**Apple Replant Disease: Microbial Consortium Responsible for Cause and Control**

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**Abstract**

The poor growth response of fruit trees after replanting on the same site which previously supported the same or closely related species is termed as “replant disease” or “replant disorder” and has been documented from all the apple growing regions of world (Tewoldemedhin *et al*., 2011a). The abiotic factors exacerbate the symptoms but the disease is primarily a biological phenomenon (Mazzola,1998). Apple replant disease (ARD) is a complex syndrome and its etiology is controversial. A multiphasic approach (conventional and molecular) has revealed that the disease is caused by a consortium of biological agents like oomycetes (*Phytophthora, Pythium*), higher fungi (*Rhizoctonia, Cylindrocarpon* etc.) and nematodes *(Pratylenchus*) and some may act synergistically (Van Schoor *et al*., 2008.,Tewoldemedhin *et al*.,2011b). Progress in ARD management has been slow due to complexity of causal factors. The potential hazards of chemical control to human health has necessitated the development of more sustainable measures. Incorporation of green manures *(Brassica sp*.) in disease management programs has received significant consideration due to their capacity to suppress plant pathogens and parasites through the release of glucosinolate hydrolysis products and through the changes in microbial community composition (Mazzola and Mullinix, 2005). Several studies have demonstrated benefits resulting from application of plant growth promoting and disease suppressive rhizobacteria to subsequent growth of apple in replant soil (Bharat, 2011). *Pseudomonas putida* strain 2 CB isolated from apple roots which to inhibit the growth of fungal complex reportedly incited replant disease, enhanced growth of M-26 root stock in multiple apple replant soils (Mazzola *et al*., 2002). Hence, the approaches that manipulate resident soil biology and induce general soil suppressiveness to apple replant disease can be a long term strategy to manage the disease (Mazzola and Manici, 2012). Engineering the rhizosphere micro-biome seems a promising strategy to manage replant disease (Winkelmann *et al*., 2019). Moreover, the use of tolerant rootstocks like G30 and CG6210 can be the best defence against replant problem*. Malus* germplasm varies in tolerance to apple replant disease and could be used in breeding and selecting clonal rootstocks for improved control of orchard replant pathogens (Isuta and Merwin,2000., Leinfelder and Merwin,2006).

**INTRODUCTION**

The poor growth response of fruit trees after replanting on a same site which previously supported the same or closely related species is termed as“replant disease” or “replant disorder”. Apple replant disease is widespread and has been documented in all the major fruit growing regions of world (Traquair, 1984). Apple replant disease is characterised by the uneven growth of young trees but, when severe disease pressure is encountered, poor growth may be exhibited by majority of the trees on the site and the death of young trees may occur. Symptoms of apple replant disease include severe stunting, shortened internodes, rosetted leaves, small root system, decayed or discoloured roots and reduced productivity (Mazzzola, 1998). Below-ground symptoms include stunted root growth that have a significant reduction in lateral root development and functional root hairs (Caruso *et al*., 1989). Examination of the root system has shown that apple replant disease is associated with premature destruction of the epidermal cells and cortical tissues (Savory, 1966a ; Hoestra, 1968). The disease is economically important as it affects the productive life of an orchard, affected trees bear fruit 2-3 years later than the normal and fail to attain yield comparable to those obtained at disease free sites (Mazzola, 1998)

In 1959, for the first time Borner reported replant problem in apple orchards even after 1 or2 years of cultivation. He attributed this to the presence of chemical compounds like phlorizin, phloretin,*p*-hydroxy hydrocinnamic acid, *p*-hydroxy benzoicacid, and phloroglucinol in root bark and released into the orchard soil after microbial decomposition of fallen root bark (Borner, 1959) and later on several others found no support for the same(Savory,1966b; Rumberger*et al*.,2007). The apple replant disease has been documented long ago but its etiology remained questionable. The factors implicated as causal agents and predisposing factors have varied considerably between the geographic regions or between orchards in the same region. Apple replant disease has been attributed to numerous abiotic factors including low or high soil pH, phytotoxins, unbalanced soil nutrition, heavy metal contamination, poor soil structure, poor drainage and cold or drought stress (Traquair, 1984; Willet*et al*.,1994). Although these factors may contribute to the growth problems, but the evidences that soil pasteurization(Jaffe*et. al.,*1982a) or fumigation (Mai and Abawi,1981., Slykhius and Li,1985) remarkably improved the plant growth provides support to the fact that this disease has biological cause biological phenomenon rather than the result of abiotic factors. However, these factors may exacerbate the symptoms.

The disease is of controversial etiology and various efforts and approaches have been used from time to time to elucidate the microbial consortia responsible for this disease. The differences continue to exist relative to functional disease causing agents, however a convergence has evolved around the fungal, oomycetes and nematode genera that appear to contribute to this disease on global basis. To ascertain etiology is the first step to have a proper management.Therefore, to have a better understanding of the disease, a multiphasic approach should be employed.

**APPROACHES TO ASCERTAIN ETIOLOGY OF APPLE REPLANT DISEASE:**

**STUDYING MICROBIAL ECOLOGY**

This disease syndrome and its etiology have been described in North America (Braun,1991; Jaffe *et al*.,1982b; Mazzola,1998) and Europe (Hoestra,1968; Manici et al.,2003;Savory,1966a) as well as many other parts of the world, including South Africa, China, NewZealand, and Tasmania (Fullerton*et al.,*1999; Tewoldemedhin*et al*.,2011a., Utkhede,2002; Van Schoor *et al*.,2009; Wilson,2004).The disease is caused by the consortia of micro-organisms and abiotic factors exacerbate the disease, but are not the primary cause of disease. So studying the etiology of the disease needs a multidisciplinary approach and the studies should be carried out in the soils that actually express the disease.

