

# Microbial Community Analysis for Identification of Novel Alfalfa Pathogens

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Rapid and uniform seedling emergence is critical for obtaining a productive and persistent stand of alfalfa. In many locations, poor seedling emergence and seedling death is caused by pathogens attacking seeds and seedlings in cold, wet soil conditions. Infection of mature plants during wet spring or fall weather causes destruction of fine feeder roots, which interferes with nitrogen fixation, nutrient uptake from the soil, and water absorption. Cumulatively, these seedling and adult plant diseases result in reduced yields, decreased winter survival, and shortened stand life. Past research identified several pathogens causing this problem; however, poor seedling establishment continues to plague alfalfa producers worldwide. Culture-based approaches to identify causal pathogens are time-consuming and may overlook organisms that are slow growing in culture. The oomycete, fungal, and bacterial communities in alfalfa roots (endosphere) and rhizosphere soil from eight locations with poor alfalfa establishment were characterized by sequencing diagnostic genes. The oomycete community in the endosphere, identified using primers targeting the rDNA ITS sequence, was dominated by *Aphanomyces euteiches* (56% abundance) and *Phytophthora medicaginis* (11% abundance). In some locations, *P. sansomeana*, not previously recognized to infect alfalfa, was highly abundant and was shown to infect alfalfa and cause root rot in seedlings. *Pythium/Globisporangium* spp. abundances varied by location with *Pythium inflatum* and *Pythium/Globisporangium sylvaticum* the most abundant across all locations and samples. Although some of these species are known pathogens of alfalfa, pathogenicity toward alfalfa is unknown for *P. aritosporum*, *P. selbyi*, *P. monospermum*, and *P. radicolica*. The most abundant fungal species in the endosphere was tentatively identified as *Fusarium waltergamsii* (28% abundance), a member of the *F. solani* species complex, one of the most abundant in soil and often associated with plant roots. Other frequently identified fungal species were *F. acutatum* and *Plectospherella* sp., *Paraphoma radicina*, and *Mycoleptodiscus terrestris*, all warranting further investigation as causal agents of seedling disease in alfalfa. The bacterial endosphere communities, identified with primers targeting the 16S V3-V4 region, were dominated by rhizobial species and actinomycetes, particularly *Streptomyces*. The only abundant pathogenic genus identified was *Pseudomonas*. The alfalfa bacterial wilt pathogen, *Clavibacter insidiosus*, was not specifically identified. Populations of *A. euteiches*, *P. medicaginis*, *Pythium irregulare*, *P. ultimum*, and *P. sylvaticum* were measured in rhizosphere soil and the endosphere using qPCR assays specific for each pathogen and confirmed the relative abundances measured by amplicon sequencing. The novel organisms identified in alfalfa roots will be targets for isolation and pathogenicity tests. The results demonstrate that community analysis is an efficient and robust method for identifying and quantifying pathogens causing poor seedling establishment.