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The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms

Jos M. Raaijmakers · Timothy C. Paulitz · Christian Steinberg · Claude Alabouvette · Yvan Moënne-Loccoz

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Abstract The rhizosphere is a hot spot of microbial interactions as exudates released by plant roots are a main food source for microorganisms and a driving force of their population density and activities. The rhizosphere harbors many organisms that have a neutral effect on the plant, but also attracts organisms that exert deleterious or beneficial effects on the plant. Microorganisms that adversely affect plant growth and health are the pathogenic fungi, oomycetes, bacteria and nematodes. Most of the soilborne pathogens are adapted to grow and survive in the bulk soil, but the rhizosphere is the playground and infection court where the pathogen establishes a parasitic relationship with the plant. The rhizosphere is also a battlefield where the complex rhizosphere community, both microflora and microfauna, interact with pathogens and influence the outcome of pathogen infection. A wide range of microorganisms are beneficial to the plant and include nitrogen-fixing bacteria, endo- and ectomycorrhizal fungi, and plant growth-promoting bacteria and fungi. This review focuses on the population dynamics and activity of soilborne pathogens and beneficial microorganisms. Specific attention is given to mechanisms involved in the tripartite interactions between beneficial microorganisms, pathogens and the plant. We also discuss how agricultural practices affect pathogen and antagonist populations and how these practices can be adopted to promote plant growth and health.

Keywords Epidemiology of soilborne pathogens · Microbial interactions · Induced systemic resistance · Antibiotic resistance in pathogens · Biofumigation and organic amendment

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J. M. Raaijmakers (✉)
Laboratory of Phytopathology, Wageningen University,
Binnenhaven 5,
6709 PD Wageningen, the Netherlands
e-mail: jos.raaijmakers@wur.nl

T. C. Paulitz
USDA-ARS,
345 Johnson Hall,
Pullman, WA 99164-6430, USA

C. Steinberg · C. Alabouvette
INRA-Université de Bourgogne, UMR-MSE,
21065 Dijon, France

Y. Moënne-Loccoz
Université Lyon 1,
Lyon 69003, France

Y. Moënne-Loccoz
UMR CNRS 5557, Ecologie Microbienne,
Villeurbanne 69622, France

Introduction

The rhizosphere is an environment that the plant itself helps to create and where pathogenic and beneficial microorganisms constitute a major influential force on

plant growth and health (Lynch 1990). Microbial groups and other agents found in the rhizosphere include bacteria, fungi, nematodes, protozoa, algae and microarthropods (Lynch 1990; Raaijmakers 2001). Many members of this community have a neutral effect on the plant, but are part of the complex food web that utilizes the large amount of carbon that is fixed by the plant and released into the rhizosphere (i.e. rhizodeposits). The microbial community in the rhizosphere also harbors members that exert deleterious or beneficial effects on the plant. Microorganisms that adversely affect plant growth and health are the pathogenic fungi, oomycetes, bacteria and nematodes, whereas microorganisms that are beneficial include nitrogen-fixing bacteria, endo- and ectomycorrhizal fungi, and plant growth-promoting rhizobacteria (PGPR) and fungi. The number and diversity of deleterious and beneficial microorganisms are related to the quantity and quality of the rhizodeposits and to the outcome of the microbial interactions that occur in the rhizosphere (Somers et al. 2004). Understanding the processes that determine the composition, dynamics, and activity of the rhizosphere microflora has attracted the interest of scientists from multiple disciplines and can be exploited for the development of new strategies to promote plant growth and health. In this review, we will focus on the epidemiology (spatial and temporal aspects) of soilborne pathogens and the economic importance of soilborne diseases. Specific attention is given to mechanisms, both offensive and defensive, involved in the interactions between soilborne pathogens and beneficial microorganisms. Also direct positive effects of rhizosphere microorganisms on the plant are addressed. Finally, we discuss the effects of agricultural practices on pathogen and antagonist populations and how these practices can be manipulated to induce soil suppressiveness and to promote plant growth and health.

Soilborne pathogens and their economic importance

In most agricultural ecosystems, soilborne plant pathogens can be a major limitation in the production of marketable yields. They are also more recalcitrant to management and control compared to pathogens that attack the above-ground portions of the plant (Bruehl 1987). Soilborne pathogens are adapted to grow and survive in the bulk soil, but the rhizosphere is the infection court where the pathogen encounters the plant and establishes a parasitic relationship. This is also where the complex rhizosphere community, both microflora and microfauna, can interact with the pathogen and influence the outcome of pathogen infection.

There are four main groups of plant pathogens (Agrios 2005), but only two of them are major players in the soil: fungi (true fungi and oomycetes) and nematodes. Only a few groups of bacteria are considered to be soilborne, probably because non-spore forming bacteria cannot survive well in soil for long periods. Bacteria also require a wound or natural opening to penetrate into the plant and cause infection. Examples are *Ralstonia solanacearum*, cause of bacterial wilt of tomato (Genin and Boucher 2004), and *Agrobacterium tumefaciens*, the well-studied causal agent of crown gall (Nester et al. 2005). Some filamentous bacteria (*Streptomyces*) can also infect plants and are better adapted to survive in the soil. Only a few viruses can infect roots. Like bacteria, they require a wound to infect the plant and are mostly transmitted by vectors. In soil, they can be transmitted by nematodes (Nepoviruses; Brown et al. 1995) or by zoosporic fungi such as *Olpidium* and *Polymyxa* (Campbell 1996).

Nematodes are complex, worm-like eukaryotic invertebrate animals and probably among the most numerous animals on the planet (Perry and Moens 2006). Most nematodes in soil are free-living, consuming bacteria, fungi, and other nematodes, but some can parasitize plants. Some feed on the outside of the root (migratory ectoparasitic), some penetrate and move in the interior of the root (migratory endoparasitic), and some set up a feeding site in the interior of the root and remain there for reproduction (sedentary endoparasites).

Fungi and oomycetes are the most important soilborne pathogens and will be the focus of this review. Fungi are eukaryotic, filamentous, multicellular, heterotrophic organisms that produce a network of hyphae called the mycelium and absorb nutrients from the surrounding substrate (Alexopoulos et al. 1996). Oomycetes have a morphology similar to fungi, but are phylogenetically more closely related to brown algae. They produce swimming spores (zoospores; Fig. 1) and contain cellulose in their cell walls as opposed to chitin in true fungi. Nevertheless,

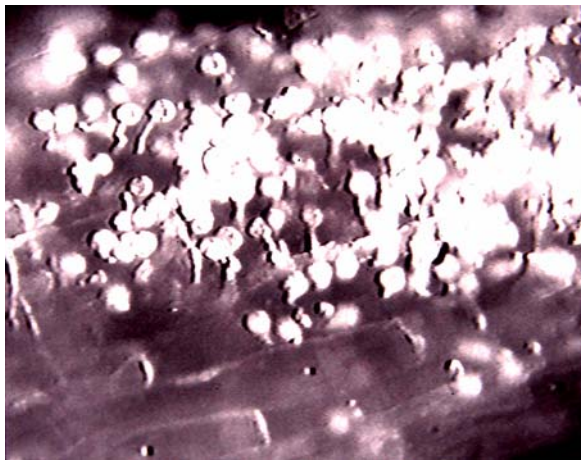

Fig. 1 Aggregation of encysted zoospores of *Pythium aphanidermatum* in the rhizoplane of roots of cucumber seedlings grown in hydroponic solution. Encysted zoospores were visualised by UV epifluorescence after staining with acridine orange and malachite green (micrograph from experiments described in Zhou and Paulitz 1993)

the mechanisms of parasitism and the diseases they cause are similar to true fungi, and therefore will be considered together in this discussion. Almost all soilborne fungi are necrotrophic, meaning they kill host tissue with enzymes and toxins in advance of the hyphae and do not require a living cell to obtain nutrients. Most of the biotrophic pathogens, such as rusts and powdery mildews, occur on the above-ground portions of the plants and require a living cell to obtain nutrients. A few root pathogens such as *Phytophthora sojae* are semibiotrophic. Surprisingly few root pathogens are biotrophic. Some examples are lower zoosporic fungi and Oomycetes, such as *Plasmodiophora brassicae* and *Plasmopara halstedii*. Most necrotrophic pathogens are generalists with a wide host range, as opposed to biotrophic pathogens with narrow host ranges that have co-evolved with the plant. Thus, there is usually no race structure within necrotrophic pathogen populations and no specific single-gene resistances in the plant.