There are several reports that are in agreement regarding the cause of replant disease, ambiguity remains because of diversity of biological entities reported as possible causative agents, many of which lack significant experimental evidence. For instance, *Trichoderma* spp. were cited as a causal agent of apple replant disease (Utkhede *et al*., 1992) but have also been reported as a potential alternative for the control of this disease syndrome (Kandula *et al*., 2010). Various fungi, and other organisms, have been implicated as causal agents without an attempt to ascertain whether they are inherently associated with apple roots in an orchard setting or whether qualitative or quantitative differences existed in such populations between orchards (or individual trees) exhibiting symptoms of replant disease and orchards free of the disease. Some microorganisms not considered as root pathogens, including *Bacillus subtilis*, *Penicillium* spp., and *Mortierella* sp., have been reported as causal agents of replant disease (Mazzola and Manici, 2012). Different approaches have been followed by many researchers to study the microbial ecology associated with apple replant disease which are as follows:

1. **Isolations and pathogenicity tests:**

The wide variety of fungi, bacteria, actinomycetes, nematodes have been isolated from the rhizosphere as well as the roots of apple trees and tested for their pathogenicity. The causal agents varied from region to region or from orchard to orchard. The pathogens, *Phytopthora cactorum, Pythium spp., Cylindrocarpon destructans and Rhizoctonia solani* were consistently isolated from the symptomatic trees in the orchrds in Washington and were pathogenic to apple. However populations of *Pratylenchus penetrans* were below the damage threshold level in eight of nine orchards surveyed (Mazzola, 1998). *Pythium intermedium*, *Rhizoctonia solani, Cylindrocarpon* spp. and *Fusarium solani*, around 75% of the root colonizing fungi, belong to the root rot complex reported to have a causal role in the development of apple replant disease. Among *Fusarium* spp., only *F.solani* showed a low pathogenicity, confirming the secondary role of *Fusarium* spp. in apple replant diseases. In south Tryole, Italy *Rhizoctonia* and *Pythium* were the most important agents of apple root rot complex, both for their root infection frequency and pathogenicity (Manici*etal*.,2003). *Phytophthora* and *pythium* were dominant in NY. Ithaca orchards (Rumberger*et al*.,2007). Van Schoor*et al*. (2009) consistently isolated *Cylindrocarpon, Fusarium, Pythium and Rhizoctonia spp*. from lesions on apple roots grown in six ARD soils of South Africa in 2000 and 2001. Tewoldemedhin*et al*. (2011c) recovered 540 *Cylindrocarpon* isolates, among these 133 were identified to the species level. In their pathogenicity tests most of them caused a significant root rot. In South Africa isolates of *Ph. cactorum, P. irregulare, P. sylvaticum and P. vexans* examined were highly virulent, and significantly affected all three measured plant parameters. *Pythium dissotocum, P. folliculosum and P. heterothallicum* were considered as moderately virulent*. P. attrantheridium* varied in pathogenicity with the one isolate only causing root rot. Among the *Fusarium* isolates, only two affected plant development; *F. avenaceum*, caused significant root rot and *F. solani* induced a significant reduction in seedling height (Tewoldemedhin *et al*., 2011b).

**Molecular Approaches**:

Traditional techniques employed to describe the composition and diversity of microbial populations in soils have commonly relied on phenotypic characteristics. Such an approach has provided an incomplete assessment of microbial diversity in soil ecosystems, as the application of phenotypic methods is restricted to culturable micro-organisms. As has been well documented, the proportion of culturable microbial community residing in environmental samples is very less, this approach may result in underestimation of diversity of soil-inhabiting microorganisms (Amman*et al*.,1995; Bridge and Spooner, 2001., Hawksworth,1991). Molecular analytical tools have recently been applied to characterize the microbial population in soil ecosystems and have provided new insights into the diversity of microbial species. Thus, application of such tools to study soil microbiology and soil borne plant pathogens may provide vast insights into the diversity, functionality of micro-organisms and their interactions with one another and with the plant roots. Some important molecular techniques used for the purpose are as follows.

**PCR METHODS**

DNA-based methods currently employed to characterize soil microbial community composition in large part rely upon use of the polymerase chain reaction for amplification of the small subunit rRNA gene. PCR amplification of rRNA genes or other ecologically significant genes generates relatively less biased information on microbial communities than do culture-based methods. PCR-based methods hold their own set of limitations and biases because of possible limits from primer design, relative differences in cell lysis efficiency among organisms, or differential amplification of the target sequence (Mazzola, 2004). However, these methodologies can still provide more information regarding microbial diversity in soils than physiological or culture-based methods. The various methods used to asses and compare the microbial community in replant infected and uninfected soils are as under:

**1)Real-Time PCR:**

Real-time PCR or quantitative PCR (qPCR) is a powerful tool for detection and quantification of microbial genera or species from soil and plant material (Schena *et al*., 2004; Lievens *et al.,* 2005; Kernaghan *et al*., 2007; Ophel-Keller *et al*., 2008). Although rRNA genes have been targeted to characterise microbial communities from environmental samples, other genomic regions may also be used. Real-time PCR can detect small quantities of target DNA from microbially complex environmental samples and has been used frequently in studies of disease risk assessment (Cullen *et al.,* 2001; Heuser and Zimmer,2002; Vandermark et al.,2002). Quantitative real-time PCR (qPCR) is more sensitive as well as quantitative in comparison to convential PCR. Therefore, qPCR is very useful for the early (when microbial load is very less in the sample) and accurate detection and quantification of soilborne plant pathogens in soil and plant material (McCartney *et al*., 2003; Lievens *et al*. 2006).

