MICROBIAL POPULATIONS RESPONSIBLE FOR SPECIFIC SOIL SUPPRESSIVENESS TO PLANT PATHOGENS[1]

David M. Weller
USDA, Agricultural Research Service, Root Disease and Biological Control Research Unit, Washington State University, Pullman, Washington 99164-6430;
e-mail: wellerd@mail.wsu.edu

Jos M. Raaijmakers
Laboratory of Phytopathology, Department of Plant Sciences, Wageningen University, Binnenhaven 9, P.O. Box 8025, 6700 EE Wageningen, The Netherlands;
e-mail: jos.raaijmakers@fyto.dpw.wau.nl

Brian B. McSpadden Gardener
Department of Plant Pathology, The Ohio State University, OARDC, Wooster, Ohio 44691-4096; e-mail: bbmg+@osu.edu

Linda S. Thomashow
USDA, Agricultural Research Service, Root Disease and Biological Control Research Unit, Washington State University, Pullman, Washington 99164-6430;
e-mail: thomasho@mail.wsu.edu

Key Words   molecular ecology, rhizosphere, biological control, *Gaeumannomyces graminis*

■ **Abstract**   Agricultural soils suppressive to soilborne plant pathogens occur worldwide, and for several of these soils the biological basis of suppressiveness has been described. Two classical types of suppressiveness are known. General suppression owes its activity to the total microbial biomass in soil and is not transferable between soils. Specific suppression owes its activity to the effects of individual or select groups of microorganisms and is transferable. The microbial basis of specific suppression to four diseases, Fusarium wilts, potato scab, apple replant disease, and take-all, is discussed. One of the best-described examples occurs in take-all decline soils. In Washington State, take-all decline results from the buildup of fluorescent *Pseudomonas* spp. that produce the antifungal metabolite 2,4-diacetylphloroglucinol. Producers of this metabolite may have a broader role in disease-suppressive soils worldwide. By

---

309

coupling molecular technologies with traditional approaches used in plant pathology and microbiology, it is possible to dissect the microbial composition and complex interactions in suppressive soils.

INTRODUCTION

Soilborne plant pathogens causing root and crown rots, wilts, and damping-off are major yield-limiting factors in the production of food, fiber, and ornamental crops. Most soilborne pathogens are difficult to control by conventional strategies such as the use of resistant host cultivars and synthetic fungicides. The lack of reliable chemical controls, the occurrence of fungicide resistance in pathogens, and the breakdown or circumvention of host resistance by pathogen populations are among the key factors underlying efforts to develop other control measures. The search for alternative strategies also has been stimulated by public concerns about the adverse effects of soil fumigants such as methyl bromide on the environment and human health. Cook et al. (38) postulated that many plant species have developed a defense strategy against soilborne pathogens that involves the selective stimulation and support of populations of antagonistic rhizosphere microorganisms. Over the past century, evidence has accumulated that such plant-associated microorganisms account for many examples in which susceptible plants remain almost free of infection despite ample exposure to virulent inoculum of soilborne pathogens. Natural disease-suppressive soils probably are the best examples in which the indigenous microflora effectively protect plants against soilborne pathogens. Suppressive soils initially become apparent because the incidence or severity of disease is lower than expected for the prevailing environment or as compared to that in surrounding soil (36). Suppressive soils have been described for many soilborne pathogens (36), including *Gaeumannomyces graminis* var. *tritici* (69, 79, 168, 184), *Fusarium oxysporum* (2, 6, 173), *Aphanomyces euteiches* (151), *Heterodera avenae* (67, 86), *H. schachtii* (44, 226), *Meloidogyne* spp. (219), *Criconemella xenoplax* (91), *Thielaviopsis basicola* (205), *Phytophthora cinnamomi* (92), *Phytophthora infestans* (14), *Pythium splendens* (83), *Pythium ultimum* (121), *Rhizoctonia solani* (119, 164, 230), *Streptomyces scabies* (115, 137), *Plasmodiophora brassicae* (140), and *Ralstonia solanacearum* (183). Suppressiveness due strictly to soil physical or chemical factors is not discussed here.

The *Glossary of Plant-Pathological Terms* (186) defines suppressive soils as "soils in which certain diseases are suppressed due to the presence in the soil of microorganisms antagonistic to the pathogen or pathogens." We prefer Baker & Cook's (20) earlier definition: "soils in which the pathogen does not establish or persist, establishes but causes little or no damage, or establishes and causes disease for a while but thereafter the disease is less important, although the pathogen may persist in the soil." In contrast, conducive (nonsuppressive) soils are soils in which disease readily occurs. The terms disease-suppressive soil and pathogen-suppressive soil often are used interchangeably. However, some consider pathogen suppression as the suppression of saprophytic growth or survival of the pathogen in

the soil, and disease suppression as the suppression of the pathogen growing parasitically (189). Numerous review articles (2, 4, 32, 49, 78, 105, 189, 228) and book chapters (3, 8, 20, 36, 77, 79, 168, 177) devoted to the topic of suppressive soils have described and catalogued the types of suppressiveness and the hypothesized mechanisms of suppression.

In this review, we focus on recent progress toward unraveling the microbial basis of suppressive soils. Because it is impossible to review all the literature relevant to this topic, we highlight four examples—specific suppression of Fusarium wilts, potato scab, apple replant disease, and take-all of wheat, with special emphasis on the molecular basis of take-all decline. We also discuss molecular approaches to dissect and identify microbial communities and the complex interactions that occur in suppressive soils.

CHARACTERISTICS OF SUPPRESSIVE SOILS

The widespread but limited ability of soils to suppress the growth or activity of soilborne pathogens has been referred to as "general suppression" (36, 69), "general" or "nonspecific antagonism" (78, 168), or "biological buffering" (81). General suppression is related to the total microbial biomass in soil, which competes with the pathogen for resources or causes inhibition through more direct forms of antagonism. General suppression often is enhanced by the addition of organic matter, certain agronomic practices, or the buildup of soil fertility (168), all of which can increase soil microbial activity. No one microorganism is responsible for general suppression (2, 36) and the suppressiveness is not transferable between soils (37, 168). When inoculum of a pathogen is added to pairs of raw and sterilized soil samples, the effect of general suppression is apparent by the greater severity of disease on a host grown in the sterilized as compared to the raw soil. "Specific suppression" (2, 36, 37, 69) is superimposed over the background of general suppression and is due, at least in part, to the effects of individual or select groups of microorganisms during some stage in the life cycle of a pathogen. Transferability is the key characteristic of specific suppression (12, 37, 69, 137, 173, 185, 190, 205, 223, 227, 230) and the term "transferable suppression" has been used synonymously with specific suppression. Suppressive soils undoubtedly owe their activity to a combination of general and specific suppression. The two function as a continuum in the soil, although they may be affected differently by edaphic, climatic, and agronomic conditions (168). Suppressive soils also have been differentiated according to their longevity. Hornby (78, 79) divided suppressive soils into the categories of "long-standing suppression" and "induced suppression." Long-standing suppression is a biological condition naturally associated with the soil, its origin is not known and it appears to survive in the absence of plants. In contrast, induced suppressiveness is initiated and sustained by crop monoculture or by the addition of inoculum of the target pathogen.

Most suppressive soils maintain their activity when brought into the greenhouse or laboratory, which facilitates assessment of their properties and mechanisms of

TABLE 1 Overview of research strategies to elucidate the microbiological basis of disease-suppressive soils: a comparison with current molecular strategies applied in microbial genetics

Research strategies	
Disease-suppressive soils	**Microbial genetics**
1. Elimination of specific microbial groups by selective heat treatment or biocides	Mutational analysis (e.g., transposon mutagenesis)
2. Isolation of microbial groups	Construction of a genomic library
3. Evaluation of the suppressiveness of microbial groups	Identification of gene(s) of interest
4. Introduction of representatives of microbial groups into conducive soils	Complementation of mutant strains with genomic library
5. Transfer of suppressiveness to conducive soils with 0.1–10% suppressive soil	Transfer and expression of gene(s) of interest in heterologous strains
6. Phenotypic characterization of microbial groups that confer suppressiveness	Sequence analysis of gene(s)
7. Analysis of genotypic diversity among and within functional groups	Phylogenetic analysis of gene(s)
8. Elucidation of mechanism(s) by which micro-organism(s) suppress disease	Functional analysis of gene(s)

suppression under more controlled and reproducible conditions. The strategy used to determine the microbiological basis of suppressive soils and to identify the contribution of specific microbial groups is summarized in Table 1. The first step is to determine whether suppressiveness can be destroyed by pasteurization (moist heat, 60°C for 30 min) (185) or selective biocides (e.g., novobiocin or chloropicrin), or whether harsher treatments (e.g., steam, methyl bromide, autoclaving, or gamma radiation) are required (83, 91, 166, 183, 219, 230). Both general and specific suppression are eliminated by autoclaving and gamma radiation. General suppression is reduced but not eliminated by soil fumigation, and usually survives 70°C moist heat (37). In many examples of specific suppression, suppressiveness was eliminated by pasteurization (2, 37, 160, 173, 185, 205, 227, 230); however, this characteristic is not a prerequisite for specific suppression. For example, the suppressiveness of a root-knot nematode–suppressive soil in Florida, which apparently resulted from the spore-forming bacterium *Pasteuria penetrans* (219), would not be eliminated by pasteurization. A second step, which allows confirmation of the biological basis of suppression, involves transfer of suppressiveness to a raw conducive, fumigated, or sterilized soil by addition of 0.1% to 10% or less (w/w) of the suppressive soil. The impact of soil edaphic factors on disease development in soil transfer studies is minimized when suppressive and conducive soils are diluted into a common background soil, allowing a direct comparison of the introduced

microbiological components. The research strategies used historically to elucidate the microbiological basis of suppressive soils are in some ways analogous to those currently applied to studies of microbial gene function (Table 1) even though the two areas of research are completely different.

EXAMPLES OF SUPPRESSIVE SOILS

Fusarium Wilt–Suppressive Soils

Fusarium wilts are caused by pathogenic *F. oxysporum*, a soilborne fungus found worldwide. Wilts cause significant yield losses in numerous crops, and fungicides and host resistance often do not give adequate and sustainable control. Natural suppressiveness of soils to Fusarium wilt was first recognized in the nineteenth century by Atkinson (18) and was later described for other soils around the globe (2, 51, 76, 150, 173, 198, 200, 213). Wilt–suppressive soils limit the incidence or severity of wilts of many plant species (3, 36). The suppressiveness is specific to Fusarium wilts and not effective against diseases caused by nonvascular *Fusarium* species including *F. roseum* and *F. solani*, or other soilborne pathogens (2, 49). Long-standing suppression operates in most Fusarium wilt–suppressive soils, although there are a few examples of induced suppression. For example, suppressiveness to *F. oxysporum* f. sp. *melonis* (200) and *F. oxysporum* f. sp. *niveum* (76, 100) was induced following continuous cropping of melon and watermelon, respectively. Interestingly, the induction of suppressiveness in these cases was associated with continuous cropping of partially resistant cultivars, whereas induction of suppressiveness against other soilborne pathogens normally involves monoculture of susceptible cultivars (228).

The microbiological nature of Fusarium wilt-suppressiveness was demonstrated by the strategies described previously (Table 1). Suppressiveness was eliminated upon treatment with moist heat, methyl bromide, or gamma radiation (2, 7, 173), and was transferred by mixing small amounts of suppressive soil into a heat-treated conducive soil (2, 173). Among the bacterial and fungal genera proposed to contribute to Fusarium wilt–suppressiveness are *Alcaligenes* sp. (236), *Bacillus*, *Trichoderma* (193), *Pseudomonas* spp. (89, 105, 174), Actinomycetes (10), and nonpathogenic *F. oxysporum* (2, 5, 98, 99, 101, 167). Although introduction of representative strains of each of these genera increased the level of soil suppressiveness in most cases, the introduction of large populations is unlikely to reproduce the microbial community structure and interactions that occur naturally in suppressive soils. The introduction of microorganisms isolated from suppressive soils into conducive soils, therefore, does not necessarily provide conclusive information about their contribution to soil suppressiveness.

The extensive studies of Fusarium wilt–suppressive soils from Chateaurenard (France) and the Salinas Valley in California (USA), however, have provided substantial insight into specific microorganisms and mechanisms involved in suppression. In these soils, natural suppressiveness is associated with a reduction in the saprophytic growth and inhibition of chlamydospore germination of pathogenic

F. oxysporum (41, 62, 105, 173, 199). This suppressiveness has been attributed mainly to the activity of nonpathogenic *F. oxysporum* and fluorescent *Pseudomonas* spp., and for both microbial groups, similar mechanisms including competition and induced systemic resistance were shown to be active (3, 55, 56, 89, 104, 106, 107, 174). Particularly interesting from the work of Lemanceau and co-workers is the intimate and complementary association between these two groups of microorganisms, which in combination, provided enhanced disease suppression mediated by competition for iron via siderophores produced by the pseudomonads and for carbon by nonpathogenic *F. oxysporum* strain Fo47 (106, 107). Work by Duijff et al. (56), using a GUS-marked strain of pathogenic *F. oxysporum* f. sp. *lini* and a *pvd-inaZ*–marked derivative *of P. putida* WCS358, supported and extended earlier observations that suppression by the nonpathogenic *Fusarium* strain is related to reductions in both population density and metabolic activity of the pathogen on the root surface, and that competition for iron contributes to the suppression by *Pseudomonas* and enhances the biological activity of the nonpathogenic *F. oxysporum* strain.

In contrast to the suppressiveness of the Chateurenard and Salinas Valley soils, the monoculture-induced suppressiveness to Fusarium wilt of watermelon (101) does not result in a reduction in saprophytic growth of the pathogen nor in inhibition of chlamydospore germination. Among a large collection of bacteria, fungi, and actinomycetes isolated from this suppressive soil, only nonpathogenic *F. oxysporum* isolates consistently suppressed the disease in both microwave-treated and natural soil. Induced systemic resistance was the primary mode of action for several of these isolates (99, 101), but it is not yet clear if the mechanism is similar to that described (115) for induced systemic resistance by rhizobacteria.

Early work by Alabouvette and co-workers (6) and later work by Larkin & Fravel (98, 99) clearly indicated that strains of nonpathogenic *F. oxysporum* differ considerably in their efficacy against Fusarium wilt. For example, strain Fo20 was the least effective of eight strains tested, whereas Fo47 proved to be the most effective in controlling Fusarium wilt (6). Furthermore, Larkin & Fravel (98, 99) showed that nonpathogenic strains differed not only in their efficacy, but also in the mechanism(s) and dose required to suppress disease. For example, strain CS-20 required doses as low as 100 chlamydospores per gram of soil to reduce disease incidence significantly, whereas strain Fo47 was effective only at doses of 10^4 to 10^5 chlamydospores per gram of soil. These studies clearly illustrate the need for knowledge of the diversity within a group of antagonistic microorganisms when studying disease-suppressive soils. Steinberg et al. (202), Abadie et al. (1), and Edel et al. (60, 61) reported considerable phenotypic and genotypic diversity within populations of nonpathogenic *F. oxysporum*. The degree of intraspecific diversity varied widely among isolates from different French soils, but not as much among isolates from the same soils collected over time (61). Edel et al. (61) observed similarities between populations of nonpathogenic *F. oxysporum* from two fields in the Chateaurenard region that were several kilometers apart. Interestingly, Fo47 and several other isolates originally isolated over 20 years ago

were recently detected again in the Chateaurenard soil (61). Collectively, these studies suggest that the composition of nonpathogenic *F. oxysporum* populations remained relatively stable over a considerable period of time, consistent with the long-standing nature (78) of the suppressiveness of these soils.

