Suppression of soilborne plant pathogens following organic amendments and soil solarization

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by

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ABSTRACT

Incorporation of the appropriate plant debris in the soil, with or without soil solarization, can be used for soil disinfestation. During the decomposition of organic material, additional chemical and biological processes take place, which in turn, may result in the evolution of "soil suppressiveness", *i.e.* low disease incidence and severity, in the presence of a potent pathogen and a susceptible host. We demonstrated the evolution of soil suppressiveness against *Fusarium oxysporum* f. sp. *radicis-cucumerinum* (FORC) following its amendment with dried leaves and stems of wild rocket (*Diplotaxis tenuifolia* [WR]). Disease incidence and severity of crown and root rot in cucumber plants, inoculated with FORC macroconidia, were reduced by 20 to 80% when seedlings were planted in the tested soils 2 to 34 months after soil amendment. Dried foliar debris of WR, *Artemisia dracunculus* (tarragon), *Salvia officinalis* (sage), and *Brassica oleracea* var. *italica* (broccoli) were the most effective at inducing soil suppressiveness, among the tested amendments. Effective soil suppressiveness was extended in WR amended soil, even after three repeated inoculations and plantings of cucumber seedlings in the same soil without additional soil treatment in-between inoculations-planting cycles. In contrast, residues of *Rosmarinus officinalis* (rosemary), *Coriandrum sativum* (coriander), *Mentha piperita* (peppermint), and *B. oleraceae* var. *botrytis* (cauliflower) induced soil suppressiveness only at the first inoculated planting, but not upon repeated inoculation and planting. Soil suppressiveness is not soil specific; soil amendments with WR induced soil suppressiveness in additional two tested soils, differing in their physical and chemical properties. Soil suppressiveness to FORC was also observed when cucumber seeds were sown in soils which were initially amended with WR residues and later infested with FORC chlamydospores Soil solarization also contributes to the evolution of soil suppressiveness, however it was inconsistent.

The above described soil suppressiveness is not pathogen-specific. We demonstrated that amending soil with WR, tarragon, peppermint or sage, with or without solarization, induced soil suppressiveness to the root knot nematode *Meloidogyne javanica* which was introduced into the soil after treatment, and reduced galling index in subsequently grown tomato, basil or snapdragon plants.

The potential of plant residues and soil solarization to induce soil suppressiveness against *M. javanica* was assessed under commercial farm conditions, in which WR was cropped in the tested soils and was used as organic amendment. Three field experiments were established in protected structures (plastic and net-houses) infested with *M. javanica*. Dried residues of WR were incorporated into the soil and then it was either mulched under shade, or solarized at moderate temperatures to only partially reduce the pathogen population. Root galling was significantly reduced on tomato roots in the following crop by WR, solarization or their combination, in two of the three experiments. Solarization, alone or combined with WR amendment, significantly reduced root knot incidence in the third experiment, but suppression of root galling in the roots of snapdragon in the following crop was not observed.

The mechanisms that are possibly involved in disease suppressiveness were studied using Fusarium disease in cucumber. We exposed nonamended soil to the volatile organic compounds (VOC) which were generated from decomposing WR in the soil. Such exposure to VOC induced soil suppressiveness to Fusarium disease in the nonamended soil. Similarly, adding small volume (5% w/w) of crude aqueous soil extract from suppressive-soil, induced suppressiveness to Fusarium disease when cucumber seedlings were transplanted into nonsuppressive soil which was amended with the soil extract. Soil exposure to VOC, as well as to aqueous-extract, which contains soluble, and suspense soil components, and concentrated microbial cells, indicates that the microbial community is involved.

We tested the relationship between soil suppressiveness and increased plant resistance to root and shoot pathogens. This was assessed by growing cucumber plants in suppressive soil and inoculating with foliar pathogens, or transplanting into nonsuppressive, FORC-infested soil. We did not find evidence for induced plant resistance against FORC or *Botrytis cinerea* in infected cucumber seedlings.

Soil suppressiveness as pathogen suppressiveness, i.e. a direct reduction in organism viability was assessed. This aspect was tested by exposure of FORC population to different soil components with or without the effect of the cucumber-host (roots activity/residues). Suppressive soils did not show any effect on the germination of macroconidia, mycelium growth nor on the production of new chlamydospores of FORC. Therefore, pathogen suppressiveness in the WR-amended soil could not be regarded a major factor in the suppressiveness mechanism.

We hypothesized that the impact of soil suppressiveness on root infection by FORC and on disease development, first occurs shortly after the penetration of FORC to the roots. This hypothesis was based on the reduction in disease onset and expression of symptoms which was noticeable already after 6 days from plant-inoculation. Therefore, we studied the microbial events in the roots during the first 6 days after inoculation. Quantitative assessment, of FORC in the cucumber roots, by real time PCR, by root maceration and plating technique indicated that the number of the initial infection units of the pathogen was not affected by the suppressive soil 3 days after seedling inoculation and transplanting. However, the establishment of the pathogen population in the roots of plants in control (nonamended) soil was three times higher within the next 3 days, compared with the suppressive (WR-amended) soils. The ratio of 66% decrease in FORC-root colonization in suppressive soil at day 6 was well correlated with the reduction in wilted plants in the suppressive soil (by 60%), 21 days from inoculation and planting in this soil. These findings suggest that disease suppressiveness occurs at the root zone, with the presence of both the pathogen the host, under suppressive conditions. Apparently, the disease suppression does not involve immediate pathogen reduction or its ability to colonize the roots.

Composition of Fungal community on cucumber roots was assessed using mass sequencing of fungal ITS. Sequences related to *Fusarium oxysporum*, *Fusarium* sp. 14005, *Chaetomium* sp. 15003, and unclassified Ascomycota comprised 96% of the total fungal sequences in all samples. The relative abundances of these major groups were highly affected by root inoculation with FORC, with a 10-fold increase in *F. oxysporum* sequences, but were not affected by the WR amendment. The dominance of saprophytic, nonpathogenic-*Fusarium* spp., did not, however, interfere with soil or root receptivity to FORC as expressed in a pronounced colonization of the roots by FORC and disease severity of plants in the nonamended soil. Hence, other mechanisms than protective *Fusarium*, play a more important role in disease suppressiveness in WR-amended soil.

Quantitative analysis and mass sequencing methods indicated a qualitative shift in the root's bacterial community composition in suppressive soil, rather than a change in bacterial numbers. The effect of FORC infection on root-bacterial community was less pronounced. Increased diversity of bacterial community characterized roots from suppressive soils, but also responded FORC inoculation, and

already before disease symptoms (3 days). Therefore, increase in diversity in root communities is in itself not necessarily an indicative of a suppressive process.

A sharp reduction in the size and root dominance of the *Massilia* population occurs in suppressive soil. These bacteria exhibited exceptional dominance in cucumber root-associated communities at early stages of plant development and are characterized as sensitive to increased microbial competition. The sharp reduction in *Massilia* numbers was accompanied by a significant increase in the relative abundance of specific populations, namely, *Rhizobium, Bacillus, Paenibacillus* and *Streptomyces*, which are frequently linked to biological control and disease suppression. Among those, *Streptomyces* was prominent and appeared to be an important component in the disease suppression process in the cucumber-FORC system. Composition of the *Streptomyces* community shifted significantly, as determined by PCR-DGGE, resulting in an increase in the dominance of a specific population in suppressive soils after only 3 days, and regardless FORC inoculation. This shift was related mainly to the increase in *S. humidus*, a group previously described as antagonistic towards phytopathogenic fungi.

We hypothesize that several mechanisms are inter-associated in soil suppressiveness following WR-amendment, including increased general competition for nutrients, and specific antagonism which take place at the root zone, following the pathogenic infestation.

Our study suggests that the generation of an appropriate soil environment with organic amendments results in a shift in bacterial communities. These, in turn, trigger disease suppressiveness to root diseases at the root zone, at early stages of root infection. These findings further validate the potential role of OA, and in certain cases solarization, in inducing soil suppressiveness, which contributes to sustainable management of soilborne pathogens.

CONTENTS

INTRODUCTION

1. Soilborne pathogens

Soil-borne plant pathogens (SBPP) belong to several different phyla: viruses, bacteria, fungi and nematodes. They usually survive in soil for extended periods. SBPP can survive actively on volunteer hosts, or as saprophytes on plant residues and organic material, or in resting structure forms until triggered for germination (Bruehl, 1987). The damage to plant from soil-borne pathogens includes stunting or seedling damping-off, root rot, and wilt, (Haas and Défago, 2005). The genera *Fusarium* and *Verticillium* constitute the greatest number of fungal wilt diseases in higher plants among all SBPP (Green, 1981).

1.1. Fusarium

Fusarium is classified in the class Hyphomycetes which belongs to the Deuteromycotina. Teleomorphs have been placed in the genera Nectria and Gibberella, order Hypocreales (Ascomycetes). The taxonomy of the genus *Fusarium* is not settled and the number of species and sections varies. A few recent classification systems of this genus exist. *Fusarium* spp. has mostly been studied in the context of their ability to cause diseases of many economically important crop plants. Some *Fusarium* species produce mycotoxins and other secondary metabolites which are harmful to humans and livestock (Zemankova and Lebeda, 2001).

F. oxysporum Schlechtend.: Fr. covers more than 120 *formae speciales*, which have been described based on specificity to host species in diverse plant families (Katan, 2012). *F. oxysporum* produces asexual spores, either micro or macro-conidia, which are intercalary or terminally produced within the hyphae. The fungus produces resting structures, (chlamydospores), which are thick-walled (Nelson, 1981). *F. oxysporum* survives in the soil during extended periods of time in the absence of the host. After germination (in the presence of a host) the fungus penetrates the host roots, and enters the plant vascular system. The xylem serves the fungus to rapidly colonize the host, thereby provoking the characteristic wilt symptoms (Beckman, 1987). Successful infection requires a number of processes such as early plant-host signaling,

root surface attachment, degradation of physical host barriers, resistance to host antifungal compounds and production of phytotoxins (Roncero et al., 2003).

F. oxysporum f. sp. *radicis*- is a relatively new formae which, in addition to wilt symptoms, causes also a severe root and hypocotyl rot of cucumber, accompanied by massive conidia formation on the exterior of the stem surface. These conidia can infect other plant and contaminate new areas (Katan, 2012). Hence, this formae can be regarded as soilborne as well as airborne pathogen. During the past 30 years few subspecies of *F. oxysporum* which belong to this new group were identified and reported; among those, *F. oxysporum* f. sp. *radicis-cucumerinum* Vakal. (FORC) (Vakalounakis, 1996), *F. oxysporum* f. sp. *radicis-lycopercisi* (FORL) and *F. oxysporum* f. sp. *basilici* (Rekah et al., 2000).

FORC causes a severe root and hypocotyl rot of cucumber, accompanied by massive conidia formation on the exterior of the stem surface. FORC has an optimum temperature for disease development of 17-20 °C. (Vakalounakis, 1996; Katan, 2012).

1.2 Root knot nematodes

Nematodes are threadlike, round worms. They are ubiquitous in freshwater, marine, and terrestrial environments and include plants and animals pathogens (Laughlin, 1971). Among plant pathogens, root-knot nematodes of the genus *Meloidogyne* are the major genus affecting plant development and yield in a wide spectrum of agricultural crops, with broad geographical distribution and under a variety of climatic conditions (Sasser, 1980). *M. javanica* (Treub.) Chitwood is the predominant representative of this group in Israel, causing substantial agricultural damage in important vegetable and floriculture crops.

The occurrence and severity of *M. javanica* has increased over the last 15 years in Israel and elsewhere, following the phase-out of methyl bromide, which was the major soil fumigant, which provided highly effective control. Soil fumigants (e.g. 1,3 dichloropropene) are the main effective measure for reducing nematode populations in the treated soil layer (Duniway, 2002; Martin, 2003). Soil solarization is effective at controlling root-knot nematodes in the upper 20 cm of the soil, where maximal temperature exceeds 40°C (Madulu and Trudgill, 1994). However, nematodes which survive in the deeper soil layers, where lower temperatures prevail, can migrate upward and re-infest the disinfested soil layer (Ogbuji, 1981). Therefore,

effective and sustainable methods of controlling nematodes, especially those that also preserve the natural soil suppressiveness, are needed. Resistant cultivars of certain crops are commercially available and depend on a single gene. However, this genetic source is adversely affected at higher soil temperature (Devran et al., 2010; Omwega et al., 1990).

2. Management of soilborne pathogens

Management of plant diseases involves the integration of four basic control approaches (Agrios, 2005; Howard, 1996; Jacobsen, 2007), namely, exclusion or avoidance (Janse and Wenneker, 2002; Waterworth and White, 1982), inoculum reduction (Duniway, 2002; Katan, 2000; Melakeberhan et al., 2006), protection (Bradley, 2008; Wilson et. al., 1999; Walters et al., 2005), and using host genetic resistance, directly (Dervan et al., 2010), in combination with other control tools Punja, 2004), or through grafting of sensitive plant onto resistant root stock (Cohen et al., 2000).

2.1 Soil disinfestation

Soil disinfestation (SD) is the major approach to controlling diseases caused by SBPP, as well as other soilborne pests, and is especially common with high-value crops (Katan, 1999). The basic principle of SD is to knock-down a wide spectrum of harmful agents in the soil before planting, usually by highly-effective chemical or physical means while attempting to minimize the damage to beneficial microorganisms, as well as to abiotic components of the soil. During 1960-2000, methyl bromide was used as the major soil fumigant due to its versatility and effectiveness against a broad spectrum of soil pests. However, its use was banned recently due to harmful effect on the ozone layer (Pizano et al., 2010). To date, alternative soil fumigants are used, such as 1,3-dichloropropene, chloropicrin, formalin and methyl isothiocyanate generators such as metam sodium. These are likely to be used in combinations, and may also be supplemented with other more specific pesticides and other biological and cultural controls. However, an increased awareness of the toxicology of soil fumigants is likely to lead to further restrictions on the use of all of the chemical soil fumigants (Duniway, 2002). Also, the global trend of reducing pesticides residues in the environment, especially in agricultural products

led to the raising in the use of non-chemical approaches as well as cultural approaches which included in pest management programs.

2.2 Non chemical soil disinfestation

Management of SBPP by non-chemical means include a variety of tools, e.g., cultural methods such as mineral fertilization, irrigation and tillage (Katan, 2000), as well as physical methods which are based on the heating of the soil to a temperature that will effectively control existing soilborne pests (Runia, 2000) or biological methods (Baker and cook, 1983). We shall concentrate on soil solarization and organic amendments which are relevant to our current research.

2.2.1. Soil solarization

Soil solarization for the control of soilborne plant pests (pathogens, weeds, and arthropods) involves physical means – solar energy which heat the soil through repeated daily cycles. The soil is wetted and tarped with transparent polyethylene sheets when climatic conditions are most favorable, e. g. high levels of solar radiation and temperatures. Pathogen control achieved either directly through physico-thermal killing, or indirectly by the stimulation of antagonistic activity (Katan and DeVay, 1991) or by weakening of the pathogen resting structures in soil, thus exposing them to antagonistic microorganisms (Freeman and Katan, 1988). The antagonistic populations can also suppress reinfestation of the soil by soilborne pathogens (Freeman et al., 1990). Additional positive side effect of soil solarization is increased growth response, which is expressed in certain crops, due to increases in soluble mineral nutrients, and promoting beneficial microorganisms (Chen et al., 1991; Gamliel and Katan, 1991). Pathogen control by soil solarization can be improved by combining with other control means (Eshel et al., 2000).

2.2.2. Organic amendments

Amending the soil with organic amendments (OAs) from various sources, e.g. plant debris, organic waste or compost for the control of soilborne pathogens (Brown and Morra, 1997; Gamliel and Stapleton, 1993b), has expanded significantly during the last 20 years (Bonanomi et al., 2007). Among the organic amendments, crop residues, including crude organic material such as green manure and vegetative plant debris (stem, root, leaves, etc.), can be suitable for the control of soilborne pathogens

(Klein et al., 2007; Klein et al., 2011a; Stapleton et al., 2010). Bonanomi et al. (2007) reviewed the contribution of soil amendment with crop residues and reported variable responses in controlling soilborne pathogens. In 45% of the reviewed articles, significant pathogen control and suppressiveness, mainly of *Fusarium* spp., *Verticillium dahliae*, *Thielaviopsis basicola* and *Phytophthora* spp., were reported. However, in 28% of the reviewed articles, increased disease incidence and conduciveness were reported following OA (Bonanomi et al., 2007). Hence, selecting the appropriate OA is crucial to achieving effective long-term pathogen control and sustainable management of soil quality and health (Abawi and Widmer, 2000).

Plant debris containing glucosinolates, e.g. crucifers, manures rich in nitrogen, and other wastes may generate biologically active products in the soil which control plant pathogens (Brown and Morra, 1997; Gamliel and Stapleton, 1993a; Gamliel and Stapleton, 1993b). For example, soil amendments with cruciferous residues have been extensively studied for their potential as OAs (Brown and Morra, 1997; Gamliel and Stapleton, 1993a; Ramirez-Villapudua and Munnecke, 1988; Wang et al., 2009). Fewer studies, however, can be found on the use of herbs as OAs for the control of soilborne pathogens (Gwinn et al., 2010; Klein et al., 2007; Yulianti et al., 2007). Herb crops involve the production of large quantities of plant debris which are either disposed of during harvesting and packing or left in the field at the end of the season. Many herb plants contain essential oils, including terpenes, phenols, alcohols, organic acids and others, some of which have biocidal activity (Echeverrigaray et al., 2010; Kordali et al., 2005; Marino et al., 1999; Paret et al., 2010; Suhr and Nielsen, 2003). Therefore, herbs have the potential to serve as OAs in soil for the control of soilborne pathogens.

Various biological (Borrero et al., 2004; Cook and Baker, 1983; Hoitink, 1980) or chemical and physical (Boehm et al., 1997; Ghorbani et al., 2008; Lazarovits et al., 2005; Steinberg et al., 2006) mechanisms are potentially involved in the decomposition of OAs, and in the related processes of pathogen control, disease management, and the development of soil suppressiveness to delay reinfestation and disease suppression. In a previous work, we found that solarization of soil amended with herb residues improves the disinfestation efficacy against soilborne pathogens (Klein et al., 2007; Klein et al., 2011a).

The mechanisms by which OAs trigger pathogen control are often biological (Cook and Baker, 1983; Hoitink and Boehm, 1999; Kasuya et al., 2006; Lodha et al.,

1997). In some cases, the efficacy of OAs is not sufficient for soilborne pest control (Blok et al., 2000; Njoroge et al., 2008; Ramirez-Villapudua and Munnecke, 1988). Combining OAs with other control methods, such as soil heating or solarization, has the potential to improve the results (Gamliel and Katan, 2009; Klein et al., 2007).

2.2.3. Solarization of organic-amended soil

Combinations of organic amendments and soil solarization have the potential to improve pathogen control and expand its spectrum of activity (Gamliel and Stapleton, 1993a; Gamliel et al., 2000; Klein et al., 2007 Lodha and Israel, 2005).

It was demonstrated that solar heating of soil amended with cabbage residues eliminates *Fusarium oxysporum* f. sp. *conglutinans* in closed containers under laboratory conditions (Ramirez-Villapudua and Munnecke, 1988). In addition, solarization of soil amended with chicken compost effectively controlled *Meloidogyne incognita* and *Pythium ultimum* (Gamliel and Stapleton, 1993b). The combination of different herb residues and soil solarization improved pathogen control at deep soil layers and in cases when organic amendment alone was not effective (Yulianti et al., 2007; Klein et al., 2011a).

3. Soil suppressiveness to plant diseases

Soil suppressiveness is defined as the capacity of a soil to control disease onset and progress in a susceptible host, even in the presence of a significant inoculum density of the pathogen (Cook and Baker, 1983). In suppressive soils, disease incidence or severity usually remain low, even under environmental conditions that favor disease development (Cook and Baker, 1983). Disease suppressiveness and pathogen suppressiveness are not necessarily the same thing, since reduction in disease incidence and severity is not always the result of a direct effect on the pathogen. Soil suppressiveness, namely, capacity of the soil to suppress reinfestation by a pathogen introduced into the soil after treatment, can evolve following various soil treatments, including incorporation of OAs (Cohen et al., 2005; Goud et al., 2004; Noble and Coventry, 2005; Stone et al., 2004; Yogev et al., 2006), but also by other agrotechnical means such as soil cultivation, monoculture or crop rotation (Cook and Baker, 1983; Pankhurst et al., 2002; Steinberg et al., 2006). Long-term soil suppressiveness against certain pathogens has been reported to evolve in some cases

following soil solarization (Gamliel and Katan, 1993; Gamliel and Katan, 2009; Greenberger et al., 1987; Martyn and Hartz, 1986). In contrast to the many studies which have demonstrated disease control by OAs combined with soil solarization (Gamliel et al., 2000; Klein et al., 2011a; Ramirez-Villapudua and Munnecke, 1988), only a few studies have dealt with their effects on soil suppressiveness.

The mechanism of pathogen control in soil following amendment with certain OAs and the evolution of soil suppressiveness might be related. OA decomposition in the soil induces shifts in chemical and physical conditions and in soil microbial populations and activities [See 2.2.2.]. The new microbial balance might be involved in the suppression of pathogen reinfestation and delay of disease onset in future cropping.

3.1. Microbial role in soil suppressiveness

Soil conditions such as nutrient supply, microbial community and structural characteristics can influence plant growth and the occurrence and severity of plant diseases (Ghorbani et al., 2008). Natural soil suppressiveness is well documented (Hornby, 1983; Mazzola et al, 2002; Persson et al., 1999), and the role of microbial populations in the process was demonstrated in many cases (Mazzola et al, 2002; Oyarzun et al., 1998). In some cases soil suppressiveness was induced by different soil treatments, including application of biocontrol agents (Stone et al., 2003; Spadaro and Gullino, 2004), organic amendments (Kasuya et al., 2006; Mazzola, 2007) and mild disinfestation methods such as soil solarization (Greenberger et al., 1987). Some of the suppressiveness mechanisms which were documented in previous studies including direct reduction of pathogenic inoculum in the soil (Freeman et al., 1990; Greenberger et al., 1987) and on the root surface (Duijff et al., 1999) or inoculum potential reduction (Knudsen et al., 1999) and increased antagonistic microbial populations (Benítez and Gardener, 2009; Duijff et al., 1999), induced crop resistance (Yogev et al., 2010), and different effects on pathogen-crop interactions (Raaijmakers et al., 2009). Studies of the biological mechanisms of soil suppressiveness include application of means which can positively or adversely affect the suppressiveness activity. Drastic soil disinfestation such as soil sterilization for the elimination of suppressiveness serves in the study of the mechanisms of suppressiveness (Scher and Baker, 1980). However, such treatments, may also cause changes in soil properties, and in turn, may limit the possibilities of recolonization with biological control agents

or microbial population (Thuerig et al., 2009). In contrast, application of means which positively affect the suppressiveness activity can be valuable for understanding the mechanism. Volatile organic compounds originated from plant residues or generated during decomposition of OA in the soil, caused quantitative and qualitative changes in the soil microflora activity and population (Gamliel and Stapleton, 1993a; Gilbert and Griebel, 1969; Kasuya et al., 2006; Klein et al., 2007; Linderman and Gilbert, 1975). Volatile compounds, as well as water soluble compounds and suppressive-soil extracts (Kao and Ko, 1983), can increase microbial competition or antagonism contribution to the eventual suppressiveness of soilborne plant pathogens. Water extracts from suppressive growing media may contain components such as suppressive bacterial populations (Postma et. al., 2005), and elimination of this fraction from the soil, or application to nonsuppressive soil, may be useful in studying the suppressiveness factors.

Natural suppressive soils to Fusarium-wilt are well documented (Steinberg et al., 2006; Weller et al., 2002). These were characterized by competition for niche and nutrients and closely related to saprophytic, non-pathogenic species of *Fusarium* (Weller et al., 2002), and the activity of native protective *Fusarium oxysporum* strains and *Pseudomonas* spp. (Duijff et al., 1999; Alabouvette et al., 2009).

Natural or induced soil suppressiveness to nematodes has been reported in several studies (Davis and Sorensen, 1986; Mcsorley et al., 2008; Olatinwo et al., 2006; Pyrowolakis et al., 2002; Westphal, 2005; Westphal and Xing, 2011). Unfortunately, this evolution of beneficial soil activity is not evident in certain agricultural Israeli soils, probably due to intensive cropping practices which involve frequent application of soil fumigants (Pyrowolakis et al., 2002; Westphal and Becker, 2001; Westphal and Xing, 2011). Achieving a shift in the soil's biological balance might contribute to more successful crop management in nematode-infested soils.

Organic amendments from a wide spectrum sources, including plant residues, are used for the control of root-knot nematodes (Abawi and Widmer, 2000; Chitwood, 2002; Westphal and Becker, 2001), and can intensify biological control of nematodes (Akhtar and Malik, 2000; Pattison et al., 2006; Sasser, 1980). The combination of OA with soil heating or solarization improves nematode control (Gamliel and Stapleton, 1993b; Klein et al., 2011a; Oka et al., 2007; Ploeg and Stapleton, 2001), with induced

soil suppressiveness to nematode reinfestation and a long-term effect being reported in some cases (Gamliel and Stapleton, 1993b; Oka et al., 2007).

4. Ecological microbiology involved in soil suppressiveness

The soil microbiome plays a significant role in natural or induced disease suppression. The possible mechanisms of induced soil suppressiveness include pathogen suppression (Chen et al., 1988; Tuitert et al., 1998), induced systemic resistance within the host (Kloepper et al., 2004; Pharand et al., 2002; Yogev et al., 2010), and microbial interaction which take place in the rhizosphere. The latter involves mechanisms such as competition for nutrient and antibiosis (Mazurier et al., 2009; Raaijmakers et al., 2009). The mechanisms of soil suppressiveness have been extensively studied by classical means such as in culture and by direct root-microbe analysis (Gamliel and Katan 1993; Shiomi et al., 1999).

Molecular methods are effective tools to characterize soil and rhizosphere microbiomes (Borneman and Becker, 2007; Borrero et al., 2004; Manici et al., 2005; Mazzola, 2004; Mendes et al., 2011) for the purpose of identifying predictive microbial parameters for disease suppressiveness and potential antagonists that can be used as biological control agents (Bonanomi et al., 2010; Borrero et al., 2004; Postma et al., 2010; Yin et al., 2003). However, adoption of metagenomic approaches for soil microbial ecology, have revealed the enormous complexity of the microbial interactions in such environments (Bardi et al., 2009; van Elsas et al., 2008). Such a detailed and accurate characterization of the microbial interactions can indicate possible suppressive mechanisms, and assist in identifying factors that enhance them.

Plant host, soil type, and physical and chemical environments are important factors in establishing the platform upon which microbial interactions that will exhibit soil suppressiveness occur (Kinkel et al., 2011). Available information clearly indicates that the microbial mechanisms of soil suppressiveness involve the activity of consortia of diverse microbial populations, rather than one specific organismal population (Mazurier et al., 2009; Mendes et al., 2011). Much effort has been invested in characterizing microorganisms and their genetic elements which are related to disease suppression in the bulk and rhizosphere of suppressive soils (Borrero et al., 2004; Kyselková et al., 2009; Mendes et al., 2011; Postma et al., 2010; van Elsas et al., 2011). In contrast, less attention had been paid to microbial events at the site of

the host-pathogen interaction- the root itself -during different stages of pathogen infection and disease development in disease-suppressive or conducive soils (Chen and Nelson, 2008; Shiomi et al., 1999; Yang et al., 2001). Early findings indicate that organisms are attracted to plant roots at early stages of seed germination and root development (Bais et al., 2006; Chen and Nelson, 2008; Nelson, 2004). Hence, a temporal analysis of the microbial interactions at different stages of pathogen infection is essential for understanding the mechanisms underlying soil suppressive interactions (Chen and Nelson, 2008). Highly sensitive methods for microbial ecology characterization, such as mass sequencing of 16S rRNA gene fragments, can increase the observational resolution of these microbial interactions in a pathosystem.

5. Objectives

The general objective of this work was to corroborate soil suppressiveness induced by soil amendments with residues from herb and other plants, with or without solarization, and to study the induced suppressiveness mechanisms in the Fusarium-cucumber pathosystem, and to evaluate the practical potential of the research under agricultural farm conditions, in root-knot nematode infested plots.

The specific objectives are:

1. To identify the physical, chemical or biological soil components that involve in soil suppressiveness, which was induced by herb-debris amendment, and to test the mechanism that enable disease reduction in pathogen-challenged plants.

2. To examine Fusarium crown and root rot establishment in cucumber roots after infestation and at symptoms appearance, under conducive and suppressive soil conditions, and to characterize the effect of induced-suppressive soil on the microbial colonization of roots, using mass sequencing of 16S rRNA and internal transcribed spacer (ITS) gene fragments, qualitative analyses with polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE), and quantitative examination of the root microbiome.

METHODOLOGY

1. Soil and organic amendments

Soil samples were collected from four agricultural field sites in different locations in Israel. Two types of soil were used: sandy soil from Rehovot, in the center of Israel (94% sand, 2% silt, 4% clay, 0.12% organic matter, pH 7.9, field capacity of 9%, measured at -33 J/kg matric potential); sandy soil from En Tamar, which was collected from the southern desert, in the Arava region (89% sand, 7% silt, 4% clay, 0.1% organic matter, pH 8.1, field capacity of 8%); sandy soil from Besor which was collected from the western Negev region (76% sand, 17% silt, 7% clay, 0.2% organic matter, pH 7.8, field capacity of 10%); loamy soil from Bet Dagan, in the center of Israel (22.5% sand, 25% silt, 52.5% clay, 1.4% organic matter, pH 7.5, field capacity of 20%). These soils represent the range of agricultural soils in Israel (Triky-Dotan et al., 2007). The soils had no history of soil disinfestation or fumigation for at least 5 years prior to sampling. Several samples were collected from the upper layer (5- to 20-cm depth) of the soil. For each site, the soil samples (total of 500 kg) were mixed into one large composite sample, air-dried, and sieved through a 1-mm screen. The soils were stored in plastic containers at room temperature pending use (up to 4 weeks after collection).