To study the etiology of apple replant disease, qPCR has been used to detect and quantify the pathogens infecting roots. Tewoldemedhin *et al*. (2011b) used qPCR for the quantification of most virulent oomycetes viz *Phytophthora* and *Pythium* species from the roots of apple seedlings. Tewoldemedhin *et al*.(2011a) again used qPCR for the quantification of *Rhizoctonia solani AG-5, Cylindrocarpon spp., Pythium sylvaticum, Pythium vexans, Pythium irregular, Pythium ultimum* and *Phytophthora spp.* There was no correlation between pathogen DNA and reduction in seedling height and weight. This could be explained by several factors. First of all, pathogens attack different sections of the seedling root systems (Agrios, 2005). Therefore, the very low root DNA concentrations of *P. ultimum, P. sylvaticum* and *P. irregulare* may be due to these pathogens mainly functioning as root pruners (Martin and Loper, 1999). In contrast, root DNA concentrations of *P. vexans* and *Phytophthora* were detected at much higher concentrations, suggesting that these pathogens may be more prone to colonize larger roots instead of being fine-root pruners. This is an interesting observation because *P. vexans* has been hypothesized as being more closely related to *Phytophthora* than to *Pythium*. Therefore, it has been suggested recently that *Pythium* species in clade K, into which *P. vexans* falls, should be placed into a new genus named *Phytopythium* (Bala *et al*., 2010; Levesque and De Cock, 2004). Secondly, the pathogens may differ in the mechanisms by which they invade roots and cause host damage. Some isolates may have a higher potential for causing host cell death through the production of cell wall enzymes, toxins or effectors, and thus do not have to establish extensive host colonization in order to reduce host growth. A quantitative real-time polymerase chain reaction (qPCR) method was developed for simultaneous detection of various *Cylindrocarpon* species from apple roots (Tewoldemedhin*et al*.,2011c).

**T-RFLP and DGGE:**

These methods were used to asses the differences in microbial communities in replant soils. Methodologies including DGGE, temperature gradient gel electrophoresis (TGGE) (Muyzer and Smalla, 1998), and terminal restriction fragment length polymorphism analysis (RELP) (Liu *et al*., 1997), among others, enable the separation of complex mixtures of PCR products generated from the amplification of DNA from environmental samples. Total group populations can be assessed or more specific elements of a community may be examined through the use of primers specific to a group of interest, such as actinomycetes (Heuer *et al*., 1997) or mycorrhizal fungi (Redecker, 2000), or primers targeted toward a specific functional gene, such as a nitrogenase gene (Tan *et al*., 2003) or antibiotic biosynthetic gene (Bergsma *et al*., 2003). The resulting profiles can be used as fingerprints for the tested soil microbial community, and functionality of the microbial communities in different soils can be compared. A more detailed information regarding the microbial population in a particular sample may be obtained by using group-specific rRNA primers or by conducting hybridization analysis with taxon-specific probes. Both alternatives may reduce the complexity of patterns produced by DGGE relative to that obtained when primers targeting the “total” bacterial or fungal community structure are used (Mazzola,2004). Laurent *et al*. (2010) used DNA fingerprinting T-RFLP to asses the rootzone fungal and bacterial communities. Soil bacterial and fungal community composition was assessed in Orchard and Bioassay soils and Bioassay roots with a DNA fingerprinting method (T-RFLP). A clone library of 267 soil bacteria was developed from sampled Orchard soils and Bioassay roots. These communities were dominated by Acidobacteria (25% of sequences), Actinobacteria (19%), δ-Proteobacteria (12%), β-Proteobacteria (10%), and these ratios differed among the ground cover management systems (Laurent*et al*.,2008).Bacterial community composition as assessed by PCR-DGGE differed between the trees grown in old grass lanes as compared to the old tree rows of the previous orchard (Rumberger*et al*.,2004).

**PLANT INDUCED CHANGES IN MICROBIAL COMMUNITY STRUCTURE:**

Replant problems occurs in soils that are utilised for apple cultivation for extended periods. However, there are evidences of symptom development after brief period of apple cultivation (Mazzola 1998, 1999). A soil microbial community capable of inciting symptoms of apple replant disease developed within 3 years of orchard establishment. There was increase in the recovery of *Cylindrocarpon*, *Phytophthora*, *Pythium*, and *Rhizoctonia* and reduction in recovery of *Burkholderia cepacia* with prolonged orchard establishment and transformation of the fluorescent *Pseudomonas* population from one dominated by *Pseudomonas putida* to one comprised almost exclusively of *Pseudomonas fluorescens* bv. III and *Pseudomonas syringae* (Mazzola *et al.,* 2002). This suggests that inoculum builds up in response to apple planting and supports the role of fungi and oomycetes in apple replant disease.

**ROLE OF ROOTSTOCK GENOTYPES**:

Rootstock genotypes have varied rhizosphere microbial community composition. Thus, studies on rootstock genotypes could lead to important insights into etiology of apple replant disease. Root-zone fungal and bacterial community composition, assessed by DNA fingerprinting (T-RFLP), differed between M.26 and CG.6210 (Laurent *et al*., 2010). There were significant differences in the composition of bacterial and actinobacterial communities between the rhizospheres of replant disease susceptible rootstocksM7 andM26 compared to tolerant rootstocks CG30 and CG210 (Rumberger *et al*., 2004). Geneva series rootstocks are less susceptible to root infection by native populations of *Pythium,* whereas M26, MM106, and MM111 are highly susceptible. Apple rootstocks from the Geneva series consistently supported lower populations of *P. penetrans* than or Malling-Merton rootstocks (Mazzola*et al*., 2009).

**INSIGHTS INTO ETIOLOGY THROUGH MANAGEMENT STRATEGIES:**

Management strategies also confirmed the biotic nature of disease since soil fumigation and pasteurisation at replant sites or in green house experiments increased plant growth (Covey *et al*., 1979., Jaffe *et al.,* 1982b., Ross *et al*., 1983., Covey *et al*., 1984). Evaluation of non-fumigant measures for disease control have also helped in ascertaining the role of multiple agents in disease development. In studies conducted in Washington state, the inability of alternative methods to achieve fumigant levels of disease control could be linked to a failure to protect against multiplecausal agents (Mazzola and Mullinix, 2005., Mazzola and Brown, 2010).)