Potato Scab Decline

Common scab is an important disease of potato caused by *Streptomyces scabies* and other *Streptomyces* species (93, 118). Pathogenic strains produce thaxtomins, phytotoxins that induce symptoms of scab when applied to tubers in the absence of the pathogen (103). Production of thaxtomin A, the major phytotoxin produced by *S. scabies*, is positively related to the ability to cause disease (88), and the amount produced by a strain in vitro is correlated with its aggressiveness in vivo (88). Thaxtomin nonproducers are nonpathogenic (117).

In the 1950s, Menzies (137) observed that potatoes grown in "old" irrigated fields in central Washington that had undergone many years of potato production were almost scab free. In contrast, potatoes grown in "new" fields that had been brought into production only in the previous 15 years suffered from scab. Where monoculture potato production was attempted, scab occurred uniformly on potatoes from new fields but did not appear on potatoes grown in the old fields. Menzies (137) put nine virgin soils and three old cultivated soils in bottomless containers in the field and planted *S. scabies*–infected seed pieces. An equivalent amount of scab developed in all of the soils in the first year. However, in each of four subsequent years when healthy seed-pieces were planted, scab increased to a high level in the virgin soils, but was suppressed in the old-field soils. Suppressiveness was eliminated by steaming the soil and transfered into scab-conducive soil by mixing 10% suppressive soil or 1% suppressive soil plus alfalfa meal with conducive soil.

Scab has declined with potato monoculture in other potato growing regions (115, 220). For example, a plot for screening scab-resistant germplasm at Grand Rapids, MN, was maintained in potato monoculture from 1943 to 1971, and in 1965 scab decline was first observed. Susceptible cultivars grown in the plot from 1985 to 1987 failed to develop scab (115), and addition of the Grand Rapids soil to a conducive plot at Becker, MN, transferred suppressiveness (29). A diverse collection of *Streptomyces* isolates from scab-free potatoes grown in the suppressive soil produced antibiotics inhibitory to *S. scabies* in vitro (59, 112, 116), and the pathogenic strains were much less inhibitory than the suppressive strains against other isolates, whether pathogenic or not (112). DNA fingerprinting by repetitive DNA sequence analysis (rep-PCR) did not distinguish between pathogenic and suppressive strains (170); cellular fatty acid analysis was more useful, but also did not distinguish perfectly between pathogens and nonpathogens (29, 88, 143).

Suppressive strains introduced into scab-infested soil reduced the severity of scab (29, 111, 112, 169). Liu et al. (111) added inocula of suppressive strain, S. *diastatochromogenes* PonSSII (nonpathogenic) or *S. scabies* PonR (weak pathogen) at 1%, 5%, and 10% (v/v) to a conducive soil naturally infested with

the pathogen. The soil mixes and appropriate controls were placed in pots buried in the field (137), and potatoes were grown for four continuous years. Both strains were isolated from the protected tubers each year, and both were equally effective at all three inoculum doses by the fourth year. The average disease reduction over all inoculum doses and all four years was 73% for PonSSII and 64% for PonR. In another study (29), PonSSII and PonR were introduced in-furrow and potatoes were grown for two years. PonSSII and PonR reduced pathogen populations at harvest by 93% and 85%, respectively, in the first year and 36% and 44%, respectively, in the second year. Scab lesions on tubers were reduced in both years. These two studies are especially notable because, as is the case with the transfer of a suppressive soil, the suppressive strains were introduced only once at the beginning of the experiment in order to initiate suppression in subsequent years. Liu et al. (112) showed that suppressive strains with more vigorous growth and antibiotic production provided better scab control than strains with less aggressive growth and antibiotic production. However, more recent studies indicate that suppressive strains function through a combination of resource competition and production of inhibitory compounds (144, 178). The picture emerging from these studies is that suppressive *Streptomyces* strains play an important role in scab decline, but the contribution of other microorganisms needs further study.

In general, *Streptomyces* spp. have received less attention than other microorganisms as agents of specific suppressiveness. Expanded studies of *Streptomyces* spp. in suppressive soils is warranted given their abundance in soil, ability to produce broad-spectrum antibiotics, and well-documented biocontrol abilities. *Streptomyces* spp. appear to have a major role in the disease suppressiveness of light-colored *Sphagnum* peat that commonly has been used as a growth medium in glasshouse cultivation in Finland (207). The biofungicide MYCOSTOP® is based on a strain of *Streptomyces griseoviridis* isolated from this peat (94).

Induction of Suppressiveness to Apple Replant Disease

The poor growth of apple trees that occurs after replanting on a site previously cropped to apples is known as replant disease. Symptoms include stunting, shortened internodes, rosetted leaves, and stunted and decayed or discolored roots (127). In Washington State, the dominant cause of replant disease is a complex of fungi including *Cylindrocarpon destructans*, *Phytophthora cactorum*, *Pythium* spp., and *Rhizoctonia solani* (127). Disease control depends largely on treatment of preplant soils with broad-spectrum pesticides such as methyl bromide that are being phased out of agricultural production.

Soils that have not undergone apple cultivation are suppressive to replant disease. However, in contrast to take-all and potato scab-suppressive soils that are induced by monoculture, orchard soils become progressively more conducive to replant disease the longer the orchard is in production. Mazzola (128) demonstrated this phenomenon by introducing inoculum of *Rhizoctonia solani* AG-5 (a member of the replant pathogen complex) (126) into soils collected from orchard blocks

in their first to fifth years of growth, and from nearby noncultivated areas. Apple seedling growth was significantly reduced in soils from the third-, fourth-, and fifth-year blocks as compared to growth in noncultivated soil or in soil from first- and second-year blocks. Concomitant with diminished soil suppressiveness was an increase in the populations of decline pathogens isolated from seedling roots and a decrease in populations of *Burkholderia cepacia* and *Pseudomonas putida*. *B. cepacia* produces multiple antibiotics and has biocontrol activity against soil-borne pathogens including *R. solani* and *Pythium* spp. (149). Isolates of *P. putida* from these soils also were highly antagonistic to *Pythium* and *Rhizoctonia* spp. (73, 129), but as their populations declined in the orchard soil, isolates of *P. syringae* and *P. fluorescens* biovar C (not inhibitory to the replant pathogens), became dominant. Introduction of strain 2C8, typical of *P. putida* isolates from the apple rhizosphere, enhanced the growth of apple seedlings in replant soil (73, 129). Mazzola & Gu (131, 132) then showed that cultivation of old orchard soils in the greenhouse with three cycles of wheat prior to planting apple seedlings induced suppressiveness, resulting in increased seedling growth, increased rhizosphere populations of *P. putida*, and decreased populations of the replant pathogens and *P. fluorescens* biovar C. These findings suggest that changes in fluorescent pseudomonad community structure play a role in suppression of replant disease (132). Interestingly, although the wheat cultivars Penewawa, Eltan, and Rely all enhanced apple seedling growth, Penewawa induced larger populations of *P. putida* and better apple seedling growth than did Eltan or Rely. These results suggest a cultivar x *Pseudomonas* interaction possibly related to differences in root exudates among the cultivars resulting in differences in growth of the antagonistic strains in the rhizosphere (132). Of particular interest now is whether specific genotypes or mixtures of subspecies of *B. cepacia* and *P. putida* contribute to suppressiveness.

Take-All Decline

Take-all, caused by the fungus *Gaeumannomyces graminis* var. *tritici*, is an important root disease of wheat worldwide. Wheat is particularly susceptible to the take-all fungus, and other Gramineae such as barley, rye, and triticale also can be infected (17). Breeding for resistance has been unsuccessful and methods of chemical control are limited. Take-all can be controlled by a combination of crop rotation and tillage, practices that reduce the inoculum potential of the pathogen, but the current trend in cereal production is toward less tillage and two or three consecutive wheat crops before a break. *G. g.* var. *tritici* is vulnerable to a number of types of soil suppressiveness (79, 189), which have been reviewed extensively over the past two decades and categorized on the basis of the requirements for the presence of the host, the pathogen, and/or severe disease in order to develop suppressiveness (78, 79, 168).

Take-all decline (TAD), the most thoroughly studied of the various types of take-all suppressiveness, requires three components: monoculture of a susceptible host, *G. g.* var. *tritici*, and at least one severe outbreak of take-all. TAD is defined

as the spontaneous decrease in the incidence and severity of take-all that occurs with monoculture of wheat or other susceptible host crops after one or more severe outbreaks of the disease (39, 77, 79, 184). TAD was first reported over 65 years ago, (71), is considered to be a field phenomenon, and occurs globally (69, 79, 184, 185, 189, 194, 217). The similarity with which TAD occurs is remarkable in view of the broad range of soil types, climates and agronomic conditions under which wheat is cultivated throughout the world. Field studies show clearly that the development of TAD follows a consistent pattern everywhere; factors such as soil type and previous cropping history seem only to modulate the extent and speed of its development (184). The number of crops of wheat or barley required before the onset of TAD typically is about four to six, but this can vary considerably depending on the location of the field, soil type, and environmental conditions (184). Suppressiveness can be reduced or eliminated by breaking monoculture with a nonhost crop (34, 184), but a field with a long history of TAD can regain suppressiveness once wheat or barley is again grown. Gerlagh (69) and Zogg & Jäggi (237) induced suppressiveness by repeatedly adding mycelium of *G. g.* var. *tritici* to soil. Whether this and other types of take-all suppressiveness (78, 79, 168) that develop without all of the classical components of TAD share a common microbial basis with TAD remains unknown.

The specific suppression associated with TAD is eliminated by treating the soil with moist heat (pasteurization, 60°C for 30 min), methyl bromide, or chloropicrin; it operates in cooler soils (10°C to 25°C) than does general suppression, and it is transferable by adding 1% to 10% TAD soil to raw conducive, fumigated, or pasteurized soil (12, 37, 69, 79, 160, 190), or from one field to another (185). Most of the mechanisms reported to be responsible for TAD involve microbiological changes in the bulk soil, the rhizosphere soil, and/or the rhizoplane, resulting in antagonism of the pathogen. Several lines of evidence support the widely held opinion that different microbial antagonists and mechanisms are responsible for TAD worldwide. First, TAD develops in multiple agroecosystems. Second, the time needed for TAD to develop differs among fields and crops grown in monoculture (79, 184). Third, the site of suppression (i.e., bulk soil, rhizosphere, root surface) and the stage in the life cycle of *G. g.* var. *tritici* when suppression is thought to occur (i.e., parasitic, saprophytic, or both) appear to differ among TAD soils (40, 190, 229). Fourth, microorganisms with biocontrol ability from TAD soils represent many different taxonomic groups (11, 79, 87, 187, 223). Fifth, the take-all fungus is sensitive to a variety of forms of antagonism including destruction of hyphae by amoebae (75); cross protection by *G. graminis* var. *graminis* or *Phialophora graminicola* (48, 232, 238); hyphal lysis by a sterile red fungus (50); and antibiosis by actinomycetes (11), *Trichoderma* spp. (54, 58, 187), *Bacillus* spp. (87), and *Pseudomonas* spp. (24, 161, 194, 221, 223). Of the many microorganisms implicated in TAD, however, several exhibit biological properties not consistent with a role in suppressiveness (35). For example, the sensitivity of TAD to pasteurization rules out the involvement of heat-resistant bacteria like *Bacillus* spp. and probably also many actinomycetes (36).

FLUORESCENT PSEUDOMONADS   Antagonistic *Pseudomonas* spp. have been implicated in TAD throughout the world (37, 160, 172, 194, 221, 223) and have characteristics consistent with their involvement in suppressiveness. They are well adapted to the rhizosphere environment; they utilize many organic substrates, they synthesize a variety of antifungal metabolites inhibitory to *G. g.* var. *tritici*, their populations increase dramatically on roots with take-all lesions, and they are eliminated by soil pasteurization (39). Efforts of Weller and co-workers to understand the role of pseudomonads in TAD have focused on TAD soils from three irrigated fields near the cities of Lind, Quincy, and Moses Lake, and a nonirrigated field near Pullman, Washington, USA. They have been compared to conducive virgin (covered by native vegetation) and nonsuppressive agricultural soils collected from sites near the TAD fields. Transferability of the suppressive factor in these TAD soils has been demonstrated in field and greenhouse studies over the past 30 years (36, 37, 160, 185, 223).

Fluorescent *Pseudomonas* spp. from the rhizosphere of wheat grown in Quincy and Moses Lake TAD soils were compared to pseudomonads from roots of wheat grown in conducive soils from Lind and Mt. Vernon. Each soil was diluted with fumigated Lind virgin soil and then amended with take-all inoculum. In the second cropping of wheat, take-all was suppressed in mixes with TAD but not conducive soils. Roots from all soil mixes had equivalent population densities of culturable aerobic bacteria, but population densities of fluorescent *Pseudomonas* spp. inhibitory to *G. g.* var. *tritici* in vitro were significantly greater on roots from mixes with Quincy and Moses Lake TAD soils than on roots from conducive soil mixes (36, 223). Furthermore, when applied as wheat seed treatments, fluorescent pseudomonads from the TAD soils provided significantly better protection against take-all than pseudomonads from conducive soils (223, 225). These findings supported those of Smiley (194), whose earlier work in Australia showed that the proportion of the population of *Pseudomonas* spp. inhibitory to *G. g.* var. *tritici* was greater from roots grown in a TAD soil near Horsham, Victoria, (over 50 years of wheat) than in a conducive soil. Furthermore, the severity of take-all in wheat grown in the TAD soil was inversely correlated with an antagonism rating for pseudomonads (194). Antagonistic fluorescent pseudomonads also were implicated in the decline of take-all patch of turfgrass caused by *G. g.* var. *avenae*, a sister phenomenon of TAD (171).

2,4-DIACETYLPHLOROGLUCINOL: A MECHANISM OF TAD   Gerlagh (69) concluded from his elegant studies of TAD in Dutch polders that specific suppression "is governed by antibiotics from soil-microorganisms." The findings that some of the most effective *Pseudomonas* biocontrol strains isolated from TAD soils produce either 2,4-diacetylphloroglucinol (2,4-DAPG) (74, 153, 216) or phenazine-1-carboxylic acid (PCA) (210, 222), that the antibiotics are synthesized in the rhizosphere (26, 211), and that they are responsible for the biocontrol activity of the strains producing them (130, 155, 210, 216, 221), prompted the hypothesis that the enrichment of producers of these antibiotics during wheat monoculture is a major

contributor to suppressiveness in some TAD soils. The availability of cloned and sequenced biosynthetic genes (22, 124) facilitated the development of specific PCR primers and hybridization probes to detect and quantify rhizosphere populations of fluorescent *Pseudomonas* spp. capable of producing either PCA or 2,4-DAPG (162). For example, primers Phl2a and Phl2b were developed from sequences within *phlD*, a key gene within the 2,4-DAPG biosynthetic operon (22), and amplify a 745-bp fragment from DNA of 2,4-DAPG-producing ($phlD^+$) *Pseudomonas* strains (162, 163). McSpadden Gardener et al. (134) subsequently developed a more rapid PCR-based method that allows for the isolation and quantification of the most abundant $phlD^+$ populations in environmental samples as well as their genotypic characterization. To date, the detection of biosynthetic genes for 2,4-DAPG or PCA in isolates of fluorescent *Pseudomonas* spp. has correlated with their ability to produce the respective antibiotics.

