Measures Analysis *Tests of Between-subjects effects,* assessed two (DISTURBANCE) soil disturbance factors: [new pasture (NP); and permanent pasture (PP)], and calculated a medium F value (F= 17.225), with a high statistical significance (sig. = 0.000).

As also illustrated by Appendix T2, SPSS outputs from the GLM Repeated Measures Analysis *Tests of Between-subjects effects,* compared (SOILSITE) factors, new pasture field-soil sample sites (NP1, NP2, NP3, NP4); and permanent pasture field-soil sample sites (PP1, PP2, PP3, PP4). These data were calculated to have a low F value (F= 0.605), with a low statistical significance (sig. = 0.725).

As illustrated by Appendix T3, SPSS outputs of General Linear Model GLM Repeated Measures Analysis *Multivariate Tests* assessed three (FUNGUS) factors as previously described: [(ARBUSCUL); (HYPHAE); and total numbers of root segment intersects observed per sample bioassay soil volume examined (ROOTS). SPSS *Multivariate tests* of these three (FUNGUS) data sets were calculated to have a high F value (F= 237.204), with a high statistical significance (sig. = 0.000).

As illustrated by Appendix T3, SPSS outputs of General Linear Model GLM Repeated Measures Analysis *Multivariate Tests* assessed three (FUNGUS) factors, and two soil-disturbance treatment factors (DISTURBA), were calculated to have a medium F value (F = 20.049), with a high statistical significance (sig. = 0.000).

As illustrated in Appendix T3, SPSS Repeated Measures analysis *Tests of Within-Subject Effects* assessed three (FUNGUS) factors: (ARBUSCUL), (HYPHAE) and (ROOTS) that were calculated to have a very high F value (F = 417.309), with a high statistical significance (sig. = 0.000).

As illustrated in Appendix T3, SPSS *Tests of Within-Subject Effects* assessed three (FUNGUS) factors: (ARBUSCUL), (HYPHAE) and (ROOTS) and soil-disturbance treatments (DISTURBA). These (FUNGUS) and (DISTURBA) data sets were calculated to have a medium F value (F = 33.493), with a high statistical significance (sig. = 0.000).

As illustrated by Appendix T3, SPSS outputs from the GLM Repeated Measures Analysis *Tests of Between-subjects effects,* two (DISTURBA) soil disturbance factors: [new pasture (NP); and permanent pasture (PP)], were calculated to have a low F value (F= 1.740), with a low or marginal statistical significance (sig. = 0.193).

Also illustrated by Appendix T3, SPSS outputs from the GLM Repeated Measures Analysis *Tests of Between-subjects effects,* assessed two (SOILSITE) factors, new pasture field-soil sample sites (NP1, NP2, NP3, NP4); and permanent pasture field-soil sample sites (PP1, PP2, PP3, PP4). These data were calculated to have a low F value (F= 0.574), with a low statistical significance (sig. = 0.749).

Soil Disturbance Treatment Effects on Root-Fungal Inhabitation ('Infectivity') ratios: RATIOA, RATIOB, and RATIOC.

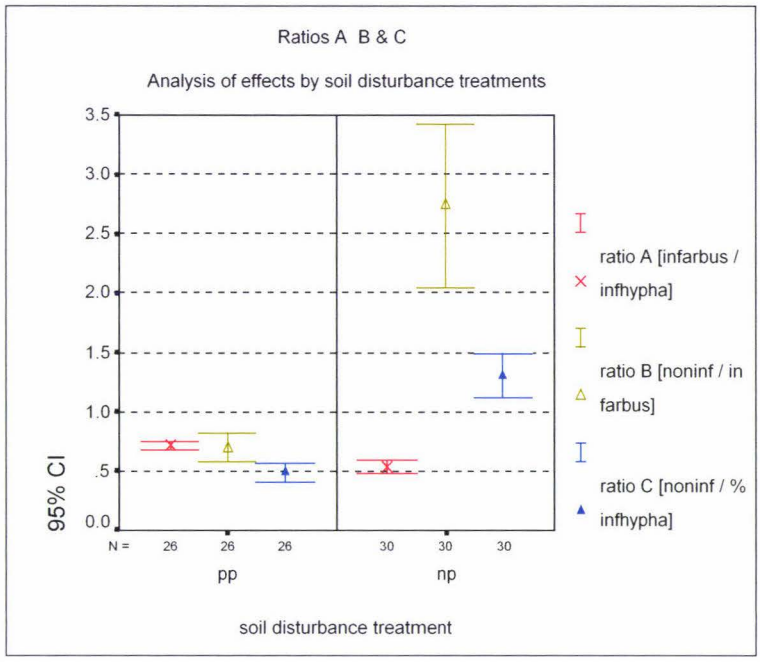


Figure 21 Analysis of effects by soil disturbance treatments.

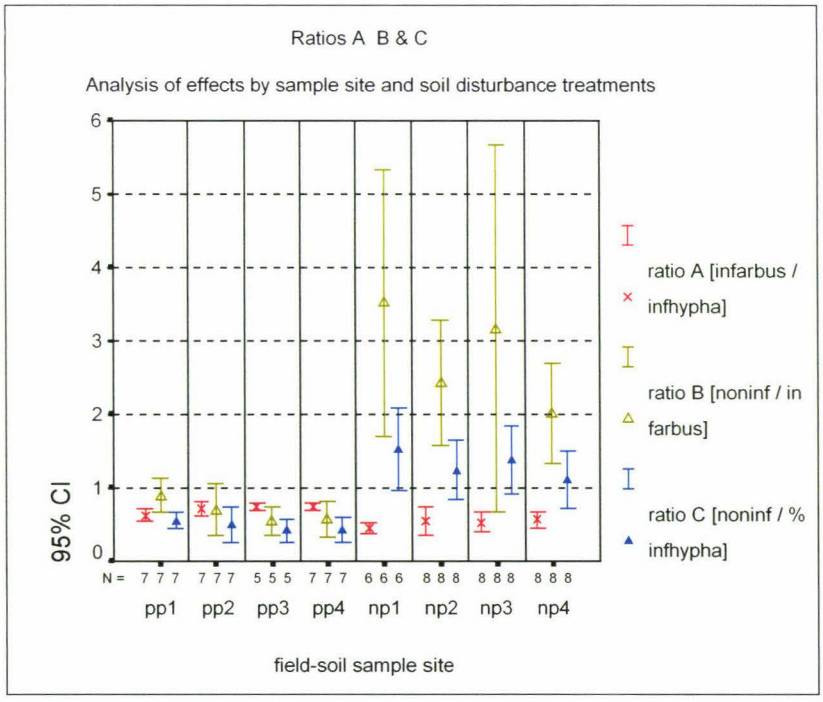


Figure 22 Analysis of effects by sample site and soil disturbance treatments.

As described in Figure 21 and Figure 22, RATIOA is a (fungal:fungal) ratio derived from the percentage (%) of observed maize seedling roots inhabited by intracellular arbuscule structures (INFARBUS), divided by, (or expressed as a ratio of) the percentage (%) of observed roots inhabited with intercellular hyphae (INFHYPHA). Thus, RATIOA describes the proportion of arbuscule intracellular inhabited roots compared to all fungal inhabited roots, ie. root intercellular fungal hyphae.

RATIOA = (INFARBUS) / (INFHYPHA)

Bioassay microscopy observations of maize roots grown in field-soil samples from permanent pasture (PP) treatments found a root-fungal inhabitation value PP RATIOA (≈0.72, sd. = 0.09). In comparison, microscopy observations of maize roots grown in new pastures found a root-fungal inhabitation NP RATIOA that was less (≈0.54, sd.= 0.16).

Thus, in comparison to permanent pasture (PP) soil disturbance treatment results, bioassays of maize seedling roots grown in new pasture (NP) soil samples had relatively a lower value (RATIOA), observed levels of arbuscule root-fungal inhabitation or 'infectivity' compared to observed levels of root-fungal intercellular hyphae derived from all fungi that inhabited maize seedling bioassay root segments.

A General Linear Model (GLM) univariate analysis of variance was used to compare the dependant variable (RATIOA); field-soil disturbance treatments (NP & PP), and field-soil sample sites (between subject effects).

As illustrated by Appendix T4, SPSS outputs from GLM Univariate Analysis, *Tests of Between-Subjects Effects* on dependant variable [RATIOA], two (DISTURBA) soil disturbance factors", (NP) and (PP), were calculated to have a medium F value (F = 25.533), with a high statistical significance (sig.= 0.000).

As also illustrated by Appendix T4, SPSS outputs from GLM Univariate Analyses *Tests of Between-Subjects Effects* on dependant root-fungal inhabitation variable [RATIOA], and (SOILSITE) field-soil sample site factors from new pastures (NP1, NP2, NP3, NP4); and permanent pasture sample sites (PP1, PP2, PP3, PP4), were calculated to have a low F value (F = 1.063), with a low statistical significance (sig. 0.398).

Soil-disturbance effects on root-fungal inhabitation (infectivity) ratios: RATIOB and RATIOC

As previously described, RATIOB describes the proportion of gridline intersects observed with maize root segments that were *not infected* with fungi, (uninhabited or asymbiotic roots) = (NONINF); divided by and in ratio to (INFARBUS) = the percentage (%) of gridline root segment intersects inhabited with intracellular arbuscules.

RATIOB = (NONINF) / (INFARBUS)

Permanent pasture (PP) soil sample bioassays had a maize root-fungal colonisation RATIOB (≈0.70, sd. = 0.30). In comparison, RATIOB was higher (≈2.74, sd.= 1.85) in root-fungal bioassays of roots grown in new pasture (NP) soil samples.

In maize seedlings grown in NP soil samples, compared to maize seedlings grown in PP soil samples, the percentages (%) of roots that were asymbiotic, not inhabited with mycorrhizae or other soil fungi (NONINF), increased in proportion to (INFARBUS) % seedling roots inhabited with intracellular arbuscules

As described in Figure 21 and Figure 22, RATIOC describes percentage (%) of gridline intersects observed with maize root segments that were not fungal inhabited, or asymbiotic (non-infected) roots (NONINF); divided by and in ratio to (INFHYPHA) the percentage (%) of root gridline intersects inhabited with intercellular fungal hyphae.

RATIOC = (NONINF) / (INFHYPHA)

Permanent pasture (PP) field-soil sample bioassays were found to have a root-fungal colonisation (RATIOC) ≈0.49, sd. = 0.18. In comparison, in root-fungal bioassays of seedling plants grown in new pasture (NP) field-soil samples, (RATIOC) was larger ≈1.31, sd.= 0.50.

In other words, in maize seedlings grown in NP field-soil samples, compared to PP soil sample root-fungal bioassays, (RATIOC), the percentage (%) of asymbiotic roots (*not infected*) with mycorrhizae or other soil fungi (NONINF), increased in proportion to the percentage of roots inhabited with intercellular hyphae (INFHYPHA).

A General Linear Model (GLM) Repeated Measures Analysis was used to compare (RATIOB) and (RATIOC), (Within-subject factors); field-soil disturbance treatments (PP or NP); and field-soil sample sites (Between-subject factors).

Appendix T5 illustrates SPSS outputs of General Linear Model GLM Repeated Measures Analysis *Multivariate Tests* and *Tests of Within Subjects Effects* assessed two (FUNGUS) factors: RATIOB] and [RATIOC]. Analyses of these two (FUNGUS) calculated a medium F value (F= 34.763), with a high statistical significance (sig. = 0.000).

As also illustrated by Appendix T5, SPSS General Linear Model GLM Repeated Measures Analysis *Multivariate Tests* and *Tests of Within Subjects Effects* assessed two (FUNGUS) factors: (RATIOB) and (RATIOC), and two soil-disturbance treatment (DISTRUBA) factors. Analyses of these (FUNGUS) and (DISTURBA) data calculated a medium low F value (F= 17.168), with a high statistical significance (sig. = 0.000).

As illustrated in Appendix T5, SPSS General Linear Model GLM Repeated Measures Analysis *Tests of Between Subjects Effects* of soil disturbance (DISTURBA) factors,

found a medium F value (F = 38.856), with a high level of statistical significance, (sig.= 0.000).

As also illustrated by Appendix T5, SPSS General Linear Model GLM Repeated Measures Analysis *Tests of Between Subjects Effects* of soil disturbance (DISTURBA) factors, and sample sites of field-soils (SOILSITE), were calculated to have a low F value (F = 0.972), and a low statistical significance (sig. = 0.454)

As illustrated in Figure 22, graphs of root-fungal inhabitation ratios: (RATIOA), (RATIOB) and (RATIOC) indicate relatively good data replication between new pasture (NP) field-soil sample sites (NP1, NP2, NP3, NP4); and between permanent pasture (PP) field-soil sample sites (PP1, PP2, PP3, & PP4).

New pasture (NP) (RATIOB) root fungal inhabitation data had the largest range of observed values. Similar results were seen for both RATIOB and RATIOC permanent pasture (PP) and new pasture (NP) soil-disturbance treatments affecting root-fungal inhabitation bioassay data.

New pasture (NP) soil disturbance treatment soil samples had a smaller but significantly increased range of values for (RATIOC), see scatter-diagrams illustrated in Appendix T5

Soil Disturbance Effects on Bioassay Maize Seedling Shoot Lengths (mm)

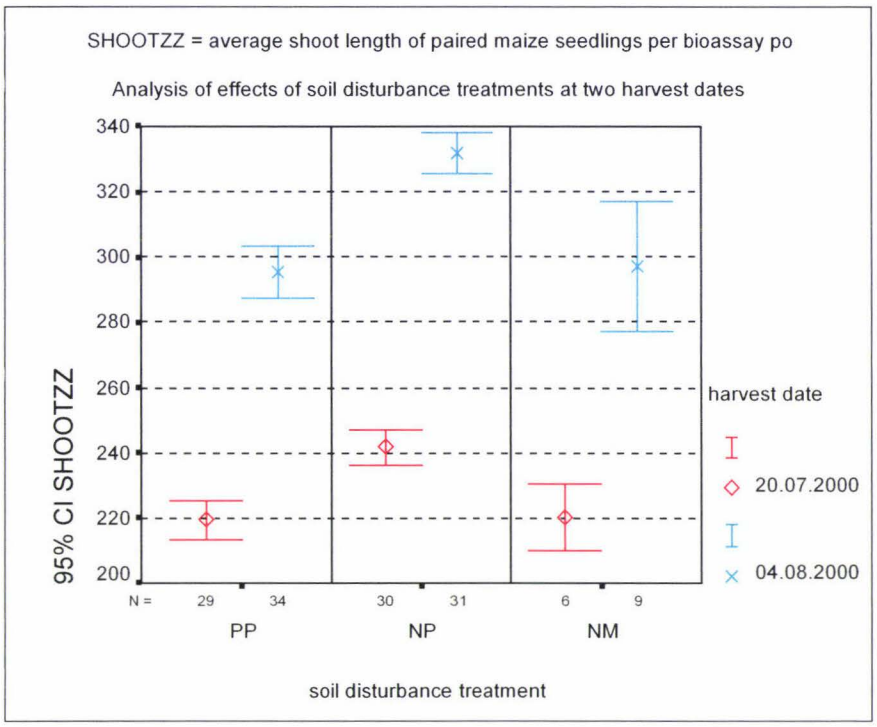


Figure 23 Analysis of effects of soil disturbance treatments at two harvest dates.

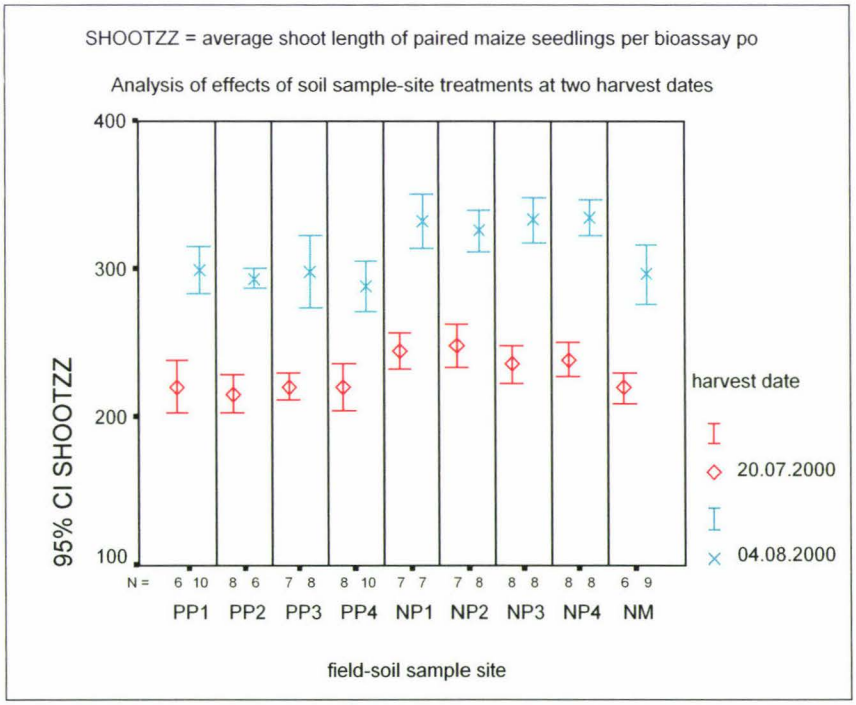


Figure 24 Analysis of effects of soil sample-site treatments at two harvest dates.

As illustrated in Figure 23, at two late-winter harvest dates (20.07.2000 and 04.08.2000), the mean shoot lengths of bioassay *Z.mays* seedlings (Crop & Food

Research CF1 maize hybrid), were compared for effects of two field-soil disturbance treatments, new pasture (NP) and permanent pasture (PP), effects of field-soil sample sites, and effects of pasteurised non-mycological (NM) control soils.

Maize seedling mean shoot length measurements of the first bioassay harvest date on 20.07.2000 showed that permanent pasture (PP) field-soil sample grown seedlings had the shortest shoot-lengths (PP_{SHOOTS} = 219.64mm, sd.= 15.06mm). This value (PP_{SHOOTS} = 219.64) is *approximately equal to* (≈) or else (slightly) *less-than* (<) the mean shoot length of maize seedlings grown in pasteurised non-mycological (NM) control soils, (NM_{SHOOTS} = 220.58mm, sd.= 9.82 mm). Mean shoot length measurements of both permanent pasture and control (PP & NM) soil grown CF1 maize seedling shoot samples were shorter-than (ie. <), the longest mean shoot-length of new-pasture grown maize seedling plants (NP_{SHOOTS} =241.90mm, sd.= 14.69mm).

As also illustrated in Figure 23, very similar trends of maize seedling shoot-length observations were observed on both the first and second bioassay harvest dates, (04.08.2000, & 20.07.2000)

Like results from the first bioassay harvest date, the second bioassay harvest date 04.08.2000, showed that maize seedlings grown in field-soil samples from permanent pasture (PP) disturbance treatment had the shortest mean shoot-length (295.60 mm, sd. = 22.17mm).

Also like results of the first harvest date, the results of second bioassay harvest date 04.08.2000 showed that permanent pasture (PP) treatments also had mean shoot-lengths that are *approximately-equal-to* (≈) or else (slightly) *less-long than* (<) the mean shoot-length of seedlings grown in pasteurised non-mycological (NM) control soils, (297.33mm, sd. = 26.18mm).

Of the three soil-disturbance treatment (DISTURBA) data sets observed, the mean shoot length of non-fungal control (NM) pasteurised soil grown maize seedlings had the largest standard deviation value (sd. = 26.18mm).

The longest mean maize seedling shoot-length (332.24 mm, sd. = 17.05mm) was observed in second-harvest seedlings grown in field-soil samples from new pasture (NP) soil disturbance treatment.

Figure 23 and Figure 24 together illustrate good separation of bioassay seedling shoot length data derived from the two field-soil disturbance treatments, permanent pasture (PP) and new pasture (NP). As also illustrated in Figure 24, at both harvest dates there was good data set replication between maize seedling shoot-lengths obtained from bioassays of soil samples from four field-soil sample sites within each pasture soil-disturbance treatment examined.

Repeated measures statistical tests of pair-planted maize shoot-lengths (SHOOT1) and (SHOOT2).

As illustrated in SPSS statistical software output tables in Appendix T6A, the General Linear Model (GLM) Repeated Measures Analysis *Multivariate Tests,* and *Tests of Within-Subjects Effects* assessed two (SHOOTS) factors, pair-planted maize seedlings (SHOOT1), and (SHOOT2). The *Multivariate Tests* and *Tests of Within-subjects Effects* of (SHOOTS) data were calculated to have a low F value = 3.305, with a medium to marginal level of statistical significance, = 0.072.

As illustrated in SPSS output tables in Appendix T6A, *Multivariate Tests* and *Tests of Within-subjects Effects* compared (SHOOTS) and (DISTURBA) data *between-subject factors* of CF1 maize hybrid seedling shoot-lengths from seedlings grown in soil samples derived from two soil-disturbance treatments, new pasture (NP), and permanent

pasture (PP). These statistical tests calculated a slightly higher F value = 4.716, with a high statistical significance sig.= 0.011.

The *Multivariate Tests* and *Tests of Within-subjects Effects* compared (SHOOTS) and (HARVEST) data, *between-subject factors* of maize shoot lengths sampled at two late winter harvest dates, (20.07.2000 and 04.08.2000). These statistical tests calculated a slightly higher F value = 5.660, with a high level of statistical significance = 0.019.

As illustrated in Appendix T6A, the GLM Repeated Measures analysis *Tests of Between-Subjects Effects* of (DISTURBA) field-soil disturbance treatment data calculated a relative larger F value = 34.364, with a high level of statistical significance = 0.000.

In contrast, GLM Repeated Measures analysis *Tests of Between-Subjects Effects* of field-soil sample site data (SOILSITE) calculated a low F value = 1.764, with a medium to marginal statistical significance = 0.112.

The GLM Repeated Measures analysis *Tests of Between-Subjects Effects* of bioassay harvest dates (HARVEST) calculated a very large F value = 672.764, with a high level of statistical significance = 0.000.

Univariate Tests of Pair-Planted Maize Seedling Shoot-length data (SHOOTZZ)

From each individual bioassay pot (POTNUM), the pair-planted maize seedling shoot-length bioassay data sets (SHOOT1) and (SHOOT2) derived an average (per- pot) maize seedling shoot-length value: [(SHOOTZZ) = (SHOOT1 + SHOOT2) /2].

As illustrated in Appendix T6B, SPSS General Linear Model (GLM) *Univariate analysis* of the dependant variable (SHOOTZZ), the averaged values of two maize seedling shoot-lengths per bioassay pot. The results of the SPSS Univariate analysis

confirmed with the previously described results of Multivariate repeated-measures tests of (SHOOT1) and (SHOOT2) as illustrated in Appendix T6A.

As illustrated in Appendix T6B, the SPSS Univariate analysis *Tests of Between-subjects Effects* of field soil-disturbance pasture treatments (DISTURBA) were calculated to have a relatively large F value = 34.364, with a high level of statistical significance = 0.000.

In contrast, SPSS Univariate analysis of the average of pair-planted shoot-length data (SHOOTZZ), and *Tests of between-subjects effects* of field-soil sample site (SOILSITE) data, both calculated a low F value =1.764, with a marginal statistical significance = 0.112.

The GLM Univariate analysis *Tests of Between-Subjects Effects* of maize harvest dates (HARVEST) again calculated a very large F value = 672.764, with a high level of statistical significance = 0.000.

Soil Disturbance Effects on Maize Seedling Shoot Mean Dry Mass (g)

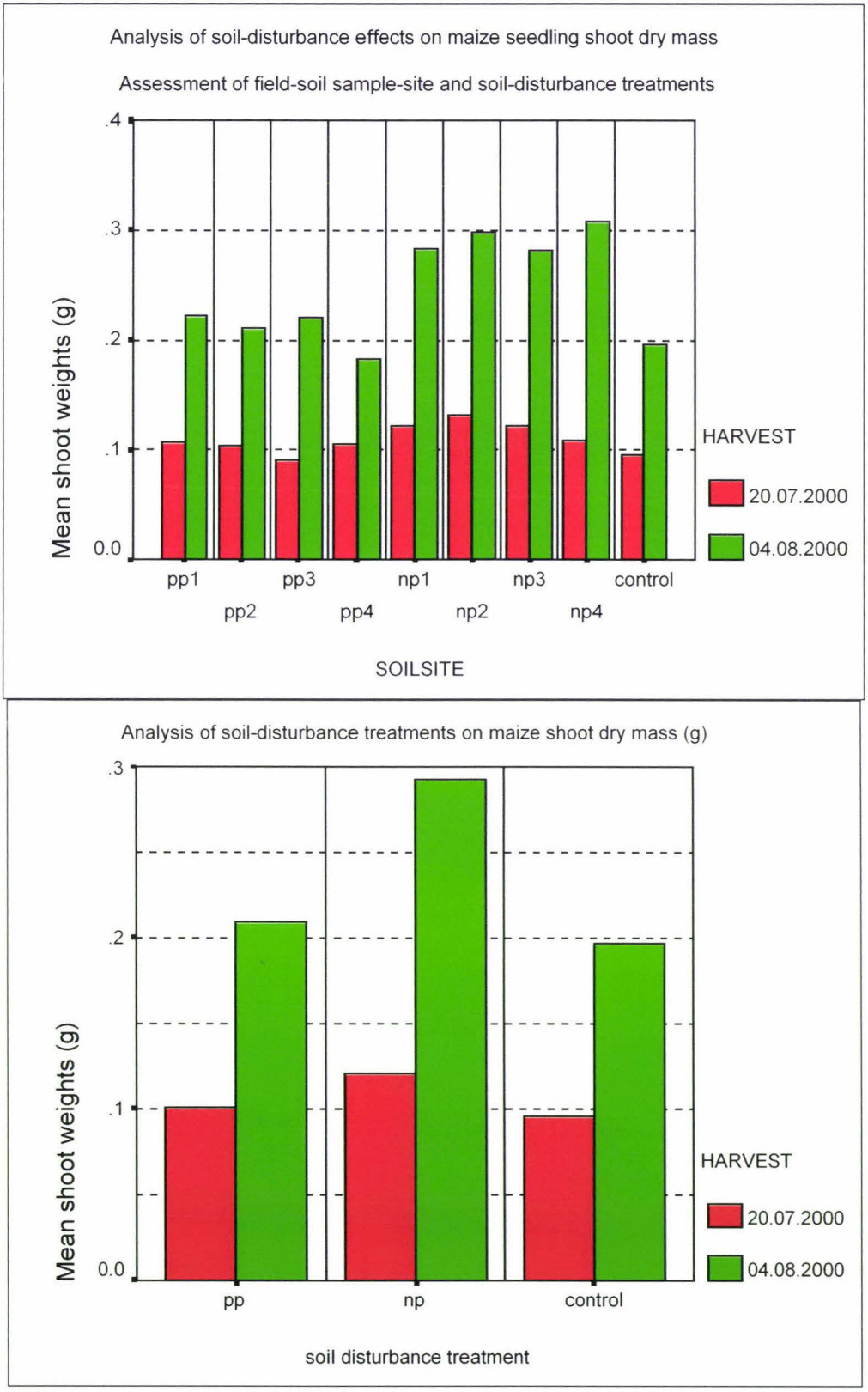


Figure 25 Analysis of soil disturbance effects and field-soil sample site on two harvest dates, on maize seedling shoot dry mass (g).