Leaves and stem debris of the following plants were tested: *Salvia officinalis* L. (sage), *Rosmarinus officinalis* L. (rosemary), *Coriandrum sativum* L. (coriander), *Diplotaxis tenuifolia* (L.) DC. (wild rocket, WR), *Mentha piperita* L. (peppermint), *Brassica oleracea* L. var. *italica* (broccoli), *B. oleraceae* L. var. *botrytis* (cauliflower) and *Artemisia dracunculus* L. (tarragon). The foliage of these crops was collected from commercial agricultural fields during crop production. The leaves and stems of each crop were separately air-dried at 25°C, then ground and sieved through a 2-mm sieve. The sieved residues of each plant species were used as OAs.

2. Inducing soil suppressiveness by OA and solarization

2.1. Soil amendment. The specified dried and ground crop residues were incorporated into 20 kg of soil sample at a rate of 1 or 2% (w/w), as indicated

(equivalent to a rate of 1 or 2 kg/m^2 in the field, respectively) (Klein et al., 2007). The soil-amendment mixture was wetted to water-holding capacity. Each amended soil mixture was packed in a porous, woven plastic net bag (total 22 kg/bag). Nonamended soil was prepared and packed similarly, and served as the respective control.

2.2. Incorporation of amended soil and soil solarization in small field plots. Small field-plot experiments were conducted during the summers of 2005 through 2009, at the experimental farm of the Hebrew University in Rehovot. In each year, the field was rotovated to 50-cm depth and then irrigated to water-holding capacity down to 50 cm. Plots (2 x 4 m) were outlined and trenches (20 cm deep) were dug in the margins of each plot. Porous plastic bags containing the amended and nonamended wetted soils were buried horizontally and flat in the center of each plot, in a layer of 10-30 cm below the soil surface. The treatments included four combinations of soil amendments and solarization; in each plot, the respective soil mixture was buried. All the plots were mulched with a transparent polyethylene sheet (100-µm thick). Nonsolarized plots were additionally covered with a shading screen (Polysak, Nir Itzhak, Israel, 90% shade) which was laid over the plastic mulch to minimize soil heating and solarization. Each experiment was set up in a randomized block design, with three replicates for each treatment. The solarized plots were exposed to solarization for a period of 28 days during the months of July or August, as indicated. Soil temperatures were recorded at depth of 20 cm, using type-T thermocouples (accuracy ±0.5ºC) connected to a micro-logger (21X, Campbell Scientific Inc., Logan, UT). Soil temperature reached 34 to 48.7ºC at 20 cm in the solarized plots, compared to 32 to 34ºC in the shaded, nonsolarized plots. Upon termination of the solarization period, the plastic sheets were removed from the plots and the soil bags were retrieved with their contents and brought to the laboratory. The soil was left to air-dry at 25ºC for 1 month prior to its use. In certain cases, the soils were stored for extended periods in the shade at room temperature.

3. Plant and soil inoculation and disease suppressiveness assay

3.1. Soil-suppressiveness assay for Fusarium disease in cucumber. *Fusarium oxysporum* (Schlechtend.) f. sp. *radicis cucumerinum* D.J. Vakal. (FORC), the causal agent of cucumber root and crown rot disease, was used as the bioassay organism, for

the suppressiveness studies and for assessment of its possible mechanisms. Infected cucumber plants were collected from a commercial cucumber greenhouse and the stems, containing macroconidia of FORC in their lower section, were used for the suppressiveness tests. Isolates of FORC were taken from each stem and tested for pathogenicity using cucumber seedlings prior to their selection for the suppressiveness assays, to validate their identity as FORC. The macroconidia of this pathogen, as a natural inoculum, were scraped into sterile water and their density was adjusted to the desired concentration with the aid of a hemocytometer. Macroconidia were assessed prior to each assay using a soil-dilution-plating technique (Klein et al., 2007). Suspensions of macroconidia which showed at least 80% germination after 24 h were used for the suppressiveness tests.

The suspensions of FORC-macroconidia were also used to test soil components for their direct effect on the pathogen and its ability to cause a disease.

Soil suppressiveness assays, in which inoculated seedlings were planted in previously treated soils, were conducted in pot experiments essentially as previously reported (Yogev et al., 2006). Pots (0.45 liter, 0.5 kg each) were filled with soil taken from the various afore-described soil-amendment treatments. One day before the experiment, the soil in the pots was irrigated to water-holding capacity. Seeds of cucumber (*Cucumis sativus* L. 'Kfir') were sown in sandy soil. Six-day-old seedlings were removed and their roots were washed, and then dipped for 2 min in a suspension of FORC macroconidia, adjusted to the indicated density. The inoculated seedlings were then transplanted into pots which were filled with soil taken from the various soil-amendment treatments. For each treatment, five pots were planted with seven inoculated seedlings per pot and repeated planting cycles were conducted at least twice. An additional pot was planted with noninoculated seedlings and served as a control. All trials were arranged in the greenhouse in a complete randomized-block design.

Disease symptoms in cucumber seedlings usually appear 7 days after inoculation and are manifested as wilt and plant collapse. Disease progress was expressed as percentage of diseased plants, area under the disease-progress curve (AUDPC) (Campbell and Madden, 1990), and when indicated, percentage of the value of the AUDPC relative to the inoculated control. The noninoculated plants remained healthy in all experiments. A reduction in disease incidence or severity, in amended soil, compared with nonamended soil was attributed to as soil

suppressiveness, since the plants were inoculated and introduced into the soil after the OA and solarization treatments had been terminated (i.e., the pathogen was not exposed to a direct control process). Soil suppressiveness was observed during the first planting cycle of which the pathogen is initially introduced. It was further evident after repeated planting and inoculations which surpass the population of FORC which exit in the soil from the previous planting.

In certain experiments (results are given in Table 1.1; Figs. 1.3 and 1.4), inoculated cucumber seedlings were repeatedly planted in the same pots to examine the long-term effect of the suppressiveness. In these experiments, all plants from the previous assay were removed and the pots were left to dry for a week. The pots were then irrigated to field capacity, followed by a second disease-suppression assay as already described.

In a few specific experiments (results are given in Table 1.4), FORC chlamydospores were used as inoculum. Chlamydospores were produced on peat (Plantobalt, peat moss, degree of decomposition h2 to h5, pH 2.5 to 3.5, water-holding capacity 55 to 75%, Estonia and Latvia) which was steam-sterilized and inoculated with FORC macroconidia suspension. The inoculated peat was incubated in the dark at 25ºC, and viability of FORC propagules was assessed at 30-day intervals for 3 months, until inoculum density stabilized at 4.075 x 10^6 CFU/g peat. Inoculum density and viability were assessed using the soil-dilution-plating technique described for the macroconidia. The infested peat was used as a source of FORC inoculum in the soil by incorporating it with the tested soil at a rate of 1, 2 or 5% (w/w). Eight cucumber seeds were planted into each pot.

3.2. Soil-suppressiveness assay for root-galling by root-knot nematodes. Soil suppressiveness to root knot caused by *Meloidogyne javanica* (Treub.) Chitwood in tomato, basil and snapdragon roots was assessed in a previously treated soils, which were brought in the bags from the field and transferred to pots, using a modification of previously described techniques (Lackey et al., 1994; Orion et al., 2001): the plant seedling being tested was planted in a soil inoculated with a suspension of chopped galled roots containing *M. javanica* eggs in their protective gelatinous matrix. Since the inoculum was introduced into the soil after termination of the treatment, any change in disease level could be attributed to a change in soil suppressiveness. The *M. javanica* inoculum was produced from galled tomato roots, which were grown for 2

months for this purpose in the greenhouse. Fresh galled roots (200 g) were chopped in 200 ml tap water in a blender for 1 min. Then J2 larvae were counted under a stereomicroscope and the suspension was brought to 80 viable juveniles per ml. Each pot was inoculated with 10 ml of suspension (total 800 J2 larvae in 0.5 kg soil per pot). Each treatment consisted of eight replicate pots. Tomato (cv. 187), basil (*Ocimum basilicum* 'Peri') or snapdragon ('Photomek', white) seedlings were planted in these pots, one plant per pot, and grown for 6 weeks. Then the plants were uprooted, washed and rated for galling on a scale of 0 (no galls) to 5 (more than 80% of the root surface galled). Results were expressed as average galling index. Root development was assessed by comparing root volume and branching of inoculated plants with non-inoculated controls, rated on scale of 1 (poorly developed) to 4 (similar to non-inoculated root).

4. Field experiments under agricultural conditions

4.1. Field experiments and soil treatments. Three field experiments were carried out to assess the use of WR residues as OA and soil solarization for the induction of soil suppressiveness against root-knot nematodes under agricultural practices. The experiments were conducted at the Besor experimental station in the western Negev region, in a sandy soil (76% sand, 17% silt, 7% clay, 0.2% organic matter, pH 7.8, field capacity of 10%). Experiments 1 and 2 were initiated in Sep 2006 and conducted through Apr 2008 in two tomato greenhouses (360 m^2 each) naturally infested with *M. javanica*. In experiment 1, the soil in the greenhouse had not been disinfested before the previous tomato crop. In experiment 2, the soil in the greenhouse had been fumigated with methyl bromide before the previous tomato crop (MB-history). Prior to setting up the experiments, both sites were planted with tomato transplants (*Solanum lycopersicum* cv. 3029 'Shanti'), from Sep 2006 to Jan 2007, to build up the nematode population and make its spatial distribution in the field more uniform. The tomato transplants were planted in two rows per plot, 10 plants per row. At the end of crop growth, all the plants were uprooted for galling evaluation. This method is giving an accurate estimation of the severity and the distribution of *Meloidogyne* in a field, and suitable for evaluating nematode potential to cause a disease in sandy soils, in which the migration of juveniles over substantial distances does not allow reproducible determination of population densities (Netscher and Sikora, 1990).

Whereas 98% of the plants in the non-disinfested plots (experiment 1) were highly infected with nematodes (80 to 100% galling), less than 1% of the plants which were grown in the MB-history plots (experiment 2) exhibited galling. At this point, the field in each experiment was demarcated into 16 plots to perform four treatments with four replicates each, in a randomized block design (each plot 3.75 x 6 m). The treatments included a non-treated control, amendment with crop residues of WR with or without soil solarization and soil solarization without WR amendment. All plots which were designated for OA were planted with WR (60 plants per m^2) and grown for 6 months (Feb 2007 to Jul 2007). The crop foliage was cut back monthly, as per common growing practice, and left to dry. The roots of WR plants which were randomly uprooted during cropping season were not galled. Six months later, all the plants were uprooted, chopped, left to dry in the amended plots and then the entire biomass (0.4 kg/m^2) was incorporated by rototilling down to 25 cm depth.

Experiment 3 was carried out during the spring and summer of 2007 in a snapdragon (*Antirrhinum majus* L.) nethouse (720 m^2) which was highly infested with *M. javanica* (as assessed in the previous crop). The field was demarcated and treated essentially as with experiments 1 and 2 (plot size was 1.5 x 7 m), namely, four treatments with four replicates. The designated plots were planted with WR and the non-amended control was left fallow. Four months later, the plants were uprooted, chopped, left to dry in the amended plots, and then the entire biomass (0.9 kg/m^2) was incorporated down to 25 cm depth. The net was removed from the structure to maintain optimal solarization conditions during the process.

After WR amendment, the soil in each of the three experiments was sprinkler irrigated to water-holding capacity down to 50 cm prior to mulching. The solarized plots were exposed to solarization for a period of 40 days during the months of August and September in the greenhouse (experiments 1 and 2), and for a period of 55 days during July and August in the nethouse (experiment 3). Soil temperatures were measured as described. In experiments 1 and 2, the range of soil temperatures at 20 cm depth was (in °C): 28.8 to 34.2, 28.8 to 31.4, 32.1 to 39.4, and 33.7 to 40.9, in bare soil, WR-amended soil, solarized soil, and WR-amended solarized soil, respectively. The range of soil temperatures at 40 cm depth was 29.7 to 31.3 and 34.0 to 37.3°C in non-solarized and solarized soils, respectively. In experiment 3, the range of soil temperatures (ºC) at 20 cm depth was: 25.2 to 35.0, 31.7 to 45.1, and 32.1 to 46.3 in

non-solarized non-amended soil, solarized soil, and WR-amended solarized soil, respectively.

4.2. Crop management. Two weeks after termination of solarization, tomato transplants (cv. 870) were planted in experiments 1 and 2 (three beds per plot) and maintained for 7 months (Oct 2007 to Apr 2008). Fruit production was assessed by harvesting mature tomato fruits and recording their weight and size. At the end of crop growth, all of the plants from the center bed of each plot were uprooted and galling and root development determined. Since the variance within the levels of root galling was low, limited scales were used in which galling index was on a scale of 0 to 2, where 0 = no galls and 2 = more than 50% of the root surface galled. Root development index on a scale of 1 to 3, where 1 = poorly root development, 2 = no development of deep roots and 3 = development of surface and deep roots.

In experiment 3, snapdragon ('Photomek', white) transplants were planted in spring 2008, 6 months after the end of the soil treatments (20 plants/m^2). Flower stems were harvested during crop growth, separated according to stem size: high quality—stem longer than 50 cm, low quality—stem shorter than 50 cm, and weighed, and total shoot biomass was recorded. Five months after planting (in Aug 2008), 40 plants from the center of each plot were uprooted for galling and root development evaluation.

4.3. Bioassaying control efficacy of the soil treatments. Samples (0.5 kg) of treated soil were taken from 20 and 40 cm depths from the center of each tested plot, immediately after termination of solarization. These samples served for an indirect assessment of nematode viability, by planting tomato seedlings in the tested soil samples and evaluating root galling (Klein et al., 2011a). This test reliably assesses nematode capability to cause a disease (Gamliel and Stapleton, 1993b; Walker and Melin, 1996). Eight soil samples from each treatment, 0.5 kg each, were taken at 20 and 40 cm depths from different locations in each plot. This 3D (dimensional) spatial sampling (plain and depth) is optimizing the coverage of the experimental plots as was offered by Bidge and Starr (2010). In addition, *F. oxysporum* f. sp. *radicis-lycopersici* Jarvis & Shoemaker (FORL) inoculum, consisting of chlamydospores in naturally infested soil, was used as a second bioassay for the control efficacy of the treatments. The inoculum was packed in nylon net bags (325 mesh), 5 g per replicate,

and was wetted to water-holding capacity 24 h before use. The bags were buried in the center of each plot, before mulching, at 20 and 40 cm depths. At the termination of solarization, the bags with FORL inoculum were retrieved and the viability of the chlamydospores was tested as previously described (Klein et al., 2007).

4.4. Testing soil suppressiveness for Fusarium disease in cucumber, in soil samples from the field experiments. Soil suppressiveness to Fusarium disease was assessed at the termination of two field experiments (tomato and snapdragon cropping seasons) in order to validate the induction of soil suppressiveness by WR amendment and solarization under agricultural conditions. Soil samples from each plot were taken from a depth of 10 to 30 cm, to evaluate soil suppressiveness under greenhouse conditions. Two soil samples, 0.5 kg each, from each plot in experiment 2 and one from each plot in experiment 3 were taken and dried at room temperature. Soil-suppressiveness assays, in which inoculated seedlings were planted in the soil samples, were conducted in pot experiments as described.

5. Possible mechanisms of induced soil suppressiveness

5.1. Soil components: volatile organic compounds (VOC) and queous extracts

5.1.1. Exposure of nonsuppressive soil to VOC generated during WR-decomposition. Nonamended soil bags were designated for exposure to the volatiles generated from the WR-amended soil, during the decomposition process. The bags containing the amended and nonamended wetted soil were buried in separate small filed plots (2x4 m). Each bag was placed horizontally flat at a depth of 20-30 cm below the soil surface. Additionally, bags which were filled with nonamended soil were placed horizontally over the bags with the amended soil (10-20 cm below the soil surface). Thus, this assembly allowed volatile compounds which were generated during the WR decomposition, to diffuse from the WR-amended soil onto the nonamended soil over it. The entire bag assembly in each location was covered with soil to level the soil surface. All plots with the buried tested soil bags were mulched with a transparent polyethylene sheets (100-µm thick) and a shading screen (Polysak, Nir Itzhak, Israel, 90% shade) which was laid 20 cm over the plastic mulch, in order to avoid soil heating and solarization. Soil temperature in the shaded plots reached 32

to 34ºC at 20 cm depth. After 28 days the plastic sheets were removed from the plots and the soil bags with their contents were brought to the laboratory and allowed to air dry at 25ºC for one month prior to their use.

5.1.2. Soil extraction. Crude aqueous soil extracts were produced using a modified technique described by Triky-Dotan et al., (2010), in order to concentrate the microbial fraction and the soluble and suspended components in a small volume of aqueous suspension. Soil sample (2 kg) was saturated with 540 ml of tap water, agitated and left for 12 h. Thereafter, the soil-water paste was filtered through a 325-mesh screen, and the filtrate suspension containing water and fine solid particles in a total volume of 100 ml (contained 15 g of dry material) was collected and used for further assays. Thus, the microbial and chemical fractions were concentrated 20 times, compared with the original soil. The crude soil extracts originated from suppressive (WR-amended) and control (nonamended) soils were each divided into four samples (24 ml). Each sample was mixed with 1 ml of macroconidial suspension of FORC to achieve a final concentration of 10^5 CFU ml^{-1}. The soil extracts with FORC were incubated in flasks for seven days in a reciprocal shaker (100 shakes min^{-1}). Viability of macroconida in the suspension was assessed immediately after setting, and 3 and 7 days later, using the plating dilution technique (Klein et al., 2007). The soil extracts with FORC were also used to inoculate cucumber seedlings which were transplanted into nonsuppressive soil (3 ml extract per seedling). Additionally, soil suppressiveness was assessed in the soil samples which were extracted, by transplanting cucumber inoculated with FORC in these soils, as described above.

5.2. Root colonization by *Fusarium* spp.. Quantification of *Fusarium* spp. in the roots of inoculated cucumber transplants was conducted by root maceration essentially as previously described (Katan, 1971). Asymptomatic and symptomatic inoculated transplants, which were grown 3 and 6 days after inoculation, respectively, in suppressive and control soil, were removed. The roots were cut and thoroughly washed in 30 ml sterile saline water, by stirring for one min, three sequential times. Then the washed roots were blotted, weighted, transferred to 20 ml sterile saline water and crushed by ultraturax for one min. the paste was plated on SQA medium, using the plating dilution technique, as previously reported (Klein et al., 2007). Results were expressed in CFU/ g fresh root weight.

5.3. Induced disease resistance in cucumber seedlings

5.3.1. Induced resistance to Fusarium disease in cucumber seedlings. Possible induced disease resistance in cucumber transplants was tested by growing seedlings first in suppressive soils, and then transplanting them to control soil followed by inoculation with FORC, essentially as described by Yogev et al., (2010). Cucumber seeds were sown and grown in pots containing 0.7 kg of either suppressive or control soil, (20 seeds per pot). The intact cucumber seedlings were removed after seven or 14 days, and the roots were rinsed with water and dipped for 2 min in a macroconidial suspension of FORC (10^5 CFU ml^{-1}). The inoculated seedlings were then transplanted into pots containing 0.7 kg nonsuppressive Rehovot soil, as described above (each treatment consisted of five pots, each containing five inoculated seedlings). The temporal separation between the seedlings which were grown in one soil, and transplanting them after inoculation in a second soil enabled us a possible acquisition of induced systemic resistance and consequent disease suppression. Noninoculated seedlings were used as control. The seedlings were irrigated and monitored daily for disease symptoms for 18 days. Disease progress was expressed as percentage of diseased plants, and area under the disease-progress curve (AUDPC) was calculated.

5.3.2. Induced resistance to gray mold in cucumber seedlings. Possible induced resistance to gray mold (caused by *Botrytis cinerea*) was tested in cucumber plants essentially as described by Yogev et al., (2010). Cucumber seeds were sown in suppressive or nonsuppressive soil, in 0.7 kg pots (4 plants in pot, 2 pots per treatment). After five weeks, leaves (from the top three - young, medium and old) of intact plants were inoculated with a culture disc of *B. cinerea* (5-mm discs of four-day old culture grown on 0.25-strength potato dextrose agar). Three inoculum discs were placed on each one of the top three leaves of each intact plant. The intact plants were then covered with polyethylene sheets to maximize the relative humidity, at 25 °C. The area of expanding necrotic diseased tissue was measured daily, two to four days after inoculation.

5.4. Survival of FORC in suppressive soil. A suspension of FORC macroconidia (10 ml) was mixed with 100 g of either suppressive or control soil, to a level of water

holding capacity, and a final concentration of $5x10^5$ CFU g $soil^{-1}$. The soils were incubated in 25 °C for 28 days. Soil samples were taken immediately (before incubation) and 28 days later. Number of FORC propagules was enumerated by the dilution plating technique. This experiment was conducted in soils immediately after soil treatment in small plots as described above, or after four inoculated plantings of cucumber (assessment of soil suppressiveness).

5.5. Spore germination and development of FORC chlamydospores in suppressive soil. Spore germination and chlamydospore formation of FORC in soils were tested using a modified technique that was described by Sztejnberg et al., (1987). The assays were conducted with soil samples which were first induced for suppressiveness as described, immediately after soil treatment, or after the soil was grown with four consecutive cucumber planting and inoculation with FORC. Soil samples (25 g) of suppressive and control soil were moistened to field capacity, placed in Petri dishes (9 cm diameter) and incubated at 25°C for 24 h. afterwards, The soil surface was covered with one layer of a sterilized dialysis membrane and 0.1 ml FORC macroconidia suspension at 10^6 CFU ml^{-1} was spread over the membrane surface. Then, the membrane with the spread macroconidia was covered with an additional sterilized dialysis membrane and covered with additional quantity of the same soil. Thus, the structure of this soil sandwich consisted of macroconidia suspension trapped between two dialysis members and covered with soil layers. The plates were incubated at 18°C for 24 or 48 h. Germination of macroconidia and formation of new chlamydospores was assessed microscopically. Values represent percent from macroconidia observed (minimum 100 spores per treatment).

Development of FORC mycelium on suppressive and control soil was assayed on Petri dishes filled with 25 g of each soil, moistened to field capacity, and incubated at 25°C for 24 h. A sterilized dialysis membranes was laid over the soil surface and culture discs (4 mm) of FORC (grown on agar culture containing 2% w/v agar, 0.025 g chloramphenicol) were placed above, 4 discs per petri-dish, and incubated at 20°C for 20 h. Hyphae growth from the discs was assessed microscopically. The lengths of five hyphae threads were measured in each of four microscopic fields per disc, 12 discs per treatment.

6. Microbial colonization of cucumber roots in suppressive soil

6.1. Root sampling. Root-microbiome analyses were conducted in cucumber transplants inoculated with FORC, planted in suppressive or control soils and grown for 3 days (before symptom appearance) or 6 days (first appearance of light chlorosis of the leaves on inoculated control plants). On each date, cucumber transplants were removed with their entire root system from the pots (three plants per replicate, five replicates per treatment). The roots were cut and washed in 30 ml sterile saline water, using orbital vortexing for 1 min in 3 intervals. The washed roots were weighed, transferred to 15-ml sterile tubes and kept at -20°C until DNA extraction.

6.2. DNA extraction. Extraction of DNA was carried out using the PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad, CA) as described by the manufacturer. DNA concentrations and quality were determined with an ND1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE).

6.3. Real-time quantitative PCR. Real-time PCR was used for the quantification of total bacteria, plant plastid, *Massilia*, *Streptomyces*, FORC and the plant *tef* gene in root samples. The plant *tef* gene, encoding translation elongation factor 1, served for data normalization as proposed by Ruppel et al. (2006). A primer pair targeting the plant plastid was applied to correct bacterial target numbers for plant plastids according to Ofek et al. (2009). The primers used for the different assays are listed in Table 1.

To verify primer specificity, a plasmid standard containing the target region was generated for each primer set. For this purpose, PCR products for each primer pair were amplified from root DNA samples as template. The PCR-amplified products were examined by gel electrophoresis to confirm the specificity of the amplification. In addition, each of the products was cloned using the TOPO TA Cloning Kit (Invitrogen, Carlsbad, CA) as instructed by the manufacturer. Plasmids were isolated and 10 randomly selected cloned inserts were sequenced from each primer set to confirm their identity and primer specificity. The specificity of FORC primers, used following Lievens et al. (2007), was also confirmed by PCR of DNA extracted from different pathogenic *Fusarium* strains, including *F. oxysporum* f. sp. *melonis*, *F. oxysporum* f. sp. *radicis-lycopersici* and *F. oxysporum* f. sp. *proliferatum*. Plasmid

DNA concentrations were determined spectrophotometrically, copy numbers were calculated accordingly and 10-fold dilution series within a range of 10^9 to 10 copies/µl were then prepared.

Real-time PCR assays were conducted in an Mx3000P QPCR System (Stratagene, La Jolla, CA), in 96-well polypropylene plates. Each well contained a 25 µl mixture consisting of 12.5 µl Absolute Blue SYBR Green ROX Mix (Thermo Fisher Scientific, Surrey, UK), 1 µl of each primer (10 µM final concentrations), 9.5 µl H_2O, and 1 µl template DNA. The PCR conditions were: 15 min at 95°C, followed by 40 cycles of 95°C for 30 s, 60ºC (or 58ºC for the bacterial primer pair) for 30 s, and 72°C for 30 s. Melting-curve analysis of the PCR products was conducted following each assay to confirm specificity. The PCR products were also examined by agarose gel electrophoresis to confirm the specificity of amplification. Results were analyzed using MxPro QPCR Software analysis tools (Stratagene).

6.4. PCR-DGGE. *Streptomyces*-specific population patterns were examined using the PCR-DGGE method. DNA extracted from different root samples served as the template for PCR amplification of *Streptomyces* 16S rRNA gene fragments using primers Strep661f and Strep1218r(GC) (Table 1), performed as previously described (Inbar et al., 2005). DGGE was performed in 6% (w/v) acrylamide gels containing a linear urea-formamide gradient ranging from 20 to 70% denaturant (with 100% defined as 7 M urea and 40% v/v formamide). Gels were run for 17 h at 100 V with the Dcode Universal Mutation System (BioRad Laboratories, Hercules, CA). DNA was visualized after staining with Gelstar (Invitrogen) by UV transillumination (302 nm) and was photographed with a Kodak KDS digital camera. DGGE images were analyzed using Fingerprinting II software (BioRad), and an unweighted pair-group method with arithmetic means (UPGMA) tree based on cosine correlation matrix was produced. Aligned densitometric curves were exported from the Fingerprint II software and multidimensional scaling analysis was performed based on 1-Pearson *r* distance matrix using STATISTICA (version 7.1) software (Stat Soft Inc., Tulsa, OK). Specific bands were excised from the gels. Band DNA was reamplified by PCR and verified on another DGGE, analyzed and cloned using the TOPO TA Cloning Kit. Plasmids were isolated and sequenced. Phylogenetic affiliation of the retrieved

sequences was determined using ARB software (Ludwig et al., 2004) and NCBI Blast analyses (Altschul et al., 1997).

6.5. Sequencing of 16S rRNA gene fragments. DNA extracted from the root samples was subjected to mass sequencing. Bacterial 16S rDNA and fungal ITS tag-encoded FLX amplicon pyrosequencing were performed by the Research and Testing Laboratory (Lubbock, TX) as described by Dowd et al. (2008). Primers are listed in Table 1. The retrieved sequences were analyzed using MOTHUR (Schloss et al., 2009). Five replicates were analyzed for each treatment at 3 and 6 days. For the root samples taken from inoculated WR-amended soil 3 days after transplantation, only four of the five replicates produced results in the fungal ITS assay (preparation of one sample library failed).

For fungal ITS, low-quality sequences and those shorter than 320 bases, were omitted from further analyses. Then, suspected chimeras were detected using the MOTHUR chimera.check module (~10% of the total sequences) and eliminated from further analysis. The sequences were aligned using ARB software (Ludwig et al., 2004) and the alignment was exported to MOTHUR where a distance matrix was calculated using the MOTHUR dis.seqs module. Sequences were then classified into operational taxonomic units (OTUs) using a 99% sequence-similarity threshold. Representatives of each OTU were classified by NCBI-Blast analysis.

From the bacterial sequences obtained, low-quality sequences and those shorter than 250 bases were omitted from further analyses, as were the suspected chimeras (~10% of the total sequences) and eliminated from further analysis. Bacterial sequences were aligned using the Silva-compatible alignment database and a distance matrix was calculated using MOTHUR dis.seq module. Sequences were then classified into OTUs using a 97% sequence similarity threshold. Representatives of each OTU were classified with the MOTHUR classify.seqs module, and affiliation was verified by NCBI Blast analysis. Sequences were deposited in the GeneBank SRA database under accession number SRA048248 (experiment SRX109547 for bacteria and experiment SRX109548 for fungal sequences).