To initially test the hypothesis that PCA or 2,4-DAPG producers are enriched in TAD soils, their frequencies were determined in bacterial populations recovered from roots of wheat grown in three TAD soils (Quincy, Moses Lake, and Lind), and four conducive soils (Quincy virgin, Moses Lake virgin, Lind virgin, and Mt. Vernon agricultural). PCA-producers were not detected on any of the roots, (162) whereas several lines of evidence indicated that 2,4-DAPG-producing ($phlD^+$) fluorescent *Pseudomonas* spp. have a key role in this suppressiveness. First, $phlD^+$ isolates were present on roots of wheat grown in the TAD soils at population densities (5×10^5 to 2×10^6 CFU g^{-1} root) above the threshold (10^5 CFU g^{-1} root) (160) required for take-all control, but were below the threshold or not detected on roots from conducive soils (162). Second, successive cultivation of wheat in Quincy TAD and Quincy virgin soils demonstrated a strong inverse association between high population densities of indigenous 2,4-DAPG producers and severity of take-all (160). Third, the specific suppression in Quincy TAD soil was lost when $phlD^+$ isolates were eliminated by soil pasteurization (160). Fourth, the addition of Quincy TAD soil to steamed Lind virgin (Figure 1) or raw Pullman conducive soil resulted in the establishment of population densities of 2,4-DAPG producers above the threshold required for disease control, and transfer of suppressiveness to the conducive soils (160). Fifth, cultivation of oats, a crop known to eliminate suppressiveness to take-all, decreased the population densities of $phlD^+$ fluorescent *Pseudomonas* spp. to levels below the threshold required for take-all control. Sixth, introduction of the 2,4-DAPG-producing strain *P. fluorescens* Q8r1-96 (from Quincy TAD soil) into steamed or raw conducive soils at low doses controlled take-all to a level similar to that of the complementary TAD soils (160). Finally, 2,4-DAPG was detected on roots of wheat grown in Quincy TAD soil at an average concentration of 19 ng g^{-1} root fresh weight, but was not detected on roots grown in Quincy virgin soil (159).

Complementary to these results were findings that $phlD^+$ fluorescent *Pseudomonas* spp. were present on roots of mature wheat collected from Pullman and Almota TAD fields at densities above the threshold required for take-all control, but were absent from roots collected from a conducive field (no-till

Figure 1   Transfer of suppressiveness from Quincy TAD and Quincy virgin soils into steamed Lind virgin soil (2 h, 95°C). Quincy TAD and virgin soils were collected in 1995 and cultivated to wheat for 8 successive cycles of 4 weeks each in the greenhouse. Wheat seeds were sown in Lind virgin soil (*A*), Lind virgin soil mixed with Quincy TAD soil (9:1 ratio, w/w) (*B*), and Lind virgin soil mixed with Quincy virgin soil (9:1 ratio, w/w) (*C*). All soils were amended with 0.5% (w/w) of an oat grain inoculum of the take-all pathogen. Plants were grown for 4 weeks under controlled conditions. Roots of wheat grown in the soil mix with Quincy TAD soil (*B*) had a population density of 2,4-DAPG producers of 6×10^5 CFU g^{-1} of root and a disease rating of 1.3 (0–8 scale); in contrast, 2,4-DAPG producers were not detected on roots from Lind virgin soil (*A*) or Lind virgin soil mixed with Quincy virgin soil (*C*), and the root disease ratings were 4.2 and 3.0, respectively. Disease ratings were significantly different among all treatments (160).
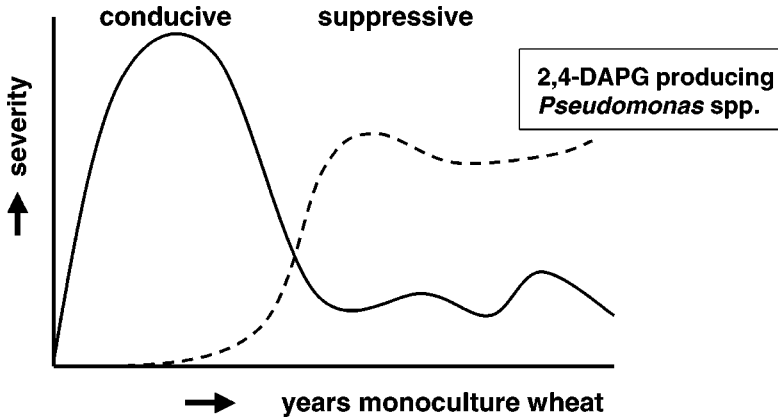
Figure 2 Hypothetical model of the role of 2,4-DAPG-producing fluorescent *Pseudomonas* spp. in take-all decline: During monoculture of a take-all susceptible host and after one or more severe outbreaks of take-all (*solid line*), populations of 2,4-DAPG producers (*dashed line*) are enriched and increase to densities above the threshold density (10^5 CFU g^{-1} root) required to control take-all.

pea-chemical fallow-wheat rotation) located near the Pullman TAD field (160). Collectively, these results provide both microbiological and biochemical evidence that 2,4-DAPG-producing fluorescent *Pseudomonas* spp. are key components of the suppressiveness of TAD soils in Washington State. We postulate that these bacteria are enriched during monoculture of a take-all susceptible host and after at least one severe outbreak of take-all (Figure 2). We think that suppression by 2,4-DAPG producers can occur during the parasitic phase of *G. g.* var. *tritici*, while the fungus is growing on the root, as well as during the saprophytic phase, while the fungus is in the debris and during growth to roots of the next host.

A Broader Role for 2,4-DAPG in Disease Suppression?

These results raise questions about how frequently 2,4-DAPG producers contribute to TAD worldwide, and whether they have a role in soils suppressive of other pathogens. To begin to address these questions, 2,4-DAPG producers were quantified in paired soil samples collected from fields in the USA and The Netherlands that had or had not undergone monoculture. In many but not all monoculture wheat field soils, *phlD*+ isolates were abundant. For example, roots from wheat grown in the greenhouse in soils from Fargo, ND (116 continuous years of wheat), Hallock, MN (10 years of wheat) (179), and Woensdrecht, The Netherlands (two fields, 14 and 27 years of wheat) (J.T. de Souza, D.M. Weller & J.M. Raaijmakers, unpublished data) supported population densities of *phlD*+ isolates greater than 10^5 CFU g^{-1} root. In contrast, populations of *phlD*+ isolates on wheat grown in

soils from adjacent fields with no history of monoculture were near or below the limit of detection (10^4 CFU g^{-1} root) by colony hybridization. These results show that threshold populations of *phlD*[+] isolates can develop at widely separated locations where wheat is grown in monoculture, and preliminary studies (J.T. de Souza, D.M. Weller & J.M. Raaijmakers, unpublished data) indicate that in the two Dutch soils, they have a key role in take-all suppressiveness.

Fluorescent *Pseudomonas* spp. that produce 2,4-DAPG inhibit many different plant pathogens (84, 122) and are enriched in other suppressive soils, especially those with a history of monoculture. For example, *P. fluorescens* strain CHA0 suppresses black root of tobacco; crown and root rot of tomato; Pythium damping-off of cucumber, wheat, and pea; and take-all of wheat (52, 84, 122, 142, 182, 205). *Pseudomonas* sp. F113 suppresses cyst nematode and soft rot of potato and Pythium damping-off of sugar beet and pea (42, 43, 65, 142, 181). Strains Q8r1-96 and Q2-87 suppress take-all and Pythium root rot of wheat (153, 160; B.B. Landa, T.C. Paulitz & D.M. Weller, unpublished). Strain CHA0 was isolated from the roots of tobacco grown in soil from a field in the Morens region near Payerne, Switzerland, that is suppressive to black root rot of tobacco (205). During tobacco monoculture for 24 years and in the presence of *Thielaviopsis basicola*, tobacco plants remained free of disease in this soil (204). As with TAD, both suppressiveness and fluorescent *Pseudomonas* spp. were eliminated by soil pasteurization and were transferred by adding suppressive soil to a conducive soil (205). Later it was shown that up to 23% of 1100 rhizosphere pseudomonads from the Morens soil were *phlD*[+] (85). Recently, Landa et al. (95) reported that 2,4-DAPG producers were enriched ($>10^5$ CFU g^{-1} root) on pea grown in a soil that had undergone over 30 years of pea monoculture and is suppressive to *F. oxysporum* f. sp. *pisi*. Antibiosis has not previously been suggested as a mechanism of suppressiveness to Fusarium wilt, but in light of these results the possibility merits further study. 2,4-DAPG producers also appear to be common in soils from Albenga, Italy and Ghana that are suppressive to Fusarium wilt of tomato (85, 208). Of special interest was the occurrence of *phlD*[+] isolates ($>10^5$ CFU g^{-1} root) on flax grown in a Fargo soil cropped to flax for 103 consecutive years (B.B. Landa, B.B. McSpadden Gardener & D.M. Weller, unpublished data). Collectively, these findings strongly suggest a role for 2,4-DAPG producers in a wide range of suppressive soils, especially those associated with crop monoculture.

Genetic Diversity Among Strains of *P. fluorescens* Producing 2,4-DAPG

Strains producing 2,4-DAPG exhibit considerable genetic and phenotypic diversity, which has been studied in relation to biological control, root colonization, and soil suppressiveness (85, 95, 125, 135, 161, 163, 182, 218). Phylogenetic comparisons based on analysis of 16S ribosomal DNA (rDNA) revealed three distinct lineages (phylogenetic groups) among 45 and 138 *phlD*[+] fluorescent *Pseudomonas* spp. studied by Keel et al. (85) and McSpadden Gardener et al. (135), respectively.

These were designated amplified ribosomal DNA restriction analysis (ARDRA) groups 1, 2, and 3 by Keel et al. (85) and correspond to groups A, B, and C as defined by McSpadden Gardener et al. (135). Picard et al. (152) identified four ARDRA groups among 167 isolates from corn roots, 89.8% of which belonged to ARDRA group 2. Primary sequence analysis of the other *Alu*I-defined ARDRA groups indicated that most *phlD*$^+$ isolates belonged to closely related species of fluorescent pseudomonads (152).

Analyses of 16S rDNA sequences indicate that members of ARDRA group 1 differ significantly from other *phlD*$^+$ *Pseudomonas* spp. and belong to a different lineage (163; B.B. McSpadden Gardener, unpublished data) within the genus as defined by Anzai et al. (15). The two major lineages (ARDRA groups 1 and 2) also are distinguished by their differential capacity to produce pyoluteorin (85), to utilize certain carbon sources for growth (135, 218), and to deamidate the ethylene precursor 1-aminocyclopropane-1-carboxylate (218). Significantly, both major lineages appear to be globally distributed, as isolates from each have been obtained from crop species grown in soils from different continents (85, 135, 218). ARDRA group 1 strains have been isolated from cotton (85), tobacco (205), tomato (85), soybean (B.B. McSpadden Gardener, unpublished), pea (95), cabbage, corn, and soybeans (B.B. McSpadden Gardener, unpublished); ARDRA group 2 strains have been found on wheat (153), cucumber, tomato (85), corn (152; B.B. McSpadden Gardener, unpublished), flax (135), pea (95), cabbage, and soybeans (B.B. McSpadden Gardener, unpublished). Strain F113 from sugar beet grown in Ireland (65) is the only known representative of ARDRA group 3.

An additional degree of variation among isolates has been observed by using fine-scale genotyping based on genomic fingerprinting by random amplified polymorphic DNA (RAPD) (85, 125, 152, 161), rep-PCR (95, 135), and *phlD* restriction fragment length polymorphism (RFLP) analyses (125, 134, 163, 218). These techniques have resolved at least 17 different genotypes within American and European collections (85, 95, 125, 134, 135, 152, 161). Genotypic groups defined by rep-PCR with the BOXA1R primer (BOX-PCR) correlated nearly perfectly with those defined by RFLP analysis of *phlD* (95, 125, 134), indicating clonal growth and a substantial degree of endemicity in geographically separated soils (135). Other examples of subspecies diversity within soil microbial populations have been reported (176), and their ecological and evolutionary significance is a major focus of current research.

CHARACTERIZING THE MICROBIAL COMPONENTS RESPONSIBLE FOR SOIL SUPPRESSIVENESS

Biocontrol of soilborne pathogens traditionally has been studied as a three-way interaction among the pathogen, the host, and the biocontrol agent, with little attention to other interspecies interactions in the rhizosphere. To fully understand the nature of soil suppressiveness, however, the ecological context within which the major players act also must be considered. With specific suppressiveness, one

or a few groups of microorganisms may be responsible for biocontrol activity, but interactions with other members of the rhizosphere community can significantly modulate the degree of suppressiveness observed. Indeed, there are probably hundreds, if not thousands, of genotypically distinct microbial species inhabiting the rhizosphere of individual plants (13), and biotic as well as abiotic variables influence both the structure (46, 70, 102, 120, 139, 195) and activities (16, 21, 146, 233) of rhizosphere populations including those of pathogens (25, 68, 130, 203) and their antagonists (23, 53, 136, 148, 192). A clear understanding of the biological factors responsible for soil suppressiveness may require broader knowledge of the identity, relative abundance, and biological activity of the phylogenetically diverse microbial populations that inhabit the rhizosphere.

Molecular Approaches to Characterizing Soil Microbial Communities

Numerous methods have been developed over the past 12 years to more fully characterize microbial communities in soils (147). Approaches such as those based on carbon source utilization (138) and lipid composition (156) are useful to distinguish among communities differing in structure, but they provide little opportunity to identify or monitor the abundance of specific microbial populations. In contrast, molecular methods based on nucleic acid composition can provide information on the relative abundance and activity of microbial populations over a range of taxonomic levels (114). These molecular approaches can be used to monitor microbial populations that modulate or contribute directly to soil suppressiveness. The choice of nucleic acid–based method depends in part on the question of interest and the availability of useful sequence information. For example, if a specific population is implicated in suppressiveness, a specific marker (i.e., gene probe) can be used to enumerate populations in suppressive soils by such methods as colony hybridization, slot-blot hybridization, or PCR, as was done for 2,4-DAPG producers in TAD soils (96, 136, 162). Alternatively, it may be of interest to find out how many different microbial populations are associated with soil suppressiveness. Such exploratory studies of microbial communities require high-throughput procedures that detect diverse populations.