As illustrated in Appendix T7, the mean dry mass of maize shoots was determined by weighing oven-dried paper bags containing a combined number of bioassay maize shoots derived from field-soil sample sites, (N1, N2, N3, N4, and PP1, PP2, PP3, PP4). A shoot dry mass was also obtained from maize seedling shoots grown in non-mycological (NM) control soils.

For each field-soil sample site and control treatment, the mean dry-weight of paper bags was subtracted from combined dry-weight of maize shoots from each treatment bag.

The oven-dried maize seedling shoot dry mass (WEIGHT) data were derived from the total dry mass of maize shoots contained in each paper bag, (g) divided by the number of dried maize shoots per treatment / sample bag (= n).

As illustrated in Figure 25 and Appendix T7, at the first harvest date (20.07.2000), bioassay maize plants grown in soil samples from new pasture (NP) soil disturbance treatment had the largest mean shoot dry-mass per maize seedling, (ie. NP mean dry mass per maize seedling shoot = 0.1221g).

The next largest mean shoot dry mass per maize seedling (0.1070g), was obtained from bioassay maize seedlings grown in field-soil samples from a permanent pasture (PP) soil disturbance treatment. The smallest mean shoot dry mass per maize seedling (0.0954g) was obtained from non-fungal control bioassay plants grown in non-mycological (NM) pasteurized soil samples.

Mean shoot dry mass (g) of maize seedlings

(at first bioassay harvest, 20.07.2000) are summarized:

$(NM_{SHOOTS} = 0.0954g) < (PP_{SHOOTS} = 0.1070g) < (NP_{SHOOTS} = 0.1221g)$

The effects of field-soil sample site and soil disturbance treatments on maize seedling shoot dry weights (g) measured at the second harvest date 04.08.2000 also have a similar pattern of shoot dry-weight measurements at the first harvest date 20.07.2000. As illustrated in Figure 25, within each pasture field-soil disturbance treatment there was relatively less separation between data obtained from soil sample bioassays of four replicate sample sites:

As also illustrated in Figure 25 the mean maize seedling shoot dry mass value of NP4 is approximately equal to (≈) the mean values of shoot dry-weights from seedlings grown in soil samples from field-soil sample sites PP1, PP2, & PP4.

As illustrated in Figure 25 there was little to distinguish between shoot dry mass data from permanent pasture (PP) field-soils and non-mycological (NM) control soils. However, the shoot dry weight value of (PP3) field-soil sample site was less than (<) the shoot dry weight value of seedlings grown in control (NM) pasteurised soils.

Chapter 4 Discussion

Ecological models and interpretations of soil-disturbance treatment effects on maize seedling AM fungal symbiosis microscopy and biomass data:

Microscopy observed levels of soil-fungi inhabiting maize seedling root segments.

As described in Appendices T1 and T2, and in chapter 3, the results of the current root-fungal bioassay method indicate good statistical correlations exist between two field-soil disturbance treatments, permanent pasture (PP) and new pasture (NP); and microscopy observed levels of maize root fungal endo-symbiotic structures.

Soil disturbance treatment effects were observed on the total numbers of root segments inhabited with intercellular fungal hyphae HYPHAE, and on percentages of roots inhabited with intercellular hyphae INFHYPHA. Soil disturbance treatment effects were also observed on the levels of intracellular inhabited root cortical tissue, total numbers of roots inhabited with fungal arbuscules ARBUSCUL, and percentages of roots inhabited with arbuscules INFARBUS.

The microscopy examinations found relatively higher levels of soil fungal structures inhabiting maize seedling roots grown in samples of field-soil cores obtained from permanent pasture (PP) soil-disturbance treatments.

The ratio of root segments inhabited with arbuscules in proportion to root segments inhabited with intercellular hyphal fungal structures, giving RATIOA.

The root-fungal inhabitation data obtained from microscopy examinations, as presented in chapter 3 results and Appendix T4, illustrate graphs and statistical analyses of the ratio of the percentages of maize seedling roots inhabited by two categories of fungal structures. In particular, the percentage of roots inhabited by intracellular arbuscular fungal structures INFARBUS, compared to the percentage of roots inhabited by intercellular hyphal fungal structures INFHYPHA, giving RATIOA.

The statistical analysis of the effects of pasture soil-disturbance treatments (NP) and (PP) on the ratio of the percentages of intracellular to intercellular fungal structures giving RATIOA data, can be discussed in fungal and plant ecological and nutritional terms as the ratio of potential mineral plant benefits (+), for example improved plant growth and or metabolism derived from phosphate (P) distributed by *arbuscule* mycorrhiza structures INFARBUS; in ratio to the percentages of root segments inhabited with root intercellular hyphae derived from soil-fungi, INFHYPHA, including mycorrhizal symbionts.

Unlike the potential for plant benefits (+) derived from arbuscule fungal structures, intercellular hyphae fungal structures may have a diverse range of ecological functional outcomes of soil-fungal and plant population interactions, (-, 0, +).

For example, there are many soil-fungi that form root intercellular hyphae, including arbuscular mycorrhizal '*Arum*' morphology type fungal hyphae that may function to actively import (+) plant carbohydrates obtained from surrounding host intercellular apoplast spaces of plant root cortical tissues and or root epidermal tissues (-). Root intercellular hyphae of biotrophic mycorrhizal fungal may thus provide a root and fungal infrastructure that allows intimate physical, genetic and biochemical endo-symbiotic interactions that enable fungal population access to, and fungal benefit (+) from plant root storage carbohydrates.

AM fungi intercellular hyphae may subsequently then take 'captured' plant carbohydrates (-) and convert them into fungal carbohydrates, that may then be transported throughout and used in the growth and metabolism of potentially extensive fungal hyphal networks of extracellular soil-fungal structures, and connected to networks of root endo-symbiotic intra-cellular and inter-cellular fungal structures, Smith & Read, (1997).

In addition, root intercellular hyphae of mycorrhizal fungi may also function as transport conduits of phosphate (P) and zinc (Zn) minerals derived from mycorrhizal soil-fungal hyphae. Subsequently, mycorrhizal soil-fungal derived minerals may be transported through extracellular soil-hyphae and via plant root intercellular networks to intracellular arbuscule fungal structures that inhabit and benefit plant root cortical tissues (+), Smith & Read, (1997).

In contrast to metabolically active intercellular fungal hyphae with nutrient uptake and transportation functions, the current methods used a non-vital CBE stain method that could not distinguish between active functioning hyphae and inactive regions and or senescent root intercellular fungal hyphae. Thus, although inactive intercellular fungal structures inhabit plant roots, the lack of interactions between such fungal and plant populations may be characterised as having neutral or negligible effects (0) *neutralism.*

Analysis of root-fungal inhabitation data from microscopy observations of maize seedling root segments showed that samples from permanent pasture (PP) soil-disturbance treatment had higher levels of potentially plant beneficial (+) fungal arbuscule structures, in proportion to levels of intercellular fungal hyphae that inhabit cortical and epidermal root tissues, (ie. PP RATIOA = 0.72, sd. = 0.09).

In comparison, analysis of root-fungal inhabitation data from microscopy observations of maize seedling root segments showed that samples from new pasture (NP) soil-disturbance treatment had statistically significant lower levels of potentially plant beneficial (+) fungal arbuscule structures, in proportion to levels of roots inhabited by intercellular fungal hyphae, (ie. NP RATIOA = 0.54, sd. = 0.16)

The ratio of maize seedling root segments not inhabited with fungi, in proportion to root segments inhabited with arbuscule fungal structures, giving RATIOB.

The root-fungal inhabitation data obtained from microscopy examinations, as presented in chapter 3 results and Appendix T5 illustrate the ratio of maize seedling root segments not inhabited with fungi, in proportion to root segments inhabited with arbuscule fungal structures, giving RATIOB.

The numbers of non-inhabited (ie. asymbiotic) root segments NONFUNGA, and the percentages of non-fungal root segment percentages NONINF, were derived from observed total numbers maize seedling root segments ROOTS; minus numbers of maize root segments inhabited with intercellular fungal hyphae HYPHAE.

Thus it may be hypothesised that as a result of asymbiosis, an absence of fungal and plant population interactions, that both the non-inhabited maize seedling root segments, and asymbiotic soil fungi, have negligible or neutral ecological effects on each other, (0) *neutralism* (Boughey, 1973).

Statistical analysis of microscopy observations of maize seedling root segments showed that new pasture (NP) soil-disturbance treatment samples had a relatively higher ratio of non-inhabited *neutral* (0) root segments, in proportion to plant mineral nutrient *benefits* (+) that may potentially be derived from networks of mycorrhizal soil-hyphal fungi connected via intercellular hyphae to intracellular fungal arbuscule structures, (ie. NP RATIOB = 2.74, sd. = 1.85).

In comparison, root segment samples from permanent pasture (PP) treatments had a statistically significant lower ratio of non-inhabited *neutral* (0) root segments; in proportion to roots inhabited with potentially beneficial (+) intracellular fungal arbuscule structures. (ie. PP RATIOB = 0.70, sd. = 0.30).

The ratio of maize seedling root segments not inhabited with fungi, in proportion to root segments inhabited with intercellular fungal hyphae, giving RATIOC.

The root-fungal inhabitation data obtained from microscopy observations, as presented in chapter 3 results and Appendix T6, illustrate the ratio of maize seedling root segments not inhabited with fungi, in proportion to root segments inhabited with intercellular hyphae fungal structures, giving RATIOC.

Statistical analysis of microscopy observations of maize seedling root segments showed that new pasture (NP) soil-disturbance treatment samples had a relatively higher ratio of non-inhabited (asymbiotic) root segments with *neutral* (0) effects; in proportion to root segments inhabited with intercellular hyphae, that as previously discussed, may potentially have a wide range of benefits and costs derived from symbiont plant and fungal population interactions, (+, 0, -). (NP RATIOC = 1.3087, sd. = 0.5056).

In comparison, root segment samples from permanent pasture (PP) treatment had a statistically significant relatively lower ratio of non-inhabited *neutral* (0) root segments; in proportion to roots inhabited with intercellular fungal hyphae that may have a range of (-, 0, +) population interaction benefits and costs. (PP RATIOC = 0.4917, sd = 0.1843).

Mean root numbers and effects of soil disturbance treatments.

As illustrated in Appendix T3 and in chapter 3, the results of the current root-fungal bioassay methods indicate a small but significant statistical correlations exist between two field-soil disturbance treatments, permanent pasture (PP) and new pasture (NP); and 'grid-line-intersect' microscopy method observed mean numbers of maize seedling root segments, ROOTS.

Microscopy observations of the mean numbers of root segments of maize seedlings grown in 120g field-soil samples from new pasture (NP) soil-disturbance treatments found slightly higher mean root segment numbers (168.20, sd.= 49.64) in nylon-mesh

bioassay pouches that contained field-soil bioassay samples obtained from new pasture (NP) sample sites. In comparison, microscopy examinations observed less root segment numbers (144.85, sd. = 38.45) in nylon-mesh bioassay pouches that contained 120g field-soil samples from permanent pastures (PP) sample sites.

Microscopy examinations of *control* (NM) or non-fungal soils found much lower numbers of maize seedling root segments in plants grown in nylon-mesh pouches that contained the same volume of pasteurised sandy-silt-loam.

Shoot biomass of maize seedlings and effects of soil disturbance treatments.

The high levels of fungal inhabitation (endo-symbiosis) in maize seedling roots grown in permanent pasture (PP) field soil samples, occurred together with, and were statistically correlated with maize seedling '*host*' plant shoot biomass observations of 'suppressed' (-) mean shoot dry weights; and shortened (-) mean seedling shoot lengths.

In comparison, bioassay examinations of the effects of pasteurised sandy-silt-loam (SSL) field-soil (non-mycological) (NM) or non-fungal *control* soil, observed that bioassay maize seedling shoots were shortened (-) and weighed less than (-) shoots grown from both pasture field-soil disturbance treatments, (PP) and (NP).

Unfortunately, for the want of more definitive shoot biomass measurements, observations of fresh leaf-area analysis were not measured. In addition, time and funding limits also prevented the use of Atomic Absorption (AA) mineral content examinations of maize seedling shoot ashes.

Initial assessments of fungal '*parasitism*' of host plant maize seedlings, (+, -).

The bioassay results of a negative (-) shoot and root biomass in response to soil fungi, including AM fungal inhabitation of maize seedlings, appear to indicate '*parasitic*' population interactions; ie. soil-fungal parasite benefit (+), and host plant biomass cost

or loss (-). Thus, such a '*parasitic*' cost and benefit analysis of plant growth response indicates a poor AM fungal *nutritional 'efficiency'*, (Smith & Smith, 1996; Odum, 1983; Boughey,1973), (see also Appendix M1).

However, such a preliminary and incomplete ecological assessment of '*parasitic*' plant and fungal symbiosis population interactions does not include a mineral analysis of plant tissue maize shoot phosphate (P) levels that may potentially be obtained from mycorrhizal fungal and root symbiosis associations, (Stribley *et al.*, 1990).

In addition, such a preliminary assessment of soil-fungal *parasitism* of maize seedlings also risks neglecting further complex considerations such as plant seedling early developmental shoot growth-stages, (ontogeny). In comparison to full sized mature photosynthetic shoots, the emergent and expanding young seedling shoots produce relatively small quantities of photosynthates or carbon sources. However, such young and actively growing meristematic, differentiating and elongating plant shoot tissues may instead function as plant carbon sinks, (Salisbury & Ross, 1985; Steeves & Sussex, 1994; Smith & Read, 1997).

Furthermore, and of particular importance to analysis of the seasonal timing of this current study, this preliminary analysis of soil-fungal population '*parasitism*' on plant seedling growth does not take into account the cost and benefit effects of winter-grown bioassay methods that examined what usually are summer-grown annual maize plants.

In regard to the discussion of the current bioassay results, it was a regrettable omission that during the greenhouse propagation period, that the mid-winter to late-winter irradiance levels and meteorological records were not recorded at the Massey University greenhouse site.

In the relatively low irradiance levels of winter, plants with mycorrhizal fungal-inhabited roots are not as likely to be growth-limited by phosphate (P). However, both plant and fungal symbionts may hypothetically be in 'resource type' (indirect) *competition* for plant photosynthates, ie. *mutually inhibitive* (-,-)., Odum, (1983). Both plant and fungus symbionts may thus be required to *share* or *divide* winter-restricted availability of carbon (C) carbohydrate nutrients.

Comparisons of results with previous mycorrhiza research into a competitive carbon economy induced by low solar irradiance effects.

The current result findings of negative plant growth responses resulting from mycorrhizal fungal '*parasitism*' by heterotrophic soil fungi appear to correspond well with existing published experimental findings of plant root and shoot biomass effects resulting from low solar irradiance levels during bioassay seedling propagation, (Buwalda & Goh, 1982; Snellgrove *et al.,* 1982; Bethlenfalvay & Pacovsky, 1983; Bethlenfalvay *et al.*, 1983; Koide, 1985; Modjo & Hendrix, 1986; Smith *et al.*, 1986; Modjo *et al.*,1987; Son & Smith, 1988; Smith *et al.*,1986; Smith & Gianinazzi-Pearson,1990; Fitter, 1991; Smith & Read,1997; Sturz, 1997).

Discussion of new and existing models and descriptions of mycorrhizal symbiosis plant and fungal population interactions

In order to further evaluate microscopy examination results of the current bioassays of maize seedling root fungal-inhabitation, together with shoot and root biomass measurements, it is necessary to evaluate both the measured, hypothesised and or un-measured mineral, energy, structural and functional *costs* (-) and *benefits* (+) for both plant and fungal symbiont populations.

As previously presented in university textbooks on basic ecology, and as illustrated by Figure 26 and Figure 27, the terms describing *two-population interactions* have been presented in a table of symbiont *cost* and *benefit* outcomes (-, +, 0), (Odum, 1983).

As illustrated in Figure 27 an adapted table demonstrates symbiont population outcomes, (-, +, 0), that may result from population interactions and non-interactions. Most of the information used to derive Figure 27was originally illustrated by Table 4-2 in Boughey (1973), which was in turn credited to derive from an earlier reference by Odum (1959), p.226.

Analysis of Two-species Population Interactions
(derived from table 7-1, Odum, 1983)

species 1	species 2	TYPE OF POPULATION INTERACTION & GENERAL NATURE OF INTERACTIONS
0	0	**Neutralism** neither population affects the other
-	-	**Competition** ❑ direct interference type: direct inhibition of each species by the other. ❑ resource use type: indirect inhibition when common resource in short supply.
-	0	**Amensalism** Population 1 inhibited, population 2 not affected
+	-	**Predation** (including herbivory, predator usually larger than prey)
+	-	**Parasitism** (parasite ~ smaller organism)
+	0	**Commensalism** Commensal population benefits whilst host is not affected
+	+	**Proto co-operation** Interaction favourable to both but not obligatory
+ *	+ *	**Mutualism** Interaction favourable to both and obligatory

KEY
(0) = no significant interaction
(+) = enhanced growth, survival benefits
(-) = inhibited ... growth, survival
(*) = obligate symbionts

Figure 26 Analysis of two-species population interactions, type of population interaction and general nature of interactions.

Analysis of Two-Species / population Interactions; Effect of Relationship on Growth and Survival of Two Populations

When not interacting		When interacting		
Specie s1	Specie s2	Species 1	Species 2	TYPE OF POPULATION INTERACTION & GENERAL NATURE OF INTERACTIONS
-*	-*	+*	+*	**Mutualism**
-*	0	+*	+	Mutual benefits & obligate* symbiosis
0	0	+	+	**Proto cooperation** Mutual benefits but non-obligate (facultative) symbiosis
-*	0	+*	0	**Commensalism**
0	0	+	+	Populations 'eating at the same table'
0	-*	0	+*	eg. a horse & a bird sharing food at a trough
0	0	0	+	
0	0	0	0	**Neutralism** Neutral or negligible population interactions, neither population affects the other population
0	0	-	0	**Amensalism** i) population 1 inhibited / population 2 neutral ii) population 1 neutral / population 2 inhibited
0	0	0	-	eg. A horse fed using a fodder-bag is detrimental to a bird that may no longer share horse food sources.
0	0	-	-	**Competition** (2 types) i) direct 'interference type', mutual inhibition of each species by the other. ii) Indirect 'resource type' when common resource in short supply
-*	0	+*	-	**Parasitism** parasite smaller than (<) host **Pathogenesis** pathogen smaller than (<) host **Predation** predator larger than (>) prey
-*	0	-*	+	**Escape / Avoidance**

eg. arbuscular mycorrhizal soil fungi and plant interactions ...

- **Species 1 = AM fungus,** Glomales order, Zygomycota (obligate* mycobiont)
- **Species 2= plant root host** cortex or epidermis tissues; either non-obligate (facultative), or else obligate* phytobiont

KEY

(0) = no significant interaction

(+) = enhanced growth / survival benefits

(-) = inhibited growth / survival

(*) = obligate symbionts

table adapted from Odum (1983) & Boughey (1973)

Figure 27 Analysis of two-species or population interactions and non-interactions; Effects of relationship on growth and survival of two populations.

The discussion of the experimental results of current soil-disturbance maize seedling bioassay studies are described in terms that aim to use scientifically accurate, easily understood and appropriate use of ecological terms that describe observed, and or hypothesised interactions, and non-interactions between two populations (eg. maize seedling plants and field-soil fungi). Thus, the current study aims to use relatively '*objective*' or '*neutral*' terminology to describe fungal populations observed within plant root tissues or cells.

Discussion of the results of current microscopy examination of fungal–plant population interactions have used terms such as fungi that '*inhabit*', '*occupy*', '*reside in*', '*live within*', '*contained within*', and observed '*levels*' of root segment '*inhabitation*' by intercellular and or intracellular fungal structures. Fungal and plant populations have also been described by the following relatively objective ecological terms: fungus symbiont (*mycobiont*); plant symbiont (*phytobiont*); plant '*host*'; '*symbiont partners*'; or plant and fungal '*partners*'; or else plant root '*fungal endo-symbionts*'.

Thus the current study aims to, but by reference to literature, has not always been able to totally avoid the use of terms that may have value-implicit 'negative' health-related, and or social-historical definitions. For example, implicit if unintentional pejorative or 'negative' terminology use such as describing soil fungi that '*infect*', '*colonise*' or '*invade*' plant roots.

However, in order to balance such a semantic debate, there are many common examples of English language-use that have more 'positive' connotations of the term '*infect*'. Many phenomena such as laughter, ideas, music, advertisements, and catch phrases are sometimes also described as '*infectious*', meaning that these phenomena tend to "*spread or take-off suddenly*".

A social context of researchers and practitioners of plant, fungal and soil health.

In general, aside from within such areas as mycorrhizal research, most scientific and farmer understandings of plant and fungal interactions occur in the areas of plant crop health, and or plant pathology studies of parasitic, saprotrophic or necrotrophic fungi. Generally, the majority of such scientific research into plant and fungal interactions is focussed within two interest areas of plant protection, pre-harvest production crop management, and post-harvest storage of economically significant crops.

Anecdotally, perhaps as a result of such research foci on plant fungal diseases, it seems that many people generally believe that fungi have only negative (-) interactions with plants. However, generally speaking most people have not heard about beneficial (+) ecological roles mediated by mycorrhizal plant and fungal associations.

In contrast to the preceding discussion, most people acknowledge the potential for human health and economic benefits available from edible fungi (+); benefits from fungi that ferment foods and beverages (+); benefits from fungi used to produce antibiotic medications (+); and benefits from fungi used to decompose or compost plant wastes (+).

In general, the predominant focus of soil health and plant health research regarding plants and fungi is usually aimed at development of commercially available anti-fungal (-) fungicidal or fungistatic chemical treatments, or else breeding for crop plant protection (+). Thus, most academic and commercial research into plant-fungal interactions aims to '*earn it's keep*' by conferring economic returns for investments into research and development of consumable products such as fungicide or fungistatic treatments; selective breeding of fungal-resistant plants, and more recently into anti-fungal GMO plant strains.

However, in contrast to most 'mainstream' research areas into plant-fungal interactions, generally most research into mycorrhizas, and likewise generally most research into organic production systems, often does not lead towards development of economic benefits (+) from sales returns and or use of consumable mycorrhizal products for use by primary producers.

Models and descriptions of symbiosis and arbuscular mycorrhizas.

In addition to the preceding discussion of a predominantly 'anti-fungal' social context surrounding scientific research into plant and fungal interactions, there may also be a common human subjective tendency or bias, including amongst many enthusiastic and supposedly 'objective' mycorrhiza researchers, to be tempted to '*generalise*', '*simplify*', '*talk-up*', '*overstate*' or '*oversell*' the benefits (+), and also sometimes if not always to '*talk-down*' the negative aspects or costs (-) associated with mycorrhizas.

For example, mycorrhizal symbiosis has previously been authoritatively described as "*normally mutualistic*" root and fungal (+, +) symbiotic associations, (Smith & Read, 1997). Mycorrhizal plant and fungal symbioses have also been authoritatively described as (+, +) ie. "*highly evolved mutualistic associations*", (Brundrett *et al,* 1996).

Thus, these two leading authorities in mycorrhiza research and literature have used ecological descriptions or definitions that may generally emphasise potentially mutually beneficial (+, +), and obligate, symbiosis aspects of fungal intercellular hyphae and or arbuscule structures. However, so as not to be unfair to these two authoritative mycorrhiza literature sources, they also considerably expand these generalised descriptions with other less beneficial aspects of a complex range of costs and benefits resulting from interactions between fungal and plant mycorrhizal symbionts.

In contrast, there are also some examples of mycorrhizal literature terminology that recognise the diverse function and structures that occur in the arbuscular mycorrhizal

symbiosis. As previously discussed in relation to negative plant growth response effects of low irradiance levels of mycorrhizal inhabited plants, and in relation to the absorptive function or intercellular hyphae, the term *'parasitism'* has previously been used to describe mycorrhizal symbiosis interactions, (Bethlenfalvay & Pacovsky, 1983; Bethlenfalvay *et al.*,1983; Smith & Smith,1996).

