AN EVALUATION OF THE SPECIFIC APPLE REPLANT PROBLEM IN WESTERN CAPE ORCHARD SOILS

By

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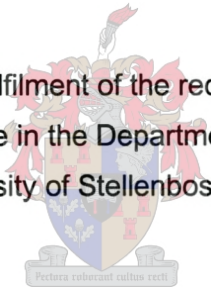

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DECLARATION

I, the undersigned hereby declare that the work contained in this thesis is my own original work and has not previously in its entirety or in part been submitted at any university for a degree.

SUMMARY

Apple replant disease (ARD) is one of the major impediments to the establishment of an economically viable apple orchard on sites previously planted to apple. In spite of extensive research on ARD, the etiology remains to be fully elucidated. A possible biological origin of ARD etiology in South Africa was investigated by the dilution of replant field soil with sterilised soil. Commercial orchards with ARD were selected for use in pot trials and disease severity evaluated after three months, by measuring shoot length, dry mass of plants as well as root discolouration. Although diluting replant soil to 25 and 50% (v/v) significantly reduced the effects of ARD, symptoms were only absent in 0% replant soil. It was clear that seedlings planted in any mixture containing replant soil, even only 25% replant soil, consistently exhibited symptoms of stunted growth and root discolouration similar to those seedlings grown in 100% replant soil. This indicates that ARD in South Africa is primarily of a biological nature.

As an initial step in formulating sustainable disease control alternatives to replace methyl bromide, pot trials were conducted to assess the impact of compost treatments as well as biological control products on ARD. Compost as well as sterilised and unsterilised compost teas (compost extract) significantly increased seedling growth even under optimum nutrient conditions when compared to the control, suggesting that they negate the effects of ARD. Results also indicated that applying high concentrations of compost does not necessarily provide additional growth benefits compared to lower concentrations. Results with biocontrol formulations were less favourable. Only one of the biocontrol formulations, a combination of *Bacillus* spp. (Biostart®) improved growth significantly compared to the control. There was, however, some inconsistency with results for the different trials conducted using this product.

Fungal as well as nematode populations associated with ARD soils were characterised to the generic level to get a clearer understanding of the etiology of ARD in South Africa. *Pythium* and *Cylindrocarpon* spp. were consistently isolated from all six replant soils in all trials that formed part of this study, indicating that these fungi may have a role in ARD etiology in South Africa. Nematodes implicated in ARD development were inconsistently associated with ARD soils used in these studies. This suggests that nematodes do not have a primary causal role in ARD etiology in South Africa.

Field trials were conducted in commercial orchards to assess the impact of organic amendments and promising biological control products, as indicated by the pot trials, on ARD severity under field conditions. These biological soil amendments were also compared with the standard chemical control methods for ARD, methyl bromide and chloropicrin. In all three trials established, compost and mulch as well as manure and mulch, consistently increased growth to the same extent as the standard chemical treatments and by combining these chemical treatments with organic amendments a significant, additional growth increase could be attained. Biocontrol formulations evaluated in field studies gave variable results. Biostart® improved growth when applied on its own, but not in combination with the chemical Herbifume (metham-sodium). Inoculating soil with effective microorganisms (EM), consisting primarily of photosynthetic bacteria, had no significant effect on growth.

Results from this study indicate that application of organic amendments could possibly substitute for soil fumigation in replanted apple orchards. However, compost quality standards need to be implemented and because few types of compost are universally effective, different types of composts should be compared in specific soil environments before recommendations can be made. Due to variable results with biocontrol products, ARD management with these biological soil amendments cannot be guaranteed at this stage and further studies are recommended.

EVALUERING VAN DIE SPESIFIEKE APPELHERVESTIGING-PROBLEEM IN BOORDE IN DIE WES-KAAP

OPSOMMING

Appelhervestiging-siekte (AHS) skep 'n groot probleem in die vestiging van jong appelbome op grond waar daar reeds voorheen appels verbou is. Ten spyte van omvangryke navorsing is die oorsaak van die probleem nog hoofsaaklik onbekend. 'n Moontlike biologiese oorsaakleer is in Suid-Afrika ondersoek deur die hervestigings-effek te probeer verminder deur die vermenging van hervestigings-grond met gesteriliseerde grond. Kommersiële boorde met 'n appelhervestigings-probleem is geselekteer en gebruik in potproewe. Die ernstigheidsgraad van die siekte is na drie maande se groei geevalueer deur lootlengte, droë massa en wortelverkleuring te meet. Alhoewel verdunning van die hervestigingsgrond tot 50 en 25% (v/v) die effek van AHS op groei betekenisvol verminder het, kon die skadelike effek van die veroorsakende faktor slegs uitgeskakel word deur saailinge in 100% gesteriliseerde grond te plant. Dit was duidelik dat saailinge wat in enige grondmengsel geplant is waarin hervestigingsgrond voorgekom het, selfs al was dit net 25%, konsekwent simptome van vertraagde groei en wortelverkleuring getoon het. Dit is 'n aanduiding dat AHS in Suid-Afrika hoofsaaklik biologies van aard is.

Potproewe is uitgevoer as 'n eerste stap in die formulering van volhoubare siektebeheer-strategieë, om die impak van kompos-behandelings en biologiese beheer produkte op AHS te ondersoek. Kompos sowel as gesteriliseerde en ongesteriliseerde kompos-tee (kompos-water) het, selfs onder optimale voedings-omstandighede, die groei van saailinge betekenisvol verbeter. Dit dui aan dat hierdie behandelings die effek van AHS kan teenwerk. Resultate het ook daarop gedui dat hoër kompos konsentrasies nie noodwendig enige addisionele voordele vir groei inhou in vergelyking met laer konsentrasies nie. Resultate met biologiese beheer produkte was minder gunstig. Slegs een van die produkte wat geëvalueer is, 'n kombinasie van *Bacillus* spp. (Biostart®), het groei betekenisvol verbeter in vergelyking met die kontrole. Resultate was egter inkonsekwent vir die verskillende proewe waarin hierdie produk gebruik is.

Swampopulasies sowel as aalwurmpopulasies wat met hervestigingsgrond geassosieer word, is geïdentifiseer tot op generiese vlak om vas te stel waardeur

AHS in Suid-Afrika veroorsaak word. *Pythium* en *Cylindrocarpon* spp. is konsekwent van al ses hervestigingsgronde geïsoleer wat daarop dui dat hierdie twee swam-genera 'n beduidende rol in AHS ontwikkeling in Suid-Afrika mag hê. Aalwurms wat aangedui is in die literatuur om 'n moontlike rol in AHS te hê, was slegs in enkele gevalle geassosieer met hervestigingsgronde waarvan in hierdie studie gebruik gemaak is. Die gevolg-trekking is dus gemaak dat aalwurms nie 'n betekenisvolle rol speel as hoof-veroorsakende organisme onder Suid-Afrikaanse toestande nie.

Veldproewe is uitgevoer in kommersiële appelboorde om vas te stel wat die effek van organiese materiaal, asook belowende biologiese beheermiddels, soos aangedui deur potproewe, op AHS onder veldtoestande is. Die biologiese grondtoedienings is ook vergelyk met die standaard chemiese beheermiddels (metielbromied en chloorpikrien). In al drie proewe wat gevestig is, het kompos met 'n deklaag, sowel as kraalmis met 'n deklaag, groei betekenisvol verbeter tot dieselfde mate as chemiese middels. Daar kon ook 'n beduidende, addisionele groeitoename gemeet word in gevalle waar chemiese middels met organiese materiaal gekombineer is. Resultate met biologiese beheer formulasies wat onder veldtoestande geëvalueer is, het gevarieer. Biostart® het groei verbeter wanneer dit alleen toegedien is, maar in kombinasie met die chemiese middel Herbifume (metham-sodium) het dit geen effek gehad nie. Die inokulering van grond met 'n oplossing van effektiewe mikro-organismes (EM) wat hoofsaaklik uit fotosinterende baterieë bestaan, het ook geen betekenisvolle effek op groei gehad nie.

Die gevolgtrekking is gemaak dat toediening van organiese materiaal moonlik as plaasvervanger vir metielbromied-beroking kan dien in die beheer van AHS. Die nodige komposkwaliteit-standaarde moet egter eers geïmplimenteer word. Omdat feitlik geen kompos universeel effektief kan wees nie, is dit ook nodig dat verskillende tipes kompos met mekaar vergelyk moet word in spesifieke grondtoestande voordat verdere aanbevelings gemaak kan word. As gevolg van variërende resultate met biologiese beheer produkte kan AHS beheer met hierdie middels nie gewaarborg word op hierdie stadium nie en verdere studies word aanbeveel.

DEDICATED TO MY PARENTS, JAPIE AND ANNA-MARIE

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CONTENTS

GENERAL INTRODUCTION

Apple replant disease (ARD) is one of the major impediments to the establishment of an economically viable apple orchard on sites previously planted to apple. In South Africa serious ARD symptoms occur in approximately 40% of replantings. This is of great economic importance because of its lasting effect on production and the problem is intensified as suitable land, not previously planted to apple becomes limited.

In spite of extensive research on ARD, the etiology remains to be fully elucidated. The problem is rarely caused by a single agent, but rather a complex of causal factors that vary across geographic regions or even between orchards in the same region. In the past, researchers have linked the poor performance of replanted apple trees to abiotic factors including unbalanced or inadequate nutrient availability, low or high soil pH, toxic residues in the soil and impaired soil structure. However, the dramatic growth improvement on ARD soils with a range of soil disinfecting treatments indicates that the causal elements are primarily biological. Furthermore, other fruit trees planted on ARD sites typically grow normal. Thus, the specificity of ARD is another strong counter-argument against abiotic factors as the main causal elements of ARD. Numerous soilborne organisms including plant parasitic nematodes, pathogenic fungi, actinomycetes and bacteria have been implicated as being potential causal factors, as well as allelopathic relationships between plants, microorganisms of the rhizosphere and soil.

No research has been conducted on the etiology of ARD in South Africa. The site-specific etiology means that elements implicated in disease development in other countries may have only a limited role locally. We investigated a possible biological origin of ARD etiology in South Africa by the dilution of replant field soil with sterilised soil. Fungal as well as nematode populations associated with ARD soils were then characterised to the generic level to establish a clearer understanding of the etiology of ARD in South Africa. The impact of the various soil amendments on fungal populations was also evaluated.

Due to the uncertain and complex etiology of ARD, control has traditionally been achieved through the use of biologically broad-spectrum soil fumigants, and in particular the application of methyl bromide. However, the high cost of chemical

control and its potential hazard to human health and the environment, necessitates the development of more sustainable means of ARD control. Furthermore, methyl bromide was declared an ozone depleting substance and its imminent phase-out has intensified the need for alternative measures to control ARD. As an initial step in formulating sustainable disease control alternatives to replace methyl bromide, pot trials were conducted to assess the impact of compost treatments as well as biological control products on ARD. Field trials were conducted in commercial orchards to assess the impact of organic amendments and promising biological control products, as indicated by the pot trials, on ARD severity under field conditions. These biological soil amendments were also compared with the standard chemical control methods for ARD (methyl bromide and chloropicrin).

CHAPTER 1

LITERATURE REVIEW

1.1 INTRODUCTION

Difficulties in replanting old apple orchard sites have troubled growers and claimed the attention of research workers across the world for more than 200 years (Mai & Abawi, 1981). Apple replant disease (ARD) is the unexplained poor growth of young apple trees, which occurs after replanting on a site that was previously planted to apple. The disease is widespread and one of the most important factors limiting production in all major apple-growing regions of the world (Traquiar, 1984). Two types of apple replant disease have been identified by Hoestra (1968). Specific ARD, which leads to apple-specific growth depression, and non-specific ARD, which affects a range of fruit trees.

Characteristics distinguishing ARD from other poor growth phenomenon are its specificity towards apple and possibly pear, and its persistence in soil after trees have been removed. An interesting case illustrating both these characteristics was reported in 1959 (Savory, 1967). A field was used as a fruit tree nursery from 1941-1953, then cultivated for 5 years with wheat and potatoes and in 1958 planted again with various fruit trees. The rows of the second planting of nursery trees were at right angles to the rows of the original planting. In the second planting, areas of poor growth appeared where closely related species were grown before, especially in the case of apples planted on apples. Since then, experimental work has been in progress to establish the cause of this problem as well as effective control measures.

The disease is not lethal, but it has great economic importance because of its lasting effect on yield. With the emphasis on early cropping to ensure a rapid return on investment it is crucial to get trees off to a good start and for trees to fill their bearing space as soon as possible. Therefore, any growth-retarding factor is adversely felt. The delayed precocity and production caused by ARD initially may decrease profitability by as much as 50% throughout the life of the orchard (Rabie, Denman & Cook, 2001). It is becoming an increasingly important problem as suitable land not previously planted to apple, becomes limited in South Africa. The tendency towards high-density plantings also intensifies the potential economic losses from this disease.

In spite of extensive research on ARD, the etiology still needs to be fully elucidated (Traquiar, 1984). Causal factors vary across geographic regions or even between orchards in the same region (Hoestra, 1968; Mazzola, 1998). It is a complex problem and the cause cannot be ascribed to one single factor. Due to this uncertain and complex etiology control has traditionally been achieved through the use of biologically broad-spectrum soil fumigants (Mai & Abawi, 1981), and in particular the application of methyl bromide. However, the high cost of chemical control and its potential hazard to human health and the environment make biological or cultural means of controlling ARD essential. Furthermore, methyl bromide was declared an ozone depleting substance and its imminent phase-out to comply with the Montreal Protocol has intensified the need for alternative measures to control ARD (WMO, 1994). This can only be achieved through a clearer understanding of the etiology of the disease (Mazzola, 1998).

1.2 GENERAL ASPECTS OF APPLE REPLANT DISEASE

1.2.1 SYMPTOMS

Affected trees can be slightly to severely damaged with aboveground symptoms including stunted growth, shortened internodes, rosetted leaves and reduction in tree vigour and productivity. Characteristically, shoot growth terminates earlier than on healthy trees (Traquiar, 1984). Trees affected by the disease begin cropping fruit 2 to 3 years later than unaffected trees and fail to attain comparable yields. Root systems display weak, necrotic roots and many decaying fine roots (Savory, 1966). ARD is associated with premature destruction of epidermal cells and primary cortex tissue of young roots as well as reduced lateral root development (Hoestra, 1968). Due to difficulty in distinguishing these ARD symptoms from other growth problems, this disease is mainly characterised by its specificity towards apple and its persistence in soil after plants have been removed.

1.2.2 CHARACTERISTICS

Before discussing the possible causal factors that play a role in ARD, it is important to note some of the characteristics of the disease complex.

- ARD persists in the soil for very long periods and cannot be avoided by delaying replanting for a few years (Hoestra, 1968). Although the problem persists in the soil, it does not seem to spread through it.

- It appears that there is some debate about the specificity of ARD. In some cases planting pears after apple has also shown poor growth suggesting that ARD is not specific to apple, but rather to pome fruit in general (Savory, 1966). However, the situation regarding this phenomenon is still unclear and needs to be investigated more intensively. Specificity was questioned by Sewell (1979) who provided evidence, which suggested that ARD is an expression of a widespread, but variable soil malaise that affects the growth of several crop plant species but is expressed most severely when replanting apple.
- Maximum disease intensity is superficial and occurs in the top 15-30cm of soil, which is usually also the main zone of feeder roots (Hoestra, 1968).
- Jaffee, Abawi and Mai (1982a) found that the factor responsible for stunting and root discolouration could not be reduced to a less damaging level by dilution of the original field soil. Strong growth reduction was also observed by Hoestra (1968) even when only 10% of ARD infested soils were mixed with fresh soil, demonstrating a possible microbial etiology.
- Acidification of soil can have a positive effect in controlling ARD. Savory (1967) reported that the ARD effect experienced is more severe if the soil pH is 6.0 or higher.
- ARD can successfully be controlled by broad-spectrum soil sterilisation.
- Nematicides and fungicides have a limited effect in controlling ARD (Hoestra, 1968).
- ARD symptoms have been noted after apples had been grown in the soil for one only year (Savory, 1966). Mazzola (1999) also found that a soil microbial community capable of inducing ARD could develop within two years of orchard establishment. This is in conflict with the general belief that ARD is most severe on sites that were planted to apple for extended periods of time (Mai & Abawi, 1981)

1.3 ETIOLOGY OF APPLE REPLANT DISEASE

The etiology of ARD varies across major fruit growing regions as well as between orchards in the same region. Numerous abiotic factors have been associated with ARD (Mai & Abawi, 1981) and biotic factors implicated include various soil-borne organisms as well as allelopathic relationships between plants, microorganisms of the rhizosphere and soil. These factors acting individually or synergistically may be involved.

1.3.1 ABIOTIC FACTORS

Many different abiotic causes have been implicated in replant diseases worldwide. In the past, people have linked the poor performance of replanted fruit trees to unbalanced or inadequate nutrient availability, low or high soil pH, heavy metal contamination, poor soil structure and drainage, and cold or drought stress (Mai & Abawi, 1981; Traquair, 1984). Although these elements may contribute to tree growth problems and disease expression, the fact that soil fumigation results in a dramatic improvement in tree growth on replant sites and that growth of other fruit trees planted on these sites is normal (Savory, 1966), indicates that the causal elements of ARD are primarily biological (Mazzola, 1998). The specificity of ARD is another strong counter-argument against abiotic factors as the main causal elements of ARD.

1.3.2 BIOTIC FACTORS

Accumulated research results, especially the effects of a wide range of soil-disinfecting treatments, suggest that soil organisms play an essential part in disease development. Plant parasitic nematodes were reported to have a major role in apple replant disease in the eastern United States (Mai & Abawi, 1981) and may also have a role in British Columbia (Utkhede, Smith & Palmer, 1992) and Australia (Dullahide *et al.*, 1994). Several investigations also point to parasitic fungi (Sewell, 1981; Jaffee, Abawi & Mai, 1982b; Braun, 1995), or phytotoxic micromycetes (Catska *et al.*, 1982) as primary causal agents, particularly a complex of pathogenic fungi with emphasis being placed on *Rhizoctonia* (Mazzola, 1998). Soil bacteria and actinomycetes have also been implicated by Savory (1966), Hoestra (1968) and Westcott, Beer and Stiles (1986). In the following section each of these agents will be discussed. It is also most probable that combinations of these biotic factors contribute towards the occurrence of ARD (Utkhede, Vrain & Yorston, 1992).

1.3.2.1 Plant pathogenic nematodes

For a long time nematodes have been associated with replant diseases in fruit growing areas throughout the world, particularly in coarse-textured soils (Hoestra & Oostenbrink, 1962; Mai & Abawi, 1978). Numerous investigators have concluded that the root lesion nematode, *Pratylenchus penetrans* (Cobb) Filipjev & Schuurmans-Stekhoven, has a causal role in ARD etiology (Jaffee, Abawi & Mai, 1982a; Merwin & Stiles, 1989; Utkhede Smith & Palmer, 1992; Dullahide *et al.*, 1994). However, the relative importance of nematodes in ARD development appears to vary among geographic

regions (Hoestra, 1968) and other researchers have laid less emphasis on the role of nematodes in ARD (Mazzola, 1998; Merwin & Stiles, 1989).

In the Granite Belt of Queensland, Australia, consistent improvement in growth of seedlings was obtained when orchard soils with replant disease were treated with the nematicide fenamiphos, suggesting that lesion nematodes were an important component of the disease complex in this region (Dullahide *et al.*, 1994). However, growth responses were greater when the soil was pasteurised, implicating that root pathogens other than nematodes were involved in replant failure (Jaffee, Abawi & Mai, 1982a; Dullahide *et al.*, 1994). Contrary to these findings, where nematodes were effectively eliminated from soil, growth of replanted apple trees was still not improved (Hoestra, 1968; Covey, Benson & Haglund, 1979; Mazzola, 1998). Mazzola (1998) demonstrated that nematicidal concentrations of fumigants did not improve growth of apple while soil pasteurisation and broad-spectrum fumigants did. Furthermore, in a separate trial carried out in New York, severe ARD symptoms and stunting were observed in apple seedlings grown in untreated soil from plots in which populations of *Pr. penetrans* were negligible (Merwin & Stiles, 1989). Also, nematode counts from healthy soils often exceed those from ARD soils (Caruso, Neubauer & Begin, 1989).