Regardless of the method chosen, thorough sampling must be conducted to identify meaningful differences in microbial community structure. Microbial communities are dynamic and vary significantly with time, space, and environmental conditions, (28, 64, 195, 235) but the significance of these natural fluctuations remains largely unknown. They may simply reflect the functional redundancy of microbial activities across broad phylogenetic lines or they may indicate chaotic patterns of microbial succession. In any case, they can be substantial. For instance, when comparing bacterial community structure in the rhizospheres of healthy and take-all–infected wheat, McSpadden Gardener & Weller (136) found that fewer than half of the significant differences were observed under all conditions tested (Figure 3). Only differences common to multiple, independent comparisons should
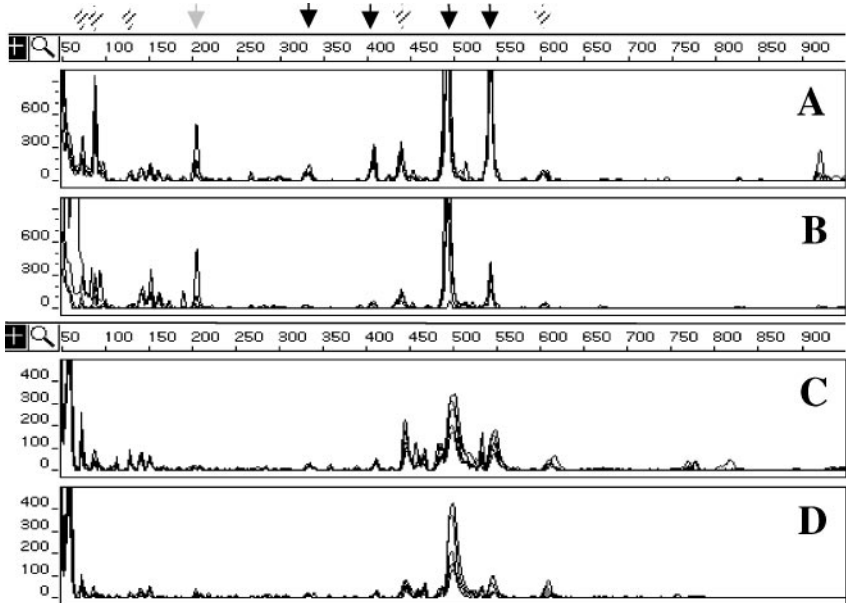
Figure 3   The structure of bacterial communities inhabiting the rhizosphere of wheat grown under different environmental conditions**.** Fluorescently tagged amplified ribosomal DNA restriction analyses (FT-ARDRA) were used to detect changes in community structure. All plants were grown in soil from Mt. Vernon, WA. Soils contained high (*A* and *C*) or low (*B* and *D*) inoculum densities of the take-all pathogen, *Gaeumannomyces graminis* var. *tritici*. Plants were grown in the growth chamber (*A* and *B*) or in the field (*C* and *D*). The community profiles were generated from *Msp*I digests of bacterial 16S sequences amplified from bacteria in rhizosphere washes. Overlaid chromatographic traces from four (*A* and *B*) or six (*C* and *D*) independent replicates of each condition are displayed in each panel. Terminal restriction fragments (TRFs) are displayed as peaks with the size in basepairs indicated by the horizontal scale at the top of the GeneScan results display, and the abundance of each is correlated with the peak area given in arbitrary fluorescence units on the vertical scale. Statistically significant differences (P $<$ 0.05) in particular TRFs are noted by arrows. Black arrows indicate ribotypes that increase in abundance in diseased rhizospheres under both growth chamber (*A* vs. *B*) and field (*C* vs. *D*) conditions. Hatched arrows indicate ribotypes that increased in abundance under both conditions shown, but not in another growth chamber experiment performed in soil taken from the same field one year later. Gray arrows indicate ribotypes that increased in abundance under one condition or the other, but not both (136).

be considered indicative of significant changes in microbial community structure (57, 136). The number of replicates required depends on the nature of the difference observed and the nature of the question asked. If the differences are small, or the occurrence of a particular microbial population relatively rare, over a dozen replicates may be needed to quantify changes in the relative abundance. However, a minimum of four to six replicates allowed identification of differences in microbial community structure in the rhizosphere of healthy and take-all infected plants (Figure 3) (136). When specific suppression is operating, microbial populations contributing most to suppressiveness should be omnipresent and identifiable, although, multiyear studies of suppressive soils may be needed to identify the common and recurring components of microbial communities that contribute to pathogen suppression.

Several approaches are now available for detailed, high-throughput analysis of the distribution and importance of microbial antagonists and root pathogens in suppressive soils. The first involves direct separation of PCR-amplified DNA products based on electrophoretic mobility. Differences in primary sequence composition can be detected under partially denaturing conditions as in denaturing- or temperature-gradient gel electrophoresis [DGGE and TGGE; see (141)] or by single-strand conformation polymorphism [SSCP (180)]. Alternatively, length polymorphisms can be assayed directly, as in amplified ribosomal intergenic spacer analysis [ARISA (66)] or by digesting amplified products with selected restriction enzymes and then characterizing the resulting fragment length polymorphisms (RFLPs). Differences in community structure can be detected by analyzing complete mixtures of restriction fragments (157, 195), but it usually is advantageous to visualize only the terminal restriction fragments (TRFs) by fluorescently labeling one of the two PCR primers (113). Because only a single band is visualized from each distinct amplification product, the number of TRFs in a T-RFLP profile indicates the species richness of the amplified sequences. T-RFLP analyses can be used to study diversity at various taxonomic levels (30). When applied to ribosomal gene sequences, RFLP analyses are more properly termed amplified ribosomal DNA (rDNA) restriction analysis [ARDRA (212)] or fluorescently tagged ARDRA [FT-ARDRA (133, 136)] (Figure 3), depending on whether all or only the terminal restriction fragments are visualized. The choice of approach depends on the degree of sequence diversity in the amplified products. FT-ARDRA is particularly useful to distinguish among larger taxonomic groups such as genera within a domain because the amplified fragments are likely to differ at multiple restriction sites. DGGE and SSCP are appropriate for diversity studies on a more limited scale, e.g., encompassing species within a genus, because the sequence differences that distinguish taxa may not correspond to most restriction sites. In the future, a third approach to characterizing microbial diversity likely will involve hybridization of amplified sequences to DNA arrays of known sequences. Early reports indicate the potential to identify several taxa within a single mixture (33, 109, 234), or individual sequences by serial hybridization (214). All of these approaches also can be adapted to examine metabolically active populations within microbial communities

by using extracted RNA to generate DNA templates by reverse-transcriptase-mediated PCR (RT-PCR) (145). Alternatively, newly synthesized template DNA, present in metabolically active microorganisms, can be isolated from cells grown in the presence of appropriate nucleotide analogues (27, 235).

The taxonomic level at which community differences are characterized depends on the oligonucleotide sequences used as amplification primers and the amount and quality of nucleotide sequence determined. Currently, most DNA-based community profiling methods rely on analyses of ribosomal sequences because they contain substantial phylogenetic information (231) and conserved primers have been designed that allow the detection of multiple taxa in a single PCR reaction (114). Profiles based on amplified ribosomal sequences have become the foundation upon which our descriptions of microbial communities are built. However, the precision with which taxonomic assignments can be made is limited by the number of nucleotides sequenced (110) and the potential for the generation of sequence heterogeneity during PCR amplification (158, 201). Given the complexity of soil microbial communities, many researchers find it convenient to use partial sequencing (250–500 bp) of a large number (>100) of rDNA clones to characterize the populations present in their clone banks (110). In lieu of sequencing, RFLP and/or T-RFLP analyses of individual clones also may be used, but their resolution limits the amount of taxonomic information that can be obtained. For community analyses involving PCR with domain-specific primers, FT-ARDRA and partial sequencing of cloned 16S sequences each provided for phylogenetic assignments roughly at the genus level (57, 136). Other genes also have been targeted for analyses of microbial diversity including bacterial RNA polymerase (45) and genes involved in nitrogen cycling (30, 80, 157). For dissection of community structure at the species or subspecies level, PCR primers also can be developed from clones of the targeted DNA fragments themselves, as was done to identify $phlD^+$ strains (134), molecular markers derived from related isolates, or sequences linked to the original target sequence and identified by screening the soil metagenome (165). Future studies will incorporate an ever-increasing number of molecular probes to evaluate the diversity of genes and their expression in complex communities.

Significant population differences detected by PCR-based approaches must be confirmed by an independent method (110) because of the limited quantitative power of profiling methods and the inherent uncertainties associated with amplification products from mixed templates. Because of differences in the efficiency with which the same set of primers will amplify different templates in a complex mixture, only monotonic differences (i.e., increases or decreases) in template abundance can be inferred from the intensity of the visualized signals. To date, the profiling methods described above have been used only to evaluate relative changes in microbial populations. Absolute differences in target abundance may be quantified by approaches such as dilution extinction PCR (134) or real-time PCR (72), but the community profiling methods now available do not offer the same degree of precision. This situation defines a certain "biological uncertainty principle:" one can assess the relative diversity of a community or the precise abundance of

a particular population, but not both with a single assay. Therefore, independent verification may involve developing target-specific PCR primers (134), probes for in situ hybridization (9), or enumerating the targeted populations by culture-based methods (209). Finally, the microorganisms identified must be characterized phenotypically, especially in relation to soil suppressiveness. If culturable, they can be studied under controlled conditions. Differential population densities in relation to soil suppressiveness also can be confirmed for nonculturable microorganisms, but until representative isolates are cultured, analyses will be limited to correlating changes in the relative abundance of molecular markers with the suspected activity of the populations within which they occur.

Functional Analysis of Key Populations Involved in Soil Suppressiveness

All populations are characterized by some degree of variation among individuals, but not all variation is functionally significant. However, among $phlD^+$ fluorescent *Pseudomonas* spp. antibiotic production (85), rhizosphere competence (95, 161), and disease suppression (161, 182) vary at the subspecies level. For example, Sharifi-Tehrani et al. (182) found that ARDRA group 2 strains were more effective than those of group 1 against Fusarium crown and root rot of tomato and Pythium damping-off of cucumber. Raaijmakers & Weller (161) examined the relationship between genotype and rhizosphere colonization by indigenous populations of 2,4-DAPG producers on roots of wheat grown in Quincy TAD soil for eight successive cycles. Of the 16 RAPD groups identified among 101 isolates, one group comprised 50% of the $phlD^+$ isolates and had an average population density of 2×10^5 CFU g^{-1} root. This genotype also was dominant on roots of wheat cycled in Moses Lake and Lind TAD soils, belongs to BOX-PCR group D, and is exemplified by *P. fluorescens* Q8r1-96 (161).

Both short- and long-term colonization studies demonstrated that Q8r1-96 (161) (Figure 4) and other group D strains (97; B.B. McSpadden Gardener, B.B. Landa & D.M. Weller, unpublished data) are much more competitive in the wheat rhizosphere than representatives of other $phlD^+$ genotypes that have so far been tested. For example, Q8r1-96 required a much lower dose (only 10 to 100 CFU seed^{-1} or g^{-1} of soil) to establish population densities of up to 10^7 CFU g^{-1} of root on wheat grown in Quincy virgin soil than did strains Q2-87 (group B) or 1M1-96 (group L). Over eight successive growth cycles, Q8r1-96 applied to Quincy virgin soil at 100 CFU g^{-1} of soil maintained rhizosphere population densities of 10^5 CFU g^{-1} of root or more, similar to the density at which D genotype isolates occur on roots of wheat cycled in Quincy TAD soil. In contrast, densities of strains Q2-87 and 1M1-96 were 100- to 1000-fold lower than those of Q8r1-96 (161). After the eighth cycle, inoculum of *G. g.* var. *tritici* was added to all of the treatments and the soil was again seeded to wheat. Take-all was controlled in soil that contained Q8r1-96 to the same extent as in Quincy TAD soil that had been cycled eight times, but no suppression occurred in the soils to which Q2-87 or 1M1-96 had been applied.
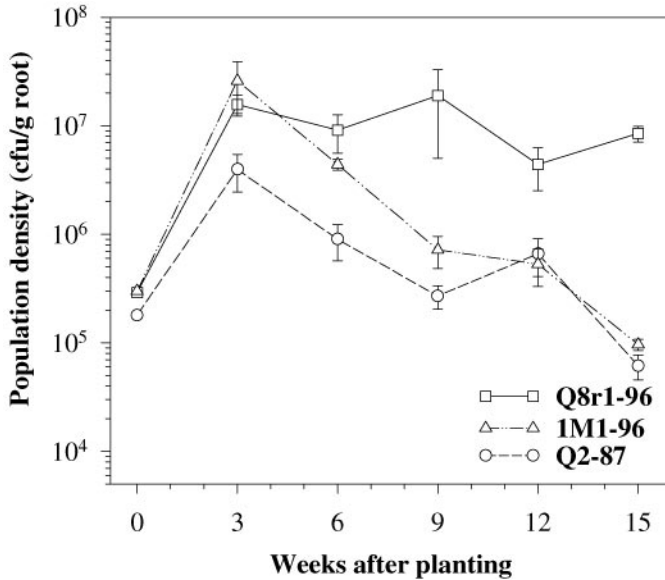
Figure 4 Population dynamics of *Pseudomonas fluorescens* Q8r1-96 (genotype D), 1M1-96 (genotype L), and Q2-87 (genotype B) on spring wheat (cv. Penawawa) grown in large pots of raw Quincy virgin soil at 15°C. Population densities at 0 weeks correspond to the dose of bacteria applied to the seed at planting.

The ability of Q8r1-96 to suppress take-all after eight months in Quincy virgin soil reflected its superior rhizosphere competence, which allowed it to maintain threshold densities throughout the cycling process. All three strains provide equivalent biocontrol if present at densities above the threshold needed for take-all control (161). The exceptional rhizosphere competence of Q8r1-96 is especially remarkable given that all three strains belong to biovar II of *P. fluorescens*, are nearly identical physiologically, and produce similar amounts of 2,4-DAPG in situ (161). Collectively, the results of these studies suggest that D genotype strains may be primarily responsible for TAD in Washington State soils, and raise the possibility of accelerating the process of TAD by introducing this specific genotype into the soil at the onset of a take-all outbreak. Field studies have verified that very low doses of D genotype strains introduced on wheat seed are sufficient to establish population densities required for root disease suppression. The populations are sustained throughout the growing season, survive between crops, and reestablish on the roots in successive years (B.B. McSpadden Gardener & D.M. Weller, unpublished data). Further studies are needed to determine whether any of the other genotypes described by McSpadden Gardener et al. (135) share the same level of rhizosphere competence.

Studies of the role of 2,4-DAPG producers in TAD fulfill four criteria that should be met to define the functional importance of a microbial population to

soil suppressiveness: (*a*) the ability of members of the population to perform a suppressive function (160); (*b*) the presence in suppressive soils of the population at densities above the threshold required for disease control (and its absence from conducive soils) (162); (*c*) duplication of the function in conducive soil by addition of populations obtained from a suppressive soil with similar physical and chemical properties (160); and (*d*) detection of a functional activity (e.g., antibiotic production) in situ (159). However, interactions among microbial populations and environmental variables are complex, and even when two soils share a common suppressive mechanism, the degree of suppressiveness may differ because local conditions affect on microbial community structure and function. For example, interactions between microbial components and soil properties including pH, clay type, and specific ions have a determinative role in Fusarium wilt–suppressive soils (8, 51, 150). Similarly, the type of clay greatly influenced the suppressive activity of *Pseudomonas* spp. in black root rot–suppressive soils (206), and fertilization with NH_4^+-N as compared to NO_3^--N resulted in a greater percentage of antagonistic pseudomonads on roots of wheat grown in take-all suppressive soils (172, 194). Production of 2,4-DAPG by biocontrol strains is influenced by their chemical and physical environment (52, 53, 146, 181); notable is the finding that expression of the 2,4-DAPG biosynthesis gene *phlA* in strain CHA0 was significantly greater in the rhizosphere of monocots (maize and wheat) than in the rhizosphere of dicots (bean and cucumber) (146). Interactions with other microorganisms also can affect the degree of root disease suppression observed. Interpopulation signaling among bacteria via *N*-acetylhomoserine lactones, which regulate the synthesis of phenazine antibiotics suppressive of take-all, has been detected in the wheat rhizosphere (154), and several recent studies indicate that increases in the abundance of 2,4-DAPG producers (by inoculation) can influence the structure of rhizosphere microbial communities (70, 139). Conversely, the effects of microbial communities on 2,4-DAPG producers are only now being explored. Altered root health was associated with multiple changes in rhizosphere bacterial community structure (136), and some of these changes might create an environment more favorable to the onset of take-all suppression. Production of fusaric acid by *F. oxysporum* f. sp. *radicis-lycopersici* repressed production of 2,4-DAPG by strain CHA0 (52), and *Pythium ultimum* decreased the ecological fitness of the 2,4-DAPG producer *P. fluorescens* F113 (63). These and numerous other observations substantiate the importance of the total rhizosphere microbial community as a modulator of the activity of suppressive microorganisms, and undoubtedly account for some of the variability in the degree of soil suppressiveness seen over time and space.