A proposed Venn diagram model of symbiont population interactions between arbuscular mycorrhizal fungi and plant host roots.

As illustrated in Figure 28 and in Appendix M2, mycorrhizal associations have previously been described in the literature with the use of a Venn diagram that depicted three essential biotic and abiotic components, plant, fungus and soil, Brundrett *et al* (1996).

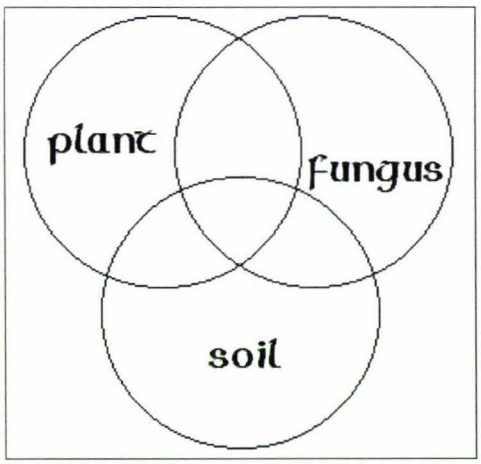


Figure 28 Venn diagram of three mycorrhizal components or entities : plant, fungus, and soils, Brundrett *et al* (1996).

In order to further the current research discussion aim to use clear ecological descriptions of two-population interaction outcomes (-, 0, +), the current thesis discussions will also elaborate on a proposed new Venn diagram '*symbiosis*' model that describes three mathematical continua of *two-population* interactions that were previously described in tables by Odum (1983), and by Boughey (1973).

The proposed new 3-way Venn-diagram *symbiosis* model of *two-population interactions* (+, -, 0) was derived in part from recognition of the learning benefits that may result from the use of the Venn diagram model of (plant-fungus-soil) mycorrhizal symbiotic associations as previously described by Brundrett *et al.*, (1996), see Figure 29.

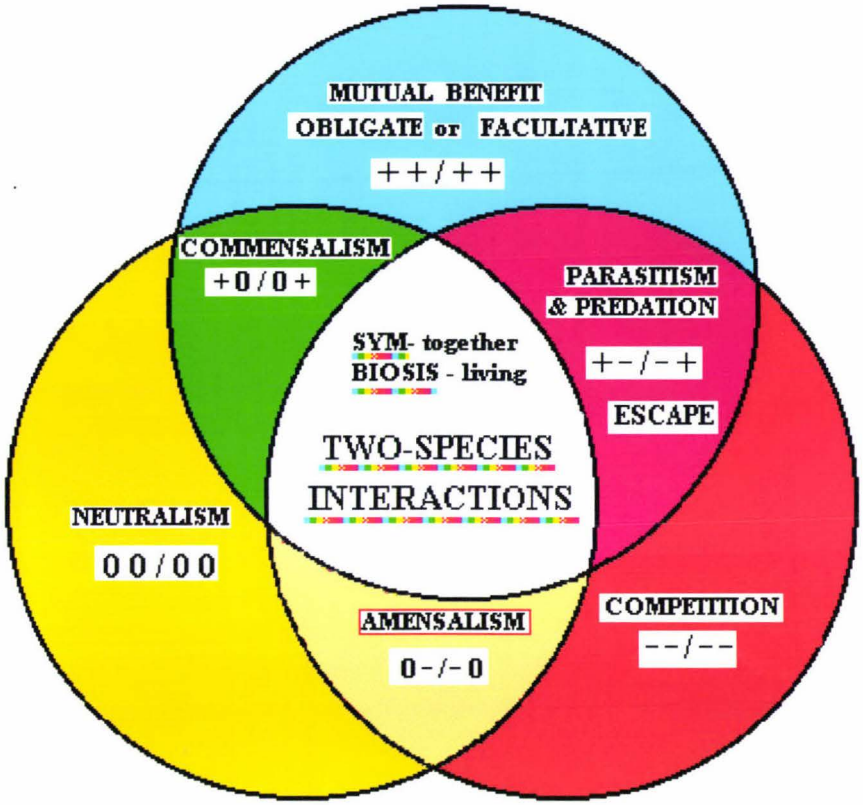


Figure 29 A proposed Venn diagram model of symbiosis and ecological outcomes derived from two-species or population interactions.

The current thesis discussion assumes that at any one point in time, or over the course of time, the overall balance of '*benefits*' and '*costs*' to both plant and mycorrhizal fungal symbionts may vary independently, according to the developmental stages (ontogeny) of plant and AM fungus, (Harris & Paul, 1987).

It is difficult to present in any particular order of importance, the biological structural and physical locations, and or the full range of possible interactions that may occur between fungi and plant symbiont populations. Thus, the reader may cross-reference

the following discussion with the previously described tables and Venn-diagram models of ecological definitions of population interactions as previously described (Odum ,1983; Boughey, 1973; Graves *et al.*,2001).

Unlike the environmental circumstances that exist within adequately sunlit autotrophic (photosynthetic) plant shoot tissues, AM fungi reside within plant roots and in soil-borne '*web-like*' fungal hyphal networks. AM fungi are heterotrophic organisms that are usually hidden endosymbiotic inhabitants of '*host*' plant root cortical and epidermal tissues. AM fungal hyphae networks also extend out from host roots into soils beyond a surrounding root-affected zone or '*rhizosphere*', thus forming a soil-fungal hyphal affected soil zone known as a '*mycorrhizosphere*', (Smith & Read, 1997).

Generally speaking, and with specific exceptions during the beginning of spring, or else except during plant seed maturation stages, most root tissues usually function as heterotrophic plant tissues, (ie. plant Carbon storage root organs or C-sinks), (Steeves & Sussex, 1994).

However, within whole plant systems, with the assistance of xylem and phloem plant vascular transport tissues, roots are physically and functionally connected to autotrophic aerial shoots. Roots with functional vascular transport systems in turn may also be connected to photosynthetic shoots that are carbon (C) sources and or aqueous mineral nutrient sinks. In addition, plant root tissues may also be physically and functionally connected to plant shoot carbon (C) sinks. Plant shoot carbon sinks may occur during early leaf-expansion growth stages, or during plant seed maturation stages, or else during the deposition of plant structural tissue such as wood, (Steeves & Sussex, 1994; Salisbury & Ross, 1985).

As biotrophic heterotrophic soil-fungal organisms, and in order to obtain and or maintain access to plant energy and carbohydrate sources, the *Zygomycete* soil fungi,

(aka *Glomales* order, aka arbuscular mycorrhiza fungi, aka 'endo-mycorrhizas'), are obligate endo-symbiont fungal partners of plant roots. In other words, in order to survive and live, AM fungi symbionts (mycobionts) have an obligate need to inhabit compatible host plant roots (phytobionts), (Smith & Read, 1997).

***Endosymbiosis*: Comparisons of symbionts residing at least partly within other symbionts.**

"Endo-symbiosis" may be defined as "*the coming together and close physical contact by a smaller organism inhabiting a larger symbiont organism*", (Margullis, 1981).

As previously introduced in chapter 1, the current study uses the following definition of fungal "*endosymbiosis*" of plant roots: "*all fungi that inhabit inner spaces*" of roots. As also previously discussed in chapter 1, many plants are facultative mycorrhizal host symbionts of obligate biotrophic AM fungi endosymbionts. Additionally AM fungi endosymbionts may have a free-living asexual spore stage that may temporarily inhabit soils outside host plant roots.

The most well known examples of '*endosymbiosis*' are eukaryotic cell 'plastid' organelles that have long ago descended from free-living (facultative) bacterial ancestors. However, with the rare exception of externally attached (disposable) mitochondria plastids of male gamete germ cells, plastid cell organelles now usually function as mutually obligate *endo-symbiotic* organelles that contain mostly ancestral bacterial origin circular DNA, (Werner, 1992; Schwemmler *et al.*,1980; Margulis & Schwartz ,1982; Margulis 1970; 1981; 1998).

Endosymbiotic plastids permanently inhabit eukaryotic host cell cytoplasm and membrane, (ie. within a host eukaryotic cell symplast). The replication of plastid cell organelle DNA usually occurs immediately prior to and as a necessary pre-condition of cell nuclear DNA division cycles of host eukaryotic cells. In exchange for living within "comparatively stable" or "more comfortable" homeostatic cytoplasm environments of

the eukaryotic cell symplast of multicellular organisms; endo-symbiotic plastid cell organelle descendants of free-living bacteria, may in turn be functionally described to '*earn their keep*' to the 'host' eukaryotic cell by generating mutually available energy benefits (+,+) to plastid cell organelles and to host eukaryotic cells and or whole organisms.

A further common example of *endo-symbiosis* and obligate (*mutualism*) mutually beneficial (+, +) nutrient-related interactions is found in populations of obligate anaerobic fermentation micro-organisms that inhabit the rumen organ and produce soluble carbohydrates for nutrient dependant needs (obligate symbiosis) of large host animal vegetation grazers or browsers. Another similar example of *endo-symbiosis* and *mutualism* is fermentation nutrient digestion provided by obligate anaerobic and methanogenic Archaebacteria that inhabit gastro-intestinal tracts of plant consuming insects including termites, (Margulis,1982;1998).

***Mutualism*: mutual benefits (+,+ / +,+) of obligate endo-symbiosis population interactions:**

As previously defined by population ecologist Eugene Odum (1983), an obligate symbiosis with mutually beneficial (+, +) population interactions is defined as '*mutualism*'.

Thus, obligate mycorrhizal symbioses with mutually beneficial (+, +) two-population interactions may occur between mycorrhizal plant and fungal partners when *both partners must receive benefits*. For example, mycorrhizal mutualism involves obligate symbiont plant derived photosynthate carbohydrates received by obligate fungus endosymbionts, in exchange for plant-available soil-fungal hyphal derived aqueous mineral nutrients, eg. of phosphate (P) or zinc (Zn).(Smith & Read, 1997).

The temporal bi-directional nutrient exchange enabled by either '*Arum*' type or '*Paris*' type intracellular AM fungal arbuscules helps to uniquely distinguish their mycorrhizal

functions as distributive fungal endosymbiont structures. Arbuscule fungal structures function quite unlike uni-directional nutrient adsorptive fungal haustoria structures that may characteristically form either parasitic, pathogenic, saprotrophic, necrotrophic or sometimes biotrophic fungal gain (+), and plant health drain, cost or loss (-) interactions, (Smith & Read, 1997).

***Proto-cooperation*: Non-obligate (*facultative*) mutual benefits (+,+) of mycorrhizal plant and fungal interactions.**

As previously defined by Odum (1983), "*proto-co-operation*" is facultative (non-obligate) symbiosis with mutually beneficial population interactions (+,+ / +,+). There are many examples of *proto-cooperation* in mycorrhizal symbioses, where facultative plant symbiont ('*phytobiont*') mycorrhizal associations that also have bi-directional or mutual benefits to both plant and fungal symbionts, (Odum, 1983).

In the analysis of two-species population interactions involving arbuscular mycorrhizal (AM) symbiosis, it may be hypothesised or inferred that symbiont *'traded'* mutual benefits (+,+) may also be associated with mutual (obligate or facultative) symbiont detrimental effects or costs (-,-). In other words, to paraphrase from economics theory, *'there is no such thing as a free lunch'*. In particular, the nature of AM fungal mycorrhizal symbiosis is the trade of fungal-derived phosphate (P); exchanged for plant (C) carbon available to AM fungi. In addition, plant symbiotic derived sources of carbon may also directly or indirectly become available to associated soil micro organisms residing in plant root '*rhizospheres*', and or residing in mycorrhizal soil-fungal affected soil zones '*mycorrhizospheres*', (Smith & Read, 1997).

As I currently understand the mycorrhiza literature in these areas, the '*Arum* type' root intercellular biotrophic fungal hyphae membranes may actively receive energy nutrients of plant photosynthate carbohydrates derived from within surrounding intercellular spaces of root epidermal and cortical cell walls (root cell apoplast). Intercellular fungal

hyphae may also be located adjacent to leaky root cell cytoplasm and membrane (root cell symplast), (Smith & Read, 1997).

I also currently understand that to date much less information is known about '*Paris*-type' intracellular hyphal coils and intracellular arbuscule structures. In particular, less is currently known about the exact *in-situ* locations of bi-directional nutrient transfers between plants root cortical cells inhabited by arbuscular mycorrhizal fungi with '*Paris* type' morphologies, (Smith & Read, 1997).

In well-lit plant seedlings grown in the longer day-lengths and warmer temperatures of summer, the early intracellular inhabitation of root cortical cells by arbuscule fungal structures may mediate an increase of plant-available phosphate (P), and thus may also enable increased rates of photosynthesis (+) and hence total production (+) of fixed carbon (C). Thus, an increase in plant mycorrhizal symbiosis mediated photosynthesis may subsequently mutually increase photosynthates available for the energy and or growth (+,+) of both plant and fungal mycorrhizal symbionts, (Smith & Read, 1997).

***Commensalism*: shared-feeding population interactions (0,+ / +,0); and *Amensalism* (non-shared) feeding by large and small symbionts (0,- / -,0).**

Commensalism population interactions have previously been described as "*a number of species sharing eating at a 'common' table*", (Werner, 1992). As an example of commensalism (0,+ / +,0) two-population interactions, a horse, a relatively large organism, and a small bird may together share eating oat grains in a localised common trough area, (Odum, 1983).

In contrast, when a change of circumstances occurs such that the larger symbiont horse is fed oats directly in a feeder bag, thereby excluding the bird from sharing the oats seed, there would predictably be detrimental effects (-) for bird access to oat seed food

supply. In such two-population interaction circumstance changes, there are negligible or neutral (0) nutritional effects to the diet of a larger horse symbiont, and detrimental effects (-) to the food availability of the smaller bird symbiont. Thus, such horse and bird symbionts may thus be defined as *amensal* (0,- / -,0) population interactions, (Odum, 1983; Boughey, 1973).

Amensalism (0,- / -,0): Costs and effects of root exudates prior to symbiosis with arbuscular mycorrhizal fungi

A 'cost and benefit' analysis of population interactions by pre-symbiotic plant roots suggests characteristics of *amensal* population interaction outcomes (-,0 / 0,-) as previously defined by (Odum, 1983; Boughey, 1973).

In the pre-symbiotic (pre-inhabitation) stages of AM plants and fungi, the gross or total plant costs (-) may also include an array of energy rich root exudates (eg. flavanoids). In pre-symbiotic plant rhizosphere soils, such root exudates may subsequently activate soil-fungal hyphal tip chemotropic gradient 'sensing', and consequently promote a change the direction of soil fungal hyphal tip growth towards such actively exuding plant roots. In contrast to this analysis of pre-symbiotic root exudate plant costs (-), there may be nil or negligible (0) immediate benefits to pre-symbiotic soil-borne AM fungi. (Smith & Read, 1997).

In pre-symbiotic plants, such root exudates are derived at a *cost* (-) of cell cytoplasm (symplast) minerals and shoot derived photosynthates lost by roots (-), at the benefit of soil rhizosphere ecosystems (+). However, plant costs or investments (-) of pre-symbiotic root exudates, may when viewed over the lifespan of a plant, may also promote and assist an increase in potential levels of mycorrhizal-derived minerals, and subsequent potential survival benefits (+) of AM root-fungal endo-symbiosis, (Smith & Read, 1997).

In addition, a body of mycorrhizal research has previously shown that when pre-symbiotic plants may uptake phosphate from mineral fertiliser inputs, or phosphate-rich soils, such plants may subsequently become so-called *'phosphate-sufficient'*. As a consequence of such plant-available nutrient rich soil media, both the quantity and the quality of root exudates, and the costs and benefits, are thus altered when compared to plants growing under nutrient poor soil conditions, (Smith & Read, 1997).

It is possible that a further example of *amensal* (0,- / -,0) population interactions between mycorrhizal plants and fungi may describe a situation in which facultative mycorrhizal plants may have constitutively expressed genetic defences against all fungal endosymbionts, including the obligate biotrophic AM fungi.

***Parasitism* (+/-) and *Escape* (-/+) population interactions**

As previously discussed regarding the current experimental findings of a negative plant growth in response to mycorrhizal symbiosis during low irradiance levels, AM fungi may be in resource competition for use of plant photosynthates, and consequently may thus form *parasitic* interactions with plant hosts, ie. fungal benefit (+) and plant detriment (-).

In low irradiance environments with *'parasitic'* fungal-plant interactions, it may be viewed that plant defences (+) against inhabitation by obligate endosymbiont fungi populations (-), may thus be characterised as "*escape*" interactions (-,+). The term "*escape*" describes two-population interactions where a *parasitised* or 'host' organism (or else *prey*) may benefit (+); ie. *get away from*, *allude*, or else *defend against* symbiont populations of *parasites* (-) and or *predators* (-). Conversely a parasite may thus *lose* or *not survive* (-) if it fails to obtain potential necessary benefits derived from a larger host organism. In a similar manner, predators may lose or fail (-) to capture and kill prey that *escapes* (+).

In summary, although it may be somewhat 'politically incorrect" to use the term '*parasitism*' to describe a maturing plant seed, a mammalian foetus, or else parent-dependant infant, however such ecological considerations as carbon sinks and sources indicates the existence of such unidirectional benefits from parents towards their immature and care-dependant offspring.

***Neutralism* (0,0 / 0,0): Observations of root segments not inhabited by soil fungi.**

Plant population interactions with fungi that have *neutral or negligible* effects to plant symbionts may thus be defined as *'neutralism'* (0,0 / 0,0), Odum (1983).

There are many economically important crop and ornamental plants that are capable of non-mycorrhizal growth in sterile potting media, or else may grow in hydroponic and aeroponic mineral nutrient solutions. Such non-obligate plant symbionts (phytobionts) that may also be potentially capable of forming mycorrhizal associations, are therefore by definition '*facultative*' plant symbionts, capable of living or surviving with (or without) arbuscular mycorrhizal fungi.

Soil biological populations such as plant roots and soil-fungi may inhabit a soil volume in close proximity to each other, but may also be *asymbiotic* (without-symbiosis), and therefore may not easily commence interactions with each other. Such asymbiotic populations may be due to timing differences of plant or fungal development; and/or due to seasonal changes in limits of resource availability; and or differences plant and fungus growth responses, (Smith & Read, 1997).

Thus, in spatial and or time separated, and or in non-interacting (*asymbiotic*) fungal and plant populations, such populations are likely to have negligible or neutral effects on each other, and thus may be defined as '*neutralism*' population (non-interactions), (0,0 & 0,0) by Odum, (1983).

***Competition* (-,- / -,-) and *Amensalism* (0,- / -,0): Analysis of plant defence responses and the decline of fungal arbuscule and appressoria structures**

At this point in time insufficient is yet known about fungal and plant population interactions that occur during the late stages, including the decline, dismantling and senescent stages of AMF arbuscule intracellular inhabitation of root cortical cells, (Smith & Read, 1997).

However, the temporal functioning nature of AM fungal arbuscules as intracellular sites of fungal-plant nutrient exchange, and subsequent the subsequent decline of arbuscules, may hypothetically be contributed to partly by 'direct inhibition' type *competition* (-/-; -/-) interactions as defined by Odum (1983).

Plant-fungal population interactions such as a variety of 'gene for gene' plant-defence mechanisms may also *directly inhibit* all fungal endosymbionts of roots, including potentially mineral beneficial arbuscule fungal structures, (Long,2000).

Plant defence mechanisms and soil-fungal interactions that occur during the early stages of soil fungal appressoria, root entry point fungal structures, may possibly be ecologically described as *amensa*l (0,-) population interactions. Plant defence against fungal appressoria structures may have neutral or negligible (0) effects to a defending plant host, but if such a defence is successful will produce detrimental (-) effects to 'unsuccessful' potential soil-fungi symbionts, (Odum, 1983).

Future requirements for deeper ecological research and wider analysis of interactions between crops and soil micro-organisms.

In conclusion to this brief discussion of current experimental results and the ecological models described above, relatively little is understood about nutrient exchanges that may occur between plants, soil-fungi and other heterotrophic micro-organisms inhabiting rhizosphere and or mycorrhizosphere soils, (Garbaye J, 1994; Lindermann, 2001).

Further mycorrhiza research and symbiosis model considerations are required in order to include a more complex analysis of above and below ground soil organism population interactions between the commonly occurring soil biotic and abiotic factors including natural soil disturbances, tillage cultivation practices, grazing animals, plant shoot and root ratios, and mycorrhizosphere associated micro-organisms.

However, the current bioassay methods and field-observations have indicated that the effects of physical soil disturbance treatments such as tillage, surface levelling earthworks and fallow have mutually '*suppressive*' (-, -) effects to pasture plants (-) and to the AM soil-fungal inhabitation levels (-) observed in maize root segments grown in greenhouse bioassay pots that contained field-soil samples.

Furthermore, it may also hypothesised that mutually detrimental *competition* (-,-) effects may also occur in some transgenic crop plants such as those carrying the PR-2 gene. This gene encodes for production of the anti-fungal enzyme, a class II acidic β-1,3 glucanase, shown to inhibit AM fungal inhabitation of roots, (Vierhielig *et al.*, 1995; Glandorf *et al.*, 1997).

Additionally, non-mycorrhizal (non-host) monoculture crops such as canola *Brassica napus* have previously been demonstrated to have 'suppressive' or 'detrimental' effects (-) on the survival of AM fungi, common soil and root inhabiting obligate biotrophic endosymbionts, (Ocampo & Hayman, 1981; Powell, 1982; Miller, 2000; Karasawa *et al*, 2000a; Karasawa *et al*, 2001; Seymour *et al*, 2001; Thompson *et al*, 2001).

In crop rotations of wheat or maize grown in soils with a cropping history of a preceding monoculture crop rotation of canola, the suppressed levels of soil populations of AM fungi that result from growing a crop rotation of non-mycorrhizal canola *B. napus* have also previously been shown to result in suppressed levels (-) of plant-

available AM fungal mediated soil minerals, eg. phosphate (P), (Ocampo & Hayman, 1981; Gavito & Miller,1998a & 1998b; Karasawa *et al*, 2000a; Seymour *et al.*, 2001).

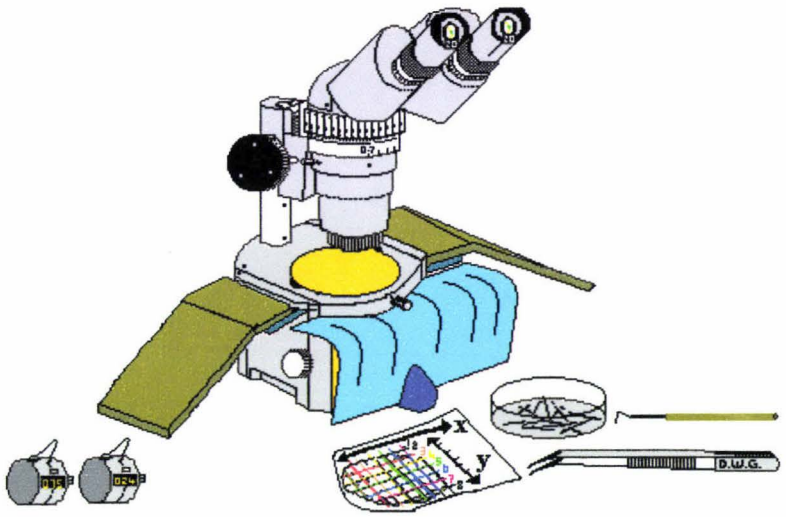
Thus, it may also be hypothesised that mutually detrimental *competition* (-,-) effects may be predicted to increase in circumstances where AM fungal potential host plants including weeds are routinely treated with herbicides (chemical tillage) during the growth of transgenic crops such as glyphosphate tolerant *Canola*.

In strongly detrimental (-) circumstances for weeds such as in glyphosphate tolerant *Canola*, crop management by chemical tillage (herbicide) may hypothetically result in the survival of only a few weeds that are negligibly affected by (0) or resistant to herbicide treatments. Thus, such a transgenic crop management may be predicted to have similar effects to intensive and repeated physical soil-disturbance treatments, further reducing the quantity and biodiversity of potential weed plant hosts (-) available for obligate biotrophic AM fungi (-), (Odum,1983; Boughey, 1973).

In conclusion to the preceding discussion of ecological models of symbiosis and mycorrhizas, it is a test of the potential use of any existing or proposed models, that most if not all known factors should 'fit' the models examined. Additionally, models of symbiotic population interactions are of further assistance if such models can also be used to predict previously unknown or unanticipated production management environmental effects (or outcomes) of two-population interactions.

Microscopy Instrument Research

Olympus SZII Light-Transmission Dissection Stereomicroscope



The current experimental aims to attain low-cost, low-heat stereomicroscopy methods were successfully met by root-fungal bioassay 'gridline-intersect' method observations that used an alternative light source, a low-cost and 'power-saving' 21W fluorescent light tube.

The re-use of a 1960's Olympus SZIII dissection stereomicroscope fitted on an Olympus 'trans-illuminator' base and frosted glass microscope stage was successfully practically tested by 'gridline-intersect' root-fungal bioassay examinations of un-squashed (intact) maize seedling root segments.

In contrast, the experimental use of root segment chambers to examine intact root-segments by transmitted light was unsuccessful. This lack of success was primarily because images of root segments were often obscured by dark-shaded irregular light refraction patterns derived from the angled slopes of the glass walls of ridged-glass microscope slides.

Fungal 'auto-fluorescence' microscopy of* in vivo *unstained root segments

The experimental methods used for UV and CLSM microscopy examinations of reflected-light fungal 'auto-fluorescence' of intact, un-stained *in-vivo* maize and leek root segments, provided a successful practical test use of ridged-glass microscope slides (see also Appendices R2, R3, R4 and R5)

Both the CLSM and UV autofluorescence microscopy methods used rapid and low-cost and minimal preparation methods to observe non-stained and intact *in vivo* root segments. However, despite the high-resolution images of autofluorescent fungal and roots visualised by CLSM methods, there are also relatively prohibitively higher operational costs associated with CLSM microscopy.

