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ABSTRACT

Certain microbial residents from composts are known to possess the ability to suppress soilborne plant pathogens. *Trichoderma* spp. and *Gliocladium* spp. are commonly found in composts and are perhaps the most wellknown hyperparasites of fungal pathogens, which may also be naturally present in compost. Trials have many times proven them to be effective against plant diseases caused by soilborne pathogens such as *Pythium* spp., *Phytophthora* spp. and *Fusarium* spp.

In this study, extracts obtained from eight different composts of various origin and maturity were applied to oilseed rape and cucumber seedlings under greenhouse conditions. The test plants (oilseed rape and cucumber) were thereafter challenged with the test pathogens. Oilseed rape was inoculated with *Verticillium longisporum* (former *V. dahliae*) and cucumber with *Pythium sylvaticum*. *V. longisporum* is a wellknown fungal soilborne plant pathogen that causes wilt disease in oilseed rape and other hosts. Since *V. longisporum* apart from being soilborne also is vascular, it is more difficult to control compared to opportunistic soilborne fungal pathogens, e.g. *Pythium* spp. Many experiments have proven compost amended media to be suppressive to several pathogens including *Pythium* spp. On the other hand, little research seems to have been done on composts regarding control of *V. longisporum.* In the present study, *P. sylvaticum* failed to infect cucumber seedlings, probably due to its weak pathogenic nature among other factors. The trials were hence focused on compost mediated suppression of *V. longisporum* in oilseed rape.

The results showed that *V. longisporum* infected oilseed rape seedlings to various degrees, depending on the compost extract applied. Both fungi and bacteria could be isolated from the compost extracts that were evaluated for their *in vitro* antagonistic activity against *V. longisporum.* Predominantly fungal isolates were demonstrated to be strongly antagonistic towards the pathogen. Most bacteria isolated from the compost extracts showed no or only slight antagonistic activity towards *V. longisporum*, with the exception of two isolates that exhibited strong antagonistic activity. The results obtained suggest that compost has potential to function as biological control of *V. longisporum*.

Keywords: Biological control, compost, *Verticillium longisporum*, oilseed rape

SAMMANFATTNING

Vissa mikrobiella invånare i kompost är kända för att besitta förmågan att hämma jordburna växtpatogener. *Trichoderma* spp. och *Gliocladium* spp. är vanliga kolonisatörer i kompost och är kanske de mest välkända hyperparasiterna på patogena svampar, vilka också kan förekomma naturligt i kompost. Försök har många gånger visat att de är effektiva mot växtsjukdomar orsakade av jordburna patogener som till exempel *Pythium* spp., *Phytophthora* spp. och *Fusarium* spp.

I föreliggande studie har filtrat från åtta komposter av varierande ursprung och mognad tillsatts till raps och gurkplantor i växthus. De patogena svamparna *Verticillium longisporum* (tidigare *dahliae*) och *Pythium sylvaticum* inokulerades därefter i jorden runt rapsplantor respektive gurkplantor. *V. longisporum* är en välkänd svamp och växtpatogen som orsakar kransmögel i raps och andra värdar. Då *V. longisporum* förutom att vara jordburen även är vaskulär är den svårare att kontrollera jämfört med opportunistiska patogena svampar, t.ex. *Pythium* spp. Många försök har bevisat att kompostberikat substrat verkar hämmande mot flera patogener, inklusive *Pythium* spp. Komposters effekt avseende kontroll av *V. longisporum* är däremot inte lika väl undersökt. Under studiens gång uteblev infektionen av *P. sylvaticum* i gurkplantorna, möjligtvis på grund av dess svaga patogena natur, bland annat. Detta försök fokuserade därför på kompostmedierad hämning av *V. longisporum* i raps.

Resultaten visade att *V. longisporum* infekterade rapsplantor i olika grad beroende på vilket kompostfiltrat som tillsatts. Både svampar och bakterier isolerades från extrakten, som utvärderades avseende antagonistiska effekter *in vitro* mot *V. longisporum*. Företrädelsevis svampisolat visade sig ha starkt antagonistisk effekt mot patogenen. De flesta bakterier som isolerades ur extrakten visade ingen eller endast svag antagonistisk effekt mot *V. longisporum*, med undantag av två isolat som var tydligt hämmande. Resultaten tyder på att kompost har potential att verka som biologisk kontroll och hämma utvecklingen av *V. longisporum*.

Nyckelord: Biologisk bekämpning, kompost, *Verticillium longisporum*, raps

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INTRODUCTION

Composting is a natural way of dealing with waste, transforming it into soil improvement and plant nutrients. It brings a spiritual satisfaction to observe how death gives birth to life. Besides waste treatment, there are other fields where composts are well known to be of value, such as in plant protection. The compost may itself act as growth medium or as a source of beneficial organisms, potential biocontrol organisms.

The rich microbial flora as such, commonly present in compost, has repeatedly proven to have suppressive effect on several plant pathogens. Likewise, several single microorganisms isolated from compost have turned out to be strongly antagonistic against certain plant pathogens.

A compost well cared for may give a contribution to biological control and hence suppression of disease caused by pathogens. Hadar & Mandelbaum (1992) define suppressive compost as "an environment in which disease development is reduced although the pathogen is introduced in the presence of a susceptible plant". Numerous composts of various origin have been investigated over the years, regarding their effects on plant pathogens; soilborne as well as foliar. This study predominantly deals with soilborne plant pathogens, and composting will be regarded as a primarily aerobic process with anaerobic features.

OBJECTIVES

The aim of the present study was to review current knowledge on use of compost as a resource in biological control and how its disease suppressive potential may be utilized. The study also describes the composting process and the parameters interfering with the final result, and thus the ability of the compost to reduce or inhibit infections by soilborne fungi. The study is led by the hypothesis that composts harbour vast microbial populations, some of which with potential to suppress disease caused by vascular soilborne plant pathogens, as well as saprophytic or opportunistic.

A compilation of literature available on compost mediated pathogen suppression constitutes a theoretical background to trials performed *in vitro* as well as *in vivo*. Extracts prepared from composts of varying composition and origin were monitored for their chemical and microbial characteristics. Isolated microorganisms from these were subsequently challenged *in vitro* with *V. longisporum*. A greenhouse trial, in which *V. longisporum* and *P. sylvaticum* served as model pathogens, was set up in order to monitor growth and disease development in oilseed rape and cucumber plants and also to discern differences, if any, between different compost extracts. Host seedlings of oilseed rape and cucumber were exposed to these two pathogens respectively, in absence and presence of the different compost extracts.

LITERATURE STUDY

THE COMPOSTING PROCESS

Composting may appear to be an easy practise, but is in theory complicated. The remarkably intricate interactions between microorganisms in compost contribute to this complexity. The process is commonly described in terms of three phases during which different conditions prevail. The organic material to be composted harbours vast numbers of microorganisms that serve as initial inoculum and a general impression is that metabolic diversity is greater among mesophilic than among thermophilic bacteria (Finstein and Hogan, 1993).