Environmental conditions in the soil are generally not favorable for fungal growth, due to high or low temperatures (frozen ground) or extremely dry conditions. Pathogens survive in the soil as resistant propagules, such as chlamydospores, sclerotia, thick-walled conidia or hyphae, or survive in plant roots and crop residues (Bruehl 1987). When conditions are favorable and when a seed or root approaches the dormant propagule, the fungus is stimulated to germinate by root or seed exudates and chemotactically grows toward the plant. The germ tube or zoospore can attach to the surface of the root, penetrate and infect the epidermal cells of the root tips, secondary roots, and root hairs, or attack the emerging shoots and radicles of seedlings. Some fast-growing pathogens, such as *Pythium* species, can attack seeds and embryos before they emerge. Fungi penetrate through intact cell walls via cell wall-degrading enzymes and mechanical turgor pressure, and colonize the root cortex. Most soilborne fungi attack young, juvenile roots as opposed to secondary woody roots. After the roots have been killed and the fungus ramifies through the cortex, it reproduces and forms spores within the root tissue. Mycelium can continue to spread up the root, internally or externally, or can spread to other roots in close proximity. A specialized group of pathogens that cause wilt diseases (e.g. *Fusarium oxysporum*, *Verticillium dahliae*) can penetrate through the endodermis into the vascular tissue and move up the xylem to above-ground parts of the plant, impeding the flow of water (Beckman 1987).

A number of diseases and symptoms can be manifested by plants infected with fungal soilborne pathogens. However, these diseases can be difficult to diagnose, because most of the symptoms occur below ground, and the above-ground symptoms may be non-distinct or similar to those caused by abiotic factors such as drought, stress, and lack of nutrients. Soilborne pathogens can cause seed decay, damping-off (both pre- and post-emergence), and can also move into the base of the stem, causing crown rot and wilt. In perennial trees, fungi can move into the collar of the tree, girdling the tree, or inoculum can splash onto the fruit, causing decay and rot. However, the primary disease is root rot. By killing root tips, root growth on that axis is eliminated. By destroying fine feeder roots and root hairs, the ability of the plant to absorb water and nutrients is diminished. This leads to reduced plant size, stunting, drought stress and nutrient deficiencies.

Economic impacts of soilborne pathogens and root diseases

Attainable yield has been defined as the potential yield in a given environment (temperature, water) without the limitations of pests and diseases (Cook

and Veseth 1991). The actual yield is that obtained after biological factors (pests, pathogens, weeds) have acted on the crop. The difference between these two yields (*attainable − actual*) is often called crop loss, but in reality it is potential yield that was never attained. But in considering the economic impact, one also has to consider the costs of control and management of diseases. How much crop losses do diseases and in particular soilborne pathogens cause? Estimating crop loss from pathogens is difficult and there are only a few well-documented studies. From 2001–2003, an average of 7% to 15% of crop loss occurred on major world crops (wheat, rice, potato, maize and soybean) due to fungi and bacteria (Oerke 2005). From 1996 to 1998, these pathogens caused an actual loss of 9.9%, but the potential loss without controls would be 14.9% (Oerke and Dehne 2004). Nematode crop losses have been estimated at 10% up to 20%, with worldwide losses exceeding $US 100 billion (Bird and Kaloshian 2003). Losses from soilborne pathogens are even more difficult to estimate, because of the difficulty of diagnosis. Some estimate that soilborne pathogens cause 50% of the crop loss in the US (Lewis and Papavizas 1991). The most accurate studies are based on replicated field plots where fumigants, fungicides and nematicides are applied and yield is compared to non-treated plots. Some studies compare resistant to susceptible varieties, but for most soil-borne pathogens there are no resistant varieties. Most studies are done with natural inoculum, and the pathogen is quantified and used as a predictor. Probably the most comprehensive studies on crop loss have been done with soilborne pathogens of cereals and serve to illustrate more realistic crop losses. Folwell et al. (1991) estimated that take-all disease caused 20% yield reduction on wheat, based on soil fumigation studies and grower surveys. Treatment with metalaxyl, a fungicide specific for oomycetes, resulted in wheat yield increases of 1–2 tons/acre, due to control of *Pythium* (Cook et al. 1980). Treatment with soil fumigation increased wheat yields by 3–36% (Cook et al. 1987). Based on detailed disease measurements, Fusarium crown rot was estimated to cause a 9.9% loss on wheat, with some losses up to 35% (Smiley et al. 2005a). With severe Rhizoctonia bare patch, close to 20% of the field can be covered with patches with essentially no yield in the patches (Cook et al. 2002). Using a combination of a nematicide (aldicarb) and tolerant and intolerant varieties, yield suppression caused by *Pratylenchus neglectus* ranged from 8% to 36% in Oregon (Smiley et al. 2005b). Aldicarb increased wheat yields 67% and 113% in soil infested with *P. thornei* (Smiley et al. 2005c). However, yield losses from combinations of pathogens and disease complexes are not well understood. For example, fungal wilt pathogens and nematodes can have synergistic interactions (Back et al. 2002).

Epidemiology of soilborne diseases: temporal and spatial aspects

Temporal spread of soilborne pathogens and diseases

Like all biological organisms, pathogens can grow, multiply and reproduce on their plant hosts, and as a consequence diseases increase over time. The increase of disease over time can be described by a disease progress curve, where disease (counts, incidence or severity) is plotted as the dependent variable measured over various times in the season. Vanderplank (1963) described two types of disease progress curves: one where inoculum multiplies many times over the season (compound interest diseases) and one where there was only one infection cycle and no increase in inoculum during the growing season (simple interest diseases). The former disease progress curve can be described by a logistic or other similar model with an S-shaped curve and an upper asymptote or plateau, because the rate slows when the density of susceptible host tissue decreases and becomes limiting. The most important part of this model is the infection rate or the slope of the line, which describes how quickly the epidemic increases and changes over time. In past literature, this type of disease progress curve was assumed to be typical of foliar diseases such as rust, which may have many cycles of infection and inoculum production (sporulation) over a single season. Soilborne diseases were assumed to be described by a monomolecular model, based on monomolecular chemical reactions of the first order, or also known as the negative exponential model. This model also has an asymptote, but the rate is more constant. This type of model has been used to describe a number of soilborne epidemics (Hao and Subbarao 2005; Stanghellini et al. 2004a). With this model, the initial inoculum plays an important role in

the outcome of the epidemic, but has a minor role in a logistic model. Thus, there is a strong relationship between the inoculum in the soil and disease, described by the inoculum density-disease incidence (ID/DI) plot. The ID/DI curve can be described by an S-shaped logistic model (Baker 1978); Baker further theorized that the slope of a linearized transformation would approach 1 for a rhizosphere effect, and 0.67 for a rhizoplane effect, based on theories from physical chemistry and packing of particles. However, Gilligan and colleagues have developed a model based on probabilistic models and proposed the "pathozone" around a root (Gilligan and Bailey 1997). The pathozone is based on the probability of infection with the distance of the inoculum from the host. Essentially, this is the zone in which a propagule can germinate and successfully infect a root. Infection efficiency, or the efficiency of a propagule, declines in an exponential or sigmoidal manner as the distance from the root increases.