7. Statistical analyses

All experiments under controlled conditions were carried out independently at least twice, with soil from different batches, yielding similar results. Peppermint amendment was performed only on 2005 field plots, therefore the suppressiveness tests in peppermint amended soil was carried out in one soil batch. The experiment in which the long-term effect was assessed was carried out only once, but with three repeat-inoculation plantings. Induced resistance tests were carried out three times, with similar results. Separate analyses of each experiment showed homogeneous variance of the experimental error between the replicates. Data were first analyzed by analysis of variance (ANOVA) to test for possible interaction among the main effects, followed by the appropriate mean separation analysis using Tukey's studentized range (honestly significant difference) test. All greenhouse trials were arranged in randomized block design. In all experiments, non-inoculated plants remained asymptomatic.

In the field experiments, Data on test-pathogen control, disease incidence, and tomato and snapdragon yield were analyzed by analysis of variance followed by mean separation using Tukey's studentized range (honestly significant difference) test. Non-parametric data such as galling index and root development index were analyzed using the rank procedure followed by analysis of variance.

When mentioned, statistical analyses in the presented figures show data from one of two experiments.

All analyses were performed with SAS software (SAS Inst., Cary, NC; release 9.01 for PC) at $P \leq 0.05$. Sample coverage was estimated according to Good (1953). For each sample, the Chao1 richness estimate (Chao, 1984) and the dominance estimate were calculated. After classification into OTUs, the count data from the different samples were Hellinger transformed, to reduce bias related to differences in the number of sequences per sample (Legendre and Gallagher, 2001). The compositions were compared by non-metric multidimensional scaling based on pairwise 1-Pearson r distance matrix using STATISTICA (version 7.1) software.

Table 1. Primers used in this study

Primer	Sequence	Gene	Target organisms	Reference
ITS1f	5′-CTTGGTCATTTAGAGGAAGTAA-3′	fungal rRNA ITS	Fungi	Buée et al., 2009
ITS2	5′-GCTGCGTTCTTCATCGATGC-3′	fungal rRNA ITS	Fungi	Buée et al., 2009
530f	5'-GTGCCAGCMGCNGCGG-3’	16S rRNA	Bacteria	Dowd et al., 2008b
1100r	5’- GGGTTNCGNTCGTTG-3’	16S rRNA	Bacteria	Dowd et al., 2008b
341f[z]	5’-GCCTACGGGAGGCAGCAG-3’	16S rRNA	Bacteria	Muyzer,et al., 1993
907r	5’-CCGTCAATTCMTTTGAGTTT-3’	16S rRNA	Bacteria	Muyzer et al., 1993
515f	5’-GTGCCAGCMGCCGCGGTAA-3’	16S rRNA	Universal	Lane, 1991
Plast_f	5’-GAGGCAATAGCTTACCAAGGCG-3’	16S rRNA	Plant plastid	Ofek et al., 2009
Plast_r	5’-CTTGGTAGTTTCCACCGCCTG-3’	16S rRNA	Plant plastid	Ofek et al., 2009
Strep661f	5’-GTAGGGGAGATCGGAATT-3’	16S rRNA	*Streptomyces*	Inbar et al., 2005
Sterp1218r[z]	5’-AGCACGTGTGCAGCCCAA-3’	16S rRNA	*Streptomyces*	Inbar et al., 2005
Oxalo225f	5’-GGGTTGGCGGCCCTCTG-3’	16S rRNA	Oxalobacteraceae	Green et al., 2007
Mass656r	5’-TTCTAGCCTTGCAGTCTCCATC-3’	16S rRNA	*Massilia*	Dohrmann & Tebbe, 2005
Tef_f	5’-ACTGTGCAGTAGTACTTGGTG-3’	Translation elongation factor1	Plants	Vieweg et al., 2005
Tef_r	5’-AAGCTAGGAGGTATTGACAAG-3’	Translation elongation factor1	Plants	Vieweg et al., 2005
FORC F5	5’-TCGTCACAATGATTTCAGCAT-3’	RAPD SCAR marker	*F. Oxysporum* f. sp. *radicis-cucumerinum*	Lievens et al, 2007
FORCR2	5’-GTGACGCAGGGTAGGCAT-3’	RAPD SCAR marker	*F. Oxysporum* f. sp. *radicis-cucumerinum*	Lievens et al, 2007

[z] For PCR-DGGE analysis a 5’ GC rich tail was added to the primer: CGCCCGCCGCGCCCCGCGCCCGTCCCGCCGCCCCCGCCC (Muyzer et al., 1993).

RESULTS

1. Soil suppressiveness tests in soils treated in the small plot experiments[(1)]

1.1. Suppression of Fusarium crown and root rot in cucumber plants by crop residues and soil solarization. Disease suppression in cucumber seedlings which were artificially inoculated with FORC was evident when they were planted in soil previously amended with residues of all of the tested plants, compared with the respective nonamended soils (Figs. 1.1 and 1.2, Tables 1.1 and 1.2). There were, however, differences among the various plant residues with respect to their potential for suppressiveness, with coriander and WR inducing the greatest level of suppressiveness. Solarization did not further contribute to the suppressiveness observed with the use of crop residues alone (Figs. 1.1 and 1.2, Tables 1.1 and 1.2). A typical example of suppressiveness, as reflected by disease progress and AUDPC is given in Fig. 1.1. Increases in growth parameters resulting from soil amendment are shown in Table 3. In nonamended soil, disease symptoms (chlorosis, plant-growth retardation and wilt) first appeared 7 to 10 days after inoculation, reaching 60 to 100% plant mortality 21 days after inoculation. In the WR-amended soils, disease symptoms usually appeared 1 to 7 days later and plant mortality was reduced by 20 to 80%. Root and shoot dry weights were significantly higher in both inoculated and noninoculated WR-amended soil compared with the respective nonamended soils (Table 3). Increasing WR concentration from 1 to 2% in the amended soil did not significantly add to the soil's suppressiveness to the disease (results not shown); disease incidence was 90% and 50% lower than in nonamended soil ($P < 0.0001$) at both WR concentrations, in the first and second inoculated planting, respectively.

1.1.1. Disease suppressiveness following repeated inoculation. The manifestation of disease suppressiveness was examined over two or three consecutive inoculation and planting cycles in the same pots. Disease incidence was significantly reduced in the soils which had been previously amended with WR, peppermint, broccoli, sage or tarragon, compared with the nonamended soils, throughout the consecutive planting cycles (Tables 1.1 and 1.2, Figs. 1.2-1.4). In contrast, solarization, in general, did not

[(1)] The results of soil suppressiveness to Fusarium disease were published in Klein et al., 2011b (Appendix).

sustain significant suppressive capacity, and in some cases increased disease incidence in the second or third planting cycle with any of the tested crops (Tables 1.1 and 1.2, Fig. 1.2). However, in one experiment, tarragon combined with solarization sustained suppressiveness capacity during the second and third crop cycles relative to either tarragon or solarization alone (Fig. 1.3). Suppressiveness was considered long term since it was evident 34 months after soil treatment and after successive plantings (Figs. 1.2 and 1.3).

1.1.2. Disease suppressiveness in soil inoculated with chlamydospores. Chlamydospores produced in infested peat (as an additional type of FORC inoculum) were mixed with the tested soils at various inoculum concentrations before seeding with cucumber. Emergence of the cucumber seedlings (70-80%) was not affected by FORC, irrespective of the soil treatment. Diseased plants showed symptoms after 9 to 11 days at the highest inoculum concentrations. The suppressiveness in WR-amended soil was more pronounced at the low rate of inoculum, in which disease symptoms first appeared after 20 days, but was still significant at the highest rate of chlamydospore inoculum (Table 4). Solarization significantly increased AUDPC relative to nonsolarized soils at the two highest inoculum concentrations in the first sowing, but had no significant effect in the second planting. In three cases out of six, combining solarization with WR significantly increased disease incidence relative to WR alone. Amendment with WR significantly reduced AUDPC only in nonsolarized soils (Table 4).

1.1.3. Induced suppressiveness in different soils. The suppressive capacity of WR amendment was evident in all three tested soils (Fig. 1.4). The nonamended soils from Bet Dagan and En Tamar expressed higher natural suppressiveness than the Rehovot soil. However, when amended with WR, the three soils exhibited similar levels of suppressiveness (Fig. 1.4). Disease suppressiveness was also sustained in all three soils during the second consecutive inoculation and planting cycle in the same pots, with the Bet Dagan soil being the most suppressive (Fig. 1.4).

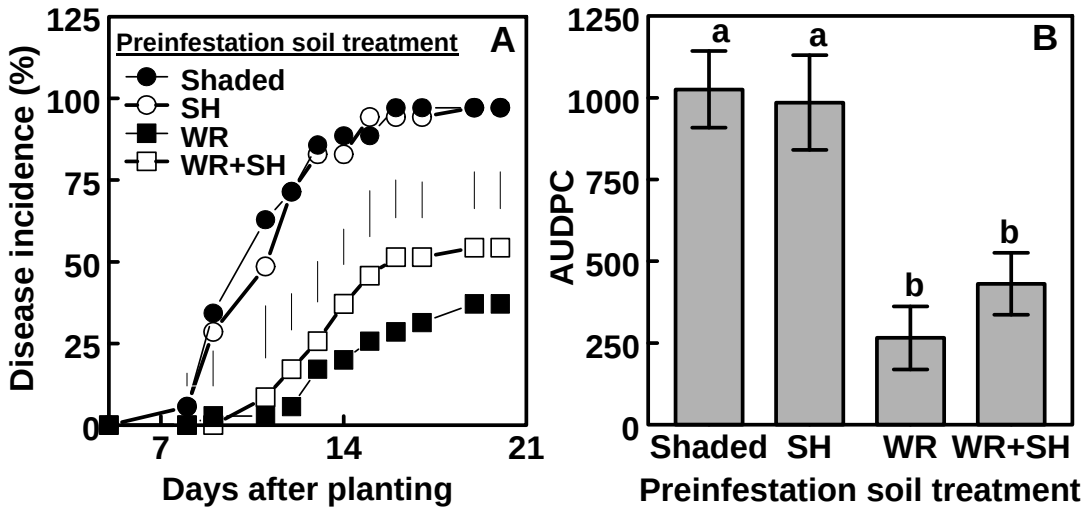

Fig. 1.1. Effect of wild rocket (WR) amendment and soil solarization (SH) on disease incidence in Fusarium crown and root rot inoculated cucumber transplants in Rehovot soil (A) or on area under the disease progress curve (AUDPC) (B). Cucumber seedlings were inoculated before planting with macroconidia of *Fusarium oxysporum* f. sp. *radicis cucumerinum* at 10^5 CFU/ml. Soil was either irrigated, mulched and shaded for 28 days under field conditions (Shaded), or irrigated, mulched, and solarized for 28 days. Soil was amended with dry WR at a rate of 1% w/w before incubation. Vertical bars indicate average standard deviations, and values followed by a different letter are significantly different according to Tukey's studentized range (honestly significant difference) test at $P \leq 0.05$.

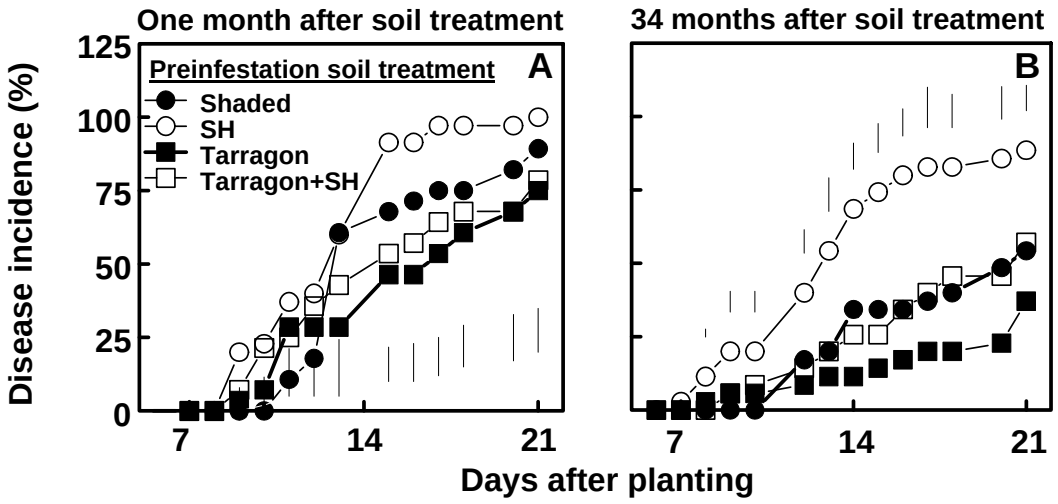

Fig. 1.2. Effect of tarragon amendment and solarization (SH) on Fusarium crown and root rot in inoculated cucumber transplants in Rehovot soil, immediately after incubation with tarragon (A) or 34 months afterwards (B). Soil was either irrigated, mulched, and shaded for 4 weeks under field conditions (Shaded), or irrigated, mulched, and solarized for 28 days. Soil was amended with dry tarragon at a rate of 1% w/w before incubation. Cucumber seedlings were inoculated before planting with macroconidia of *Fusarium oxysporum* f. sp. *radicis cucumerinum* at 1.5×10^5 or 1×10^5 CFU/ml in A and B, respectively. Vertical bars indicate average standard deviations. Amendment and solarization main effects were significant by ANOVA of the area under the disease progress curve (AUDPC). First suppressiveness test: amendment F value = 12.36; Pr > F = 0.0038; solarization F value = 7.17; Pr > F = 0.0190. Second suppressiveness test: amendment F value = 17.65; Pr > F = 0.0007; solarization F value = 15.44; Pr > F = 0.0012.

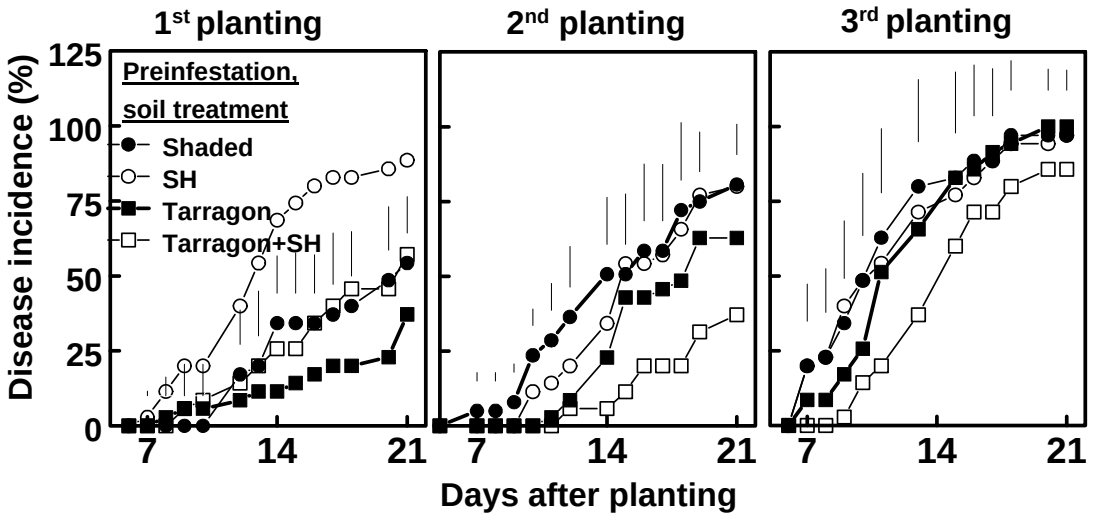

Fig. 1.3. Effect of tarragon amendment and solarization (SH) on Fusarium crown and root rot of cucumber transplants in Rehovot soil. Three repeat-inoculated plantings were carried out, 34 months after soil treatment. Soil was either irrigated, mulched, and shaded for 28 days under field conditions (Shaded), or irrigated, mulched, and solarized for 28 days. Soil was amended with dry tarragon at a rate of 1% w/w before incubation. Cucumber seedlings were inoculated before each planting with macroconidia of *Fusarium oxysporum* f. sp. *radicis cucumerinum* at 1×10^5 CFU/ml. Vertical bars indicate average standard deviations. Amendment and solarization main effects were significant by ANOVA of the area under the disease progress curve (AUDPC) of each inoculated planting. First planting: amendment F value = 17.65; Pr > F = 0.0007; solarization F value = 15.44; Pr > F = 0.0012. Second planting: amendment F value = 19.0; Pr > F = 0.0005; solarization effect was not significant. Third planting: amendment F value = 6.39; Pr > F = 0.0224; solarization effect was not significant.

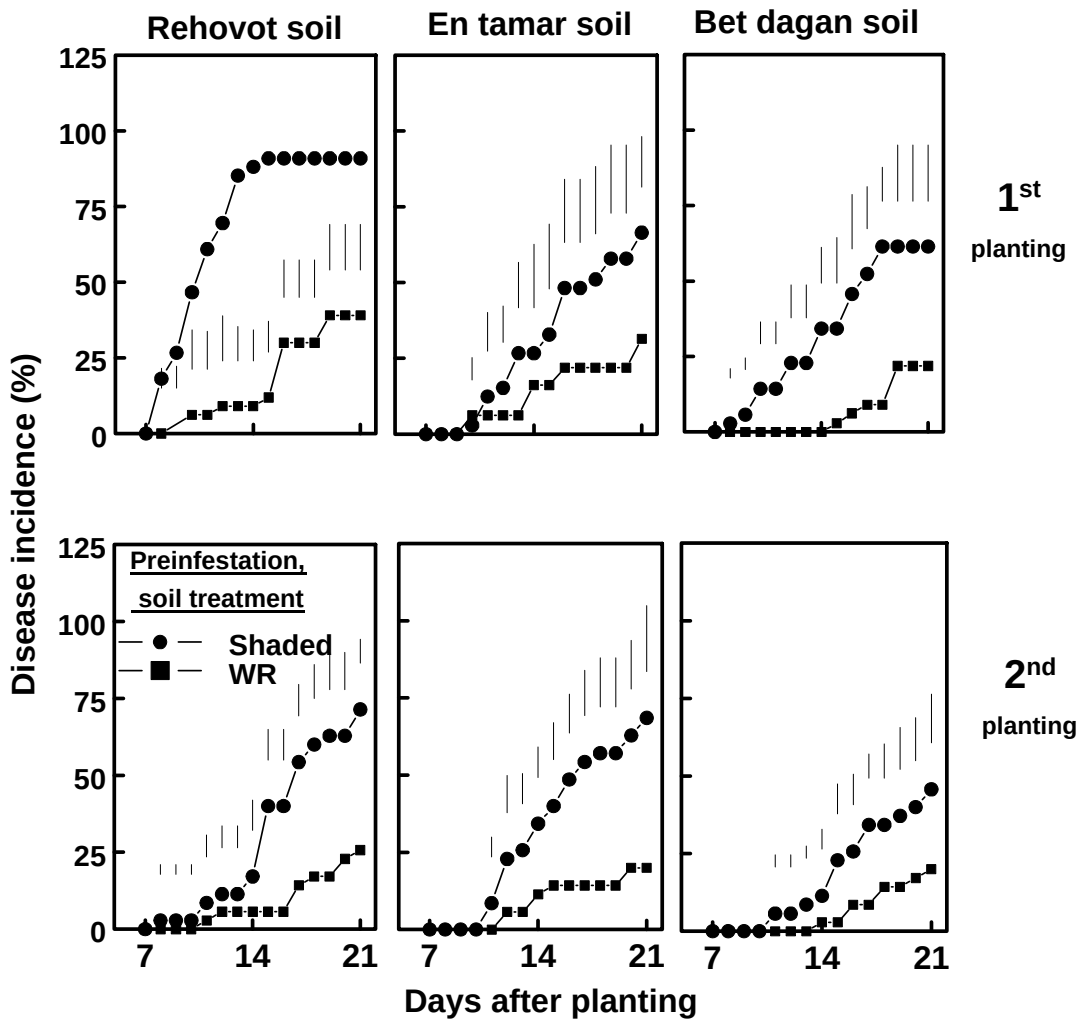

Fig. 1.4. Effect of wild rocket (WR) amendment on Fusarium crown and root rot of cucumber transplants, in Rehovot, En Tamar, and Bet Dagan soils. Soil was irrigated, mulched and shaded for 28 days under field conditions, or amended with dry WR at a rate of 1% w/w before incubation. Two repeat-inoculated plantings were carried out. Cucumber seedlings were inoculated before each planting with macroconidia of *Fusarium oxysporum* f. sp. *radicis cucumerinum* at 1.5 x 10^5 CFU/ml. Vertical bars indicate average standard deviations. In the first inoculated planting, soil x amendment interaction was significant by ANOVA of the area under the disease progress curve (AUDPC): F value = 7.77; Pr > F = 0.0025. In the second inoculated planting, soil and amendment main effects were significant by the analysis of variance (ANOVA) of the AUDPC: soil F value = 5.46; Pr > F = 0.0111; AUDPC in Bet Dagan soil significantly decreased compared with the other soils. Amendment main effect F value = 67.03; Pr > F < 0.0001.

Table 1.1. Effect of organic amendments and solarization on area under the disease progress curve (AUDPC) of Fusarium crown and root rot in inoculated[w] cucumber seedlings in Rehovot soil.

		AUDPC, first planting[w]		AUDPC, second planting[w]	
Experiment[x]	Amendment	Shaded[y]	Solarized[y]	Shaded	Solarized
1	Nonamended	738.6[z] A	597.1 A	538.6 A	447.1 A
1	Coriander	125.7 C	128.6 B	547.1 A	644.3 A
1	Peppermint	370.0 B	199.0 B	261.4 B	484.3 A
1	Rosemary	278.6 B	250.0 B	455.7 AB	604.3 A
2	Nonamended	825.7 A	640.0 A	788.6 A	765.7 A
2	Broccoli	333.6 B	202.1 B	531.2 AB	473.8 B
2	Cauliflower	284.1 B	280.1 B	530.0 AB	672.9 AB
2	Sage	639.3 B	218.3 B	241.1 B	195.7 C

[w] Cucumber seedlings were inoculated with *Fusarium oxysporum* f. sp. *radicis-cucumerinum* macroconidia at 1.5×10^5 CFU/ml and planted in the previously treated soils.

[x] Data from each experiment represents the combined analysis of two trials that were pooled.

[y] Solarization or shading under field conditions was conducted for 28 days during July 2005. Plant residues were applied to the soil at 10 g/kg (1% w/w).

[z] For each experiment, values within a column followed by different letters are significantly different according to Tukey's studentized range (honestly significant difference) test at $P \leq 0.05$ (Table 2).

Table 1.2. Statistical ANOVA of area under the disease progress curve (AUDPC) of Fusarium crown and root rot in inoculated cucumber seedlings[x] which were grown in amended Rehovot soil[y]

		ANOVA of AUDPC ($P < 0.05$)					
		AUDPC, first planting[x]			AUDPC, second planting[x]		
Experiment	Soil treatment[y]	DF	F value	Pr > F	DF	F value	Pr > F
1	Amendment[z]	3	44.38	<0.0001	3	3.12	0.0397
1	Solarization	1	5.89	0.0210	1	3.16	0.0847
1	Amendment x Solarization	3	1.48	0.2398	3	1.60	0.2084
2	Amendment[z]	3	15.02	<0.0001	3	19.90	<0.0001
2	Solarization	1	10.76	0.0026	1	0.01	0.9339
2	Amendment x Solarization	3	2.28	0.0986	3	0.84	0.4813

[x] Cucumber seedlings were inoculated with *Fusarium oxysporum* f. sp. *radicis-cucumerinum* macroconidia at 1.5×10^5 CFU/ml and planted in the previously treated soils.

[y] Solarization or shading under field conditions was conducted for 28 days. Soil was amended with different plant residues, before planting, as indicated in Table 1.

[z] In each experiment, different amendments were tested. In the first experiment, amendments were coriander, peppermint and rosemary. In the second experiment, amendments were broccoli, cauliflower and sage.

Table 1.3. Effect of wild rocket-amended soil (WR)[w] and Fusarium crown and root rot inoculation[x] on cucumber root and shoot dry weight[y]

Soil amendment	Root dry weight (g/plant)		Shoot dry weight (g/plant)	
	Noninoculated	Inoculated	Noninoculated	Inoculated
Nonamended	0.0479 Ba[z]	0.0467 Ba	0.1567 Ba	0.1100 Ba
WR	0.0777 Ab	0.1159 Aa	0.3786 Aa	0.3333 Aa

[w] Shading of Rehovot soil under field conditions was conducted for 28 days during July 2009. WR applied to the soil at 10 g/kg (1% w/w).

[x] Cucumber seedlings were inoculated with *Fusarium oxysporum* f. sp. *radicis-cucumerinum* macroconidia at 10^5 CFU/ml and planted in the previously treated soils.

[y] Shoot and root weights were recorded on day 22 after planting.

[z] Different uppercase letters denote significant difference in amendment level; different lowercase letters denote significant difference in inoculation level, according to ANOVA of the root or shoot dry weight, Tukey's studentized range (honestly significant difference) test at $P \leq 0.05$. For root dry weight, inoculation x amendment F value = 8.9; Pr > F < 0.0054. For shoot dry weight, inoculation F value = 7.8; Pr > F = 0.0087; amendment F value = 182.9; Pr > F < 0.0001; the inoculation x amendment interaction was not significant.

Table 1.4. Effect of wild rocket amendment (WR)[v] and solarization on area under the disease progress curve (AUDPC) of Fusarium crown and root rot in cucumber which was sown or planted in a Rehovot soil inoculated with chlamydospores of *Fusarium oxysporum* f. sp. *radicis-cucumerinum*

		Planting[w]			
		First (sowing)		Second (planting)	
Chlamydospore concentration	Solarization	Nonamended	WR	Nonamended	WR
4.075×10^4	Shaded	386.8[x,y] B	53.0 C	775.0[x,z]AB	452.4 B
4.075×10^4	Solarized	717.9 B	417.7 BC	368.6 B	785.7 B
8.15×10^4	Shaded	479.2 B	138.5 C	835.7 AB	527.1 B
8.15×10^4	Solarized	1212.0 A	707.2 AB	1292.9 A	920.0 B
2.375×10^5	Shaded	601.7 B	409.3 BC	1040.0 A	461.7 B
2.375×10^5	Solarized	1659.7 A	1012.7 A	1257.1 A	1635.7 A
Min. Sign. Diff. ($P < 0.05$)		478.7	429.5	604.1	705.4

[v] Solarization or shading under field conditions was conducted for 28 days during July 2008. WR applied to the soil at 10 g/kg (1% w/w).

[w] In the first suppressiveness test, cucumber seeds were sown in the tested soils; in the second (repeat) planting, cucumber seedlings were planted in the same soils without additional inoculation. Cucumber was grown for 28 days in each cycle.

[x] AUDPC means within a column followed by different letters are significantly different, according to Tukey's studentized range (honestly significant difference) test at $P \leq 0.05$.

[y] Amendment main effect and the chlamydospore concentration x solarization interaction were significant by ANOVA of the AUDPC: amendment F value = 41.78; Pr > F < 0.0001; chlamydospore concentration x solarization F value = 5.66; Pr > F = 0.0063.

[z] The inoculum concentration x solarization and solarization x amendment interactions were significant by ANOVA of the AUDPC: chlamydospore concentration x solarization F value = 6.17; Pr > F = 0.0042; solarization x amendment F value = 9.80; Pr > F = 0.0030.

1.2. Suppressiveness *of Meloidogyne javanica* root galling in tomato, basil and snapdragon plants by crop residues and soil solarization. Suppression of root galling in tomato, basil, and snapdragon transplants artificially inoculated with *Meloidojyne javanica* was evident in the pot experiment when they were planted in soil samples previously amended in the field with residues of the tested herb crops, compared with the respective non-amended soils (Tables 2.1 and 2.2). This effect was expressed as reduced galling index accompanied by increased root development, foliage height, and total biomass. Solarization in this experiment did not induce soil suppressiveness to nematode galling, nor did it affect root or plant development.

2. Inducing soil suppressiveness to *M. javanica* root-knot and Fusarium disease by WR and soil solarization under agricultural practice – field experiments

2.1. Survival of *M. javanica* and FORL in soil following treatments. The objective in the three field experiments was to induce soil suppressiveness without severely affecting the nematode population, in order to exclude a possible effect of pathogen reduction by the treatment. Indeed, in field experiments 1 and 2, which involved moderate disinfestation (low dosage of OA and moderate soil temperatures) by WR amendment and solarization, *M. javanica* root-knot incidence was not significantly reduced by the soil treatments, as evaluated in bioassay tests (Table 2.3). There was no significant effect on the re-infestation and nematode galling in experiments 1 and 2, in which the initial nematode potential to root galling was significantly different (Table 2.3). However, the survival of *Fusarium oxysporum* F. SP. *radicis-lycopersici* (FORL), which was introduced as a test organism, was partially reduced in the solarized plots (Table 2.3), indicating an effect of solarization on soil microbial population. In experiment 3, however, the disinfestation conditions, which included a higher rate of amendment and intensified soil heating (higher maximal temperature and longer period of application than in experiments 1 and 2) resulted in significant reductions in nematode survival in all solarized plots (Table 2.4). This effect was more pronounced with the combination of solarization and WR amendment, which eliminated root galling to a depth of 40 cm.

2.2 Suppressiveness of root galling in tomato plants (experiments 1 and 2). A significant reduction in galling severity on roots of tomato plants was evident in the

solarized plots, alone or in combination with WR amendment, in experiment 1 (Table 2.5). The reduction in galling severity was not significant in experiment 2, apparently due to the lower initial nematode density. However, separate analysis of each galling level revealed, in both experiments, a significant reduction in the highly galled roots. For example, a significant reduction of about 50% was recorded in roots from the amended or solarized plots compared with the non-amended non-solarized plots (data not shown). Root development in experiments 1 and 2 was not affected by the treatments (Table 2.5).