Composting is a predominantly thermophilic and aerobic process under controlled circumstances involving microbial breakdown and stabilization of organic materials from which the main products released are carbon dioxide, water, ammonia and heat. Nitrate and methane may also be formed. Anaerobic elements usually exist where oxygen depletion prevails, for example in the interior of organic matter. The composting process as a whole may be considered as waste stabilization requiring special conditions (McKinley *et al*., 1985a; Hoitink & Fahy, 1986; Hadar & Mandelbaum, 1992). Microbial metabolic activity constantly changes the chemical and physical properties of the surrounding environment, which also in turn affects the ability of microorganisms to grow, metabolize and survive (McKinley *et al*., 1985a). To ensure a suitable end product, there are a number of factors of utmost importance for the composting process to succeed. Composition of raw materials, oxygen availability, moisture and temperature should all be taken into account (Eklind, 1998). pH is also critical in the outcome of the composting process and a pH varying between 6,5 to 8,5 is optimal (Hoitink, 1980). How these factors affect the composting process is discussed below. Temperature is elevated during composting since organic material works as a self-insulating mass that retains heat generated due to microbial activity and heat might thus be regarded as a metabolic waste product (McKinley *et al*., 1985a; Finstein & Hogan, 1993). This heat generation is crucial for the succession of microbes through the process, and thereby for natural suppression of plant pathogens.

Three Distinct Phases

Initial Phase

The initial phase during the first few days is a lag phase with a rather low microbial activity due to adaptation of the metabolism to the food bases present (Åkesson & Gustafsson, 1993). The conditions prevailing during the initial phase favour mesophilic growth, decomposition and heat elevation. Mesophilic activity slows down at approximately 35°C, but temperatures continue to rise regardlessly, only more slowly. When reaching 40-50°C the mesophilic community self destructs (Finstein & Hogan, 1993). During this first mesophilic phase easily degradable substances such as sugars, proteins, amino acids, nucleic acids, lipids and starches are degraded (Hoitink *et al*., 1991).

Peak Heating Phase

Mesophilic decline and the rise of temperatures (40-55°C) initiate a new burst of activity by thermophilic microorganisms. The activity of the thermophilic community begins to decline at approximately 55°C and heat elevation is continued in the same manner as in the

mesophilic phase, but now partly due to an increased rate of microbial degradation of organic material (Hoitink, 1980) and temperature ranges from 55 to 70° C. At 75 to 80°C the system brings itself close to microbial extinction. Peak temperature is maintained while readily metabolized compounds are slowly depleted (Finstein & Hogan, 1993; Hoitink *et al*., 1997a). This second phase during which less easily degradable substances such as cellulose and hemicellulose are degraded (Alm *et al*., 1997; Hoitink *et al*., 1997a), is characterized by the continued heat elevation, i.e. peak heating. Thermophilic microorganisms predominate in the peak heating phase. *Bacillus* spp. seems to dominate the early stages, to be subsequently outnumbered by thermophilic actinomycetes (Hoitink & Fahy, 1986). Even though thermophilic microbes are adapted to grow at high temperatures as in the peak heating phase, they are not as effective as mesophiles in their growth and respiration. It seems as if metabolic efficiency is somewhat sacrificed to endure heat (McKinley *et al*., 1985b). Few fungi can grow at peak heating temperatures (Finstein & Hogan, 1993) and fungal as well as bacterial plant pathogens are killed during this phase. Unfortunately, that is also the fate of biocontrol agents (Hoitink *et al*., 1991). A terminal decline in temperature and slow decomposition now follows (Finstein & Hogan, 1993).

Curing Phase

The third phase is usually referred to as a ”curing” phase during which gradual stabilization proceeds as the concentration of readily degradable substances decline (Hoitink *et al*., 1991). Decomposition rates decrease, temperatures decline, mesophilic microorganisms recolonize the compost (Hoitink & Fahy, 1986) and the humic fraction increases due to degradation of lignins and dead microorganisms (Åkesson & Gustafsson, 1993). Immediately after peak heating a biological vacuum prevails and the compost may be rapidly infested with opportunistic plant pathogens such as *Pythium* spp. (Hoitink *et al*., 1993). This is a major reason why composts ought to be given time to cure. When curing begins, mesophilic microorganisms from the outer cooler layers migrate into the compost centre (Hoitink *et al*., 1991) and recolonize it in succession to the thermophiles. Very few known beneficial microorganisms survive in high temperature areas (Hoitink *et al*., 1997b) and the pathogen- and disease suppressive properties of compost are largely induced during curing since most biocontrol agents recolonize after peak heating (Hoitink *et al*., 1997a). Several known biocontrol agents have been identified as recolonizers of composts after peak heating; e.g. *Bacillus* spp., *Enterobacter* spp., *Flavobacterium balustinum*, *Pseudomonas* spp., among bacterial genera, and *Trichoderma* spp., *Streptomyces* spp., *Gliocladium* spp., *Penicillium* spp., among fungal genera (Hoitink *et al*., 1997a). It may be of importance to allow for curing not only to promote recolonization and induction of disease suppressiveness, but also to eliminate or reduce negative plant responses (Hoitink & Fahy, 1986).

Influence of Physical Factors

Physical properties during the composting process extensively affect the predominating microflora and hence also indirectly and subsequently affect the disease suppressive qualities. Organic materials used, moisture, oxygen and temperature all seem to have a great impact on the composting process.

Organic Materials

The composition of organic materials is of fundamental importance for the outcome of the composting process. Finstein & Hogan (1993) describe the role of the organic material as follows; ”In composting, a solid-phase organic material serves as physical support, gas exchange matrix, source of organic and inorganic nutrients, water, and diverse indigenous microbes, a sink for metabolic waste products, and thermal insulation”.

Microorganisms need carbon and nitrogen for their metabolism; breaking of carbon-bonds rich in energy supplies microorganisms with energy, while nitrogen is incorporated into amino acids to build proteins (Eklind, 1998). The microbial consumption of nitrogen varies during the composting process. Microorganisms need nitrogen early in the process to be able to proliferate and work with the organic material, and nitrogen levels decrease. Once decomposing activity is established nitrogen levels are stabilized since microorganisms themselves produce nitrogen in equal rates as they consume it. As decomposition rates decline and microorganisms die, nitrogen levels are increased (Alm, 1978). High C/N ratios (larger than 30:1) usually result in slow decomposition due to nitrogen deficiency and hence slow population growth. Low C/N ratios (smaller than 15:1) on the other hand, i.e. when nitrogen is in excess relative to carbon, may lead to gaseous nitrogen losses. Stabilized C/N ratios in between 15:1 and 30:1 generally work well for wastes (Miller, 1993; Eklind, 1998). A balance in the C/N ratio is thus a necessity for microbial life to thrive (Olausson, 1994).

However, the total C/N ratio does not reveal the amount of carbon available to microorganisms and is therefore a rather coarse measurement of decomposability (Eklind, 1998).