It is apparent that there is conflicting evidence to the hypothesis that nematodes are the primary causal agent of ARD. However, because nematodes are not consistently associated with ARD they do not seem to be the main cause of ARD, although high populations of nematodes can cause direct root destruction and eventual growth and yield reduction in specific sites. It can therefore only be seen as a complicating factor that can aggravate a replant situation. Additional damage to fruit trees is undoubtedly caused by interaction among nematodes and other soil-borne organisms and among nematodes and unfavourable environmental factors. It is evident that necrotic lesions induced by nematodes on feeder roots provide ports of entry for fungal pathogens and can have an important role in certain disease complexes (Powell, 1971).

1.3.2.2 Actinomycetes

The actinomycetes are filamentous or rod-shaped bacteria tending strongly to the development of branches and true mycelium. They are gram-positive organisms and are sometimes called 'higher bacteria', organisms possessing properties intermediate between the fungi and bacteria (Alexander, 1961).

Hoestra (1968) suggested that actinomycetes were involved in the etiology of ARD in The Netherlands based on the failure of nematicides and fungicides to control the disease. In environments of high pH a large proportion of the microbial population consists of actinomycetes. Savory (1967) observed lower incidence of ARD in acidic soils and noted that actinomycetes were also less damaging in these acidic soils. Otto and Winkler (cited by Westcott, Beer & Stiles, 1986) first presented evidence implicating the involvement of actinomycetes in ARD. They found that the extent of colonisation of apple root epidermal tissue by actinomycete-like organisms was positively correlated with ARD severity. In experiments conducted by Westcott, Beer and Israel (1987) apple seedling roots planted in soil conducive to ARD were consistently infected while those planted in steamed soil were not. Histological studies showed that actinomycetes invade the cortex of rootlets by penetrating the epidermal cells (Otto & Winkler, 1998), and reduce the efficiency of the rootlet system by damaging the root hairs (Westcott, Beer & Israel, 1987; Otto & Winkler, 1993).

Szabo *et al.* (1998) suggested that the infection by actinomycetes is a primary one and that these organisms are therefore pathogenic and may be responsible for ARD. They reported that the portion of rootlets infected exclusively by actinomycetes exceeded 50 % of the total number of infected rootlets. However, pathogenicity of actinomycetes has not yet been demonstrated by inoculation of test plants and re-isolation of the pathogenic actinomycetes.

Attempts to isolate these pathogenic actinomycetes have so far remained without success (Westcott, Beer & Israel, 1987; Mazzola & Gu, 2000). To prove that actinomycetes are causal agents of ARD will require isolation of the organism, axenic cultivation and controlled infestation of soils that are not conducive to replant disease. Furthermore, soil used in previous studies contained many other microorganisms and it could not be determined whether actinomycetes possessed the capacity to infect plants in the absence of these organisms (Westcott, Beer & Israel, 1987). However, until counter evidence is found, actinomycetes cannot be dismissed as possible causal agents of ARD.

1.3.2.3 Bacteria

The involvement of bacteria in ARD had been hypothesised by Savory (1966) as well as Hoestra (1968). However, bacteria tested by Dullahide *et al.* (1994) were not pathogenic to apple seedlings and did not have a role in the disease phenomenon observed in

Queensland. Findings by Mazzola (1998) also suggested that bacteria do not have a significant causal role in the etiology of ARD in Washington, since applications of a broad-spectrum antibiotic reduced soil populations of bacteria but failed to improve growth of apple transplants. Furthermore, he reported that enhanced growth was achieved at pasteurisation temperatures that did not alter the bacterial community recovered from apple roots.

In contrast to these findings, bacteria including mainly the fluorescent pseudomonads have been reported to contribute to ARD (Bunt & Mulder cited by Mazzola, 1998). Waschkies, Schropp & Marscher (1994) also suggested a direct or indirect role of fluorescent pseudomonads in replant diseases of grapevine. Furthermore, *Bacillus subtilis* has on occasion been reported to contribute to the development of the ARD complex (Utkhede, Vrain & Yorston, 1992). Four strains of *B. subtilis* isolated from ARD soils in British Columbia stunted the growth of apple seedlings. However, generally isolates of *B. subtilis* are not pathogenic to plants unless their populations are very high.

1.3.2.4 Fungi

Several investigations in England and USA point to *Pythium* and *Phytophthora* species as primary causal agents of ARD (Sewell, 1981; Matherton, Young & Matejka, 1988). Furthermore, *Fusarium* spp. (Dullahide *et al.*, 1994) *Cylindrocarpon* spp. (Braun, 1995; Mazzola, 1998), and *Rhizoctonia* spp. (Mazzola, 1998) have also been implicated in disease development. Recent findings indicate that a complex of fungi in orchard soils contributes to ARD rather than individual fungi (Braun, 1995; Mazzola, 1998). Another important biological factor associated with ARD is the soil and rhizosphere saprophytic microflora and increased attention has been paid to the phytotoxic micromycetes (Catska *et al.*, 1982). Micromycetes are fungi of small size that produce microscopic sporiferous structures (Ulloa & Hanlin, 2000).

1.3.2.4.1 Phytotoxic micromycetes

Root exudates in the rhizosphere of apple monoculture may lead to the dominance of certain saprophytic phytotoxic microorganisms that affect the development of young plants negatively (Catska *et al.*, 1982). Depending on the apple-growing area, some of these microfungi appear to be responsible for ARD.

Catska *et al.* (1982) reported that fungi of the genus *Penicillium*, as well as *Alternaria*, may produce phytotoxins, such as patulin, that become prevalent in the rhizosphere

during apple monoculture. These saprophytic micromycetes not only affect the growth and health of plants negatively, but also the beneficial microflora in the rhizosphere. By inoculating apple seedlings with the phytotoxic fungus *Penicillium claviforme* Bainier, Catska *et al.* (1988) induced ARD symptoms. In contrast to this, in the rhizoplane of seedlings grown in soil not conducive to ARD, micromycetes of the genera *Mucor* and *Trichoderma* occurred at high levels. This suggestes that the type and amount of phytotoxic micromycetes in the soil could be used as an indicator of the degree of ARD.

1.3.2.4.2 Pythium

Several investigations in England implicate *Pythium* species as primary causal agents of ARD (Sewell, 1981). In pathogenicty tests isolates of *Pythium sylvaticum* Campbell & Hendrix (Braun, 1995; Sewell, 1981; Mazzola, 1998) as well as *P. ultimum* Trow (Mazzola, 1998) caused extensive root rot of apple transplants and significant reductions in plant biomass. Studies carried out by Utkhede, Smith and Palmer (1992) confirmed that *P. ultimum* significantly reduced seedling length, but indicated that *P. sylvaticum* had no effect on seedling growth. Sewell (1981) and Braun (1995) found that certain isolates *of P. coloratum* Vaartaja, *P. echinulatum* Matthews, *P. irregulare* Buisman, and *P. oligandrum* Drechlser, also significantly reduced growth of apple seedlings. Jaffee, Abawi and Mai (1982b) consistently isolated *P. irregulare* from roots of seedlings grown in ARD soil. It therefore seems that the species implicated in ARD varies between different regions and that not all isolates of the same species cause the same response in apple seedlings.

Most evidence against a causal role for *Pythium* spp. is derived from failure to control the disease by the use of fungicides that are generally effective in controlling *Pythium*-induced damping-off diseases (Hoestra, 1968; Mai & Abawi, 1978). The evidence indicating that *Pythium* spp. are not the main cause of ARD does not exclude the possibility that they may be components of a disease complex. This aspect will be discussed in a following section.

1.3.2.4.3 Phytophthora

Investigations by Utkhede, Smith & Palmer (1992) showed that *Phytophthora cactorum* (Leb. and Cohn) Schröeter and *Ph. cinnamomi* Rands significantly reduced plant height in sterilised replant soil, while *Ph. cambivora* (Petri) Buisman was extremely virulent to young apple trees and killed all trees tested. It appears that this aggressive species of *Phytophthora* is widely distributed in the USA and could be associated with the apple

replant problem (Harris, 1991). However, it was not isolated by Mazzola (1998) in Washington State, USA and has also not been isolated from apple orchards in British Columbia (Utkhede, Smith & Palmer, 1992). Mazzola (1998) consistently recovered isolates of *Ph. cactorum* from ARD symptomatic apple roots. Furthermore, Matherton, Young and Matejka (1988) indicated that *Ph. Parasitica* Dastur (now *Ph. nicotianae* Breda de Haan) may have a role in ARD. In the investigation by Utkhede, Smith & Palmer (1992) *Ph. parasitica* reduced plant growth only when it was present together with the lesion nematode *Pr. penetrans*, again indicating that ARD is caused by a complex of soil microorganisms.

1.3.2.4.4 Cylindrocarpon

Jaffee, Abawi and Mai (1982b) showed that *Cylindrocarpon lucidum* Booth, isolated from seedlings grown in ARD soil, was pathogenic to apple seedlings and caused stunting and black lesions on feeder roots. This is in agreement with results from Braun (1995) who also implicated a role for *C. lucidum* in ARD. In contrast with these findings, Dullahide *et al.* (1994) and Mazzola (1998) consistently isolated the species *C. destructans* (Zins.) Scholten from discoloured roots grown in replant soil.

1.3.2.4.5 Rhizoctonia

Studies conducted by Mazzola (1997, 1998) were the first to substantiate a role for *Rhizoctonia* in ARD development. *Rhizoctonia solani* Kühn AG 5 and AG 6 were isolated from stunted trees, but not healthy trees, in an orchard that exhibited severe symptoms of ARD (Mazzola, 1997). He also found that soils not previously cultivated to apple were suppressive towards the development of *Rhizoctonia* root rot caused by an introduced pathogenic strain, while soils that had been planted to apple for two years or longer were conducive to disease development (Mazzola, 1998).

1.3.2.4.6 Involvement of a fungal complex

Recent findings by Mazzola (1998) demonstrated that a complex that included species of *Rhizoctonia, Fusarium, Cylindrocarpon, Pythium* and *Phytophthora* were isolated from apple roots grown in ARD soils in Washington State. The relative dominance of these individual species in the fungal community varied among orchards. In pathogenicity tests, isolates of *C. destructans, Ph. cactorum, P. ultimum, P. sylvaticum* and *R. solani* AG 5 caused extensive root rot of apple transplants (Mazzola, 1998). Isolates of *Fusarium*, however, were not pathogenic or were only weakly virulent. This soil microbial community capable of inducing ARD can develop within two years of orchard

establishment (Mazzola, 1999). Mazzola (1999) found that extensive modification of soil microbial communities occurs during apple monoculture. The increase in pathogenic fungi were associated with reductions in the relative populations of some of the soil bacteria *viz*, *Burkhoderia cepacia*, and *Pseudomonas putida* in the rhizospere of apple. He therefore concluded that the resident soil microflora is transformed from one that supports optimal growth of apple to one that induces symptoms of replant disease.

Dullahide *et al.* (1994) concluded that *Fusarium tricinctum* (Corda) Sacc., *Cylindrocarpon destructans* and *Pythium* spp. were implicated in the replant problem because they were consistently recovered from discoloured roots in Queensland. In agreement with these results, Merwin & Stiles (1989) isolated *Fusarium* and *Cylindrocarpon* spp. from severly stunted apple seedlings with root necrosis. In contrast to this, as was found in Washington (Mazzola, 1998), Utkhede, Smith and Palmer (1992) found that *F. solani* (Mart.) Sacc. and *F. oxysporum* Schlectend. did not affect seedling growth in sterilised soil.

Severe disease has been reported to result from interactions between *Pythium* and *Rhizoctonia* (Sewell, 1981). *Pythium* and *Rhizoctonia* were predominantly recovered from stunted trees or trees near death and were rarely present on the roots of healthy trees from the same site (Mazzola, 1998). According to Braun (1995) growth suppression was also greater with all combinations of *Pythium* spp. with *Cylindrocarpon lucidum* than with the *Pythium* or the *Cylindrocarpon* isolates individually. *C. lucidum* combined with *P. ultimum* or *P. irregulare* caused more than 50% suppression in shoot height, the greatest suppression being observed with *C. lucidum* and *P. irregulare* (68%). This combination caused replant disease-like symptoms in apple and pear, but had no significant effect on plum or peach (Braun, 1995). Isolations from roots made by Jaffee, Abawi and Mai (1982b) from seedlings grown in steamed field soil amended with feeder roots obtained from seedlings previously grown in untreated field soil, consistently yielded both *C. lucidum* and *P. irregulare*.

1.3.2.5 Interaction of fungi, bacteria and nematodes

It was suggested that fungi, bacteria and nematodes in combination might contribute towards the occurrence of ARD. In some cases, infection by actinomycetes is accompanied by the occurrence of fungal hyphae as well as nematodes (Otto & Winkler, 1993). Utkhede & Li (1988) found that fungi, bacteria and their interactive effect might be involved in the ARD complex in British Columbia. Previous studies indicated that

nematode activity could predispose roots to attack by other soil microorganisms (Mountain & Patrick, 1959). The combination of *Pr. penetrans* and *B. subtilis,* or *Pr. penetrans* and fungi and bacteria significantly reduced plant height and root weight when present together in soil (Utkhede, Smith & Palmer, 1992). The combination of nematodes plus bacteria affected plant growth more severely compared with the nematodes plus fungi combination. It is possible that certain microorganisms are destructive only when they occur in combination with other microorganisms.

Histological studies showed that nematodes and hyphae of *Rhizoctonia, Phytophthora* and *Pythium* spp. are found together in roots of trees planted in replant soils (Caruso, Neubauer & Begin, 1989). Utkhede, Smith and Palmer (1992) also showed that interactions between *Phytophthora* spp. and *Pr. penetrans* may be associated with replant disease. *Ph. parasitica* alone did not affect tree growth but in combination with *Pr. penetrans* significantly reduced young tree growth compared to nematodes alone in the ARD soil. There was also a synergistic effect between root lesion nematodes and the soil fungi *Ph. cactorum* and *Ph. cinnamomi.*

1.3.3 ALLELOPATHIC RELATIONSHIPS

Allelopathy was defined by Rice (1984) as any direct or indirect harmful effect by one plant (including microorganisms) on another through chemical compounds that were produced by the plant and added to the environment. A supposition that has been made is that toxins produced directly by living plant roots, or through microbial decomposition of residues from the plant, can remain in the soil and decrease the growth of a second crop of the same species, thus playing a causal role in ARD (Patrick, Tousson & Koch, 1963). The mode of action of allelochemicals can broadly be divided into direct and indirect action (Rizvi *et al.*, 1992). Indirect action includes effects that alter the chemical and biological properties of soil including, its nutritional status and the population size and/or activity of beneficial or harmful microorganisms. The direct mode of action includes effects of allelochemicals on various aspects of plant growth and metabolism.

Research investigating the role of allelopathic substances in ARD is limited to experiments carried out in the 1960's by the pioneers of ARD, Savory (1966) and Hoestra (1968).

1.3.3.1 Leachate from apple soil

Contradictory results were attained from experiments where ARD soils were leached and treated with leachate. Fastabend (cited by Savory, 1966) reported that growth of apples in old apple soil was much improved by previously leaching the soil with moderate amounts of distilled water over a long period. Conversely, fresh soil treated with some of the leachate from the old apple soil or with added crushed apple roots, reduced growth of apples to about the same level obtained in old apple soil. Results from experiments carried out by Hoestra (1968) showed that no appreciable growth reduction resulted from addition of soil leachate to apple seedlings grown in healthy soil. Furthermore, the leached soil did not loose its capability of reducing growth of seedlings.

However, in both of these experiments no attempt was made to identify toxins and to show their transfer or decreased concentration in soil. Furthermore, it is possible that bacteria or even fungi were leached out of the soils and not necessarily toxins. If the causal factor were so easily washed out of the soil, persistence, which is one of the most important characteristics of ARD, would not occur.

1.3.3.2 Toxins produced by microbial degradation of plant residues in soil

Toxic compounds may be produced by common soil organisms that decompose apple roots. Borner's work on phloridzin is important in this connection and was reviewed by Hoestra (1968) and Savory (1969). Phloridzin is a glucoside present in high concentrations in different apple tissues and is especially high in the root cortex. When microorganisms degrade root residues this glycoside is released into the soil. Borner found that under laboratory conditions phloridzin had a toxic effect on apple seedlings grown in water cultures, but later showed that under field conditions it was not directly responsible for ARD. Hoestra's work confirmed that phloridzin has no direct effect on the growth of apple when the pure chemical was added to the soil, nor is growth affected by adding cut pieces of apple roots to the soil. Hudska (1988) also found that when the effect of phloridzin at concentrations found in the field was tested on roots of apple trees, no inhibition was observed.

Another theory is that many root rots are initiated by direct toxic action of plant residues (Cochrane, 1948). Toxins produced can predispose roots to infection by various pathogens. This hypothesis implies that the activities of the soil organism are secondary and are incident upon an initial injury that is of chemical origin.

1.3.3.3 Problems with the toxin hypothesis

Chemicals with allelopathic potential are present in virtually all plant tissues, whether these compounds are released into the environment in sufficient quantities and with enough persistence to affect succeeding plants remains a critical question (Putnam & Tang, 1986). Evidence of production of effective concentrations of toxins, especially under field conditions, has been less convincing partly because the detection and assay of toxins are extremely difficult and complex processes (Rice, 1984).

Arguments against abandoning the idea that allelopathy contributes to ARD include the fact that insufficient attention has been given to the role of strict chemical reactions on the transformation and fate of allelochemicals in the soil. Chemical transformation processes, such as oxidation, reduction, hydrolysis, substitution, complexation and polymerisation, can play a significant role in reducing the allelopathic potential of certain chemicals (Cheng, 1992). Furthermore, the ARD symptoms caused by allelopathogens may not be manifested at the time that plant damage actually occurs and by the time symptoms are observed, the chemical may no longer be present (Cheng, 1992), making its detection extremely difficult. Thus, at this stage there is insufficient scientific evidence to completely abandon the possible role of allelopathic toxins in ARD and research in this direction should be encouraged.

1.4 CONTROL OF APPLE REPLANT DISEASE

Progress towards the control of ARD has been impeded by difficulties in recognising the primary causal agent within a background of complex interacting factors. At present, there are few satisfactory alternatives to the long-standing practice of soil fumigation because of the broad-spectrum biocidal activity of the fumigants used (Mai & Abawi, 1981). The most effective fumigant is methyl bromide, which currently plays an indispensable role in establishing an economically viable orchard on a site that was previously planted to apple. However, growers have also had some success with other chemicals. Although these alternative chemicals have provided some form of control of ARD, the high cost of chemical control and the potential hazard to human health and the environment make it essential to develop more sustainable means of ARD control. Use of a diversity of management practices that include less dependence on single-chemical strategies and greater use of biological and cultural management strategies could enhance grower options (Ristaino & Thomas, 1997).

1.4.1 CHEMICAL SOIL DISINFESTATION

1.4.1.1 Soil fumigation with methyl bromide

Fumigation with a broad-spectrum soil sterilant is currently the most effective way of combating ARD. However, scientists cannot entirely explain the powerful effect of fumigation. Increase in plant growth is only partly accounted for by the elimination of pathogenic soilborne organisms (McKenry *et al.*, 1994). An alteration of the nitrogen content of soil has been proposed by Jackson (1979) to be responsible for the increased growth response. However, Cook (1992) provided evidence that the increased growth response to soil fumigation results from improved root health and not from increased nitrogen in soil. Furthermore, Mazzola (pers. comm.) quantified the impact of soil pasteurisation on soil N-content, and subsequent N-content in apple leaves grown in such soils and documented no differences induced by pasteurisation.

Methyl bromide is a broad-spectrum biocide and since the first reports of its fungicidal properties its use has become indispensable in all major apple-growing areas. It is a highly poisonous gas and because it has no smell and to prevent injury, 1-2% tear gas (chloropicrin) is added as a warning agent. This fumigant effectively destroys most plant pathogenic pathogens as well as eradicating weeds and soil insect pests. For this reason methyl bromide has become one of the most widely used fumigants (De Ceuster & Hoitink, 1999). Unfortunately methyl bromide also has a direct negative effect on mycorrhizal fungi (Menge *et al.*, 1978). Furthermore, evidence was obtained implicating this chemical as a potent contributor to ozone depletion (Ristaino & Thomas, 1997). For this reason it is scheduled to be phased out by 2005 in developed countries, 2015 in developing countries and 2010 in South Africa, as agreed by signatories to the Montreal Protocol (WMO, 1994). Consequently, there is great urgency to find alternative methods for controlling ARD.