After reviewing the results from several researchers, it can be concluded that the pathogenic species that have evolved as incitants of apple replant disease fall in genera viz, *Phytophthora, Pythium, Rhizoctonia, Cylindrocarpon, Fusarium, Pratylenchus,* (Table I) varying from region to region and some may act synergistically

**Table I:Pathogenis species involved in ARD complex**

|  |  |
| --- | --- |
| PATHOGEN | REFERENCE |
| OOMYCETES | |
| *Phytophthora cactorum* | Manici*et al*.(2003) |
| *Pythium sylvaticum* | Manici*et al.*(2003) |
| *Pythium irregular* | Manici *et al.*(2003) |
| *Pythium ultimum* | Manici *et al.*(2003) |
| *Pythium vexans* | Manici *et al.*(2003) |
| *Pythium intermedium* | Manici*et al*. (2003) |
| *Pythium littorale* | Manici*et al.*(2003) |
| HIGHER FUNGI | |
| *Fusarium avenaceum* | Tewoldemedhin *et.al*.(2011) |
| *Fusarium solani* | Tewoldemedhin *et.al*.(2011) |
| *Rhizoctonia solani AG-5* | Manici *et al.*(2003) |
| *Rhizoctonia solani AG-6* | Manici *et al.*(2003) |
| *Cylindrocarpon destructans* | Manici *et al.*(2003) |
| *Cylindrocarpon lucidum* | Jaffe *et al*.(1982b) |
| *Cylindrocarpon macrodidymum* | Tewoldemedhin *et al*.(2011) |
| *Cylindrocarpon liriodendra* | Tewoldemedhin *et al*.(2011) |
| *Cylindrocarpon pauciseptatum* | Tewoldemedhin *et al*.(2011) |
| NEMATODES | |
| *Pratylenchus penetrans* | Jaffe *et al*.(1982b), Mazzola *et al*.(1999) |
| *Pratylenchus scribneri* | Tewoldemedhin *et al*. (2011) |
| *Pratylenchus detallrie* | Tewoldemedhin *et al*. (2011) |
| *Pratylenchus sp.* (from kashmir) | Zaki and Mantoo(2003), Askary *et al*(2012) |

**MANAGEMENT STRATEGIES:**

* Chemical control(Fumigants, nematicides, fungicides)
* Physical control(Pasteurization)
* Cultural control(Planting position, ground covers,)
* Soil amendments (Compost, Seed meal)
* Biological control
* Soil suppression
* Host genetics

**CHEMICAL CONTROL**

The general biocides like methyl bromide or chloropicrin, volrex have been found effective against apple replant disease and the nematicides like 1, 3-dichloropropene (1, 3-D) and ethylene dibromide is effective against root lesion nematodes (Benson *et al*., 1978., Sewell and White, 1979., Mai and Abawi, 1981., Ross *et al*., 1983 and Covey *et al*., 1984). The broad spectrum fungicides like difenaconazole and metalaxyl were effective in controlling higher fungi and oomycetes involved in this complex, respectively. Fenamiphos controlled nematode populations (Mazzola, 1998., Tewoldemedhin *et al*., 2011a). Methyl bromide fumigation has satisfactorily controllled the disease. However, this chemical was declared an ozone depleting substance and its removal from the market in compliance with the Montreal Protocol has intensified the need for alternative measures of ARD control ([WMO, 1994](http://www.sciencedirect.com/science/article/pii/S0304423808003105#bib54)). The high cost of chemical control and its concerns reagrding human health and the environment has necessitated the development of alternative and environment friendly means of control.

**PHYSICAL CONTROL**

In various greenhouse and pot experiments, pasteurisation of field soil prior to planting was found to increase the plant growth compared to control. Apple seedlings (Northern Spy) exhibited severe stunting and root discolouration when grown in steamed (75ºC for 30 min.) field soil amended with 5% (v/v) untreated field soil obtained from orchard with a history of apple replant disease (Jaffe *et al*., 1982b). Treatment of field soil prior to planting with gamma radiations or heating (60ºCor higher for 30 min.) improved plant growth and reduced root discolouration (Jaffe *et al*., 1982a). Pasteurization of soil at 50°C enhanced growth of apple in all four orchard soils tested, and this corresponded with significant reductions in populations of soil fungi. In addition, dramatic changes in the fungal community isolated from seedlings grown in these soils were observed in response to pasteurization temperatures that enhanced plant growth. Mazzola, (1998) reported that species of *Cylindrocarpon*, *Fusarium*, and *Rhizoctonia* comprised most isolates recovered from apple roots in natural soils, while *Trichoderma* spp. Were predominant fungal agents present in the roots of apple grown in pasteurized soils.

**CULTURAL CONTROL:**

**Planting Position:**

Planting position has got marked effect on growth of replanted trees and on microbial community composition as well. Rumberger *et al*.,( 2004) reported that there is significant difference in growth of fruit trees and composition of rhizosphere bacteria and actinobacteria in old tree rows and grass lanes. In a field trial at the site that had history of apple cultivation for more than 90 years , tree planting position affected tree growth more strongly over the first three years (Leinfelder and Merwin, 2006). There are evidences that replanting in inter-row can minimize replant disease but potential fungal pathogens are endemic to soil, therefore replanting should be associated with strategies increasing soil suppressiveness in established orchards (Kelderer *et al.,* 2012).