Oxygen

Composting is to a great extent due to aerobic microbial respiration (Finstein & Hogan, 1993) and a flow of oxygen in appropriate amounts is crucial, which is why material about to be composted should be packed lightly to allow oxygen availability throughout the compost (Olausson, 1994). Gas transfer is also greatly affected by the physical structure of the composting material (Miller, 1993). An excess of air may render a compost too dry while limitation of air may result in anaerobic respiration with increases in unpleasant odours such as sulfides and volatile fatty acids (Miller, 1993; Olausson, 1994). The degree of access to oxygen in the compost determines microbial metabolism and hence the course of the process and final products produced (Miller, 1993). Optimum oxygen levels for maximum rates of thermophilic composting are 5 to 12% and mechanical agitation may facilitate regulation of oxygen to appropriate levels (Hoitink, 1980; Finstein & Hogan, 1993). The microbial consumption of oxygen is closely connected to temperature elevation as reviewed by Finstein & Hogan (1993), i.e. as air flows through the composting material, oxygen is consumed by microorganisms actively releasing heat from the organic material. Energy is thus transferred from the solid phase to the flowing airstream as sensible (10 to 20%) and latent (80 to 90%) heat.

Moisture

”A compost should have a consistency as that of a squeezed sponge” (Olausson, 1994).

The moisture content in the compost is indeed critical for microbial diversity and activity and should be present in appropriate amounts throughout the composting cycle (Finstein & Hogan, 1993). About 65 to 70% is the recommended level of moisture content (w/w) during

the most intense phase of the composting process (Eklind, pers. comm.). A dry and dusty compost results in formation of an abundance of moulds which may cause difficulties in retaining moisture, since fungal masses tend to repel water (Hoitink *et al*., 1997b). Transport of nutrients through the compost is also retarded by low moisture levels. In a wet compost on the other hand, microbial migration and colonization along with diffusion of nutrients and metabolic wastes is simplified, but the essential gas exchange is impeded since water tends to plug pores (Finstein & Hogan, 1993; Olausson, 1994). There is thus need for a balance between the requirements for available water and gas exchange to be maintained (Finstein & Hogan, 1993).

For a compost to induce disease suppression the moisture content has to be high enough (Hoitink *et al*., 1997a) and should be more than 35% and preferably over 45% for beneficial microorganisms to colonize compost (Hoitink *et al*., 1997b). In such conditions bacteria as well as fungi are able to colonize. A compost with a moisture content less than 35% is usually colonized predominantly by fungi and has shown to be consequently conducive to diseases caused by *Pythium* spp. (Hoitink *et al*., 1997a). The optimum moisture level ranges between 50 to 65% (wet weight) for thermophilic activity (Hoitink, 1980). The microbes grow as biofilms on the surface of organic matter and the ability to do so is greatly restricted by moisture content. This is especially true for bacteria since they are dependent on water films to colonize readily and the moisture content may be especially critical after peak heating, which affects bacterial mesophilic potential to recolonize (Hoitink *et al*., 1997a; Hoitink *et al*., 1997b).

Temperature

Temperature before start of microbial degradation and self heating is a factor that primarily is of importance in the initial stage of composting. Microorganisms need to be active to decompose and hence generate heat. Fungi and bacteria responsible for self heating of composts need at least 10 to 12°C to be active and get the composting process going. Once the process is started heat is generated by microbial activities. Heat in compost is due to metabolic activity of microbial origin and tends to be retained within the compost, causing a temperature elevation, i.e. self heating, which is characteristic of the composting process (Finstein & Hogan, 1993).

At temperatures from 35 to 55°C prerequisites of decomposition are at their best, and from 40 to 55°C well balanced organic matter is decomposed most rapidly (Hoitink, 1980; Olausson, 1994). A study on temperature effects during composting of municipal sludge conducted by McKinley *et al*. (1985a) showed that microbial activity decreased dramatically as temperatures rose to 55°C. When temperatures exceeded 60°C microbial activity nearly collapsed. They examined several factors apart from temperature; moisture content, pH, organic content, total nitrogen, total carbon, C/N ratios and protein, but temperature undoubtedly had the most dramatic effect on microbial activity. The highest microbial activity was generally detected in samples from the 35 to 45°C areas (McKinley *et al*, 1985b).

Temperature and its effect on microorganisms is further discussed below under ”Heat” in the following section.

ALTERATIONS IN VIABILITY OF PLANT PATHOGENS DURING COMPOSTING

The nature of the organic material, which composting process used, and maturity of the compost affect any compost amendment regarding disease control (Hoitink & Fahy, 1986). *Phytophthora cinnamomi*, *Pythium irregulare*, *Rhizoctonia solani*, *Botrytis cinerea* and *Erwinia carotovora* var. *chrysantemi* in infested plant tissues have been found to be eradicated when buried and composted in hardwood bark (Hoitink *et al*., 1976). The eradication or inactivation of plant pathogens occurs as a result of several interacting factors.

Inactivation

According to Finstein & Hogan (1993), three objectives should be pursued during composting with respect to pathogenic microbes, namely prevention of growth and dissemination of pathogens, destruction of pathogens originally present, and to render the compost inhospitable for their regrowth. Conditions prevailing in composts based on plant residues are generally highly detrimental to most plant pathogens, although sensitivity to composting differs somewhat between pathogenic groups (Bollen, 1993).

Factors Involved in Inactivation

At least three factors are involved in eradication of pathogens during composting; (1) high-temperature exposure, (2) release of toxic products during or after the self-heating process and (3) microbial antagonism (Hoitink & Fahy, 1986). These factors are believed to be of unequal importance and they operate in succession or at the same time. Heat is however the best basis for estimating sanitation since toxins and microbial antagonism are more difficult to monitor (Bollen, 1993). The three factors are reviewed separately below.

The work described below on plant pathogen survival during composting is mainly based on work reviewed by Bollen (1993). Results from experiments based on infected plant material placed in nylon fabric nets or perforated metal containers and incorporated in compost heaps are presented. Fragments of the results are included in the following sections.

Heat

Inactivation of pathogens in naturally infested plant tissues can mostly be explained by periods of exposure to heat and the earliest report on the subject is from 1940 in a Russian article on killing of tobacco pathogens (Hoitink & Fahy, 1986). Baker *et al*. (1967) recommended 60°C for 30 minutes as a reasonable compromise if using aerated steam to eradicate pathogens, while 100°C for 30 minutes destroys most of the antagonists as well. Temperature is as previously mentioned to be elevated during the self-heating process, and in combination with formation of toxic substances it is believed to be extremely effective in eradication of pathogens of all kinds (Bollen, 1993).

Microorganisms generally handle temperatures slightly below growth optima far better than they handle temperatures slightly above, and a microbe exposed to temperatures exceeding its optimal growth temperature quickly suffers from severe stress (McKinley *et al*., 1985b). Fungi, bacteria, nematodes and viruses generally differ in sensitivity to heat; bacteria and nematodes are usually more sensitive to heat than fungal plant pathogens according to

Hoitink & Fahy (1986). Bollen (1969) on the other hand, claims that fungal plant pathogens are more heat sensitive than bacterial plant pathogens and also that pathogenic fungi are less heat resistant than saprophytic fungi.
Temperature and time of exposure required to inactivate a vast number of pathogens have been investigated (Bollen, 1969; 1993), and Åkesson & Gustafsson (1993) have compiled data from scientific articles concerning temperature and time of exposure for thermal kill of 44 plant pathogens in ”Smittar komposten?”. Most plant pathogens are however dead after exposure to 55°C for 30 minutes (Bollen, 1969). In contrast, in an interview in BioCycle, Harry Hoitink (1988) says that it takes three days at 55°C to kill possible faecal pathogens in municipal sludge compost. Prolonged exposure to sublethal temperatures may well weaken the resting structures of plant pathogenic fungi and render them more vulnerable to antagonistic attack as in the case of propagules of *Fusarium oxysporum* f. sp. *vasinfectum* and sclerotia of *Sclerotium rolfsii* (Tjamos & Fravel, 1995).