1.4.1.2 Alternative chemicals

Several alternatives to methyl bromide are being developed. One approach is to substitute methyl bromide with another less problematic but still effective fumigant. The main alternatives are combinations of chemicals of which the nematicide, Telone, mixed with Chloropicrin is most likely the front-runner. At this stage methyl iodide as well as metham-sodium also seem to be suitable substitutes (Ohr *et al.*, 1996). Metham-sodium (Vapam/ Dazomet/ Metham/ Herbifume) is a methyl isothiocyanate (MIT)-generating formulation that has shown promise as an alternative to methyl bromide

especially in the strawberry industry in California (Duniway *et al.*, 1999; Porter, Brett & Wiseman, 1999). Although this chemical is already commercially used and some producers are satisfied with the results they are obtaining, a more practical application method is needed (McKenry, *et al.*, 1994; De Ceuster & Hoitink, 1999). This fumigant may also be useful at low rates in combination with biocontrol agents. Furthermore, Dazomet is not as effective against bacteria, actinomycetes and weeds (Sewell *et al.*, 1986). Vadachter (1979) also found that for fungi forming resistant structures, MIT-generating fumigants did not significantly reduce the number of fungal colonies.

Many other chemical alternatives are deficient for various reasons. Nematicides such as 1,3-dichloropropene (1,3-D) control nematodes but are not efficient against weeds or fungal pathogens (Hoestra, 1968). Chloropicrin, another broad-spectrum fumigant, is best known for its efficacy against soilborne fungi (Jackson, 1979). It also provides some control of root destroying insects and free-living nematodes. However, because of its marginal activity against pathogenic nematodes, on its own it cannot replace methyl bromide. Furthermore, it is extremely unpleasant to work with and corrosive to machinery and other implements.

Formalin (containing 38% formaldehyde) is also a broad-spectrum biocide that has been used in the past to control ARD (Sewell & White, 1979). It is relatively inexpensive and with application at one-quarter of the recommended rate (about 150 ml/m^2) or perhaps even less, may provide an economical and far less hazardous alternative (Covey *et al.*, 1984). The main difficulty in the field is the apparent requirement for its application in large volumes of water, because the chemical moves in the soil water phase. Hoestra (1968) however, found formalin to be only moderately effective. Thus its efficiency could vary with different soil types. Xue and Yao (1998) observed that combining formaldehyde fumigation and vesicular arbuscular mycorrhizal (VAM) fungi inoculation was very effective in controlling ARD.

Field-testing has shown fungicides not to be very effective in controlling ARD, probably because of their narrow spectrum of activity (Sewell & White, 1979; Tranquiar, 1984). Although a small growth increase of marginal significance is sometimes found, the resulted growth increase is nowhere near that which is reached with methyl bromide. In contradiction, however, some positive results have been found. Sewell (1978) showed some success with furalaxyl at low rates of application, which directly effects *Phytophthora* and *Pythium* species. Mazzola (1998) recently also showed that the

application of semiselective biocides can enhance growth of transplanted apples. He observed that a combination of metalaxyl (specifically suppressing *Phytophthora* and *Pythium* spp.) and difenconazole (eliminating species including *Cylindrocarpon, Fusarium, Rhizoctonia* and *Trichoderma*) enhanced growth of apple. The response of apple seedlings to an application of these two fungicides combined were equivalent to that obtained in soil pasteurised at 95°C.

Success with these narrow spectrum fungicides is dependent on the precise identification of the causal agents of ARD as well as predisposing environmental factors. Furthermore, the mechanisms of action of fungicides are not entirely known (Szczygiel & Zepp, 1998). Fungicides may eliminate some fungi participating in the metabolism of substances liberated from decomposed roots that remain in soil after the removal of previous orchards. These metabolised substances could be toxic to the developing roots of young trees resulting in the typical stunted growth associated with ARD. Negative results with fungicides may also be caused by failure of the respective fungicides to adequately penetrate the soil.

In South Africa methyl bromide is still the standard treatment to control ARD. However, other compounds such as formaldehyde, Telone, Bacfume (chloropicrin), Enzone (sodium tetrathiocarbonate) and Herbifume (metham-sodium) have been tested extensively, of which only Bacfume and Herbifume have shown to be effective (Honeyborne & Groenewald, 1997). However, as previously mentioned, effective application of metham-sodium remains a problem.

1.4.2 BIOLOGICAL CONTROL

Baker and Cook (1974) have defined biological control as the reduction of inoculum density or disease producing activities of a pathogen in its active or dormant state, by one or more organisms. This can be accomplished naturally by manipulation of the environment, host or resident antagonist or by mass introduction of one or more antagonists or other beneficial organism (Catska, 1988; Catska & Taube-Baab, 1994). In this section some approaches toward biological control of ARD will be discussed.

1.4.2.1 Introduction of antagonistic or beneficial bacteria

Some microorganisms are known to produce antagonistic metabolites that can control soilborne pathogens. These metabolites include antibiotics (*Agrobacterium radiobacter, Bacillus subtilis, Trichoderma*) siderophores (*Pseudomonas* spp.) and enzymes (*A.*

radiobacter, Bacillus spp., *Pseudomonas* spp.). Furthermore, mycoparasitic fungi (for example *Trichoderma*) can be used for general or specific control of soil-borne pathogens as well as saprophytic phytotoxic microorganisms, especially the micromycetes (Catska, 1993). It has been shown that inoculation of apple seedlings or apple rootstocks growing in soil with some bacterial antagonists can be used for suppressing ARD.

Utkhede & Smith (1992; 2000) suggested that the bacterium *Bacillus subtilis* strain EBW-4 has potential to control ARD in orchards in British Columbia. However, in their experiments no attempt was made to determine if the introduced isolates were present on the plants or persisted after introduction. It is therefore not possible to state with certainty that the results obtained with EBW-4 were in fact a function of the introduced strain. Results were also variable when these bacteria where applied in combination with other treatments, for example with metham-sodium and the VAM fugus *Glomus intraradices*.

Apple replant disease can also be reduced by plant growth promoting rhizobacteria (PGPR). These rhizobacteria enhance the plants defence by stimulating plant growth. This can lead to disease escape by shortening the time that the plant is in a susceptible state. Furthermore, rhizobacteria can be antagonists to pathogens and colonise the roots to prevent invasion. In most cases control results from metabolites produced by bacteria that directly inhibit the pathogen. Experiments by Biro *et al.* (1996) provided evidence supporting the use of antagonistic *fluorescens-putida* type *Pseudomonas* rhizobacteria for controlling ARD. However, effective control strongly depended on the type of soil as well as the environmental conditions. Furthermore, inoculation of replant soil and steam sterilised soil resulted in the same rate of growth stimulation, suggesting that factors other than antagonistic ability, for example hormone production, could contribute to the beneficial effects. *Pseudomonas putida* is also being field tested in the USA as a potential biocontrol agent against ARD (Warner, 1999).

According to Catska (1993) *Agrobacterium radiobacter* can to some extent suppress replant disease. Inoculation with *A. radiobacter* may affect the plants by changing the composition of the rhizosphere microflora in reducing the number of colony forming units of phytotoxic micromycetes which might contribute to ARD (Catska & Hudska, 1993). This bacterium inhibits the growth of phytotoxic micromycetes *in vitro,* such as *Penicillium claviforme, P. expansum* Link, *P. griseofulvum* Dierckx, *Alternaria alternata*

(Fries:Fries) von Keissler as well as some of the phytopathogenic fungi. *A. radiobacter* can also suppress these harmful microorganisms directly in the rhizosphere, due to its antibiotic activity and ability to persist for several years in the rhizosphere.

Although biocontrol agents are not always very effective on their own, great potential lies in combining them with other treatments. When applying microbial preparations of antagonists to soil, treatments usually need to be repeated regularly because the microbes are not persistent due to low concentration of nutrient and energy sources in the soil. However, application of these antagonists or beneficial organisms in conjunction with other management practices such as addition of organic amendments, might improve the persistence of these agents and be beneficial to suppression of ARD.

1.4.2.2 Introduction of mycorrhizae

Mycorrhizal symbioses can improve nutrient uptake, particularly that of immobile ions such as phosphates. As a result of the increased uptake of mineral nutrients from soil, mycorrhizal plants grow more vigorously especially in nutrient deficient soils. However, these fungi are usually eliminated from soil when ARD is controlled by fumigation (Ohr *et al.*, 1996; Utkhede & Smith, 2000). Thus, addition of mycorrhizal fungi usually eliminates stunting of plant growth following fumigation (Menge *et al.*, 1978). However, mycorrhizal fungi may also exert a biological control effect on soil pathogens. Uthkede and Smith (2000) observed that application of the VAM fungus *Glomus intraradices* increased fruit yield and tree growth in ARD soils and reduced root infection by *Pythophthora cactorum* and *Pythium ultimum*. This protection provided against ARD pathogens may be due to improved plant nutrition, particularly phosporous. Greenhouse trials showed a significant increase in seedling growth when *G. mossea* was mixed with ARD soil (Utkhede, 1992). These VAM fungi could be inoculated at the time of replanting or even in apple nurseries.

1.4.2.3 Application of organic matter

Another approach towards ARD control is the return to a practice that replaces soil sterilisation with soil organic matter management. Several scientists ask the question as to whether this process should be labelled as biological control, cultural management or chemical control. Since suppression of pathogen activity is the result of biologically mediated metabolism, according to Lazarovits (2001), this is biological control. Examples include application of animal manures, green manures, compost or biocontrol-agent–fortified composts which can provide effective control of diseases as well as

insects and weeds if combined with specific cultural practices (De Ceuster & Hoitink, 1999). Organic substrates influence soil structure and moisture but may also modify the composition of the microflora so that it benefits growth of young roots.

Compost has long been recognised to provide a degree of control of diseases caused by soilborne pathogens (Hoitink & Fahy, 1986). For this reason disease-suppressive effects of compost have been investigated intensively over the last decade and the use of compost for disease control is increasing rapidly. Compost contains its own complex of microflora and by adding it to soils a whole microbial community that may be antagonistic to existing soilborne pathogens is introduced into the environment (De Ceuster & Hoitink, 1999). The more diverse the microflora, the greater the chance that the right selective conditions will be created which are needed for beneficial organisms to protect the roots. Compost has been used to successfully control *Pythium* and *Phytophthora* in composted bark-amended container media (Hoitink, Stone & Han, 1997). Unfortunately the mechanism of their action is not entirely known. Furthermore, Lazarovits (2001) observed that amendments worked well in some soils but had little impact in others. It is therefore necessary to elucidate the mechanisms of disease control provided by these amendments in order to apply it over a wide geographical region with different soil conditions.

Application of biohumus at dosages of 10-20% as well as peat and decomposted bark was very effective in experiments carried out by Szczygiel and Zepp (1998). The beneficial effect of certain dispersed organic matter such as peat or compost on replanted apple sites may be due to the absorption of harmful compounds secreted by the causal organisms (Gur, Luzzati, & Katan, 1998). According to Kummeler cited by Gur, Luzzati & Katan (1998), ethylene is such a substance, but it is not the only compound that is involved. The ethylene component of the soil and root atmosphere of replanted apple plants is decreased by soil fumigation as well as by adding activated charcoal. Apparently fumigants reduce the population of ethylene forming soil microorganisms, whereas organic matter and activated charcoal act by absorbing the ethylene.

Manures tested have given extremely variable results. In some soils, cow or horse manure with mono-ammonium phosphate (MAP) or pig manure alone were equal to or more beneficial than fumigants for increasing growth of seedlings, while in other soils

they were detrimental to survival and growth (Slykhuis, 1988). Some manure also appears to carry persistent herbicides.

Fumigants or chemical control alternatives are typically applied only as a once-off precautionary measure or can be used successfully when pathogens have reached populations that cause major losses. The same strategy unfortunately cannot be adopted if manures or compost are used for disease control. Composts typically suppress or eradicate pathogens slowly over a long period of time and therefore need to be applied well before pathogens reach populations capable of causing losses (De Ceuster & Hoitink, 1999). Furthermore quality factors have to be standardised to reduce variability and obtain consistent results with these amendments (Hoitink & Fahy, 1986). According to Hoitink, Stone and Hun (1997) controlled inoculation of composts with biocontrol agents is a procedure that can induce consistent levels of disease suppression on a commercial scale. Nevertheless, few types of compost are universally effective (Van Dyk, Cronje & Wehner, 2001) and it is therefore necessary to determine specific compost types for various biological as well as chemical and physical soil conditions. Researchers are also studying compost not as a single replacement for methyl bromide, but as part of a system of ARD management (Naegely, 2000).

1.4.3 CULTURAL PRACTICES

Without broad-spectrum fumigants, management of ARD is becoming increasingly complex and individual methods of disease control need to be integrated. In this situation cultural practices can provide a practical mean of disease management. Cultural practices affect the severity of several soilborne diseases by either directly acting on the pathogen, or by interfering with the microbiological and environmental factors (Gullino & Mezzalama, 1993). Simple modification of fertilisation, crop rotation or cultivation can have dramatic effects on disease development. Cultural practices such as planting new trees in the aisles of the old orchard seem to lessen the replant effect to some degree. In a field trial by Mazzola in Washington State (Warner, 1999) this approach enhanced tree growth almost as well as fumigating with methyl bromide.

1.4.3.1 Physical soil disturbance

Growers have claimed that soil profile disruption, i.e. cross ripping or ploughing, can greatly minimise ARD in some cases. Soil excavation, where the soil is spread from the tree row to the aisles just after trees are removed, are used in countries with a cold climate where the soil is then subjected to freezing and thawing (Warner, 1999). This

may prove beneficial in controlling certain pathogens such as *Rhizoctonia solani* through reduced inoculum potential. Some anastomosis groups of *R. solani* persists predominantly as mycelium and by disrupting this mycelium 'mat', viability of the fungus is reduced.

1.4.3.2 Crop rotation and cover crops

Crop rotation can in most cases effectively control soilborne diseases, but it is not feasible for control in orchard systems because of the perennial nature of the crop and the high value of land. However, cultivation of alternative crops during orchard renovation has been suggested (Mazzola & Gu, 2000). These cropping systems can promote growth through enhanced nutrient availability, suppression of plant pathogenic nematodes, or by preventing a build up of detrimental microorganisms. In Washington some soils previously cultivated with wheat, foster growth of a beneficial fluorescent pseudomonad bacterium, *Pseudomonas putida*, which protects young apple roots against root pathogens involved in ARD (Mazzola & Gu, 2000). However, although the relative growth response was consistent across multiple replant soils, the magnitude of this growth response varied among different wheat cultivars grown prior to planting. Rape seed, which produces isothiocyanate as a breakdown product, also might have an effect on reducing ARD. Preplant fallow periods have provided limited control of ARD in New York (Merwin & Stiles, 1989).

Marigold (*Tagetes* spp.) is known to suppress various nematode species (Merwin & Stiles, 1989) including *Pr. penetrans* when grown as a cover crop or as a preceding crop. Therefore, in orchards where nematodes pose a problem it may provide an effective alternative to soil fumigation when combined with other management practices.

1.4.3.3 Mono-ammonium phosphate (MAP)

Another proposed way of combating ARD is the application of phosphorous fertiliser (Utkhede & Li, 1989; Neilsen & Yorston, 1991). High rates of MAP incorporated into planting holes have improved tree vigour and precocity of apple trees. This is generally recommended in fumigated soil because mycorrhiza, which improve phosphate uptake in these soils, are usually eliminated in the fumigation process. Adding MAP to the soil also increases the efficacy of pasteurisation (Slykhuis & Li, 1985). However, in many orchards application of MAP is effective even in unfumigated or unsterilised soil.

The most plausible hypothesis concerning this positive role of MAP is that, in soil with replant disease development of mycorrhiza is inhibited. Mycorrhiza is necessary for supplying the roots with phosphorous usually not directly available to the plant. Thus the introduction of available phosphate enables young roots to use it without mycorrhiza. This also explains the efficacy of MAP with different soil sterilisation methods. Addition of MAP also significantly increases the soluble P, Ca, Mg and N-NO_3 content of the soil and lowers its pH (Gur, Luzzati & Katan, 1998). Low pH soils are less heavily infested with pathogens than near-neutral soils (Hoestra, 1968). Thus it is possible that the lower pH can have a detrimental influence on the causal agent of ARD. Soil pH can also influence the antagonistic microflora (Gullino & Mezzalama, 1993).

In some pot experiments conducted by Gur, Luzzati and Katan (1998) MAP was the dominant factor in stimulating growth of apple plants grown in ARD soils. Fumigation without MAP did not result in growth stimulation, whereas addition of MAP to fumigated soil gave no advantage over non-fumigated soil with added MAP. However, the greatest improvement in growth in most orchard soils resulted from treatment with heat or a fumigant and mixing MAP in the soil at rates of 1.5g/L before planting (Slykhuis, 1988). The application of MAP is also compatible with treatments with antagonistic bacterial strains (Utkhede & Li, 1989). Similar but less spectacular responses occur in the orchard. Apple seedlings used for pot experiments are grown under sterile conditions before planting them in soil from aged apple plots, whereas nursery apple plants were always VAM infected, thus providing less of a chance to obtain a P effect.

1.4.3.4 Rootstock selection

According to Isutsa and Merwin (2000) the commonly used dwarfing rootstocks are susceptible to ARD and the more vigorous rootstocks with partial ARD tolerance are not suitable for the preferred high-density plantings. They tested seedling lots and clonal accessions representing 941 genotypes and 19 species or interspecifc hybrids of *Malus* for their resistance or tolerance to ARD in a mixture of New York soils with known replant disease. They concluded that sources of genetic tolerance to ARD exist in *Malus* germplasm collections and could be used in breeding and selecting new clonal rootstocks for improved control of ARD.

1.4.4 PHYSICAL SOIL DISINFESTATION

Physical soil disinfestation can be carried out using different methods. High temperatures have long been used to kill pathogens. Various techniques to heat soil

have been devised but steam, more recently aerated steam (Gullino & Mezzalama, 1993) as well as solarisation (Katan *et al.*, 1976), is the most widely adopted.

1.4.4.1 Steam sterilisation

The traditional approach is to heat the soil with steam at 100°C. The sheet steaming method is the most frequently used until now and requires a temperature of 95°C at a depth of 30cm (Nederpel, 1979; Wilkie, 1997). Steaming alters the chemical composition of the soil to some extent, the most important hazard being the possibility of releasing toxic levels of manganese. This makes it essential to add lime to decrease the accumulation of high concentrations of water-soluble and exchangeable manganese (Dawson *et al.*, 1965).

Aerated steam provides an opportunity to treat soil at lower temperatures (65°C) and thus has several important advantages (Gullino & Mezzalama, 1993). It offers the possibility of eliminating pathogens, without affecting a large part of the resident saprophytic microflora (Baker, 1970). Also, soil treatments at moderate temperatures (50-70°C) avoid toxicity problems associated with treatments at higher temperatures and thus liming might be unnecessary. Although steam sterilisation can cause changes in the nutrient status of the soil, Merwin and Stiles (1989) suggested that the biotic effects of steam sterilisation on soil microflora are probably more important than its abiotic effects on soil nutrients.

Steam is a relatively high cost alternative to fumigation, but more mobile and energy-efficient steam generating equipment is becoming available (Wilkie, 1997). Although this may not be practical to use for extensive orchard sterilisation, it could be of use in nurseries as well as in cases where the whole orchard is not affected.

1.4.4.2 Soil solarisation

Solarisation involves the thermal heating of moistened soil by sunlight under clear plastic mulch to temperatures that are lethal to a broad spectrum of soilborne pathogens, insects and weeds (Katan, 1981). It is generally conducted for three to six weeks in the hottest part of the year and is most effective where there is sufficient sunshine and soil conditions are favourable. The tarps prevent heat losses from the soil caused by evaporation and convection and trap long-wave radiation creating a greenhouse effect (Katan, 1980; Porter & Merriman, 1983).

Although solar heating is similar in principle to that of artificial heating by aerated steam, it is carried out at relatively low temperatures, thus its effects on living and non-living soil components are less severe. The negative side effects observed with soil steaming have not been reported for solarisation, but should not be excluded. Maximal temperatures reached in the mulched soils usually range from 49 to 52°C (Katan, 1980). With increasing soil depths, maximal soil temperatures decrease, but the peaks last longer. These temperatures achieved in the upper soil layers are in the range of those found to be lethal to pathogens (Porter & Merriman, 1983). Thirty minutes at 65°C will kill most of the important plant pathogenic bacteria and fungi as well as insects and weeds.

