

Nematodes contributing to apple replant disease, and as indicators of soil quality

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Understanding the etiology of apple replant disease (ARD) is challenging owing to the fact that many agents including fungi, bacteria and nematodes play adverse roles in the development of the disease. Nematodes occupy a key position in the ecosystem and as such are involved in ecosystem functioning as well as being indicators for soil quality. This study seeks to elucidate the contribution of nematodes to ARD by investigating the long term soil decline in apple fields, to examine the changes in nematode community structure and how they influence the plant-associated microbial community. Two samplings per year will be carried out from three different apple fields, each with four replicate ARD plots and control plots. In the ARD plots apple was planted yearly since 2009. The control plots were covered by grass and have been switched to apple in 2016. We will utilize both morphological and molecular techniques such as PCR-DGGE, qPCR and amplicon NGS on the samples to compare nematode communities among ARD and control soils, and to correlate ARD severity to nematode species abundance. The NGS approach

using Illumina Miseq 18S rRNA gene sequencing will be combined with SMRT-CCS sequencing of large fragments to resolve the nematode diversity in both ARD and control plots. This will enable us to assess soil quality based on ecological indices, to find indicator species and their associated microbes that may contribute to ARD. A preliminary sampling in Ellerhoop in December 2015 revealed that *Paratylenchus spp.* were the most abundant nematodes in the ARD plots while *Tylenchorynchus spp.* were mostly associated with control plots. These species feed on root surfaces and may not pose serious economic damage to apple plants as single entities. However, their potential synergistic role in the disease will be investigated. A colonizer-persister analysis on the free-living nematodes in both ARD and grass fields suggested a gradual shift from a stable environment with N enriched, conducive environment to more C enriched, less stable (stressed) environment with time. Additional sampling data coupled with molecular analysis may give us better understanding about the role of nematodes in ARD.