Hoitink & Fahy (1986) have reviewed several examples of plant pathogens which are inactivated by heat; *Rhizoctonia solani*, *Verticillium albo-atrum*, *Verticillium longisporum*, *Phytophthora cinnamomi*, *Phytophthora cryptogea*, *Pythium irregulare*, *Botrytis cinerea*, *Botrytis alli*, *Sclerotium cepivorum*, *Sclerotium rolfsii*, *Armillaria mellea*, *Didymella lycopersici*, *Plasmodiophora brassicae*, *Phomopsis sclerotioides*, *Sclerotinia sclerotiorum*, *Stromatinia gladioli*, *Dickeya* sp., *Pseudomonas syringae* pv. *phaseolicola* and tobacco necrosis virus. Inactivation of resting spores of *P. brassicae* requires alkaline pH and 60 to 80% moisture, besides high temperature (Hoitink & Fahy, 1986). For composts prepared from residues heavily infested with obligate root parasitic fungi or *Fusarium oxysporum* a temperature of at least 60°C must be attained during the heating phase (Bollen, 1993).

Katan (1981) has listed pathogens for which disease severity was readily reduced by solarization of soil. This information could probably be referred to when considering low temperature inactivation during the composting process, since it is a similar situation involving prolonged exposure to moderate temperatures (Bollen, 1993). The following pathogens were effectively controlled by soil solarization according to Katan (1981): *Verticillium* spp., *Rhizoctonia solani, Sclerotium rolfsii, Pyrenochaeta lycopersici*, *Pyrenochaeta terrestris*, *Fusarium* spp., *Pratylenchus thornei*, *Orobanche* spp. and pod rots.

Fungal pathogens generally do not survive high temperature during composting (Hoitink & Fahy, 1986). However, many of them have one feature in common; their reproductive structures are usually more heat resistant than their vegetative structures. A few of the more heat resistant fungi have been examined regarding thermal kill; a few *formae speciales* of *Fusarium oxysporum* survived exposure to more than 55°C for 30 minutes and thick-walled resting spores of *Olpidium brassicae* and *Synchytrium endobioticum* also survived. Resting spores of *Plasmodiophora brassicae* survived 65°C for 10 minutes. When exposed for 30 minutes thermal kill approached to 55°C (Bollen, 1993). From these examples it is easily understood that time of exposure needed for inactivation varies with temperature. One exception to heat resistant resting structures is *Phytophthora infestans* with a thermal deathpoint for spores at 25°C and for mycelium at 45°C (Golueke, 1982). However, fungal activity generally decreases at temperatures exceeding 55°C (Miller, 1993) and survival in compost at relatively high temperatures could be explained by low moisture conditions under which more heat is needed for thermal kill (Bollen, 1993). Alkalinity is possibly an

important factor as well, and pathogens might be more sensitive to composting at low pH values than at high, as reviewed by Bollen (1993).

Bacteria are relatively sensitive to heat, with the exception of spore-forming species. The only known spore-forming bacterial plant pathogen is *Bacillus cereus*, a tobacco pathogen. Experiments with bacteria suspended in water or physiological salt solutions indicate that most bacterial plant pathogens have thermal death points near 50°C. A thermal deathpoint of 53°C makes *Corynebacterium michiganense* one of the most heat resistant bacterial plant pathogens.
On basis of bacterial heat sensitivity though, bacterial plant pathogens are unlikely to survive composting, where temperature normally rises above 50°C (Bollen, 1993).

Nematodal heat endurance has not been extensively reviewed and there is a limited availability of information concerning nematode inactivation during composting. Nevertheless, cyst-forming as well as root-knot nematodes, which are more persistent than nematodes in general, are indeed sensitive to composting temperatures and hence easily eradicated as reviewed by Bollen (1993).

Viral pathogen inactivation may involve vector inactivation as well depending on virus, and the majority of soilborne viruses are more heat resistant than the average pathogen. Most soilborne viruses infect host roots aided by vectors and successful infection is likely to occur only if both virus and vector survive the composting process, alternatively if vectors already present in soil are able to pick up the virus from the compost-amendment (Bollen, 1993). Tobacco rattle virus (TRV), tobacco necrosis virus (TNV), and tobacco mosaic virus (TMV) are three examples of viruses that may not easily be inactivated during composting (Hoitink & Fahy, 1986; Bollen, 1993). Despite their common heat resistance and uncertain eradication during composting their possible survival in compost is regarded unequally serious since vector heat-resistance differs and not all are vector-mediated.
TRV and TNV are both transmitted by vectors that are less heat resistant than the viruses themselves. TRV is transmitted by nematodes of the genera *Trichodorus* spp. and *Paratrichodorus* spp.. Since plant parasitic nematodes are highly heat sensitive they are likely to be killed during composting. TNV is on the other hand transmitted by the fungus *Olpidium brassicae*, which is one of the most heat resistant root-infecting fungi (Bollen, 1993).
TMV does not need any vector to infect roots on host plants and it has furthermore a wide host range. Additionally, TMV is the most heat resistant plant virus and could still be detected after ten weeks of exposure to 68°C or five weeks to 75°C (Bollen, 1993). Hence it is not surprising that attempts to eradicate TMV from infested stems and leaves from tobacco have not been as successful as eradication of the above mentioned fungal plant pathogens. Extracts prepared from TMV infested plant tissues buried in composted hardwood bark (CHB) and exposed to 50 to 75°C for six weeks were still infective (Hoitink, 1980).

Toxic Products
Composts undergoing decomposition form and release a variety of substances toxic to pathogens. Ethanol, methanol, formaldehyde, ammonia and ethyl esters have all been identified as such substances (Bollen, 1993). Reproductive activities among microorganisms

may be altered by these substances; low concentrations might stimulate growth while high concentrations might inhibit microbial development. The composition of raw material and prevalence of the different toxic substances are crucial to how different microorganisms are affected, e.g. *Fusarium culmorum*, *Botrytis cinerea* and *Verticillium longisporum* were all suppressed in compost containing pea residues (Åkesson & Gustafsson, 1993). Toxic substances and their effect on pathogens are discussed further below in ”Inhibitors Released by Compost”.

Microbial Antagonism

According to Bollen (1993) ”exposure to elevated but sublethal temperatures or to toxic agents may weaken pathogens and make them more vulnerable to microbial antagonism”, but ”experimental evidence for an essential contribution to pathogen inactivation is lacking” and data on the role of microbial antagonism in inactivation of pathogens with less heat resistant structures is not available.