However, the simple interest or monomolecular models may be overly simplistic for most soilborne pathogens, although they may fit a disease such as Fusarium wilt where there is little transmission from plant to plant. These models assume there is a uniform environment over time, that the population of pathogen and host are uniformly virulent and susceptible, and that the spatial pattern of the pathogen is uniform or random. However, soil temperature may increase over the season, host roots may become more resistant over time, and pathogens are often aggregated or clustered. In most soilborne diseases, there is a primary infection step with new infection of healthy plants from a reservoir of inoculum in the soil. But once a root is infected, the pathogen can spread to adjacent roots in the same season. For example, zoosporic pathogens such as *Pythium* and *Phytophthora* can produce zoospores that can swim to and encyst on adjacent roots (Fig. 1). Mycelium of *Rhizoctonia* or *Gaeumannomyces* can grow into the soil from an infected root and can spread to adjacent healthy roots. Gilligan has produced a series of elegant models and experiments with take-all (caused by *Gaeumannomyces graminis* var. *tritici*) that demonstrated in the early part of the epidemic, the proportions of diseased roots increased monotonically to an initial plateau and then increased sigmoidally to an asymptotic level (Bailey et al. 2005; Bailey and Gilligan 1999, 2004). Their model has components for primary infection, secondary infection, and also accounts for root production and decay of inoculum. As roots grow and move through the soil, they encounter more inoculum and infected roots. The disease itself will alter the density of roots and in some cases increase root production (Bailey et al. 2006). Over time, inoculum will decay as it exhausts endogenous nutrients and propagules are parasitized and colonized by other microbes and antagonists or consumed by predators. Pathogens have a latent period (period of time from infection to production of inoculum) and an infectious period (time during which inoculum is produced), which must also be considered in models. For a more in-depth review of the temporal aspects of root disease epidemics we refer to Gilligan (1994).

Spatial aspects of soilborne pathogens and root diseases

Soilborne pathogens not only spread through time but also through space. However, the dynamics of spread and spatial patterns of soilborne pathogens are very different from foliar pathogens, which produce spores or propagules that can rapidly spread aerially by wind and rain over large distances or by water splash over small distances. Soilborne pathogens are confined within the soil, a three-dimensional matrix of mineral soil particles, pores, organic matter in various stages of decomposition, and a biological component. Thus, the spread of soilborne pathogens over time and space is more limited. Some soilborne pathogens in infected crop debris or soil can be spread by wind that blows during harvesting or cultivation. Some soilborne pathogens, such as *Sclerotinia sclerotiorum* or *Rhizoctonia solani*, produce aerial sexual spores that are ejected into the air and spread by wind. Pathogens can move above ground with irrigation water or rain runoff, which can carry soil particles into adjacent fields. The oomycetes, which produce motile swimming zoospores (Fig. 1), are especially adapted for movement in water. Both *Pythium* and *Phytophthora* have frequently been recovered from lakes, streams and irrigation ponds by using baits or molecular methods. Recent work with *Phytophthora ramorum*, an introduced pathogen and causal agent of sudden oak death, has documented movement in soil and streams in natural ecosystems (Davidson et al. 2005). However, most soilborne pathogens move and spread directly

through the soil profile as mycelium. Soil texture and water (matric) potential are probably the two most important factors that determine spread, based on the size of the soil pores. For example, *Rhizoctonia* growth is restricted at high matric potentials and spreads faster in soils with high porosity with larger pores (Otten et al. 1999, 2004; Harris et al. 2003), an observation confirmed in the field in Australia (Gill et al. 2000).

How does the biology of soilborne pathogens affect the spatial patterns or distribution of plant diseases? In general, soilborne pathogens tend to be more aggregated or clustered, compared to foliar pathogens. An aggregated distribution has been demonstrated with pathogens such as *Phytophthora*, *Verticillium*, *Gaeumannomcyes*, and *Macrophomina* (Ristaino et al. 1993; Johnson et al. 2006; Gosme et al. 2007; Mihail and Alcorn 1987). These patterns are also more likely to be preserved from year to year. For example, the aggregated patterns of *Rhizoctonia oryzae* in wheat over a 30-acre field were evident from sampling the following year (Paulitz et al. 2003). The distance of spread each year is also likely to be less than that for aerial pathogens. However, long-distance movement can occur from soil attached to cultivation or harvesting equipment or by movement of nursery material across a country. A classic example was the movement of *P. ramorum* across the US in 2004 on infected potted camellias (Stokstad 2004).

How are the spatial patterns or distributions described or quantified? How does one determine whether the pathogen is clustered or randomly distributed? It is beyond the scope of this review to cover this in detail and for more information on this topic we refer to reviews by Campbell and Madden (1990), Campbell and Benson (1994) and Madden et al. (2007). Basically, disease or pathogens are sampled in regular quadrats, transects, or rows of plants, and the spatial location of each sample is recorded. The data can be mapped and the frequency distributions can be fitted to various models. For example, a Poisson distribution would indicate randomness, whereas fitting to a beta binomial distribution (Madden and Hughes 1994) would indicate a clustered pattern. Indices of dispersion can be calculated, the simplest being the ratio of the variance to the mean, which should be greater than 1 for an aggregated pattern, and equal to 1 for a random distribution. Other widely used indices, including Lloyd's Index of Patchiness and Morista, are based on the mean and variance. With intensively-mapped or quadrat data, the spatial information can be utilized to pick out patterns, such as with distance-based or nearest neighbor analyses (Madden et al. 2007). If the distribution is random, the distance between neighboring samples should not have any effect. A similar reasoning is used for spatial autocorrelation techniques and geostatistics, which have been widely used in earth sciences. The assumption is that plants or samples that are close together will be more similar than samples that are further apart. Another technique that is becoming more widely used is SADIE (Spatial analysis by distance indices; Perry 1998). This method is based on how much samples must be moved in a grid to attain a regular pattern, based on random rearrangements, called the distance to regularity. With a more clustered distribution, more rearrangements are required. One overriding factor in spatial analysis is the scale of measurement. Most studies in agriculture are done at the scale of a few meters in replicated agronomic plots. Very few studies have looked at a microscale (millimeter or micrometer) of a rhizosphere or at a mesoscale (kilometer) of a county, district, province, or country. The spatial patterns may vary with the grain or resolution of the measurement. For example, at a microscale level (square millimeter), propagules of *Macrophomina phaseolini* in the maize rhizosphere exhibited a random distribution (Olanya and Campbell 1989), but at a larger scale in the field (square meter) the pattern was aggregated (Mihail and Alcorn 1987). On the other hand, Paulitz and Rossi (2004) found that *R. solani* and *R. oryzae* showed a similar pattern of aggregation at a 30, 3, or 0.3 m scale. Large patches of *Rhizoctonia* in the field were composed of smaller patches, which themselves are composed of smaller patches.

