

# APPLE REPLANT DISEASE: MICROBIAL CONSORTIUM RESPONSIBLE FOR CAUSE AND CONTROL

## Abstract

“Replant disease” or “replant disorder” may be defined as poor growth response of fruit trees When replanting is done on the same site which previously supported the same or closely related species. It has been reported from different regions of world, wherever the apple is grown (Tewoldemedhin *et al.*, 2011a). The abiotic factors exacerbate the symptoms but the disease seems to have biotic cause (Mazzola,1998). Apple replant disease (ARD) is a disease complex and its etiology is controversial. A multiphasic approach (conventional and molecular) has revealed that ARD is caused by a consortium of microbes 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). Since ARD is a disease complex, its management has always been a challenging task. The potential hazards of chemical control to human health has necessitated the development of more sustainable measures. Lately, the use of *Brassica sp.* as green manures in disease management has received great attention. Their use suppresses plant pathogens via release of glucosinolates and manipulating in microbial community composition (Mazzola and Mullinix, 2005). Plant growth promoting rhizobacteria have also been found effective in growth promotion of replanted trees (Bharat, 2011). *Pseudomonas putida* strain 2 CB isolated from apple roots is reported inhibit the growth of fungal complex responsible replant disease, enhanced growth of M-26 apple root stock in replanted orchards (Mazzola *et al.*, 2002). Hence, the

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approaches that manipulate resident soil biology and induce soil suppressiveness can be a long-term strategy for ARD management (Mazzola and Manici, 2012). Engineering rhizosphere micro-biome also seems a promising for its management (Winkelmann *et al.*, 2019). Moreover, the tolerant rootstocks like G30 and CG6210 also prove to be the best defence against replant problem. Evaluation of *Malus* germplasm against apple replant disease may also pave the way in identifying the tolerant genotypes that could be used in breeding programs for producing tolerant clonal rootstocks for replant disease management (Isuta and Merwin,2000., Leinfelder and Merwin,2006).

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## I. INTRODUCTION

Replant disease manifests the poor growth of fruit trees when planted in the orchard that had a history of growing same tree species. Apple replant disease has worldwide occurrence and has been found in all the areas of the world, wherever the fruits are grown (Traquair, 1984). Apple replant disease is characterised by the uneven growth of young trees but, when the disease pressure is high, most of the trees show stunted growth and eventually the trees may die. The major symptoms include stunted growth, rosetted leaves, internodes become shortened, under-developed root system and root discolouration (Mazzola, 1998; Caruso *et al.*, 1989). A premature destruction of epidermal cells and cortical tissues was observed when ARD affected roots were examined (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)

Replant problem in apple orchards was reported for the first time by Borner even within few years of replantation in an old orchard. It was presumed that chemical compounds like *p*-hydroxy benzoic acid, phlorizin, phloretin, *p*-hydroxy hydrocinnamic acid, phloroglucinol etc. released into the orchard soil after microbial decomposition of fallen root bark may have led to this problem (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. Several biotic and abiotic factors have been reported to cause this disease, but there is a discrepancy as far as different regions or different orchards in the same region are concerned. Abiotic stresses like temperature stress, low or high soil pH, nutrient excess or deficiencies, auto allelopathy, heavy metal contamination, poor soil structure, poor drainage etc. have been associated with ARD (Traquair, 1984; Willet *et al.*, 1994). The abiotic factors may exacerbate the symptoms, however it has been observed that soil pasteurization and fumigation alleviates the problem and improves growth conditions of fruit trees (Jaffe *et al.*, 1982a; Mai and Abawi, 1981). It implies that this disease has a biological cause, and abiotic factors only 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. There is a lot of research that supports the involvement of different causal agents for this disease, however there is a consensus on various fungal species and nematodes responsible for causing this problem. To ascertain etiology is the first step to have a proper management. Therefore, to have effective management, a multiphasic approach should be employed to establish the cause of this disease.

## II. APPROACHES TO ASCERTAIN THE CAUSE OF ARD: STUDYING MICROBIAL AGENTS RESPONSIBLE FOR THE DISEASE

This disease syndrome and its etiology have been described in North America, Europe (Braun, 1991; Jaffe *et al.*, 1982b; Mazzola, 1998; Hoestra, 1968; Manici *et al.*, 2003; Savory, 1966a) as well as many other parts of the world, including South Africa, China, New Zealand, and Tasmania (Fullerton *et al.*, 1999; Tewoldemedhin *et al.*, 2011a., Uthkhe, 2002; Van Schoor *et al.*, 2009; Wilson, 2004). ARD 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.