COMPETING IN A HOSTILE ENVIRONMENT

Specific suppression is a remarkable biological phenomenon in view of the intensely competitive microbial environment in which it occurs. Microbial population densities in the rhizosphere are 10 to 20 times greater than in the bulk soil

and can reach 1×10^9 cells/cm^2 (47, 224). The overwhelming impact of biological buffering is demonstrated by the population dynamics typical of rhizobacteria introduced on seeds or into the soil: They initially establish high population densities, but these densities cannot be sustained and subsequently decline (90, 224).

How do the microorganisms involved in specific suppression sidestep this formidable gauntlet of competitive microbial activity to reach and sustain the densities required to control target pathogens? One answer may lie in avoidance strategies such as those used by soilborne pathogens, rhizobia, and arbuscular mycorrhizal fungi that occupy favorable niches in or on roots, essentially "insulated" from the rest of the rhizosphere. It has been proposed that these microorganisms utilize specific recognition mechanisms governed by corresponding genes in the plant and the microorganism (196) to initiate the interactions with their hosts that ultimately lead to disease or the establishment of symbiotic relationships. Nonpathogenic *Fusarium oxysporum* and *Streptomyces* spp. have considerable niche overlap with the pathogens they control, giving them access to the same protected sites and resulting in a highly targeted mode of attack. Nonpathogenic *F. oxysporum* readily invades cortical tissues of roots and below-ground stems, and the failure of rep-PCR to distinguish between pathogenic and scab-suppressive *Streptomyces* strains (170) highlights how genetically similar these two functionally distinct bacterial populations are. Both nonpathogenic *F. oxysporum* and *Streptomyces* spp. also are able to maintain stable populations in agricultural soils between crops because they produce spores. The ability of *Streptomyces* spp. to grow in dry soils also may contribute to avoidance of competition with many rhizosphere bacteria that are less tolerant of desiccation. Antibiotic-producing *Trichoderma* spp., especially *T. koningii*, which are responsible for the suppressiveness of TAD soils in Western Australia where ammonium sulfate had been used as a nitrogen fertilizer, present an especially interesting example of avoidance of competition (188, 189). The repeated application of ammonium sulfate to these soils had resulted in acidification, reducing the level of general microbial activity (general suppression) and providing an environment highly favorable to *Trichoderma*. Not surprisingly, when these soils were treated with lime, both the activity of *Trichoderma* and suppression of take-all were reduced (191).

Subspecies of *P. fluorescens* such as the 2,4-DAPG producers responsible for TAD would appear to face even greater challenges than nonpathogenic *F. oxysporum* and *Streptomyces* spp. because the rhizosphere environment is dominated by fast-growing, nutritionally versatile Gram-negative bacteria that compete for root exudates. It would be difficult to defend the hypothesis that specific suppression is due to a single subspecies of one of the most abundant genera comprising rhizosphere populations if it is assumed that saprophytic rhizosphere bacteria maintain a nonspecific relationship with the plant root, merely mopping up nutrients released by rhizodeposition. However, if plants selectively stimulate certain saprophytic populations as a first line of defense against root pathogens, as hypothesized by Cook et al. (38), then it is reasonable to envision a hierarchy of root-microorganism

interactions within the milieu of the saprophytic rhizosphere microbial community. Several lines of evidence indicate clearly that some bacteria do indeed maintain surprisingly sophisticated relationships with the roots of their host plant hosts. For example, certain nonpathogenic rhizobacteria can induce a systemic resistance response in plants similar to pathogen-induced systemic acquired resistance (215). Numerous studies (19, 108, 123) have shown that the plant genotype influences both the quantity and the composition of microorganisms in the rhizosphere, but the work of Smith et al. (196, 197), who found that the genotype of tomato significantly influences the growth of *Bacillus cereus* UW85 and its ability to suppress damping-off caused by *Pythium torulosum*, has redefined how close this relationship can be. Genetic analysis of recombinant inbred lines of tomato revealed that three quantitative trait loci associated with biocontrol by *B. cereus* explained 38% of the phenotypic variation found. The results of Raaijmakers & Weller (161) and Landa et al. (95), showing substantial differences in the ability of genetically distinct strains of 2,4-DAPG producers to colonize the roots of wheat and pea, indicate that specific bacterial genes may have a complementary role in supporting these interactions. We suggest that more highly sophisticated relationships than those originally envisioned by Cook et al. (38) may have evolved between some nonpathogenic fluorescent *Pseudomonas* spp. and their plant hosts, resulting in a novel mechanism of protection against diseases caused by certain soilborne fungal pathogens.

CONCLUDING COMMENTS AND FUTURE DIRECTIONS

Suppressive soils represent an underutilized resource for the control of soilborne pathogens of food, fiber, and ornamental crops. Early research identified the characteristics of soil suppressiveness and the major groups of microorganisms involved, but only during the past decade has the coupling of molecular and traditional approaches made it possible to begin to dissect the genetic and functional determinants underlying the activity of some biologically suppressive soils. These studies provide a foundation for more widespread integration of suppressive soils into organic and conventional cropping systems. For example, thousands of acres of wheat in the Northwestern USA currently are managed in take-all decline with the confidence that yields can be maintained in monoculture nearly as well as with long crop rotations. Our understanding of the microbial basis of TAD in Washington State suggests that it may be possible to reduce or eliminate the years of severe disease that growers currently must endure before their soils become suppressive.

From a more general perspective, the diversity within populations of antagonistic microorganisms with a common biocontrol trait is a means to improving biocontrol. This approach builds on existing knowledge of mechanisms while exploiting genetic differences that have evolved to enable microbial populations to compete successfully in diverse soil and rhizosphere environments. Understanding the diversity within populations of biocontrol agents holds the promise of pairing

specific genotypes with their most supportive plant hosts or soil environments to maximize root colonization and disease suppression. Such knowledge also can streamline the process of selecting new biocontrol agents because targeted molecular screens can be used to identify isolates with specific traits such as superior competitiveness or the production of 2,4-DAPG.

Numerous questions remain unanswered in relation to the suppressive soils already under investigation as well as those not yet studied. For example, 2,4-DAPG producers of the D genotype have been isolated from monoculture wheat fields throughout the United States, but they have not yet been reported in European soils. In the Dutch soils studied to date (J.T. de Souza, D.M. Weller & J.M. Raaijmakers, unpublished data), only genotypes such as F and M have been detected (135). Given that strains of the A genotype occur in both Europe and the USA, did the D genotype arise as a result of intensive cereal cultivation in the USA? How many other genotypes share the same level of rhizosphere competence on wheat as the D genotype? Do specific genotypes exhibit a preference for particular plant hosts or cultivars? How frequently do 2,4-DAPG producers have a role in the suppressiveness of soils to other pathogens? Why does crop monoculture seem to enrich for 2,4-DAPG producers? Do nonculturable microorganisms have a role in specific suppressiveness?

Finally, establishing the presence and functionality of individual populations within a particular soil is just one first step toward fully understanding the nature of suppressiveness within that soil. Ultimately, the parameters within which the activities of functionally important microbial populations combine to produce a suppressive soil also must be defined. To identify those parameters, new and more detailed studies will be required to characterize the soil structure and composition, the environmental conditions under which suppression occurs, the molecular interactions among functionally important populations under different conditions, and the biogeography and population dynamics of beneficial as well as pathogenic microbial populations in the field. Because of the complexity of field soils, high-throughput methods will be required to adequately characterize these populations, but the pay-off will be worth the effort. We are confident that future studies of biologically based soil suppressiveness will present new insights into the microbial ecology of agricultural soils and lay the foundation for the development of creative management strategies for the suppression of soilborne diseases.

The *Annual Review of Phytopathology* is online at http://phyto.annualreviews.org

LITERATURE CITED

1. Abadie C, Edel V, Alabouvette C. 1998. Soil suppressiveness to Fusarium wilt: influence of a cover-plant on density and diversity of Fusarium populations. *Soil Biol. Biochem.* 30:643–49

2. Alabouvette C. 1986. Fusarium wilt-suppressive soils from the Chateaurenard region: review of a 10-year study. *Agronomie* 6:273–84

3. Alabouvette C. 1990. Biological control of Fusarium wilt pathogens in suppressive soils. In *Biological Control of Soilborne Plant Pathogens*, ed. D Hornby, pp. 27–43. Wallingford, UK: CAB Int. 479 pp.

4. Alabouvette C. 1999. Fusarium wilt suppressive soils: an example of disease-suppressive soils. *Australas. Plant Pathol.* 28:57–64

5. Alabouvette C, Couteaudier Y, Louvet J. 1984. Recherches sur la résistance des sols aux maldies. IX. Dynamique des populations *Fusarium* spp. et *Fusarium oxysporum* f. sp. *melonis* dans un sol résistant et un sol sensible aux fusarioses vasculaires. *Agronomie* 4:729–33

6. Alabouvette C, Lemanceau P, Steinberg C. 1993. Recent advances in the biological control of Fusarium wilts. *Pestic. Sci.* 37:365–73

7. Alabouvette C, Rouxel F, Louvet J. 1977. Recherches sur la résistance des sols aux maldies. III. Effets du rayonnement y sur la microflore d'un sol et sa résistance a la fusariose vasculaire du melon. *Ann. Phytopathol.* 9:467–71

8. Alabouvette C, Hoeper H, Lemanceau P, Steinberg C. 1996. Soil suppressiveness to diseases induced by soilborne plant pathogens. In *Soil Biochemistry*, ed. G Stotzky, J-M Bollag, pp. 371–413. New York: Marcel Dekker

9. Amann RI, Ludwig W, Schleifer K-H. 1995. Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. *Microbiol. Rev.* 59:143–69

10. Amir H, Amir A. 1989. Influence de la desinfection et du type de sol sur l'antagonisme d'un actinomycete vis-à-vis d'une souche de *Fusarium oxysporum* f. sp. *albedinis*. *Rev. Ecol. Biol. Sol* 26:57–74

11. Andrade OA, Mathre DE, Sands DC. 1994. Natural suppression of take-all of wheat in Montana soils. *Plant Soil* 164:9–18

12. Andrade OA, Mathre DE, Sands DC. 1994. Suppression of *Gaeumannomyces graminis* var. *tritici* in Montana soils and its transferability between soils. *Soil Biol. Biochem.* 26:397–402

13. Andrews JH, Harris RF. 2000. The ecology and biogeography of microorganisms on plant surfaces. *Annu. Rev. Phytopathol.* 38:145–80

14. Andrivon D. 1994. Dynamics of the survival and infectivity to potato tubers of sporangia of *Phytophthora infestans* in three different soils. *Soil Biol. Biochem.* 26:945–52

15. Anzai Y, Kim H, Park J-Y, Wakabayashi H, Oyaizu H. 2000. Phylogenetic affiliation of the pseudomonads based on 16S rRNA sequences. *Int. J. Syst. Evol. Microbiol.* 50:1563–89

16. Aon MA, Colaneri AC. 2001. Temporal and spatial evolution of enzymatic activities and physico-chemical properties in an agricultural soil. *Appl. Soil Ecol.* 18:255–70

17. Asher MJC, Shipton PJ, eds. 1981. *Biology and Control of Take-all*. New York: Academic. 538 pp.

18. Atkinson GF. 1892. Some diseases of cotton. *Al. Agric. Exp. Stn. Bull.* 41. 65 pp.

19. Atkinson TG, Neal JL Jr, Larson RI.

1975. Genetic control of the rhizosphere microflora of wheat. See Ref. 31, pp. 116–22

20. Baker KF, Cook RJ. 1974. *Biological Control of Plant Pathogens*. San Francisco: Freeman. 433 pp.

21. Bandick A, Dick R. 1999. Field management effects on soil enzyme activities. *Soil Biol. Biochem.* 31:1471–79

22. Bangera MG, Thomashow LS. 1999. Identification and characterization of a gene cluster for synthesis of the polyketide antibiotic 2,4-diacetylphloroglucinol from *Pseudomonas fluorescens* Q2-87. *J. Bacteriol.* 181:3155–63

23. Barea JM, Andrade G, Bianciotto V, Dowling D, Lohrke S, et al. 1998. Impact on arbuscular mycorrhiza formation of *Pseudomonas* strains used as inoculants for biocontrol of soil-borne fungal plant pathogens. *Appl. Environ. Microbiol.* 64:2304–7

24. Barnett SJ, Singleton I, Ryder M. 1999. Spatial variation in populations of *Pseudomonas corrugata* 2140 and pseudomonads on take-all diseased and healthy root systems of wheat. *Soil Biol. Biochem.* 31:633–36

25. Bockus W, Shroyer J. 1998. The impact of reduced tillage on soilborne plant pathogens. *Annu. Rev. Phytopathol.* 36:485–500

26. Bonsall RF, Weller DM, Thomashow LS. 1997. Quantification of 2,4-diacetylphloroglucinol produced by fluorescent *Pseudomonas* spp. in vitro and in the rhizosphere of wheat. *Appl. Environ. Microbiol.* 63:951–55

27. Borneman J. 1999. Culture-independent identification of microorganisms that respond to specific soil stimuli. *Appl. Environ. Microbiol.* 65:3398–400

28. Bosio D, Scow K, Gunapala N, Graham KJ. 1998. Determinants of soil microbial communities: effects of agricultural management, season and soil type on phospholipid fatty acid profiles. *Microb. Ecol.* 36:1–12

29. Bowers JH, Kinkel LL, Jones RK. 1996. Influence of disease-suppressive isolates of S*treptomyces* on the native *Streptomyces* community in soil as determined by the analysis of cellular fatty acids. *Can. J. Microbiol.* 42:27–37

30. Braker G, Ayala-del-Rio HL, Devol AH, Fesefeldt A, Tiedje JM. 2001. Community structure of denitrifiers, *Bacteria*, and *Arcahe* along redox gradients in Pacific Northwest marine sediments by terminal restriction fragment length polymorphism analysis of amplified nitrite reductase (*nirS*) and 16S rRNA genes. *Appl. Environ. Microbiol.* 67:1893–901

31. Bruehl GW. 1975. *Biology and Control of Soil-Borne Plant Pathogens*. St. Paul: Am. Phytopathol. Soc.

32. Campbell R. 1994. Biological control of soil-borne diseases: some present problems and different approaches. *Crop Prot.* 13:4–13

33. Cho J-C, Tiedje JM. 2001. Bacterial species determination from DNA-DNA hybridization by using genome fragments and DNA microarrays. *Appl. Environ. Microbiol.* 67:3677–82

34. Cook RJ. 1981. The influence of rotation crops on take-all decline phenomenon. *Phytopathology* 71:189–92

35. Cook RJ. 1985. Biological control of plant pathogens, with special reference to the take-all fungus in suppressive soils. *Taiwan Agric. Res. Inst. Plant Prot. Bull.* 27:179–98

36. Cook RJ, Baker KF. 1983. *The Nature and Practice of Biological Control of Plant Pathogens*. St. Paul, MN: Am. Phytopathol. Soc. 539 pp.