To whatever extent microbial antagonism does contribute to pathogen inactivation it probably occurs mainly in the sublethal outer temperature zones of piles or later during curing due to the increased activity of antagonists in mesophilic areas during curing (Hoitink & Fahy, 1986). However, Yuen (1984) discovered that sclerotia of *Sclerotium rolfsii* lost viability after roughly one week in the sublethal temperature areas during composting and suggested microbial antagonism as a possible contributing factor. Increased microbial activity has also been observed near dried sclerotia of *S. rolfsii* (Henis & Papavizas, 1983). Exposure to sublethal heat, alternatively rewetting of dried sclerotia, causes leakage of nutrients from the sclerotia (Katan, 1981), which may have enhanced microbial antagonism (Yuen, 1984).

While sclerotia of *S. rolfsii* died during the maturing process after peak heating, resting spores of *Plasmodiophora brassicae* and *Verticillium albo-atrum* survived (Yuen, 1984; Hoitink & Fahy, 1986).

FACTORS AFFECTING PATHOGEN SUPPRESSIVENESS OF COMPOSTS

Physical & Chemical factors

Several chemical as well as physical factors are known to affect the incidence of disease caused by soilborne plant pathogens. Elements being of chemical or physical origin involved in biological control induced by compost are air capacity, pH, carbon availability, nitrogen content, conductivity and release of inhibitory toxins (Hoitink & Fahy, 1986; Hoitink *et al*., 1993). The impact of each of these six elements is reviewed below.

Air Capacity

Particle size has an impact on air capacity and low air capacity in container media renders rhododendrons more susceptible to Phytophthora root rot as compared to rhododendrons in tree bark media with higher air capacity. The suppressive effect of the tree bark media was removed when the air capacity was lowered with silica sand amendments. High rates of decomposition lead to a reduction in particle size and some media need to be amended with neutral aggregates for structure and maintained air capacity (Hoitink & Fahy, 1986).

pH

pH has an impact on disease suppression, and acidic as well as alkaline pH values may have advantages. Alkaline conditions favour formation and release of the antimicrobial agent ammonia and reduce disease incidence caused by *Pythium* spp. (Mandelbaum & Hadar, 1990). An acidic pH, below 4,0, is suppressive to *Phytophthora cinnamomi* due to reduced sporangium formation, zoospore release and motility. It is however difficult to take advantage of it in practice, since very few plants have their growth optimum at such a low pH, which renders low pH an impractical disease control (Hoitink & Fahy, 1986).

As pH rises, solubility of trace elements decreases and microbial growth is limited. Some microorganisms produce siderophores, keeping the micronutrients in solution even at high pH, and thus concentrations of available iron, manganese and zinc may be high enough even at pH values above 7 (Hoitink *et al*., 1997b).

Carbon availability

The carbon availability or lignin/cellulose ratio affects the duration of the composting process (Hoitink & Fahy, 1986); the higher cellulose content the longer the composting process. The cellulose/lignin ratio varies with age and species of the organic material being composted (Hoitink, 1980). It also has an impact on disease severity (see ”Stage of Decomposition” below) since an excess of readily available carbon increases disease incidence of e.g. *Pythium aphanidermatum* (Mandelbaum & Hadar, 1990) and *Rhizoctonia solani*, while *Phytophthora* spp. may be inhibited (Hoitink & Fahy, 1986). Amendment of glucose/asparagine to container media delayed hyphal lysis of *P. aphanidermatum* and also rendered the media more conducive to bacteria than fungi in general, according to Mandelbaum & Hadar (1990).

Nitrogen Content

Attention ought to be paid to the nitrogen content of composts, since excessive nitrogen in combination with other factors favourable for disease conduciveness might cause severe plant pathogen epidemics. Excessive amendment with composts rich in nitrogen (low C/N ratio), e.g. composted municipal sludge (CMS), enhances *Erwinia amylovora*, Phytophthora diebacks and Fusarium wilts (Hoitink *et al*., 1993; Hoitink *et al*., 1997b). The reason why is that the form in which nitrogen prevails affects severity caused by, among others *Fusarium* spp., and high concentrations of ammonium in comparison to nitrate increases infection by *Fusarium* spp. (Trillas-Gay *et al*., 1986; Hoitink *et al*., 1993; Hoitink *et al*., 1997a). These composts are conducive to *Fusarium* spp. while composts with high C/N ratio, e.g. tree barks, immobilize nitrogen and suppress *Fusarium* spp., if colonized with an appropriate microflora (Hoitink *et al*., 1993; Hoitink *et al*., 1997a).

Conductivity

Any compost can have high salt concentrations (Hoitink *et al*., 1997b), i.e. be high in electrical conductivity, and bark composts tend to be lower in salinity compared with dairy manure or sludge composts, that can have consistently high levels of salinity (Hoitink *et al*., 1991; Hoitink *et al*., 1997b). Mineralization proceeds as a compost matures, which means that salts accumulate with time in composts. Fungal pathogens aggravated by salinity are *Phytophthora* spp. and *Pythium* spp. (Hoitink *et al*., 1997b), and will hence be enhanced unless the composts are applied ahead of planting to leach (Hoitink *et al*., 1997a). Composted municipal sludge (CMS) applied four months ahead of planting of soybean to

allow leaching of salts increased yields and suppressed Phytophthora root rot in a trial made by Schmitthenner and Hoitink (unpublished information). Amendment of exactly the same compost immediately before planting decreased yields, and treatment with metalaxyl prevented the yield decrease and Phytophthora root rot. Sodium chloride alone applied in the same amount as that present in the CMS decreased yields if applied just before planting, but not if applied in advance to allow for leaching. This effect of salinity on Phytophthora root rot has been known for decades (Hoitink *et al*., 1993).

Inhibitors Released by Compost

In cases when pathogens are inactivated and temperature is below the critical points for thermal kill, toxic conversion products formed from decomposing plant residues or microbial antagonism may be attributed (Bollen, 1993). Bollen (1993) reviewed a report by Berestetsky and Kravchenko from 1984 on soil volatiles formed during decomposition of crop residues. Major components of the volatiles were ethanol, methanol and formaldehyde, which all exhibited suppressive effects on germination of *Verticillium longisporum* sclerotia as well as inhibition of sporulation of other parasitic and saprophytic fungi.

Ammonia is often formed early during the composting process and may have detrimental effects at high concentrations on some fungal pathogens, e.g. *Phytophthora cinnamomi*. *P. cinnamomi* infested avocado roots were decontaminated in soil with high concentration of ammonia but not in soil with low concentration. Additionally, zoospore germination was prevented and mycelium killed when put in a 17 ppm ammonia phosphate buffer (Gilpatrick, 1969). Ammonia probably contributes to pathogen inactivation during the decomposition of residues rich in nitrogen. No effect of ammonia is assumed later in the process since most of it is converted into nitrate (Bollen, 1993). Ammonia is predominantly released under aerobic conditions, since organic acid formation under anaerobic conditions lowers pH to acid and then ammonium is formed instead of ammonia. The ammonium ion is not volatile (Miller, 1993).

Formation of toxic agents during composting can also be inferred from leachates and extracts from composts (Bollen, 1993). Extracts of tree bark compost have been found to release compounds toxic to fungi, i.e. inhibitors that lyse zoospores and sporangia of *Phytophthora* spp. (Hoitink *et al*., 1993). Ethyl esters of hydroxy-oleic acids were the most toxic compounds in six-month old composted hardwood bark suppressive to *Phytophthora* spp. (Hoitink & Fahy, 1986).