In comparison, the UV microscopy methods used to visualise fungal autofluorescence had relatively very low financial cost. Additionally, fungal autofluorescence observed by UV microscopy methods also provided a useful time saving and CLSM Ar/Kr laser resource saving method for visual screening or 'scanning' of root segments prior to selecting individual fungal arbuscule structures for examination by CLSM fungal and plant autofluorescence, (Long, 2000).

In comparison to previously published UV autofluorescent fungal microscopy images by Ames *et al.* (1982) and Jabaji-Hare *et al.* (1984), the current study visualised similar quality images of fungal structures observed in *in-vivo* root segments of maize and white clover seedlings, (see photos Appendix R3).

There may also be some potential for future research into low-cost UV fungal autofluorescence examinations of relatively large quantities of fresh *in-vivo* root segments using high-resolution images obtained by modifying 'flat-bed' or hand-held digital scanner hardware.

In comparison to previously published CLSM autofluorescent fungal and *in-vivo* plant root images by Vierheilig *et al* (1999) & Vierheilig *et al* (2001), the current study provided equal if not better quality images of CLSM autofluorescent AM fungal arbuscule structures, with a clear background and foreground of minimally autofluorescent *in vivo* plant root tissues (see Appendices R4 & R5).

Recently published research into CLSM fungal-autofluorescence within *in-vivo* root segments has found that autofluorescence is largely associated with senescent and declining arbuscule structures, Vierheilig *et al.* (2001).

Squashed root segment examinations

At the commencement of this thesis project prototype the bioassay microscopy methods quantitatively examined cleared and stained squashed maize seedling root segments permanently mounted between a microscope slide and a long cover slip, as described by Brundrett et al, (1996), Varma (1998). Considerable practical experience was necessary for the effective use of hand tools required for careful handling of squashed or intact root segment samples prepared for microscopy examinations.

In comparison to later microscopy examinations of cleared and stained maize root segments temporarily mounted Petri dish, the 'squashed-root' sample microscopy preparation method is relatively time consuming, resource intensive and more expensive. However, the 'squashed-root' microscopy preparation methods are relatively less time-consuming or resource intensive if compared to compound microscopy sample preparations that may involve a number of complex procedures including: drying, freezing, embedding, and sectioning.

Chapter 5 Conclusions

The observed effects of field-soil disturbance treatments.

The dry weight biomass of maize seedling shoot tissues was negatively correlated with increased soil-fungal inhabitation by both intercellular and intra-cellular fungal structures examined.

This result finding of soil-fungal inhabited plant growth suppression, or fungal 'parasitism' of plant growth, appears to agree with similar results of mycorrhiza researchers that have previously examined bioassay seedlings grown under winter day-length and 'sun strength' limited lower light environments.

This plant-growth parasitic fungal effect has previously been reported to be a consequence of winter-grown annual summer crop plants such as maize seedlings with roots inhabited by arbuscular mycorrhizal fungi. It has previously been hypothesised that symbiotic plant and mycorrhizal fungal metabolism is consequently not as limited by, and nor are mycorrhizal plants growth-restricted by, plant available soil phosphates (P).

It has previously been hypothesised that as a result of winter seasonal effects on light quantity and quality, that in plants in symbiosis with arbuscular mycorrhizal (AM) fungi and other soil-borne fungi, both plant and fungal species may be growth restricted by being required to share or compete for less-available plant photosynthetic fixed Carbon.

In an unexpected observed result finding, the observed effects of soil disturbance treatments resulted in statistically significant decreased numbers of maize seedling root segments per uniform soil-volume. There were slightly fewer root segments observed from seedling maize plants with the most fungal inhabited root segments grown in samples of Permanent Pasture (PP) field-soil, and contained in a nylon mesh pouch

within each bioassay pot. This similar and related result finding of negatively correlated reduced seedling growth, ie. less root segment numbers observed in soil volume samples form PP permanent pasture sites may also potentially be explained by the Carbon-limited plant growth hypothesis explaining the fungal 'parasitism' effect of plant-symbiont shared Carbon.

The current observations of a plant 'carbon-drain' effect are possibly also result from a time-determined snap-shot measurement of mycorrhiza and other soil-fungal symbiosis interactions derived during relatively early plant and soil-fungal stages of winter-grown mycorrhizal inhabited maize seedling root segments.

These result findings of fungal parasitism of two plant growth parameters are however qualified in the current experiment by the as yet untested possibility (and hypothesised probability) of bi-directional or mutual nutrient benefits, ie. AM fungal-mediated increased plant phosphate levels.

Ecological models of interactions between two populations.

Whilst presenting a mycorrhiza conference poster at ICOM3 in Adelaide in July 2001, I received encouraging discussion feedback regarding the herein proposed 3-way Venn-diagram model of (+, -, 0) two-population (plant-fungus) symbiosis interactions, including from Dr Mark Brundrett, author of the previously published 3-way Venn diagram model of mycorrhiza (*soil-fungal-plant*) symbiotic interactions. It was recommended to me that I should present my poster topic as a short journal article for publication in *Trends in Ecology & Evolution.*

Microscopy instrument research

I was fortunate to be offered to purchase an excellent ex-teaching stereomicroscope, an Olympus SZIII dissection microscope. This important personal purchase thus enabled a

freedom of choice to make minor adaptations to the microscope light sources as described by the current microscopy instrument research. This instrument property ownership point of difference was a personally helpful research option that enabled microscope modifications 'outside the square' of what conventionally may be expected by students or staff working with University or institutional owned research instruments, under warranty, service contract or not. After only minor electrical wiring safety microscope adaptations, a relatively cool temperature 21W fluorescent light source was used instead of the original 20W incandescent Olympus SZIII microscope light transmission source. Thus, the new brighter and cool temperature light-source successfully enabled many continuous hours of 'grid-line intersect' method microscopy examinations of samples of intact (unsquashed) maize seedling root segments.

I was successful in preparing and using microscope glass slides as sealed chambers for direct UV and CLSM microscopy examination of intact and *in vivo* root segments of maize seedlings. Further instrument research is possibly desirable into scaling up the ease and speed of examinations, especially research into the relatively low-cost UV-induced autofluorescence of fungi inhabiting *in-vivo* root segments.

SPSS

As a software package, SPSS offered very good user flexibility for statistical analysis of effects between fungal structure types and soil disturbance treatments examined. Statistically significant replicate numbers were examined for analysis of root-fungal inhabitation bioassays.

Appendices

The costs of methods and materials described and the details of suppliers of materials or time are annotated as footnotes.

APPENDIX F: Pasture Field-soil Sample Sites

Vegetation Photographs of New Pasture (NP) Field-soil Sample Sites

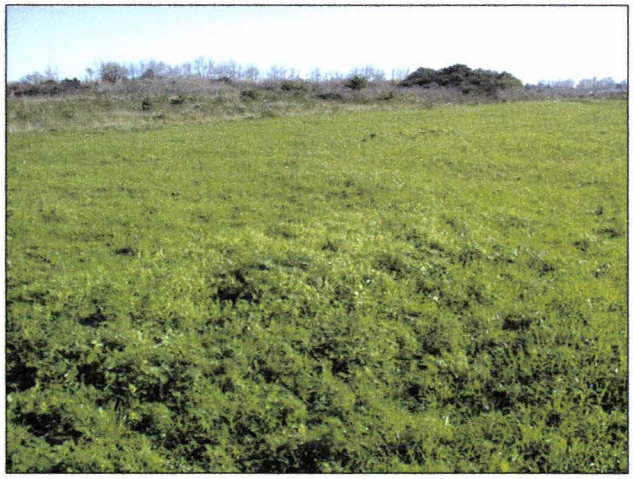


Figure 30 Un-grazed new pasture vegetation, facing S.W. towards sandy dunes and Manawatu River.

Figure 31 Close-up of un-grazed new pasture vegetation.

Figure 32 Un-grazed new pasture vegetation

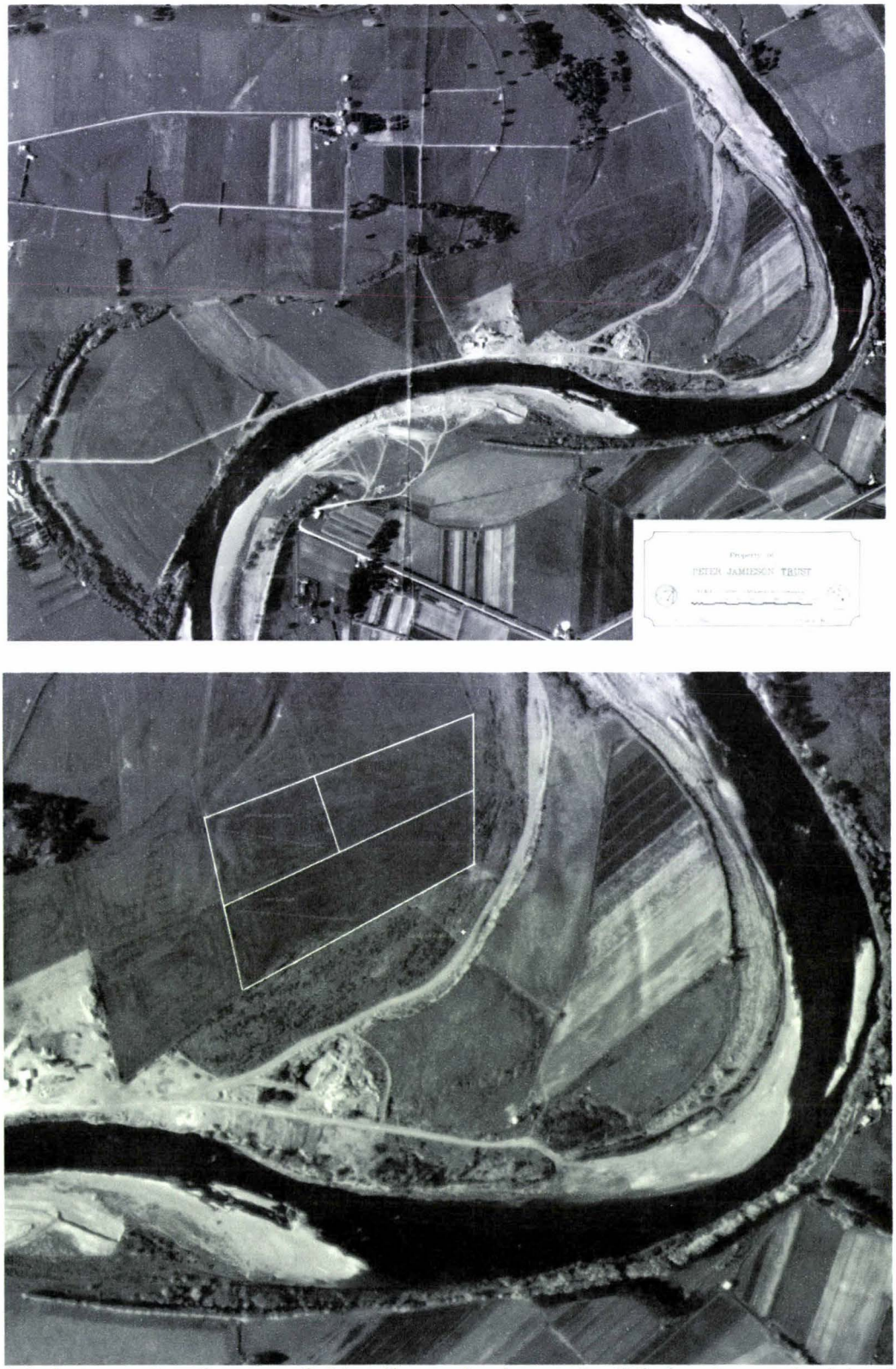
Figure 33 Legumes and other un-grazed vegetation in new pasture soil disturbance treatments

Vegetation Photographs of Permanent Pasture Field-soil Sample Sites.

Figure 34 Permanent pasture vegetation, facing NW towards the Manawatu River and Tararua Ranges.

Figure 35 Close-up of permanent pasture vegetation.

APPENDIX AP: Aerial Photographs of Pasture Field-soil Sample Sites



Aerial photographs of pasture field-soil sample sites at the 'Biofarm' organic dairy farm at Whakarongo, adjacent to the Manawatu River near Palmerston North, New Zealand

APPENDIX P: Pasteurisation of Low-Nutrient Soils as Bioassay Growing Media

As previously discussed, AM seedling bioassay propagation methods used surface-sterilised maize seeds sown directly into nylon mesh pouches containing field soil. In all prototype and current bioassay methods, seedling roots required a larger one-Litre soil volume to grow in, and to contain the 120g field-soil samples and or control (NM) sandy silt-loam soils supplied by the soil pouch alone.

Thus, it was essential to remove any potential contamination effects from soil fungi derived from the sandy silt loam growing media. Effective pasteurisation of sandy silt loam bioassay propagation media was thus obligatory and provided control (NM) soil media in which to grow maize bioassay plant roots. The three different soil pasteurisers used in these studies all used standardised protocols for media pasteurising as described by Brundrett et al (1996).

Sandy silt-loam soil was collected from beneath a small dune in a Biofarm pasture site adjacent to the Manawatu River at Whakarongo as shown in appendix (AP). The uppermost 30cm surface layer of sandy topsoil was removed to enable collection of a relatively homogenous sandy silt-loam soil from between 30cm to 60cm deep. The sandy soil was collected and transported in clean plastic rubbish bags.

In order to ensure that only maize bioassay roots were present in the pasteurised growing medium at harvest, it was necessary to thoroughly sieve the freshly dug sandy silt-loam soils with a 5mm mesh to remove mature pasture plant roots.

Pasteurised soil steam formation and increased thermal conductivity of sandy silt-loam soils was assisted by wetting soils with distilled water before each heat treatment. Soils were maintained at temperatures of more than 70°C for approximately one hour. Soils were then cooled for one day before soil mixing, followed by a second and final 70°C soil heat-treatment.

As shown in Figure 36, the Crop & Food soil pasteuriser was mounted on a trailer. The soil-pasteuriser chamber enclosed a rotary soil mixer operated by a tractor-PTO connection. Soil media received steam carried through pressure hose fittings from an adjacent stationary electric boiler.

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 .

Soil media was added via the top of the soil-mixer through a safety grill. After rotary soil mixing, the grill was replaced and the lid fitted securely. The soil-mixing chamber was then inverted so the lid became the base of the soil-steaming chamber during steaming. To assist drainage of wet soils during steaming, and to provide a separate steaming compartment under the soil media, the inside of the soil chamber base (or lid) was fitted with a stainless-steel sheet metal grill.

Compared to sandy silt-loam soils used here, most potting media pasteurised are relatively coarse materials. Therefore, in order to ensure effective pasteurising of relatively fine textured sandy silt loam soils, it was necessary to modify the container base grill to prevent the sandy soil media from falling through and clogging the basal compartment of the media steaming chamber. The grill was removed from the lid

and covered with a single size 'fitted' cotton sheet[29]. The sheet covered grill was then secured inside the lid.

The soil was steam heated to 70o C for one hour before cooling for one day. Following cooling, the soil pasteuriser was inverted back to the 'upright' soil mixer position, soils were rotary-mixed for 2 minutes. The chamber was then returned to the pasteuriser position before soils received a further hour of steam heating.

Pasteurised soils were cooled for a further day before being measured into one-litre volumes into clear 'MB' bioassay pots[30] lined with black PB2 planter bags[31]. A one-litre volume soil scoop was especially crafted using recycled sheet metal, wooden dowel, and plywood.[32]

[29] single bed fitted-sheet cost $9.95

[30] "MB" pots are recycled HDPE 2-litre"milk bottles", cut down to contain ~1.1 litres, Palmerston North City Council, PNCC recycling centres. cost $ ... nil public cost to re-use & recycle

[31] "PB2" planter bags, INR 4 Pkts @ $5,10, = $20.40

[32] One litre scoop constructed from sheet metal and wood. Materials and manufacture supplied *GRATIS* by Jens Jorgensen, INR, Ecology Department. nil cost $ ... gratis materials & time. Tena koe Jens, thankyou again.

APPENDIX A: Wet-stability soil aggregate tests

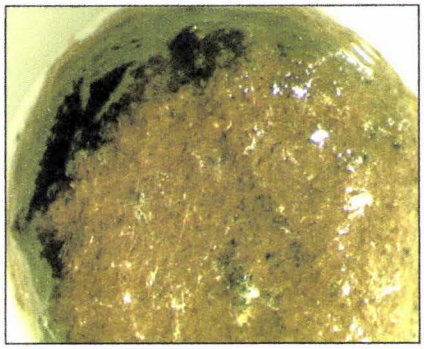


Figure 37 Wet-stable soil aggregate and soil fungal hyphae

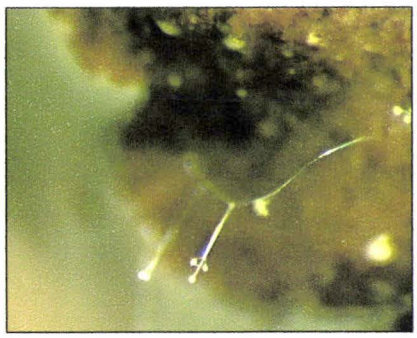


Figure 38 Soil fungal hyphae extending from wet-stable soil aggregate

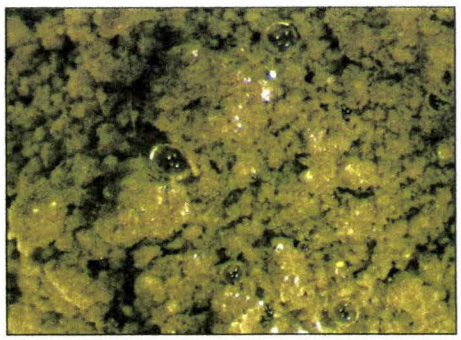


Figure 39 New pasture artificial soil aggregate - control no added nutrients, soil structure is slaking with soaking in 50% ethanol solution

Figure 40 Permanent pasture artificial soil aggregate – no added nutrients, soil structure is slaking in 25% ethanol solution

APPENDIX C: Chemical laboratory tests of permanent pasture, new pasture and sandy-silt-loam field-soil samples

Soil Sample	pH	Olsen P µg P / g	SO_4 µg S / g	K me / 100g	Ca me / 100g	Mg me / 100g	Na me / 100g	CEC me / 100g	Soil volume Adj. Factor	C %	N %
np1	5.4	13.5	6.3	0.68	4.2	1.12	0.05	8	1.05	1.94	0.20
np2	5.5	19.9	4.0	0.51	3.9	1.10	0.08	8	1.12	1.90	0.20
np3	5.3	15.0	4.5	1.67	8.5	2.68	0.17	15	1.11	2.31	0.22
np4	5.4	16.2	3.5	0.65	7.4	1.80	0.19	12	1.05	2.24	0.22
pp1	6.1	28.4	3.8	0.65	4.0	1.16	0.08	7	0.84	5.26	0.49
pp2	6.2	22.7	2.8	0.75	3.8	1.10	0.07	7	0.87	4.66	0.44
pp3	6.3	34.2	4.5	1.40	8.7	2.96	0.09	15	0.86	4.38	0.43
pp4	6.3	29.9	3.3	1.60	7.7	2.48	0.10	15	0.85	4.95	0.48
SSL	5.9	19.2	0.5	0.30	1.1	0.59	0.10	4	1.14	0.60	0.06
NP 6	5.8	6.6	3.3	0.52	7.1	1.62	0.11	17	1.02	2.46	0.225
PP 6	5.8	10.4	5.5	0.56	9.2	2.13	0.16	19	0.86	3.65	0.33

APPENDIX S: Microscopy examinations of squashed maize root segments

Using 'squashed-root' microscopy preparation methods described by Brundrett et al. (1996), permanently mounted 'cleared and squashed' root segments were sealed by clear nail varnish adhesive[33], on glass slides[34] fitted with long cover slips[35].

Phase Contrast Microscopy

A Zeiss Axiophot compound microscope was used in Phase Contrast mode to examine squashed root samples of *Stelaria media* (chickweed), *Z.mays* (CF1 maize hybrid & 'Early Gem' dwarf sweet corn), and *Trifolium repens* (white clover).

Phase-contrast methods used a 10X eyepiece, in combinations with 10X, 20X, 40X standard objective lenses, and a 100X oil immersion objective lens. Phase-contrast microscope images were captured on a JVC video camera CCD system connected to a Silicon Graphics Indy workstation. The Silicon Graphics systems provided an image resolution equivalent to (1028 X 1028) pixels.

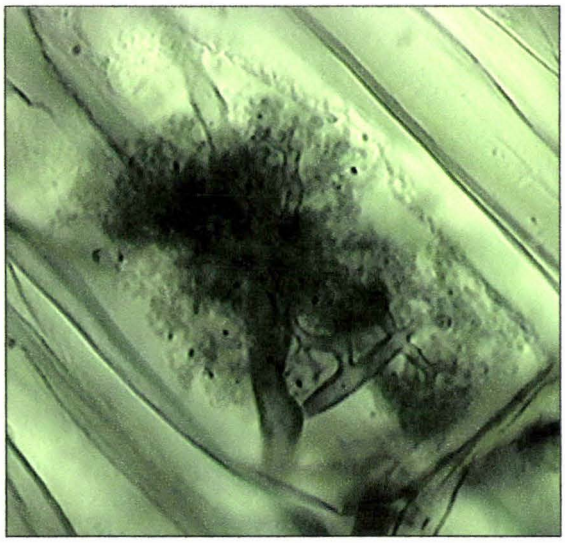


Figure 41 A phase-contrast microscope image of a CBE Black-stained dendritic 'branch' or 'small-tree' shaped fungal *arbuscule* structure inhabiting a rectangular-shaped maize root cortical cell.

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[33] Clear nail varnish used as adhesive for permanent mounted squashed root samples $5.00 each (X5)
[34] Microscope clear glass slides (1"X3", or 25.4mm X 76.2mm) 1mm-1.2mm thick, estimated cost $70.00
[35] Glass cover slips for microscope slides, (22mm X 60mm) one ounce No.1, Biolab Scientific , $30.00

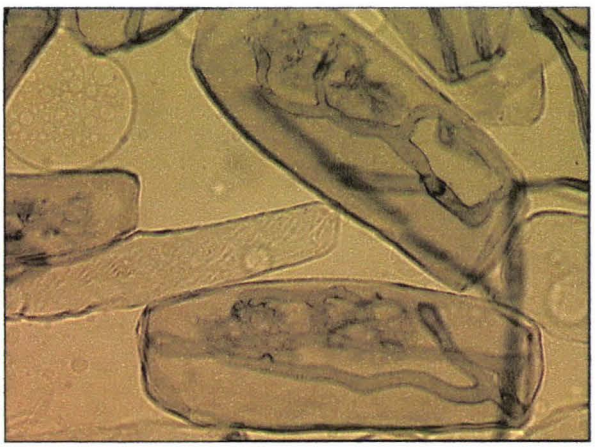

Figure 42 '*Paris*-type' intracellular arbuscules and thicker intra-cellular coiled hyphae.

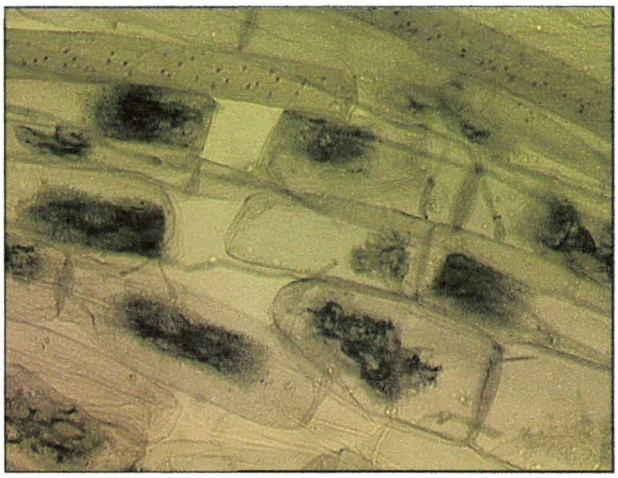

Figure 43 '*Arum*-type' fungal arbuscules inhabiting rectangular-shaped root cortical cells, and adjacent to distinctive xylem vessels.

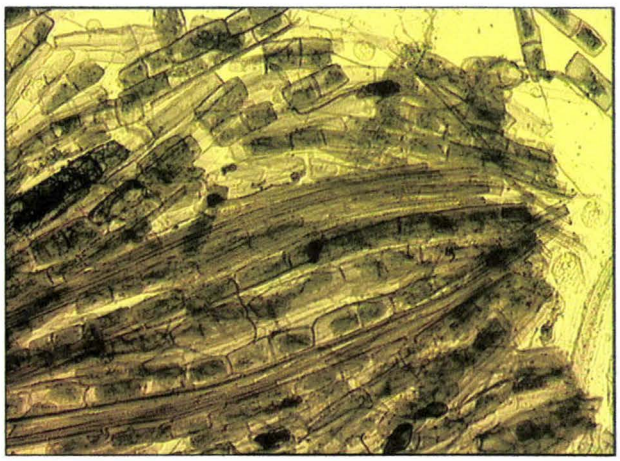

Figure 44 A Phase-Contrast microscope image of a densely fungal arbuscule inhabited 'squashed-root' prepared segment of a maize seedling.