Although the most pronounced effect is physical, i.e. increasing the soil temperature, continuously accumulating evidence for the involvement of accompanying processes may explain the surprisingly good control and the improved growth response (IGR) achieved even with temperatures not sufficiently high enough to justify such control (Katan, 1987). These accompanying processes include shifts in the microbial population in favour of the beneficial microorganisms and changes in the chemical composition of the soil (increased NO_3, NH_4, Ca, K and soluble organic matter) (Gullino & Mezzalama, 1993). Neutralisation of toxins in the soil may also contribute to IGR (Gullino & Mezzalama, 1993).

Pullman *et al.* (1981) showed that the mycorrhizal fungus *Glomus fasciculatus* survived soil solarisation. This higher thermal tolerance of mycorrhizal fungi increases the potential usefulness of soil solarisation for ARD control as compared to fumigation. Although soil solarisation is most successful in arid and semi-arid areas, which have intense sunshine, few cloudy days and minimal rainfall, it has been used successfully alone or in combination with other control measures under a wide range of conditions. Extension of the mulching period and lack of efficacy against some parasites are regarded as the major limitations of soil solarisation in marginally suitable areas. Recent studies by Pinkerton *et al.* (2000) demonstrated that conditions in the temperate climate of Oregon were adequate for solarisation and provide an additional management alternative to several important soilborne pathogens. The added benefit of weed control make solarisation an attractive alternative or supplement to chemical or biological control of pest and pathogens.

1.5. PREDICTION OF ARD SEVERITY

An essential part of any control programme is to know when it is necessary to take measures. Apple replant disease does not affect all apple soil and where the disease is present, the severity of replant effects varies considerably from site to site (Hoestra, 1968). Soil fumigation prior to planting an orchard is very expensive and may not be necessary because of the uneven incidence of apple replant disease. The condition of the old plantation unfortunately does not offer any insight into the occurrence of the disease in a following planting.

A system for testing soils for their response to fumigation has therefore been developed to determine the severity of ARD and whether fumigation is economically justifiable. Seedling bioassay tests to predict potential replant problems were originally developed on a nation-wide scale in the Netherlands (Hoestra, 1968) to develop recommendations regarding corrective soil amendments prior to replanting apples on old orchard soils. Similar soil-testing services were subsequently adopted in Australia (Cobran, 1970 sited by Gilles, 1974), Belgium (Gilles, 1974), England, Bristish Columbia (Slykhuis & Li, 1985), South Africa (McVeigh, 1987; Van Zyl & Nolte, 1987), and other countries.

The essential principle of this test is to compare the growth of small test plants in pots containing soil from the potential orchard with their growth in pots containing soil free from replant disease either by origin or as a result of soil sterilisation. The percent growth response (R) to soil fumigation or any other soil treatment can be calculated from the formula

$$\% R = 100\ F/U$$

where F and U respectively represent the mean shoot lengths in fumigated and untreated soils. Interpretation of the results follow the recommendations of Hoestra (1968), based on direct comparisons of the results from seedling bioassays and field studies on the growth of apple rootstocks or grafted trees. If the growth response is less than 150% the disease problem is considered slight and would not justify the expense of fumigation. If the growth response is more than 150% replant disease can be expected to be moderate to severe and field fumigation is considered economically beneficial.

In bioassays the absence of VAM fungi in apple seedlings grown in fumigated soil is an artefact that would not normally occur in the field, where trees are planted that have

already become mycorrhizal during several years' previous growth in the nursery (Sewell, Preece & Elsey, 1988). This feature may largely account for the poor agreement between bioassay results and subsequent field observations. Therefore Sewell, Roberts and Elsey (1992) recommended that this method must not be used as a diagnostic tool without addition of phosphorus to compensate for the eradication of VAM fungi by soil sterilisation.

It can be questioned whether bioassays with apple seedlings grown in small containers of soil in a greenhouse can give a reliable indication of benefits or hazards of treatments for trees transplanted in the orchard. Very young seedlings are sensitive and may respond more than an older plant, but since they belong to the same species, their response probably is the best indicator of the needs of the apple tree. To experiment with treatments in the orchard is expensive in time and land and is also complicated by land variability (Slykhuis, 1988) and many interacting factors. Gilles & Bal (1988) showed that for chloropicrin and methyl bromide a good correlation exists between the results of the biotest and those of field trials in 57% of the trials. They also noted that the reliability of the test is much better in cases of moderate to high replant disease (78%). Similar conclusions were made by Neilsen *et al.* (1991). They reported that the bioassay successfully predicted treatments that increased first year shoot growth in 23 out of 30 cases. However, the technique of the bioassay should be refined if possible, in order to further improve reliability.

1.6 CONCLUSION

The etiology of apple replant disease is extremely difficult to investigate because of its complexity due to interactions among various soil organisms and soil parameters. The cause of the disease also seems to vary between different regions as well as orchards within the same region. This site-specific etiology of the disease makes identification of the causal factors even more difficult. Results obtained so far, especially the effects of a wide range of soil sterilisation treatments, suggest that the causal elements of ARD are primarily biological. During apple monoculture the biological soil population is selected by the specific composition of root exudates and other plant residues. It seems that cultivation with apple leads to the dominance of certain pathogenic fungi that affect the development of young apple plants negatively.

Nematodes are not consistently associated with ARD and therefore do not seem to be the main cause, although they undoubtedly contribute to disease severity when they are present. The role of factors such as actinomycetes and allelopathic toxins still remain unclear, and at this stage there is insufficient scientific evidence to abandon their possible role in ARD. Abiotic factors such as unbalanced soil nutrition, together with site-specific problems contribute to additional tree growth problems and therefore remain complicating factors that need to be managed in addition to ARD.

At present, there are few satisfactory alternatives to the long-standing practice of soil fumigation, especially the use of methyl bromide. However, the harmful effect of these broad-spectrum soil fumigants on human health as well as the environment, and in particular the phasing out of methyl bromide necessitates the substitution of soil fumigants by other environmentally more acceptable methods. Although growers have had some success with other chemicals as a short term alternative, use of a diversity of management practices that include less dependence on single-chemical strategies and greater use of biological and cultural management strategies could enhance grower options. Incorporation of biological control organisms into sterilised as well as un-treated soils also show promise for control of a number of soilborne pathogens implicated in ARD development. These biological control organisms can be used as part of an integrated pest management program to target specific problem areas in the field. Many types of biocontrol agents and plant growth promoting microorganisms have been identified over the past few decades and together with management practices such as MAP fertilisation and application of organic material may provide sustainable alternatives for ARD control. In future special emphasis on management of all factors concerning replant, including soil preparation, quality of nursery material, rootstock, time of fumigation and planting, fertilisation, irrigation and weed control will be needed to secure successful new planting on old orchard soils.

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CHAPTER 2

ELUCIDATING THE ETIOLOGY OF APPLE REPLANT DISEASE BY DILUTING APPLE REPLANT SOIL

ABSTRACT

The possible biological origin of ARD etiology in South Africa was investigated by the dilution of replant field soil with fumigated soil. Seven commercial orchards with ARD, located in representative apple growing regions were selected for use in pot trials. Soils were sterilised with methyl bromide and increased portions (25%, 50%, 75% and 100%) added to the original replant soils. Disease severity was evaluated after three months, by measuring shoot length, dry mass of the plants as well as root discolouration. It was clear that seedlings planted into only 25% replant soil (i.e., 75% fumigated soil), consistently exhibited symptoms similar to those grown in 100% replant soil. The elements responsible for stunted growth and root discolouration could, therefore, not be reduced to a level having no negative effect on apple seedlings by dilution of the original ARD soils from 100 to 25%. This indicates that ARD in South Africa is primarily of a biological nature and is a strong argument against abiotic factors as the main cause of ARD.

INTRODUCTION

Apple replant disease (ARD) is the unexplained poor growth of young apple trees, which occurs after replanting on a site that was previously planted to apple. It is mainly characterised by its specificity towards apple and its persistence in soil, irrespective of intervening crops or rest periods, after plants have been removed. ARD does not invariably affect all replanted trees and the severity of replant effects experienced can vary from site to site (Hoestra, 1968; Sewell, 1981). Aboveground symptoms include stunted growth, shortened internodes, rosetted leaves and reduction in tree vigour and productivity. Characteristically, shoot growth terminates earlier than on healthy trees (Traquiar, 1984). Trees affected by the disease start cropping two to three years later than unaffected trees and fail to attain comparable yields. Root systems are typically small with discoloured roots, few functional root hairs and a marked reduction in lateral root development (Savory, 1966; Hoestra, 1986; Mai & Abawi, 1981). Although the disease is not lethal, it has great economic importance due to its lasting effect on yield.

In South Africa serious ARD symptoms occur in approximately 40% of replantings (Honeyborne, 1995). The delayed precocity and production caused by ARD may decrease profitability by as much as 50% throughout the life of the orchard (Rabie, Denman & Cook, 2001). It is becoming an increasingly important problem as producers are forced to replant old orchard soil due to limited virgin soil suitable for apple production.

In spite of extensive research on ARD, the etiology remains to be fully elucidated (Traquiar, 1984). Causal factors vary across geographic regions or even between orchards in the same region. It is a complex problem, for which no single factor can be found responsible. Biotic or abiotic factors acting individually or synergistically may be involved. In the past, researchers have linked the poor performance of replanted fruit trees to abiotic factors including unbalanced or inadequate nutrient availability, low or high soil pH, toxic residues in the soil and impaired soil structure (Mai & Abawi, 1981; Traquair, 1984). However, the dramatic growth improvement on ARD soils with a range of soil disinfecting treatments indicates that the causal elements are primarily biological (Savory, 1966; Mazzola, 1998). Furthermore, other fruit trees planted on ARD sites typically grow normal. Thus, the specificity of ARD is another strong counter-argument against abiotic factors as the main causal elements of ARD.

Numerous soilborne organisms have been implicated as being potential causal factors, as well as allelopathic relationships between plants, microorganisms of the rhizosphere and soil. Plant parasitic nematodes have been reported to have a major role in ARD (Mai & Abawi, 1981; Utkhede, Smith & Palmer, 1992; Dullahide *et al.*, 1994). It is however, generally accepted that nematodes are not the primary cause of ARD, although they remain a complicating factor in the causal complex. Several investigations also point to parasitic fungi as primary causal agents (Sewell, 1981), particularly a complex of pathogenic fungi (Braun, 1995; Mazzola, 1998). Recent findings in Washington State clearly demonstrate that a complex of the fungal genera *viz*, *Rhizoctonia, Cylindrocarpon, Pythium* and *Phytophthora*, are the dominant causal agents and to a varying degree play a significant role in the etiology of ARD in this state. Furthermore, soil bacteria, mainly the fluorescent pseudomonads and actinomycetes have been implicated by Hoestra (1968), Savory (1966) and Westcott, Beer & Stiles (1986).

As an initial step to study ARD etiology in South Africa, we assessed the effect of diluting ARD soil with sterile soil so that the status of the role microorganisms play in ARD in

South Africa, could be determined. The investigation also partly served as an ARD bioassay to determine the presence of ARD in the various replant soils used.

MATERIALS AND METHODS

Soils used

Due to the variability of the replant effect experienced in different soils (Savory, 1966; Hoestra, 1968; Sewell, 1981; Mazzola, 1998), a number of commercial orchards with ARD located in representative apple growing regions of South Africa (Grabouw/Elgin and Vyeboom regions) were identified to ensure representatives of different soil types, and seven soils were selected for this study. Selection was mainly based on standard ARD bioassays conducted for growers by ARC Infruitec-Nietvoorbij to predict replant disease potential in orchard soil (McVeigh, 1987; Van Zyl & Nolte, 1987). This bioassay is a modification of the one used in Europe (Hoestra, 1968; Gilles, 1974) that has been shown to reliably predict ARD in orchards (Gilles & Bal, 1988; Neilsen *et al.*, 1991). Soils were also collected from orchards exhibiting severe replant disease symptoms, and these soils were selected through consultation with technical advisers and growers. These sites had been in continuous apple production for between 8 to 40 years, and at sampling time included mature bearing, recently fallowed as well as newly replanted orchards.

In taking soil samples, vegetation was scraped off the soil surface and soil collected within the root zone at a depth of 10-30 cm from twelve randomly selected sites within each of the affected areas in the seven orchards. Composite soil samples were prepared by mixing the soil from the twelve sub-samples thoroughly for each soil. Samples were stored in 50kg plastic bags at 4°C and used as needed. Sub-samples of all soils were analysed for chemical and physical soil properties according to standard ARC Infruitec-Nietvoorbij procedures (Appendix 1 and 2 respectively) (Kotzé, 2001). Where nutrient deficiencies occurred, soils were fertilised according to standard industry recommendations (Kotzé, 2001) and then used in pot trials.

Plant material

Seeds were collected from open pollinated 'Golden Delicious' apples, surface disinfested in 1% sodium hypochlorite (NaOCl) for five minutes, treated with a mixture of captan (50% a.i., WP) and thiram (75% a.i., WP) broadspectrum fungicides and stratified at 4°C under moist conditions. Sprouted seeds were selected for uniformity and sown into seedling trays containing sterile perlite and peat moss (1:1). Three-week-old seedlings were transplanted, one per pot, into 10cm deep, 500mL plastic pots containing the ARD

field soils and dilutions thereof with sterile soil. Seedlings were watered daily with municipal water during summer and every second or third day during colder months. A commercial multi-nutrient was applied every two weeks providing essential macro- and micro-nutrients. Plants were grown under shade net during summer and in a greenhouse without artificial lighting during winter. Temperature in the greenhouse ranged from 12°C at night to 28°C during the day. When necessary, powdery mildew (*Podosphaera leucotricha* Ellis & Everh.) and aphids were controlled with buprimate (Nimrod)(233g a.i./L) systemic fungicide and chlorpiriphos (Dursban)(480g a.i/L) insecticide sprays applied at the recommended rates. Weeds were removed soon after emergence to avoid competition.

Plants were harvested 3-4 months after transplanting. Roots were washed gently under running tap water and blotted dry. Shoots and leaves were then separated from roots and their fresh masses recorded separately. Roots were also rated visually on discolouration, as a further indicator of ARD severity. Roots were rated on a scale from 1-3, where 1 = white, healthy roots and 3 = severely discoloured, necrotic roots (Figure 1). Plant material was placed in paper bags and dried at 60°C to record dry mass.

Experimental design and treatments

The trial was laid out in a randomised complete split-plot design with seven block replicates and one seedling per pot as the experimental unit. The main plot treatments consisted of seven ARD soils. Subplot treatments consisted of the different ratio's of replant soils.

A portion of each of the seven soils was sterilised using the standard ARC Infruitec-Nietvoorbij fumigation procedure for ARD bioassays (Van Zyl & Nolte, 1987). Soils were fumigated through pressure injection (probing) with methyl bromide in 25L plastic containers, sealed immediately for 4-6 days to ensure effective fumigation in all soil samples and then opened and spread for aeration. By mixing the soil daily for at least two weeks before planting, toxicity was avoided. Increased portions of sterilised soil (25%, 50%, 75% and 100% v/v) were then added to the replant soils and mixed thoroughly. To establish the degree of ARD present in the seven soils evaluated and confirm the variable nature of ARD, data from seedlings planted in original ARD soil (control) and seedlings planted in 100% sterilised soil were used to calculate the percentage growth response of apple seedlings to methyl bromide in these soils respectively.

Statistical analysis

Recognising the variable and site-specific etiology of ARD (Hoestra, 1968; Mai & Abawi, 1981; Sewell, 1981; Mazzola, 1998), averages over the seven soils were taken for the various parameters measured and an analysis of variance performed on the data using the General Linear Models (GLM) procedure of the Statistical Analyses System (SAS) V8.11 Statistical Software (SAS, 1990). Student's t-LSD (least significant difference, $P \leq 0.05$) was calculated at a 5% significance level to compare the treatment means. Single degree of freedom polynomial contrasts were fitted to test for linear or quadratic trends.

RESULTS AND DISCUSSION

Indication of apple replant disease

The percentage growth response of apple seedlings to soil sterilisation with methyl bromide varied from 153-310% in the seven soils evaluated (Table 1). This is in agreement with the variable nature of ARD severity (Savory 1966; Hoestra, 1968; Sewell, 1981, Mazzola, 1998). These results indicate that all the soils tested had moderate to severe ARD (Hoestra, 1968) and could be used for further studies on ARD.

Effect of dilution of replant soil

Diluting replant soil to 75% had no significant effect on any of the growth parameters measured (Table 2). However, diluting the original replant soil to 50% and 25% as well as planting seedlings in 0% replant soil, increased shoot length as well as shoot dry mass significantly when compared to 100% replant soil. When no replant soil was present (i.e., 100% fumigated soil), shoot length and shoot mass values were significantly higher in comparison to all the other treatments. Similar results were obtained for total dry mass measurements. However, the effect was more pronounced, with 0% replant soil doubling the total dry mass of the plant compared to seedlings planted in 100% replant soil. With root dry mass evaluation, significant differences from the control occurred only when no replant soil was present. Visual inspection of roots indicated that seedlings planted in any mixture containing replant soil, even only 25% replant soil, consistently showed pronounced root discolouration, rating 2 and 3, in comparison with seedlings planted in 100% fumigated soil rating 1 (Figure 1). Therefore, although diluting replant soil to 25% and 50% significantly reduced the effect of ARD, symptoms were only absent in the 0% replant soil. A linear response fit the shoot, root as well as total dry mass data (Table 2), which shows that there is a negative effect on growth as soon as replant soil is added to the fumigated soil. Results are in agreement with that of Hoestra (1968) who observed moderately strong growth reduction when 10% of ARD infested soil was mixed with ARD

free soil. Jaffee, Abawi and Mai (1982) also found that the factor responsible for stunting and root discolouration could not be reduced to a less damaging level by dilution of the ARD field soil.

To conclude, in all soils as well as all parameters measured, growth was significantly better in 100% fumigated soil compared to all other treatments. Furthermore, it was clear that seedlings planted into only 25% replant soil (i.e. 75% fumigated soil), continued to exhibit symptoms similar to those occurring in 100% replant soil. The elements responsible for stunting and root discolouration could not be reduced through soil dilution to a level that did not damage apple seedlings. This indicates that ARD in South Africa is primarily of a biological nature and is a strong argument against abiotic factors as the main cause of ARD. Although abiotic elements may contribute to additional tree growth problems and pronounced disease expression, they are merely non-specific complicating factors that need to be managed in addition to ARD.

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TABLE 1. Growth response to methyl bromide fumigation of seven orchard soils tested for apple replant disease (ARD).

Orchard soil no.	Shoot length (mm) in unfumigated soil	Shoot length (mm) in fumigated soil	Growth response (%R)[a]	ARD test result[b]
1	48	149	310	Severe
2	108	165	153	Moderate
3	72	114	158	Moderate
4	49	105	214	Severe
5	72	110	153	Moderate
6	41	108	263	Severe
7	94	146	155	Moderate

[a] $\%R = \frac{\text{Shoot length in fumigated soil}}{\text{Shoot length in unfumigated soil}} \times 100$

[b] ARD test result = Severe %R > 200
Moderate 200 > %R > 150
Slight %R < 150

TABLE 2. Effect of adding increased ratios of fumigated soil to seven apple replant disease soils on mean growth of apple seedlings planted in these ARD soils.