2.3 Induced suppressiveness in snapdragon plots (experiment 3). Soil suppressiveness to root galling by *M. javanica* was not evident with the snapdragon plants in experiment 3 (Table 2.6). Although an effective reduction in nematode infectivity was achieved by WR amendment and extended solarization (Table 4), this was not reflected in galling reduction, or in improved root development or yield quality of snapdragon flowers. Apparently, snapdragon is highly sensitive to *M. javanica* and extending the crop to five months of growth masked any potential suppressive effect.

2.4. Suppression of Fusarium disease in cucumber plants grown in soil samples taken after the cropping season. Soil samples which were taken from all treatments in experiments 2 and 3 (tomato and snapdragon) at the end of the cropping season were bioassayed to assess the sustainability of soil suppressiveness against FORC in cucumbers seedlings. Soil samples from solarized plots in both experiments reduced Fusarium disease in inoculated cucumber seedlings to various levels (Table 2.7). Factorial analysis showed that solarization as main effect significantly increased the survival of cucumber seedlings 14 days from transplanting and significantly reduced AUDPC at 28 days, compared with the non-solarized treatments, in both experiments. In contrast, amended soil did not exhibit soil suppressiveness to Fusarium disease in cucumber.

Table 2.1. Effect of organic amendments and solarization[a] on soil suppressiveness to tomato or basil root galling caused by inoculation[b] with *Meloidogyne javanica*, in Rehovot soil, in the small plot experiment

		Galling index (0–5)[c]		Root development index (1–4)[c]	
Tested plant	Amendment	Shaded	Solarized	Shaded	Solarized
Tomato	Non-amended	4.38[d] A	4.75 A	1.63 B	1.75 B
Tomato	Sage	2.63 B	2.75 B	3.50 A	3.63 A
Tomato	Tarragon	2.13 B	2.63 B	3.63 A	4.00 A
Tomato	Wild rocket	2.88 B	2.88 B	3.63 A	3.88 A
Basil	Non-amended	3.75 A	Nt[e]	4.00 A	Nt
Basil	Peppermint	2.03 B	Nt	4.00 A	Nt

[a] Solarization or shading under field conditions was conducted for 28 days during July 2005 (peppermint) or July 2006. Plant residues were applied to the soil at 10 g/kg (1% w/w). Soil samples from the treated plots were used for pot experiments in the greenhouse.

[b] Tomato or basil seedlings were inoculated with *M. javanica* at transplanting into the previously treated soils at 800 J2/plant. Data from each experiment represent the combined analysis of two trials that were pooled.

[c] Galling index on a scale of 0 to 5, where 0 = no galls and 5 = more than 80% of the root surface galled. Root development index on a scale of 1 to 4, where 1 = poorly root development and 4 = maximal root volume. The statistical analysis was done on ranked values. Data represent the combined analysis of two trials that were pooled.

[d] For each crop, values within each column followed by different letters are significantly different according to Tukey test at $P \leq 0.05$. There were no significant differences between non-solarized and solarized treatments at $P \leq 0.05$.

[e] Not tested.

Table 2.2. Effect of wild rocket (WR) amendment and solarization[a] on soil suppressiveness to snapdragon root galling caused by inoculation[b] with *Meloidogyne javanica*, in Rehovot soil, in the small plot experiment

Soil treatment	Galling index (0–5)[c]	Root development index (1–4)[c]	Plant height (cm)	Shoot dry weight (g)
Control	5.00[d] A	1.75 B	22.14 B	0.56 B
Solarized	5.00 A	2.00 B	20.10 B	0.52 B
WR	3.00 B	3.13 A	64.81 A	1.84 A
WR-solarized	3.75 B	2.63 AB	69.38 A	2.01 A

[a] Solarization or shading under field conditions was conducted for 28 days during July 2006. Plant residues were applied to the soil at 10 g/kg (1% w/w). Soil samples from the treated plots were used for pot experiments in the greenhouse.

[b] Snapdragon seedlings were inoculated with *M. javanica* at transplanting into the previously treated soils at 800 J2/plant.

[c] Galling index on a scale of 0 to 5, where 0 = no galls and 5 = more than 80% of the root surface galled. Root development index on a scale of 1 to 4, where 1 = poorly root development and 4 = maximal root volume. The statistical analysis was done on ranked values. Data represent the combined analysis of two trials that were pooled.

[d] Values within each column followed by different letters are significantly different according to Tukey test at $P \leq 0.05$. There were no significant differences between non-solarized and solarized treatments at $P \leq 0.05$.

Table 2.3. Survival of *Meloidogyne javanica*[a] and *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) under farm conditions, following soil solarization (SH) and wild rocket (WR) amendment[b], in tomato greenhouses at the Besor experimental station in the western Negev region

Experiment[c]	Soil treatment	*M. javanica* galling index (0–5)				FORL viability (CFU/g dry soil)			
		Depth (20 cm)		Depth (40 cm)		Depth (20 cm)		Depth (40 cm)	
		Mean	SD[d]	Mean	SD	Mean	SD	Mean	SD
1	Control	3.50[e] A	1.51	2.50 A	1.51	2817 A	519	6088 A	3535
1	SH	3.75 A	1.04	3.38 A	1.41	301 BC	129	402 A	119
1	WR	2.88 A	1.64	3.57 A	1.40	1215 B	712	3743 A	1355
1	WR+SH	3.63 A	1.06	3.38 A	1.19	218 C	166	1481 A	1243
2	Control	0.13 A	0.35	0.38 A	1.06	2079 A	704	3617 A	1455
2	SH	0.00 A	0.00	0.00 A	0.00	622 B	459	1002 B	630
2	WR	0.50 A	1.41	0.50 A	1.07	1383 AB	582	3015 AB	758
2	WR+SH	1.00 A	1.85	0.25 A	0.46	382 B	75	751 B	56

[a]Assessed by planting tomato plants in soil samples from treated plots, and evaluating galling index on a scale of 0 to 5, where 0 = no galls and 5 = more than 80% of the root surface galled. The statistical analysis was done on ranked values for each experiment separately.

[b] Solarization was conducted for 40 days during Aug–Sep 2007. WR residues were incorporated into the soil at 0.4 kg/m^2 (0.4% w/w).

[c] Two experiments were carried out in tomato greenhouses (310 m^2) naturally infested with *M. javanica*. The first (Exp. 1) had no history of methyl bromide disinfestation. The second (Exp. 2) had been disinfested with methyl bromide before the previous crop.

[d] Standard deviation.

[e] In each experiment, values within each column followed by different letters are significantly different according to Tukey test at $P \leq 0.05$.

Table 2.4. Survival of *Meloidogyne javanica*[a] under farm conditions, following soil solarization (SH) and wild rocket (WR) amendment[b], in a snapdragon nethouse at the Besor experimental station in the western Negev region (experiment 3)

Soil treatment	*M. javanica* galling index			
	Depth (20 cm)		Depth (40 cm)	
	Mean	SD[c]	Mean	SD
Control	1.50[d] A	1.00	2.00 AB	1.83
SH	0 B	0	0.25 B	0.50
WR	2.75 A	0.50	2.75 A	1.26
WR+SH	0 B	0	0 B	0

[a] Assessed by planting tomato plants in soil samples from treated plots and evaluating galling index on a scale of 0 to 5, where 0 = no galls and 5 = more than 80% of the root surface galled. The statistical analysis was done on ranked values.

[b] Solarization was conducted for 55 days during Jul–Aug 2007. WR residues were applied to the soil at 0.9 kg/m^2 (0.4% w/w).

[c] Standard deviation.

[d] Values within a column followed by different letters are significantly different according to Tukey test at $P \leq 0.05$. SH main effect significantly reduced AUDPC compared with the non-solarized treatments according to the factorial analysis at $P \leq 0.0001$. No significant effect was found for depth, WR or interactions between the main effects according to the factorial analysis at $P \leq 0.05$.

Table 2.5. Effect of soil solarization (SH)[a] and wild rocket (WR) amendment on soil suppressiveness to *Meloidogyne javanica*[b], tomato root development and yield under farm conditions in tomato greenhouses (experiments 1 and 2)

Experiment[c]	Soil treatment	*M. javanica* galling index (0–2)		Root development index (1–3)		Yield[d] (kg/m^2)	
		Mean	SD[e]	Mean	SD	Mean	SD
1	Control	1.75[f] A	0.19	1.93 A	0.44	8.22 A	1.01
1	SH	1.39 B	0.21	2.44 A	0.27	8.70 A	2.08
1	WR	1.49 AB	0.16	2.30 A	0.45	9.12 A	2.08
1	WR+SH	1.48 B	0.19	2.45 A	0.39	8.27 A	0.82
2	Control	0.80 A	0.79	2.74 A	0.46	8.56 A	1.72
2	SH	0.38 A	0.29	2.89 A	0.14	9.27 A	1.95
2	WR	0.61 A	0.46	2.89 A	0.14	7.66 A	0.48
2	WR+SH	0.46 A	0.46	2.82 A	0.26	8.54 A	1.07

[a] Solarization was conducted for 40 days during Aug–Sep 2007. WR residues were applied to the soil at 0.4 kg/m^2 (0.4% w/w).

[b] Assessed by galling index and root development index of tomato plants which were grown for 7 months in the plots after soil treatments. Galling index on a scale of 0 to 2, where 0 = no galls and 2 = more than 50% of the root surface galled. Root development index on a scale of 1 to 3, where 1 = poorly root development, 2 = no development of deep roots and 3 = development of surface and deep roots. The statistical analysis was done on ranked values for each experiment separately.

[c] Two experiments were carried out in tomato greenhouses (310 m^2) naturally infested with *M. javanica*. The first (Exp. 1) was with no history of methyl bromide disinfestation. The second (Exp. 2) had been disinfested with methyl bromide before the previous crop.

[d] Tomato yield was assessed by total fruit biomass. There were no significant differences in fruit quality, i.e. number of fruits at each size scale, or in the biomass of the different size groups.

[e] Standard deviation.

[f] In each experiment, values within a column followed by different letters are significantly different according to Tukey test at $P \leq 0.05$.

Table 2.6. Effect of soil solarization (SH) and wild rocket (WR) amendment[a], on soil suppressiveness to *Meloidogyne javanica*[b], snapdragon root development and yield under farm conditions in a snapdragon nethouse (experiment 3)

Soil treatment	*M. javanica* galling index (0–5)		Root development index (1–4)		Yield[c] (kg/m^2)	
	Mean	SD[d]	Mean	SD	Mean	SD
Control	3.30[e] AB	0.54	2.19 A	0.38	25.65 A	2.60
SH	3.94 A	0.94	2.29 A	0.21	24.21 A	2.49
WR	2.87 B	0.79	2.31 A	0.42	24.79 A	2.32
WR+SH	3.85 A	1.13	2.00 A	0.38	24.19 A	3.42

[a] Solarization was conducted for 55 days during Jul–Aug 2007. WR residues were applied to the soil at 0.9 kg/m^2 (0.9% w/w).

[b] Assessed by galling index and root development index of snapdragon plants which were grown for 5 months in the plots after soil treatments. Galling index on a scale of 0 to 5, where 0 = no galls and 5 = more than 80% of the root surface galled. Root development index on a scale of 1 to 4, where 1 = poorly root development and 4= maximal root volume. The statistical analysis was done on ranked values.

[c] Snapdragon yield was assessed by total cut-flower biomass. There were no significant differences in flower quality, i.e. number of flowers with stems $\geq$ 50 cm (A class), $\leq$ 50 cm (B class), or in the biomass of the different quality groups.

[d] Standard deviation.

[e] Values within a column followed by different letters are significantly different according to Tukey test at $P \leq 0.05$.

Table 2.7. Effect of soil solarization (SH) and wild rocket (WR) amendment[a] on plant survival after inoculation[b] and area under the disease progress curve (AUDPC) of Fusarium crown and root rot in inoculated cucumber seedlings in soil samples from the tomato greenhouse (experiment 2) and snapdragon nethouse (experiment 3) after the cropping season

	Soil	Cucumber survival (%)		AUDPC[b]	
Experiment	treatment	Mean	SD[c]	Mean	SD
2	Control	12.5[d] B	11.9	1687.3 A	284.8
2	SH	50.0 A	27.5	1383.0 A	370.1
2	WR	17.9 B	18.3	1576.8 A	366.4
2	WR+SH	40.7 AB	30.0	1333.8 A	452.0
3	Control	40.2 AB	21.65	1345.1 AB	318.0
3	SH	51.2 AB	22.4	1049.6 BC	363.3
3	WR	34.4 B	17.5	1434.6 A	329.2
3	WR+SH	57.9 A	28.6	986.1 C	453.1

[a] In experiment 2, solarization was conducted for 40 days during Aug–Sep 2007. WR residues were applied to the soil at 0.4 kg/m^2 (0.4% w/w). In experiment 3, solarization was conducted for 55 days during Jul–Aug 2007. WR residues were applied to the soil at 0.9 kg/m^2 (0.9% w/w).

[b] Cucumber seedlings were inoculated with *Fusarium oxysporum* f. sp. *radicis-cucumerinum* macroconidia at 1.5 x 10^5 CFU/ml and planted in the soil samples from each field experiment. Cucumber survival is given for day 14, since after 28 days most of the plants had wilted. The plants were grown for 28 days and area under the disease progress curve (AUDPC) was calculated.

[c] Standard deviation.

[d] In each experiment, values within a column followed by different letters are significantly different according to Tukey test at $P \leq 0.05$. In both experiments, SH as main effect significantly increased survival and reduced AUDPC relative to the non-solarized treatments according to the factorial analysis at $P \leq 0.05$.

3. Induced soil suppressiveness – possible mechanisms

3.1. Examination of suppressiveness by different soil components. The suppressiveness characterization of wild rocket (WR)-amended soil reflected in significant reduction in diseased plants which were inoculated with FORC (Fig. 3.1). Therefore, this soil was served for studying possible mechanisms with regard to soil components, which were produced from WR-amended soil and involved in the suppressive phenomenon.

3.1.1. Induced soil suppressiveness by volatiles generated during organic amendment decomposition in the soil. Exposure of soil to volatile organic compounds from WR-amended soil during decomposition, significantly suppressed Fusarium crown and root rot incidence and AUDPC in inoculated cucumber transplants (Fig 3.1), although this reduction (by 53% in the AUDPC) was less pronounced compared to the AUDPC reduction in the WR-amended soil.

3.1.2. Effect of components of the aqueous soil extract on soil suppressiveness. No reduction of the pathogen by suppressive-soil extract was observed after 7 days of incubation; the figures of viable macroconidia were $4.2x10^4$ and $4.8x10^4$, in extracts from suppressive (WR-amended) and control (nonamended) soils, respectively. Aqueous extract from soil amended with WR, which includes soluble and suspended components (including microorganisms and extracellular enzymes), significantly reduced AUDPC of Fusarium crown and root rot when added to nonamended nonsuppressive soil (Fig 3.2A). This suppression, however, was at a lower extent than that observed when the inoculated seedlings were transplanted directly into suppressive soil (Fig. 3.1). The results presented in Fig 3.2B indicate that a certain fraction of the suppressive components was not removed from the suppressive soil from which the extraction was made, since the extracted soil still maintained a certain level of suppressiveness.

3.2. Induced disease resistance in the host. The capacity of suppressive soil to induce cucumber resistance to Fusarium crown and root rot was assessed by inoculation of plants which were first grown in the suppressive soil and afterwards inoculated and transplanted into nonsuppressive soil. Disease incidence of cucumber

wilt was similar in both soils indicating lack of induced resistance in transplants which were grown in suppressive soil for two weeks before inoculation and transplanting to nonsuppressive soil, compared with control nursery (AUDPC of 700±102 and 702±126, respectively). Similarly, No induced resistance to gray mold was found when cucumber plants were inoculated with *B. cinerea* and grown in suppressive soil, compared with plants in control soil (Fig. 3.3).

3.3. Pathogenic population dynamics in suppressive soil and root colonization by *Fusarium* spp.

3.3.1. Spore germination and development of FORC in the soil. The direct effect of WR-amended soil on pathogen germination and development, (i.e. pathogen suppressiveness), was tested in soil immediately after incubation with WR amendment, or after four repeated plantings and growing of inoculated cucumber transplants in the same soil. A sharp reduction (by 40%) in the viability of introduced FORC macroconidia was observed in soils, either WR-amended or not (Fig. 3.4A). The suppressive soil, which was used for repeated inoculated plantings, further and significantly increased the reduction in viability of macroconidia by additional 50%, 28 days after inoculation, compared with control (Fig 3.4B).

Germination of macroconida and production of new chlamydospores in the suppressive soil was not significantly different from the control soil after 24 and 48 hours (Fig 3.5). A similar trend was observed when previously inoculated and planted soils were used for incubation of macroconidia (Fig. 3.6), although formation of new chlamydospores was delayed and observed only at day 6 from inoculation (data not shown). However, no significant differences were observed between the germination and the formation chlamydospores between suppressive and control soils, and FORC mycelia growth on soils after incubation with or without WR amendment was similar (hyphae length of 743±226 and 628±24 μm, respectively, after 24h at 25 °C), namely, no inhibition was observed.

3.3.2. Root colonization of cucumber by *Fusarium* spp. following inoculation with FORC. In the present patho-system, we observed that disease suppression is expressed as a delayed disease onset at the early stages (Fig. 3.1). Disease symptoms (chlorosis) initiated in nonamended soil 6 days after inoculation and transplantation,

while in WR-amended soil, first symptoms appeared only after 14 days. Hence, we examined root colonization by FORC in suppressive compared with the control soil, concentrates the early stages after seedling inoculation. Cucumber seedlings, with or without inoculation with FORC macroconidia, were grown in the tested soils for 3 days (before symptom appearance in the inoculated transplants) and 6 days (when first symptoms appeared in the inoculated transplants in nonsuppressive soil) and root colonization with *Fusarium* spp. was evaluated. Inoculated cucumber roots were similarly colonized by *Fusarium* spp. 3 days after transplanting, in suppressive or in control soil. However, after 6 days, rate of colonization by *Fusarium* spp. was significantly lower (by 64%) from that in roots in the control soil (Table 3.1). Colonization of roots of the noninoculated transplants was low; it consisted 1.6-11% of the respective inoculated transplants (Table 3.1), therefore we assume that the majority of *Fusarium* propagules in the inoculated roots are FORC. The ratio of 64% decrease in root colonization by *Fusarium* spp. in suppressive soil at day 6 is in accordance with the reduction in wilted plants in the suppressive soil (60%), 21 days after inoculation and planting, compared with wilted plants in control soil (Fig 3.1).

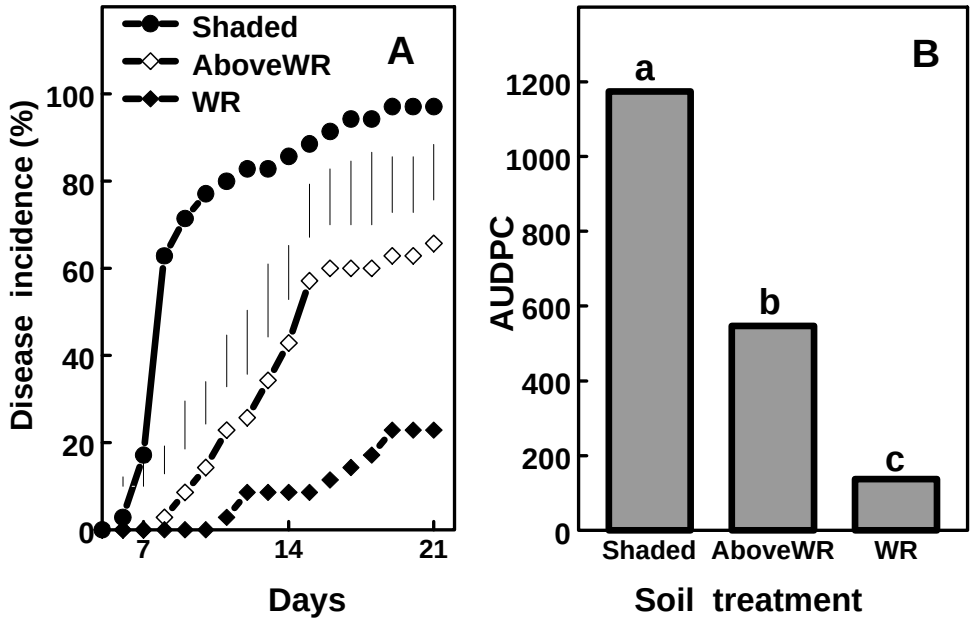

Fig. 3.1. Effect of incorporation of wild rocket (WR) in the soil, or exposure to volatile organic compounds generated from the amended soil (Above WR), on incidence (A) and area under disease progress curve (AUDPC) of Fusarium crown and root rot in cucumber (B). Cucumber seedlings were inoculated with macroconidia of *Fusarium oxysporum* f. sp. *radicis-cucumerinum* at 10^5 CFU ml^{-1}, before transplanting. Different letters in AUDPC values denotes a significant difference between the treatments. F Value=68.51; Pr>F <0.0001; Minimum significant difference 238.08. Vertical lines represent average SD.

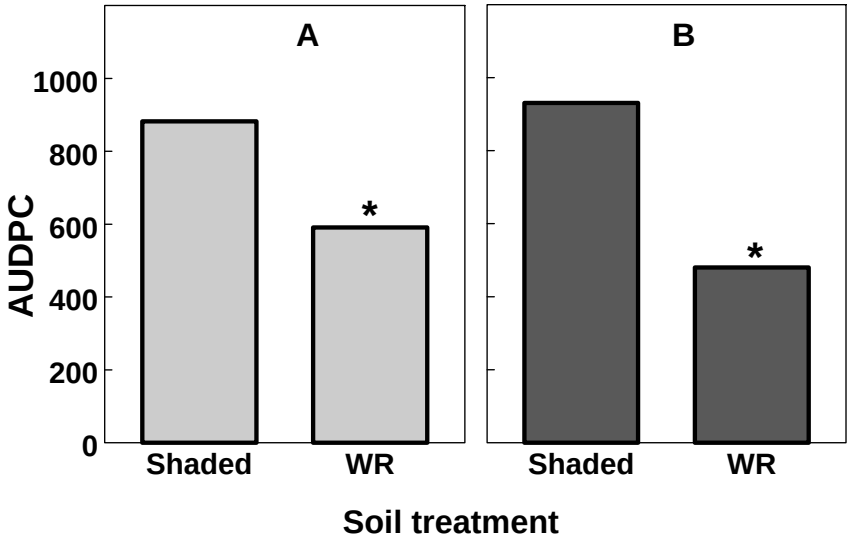

Fig. 3.2. Effect of application of crude aqueous extract from a suppressive soil (amended with wild rocket [WR]), or nonamended (Shaded) on Fusarium crown and root rot incidence in inoculated cucumber transplants. Macroconidia of *Fusarium oxysporum* f. sp. *radicis-cucumerinum* were first suspended in the soil extracts at 10^5 CFU ml^{-1}, and afterwards the suspension was used to infest nonamended soils before cucumber transplanting. A – The transplants were grown in soil inoculated with a pathogen suspension and soil extract from suppressive (WR) or nonsuppressive (Shaded) soil. B – The transplants were grown in soil from which the extraction was removed and inoculated with a pathogen suspension. Asterisk denotes a significant difference between soil extracts (F Value=123.55; Pr>F <0.0001; Minimum significant difference 67.45) (A) or extracted soils (F Value=130.31; Pr>F <0.0001; Minimum significant difference 96.46) (B).

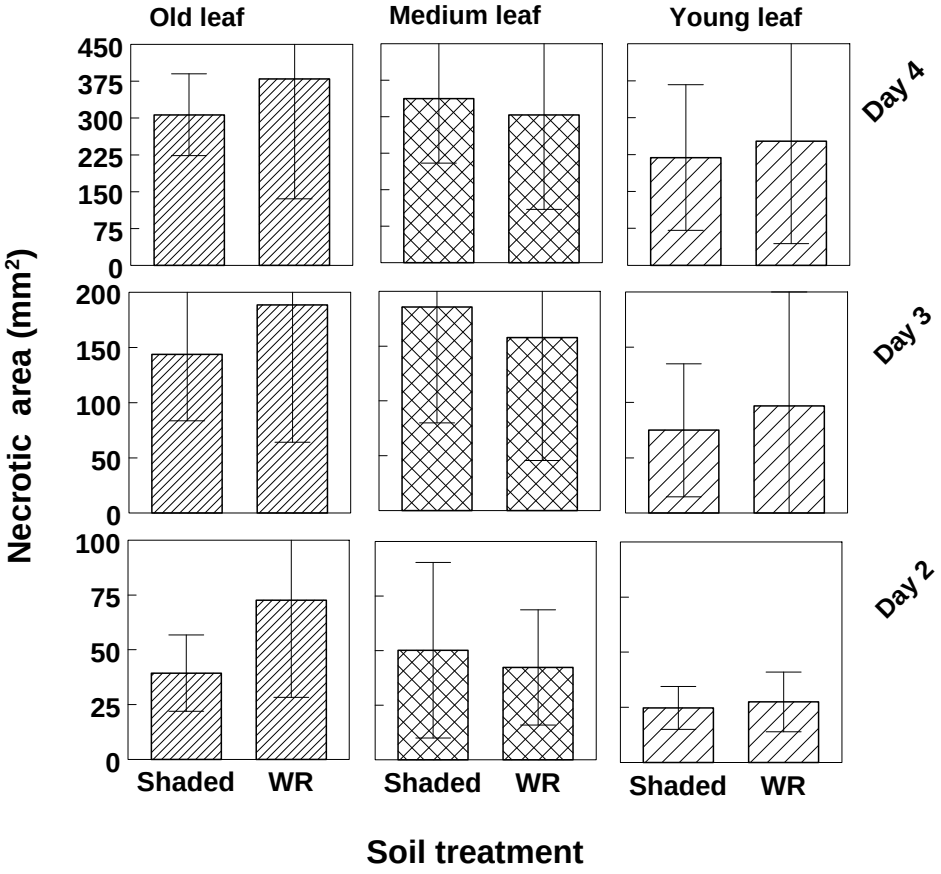

Fig. 3.3. The effects of growing cucumber transplants in suppressive (WR-amended [WR]) soil or nonamended soil (Shaded) on the size of the necrotic area infected by *Botrytis cinerea*, on inoculated leaves of cucumber, 5 weeks after sowing. Inoculation with the pathogen was performed on intact plants on each leaf from the top three (young, medium and old). The area of expanding necrotic diseased tissue was measured daily, 2 to 4 days after inoculation. No significant differences were observed between shaded or WR treatments.

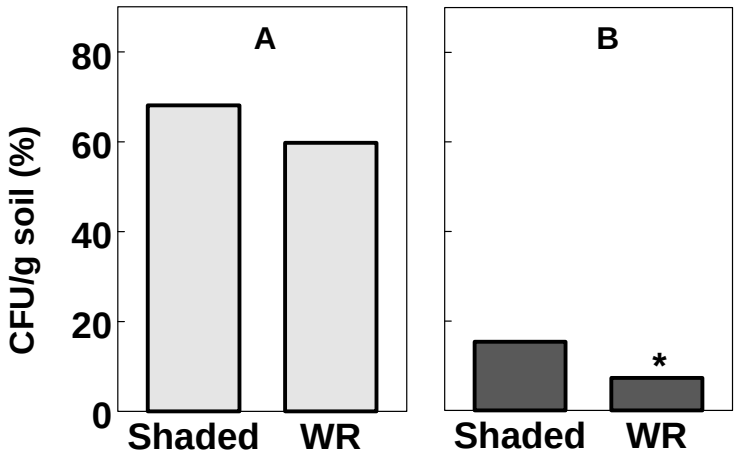

Fig. 3.4. Effect of suppressive (wild rocket amended [WR]) soil on the survival of macroconidia of *Fusarium oxysporum* f. sp. *radicis-cucumerinum*. A - Assay was done 28 days after soil incubation with WR or nonamended (Shaded), before the first crop cycle; B – Soil was assayed after four repeated plantings with FORC-inoculated cucumber transplants. Asterisk denotes a significant difference between the two soil treatments (F Value=78.21; Pr>F =0.0001; Minimum significant difference 2.23).

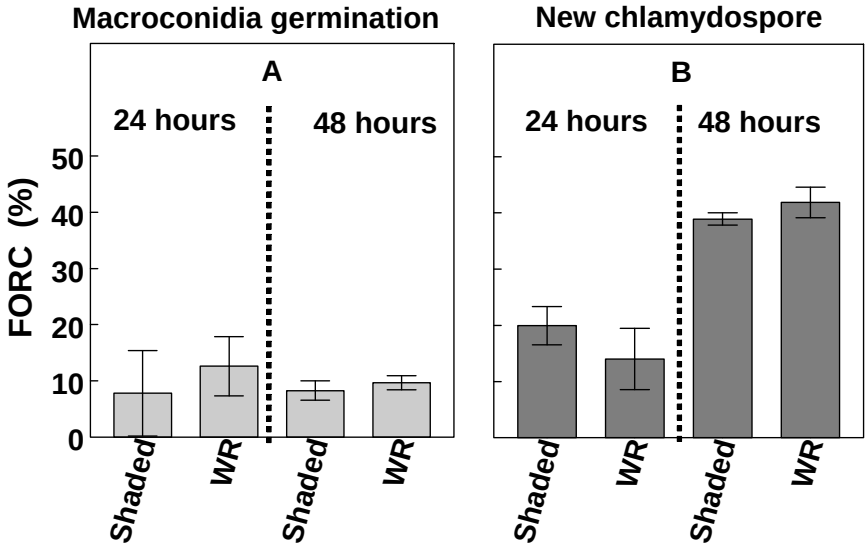

Fig. 3.5. Effect of suppressive (wild rocket amended [WR]) or nonamended (Shaded) soil on the viability of *Fusarium oxysporum* f. sp. *radicis-cucumerinum* (FORC) macroconidia, (A) and chlamydospores formation (B). FORC macroconidia were put on dialysis bags above the tested soil and germination and development were measured 24 and 48 hours afterwards. Values represent percent from macroconidia observed (minimum 100 CFU per treatment). No significant differences were observed between the two soil treatments.