Biological factors

Stage of Decomposition

The activity of antagonists is affected by the maturity of composts and the stage of decomposition has a major impact on disease suppression (Hoitink & Fahy, 1986; Hoitink *et al*., 1997a). The fact that green composted hardwood bark (CHB) is conducive to Fusarium wilts while mature CHB is suppressive (Chef *et al*., 1983) indicates its significance. Organic matter neither adequately stabilized nor fully colonized by soil microorganisms capable of inducing microbiostasis tends to serve as a direct food base for both beneficial microorganisms and plant pathogens. Plant pathogens often possess a high competitive saprophytic ability and immature composts may increase the pathogen population, which

results in the opposite effect desired (Hoitink *et al*., 1997b; Hoitink & Boehm, 1999). Additionally, many biocontrol agents grow strictly as saprophytes in fresh organic matter (Hoitink *et al*., 1997b), which is why composts in order to induce suppression must be stabilized enough to attain a decomposition level where biological control is feasible (Hoitink *et al*., 1991). Grebus *et al*. (1994) successfully used radish as an indicator of compost maturity. As the compost matured, suppression of Pythium damping-off increased while the conduciveness to Rhizoctonia damping-off remained. *Pythium* spp. has a high competitive saprophytic ability and can utilize fresh plant residues, which explains why the nature of fresh waste generally is conducive. The same applies to *Rhizoctonia* spp.. Mature composts, however, that are fully recolonized by mesophilic bacteria as well as fungi, whether prepared from sewage sludge or tree bark, consistently induce suppression of Pythium damping-off (Grebus *et al*., 1994). There are however exceptions; in an experiment carried out by Aryantha *et al*. (2000) fresh chicken manure turned out to be just as suppressive as five weeks´ composted chicken manure to *Phytophthora cinnamomi* on *Lupinus albus* seedlings, whereas neither fresh nor composted cow, sheep, or horse manure proved to be suppressive. One likely explanation was the fact that only chicken manure stimulated activity of endospore forming bacteria, which were strongly associated with seedling survival.
The suppression or non-suppression of *R. solani* illustrates the importance of adequately stabilized composts to obtain satisfactory control, and is described below.

Initial stage
R. solani is a pathogen able to survive on fresh organic matter and is quite competitive as a saprophyte (Hoitink *et al*., 1991; Hoitink *et al*., 1997b). Although fresh hardwood bark high in cellulose is toxic to *Phytophthora* spp., it is conducive to *Rhizoctonia* spp. Fresh pine bark is also conducive to *Rhizoctonia* spp. (Hoitink & Fahy, 1986).

Trichoderma spp. and *Gliocladium* spp. have both been identified as antagonists to *R. solani* (Beagle-Ristaino & Papavizas, 1985; Lewis & Papavizas, 1985; Chung & Hoitink, 1990). *Trichoderma* spp. is however able to colonize fresh composted hardwood bark as well as a mature compost. Hence both the pathogen and the antagonist grow as saprophytes in fresh matter and *R. solani* is free to cause disease (Hoitink *et al*., 1997a, Hoitink & Boehm, 1999). A significantly higher cellulase activity has been recorded in green CHB as compared to mature CHB (Chung *et al*., 1988) and addition of cellulose to mature and suppressive *Trichoderma*-infested hardwood bark compost renders it conducive to *R. solani* despite increased *Trichoderma* spp. populations (Hoitink & Fahy, 1986). The reason why *Trichoderma* spp. does not suppress *R. solani* in either immature compost or cellulose-amended mature compost is attributed to repression of the transcriptional activity of the degrading enzymes such as chitinases, β-1,3- and β-1,6-glucanases, proteases and lipases in presence of the highly favoured cellulosic substrate (Hoitink & Boehm, 1999).

Just a few weeks of composting is usually enough for organic material to be adequately stabilized for most diseases to be controlled, with variations depending on the type of organic material to be composted (Hoitink *et al*., 1997b).

Intermediate stage
Nutrients are not as abundant in partially decomposed mulch as they are in fresh mulch (Chen *et al*., 1988b). Microorganisms have to compete for nutrients and while doing so, secondary metabolites are released. These secondary metabolites are commonly inhibitory or fatal to plant pathogens. A competition like this does not occur in fresh matter. The hyperparasite *Trichoderma* spp. is one of those biocontrol agents that behaves as a saprophyte in fresh mulches rich in cellulose and lignin, and as a competitor with a battery of antagonistic enzymes in partially decomposed mulches where nutrient availability is becoming limited (Hoitink *et al*., 1997a; Hoitink *et al*., 1997b). *Trichoderma* spp. and *Gliocladium* spp. are both excellent colonizers of mature composts and also parasites on sclerotia of *R. solani* (Hoitink *et al*., 1991). As degradation of organic material proceeds and the concentration of available cellulose decreases, saprophytic competitiveness increases. The chitin degrading enzymes in *Trichoderma* spp. are no longer repressed but transcribed, and ability of suppression and occasional eradication of *R. solani* through hyperparasitism of sclerotia prevails (Hoitink & Boehm, 1999).

Mature bark compost is low in cellulose and because of that, *Rhizoctonia* spp. is disadvantaged as regards colonization (Hoitink & Fahy, 1986; Hoitink *et al*., 1991) and falls victim for microbial antagonism, which is the case in composted pine bark. Biological control usually prevails in composts that have stabilized to non toxic levels and that becomes colonized by an appropriate microflora (Hoitink *et al*., 1997a). In addition, plant pathogens are usually poor competitors for resources in high carrying capacity composts (Hoitink & Boehm, 1999). Carrying capacity is defined below.

Final stage
Excessively stabilized organic material is the result of prolonged microbial degradation and gradual accumulation of humic substances, and the availability of organic matter for microorganisms becomes increasingly limited (Hoitink & Boehm, 1999). The compost is no longer able to adequately support activity of beneficial microorganisms and biocontrol begins to decline.
For how long time microbial activity is supported has not yet been investigated according to Hoitink *et al*. (1997a). They also state that duration presumably varies with temperature, soil characteristics and type of organic matter from which the compost is prepared.

Carrying Capacity
The carrying capacity of a substrate limits its suppressiveness to pathogens that rely on exogenous nutrients for germination and infection (Hoitink *et al*., 1993), and Hoitink *et al*. (1991) describe the carrying capacity of a substrate as ”the potential for a soil or potting mix to support sustained microbial activity”. A mature compost is largely composed of lignins, humic substances and biomass (Hoitink *et al*., 1991). Organic nutrients are tied up and released slowly, microbial activity is supported, and biocontrol sustained. This may also be the case for light sphagnum peat, although sustenance of microbial activity and biocontrol generally declines earlier (Hoitink *et al*.1993).

Compost-Amended Media
The antagonists capable of inducing suppression of Rhizoctonia and Pythium damping-off and Fusarium wilt have a short-term effect in peat amended substrates compared to their

effect in compost amended substrates (Hoitink & Fahy, 1986), which might be a consequence of the higher carrying capacity in composts than in peat.
Peat is typically a common component in container media, but unfortunately not a very disease suppressive component since its high resistance to decomposition renders it a poor medium for high microbial activity (Hoitink *et al*., 1991). Plant pathogens that are not suppressed in peat media are for example *Phytophthora* spp., *Fusarium* spp., *Pythium* spp. and *R. solani*, as well as nematodes (Hoitink & Fahy, 1986). Besides resistance to decomposition the acidic pH (3,2 to 4,0) of peat greatly limits microbial diversity (Hoitink & Fahy, 1986; Hoitink *et al*., 1993).