Treatments[a]	Mean[b] shoot length (mm)	Dry shoot mass (g)	Dry root mass (g)	Total dry mass (g)
Replant soil 0%	126.91 a	1.59 a	1.72 a	3.31 a
Replant soil 25%	101.90 b	1.10 b	1.12 b	2.23 b
Replant soil 50%	99.95 bc	1.05 bc	1.09 b	2.13 b
Replant soil 75%	85.59 cd	0.87 cd	0.98 b	1.85 bc
Replant soil 100% (Control)	72.17 d	0.78 d	0.86 b	1.64 c
LSD[c]	*15.43*	*0.18*	*0.31*	*0.43*
	df	Significance (Pr>F)		
Treatment	4	<.0001	<.0001	<.0001	<.0001
Linear	1	<.0001	<.0001	<.0001	<.0001
Quadratic	1	0.5908	0.0097	0.0354	0.0095

Means in a column followed by the same letter are not significantly (P = 0.05) different

[a] % volume:volume

[b] means were calculated from seven blocks and seven soils

[c] Student's t-LSD (least significant difference) was calculated at a 5% significance level

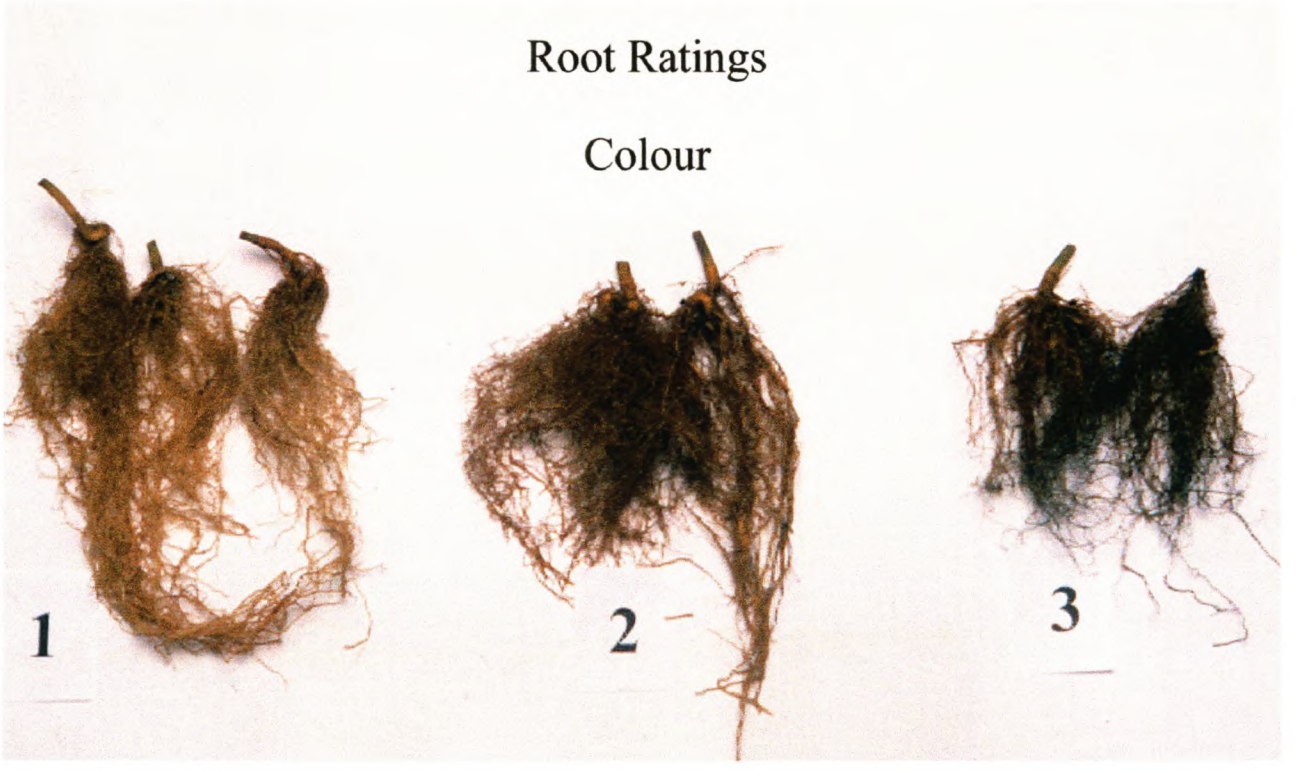

FIGURE 1. Rating system used to assess discolouration caused by apple replant disease. Roots were rated on a scale from 1 to 3, where 1 = white, healthy roots and 3 = severely discoloured, necrotic roots.

CHAPTER 3

EVALUATION OF BIOLOGICAL METHODS TO CONTROL APPLE REPLANT DISEASE USING A POT TEST

ABSTRACT

Apple replant disease (ARD) is one of the major impediments to the establishment of an economically viable apple orchard on sites previously planted to apple. In South Africa serious ARD symptoms occur in approximately 40% of replantings. As an initial step in formulating sustainable disease control alternatives to replace methyl bromide, pot trials were conducted to assess the impact of compost treatments as well as biological control products on ARD severity. The application of compost as well as sterilised and unsterilised compost teas to replant soils significantly increased growth, even under optimum nutrient conditions. Results also indicated that applying high concentrations of compost does not necessarily provide additional growth benefits compared to lower concentrations. Results with biocontrol formulations were less favourable. Only one of the biocontrol formulations, a combination of *Bacillus* spp. (Biostart®), improved growth significantly compared to the control, but there was some inconsistency with results for the different trials conducted using this product. *Pythium* and *Cylindrocarpon* spp. were consistently isolated from all six replant soils in all four trials that formed part of this study, indicating that these fungi may have a role in ARD etiology in South Africa. Furthermore, nematodes implicated in ARD were inconsistently associated with the ARD soils tested, indicating that nematodes are not the primary causal factor in ARD etiology locally.

INTRODUCTION

Apple replant disease (ARD) is the unexplained poor growth of young apple trees, which occurs after replanting on a site that was previously planted to apple. It is mainly characterised by its specificity towards apple and its persistence in soil after plants have been removed. ARD does not invariably affect all replanted trees and where the disease is present, the severity of replant effects can vary from site to site (Hoestra, 1968; Mazzola, 1998). Aboveground symptoms include reduction in tree vigour and yield (Traquiar, 1984) and affected trees start cropping fruit 2 to 3 years later than unaffected trees. Root systems are typically small with discoloured roots and few functional root hairs (Savory, 1966; Hoestra, 1968; Jaffee, Abawi & Mai, 1982a). In South Africa serious ARD symptoms occur in approximately 40% of replantings (Honeyborne, 1995). The

delayed precocity and production caused by ARD may decrease profitability by as much as 50% throughout the life of the orchard (Rabie, Denman & Cook, 2001). The problem is intensified as suitable land, not previously planted to apple becomes limited in South Africa.

In spite of extensive research on ARD, the etiology still needs to be fully elucidated. Causal factors vary across geographic regions or even between orchards in the same region. Biotic or abiotic factors acting individually or synergistically may be involved. In the past, researchers have linked ARD to abiotic factors such as inadequate nutrient availability or toxic residues in the soil (Mai & Abawi, 1981; Traquair, 1984). However, the dramatic growth improvement on replant soils with a range of soil sterilisation treatments indicates that the causal elements are primarily biological (Savory, 1966; Mazzola, 1998). Numerous soilborne organisms including plant parasitic nematodes (Mai & Abawi, 1981; Utkhede, Smith & Palmer, 1992; Dullahide *et al.*, 1994), pathogenic fungi (Sewell, 1981; Braun, 1995; Mazzola, 1998), actinomycetes (Westcott, Beer & Stiles, 1986) and bacteria (Savory, 1966; Hoestra, 1968) have been implicated as being potential causal factors, as well as allelopathic relationships between plants, microorganisms of the rhizosphere and soil. In Chapter 2 the microbial origin of ARD etiology in South Africa was confirmed through inability to eliminate ARD symptoms by dilution of replant soil with sterilised soil, which was in agreement with conclusions from Hoestra (1968) and Jaffee, Abawi and Mai (1982a).

Progress towards the control of ARD has been hampered by difficulties in recognising the primary causal agent(s). At present, there are few satisfactory alternatives to the long-standing practice of soil fumigation with methyl bromide (Mai & Abawi, 1981). However, this chemical was declared an ozone depleting substance and its imminent phase-out to comply with the Montreal Protocol has intensified the need for alternative measures to control ARD (WMO, 1994). The high cost of chemical control and its potential hazard to human health and the environment make it essential to develop more sustainable means of control. The disease-suppressive effects of compost have been investigated intensively over the past two decades and due to the biological nature of ARD etiology, compost may also have a role in controlling ARD (De Ceuster & Hoitink, 1999; Naegely, 2000). The concept of inoculating soils and plants with beneficial microorganisms such as *Bacillus subtilis* (Utkhede & Smith, 2000), *fluorescens-putida* type *Pseudomonas* (Biro *et al.,* 1996; Mazzola & Gu, 2000) and effective microorganisms (EM) (Higa, 1998) to create a more favourable microbiological environment for plant growth has also shown promise (Baker & Cook, 1974; Catska *et al.*, 1982). The beneficial influences of these organisms on plants include, increased efficiency of organic materials as fertilisers due to nutrient

release from rapid decomposition of organic matter, better plant establishment, enhanced photosynthetic capacity of crops, improved physical and biological environments in the soil and suppression of soilborne pathogens and pests through increased competitive and antagonistic abilities of microorganisms (Catska, 1993; Parr, Hornick & Papendick, 1998).

The objective of this study was to assess the impact of compost treatments as well as other biological amendments on ARD severity as an initial step in formulating sustainable disease control alternatives to methyl bromide. In addition, the fungal populations associated with ARD soils were characterised and the impact of the soil amendments on these fungal populations evaluated.

MATERIALS AND METHODS

Soils used

Due to the variability of the replant effect experienced in different soils (Savory, 1966; Hoestra, 1968; Sewell, 1981), soils from ten commercial orchards with ARD located in apple growing regions of South Africa (Grabouw/Elgin and Vyeboom) were selected for this study to ensure representatives of different soil types. Selection of seven of the ten orchards was based on standard ARD bioassays conducted for growers by ARC Infruitec-Nietvoorbij to predict replant disease in orchard soil (McVeigh, 1987; Van Zyl & Nolte, 1987). This bioassay is a modification of the one used in Europe (Hoestra, 1968; Gilles, 1974) that has been shown to reliably predict ARD in orchards (Gilles & Bal, 1988; Neilsen *et al.,* 1991). The other three orchards used showed severe replant disease symptoms and were selected through consultation with technical advisers and growers. These sites had been in continuous apple production for 8 to 40 years, and at sampling time included mature bearing, recently fallowed as well as newly replanted orchards. Initially soils from all ten ARD orchards were used. Subsequently soils from the six orchards showing most severe ARD symptoms were selected for further experiments (Appendix 1).

To minimise the effect of long-term cold storage on soilborne inoculum, soil samples were collected on four occasions: May 1999, December 1999, April 2000 and February 2001. In taking samples vegetation was scraped off the soil surface and soil collected within the root zone at a depth of 10-30 cm from twelve randomly selected sites within each of the ten ARD affected areas. Composite soil samples were then prepared by mixing the twelve sub-samples thoroughly for each soil. Samples were stored in 50kg plastic bags at 4°C in the dark for no more than six months and used as needed. Sub-samples of all

soils were analysed for chemical and physical soil properties according to standard ARC Infruitec-Nietvoorbij procedure (Kotzé, 2001) (Appendix 1 and 2 respectively). Where nutrient deficiencies occurred, soils were fertilised according to standard industry fertiliser recommendations (Kotzé, 2001) and then used in pot trials. Populations of plant parasitic nematodes were determined from soil and root samples of the six most severely affected ARD soils, using the standard procedure of the ARC Infruitec-Nietvoorbij laboratory (Hugo, 1984).

Plant material

The same procedure was used for all pot trials conducted in this study. Seeds were collected from open pollinated 'Golden Delicious' apples, surface disinfested in 1% sodium hypochlorite (NaOCl) for five minutes, treated with a mixture of captan (50% a.i., WP) and thiram (75% a.i., WP) broad-spectrum fungicides and stratified at 4°C under moist conditions. Sprouted seeds were selected for uniformity and sown into seedling trays containing sterile perlite and peat moss (1:1). Three-week-old seedlings were transplanted one per pot into 10cm deep, 500mL plastic pots containing the treated and untreated ARD soils. Seedlings were watered daily with municipal water during summer and every second or third day during colder months. A commercial multi-nutrient solution was applied every two weeks providing essential macro- and micro-nutrients. Plants were grown under shade net during summer and in a greenhouse without artificial lighting during winter. Temperature in the greenhouse ranged from 12°C at night to 28°C during the day. When necessary, powdery mildew (*Podosphaeria leucotricha* Ellis & Everh.) and aphids were controlled with buprimate (Nimrod)(233g a.i./L) systemic fungicide and chlorpiriphos (Dursban)(480g a.i/L) insecticide sprays applied at the recommended rates. Weeds were removed soon after emergence to avoid competition.

Plants were harvested 3-4 months after transplanting. Seedlings were removed from the pots and the soil from each soil x treatment combination was bulked, mixed well and stored at 4°C for microbial studies. Roots were washed carefully under running tap water and blotted dry. Seedling length was measured, the shoots and leaves were then separated from roots and their fresh masses recorded separately. The plant material was placed in paper bags and dried at 60°C for dry mass measurements.

Treatments

Compost trials

In the first compost trial compost was added to soils at five concentrations (0%, 12.5%, 25%, 37.5% and 50% v/v). The compost was mixed thoroughly into the ARD soils and seedlings planted as described above. The compost used was fully aerobically produced

and consisted of wheat straw (70%), chicken manure (10%) and cow manure (20%) and was inoculated with effective microorganisms (EM). The EM mixture is a cocktail of beneficial microorganisms consisting primarily of photosynthetic and lactic acid bacteria as well as yeasts, actinomycetes and fermenting fungi (Higa, 1994).

In the second compost trial only a low concentration of compost (10% v/v) was used and treatments with sterilised or unsterilised compost extract (tea) added, respectively. Compost tea is a liquid extract of compost that was prepared by mixing 2L compost with 15L municipal tap water and allowing it to stand for 16h. The liquid was then separated from the solid compost (unsterilised compost tea) and auotclaved at 120°C for 15 min as required to make sterilised compost tea. Plants were watered twice a week with freshly made compost teas. Osmocote plus (31), a 3-4 month controlled release fertiliser, was applied on its own as a treatment as well as to all of the compost treatments, to establish the effects of compost under optimum nutrient conditions. Osmocote contains 15% N, 4% P, 10% K and 1.2% Mg, as well as all the essential micro-elements. The same trial was repeated in the following year (2001), using new compost.

Biocontrol trials

Two trials were conducted. In the first trial soils were amended with one of four commercial biocontrol products. Biostart® (Microbial Solutions (Pty) Ltd.), a liquid microbial soil inoculant consisting of three species of bacteria, *Bacillus laterosporus, B. chitinosporus* and *B. licheniformis* was applied as a soil drench, with 40mL of the solution per pot, containing 10^9 colony forming units (CFUs) and 1g of carbon-based Microboost® activator. Control plants were drenched with pure water. The Biostart® was applied every week for the first month and then every second week over the next two months. Rootshield® (Microbial Solutions (Pty) Ltd.), which is a granular microbial soil inoculant consisting of 1.15% *Trichoderma harzianum* (Rifai strain KRL-AG2) and 98.85% inert ingredients, was also tested. It was applied once off as a soil drench at planting at the recommended rate of 150g/170L. An organic product comprised of different endomycorrhizal fungi and being traded under the name Biocult®, was applied at planting by adding 10mL of the formulation to the planting hole. Another commercial product consisting of effective microorganisms (EM) was also included. A diluted suspension (1:1000) of stock EM was prepared and applied as a soil drench at 40mL per pot twice a week. Finally, a combination of Biocult® and EM was applied at the same concentrations as described above.

In the second trial a single product, Biostart®, was tested at different application rates. The concentrations tested were the recommended concentration (used in the first biocontrol trial) and 50%, 25%, 12.5% and 6.25% of the recommended concentration.

Two additional treatments where Osmocote was applied on its own, as well as in combination with the full Biostart® concentration were also included in this trial. At planting, the soil was drenched with the various concentrations and control plants were drenched with pure water. The frequency of applications was the same as for the previous trial. The trial was repeated, again containing only the recommended Biostart® concentration as well as the Osmocote treatments.

Experimental design

All trials were laid out as randomised complete split-plot designs with either five or seven block replicates, depending on the number of treatments, with one seedling per pot as the experimental unit. The main plot treatments consisted of six or ten ARD soils. For the initial compost and Biostart® trial all ten soils were used and for the other trials only the six selected soils were used. Subplot treatments consisted of the various soil amendments.

Characterisation of soil and rhizosphere microbial communities

Fungi from plant roots

The composition of fungal populations in the rhizosphere of apple seedlings grown in original or treated ARD orchard soils was only determined in the compost and Biostart® trials. Five seedlings were randomly selected from each soil x treatment combination, their roots were washed and surface sterilised in 1% NaOCl for two minutes and then dipped in 70% ethanol for 30 seconds and rinsed in distilled water for two minutes. Roots were allowed to air dry and four root segments from each seedling were plated onto the following media: potato dextrose agar (PDA; Difco) amended with streptomycin; water agar (Difco); a selective medium for *Phytophthora* (PH) (Solel & Pinkas, 1984) and a selective medium for *Pythium* (P) (the same as the PH medium, but without adding hymexazol). After incubation at 21°C on a laboratory bench for four days root segments were examined and hyphae emanating from these tissue were subcultured to divided plates containing PDA in one half of the dish and carnation leaf agar in the other (Fisher *et al.*, 1982). Fungi were identified to generic levels by microscopic examination and the frequency of isolation was recorded.

Fungi from soil

Fungal populations were estimated using the soil dilution plate technique (Ali-Shtayeh, Ho & Dick, 1986). Soil suspensions were prepared from the bulked soil for each soil-treatment according to the method described by Swart and Denman (2000) and five 1mL aliquots of each dilution were plated onto P and PH selective media. The number of

CFUs were counted after 3–6 days. Only *Pythium* and *Phytophthora* populations in soil were assessed due to a lack of success with other selective media and fungal genera.

Statistical analysis

Recognising the variable and site-specific etiology of ARD (Hoestra, 1968; Mai & Abawi, 1981; Sewell, 1981; Mazzola, 1998), averages over the six or ten soils were taken for the various parameters measured and an analysis of variance performed on the data using the General Linear Models (GLM) procedure of the Statistical Analyses System (SAS) V8.11 Statistical Software (SAS, 1990). Student's t-LSD (least significant difference, $P \leq 0.05$) was calculated at a 5% significance level to compare the treatment means. Single degree of freedom polynomial contrasts were fitted to test for linear or quadratic trends where different concentrations of compost were used.

RESULTS AND DISCUSSION

Plant parasitic nematodes associated with ARD soil

In this study, plant parasitic nematodes extracted from soil collected at the six ARD orchards consisted primarily of the genera *Pratylenchus* and *Xiphenema*. In general, soil populations of *Pratylenchus* were either absent or low (Table 1) and in only one soil did it exceed 100 per gram of root sample. Populations of *Xiphenema* were high in two of the orchard soils surveyed, 84 and 200 per $100cm^3$ respectively (Table 1), but this species is not commonly related to ARD. Although nematodes were not identified to species level, according to Hugo (1984) the most common *Pratylenchus* sp. in the South African apple-growing region is *P. flakkensis* Seinhorst. *P. penetrans* (Cobb) Filipjev & Schuurmans-Stekhoven is less common.

Some investigators have concluded that the root lesion nematode, *P. penetrans* has an important role in ARD etiology (Jaffee, Abawi & Mai, 1982a; Merwin & Stiles, 1989; Utkhede Smith & Palmer, 1992; Dullahide *et al.,* 1994). However, nematodes implicated in ARD were inconsistently associated with the ARD soils used in our studies and this indicates that nematodes do not have a causal role in ARD etiology in South Africa. Similar conclusions have been reached by Covey, Benson and Haglund (1979) and Mazzola (1998) who demonstrated that *P. penetrans* has a minor, if any role in the etiology of ARD in Washington State, USA, and were frequently absent from replant soils (Savory, 1966; Hoestra, 1968; Mazzola, 1998). The argument against the role of nematodes as primary causal agents of ARD is furthered by the fact that when nematodes were effectively eliminated from soil, growth of apple trees was still not improved (Hoestra, 1968; Covey, Benson & Haglund, 1979; Mazzola, 1998).

Furthermore, nematode counts from healthy soils often exceed those from ARD soils (Caruso, Neubauer & Begin, 1989). However, nematodes undoubtedly contribute to disease severity when they are present in ARD soils.

Effect of biological soil amendments on growth

Compost trials

In the first trial, for shoot length as well as shoot and total mass, compared to the control growth was significantly increased by all four concentrations of compost (Table 2). However, for root mass, only the lower concentrations (12.5%, 25% and 37.5%) increased growth significantly. Shoot parameters measured was significantly increased by the 25% and 37.5% treatments, but not by the 50% compost when compared to the 12.5% treatment. Root- and total mass, were highest at the three lower concentrations of compost and applying 25% or 37.5% did not result in an additional growth increase compared to the 12.5% treatment. The more favourable effect of higher compost concentrations on shoot growth compared to root growth can probably be attributed to an increase in nitrogen with the higher concentrations of compost, stimulating shoot growth more than root growth. In general growth was slightly retarded at the highest concentration of compost. At the two highest compost ratio's, survival of seedlings was significantly lower than at the lower ratio's (Figure 2). Visual inspection of roots indicated that this was probably due to root burn. A quadratic response fit the root and the total mass data, while both linear and quadratic responses fit the shoot data (Table 2). This indicates that application of more compost is not necessarily better and too much compost can be detrimental.