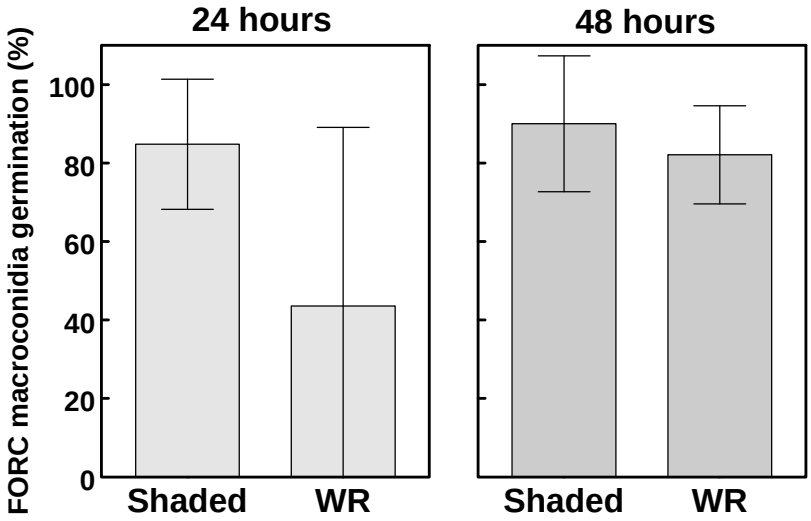

Fig. 3.6. Effect of suppressive (wild rocket amended [WR]) or nonamended (Shaded) soil on the germination of *Fusarium oxysporum* f. sp. *radicis-cucumerinum* (FORC) macroconidia. FORC macroconidia were put on dialysis bags above the soil and germination and development were measured 24 and 48 hours afterwards. The values represent percent from macroconidia observed (minimum 100 CFU per treatment). No significant differences were observed. New chlamydospores were observed only at day 6, without significant differences between the two soil treatments.

Table 3.1. Cucumber root colonization by *Fusarium* spp., following inoculation[x] and planting in suppressive (wild rocket amended, WR), or nonamended (Shaded) soil[y]

		Days after planting	
		3	6
Transplants inoculation[x]	Soil treatment[y]	CFU /g fresh root	CFU / g fresh root
None	Shaded	2,175[z] Ba	1,381 Ba
None	WR	563 Ba	1,828 Ba
Inoculated	Shaded	19,320 Aa	86,652 Aa
Inoculated	WR	24,222 Aa	31,188 Ab

[x] Cucumber seedlings were inoculated with *Fusarium oxysporum* f. sp. *radicis-cucumerinum* macroconidia at 10^5 CFU/ml and planted in the previously treated soils.

[y] Shading under field conditions was conducted for 28 days during July 2009. Plant residues were applied to the soil at 10 g/kg (1% w/w).

[z] For each day, values within a column followed by different letters are significantly different according to GLM and Tukey's studentized range (honestly significant difference) test at $P \leq 0.05$. Different uppercase letters denote significant difference in inoculation level; different lowercase letters denote significant difference in soil treatment level. Values of inoculated plants in the shaded treatment at day 6 are significantly different from the corresponded values at day 3 (Minimum significant difference= 45,020).

4. Root-associated microbiome under suppressive soil environment

4.1. Cucumber root colonization by FORC following transplant inoculation. Real-time PCR quantification of FORC in the roots 3 days after inoculation (in plants with no expressed symptoms) showed similar target numbers for both WR-amended and nonamended soils (Fig. 4.1A), indicating similar inoculum density in the root and infection potential. In contrast, target numbers of FORC were significantly lower (by 66%, $P < 0.01$) 6 days after inoculation and transplanting in the suppressive soil, compared with the nonamended one (Fig. 4.1B). No FORC targets were detected in control, noninoculated plants.

4.2. Impact of root inoculation by FORC and transplantation in suppressive soil on the composition of root fungal community. Fungal colonization of cucumber roots in the suppressive soils was assessed using mass sequencing. A set of 182,204 high-quality fungal ITS sequences were analyzed (Table 4.1). These sequences were grouped into 33 OTUs, using a 99% sequence-similarity threshold. The fungal community was characterized by low species diversification in roots of all treatments (Tables 4.1 and 4.2): 4 out of 33 OTUs comprised 96% of the total fungal sequences in all samples. These OTUs were affiliated with *Fusarium oxysporum*, *Fusarium* sp. 14005, *Chaetomium* sp. 15003, and an unclassified Ascomycota (Table 4.2). Factorial analysis demonstrated the relative abundances of these major groups, which were highly affected by root inoculation with FORC ($P < 0.001$), but were not affected by WR amendment or day of sampling. As expected, the relative abundance of sequences affiliated with *F. oxysporum* increased 10-fold following root inoculation with FORC, while the relative abundances of the other major groups were reduced. This reduction was similar for the additional detected fungal OTUs (data not shown).

Root inoculation with FORC affected fungal community composition in both suppressive and control soils, 3 and 6 days after inoculation and transplanting (Fig. 4.2A). Whereas fungal communities in samples from inoculated roots were closely clustered, samples from noninoculated roots were scattered (regardless of the soil), indicating that FORC inoculation is the major factor affecting the composition of the root fungal community. When the *F. oxysporum*-affiliated OTUs were removed from the analysis (Fig. 4.2B), there was no evident clustering of the samples with respect to

either soil type or FORC inoculation. Some separation could be observed between 3-day and 6-day samples (Fig. 4.2B). However, this trend was not consistent with the total OTU analysis (Fig. 4.2A).

4.3. Impact of root inoculation with FORC and transplantation in suppressive soil on root colonization by bacteria. Real-time quantitative PCR revealed no significant differences in the abundance of total bacteria associated with cucumber roots 3 and 6 days after transplantation in suppressive or control soils, regardless of FORC inoculation (Table 4.3).

Mass sequencing of bacterial 16S rRNA gene fragments showed a high diversity of bacteria associated with the roots. Based on a data set of 81,731 high-quality sequences, 3,490 OTUs were defined using a 97% sequence-similarity threshold, which were affiliated with 792 genera. Based on genus classification, root inoculation with FORC significantly increased the taxonomic richness (Chao1 estimate) and moderately reduced community dominance levels 3 days after transplantation in both nonamended and suppressive soils (Table 4.4). This effect continued to 6 days after transplantation in the nonamended soil. In contrast, a sharp decrease in the dominance levels of bacterial communities was evident 6 days after transplantation in the suppressive soil, regardless of FORC inoculation (Table 4.4). Comparative analysis of root bacterial community compositions revealed divergence in root assemblages from nonamended and suppressive soils, 6 days after transplantation (Fig. 4.3).

The effect of FORC inoculation on bacterial community composition only became apparent 6 days after transplantation. Proteobacteria were the dominant bacterial phylum colonizing the cucumber roots (75 to 93% of the sequences in all treatments) (Table 4.5). Among the Proteobacteria, β-proteobacteria and α-proteobacteria were the most dominant (19 to 73% and 11 to 46%, respectively). Suppressive soil significantly altered the relative abundance of the dominant bacterial groups due to a reduction in *Massilia* (β-proteobacteria), the single most dominant genus in the dataset ($P < 0.05$), compared with the root-associated bacteria from nonamended soil (Table 4.5). After 6 days, the relative abundance of root-associated *Massilia* was four to five times lower in the WR-amended, suppressive soil relative to

nonamended soils. The relative abundance of *Massilia* in the FORC-inoculated roots was further reduced in the suppressive soil plants ($P < 0.05$). In contrast, the relative abundance of *Rhizobium*, *Devosia* and other α-proteobacteria in the roots significantly increased in suppressive soil compared with the nonamended one ($P < 0.05$).

Quantitative analysis of root-associated *Massilia* revealed one order of magnitude lower abundance on roots of suppressive soil, compared with nonamended soil, after 6 days of growth (Table 4.3). These quantitative real-time PCR results were consistent with those obtained by mass sequencing.

The relative abundance of root-associated actinobacteria, including *Streptomyces*, was significantly higher in suppressive vs. nonamended soil, with no significant effect of FORC inoculation (Table 4.5). Three days after transplantation, the number of *Streptomyces*, which was the dominant taxon in the actinobacteria, was significantly higher in the suppressive vs. nonamended soil (Table 4.3). However, its quantity in the roots of FORC-inoculated transplants decreased significantly after 6 days compared with the noninoculated transplants in the nonamended soil (Table 4.3).

The relative abundance of members of the Firmicutes, namely, *Bacillus* and *Paenibacillus,* also increased in the suppressive soils compared with the nonamended soils, for both 3- and 6-day-old transplants (Table 4.5).

Root infection with FORC significantly affected the relative abundance of some bacterial taxons. For example, *Mitsuaria* (β-proteobacteria) was detected almost exclusively in FORC-inoculated nonamended soil and the relative abundance of *Methylophilus* (β-proteobacteria) increased following FORC inoculation, irrespective of soil type (Table 4.5).

4.4. Composition of root-associated *Streptomyces*. The relative abundance of root-associated *Streptomyces* increased in the suppressive soils compared with the nonamended soils as early as 3 days post-FORC inoculation and transplantation (Table 4.5). A significant shift in composition of the root-associated *Streptomyces* populations between control and suppressive soils was found 6 days after transplantation according to PCR-DGGE analysis (Fig. 4.4). In contrast, FORC inoculation had no visible effect on the community fingerprint, in either suppressive or nonamended soils. Similar results were found in roots 3 days after transplantation

(Fig. 4.5). The difference between root *Streptomyces* community fingerprints in WR-amended and nonamended soils was mainly attributed to a dominant band (Figs. 4.4 and 4.5, marked with arrow) that appeared in all samples from WR-amended soil. This band also appeared in some samples from nonamended soil, but in most of them, at much lower intensity. Sequence analysis of this band was performed for eight excised bands representing all of the treatments. All obtained sequences were identical and were 99.9% similar to the *Streptomyces humidus*-related population (HQ607425) according to NCBI-Blast analysis.

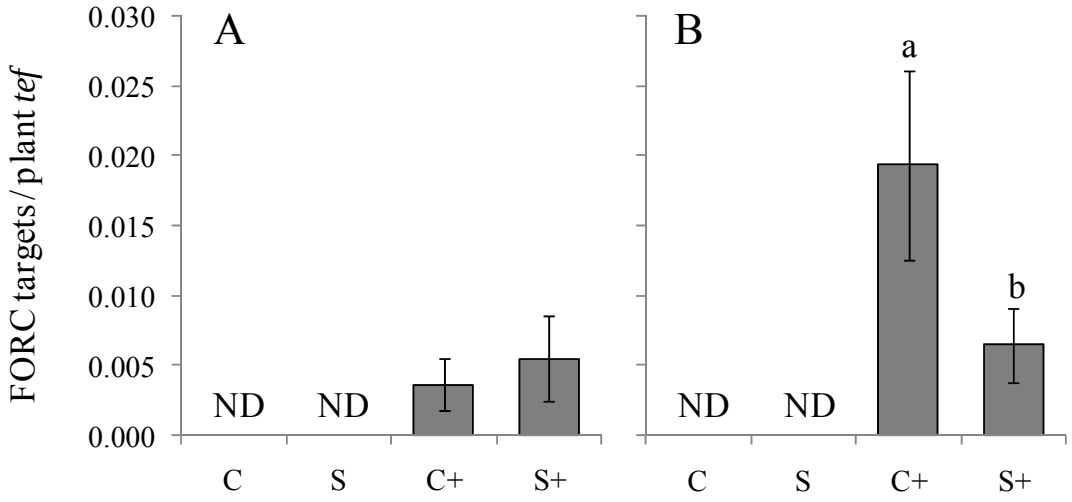

Fig. 4.1. Quantification of *Fusarium oxysporum* f. sp. *radicis-cucumerinum* (FORC) in roots of cucumber grown for 3 (A) or 6 (B) days in wild rocket-amended, suppressive soil (S) or control soil (C). The seedlings were inoculated with macroconidia of FORC at 10^5 CFU/ml (+), or noninoculated, before transplanting. FORC was quantified using a specific primer pair and normalized using the reference plant *tef* gene. ND: not detected. Different letters indicate significant differences between the means according to Kruskal-Wallis ANOVA at $P < 0.01$.

Table 4.1. Analysis of fungal community structure based on mass sequencing of fungal ITS gene fragments. Coverage and diversity indices were calculated for the different samples after classification of the sequence data at the genus level. Means and standard deviations (n = 5) are presented

Day	Soil	FORC[x]	Σ Sequences[y]	Number of 99% OTUs[z]
3	Control	-	9,112	9±3
3	Suppressive	-	11,315	9±3
3	Control	+	17,962	9±2
3	Suppressive	+	19,989	7±1
6	Control	-	17,071	14±2
6	Suppressive	-	22,325	11±2
6	Control	+	46,100	8±2
6	Suppressive	+	38,330	7±2

[x] Cucumber seedlings were inoculated with macroconidia of *Fusarium oxysporum* f. sp. *radicis-cucumerinum* (FORC) at 10^5 CFU/ml (+), or noninoculated (-), before transplanting.

[y] Number of sequences. Coverage estimates, calculated after Good, 1953 were >99.9% for all samples. C_{Good} = 1-F1/N, F1: number of singletons, N: number of sequences.

[z] Operational taxonomic units; classification using 99% similarity threshold.

Table 4.2. Composition and relative abundance of fungi associated with cucumber roots, in control (C) and suppressive (S) soils, as affected by *Fusarium oxysporum* f. sp. *radicis-cucumerinum* (FORC) inoculation at 10^5 CFU/ml (+). The composition was determined by mass sequencing of the fungal ITS genes. Data from seedling 3 and 6 days after inoculation and transplantation were combined. Mean relative abundances of the four major OTUs (99% similarity threshold) are presented. Mean relative abundances were compared by non-parametric Kruskal-Wallis test. Different letters in a line indicate significant differences at $P < 0.05$

Closest NCBI relative		% Identity	Relative abundance (%) on roots from soil			
			C	S	C+	S+
Fusarium oxysporum	JF440593	99	13.5b	2.9b	89.0a	88.2a
Fusarium sp. 14005	EU750680	99	46.3a	68.0a	3.4b	6.4b
Chaetomium sp. 15003	EU750691	98	21.1a	15.9a	6.4b	2.8b
Unclassified Ascomycota	EU437434	98	3.5ab	8.2a	0.7b	2.3ab
		Total	84.3	94.9	99.4	99.7

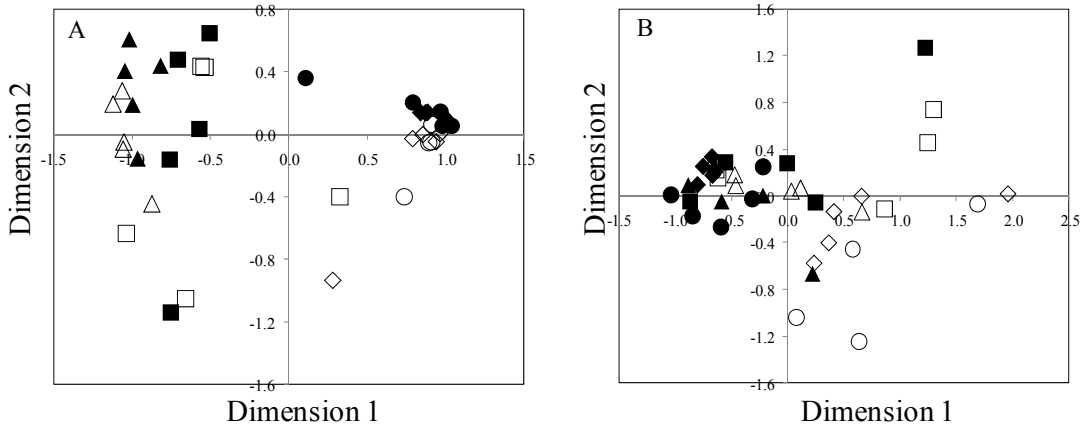

Fig. 4.2. Comparison of root-associated fungal community based on mass sequencing of ribosomal internal transcribed spacer (ITS) by 454-pyrosequencing. Fungal community composition was determined for cucumber roots grown for 3 (open symbols) or 6 (filled symbols) days after inoculation with macroconidia of *Fusarium oxysporum* f. sp. *radicis-cucumerinum* (FORC) at 10^5 CFU/ml or noninoculation, and transplantation in: ■ Control soil; ▲ Wild rocket-amended soil; ◆ Control soil + FORC; ● Wild rocket-amended soil + FORC. Sequences were classified into OTUs using a 99% similarity threshold. After Hellinger transformation of the count data, a 1-Pearson r distance matrix between the different samples was calculated. The matrix was used for nonmetric multidimensional scaling analysis by STATISTICA. The first and second dimensions are presented for (A) the complete dataset (Stress = 0.065) and (B) after omitting *Fusarium oxysporum*-affiliated sequences (Stress = 0.056).

Table 4.3. Quantification of total bacteria, *Streptomyces* and *Massilia* on cucumber roots 3 and 6 days after inoculation with *Fusarium oxysporum* f. sp. *radicis-cucumerinum* (FORC) and transplantation. Quantification based on real-time quantitative PCR using the plant *tef* gene for normalization of the data. The means (n = 5) are presented. Means were compared by factorial ANOVA. Only statistically significant sources and interactions are indicated. Different letters in a column indicate significant differences at $P < 0.05$

			Log targets / plant *tef*		
Day	Soil	FORC	Total bacteria	*Streptomyces*	*Massilia*
3	Control	-	2.14 ± 0.16	-0.18 ± 0.18 b	1.40 ± 0.20
3	Suppressive	-	2.20 ± 0.29	0.20 ± 0.22 a	1.09 ± 0.25
3	Control	+	1.96 ± 0.37	0.08 ± 0.11 ab	1.41 ± 0.43
3	Suppressive	+	1.88 ± 0.78	0.28 ± 0.29 a	0.96 ± 0.79
6	Control	-	2.25 ± 0.79	1.79 ± 0.44 a	2.20 ± 0.45 a
6	Suppressive	-	2.24 ± 0.16	1.42 ± 0.13 ab	0.78 ± 0.36 b
6	Control	+	2.54 ± 0.14	1.53 ± 0.22 ab	2.06 ± 0.23 a
6	Suppressive	+	2.36 ± 0.19	1.23 ± 0.09 b	0.58 ± 0.45 b

Table 4.4. Analysis of bacterial community structure based on mass sequencing of 16S rRNA gene fragments. Coverage and diversity indices were calculated for the different samples after classification of the sequence data at the genus level. Means and standard deviations (n = 5) are presented for coverage, richness, dominance and Shannon H' diversity estimators

Day	Soil	FORC[t]	Σ Sequences[u]	97% OTUs	Genera	Coverage[v,w]	Diversity indices		
							Chao1[x,w]	Dominance[y]	H'[z]
3	Control	-	11,260	524±54	116±16	0.979	173	0.285	2.38
3	Suppressive	-	7,967	466±63	122±16	0.968	189	0.205	2.79
3	Control	+	15,300	628±99	168±26	0.979	239	0.185	2.94
3	Suppressive	+	11,872	472±36	145±17	0.977	200	0.129	3.13
6	Control	-	12,246	572±54	158±13	0.975	222	0.266	2.67
6	Suppressive	-	5,917	429±76	135±10	0.974	206	0.071	3.52
6	Control	+	10,637	499 ±78	142±22	0.975	206	0.155	3.06
6	Suppressive	+	6,496	415±36	137±10	0.958	216	0.059	3.66
Neuman-Keuls critical range							43	0.071	0.37

[t] Cucumber seedlings were inoculated with macroconidia of *Fusarium oxysporum* f. sp. *radicis-cucumerinum* (FORC) at 10^5 CFU/ml (+), or noninoculated (-), before transplanting.

[u] Number of sequences**.**

[v] Sample coverage estimate calculated after Good (1953): $C_{Good} = 1-F1/N$, F1: number of singletons, N: number of sequences.

[w] No significant differences according to ANOVA and Tukey HSD test (P = 0.05).

[x] Chao1 taxa richness estimate (Chao, 1984).

[y] Dominance $D = \Sigma((n_i/N)^2)$, n_i: number of sequences assigned to the *i*th genus.

[z] Shannon H' index of diversity $H' = -\Sigma p_i \cdot \ln(p_i)$, p_i: relative abundance of the *i*th genus.

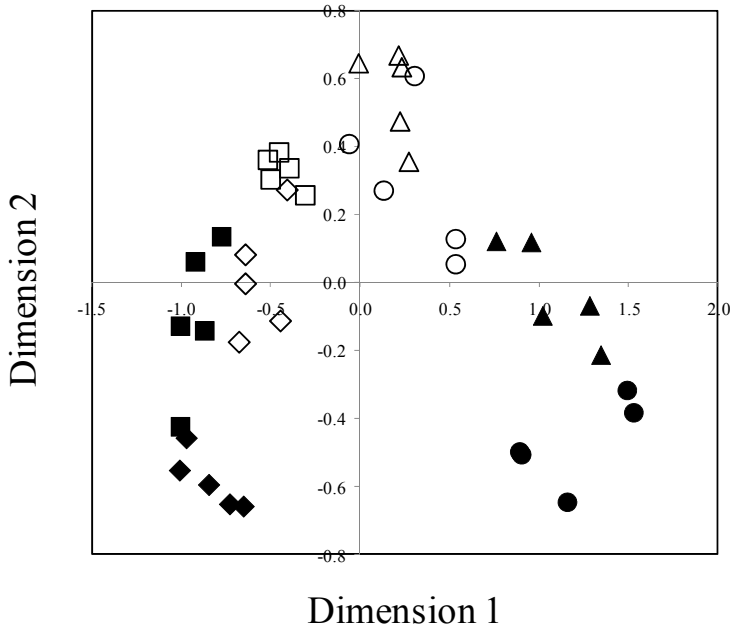

Fig. 4.3. Comparison of the bacterial community composition on roots based on mass sequencing of bacterial 16S rRNA gene fragments. Bacterial community composition was determined for cucumber roots grown for 3 (open symbols) or 6 (filled symbols) days after inoculation with macroconidia of *Fusarium oxysporum* f. sp. *radicis-cucumerinum* (FORC) at 10^5 CFU/ml, or noninoculation, and transplantation in: ■ Control soil; ▲ Suppressive soil; ◆ Control soil + FORC inoculation; ● Suppressive soil + FORC inoculation. Sequences were classified into OTUs using a 97% similarity threshold. After Hellinger transformation of the count data, a 1-Pearson r distance matrix between the different samples was calculated. The matrix was used for nonmetric multidimensional scaling analysis by STATISTICA (Stress = 0.038). The first and second dimensions are presented.

Table 4.5. Composition and relative abundance (%) of the major taxonomic groups identified in cucumber root bacterial communities by mass sequencing of 16S rRNA gene fragments by 454-pyrosequencing

	3 days				6 days			
	Null[w]		FORC[w]		Null		FORC	
Taxonomy	C	S	C	S	C	S	C	S
Proteobacteria	93.4	85.6	90.2	86.5	89.2	74.8	86.2	76.7
β-proteobacteria	69.0	56.7	64.3	45.2	73.3	21.9	61.3	19.3
Massilia [bc][x]	51.9	42.5	41.0	28.3	50.7	12.5	34.3	6.7
Janthinobacterium [c]	1.7	0.8	2.1	0.3	4.2	0.3	4.5	0.5
Methylophilus [b]	1.6	1.8	2.5	2.8	1.5	0.9	3.2	4.0
Methylobacillus [c]	ND[y]	0.2	ND	0.4	<0.1%	1.64	ND	1.6
Mitsuaria [d]	ND	ND	1.6	<0.1	ND	ND	3.1	ND
Thauera [f]	0.1	0.3	0.1	0.2	<0.1	0.2	0.7	1.5
α-proteobacteria	20.8	23.3	21.1	33.3	10.7	43.2	19.6	45.8
Rhizobium [c]	7.2	9.8	6.0	10.3	4.3	17.1	7.0	16.6
Brevundimonas [f]	4.2	2.9	3.4	6.1	0.9	4.4	4.2	6.1
Devosia [c]	0.7	2.3	0.6	2.4	0.3	6.0	0.3	4.9
Shinella_genera [c]	0.4	1.5	0.5	0.9	0.3	4.5	0.4	5.3
Mesorhizobium [c]	1.3	1.5	1.1	1.4	0.9	1.9	0.8	1.9
Sphingopyxis [c]	0.1	0.4	<0.1	0.3	<0.1	1.3	0.2	1.5
Phenylobacterium [ab]	0.2	0.3	0.4	0.7	0.2	0.7	0.4	1.0
γ-proteobacteria	3.5	5.4	4.8	7.7	4.7	9.5	5.2	11.5
Pseudomonas	1.9	2.0	2.7	3.2	1.9	1.6	2.4	3.4
Cellvibrio	<0.1	0.1	<0.1	0.4	0.2	2.8	<0.1	2.3
Colwellia [c]	0.2	0.6	0.2	0.6	<0.1	1.5	0.2	1.8
Stenotrophomonas [e]	0.2	0.3	0.1	0.4	0.2	0.2	0.7	1.1
Bacteroidetes	2.5	2.8	4.7	2.7	4.7	4.2	10.8	5.6
Chryseobacterium [d]	0.1	<0.1	0.7	<0.1	0.2	ND	5.5	ND
Chitinophagaceae [a]	1.7	0.7	2.3	0.8	3.4	1.0	4.6	2.0
Flavisolibacter [c]	0.1	<0.1	0.2	<0.1	1.2	0.3	0.8	0.4
Sphingobacterium [a]	0.5	0.9	0.9	0.8	0.4	1.1	0.3	1.0
Actinobacteria	2.6	4.6	3.4	4.3	3.5	9.6	1.4	4.8
Streptomyces [d]	0.4	2.0	1.2	2.4	1.9	6.2	0.6	2.6
Firmicutes	1.1	5.7	1.4	4.3	2.1	8.5	1.4	9.7
Bacillus [a]	<0.1	1.53	<0.1	1.2	ND	1.0	ND	1.0
Paenibacillus [c]	0.5	2.0	0.8	1.8	1.6	5.9	0.7	7.6
Planococcaceae [a]	<0.1	1.1	<0.1	0.5	ND	0.9	ND	0.7
Other phyla[z]	0.4	1.5	0.3	2.5	1.1	3.2	0.3	3.2

[w] Null and FORC: noninoculated (Null) and *Fusarium oxysporum* f. sp. *radicis-cucumerinum* (FORC) inoculation at 10^5 CFU/ml, respectively. C: control soil; S: suppressive soil.

[x] For each indicated taxon, the mean relative abundances were compared by factorial ANOVA followed by Tukey HSD test. The letters indicate significant differences ($P < 0.05$) between the factors: day (a), soil (b), FORC (c), day x soil (d), soil x FORC (e), day x FORC (f), day x soil x FORC (g).

[y] Not detected.

[z] Combination of phyla in which the total taxonomic groups detected had relative abundance <1%.

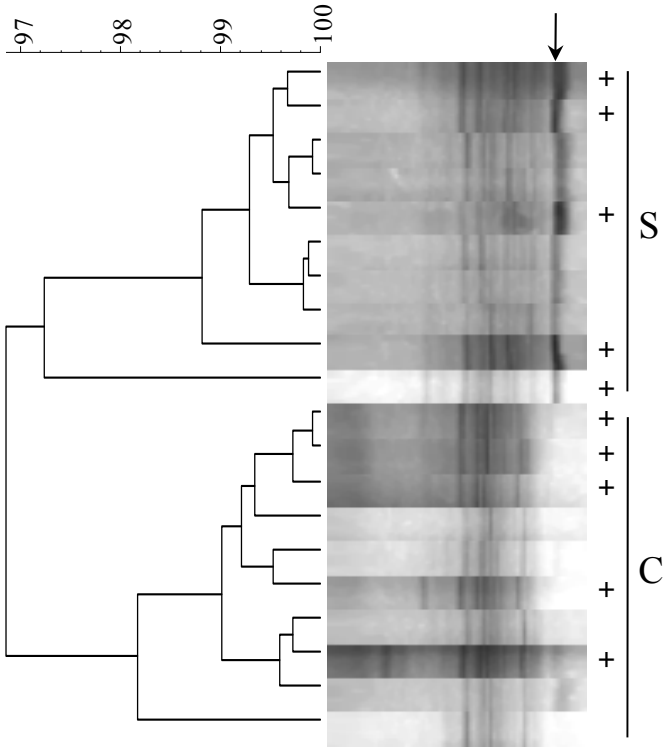

Fig. 4.4. Community fingerprint of root *Streptomyces* in cucumber seedlings 6 days after transplantation into suppressive soil (S) compared with control soil (C). Cucumber seedlings were inoculated with macroconidia of *Fusarium oxysporum* f. sp. *radicis-cucumerinum* (FORC) at 10^5 CFU/ml (+), or noninoculated before planting. *Streptomyces* community compositions were compared based on PCR-DGGE patterns of 16S rRNA gene fragments amplified with a *Streptomyces*-specific primer pair. PCR-DGGE patterns were analyzed using Fingerprint II software. A UPGMA tree was calculated from cosine correlation distance matrix. The arrow indicates bands corresponding to *Streptomyces* populations identified by sequence analysis of excised bands.