There is a noticeable difference in carrying capacity between light peat and dark peat. Dark peat is collected from 1,2 m or deeper layers in bogs (Hoitink *et al*., 1997a) and is lower in cellulose and more stabilized in comparison to light peat. Light peat is collected from superficial bog layers and harbours a larger microbial diversity than dark peat since it is less decomposed. Consequently, light peat has a higher carrying capacity than dark peat, and may contain large populations of miscellaneous antagonists such as *Trichoderma viride* and *Streptomyces* spp., apt to suppress *Pythium* spp., Rhizoctonia damping-off and Fusarium wilt. All light peats do not have similar capacity and the suppressive effect is moreover not very durable. Dark peat is presumably too stabilized for general microbial and antagonistic activity to be sustained (Hoitink & Fahy, 1986; Hoitink & Boehm, 1993; Hoitink *et al*., 1997a).

Despite of being a non-renewable resource, peat has desirable physical and relatively inert biological properties advantageous to many other potting media, and efforts have been made to confer disease suppressive properties to peat. Three approaches tested are 1) treatment with aerated steam followed by inoculation with specific biocontrol agents, 2) suppressive soil amendments and 3) suppressive compost amendments. Addition of compost to peat media has repeatedly proven to control a diversity of soilborne plant pathogens (Hoitink & Fahy, 1986).

To cite Hoitink & Boehm (1999): ”Composts can serve as ideal food bases for biocontrol agents and offer opportunities to introduce and establish specific biocontrol agents into soils, which in turn leads to sustained biological control based on activities of a microbial community”. How peat and other potting media can attain a higher carrying capacity and increased disease suppressiveness as a result of various compost amendments is accounted for below.

Composted Hardwood Bark Amendment

Composted hardwood bark (CHB) is a light weight growth medium with fungicidal properties. Its suppressive effect on a soilborne disease was first reported in 1962 when CHB incorporated into soil decreased incidence of strawberry red stele caused by *Phytophthtora fragaria*. The suppressive effect declined with time and vanished after four years, very likely due to loss of carrying capacity as a result of excessive decomposition. The first report on suppression of a specific plant pathogen by incorporation of tree bark in container media dates back to 1973. The incidence of Japanese holly root rot caused by *Pythium irregulare* decreased with increased amount of bark amendment, regardless of the size of the *P. irregulare* population (Hoitink, 1980). Since then, CHB has been widely investigated and

utilized as an amendment in container media to render them disease suppressive (Hoitink, 1980; Kuter *et al*., 1983). CHB has been successfully utilized in potting mixes for suppression of several soilborne pathogens; *Pythium* spp., *Phytophthora* spp., *R. solani* and *Fusarium* spp. (Trillas-Gay *et al*., 1986). *Thielaviopsis basicola* is also suppressed by CHB, according to Hoitink (1980).

Nelson *et al*. (1983) isolated 331 fungi from CHB and tested their ability to suppress *R. solani*. *Trichoderma* spp., *Gliocladium* spp., *Penicillium* spp., *Mortierella* spp., *Paecilomyces* spp., *Geomyces* spp. and *Ophiostoma* spp. were among the most effective genera and *Trichoderma harzianum* and *Trichoderma hamatum* were generally most efficient.
Trichoderma spp. and *Gliocladium* spp. are usually the most abundant and effective fungal antagonists in CHB. According to Kuter *et al*. (1983) *R. solani* was suppressed in CHB amended media with high prevalence of *Trichoderma* spp. populations but not in batches low in *Trichoderma* spp.. Nelson & Hoitink (1983) suggested that suppression of *R. solani* in CHB media is due to microbial activity.
Bacterial antagonists also play a role and seem to beneficially interact with the fungal hyperparasites. *Flavobacterium balustinum*, *Pseudomonas putida* and *Xanthomonas maltophilia* are three bacteria that have been observed to interact with *Trichoderma* spp., and they are all rapid colonizers of organic matter (Hoitink & Fahy, 1986). *Enterobacter cloacae, Flavobacterium balustinum, Pseudomonas fluorescens, P. putida* or *Pseudomonas stutzeri* combined with *T. hamatum* were consistently more effective than *T. hamatum* alone in suppression of *R. solani* (Kwok *et al*., 1987). Other bacterial antagonists inducing suppressiveness toward *R. solani* in the same trial were *Bacillus cereus, Janthinobacterium lividum* and *X. maltophilia*. Thermophilic fungi are also believed to interact with *Trichoderma* spp. in high temperature areas, although detrimentally, probably interfering with the antagonistic abilities of *Trichoderma* spp.. Chung & Hoitink (1990) observed poor antagonistic activity of *T. hamatum* in 40 to 50°C areas in CHB. Large populations of *Humicola* spp. were found in the same temperature area and some isolates had significantly detrimental effects on antagonism conferred by *T. hamatum*, though its populations were not reduced. No similar interactions between *T. hamatum* and termophilic bacteria were detected.

Fusarium oxysporum f.sp. *conglutinans* was suppressed in CHB incubated at 25°C but not when heated, which indicates that the suppressive mechanism is biotic in nature and not pH dependent (Trillas-Gay *et al*., 1986) as in the suppressive soils of Châteaurenard, where most *Fusarium* suppressive soils have a high pH (>7) although the suppressiveness is essentially microbiological in nature (Alabouvette, 1986). A mixture of *T. hamatum* and *Flavobacterium balustinum* was more suppressive than either of the antagonists added singly (Trillas-Gay *et al*., 1986). CHB amended potting mixes also act suppressive to Fusarium wilts of chrysantemum and flax (Chef *et al*., 1983).

Phytophthora cinnamomi was controlled in hardwood bark-sand compost. This was believed to be due to biotic and chemical properties of the compost, since it was not related to drainage, and since leachates from fresh bark compost contained inhibitors that lysed zoospores and cysts of *P. cinnamomi*. Additionally, these inhibitors were not found in

leachates from bark compost in which rhododendrons had been grown for two years (Hoitink *et al*., 1977).

Pythium ultimum was suppressed in container media amended with CHB from the low temperature areas (Chen *et al*., 1987). Likewise, Hoitink (1980) mentions that Pythium crown and root rot were suppressed in CHB-amended peat but not in non-amended peat. CHB suppressive to *Pythium* spp. is characterized by coexistence of large populations of mesophilic microbes, great microbial activity, low concentration of available nutrients and a high degree of microbiostasis, hence, the general microflora is of utmost importance (Chen *et al*., 1988a; Chen *et al*., 1988b).

Development in populations of *Pratylenchus penetrans* and *Trichodorus christiei* have been observed to be inhibited in CHB. Likewise, lower incidence of tomato root knot caused by *Meloidogyne hapla* and *Meloidogyne incognita* was detected in CHB, as compared to peat (Hoitink, 1980).