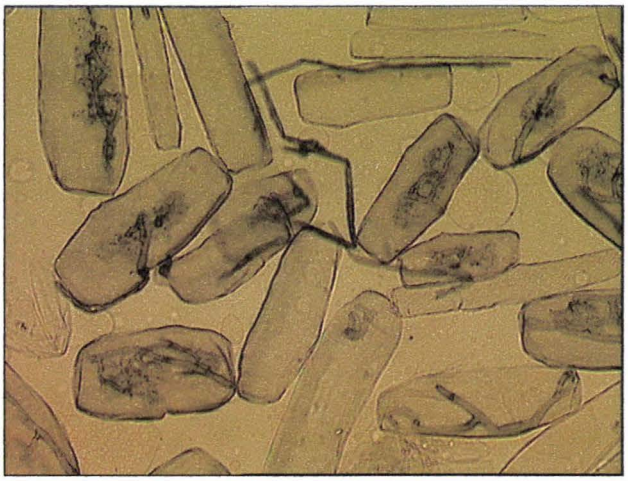

Figure 45 'Squashed-root' method microscopy examination of CBE black-stained fungal arbuscules and intercellular hyphae that inhabited squashed root cortical cells.

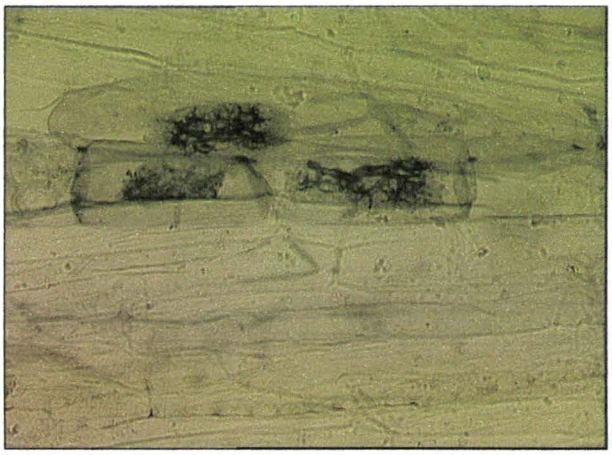

Figure 46 A group or colony of three fungal arbuscules inhabiting root cortical cells.

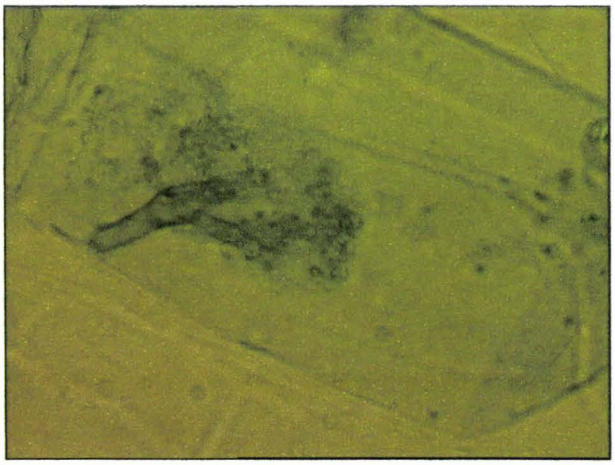

Figure 47 A single '*Arum*-type' fungal arbuscule structure, characteristic of the 'small-tree-shaped' dendritic branching morphologies associated with transfer of soil Phosphate (P) from AM fungi to plant root cortical cells and whole plants.

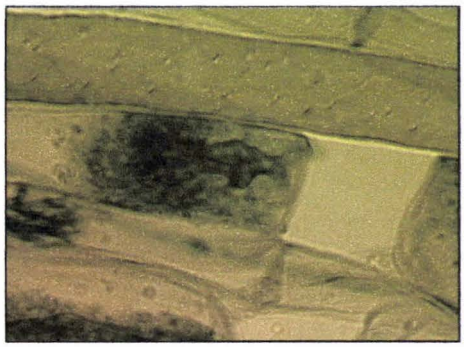

Figure 48 A close-up view of an *'Arum*-type' fungal arbuscule contained in a rectangular-shaped root cortical cell and adjacent to a xylem vessel.

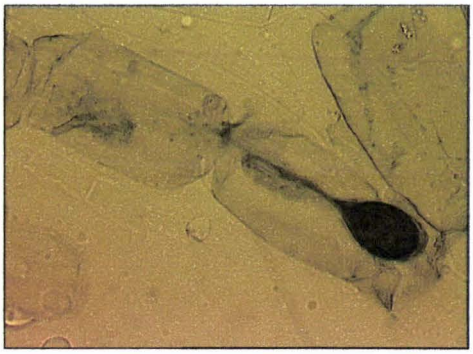

Figure 49 An *'Arum*-type' arbuscule and a vesicle occupying adjacent rectangular-shaped root cortical cells.

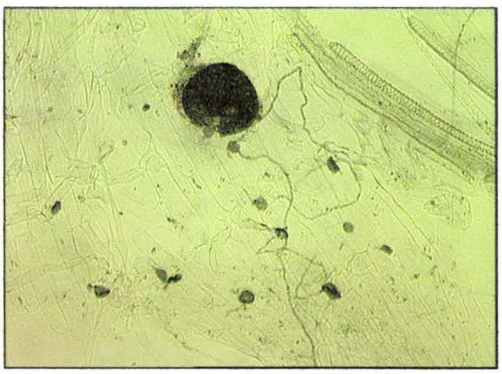

Figure 50 A fungal sporocarp, several spores and hyphae adjacent to a *'squashed-root'* microscopy method sample.

Differential Interference Microscopy, or Normarski Microscopy

A Zeiss Axiophot microscope was used in "Differential Interference" or "Normarski" mode. Normarski microscope images were viewed with a 10X eyepiece magnification used in combinations with 10X, 20X, and 40X objectives lenses.

As illustrated in Figure 51, the transmitted light birefringence colouring effects of Normarski microscopy were used to visually differentiate between fungal hyphae and surrounding plant root wall materials. Normarski microscopy methods allowed rapid visual differentiation between plant and fungal tissues using the light refractional and colourising effects of squashed root tissues, in contrast to black stained root borne fungal tissues.

In prototype bioassay methods the entire length of each squashed root segment was scored for the presence of CBE stained fungal structures. Normarski microscopy provided a relatively quick scanning and counting method for squashed Z.mays root segments containing CBE-stained fungal arbuscules. Squashed root microscopy examination methods were derived from (Brundrett et al. 1996; Clapp et al. 1997; Dodd & Thomson 1994; Kormanik & McGraw 1982; Kucey & Paul 1982).

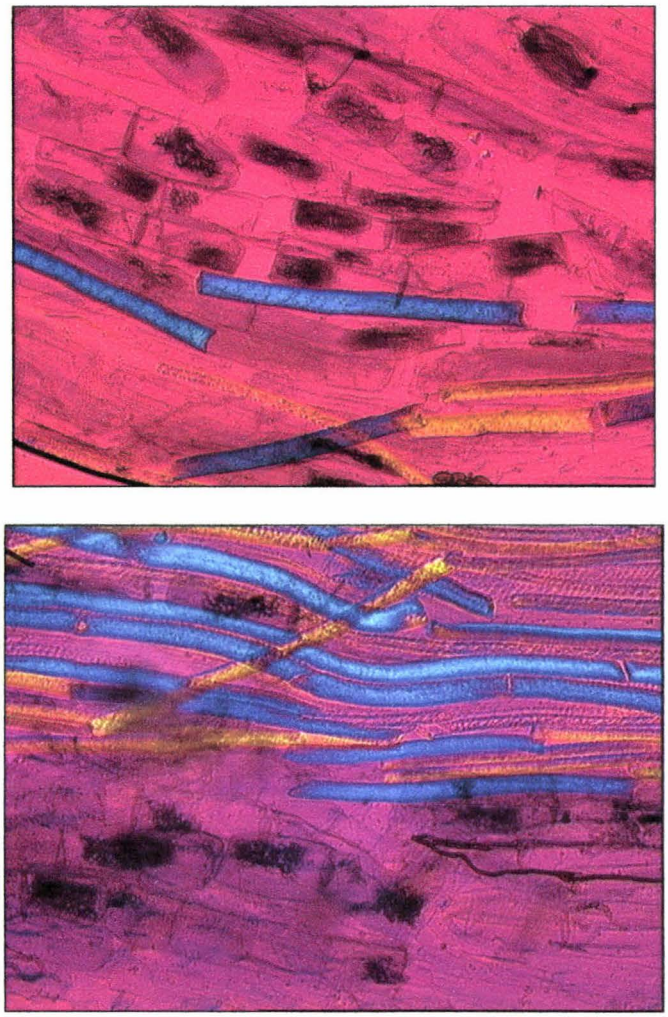

Figure 51 'Normarski' or 'Differential-interference' microscope image of colourised plant tissues and CBE black-stained fungal arbuscules inhabiting a squashed maize root segment.

APPENDIX R: Intact Root Segment Microscopy Examinations

APPENDIX R1: Transmitted light microscopy examinations of KOH cleared & CBE stained intact root segments.

Intact root segments were examined at image magnifications between 28X and 160X. Higher magnification images had a relatively limited depth-of-field. This transmitted-light dissection microscope and root-chamber slide method was unsuitable for useful examinations of roots, mostly due to transmitted-light diffraction from the diagonally angled walls of individual root-chambers that caused light and dark image interference effects.

As illustrated by Brundrett *et al* (1996), '*gridline-intersect*' root-fungal bioassay microscopy methods used a modified light-source in an Olympus SZIII transmitted-light dissection stereomicroscope to examine intact root segments drained of storage liquid (lactoglycerol), and evenly distributed across a Petri dish base.

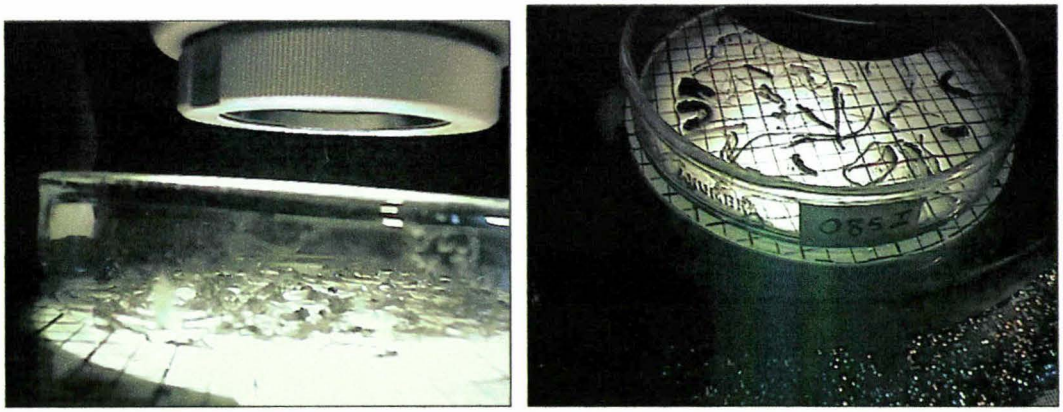

Figure 52 Side views of Olympus SZIII microscope fitted with 2X objective lens for viewing intact maize root segments contained in a Petri-dish.

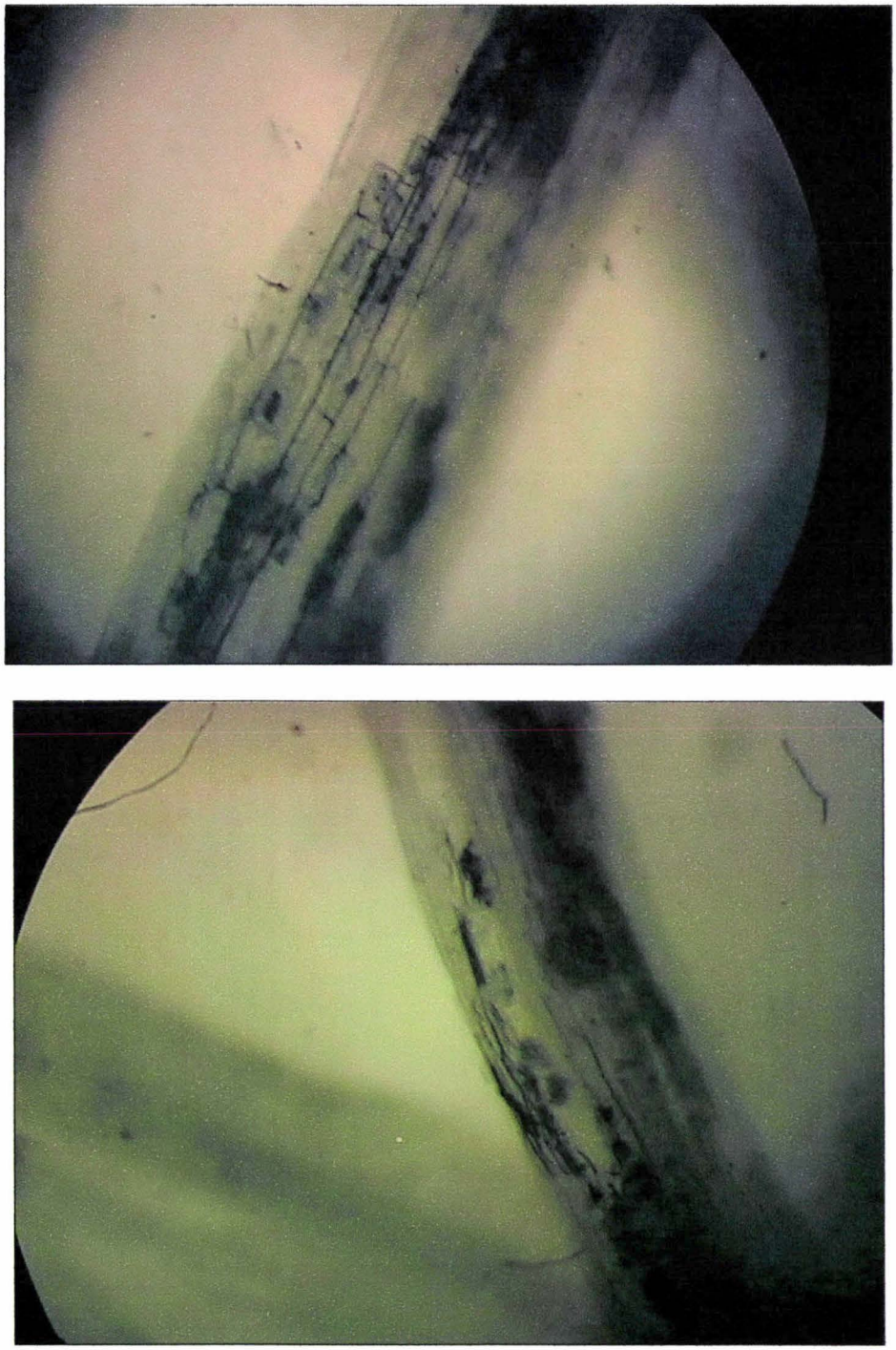

Figure 53 Transmitted-light microscopy images of KOH cleared and CBE stained intact maize root segments, (160 X magn.).

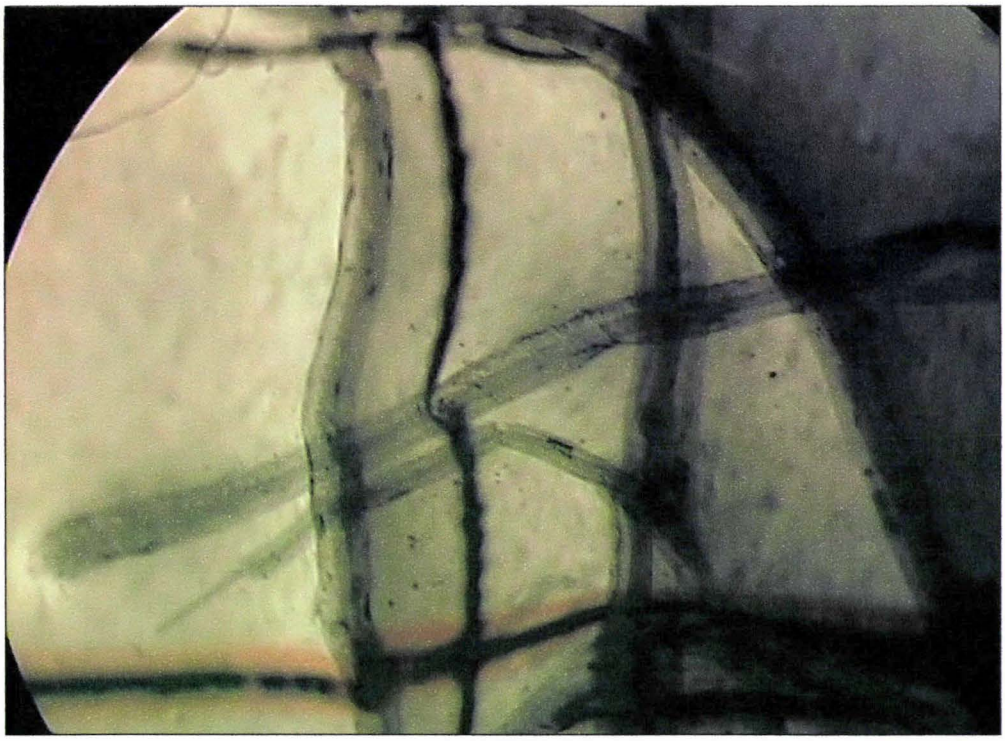

Figure 54 Low magnification image (28X magn.) of maize roots observed using gridline-intersect method described by Brundrett *et al* (1996)

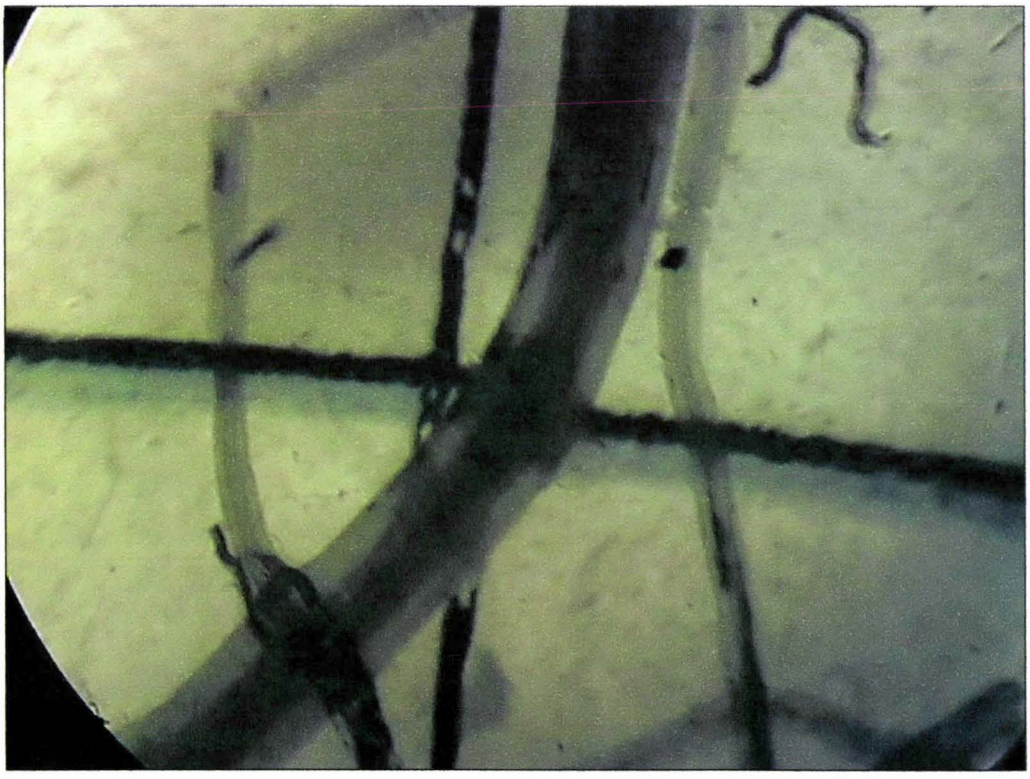

Figure 55 A higher-magnification image of maize root segments observed by in gridline-intersect method as described by Brundrett *et al* (1996).

APPENDIX R2: Root-chamber glass slide examinations of intact maize root segments.

The development of a reliable leak-proof root-chamber microscope slide was necessary to make it feasible to use UV and CLSM microscopes to qualitatively and quantitatively examine fungal structures in fresh in-vivo roots of *Z.mays* and *T. repens.* As shown in Figure 56 'grooved' glass slide sealed root-chamber methods tested here enabled microscope examinations of individual root segments aligned in six parallel root-segment 'chambers' or 'tanks'.

Figure 56 Oblique view of grooved-glass 'root chamber' glass microscope slide containing maize root segments

"Reeded" glass sheets[36] were cut to form (75mm X 25mm) grooved cavity slides, for use as root "chambers", or root "tank" microscope slides. Intact root segments were temporarily mounted in either distilled-water or else optical grade glycerol[37]. Earlier root chamber methods used a syringe to apply petroleum jelly around the outer edges of a (25mm X 75mm) "Reeded" glass slide. A long cover slip, (22mm X 60mm) was temporarily mounted and adhered onto the "Reeded" glass slide surface using

[36] 'Reeded' glass sheet, cost $19.80
[37] optical grade glycerol cost $ 25.00 per 100ml

petroleum jelly[38] applied by syringe. This early method 'worked', in that the root chamber slide was leak-proof when roots were examined on an inverted microscope. Later prototype root chamber slides improved the potential for petroleum jelly mess problems by forming rectangular shaped slide chamber walls formed by a waterproof silicon glass adhesive sealant[39].

Intact root segments mounted in root chambers were examined by transmitted light microscopy, UV-microscopy, and Confocal-microscopy of in-vivo untreated freshly harvested soil-fungal colonised plant roots.

APPENDIX R3: UV-Microscopy of Fungal Autofluorescence of In-vivo *Root Segments*

Root segments were mounted in distilled water or optical quality glycerol contained in Reeded glass root chamber slides. Three (3) separate UV microscope instruments, in combination with FITC filter sets, were used to visualise ultra-violet induced fungal autofluorescence in (or on), fresh *in vivo* root segments. The UV-autofluorescence root-fungal microscopy methods used here were derived from Ames *et al* (1982).

Using a Nikon Diaphot TMD inverted microscope, and FITC filter set C, allowed UV-induced visualisation of fungal infected root segments.

The sealed root chamber grooved-glass slide was used to examine UV auto-fluorescence of intact root segments by both inverted and upright UV microscopes. When the slide root-chamber was inverted the roots segments laid against the inside surface of the inverted slide cover slip.

In a totally darkened room background, a twenty second (20s) exposure time provided good quality yellow autofluorescent biological (fungal?) images, with dark backgrounds photographed on 100 ASA Kodak Elite slide film.

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[38] 'Vaseline'petroleum jelly $12.00 200g

[39] 'Silaflex' silicon glass adhesive $20.00 tube

An Olympus BX66 digital-image microscope using UV epifluorescence illumination, and an FITC filter set, obtained digital images of fungal arbuscule colonised roots. Digital images used a relatively low power (10X) objective lens, and had a relatively short exposure time of 4 seconds (4s), (see Figure 57 and Figure 58).

A Leica DMRBE TCS4D microscope fitted with Leica I3 (450-490nm) excitation filter set was also used to visualise the bright yellow autofluorescence of fungal structures in and on fresh in-vivo roots.

The Leica microscope method for visualisation of fungal UV-autofluorescence provided an effective pre-screening method to locate fungal structures prior to fungal autofluorescence visualisation by CLSM (laser) illumination of the same image field of view on the same microscope, as described by Long (2000).

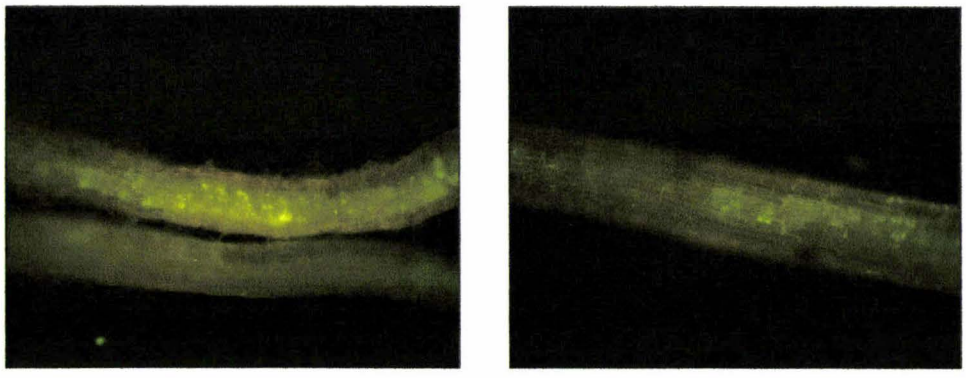


Figure 57 UV-induced yellow autofluorescence of unstained living fungi inhabiting fresh untreated maize root segments mounted in root-chambers containing optical quality glycerol.

Figure 58 Image software brightness and contrast intensified root-fungal image

APPENDIX R4: Confocal laser-induced fungal autofluorescence of unstained* in-vivo *root segments

Freshly harvested *T. repens* white clover roots were obtained from outside grown pots of *A.porum* leek plants (cv. 'Welsh wonder'), originally grown for a prototype bioassay.

On Reeded-glass root chamber slides, the uppermost surfaces of silicon sealant root chamber walls were smeared with a thin layer of petroleum jelly. Root segments 3-4cm long were placed into root chambers. Optical quality glycerol mountant was added to the root chamber until nearly full, thereby minimising bubbles in root chambers, and minimising risks of messy excess mountant.

Using adapted confocal laser-scanning microscope (CLSM) methods of Vierheilig *et al.* (1999), a Leica DMRBE TCS4D Kr/Ar (CLSM) used 488nm excitation wavelength to visualise autofluorescence of fungal structures in fresh in-vivo roots of *T. repens,* white clover. The 3-D composite image illustrated in Figure 59 was obtained using (63X) oil-immersion objective lens with a numerical aperture (=1.4, and an electronic zoom factor of (1.3X).