There are several reports that are in line regarding the etiology of replant disease, ambiguity remains because several non-pathogenic species, *Trichoderma* spp., *Bacillus subtilis*, *Penicillium* spp. have been reported as a cause of replant disease (Utkhede *et al.*, 1992; Mazzola and Manici, 2012), but these have also been reported as biocontrol agents against this disease (Kandula *et al.*, 2010). Various micro-organisms have been reported as causal agents of this disease without ascertaining whether they are really associated with ARD infected apple roots or whether there is difference in microbial composition between healthy orchards and replant infected orchards (Mazzola and Manici, 2012). Different approaches have been followed by many researchers to study the microbial entities responsible in causing the 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, *Phytophthora cactorum*, *Pythium* spp., *Cylindrocarpon destructans* and *Rhizoctonia solani* were consistently isolated from the symptomatic trees in the orchards 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 *et al.*, 2003). *Phytophthora* and *pythium* were dominant in NY. Ithaca orchards (Rumberger *et al.*, 2007). Van Schooret *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. Numerous isolates of *Cylindrocarpon* spp., were also reported to cause root rot in replanted trees (Tewoldemedhin *et al.*, 2011c). Among various *Phytophthora* species, *P. cactorum*, *P. irregulare*, *P. sylvaticum* and *P. vexans* examined were found virulent. *Pythium dissotocum*, *P. folliculosum* and *P. heterothallicum* were considered as moderately virulent. *Fusarium* species are reportedly associated with ARD (Tewoldemedhin *et al.*, 2011b).
- 2. Molecular Approaches:** Culture based rely mainly on phenotypic parameters and are restricted only to those micro-organisms that can be cultured. It leads to under estimation of the diversity present in rhizosphere because culturable micro-organisms only contribute a portion of the total microbial diversity in soil ecosystem (Ammanet *al.*, 1995; Bridge and Spooner, 2001., Hawksworth, 1991). Advanced molecular technique have recently gained popularity in characterizing the resident soil microbial diversity. Therefore, application of such techniques to study the dynamics of microbial population in soil 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.

**3. Molecular Methods Based on PCR:** DNA-barcoding has helped in identification of vast diversity of microbes in different ecosystems including soil ecosystems. PCR amplification using rRNA and other barcodes are the best tools to generate the data regarding soil microbial diversity than the culture based methods (Mazzola, 2004). These methodologies can provide more information regarding microbial diversity in soils than physiological or culture-based methods. The various methods used to assess and compare the microbial community in replant infected and uninfected soils are as under:

- **Real-Time PCR:** Real-time PCR has gained popularity as a best tool for detection and quantification of microbes in different environments (Sचना *et al.*, 2004; Kernaghan *et al.*, 2007; Ophel-Keller *et al.*, 2008). It can detect relatively small amounts of target DNA in the sample. Mostly rRNA genes have been targeted to identify species diversity from different environments but efforts are continuously made to characterise them based on other barcode regions. Real-time PCR has been used to detect small quantities of target DNA from complex environmental samples (Cullen *et al.*, 2001; Vandermark *et al.*, 2002). Quantitative real-time PCR (qPCR) has an advantage over conventional PCR as it is highly sensitive and helps in quantitative detection of the target right from the beginning (McCartney *et al.*, 2003; Lievens *et al.* 2006). Therefore, it facilitates early detection (when microbial load is very less in the sample) as well as the quantification of the microbes present in a sample.

qPCR has also been used to detect and quantify the pathogens causing apple replant problem. Tewoldemedhin *et al.* (2011b) used qPCR for the quantification of most virulent oomycetes viz *Phytophthora* and *Pythium* species associated with apple roots in replanted orchards. Tewoldemedhin *et al.* (2011a) again used qPCR for the quantification of *Rhizoctonia solani*, *Cylindrocarpon spp.*, *Pythium sylvaticum*, *Pythium vexans*, *Pythium irregular*, *Pythium ultimum* and *Phytophthora spp.* No correlation was found between amount of pathogen DNA and reduction in seedling height and weight. This could be explained by several factors. The probable reason may be the fact pathogens attack different sections of root (Agrios, 2005). This explains the low root DNA concentrations of *P. ultimum*, *P. sylvaticum* and *P. irregulare* as they have been reported to function as root pruners (Martin and Loper, 1999). The higher concentrations of *P. vexans* and *Phytophthora* indicated that these function as root colonizers. were detected at much higher concentrations, suggesting that these pathogens may be more prone to colonize larger roots instead of being fine-root pruners. Also, the pathogens may vary in potential to colonize roots. Tewoldemedhin *et al.* (2011c) has standardised qPCR protocol for simultaneous detection of various *Cylindrocarpon* species from apple roots.