In conclusion, although we know more about how soilborne pathogens are distributed on scales applicable to agriculture, we know little about how pathogens are arranged or interact with beneficial microorganisms at the microscale of the rhizosphere. Advances in molecular techniques, fluorescence labeling, and imaging such as confocal laser microscopy, may be useful in the future. These techniques have been used to study colonization of roots by biocontrol bacteria and fungi (Gamalero et al. 2005; Bloemberg et al. 2000; Lu et al. 2004) and infection of roots by *Fusarium* (Lagopodi

et al. 2002; Bolwerk et al. 2005; Olivain et al. 2006), but these have been descriptive and have not employed spatial statistics.

Interactions between beneficial microorganisms and soilborne pathogens

The rhizosphere is the playground and infection court where soilborne pathogens establish a parasitic relationship with the plant. However, the rhizosphere is also a battlefield where the complex rhizosphere community, both microflora and microfauna, interact with soilborne pathogens and influence the outcome of pathogen infection. The growth or activity of soilborne pathogenic fungi, oomycetes, bacteria, and/or nematodes can be inhibited by several beneficial rhizosphere microorganisms. The activity and effects of beneficial rhizosphere microorganisms on plant growth and health are well documented for bacteria belonging to the Proteobacteria (noticeably *Pseudomonas* and *Burkholderia*) and Firmicutes (*Bacillus* and related genera), and for fungi from the Deuteromycetes (e.g. *Trichoderma*, *Gliocladium* and non-pathogenic *F. oxysporum*). In the remainder of this section, these beneficial microorganisms will be referred to as biocontrol microorganisms or biocontrol agents.

Biocontrol microorganisms may adversely affect the population density, dynamics (temporal and spatial) and metabolic activities of soilborne pathogens via mainly three types of interactions, which are competition, antagonism and hyperparasitism. In the rhizosphere, competition takes place for space at the root surface (Fig. 2) and for nutrients, noticeably those released as seed or root exudates. Competitive colonisation of the rhizosphere and successful establishment in the root zone is a prerequisite for effective biocontrol, regardless of the mechanism(s) involved (Weller 1988; Raaijmakers et al. 1995). In the case of biocontrol bacteria, this is explained in part by the fact that production of several antagonistic traits and compounds is subjected to cell-density dependent regulation or quorum sensing (Pierson et al. 1998; Pierson and Pierson 2007). In addition, competition can in itself be a biocontrol mechanism, often for organic compounds necessary for reactivation of propagules and/or subsequent proliferation and root colonisation by the pathogen (Paulitz et al. 1992; Van Dijk and Nelson 2000; Fravel et al. 2003). Competition can also take place for micronutrients, especially iron, that are essential for growth and activity of the pathogen. Competition for soluble ferric iron is based on production and/or utilisation of high-affinity chelators termed siderophores (Lemanceau et al. 1992; Neilands 1995). Once complexed with iron,

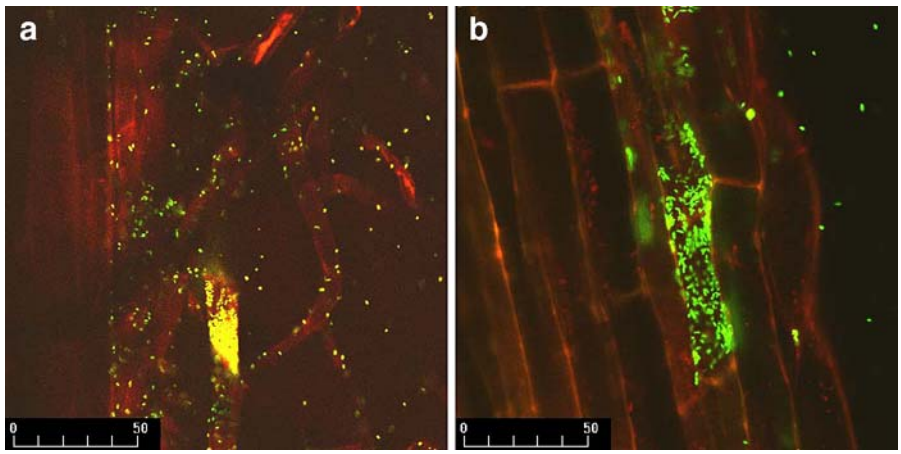

Fig. 2 Confocal laser scanning microscopy of wheat roots colonized by *Pseudomonas fluorescens* Q8r1-96 tagged with the green fluorescent protein (*P. fluorescens* Q8r1-96-*gfp*). Wheat seeds were surface-sterilized, pre-germinated for 2 days and inoculated with a suspension of *P. fluorescens* Q8r1-96-*gfp*. Plants were grown under controlled conditions in a mixture of quartz sand and clay pellets, and harvested 10 days after inoculation. Root samples were stained for 20 min with propidium-iodide. Microcolonies of *P. fluorescens* Q8r1-96-*gfp* on mature root hairs (**a**) and along the junctions of epidermal cells (**b**). Courtesy of Olga Mavrodi and Dmitri Mavrodi, Department of Plant Pathology, Washington State University, USA

siderophores are taken up via specific membrane receptors. Competition for iron as well as competition for carbon are documented as important modes of action for several biocontrol bacteria and fungi (Lemanceau et al. 1992; Alabouvette et al. 2006), with iron competition being particularly significant in calcareous soils where high pH leads to low iron solubility.

Antagonism is usually mediated by the production of secondary antimicrobial metabolites (antibiosis), lytic enzymes and/or effectors. Often, antagonistic microorganisms can produce a range of different antimicrobial secondary metabolites, e.g. 2,4-diacetylphloroglucinol (DAPG), pyrrolnitrin, pyoluteorin, phenazines, cyclic lipopeptides and hydrogen cyanide in the case of certain fluorescent pseudomonads (Raaijmakers et al. 2002, 2006; Picard and Bosco 2008; Cook et al. 1995; Weller 2007). Antimicrobial secondary metabolites are also involved in antagonistic effects of fungi such as *Trichoderma* and *Gliocladium* (Kubicek et al. 2001). The concentration at which these compounds are toxic towards pathogenic bacteria, fungi and nematodes depends on the compound and the target. In fungal pathogens, they may affect the electron transport chain (phenazines, pyrrolnitrin), metalloenzymes such as copper-containing cytochrome *c* oxidases (hydrogen cyanide), membrane integrity (biosurfactants), or cell membrane and zoospores (DAPG, biosurfactants; Haas and Défago 2005; Raaijmakers et al. 2006), but the modes of action of many known antimicrobial metabolites are still poorly understood. The role of several antimicrobial secondary metabolites in plant protection has been demonstrated by comparing wild-type strains and mutant derivatives. Results of these studies have indicated that multiple antimicrobial metabolites can play an important role in the same pathosystem (Haas and Défago 2005). For instance, both the abilities of *Pseudomonas* sp. CHA0 to produce hydrogen cyanide (Voisard et al. 1989) and DAPG (Keel et al. 1992) contribute to suppression of *Thielaviopsis basicola*-mediated black root rot of tobacco. However, population-level comparisons of biocontrol strains indicated that some of these compounds play a more significant role than others in plant protection (Sharifi-Tehrani et al. 1998; Ellis et al. 2000; Rezzonico et al. 2007). Antimicrobial secondary metabolites have received extensive research attention, in part because they are thought to contribute largely to soil disease suppressiveness. This is particularly the case of DAPG-producing pseudomonads, which are involved in soil suppressiveness in different pathosystems (Weller et al. 2002), noticeably black root rot of tobacco (Stutz et al. 1986) and take-all of wheat (Raaijmakers and Weller 1998; Raaijmakers et al. 1997, 1999).