The CLSM 3-D composite image illustrated by Figure 59 and Appendix T5 is comprised of forty-six (46) CLSM optical sections, with 30 μm between each section. The depth of the Confocal image (z) is thus (46 X 30(μm) = 1380 μm. CLSM image scanning resolution was 512 X 512 pixels = 262 144 pixels.

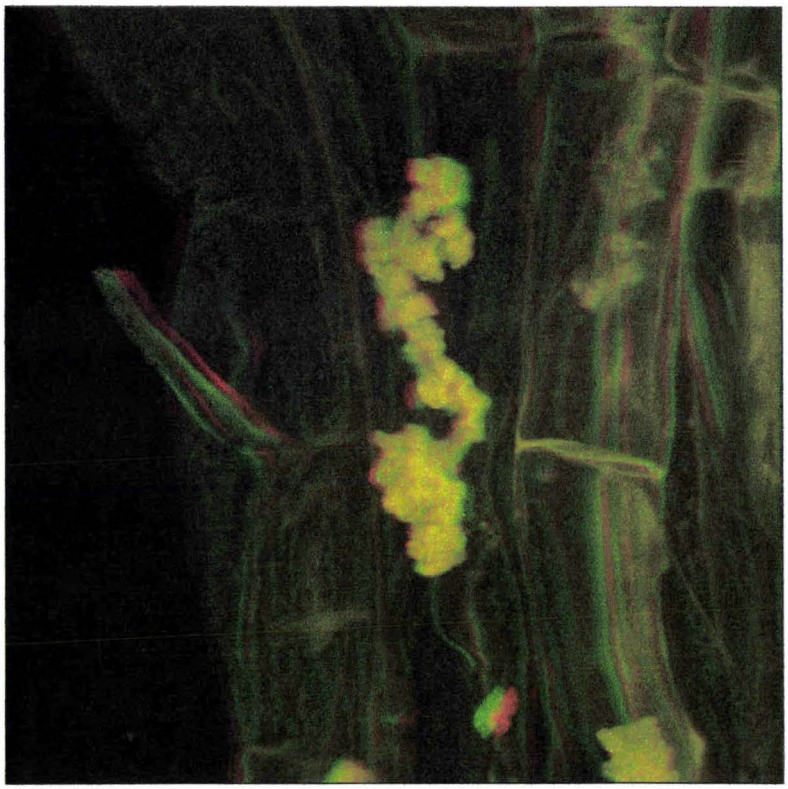
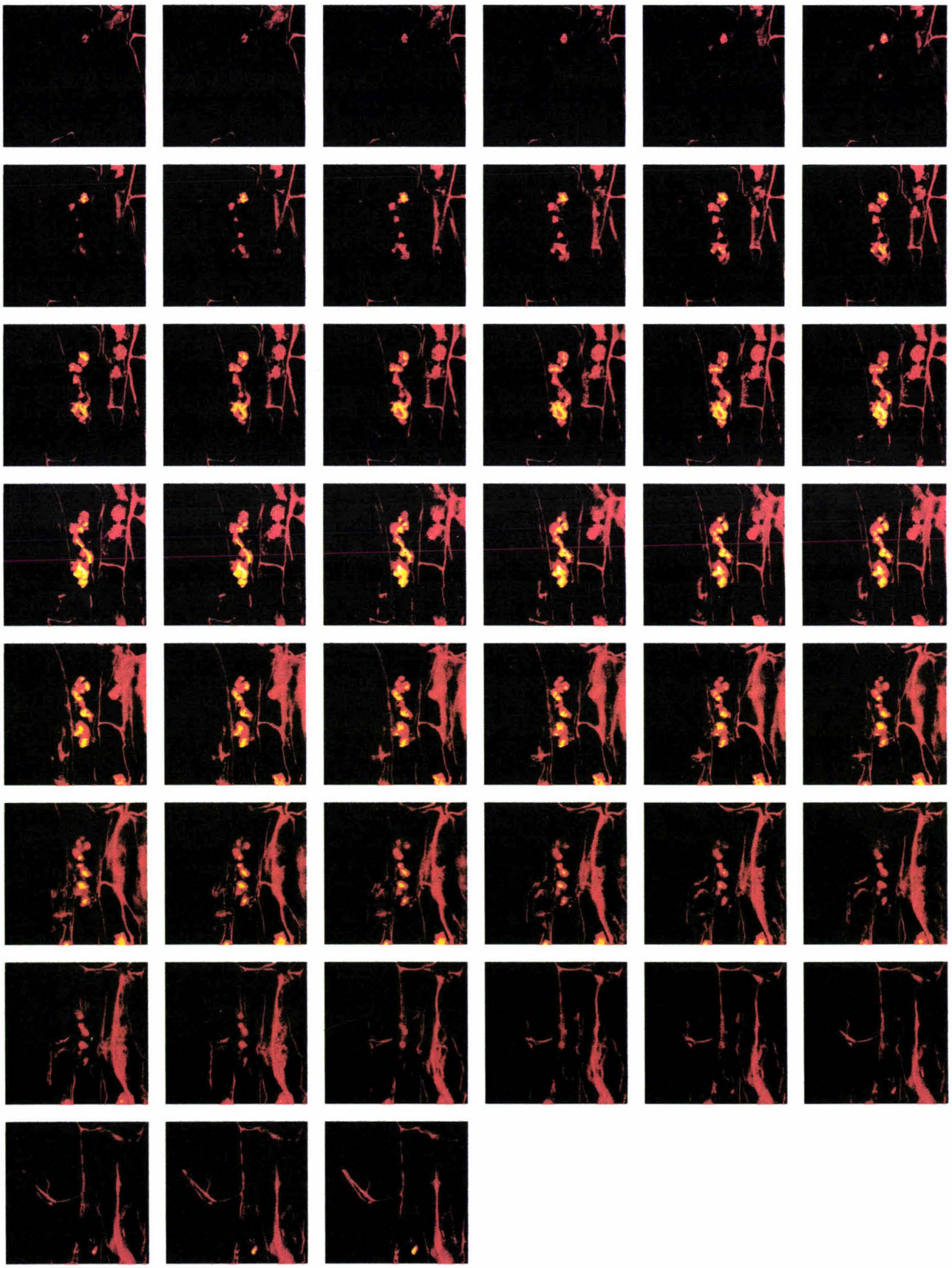


Figure 59 CLSM 3-D image of a living fungal arbuscule structure inhabiting a white clover root

APPENDIX R5: CLSM Z-series

A 'Z-series' or gallery of forty (40) digital images from optical sections observed by confocal laser scanning microscopy (CLSM) autofluorescence of unstained *in-vivo* fungal and root segment structures.



APPENDIX M: Models of Two-Population Interactions

APPENDIX M1: Analysis of Two-species Population Interactions

(derived from table 7-1, Odum, 1983)

species 1	species 2	TYPE OF POPULATION INTERCATION & GENERAL NATURE OF INTERACTIONS
0	0	**Neutralism** neither population affects the other
- -	- -	**Competition** ❑ direct interference type: direct inhibition of each species by the other. ❑ resource use type: indirect inhibition when common resource in short supply.
-	0	**Amensalism** Population 1 inhibited, population 2 not affected
+ +	- -	**Predation** (including herbivory, predator usually larger than prey) **Parasitism** (parasite ~ smaller organism)
+	0	**Commensalism** Commensal population benefits whilst host is not affected
+	+	**Proto co-operation** Interaction favourable to both but not obligatory
+ *	+ *	**Mutualism** Interaction favourable to both and obligatory

KEY

(0) = no significant interaction
(+) = enhanced growth, survival benefits
(-) = inhibited ... growth, survival
(*) = obligate symbionts

Figure 60. Analysis of two-specie population interactions, type of population interaction and general nature of interactions.

Analysis of Two-Species / population Interactions;
Effect of Relationship on Growth and Survival of Two Populations

When not interacting		When interacting		
Species1	**Species2**	**Species 1**	**Species 2**	**TYPE OF POPULATION INTERACTION & GENERAL NATURE OF INTERACTIONS**
-*	-*	+*	+*	**Mutualism**
-*	**0**	+*	+	Mutual benefits & obligate* symbiosis
0	**0**	+	+	**Proto cooperation** Mutual benefits but non-obligate (facultative) symbiosis
-*	**0**	+*	**0**	**Commensalism**
0	**0**	+	+	Populations 'eating at the same table'
0	-*	**0**	+*	eg. a horse & a bird sharing food at a trough
0	**0**	**0**	+	
0	**0**	**0**	**0**	**Neutralism** Neutral or negligible population interactions, neither population affects the other population
0	**0**	-	**0**	**Amensalism** iii) population 1 inhibited / population 2 neutral iv) population 1 neutral / population 2 inhibited
0	**0**	**0**	-	eg. A horse fed using a fodder-bag is detrimental to a bird that may no longer share horse food sources.
0	**0**	-	-	**Competition** (2 types) iii) direct 'interference type', mutual inhibition of each species by the other. iv) Indirect 'resource type' when common resource in short supply
-*	**0**	+*	-	**Parasitism** parasite smaller than (<) host **Pathogenesis** pathogen smaller than (<) host **Predation** predator larger than (>) prey
-*	**0**	-*	+	**Escape / Avoidance**

eg. arbuscular mycorrhizal soil fungi and plant interactions ...

- **Species 1 = AM fungus,** Glomales order, Zygomycota (obligate* mycobiont)
- **Species 2= plant root host** cortex or epidermis tissues; either non-obligate (facultative), or else obligate* phytobiont

KEY

(0) = no significant interaction

(+) = enhanced growth / survival benefits

(-) = inhibited growth / survival

(*) = obligate symbionts

table adapted from
Boughey (1973) after Odum (1959)

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.

APPENDIX M2: Venn-diagram Models of Mycorrhizas and Symbiosis

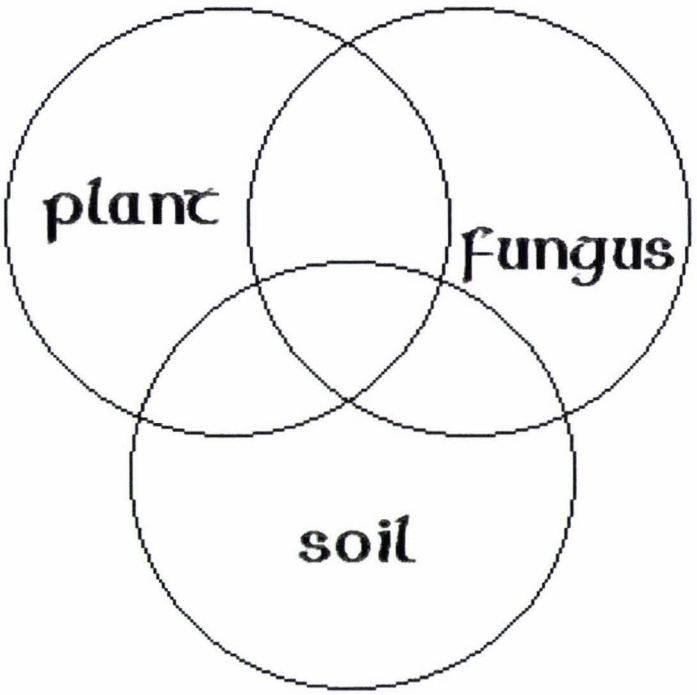


Figure 62 Venn-Diagram of fungal and plant (*biotic*), and soil (*abiotic* and *biotic*) mycorrhizal associations as illustrated by Brundrett *et al.* (1996)[40].

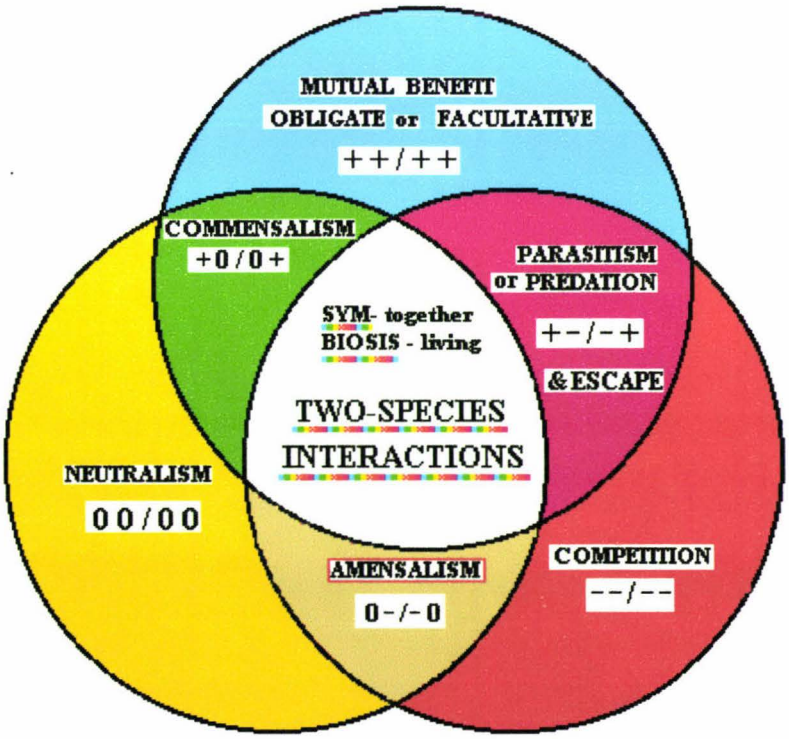


Figure 63 Proposed Venn-diagram model of symbiosis and twos-species population interactions.

[40] many thanks for advantages of travel and the resulting feedback, testing and encouragement that meeting, communicating and being judged by one's academic peers @ ICOM3 in Adelaide July 2001

APPENDIX T: SPSS Tables of Statistical Analysis of Results

APPENDIX T1: Soil Disturbance Effects on Percentage Fungal-Infected Maize Seedling Roots

SPSS General Linear Model: Repeated Measures Analysis.

Within-Subjects Factors

Measure: MEASURE_1

FUNGUS	Dependent Variable
1	INFARBUS
2	INFHYPHA

Between-Subjects Factors

		N
soil disturbance treatment	np	30
	pp	26
field-soil sample site	np1	6
	np2	8
	np3	8
	np4	8
	pp1	7
	pp2	7
	pp3	5
	pp4	7

Descriptive Statistics

	soil disturbance	field-soil sample site	Mean	Std. Deviation	N
% arbuscule infected roots	np	np1	19.2667	6.3019	6
		np2	25.1550	9.5303	8
		np3	24.9325	10.2114	8
		np4	27.9125	8.1465	8
		Total	24.6533	8.8873	30
	pp	pp1	41.4143	6.5373	7
		pp2	50.0943	12.6485	7
		pp3	53.2260	3.7765	5
		pp4	53.6529	8.9461	7
		Total	49.3177	9.8040	26
	Total	np1	19.2667	6.3019	6
		np2	25.1550	9.5303	8
		np3	24.9325	10.2114	8
		np4	27.9125	8.1465	8
		pp1	41.4143	6.5373	7
		pp2	50.0943	12.6485	7
		pp3	53.2260	3.7765	5
		pp4	53.6529	8.9461	7
		Total	36.1046	15.4724	56
% intercellular hyphae infected roots	np	np1	40.8300	8.6059	6
		np2	46.1113	9.1502	8
		np3	43.6838	8.5161	8
		np4	48.9525	9.1169	8
		Total	45.1653	8.9020	30
	pp	pp1	64.7471	4.6415	7
		pp2	67.9100	10.6858	7
		pp3	70.2300	6.5221	5
		pp4	70.3614	9.0476	7
		Total	68.1646	8.0325	26
	Total	np1	40.8300	8.6059	6
		np2	46.1113	9.1502	8
		np3	43.6838	8.5161	8
		np4	48.9525	9.1169	8
		pp1	64.7471	4.6415	7
		pp2	67.9100	10.6858	7
		pp3	70.2300	6.5221	5
		pp4	70.3614	9.0476	7
		Total	55.8436	14.3203	56

Multivariate Tests[b]

Effect		Value	F	Hypothesis df	Error df	Sig.
FUNGUS	Pillai's Trace	.898	421.345[a]	1.000	48.000	.000
	Wilks' Lambda	.102	421.345[a]	1.000	48.000	.000
	Hotelling's Trace	8.778	421.345[a]	1.000	48.000	.000
	Roy's Largest Root	8.778	421.345[a]	1.000	48.000	.000
FUNGUS * DISTURBA	Pillai's Trace	.015	.746[a]	1.000	48.000	.392
	Wilks' Lambda	.985	.746[a]	1.000	48.000	.392
	Hotelling's Trace	.016	.746[a]	1.000	48.000	.392
	Roy's Largest Root	.016	.746[a]	1.000	48.000	.392
FUNGUS * SOILSITE	Pillai's Trace	.086	.748[a]	6.000	48.000	.614
	Wilks' Lambda	.914	.748[a]	6.000	48.000	.614
	Hotelling's Trace	.094	.748[a]	6.000	48.000	.614
	Roy's Largest Root	.094	.748[a]	6.000	48.000	.614
FUNGUS * DISTURBA * SOILSITE	Pillai's Trace	.000	.[a]	.000	.000	.
	Wilks' Lambda	1.000	.[a]	.000	48.000	.
	Hotelling's Trace	.000	.[a]	.000	2.000	.
	Roy's Largest Root	.000	.000[a]	1.000	47.000	1.000

a. Exact statistic

b. Design: Intercept+DISTURBA+SOILSITE+DISTURBA * SOILSITE
Within Subjects Design: FUNGUS

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type I Sum of Squares	df	Mean Square	F	Sig.
FUNGUS	Sphericity Assumed	10909.508	1	10909.508	421.345	.000
	Greenhouse-Geisser	10909.508	1.000	10909.508	421.345	.000
	Huynh-Feldt	10909.508	1.000	10909.508	421.345	.000
	Lower-bound	10909.508	1.000	10909.508	421.345	.000
FUNGUS * DISTURBA	Sphericity Assumed	19.308	1	19.308	.746	.392
	Greenhouse-Geisser	19.308	1.000	19.308	.746	.392
	Huynh-Feldt	19.308	1.000	19.308	.746	.392
	Lower-bound	19.308	1.000	19.308	.746	.392
FUNGUS * SOILSITE	Sphericity Assumed	116.271	6	19.378	.748	.614
	Greenhouse-Geisser	116.271	6.000	19.378	.748	.614
	Huynh-Feldt	116.271	6.000	19.378	.748	.614
	Lower-bound	116.271	6.000	19.378	.748	.614
FUNGUS * DISTURBA * SOILSITE	Sphericity Assumed	.000	0	.	.	.
	Greenhouse-Geisser	.000	.000	.	.	.
	Huynh-Feldt	.000	.000	.	.	.
	Lower-bound	.000	.000	.	.	.
Error(FUNGUS)	Sphericity Assumed	1242.820	48	25.892		
	Greenhouse-Geisser	1242.820	48.000	25.892		
	Huynh-Feldt	1242.820	48.000	25.892		
	Lower-bound	1242.820	48.000	25.892		

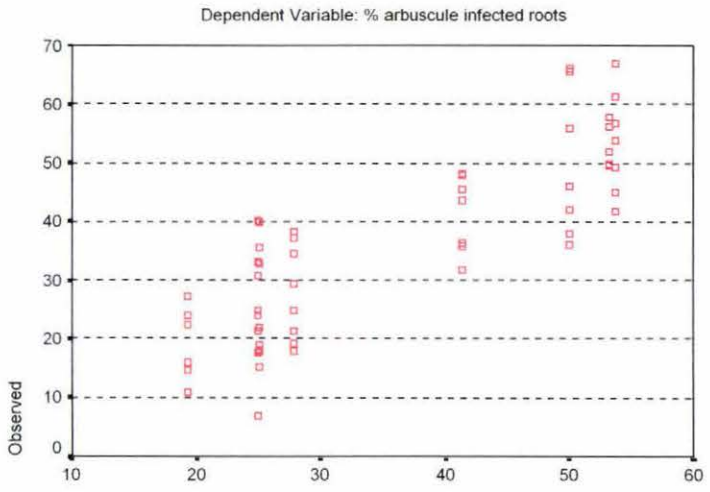
Tests of Between-Subjects Effects

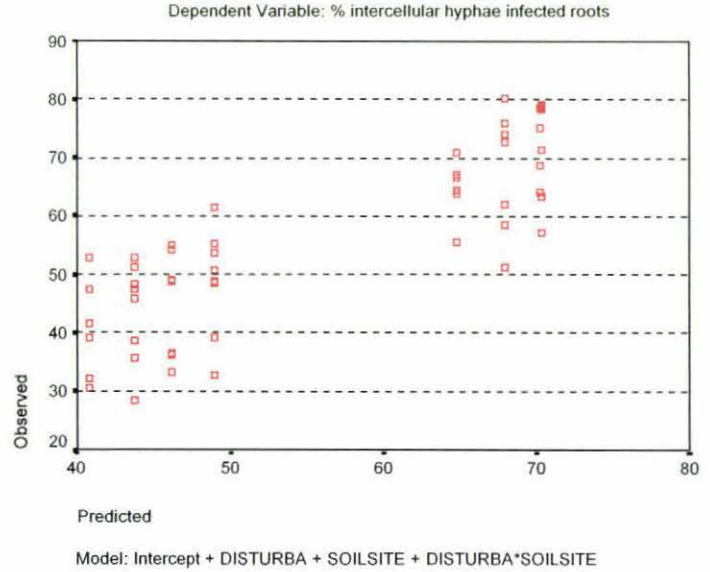
Measure: MEASURE_1

Transformed Variable: Average

Source	Type I Sum of Squares	df	Mean Square	F	Sig.
Intercept	236725.275	1	236725.275	1874.695	.000
DISTURBA	15821.622	1	15821.622	125.296	.000
SOILSITE	1184.388	6	197.398	1.563	.178
DISTURBA * SOILSITE	.000	0	.	.	.
Error	6061.154	48	126.274		

Observed * Predicted * Std. Residual Plots





APPENDIX T2 Soil-Disturbance Effects on Mean Numbers of Fungal Inhabited Root Segments

SPSS General Linear Model : Repeated Measures Analysis.