- **T-RFLP and DGGE:** Temperature gradient gel electrophoresis (TGGE), and terminal restriction fragment length polymorphism analysis (RELPA) have been used to assess the differences in microbial communities in replant soils. These methods separate the mixtures of PCR products of various environmental samples wherein different universal primers are used (Muyzer and Smalla, 1998; Liu *et al.*, 1997). These methods can also target a specific group when the primers belonging to that group are used (Heuer *et al.*, 1997; Redecker, 2000). These can also be used to identify functional diversity when primers of that functional gene are used e.g.

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. DGGE analysis gets simplified when group-specific rRNA primers are used as compared to universal primers (Mazzola, 2004). Laurent *et al.* (2010) used DNA fingerprinting T-RFLP to assess the rootzone fungal and bacterial communities. Laurent *et al.* (2008) assessed Soil bacterial and fungal community composition in an Orchard and found that bacteria dominated the community composition. Acidobacteria (25% of sequences), Actinobacteria (19%),  $\delta$ -Proteobacteria (12%) and  $\beta$ -Proteobacteria (10%) were found. Bacterial community composition was found to differ between the trees grown in old grass lanes and the old tree rows of the previous orchard using DGGE analysis (Rumberger *et al.*, 2004).

### III. PLANT INDUCED VARIATIONS MICROBIAL COMMUNITY COMPOSITION:

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). Populations of microbes that are associated with this disease were found considerable after brief period of orchard establishment. Increase in *Cylindrocarpon*, *Phytophthora*, *Pythium*, and *Rhizoctonia* was observed. However, there was a decrease in populations of *Burkholderia cepacia* years after orchard establishment. Initially, *Pseudomonas putida* dominated the population and then *Pseudomonas fluorescens* and *Pseudomonas syringae* dominated the population during later years (Mazzola *et al.*, 2002). This suggests that inoculum builds up in response to apple planting and provides an evidence that biological incitants are responsible for this disease.

### IV. ROLE OF ROOTSTOCK GENOTYPES

Rootstock genotypes have varied rhizosphere microbial community composition. Thus, studies on rootstock genotypes have provided new insights into etiology of apple replant disease. The microbial composition rhizosphere differed between M.26 and CG.6210 rhizosphere (Laurent *et al.*, 2010). Different species of bacteria and actinobacteria were found in the rhizospheres of ARD susceptible rootstocks M7 and M26 compared to tolerant rootstocks CG30 and CG210 (Rumberger *et al.*, 2004). Geneva series rootstocks have been found less susceptible than or Malling-Merton rootstocks like M26, MM106, and MM111 (Mazzola *et al.*, 2009).

### V. 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 (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 1:** Pathogenis species involved in ARD complex

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

## VI. MANAGEMENT STRATEGIES

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

**1. 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 controlled the disease. However, this chemical was removed from market based on Montreal Protocol because of its contribution in ozone layer depletion (WMO, 1994). The high cost of chemical control and its concerns regarding human health and the environment has necessitated the development of alternative and environment friendly means of control.

**2. 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 ARD symptoms when grown in pre-sterilized field soil amended with 5% (v/v) sick soil (Jaffe *et al.*, 1982b). Treatment of field soil prior to planting with gamma radiations or heating (60°C or higher for 30 min.) improved plant growth and reduced root discoloration (Jaffe *et al.*, 1982a). There are several reports indicating that pasteurization helps in reduction of population of ARD pathogens in sick soils which again provides an evidence of biotic nature of apple replant disease. Mazzola, (1998) reported that species of *Cylindrocarpon*, *Fusarium*, and *Rhizoctonia* dominated the fungal in natural sick soils, while *Trichoderma* spp. Were predominant species present when apple trees were grown in pasteurized soils.