**Cover Crops:**

Various attempts have been made to manage the orchard floor to manipulate soil biology. Because of the complex nature of replant disease, such strategy may not be appropriate in all the cases. Among specific replant disease pathogens, the use of cover crops to control *P. penetrans* has received a great attention. Several studies have investigated the use of non-host plant species or species that directly suppress nematodes populations via the production of allelochemicals. Apple was found to grow well after a cover crop of prairie grass or oats, but trees were severely stunted when grown in the same soils after a cover crop of rye or blue lupin. This differential response was attributed to the fact that rye and lupin were much better hosts for the root-lesion nematode than were prairie grass or oats (Colbran,1979). A diversity of cover crops have been evaluated against *P. penetrans* populations (Pruyne *et al.,* 1994); however, it was found that the efficacy may vary from one orchard to another (Merwin, 1995). Long-term soil treatments with different cover crops influenced the apparent severity of apple replant disease. Apple seedling growth was closely linked with bacterial community composition, but seedling growth responses were not closely correlated with widely accepted indicators of soil quality such as soil organic matter content, macro- and micro-nutrient availability, and pH (Laurent *et. al*., 2008). Wheat cover crops also reportedly have an effect on microbial community composition. Wheat was the chosen cover crop, as it was observed that soil collected from a field formerly cropped to continuous wheat monoculture was suppressive to disease incited by the apple pathogen R. solani ([Mazzola, 1999](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2586500/#B26)). In greenhouse trials, application of successive short-term wheat cropping sequences to soils enhanced growth of apple seedlings subsequently planted into these orchard soils due to reduction in root infestation by R. solani and Pratylenchus penetrans, as well as significant increases in rhizosphere populations of fluorescent pseudomonads and a transformation in composition of the population to one dominated by P. putida (Mazzola and Gu, 2000) In green house trials, disease suppression was induced in a wheat cultivar-specific manner (‘Penawawa’ > ‘Rely’ > ‘Eltan’) (Mazzola *et al*., 2002). In field trials in Washington, 1-year wheat cover crop consisting of three short-term cropping periods with plant material removed at the end of each growth period and a 3-year *B. napus* green manure significantly enhanced vegetative growth and yield of Gala/M26. However, in each instance, the resulting disease control and growth response were inferior to that achieved through preplant methyl bromide soil fumigation. This is due to the fact that cover crops reduced the infection by *Rhizoctonia solani* AG-5 and *Pratylenchus penetrans* but failed to reduce the infection by *Cylindrocarpon sp*.(Mazzola and Mullinix, 2005). Wheat cultivation during orchard renovation appears to hold promise as means to suppress most but possibly not all components of thepathogen complex that incites replant disease. Integration of this approach with biocidal plant products or cultural practices may provide effective and economically feasible disease control on replant sites (Mazzola *et al*., 2002).

**SOIL AMENDMENTS:**

**Compost amendments:**

In addition to being able to offer nutrients and increase the soil's capacity to store water, organic amendments have a number of other advantageous qualities. Compost has been shown to have disease-suppressive properties (Hotink et al., 1997; Noble and Coventry, 2005), and their innate microbial activities have been primarily implicated in the mechanisms of disease suppression (Ristaino and Thomas, 1997). Additionally, adding a carbon source to the soil stimulates its overall biological activity (Campbell, 1989; Magarey, 1999); and soils rich in a variety of health-promoting microbes are more likely to be disease-suppressive (Lazarovits, 2001).

Compost amendments improved growth in newly planted apple orchards ([Moran and Schupp, 2001](http://www.sciencedirect.com/science/article/pii/S0304423808003105#bib33)), and [Neilsen *et al*. (2003)](http://www.sciencedirect.com/science/article/pii/S0304423808003105#bib36) found that a variety of organic material benefited growth of young apple trees in high-density plantings. On the other hand, compost and other organic amendments have also been reported to be mainly ineffective in controlling ARD in some studies (Granatstein and Mazzola, 2001., Rumberger *et al*., 2004., Neilsen *et al*., 2004., Wilson *et al*., 2004 and Leinfelder and Merwin, 2006). However in certain cases compost amendments have been found to be effective in ARD management. Van Schoor *et al*. (2009) obtained positive results with compost amendments in both pot as well as field experiments. The disease-suppressive effects of compost have generally been attributed to biological effects, either direct or indirect. Compost is therefore a dynamic medium and compost quality factors need to be controlled to ensure consistent results. Much of the variability reported from different studies may be attributed to different experimental conditions and compost types used. Composts used in trials with positive results were aerobically produced mainly from a combination of straw and manure and inoculated with beneficial microorganisms. Thus, compost quality standards need to be implemented.

**Seed meal amendment:**

Biologically based treatments such as the use of soil organic residue amendments have been promoted as alternatives to the use of broad-spectrum biocides for the management of soilborne plant pathogens. Members of the plant family Brassicaceae, including *Brassicanapus*, produce glucosinolates which, upon hydrolysis, yield biologically active compounds, including isothiocyanates. Isothiocyanates have a remarkable antimicrobial activity; therefore, these plants have been used as “biofumigants”, release active hydrolysis products when added to soil (Angus *et al*., 1994., Brown *et al*., 1997). However, some studies suggest that these plant residues may suppress fungal pathogens via a different mechanisms. For example, *Brassica napus* residues have been found effective against soilborne plant pathogens (Mazzola *et al*., 2001., Cohen *et al*., 2005., Mazzola and Mullinix, 2005). In contrast, some reports suggest that these plant residues yield ITCs having relatively low antimicrobial activity (Manici *et al*.,1997). Brassica seed meal amendment has got nematicidal or nematistatic effect and bring about shifts in microbial community composition. Control of *Rhizoctonia solani* and *P. penetrans* was obtained via the incorporation of rapeseed meal (RSM) regardless of the glucosinolate content of the amendment (Mazzola *et al*.,2001). RSM is a high-nitrogen-containing product, and suppression of lesion nematodes may be attributed to the oftencited nematicidal or nematistatic effect of nitrogenous amendments (Rodriguez-Kabana, 1986., Oka and Pivonia, 2002). However, RSM-induced control of *R. solani* does not appear to operate via chemical inhibition of hyphal growth in soil (Cohen *et al*., 2005) but, through an influence on the structure soil microbial community (Cohen and Mazzola, 2006., Mazzola*et al*., 2007). Mazzola*et al*. (2007) found that *B. juncea* seed meal amendment suppressed *R. solani* suppression in a temporal manner, which initially was associated with the generation of allyl isothiocyanate and lateron, via proliferation of resident *Streptomyces* spp. and not because of qualitative or quantitative attributes of seed meal glucosinolate content. Preplant RSM amendment in conjunction with a postplant mefenoxam soil drench provided effective suppression of ARD, and the resulting tree growth and yield were comparable with that attained in response to fumigation in one orchard. At a second orchard, the growth response attained with the alternative treatment was inferior to preplant soil fumigation, which was associated with an apparent re-infestation of RSM-treated soils and tree roots by *Pratylenchus* spp. Application of RSM after wheat cropping or in conjunction with soil solarization provided an intermediate level of disease control and a corresponding reduction in growth and yield of apple relative to preplant fumigation at both sites (Mazzola and Mullinix, 2005). The utilization of brassicaceous seed meal amendments for replant disease suppression must employ an appropriate rootstock in order to achieve optimal disease control as *B. juncea* SM suppressed lesion nematode root populations irrespective of rootstock while as nematode suppression in response to *B. napus* or *S. alba* SM was only observed when used in concert with a tolerant rootstock (Mazzola *et al*., 2009). The problem with some *Brassica*SM amendments is that these stimulate the populations of *Pythium sp.*and the infection of apple roots by them. Among those tested, only *B. juncea* seed meal did not stimulate orchard soil populations of *Pythium.* Although application of *B. napus* seed meal alone consistently induced an increase in *Pythium* spp. populations, no significant increase in *Pythium* spp. populations was observed in response to a composite *B. juncea* and *B. napus* seed meal amendment. Therefore, the use of a composite *B. juncea* and *B. napus* seed meal mixture can provide superior control of the pathogen complex inciting apple replant disease relative to either seed meal used alone (Mazzola *et al*., 2007).A series of trials have also demonstrated that *Brassicaceae* SM soil amendments have predictable impacts on the overall soil microbial community and provide control of specific soilborne pathogens of apple in a consistent manner. In these studies, *Brassicaceae* SMs were applied at a rate of 8–10 t ha−1(Mazzola *et al*., 2001., Cohen et al., 2005., Cohen and Mazzola, 2006 ).