Within-Subjects Factors

Measure: MEASURE_1

FUNGUS	Dependent Variable
1	ARBUSCUL
2	HYPHAE

Between-Subjects Factors

		N
soil disturbance treatment	np	30
	pp	26
field-soil sample site	np1	6
	np2	8
	np3	8
	np4	8
	pp1	7
	pp2	7
	pp3	5
	pp4	7

Descriptive Statistics

	soil disturbance	field-soil sample site	Mean	Std. Deviation	N
# intracellular arbuscule infected roots	np	np1	30.33	9.89	6
		np2	43.75	21.30	8
		np3	43.88	20.70	8
		np4	45.00	16.12	8
		Total	41.43	18.02	30
	pp	pp1	65.57	22.85	7
		pp2	83.29	33.37	7
		pp3	66.60	14.03	5
		pp4	72.29	31.63	7
		Total	72.35	26.78	26
	Total	np1	30.33	9.89	6
		np2	43.75	21.30	8
		np3	43.88	20.70	8
		np4	45.00	16.12	8
		pp1	65.57	22.85	7
		pp2	83.29	33.37	7
		pp3	66.60	14.03	5
		pp4	72.29	31.63	7
		Total	55.79	27.19	56
# intercellular hyphae infected roots	np	np1	64.50	18.61	6
		np2	80.00	30.19	8
		np3	76.38	25.08	8
		np4	76.75	19.95	8
		Total	75.07	23.63	30
	pp	pp1	99.86	28.37	7
		pp2	111.00	33.28	7
		pp3	88.40	22.14	5
		pp4	95.00	41.43	7
		Total	99.35	31.89	26
	Total	np1	64.50	18.61	6
		np2	80.00	30.19	8
		np3	76.38	25.08	8
		np4	76.75	19.95	8
		pp1	99.86	28.37	7
		pp2	111.00	33.28	7
		pp3	88.40	22.14	5
		pp4	95.00	41.43	7
		Total	86.34	30.10	56

Multivariate Tests[b]

Effect		Value	F	Hypothesis df	Error df	Sig.
FUNGUS	Pillai's Trace	.839	250.957[a]	1.000	48.000	.000
	Wilks' Lambda	.161	250.957[a]	1.000	48.000	.000
	Hotelling's Trace	5.228	250.957[a]	1.000	48.000	.000
	Roy's Largest Root	5.228	250.957[a]	1.000	48.000	.000
FUNGUS * DISTURBA	Pillai's Trace	.058	2.942[a]	1.000	48.000	.093
	Wilks' Lambda	.942	2.942[a]	1.000	48.000	.093
	Hotelling's Trace	.061	2.942[a]	1.000	48.000	.093
	Roy's Largest Root	.061	2.942[a]	1.000	48.000	.093
FUNGUS * SOILSITE	Pillai's Trace	.068	.587[a]	6.000	48.000	.739
	Wilks' Lambda	.932	.587[a]	6.000	48.000	.739
	Hotelling's Trace	.073	.587[a]	6.000	48.000	.739
	Roy's Largest Root	.073	.587[a]	6.000	48.000	.739
FUNGUS * DISTURBA * SOILSITE	Pillai's Trace	.000	.[a]	.000	.000	.
	Wilks' Lambda	1.000	.[a]	.000	48.000	.
	Hotelling's Trace	.000	.[a]	.000	2.000	.
	Roy's Largest Root	.000	.000[a]	1.000	47.000	1.000

a. Exact statistic

b.
Design: Intercept+DISTURBA+SOILSITE+DISTURBA * SOILSITE
Within Subjects Design: FUNGUS

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type I Sum of Squares	df	Mean Square	F	Sig.
FUNGUS	Sphericity Assumed	26138.580	1	26138.580	250.957	.000
	Greenhouse-Geisser	26138.580	1.000	26138.580	250.957	.000
	Huynh-Feldt	26138.580	1.000	26138.580	250.957	.000
	Lower-bound	26138.580	1.000	26138.580	250.957	.000
FUNGUS * DISTURBA	Sphericity Assumed	306.436	1	306.436	2.942	.093
	Greenhouse-Geisser	306.436	1.000	306.436	2.942	.093
	Huynh-Feldt	306.436	1.000	306.436	2.942	.093
	Lower-bound	306.436	1.000	306.436	2.942	.093
FUNGUS * SOILSITE	Sphericity Assumed	367.024	6	61.171	.587	.739
	Greenhouse-Geisser	367.024	6.000	61.171	.587	.739
	Huynh-Feldt	367.024	6.000	61.171	.587	.739
	Lower-bound	367.024	6.000	61.171	.587	.739
FUNGUS * DISTURBA * SOILSITE	Sphericity Assumed	.000	0	.	.	.
	Greenhouse-Geisser	.000	.000	.	.	.
	Huynh-Feldt	.000	.000	.	.	.
	Lower-bound	.000	.000	.	.	.
Error(FUNGUS)	Sphericity Assumed	4999.460	48	104.155		
	Greenhouse-Geisser	4999.460	48.000	104.155		
	Huynh-Feldt	4999.460	48.000	104.155		
	Lower-bound	4999.460	48.000	104.155		

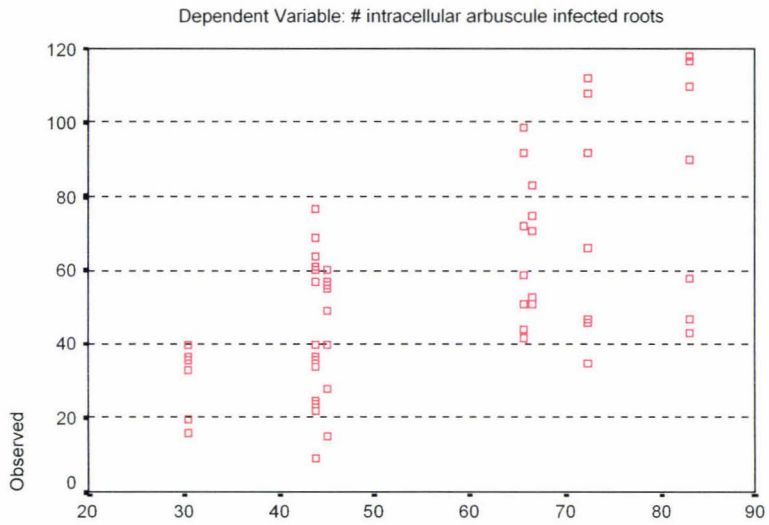
Tests of Between-Subjects Effects

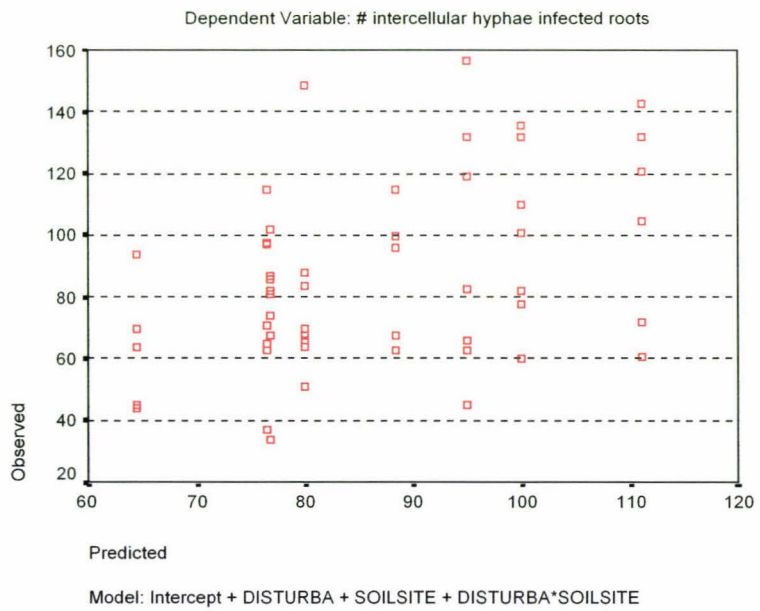
Measure: MEASURE_1

Transformed Variable: Average

Source	Type I Sum of Squares	df	Mean Square	F	Sig.
Intercept	565586.438	1	565586.438	459.212	.000
DISTURBA	21214.543	1	21214.543	17.225	.000
SOILSITE	4473.567	6	745.594	.605	.725
DISTURBA * SOILSITE	.000	0	.	.	.
Error	59118.952	48	1231.645		

Observed * Predicted * Std. Residual Plots





APPENDIX T3: Soil Disturbance Effects on Total Numbers of Maize Root Segments per Sample Soil-Volume.

SPSS General Linear Model : Univariate Analysis

Between-Subjects Factors

		N
soil disturbance treatment	np	30
	pp	26
field-soil sample site	np1	6
	np2	8
	np3	8
	np4	8
	pp1	7
	pp2	7
	pp3	5
	pp4	7

Descriptive Statistics

Dependent Variable: maize root total numbers per potnum transect

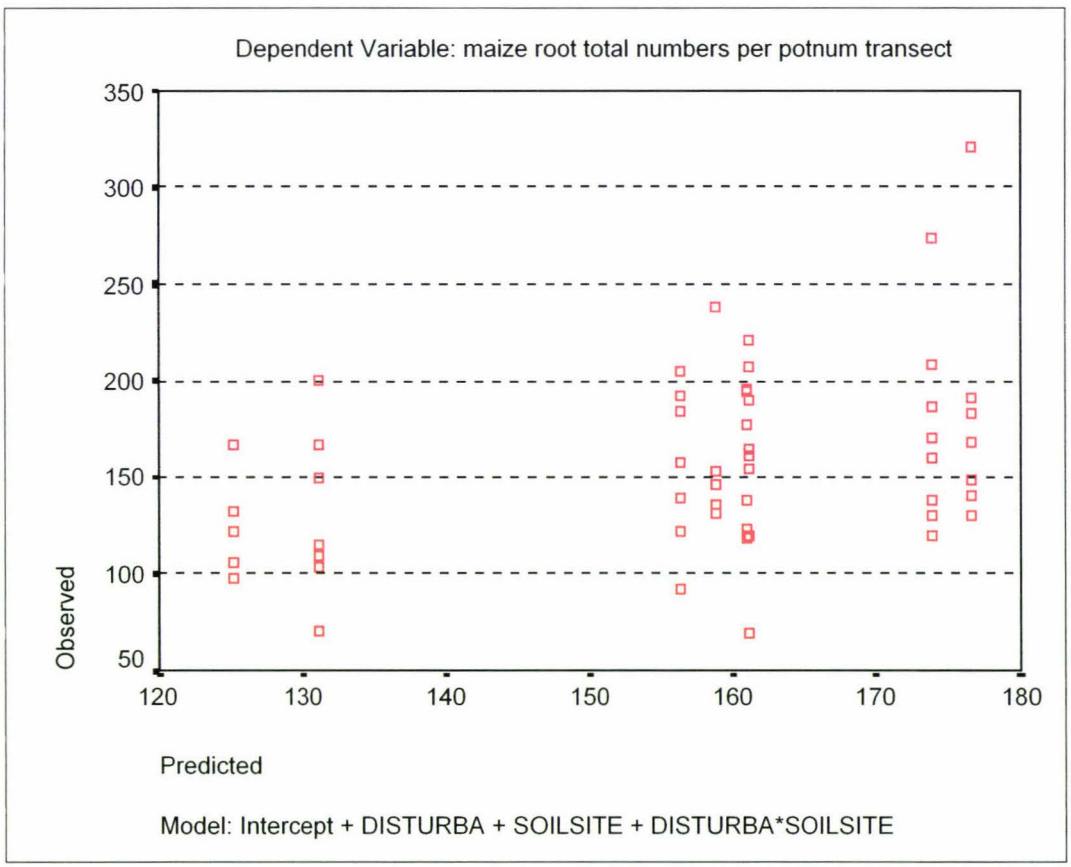
soil disturbance	field-soil sample site	Mean	Std. Deviation	N
np	np1	158.83	40.02	6
	np2	173.88	50.13	8
	np3	176.63	62.73	8
	np4	161.13	48.65	8
	Total	168.20	49.64	30
pp	pp1	156.43	40.66	7
	pp2	161.00	33.41	7
	pp3	125.20	27.05	5
	pp4	131.14	43.61	7
	Total	144.85	38.45	26
Total	np1	158.83	40.02	6
	np2	173.88	50.13	8
	np3	176.63	62.73	8
	np4	161.13	48.65	8
	pp1	156.43	40.66	7
	pp2	161.00	33.41	7
	pp3	125.20	27.05	5
	pp4	131.14	43.61	7
	Total	157.36	45.93	56

Tests of Between-Subjects Effects

Dependent Variable: maize root total numbers per potnum transect

Source	Type I Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	15359.027[a]	7	2194.147	1.046	.412
Intercept	1386631.1	1	1386631.143	661.154	.000
DISTURBA	7596.673	1	7596.673	3.622	.063
SOILSITE	7762.355	6	1293.726	.617	.716
DISTURBA * SOILSITE	.000	0	.	.	.
Error	100669.830	48	2097.288		
Total	1502660.0	56			
Corrected Total	116028.857	55			

a. R Squared = .132 (Adjusted R Squared = .006)



APPENDIX T4: Soil Disturbance Effects on Root-Fungal RATIOA

SPSS General Linear Model: Univariate Analysis of Variance

Between-Subjects Factors

		N
soil disturbance treatment	np	30
	pp	26
field-soil sample site	np1	6
	np2	8
	np3	8
	np4	8
	pp1	7
	pp2	7
	pp3	5
	pp4	7

Descriptive Statistics

Dependent Variable: ratio A [infarbus / infhypha]

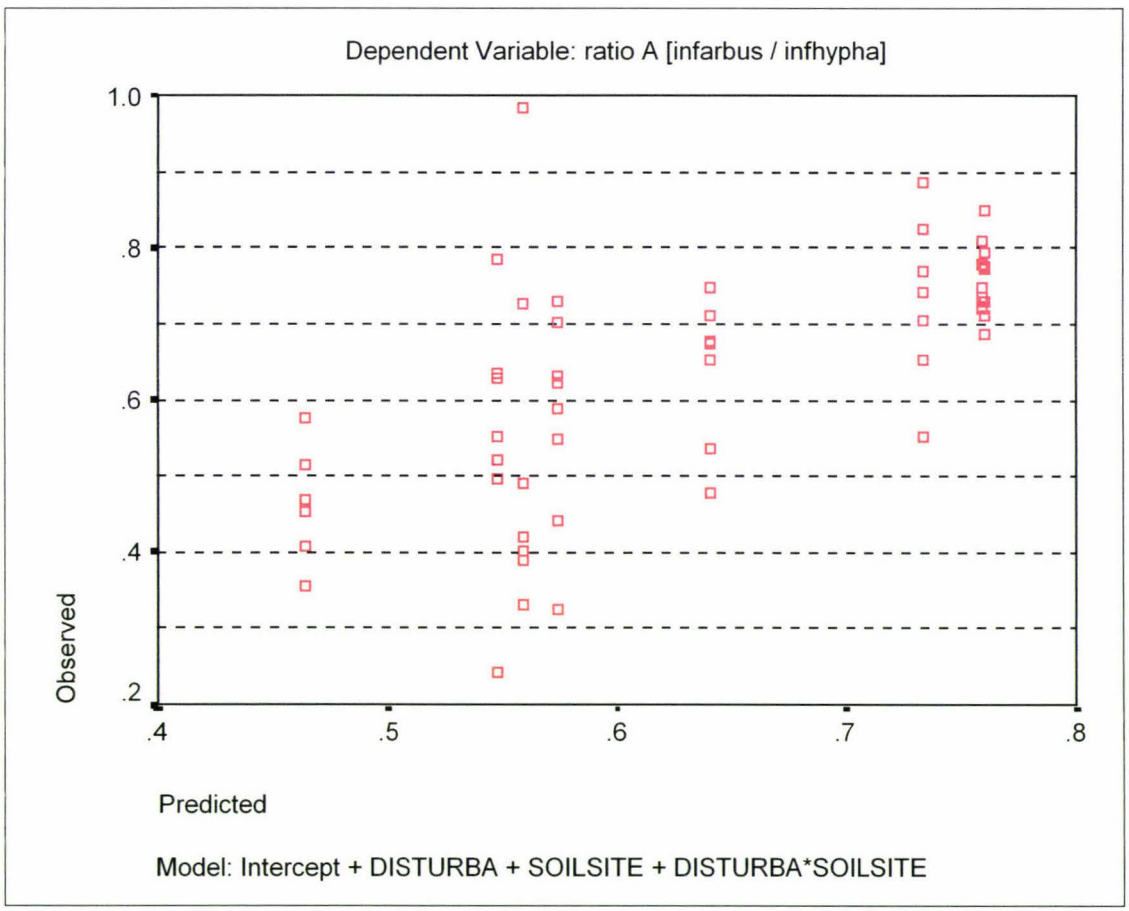
soil disturbance	field-soil sample site	Mean	Std. Deviation	N
np	np1	.4636	7.822E-02	6
	np2	.5595	.2287	8
	np3	.5483	.1548	8
	np4	.5741	.1350	8
	Total	.5412	.1597	30
pp	pp1	.6409	9.764E-02	7
	pp2	.7335	.1105	7
	pp3	.7596	3.506E-02	5
	pp4	.7606	5.466E-02	7
	Total	.7209	9.333E-02	26
Total	np1	.4636	7.822E-02	6
	np2	.5595	.2287	8
	np3	.5483	.1548	8
	np4	.5741	.1350	8
	pp1	.6409	9.764E-02	7
	pp2	.7335	.1105	7
	pp3	.7596	3.506E-02	5
	pp4	.7606	5.466E-02	7
	Total	.6246	.1600	56

Tests of Between-Subjects Effects

Dependent Variable: ratio A [infarbus / infhypha]

Source	Type I Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.562[a]	7	8.028E-02	4.559	.001
Intercept	21.850	1	21.850	1240.887	.000
DISTURBA	.450	1	.450	25.533	.000
SOILSITE	.112	6	1.872E-02	1.063	.398
DISTURBA * SOILSITE	.000	0	.	.	.
Error	.845	48	1.761E-02		
Total	23.258	56			
Corrected Total	1.407	55			

a. R Squared = .399 (Adjusted R Squared = .312)



APPENDIX T5: Soil Disturbance Effects on Root-Fungal Infectivity RATIOB & RATIOC

SPSS General Linear Model: Repeated Measures Analysis

Within-Subjects Factors

Measure: MEASURE_1

FUNGUS	Dependent Variable
1	RATIOB
2	RATIOC

Between-Subjects Factors

		N
soil disturbance treatment	np	30
	pp	26
field-soil sample site	np1	6
	np2	8
	np3	8
	np4	8
	pp1	7
	pp2	7
	pp3	5
	pp4	7

Descriptive Statistics

	soil disturbance	field-soil sample site	Mean	Std. Deviation	N
ratio B [noninf / infarbus]	np	np1	3.5266	1.7287	6
		np2	2.4339	1.0188	8
		np3	3.1811	2.9965	8
		np4	2.0175	.8167	8
		Total	2.7407	1.8563	30
	pp	pp1	.9064	.2477	7
		pp2	.7139	.3883	7
		pp3	.5674	.1551	5
		pp4	.5887	.2719	7
		Total	.7039	.3024	26
	Total	np1	3.5266	1.7287	6
		np2	2.4339	1.0188	8
		np3	3.1811	2.9965	8
		np4	2.0175	.8167	8
		pp1	.9064	.2477	7
		pp2	.7139	.3883	7
		pp3	.5674	.1551	5
		pp4	.5887	.2719	7
		Total	1.7950	1.7056	56
ratio C [noninf / % infhypha]	np	np1	1.5424	.5374	6
		np2	1.2525	.4850	8
		np3	1.3822	.5541	8
		np4	1.1164	.4600	8
		Total	1.3087	.5056	30
	pp	pp1	.5672	.1187	7
		pp2	.5070	.2576	7
		pp3	.4335	.1305	5
		pp4	.4424	.1929	7
		Total	.4917	.1843	26
	Total	np1	1.5424	.5374	6
		np2	1.2525	.4850	8
		np3	1.3822	.5541	8
		np4	1.1164	.4600	8
		pp1	.5672	.1187	7
		pp2	.5070	.2576	7
		pp3	.4335	.1305	5
		pp4	.4424	.1929	7
		Total	.9294	.5651	56

Multivariate Tests[b]

Effect		Value	F	Hypothesis df	Error df	Sig.
FUNGUS	Pillai's Trace	.420	34.763[a]	1.000	48.000	.000
	Wilks' Lambda	.580	34.763[a]	1.000	48.000	.000
	Hotelling's Trace	.724	34.763[a]	1.000	48.000	.000
	Roy's Largest Root	.724	34.763[a]	1.000	48.000	.000
FUNGUS * DISTURBA	Pillai's Trace	.263	17.168[a]	1.000	48.000	.000
	Wilks' Lambda	.737	17.168[a]	1.000	48.000	.000
	Hotelling's Trace	.358	17.168[a]	1.000	48.000	.000
	Roy's Largest Root	.358	17.168[a]	1.000	48.000	.000
FUNGUS * SOILSITE	Pillai's Trace	.092	.806[a]	6.000	48.000	.570
	Wilks' Lambda	.908	.806[a]	6.000	48.000	.570
	Hotelling's Trace	.101	.806[a]	6.000	48.000	.570
	Roy's Largest Root	.101	.806[a]	6.000	48.000	.570
FUNGUS * DISTURBA * SOILSITE	Pillai's Trace	.000	.[a]	.000	.000	.
	Wilks' Lambda	1.000	.[a]	.000	48.000	.
	Hotelling's Trace	.000	.[a]	.000	2.000	.
	Roy's Largest Root	.000	.000[a]	1.000	47.000	1.000

a. Exact statistic

b. Design: Intercept+DISTURBA+SOILSITE+DISTURBA * SOILSITE
Within Subjects Design: FUNGUS

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type I Sum of Squares	df	Mean Square	F	Sig.
FUNGUS	Sphericity Assumed	20.980	1	20.980	34.763	.000
	Greenhouse-Geisser	20.980	1.000	20.980	34.763	.000
	Huynh-Feldt	20.980	1.000	20.980	34.763	.000
	Lower-bound	20.980	1.000	20.980	34.763	.000
FUNGUS * DISTURBA	Sphericity Assumed	10.361	1	10.361	17.168	.000
	Greenhouse-Geisser	10.361	1.000	10.361	17.168	.000
	Huynh-Feldt	10.361	1.000	10.361	17.168	.000
	Lower-bound	10.361	1.000	10.361	17.168	.000
FUNGUS * SOILSITE	Sphericity Assumed	2.919	6	.486	.806	.570
	Greenhouse-Geisser	2.919	6.000	.486	.806	.570
	Huynh-Feldt	2.919	6.000	.486	.806	.570
	Lower-bound	2.919	6.000	.486	.806	.570
FUNGUS * DISTURBA * SOILSITE	Sphericity Assumed	.000	0	.	.	.
	Greenhouse-Geisser	.000	.000	.	.	.
	Huynh-Feldt	.000	.000	.	.	.
	Lower-bound	.000	.000	.	.	.
Error(FUNGUS)	Sphericity Assumed	28.968	48	.604		
	Greenhouse-Geisser	28.968	48.000	.604		
	Huynh-Feldt	28.968	48.000	.604		
	Lower-bound	28.968	48.000	.604		

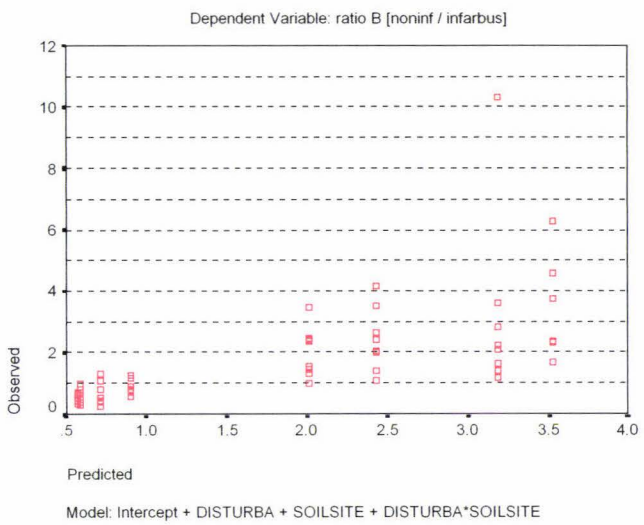
Tests of Between-Subjects Effects

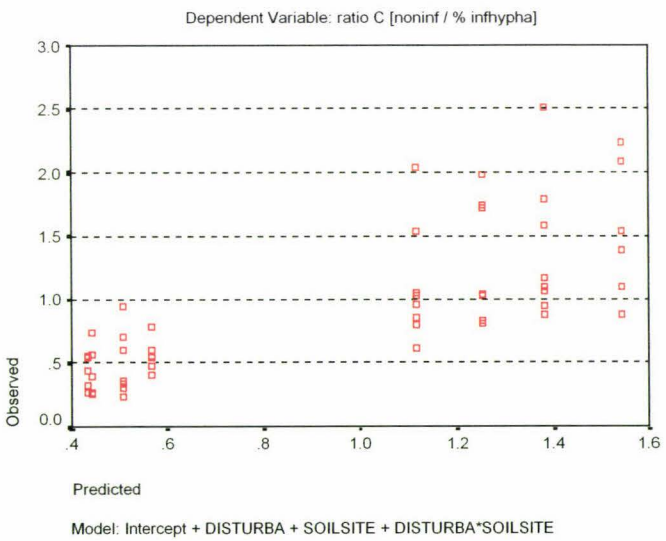
Measure: MEASURE_1

Transformed Variable: Average

Source	Type I Sum of Squares	df	Mean Square	F	Sig.
Intercept	207.825	1	207.825	142.367	.000
DISTURBA	56.721	1	56.721	38.856	.000
SOILSITE	8.518	6	1.420	.972	.454
DISTURBA * SOILSITE	.000	0	.	.	.
Error	70.070	48	1.460		

Observed * Predicted * Std. Residual Plots





APPENDIX T6A: Soil Disturbance Effects on Bioassay Maize Seedling Shoot-Lengths (SHOOT1) & (SHOOT2).

SPSS General Linear Model Repeated Measures Analysis.