Production of extracellular lytic enzymes is quite common among antagonistic microorganisms (Adesina et al. 2007), but it does not contribute to antagonism in all cases (Sharifi-Tehrani et al. 1998; Dunne et al. 1997). Extracellular lytic enzymes act in different ways: many of them can affect the cell wall of pathogens, and this is documented for cellulases, chitinases and proteases produced by various bacteria. Inactivation of genes involved in their biosynthesis has been used to provide evidence for their contribution in biocontrol *in planta* (Dunne et al. 1997; Kobayashi et al. 2002). Other lytic enzymes from Proteobacteria target virulence factors, such as the phytotoxin fusaric acid produced by *F. oxysporum*, thereby enabling protection of tomato plants from wilt disease (Toyoda et al. 1988).

Antagonism can also implicate effectors (not yet identified) secreted by the type III secretion system of biocontrol bacteria, leading to reduced virulence in certain pathogens (Rezzonico et al. 2005). Type III protein secretion systems, first discovered in pathogenic bacteria (Stuber et al. 2003), enable direct introduction of effectors into eukaryotic host cells. Type III secretion genes are also present in many saprophytic pseudomonads, including biocontrol strains (Preston et al. 2001; Mazurier et al. 2004; Rezzonico et al. 2004). Inactivation of the type III secretion gene *hrcV* impaired the ability of *P. fluorescens* KD to diminish polygalacturonase activity of *P. ultimum* in vitro, and reduced its biocontrol efficacy against this pathogen on cucumber (Rezzonico et al. 2005). This gene is upregulated in presence of the pathogen rather than the plant, which further suggests that the Oomycete is the target of the type III secretion system in this pseudomonad.

In addition to competition and antagonism, direct biocontrol effects on soilborne plant pathogens can result from hyperparasitism. This is mainly documented for *Trichoderma* and *Gliocladium*, and it affects various fungal pathogens, such as *Rhizoctonia*, *Sclerotinia*, *Verticillium* and *Gaeumannomyces* (Harman et al. 2004). Hyperparasitism by *Trichoderma* involves secretion of chitinases and cellulases, which release

small molecules from the target pathogen and trigger chemotropism towards the latter (Zeilinger et al. 1999). Contact is followed with coiling of hyphae around the hyphae of the pathogen, further enzymatic digestion of its cell wall, and penetration by *Trichoderma* (Djonović et al. 2006; Woo et al. 2006). Cell wall damage caused by endochitinases was also shown to play an important role in the activity of *Gliocladium virens* against *Botrytis cinerea* (Di Pietro et al. 1993). Hyperparasitim enables also the Firmicute *Pasteuria penetrans* to control the plant parasitic nematode *Meloidogyne* (Duponnois et al. 1999), but the mechanisms involved are still poorly understood.

Direct positive effects of rhizosphere microorganisms on the plant

Next to the biocontrol activity of rhizosphere microorganisms, several can have a direct positive effect on plant growth and health. Often, it is one of several modes of actions by which these microorganisms can benefit plant health. First, phytostimulatory and biofertilising microbes can promote plant health by making the plant 'stronger'. Second, many rhizosphere microorganisms can induce a systemic response in the plant, resulting in the activation of plant defence mechanisms (Pieterse et al. 2003). This capacity has been identified in a wide range of bacteria (Van Loon et al. 1998; Haas and Défago 2005), including endophytes (Compant et al. 2005) as well as saprophytic (Fuchs et al. 1997), hyperparasitic (Woo et al. 2006) and arbuscular mycorrhizal fungi (Pozo et al. 2002). Induced systemic resistance (ISR) does not confer complete protection, but it does protect the plant from various types of phytopathogens (including root pathogens), without requiring direct interaction between the resistance-inducing microorganisms and the pathogen (Van Loon et al. 1998; Zehnder et al. 2001). In addition, ISR can be effective under field conditions and in commercial greenhouses (Zehnder et al. 2001; Pieterse et al. 2003).

ISR exhibits similarities but also several differences with systemic acquired resistance (SAR), which is the plant response triggered upon exposure to pathogens. ISR involves jasmonate and ethylene signals, as evidenced in experiments with specific *Arabidopsis* mutants (Pieterse et al. 2003). Unlike SAR, ISR is typically salicylate-independent, although certain plant-beneficial microorganisms can activate a salicylate-dependent pathway in the plant (De Meyer et al. 1999). Several cell surface constituents of biocontrol bacteria, i.e. lipopolysaccharides and flagella, can trigger ISR (Pieterse et al. 2003; Haas and Défago 2005). ISR can also take place following exposure of the plant to compounds produced by plant-beneficial bacteria, e.g. the volatile 2,3-butanediol (Ryu et al. 2004), the siderophore pyoverdine (Maurhofer et al. 1994), DAPG (Iavicoli et al. 2003), and cyclic lipopeptide surfactants (Ongena et al. 2007; Tran et al. 2007).

Adaptation and defense of plant pathogens to microbial antagonism

To date, microbial interactions in the rhizosphere are mostly viewed from the perspective of how beneficial microorganisms inhibit the growth or activity of pathogenic microorganisms. However, also pathogens have a diverse array of mechanisms to counteract antagonism, including active efflux and degradation of antimicrobial compounds, and interference with the regulation and biosynthesis of enzymes and antimicrobial metabolites produced by antagonistic microorganisms (reviewed in Duffy et al. 2003). Resistance development in pathogen populations to chemical control agents is a common and well-studied phenomenon. In contrast, resistance in pathogens to antimicrobial compounds produced by antagonistic microorganisms is presumed not to develop or at least relatively slowly, because antagonistic microorganisms operate in microsites in the rhizosphere where only a small fraction of the pathogen population is exposed to the antimicrobial compounds during a short period of its life cycle (Handelsman and Stabb 1996). Furthermore, in contrast to the inundative application of chemical pesticides, only minute amounts of the antimicrobial compounds are produced by the antagonistic microorganisms in the rhizosphere (Séveno et al. 2002; Duffy et al. 2003). Nevertheless, a number of studies have shown that substantial variation in sensitivity against antimicrobial compounds exists within pathogen populations and that pathogens harbour a wide range of defense mechanisms against microbial antagonism.

Variation in sensitivity of pathogen populations to antimicrobial compounds

Studies on the effects of antimicrobial compounds produced by antagonistic microorganisms on plant pathogens often consider only one single strain and one specific stage in the life cycle of the pathogen. Most pathogen populations and life cycles, however, are diverse and comprise numerous structures that allow pathogens to respond adequately to selection pressure exerted by competing microorganisms. Several studies have addressed the variation in sensitivity of pathogenic fungi, oomycetes and bacteria to several antimicrobial compounds, including agrocin 84 (Cooksey and Moore 1982; Stockwell et al. 1996), the volatile hydrogen cyanide (Mackie and Wheatley 1999), phenazines (Gurusiddaiah et al. 1986; Mazzola et al. 1995), the phenolic antibiotic DAPG (Mazzola et al. 1995; De Souza et al. 2003; Schouten and Raaijmakers 2004), gliotoxin (Jones and Hancock 1988), kanosamine (Milner et al. 1996), and the cyclic lipopeptide massetolide A (Mazzola et al. 2007).

