BIOASSAYS FOR DETECTION AND ANALYSIS OF *PRUNUS* REPLANT SUPPRESSION

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Replant suppression of pome, stone fruits and related ornamentals occurs in all major crop-growing regions of the world (Traquair, 1984). Symptoms are expressed as a strong reduction in plant growth with a subsequent reduction in yield. While biotic agents are typically thought to be responsible, causes of replant suppression are complex and may differ among regions. The frequently unsuccessful search for causal agents has frustrated researchers for many years but was inconsequential to growers as long as broad-spectrum soil fumigants provided an affordable remedy for the malaise. The ban of methyl bromide and the difficulties to develop effective as well as environmentally and economically acceptable replacement strategies has renewed the quest for identifying the cause of replant suppression (Schneider et al., 2003). This key information might not only help to predict which orchard sites would most likely benefit from remediation, but will foster the development of targeted management strategies that fit regulatory requirements and production needs. The identification of such organisms has been hindered by methodological limitations, such as the difficulty to obtain thorough descriptions of rhizosphere microbial composition in a cost effective and timely manner. In addition, it is difficult to analyze the functions of microorganisms in soil.

To address these limitations, we developed an experimental approach that enables the identification of organisms involved in specific functions in their soil environment (Valinsky et al., 2002). This approach utilizes a population-based strategy comprised of three phases: 1) utilization of oligonucleotide fingerprinting of ribosomal RNA genes (OFRG) to identify the microorganisms whose population levels correlate with a functional parameter, 2) confirmation of the population trends with quantitative PCR and, 3) substantiation of the role of the identified organisms by reintroducing them into non-suppressive soil. Recently, we demonstrated the usefulness of this approach during the analysis of a soil suppressive to the plant parasitic nematode *Heterodera schachtii* (Borneman et al., 2004, Olatinwo et al., 2006).

Essential for utilizing this approach in replant suppression was the development of a suitable bioassay. Our goal was to run replant detection tests throughout the year, which required a greenhouse assay. In the absence of specific disease symptoms or significant population densities of known major pathogens, we based the detection of replant suppression on the comparison of *Prunus* growth parameters in non-treated and pasteurized or fumigated replant soil. Although plant parasitic nematodes were not involved in *Prunus* replant suppression in a recent California study (Browne et al., 2006), we monitored their population densities if significant numbers were detected in non-treated soils. Initially, test seedlings (cv. Nemaguard) were derived from rooted

cuttings or germinated seeds. Both methods resulted in planting material that varied considerably in size, vigor and root development. Clonal plants, however, provided vastly superior seedlings of same age, compact size and with vigorous root systems. While increased plant vigor and growth are often recorded after soil pasteurization or fumigation, replant suppression is genus specific. Consequently, citrus seedlings were included in the tests as a non-*Prunus* control to help to distinguish replant suppression from non-specific effects.

Another key element of our research approach was the establishment of microbial gradients in the test soils. Replant soil was exposed to chemical or physical agents or forces that caused qualitative and quantitative changes in the microbial composition. These modifications affected replant suppression, as indicated by improved *Prunus* growth parameters compared to the non-treated check. Currently, ongoing OFRG analysis of rhizosphere soil and root tips will allow comparisons among the microbial microflora from soils with various levels of suppressiveness. Multiple comparisons from several different gradients are expected to eliminate most organisms that are not involved and to focus the attention to those groups that are potentially responsible for replant suppression.

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