### **3. Cultural Control:**

- **Planting Position:** Planting position has got marked effect on growth of replanted trees and on rhizospheric microbiota 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 position of the previous orchard can minimize replant disease because inoculum load will be lesser in those positions. However, it may build up in the upcoming period and it is suggested that replanting in inter-row should be coupled with other management strategies (Kelderer *et al.*, 2012).
- **Cover Crops:** Various attempts have been made to manage ARD by using different cover crops to manipulate rhizosphere microbial population dynamics. However, such strategy may not be appropriate in all the cases. The use of cover crops to control *P. penetrans* has received a great attention and positive results were obtained. Several studies have investigated the use of non-host plant species that directly suppress nematodes populations via the production of allelochemicals. Different cover crop species are reportedly having varied effects. For example, prairie grass and oats had a positive effect on growth of apple seedlings whereas, blue lupin was found to have detrimental effects on growth of apple seedlings. The reason for this differential response could be that some cover crops may have allelopathic effect and some may attract disease causing nematodes and microbes (Colbran, 1979). Several 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. Laurent *et al.* (2008) reported that apple seedling growth was positively correlated with microbial composition, but not with soil quality parameters like soil organic matter content, macro- and micro-nutrient availability, and pH. Wheat has also been identified as an efficient cover crop that changes microbial composition in soils where it is grown community composition. It has been found to suppress the populations of *R. solani* (Mazzola, 1999). In greenhouse trials, wheat cropping has improved apple seedlings growth subsequently by reducing the rhizosphere populations of *R. solani* and *Pratylenchus penetrans* increases in of *Pseudomonas fluorescens* and *P. putida*. Also wheat cultivars were having a marked effect on population dynamics of different microbial agents (Mazzola and Gu, 2000; Mazzola *et al.*, 2002). In field trials in Washington, wheat cover crop with three short-term cropping periods coupled with 3-year *B. napus* green manure significantly improved growth and yield of Gala/M26. However, the disease control was still less than that obtained using soil fumigant, methyl bromide. It can be attributed to the fact that cover crops could the populations of *Rhizoctonia solani* and *Pratylenchus penetrans* but not the infection by *Cylindrocarpon* species (Mazzola and Mullinix, 2005). Wheat cultivation therefore, proved effective in suppressing the ARD but it does not suppress all the disease-causing agents. Integration of wheat cropping coupled with other management practices may provide effective ARD control (Mazzola *et al.*, 2002).

#### 4. 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).

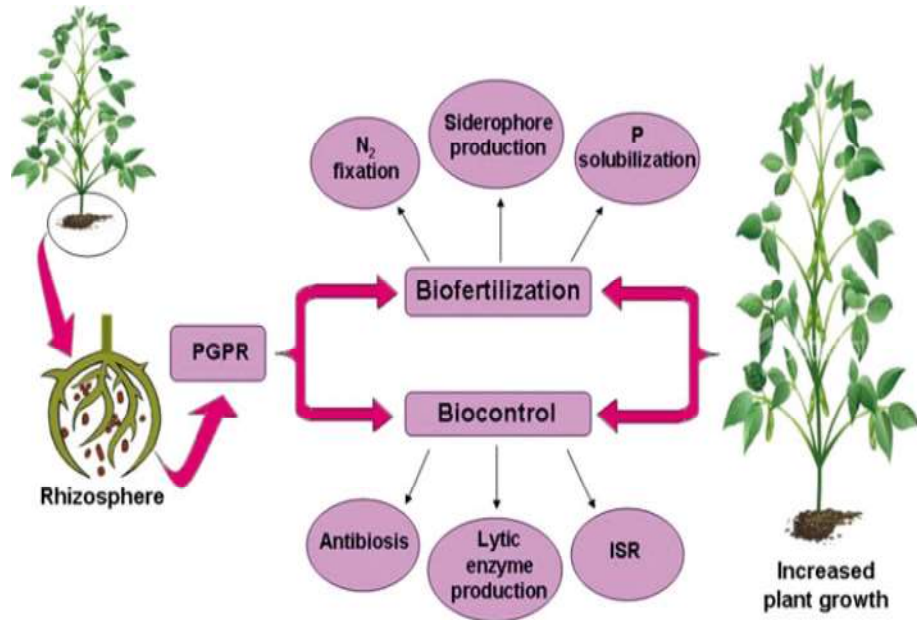
Compost amendments have been reported to improve apple plant growth in newly established orchards and also in high-density plantation (Moran and Schupp, 2001; Neilsen *et al.* 2003). In addition, these amendments have proven ineffective in managing apple replant disease as has been reported by several researchers (Granatstein and Mazzola, 2001., Neilsen *et al.*, 2004., Wilson *et al.*, 2004 ). 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. Composts improve plant growth by improving soil properties and supporting the growth of beneficial microbes. Discrepancies in different studies may be attributed to compost types and experimental conditions, which needs to be standardised.