One of the most detailed studies on the variation in sensitivity to antimicrobial compounds produced by antagonistic microorganisms was performed by Mazzola et al. (1995), who screened a total of sixty-six individual isolates of the take-all fungus *G. graminis* var. *tritici* (*Ggt*) for sensitivity to DAPG and phenazine-1-carboxylic acid (PCA). Substantial variation in sensitivity to both antimicrobials was observed among a range of Ggt isolates obtained from a single wheat field. In interactions with antagonistic *Pseudomonas* strains producing either DAPG or PCA, the antibiotic-insensitive *Ggt* isolates could not be controlled effectively in the rhizosphere of wheat plants. At least one of the PCA-insensitive *Ggt* isolates was also insensitive to DAPG, suggesting similar mechanisms of resistance to both antimicrobial compounds. Studies by Schouten and Raaijmakers (2004) showed that also among pathogenic and non-pathogenic *F. oxysporum*, substantial variation in sensitivity to DAPG exists. There was no clear relationship between DAPG insensitivity and geographical origin or formae speciales of *F. oxysporum*, suggesting that the traits responsible for DAPG insensitivity are relatively ancient, have developed independently, or are easily transferred within and between populations.

Mechanisms of resistance in plant pathogens against antimicrobial compounds

Pathogenic fungi, oomycetes and bacteria have developed a range of strategies to tolerate or resist the deleterious effects of antimicrobial compounds produced by antagonistic microorganisms (Duffy et al. 2003). One of the best studied examples in plant pathogenic bacteria is resistance of the crown gall pathogen *A. tumefaciens* to agrocin 84 produced by *Agrobacterium rhizogenes* strain K84. Agrocin 84 is believed to inhibit DNA replication and is transported into cells of *A. tumefaciens* via agrocinopine permease, a periplasmic protein encoded by genes carried on certain types of the Ti plasmid present in sensitive strains of *A. tumefaciens* (Stockwell et al. 1996). In strain K84, agrocin 84 biosynthesis and resistance genes are located on the conjugative plasmid pAgK84 (Ryder et al. 1987). Among 65 strains and isolates of *A. tumefaciens*, all of the biotype 3 strains tested were resistant to K84, whereas many of the biotype 1 and 2 strains were susceptible (Van Zyl et al. 1986). Cooksey and Moore (1982) earlier showed that in three *A. tumefaciens* strains and one *A. rhizogenes* strain, mutation rates for resistance to agrocin 84 were relatively high ranging from 2.5×10^{-3} to 4.2×10^{-4}. Conjugal transfer of plasmid pAgK84 was demonstrated in vitro and in crown gall tissue of infected plants and is regarded as one of the main mechanisms of agrocin 84 resistance in pathogenic *A. tumefaciens* strains. The fact that agrocin 84 resistant *A. tumefaciens* strains were isolated from different soils worldwide (Van Zyl et al. 1986) suggested that conjugal transfer may also have occurred in natural environments. Stockwell et al. (1996) carried out a field experiment with cherry seedlings treated with K84 and *A. tumefaciens* and showed that transconjugants were detected in four out of 13 galls and estimated that the frequency of pAgK84 transfer was approximately 10^{-4} transconjugants per recipient. A transconjugant strain retained the plasmid for up to seven months in the rhizosphere of plants grown in the field, colonized the rhizosphere of cherry plants to the same extent as its parental strain and caused crown gall disease.

Studies on resistance mechanisms of soilborne pathogenic fungi to antimicrobial compounds produced by antagonistic microorganisms is limited and fragmentary (Duffy et al. 2003). Although most of the

studies in this research area have been performed with pathogenic fungi infecting aerial plant parts, several of the resistance mechanisms described below most likely also operate in soilborne fungi. Work by Levy et al. (1992) suggested that resistance in *Mycosphaerella graminicola* against phenazines produced by *Pseudomonas aeruginosa* is based, in part, on degradation of these antimicrobials and on the presence of superoxide dismutase and catalase, enzymes involved in the detoxification of oxygen radicals resulting from the oxidative stress generated by the phenazines. Also for the nematode *Caenorhabditis elegans*, Mahajan-Miklos et al. (1999) reported that a mutant with increased levels of catalase and superoxide dismutase was more resistant to fast killing by the phenazine pyocyanin. Degradation of antimicrobial compounds produced by antagonistic microorganisms was shown to be an important mechanism of DAPG tolerance in several *F. oxysporum* isolates (Schouten and Raaijmakers 2004). DAPG tolerance was correlated with the ability of the *F. oxysporum* isolates to convert this antimicrobial metabolite, via deacetylation, into the less toxic derivatives monoacetylphloroglucinol and phloroglucinol (Schouten and Raaijmakers 2004).

Among the non-degradative resistance mechanisms, membrane-bound efflux transporters not only enables target pathogens to resist exogenous toxic compounds, but also play an important role in preventing self-intoxication in antimicrobial metabolite-producing microorganisms (De Waard 1997; Stergiopoulos et al. 2002). Schoonbeek et al. (2002) demonstrated that in *B. cinerea* the efflux pump BcAtrB (*Botrytis cinerea* ABC transporter B) plays an important role in defense against phenazines produced by antagonistic *Pseudomonas* strains: several phenazines induced expression of *BcatrB* in a dose-dependent manner and *BcatrB* replacement mutants were more sensitive to phenazines and phenazine-producing *Pseudomonas* strains than their parental strain. BcATRB also confers increased tolerance of *B. cinerea* to the phytoalexin resveratrol and the phenylpyrrole fungicides fenpiclonil and fludioxinil (Schoonbeek et al. 2001). Recent studies by Schouten et al. (2008) showed that DAPG also induces expression of *BcatrB* and that *BcatrB* replacement mutants are more sensitive to DAPG. Collectively, these results indicate that plant pathogens harbor efflux transporters that confer resistance to multiple and structurally different antimicrobials produced by antagonistic microorganisms.

Interference with the biosynthesis of antimicrobial compounds in rhizosphere microorganisms

The first example of interference of a soil-borne pathogenic fungus with the biosynthesis of an antimicrobial compound in a beneficial bacterium was described by Duffy and Défago (1997). In their study, DAPG biosynthesis in fluorescent *Pseudomonas* strain CHA0 was repressed by *F. oxysporum* f.sp. *radicis-lycopersici*. Fusaric acid produced by *Fusarium* was shown to be the fungal metabolite that specifically repressed DAPG biosynthesis (Duffy and Défago 1997; Notz et al. 2002): blocking fusaric acid production in *Fusarium* by addition of zinc relieved repression of the *phlA* gene and improved the activity of strain CHA0. Subsequent studies showed that among a collection of genotypically different DAPG-producing *Pseudomonas* strains, several were relatively insensitive to fusaric acid-mediated repression of DAPG biosynthesis (Duffy et al. 2004).

Another example of pathogen-antagonist signalling was described for the interaction between mycotoxigenic *Fusarium* and mycoparasitic *Trichoderma* (Lutz et al. 2003). Their study showed that the mycotoxin deoxynivalenol produced by *Fusarium culmorum* and *Fusarium graminearum* acts as a negative signal repressing the expression of the *nag1* chitinase gene in *Trichoderma atroviridae*. Repression appeared to be specific for *nag1* since no adverse effect was observed on the expression of *ech42*, another important chitinase gene in *T. atroviridae* (Lutz et al. 2003).