**BIOLOGICAL CONTROL:**

Biological control refers to the purposeful utilization of introduced or resident living organisms, other than disease resistant host plants, to suppress the activities and populations of one or more plant pathogens. This may involve the use of microbial inoculants to suppress a single plant pathogen or this may involve managing soils to promote the combined activities of native soil- and plant-induced microbial diversity to increase general disease suppression (Pal and Gardner, 2006). Chemical inputs in agriculture must be decreased due to worries about how they may affect both human health and the environment. It is possible to maintain greater biological diversity and balance in the environment using appropriate biological controls for the management of plant pathogens, which could result in more long-term sustainable agricultural production practices and, in some cases, more effective disease control than is currently possible. (Larkin *etal*., 1998). Many factors are responsible for the poor transition of biocontrol agents from *in vitro* test strains to commercial products (Roberts and Lohrke, 2003). Within the context of soilborne plant disease management, attempts to employ microbial biological control have typically involved the inundative release of non-native microorganisms into soil systems. Such an approach assumes that the introduced microbial agent or mixture will effectively compete with the resident microbial community, efficiently colonize the rhizosphere of the targeted plant and persist in the rhizosphere at the threshold population required for activity during the period of plant susceptibility, and also that the active mechanism is operative in the environment into which it has been applied. Soil dwelling microbial antagonists of plant pests and pathogens have been studied extensively asto their role in the development of soil suppressiveness (Weller *et al.*, 2002). Chemical control with soil fumigants is the most adopted method of controlling replant disease all over the world. But it is not an attractive approach as the effectiveness of volatile fumigants is influenced by temperature and moisture. Also the application of fumigants is difficult, expensive and hazardous and is believed to destroy the natural equilibrium between pathogens and antagonistic microorganisms in soil. In this regards attempts have been made to develop a biological control of replant problem. A number of studies have demonstrated benefits resulting from application of plant growth promoting and disease suppressive rhizobacteria to subsequent growth of apple in replant soil (Bharat, 2011). The various biocontrol agents of replant pathogens can be:

* *Trichoderma*
* Plant growth promoting Rhizobacteria.
* VAM (Vesicular arbuscular mycorrhiza)
* Other hyperparasites and endophytes.

***Trichoderma***

Several strains of the fungus *Trichoderma* species have been isolated and found to be effective biocontrol against various soil borne plant pathogenic fungi under greenhouse and field conditions. *Trichoderma viride* are having ability to inhibit soil borne pathogen of different crops like *Rhizoctonia solani* and most commonly used fungal biocontrol agent &have long been known as effective antagonist against plant pathogen (Gaigole, 2011). Application of *Trichoderma* spp. enhanced AM colonisation in SARD (specifi apple replant disease soils) in pot experiment and could be exploited to improve the root quality (Kendula *et al.,* 2006).

**Plant growth promoting Rhizobacteria**

Rhizobacteria that exert beneficial effects on plant growth and development are referred to as plant growth promoting rhizobacteria (PGPR) (Ashrafuzzaman *et al*., 2009). They possess the ability to colonize the roots and either promote plant growth through direct action or via biological control of plant diseases (Kloepper and Schroth 1978). They are associated with many plant species and are commonly present in varied environments. Strains with PGPR activity, belonging to genera *Azoarcus, Azospirillum, Azotobacter, Arthrobacter, Bacillus, Clostridium, Enterobacter, Gluconacetobacter, Pseudomonas, and Serratia,* have been reported (Hurek and Reinhold-Hurek, 2003) Among these, species of *Pseudomonas* and *Bacillus* are the most extensively studied.The principal mechanisms of growth promotion (fig I) include production of growth stimulating phytohormones, solubilization and mobilization of phosphate, asymbiotic N- fixation, siderophore production, antibiosis, i.e., production of antibiotics, production of lytic enzymes (Proteases, chitinases, glucanases etc.), inhibition of plant ethylene synthesis, and induction of plant systemic resistance to pathogens (Richardson *et al*. 2009; Idris et al. 2007; Gutierrez-Manero et al. 2001; Whipps 2001., Sarvanakumar*et al*., 2007 ).To date, many studies on biological control of plant pathogens by antagonistic bacteria focus on the suppressive effects of single strains introduced repeatedly into soil or on



**Figure I: Schematic illustration of important mechanisms known for plant growth promotion by PGPR (pic source: internet)**.