- **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 *Brassic napus*, 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 nematostatic 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 nematostatic 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 coupled with a postplant mefenoxam soil drench provided effective suppression of ARD comparable to that provided with pre-plant soil fumigation. (Mazzola and Mullinix, 2005). The use of seed meal amendments for replant disease suppression must be coupled with the use of 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). There are a number of reports that determine the role of seed amendments in controlling soil borne diseases (Mazzola *et al.*, 2001., Cohen *et al.*, 2005., Cohen and Mazzola, 2006 ).

**5. Biological Control:** Biological control uses micro-organisms or their products, manipulation in the cropping systems to suppress the pathogen and minimise the disease incidence. 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 *et al.*, 1998). Many factors are responsible for the poor transition of biocontrol agents from *in vitro* test strains to commercial products (Roberts and Lohrke, 2003). To manage the soil borne diseases, several attempts have been made to employ microbial biological control through the introduction of non-native microorganisms into soil systems. This microbial agent competes with the resident microbes and colonize the roots, thus provide optimum level of soil suppressiveness (Weller *et al.*, 2002). Although, chemical control is most effective in controlling replant diseases, but it has environment and human health concerns. Therefore, alternative management strategies that are less harmful to environment have been explored from time to time (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:*** *Trichoderma* species serve as an effective biocontrol against various soil borne diseases. *Trichoderma viride* has reportedly found effective against various soil borne pathogen of different crops like *Rhizoctonia solani* etc. (Gaigole, 2011). Application of *Trichoderma* spp. has been found to increase AM colonisation in SARD (specific apple replant disease soils) in pot experiment and therefore could be used to improve root health (Kendula *et al.*, 2006).
- **PGPRS-Plant Growth Promoting Rhizobacteria as Biocontrol:** Plant growth promoting rhizobacteria (PGPR) are the bacteria associated with rhizosphere and are beneficial in promoting plant growth (Ashrafuzzaman *et al.*, 2009). They colonize the roots and promote plant growth through various mechanisms (Kloepper and Schroth 1978). *Azoarcus*, *Azospirillum*, *Azotobacter*, *Arthrobacter*, *Bacillus*, *Clostridium*, *Enterobacter*, *Gluconacetobacter*, *Pseudomonas*, and *Serratia*, have been reported as the bacteria that promote plant growth (Hurek and Reinhold-Hurek, 2003) Out of these genera, *Pseudomonas* and *Bacillus* are the most thoroughly studied. PGPRS promote growth via different mechanisms of action (fig I) including production of phytohormones that enhance plant growth, 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 inducing systemic resistance by jasmonic

acid pathway (Richardson et al. 2009; Idris et al. 2007; Gutierrez-Manero et al. 2001; Whipps 2001., Sarvanakumaret 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 1:** 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). PGPRs have reportedly found effective in growth improvement of apple in replanted orchards (Caesar and Burr, 1987; 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 effective biological agent against *Phytophthora cactorum*, one of the causal agents of ARD (Utkhede and Smith, 1991). *E. aerogenes* improved the growth of apple seedlings in replant soil (Utkhede and Li, 1989a). In another study, *Bacillus subtilis* and *E. aerogenes* applied as soil drench increased plant growth over and above that of formalin fumigation (Utkhede and Li, 1989b). *Agrobacterium radiobacter* has also been found effective as biocontrol agent against replant disease under green house conditions as well as in the nursery (Catska and Hudska, 1990). *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). Fluorescent pseudomonads were able to improve growth of apple in replant soils via reducing the population of *Cylindrocarpon destructans*, one of the causal pathogens

replant disease (Jaffe *et al.*, 1982a; Mazzola, 1998; Casear and Burr 1987). 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 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:** VAM fungi facilitate the uptake of one of the important nutrients, phosphorus to the plants. Young plants require Phosphorus for proper growth and development. VAM-Plant associations can provide improved P uptake from immobile phase (Mosse, 1973). Consequently, VAM associated plants show better growth than those that do not have mycorrhizal associations.. Inoculation of apple seedlings with arbuscular mycorrhizal fungi (AMF) increased their growth in 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 roots were 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 *Pratylenchus penetrans* (Stirling, 1991) and *Pseudomonas chlororaphis* is also an antagonist of *Pratylenchus penetrans* (Hackenberg *et al.*, 2000). *B. subtilis* ZZ120 have also shown strong activity against the root rot pathogens by producing various bioactive compound, therefore could be integrated into various management programs 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 2).