37. Cook RJ, Rovira AD. 1976. The role of bacteria in the biological control of *Gaeumannomyces graminis* by suppressive soils. *Soil Biol. Biochem.* 8:269–73

38. Cook RJ, Thomashow LS, Weller DM, Fujimoto D, Mazzola M, et al. 1995. Molecular mechanisms of defense by rhizobacteria against root disease. *Proc. Natl. Acad. Sci. USA* 92:4197–201

39. Cook RJ, Weller DM. 1987. Management of take-all in consecutive crops of wheat or barley. In *Innovative Approaches to Plant Disease Control*. ed. I Chet, pp. 41–76. New York: Wiley. 372 pp.

40. Cook RJ, Wilkinson HT, Aldredge JR. 1986. Evidence that microorganisms in suppressive soil associated with wheat take-all decline do not limit the number of lesions produced by *Gaeumannomyces graminis* var. *tritici*. *Phytopathology* 76:342–45

41. Couteaudier Y, Alabouvette C. 1990. Quantitative comparison of *Fusarium oxysporum* competitiveness in relation to carbon utilization. *FEMS Microbiol. Ecol.* 74:261–67

42. Cronin D, Moënne-Loccoz Y, Fenton A, Dunne C, Dowling DN, O'Gara F. 1997. Role of 2,4-diacetylphloroglucinol in the interaction of the biocontrol pseudomonad strain F113 with the potato cyst nematode *Globodera rostochiensis*. *Appl. Environ. Microbiol.* 63:1357–61

43. Cronin D, Moënne-Loccoz Y, Fenton A, Dunne C, Dowling DN, O'Gara F. 1997. Ecological interaction of a biocontrol *Pseudomonas fluorescens* strain producing 2,4-diacetylphloroglucinol with the soft rot potato pathogen *Erwinia carotovora* subsp. *atroseptica*. *FEMS Microbiol. Ecol.* 23:95–106

44. Crump DH, Kerry BR. 1987. Studies on the population dynamics and fungal parasitism of *Heterodera schachtii* in soil from sugar-beet monoculture. *Crop Prot.* 6:49–55

45. Dahllof I, Baillie H, Kjelleberg S. 2000. *rpoB*-based microbial community analysis avoids limitations inherent in 16s rRNA gene intraspecies heterogeneity. *Appl. Environ. Microbiol.* 66:3376–80

46. Dalmastri C, Chiarini L, Cantale C, Bevivino A, Tabacchioni S. 1999. Soil type and maize cultivar affect the genetic diversity of maize root-associated *Burkholderia cepacia* populations. *Microb. Ecol.* 38:273–84

47. Dandurand L-MC, Knudsen GR. 2002. Sampling microbes from the rhizosphere and phyllosphere. See Ref. 82, pp. 516–26

48. Deacon JW. 1976. Biological control of the take-all fungus, *Gaeumannomyces graminis*, by *Phialophora radicicola* and similar fungi. *Soil Biol. Biochem.* 8:275–83

49. Deacon JW, Berry LA. 1993. Biocontrol of soil-borne plant-pathogens—concepts and their application. *Pestic. Sci.* 37:417–26

50. Dewan MM, Sivasithamparam K. 1989. Growth promotion of rotation crop species by a sterile fungus from wheat and effect of soil temperatures and water potential on its suppression of take-all. *Mycol. Res.* 93:156–60

51. Domínguez J, Negrin MA, Rodríguez CM. 2001. Aggregate water-stability, particle size and soil solution properties in conducive and suppressive soils to Fusarium wilt of banana from Canary islands (Spain). *Soil Biol. Biochem.* 33:449–55

52. Duffy BK, Défago G. 1997. Zinc improves biocontrol of Fusarium crown and root rot of tomato by *Pseudomonas fluorescens* and represses the production of pathogen metabolites inhibitory to bacterial antibiotic biosynthesis. *Phytopathology* 87:1250–57

53. Duffy BK, Défago G. 1999. Environmental factors modulating antibiotic and siderophore biosynthesis by *Pseudomonas fluorescens* biocontrol strains. *Appl. Environ. Microbiol.* 65:2429–38

54. Duffy BK, Simon A, Weller DM. 1996. Combinations of *Trichoderma koningii* with fluorescent pseudomonads for control of take-all on wheat. *Phytopathology* 86:188–94

55. Duijff BJ, Pouhair D, Olivain C, Alabouvette C, Lemanceau P. 1998. Implication of systemic induced resistance in the

suppression of fusarium wilt of tomato by *Pseudomonas fluorescens* WCS417r and by nonpathogenic *Fusarium oxysporum* Fo47. *Eur. J. Plant Pathol.* 104:903–10

56. Duijff BJ, Recorbet G, Bakker PAHM, Loper JE, Lemanceau P. 1999. Microbial antagonism at the root level is involved in the suppression of Fusarium wilt by the combination of nonpathogenic *Fusarium oxysporum* Fo47 and *Pseudomonas putida* WCS358. *Phytopathology* 89:1073–79

57. Dunbar J, Ticknor LO, Kuske CR. 2001. Phylogenetic specificity and reproducibility and new method for analysis of terminal restriction fragment profiles of 16S rRNA genes from bacterial communities. *Appl. Environ. Microbiol.* 67:190–97

58. Dunlop RW, Simon A, Sivasithamparam K, Ghisalberti EL. 1989. An antibiotic from *Trichoderma koningii* active against soilborne plant pathogens. *J. Nat. Prod.* 52:67–74

59. Eckwall EC, Schottel JL. 1997. Isolation and characterization of an antibiotic produced by the disease-suppressive *Streptomyces diastatochromogenes* strain Pon SSII. *J. Ind. Microbiol. Biotechnol.* 19:220–25

60. Edel V, Steinberg C, Gautheron N, Alabouvette C. 1997. Populations of nonpathogenic *Fusarium oxysporum* associated with roots of four plant species compared to soilborne populations. *Phytopathology* 87:693–97

61. Edel V, Steinberg C, Gautheron N, Recorbet G, Alabouvette C. 2001. Genetic diversity of *Fusarium oxysporum* populations isolated from different soils in France. *FEMS Microbiol. Ecol.* 36:61–71

62. Elad Y, Baker R. 1985. The role of competition for iron and carbon in suppression of chlamydospore germination of *Fusarium* spp. by *Pseudomonas* spp. *Phytopathology* 75:1053–59

63. Fedi S, Tola E, Moënne-Loccoz Y, Dowling DN, Smith LM, O'Gara F. 1997. Evidence for signaling between the phytopathogenic fungus *Pythium ultimum* and *Pseudomonas fluorescens* F113: *P. ultimum* represses the expression of genes in *P. fluorescens* F113, resulting in altered ecological fitness. *Appl. Environ. Microbiol.* 63:4261–66

64. Felske A, Wolterink A, van Lis R, de Vos W, Akkermans ADL. 2000. Response of a Soil bacterial community to grassland succession as monitored by 16S rRNA levels of the predominant ribotypes. *Appl. Environ. Microbiol.* 66:3998–4003

65. Fenton AM, Stephens PM, Crowley J, O'Callaghan M, O'Gara F. 1992. Exploitation of gene(s) involved in 2,4-diacetylphloroglucinol biosynthesis to confer a new biocontrol capability to a *Pseudomonas* strain. *Appl. Environ. Microbiol.* 58:3873–78

66. Fisher MM, Triplett EW. 1999. Automated approach for ribosomal intergenic spacer analysis of microbial diversity and its application to freshwater bacterial communities. *Appl. Environ. Microbiol.* 65:4630–36

67. Gair R, Mathias PL, Harvey PN. 1969. Studies of cereal nematode populations and cereal yields under continuous or intensive culture. *Ann. Appl. Biol.* 63:503–12

68. Gavassoni WL, Tylka GL, Munkvold GP. 2001. Relationships between tillage and spatial patterns of *Heterodera glycines*. *Phytopathology* 91:534–45

69. Gerlagh M. 1968. Introduction of *Ophiobolus graminis* into new polders and its decline. *Neth. J. Plant Pathol.* 74:(Suppl. 2)1–97

70. Girlanda M, Perotto S, Moënne-Loccoz Y, Bergero R, Lazzari A, et al. 2001. Impact of biocontrol *Pseudomonas fluorescens* CHA0 and a genetically modified derivative on the diversity of culturable fungi in the cucumber rhizosphere. *Appl. Environ. Microbiol.* 67:1851–64

71. Glynne MD. 1935. Incidence of take-all on wheat and barley on experimental plots at Woburn. *Ann. Appl. Biol.* 22:225–35

72. Gruntzig V, Nold C, Zhou J, Tiedje JM. 2001. *Pseudomonas stutzeri* nitrite reductase gene abundance in environmental samples measured by real-time PCR. *Appl. Environ. Microbiol.* 67:760–68

73. Gu Y-H, Mazzola M. 2001. Impact of carbon starvation on stress resistance, survival in soil habitats and biocontrol ability of *Pseudomonas putida* strain 2C8. *Soil Biol. Biochem.* 33:1155–62

74. Harrison LA, Letendre L, Kovacevich P, Pierson EA, Weller DM. 1993. Purification of an antibiotic effective against *Gaeumannomyces graminis* var *tritici* produced by a biocontrol agent, *Pseudomonas aureofaciens*. *Soil Biol. Biochem.* 25:215–21

75. Homma Y, Sitton JW, Cook RJ, Old KM. 1979. Perforation and destruction of pigmented hyphae of *Gaeumannomyces graminis* by vampyrellid amoebae from Pacific Northwest wheat field soils. *Phytopathology* 69:1118–22

76. Hopkins DL, Larkin RP, Elmstrom GW. 1987. Cultivar-specific induction of soil-suppressiveness to Fusarium wilt of watermelon. *Phytopathology* 77:607–11

77. Hornby D. 1979. Take-all decline: a theorist's paradise. See Ref. 175, pp. 133–56

78. Hornby D. 1983. Suppressive soils. *Annu. Rev. Phytopathol.* 21:65–85

79. Hornby D. 1998. *Take-All of Cereals: A Regional Perspective*. Wallingford, UK: CAB Int. 384 pp.

80. Horz HP, Rottahauwe JH, Lickow T, Liesack W. 2000. Identification of the major subgroups of ammonia-oxidizing bacteria in environmental samples by T-RFLP analysis of *amoA* PCR products. *J. Microb. Methods* 39:4516–22

81. Huber DM, Watson RD. 1970. Effect of organic amendment on soil-borne plant pathogens. *Phytopathology* 60:22–26

82. Hurst CJ, Crawford RL, Knudsen GR, McInerney MJ, Stetzenbach LD, eds. 2002. *Manual of Environmental Microbiology*. Washington DC: ASM Press. 1138 pp. 2nd ed.

83. Kao CW, Ko WH. 1983. Nature of suppression of *Pythium splendens* in a pasture soil in South Kohala, Hawaii. *Phytopathology* 73:1284–89

84. Keel C, Schnider U, Maurhofer M, Voisard C, Burger D, et al. 1992. Suppression of root diseases by *Pseudomonas fluorescens* CHA0: Importance of the bacterial secondary metabolite 2,4-diacetylphloroglucinol. *Mol. Plant-Microbe Interact.* 5:4–13

85. Keel C, Weller D, Natsch A, Défago G, Cook RJ, Thomashow LS. 1996. Conservation of the 2,4-diacetylphloroglucinol biosynthesis locus among fluorescent *Pseudomonas* strains from diverse geographic locations. *Appl. Environ. Microbiol.* 62:552–63

86. Kerry BR. 1988. Fungal parasites of cyst nematodes. *Agric. Ecosyst. Environ.* 24:293–95

87. Kim D-S, Cook RJ, Weller DM. 1997. *Bacillus* sp. L324-92 for biological control of three root diseases of wheat grown with reduced tillage. *Phytopathology* 87:551–58

88. Kinkel LL, Bowers JH, Shimizu K, Neeno-Eckwall EC, Schottel JL. 1998. Quantitative relationship among thaxtomin A production, potato scab severity, and fatty acid composition in *Streptomyces*. *Can. J. Microbiol.* 44:768–76

89. Kloepper JW, Leong J, Teintze M, Schroth MN. 1980. *Pseudomonas* siderophores: a mechanism explaining disease suppressive soils. *Curr. Microbiol.* 4:317–20

90. Kluepfel DA. 1993. The behavior and tracking of bacteria in the rhizosphere. *Annu. Rev. Phytopathol.* 31:441–72

91. Kluepfel DA, McInnis TM, Zehr EI. 1993. Involvement of root-colonizing bacteria in peach orchard soils suppressive of the nematode *Criconemella*

xenoplax. *Phytopathology* 83:1240–45

92. Ko WH, Shiroma SS. 1989. Distribution of *Phytophthora cinnamomi*-suppressive soil in nature. *J. Phytopathol.* 127:75–80

93. Kreuze JF, Suomalainen S, Paulin L, Valkonen JPT. 1999. Phylogenetic analysis of 16S rRNA genes and PCR analysis of *nec1* gene from *Steptomyces* spp. causing common scab, pitted scab, and netted scab in Finland. *Phytopathology* 89:462–69

94. Lahdenperä M-L, Simon E, Uoti J. 1991. Mycostop—a novel biofungicide based on *Streptomyces* bacteria. In *Biotic Interactions and Soil-Borne Diseases*, ed. ABR Beemster, GJ Bollen, M Gerlagh, MA Ruissen, B Schippers, A Tempel, pp. 258–63. Amsterdam: Elsevier

95. Landa BB, Mavrodi OV, Raaijmakers JM, McSpadden Gardener BB, et al. 2002. Differential ability of genotypes of 2,4-diacetylphloroglucinol-producing *Pseudomonas fluorescens* strains to colonize the roots of pea plants. *Appl. Environ. Microbiol.* In press

96. Landa BB, de Werd HAE, McSpadden Gardener BB, Weller DM. 2002. Comparison of three methods for monitoring populations of different genotypes of 2,4-diacetylphloroglucinol-producing *Pseudomonas fluorescens* in the rhizosphere. *Phytopathology* 92:129–37

97. Landa BB, Weller DW. 2001. Crop preference by genotypes of 2,4-diacetylphloroglucinol (DAPG)-producing *Pseudomonas* spp. *Phytopathology* 91(Suppl.):S52

98. Larkin RP, Fravel DR. 1998. Efficacy of various fungal and bacterial biocontrol organisms for control of Fusarium wilt of tomato. *Plant Dis.* 82:1022–28

99. Larkin RP, Fravel DR. 1999. Mechanisms of action and dose-response relationships governing biological control of fusarium wilt of tomato by nonpathogenic *Fusarium* spp. *Phytopathology* 89:1152–61

100. Larkin RP, Hopkins DL, Martin FN. 1993. Effect of successive watermelon plantings on *Fusarium oxysporum* and other microorganisms in soils suppressive and conducive to fusarium-wilt of watermelon. *Phytopathology* 83:1097–105