planting material at relatively high densities. Contrary to this inundative strategy, crop rotation and organic amendments have been used to manage and manipulate naturally occurring antagonistic microorganism communities. Even though these tactics have produced extremely effective biological control methods, they have received relatively less attention. (Hoitink & Boehm 1999). Several studies have demonstrated benefits resulting from application of plant growth promoting and disease suppressive rhizobacteria to subsequent growth of apple in replant soil (Caesar and Burr, 1987; Utkhede and Li, 1989b; Janisiewicz and Covey, 1983). A diversity of bacterial species has been identified that suppress individual causal agents and enhance growth of plants in replant soil. *Enterobacter aerogenes* has been found to be an effectivebiological against *Phytophthora cactorum* which contributes to replant disease (Utkhede and Smith, 1991). An increase in growth of apple seedlings in replant soil was found with the application of strain B8 of *E. aerogenes* (Utkhede and Li, 1989a). Similarly, BACT-1, EBW and B10 strains of *Bacillus subtilis* and B8 strain of *E. aerogenes* applied as soil drench increased plant growth over and above that of formalin fumigation (Utkhede and Li, 1989b). The inoculation of roots of young apple plants with *Agrobacterium radiobacter* has eliminated replant problems in green house and nursery experiments (Catska and Hudska, 1990). This biocontrol agent reduced the number of colonies of phytotoxic micromycetes which contribute towards replant disease. *Pseudomonas putida strain 2 CB,* which was isolated from apple roots, was found to promote the growth of M-26 root stock in numerous apple replant soil samples while inhibiting the growth of every component of the fungal complex thought to cause replant disease(Mazzola *et al*., 2002). Casear and Burr (1987) identified two fluorescent pseudomonads and an enteric bacterium possessing the ability to promote growth of apple in replant soils. Enhanced growth by these rhizobacteria was associated with a reduction in root infection of *Cylindrocarpon destructans*, a fungal pathogen known as one of the causal agents of replant disease (Jaffe *et al*., 1982a; Mazzola, 1998). Application of *Bacillus subtilis* and *Enterobacter aerogenes* has also been found effective in promoting growth of apple plants under filed condition in British Columbia, Canada (Utkhede and Smith, 1994). *Burkholderia cepacia* was obtained obtained only from CG.6210 (tolerant rootstock) soil and not from the rhizosphere of susceptible rootstocks which indicate that these species of rhizobacteria have a potential for biological control of replant disease. (Laurent *et al.,* 2010).

**VAM Fungi**

Various studies have indicated that phosphorus is an essential nutrient for early growth of young plants in replant soil Improved nutrient uptake can result from mycorrhizae symbiosis, especially for immobile ions like phosphate. (Mosse, 1973). As a result of increased uptake of mineral nutrients from soil, mycorrihizal plants grow more vigorously and appear healthier than those that do not have mycorrhizal associations. However, these beneficial fungi are eliminated when replant disease is controlled by soil fumigation (Nemec, 1980). Inoculation of apple seedlings with arbuscular mycorrhizal fungi (AMF) increased their growth is replant disease soil (Catska and Taube-Baab, 1994). Inoculation of apple seedlings with AMF *Glomus fasciculatum* and *G. macrocarpus* suppressed the population of phytotoxic micromycetes, responsible for replant disease and subsequently increased plant biomass (Catska, 1994). In replant soil, two AMF, Glomus intraradices and G. mosseae, markedly enhanced total shoot length and the number of shoots per rootstock. The seedlings that received the G. mosseae inoculation grew more quickly in the unfertilized and pasteurized replant soil. (Utkhede *et al*., 1992). After sterilizing the soil prior to planting and inoculating it with AMF, Glomus epigaeum considerably reduced the problem of apple and peach replanting. It was found that autoclaved replant soil had greater growth promotion from AMF inoculation. (Bingye and Shengrui, 1998).In the field, population densities of *P. penetrans* in root zone soil and rootswere less for *G. mosseae-*inoculated plants than for non-inoculated plants (Forge *et al.,* 2001). In an experiment, out of different VAM fungi inoculated, *Scutellospora calospora* had the greatest beneficial effect in improving shoot and root dry weight and shoot length in specific apple replant disease soil (Ridgway *et al*., 2008). In a pot experiment with treatments comprising two commercial formulations of Trichoderma spp. in soil conducive to apple replant disease, AMF colonization enhanced plant growth, indicating that there may be interaction between these two groups that can be utilized to treat apple replant disease. (Kandula *et al.,* 2006).

**Other hyperparasites and endophytes**

*Pasteuria* *penetrans* is a bacterium that parasitizes *Pratylennchus penetrans* (Stirling, 1991) and *Pseudomonas chlororaphis* is also an antagonist of Pratylenchus penetrans (Hackenberg *et al*., 2000). Moreover, strong antifungal activity suggested that the endophytic *B. subtilis* ZZ120 and its bioactive components might provide an alternative agent for the biocontrol of replant diseases (Li *et al*., 2012). Various biocontrol agents for replant pathogens that have been reported so far are summarised in given table (Table II).

**Table II: Biocontrol agents of replant pathogens**

|  |  |
| --- | --- |
| **BIOCONTROL AGENTS** | **REFERENCE** |
| *Trichoderma* | Kendula*et al*., 2006 |
| *Pseudomonas flourescence* | Casear and Burr, 1987 |
| *Pseudomonas putida 2C8* | Mazzola*et al*., 2002 (patent) |
| *Pseudomonaschlororaphis (*against *Pratylenchus)* | Hackenberg*et al*., 2000 |
| *Bascillus subtilis* | Utkhede and Li,1989b |
| *Endophytic Bascillus subtilis ZZ120* | Li *et al*., 2012 |
| *Burkhloderia cepacia* | Mazzola*et al*., 2002; Laurent*et al*.,2010 |
| *Frateuria* | Laurent*etal*., 2010 |
| *Agrobacterium radiobacter* | Catske and Hudska, 1990 |
| *Enterobacter aerogenes* | Utkhede and Smith., 1994 |
| *Streptomyces spp* | Mazzola,2007 |
| *Pasteuria penetrans(*against nematodes*)* | Sterling,1991 |
| *Glomus mosseae* | Forge*et al*., 2001 |
| *Glomus intraradices* | Forge*et al*.,2001 |
| *Scutellospora calospora* | Ridgway*et al*., 2008 |
| *Glomus epigaeum* | Bingye and Shengrui, 1998 |
| *Glomus fasciculatum* | Catska, 1994 |
| *Glomus macrocarpus* | Catska, 1994 |