Within-Subjects Factors

Measure: MEASURE_1

SHOOTS	Dependent Variable
1	SHOOT1MM
2	SHOOT2MM

Between-Subjects Factors

		N
soil disturbance treatment	NM	15
	NP	62
	PP	62
field-soil sample site	NM	15
	NP1	14
	NP2	15
	NP3	16
	NP4	16
	PP1	16
	PP2	14
	PP3	15
	PP4	18
harvest date	20.07.2000	65
	04.08.2000	74

Descriptive Statistics

	soil disturbance	field-soil sample site	harvest date	Mean	Std. Deviation	N
shoot 1 length (mn	NM	NM	20.07.2000	214.50	12.39	6
			04.08.2000	290.33	36.60	9
			Total	260.00	47.95	15
		Total	20.07.2000	214.50	12.39	6
			04.08.2000	290.33	36.60	9
			Total	260.00	47.95	15
	NP	NP1	20.07.2000	241.00	9.54	7
			04.08.2000	345.43	15.23	7
			Total	293.21	55.54	14
		NP2	20.07.2000	246.00	13.77	7
			04.08.2000	329.62	25.88	8
			Total	290.60	47.76	15
		NP3	20.07.2000	241.00	22.28	8
			04.08.2000	338.75	20.61	8
			Total	289.88	54.57	16
		NP4	20.07.2000	246.13	17.77	8
			04.08.2000	344.38	13.59	8
			Total	295.25	52.99	16
		PP2	20.07.2000	247.00	.	1
			Total	247.00	.	1
		Total	20.07.2000	243.65	15.89	31
			04.08.2000	339.35	19.64	31
			Total	291.50	51.39	62
	PP	PP2	20.07.2000	215.14	8.69	7
			04.08.2000	293.50	20.09	6
			Total	251.31	43.11	13
		PP1	20.07.2000	218.00	16.55	6
			04.08.2000	305.40	23.58	10
			Total	272.62	48.32	16
		PP3	20.07.2000	220.71	19.31	7
			04.08.2000	297.38	31.83	8
			Total	261.60	47.26	15
		PP4	20.07.2000	214.38	21.19	8
			04.08.2000	300.40	21.61	10
			Total	262.17	48.65	18
		Total	20.07.2000	216.93	16.54	28
			04.08.2000	299.94	23.94	34
			Total	262.45	46.54	62
	Total	NM	20.07.2000	214.50	12.39	6
			04.08.2000	290.33	36.60	9
			Total	260.00	47.95	15
		NP1	20.07.2000	241.00	9.54	7
			04.08.2000	345.43	15.23	7
			Total	293.21	55.54	14
		NP2	20.07.2000	246.00	13.77	7
			04.08.2000	329.62	25.88	8
			Total	290.60	47.76	15
		NP3	20.07.2000	241.00	22.28	8
			04.08.2000	338.75	20.61	8
			Total	289.88	54.57	16
		NP4	20.07.2000	246.13	17.77	8
			04.08.2000	344.38	13.59	8
			Total	295.25	52.99	16
		PP2	20.07.2000	219.13	13.84	8
			04.08.2000	293.50	20.09	6
			Total	251.00	41.44	14
		PP1	20.07.2000	218.00	16.55	6
			04.08.2000	305.40	23.58	10
			Total	272.62	48.32	16
		PP3	20.07.2000	220.71	19.31	7
			04.08.2000	297.38	31.83	8
			Total	261.60	47.26	15
		PP4	20.07.2000	214.38	21.19	8
			04.08.2000	300.40	21.61	10
			Total	262.17	48.65	18
		Total	20.07.2000	229.45	20.81	65
			04.08.2000	315.28	31.57	74
			Total	275.14	50.75	139
shoot 2 length (mn	NM	NM	20.07.2000	226.67	16.78	6
			04.08.2000	304.33	26.44	9
			Total	273.27	45.29	15
		Total	20.07.2000	226.67	16.78	6
			04.08.2000	304.33	26.44	9
			Total	273.27	45.29	15
	NP	NP1	20.07.2000	248.57	21.93	7
			04.08.2000	321.14	32.53	7
			Total	284.86	46.13	14
		NP2	20.07.2000	251.00	23.61	7
			04.08.2000	323.38	15.04	8
			Total	289.60	41.82	15
		NP3	20.07.2000	231.00	17.32	8
			04.08.2000	328.63	25.62	8
			Total	279.81	54.66	16
		NP4	20.07.2000	232.88	12.02	8
			04.08.2000	326.88	31.50	8
			Total	279.88	53.73	16
		PP2	20.07.2000	241.00	.	1
			Total	241.00	.	1
		Total	20.07.2000	240.29	19.80	31
			04.08.2000	325.13	25.65	31
			Total	282.71	48.43	62
	PP	PP2	20.07.2000	208.71	14.82	7
			04.08.2000	295.33	12.85	6
			Total	248.69	46.89	13
		PP1	20.07.2000	224.17	21.45	6
			04.08.2000	295.00	25.03	10
			Total	268.44	42.23	16
		PP3	20.07.2000	221.43	16.73	7
			04.08.2000	300.38	29.32	8
			Total	263.53	47.03	15
		PP4	20.07.2000	227.63	19.12	8
			04.08.2000	277.80	30.91	10
			Total	255.50	36.26	16
		Total	20.07.2000	220.61	18.58	28
			04.08.2000	291.26	26.85	34
			Total	259.35	42.42	62
	Total	NM	20.07.2000	226.67	16.78	6

Multivariate Tests[b]

Effect		Value	F	Hypothesis df	Error df	Sig.
SHOOTS	Pillai's Trace	.027	3.277[a]	1.000	120.000	.073
	Wilks' Lambda	.973	3.277[a]	1.000	120.000	.073
	Hotelling's Trace	.027	3.277[a]	1.000	120.000	.073
	Roy's Largest Root	.027	3.277[a]	1.000	120.000	.073
SHOOTS * DISTURBA	Pillai's Trace	.072	4.677[a]	2.000	120.000	.011
	Wilks' Lambda	.928	4.677[a]	2.000	120.000	.011
	Hotelling's Trace	.078	4.677[a]	2.000	120.000	.011
	Roy's Largest Root	.078	4.677[a]	2.000	120.000	.011
SHOOTS * SOILSITE	Pillai's Trace	.029	.511[a]	7.000	120.000	.825
	Wilks' Lambda	.971	.511[a]	7.000	120.000	.825
	Hotelling's Trace	.030	.511[a]	7.000	120.000	.825
	Roy's Largest Root	.030	.511[a]	7.000	120.000	.825
SHOOTS * HARVEST	Pillai's Trace	.045	5.693[a]	1.000	120.000	.019
	Wilks' Lambda	.955	5.693[a]	1.000	120.000	.019
	Hotelling's Trace	.047	5.693[a]	1.000	120.000	.019
	Roy's Largest Root	.047	5.693[a]	1.000	120.000	.019
SHOOTS * DISTURBA * SOILSITE	Pillai's Trace	.000	.[a]	.000	.000	.
	Wilks' Lambda	1.000	.[a]	.000	120.000	.
	Hotelling's Trace	.000	.[a]	.000	2.000	.
	Roy's Largest Root	.000	.000[a]	1.000	119.000	1.000
SHOOTS * DISTURBA * HARVEST	Pillai's Trace	.008	.474[a]	2.000	120.000	.623
	Wilks' Lambda	.992	.474[a]	2.000	120.000	.623
	Hotelling's Trace	.008	.474[a]	2.000	120.000	.623
	Roy's Largest Root	.008	.474[a]	2.000	120.000	.623
SHOOTS * SOILSITE * HARVEST	Pillai's Trace	.083	1.802[a]	6.000	120.000	.104
	Wilks' Lambda	.917	1.802[a]	6.000	120.000	.104
	Hotelling's Trace	.090	1.802[a]	6.000	120.000	.104
	Roy's Largest Root	.090	1.802[a]	6.000	120.000	.104
SHOOTS * DISTURBA * SOILSITE * HARVEST	Pillai's Trace	.000	.[a]	.000	.000	.
	Wilks' Lambda	1.000	.[a]	.000	120.000	.
	Hotelling's Trace	.000	.[a]	.000	2.000	.
	Roy's Largest Root	.000	.000[a]	1.000	119.000	1.000

a. Exact statistic

b. Design: Intercept+DISTURBA+SOILSITE+HARVEST+DISTURBA * SOILSITE+DISTURBA * HARVEST+SOILSITE * HARVEST+DISTURBA * SOILSITE * HARVEST
Within Subjects Design: SHOOTS

Tests of Within-Subjects Effects

Measure: MEASURE_1

Source		Type I Sum of Squares	df	Mean Square	F	Sig.
SHOOTS	Sphericity Assumed	1041.165	1	1041.165	3.277	.073
	Greenhouse-Geisser	1041.165	1.000	1041.165	3.277	.073
	Huynh-Feldt	1041.165	1.000	1041.165	3.277	.073
	Lower-bound	1041.165	1.000	1041.165	3.277	.073
SHOOTS * DISTURBA	Sphericity Assumed	2971.521	2	1485.761	4.677	.011
	Greenhouse-Geisser	2971.521	2.000	1485.761	4.677	.011
	Huynh-Feldt	2971.521	2.000	1485.761	4.677	.011
	Lower-bound	2971.521	2.000	1485.761	4.677	.011
SHOOTS * SOILSITE	Sphericity Assumed	1135.672	7	162.239	.511	.825
	Greenhouse-Geisser	1135.672	7.000	162.239	.511	.825
	Huynh-Feldt	1135.672	7.000	162.239	.511	.825
	Lower-bound	1135.672	7.000	162.239	.511	.825
SHOOTS * HARVEST	Sphericity Assumed	1808.421	1	1808.421	5.693	.019
	Greenhouse-Geisser	1808.421	1.000	1808.421	5.693	.019
	Huynh-Feldt	1808.421	1.000	1808.421	5.693	.019
	Lower-bound	1808.421	1.000	1808.421	5.693	.019
SHOOTS * DISTURBA * SOILSITE	Sphericity Assumed	.000	0	.	.	.
	Greenhouse-Geisser	.000	.000	.	.	.
	Huynh-Feldt	.000	.000	.	.	.
	Lower-bound	.000	.000	.	.	.
SHOOTS * DISTURBA * HARVEST	Sphericity Assumed	301.394	2	150.697	.474	.623
	Greenhouse-Geisser	301.394	2.000	150.697	.474	.623
	Huynh-Feldt	301.394	2.000	150.697	.474	.623
	Lower-bound	301.394	2.000	150.697	.474	.623
SHOOTS * SOILSITE * HARVEST	Sphericity Assumed	3435.345	6	572.558	1.802	.104
	Greenhouse-Geisser	3435.345	6.000	572.558	1.802	.104
	Huynh-Feldt	3435.345	6.000	572.558	1.802	.104
	Lower-bound	3435.345	6.000	572.558	1.802	.104
SHOOTS * DISTURBA * SOILSITE * HARVEST	Sphericity Assumed	.000	0	.	.	.
	Greenhouse-Geisser	.000	.000	.	.	.
	Huynh-Feldt	.000	.000	.	.	.
	Lower-bound	.000	.000	.	.	.
Error(SHOOTS)	Sphericity Assumed	38120.480	120	317.671		
	Greenhouse-Geisser	38120.480	120.000	317.671		
	Huynh-Feldt	38120.480	120.000	317.671		
	Lower-bound	38120.480	120.000	317.671		

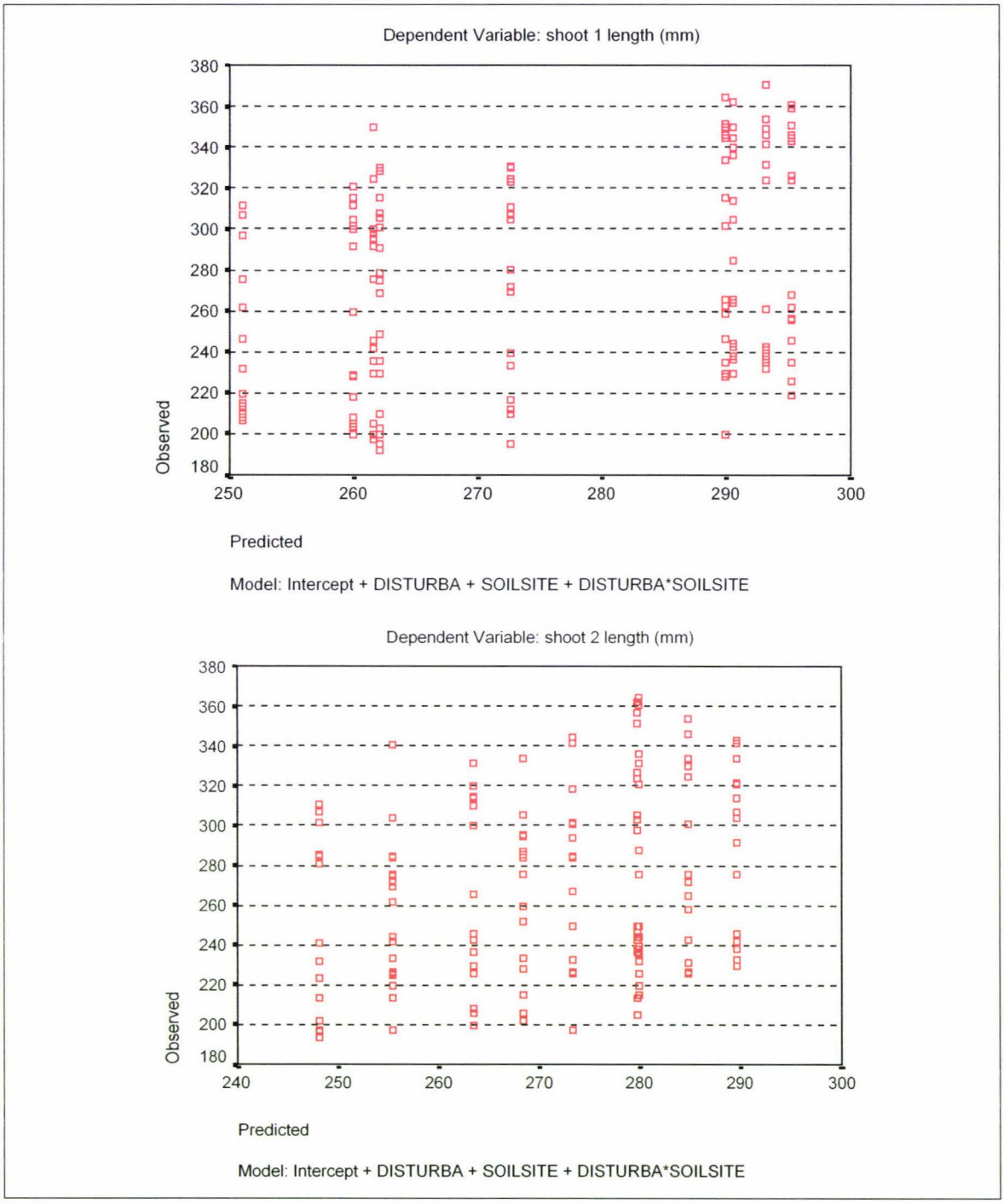
Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type I Sum of Squares	df	Mean Square	F	Sig.
Intercept	20750742	1	20750742.10	30606.845	.000
DISTURBA	44018.457	2	22009.228	32.463	.000
SOILSITE	10554.382	7	1507.769	2.224	.037
HARVEST	464197.590	1	464197.590	684.680	.000
DISTURBA * SOILSITE	.000	0	.	.	.
DISTURBA * HARVEST	3282.415	2	1641.208	2.421	.093
SOILSITE * HARVEST	2781.796	6	463.633	.684	.663
DISTURBA * SOILSITE * HARVEST	.000	0	.	.	.
Error	81357.259	120	677.977		

Observed * Predicted * Std. Residual Plots



APPENDIX T6B: Soil disturbance effects on maize seedling shoot lengths: SPSS Univariate Analysis of Variance: Maize Shoot Length (SHOOTZZ) data

Between-Subjects Factors

		N
soil disturbance treatment	NM	15
	NP	62
	PP	62
field-soil sample site	NM	15
	NP1	14
	NP2	15
	NP3	16
	NP4	16
	PP1	16
	PP2	14
	PP3	15
	PP4	18
harvest date	20.07.2000	65
	04.08.2000	74

Descriptive Statistics

Dependent Variable: SHOOTZZ

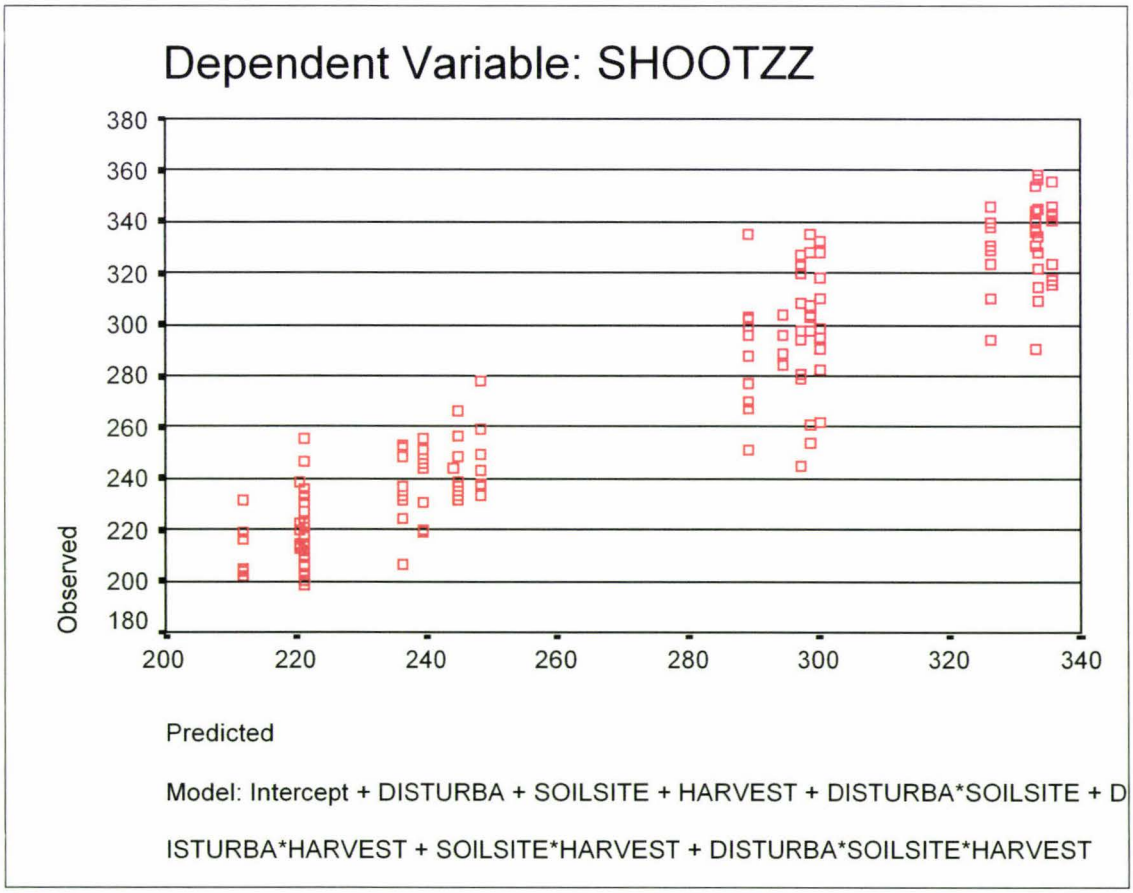
soil disturbance	field-soil sample site	harvest date	Mean	Std. Deviation	N
NM	NM	20.07.2000	220.5833	9.8154	6
		04.08.2000	297.3333	26.1821	9
		Total	266.6333	44.0549	15
	Total	20.07.2000	220.5833	9.8154	6
		04.08.2000	297.3333	26.1821	9
		Total	266.6333	44.0549	15
NP	NP1	20.07.2000	244.7857	13.0475	7
		04.08.2000	333.2857	20.0143	7
		Total	289.0357	48.7046	14
	NP2	20.07.2000	248.5000	15.6205	7
		04.08.2000	326.5000	16.8608	8
		Total	290.1000	43.2333	15
	NP3	20.07.2000	236.0000	15.5793	8
		04.08.2000	333.6875	18.5990	8
		Total	284.8438	53.0986	16
	NP4	20.07.2000	239.5000	14.0814	8
		04.08.2000	335.6250	14.7691	8
		Total	287.5625	51.5590	16
	PP2	20.07.2000	244.0000	.	1
		Total	244.0000	.	1
	Total	20.07.2000	241.9677	14.4505	31
		04.08.2000	332.2419	17.0504	31
		Total	287.1048	48.1293	62
PP	PP2	20.07.2000	211.9286	11.1296	7
		04.08.2000	294.4167	6.7928	6
		Total	250.0000	43.7388	13
	PP1	20.07.2000	221.0833	17.1680	6
		04.08.2000	300.2000	22.2439	10
		Total	270.5313	44.2717	16
	PP3	20.07.2000	221.0714	10.1465	7
		04.08.2000	298.8750	28.6590	8
		Total	262.5667	45.4866	15
	PP4	20.07.2000	221.0000	18.7693	8
		04.08.2000	289.1000	23.7227	10
		Total	258.8333	40.6871	18
	Total	20.07.2000	218.7679	14.5771	28
		04.08.2000	295.6029	22.1745	34
		Total	260.9032	42.9664	62
Total	NM	20.07.2000	220.5833	9.8154	6
		04.08.2000	297.3333	26.1821	9
		Total	266.6333	44.0549	15
	NP1	20.07.2000	244.7857	13.0475	7
		04.08.2000	333.2857	20.0143	7
		Total	289.0357	48.7046	14
	NP2	20.07.2000	248.5000	15.6205	7
		04.08.2000	326.5000	16.8608	8
		Total	290.1000	43.2333	15
	NP3	20.07.2000	236.0000	15.5793	8
		04.08.2000	333.6875	18.5990	8
		Total	284.8438	53.0986	16
	NP4	20.07.2000	239.5000	14.0814	8
		04.08.2000	335.6250	14.7691	8
		Total	287.5625	51.5590	16
	PP2	20.07.2000	215.9375	15.3214	8
		04.08.2000	294.4167	6.7928	6
		Total	249.5714	42.0535	14
	PP1	20.07.2000	221.0833	17.1680	6
		04.08.2000	300.2000	22.2439	10
		Total	270.5313	44.2717	16
	PP3	20.07.2000	221.0714	10.1465	7
		04.08.2000	298.8750	28.6590	8
		Total	262.5667	45.4866	15
	PP4	20.07.2000	221.0000	18.7693	8
		04.08.2000	289.1000	23.7227	10
		Total	258.8333	40.6871	18
	Total	20.07.2000	230.0000	18.1090	65
		04.08.2000	311.1622	27.2379	74
		Total	273.2086	46.8652	139

Tests of Between-Subjects Effects

Dependent Variable: SHOOTZZ

Source	Type I Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	262417.320[a]	18	14578.740	43.007	.000
Intercept	10375371	1	10375371.05	30606.845	.000
DISTURBA	22009.228	2	11004.614	32.463	.000
SOILSITE	5277.191	7	753.884	2.224	.037
HARVEST	232098.795	1	232098.795	684.680	.000
DISTURBA * SOILSITE	.000	0	.	.	.
DISTURBA * HARVEST	1641.208	2	820.604	2.421	.093
SOILSITE * HARVEST	1390.898	6	231.816	.684	.663
DISTURBA * SOILSITE * HARVEST	.000	0	.	.	.
Error	40678.629	120	338.989		
Total	10678467	139			
Corrected Total	303095.950	138			

a. R Squared = .866 (Adjusted R Squared = .846)



APPENDIX T7: Soil Disturbance Effects on Mean Dry Mass of Maize Seedling Shoots: Univariate Analysis of Variance.

Between-Subjects Factors

		N
soil disturbance treatment	control	2
	np	8
	pp	8
SOILSITE	control	2
	np1	2
	np2	2
	np3	2
	np4	2
	pp1	2
	pp2	2
	pp3	2
	pp4	2

Descriptive Statistics

Dependent Variable: shoot weights (g)

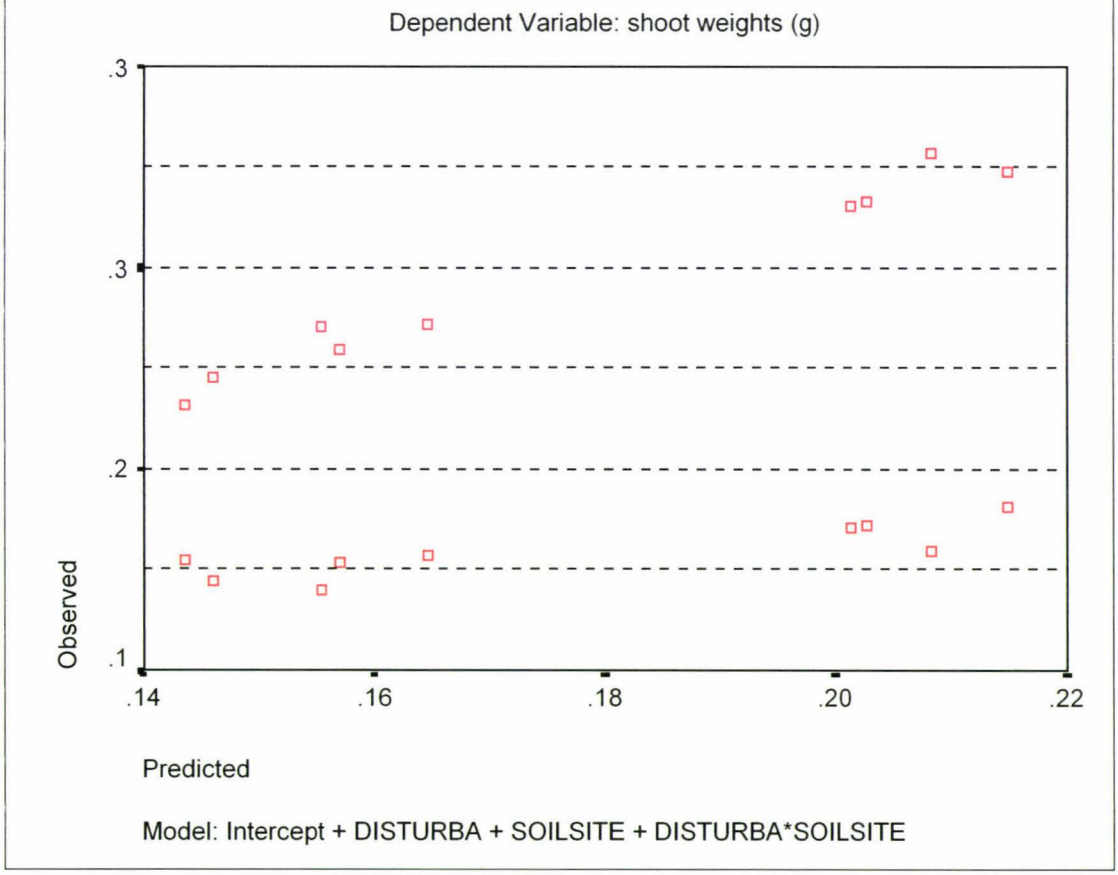
soil disturbance	SOILSITE	Mean	Std. Deviation	N
control	control	.145940	7.15026E-02	2
	Total	.145940	7.15026E-02	2
np	np1	.202825	.114092	2
	np2	.214990	.118384	2
	np3	.201310	.112982	2
	np4	.208430	.139965	2
	Total	.206889	9.22867E-02	8
pp	pp1	.164585	8.14799E-02	2
	pp2	.157030	7.50523E-02	2
	pp3	.155430	9.27300E-02	2
	pp4	.143595	5.45957E-02	2
	Total	.155160	5.89233E-02	8
Total	control	.145940	7.15026E-02	2
	np1	.202825	.114092	2
	np2	.214990	.118384	2
	np3	.201310	.112982	2
	np4	.208430	.139965	2
	pp1	.164585	8.14799E-02	2
	pp2	.157030	7.50523E-02	2
	pp3	.155430	9.27300E-02	2
	pp4	.143595	5.45957E-02	2
	Total	.177126	7.74315E-02	18

Tests of Between-Subjects Effects

Dependent Variable: shoot weights (g)

Source	Type I Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.358E-02[a]	8	1.697E-03	.173	.989
Intercept	.565	1	.565	57.527	.000
DISTURBA	1.289E-02	2	6.446E-03	.657	.542
SOILSITE	6.836E-04	6	1.139E-04	.012	1.000
DISTURBA * SOILSITE	.000	0	.	.	.
Error	8.835E-02	9	9.817E-03		
Total	.667	18			
Corrected Total	.102	17			

a. R Squared = .133 (Adjusted R Squared = -.637)



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