101. Larkin RP, Hopkins DL, Martin FN. 1996. Suppression of Fusarium wilt of watermelon by nonpathogenic *Fusarium oxysporum* and other microorganisms recovered from a disease-suppressive soil. *Phytopathology* 86:812–19

102. Latour X, Corberand T, Laguerre G, Allard F, Lemanceau P. 1996. The composition of fluorescent pseudomonad populations associated with roots is influenced by plant and soil type. *Appl. Environ. Microbiol.* 62:2449–56

103. Lawrence CH, Clark MC, King RR. 1990. Induction of common scab symptoms in aseptically cultured potato tubers by the vivotoxin, thaxtomin. *Phytopathology* 80:606–8

104. Lemanceau P, Alabouvette C. 1991. Biological control of fusarium diseases by fluorescent pseudomonas and nonpathogenic *Fusarium*. *Crop Prot.* 10:279–86

105. Lemanceau P, Alabouvette C. 1993. Suppression of Fusarium wilt by fluorescent pseudomonads—mechanisms and applications. *Biocont. Sci. Tech.* 3:219–34

106. Lemanceau P, Bakker PAHM, Dekogel WJ, Alabouvette C, Schippers B. 1992. Effect of pseudobactin 358 production by *Pseudomonas putida* WCS358 on suppression of fusarium wilt of carnations by nonpathogenic *Fusarium oxysporum* Fo47. *Appl. Environ. Microbiol.* 58:2978–82

107. Lemanceau P, Bakker PAHM, Dekogel WJ, Alabouvette C, Schippers B. 1993. Antagonistic effect of nonpathogenic *Fusarium oxysporum* Fo47 and pseudobactin 358 upon pathogenic *Fusarium oxysporum* f. sp. *dianthi*. *Appl. Environ. Microbiol.* 59:74–82

108. Lemanceau P, Corberand T, Gardan L, Latour X, Laguerre G, et al. 1995. Effect of two plant species, flax (*Linum usitatissinum* L.) and tomato (*Lycopersicon esculentum* Mill.), on the diversity of soilborne populations of fluorescent pseudomonads. *Appl. Environ. Microbiol.* 61:1004–12

109. Lévesque CA. 2001. Molecular methods for detection of plant pathogens: What is the future? *Can. J. Plant Pathol.* 23:333–36

110. Liesack W, Janssen PH, Rainey FA, Ward-Rainey NL, Stackenbrandt E. 1997. Microbial diversity in soil: the need for a combined approach using molecular and cultivation techniques. In *Modern Soil Microbiology*, ed. JD van Elsas, J Trevors, M Wexler, pp. 375–440. New York: Marcel Dekker

111. Liu D, Anderson NA, Kinkel LL. 1995. Biological control of potato scab in the field with antagonistic *Streptomyces* scabies. *Phytopathology* 85:827–31

112. Liu D, Anderson NA, Kinkel LL. 1996. Selection and characterization of strains of *Streptomyces* suppressive to the potato scab pathogen. *Can. J. Microbiol.* 42:487–502

113. Liu W, Marsh TL, Cheng H, Forney JL. 1997. Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. *Appl. Environ. Microbiol.* 63:4516–22

114. Liu W-T, Stahl D. 2002. Molecular approaches for the measurement of density, diversity, and phylogeny. See Ref. 82, pp. 114–34

115. Lorang JM, Anderson NA, Lauer FI, Wildung DK. 1989. Disease decline in a Minnesota potato scab plot. *Am. Potato J.* 66:531

116. Lorang JM, Liu D, Anderson NA, Schottel JL. 1995. Identification of potato scab inducing and suppressive species of *Streptomyces*. *Phytopathology* 85:261–68

117. Loria R, Bukhalid RA, Creath RA, Leiner RH, Olivier M, Steffens JC. 1995. Differential production of thaxtomins by pathogenic *Streptomyces* species in vitro. *Phytopathology* 85:537–41

118. Loria R, Bukhalid RA, Fry BA, King RR. 1997. Plant pathogenicity in the genus *Streptomyces. Plant Dis.* 81:836–46

119. Lucas P, Smiley RW, Collins HP. 1993. Decline of Rhizoctonia root rot on wheat in soils infested with *Rhizoctonia solani* AG-8. *Phytopathology* 83:260–65

120. Lukow T, Dunfield PF, Liesack W. 2000. Use of the T-RFLP technique to assess spatial and temporal changes in the bacterial community structure within an agricultural soil planted with transgenic and non-transgenic potato plants. *FEMS Microbiol. Ecol.* 32:241–47

121. Martin FN, Hancock JG. 1986. Association of chemical and biological factors in soils suppressive to *Pythium ultimum. Phytopathology* 76:1221–31

122. Maurhofer M, Keel C, Haas D, Défago G. 1995. Influence of plant species on disease suppression by *Pseudomonas fluorescens* CHA0 with enhanced antibiotic production. *Plant Pathol.* 44:44–50

123. Mavingui P, Laguerre G, Berge O. 1992. Genetic and phenotypic diversity of *Bacillus polymyxa* in soil and in the wheat rhizosphere. *Appl. Environ. Microbiol.* 58:1894–903

124. Mavrodi DV, Ksenzenko VN, Bonsall RF, Cook RJ, Boronin AM, Thomashow LS. 1998. A seven-gene locus for synthesis of phenazine-1-carboxylic acid by *Pseudomonas fluorescens* 2-79. *J. Bacteriol.* 180:2541–48

125. Mavrodi OV, McSpadden Gardener BB, Mavrodi DV, Bonsall RF, Weller DM, Thomashow LS. 2001. Genetic diversity of *phlD* from 2,4-diacetylphloroglucinol-producing fluorescent *Pseudomonas* species. *Phytopathology* 91:35–43

126. Mazzola M. 1997. Identification and

pathogenicity of *Rhizoctonia* spp. isolated from apple roots and orchard soils. *Phytopathology* 87:582–87

127. Mazzola M. 1998. Elucidation of the microbial complex having a causal role in the development of apple replant disease in Washington. *Phytopathology* 88:930–38

128. Mazzola M. 1999. Transformation of soil microbial community structure and *Rhizoctonia*-suppressive potential in response to apple roots. *Phytopathology* 89:920–27

129. Mazzola M. 1999. Control of replant disease of tree fruits with *Pseudomonas putida*. *U.S. Patent No. 5,948,671*

130. Mazzola M, Fujimoto DK, Thomashow LS, Cook RJ. 1995. Variation in sensitivity of *Gaeumannomyces graminis* to antibiotics produced by fluorescent *Pseudomonas* spp. and effect on biological control of take-all of wheat. *Appl. Environ. Microbiol.* 61:2554–59

131. Mazzola M, Gu Y-H. 2000. Impact of wheat cultivation on microbial communities from replant soils and apple growth in greenhouse trials. *Phytopathology* 90:114–19

132. Mazzola M, Gu Y-H. 2000. Phytomanagement of microbial community structure to enhance growth of apple in replant soils. *Acta Hortic.* 532:73–78

133. McSpadden Gardener BB. 1998. *Assessing the potential of creating biased rhizospheres based on inositol rhizopines*. PhD thesis. Mich. State Univ., East Lansing. 175 pp.

134. McSpadden Gardener BB, Mavrodi DV, Thomashow LS, Weller DM. 2001. A rapid PCR-based assay characterizing rhizosphere populations of 2,4-DAPG-producing bacteria. *Phytopathology* 91:44–54

135. McSpadden Gardener BB, Schroeder KL, Kalloger SE, Raaijmakers JM, Thomashow LS, Weller DM. 2000. Genotypic and phenotypic diversity of *phlD*-containing *Pseudomonas* isolated from the rhizosphere of wheat. *Appl. Environ. Microbiol.* 66:1939–46

136. McSpadden Gardener BB, Weller DM. 2001. Changes in populations of rhizosphere bacteria associated with take-all of wheat. *Appl. Environ. Microbiol.* 67:4414–25

137. Menzies JD. 1959. Occurrence and transfer of a biological factor in soil that suppresses potato scab. *Phytopathology* 49:648–52

138. Mills AL, Garland JL. 2002. Application of physiological profiles to assessment of community properties. See Ref. 82, pp. 135–46

139. Moënne-Loccoz Y, Tichy HV, O'Donnell A, Simon R, O'Gara F. 2001. Impact of 2,4-diacetylphloroglucinol-producing biocontrol strain *Pseudomonas fluorescens* F113 on intraspecific diversity of resident culturable fluorescent pseudomonads associated with the roots of field-grown sugar beet seedlings. *Appl. Environ. Microbiol.* 67:3418–25

140. Murakami H, Tsushima S, Shishido Y. 2000. Soil suppressiveness to clubroot disease of Chinese cabbage caused by *Plasmodiophora brassicae*. *Soil Biol. Biochem.* 32:1637–42

141. Muyzer G, Smalla K. 1998. Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. *Antonie van Leeuwenhoek J. Microbiol. Serol.* 73:127–41

142. Naseby DC, Way JA, Bainton NJ, Lynch JM. 2001. Biocontrol of *Pythium* in the pea rhizosphere by antifungal metabolite producing and non-producing *Pseudomonas* strains. *J. Appl. Microbiol.* 90:421–29

143. Ndowora TCR, Kinkel LL, Jones RK, Anderson NA. 1996. Fatty acid analysis of pathogenic and suppressive strains of S*treptomyces* species isolated in Minnesota. *Phytopathology* 86:138–43

144. Neeno-Eckwall EC, Kinkel LL, Schottel

JL. 2001. Competition and antibiosis in the biological control of potato scab. *Can. J. Microbiol.* 47:332–40

145. Nogales B, Moore E, Llobet-Brossa E, Rossello-Mora R, Amann R, Timmis N. 2001. Combined use of 16S ribosomal DNA and 16S rRNA to study the bacterial community of polychlorinated biphenyl-polluted soil. *Appl. Environ. Microbiol.* 67:1874–84

146. Notz R, Maurhofer M, Schnider-Keel U, Duffy B, Haas D, Défago G. 2001. Biotic factors affecting expression of the 2,4-diacetylphloroglucinol biosynthesis gene *phlA* in *Pseudomonas fluorescens* biocontrol strain CHA0 in the rhizosphere. *Phytopathology* 91:873–81

147. Ogram A, Sharma K. 2002. Methods of soil microbial community analysis. See Ref. 82, pp. 554–63

148. Ownley BH, Weller DM, Thomashow LS. 1992. Influence of in situ and in vitro pH on suppression of *Gaeumannomyces graminis* var. *tritici* by *Pseudomonas fluorescens* 2-79. *Phytopathology* 82:178–84

149. Parke JL, Gurian-Sherman D. 2001. Diversity of the *Burkholderia cepacia* complex and implications for risk assessment of biocontrol strains. *Annu. Rev. Phytopathol.* 39:225–58

150. Peng HX, Sivasithamparam K, Turner DW. 1999. Chlamydospore germination and Fusarium wilt of banana plantlets in suppressive and conducive soils are affected by physical and chemical factors. *Soil Biol. Biochem.* 31:1363–74

151. Persson L, Larsson-Wikström M, Gerhardson B. 1999. Assessment of soil suppressiveness to Aphanomyces root rot of pea. *Plant Dis.* 83:1108–12

152. Picard C, Di Cello F, Ventura M, Fani R, Guckert A. 2000. Frequency and biodiversity of 2,4-diacetylphloroglucinol-producing bacteria isolated from the maize rhizosphere at different stages of plant growth. *Appl. Environ. Microbiol.* 66:948–55

153. Pierson EA, Weller DM. 1994. Use of mixtures of fluorescent pseudomanads to suppress take-all and improve the growth of wheat. *Phytopathology* 84:940–47

154. Pierson EA, Wood DW, Cannon JA, Blachere FM, Pierson LS III. 1998. Interpopulation signaling via N-acyl-homoserine lactones among bacteria in the wheat rhizosphere. *Mol. Plant-Microbe Interact.* 11:1078–84

155. Pierson LS III, Thomashow LS. 1992. Cloning and heterologous expression of the phenazine biosynthetic locus from *Pseudomonas aureofaciens* 30-84. *Mol. Plant-Microbe Interact.* 5:330–39

156. Pinkart HC, Ringlelberg DB, Piceno YM, Macnaughton SJ, White DC. 2002. Biochemical approaches to biomass measurements and community structure analysis. See Ref. 82, pp. 101–13

157. Poly F, Rnajard L, Nazaret S, Gourbiere F, Jocteur Nonrozier L, 2001. Comparison of *nifH* gene pools in soils and soil microenvironments with contrasting properties. *Appl. Environ. Microbiol.* 67:2255–62

158. Qiu X, Wu L, Huang H, McConel PE, Palumbo AV, et al. 2001. Evaluation of PCR-generated chimeras, mutations, and heteroduplexes with 16S rRNA gene-based cloning. *Appl. Environ. Microbiol.* 67:880–87

159. Raaijmakers JM, Bonsall RF, Weller DM. 1999. Effect of population density of *Pseudomonas fluorescens* on production of 2,4-diacetylphloroglucinol in the rhizosphere of wheat. *Phytopathology* 89:470–75

160. Raaijmakers JM, Weller DM. 1998. Natural plant protection by 2,4-diacetylphloroglucinol-producing *Pseudomonas* spp. in take-all decline soils. *Mol. Plant-Microbe Interact.* 11:144–52

161. Raaijmakers JM, Weller DM. 2001. Exploiting the genetic diversity of *Pseudomonas* spp: characterization of superior colonizing *P. fluorescens* strain

Q8r1-96. *Appl. Environ. Microbiol.* 67:
2545–54

162. Raaijmakers JM, Weller DM, Thoma-
show LS. 1997. Frequency of antibiotic
producing *Pseudomonas* spp. in natural
environments. *Appl. Environ. Microbiol.*
63:881–87

163. Ramette A, Moënne-Loccoz Y, Défago
G. 2001. Polymorphism of the poly-
ketide synthase gene *phlD* in bio-
control fluorescent pseudomonads pro-
ducing 2,4-diacetylphloroglucinol and
comparison of PhlD with plant polyke-
tide synthases. *Mol. Plant-Microbe In-
teract.* 14:639–52

164. Roget DK. 1995. Decline in root rot
(*Rhizoctonia solani* AG-8) in wheat in a
tillage and rotation experiment at Avon,
South Australia. *Aust. J. Exp. Agric.*
35:1009–13

165. Rondon MR, August PR, Bettermann
AD, Brady SF, Grossman TH, et al. 2000.
Cloning the soil metagenome: a strategy
for accessing the genetic and functional
diversity of uncultured microorganisms.
Appl. Environ. Microbiol. 66:2541–47

166. Rouxel F, Alabouvette C, Louvet J. 1977.
Recherches sur la résistance des sols
aux maladies. II. Incidence de traite-
ments thermiques sur la résistance mi-
crobiologique d'un sol à la Fusariose
vasculaire du melon. *Ann. Phytopathol.*
9:183–92

167. Rouxel F, Alabouvette C, Louvet J. 1979.
Recherches sur la résistance des sols aux
maladies. IV. Mise en evidence du role
des Fusarium autochtones dan la résis-
tance d'un sol a la fusariose vasculaire du
melon. *Ann. Phytopathol.* 11:199–207

168. Rovira AD, Wildermuth GB. 1981. The
nature and mechanisms of suppression.
In *Biology and Control of Take-all.* ed.
MJC Asher, P Shipton, pp. 385–415.
London: Academic. 538 pp.