The second compost trial was replicated in two consecutive years (2000 and 2001) to confirm results. Although results were similar for the two trials, overall growth in the 2001 trial was much less in comparison with the first trial. The growth difference between the two years could be due to the different batches of compost used or different growth conditions prevailing as the trials were carried out at different times of the year. This demonstrates the importance of standardisation in order to obtain consistent results with compost. Compost quality needs to be controlled to eliminate this as a variable and ensure consistent performance. In both trials all amendments significantly increased all growth parameters compared to the control (Table 3). In general the Osmocote applied in combination with compost or sterilised or unsterilised compost tea resulted in more growth than with the Osmocote alone. The compost and the teas therefore had an additional effect to supplying nutrients, as soils were at optimum nutrient levels because of the Osmocote added. Furthermore, sterilised compost tea provided a growth response equivalent to that obtained with the unsterilised tea. This suggests that the compost tea

could function through microbe produced metabolites extracted from the compost, growth promotion, either directly or indirectly through alteration of the soil nutritional status, or an altered population or activity of beneficial or damaging organisms.

In 2001 there was some variation in treatment performance for the various growth parameters measured (Table 3). When looking at root- and total mass, compost and unsterilised tea in combination with Osmocote, increased growth significantly compared to Osmocote on its own. For shoot mass, only compost with Osmocote increased growth significantly compared to Osmocote alone and for shoot length no additional growth increase to Osmocote alone was recorded in any of the treatments.

Our results with compost treatments are in contrast to results from pot trials reported by Daemen (1995), which indicated that compost gave insufficient protection of apple seedlings against ARD. However, application of humus at dosages of 10-20% as well as peat and decomposted bark was very effective in experiments done by Szczygiel and Zepp (1998), suggesting a role for organic amendments in ARD control. Furthermore, it has to be noted that in this study, compost was combined with Osmocote. Although compost had a significant additional effect to Osmocote on most growth parameters measured in the two years, results with Osmocote applied on its own indicates that nutrition needs to be considered when formulating ARD control measures. Furthermore, composts used in these trials were inoculated with EM. Although Hoitink, Stone and Hun (1997) maintained that controlled inoculation of composts with biocontrol agents is a procedure that can induce consistent levels of disease suppression on a commercial scale, Van Dyk, Cronje and Wehner, (2001) argue that few composts are universally effective. It is therefore necessary to determine which microbial mixtures provide effective disease suppression in specific soil environments and then to formulate and apply these various compost types for use in the appropriate environments.

Biocontrol trials

Only one of the biocontrol formulations, Biostart® improved growth significantly, more or less doubling seedling length, root mass as well as total mass compared to the control and the other biocontrol treatments (Table 4). Rootshield®, Biocult® and EM had no significant effect on growth, shoot length or plant mass. It was therefore decided to continue further experiments only with the Biostart®.

A second trial assessing the effect of lower concentrations of Biostart® in combination with Osmocote fertiliser was conducted in 2000 and repeated in 2001. All growth parameters measured in the first year (2000) were significantly increased by Osmocote,

the recommended concentration of Biostart®, the combination of the two as well as half the recommended concentration of Biostart® (Table 5). Significantly more growth was obtained using the recommended dose compared to that obtained with 50% of the recommended dose. None of the lower concentrations (25%, 12,5% and 6.25% Biostart®) had a significant effect on growth. In 2000, shoot growth parameters and total mass were highest in seedlings treated with Osmocote in combination with Biostart®, and Osmocote on its own and there were no significant differences in growth between these two treatments. This may suggest that the negative impact of the causal organism(s), as they affect root hairs and the fine root system, primarily act through the inability of the plant to attain sufficient mineral nutrition due to a dysfunctional root system. When looking only at root mass, both Biostart® and Osmocote on their own as well as in combination, significantly increased growth compared with the controls. Plants treated with Osmocote only, had significantly higher root mass than those treated with the combination of Biostart® and Osmocote, or Biostart® on its own, the latter being only the third best treatment (Table 5). Biostart® also gave a less substantial shoot and root growth increase when applied alone, than when combined with Osmocote.

In contrast with the positive results of the 2000 trial, in 2001 for all growth parameters measured, application of Biostart® on its own had a negative effect on shoot growth parameters and total dry mass even when compared to the control (Table 6). However, Osmocote on its own and Biostart® in combination with Osmocote still increased all growth parameters significantly and to the same extent as each other. It was concluded that in the 2000 trial Osmocote was the main contributor to increased growth. Furthermore, overall growth of all plants was lower in the 2001 trial, as was found for the 2001 compost trial (Table 3). These two trials were conducted simultaneously, under similar growth conditions and from soil samples collected at the same time. It is therefore possible that there was some factor inherent to the soil samples used in 2001 or prevailing environmental conditions during this season that affected growth negatively in the 2001 trials. Utkhede and Smith (2000) noted that control with biocontrol agents was strongly dependent on environmental conditions. Furthermore, many quality factors have to be standardised and mechanisms of control determined to obtain consistent effects with these biocontrol products.

Effect of soil amendments on fungal populations

Compost trials

From plants

Fungi consistently isolated from lesions on apple roots from all six ARD soils, consisted primarily of a complex of *Cylindrocarpon, Fusarium* and *Pythium* spp. Among the

Fusarium species identified, *F. oxysporum* was the most prevalent and secondly *F. solani*. The remaining Fusarium species were not identified to species level since their occurrence was sporadic and low numbers were isolated. Mazzola (1998) found that isolates of *Fusarium* were not pathogenic or only weakly virulent to apple seedlings. Utkhede, Smith and Palmer (1992) also found that *F. solani* and *F. oxysporum* did not affect seedling growth when added to sterilised soil. Therefore, attention was focussed on *Cylindrocarpon* and *Pythium* spp. which have been reported to have a role in ARD etiology (Sewell, 1981; Jaffee, Abawi & Mai, 1982b; Merwin & Stiles, 1989; Dullahide *et al.*, 1994; Braun, 1995). In general the dominance of the two genera varied over the two consecutive years that the trial was conducted. *Cylindrocarpon* infection was high in 2000 and low in 2001, in contrast to this *Pythium* infection was higher in 2001 and lower in 2000 (Table 7). This was reflected in seedling growth where there was a marked reduction in overall growth in 2001 (Table 3) since the growth retarding effects *Pythium* spp. have on apple seedlings and similar effects have been documented (Sewell, 1981; Braun, 1995).

In both years, the incidence of *Cylindrocarpon* infection of plants was not affected by any of the treatments (Table 7). In 2000 the percentage *Pythium* isolated from seedlings was also unaffected by any of the treatments except for sterilised tea where no *Pythium* was isolated. However, the following year (2001) in the repeat of the trial, there was an increase in the incidence of *Pythium* isolated from roots treated with compost plus Osmocote or unsterilised compost tea plus Osmocote. In spite of the increased incidence of *Pythium* there was still an increase in growth of plants in these treatments relative to the controls (Table 3). It is therefore concluded that the treatments either did not affect the fungal populations and their infecting ability or in some cases increased them, but did not exert a reducing effect on growth. High percentages of *Cylindrocarpon* infection together with increased *Pythium* populations were not reflected in growth measurements from compost treated plants. It therefore seems that in some cases the application of compost had an overriding effect on pathogens. However, this hypothesis needs to be confirmed.

From soil

No *Phytophthora* was isolated from the ARD soils, although it was isolated from the plants occasionally. There were significant differences between the two trials in the number of CFUs of *Pythium* in the soil (Table 8). In 2000, all treatments increased *Pythium* counts in the soil significantly and there were significantly higher numbers of CFUs with the compost in combination with Osmocote compared to the other treatments. However, in the repeated trial no significant differences were recorded in *Pythium* counts

in soils amended with the various treatments. In general the *Pythium* counts in 2000 were higher than those in 2001, except in the control treatment. These results from the soil cannot be viewed in relation with *Pythium* isolated from plants because no pathogenicity tests were performed and identifications were not carried out to species level for the purpose of this study. Recently McKellar and Nelson (2001) reported that *Pythium* suppressive composts existed and were characterised by high populations of fatty acid metabolising bacteria. Our results suggest that the composts used in the present trials tended to stimulate *Pythium*. Thus, as mentioned previously, there are differences in composts and there is no single compost that is universally suitable for disease suppression of all soil-borne pathogens. Therefore it is important to establish the nature of the microbial population that will suppress disease in the particular soil of interest and to source suitable composts that will enhance populations of these organisms.

Biostart® trials

From plants

Fungi consistently isolated from lesions on apple roots primarily included species of *Cylindrocarpon, Fusarium* and *Pythium.* This could be expected since the same six soils were used for the Biostart® pot trials as for compost trials. In the first run of the trial as well as for the repeat run, *Cylindrocarpon* populations were high (Table 9). *Pythium* populations were low for the 2000 trial, but higher in the 2001 trial. In the repeat trial overall growth was again less than in the 2000 trial (Table 5 and 6) as with the repeat compost trial (Table 3).

In 2000, none of the treatments applied affected the incidence of *Pythium* infection in plants but the percentage isolates of *Cylindrocarpon* was significantly decreased by Biostart® in combination with Osmocote when compared to the control and Biostart® on its own (Table 9). There was also a significant increase in plant growth with Biostart® in combination with osmocote compared to the control and Biostart only treatments (Table 5). For the 2001 trial, *Cylindrocarpon* infection was again decreased with Biostart® in combination with Osmocote, although not significantly, but this time Osmocote on its own significantly decreased infection when compared to the control (Table 9). In 2001 these two treatments were the only ones to significantly increase growth (Table 6). This suggests that with lower levels of *Cylindrocarpon* infection there is an increase in plant growth. In the repeat trial *Pythium* levels for the different treatments did not differ significantly from each other, however % *Pythium* isolated from Biostart® treated soils was double that of the control. The negative effect of Biostart® in 2001 on shoot growth and total mass (Table 6) could possibly be ascribed to the higher levels of *Pythium*

infection associated with the Biostart® treatment (Table 9). Also, for this treatment, infection levels of both *Cylindrocarpon* and *Pythium* were high.

From soil

As with the compost trials, no *Phytophthora* was isolated from the ARD soils. Although there were differences between the two trials in the number of CFUs of *Pythium* in the soil, no significant differences in *Pythium* counts were recorded with the various treatments (Table 10). In the first trial, *Pythium* counts were much lower than in the repeated trial. As mentioned previously, *Pythium* counts from the soil cannot be related to isolations from plants without knowing the pathogenicity and the different species involved. However, it does seem that the higher counts from the soil may have led to higher infection levels in the plant, which explains the reduced effect of treatments in 2001.

Microorganisms as role players in ARD etiology

Reduced plant growth in ARD orchards in Washington State was associated with a complex of the plant pathogenic fungal genera, *Rhizoctonia, Cylindrocarpon, Pythium* and *Phytophthora* (Mazzola, 1998). This study was the first to substantiate a role for *Rhizoctonia* in ARD development. Isutsa and Merwin (2000) isolated *Pythium* spp. from 29% of all root samples, *Fusarium* from 26%, *Phytophthora* from 23%, *Cylindrocarpon* from 13% and *Rhizoctonia* from 1% of all root samples grown in a mixture of five New York soils with known ARD problems. In the South African study *Pythium* and *Cylindrocarpon* spp. were consistently isolated from all six replant soils, indicating that they play a role in ARD etiology in South Africa. In contrast to studies in the USA, no evidence could be found that *Rhizoctonia* spp. had an important role in ARD etiology in South Africa, as it was only sporadically isolated. There was also no indication of the involvement of *Phytophthora*. However, we have to keep in mind that this study involved only 3-month-old seedlings. It is possible that the most highly r-selected organisms would be the first primary colonisers of root tissues and later in the season when the seedlings are slightly older, different organisms would dominate (Cooke & Rayner, 1984). If this is the case it suggests that *Pythium* and *Cylindrocarpon* are well-adapted primary colonisers of apple seedling roots. Evidence has been provided confirming that *Pythium* spp. are r-selected, primary colonisers of plant tissues (Campbell, 1989) thereby supporting the latter idea. Consequently, the low incidence of *Rhizoctonia* revealed in this study may underestimate its role in ARD etiology in South Africa. Botha *et al.* (2001) found that there was a seasonal succession of the main causal agents involved in the black-root-rot disease-complex of strawberries in South Africa. *Pythium* spp. predominated in the mid-winter, but the incidence of *Rhizoctonia* spp. increased in the

late spring and by the end of the season it was the most prevalent pathogen isolated. It is possible that the situation is similar with ARD. However, this hypothesis needs to be confirmed and further research is recommended.

The involvement of niche ecology in fungal complexes is reflected in results from this study, where high *Pythium* infection could usually be associated with low *Cylindrocarpon* infection and *vice versa*. *Cylindrocarpon* directly competes with pythiaceous fungi for colonisation sites in the apple rhizosphere (Mazzola, 1998). This suggests that if *Cylindrocarpon* is reduced *Pythium* increases and colonises the niche area and resources that the *Cylindrocarpon* had occupied.

CONCLUSION

Application of organic amendments was identified as a promising alternative to methyl bromide in controlling ARD. Compost as well as sterilised and unsterilised compost teas significantly increased seedling growth even under optimum nutrient conditions when compared to the control, suggesting that they negate the effects of ARD. Results also indicated that applying high concentrations of compost does not necessarily provide any additional growth benefits compared to lower concentrations. Field verification of results with these biological soil amendments is of extreme importance. Furthermore quality factors have to be standardised to reduce variability and obtain consistent results with these amendments.

Results with biocontrol formulations were inconsistent. Biostart® increased growth significantly in two trials, but results were less favourable for the second trial and when repeating this trial Biostart® had a negative effect on growth compared to the control. Therefore, although this is a cost-effective alternative to methyl bromide ARD management with this biocontrol formulation cannot be guaranteed at this stage.

Although Osmocote was not intended to be an alternative option for ARD control, positive results achieved with this slow release fertiliser revealed that nutritional factors need to be considered in formulating alternative strategies to manage ARD. Although substantial growth increases with Osmocote in pot trials cannot be used to predict nutritional effects under field conditions, it does stress the importance of optimum nutrient conditions in an ARD management programme and that emphasis on management of all factors concerning replant will be needed to ensure successful new plantings on old orchard soil.

Nematodes implicated in ARD were inconsistently associated with the ARD soils used in this study, indicating that nematodes do not have a primary causal role in ARD etiology in South Africa, although they may be a complicating factor in some areas. *Pythium* and *Cylindrocarpon* spp. were consistently isolated from all six replant soils tested as well as all four trials that formed part of this study, indicating that these fungi may have a role in ARD etiology locally.

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TABLE 1. Nematode populations from soil and root samples of six apple replant disease soils.

ARD soil	Genus	
	Pratylenchus [a]	*Xiphinema* [b]
1	2	100
2	154	210
3	64	500
4	20	< 10
5	0	< 10
6	0	< 10

[a] Counts per gram of roots

[b] Counts per 250cm^3 of soil

TABLE 2. Mean shoot and root growth of apple seedlings grown in apple replant disease soils amended with various concentrations of compost.

%Compost [a]		Shoot length (mm)[b]	Fresh shoot mass (g)	Fresh root mass (g)	Total fresh mass (g)
0		45.10 c	0.98 b	2.12 b	3.10 c
12.5		123.64 b	3.75 a	6.30 a	10.05 a
25		179.30 a	4.81 a	5.69 a	10.50 a
37.5		173.30 a	4.73 a	4.82 a	9.55 a
50		149.34 ab	3.68 a	3.02 b	6.70 b
LSD [c]		*42.14*	*1.35*	*1.54*	*2.71*
	df		Significance (Pr>F)		
Treatment	4	<.0001	0.0003	0.0003	0.0003
Linear	1	<.0001	0.0006	0.8346	0.0330
Quadratic	1	0.0002	0.0002	<.0001	<.0001

Means in a column followed by the same letter are not significantly (P = 0.05) different

[a] % v/v of compost added to replant soil

[b] means of 50 seedlings (5 block replicates and 10 soils)

[c] Student's t-LSD (least significant difference) was calculated at a 5% significance level

TABLE 3. Mean shoot and root growth of apple seedlings planted in six replant soils amended with compost and compost tea in combination with Osmocote, a slow-release fertiliser. Pot trials were conducted in two consecutive years.

Treatment	Mean Shoot length (mm)[a]		Fresh shoot mass (g)		Fresh root mass (g)		Total fresh mass (g)	
Year	2000	2001	2000	2001	2000	2001	2000	2001
Control	34.93 c	35.83 b	0.61 c	0.89 c	1.45 c	0.87 c	2.06 c	1.76 d
Osmocote	270.70 b	115.67 a	7.86 b	3.38 b	5.93 b	2.18 b	13.79 b	5.56 c
Compost + Osmocote	324.69 a	114.67 a	11.21 a	4.50 a	7.81 a	2.83 a	19.02 a	7.32 a
Unsterilised Tea + Osmocote	341.05 a	110.52 a	11.29 a	3.96 ab	8.50 a	2.77 a	19.80 a	6.73 ab
Sterilised Tea + Osmocote	313.81 ab	108.17 a	10.08 a	3.79 ab	8.38 a	2.21 b	18.47 a	6.01 bc
LSD [b]	*46.15*	*14.94*	*1.38*	*0.71*	*1.17*	*0.50*	*2.29*	*1.09*
	Significance (Pr>F)							
Treatment	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Means in a column followed by the same letter are not significantly (P = 0.05) different.

[a] Means of 42 seedlings(7 block replicates and 6 soils)

[b] Student's t-LSD (least significant difference) was calculated at a 5% significance level

TABLE 4. Mean shoot and root growth of apple seedlings planted in ten replant soils amended with various commercially formulated biological control products in 1999.

Treatment	Shoot length (mm)[a]	Fresh shoot mass (g)	Fresh root mass (g)	Total fresh mass (g)
Control	50.17 b	1.57 b	4.28 bc	5.86 b
Biostart®	175.49 a	5.27 a	8.86 a	14.13 a
Rootshield®	56.30 b	1.63 b	4.48 b	6.11 b
Biocult®	47.34 b	1.32 b	4.05 bcd	5.37 bc
Effective microorganisms (EM)	53.00 b	1.46 b	3.84 cd	5.30 bc
Biocult® + EM	44.40 b	1.46 b	3.48 d	4.89 c
LSD[b]	*20.48*	*0.44*	*0.61*	*0.96*
	Significance (Pr>F)			
Treatment	<.0001	<.0001	<.0001	<.0001

Means in a column followed by the same letter are not significantly (P = 0.05) different.

[a] Means of 50 seedlings (5 block replicates and 10 soils)

[b] Student's t-LSD (least significant difference) was calculated at a 5% significance level

TABLE 5. Effect of different concentrations of Biostart® as well as Biostart® in combination with Osmocote on growth of apple seedlings grown in six replant soils in a pot trial conducted in 2000.

Treatment	Shoot length (mm)[a]	Dry shoot mass (g)	Dry root mass (g)	Total dry mass (g)
Control	34.93 d	0.24 d	0.48 d	0.72 d
Osmocote	221.14 a	3.38 a	3.56 a	6.74 a
Biostart® [b]	129.90 b	1.35 b	1.36 c	2.72 b
Biostart®+ Osmocote	219.17 a	3.31 a	2.95 b	6.30 a
50% Biostart®	68.26 c	0.71 c	0.87 d	1.57 c
25% Biostart®	51.36 cd	0.48 cd	0.70 d	1.18 cd
12.5% Biostart®	41.05 cd	0.30 d	0.56 d	0.85 d
6.25% Biostart®	36.26 d	0.28 d	0.54 d	0.84 d
LSD [c]	*31.42*	*0.36*	*0.40*	*0.68*
	Significance (Pr>F)			
Treatment	<.0001	<.0001	<.0001	<.0001

Means in a column followed by the same letter are not significantly (P = 0.05) different.