For many antagonistic bacteria living in the rhizosphere, expression of a range of genes is regulated by autoinducers, such as *N*-acylhomoserine lactones (AHLs), which act as intercellular signals (reviewed in Somers et al. 2004; Zhang and Dong 2004). This phenomenon of cell to cell communication, also referred to as quorum sensing, drives the expression of several beneficial traits in rhizosphere bacteria and of a range of virulence traits in human and plant pathogenic bacteria (Pierson et al. 1998; Zhang and Dong 2004). The ability of antagonists to interfere with quorum sensing in plant pathogens provides a means to control plant diseases and to promote plant health. Conversely, soilborne plant

pathogens can utilize similar strategies to interfere with quorum-regulated antibiotic biosynthesis as a defense strategy against microbial antagonism. Several strategies of quorum sensing inhibition, also refered to as quorum quenching, have been unraveled in the past decade and include repression or blockage of the production of signal molecules, inactivation of the signal molecules or interference with signal perception (reviewed in Zhang and Dong 2004; Rasmussen and Givskov 2006). To date, two types of enzymes that inactivate AHLs have been identified in a range of bacterial species and genera; these include the AHL-lactonases that hydrolyse the lactone ring to yield acyl homoserines with reduced biological activity, and the AHL-acylases that break the amide linkage of AHLs resulting in homoserine lactone and fatty acids, which do not exhibit biological activity (reviewed in Zhang and Dong 2004; Uroz et al. 2007). Work by Molina et al. (2003) elegantly demonstrated that lactonolysis of AHLs by the soil bacterium *Bacillus* sp. A24 or by the rhizosphere isolate *P. fluorescens* P3 modified with the lactonase gene *aiiA*, significantly reduced potato soft rot caused by *Pectobacterium carotovorum* and crown gall of tomato caused by *A. tumefaciens*. Other studies, including those by Uroz et al. (2003) and Jafra et al. (2006), have shown that various other bacterial species are able to degrade AHLs.

Finally, plant pathogens can also fight back without targeting pathways involved in the biosynthesis of specific antimicrobial compounds in biocontrol microorganisms. However, these mechanisms are comparatively much less documented. In the case of *P. fluorescens* strain F113, genes necessary for competitive colonisation of sugar beet roots are downregulated by signal(s) released by the oomycete pathogen *Pythium ultimum*, and thus strain F113 does not reach population densities in the rhizosphere high enough for effective biocontrol of *P. ultimum* (Fedi et al. 1997). Some of the genes targeted in *P. fluorescens* F113 include rRNA genes (Smith et al. 1999), which play a key role in cell physiology during growth.

Influence of agricultural practices on pathogen and antagonist populations

Management of the biotic and abiotic properties of a soil is an important approach to promote the activities of beneficial microorganisms in the rhizosphere and thus limiting the densities and activities of soilborne pathogens to a tolerable level (Janvier et al. 2007). Adaptation of cultural practices has been proposed as a means to decrease the soil inoculum potential or increase the level of suppressiveness to diseases (Steinberg et al. 2007). Indeed, disease suppressiveness has been obtained through crop rotation (Cook et al. 2002), intercropping (Schneider et al. 2003), residue destruction (Baird et al. 2003), organic amendments (Tilston et al. 2002), tillage management practices (Sturz et al. 1997; Pankhurst et al. 2002) and a combination of those regimes (Hagn et al. 2003; Garbeva et al. 2004). Forty years ago, the use of heat-treatment of soils by steaming was a common practice in intensive vegetable cultivation in greenhouses. Most of the pathogens are highly susceptible to heat, the lethal temperatures for pathogenic fungi being reached at 55–65°C for 15 to 30 min (Bollen 1969). With the oil crisis, soil steaming became too expensive and the growers moved to application of chemical biocides which are hazardous for man and environment. These biocides kill not only the pathogens but also most of the beneficial microorganisms, leading to an unbalanced equilibrium in soil and rhizosphere environments. Many of these biocides, except methyl bromide, are still in use and produce ephemeral results including uncontrolled side effects on both existing and forthcoming microbial communities, leading to the infernal circle of applying repeatedly the same treatments. Fortunately, less drastic techniques of pathogen eradication have been proposed that do not kill every soil microorganism, but instead modify the microbial balance in a positive direction for pathogen control and stimulation of plant growth and health (Mazzola 2004).

Solarisation

Solarisation or solar heating is a method that uses the solar energy to enhance the soil temperature to levels at which many plant pathogens will be killed or sufficiently weakened to obtain significant control of the diseases. Solarisation does not destroy all soil microorganisms, but modifies the microbial balance in favour of the beneficial microorganisms. Many studies report that the efficacy of soil solarisation is not only due to a decrease of pathogen populations, but also to an increase of the density and activity of populations of

antagonistic microorganisms such as *Bacillus* spp., *Pseudomonas* spp. and *Talaromyces flavus*. Several review papers are available that describe both the technology of solar heating and mechanisms involved in the control of pests, pathogens and weeds by solarisation (DeVay 1995; Katan 1996).

Solarisation is a hydrothermal process; its effectiveness is not only related to the temperature but also to the soil moisture. Temperature maxima are obtained when the soil water content is about 70% of the field capacity in the upper layers and the soil should be moist to a depth of 60 cm. The duration of solarisation is an important factor determining the effectiveness of the treatment. The longer the mulching period, the greater the depth of effective activity and the higher the pathogen killing rates are. In Mediterranean areas, four weeks are usually required to achieve disease control. An important characteristic of soil solarisation is its broad spectrum of activity, including activity against fungi, nematodes, bacteria, weeds, arthropod pests and some unidentified agents. It should be noted, however, that not all of the pathogens present the same susceptibility to solar heating and that failures have been reported. Solarisation often results in increased yield when applied to monoculture soils where specific pathogens have not been identified. In this case, solarisation probably controls the weak pathogens or deleterious microorganisms responsible for "soil sickness". Another interesting property of solarisation is its long-term effect. Disease control and yield increase have been reported two and sometimes three years after solarisation (Gallo et al. 2007). This long term effect is probably due to both the reduction of the inoculum density and some induced level of disease suppressiveness of the soil. The efficiency of the process can be improved by combining soil solarization and organic amendments, leading to an accumulation of ammonium/ammonia in the soil which reduces the inoculum densities and may weaken the remaining inoculum, including nematodes (Ndiaye et al. 2007; Oka et al. 2007). Obviously, solarisation is effective in warm and sunny areas in the world and, in Europe, adopted in the Mediterranean area (Katan 1996).

Biofumigation or biodisinfection

A strategy better adapted to the cooler regions of the world is biological soil disinfection, which is based on plastic mulching of the soil after incorporation of fresh organic matter (Blok et al. 2000). The mechanisms involved are not fully understood yet, but two main mechanisms have been proposed to contribute to the efficacy of biological soil disinfection: the fermentation of organic matter under plastic results in (a) anaerobic conditions in soil and (b) in the production of toxic metabolites, and both processes contribute to the inactivation or destruction of pathogenic fungi. Based on the type of mechanisms involved, two definitions have been proposed by Lamers et al. (2004): biofumigation corresponds to the use of specific plant species containing identified toxic molecules, whereas biodisinfection refers to the use of high quantities of organic matter which, after soil tarping, result in anaerobic conditions mainly responsible for the destruction of pathogens.

Many species of *Brassicaceae* (*Cruciferae*) produce glucosinolates, a class of organic molecules that may represent a source of allelopathic control of various soilborne plant pathogens (Kirkegaard and Sarwar 1998). Toxicity is not attributed to glucosinolates but to products such as isothiocyanates, organic cyanides or ionic thiocyanates resulting from their enzymatic degradations achieved by a group of enzymes called myrosinases. Myrosinase and glucosinolates are separated from each other in intact plant tissues. When the *Brassicaceae* (cabbage, mustard, horseradish) are grown as an intermediate crop and subsequently buried into soil as green manure, the disruption of cellular tissues allows mixing of glucosinolates and myrosinases resulting in the rapid release of glucosinolate degradation products. The hydrolysis products have a broad biocidal activity towards nematodes, insects and fungi as well as putative phytotoxic effects. They act either as selective fungicides or as fungistatic compounds thereby limiting the development and activity of fungal populations, some of them being pathogenic on the forthcoming crop (Sarwar et al. 1998). Also other plant families, including the *Alliacae*, release toxic compounds. Degradation of garlic, onion, and leek tissues releases sulfurous volatiles such as thiosulfinates and zwiebelanes which are converted into disulfides that have biocidal activities against fungi, nematodes and arthropods (Arnault et al. 2004).