169. Ryan AD, Kinkel LL. 1997. Inocu-
lum density and population dynamics
of suppressive and pathogenic *Strepto-
myces* strains and their relationship of

biological control of potato scab. *Biol.
Control* 10:180–86

170. Sadowsky MJ, Kinkel LL, Bowers
JH, Schottel JL. 1996. Use of repeti-
tive intergenic DNA sequences to clas-
sify pathogenic and disease-suppressive
S*treptomyces* strains. *Appl. Environ. Mi-
crobiol.* 62:3489–93

171. Sarniguet A, Lucas P. 1992. Evalua-
tion of populations of fluorescent pseu-
domonads related to decline of take-all
patch on turfgrass. *Plant Soil* 145:11–15

172. Sarniguet A, Lucas P, Lucas M. 1992.
Relationship between take-all, soil con-
duciveness to the disease, populations of
fluorescent pseudomonads and nitrogen
fertilizer. *Plant Soil* 145:17–27

173. Scher FM, Baker R. 1980. Mecha-
nism of biological control in a Fusa-
rium-suppressive soil. *Phytopathology*
70:412–17

174. Scher FM, Baker R. 1982. Effect of
Pseudomonas putida and a synthetic iron
chelator on induction of soil suppressive-
ness to Fusarium wilt pathogens. *Phy-
topathology* 72:1567–73

175. Schippers B, Gams W, eds. 1979. *Soil-
Borne Plant Pathogens.* New York: Aca-
demic

176. Schloter M, Lebuhn M, Heulin T, Hart-
mann A. 2000. Ecology and evolution of
bacterial microdiversity. *FEMS Micro-
biol. Rev.* 24:647–60

177. Schneider RW, ed. 1982. *Suppressive
Soils and Plant Disease.* St. Paul, MN:
Am. Phytopathol. Soc. 88 pp.

178. Schottel JL, Shimuzu K, Kinkel LL.
2001. Relationships of in vitro pathogen
inhibition and soil colonization to potato
scab biocontrol by antagonistic *Strepto-
myces* spp. *Biol. Control* 20:101–12

179. Schroeder KL, Raaijmakers JM,
Kalloger SE, Mavrodi DV, Thomashow
LS, et al. 1998. Distribution of 2,4-
diacetylphloroglucinol-producing *Pseu-
domonas* spp. with extended monocul-
ture. *Phytopathology* 88(Suppl.):S80

180. Schwieger F, Tebbe CC. 2000. Effect

of field inoculation with *Sinorhizobium meliloti* L33 on the composition of bacterial communities in rhizospheres of a target plant (*Medicago staiva*) and a non-target plant (*Chenopodium album*)—linking of 16S rRNA gene-based single-strand conformation polymorphism community profiles to the diversity of cultivated bacteria. *Appl. Environ. Microbiol.* 66:3556–65

181. Shanahan P, O'Sullivan DJ, Simpson P, Glennon JD, O'Gara F. 1992. Isolation of 2,4-diacetylphloroglucinol from a fluorescent pseudomonad and investigation of physiological parameters influencing production. *Appl. Environ. Microbiol.* 58:353–58

182. Sharifi-Tehrani A, Zala M, Natsch A, Moënne-Loccoz Y, Défago G. 1998. Biocontrol of soil-borne fungal plant diseases by 2,4-diacetylphloroglucinol-producing fluorescent pseudomonads with different restriction profiles of amplified 16S rDNA. *Eur. J. Plant Pathol.* 104:631–43

183. Shiomi Y, Nishiyama M, Onizuka T, Marumoto T. 1999. Comparison of bacterial community structures in the rhizoplane of tomato plants grown in soils suppressive and conducive toward bacterial wilt. *Appl. Environ. Microbiol.* 65:3996–4001

184. Shipton PJ. 1975. Take-all decline during cereal monoculture. See Ref. 31, pp. 137–44

185. Shipton PJ, Cook RJ, Sitton JW. 1973. Occurrence and transfer of a biological factor in soil that suppresses take-all of wheat in eastern Washington. *Phytopathology* 63:511–17

186. Shurtleff MC, Averre CW III. 1997. *Glossary of Plant-Pathological Terms*. St. Paul, MN: Am. Phytopathol. Soc. Press. 361 pp.

187. Simon A. 1989. Biological control of take-all of wheat by *Trichoderma koningii* under controlled environmental conditions. *Soil Biol. Biochem.* 21:323–26

188. Simon A, Sivasithamparam V. 1988. Interactions among *Gaeumannomyces graminis* var. *tritici*, *Trichoderma koningii*, and soil bacteria. *Can. J. Microbiol.* 34:871–76

189. Simon A, Sivasithamparam V. 1989. Pathogen-suppression: a case study in biological suppression of *Gaeumannomyces graminis* var. *tritici* in soil. *Soil Biol. Biochem.* 21:331–37

190. Simon A, Sivasithamparam V, MacNish GC. 1987. Biological suppression of the saprophytic growth of *Gaeumannomyces graminis* var. *tritici in soil. Can. J. Microbiol.* 33:515–19

191. Simon A, Sivasithamparam V, MacNish GC. 1988. Effect of application to soil of nitrogenous fertilizers and lime on biological suppression of *Gaeumannomyces graminis* var. *tritici. Trans. Br. Mycol. Soc.* 91:287–94

192. Simon H, Smith K, Dodsworth J, Guenthner B, Handelsman J, Goodman R. 2001. Influence of tomato genotype on growth of inoculated and indigenous bacteria in the spermosphere. *Appl. Environ. Microbiol.* 67:514–20

193. Sivan A, Chet I. 1989. The possible role of competition between *Trichoderma harzianum* and *Fusarium oxysporum* on rhizosphere colonization. *Phytopathology* 79:198–203

194. Smiley RW. 1979. Wheat-rhizoplane pseudomonads as antagonists of *Gaeumannomyces graminis*. *Soil Biol. Biochem.* 11:371–76

195. Smit E, Leeflang P, Gommans S, Van Den Broek J, Van Mil S, Wernars K. 2001. Diversity and seasonal fluctuations of the dominant members of the bacterial soil community in a wheat field as determined by cultivation and molecular methods. *Appl. Environ. Microbiol.* 67:2284–91

196. Smith KP, Goodman RM. 1999. Host variation for interactions with beneficial plant-associated microbes. *Annu. Rev. Phytopathology* 37:473–91

197. Smith KP, Handelsman J, Goodman RM. 1999. Genetic basis in plants for interactions with disease-suppressive bacteria. *Proc. Natl. Acad. USA* 96:4786–90

198. Smith SN, Snyder WC. 1971. Relationships of inoculum density and soil types to severity of fusarium wilt of sweet potato. *Phytopathology* 61:1049–51

199. Sneh B, Dupler M, Elad Y, Baker R. 1984. Chlamydospore germination of *Fusarium oxysporum* f. sp. *cucumerinum* as affected by fluorescent and lytic bacteria from fusarium-suppressive soil. *Phytopathology* 74:1115–24

200. Sneh B, Pozniak D, Salomon D. 1987. Soil suppressiveness to Fusarium wilt of melon induced by repeated croppings of resistant varieties of melons. *J. Phytopathol.* 120:347–54

201. Speksnijder AGCL, Kowalchuk GA, de Jong S, Kline E, Stephen JR, Laanbroek HJ. 2001. Microvariation artifacts introduced by PCR and cloning of closely related 16S rRNA gene sequences. *Appl. Environ. Microbiol.* 67:469–72

202. Steinberg C, Edel V, Gautheron N, Abadie C, Vallaeys T, Alabouvette C. 1997. Phenotypic characterization of natural populations of *Fusarium oxysporum* in relation to genotypic characterization. *FEMS Microbiol. Ecol.* 24:73–85

203. Stone A, Traina S, Hoitink H. 2001. Particulate organic matter composition and Pythium damping-off of cucumber. *Soil Sci. Soc. Am. J.* 65:761–70

204. Stutz EW, Défago G, Hantke R, Kern H. 1985. Effect of parent materials derived from different geological strata on suppressiveness of soils to black root rot of tobacco. In *Ecology and Management of Soilborne Plant Pathogens*, ed. CA Parker, AD Rovira, KJ Moore, PTW Wong, JF Kollmorgen, pp. 215–17. St. Paul, MN: Am. Phytopathol. Soc.

205. Stutz EW, Défago G, Kern H. 1986. Naturally occurring fluorescent pseudomonads involved in suppression of black root rot of tobacco. *Phytopathology* 76:181–85

206. Stutz EW, Kahr G, Défago G. 1989. Clays involved in suppression of tobacco black root rot by a strain of *Pseudomonas fluorescens. Soil Biol. Biochem.* 21:361–66

207. Tahvonen R. 1982. The suppressiveness of Finnish light coloured Sphagnum peat. *J. Sci. Agric. Soc. Finl.* 54:345–56

208. Tamietti G, Ferraris L, Matta A, Gentile IA. 1993. Physiological responses of tomato plants grown in *Fusarium* suppressive soil. *J. Phytopathol.* 138:66–76

209. Tanner RS. 2002. Cultivation of bacteria and fungi. See Ref. 82, pp. 62–71

210. Thomashow LS, Weller DM. 1988. Role of a phenazine antibiotic from *Pseudomonas fluorescens* in biological control of *Gaeumannomyces graminis* var. *tritici. J. Bacteriol.* 170:3499–508

211. Thomashow LS, Weller DM, Bonsall RF, Pierson LS III. 1990. Production of the antibiotic phenazine-1-carboxylic acid by fluorescent *Pseudomonas* species in the rhizosphere of wheat. *Appl. Environ. Microbiol.* 56:908–12

212. Tiedje J, Asuming-Brempong S, Nusslein K, Marsh T, Flynn S. 1999. Opening the black box of soil microbial diversity. *Appl. Soil Ecol.* 13:109–22

213. Toussoun TA. 1975. Fusarium-suppressive soils. See Ref. 31, pp. 145–51

214. Valinsky L, Scupham L, Vedova GD, Chrobak M, Jinag T, et al. 2001. A DNA array approach for analysis of microbial communities using oligonucleotide fingerprinting of ribosomal RNA genes. *Phytopathology* 91(Suppl.):S90–91

215. van Loon LC, Bakker PAHM, Pieterse, CMJ. 1999. Systemic resistance induced by rhizosphere bacteria. *Annu. Rev. Phytopathol.* 36:453–83

216. Vincent MN, Harrison LA, Brackin JM, Kovacevich PA, Mukerji P, et al. 1991. Genetic analysis of the antifungal activity of a soilborne *Pseudomonas*

aureofaciens strain. *Appl. Environ. Microbiol.* 57:2928–34

217. Vojinovic ZD. 1972. Biological antagonism as the cause of decline of *Ophiobolus graminis* Sacc. in prolonged wheat monoculture. *J. Sci. Agric. Res.* 25:31–41

218. Wang C, Ramette A, Punjasamarnwong P, Zala M, Natsch A, et al. 2001. Cosmopolitan distribution of *phlD*-containing dicotyledonous crop-associated biocontrol pseudomonads of worldwide origin. *FEMS Microb. Ecol.* 37:105–16

219. Weibelzahl-Fulton E, Dickson DW, Whitty EB. 1996. Suppression of *Meloidogyne incognita* and *M. javanica* by *Pasteuria penetrans* in field soil. *J. Nematol.* 28:43–49

220. Weinhold AR, Oswald JW, Bowman T, Bishop J, Wright D. 1964. Influence of green manures and crop rotation on common scab of potato. *Am. Potato J.* 41:265–73

221. Weller DM. 1988. Biological control of soilborne plant pathogens in the rhizosphere with bacteria. *Annu. Rev. Phytopathol.* 26:379–407

222. Weller DM, Cook RJ. 1983. Suppression of take-all of wheat by seed treatments with fluorescent pseudomonads. *Phytopathology* 73:463–69

223. Weller DM, Howie WJ, Cook RJ. 1988. Relationship between in vitro inhibition of *Gaeumannomyces graminis* var. *tritici* and suppression of take-all of wheat by fluorescent pseudomonads. *Phytopathology* 78:1094–100

224. Weller DM, Thomashow LS. 1994. Current challenges in introducing beneficial microorganisms into the rhizosphere. In *Molecular Ecology of Rhizosphere Microorganisms*, ed. F O'Gara, DN Dowling, B Boesten, pp. 1–18. Weinheim: VCH

225. Weller DM, Zhang B-X, Cook RJ. 1985. Application of a rapid screening test for selection of bacteria suppressive to take-all of wheat. *Plant Dis.* 69:710–13

226. Westphal A, Becker JO. 1999. Biological suppression and natural population decline of *Heterodera schachtii* in a California field. *Phytopathology* 89:434–40

227. Westphal A, Becker JO. 2000. Transfer of biological soil suppressiveness against *Heterodera schachtii*. *Phytopathology* 90:401–6

228. Whipps JM. 1997. Developments in the biological control of soil-borne plant pathogens. *Adv. Bot. Res.* 26:1–134

229. Wildermuth GB, Rovira AD, Warcup JH. 1979. Mechanism and site of suppression of *Gaeumannomyces graminis* var. *tritici* in soil. See Ref. 175, pp. 157–64

230. Wiseman BM, Neate SM, Keller KO, Smith SE. 1996. Suppression of *Rhizoctonia solani* anastomosis group 8 in Australia and its biological nature. *Soil Biol. Biochem.* 28:727–32

231. Woese DR, Kandler O, Wheelis ML. 1990. Towards a natural system of organisms: proposal for the domains *Archae*, *Bacteria*, and *Eucarya*. *Proc. Natl. Acad. Sci. USA* 87:4576–79

232. Wong PTW, Mead JA, Holley MP. 1996. Enhanced field control of wheat take-all using cold tolerant isolates of *Gaeumannomyces graminis* var. *graminis* and *Phialophora* sp. (lobed hyphopodia). *Plant Pathol.* 45:285–93

233. Workneh F, Yang XB, Tylka GL. 1999. Soybean brown stem rot, *Phytophthora sojae*, and *Heterodera glycines* affected by soil texture and tillage relations. *Phytopathology* 89:844–50

234. Wu L, Thompson DK, Li G, Hurt RA, Tiedje JM, Zhou J. 2001. Development and evaluation of functional gene arrays for detection of selected genes in the environment. *Appl. Environ. Microbiol.* 67:5780–90

235. Yin B, Crowley D, Sparovek, G Wanderley JDM, Borneman J. 2000. Bacterial functional redundancy along a soil reclamation gradient. *Appl. Environ. Microbiol.* 66:4361–65

236. Yuen GY, Schroth MN, McCain AH. 1986. Inhibition of *Fusarium oxysporum* f. sp. *dianthi* by iron competition with an *Alcaligines* sp. *Phytopathology* 76:171–76

237. Zogg H, Jäggi. 1974. Studies on the biological soil disinfection: VII. Contribution to the take-all decline (*Gaeumannomyces graminis*) imitated by means of laboratory trials and some of its possible mechanisms. *Phytopathol. Z.* 81:160–69

238. Zriba N, Sherwood JE, Mathre DE. 1999. Characterization and effectiveness of *Phialophora* spp. isolated from a Montana take-all suppressive soil in controlling take-all disease of wheat. *Can. J. Plant Pathol.* 21:110–18