**SOIL SUPRESSION**:

Disease suppressive soils have been defined as those in which disease development is minimal even in the presence of a virulent pathogen and a susceptible host. The concept of disease suppressive soil has been described in terms of both general suppression and specific suppression. Every natural soil possesses some ability to suppress the activity of plant pathogens due to the presence and activity of its complement of resident soil microorganisms (Cook and Baker, 1983). The general suppression and is directly related to the total amount of microbial activity in a given soil rather than operating through the action of a specific microorganisms. While general suppression is a phenomenon that occurs as a result of biological factors, researchers and crop producers have perhaps more frequently sought to manipulate or exploit these biological factors when developing a disease management strategy. By showing that the disease suppressive component may be transferred to a conducive soil through the introduction of very small amounts of the suppressive soil, the microbial contribution to disease suppression is established. The discovery that soil pasteurization could abolish the suppressive component further confirmed the importance of soil microorganisms in disease suppression. (Mazzola, 2010). Soils that have not undergone apple cultivation are suppressive to replant disease. However, in contrast to take-all and potato scab-suppressive soils that are induced by monoculture, orchard soils become progressively more conducive to replant disease the longer the orchard is in production (Weller et al., 2002). Management of resident plant-beneficial rhizobacteria may be a viable method for control of specific soilborne plant pathogens (Mazzola, 2007). Different approches that can lead to the development of supressive soils are as follows:

* application of individual or mixtures of microbial strains.
* Organic amendments.
* Cropping systems.

**HOST GENETICS IN DISEASE MANAGEMENT:**

Host resistance is an effective and economical component of integrated pest management programs. Apple rootstocks vary in their tolerance or susceptibility to apple replant disease (Table III). Recent findings have suggested that novel rootstock clones of Cornell-Geneva may be reasonably tolerant to this soil-borne disease, and apple rootstocks with intrinsic resistance or tolerance to ARD could provide a viable way for treating this disease. (Isuta and Merwin, 2000., Leinfelder and Merwin, 2006). Rootstock genotype has a dominant influence on root characteristics (lifespan, distribution etc.) than any other factor (Yao *et al*., 2006) which can explain their tolerance or succeptibility to replant problem. Rootstocks also structures the microbial community composition. The species composition of rhizosphere bacteria and actinobacteria differed significantly between the planting positions and between the rootstocks M7 and M26compared to CG30 and CG210 (Rumberger *et al*., 2004). The bacterial and fungal rhizosphere community compositions of susceptible rootstocks, differed from those of the tolerant rootstocks, as assessed by T-RFLP (Rumberger *et al*, 2007). Apple rootstock resistance or susceptibility to ARD is correlated with genotype-specific interactions with soil microbial consortia.Four sequences obtained from the CG.6210 root-zone represented the *Burkholderia cepacia* complex, while no sequences from *Burkholderiaceae* were obtained from M.26 soil (Laurent *et al*., 2010). According to reports, a number of Burkholderia cepacia strains, including several that have been linked to ARD, are suppressive to fungi and oomycete root infections. (Bevivino *et al*. 1998., Hebbar *et al*., 1998., Mazzola, 1998). These findings suggest that a diversity of associated antagonistic rhizosphere bacteria might be contributing to the tolerance of CG.6210 to replant disease. While M26, MM106, and MM111 are very sensitive, Geneva series rootstocks have been proven to be less vulnerable to root infection by natural populations of Pythium. P. penetrans populations were consistently lower on apple rootstocks from the Geneva series than on Malling-Merton rootstocks. (Mazzola *et. al*., 2009) Malus germplasm collections contain sources of genetic resistance to ARD that could be employed in breeding and clonal rootstock selection for better management of orchard replant diseases. (Isuta and Merwin, 2000).

**Table III: Apple replant disease tolerant and susceptible rootstocks**

|  |  |
| --- | --- |
| **TOLERANT ROOTSTOCKS** | **REFERENCE** |
| G 30 | Isuta and Merwin,2000. Leinfelder and Merwin, 2006. Laurent*et al*.,2010 |
| CG 6210 | Isuta and Merwin,2000. Leinfelder and Merwin, 2006. Laurent*et al*., 2010 |
| Merton I 793 | Soni*et al*., 2011 |
| CG 5935 | Robinson, 2004 |
| CG 4204 | Robinson, 2004 |
| **SUSCEPTIBLE ROOTSTOCKS** | **REFERENCE** |
| G 65 | Laurent2010 |
| CG 16 | Rumberger*et al.*, 2004 |
| M7 | Rumberger *et al*., 2004 |
| M26 | Laurent*et al*.,2010, Rumberger*et al*.,2007.Mazzola,2003 |
| MM 106 | Mazzola*et al.,*2009 |
| MM 111 | Mazzola*et al*.,2009 |

**CONCLUSION:**

Apple replant disease is a complex syndrome and has been documented from all the apple growing regions. The abiotic factors can exacerbate the symptoms but the disease is primarily a biological phenomenon. Various pathogenic genera have evolved as incitants of this disease like *Pythium, Phytophthora, Fusarium, Rhizoctonia, Cylindrocarpon and Pratylenchus*. However, these may vary from region to region and some may act synergistically. So, studying microbial incitants in a particular region is important for its management. A microbial consortium is responsible for causing the disease and another set of microbes have the potential of biological control of this disease. Thus, the approaches that manipulate functional soil biology and induce general soil suppressiveness can be a long term strategy to manage this disease. Also, use of tolerant rootstocks can be the best defence against this problem. Management of ARD is of serious concern as the land suitable for orchards is limited and replantation has to be done on the same piece of land and thus there is no scope of practices like crop rotation. Due to complex nature of disease, no single alternative strategy for controlling apple replant disease can currently provide the level and consistency of control obtained with methyl bromide or other broad-spectrum soil fumigants. The biological cause of replant disease can vary substantially among orchards, soil types, and regions and biologically based controls are likely to be less consistent in their effectiveness than chemical biocides. Therefore, researchers must now work with the promising alternatives and integrate different approaches into various configurations for eventual field testing.

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