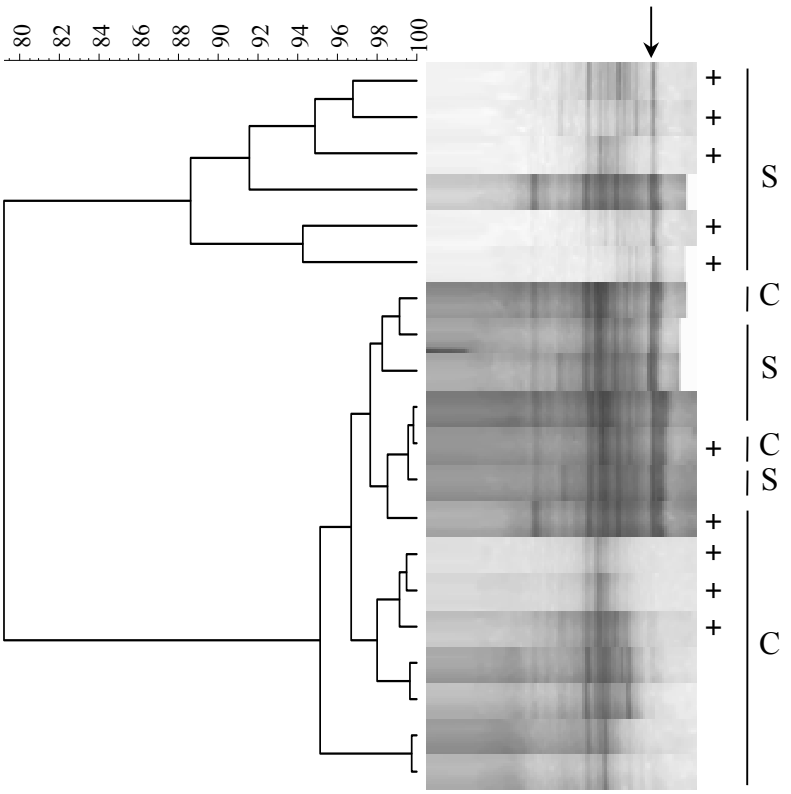

Fig. 4.5. Community fingerprint of root *Streptomyces* in cucumber seedlings 3 days after transplantation into suppressive soil (S) compared with control soil (C). Cucumber seedlings were inoculated with macroconidia of *Fusarium oxysporum* f. sp. *radicis-cucumerinum* (FORC) at 10^5 CFU/ml (+), or noninoculated before planting. *Streptomyces* community compositions were compared based on PCR-DGGE patterns of 16S rRNA gene fragments amplified with a *Streptomyces*-specific primer pair. PCR-DGGE patterns were analyzed using the Fingerprint II software. A UPGMA tree was calculated from cosine correlation distance matrix. The arrow indicates bands corresponding to *Streptomyces* populations identified by sequence analysis of excised bands.

DISCUSSION AND CONCLUSIONS

In the present study, we focused on soil suppressiveness, i.e. the reduction in disease level when a pathogen is introduced into a soil after it has been amended and incubated with various OAs or disinfested by solarization (Figs. 1.1 and 1.2, Tables 1.1 and 1.2). A direct impact on the pathogen and disease reduction which results from direct action on the pathogen during the incubation process was not evaluated in these studies. These aspects have been discussed in previous publications (Klein et al., 2007; Klein et al., 2011a). The suppressive capacity of soil is not necessarily related to direct pathogen control, which occurs during the incubation process and has been documented in numerous studies dealing with disease control by OAs (Blok et al., 2000; Lodha et al., 1997; Ramirez-Villapudua and Munneke, 1988; Subbarao et al., 1999). The combination of solarization and OAs, while showing an added value in pest control (Gamliel and Stapleton, 1993b; Klein et al., 2007), does not necessarily provide a higher suppressiveness potential. The present study demonstrates that crop residues alone provide the most significant suppressive effect (Figs. 1.1 and 1.2; Table 1.1). Apparently, the mechanisms involved in the two processes, i.e. pest control using solarization and OAs and disease suppressiveness, may not be related, since solarization had no significant effect on suppressiveness in the repetitive inoculated planting cycles (Fig. 1.3).

1. Soil suppressiveness to Fusarium crown and root rot in inoculated cucumber seedlings

The evolution of soil suppressiveness to FORC, as reflected in reduced disease incidence and AUDPC, was evident in three tested soils (Fig. 1.4), with two types of inoculum (Fig. 1.1, Table 1.4) and various OAs (Tables 1.1 and 1.2, Figs. 1.1 and 1.2). Disease suppressiveness was reflected in the OA-treated soil by a delay in disease onset and 20 to 80% lower plant mortality (Figs. 1.1-1.4), phenomena which are associated with disease suppressiveness (Hornby, 1983; Steinberg et al., 2006; Yogev et al., 2006). Moreover, a long-term effect (lasting 34 months, Figs. 1.2 and 1.3, and with repeated plantings, Fig. 1.3, Tables 1.1 and 1.2) was also recorded, further indicating the evolution of soil suppressiveness. Soil suppressiveness against FORC, as also manifested with other formae specialis of *Fusarium*, was demonstrated

following consecutive inoculation and cucumber plantings in compost-amended soil by Yogev et al. (2006). The long-term effect of OAs in producing suppressiveness, as observed in our study, was more pronounced than that reported previously in other studies (Motisi et al. 2009; Yulianti et al. 2007). Yulianti et al. (2007) suggested that an increase in microbial activity following the decomposition of WR or *B. nigra* manures plays a dominant role in suppressing *Rhizoctonia solani*. Motisi et al. (2009) found a suppressive effect using *B. juncea* which lasted for 13 days, while the predominant glucosinolates, which may have a direct effect on the pathogen, were hydrolyzed in less than 3 days.

Most of the plant residues tested for suppressiveness had been previously found effective in controlling soilborne pathogens or to have the potential to control pathogens as a result of the generation of biotoxic molecules, e.g. essential oils or glucosinolates (Bennett et al., 2006; Blok et al., 2000; Daferera et al., 2003; Lodha et al., 1997; Ploeg and Stapleton, 2001; Postma et al., 2010; Subbarao et al., 1999). In the present study, the decomposition of OAs in soil prior to infestation was carried out under aerobic conditions (Klein et al., 2007). Generally, under such conditions, the effect of decomposition products of plant residues on the pathogen population is variable. In contrast, under anaerobic conditions, a significant reduction in pathogen populations was found irrespective of the type of residues incorporated (Bonanomi et al., 2007). The decomposition of OAs under anaerobic conditions, although directly controlling soilborne pathogens, did not induce soil suppressiveness in some trials while it did in others (Blok et al., 2000; Goud et al., 2010), similar to the variable effects seen when using solarization. This further supports the hypothesis that the native microflora plays an important role in developing suppressiveness (Cook and Baker, 1983; Hornby, 1983; Momma et al., 2010).

The potential of different plant residues, as well as composts, to induce soil suppressiveness against plant diseases has been documented (Kuter et al., 1983; Mazzola, 2004; Noble and Coventry, 2005; Raaijmakers et al., 2009). In most cases, it was correlated with antagonistic or competitive microbial activity. Soil suppressiveness will persist for longer periods if the OA is functioning through enhanced activity of the resident soil microbial community, rather than through the introduction of a novel active community (Mazzola, 2004). However, this activity may be nullified by antimicrobial means such as soil disinfestation (Baker, 1987).

In the present study, the effect of soil solarization, alone or combined with OAs, on soil suppressiveness to Fusarium disease in cucumber was variable (Tables 1.1, 1.2, 1.4, Fig. 1.2). This is in contrast to previous studies in which solarization-induced soil suppressiveness toward various pathogens was reported (Freeman et al., 1990; Greenberger et al., 1987; Kassaby, 1985; Martyn and Hartz, 1986), and also to the findings in the field studies (discussed later in 'soil suppressiveness to root knot galling by *Meloidogyne javanica*'). Greenberger et al. (1987) found that solarization increased suppressiveness to *F. oxysporum* f. sp. *lycopersici* in eight out of 12 tested soils. In contrast, Mihail and Alcorn (1984) found no reduction in a population of *Macrophomina phaseolina* following solarization, probably due to the treatment's elimination of antagonistic or competitive microflora in the soil. The inconsistency in suppressiveness following solarization can be related to differences within a given soil or among soils from different locations. For example, when soil samples were taken from different locations of Rehovot soil (Greenberger et al., 1987), one sample of solarized soil increased suppressiveness whereas another sample of this soil slightly reduced it. Deadman et al. (2006) found that solarization improved soil suppressiveness when combined with OAs. Solarization of cabbage-amended soil reduces the inoculum potential of *Pythium aphanidermatum* during the following winter growing season, leading to a 40% reduction in damping-off of cucumber plants compared to solarization alone, although inoculum density was similar in both treatments. In that study, solarization alone did not contribute to suppressiveness. It is also possible that soil suppressiveness following OA and solarization depends on the challenged pathogen in a given soil (in this study mainly FORC, but also discussed later in 'soil suppressiveness to root knot galling by *Meloidogyne javanica*').

The level of suppressiveness induced by WR was reduced by increased inoculum density (Table 4). Similarly, infection rates of Fusarium wilt of pea in chitin-amended soil were found to be directly correlated with chlamydospore density, in comparison with nonamended controls (Guy and Baker, 1977). Smith and Snyder (1971) distinguished suppressive soils by the rate of chlamydospores needed to induce Fusarium wilt on sweet potatoes. They found that in a suppressive soil, the inoculum density required to cause disease was twice that in conducive or noncultivated-suppressive soils. In addition, a higher percentage of chlamydospores of saprophytic *Fusarium* germinated in wilt-suppressive soils than did chlamydospores of the tested pathogens (Smith and Snyder, 1972).

2. Soil suppressiveness to root knot galling by *Meloidogyne javanica*, under controlled and agricultural conditions

In the small plot experiment, soil amendment with herb residues before inoculation with *M. javanica* reduced root-knot severity by 35 to 50% in tomato and basil, and by up to 40% in snapdragon, while solarization had no significant effect on disease reduction (Tables 2.1 and 2.2). This is in agreement with the results which were obtained in the FORC - cucumber pathosystem (Klein et al., 2011b). The contribution of OA to nematode suppressiveness has also been described in previous studies (Akhtar and Malik, 2000; Pandey, 2005; Rodriguez-Kabana et al., 1987), deriving mainly from antagonistic microbial activity. Increased root development of tomato and snapdragon (Tables 2.1 and 2.2), as well as increased height and weight in the latter, are beneficial side effects that should be considered but are not necessarily correlated with disease suppressiveness. Increased growth response following WR amendment has also been found in cucumber (Klein et al., 2011b).

Soil suppressiveness in *M. javanica*-infested plots, expressed as reduced root-knot severity in tomato, was significant in the solarized plots under agricultural conditions, in experiment 1 (Table 2.5), which was carried out in highly infested plots (Table 2.3). It was particularly evident in the highly galled plants, in which galling was significantly reduced following soil solarization, soil amendment with WR or their combination. Reduced galling was also observed in experiment 2 under low infestation levels (Tables 2.3 and 2.5). Induction and assessment of soil suppressiveness under farm conditions is a challenging task, since it requires the application of two conflicting measures toward a similar goal: the soil disinfestation treatment is aimed, by definition, at reducing pathogen populations in the soil; on the other hand, assessing suppressiveness under cropping conditions requires survival of the pathogen which has the potential for infection and disease onset. To overcome this conflict, we applied a moderate solarization treatment in the field experiments. In addition, a relatively lower rate of WR (0.4% w/w) was used to amend the plots. These two factors resulted in a partial reduction in the viability of the test organism (FORL), but did not control *M. javanica* (Table 2.3), enabling its use to test soil suppressiveness. In experiments 1 and 2, root galling was not reduced by the treatment (Table 2.3), but soil suppressiveness in the tomato crop was evident (Table 2.5). However, it was not evident with the snapdragon crop in the third experiment

(Table 2.6). Similarly, suppression of the proportion of plant-parasitic nematodes in a biosolids-amended soil was not matched by a reduction in the number of *Radophulus similis* in the roots of bananas (Pattison et al., 2006). Snapdragon is highly vulnerable to root-knot nematodes (McSorley and Frederick, 1994), and the cropping season was extended until plants were highly infected (5 months after planting), apparently reducing the expression of soil suppressiveness. In previous studies, increasing inoculum level has been shown to dampen soil suppressiveness (Klein et al., 2011b; Zhou and Everts, 2007).

Soil suppressiveness following soil disinfestation by solarization, with or without OA, has been induced in some cases but not in others (Greenberger et al., 1987; Klein et al., 2011b; Ozores-Hampton et al., 2004). Thus, many factors appear to be involved in the evolution of this phenomenon and more importantly, in its expression during crop production. Soil suppressiveness can be first tested in a greenhouse bioassay (Klein et al., 2011b). Indeed, in our study, soil suppressiveness by solarization, with or without OA, was confirmed before and after the cropping season in controlled greenhouse tests in which soil samples from the treated plots were planted with FORC-inoculated cucumber (Table 2.7). The significant soil suppressiveness obtained in the greenhouse bioassay did not match the mild response under field conditions. This can be attributed to the fact that in the field, the roots are exposed to continuous and extended periods of root infestation from deeper layers, and the long cropping season also involves continuous infestation from the deeper layers. The susceptibility of the tested crop (McSorley and Frederick, 1994, Pegard et al., 2005) also significantly influences the expression of soil suppressiveness (Zhou and Everts, 2007), as was evident here in the snapdragon experiment (Table 2.6), as well as in other studies (Davis and Sorensen, 1986).

We demonstrated, in certain cases, soil suppressiveness to root-knot nematode following herb amendment and soil solarization. This demonstration was carried out in small field plots and in farm plots infested with *M. javanica*, as a component of agrotechnical practice in which the required amendment is cropped and incorporated into infested soils. Soil suppressiveness is essential as a complementary process of soil disinfestation for sustainable and long-term pest control. In contrast, soil disinfestation that is too drastic creates a "biological vacuum" which facilitates rapid reinfestation (Pyrowolakis et al., 2002; Weller et al., 2002; Westphal and Becker, 2001; Westphal and Xing, 2011). Thus, under regular, more effective solarization,

disease control is expected to be more evident. In some studies, a combination of OA and soil solarization has been shown to improve nematode control (Klein et al., 2011a; Oka et al., 2007; Ploeg and Stapleton, 2001; Stapleton et al., 2010). The suppressiveness to root-knot nematodes expressed in this study after a single soil treatment is a promising step in this direction which, nevertheless, requires further substantiation. Studies under farm conditions, in which long-term nematode suppressiveness was assessed, have indicated that its expression follows a lag period after inducing the phenomenon, before a significant suppressive effect is evident (Pattison et al., 2006; Stapleton and De Vay, 1983); in pot experiments on the other hand, the suppressiveness was expressed immediately after soil treatment (Pattison et al., 2006). The findings in our study indicate that soil suppressiveness in a given soil can be maintained, and repeated application of appropriate treatments might further improve the results.

3. Pathogen and disease suppressiveness by soil components and possible suppressiveness mechanism

Soil suppressiveness to Fusarium disease in cucumber was examined by studying the effect of different soil components on disease development and on the pathogen development. We concentrated on WR-amended soil, since this soil expressed reproducible and significant suppressiveness at various conditions, as also observed in previous studies (Fig. 3.1; Klein et al., 2011b). In this study, we demonstrated the microbial origin of soil suppressiveness by exposure of nonamended-nonsuppressive soil to volatile organic compounds (VOC) which were generated during decomposition of WR in the soil. Since VOC, originated from plant residues or generated during decomposition of OA in the soil, cause quantitative and qualitative changes in the soil microflora activity and population (Gamliel and Stapleton, 1993a; Gilbert and Griebel, 1969; Kasuya et al., 2006; Linderman and Gilbert, 1975), we assume that the suppressiveness which was found in nonamended soil after exposure to these VOC (Fig. 3.1) is intrinsically microbial, and connected to WR decomposition. Similarly, adding low amount (5% w/w) of aqueous soil extraction from suppressive-soil (WR-amended), which contains soluble and a suspense components, as well as microbial cells, to nonsuppressive soil, also induced suppressiveness to Fusarium disease in the inoculated cucumber seedlings (Fig 3.2), thus further emphasizing the microbial origin of the suppressiveness. This

corresponds with other studies in which soil suppressiveness was transferred to nonsuppressive soil by mixing soils (Kao and Ko, 1983; Raaijmakers and Weller, 1998). Reduction in disease progress can derive from increase in host defense and resistance (Stone et al., 2003; Yogev et al., 2010), reduction in inoculum level or potential (Kao and Ko, 1983; Stapleton and DeVay, 1983; Westphal and Xing, 2011), following exposure to suppressive soil, or a beneficial shift in pathogen-host interaction, in the presence of suppressive factors (Oyarzun et al., 1998; Raaijmakers and Weller, 1998). The capability of suppressive soil to increase plant resistance to root and shoot pathogens was assessed by growing cucumber in the suppressive soil and afterwards inoculating them, as well as using a foliar pathogen as an indicator. Based on the used assays, no evidence for induced resistance to FORC or *B. cinerea* was detected (Fig. 3.3) Pathogen suppressiveness, i.e. the reduction in pathogenic inoculum density or potential, was evaluated by exposure of pathogen population to different soil components with or without the effect of the cucumber-host (roots activity/residues). FORC-macroconidia density declined significantly in soil which was used for repeated inoculated transplanting, but not in soil which was used immediately after incubation with WR (Fig. 3.4). Suppressive soil significantly increased macroconidia decline by 50%, 28 days after inoculation, compared with control soil, following inoculation of the used soils with FORC (Fig 3.4). No effect on conidia germination and production of new chlamydospores, was found in suppressive soils, compared with nonamended soils (Fig 3.5 and 3.6), and FORC mycelia growth on the suppressive soils was similar to growth in nonamended soil, namely, no inhibition was observed. Therefore, in the current study, pathogen suppressiveness in the early stages of infection cannot be a major factor in the suppressiveness mechanism. In another study which was carried out in a suppressive solarized soil, a reduction in inoculum level and potential of *Rosellinia necatrix* was found (Freeman et al., 1990). Changes in pathogen population in the tissues of the cucumber host were examined. Root colonization by *Fusarium* spp. in suppressive vs. control soils, was evaluated 3 days after inoculation (before symptom appearance) and 6 days after transplanting (first symptom appearance in inoculated seedlings in nonamended soil). *Fusarium* spp. population (mostly FORC) on the roots was much reduced in the suppressive soil, only at the later stage (Table 3.1). This finding further indicates that the main suppressiveness mechanism is not involved with initial pathogen decline in the soil. The reduction by 64% in *Fusarium* spp. colonization of

roots in suppressive soil at day 6 corresponds with the reduction by 60% in wilted plants in the suppressive soil, 21 days from inoculation and planting, compared with diseased plants in control soil (Fig 3.1). Therefore, the pathogenic capacity of the pathogen was suppressed only at later stages, after exposure of both the pathogen and the host to both biotic and abiotic components of the amended soil. Possble mechanisms, in which WR-amended soil suppress the FORC establishment on cucumber roots are microbial competition (Mazurier et al., 2009; Raaijmakers et al., 2009) or structural and biochemical barriers elaborated in root tissues (Pharand et al., 2002).

4. Shifts in root-associated microbiome following FORC inoculation and transplantation of cucumber into suppressive soil

Disease suppression was maintained for 21 days after FORC inoculation of cucumber seedlings and transplanting into the WR-amended soil. Delayed disease onset and reduced symptom expression in the suppressive soil were noticeable as early as 6 days postinoculation. We therefore hypothesize that the impact of soil suppressiveness on root infection by FORC and disease development begins shortly after root inoculation by the pathogen. This impact may involve direct suppression of fungal survival and growth, or an indirect effect via shifts in the microbial population and assembly in the rhizosphere and roots.

Quantitative assessment of FORC in the cucumber roots indicated that the initial infection units of the pathogen were not affected by the suppressive soil 3 days after seedling inoculation and transplanting (Fig. 4.1). However, after an additional 3 days, the buildup of pathogen population in the roots of plants in control, nonamended soil was three times higher than in the suppressive soil. This suggests that the suppressive soil itself does not initially directly reduce the number of pathogen propagules or their capacity to colonize the roots. It also emphasizes that disease suppressiveness occurs in the root zone, with the presence of both pathogen and host, under suppressive conditions (Chen and Nelson, 2008; Raaijmakers et al., 2009; Shetty et al., 2000).

Soil suppressiveness to root diseases may result from specific antagonism to the pathogen. For example, soils that are naturally suppressive to Fusarium wilt have been well documented (Steinberg et al., 2006; Weller et al., 2002): they are characterized by competition for niche and nutrients that is closely related to

saprophytic, nonpathogenic species of *Fusarium* (Weller et al., 2002) and the activity of native protective *F. oxysporum* strains and *Pseudomonas* spp. (Alabouvette et al., 2009; Duijff et al., 1999). In the roots of cucumber transplants, native *Fusarium* species constituted the dominant root fungal population in all tested soils, regardless of previous soil treatment (suppressive or not) or transplant inoculation with FORC (Table 4.2). This dominance of saprophytic *Fusarium* species did not, however, interfere with soil or root receptivity to FORC, as reflected by its pronounced colonization of the roots and disease severity of plants in the nonamended soil (Figs. 1.1 and 3.1). Moreover, the root-associated fungal community composition was not affected by the suppressive soil during the time intervals examined ($P > 0.05$). However, FORC inoculation increased the relative abundance of *F. oxysporum* in roots from between 3 and 14% to between 88 and 89% in WR-amended as well as nonamended soils (Fig. 4.2, Table 4.2). Hence, mechanisms other than the presence of protective nonpathogenic *Fusarium* play a more important role in disease suppressiveness in WR-amended soil. Such mechanisms may contribute to the overall suppressiveness, and should be examined later by inoculation of nonamended soils with different isolates from suppressive soils and rhizospheres, following by suppressiveness tests (Sneh et al., 1987).

The evolution of soil suppressiveness has been related to shifts in the soil- and root-associated microbiome (Duijff et al., 1999; Mazzola, 2007). In many cases, soil suppressiveness is biologically derived from shifts in the bacterial balance in the rhizosphere, including its density and functional diversity (Liu et al., 2007; Raaijmakers et al., 2009; Yin et al., 2003). Indeed, the composition of the fungal community in the root zone was not affected in the suppressive soils during our study ($P > 0.05$). Moreover, it has been suggested that consortia of microorganisms and mechanisms, rather than a single group, are involved in disease suppressiveness (Mazurier et al., 2009; Mendes et al., 2011; Singh et al., 1999). Therefore, the WR-mediated suppression of FORC development observed in infected roots 6 days postinoculation seems to be associated with changes in root bacterial community properties.

Quantitative analysis and mass-sequencing methods indicated that the shift in bacterial communities in suppressive soil is qualitative, i.e., a shift in the composition of the bacterial community rather than in the total number of bacteria (Table 4.3, Fig. 4.3). Previous studies have positively correlated density of cultivable heterotrophic

bacterial populations with soil suppressiveness (Bonanomi et al., 2010). Our finding, however, is based on a quantitative assessment that was not affected by the bias of cultivable heterotrophy; therefore, the suppression mechanism may stem from the interaction with more groups of microorganisms.

Bacterial community composition and structure in the rhizosphere and root have been previously associated with suppressiveness (Benítez et al., 2007; Borrero et al., 2004; Hallmann et al., 1999). In our study, an increase in diversity of the root bacterial communities was evident in the suppressive soil (Table 4.4). However, an increase in the diversity of the bacterial community also characterized roots as a response to FORC inoculation, prior to the appearance of disease symptoms (3 days). Postma et al. (2000) found that qualitative, rather than quantitative shifts in the bacterial community correlate with disease suppressiveness. Moreover, disease suppression has been correlated with increased soil (Garbeva et al., 2004; Manici et al., 2005; van Elsas et al., 2002) and endophytic (Shiomi et al., 1999) bacterial diversity. Yang et al. (2001) also reported increased bacterial diversity on *Phytophthora*-infected avocado roots, compared with healthy ones. Therefore, it is still unknown whether an increase in root community diversity is in itself indicative of a suppressive process.

A sharp reduction in the population of *Massilia* (β-proteobacteria) in the root bacterial community (Tables 4.3 and 4.5) was the key determinant of the observed structural changes in root bacterial communities in suppressive soil (Table 4.4). Members of this genus, first isolated from clinical samples, are characterized as Gram-negative, aerobic, flagellated and non-spore-forming rods (Kämpfer et al., 2010; La Scola et al., 1998). *Massilia* have been detected in the rhizosphere of a variety of plant species (Abou-Shanab et al., 2007; Compant et al., 2011; Grönemeyer et al., 2011; Hrynkiewicz et al., 2010), including cucumber (Green et al., 2007; Ofek et al., 2009, 2011). *Massilia* have been found to exhibit exceptional dominance in cucumber root-associated communities at early stages of plant development and to be sensitive to increased microbial competition (Hrynkiewicz et al., 2010; Ofek et al., 2009).

The reduction in the *Massilia* population's size and root dominance in the suppressive soil was accompanied by significant changes in the relative abundance of additional bacteria (Table 4.5). Most notable was the increase in relative abundance of

specific populations, namely *Rhizobium, Bacillus, Paenibacillus* and *Streptomyces*, members of which are frequently linked to plant health, biological control and disease suppression (Borrero et al., 2004; Katan and Gamliel, 2012; Mazzola, 2007; Mendes et al., 2011; Nautiyal, 1997; Postma et al., 2010; Singh et al., 1999; Workneh and van Bruggen, 1994; Yin et al., 2003). Among these, *Streptomyces* seems to be an important component in the disease-suppression process in the cucumber-FORC system (Hammad and El-Mohandes, 1999; Singh et al., 1999). The composition of the *Streptomyces* community, determined by PCR-DGGE examination, shifted significantly, resulting in a strong increase in the dominance of a specific population in suppressive soils after 3 days (Fig. 4.5) and on day 6 (Fig. 4.4). This shift was related mainly to a *S. humidus*-related population (99.9% similarity according to sequence analysis of excised bands). Reports have indicated that some strains of *S. humidus* are antagonistic to different phytopathogenic fungi (Lim et al., 2000), and suppress *Phytophthora capsici in vivo* through direct antibiosis (phenylacetic acid and sodium phenylacetate) (Hwang et al., 2001).

In naturally developed suppressive soils, abundance of the native antagonistic microorganisms may increase in the presence of a particular pathogen (Mazzola, 2002; Weller et al., 2002; Westphal and Becker, 2001). The increase in populations of potential antagonists, such as *Streptomyces*, in suppressive soil is therefore restricted to specific pathosystems. However, in the system studied here, in which suppressiveness was induced, the presence of FORC triggered little change in the root bacterial community (Table 4.4). Acquisition of *Pythium*-suppressive microbial consortia by cucumber seeds in suppressive compost has been shown to be independent of the pathogen's presence (McKellar and Nelson, 2003; Ofek et al., 2011). We hypothesize that several mechanisms are associated with soil suppressiveness following WR amendment, including a change in root bacterial composition, increased diversity that sustains general competition for nutrients, and specific antagonism which may take place in the root zone, following the pathogenic infection. Further studies should be carried out in characterizing the factors that are involved in the long-term suppressiveness in organic amended soil.

5. Conclusions

The evolution of soil suppressiveness to Fusarium disease in cucumber and to root knot nematodes by M. *javanica* was demonstrated following various herb amendment and soil solarization. It was reflected as reduced disease incidence and obtained in different soils and crops. Soil suppressiveness to root-knot nematode was also demonstrated in farm plots infested with *M. javanica*, as a component of agrotechnical practice in which the required amendment is cropped and incorporated into infested soils.

Definition and revealing the factors that enhance soil suppressiveness can contribute to the efforts to increase soil or growing media suppressiveness and to improve integrated pest management. Manipulation of soil microbiota to create soil suppressiveness has more chances of success than the introduction of a biocontrol agent into a hostile and competitive environment. In this study, generating an appropriate soil environment with organic amendments resulted in a shift in root bacterial communities. Under the new microbial equilibrium, root infection by a virulent pathogen was contained by the root microbiome and disease severity was reduced.

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APPENDIX

Soil Suppressiveness to Fusarium Disease Following Organic Amendments and Solarization

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Abstract

Klein, E., Katan, J., and Gamliel, A. 2011. Soil suppressiveness to Fusarium disease following organic amendments and solarization. Plant Dis. 95:1116-1123.

Soil suppressiveness to soilborne pathogens can evolve following the incorporation of plant residues in the soil and solarization. We studied its occurrence by assessing disease incidence and severity in sandy soil which was infested after the disinfestation treatment. Disease incidence and severity of crown and root rot in cucumber plants inoculated with *Fusarium oxysporum* f. sp. *radicis-cucumerinum* macroconidia were reduced by 20 to 80% when seedlings were planted in the tested soils 2 to 34 months after soil amendment. Residues of *Diplotaxis tenuifolia* (wild rocket [WR]), *Artemisia dracunculus* (tarragon), *Salvia officinalis* (sage), and *Brassica oleracea* var. *italica* (broccoli) were most effective for inducing soil suppressiveness. Effective soil suppressiveness continued to be evident after repeated inoculations and plantings in the same soil without additional treatment between inoculations. Moreover, residues of WR induced soil suppressiveness in two additional tested soils differing in their physical and chemical properties. Residues of *Rosmarinus officinalis* (rosemary), *Coriandrum sativum* (coriander), *Mentha piperita* (peppermint), and *B. oleraceae* var. *botrytis* (cauliflower) induced disease suppression at the first inoculated planting but not upon repeated inoculation and planting. The contribution of soil solarization to the evolution of soil suppressiveness, albeit evident, was inconsistent. Soil suppressiveness to Fusarium crown and root rot was also observed when cucumber seed were sown in soils which were initially amended with WR residues and later infested with *F. oxysporum* f. sp. *radicis-cucumerinum* chlamydospores. There is a potential for the use of plant residues for inducing soil suppressiveness and further contributing to the control of diseases caused by soilborne pathogens.

