Biological control of plant diseases can result from the activity of indigenous microorganisms naturally associated with the plant and/or introduced biocontrol agents (BCA) that are applied to the plant or soil. Unlike for many foliar diseases, crop plants often lack resistance to soilborne diseases, and as a result, indigenous antagonists often constitute the first and sometimes the only line of defense against them (Weller et al., 2007). Disease-suppressive soils provide some of the best examples of indigenous BCAs protecting plants against pathogens (Weller et al., 2002). Suppressive soils are soils in which the pathogen does not establish or persist, establishes but causes little or no damage, or establishes and causes disease for awhile but thereafter the disease is less important (Weller et al., 2002). Natural disease suppressiveness is highly effective, transferable between soils, and often due to the effects of individual or select groups of microorganisms (Weller et al., 2002).

The observation about 75 years ago that *Trichoderma* spp. were able to attack other fungi and produce antibiotics that inhibited other microorganisms, ushered in the era of biological control of plant pathogens and opened the possibility of augmenting and reinforcing natural microbial-based defenses against diseases (Baker and Cook, 1974). Thousands of putative BCAs have been intensively tested, and some strains have been commercialized, but biocontrol studies have concentrated on three genera: *Bacillus*, *Trichoderma* and *Pseudomonas* (McSpadden Gardener and Driks, 2004). *Bacillus* and *Trichoderma* spp. are the bacteria and fungi of choice, respectively, for development into commercial BCAs (Kloepper et al., 2004). However, *Pseudomonas* spp. are the organisms of choice for fundamental studies of plant colonization, mechanisms of action, and molecular biology of BCAs but few have been commercialized.

In this paper, we will discuss the tools that are available to document the establishment and persistence of BCAs and will do that by focusing on agents, especially *Pseudomonas* spp. that control soilborne diseases. Monitoring BCAs in the soil or rhizosphere is especially challenging because of the complexity of these environments and the diverse and complex microbial milieu surrounding and competing with the BCAs.

Whether BCAs are indigenous or introduced, establishment of an agent on the plant or in the soil is a critical part of the biocontrol process and generally is thought to be required for successful disease suppression. Lack of establishment of BCAs often has been cited as an explanation for inconsistent performance, a historic and chronic problem of biocontrol. However, the problem of inconsistent performance also stems from a lack of fundamental understanding of the complex *in situ* interactions among the BCA, host plant, pathogen, indigenous organisms, and the environment. Even if a BCA successfully establishes, biocontrol traits and genes must be expressed at the critical time needed for pathogen suppression or induction of host resistance mechanisms.
During the last 35 years, dramatic improvements have occurred in the tools available to directly visualize BCAs on the plant or in the rhizosphere and to quantify their population densities and dynamics (Sørensen et al., 2009). Direct microscopy methods have included techniques such as scanning electron microscopy (SEM) (Fukui et al., 1994), epifluorescence microscopy (EFM), and confocal laser scanning microscopy (CLSM), an especially powerful tool that can provide high resolution, 3-D reconstructions of the structural and spatial composition of microbial communities on plant surfaces and in the rhizosphere (Sørensen et al., 2009). The use of molecular staining and tagging systems along with advanced fluorescence microscopy has made it possible to detect single cells of a BCA in the microbial milieu of the phyllosphere and rhizosphere. For example, CLSM along with strain specific fluorescent antibody staining were used in early studies of wheat and barley colonization by *Azospirillum brasilense* and *P. fluorescens* (Schloter et al., 1993; Hansen et al., 1997). Especially powerful has been the use of CLSM to visualize in situ BCAs with reporter genes such as *lux* or *gfp* genes (bioluminescence or green fluorescence protein) under the control of a constitutive promoter (Bloemberg et al., 2000). For example, CLSM was used to track GFP marked *P. fluorescens* strains on the roots of a wide variety of crops including barley (Normander et al., 1999) and tomato (Bloemberg et al., 2000).

Culture-based methods to monitor the population densities and dynamics of indigenous and introduced BCAs have included the following approaches used either individually or in combinations: selective media (Grant and Holt, 1977), antibiotic resistant strains (Mahaffee et al., 1997), immunofluorescent colony staining (Mahaffee et al., 1997), colony hybridization (Landa et al., 2002) and PCR-based dilution-endpoint method (McSpadden Gardener, 2001). The effectiveness of each method to detect BCAs has been compared (Landa et al., 2002; Mahaffee et al., 1997) and each method has its strengths and weaknesses. Culture-independent quantitative PCR: (Rezzonico et al., 2003; Mavrodi et al., 2007) allows detection and quantification of BCAs by only isolating DNA from the environment being sampled without isolating agents (Mavrodi et al., 2007; Rezzonico et al., 2003).

Solving problems that are barriers to biocontrol reaching its full potential as an integral part of sustainable agriculture will require multi-disciplinary research focusing on the biocontrol process at all levels, from the genome to ecosystem scale. Developing tools to track the establishment and fate of BCAs will continue to be an important component of the multi-disciplinary research effort.

**References:**