[a] Means of 42 seedlings(7 block replicates and 6 soils)

[b] Full rate (Biostart® 100%)

[c] Student's t-LSD (least significant difference) was calculated at a 5% significance level

TABLE 6. Effect of Biostart® in combination with Osmocote on growth of apple seedlings planted in six replant soils for a repeat pot trial conducted in 2001.

Treatment	Mean shoot length (mm)[a]	Fresh shoot mass (g)	Fresh root mass (g)	Total fresh mass (g)
Control	59.67 b	1.07 b	0.70 b	1.76 b
Osmocote	76.81 a	2.06 a	1.59 a	3.65 a
Biostart® [b]	29.23 c	0.44 c	0.67 b	1.11 c
Biostart® + Osmocote	82.47 a	2.00 a	1.48 a	3.48 a
LSD [c]	*10.76*	*0.30*	*0.25*	*0.42*
	Significance (Pr>F)			
Treatment	<.0001	<.0001	0.0002	<.0001

Means in a column followed by the same letter are not significantly (P = 0.05) different.

[a] Means of 42 seedlings(7 block replicates and 6 soils)

[b] Applied at full rate

[c] Student's t-LSD (least significant difference) was calculated at a 5% significance level

TABLE 7. Effect of compost, compost tea and Osmocote on average frequency of recovery of dominant fungal genera from apple seedling roots planted in six apple replant disease soils. Two independent trials were conducted in consecutive years.

Treatment	Fungi			
	Cylindrocarpon (%)[a]		*Pythium* (%)	
	2000	2001	2000	2001
Control	29.7 a	6.7 a	4.3 ab	9.5 b
Osmocote	19.8 a	1.5 a	7.2 a	16.2 ab
Compost + Osmocote	32.8 a	3.5 a	5.0 a	26.2 a
Tea A[b] + Osmocote	16.3 a	4.3 a	5.3 a	24.0 a
Tea B[c]+ Osmocote	20.2 a	1.7 a	0.0 b	12.0 b
LSD [d]	*20.5*	*7.4*	*4.7*	*11.8*
	Significance (Pr>F)			
Treatment	0.4143	0.5945	0.0522	0.0306

Means in a column followed by the same letter are not significantly (P = 0.05) different

[a] percentage calculated from the total number of root segments from which fungi of a given genus were isolated. Fungi were isolated from four root segments for five plants of each soil x treatment combination.

[b] Unsterilised compost tea

[c] Sterilised compost tea

[d] Student's t-LSD (least significant difference) was calculated at a 5% significance level

TABLE 8. Effect of compost, compost teas and Osmocote on the number of *Pythium* colonies in replant soils planted with apple seedlings between 2000–2001.

Treatment	*Pythium* colonies per gram of soil per year	
	2000	2001
Control	13 c	33 a
Osmocote	43 b	33 a
Compost + Osmocote	75 a	18 a
Tea A[a] + Osmocote	40 b	28 a
Tea B[b] + Osmocote	38 b	33 a
LSD [c]	*22*	*20*
	Significance (Pr>F)	
Treatment	0.0004	0.4922

Means in a column followed by the same letter are not significantly (P = 0.05) different

[a] Unsterilised compost tea

[b] Sterilised compost tea

[c] Student's t-LSD (least significant difference) was calculated at a 5% significance level

TABLE 9. Effect of Biostart® and Osmocote on average frequency of recovery of dominant fungal genera from apple seedling roots plant in six apple replant disease soils. Two independent trials were conducted in consecutive years.

Treatment	Fungi			
	Cylindrocarpon (%)[a]		*Pythium* (%)	
	2000	2001	2000	2001
Control	29.7 a	27.2 a	4.3 a	7.3 a
Osmocote	28.0 ab	11.5 b	0.8 a	10.1 a
Biostart® + Osmocote	14.7 b	17.5 ab	3.3 a	8.0 a
Biostart®	34.5 a	24.0 a	2.2 a	15.5 a
LSD [b]	*13.7*	*11.2*	*7.2*	*9.8*
	Significance (Pr>F)			
Treatment	0.0426	0.0413	0.7574	0.3115

Means in a column followed by the same letter are not significantly (P = 0.05) different

[a] percentage calculated from the total number of root segments from which fungi of a given genus were isolated. Fungi were isolated from four root segments for five plants of each soil x treatment combination.

[b] Student's t-LSD (least significant difference) was calculated at a 5% significance level

TABLE 10. Effect of Biostart ® and Osmocote on the number of *Pythium* colonies in replant soils planted with apple seedlings between 2000-2001.

Treatment	*Pythium* colonies per gram of soil per year	
	2000	2001
Control	17 a	55 a
Osmocote	34 a	48 a
Biostart® + Osmocote	37 a	43 a
Biostart®	20 a	52 a
LSD [a]	*21*	*23*
	Significance (Pr>F)	
Treatment	0.1326	0.7503

Means in a column followed by the same letter are not significantly (P = 0.05) different

[a] Student's t-LSD (least significant difference) was calculated at a 5% significance level

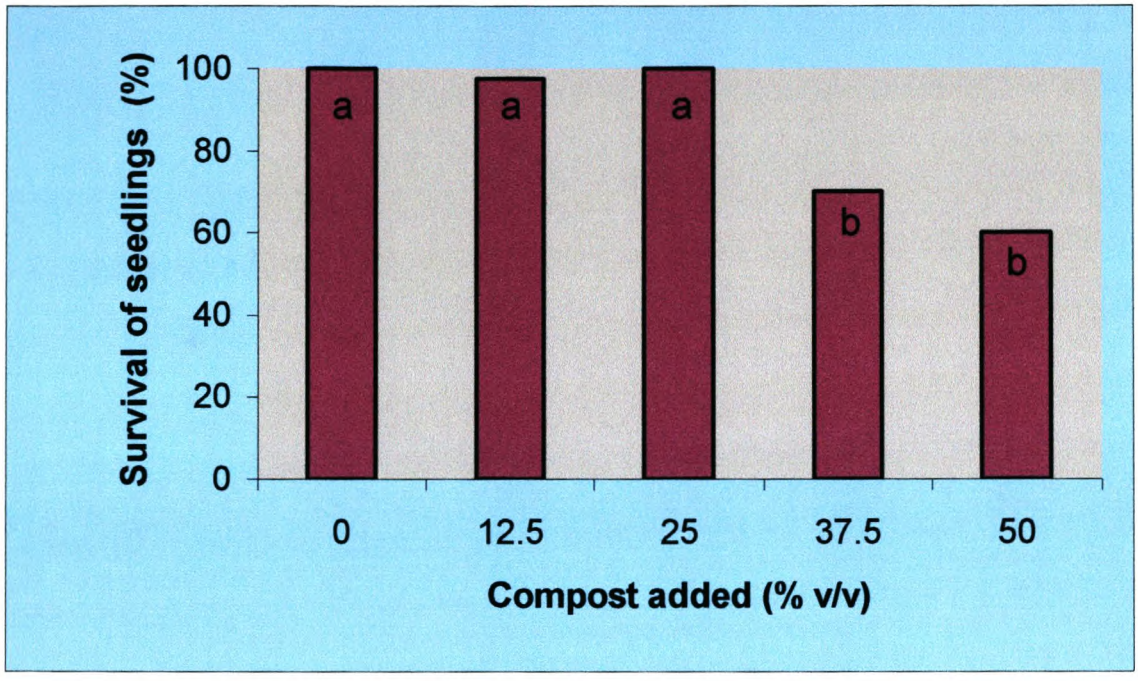

FIGURE 2. Effect of increased compost concentrations on survival of seedlings planted into ten compost-amended apple replant disease soils.

CHAPTER 4

EVALUATION OF BIOLOGICAL METHODS TO CONTROL APPLE REPLANT DISEASE UNDER FIELD CONDITIONS

ABSTRACT

Three field trials were conducted in commercial orchards in the Elgin region (34°S, 300m) to assess the impact of organic amendments as well as promising biological control products, as implicated in pot trials, on ARD severity under field conditions in comparison with the standard chemical control methods for ARD (methyl bromide and chloropicrin). In all three trials established, compost and mulch as well as manure and mulch consistently increased growth to the same extent as the standard chemical treatments. Furthermore, Biostart®, a microbial soil inoculant consisting of beneficial bacteria, as well as effective microorganisms (EM) in combination with compost, manure and mulch also significantly improved growth. These soil amendments could possibly substitute for soil fumigation in replanted apple orchards. However, only when quality factors have been implemented and optimum rates established, can consistent results with these biological soil amendments be obtained.

INTRODUCTION

Apple replant disease (ARD) is the unexplained poor growth of young apple trees, which occurs after replanting on a site that was previously planted to apple. Aboveground symptoms include stunted growth, shortened internodes, rosetted leaves and reduction in tree vigour and yield. Characteristically, shoot growth terminates earlier than on healthy trees (Traquiar, 1984). Trees affected by the disease start cropping fruit 2 to 3 years later than unaffected trees and continue to produce relatively low yields ten or more years after the trees have filled their allocated orchard space (Smith, 1993). Root systems are typically small with discoloured roots, few functional root hairs and a marked reduction in lateral root development (Savory, 1966; Hoestra, 1968).

Although the disease is not lethal, it has great economic importance because of its continuous influence on production. In South Africa serious ARD symptoms occur in approximately 40% of replantings (Honeyborne, 1995). With the emphasis on early cropping to ensure an early return on investment any growth-retarding factor is

adversely felt. The delayed precocity and production caused by ARD may decrease profitability by as much as 50% throughout the life of the orchard (Rabie, Denman & Cook, 2001). It is becoming an increasingly important problem as the release of new cultivars and rootstocks necessitates new plantings and producers are forced to replant old orchard soil due to limited virgin soil suitable for apple production.

In spite of extensive research on ARD, the etiology still needs to be fully elucidated. Causal factors vary across geographic regions or even between orchards in the same region. In the past, researchers have linked ARD to abiotic factors such as inadequate nutrient availability or toxic residues in the soil (Mai & Abawi, 1981; Traquair, 1984). However, the dramatic growth improvement on replant soils with a range of soil sterilisation treatments indicates that the causal elements are primarily biological (Savory, 1966; Mazzola, 1998). Numerous soilborne organisms including plant parasitic nematodes (Mai & Abawi, 1981; Utkhede, Smith & Palmer, 1992; Dullahide *et al.*, 1994), pathogenic fungi (Sewell, 1981; Braun, 1995; Mazzola, 1998), actinomycetes (Westcott, Beer & Stiles, 1986) and bacteria (Savory, 1966; Hoestra, 1968) have been implicated as being potential causal factors, as well as allelopathic relationships between plants, microorganisms of the rhizosphere and soil. In Chapter 2 the microbial origin of ARD etiology in South Africa was confirmed through inability to eliminate ARD symptoms by dilution of replant soil with sterilised soil, which was in agreement with conclusions from Hoestra (1968) and Jaffee, Abawi and Mai (1982).

Progress towards the control of ARD has been hampered by difficulties in recognising the primary causal agent within a background of complex interacting factors. At present, there are few satisfactory alternatives to the long-standing practice of soil fumigation (Mai & Abawi, 1981). The most effective fumigant is methyl bromide, which is currently indispensable in establishing an economically viable orchard on a site that was previously planted to apple. However, methyl bromide was declared an ozone depleting substance and its imminent phase-out to comply with the Montreal Protocol has intensified the need for alternative measures to control ARD (WMO, 1994). Although alternative chemicals have provided some form of ARD control, the high cost of chemical control and the potential hazard to human health and the environment make it essential to develop more sustainable means of ARD control. The disease-suppressive effects of compost have been investigated intensively over the last decade and due to the biological nature of ARD etiology, compost may also have a role in controlling ARD (De Ceuster & Hoitink, 1999; Naegely, 2000). The concept of inoculating soils and plants with beneficial microorganisms such as *Bacillus subtilis* (Utkhede & Smith, 2000) and

fluorescens-putida type *Pseudomonas* (Biro *et al.,* 1996; Mazzola & Gu, 1999) to create a more favourable microbiological environment for plant growth has also shown promise (Baker & Cook, 1974; Catska, 1993). However, the utilisation of these biological and cultural amendments for control of soilborne plant pathogens has often been considered at best variable (Lazarovits, 2001), and has yet to meet expectations for disease control efficacy under field conditions.

Good results were achieved with compost and Biostart®, a microbial soil inoculant consisting of beneficial bacteria, in pot trials as an initial step in finding sustainable disease control alternatives to control ARD (Chapter 3). The objective of this study was to assess the impact of organic amendments and biological control products, that demonstrated disease reduction in pot trials, on ARD severity under field conditions, and to compare them with the standard chemical control methods for ARD.

MATERIALS AND METHODS

Plant material

Three field trials were conducted on ARD sites in commercial orchards in Elgin (34°S, 300m, Mediterranean climate), a major apple growing region in South Africa. In the first two trials 'Golden King' (an Applethwaite early flowering mutation from 'Golden Delicious') apple nursery whips on M793 rootstock were planted in August 1999 on a sandy soil, following two months of cold-storage, with a spacing of 4.5m between rows and 1.5m between trees. The orchard was previously planted with 'Golden Delicious' on seedling rootstock. In the third trial 'Fuji' nursery trees on M793 rootstock were planted in August 2000 on an ARD site previously planted with 'Golden Delicious' on M793 rootstock.

Treatments and experimental design

The three trials were conducted in a completely randomised complete split-plot design with 10 block replications (Table 1). An experimental unit consisted of a plot of three trees in the second trial and a plot of four trees in trials one and three. Old orchards were removed one year prior to planting and fumigation treatments with methyl bromide (300g/running m) and chloropicrin (50mL/m^2) applied in late summer 1999. Herbifume (metham-sodium) was applied in March 2000 at 100mL/100L of water per tree, as a soil drench. Both types of compost used were produced by fully aerobic composting procedures. Compost2 consisted of 20% cow manure, 70% wheat straw and 10% chicken manure and was inoculated with effective microorganisms (EM). Compost1

consisted of 90% of the Compost2 mixture, with 10% bokashi (dried EM) added. Bokashi is a mixture of wheat straw and wheat bran, inoculated with EM mixed with molasses and anaerobically fermented for three weeks. The mulch used was wheat straw. The composts and mulches were applied one week after planting. Compost was applied to the surface at 15kg per tree and then covered with a layer of mulch ca. 5-10 cm thick.

Where EM was applied on its own, a diluted suspension (1:1000) of stock EM was prepared and applied as a soil inoculant through the micro-irrigation system at 20mL per tree twice a week for the first growing season. Suspensions of EM are mixed cultures of naturally occurring beneficial microorganisms, consisting primarily of photosynthetic and lactic acid bacteria as well as yeasts, actinomycetes and fermenting fungi (Higa, 1994). Biostart® (Microbial Solutions), is a liquid microbial soil inoculant consisting of three bacteria, *Bacillus laterosporus, B. chitinosporus* and *B. licheniformis*. At planting roots were dipped into a solution containing 20mL of Biostart® inoculant, 20g of activator and 100 liters of water. A further soil drench was applied at 2mL/2g in 10 liters of water per tree at planting and repeated monthly at a lower concentration of 1mL/1g per 10 liters of water for the remainder of the growing season. Control trees were dipped and drenched with water. All trees received fertilisation according to industry norms.

Data collected

Data were collected from the centre tree(s) in each plot during May 2000 and 2001 respectively, at the end of the growing season after shoot growth had terminated. The following data were recorded and used to assess ARD severity: (1) main leader length, (2) number of shoots per tree longer than 5 cm, (3) number of shoots per tree shorter than 5 cm, (4) total number of shoots (budburst), (5) total new shoot growth per tree.

Statistical analysis

A standard split-plot analysis of variance was performed on the data of all three field trials using SAS V8.11 Statistical Software (SAS, 1990). Student's t-LSD was calculated at a 5% significance level to compare the treatment means.

RESULTS

'Golden King' Trial 1

Similar results were obtained for both growing seasons. There were no significant interactions between the main and the sub treatments (Table 2 and 3), indicating that compost and mulch increased growth parameters significantly irrespective of whether it

was applied in combination with chloropicrin (CP), methyl bromide (MeBr) or on its own. In the first growing season (Table 2) both fumigation treatments as well as application of compost and mulch significantly, and to the same extent, increased total growth when compared to the control. However, in the second growing season CP did not increase total growth significantly (Table 3). Although budburst (total number of shoots) was not affected by any of the treatments during the first growing season (Table 2), the proportion of long shoots (>5cm) was significantly increased with all treatments. In the second year after planting, however, the total shoot number was significantly increased by all treatments except MeBr fumigation, while the number of long shoots was again significantly increased by all treatments (Table 3).

The combination of soil fumigation with compost and mulch application gave a substantial and significant total growth increase in addition to fumigation on its own. In the second year (Table 3) total new growth was increased, but not significantly, by CP or compost and mulch applied individually. However, the number of long shoots was significantly increased by all treatments. The MeBr with compost sub treatment still increased growth considerably in the second growing season.

'Golden King' Trial 2

There was no significant interaction between the main and the sub treatments (Table 4 and 5). While EM application tended to increase growth in both growing seasons, compared to the control this effect was not significant (Table 4). However, results for the first growing season showed that both composts and manure with mulches significantly improved total growth consistently. Results for the second growing season were similar, except that Compost2 and mulch increased total new growth, but not significantly (Table 5). Furthermore, the growth increase with kraal manure was more pronounced in the second growing season. For both growing seasons, the treatments did not have a significant effect on total shoot number, while the number of long shoots was significantly increased by the composts or manure applied with the mulch. Mulch applied on its own had no effect on any of the growth parameters measured.

'Fuji' Trial

The effect of chemical treatment, Biostart® and compost and mulch on first year growth of 'Fuji' nursery trees is presented in Table 6. There were no significant interactions between the sub and main treatments. When looking at the main effects no significant differences were recorded between the sub treatment with Biostart® and the control. For the main treatments, total new growth and number of long shoots were significantly

increased by the compost and mulch, but not by the Herbifume. Results from individual treatments showed that neither Herbifume on its own nor in combination with Biostart® improved growth when compared to the control (Table 6). In contrast to results indicated by the main effects, Biostart® applied on its own, improved growth to the same extent as compost and mulch. When these two treatments were combined an additional, although not significant, growth increase was recorded when compared to either of the treatments alone.

DISCUSSION

Results from field trials in this study confirmed positive results achieved with the use of compost and biological control formulations evaluated in pot trials (Chapter 3). In all three trials established, compost and mulch consistently increased growth significantly compared to the control and the effect could still be measured in the second growing season. Manure with mulch also improved growth, particularly in the second year. The delayed effect may be due to slower release of nutrients by compounds in the manure or due to build up of reserves in the trees. The fact that straw mulch on its own did not have a significant effect on growth in either of the growing seasons indicates that where compost and manure were applied with a mulch, growth promotion was the effect of the compost. In general, Compost1 performed best, possibly due to the bokashi added to this compost. Bokashi is fermented wheat bran inoculated with EM. EM in combination with Compost1 resulted in more growth than Compost1 alone. Apparently, increasing the amount of beneficial organisms present in a replanted soil enhances apple tree growth.

Results from this study also indicated that application of compost and mulch increased growth to the same extent as the standard chemical treatments, MeBr and CP and that by combining these chemical treatments with organic amendments an additional growth increase could be obtained. Herbifume, however, did not improve growth significantly compared to the control. This may be due to ineffective application of the chemical, or its variable activity against soilborne pests. Both problems have been documented previously (McKenry, *et al.*, 1994; De Ceuster & Hoitink, 1999).

These positive results with organic amendments are in contradiction with field trial results in Washington State, USA, where testing various types of compost in six ARD orchards revealed no significant differences on tree growth (Granatstein, 1999). Compost must be of consistent quality to be used successfully in biological control of diseases of horticultural crops. Variability in compost type and stability is one of the principal factors that lead to inconsistent results with these organic amendments

(Hoitink, Stone & Hun, 1997). There is an increasing awareness that organic residues have a variety of agriculturally beneficial properties in addition to their ability to supply nutrients (Wooldridge & Nell, 1998). Soils that are mulched with organic materials remain cooler, leading to reduced evaporative losses. Mulching also physically protects the soil surface against sealing and compacting, thereby improving water infiltration and oxygen availability. The general biological activity of the soil is furthermore stimulated by addition of an available carbon source (Campbell, 1989; Magarey, 1999) and soils with a diversity of beneficial microorganisms are more likely to be suppressive to disease development than are soils that have little or no biological diversity (Lazarovits, 2001). Compost has been shown to suppress plant disease due to the microbial activities inherent to them and may modify the composition of the microflora so that it benefits the growth of young roots. The soil microflora becomes rich and well balanced with beneficial microorganisms, and pathogenic microorganisms do not dominate (Ristaino & Thomas, 1997). A possible overriding effect of compost over *Pythium* and *Cylindrocarpon* spp., both pathogens implicated in ARD, was observed in compost pot trials in Chapter 2. This suggests that compost either stimulates plant growth, leading to disease escape by shortening the time that the plant is in a susceptible state or they contain microorganisms that can colonise the roots to prevent invasion by the pathogen (competitive exclusion). In some cases control can also result from production of metabolites, which directly inhibit the pathogen.