Organic amendments (OAs) from various sources (e.g., plant debris, organic waste, or compost) are added to the soil for the control of soilborne pathogens (6,8,20). Among these, crop residues, including crude organic material such as green manure and vegetative plant debris (stem, root, leaves, and so on), can be suitable for the control of soilborne pathogens (28,29,46). Bonanomi et al. (6) reviewed the contribution of soil amendment with crop residues and reported variable responses in controlling soilborne pathogens. In 45% of the reviewed articles, significant pathogen control and suppressiveness, mainly of *Fusarium* spp., *Verticillium dahliae*, *Thielaviopsis basicola*, and *Phytophthora* spp., were reported. However, in 28% of the reviewed articles, increased disease incidence and conduciveness were reported following OA (6). Hence, selecting the appropriate OA is crucial to achieving effective long-term pathogen control and sustainable management of soil quality and health (1). Various biological (7,12,24) or chemical and physical (5,31,47) mechanisms are potentially involved in the decomposition of OAs and in the related processes of pathogen control, disease management, and the development of soil suppressiveness to reinfestation and disease suppression. In a previous work, we found that solarization of soil amended with herb residues improves the disinfestation efficacy against soilborne pathogens (28,29).

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Soil suppressiveness is defined as the soil's ability to detain disease onset in a susceptible host, even in the presence of a significant inoculum density of the pathogen (12). In suppressive soils, disease incidence or severity usually remain low, even under environmental conditions that favor disease development (12). Disease suppressiveness and pathogen suppressiveness are not necessarily the same thing, because reduction in disease incidence and severity is not always the result of a direct effect on the pathogen. Soil suppressiveness—namely, capacity of the soil to suppress reinfestation by a pathogen introduced into the soil after treatment—can evolve following various soil treatments, including incorporation of OAs (11,21,38,48,51), but also by other agrotechnical means such as soil cultivation, monoculture, or crop rotation (12,39,47). Long-term soil suppressiveness against certain pathogens has been reported to evolve in some cases following soil solarization (18,19,22,33). In contrast to the many studies which have demonstrated disease control by OAs combined with soil solarization (16,29,43), only a few studies have dealt with their effects on soil suppressiveness.

The mechanism of pathogen control following amendment with certain OAs and the evolution of soil suppressiveness might be related. OA decomposition in the soil induces shifts in chemical and physical conditions and in soil microbial populations and activities. The new microbial balance might be involved in the suppression of pathogen reinfestation and delay of disease onset in future cropping. The objective of this work was to assess soil suppressiveness by soil amendments with residues from herb and other plants, with or without solarization, and its potential to suppress a disease caused by a soilborne pathogen introduced into the soil following the treatment.

Materials and Methods

Soil and OAs. Soil samples were collected from three agricultural field sites in different locations in Israel. Two types of soil

were used: sandy soil from Rehovot, in the center of Israel (94% sand, 2% silt, 4% clay, 0.12% organic matter, pH 7.9, field capacity of 9%, measured at –33 J/kg matric potential); sandy soil from En Tamar, which was collected from the southern desert, in the Arava region (89% sand, 7% silt, 4% clay, 0.1% organic matter, pH 8.1, field capacity of 8%); and loamy soil from Bet Dagan, in the center of Israel (22.5% sand, 25% silt, 52.5% clay, 1.4% organic matter, pH 7.5, field capacity of 20%). These soils represent the range of agricultural soils in Israel (50). The soils had no history of soil disinfestation or fumigation for at least 5 years prior to sampling. Several samples were collected from the upper layer (5- to 20-cm depth) of the soil. For each site, the soil samples (total of 500 kg) were mixed into one large composite sample, air dried, and sieved through a 1-mm screen. The soils were stored in plastic containers at room temperature pending use (up to 4 weeks after collection).

Leaves and stem debris of the following plants were tested: *Salvia officinalis* L. (sage), *Rosmarinus officinalis* L. (rosemary), *Coriandrum sativum* L. (coriander), *Diplotaxis tenuifolia* (L.) DC. (wild rocket [WR]), *Mentha piperita* L. (peppermint), *Brassica oleracea* L. var. *italica* (broccoli), *B. oleraceae* L. var. *botrytis* (cauliflower), and *Artemisia dracunculus* L. (tarragon). The foliage of these crops was collected from commercial agricultural fields during crop production. The leaves and stems of each crop were separately air dried at 25°C, then ground and sieved through a 2-mm sieve. The sieved residues of each plant species were used as OAs.

Soil amendment. The specified dried and ground crop residues were incorporated into 20 kg of soil sample at a rate of 1 or 2% (wt/wt), as indicated (equivalent to a rate of 1 or 2 kg/m^2 in the field, respectively; 28). The soil-amendment mixture was wetted to water-holding capacity. Each amended soil mixture was packed in a porous, woven plastic bag (total of 22 kg/bag). Nonamended soil was prepared and packed similarly and served as the control.

Incorporation of amended soil and soil solarization in small field plots. Small field-plot experiments were conducted during the summer in 2005 through 2009, at the experimental farm of the Hebrew University in Rehovot. In each year, the field was rotovated to 50-cm depth and then irrigated to water-holding capacity down to 50 cm. Plots (2 by 4 m) were outlined and trenches (20 cm deep) were dug in the margins of each plot. Porous plastic bags containing the amended soils were buried horizontally and flat in the center of each plot, in a layer 10 to 30 cm below the soil surface. The treatments included four combinations of soil amendments and solarization; in each plot, the respective soil mixture was buried. All plots were mulched with a transparent polyethylene sheet (100 μm thick). Nonsolarized plots were additionally covered with a shading screen (90% shade; Polysak, Nir Itzhak, Israel) which was laid over the plastic mulch to avoid soil heating and solarization. Each experiment was set up in a randomized block design, with three replicates for each treatment. The solarized plots were exposed to solarization for a period of 28 days during the months of July or August, as indicated. Soil temperatures were recorded at a depth of 20 cm, using type-T thermocouples (accuracy ± 0.5°C) connected to a micrologger (21X; Campbell Scientific Inc., Logan, UT). Soil temperature reached 34 to 48.7°C at 20 cm in the solarized plots, compared with 32 to 34°C in the shaded, nonsolarized plots. Upon termination of the solarization period, the plastic sheets were removed from the plots and the soil bags with their contents were brought to the laboratory and allowed to air dry at 25°C for 1 month prior to their use. In certain cases, the soils were stored for extended periods in the shade at room temperature.

Plant inoculation and disease suppressiveness assay. *Fusarium oxysporum* f. sp. *radicis-cucumerinum* Vakal., the causal agent of cucumber root and crown rot disease, was used as the bioassay organism. Infected cucumber plants were collected from a commercial cucumber greenhouse and the stems, containing macroconidia of *F. oxysporum* f. sp. *radicis-cucumerinum* in their lower section, were used for the suppressiveness tests. Isolates of *F. oxysporum* f. sp. *radicis-cucumerinum* were taken from each stem and tested for pathogenicity using cucumber seedlings prior to their selection for the suppressiveness assays, to validate their identity as *F. oxysporum* f. sp. *radicis-cucumerinum*. The macroconidia of this pathogen, as a natural inoculum, were scraped into sterile water and their density was adjusted to the desired concentration with the aid of a hemacytometer. Macroconidia were assessed prior to each assay using a soil-dilution-plating technique (28). Suspensions of macroconidia which showed at least 80% germination after 24 h were used for the suppressiveness tests.

Soil suppressiveness assays in which inoculated seedlings were planted in previously treated soils were conducted in pot experiments essentially as previously reported (51). Pots (0.45-liter, 0.5 kg each) were filled with soil taken from the various aforedescribed soil-amendment treatments. One day before the experiment, the soil in the pots was irrigated to water-holding capacity. Seed of cucumber (*Cucumis sativus* L. 'Kfir') were sown in sandy soil. Six-day-old seedlings were removed and their roots were washed, then dipped for 2 min in a suspension of *F. oxysporum* f. sp. *radicis-cucumerinum* macroconidia, adjusted to the indicated density. The inoculated seedlings were then transplanted into pots which were filled with soil taken from the various soil-amendment treatments. For each treatment, five pots were planted with seven inoculated seedlings per pot and repeated planting cycles were conducted at least twice. An additional pot was planted with noninoculated seedlings and served as a control. All trials were arranged in the greenhouse (24–27°C) in a complete randomized-block design.

Disease symptoms in cucumber seedlings usually appear 7 days after inoculation and are manifested as wilt and plant collapse. Disease progress was expressed as percentage of diseased plants, area under the disease-progress curve (AUDPC; 9), and, when indicated, percentage of the value of the AUDPC relative to the inoculated control. The noninoculated plants remained healthy in all experiments. A reduction in disease incidence or severity in amended soil compared with nonamended soil reflected soil suppressiveness, because the plants were inoculated and introduced into the soil after the OA and solarization treatments had been terminated (i.e., the pathogen was not exposed to a direct control process). Soil suppressiveness was observed during the first planting cycle in which the pathogen was initially introduced. It was further evident after repeated planting and inoculations which surpassed the population of *F. oxysporum* f. sp. *radicis-cucumerinum* which existed in the soil from the previous planting.

In certain experiments (Table 1), inoculated cucumber seedlings were repeatedly planted in the same pots to examine the long-term effect of the suppressiveness. In these experiments, all plants from the previous assay were removed and the pots were left to dry for a week. The pots were then irrigated to field capacity, followed by a second disease-suppression assay as already described.

In a few specific experiments, chlamydospores were used as inoculum. Chlamydospores were produced on peat (Plantobalt, peat moss, degree of decomposition h2 to h5, pH 2.5 to 3.5, water-holding capacity 55 to 75%, Estonia and Latvia) which was steam sterilized and inoculated with an *F. oxysporum* f. sp. *radicis-cucumerinum* macroconidia suspension. The inoculated peat was incubated in the dark at 25°C, and viability of *F. oxysporum* f. sp. *radicis-cucumerinum* propagules was assessed at 30-day intervals for 3 months, until inoculum density stabilized at 4.075×10^6 CFU/g peat. Inoculum density and viability were assessed using the soil-dilution-plating technique described for the macroconidia. The infested peat was used as a source of *F. oxysporum* f. sp. *radicis-cucumerinum* inoculum in the soil by incorporating it with the tested soil at a rate of 1, 2, or 5% (wt/wt). Eight cucumber seeds were planted into each pot.

Statistical analyses. All experiments were carried out at least twice, yielding similar results, except for the experiment in which the long-term effect was assessed, which was carried out only once but with three repeat-inoculation plantings. Separate analyses of

each experiment showed homogeneous variance of the experimental error between repetitions. Data were first analyzed by analysis of variance (ANOVA) to test for possible interaction among the main effects, followed by the appropriate mean separation analysis using Tukey's studentized range (honestly significant difference) test. All analyses were performed with SAS software (release 9.1 for PC; SAS Institute, Cary, NC) with significance at $P \leq 0.05$.

Results

Suppression of Fusarium crown and root rot in cucumber plants by crop residues and soil solarization. Disease suppression in cucumber seedlings which were artificially inoculated with *F. oxysporum* f. sp. *radicis-cucumerinum* was evident when they were planted in soil previously amended with residues of all of the tested plants, compared with the respective nonamended soils (Figs. 1 and 2; Tables 1 and 2). There were, however, differences among the various plant residues with respect to their potential for suppressiveness, with coriander and WR inducing the greatest level of suppressiveness. Solarization did not further contribute to the suppressiveness observed with the use of crop residues alone (Figs. 1 and 2; Tables 1 and 2). A typical example of suppressiveness as reflected by disease progress and AUDPC is given in Figure 1. Increases in growth parameters resulting from soil amendment are shown in Table 3. In nonamended soil, disease symptoms (chlorosis, plant-growth retardation, and wilt) first appeared 7 to 10 days after inoculation, reaching 60 to 100% plant mortality 21 days after inoculation. In the WR-amended soils, disease symptoms usually appeared 1 to 7 days later and plant mortality was reduced by 20 to 80%. Root and shoot dry weights were significantly higher in both inoculated and noninoculated WR-amended soil compared with the respective nonamended soils (Table 3). Increasing WR concentration from 1 to 2% in the amended soil did not significantly add to the soil's suppressiveness to the disease (*results not shown*); disease incidence was 90 and 50% lower than in nonamended soil ($P < 0.0001$) at both WR concentrations in the first and second inoculated planting, respectively.

Disease suppressiveness following repeated inoculation. The manifestation of disease suppressiveness was examined over two or three consecutive inoculation and planting cycles in the same pots. Disease incidence was significantly reduced in the soils which had been previously amended with WR, peppermint, broccoli, sage, or tarragon compared with the nonamended soils, throughout the consecutive planting cycles (Tables 1 and 2; Figs. 2–4). In contrast, solarization, in general did not sustain significant suppressive capacity and, in some cases, increased disease incidence was observed in the second or third planting cycle with many of the tested crops (Tables 1 and 2; Fig. 2). However, in one experiment, tarragon combined with solarization sustained suppressiveness capacity during the second and third crop cycles relative to either tarragon or solarization alone (Fig. 3). Sup-

Table 1. Effect of organic amendments and solarization on area under the disease progress curve (AUDPC) of Fusarium crown and root rot in inoculated cucumber seedlings in Rehovot soil[x]

		AUDPC, first planting[y]		AUDPC, second planting[y]	
Experiment[z]	Amendment	Shaded	Solarized	Shaded	Solarized
1	Nonamended	738.6 A	597.1 A	538.6 A	447.1 A
1	Coriander	125.7 C	128.6 B	547.1 A	644.3 A
1	Peppermint	370.0 B	199.0 B	261.4 B	484.3 A
1	Rosemary	278.6 B	250.0 B	455.7 AB	604.3 A
2	Nonamended	825.7 A	640.0 A	788.6 A	765.7 A
2	Broccoli	333.6 B	202.1 B	531.2 AB	473.8 B
2	Cauliflower	284.1 B	280.1 B	530.0 AB	672.9 AB
2	Sage	639.3 B	218.3 B	241.1 B	195.7 C

[x] Cucumber seedlings were inoculated with *Fusarium oxysporum* f. sp. *radicis-cucumerinum* macroconidia at 1.5×10^5 CFU/ml and planted in the previously treated soils. For each experiment, values within a column followed by different letters are significantly different according to Tukey's studentized range (honestly significant difference) test at $P \leq 0.05$.

[y] Solarization or shading under field conditions was conducted for 28 days during July 2005. Plant residues were applied to the soil at 10 g/kg (1%, wt/wt).

[z] Data from each experiment represent the combined analysis of two trials that were pooled.

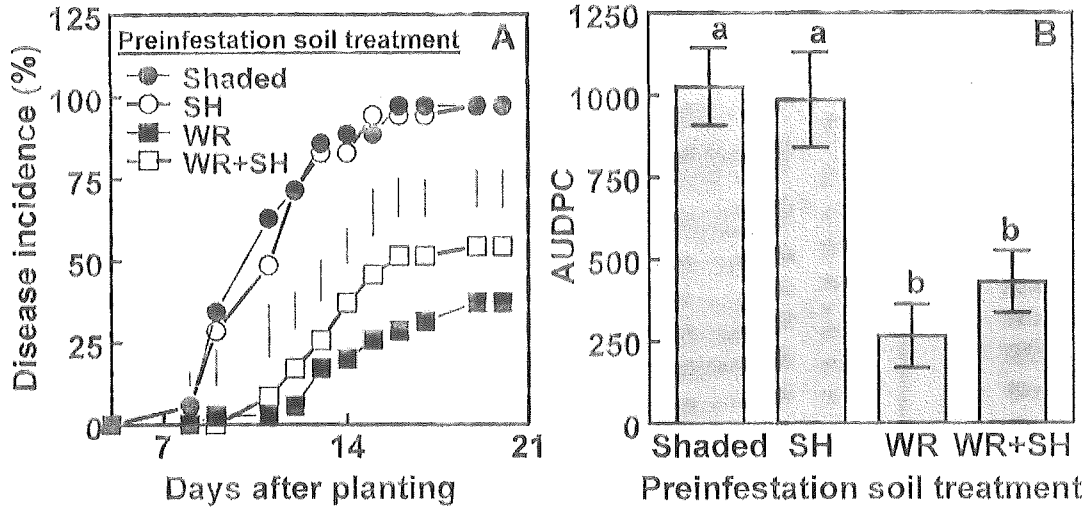

Fig. 1. Effect of wild rocket (WR) amendment and soil solarization (SH) on **A**, disease incidence in Fusarium crown and root rot-inoculated cucumber transplants in Rehovot soil or **B**, area under the disease progress curve (AUDPC). Cucumber seedlings were inoculated before planting with macroconidia of *Fusarium oxysporum* f. sp. *radicis cucumerinum* at 10^5 CFU/ml. Soil was either irrigated; mulched and shaded for 28 days (during July 2009) under field conditions (Shaded); or irrigated, mulched, and solarized for 28 days. Soil was amended with dry WR at a rate of 1% (wt/wt) before incubation. Vertical bars indicate average standard deviations, and values followed by a different letter are significantly different according to Tukey's studentized range (honestly significant difference) test at $P \leq 0.05$.

pressiveness was considered long term because it was evident 34 months after soil treatment and after successive plantings (Figs. 2 and 3).

Disease suppressiveness in soil inoculated with chlamydospores. Chlamydospores produced in infested peat (as an additional type of *F. oxysporum* f. sp. *radicis-cucumerinum* inoculum) were mixed with the tested soils at various inoculum concentrations before seeding with cucumber. Emergence of the cucumber seedlings (70 to 80%) was not affected by *F. oxysporum* f. sp. *radicis-cucumerinum*, regardless of the soil treatment. Diseased plants showed symptoms after 9 to 11 days at the highest inoculum concentrations. The suppressiveness in WR-amended soil was more pronounced at the low rate of inoculum, in which disease symptoms first appeared after 20 days, but was still significant at the highest rate of chlamydospore inoculum (Table 4). Solarization significantly increased AUDPC relative to nonsolarized soils at the two highest inoculum concentrations in the first sowing but had no significant effect in the second planting. In three of six cases, combining solarization with WR significantly increased disease incidence relative to WR alone. Amendment with WR significantly reduced AUDPC only in nonsolarized soils (Table 4).

Induced suppressiveness in different soils. The suppressive capacity of WR amendment was evident in all three tested soils (Fig. 4). The nonamended soils from Bet Dagan and En Tamar expressed higher natural suppressiveness than the Rehovot soil. However, when amended with WR, the three soils exhibited similar levels of suppressiveness (Fig. 4). Disease suppressiveness was also sustained in all three soils during the second consecutive inoculation

Table 3. Effect of wild rocket-amended soil (WR) and Fusarium crown and root rot inoculation on cucumber root and shoot dry weight[x]

	Root dry weight (g/plant)[y]		Shoot dry weight (g/plant)[z]	
Soil	Noninoculated	Inoculated	Noninoculated	Inoculated
Nonamended	0.0479 Ba	0.0467 Ba	0.1567 Ba	0.1100 Ba
WR	0.0777 Ab	0.1159 Aa	0.3786 Aa	0.3333 Aa

[x] Shading of Rehovot soil under field conditions was conducted for 28 days during July 2009. WR was applied to the soil at 10 g/kg (1%, wt/wt). Cucumber seedlings were inoculated with *Fusarium oxysporum* f. sp. *radicis-cucumerinum* macroconidia at 10^5 CFU/ml and planted in the previously treated soils. Shoot and root weights were recorded on day 22 after planting. Different uppercase letters denote significant difference in amendment level; different lowercase letters denote significant difference in inoculation level, according to analysis of variance of the root or shoot dry weight, Tukey's studentized range (honestly significant difference) test at $P \leq 0.05$.

[y] For root dry weight, inoculation–amendment F value = 8.9; $P > F < 0.0054$.

[z] For shoot dry weight, inoculation F value = 7.8; $P > F = 0.0087$; amendment F value = 182.9; $P > F < 0.0001$; inoculation–amendment interaction was not significant.

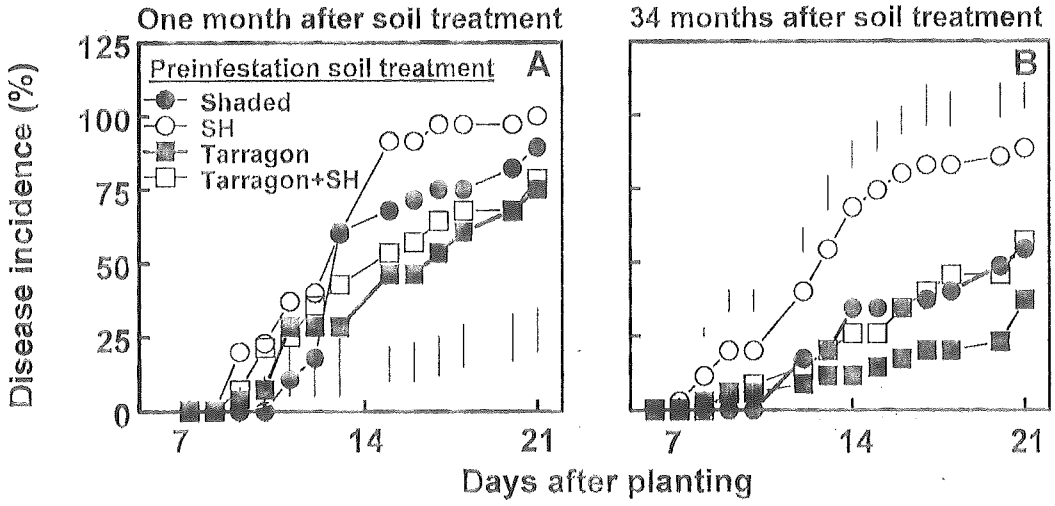

Fig. 2. Effect of tarragon amendment and solarization (SH) on Fusarium crown and root rot in inoculated cucumber transplants in Rehovot soil A, immediately after incubation with tarragon or B, 34 months afterward. Soil was either irrigated, mulched, and shaded for 4 weeks under field conditions (Shaded) or irrigated, mulched, and solarized for 28 days (during July 2009). Soil was amended with dry tarragon at a rate of 1% (wt/wt) before incubation. Cucumber seedlings were inoculated before planting with macroconidia of *Fusarium oxysporum* f. sp. *radicis cucumerinum* at 1.5×10^5 or 1×10^5 CFU/ml in A and B, respectively. Vertical bars indicate average standard deviations. Amendment and solarization main effects were significant by analysis of variance of the area under the disease progress curve. First suppressiveness test: amendment F value = 12.36, $P > F = 0.0038$; solarization F value = 7.17, $P > F = 0.0190$. Second suppressiveness test: amendment F value = 17.65, $P > F = 0.0007$; solarization F value = 15.44, $P > F = 0.0012$.

Table 2. Statistical analysis of variance (ANOVA) of area under the disease progress curve (AUDPC) of Fusarium crown and root rot in inoculated cucumber seedlings which were grown in amended Rehovot soil[y]

		ANOVA of AUDPC ($P < 0.05$)					
		AUDPC, first planting			AUDPC, second planting		
Experiment	Soil treatment[z]	df	F value	$P > F$	df	F value	$P > F$
1	Amendment	3	44.38	<0.0001	3	3.12	0.0397
1	Solarization	1	5.89	0.0210	1	3.16	0.0847
1	Amendment × solarization	3	1.48	0.2398	3	1.60	0.2084
2	Amendment	3	15.02	<0.0001	3	19.90	<0.0001
2	Solarization	1	10.76	0.0026	1	0.01	0.9339
2	Amendment × solarization	3	2.28	0.0986	3	0.84	0.4813

[y] Cucumber seedlings were inoculated with *Fusarium oxysporum* f. sp. *radicis-cucumerinum* macroconidia at 1.5×10^5 CFU/ml and planted in the previously treated soils.

[z] Solarization or shading under field conditions was conducted for 28 days during July 2005. Soil was amended with different plant residues before planting. In each experiment, different amendments were tested. In the first experiment, amendments were coriander, peppermint, and rosemary. In the second experiment, amendments were broccoli, cauliflower, and sage.

and planting cycle in the same pots, with the Bet Dagan soil being the most suppressive (Fig. 4).

Discussion

In the present study, we focused on soil suppressiveness (i.e., the reduction in disease level when a pathogen is introduced into a soil after it has been amended and incubated with various OAs or disinfested by solarization) (Figs. 1 and 2; Tables 1 and 2). Direct impact on the pathogen and disease reduction which results from direct action on the pathogen during the incubation process was not evaluated in these studies. These aspects have been discussed in previous publications (28,29). The suppressive capacity of soil is not necessarily related to direct pathogen control, which occurs during the incubation process and has been documented in numerous studies dealing with disease control by OAs (4,32,43,49). The combination of solarization and OAs, while showing an added value in pest control (20,28), does not provide higher suppressiveness potential. The present study demonstrates that crop residues alone provide the most significant suppressive effect. Apparently, the mechanisms involved in the two processes, (i.e., pest control using solarization and OAs and disease suppressiveness) may not be related, because solarization had no significant effect on suppressiveness in the repetitive inoculated planting cycles (Fig. 3).

The evolution of soil suppressiveness to *F. oxysporum* f. sp. *radicis-cucumerinum*, as reflected in reduced disease incidence and AUDPC, was evident in three tested soils (Fig. 4), with two types of inoculum (Fig. 1; Table 4) and various OAs (Tables 1 and 2; Figs. 1 and 2). Disease suppressiveness was reflected in the OA-treated soil by a delay in disease onset and 20 to 80% lower plant mortality (Figs. 1–4), phenomena which are associated with disease suppressiveness (25,47,51). Moreover, a long-term effect (lasting 34 months [Figs. 2 and 3] or with repeated plantings [Fig. 3; Tables 1 and 2]) was also recorded, further indicating the evolution of soil suppressiveness. Soil suppressiveness against *F. oxysporum* f. sp. *radicis-cucumerinum*, as also manifested with other formae specialis of *Fusarium*, was demonstrated following consecutive inoculation and cucumber plantings in compost-amended soil by Yogev et al. (51). The long-term effect of OAs in producing suppressiveness, as observed in our study, was more pronounced than that reported previously in other studies (38,52). Yulianti et al. (52) suggested that an increase in microbial activity following the decomposition of WR or *B. nigra* manures plays a dominant role in suppressing *Rhizoctonia solani*. Motisi et al. (37) found a suppressive effect using *B. juncea* which lasted for 13 days, whereas the predominant glucosinolates, which may have a direct effect on the pathogen, were hydrolyzed in less than 3 days.

Most of the plant residues tested for suppressiveness had been previously found effective in controlling soilborne pathogens or to have the potential to control pathogens as a result of the generation of biotoxic molecules (e.g., essential oils or glucosinolates; 3,4,13,32,40,41,49). In the present study, the decomposition of OAs in soil prior to infestation was carried out under aerobic conditions (28). Generally, under such conditions, the effect of decomposition products of plant residues on the pathogen population is variable. In contrast, under anaerobic conditions, a significant reduction in pathogen populations was found regardless of the type of residues incorporated (6). The decomposition of OAs under anaerobic conditions, although directly controlling soilborne pathogens, did not induce soil suppressiveness in some trials while it did in others (4,21), similar to the variable effects seen when using solarization. This further supports the hypothesis that the native microflora play an important role in developing suppressiveness (12,25,36).

The potential of different plant residues as well as composts to induce soil suppressiveness against plant diseases has been documented (30,34,38,42). In most cases, it was correlated with antagonistic or competitive microbial activity. Soil suppressiveness will persist for longer periods if the OA is functioning through enhanced activity of the resident soil microbial community rather than through the introduction of a novel active community (34). However, this activity may be nullified by antimicrobial means such as soil disinfestation (2).

