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# Microbial community development in the rhizosphere of apple trees at a replant disease site

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## Abstract

Apple replant disease (ARD) is a complex syndrome that affects young trees in replanted orchard sites causing necrotic lesions on feeder roots, stunted tree growth and reduced cumulative yields. Use of ARD-tolerant rootstocks is an emerging control strategy. We studied the bacterial, fungal, and oomycetes populations in the rhizosphere of five rootstock cultivars (M.7, M.26, G.16, G.30 and CG.6210) planted into the old tree row or grass lanes of a previous orchard in Ithaca, NY, to better understand the role of rhizosphere microbial communities in the prevalence and control of ARD. The possible involvement of antagonistic *Pseudomonas* species, *Pythium* spp., *Phytophthora* spp. and rhizosphere cyanide concentrations in ARD were also examined. The rootstocks M.7, M.26 and G.16 were susceptible to ARD, while G.30 and CG.6210 were more tolerant. Tree growth on the rootstocks M.7, M.26 and G.16 was reduced by 10% when planted in the old tree rows, but this did not significantly reduce yields in the first fruiting year. The susceptible rootstocks, M.7 and M.26, supported higher densities of culturable rhizosphere fungi and bacteria than G.16, G.30 and CG.6210. Over 2 years, microbial densities were highest in July, lower in May and lowest in September. The composition of bacterial and fungal communities in the rhizosphere was highly variable and changed over seasons and years, as assessed by terminal restriction fragment length polymorphism (T-RFLP) analyses. Initial differences in fungal rhizosphere communities between the two planting positions converged 2 years after the trees were replanted. In contrast, the bacterial rhizosphere community composition still differed significantly between the two planting positions 3 years after the orchard was replanted. The bacterial and fungal rhizosphere community compositions of susceptible rootstocks, M.7 and M.26, differed from those of the tolerant rootstocks, G.30 and CG.6210; G.16, differed from all the other rootstocks. The observed effects of rootstocks, planting positions and time on microbial community composition were small relative to the high variability observed overall. *Pythium* spp. and *Phytophthora* spp. infestations were high and similar for all rootstocks and planting positions. Neither potentially antagonistic *Pseudomonas* nor rhizosphere cyanide concentrations appeared to be involved in the ARD-complex at the studied site. Avoiding replanting into the old tree rows coupled with use of tolerant rootstocks appear to be the best strategies for reducing ARD in replanted orchards. Changes in rhizosphere microbial communities are among the many factors that contribute to improved tree growth when these management strategies are used.

*Keywords:* Apple replant disease; Cyanide; Disease tolerant rootstocks; Oomycetes; *Pseudomonas*; Rhizosphere bacteria; Rhizosphere fungi; Rootstocks

## 1. Introduction

Apple replant disease (ARD) is a complex syndrome that occurs in young apple trees (*Malus* X *domestica* Borkh.) in replanted orchard sites (Mai and Abawi, 1981). Symptoms include death of fine feeder roots, stunted growth above- and below-ground, and reduced fruit yields. In most sites, biotic factors have been implicated in ARD, with soil-borne fungi, bacteria, nematodes, actinobacteria and oomycetes variously cited as causal pathogens in

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*Abbreviations:* AMMI, additive main effects and multiplicative interaction model; ARD, apple replant disease; DAPG, 2, 4-diacetylphloroglucinol; DGGE, denaturing gradient gel electrophoresis; OTU, operational taxonomic unit; PCA, principal component analysis; PCR, polymerase chain reaction; T-RFLP, terminal restriction fragment length polymorphism.

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site-specific combinations (Mai et al., 1994; Mazzola, 1998). Replant problems have reportedly been more severe in old tree rows than in the grass lanes of previous orchards (Buszard and Jensen, 1986; Facteau et al., 1996; Leinfelder and Merwin, 2006). After several years, trees may recover from the initial growth depression and eventually reach the size and annual yields of unaffected trees (Arneson and Mai, 1976; Foy et al., 1996). Despite this partial recovery, cumulative yields and profitability in ARD-affected orchards usually remain lower than in unaffected orchards (Peterson and Hinman, 1994).