However, not all pathogens are equally susceptible to volatile compounds. For example, soil amendment

with *Brassica napus* seed meal controlled root infection by *Rhizoctonia* spp. and the nematode *Pratylenchus penetrans*, but did not consistently suppress soil populations of *Pythium* spp. and control apple root infection (Mazzola et al. 2001). Mazzola (2007) suggested that the role of the isothiocyanates could be mediated by select groups of indigenous populations of microorganisms whose presence and sufficient population density are necessary to achieve disease control. A plant systemic protection was proposed to explain, at least in part, the positive relation observed between the increase in population densities and activity of *Streptomyces* spp. and disease control obtained in a soil amended with *B. napus* seed meal (Cohen and Mazzola 2006).

Crop rotation versus mono-cropping

In general, continuous cropping with a susceptible host causes the build-up of populations of specific plant pathogens resulting in increases in disease incidence and/or severity. In contrast, rotation with non-host plants or plants that are less susceptible to the pathogen will limit the build-up of pathogen populations, and in some cases may even lead to a decrease of the pathogen inoculum density. Some non-host plants are able to trigger the germination of pathogen survival structures (sclerotia, chlamydospores, oospores) and in the absence of a susceptible host, some pathogens are not able to survive saprophytically in soil. Therefore, cropping of a non-host plant will result in a decrease of the inoculum potential of the soil. Moreover, crops in a rotation scheme may also stimulate antagonistic microbial populations that adversely affect the growth or activity of the pathogens. For example, Mazzola (1999) showed that growing wheat in orchard soil prior to planting apple seedlings significantly reduced infection by a complex of pathogens including *Cylindrocarpon destructans*, *Phytophthora* and *Pythium* spp., and *R. solani*. This beneficial effect correlated with an increased population of specific antagonistic populations of fluorescent pseudomonads making the soil more suppressive towards *R. solani*.

The case of take-all decline of wheat, however, illustrates that longterm monocropping may also be beneficial to plant health. In this case, monoculture of wheat or barley results first in an increase of take-all disease which in turn stimulates antagonists of the take-all pathogen. Therefore, take-all disease of wheat can be naturally controlled by monocropping wheat or barley provided that monoculture lasts for more than 4 years (Dulout et al. 1997; Weller et al. 2002). Take-all suppressiveness was related to the development of populations of DAPG-producing fluorescent pseudomonads in the rhizosphere which adversely affect the growth and activity of the take-all pathogen (Raaijmakers and Weller 1998; Weller et al. 2002). It should be noted that the best yields following take-all decline are rarely equal to those achieved with crop rotation. Nevertheless, in several countries wheat monoculture is a common practise and preferred by growers (Cook 2003).

Residue management

Plant residues left on or near the soil surface may contribute to an increase of disease suppressiveness through the promotion of the general microbial activity. In some cases, however, the debris not only promotes the microbial activity but also helps to preserve the pathogens, preventing a decrease of the inoculum density. This is the case for *Macrophomina phaseolina* causing charcoal rot in soybean (Baird et al. 2003), *Fusarium* sp. causing root and crown rot on maize (Cotten and Munkvold 1998), and *R. solani* causing crown and root rot on sugar beet (Guillemaut 2003). Some practices used by growers to kill living plants at crop termination (e.g. foliar application of herbicide and mechanical destruction of the vines) could be counterproductive with respect to disease management. Indeed, such strategies might enhance the fungal reproduction and increase the soil inoculum as it was shown in the case of the root-infecting fungus *Monosporascus cannonballus* causing vine decline of melons. In such cases, destruction of infected roots prior to pathogen reproduction would be a method of preventing inoculum build-up in soil (Stanghellini et al. 2004b). Therefore, attention should be paid to residue management by burial through tillage practices or promotion of rapid decomposition (Toresani et al. 1998). When residues are buried, the pathogens are displaced from their niche to deeper layers in the soil and their ability to survive is severely decreased. Repeated incorpora-

tions of crop residues can affect a change in the activity of residue-borne microorganisms that in turn influence the decomposition of crop residues. Carbon released from this decomposition contributes to an increase of soil microbial activity and thereby enhances the level of general suppression. Developing disease suppressive soils by introducing organic amendments and crop residue management takes time, but the benefits accumulate across successive years leading to an improvement of soil health and structure (Bailey and Lazarovits 2003).

Soil tillage

It is difficult to assess the role of tillage on disease suppression as its evaluation is often combined with the effects of other agricultural practices such as organic amendments and green manure burial, residue management or crop rotations (Bailey and Lazarovits 2003). Therefore tillage appears as giving conflicting effects on disease suppression. Conventional tillage results in considerable disturbance of the soil but removes residue from the surface. Tillage also disrupts hyphae thereby affecting the ability of fungi such as *R. solani* to survive (Roget et al. 1996; Bailey and Lazarovits 2003). Reduced tillage can also favour pathogens by protecting the pathogen's refuge in the residue from microbial degradation, lowering soil temperature, increasing soil moisture, and leaving soil undisturbed (Bockus and Shroyer 1998). The impact of tillage practices depends on specific pathogen–soil–crop–environment interactions, with the environment being sometimes the most important factor limiting disease severity regardless of tillage or crop rotation practices (Bailey et al. 2000).

Organic amendments

Some years ago organic amendments were proposed to control soilborne diseases (Lumsden et al. 1983). Although their effects were not studied in relation to the induction of suppressiveness in soil, many papers reported that organic amendment can reduce disease incidence or severity. Hoitink (1980) developed a growth medium based on composted bark to grow rhododendron and azaleas. This substrate is suppressive towards root rots induced by several species of *Pythium* and *Phytophthora*. After heating, the compost can be colonized by a great diversity of microorganisms some being antagonistic to the pathogens. The level of disease control obtained depends on many factors such as the chemical properties of the parent material, the composting process and obviously the type of microorganisms present. This is probably why such contrasted data have been published regarding the efficacy of disease control obtained by organic amendments of soil (Termorshuizen et al. 2006). To enhance the suppressive potential of composts and thus to improve the efficacy of disease control, it has been proposed to inoculate these composts after peak heating with specific strains of antagonistic microorganisms. Although promising, this strategy has not yet been successfully applied. Composts can also mimic a non-host plant: an interesting example is provided by the incorporation of onion wastes into the soil to control Allium white rot due to *Sclerotium cepivorum*. This fungus is an obligatory parasite which can survive as dormant sclerotia in the soil for many years but can only germinate in the presence of the host plants. The stimulus for germination is the exudation of alk(en)yl cysteine sulphoxides by the roots of *Allium* species. Properly composted, onion wastes contained some sulphoxides (di-*n*-propyl disulphide) which trigger the dormant sclerotia to germinate in absence of the root. These germinated sclerotia are unable to survive without the living host, which contributes to the decrease in the primary inoculum faced by the next onion crop (Coventry et al. 2002).

To date, compost amendment has been successfully used to increase soil suppressiveness to diseases in agricultural crops, including nematode diseases (Erhart et al. 1999; Lumsden et al. 1983; Oyarzun et al. 1998; Serra-Wittling et al. 1996; Steinberg et al. 2004; Widmer et al. 2002), as well as disease suppression in horticultural crops (Cotxarrera et al. 2002; Hoitink and Boehm 1999). The mechanisms involved, however, are not fully understood yet and subject of ongoing studies.

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