In the present study, the effect of soil solarization, alone or combined with OAs, on soil suppressiveness was variable (Tables 1, 2, and 4; Fig. 2). This is in contrast to previous studies, in which solarization-induced soil suppressiveness toward various pathogens was reported (15,22,27,33). Greenberger et al. (22) found that solarization increased suppressiveness to *F. oxysporum* f. sp. *lycopersici* in 8 of 12 tested soils. In contrast, Mihail and Alcorn (35) found no reduction in a population of *Macrophomina phaseolina* following solarization, probably due to the treatment's elimination of antagonistic or competitive microflora in the soil. The inconsistency in suppressiveness following solarization can be related to differences within a given soil or among soils from different loca-

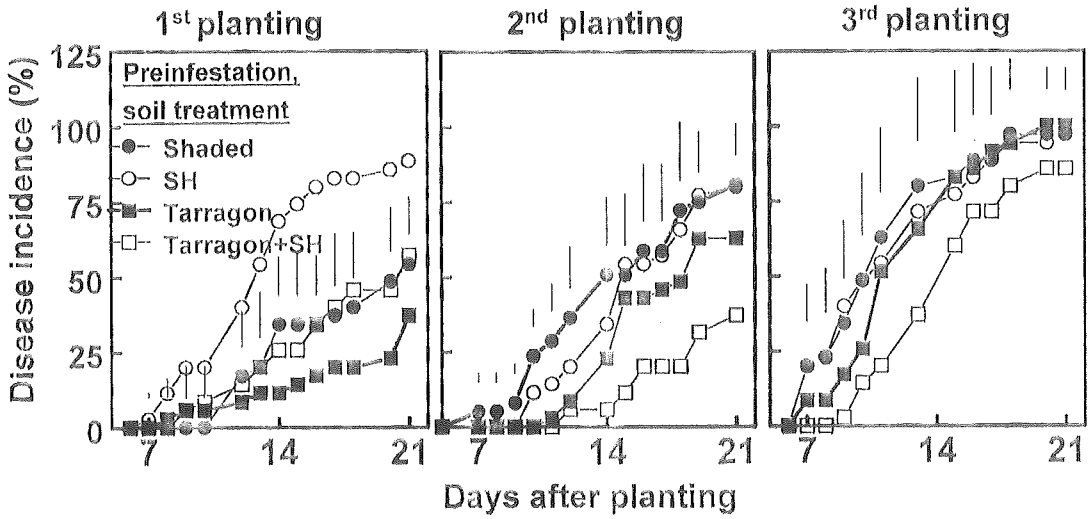

Fig. 3. Effect of tarragon amendment and solarization (SH) on Fusarium crown and root rot of cucumber transplants in Rehovot soil. Three repeat-inoculated plantings were carried out, 34 months after soil treatment. Soil was either irrigated, mulched, and shaded for 28 days under field conditions (Shaded) or irrigated, mulched, and solarized for 28 days. Soil was amended with dry tarragon at a rate of 1% (wt/wt) before incubation. Cucumber seedlings were inoculated before each planting with macroconidia of *Fusarium oxysporum* f. sp. *radicis cucumerinum* at 1×10^5 CFU/ml. Vertical bars indicate average standard deviations. Amendment and solarization main effects were significant by analysis of variance of the area under the disease progress curve of each inoculated planting. First planting: amendment F value = 17.65, $P > F = 0.0007$; solarization F value = 15.44, $P > F = 0.0012$. Second planting: amendment F value = 19.0, $P > F = 0.0005$; solarization effect was not significant. Third planting: amendment F value = 6.39, $P > F = 0.0224$; solarization effect was not significant.

tions. For example, when soil samples were taken from different locations of Rehovot soil (22), one sample of solarized soil increased suppressiveness, whereas another sample of this soil slightly reduced it. Deadman et al. (14) found that solarization improved soil suppressiveness when combined with OAs. Solarization of cabbage-amended soil reduced the inoculum potential of *Pythium aphanidermatum* during the following winter growing season, leading to a 40% reduction in damping-off of cucumber plants

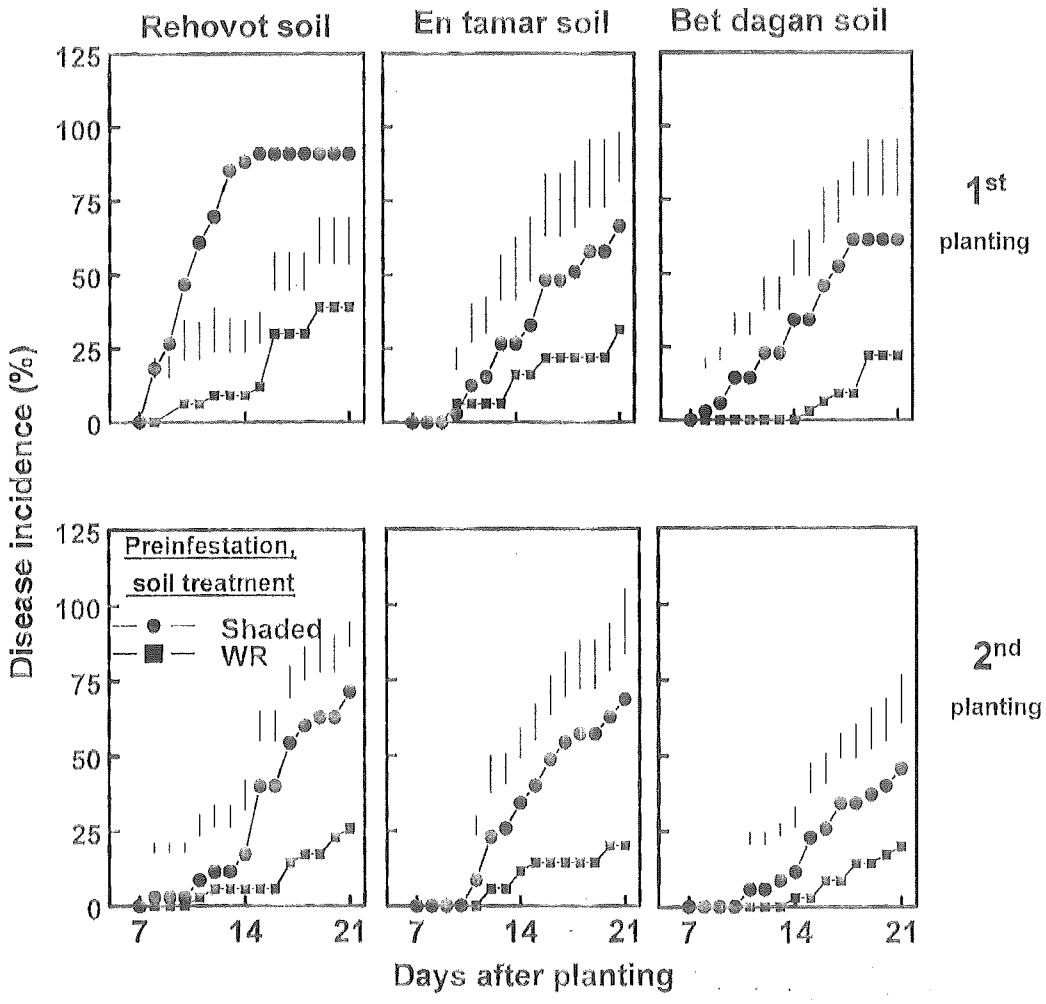

Fig. 4. Effect of wild rocket (WR) amendment on Fusarium crown and root rot of cucumber transplants in Rehovot, En Tamar, and Bet Dagan soils. Soil was irrigated, mulched, and shaded for 28 days (during July 2009) under field conditions or amended with dry WR at a rate of 1% (wt/wt) before incubation. Two repeat-inoculated plantings were carried out. Cucumber seedlings were inoculated before each planting with macroconidia of *Fusarium oxysporum* f. sp. *radicis cucumerinum* at 1.5×10^5 CFU/ml. Vertical bars indicate average standard deviations. In the first inoculated planting, the soil–amendment interaction was significant by analysis of variance (ANOVA) of the area under the disease progress curve (AUDPC): F value = 7.77, $P > F = 0.0025$. In the second inoculated planting, soil and amendment main effects were significant by ANOVA of the AUDPC: soil F value = 5.46, $P > F = 0.0111$. AUDPC in Bet Dagan soil significantly decreased compared with the other soils. Amendment main effect F value = 67.03, $P > F < 0.0001$.

Table 4. Effect of wild rocket amendment (WR) and solarization on area under the disease progress curve (AUDPC) of Fusarium crown and root rot in cucumber which was sown or planted in a Rehovot soil inoculated with chlamydospores of *Fusarium oxysporum* f. sp. *radicis-cucumerinum*

		Planting[x]			
		First (sowing)		Second (planting)	
Chlamydospores[y]	Solarization[z]	Nonamended	WR	Nonamended	WR
4.075×10^4	Shaded	386.8 B	53.0 C	775.0 AB	452.4 B
4.075×10^4	Solarized	717.9 B	417.7 BC	368.6 B	785.7 B
8.15×10^4	Shaded	479.2 B	138.5 C	835.7 AB	527.1 B
8.15×10^4	Solarized	1,212.0 A	707.2 AB	1,292.9 A	920.0 B
2.375×10^5	Shaded	601.7 B	409.3 BC	1,040.0 A	461.7 B
2.375×10^5	Solarized	1,659.7 A	1,012.7 A	1,257.1 A	1,635.7 A
MSD ($P < 0.05$)	...	478.7	429.5	604.1	705.4

[x] In the first suppressiveness test, cucumber seed were sown in the tested soils; in the second (repeat) planting, cucumber seedlings were planted in the same soils without additional inoculation. Cucumber was grown for 28 days in each cycle. AUDPC means within a column followed by different letters are significantly different according to Tukey's studentized range (honestly significant difference) test at $P \leq 0.05$. Amendment main effect and the chlamydospore concentration–solarization interaction were significant by analysis of variance (ANOVA) of the AUDPC: amendment F value = 41.78, $P > F < 0.0001$; chlamydospore concentration–solarization F value = 5.66, $P > F = 0.0063$. Inoculum concentration–solarization and solarization–amendment interactions were significant by ANOVA of the AUDPC: chlamydospore concentration–solarization F value = 6.17, $P > F = 0.0042$; solarization–amendment F value = 9.80, $P > F = 0.0030$.

[y] Chlamydospore concentration. MSD = minimum significant difference.

[z] Solarization or shading under field conditions was conducted for 28 days during July 2008. WR was applied to the soil at 10 g/kg (1%, wt/wt).

compared with solarization alone, although inoculum density was similar in both treatments. In that study, solarization alone did not contribute to suppressiveness. It is also possible that soil suppressiveness following OA and solarization depends on the challenged pathogen in a given soil (in this study, *F. oxysporum* f. sp. *radicis-cucumerinum*).

The level of suppressiveness induced by WR was reduced by increased inoculum density (Table 4). Similarly, infection rates of Fusarium wilt of pea in chitin-amended soil were found to be directly correlated with chlamydospore density, in comparison with nonamended controls (23). Smith and Snyder (44) distinguished suppressive soils by the rate of chlamydospores needed to induce Fusarium wilt on sweet potato. They found that, in a suppressive soil, the inoculum density required to cause disease was twice that in conducive or noncultivated-suppressive soils. In addition, a higher percentage of chlamydospores of saprophytic *Fusarium* spp. germinated in wilt-suppressive soils than did chlamydospores of the tested pathogens (45).

The induced suppressiveness in OA-amended soils may be related to chemical or physical changes in the soil which, in turn, affect plant response to pathogens (10), or to stimulation of beneficial microbes which either directly affect the pathogens or induce resistance in the plant (17). Induced soil suppressiveness is only one component in disease management by OAs or solarization, and the other components (e.g., reduction in pathogen population and in its capacity to induce disease) should also be considered.

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תקציר

הצנעת שיירי צמחים מתאימים בקרקע, עם או ללא חיטוי סולרי, משמשת לחיטוי קרקע ולהדברת פגעים בחקלאות. במהלך התפרקות החומר האורגני, מתרחשים בקרקע תהליכים כימיים ומיקרוביאליים רבים שחלקם גורמים בסוף התהליך להיווצרות קרקע מדכאת מחלה (סופרסיבית). בקרקע סופרסיבית שכיחות המחלה וחומרתה נמוכים בנוכחותם של פתוגן חיוני ופונדקאי רגיש. בעבודה זו תיעדנו התפתחות סופרסיבית בקרקע, בעקבות הצנעת שיירי הצמח רוקט (*Diplotaxis tenuifolia*) בקרקע, כנגד מחלת ריקבון הכתר והשורשים במלפפון (*Cucumis sativus* L. 'Kfir'), שנגרמת ע"י הפטריה *Fusarium oxysporum* f. sp. *radicis-cucumerinum* (FORC). תיעוד הסופרסיביות בקרקע בוצע באמצעות הערכת שכיחות המחלה וחומרתה בצמחים שאולחו בפתוגן ונשתלו בקרקע הנבחנת, או בקרקע חולית שאולחה בפתוגן לאחר חיטויה. שכיחות ריקבון הכתר והשורשים במלפפון שאולח במאקרוקונידיה של FORC ונשתל בקרקע סופרסיבית הופחתה ב-20-80% (בהשוואה לקרקע ביקורת ללא הצנעת שיירי צמחים). דיכוי מחלת ריקבון הכתר התפתח גם כאשר מלפפון נזרע בקרקעות שטופלו בשיירי רוקט ולאחר מכן אולחו בכלמידוספורות של FORC. כושר הסופרסיביות של הקרקע נשמר גם כאשר הנבטים נשתלו בקרקע הנבדקת 34 חודשים לאחר חיטוייה.

בחנו את כושרם של שיירי צמחים שונים להשרות היווצרות קרקע סופרסיבית. שיירי הצמחים רוקט, טרגון (*Artemisia dracunculus*), מרווה (*Salvia officinalis*) וברוקולי (*Brassica oleracea* var. *italica*) היו היעילים ביותר בהשראת קרקע סופרסיבית. דיכוי מובהק של המחלה בקרקע סופרסיבית התקבל גם לאחר שתילה חוזרת של צמחים מאולחים באותן קרקעות, וללא טיפולים נוספים בין השתילות. מצאנו כי השראת הסופרסיביות בקרקע אינה סגולית לקרקע חולית. כך לדוגמא, הצנעת שיירי רוקט הפעילה סופרסיביות בשתי קרקעות נוספות (בית דגן, ועין תמר) שנבדלות זו מזו בתכונותיהן הפיזיקאליות והכימיות. הצנעת עלים וגבעולים של רוזמרין (*Rosmarinus officinalis*), גד השדה (כוסברה [*Coriandrum sativum*]), מנטה חריפה (*Mentha piperita*) וכרובית (*B. oleraceae* var. *botrytis*) גררה יצירת דיכוי מחלה בקרקע. אולם בניגוד להצנעת רוקט, סופרסיביות הקרקע לא נשמרה במחזורי אילוח ושתילה נוספים. חיטוי סולרי לבדו, השרה סופרסיביות בקרקע במספר מקרים, אולם הסופרסיביות הזו לא הייתה הדירה.

בחנו כושר הסופרסיביות של הקרקעות בדיכוי התפתחות הנמטודה *Meloidogyne javanica* והיווצרות העפצים בצמחי עגבניה (*Solanum lycopersicum* cv. 870), לוע ארי (*Antirrhinum majus* L. 'Photomek', white) ובזיל (*Ocimum basilicum* 'Peri'). הצנעת שיירי רוקט, טרגון, מנטה חריפה או מרווה בקרקע, עם וללא שילוב חיטוי סולרי, גררו דיכוי התפתחות עפצים בשורשי עגבניה, בזיל או לוע ארי, שאולחו בביצים של *M. javanica*.

ביצענו שלושה ניסויי שדה על מנת לבחון את סופרסיביות הקרקע בדיכוי נמטודות יוצרות עפצים בתנאי גידול מסחריים. בניסויים אלה גידלנו צמחי רוקט בקרקע בחלקה הנבחנת, ובתום הגידול

(לאחר יבוש) הצנענו אותו לקרקע. ולאחר מכן בוצע חיטוי סולרי מתון על מנת להפחית את מידבק הפתוגן בקרקע, ללא הכחדתו. החיטוי הסולרי המתון יצר פוטנציאל אינוקולום שאפשר לנמטודות לשמש כפתוגן בוחן לדיכוי מחלה. בשני הניסויים שבהם גודלה עגבנייה לאחר החיטוי, הופחתה הנגיעות בדרגה החמורה עד 50% בהשוואה להיקש, בחלקות שטופלו ברוקט, בחיטוי סולרי או בשילוב שלהם. בניסוי השלישי חיטוי סולרי לכשלעצמו, או בשילוב עם הצנעת רוקט, הפחית באופן מובהק את הנגיעות בעפצים בעגבניה ששמשה כצמח בוחן, אולם לא נמצא דיכוי יצירת עפצים בשורשי לוע ארי בעונת הגידול שלאחר מכן.

חקרנו את מנגנוני הסופרסיביות בקרקע לאחר הצנעת רוקט. דיכוי ריקבון הכתר במלפפון שנגרם על ידי FORC והמנגנונים המעורבים בה נחקרו ע"י לימוד השפעתם של רכיבי הקרקע השונים על התפתחות הפתוגן והתקדמות המחלה. חשיפת קרקע שאינה מדכאת מחלה, לתרכובות אורגניות נדיפות שנוצרו במהלך התפרקות רוקט בקרקע, השרתה סופרסיביות ודיכוי של מחלת ריקבון הכתר בנבטי מלפפון שאולחו בפתוגן טרם שתילתם בקרקע הנבחנת. באופן דומה, הוספת כמות קטנה של מיצוי מימי מקרקע סופרסיבית לקרקע שאינה מדכאת מחלה, גם כן השרתה דיכוי ריקבון הכתר בנבטי מלפפון מאולחים ב-FORC. המיצוי מכיל מרכיבים מסיסים ורחיפים, כמו גם אוכלוסיות מיקרוביאליות. ממצאים שהתקבלו בבחינת מנגנונים אפשריים לסופרסיביות, מחזקים את הנחתנו שמקור דיכוי המחלה מיקרוביאלי.

בחנו האם כושרה של הקרקע הסופרסיבית לדכא התפתחות מחלות מתבטא גם בהגברת עמידותם של צמחים לפתוגנים של השורש או למחוללי מחלות נוף. בחנו השערה זאת באמצעות גידול מלפפון בקרקע סופרסיבית לפני אילוח הנוף או השורש בפתוגן. לא נמצאה השראת עמידות בנבטים שגודלו בקרקע סופרסיבית לפני האילוח ב-FORC ושלאחריו הועתקו לקרקע שאינה מדכאת מחלה, בהשוואה לצמחי הביקורת. כמו כן לא נמצא עיכוב בהתפתחות עובש אפור בעלווה של צמחים שנשתלו בקרקע סופרסיבית ואולחו במחולל מחלת העובש האפור, *Botrytis cinerea* .

הביטוי של דיכוי מחלה בצמח כתוצאה של דיכוי הפתוגן בקרקע, כלומר הפחתה בצפיפות המידבק או בכושרו לחולל מחלה, נבחן באמצעות חשיפת אוכלוסיית הפתוגן למרכיבי קרקע שונים, בנוכחות או ללא השפעת הפונדקאי (פעילות שורשים או אקסודטים). קרקע סופרסיבית, לאחר גידול מלפפון הגבירה באופן מובהק את דעיכת מאקרוקונידיה של FORC (שהודגרו בה) ב-50%, 28 ימים לאחר האילוח, בהשוואה לקרקע ביקורת. לא נמצאה השפעה מובהקת של הקרקע מדכאת המחלה על כושרו של המדבק לנבוט וליצור כלמידוספורות חדשות, בהשוואה לקרקע ביקורת. כמו כן קצב התפתחות התפטיר של FORC על גבי קרקע סופרסיבית נמצא דומה לזה שבקרקע הביקורת. היות וההפחתה בצפיפות המידבק בהעדר פונדקאי לא נמצאה מיד לאחר השראת דיכוי מחלה בקרקע ועם זאת נמצאה הפחתת מחלה בצמחים מאולחים שנשתלו באותה קרקע, הפחתת המדבק אינה יכולה להוות מנגנון בלעדי או משמעותי בדיכוי המחלה. לכן, נבדקה האפשרות שהפחתת המחלה היא תוצאה של תהליך המתרחש בנוכחות הפונדקאי.

שיערנו שההפחתה בהדבקה של הפונדקאי ו/או דיכוי המחלה בצמח מתרחשים זמן קצר לאחר חדירת הפתוגן לשורש. הערכה כמותית של FORC בשורשי המלפפון, באמצעות real time PCR, שלושה ימים לאחר אילוחם מצביעה שההדבקה הראשונית של הפונדקאי בפתוגן לא הושפעה ע"י הקרקע הסופרסיבית, והייתה דומה לזו שבקרקע ביקורת. לעומת זאת, שישה ימים לאחר האילוח, התבססות הפתוגן בשורשי המלפפון בקרקע שאינה מדכאת מחלה הייתה פי 3 גדולה יותר, בהשוואה לקרקע הסופרסיבית. כמו כן, נתקבלו ממצאים דומים כאשר אוכלוסית הפתוגן בשורש הוערכה בשיטת המיהול הקלאסית. ממצאים אלו מעידים על כך שהקרקע הסופרסיבית אינה מפחיתה את מספר גופי הריבוי של הפתוגן מיד לאחר האילוח, או את כושרו לאכלס את שורשי הפונדקאי. ההפחתה של 66% באיכלוס השורש בפתוגן, שנמצאה בקרקע הסופרסיבית שישה ימים לאחר אילוח ושתילה, הייתה דומה לזו שבמספר הצמחים הנובלים (60%), 21 ימים לאחר האילוח והשתילה.

במקביל לבחינת התבססות הפתוגן בשורש, נבחנה חברת המיקרואורגניזמים המאכלסת את השורש (Microbiome), הכוללת אוכלוסיות של חיידקים ופטריות. הסוג *Fusarium* spp. היווה את קבוצת הפטריות הדומיננטית בשורשיהם של נבטי המלפפון, ללא תלות בטיפול שניתן לקרקע (עם או ללא השראת דיכוי מחלה) או באילוח הנבטים ב-FORC. דומיננטיות זו של פוזריום ספרופיטי לא השפיעה על התפתחות הפוזריום הפתוגני, כפי שהתבטאה בצמחים שגודלו בקרקע ללא הצנעת רוקט, בהם נמצא אילוח משמעותי של השורש ב-FORC וחומרת המחלה גבוהה. יתרה מזאת, הרכב חברת הפטריות בשורש לא הושפע מסופרסיביות הקרקע בפרקי הזמן שנבדקו, אולם אילוח הנבטים בפתוגן, הגביר את השכיחות היחסית של *F. oxysporum* בשורשים מ-3-14% ל-88-89% בקרקע מדכאת מחלה, כמו גם בקרקע הביקורת. לכן, מנגנונים נוספים, שאינם מבוססים על התבססות פוזריום פרוטקטנטי, מעורבים בדיכוי המחלה בקרקע שהוצנעו בה שיירי רוקט.

השימוש בשיטות של קביעה כמותית של רצפי חומצות גרעין (16S rRNA), כמו גם בשיטות לקביעת רצף באופן מאסיבי (16S rRNA and fungal ITS pyrosequencing), אפשר לאפיין את השינויים בחברת החיידקים בשורש של צמח שגודל בקרקע הסופרסיבית. שינויים אלו היו איכותיים, כלומר שינוי בהרכב אוכלוסיות החיידקים ולא במספר כלל החיידקים. מגוון החיידקים בשורש הוגבר בקרקע הסופרסיבית, אולם הגברה דומה במגוון נמצאה גם בעקבות אילוח בפתוגן וכן כבר שלושה ימים לאחר אילוח ושתילה. לכן, לא ניתן לקבוע האם ההגברה במגוון החיידקים לכשלעצמה היא מאפיין של התהליך הסופרסיבי.

השינוי העיקרי בחברת החיידקים בשורשים שגודלו בקרקע סופרסיבית נגרם עקב הפחתה חדה בשכיחות היחסית של חיידקי *Massilia* (β-proteobacteria) בחברת החיידקים בשורש. ההפחתה המובהקת באוכלוסייה זו היוותה גורם מפתח בשינויים המבניים שנמצאו בחברת החיידקים בשורש בקרקע הסופרסיבית. חיידקי *Massilia* הינם דומיננטים בין סוגי החיידקים המאכלסים שורשי מלפפון בשלבים המוקדמים של התפתחות הצמח, ורגישים במיוחד להגברה בתחרות מיקרוביאלית.

במקביל להפחתה בגודל המוחלט של אוכלוסיית ה-*Massilia* בשורש ובדומיננטיות שלה כאשר הצמחים גדלו בקרקע סופרסיבית, נמצאו שינויים מובהקים בשכיחות היחסית של חיידקים נוספים. העיקריים שבהם היו *Rhizobium*, *Bacillus*, *Paenibacillus* ו-*Streptomyces*, שכולם קשורים לתהליכי הדברה ביולוגית ודיכוי מחלות צמחים. מבין אלה, הסטרפטומיצטים, שמהווים מרכיב חשוב בדיכוי מחלה במערכת של פוזריום במלפפון, היו בולטים. הרכב אוכלוסיית הסטרפטומיצטים (שנבחן באמצעות PCR-DGGE) השתנה באופן מובהק כאשר קבוצת סטרפטומיצטים ספציפית הפכה לדומיננטית בשורשים שגודלו בקרקע הסופרסיבית, כבר לאחר שלושה ימים משתילה. שינוי זה יוחס בעיקר למין *Streptomyces humidus* שהינו מין אנטגוניסטי למגוון פטריות פתוגניות לצמחים.

במערכת ניסויית זו של השראת דיכוי מחלה בקרקע, נוכחות הפתוגן FORC גרמה לשינויים משניים בלבד בחברת החיידקים בשורש הפונדקאי.

אנו מניחים כי מספר מנגנונים שונים מעורבים בדיכוי מחלה בקרקע בעקבות הצנעת שיירי רוקט. מנגנונים אלו כוללים הגברת תחרות כללית על מקורות מזון וכן אנטגוניזם ספציפי המתרחש בסביבת השורש זמן קצר לאחר האילוח בפתוגן.

המחקר הנוכחי מדגים ומדגיש כי שינוי התנאים בקרקע באמצעות שיירי צמחים ויצירת מאזן חדש, גורמת לשינויים בחברה המיקרוביאלית המאכלסת את שורשי הפונדקאי. שינויים אלה מתבטאים גם בתהליך של דיכוי התבססות הגורם הפתוגני בשורש ומובילים לדיכוי מחלה כבר בשלבים המוקדמים, אך לא המיידיים, של הדבקת השורש בפתוגן.

דיכוי ההתבססות של מחלות צמחים ע"י תוספים אורגניים לקרקע וחיטוי סולרי מהווה ערך מוסף להיותם אמצעי יעיל בהדברה המשולבת של פגעי קרקע. זהו תהליך משלים בפעולת ההדברה אשר עשוי להבטיח את יעילותה לפרק זמן ארוך יותר, לעכב את התבססות הפגעים בשורשי הצמח הפונדקאי ולהגן על הקרקע בזמן הגידול שלאחר פעולת החיטוי.

שלמי תודות

ברצוני להביע את הערכתי העמוקה ותודתי לפרופ' יעקב קטן ופרופ' אברהם גמליאל. מחוייבותם והדוגמה האישית עודדו אותי והנחייתם הייתה מקור להשראה ולהתלהבות.

לעונג הוא לי להודות לחברי המעבדה שעמם הייתה לי הזכות לעבוד. נכונותכם לסייע ולעזור וחברתכם הנעימה ראויים לציון. תודה מיוחדת למרינה בניחיס, ברכה שטיינר ויהודית ריבן עבור העזרה והעידוד במהלך עבודה זו; לשחף טריקי-דותן, הראל כדורי ורוני אלון עבור פועלם וחברותם היקרה מפז.

תודות מקרב לב לד"ר דרור מינץ עבור ההדרכה, שיתוף הפעולה ועצות מועילות ביותר; תודה לכל חברי מעבדת 'מינצומונס' עבור האירוח.

תודה מיוחדת לד"ר מיה אופק שהדרכתה ועזרתה הרבה במבחנים המולקולאריים איפשרו לי להגיע לתובנות בנושא המיקרוביום של השורש.

אני אסיר תודה לפרופ' יגאל אלעד עבור עצותיו המועילות; דליה רב-דויד, עבור ההדרכה בנושא מחלות הנוף; שרה ארז, עבור הסיוע עם הפתוגנים שוכני הקרקע; פרופ' דניאל אוריון, עבור הייעוץ; פרופ' יצחק הדר וד"ר שאול בורדמן, חברי הועדה המלווה עבור עצותיהם המועילות.

רוב תודות לעירית דורי, חנה יחזקאל וצוות תחנת הנסיונות בשור עבור שיתוף הפעולה בניסויי השדה; משתלות 'חישתיל' ו'שורשים' עבור אספקת שתילים; משה אלפרט ושותפות יע"ף (מחולה, ישראל) עבור אספקת תבלינים טריים; הילרי פוט, עבור ייעוץ בנושא הניתוחים הסטטיסטיים; יוסי גוטליב וצוות חוות הפקולטה לחקלאות ברחובות עבור שיתוף הפעולה.

אני חייב את הכרת התודה העמוקה לאשתי, סיגל, שותפתי לחיים וחברתי עבור היותה אם הבית וההורה הבכיר במהלך ההיעדרויות הממושכות שלי מהבית. תודה ענקית ליונתן, דניאלה ואמיתי שמהווים בשבילי מקור אור, אושר ושמחה. ברצוני להודות למשפחתי שתמיד ניצבה לצידי במהלך תקופה מאתגרת זו בחיי, במיוחד להורי שוש ואיציק עבור אהבתם שאינה תלויה בדבר ותמיכתם.

מחקר זה מומן בחלקו באמצעות מענקים מטעם המדען הראשי של משרד החקלאות ופיתוח הכפר, מלגת הצטיינות מטעם החברה הישראלית למחלות צמחים, קרנות "קנט", "האורגניזם השלם בחקלאות" ו"הקרן ע"ש אוגניה ופרופ' ג. מינץ" מטעם מנהל המחקר החקלאי.

עבודה זו נעשתה בהדרכתם של

פרופ' **יעקב קטן**

האוניברסיטה העברית בירושלים, המחלקה למחלות צמחים ומיקרוביולוגיה, הפקולטה לחקלאות, מזון וסביבה ע"ש רוברט ה. סמית, רחובות.

פרופ' **אברהם גמליאל**

המעבדה לחקר היישום של שיטות הדברה, המכון להנדסה חקלאית, מנהל המחקר החקלאי, מרכז וולקני, בית-דגן.

דיכוי התבססות של מחלות צמחים בקרקע בעקבות העשרתה בתוספים צמחיים וחיטוי סולרי

חיבור לשם קבלת תואר דוקטור לפילוסופיה

מאת

איל קליין

הוגש לסנט האוניברסיטה העברית בירושלים
דצמבר 2011