**Table 2:** Biocontrol agents of replant pathogens

BIOCONTROL AGENTS	REFERENCE
<i>Trichoderma</i>	Kendulaet <i>al.</i> , 2006
<i>Pseudomonas fluorescence</i>	Casear and Burr, 1987
<i>Pseudomonas putida 2C8</i>	Mazzolaet <i>al.</i> , 2002 (patent)
<i>Pseudomonas chlororaphis</i> (against <i>Pratylenchus</i> )	Hackenberget <i>al.</i> , 2000
<i>Bacillus subtilis</i>	Utkhede and Li, 1989b
Endophytic <i>Bacillus subtilis</i> ZZ120	Li <i>et al.</i> , 2012
<i>Burkholderia cepacia</i>	Mazzolaet <i>al.</i> , 2002; Laurentet <i>al.</i> , 2010
<i>Frateuria</i>	Laurentet <i>al.</i> , 2010
<i>Agrobacterium radiobacter</i>	Catske and Hudska, 1990
<i>Enterobacter aerogenes</i>	Utkhede and Smith., 1994
<i>Streptomyces spp</i>	Mazzola, 2007
<i>Pasteuria penetrans</i> (against nematodes)	Sterling, 1991
<i>Glomus mosseae</i>	Forgeet <i>al.</i> , 2001
<i>Glomus intraradices</i>	Forgeet <i>al.</i> , 2001
<i>Scutellospora calospora</i>	Ridgwayet <i>al.</i> , 2008
<i>Glomus epigaeum</i>	Bingye and Shengrui, 1998
<i>Glomus fasciculatum</i>	Catska, 1994
<i>Glomus macrocarpus</i>	Catska, 1994

**6. Soil Suppression:** 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. It can be both general suppression or specific suppression. Soil suppressiveness is usually result of the activity of micro-organisms inherent to soil ecosystems (Cook and Baker, 1983). The general suppression results from the total activity of microbial population while as specific suppression is a function of specific group of 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 by adding 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 role of soil microorganisms in disease suppression. (Mazzola, 2010). In apple cultivation, it has been seen that soils become conducive to replant disease with time. However, in case of take-all and potato scab, the soils become suppressive when there is monoculture of the crop (Weller et al., 2002). Management strategies oriented to manipulate plant-beneficial rhizobacteria can prove very effective in managing soilborne plant diseases (Mazzola, 2007). Different approaches that can lead to the development of suppressive soils are as follows:

- Application of specific or mixtures of different microbial strains.
- Organic amendments.
- Cropping systems.

**7. 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 susceptibility to replant problem. Rootstocks also structures the microbial community composition. There were significant differences in composition of microbial composition associated with the rhizosphere of M7 and M26, CG30 and CG210 rootstocks which indicates that susceptible and tolerant rootstocks differently support the rhizospheric composition of microbial population (Rumberger *et al.*, 2004; Rumberger *et al*, 2007). Therefore, apple rootstock resistance or susceptibility to ARD strongly correlates with their interaction with soil microbial population. *Burkholderia cepacian* has been recovered from the rhizosphere of rootstock CG.6210 while no such species was recovered from susceptible one, M.26 (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 which supports the fact that rootstocks actually alter the microbial composition in rhizosphere ( Hebbbar *et al.*, 1998., Mazzola, 1998). While M26, MM106, and MM111 are very sensitive, Geneva series rootstocks have been proven to be less vulnerable to root infection by *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 programs and clonal rootstock selection for better management of orchard replant diseases. (Isuta and Merwin, 2000).

**Table 3:** Apple replant disease tolerant and susceptible rootstocks

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

## VII. CONCLUSION

Apple replant problem is a disease complex and has been reported from all the apple growing regions. The abiotic factors can exacerbate the symptoms but the disease is primarily caused by biotic agents. 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 strategy can provide the optimum level of disease control. Therefore, researchers must explore integrated approach for the management of this disease.

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