Trees and herbaceous plants differentially affect the composition and growth of microbial communities in their rhizosphere (Marschner et al., 2001; DeLong et al., 2002). Among the factors that influence the rhizosphere community are the quality and quantity of organic substances released by the roots that serve as carbon (C) and energy sources for microorganisms (Bazin et al, 1990). Biocidal compounds released by roots may also significantly influence the composition of rhizosphere microbial communities, even in small amounts (Rumberger and Marschner, 2003). Apples and peaches (*Prunus persica* BATSCH.) contain cyanogenic glycosides in the cortex of their twigs and roots (Mendel et al., 2003). Cyanide (HCN) is a cell toxin and strong chelator, which inhibits the activity of several metallo-enzymes, thus inhibiting respiratory pathways and other cellular functions (Way et al., 1988). Gur and Cohen (1989) reported that apple and peach seedlings had similar effects on cyanide development by soil bacteria in a peach replant soil. It is not yet known whether cyanide is involved in the ARD-complex. In the context of rhizosphere cyanide production and ARD, cyanide may perform two opposing functions: (i) cyanide released from cyanogenic glycosides may exacerbate replant disorders as has been observed for peach (Gur and Cohen, 1989) or (ii) cyanide produced by *Pseudomonas* strains or other soil bacteria may be a mechanism of protective antagonism against root pathogens (Blumer and Haas, 2000; Pal et al., 2000).

Several studies have shown that rhizosphere bacterial communities may suppress root infection by soil-borne pathogens. For example, inoculation with an antagonistic *Bacillus subtilis* strain reportedly protected young apple trees from *Phytophthora cactorum* crown and root rot (Utkhede et al., 2001). Mazzola and Gu (2002) showed that soil suppressiveness to *Rhizoctonia* root rot in apple developed after repeated monoculture with certain wheat cultivars (*Triticum* sp.), and was linked to an increase in the number of antagonistic psuedomonads isolated from the wheat rhizosphere. *Pseudomonas* isolates producing the antibiotics phenazine-1-carboxylic-acid (*PCA*) and 2, 4-diacetylphloroglucinol (DAPG) were implicated in the observed soil suppressiveness (Mazzola, 1999). Recently, Yao et al. (2006a) showed that rhizosphere *Pseudomonas* community composition of ARD-susceptible rootstocks differed from that of ARD-tolerant rootstocks.

In our studies, 'Royal empire' apple scions were grafted onto five different rootstock cultivars that were planted into either old tree rows or grass-covered drive lanes of the previous orchard (Rumberger et al., 2004; Leinfelder and Merwin, 2006). Growth of trees on three of these rootstocks (M.7, M.26 and G.16) was significantly reduced and this was exacerbated in the old row planting position. The two other rootstocks (G.30 and CG.6210), which had been previously rated as ARD-tolerant by Isutsa and Merwin (2000), showed no ARD symptoms and performed equally well in both planting positions. In the year after replanting, the rhizosphere bacterial community composition differed among rootstocks and between the two orchard replant positions, while *Pythium* spp. and *Phytophthora* spp. infestation was similar among rootstocks and planting positions (Rumberger et al., 2004).

In this study, we monitored the development of rhizosphere microbial communities in the same orchard over the 3 years following replanting, up to the first apple harvest. We used both culture-dependent and PCR-based molecular methods to characterize the bacterial, fungal and oomycete communities in the apple rhizosphere. Our aim was to gain a better understanding of the etiology, duration, and potential for control of ARD under different orchard management practices by: (i) monitoring how rhizosphere bacterial, fungal and oomycete communities changed over time and in relation to orchard planting position (old tree row versus grass lane); (ii) comparing bacterial and fungal communities in the rhizospheres of disease-susceptible versus tolerant apple rootstocks; and (iii) assessing if cyanide produced by apple roots or associated *Pseudomonas* spp. may be involved in the ARD syndrome.

## 2. Material and methods

### 2.1. Orchard site

A detailed description of the test site, apple rootstock genotypes and soil treatments was published recently (Rumberger et al., 2004). The test orchard is operated by the Department of Horticulture, Cornell University, Ithaca, NY. The site soil is a glacial, lacustrine soil characterized as mixed mesic *Udic Hapludalfs* (Cline and Bloom, 1965; Cline and Marshall, 1977). The orchard site was originally planted with apple trees around 1910 and then replanted in 1981 with trees grafted on M.9/M.106 and M.9/M.111 rootstocks (M.111 and M.106 roots with an M.9 interstem). The second planting established poorly and exhibited many symptoms of ARD (Mai et al., 1994). Over a 20-year period, about 25% of the trees in the first replanting died from various causes, including winter injury and *Phytophthora* crown rot.

In September 2001, the old trees were removed and the site was chiseled to 50 cm depth to remove as many old roots as possible from the soil. The location of each previous tree row was mapped precisely. Detailed nutrient analyses of soil sampled from the site in July 2001 were described in Rumberger et al. (2004). Soil nutrient analyses from 2002 and 2003 were reported by Leinfelder and