All composts used in this study were inoculated with beneficial microorganisms and were applied in combination with mulch, which may explain the significant growth increases with these amendments in contrast with results from Granatstein (1999). According to Hoitink, Stone and Hun (1997) controlled inoculation of composts with biocontrol agents is a procedure that can induce consistent levels of disease suppression on a commercial scale. Nevertheless, few types of compost are universally effective and it is therefore necessary to determine specific compost types for various biological as well as chemical and physical soil conditions.

Biocontrol formulations evaluated in this study gave variable results. Biostart® improved growth significantly applied on its own, but did not show any effect when applied after the chemical, Herbifume. This, however, could probably be ascribed to the ineffectiveness of the Herbifume itself or possible incompatibility of this biocontrol formulation with the chemical. Furthermore, there was a tendency for Biostart® in combination with compost and mulch, to increase total new growth when compared to either of these treatments alone. This tendency may become significant during the

second growth year and measurements should continue at least for the next growing season.

Despite positive results with EM from studies in other countries (Higa, 1994,1998; Parr, Hornick & Papendick, 1998), in our study inoculating soil with EM solution on its own had no significant effect. However, we have only recently started to make use of this technology in South Africa, and more research is needed on dosage for different soil types and crops as well as improved application methods before this product can be discarded. Furthermore, as with Biostart® there was again a tendency for application of EM in combination with other organic amendments to increase total new growth. The action of these biocontrol agents usually does not occur in isolation and requires complex organic compounds of carbon and nitrogen for metabolism and biosynthesis. Organic carbon is the dominant food reservoir in soil and is needed to sustain microbial development (Alexander, 1977). Thus, the effectiveness and benefits of biocontrol agents are maximised when it is applied in combination with supporting ecologically effective management practices such as adding organic amendments. This is in agreement with the positive effects of EM and Biostart® in combination with compost, manure and mulch on growth parameters measured.

In some studies the effect of these biocontrol products has shown variation with soil type, kinds and amounts of organic matter used, as well as crop species and varieties (Lou, 1997; Lazarovits, 2001). Utkhede and Smith (2000) also noted that control with biocontrol agents was strongly dependent on soil type and environmental conditions. It is therefore necessary to elucidate the mechanisms of disease control of these soil amendments in order to apply it over a wide geographical region. These beneficial organisms can function trough suppression of plant pathogens and diseases, enhanced nutrient availability, blocking of toxic elements, stimulated plant growth (i.e., auxin-mediated effects), and improved root surface-rhizosphere relationships (Parr, Hornick & Papendick, 1998).

CONCLUSION

Over the past two decades, considerable progress has been made in the reintroduction of cultural practices into agriculture that offers opportunities for biological control of diseases. Extensive modification of soil microbial communities occurs during apple monoculture. It seems that by adding these biological amendments microbial diversity is increased leading to the establishment of a new soil microbiological equilibrium that restores soil microflora to conditions that is again conducive to apple tree growth.

In all three trials established, either compost or manure combined with mulch consistently increased growth compared to the control. Furthermore, results indicated that application of these organic amendments increased growth to the same extent as the standard chemical treatments, methyl bromide and chloropicrin and that by combining these chemical treatments with organic amendments a significant, additional growth increase could be attained. However, there are many different sources of compost and not all compost function in the same way. Therefore, it is important to find a reliable source and to establish which types of compost work and why they work under specific conditions. Compost quality standards should to be implemented, optimum rates established and different types of compost compared before recommendations can be made.

Biocontrol formulations evaluated in this study gave variable results. Biostart® improved growth when applied on its own, but not in combination with metham-sodium (Herbifume). Furthermore, inoculating soil with EM solution had no significant effect on growth. However, success in biological control of diseases with soil amendments is possible only if all factors involved in its production and use are defined and kept consistent. These biocontrol products also need to be evaluated in different soil types and environmental conditions. Furthermore, elucidation of the mechanisms for disease control is necessary for implementation of these soil amendments into disease control strategies over a wide geographical area.

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TABLE 1. Treatments as conducted in all three field trials.

	Main treatments	Sub-treatments
'Golden King' Trial 1	1. Control (No amendments) 2. Methyl bromide (MeBr) 3. Chloropicrin (CP)	1. Control 2. Compost1 + Mulch (C+M)
Golden King' Trial 2	1. Control 2. Effective microorganisms (EM)	1. Control 2. Mulch (M) 3. Compost1 + Mulch (C1+M) 4. Compost2 + Mulch (C2+M) 5. Kraal manure + Mulch (KM+M)
'Fuji' Trial	1. Control 2. Herbifume (metham-sodium) 3. Compost1 + Mulch	1. Control 2. Biostart®

TABLE 2. Effect of compost and mulch as well as standard chemical treatments on first year growth of 'Golden King' apple whips planted in 1999 on a site with apple replant disease.

Treatments		Total new growth (cm)	Number of shoots >5cm	Number of shoots <5cm	Total number of shoots
Main effect[a]					
Main treatments					
Control		331.1 c	11 b	11 a	22 a
Chloropicrin		691.4 a	15 a	7 b	22 a
Methyl bromide		613.8 b	15 a	7 b	22 a
LSD (5%)		*134.8*	*2*	*2*	*2*
Sub treatments					
Control		445.1 b	12 b	9 a	22 a
Compost + Mulch (C+M)		645.8 a	14 a	7 b	22 a
LSD (5%)		*47.0*	*2*	*1*	*2*
Split effect[b]					
Main	**Sub**				
Control	Control	255.9 e	9 e	13 a	22 a
	C+M	406.3 d	12 d	10 b	22 a
Chloropicrin	Control	572.1 c	13 cd	8 c	21 a
	C+M	810.8 a	16 a	6 d	22 a
Methyl bromide	Control	507.3 c	14 bc	8 c	22 a
	C+M	720.4 b	15 ab	7 c	22 a
LSD (5%)		*83.4*	*2*	*2*	*2*
		Significance (Pr>F)			
Main treatment		<.0001	0.0010	<.0001	0.9635
Sub treatment		<.0001	0.0020	0.0026	0.6644
Interaction		0.2857	0.2634	0.5673	0.6336

Means in a column followed by the same letter are not significantly (P = 0.05) different

[a] Average of pooled values from the individual treatments as in Table 1

[b] Individual treatment effects

TABLE 3. Effect of compost and mulch compared to standard chemical treatments on second year growth of 'Golden King' apple whips planted in 1999 on a site with apple replant disease.

Treatments		Total new growth (cm)	Number of shoots >5cm	Number of shoots <5cm	Total number of shoots
Main effect[a]					
Main treatments					
Control		354.9 b	15 b	26 b	41 b
Chloropicrin		429.6 b	19 a	27 b	58 a
Methyl bromide		566.4 a	20 a	38 a	46 b
LSD (5%)		*83.3*	*2*	*8*	*9*
Sub treatments					
Control		382.5 b	15 b	26 b	41 b
Compost + Mulch (C+M)		518.0 a	20 a	35 a	55 a
LSD (5%)		*55.8*	*2*	*7*	*8*
Split effect[b]					
Main	**Sub**				
Control	Control	318.9 d	12 d	22 c	34 c
	C+M	390.8 bcd	17 c	31 bc	48 b
Chloropicrin	Control	372.5 cd	18 bc	33 ab	51 b
	C+M	486.7 b	21 ab	43 a	64 a
Methyl bromide	Control	456.3 b	16 c	24 c	40 bc
	C+M	676.4 a	22 a	30 bc	52 ab
LSD (5%)		*98.9*	*3*	*11*	*13*
		Significance (Pr>F)			
Main treatment		0.0002	0.0001	0.0085	0.0023
Sub treatment		0.0001	0.0001	0.0139	0.0020
Interaction		0.0907	0.5187	0.8258	0.9609

Means in a column followed by the same letter are not significantly (P = 0.05) different

[a] Average of pooled values from the individual treatments as in Table 1

[b] Individual treatments effects

TABLE 4. Effect of compost, manure, mulch and effective microorganisms (EM) on first year growth of 'Golden King' apple whips planted in 1999 on an apple replant disease site.

Treatments **Main effect**[a]		Total new growth (cm)	Number of shoots >5cm	Number of shoots <5cm	Total number of shoots
Main treatment					
Control		536.3 a	13 a	8 a	21 a
Effective Microorganisms (EM)		552.7 a	13 a	8 a	21 a
LSD (5%)		*112.3*	*1*	*1*	*2*
Sub treatment					
Control		420.8 c	12 bc	10 a	22 a
Mulch		484.0 bc	12 bc	8 ab	20 a
Compost1 + Mulch (C1+M)		657.7 a	14 a	7 b	21 a
Compost2 + Mulch (C2+M)		610.5 a	13 ab	6 b	19 a
Kraal manure +Mulch (KM+M)		549.5 ab	14 a	8 ab	22 a
LSD (5%)		*113.7*	*2*	*2*	*3*
Split effect[b]					
Main	**Sub**				
Control	Control	406.8 cd	11 b	10 a	22 a
	Mulch	467.7 cd	12 ab	8 ab	20 a
	C1+M	608.1 ab	14 a	7 b	21 a
	C2+M	633.2 ab	13 ab	6 b	19 a
	KM+M	565.5 abc	14 a	8 ab	22 a
EM	Control	434.7 cd	12 ab	9 a	21 a
	Mulch	500.3 bcd	12 ab	8 ab	20 a
	C1+M	707.2 a	14 a	7 b	21 a
	C2+M	587.7 abc	13 ab	7 b	20 a
	KM+M	533.6 bcd	14 a	8 ab	22 a
LSD (5%)		*160.5*	*3*	*3*	*4*
		Significance (Pr>F)			
Main treatment		0.7436	0.9465	0.6525	0.7507
Sub treatment		0.0005	0.0486	0.0496	0.6990
Interaction		0.7253	0.9664	0.6645	0.9209

Means in a column followed by the same letter are not significantly (P = 0.05) different

[a] Average of pooled values from the individual treatments as in Table 1

[b] Individual treatment effects

TABLE 5. Effect of compost, manure, mulch and effective microorganisms (EM) on second year growth of 'Golden King' apple whips planted in 1999 on an apple replant disease site.

Treatments **Main effect**[a]		Total new growth (cm)	Number of shoots >5cm	Number of shoots <5cm	Total number of shoots
Main treatment					
Control		381.4 a	16 a	43 a	59 a
Effective Microorganisms (EM)		420.1 a	17 a	42 a	59 a
LSD (5%)		*77.6*	*2*	*7*	*8*
Sub treatment					
Control		307.3 c	13 c	40 a	53 a
Mulch		377.0 c	15 bc	41 a	56 a
Compost1 + Mulch (C1+M)		442.3 ab	18 a	45 a	63 a
Compost2 + Mulch (C2+M)		401.6 abc	16 ab	42 a	58 a
Kraal manure + Mulch (KM+M)		478.3 a	18 a	44 a	62 a
LSD (5%)		*95.6*	*3*	*11*	*12*
Split effect[b]					
Main	**Sub**				
Control	Control	270.1 c	12 e	34 b	46 b
	Mulch	353.4 bc	15 cde	43 ab	58 ab
	C1+M	417.3 abc	16 bcd	45 ab	61 ab
	C2+M	427.6 ab	17 abcd	41 ab	58 ab
	KM+M	438.5 ab	18 abc	51 a	69 a
EM	Control	344.5 bc	14 de	45 ab	59 ab
	Mulch	400.7 abc	15 cde	40 ab	55 ab
	C1+M	467.2 ab	19 ab	46 ab	65 a
	C2+M	375.7 abc	16 bc	42 ab	58 ab
	KM+M	522.6 a	20 a	36 b	56 ab
LSD (5%)		*152*	*4*	*15*	*17*
		Significance (Pr>F)			
Main treatment		0.2538	0.2674	0.7700	0.8709
Sub treatment		0.0054	0.0004	0.8497	0.3148
Interaction		0.5642	0.5258	0.2458	0.3523

Means in a column followed by the same letter are not significantly (P = 0.05) different

[a] Average of pooled values from the individual treatments as in Table 1

[b] Individual treatment effects

TABLE 6. Effect of chemical treatment, Biostart® and compost and mulch on first year growth of 'Fuji' nursery trees planted in 2000 on an apple replant disease site.

Treatments		Total new growth (cm)	Number of shoots >5cm	Number of shoots <5cm	Total number of shoots
Main effect[a]					
Main treatment					
Control		220.8 b	6 b	12 a	18 a
Herbifume		186.0 b	5 b	10 a	15 a
Compost + Mulch (C+M)		321.3 a	8 a	10 a	18 a
LSD (5%)		*68.1*	*2*	*4*	*4*
Sub treatment					
Control		215.9 a	6 a	11 a	17 a
Biostart		269.5 a	7 a	10 a	17 a
LSD (5%)		*66.9*	*2*	*2*	*3*
Split effect[b]					
Main	**Sub**				
Control	Control	164.7 c	5 b	13 a	18 a
	Biostart	276.9 ab	7 a	11 ab	18 a
Herbifume	Control	203.6 bc	6 b	11 ab	17 ab
	Biostart	168.3 bc	5 b	9 b	14 b
C + M	Control	279.4 ab	8 a	10 ab	18 a
	Biostart	363.2 a	8 a	10 ab	18 a
LSD (5%)		*112.1*	*2*	*4*	*4*
		Significance (Pr>F)			
Main treatment		0.0018	0.0022	0.4872	0.3251
Sub treatment		0.1113	0.4724	0.1941	0.4566
Interaction		0.1652	0.6266	0.5102	0.6089

Means in a column followed by the same letter are not significantly (P = 0.05) different

[a] Average of pooled values from the individual treatments as in Table 1

[b] Individual treatment effects

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSION

Etiology of apple replant disease in South Africa

We investigated a possible biological origin of ARD etiology in South Africa by the dilution of replant field soil with fumigated soil. Seedlings planted into only 25% replant soil, still consistently exhibited ARD symptoms similar to those occurring in 100% replant soil. The elements responsible for stunted growth and root discolouration could therefore not be reduced to a non-damaging level by dilution of the original ARD soil from 100 to 25%. This indicates that ARD in South Africa is primarily of a biological nature.

Fungal populations associated with ARD soils were characterised to the generic level and the impact of soil amendments on these fungal populations evaluated. *Pythium* and *Cylindrocarpon* spp. were consistently isolated from all six replant soils in all four trials that formed part of this study, indicating that these fungi may have a role in ARD development in South Africa. However, we have to keep in mind that this study involved only 3-month-old seedlings. It is possible that *Pythium* and *Cylindrocarpon* are well adapted primary colonisers on apple seedlings. Consequently, the low incidence of other pathogens revealed in this study may underestimate their role in ARD etiology in South Africa. Therefore, further studies are recommended. Furthermore, because identifications were not made to species level and no pathogenicity tests were performed, it is difficult to correlate growth of treated plants with the frequency of isolation of fungi from these plants. High percentages of *Cylindrocarpon* infection together with increased *Pythium* populations were not reflected in growth measurements from compost treated plants. It therefore seems that in some cases the application of compost had an overriding effect on pathogens. However, this hypothesis needs to be confirmed.

Nematodes implicated in ARD development were inconsistently associated with the ARD soils used in this study, indicating that nematodes do not have a primary causal role in ARD etiology in South Africa.

Alternative control measures

Results from pot trials as well as field trials indicate that application of organic amendments could possibly substitute soil fumigation in replanted apple orchards.

Compost as well as sterilised and unsterilised compost teas significantly increased seedling growth even under optimum nutrient conditions when compared to the control, suggesting that they negate the effects of ARD. Results also indicated that applying high concentrations of compost does not necessarily provide any additional growth benefits compared to lower concentrations. In all three field trials established compost or manure combined with mulch consistently increased growth compared to the control. Furthermore, application of these organic amendments increased growth almost to the same extent as the standard chemical treatments, methyl bromide and chloropicrin.

However, compost quality standards need to be implemented to obtain consistent results with organic amendments. There are differences in the composition of various composts and there is no single compost that is universally suitable for disease suppression of all soil-borne pathogens. It is therefore necessary to determine which microbial mixtures provide effective disease suppression in specific soil environments and then to formulate and apply these various compost types for use in the appropriate environments.

Replacement technology can only compete if it is less costly and provides long-term disease suppression. Application of these biological soil amendments results in an increase in microbial diversity in the soil, leading to the establishment of a new soil microbiological equilibrium that restores the soil microflora to conditions that is again conducive to apple tree growth. This effect can last for years if the beneficial organisms are sustained through other ecologically effective management practices. Results showed that surface application of a small amount of compost per tree was sufficient to achieve a significant increase in first year as well as second year growth. There is an indication that applying compost costs only a third of methyl bromide fumigation. In addition, the increasing costs of synthetic fertilisers, also make the use of organic amendments more cost competitive.

Results with biocontrol products were variable. Therefore ARD management with these biological soil amendments cannot be guaranteed at this stage and further studies is recommended. Elucidation of the exact mechanisms of disease control is necessary for implementation of biocontrol products into disease control strategies over a wide geographical area.

Appendix 1. Chemical analysis for the topsoil (0-30 cm) of the ten soils in the study.

Soil	pH (KCl)	Resistance (ohms)	P (mg kg^{-1})	K (mg kg^{-1})	Ca ($cmol_c kg^{-1}$)	Mg ($cmol_c kg^{-1}$)	K ($cmol_c kg^{-1}$)	Na ($cmol_c kg^{-1}$)	S-value ($cmol_c kg^{-1}$)	Cu (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	B (mg kg^{-1})
1*	6.4	1360	178	196	11.27	1.68	0.50	0.15	13.60	6.46	8.7	35.0	0.95
2	6.4	880	142	94	9.62	1.31	0.24	0.09	11.26	4.44	10.6	22.8	0.77
3	5.5	1350	23	163	5.46	1.11	0.42	0.18	7.17	4.20	2.3	14.2	0.90
4	5.7	950	47	184	8.07	1.11	0.47	0.10	9.75	4.34	3.9	26.5	1.23
5	5.7	1440	18	176	5.03	1.33	0.45	0.14	6.95	5.43	7.7	23.2	0.89
6	6.0	1200	14	301	11.49	1.66	0.77	0.11	14.03	5.11	8.5	23.4	1.07
7	6.7	720	13	141	6.00	0.60	0.36	0.05	7.01	6.07	4.7	16.2	0.55
8	5.8	510	22	211	6.77	1.09	0.54	0.11	8.51	5.22	18.4	24.3	1.42
9	6.6	700	73	160	6.00	0.61	0.41	0.07	7.09	6.12	4.1	15.3	0.55
10	6.9	860	34	164	12.11	0.95	0.42	0.08	13.56	8.92	4.1	36.9	0.72

* Soils 1-7 used in Chapter 2 and soils 1-6 as well as all ten soils, used in Chapter 3.

Appendix 2. Particle size distribution (%) and available moisture for the topsoil (0-30 cm) of the ten soils in the study.

Soil	Clay (%)	Silt (%)	Fine sand (%)	Medium sand (%)	Coarse sand (%)	Gravel and stone (%)	Available moisture (mm m^{-1})
1	25.6	26.0	31.8	4.6	12.0	53.0	90.3
2	5.2	13.1	23.3	32.9	25.5	0	124.0
3	35.2	36.8	21.4	1.4	5.2	40.3	161.5
4	22.6	19.8	37.2	2.0	18.4	59.6	78.4
5	17.4	16.2	57.8	4.2	4.4	30.6	172.3
6	19.0	30.8	29.4	8.0	12.8	56.9	99.7
7	10.9	10.1	31.0	33.0	15.0	22.0	81.8
8	29.4	36.0	25.2	3.2	6.2	35.5	86.3
9	8.2	22.0	26.2	27.2	16.4	13.8	99.6
10	17.0	16.0	58.2	3.0	5.8	41.4	108.7