Composted Municipal Sludge Amendment

Very little was known about the effect of sludge compost on plant disease development when Lumsden *et al*. (1983) conducted their greenhouse trials on plant pathogen suppression with composted municipal sludge (CMS) amendment to soil. They found that some pathogens were suppressed immediately, others only with time and still others were unaffected, rather, disease severity increased. *Aphanomyces euteiches*, *Sclerotinia minor* and *R. solani* were suppressed shortly after amendment. Fusarium root rot of pea and Thielaveopsis root rot of bean were aggravated by CMS amendment. Thielaveopsis root rot of cotton was not aggravated. Suppression of *Pythium* spp. was not consistent; damping-off was either stimulated, not affected or decreased depending on pathogen species, the host, the environment and possibly non-uniformity of the CMS. Suppressiveness was however clearly enhanced with time after incorporation into soil.

Experiments a few years later claimed that CMS suppresses *Sclerotinia minor* and decreases incidence of Sclerotinia lettuce drop during a four-year period. The suppressive effect was suggested to be correlated with increased soil microbial activity, total nitrogen (high levels of available nitrogen enhances *S. minor*) and organic matter content of the soil as well as its improved physical structure. Mycoparasitism was not believed to contribute, instead, nutrients and fungistatic compounds were suggested to affect pathogenesis of *S. minor* (Lumsden *et al*., 1986).

Kuter *et al*. (1988) conducted experiments with CMS amendment formulated into container media. It was initially conducive to *P. ultimum* and *R. solani*. Four months´ curing rendered the media consistently suppressive to *P. ultimum* but not to *R. solani*. Additional four weeks´ storage of the cured media rendered it consistently suppressive to both pathogens. Similarly, amendment with four months old CMS removed from low temperature areas was suppressive to *P. ultimum* in an experiment by Chen *et al*. (1987).

CMS seems to be conducive to plant pathogens immediately after production, probably due to the high temperature reached during the composting process which is needed to eradicate

all faecal pathogens. Recontamination, controlled or natural, with antagonists is necessary to render them suppressive (Hoitink & Fahy, 1986).

Miscellaneous Compost Amendments

Various composts suppress different pathogens and apart from amendment with CHB and CMS, miscellaneous compost varieties have been tested for disease suppressiveness (Gorodecki & Hadar, 1990).

Container media amended with composted liquorice roots suppressed *Pythium aphanidermatum* in cucumber, as did composted grape marc (Chen *et al*., 1987, Hadar & Mandelbaum, 1986). Gorodecki & Hadar (1990) found composted grape marc to be suppressive to *R. solani* in radish and pothos and *S. rolfsii* in beans and chickpea. Composted separated cattle manure suppressed *R. solani* in radish and *Sclerotium rolfsii* in beans and chickpeas, similarly to composted grape marc (Gorodecki & Hadar, 1990). Similar observations concerning composted grape marc and separated cattle manure have been reviewed by Hadar & Mandelbaum (1992).

Schüler *et al*. (1989) were able to reduce incidence of disease caused by *P. ultimum* and *R. solani* by adding 8, 10 or 30% composted organic household waste to different varieties of host plants.

Pythium graminicola was suppressed by a variety of composts in trials performed by Craft & Nelson (1996) and batches of brewery sludge compost and biosolids compost were among the most suppressive composts. Batches that were initially non-suppressive gained suppressiveness with increasing age. Several of the composts tested such as leaf, yard waste, food, spent mushrooms, certain biosolids, cow manure, chicken-cow manure and leaf-chicken manure were not suppressive to *P. graminicola*. Brewery sludge baths consistently contained the largest populations of heterotrophic fungi and antibiotic-producing actinomycetes. Craft & Nelson (1996) related suppression of *P. graminicola* directly to high microbial populations and activity.

MECHANISMS OF DISEASE SUPPRESSION

Suppressive effects of composts seem in most cases to be due to microbial activity, since heating or sterilization of suppressive composts renders them conducive. Re-inoculation of beneficial microorganisms, artificially or naturally, into heated or sterilized composts re-establishes suppressiveness and thus supports the assumptions that disease suppression in compost is mainly of biological origin (Nelson & Hoitink, 1983; Trillas-Gay *et al*., 1986; Chen *et al*., 1987; Gorodecki & Hadar, 1990; Craft & Nelson, 1996). Even though the fundamental mechanism of suppression is biological, the specific mechanisms observed in relation to various plant pathogens differ (Hadar & Mandelbaum, 1992). The suppression of plant pathogens by beneficial microorganisms in composts or compost-amended substrates might initially be divided into two modes of action; general and specific suppression (Hoitink *et al*., 1997a).

General versus Specific Suppression

General suppression is based on the carrying capacity of the substrate and has been extensively described as the major mechanism involved in suppression of e.g. *Pythium* spp. and *Phytophthora* spp. (Chen *et al*., 1988b; Hoitink *et al*., 1993; Hoitink *et al*., 1997a). *Pythium* spp. and *Phytophthora* spp. both have small, nutrient-dependent propagules that rely on exogenous nutrient sources for germination and they are also sensitive to microbiostasis (Hoitink *et al*., 1993). Composts that have high carrying capacity harbour a high microbial activity and biomass that is constituted by the general microflora. The vast microbial activity acts as a nutrient sink and hinders germination of pathogen propagules. Infection is thereby avoided. The propagules either remain resting or die due to lack of nutrients in composts with these general suppressive qualities towards *Pythium* spp. and *Phytophthora* spp. (Hoitink *et al*., 1993; Hoitink *et al*., 1997a). The carrying capacity of the substrate determines the potential of its ability to sustain microbial activity (Hoitink *et al*., 1991) and hence also its general inhibitory effect towards certain plant pathogens. The general suppression phenomenon does consequently not actively contribute to eradication of these pathogens, it merely controls them through fungistasis.

Specific suppression involves microbial control or eradication of plant pathogens with nutrient-independent propagules. The fact that the mechanism behind general suppression is conducted by the joined microbial activity in a substrate renders it close at hand to understand that the specific suppression is due to a smaller group of antagonists (Hoitink *et al*., 1997a).

Rhizoctonia solani and *Sclerotium rolfsii* are well-known examples of plant pathogens suppressed specifically. They produce large sclerotia, independent of exogenous nutrients (Hoitink *et al*., 1991), and are inhibited by a restricted number of microorganisms, e.g. *Trichoderma* spp. and *Gliocladium* spp., commonly found in lignocellulosic matter (Hoitink *et al*., 1993; Hoitink *et al*., 1997a). These hyperparasites colonize and reduce inoculum potential of sclerotia of *R. solani* and *S. rolfsii*. A synergism between bacteria and *Trichoderma* spp. against *R. solani* has been observed (Chung & Hoitink, 1990). *Flavobacterium* spp., *Enterobacter* spp., *Pseudomonas* spp. and *Xanthomonas* spp. have all been observed to interact with *Trichoderma* spp. for suppression of *R. solani* (Hoitink *et al*., 1993). *Penicillium* spp. and *Fusarium* spp. have been found to colonize and readily reduce inoculum potential of *S. rolfsii* when baiting composted grape pomace with *S. rolfsii* sclerotia. *Trichoderma* spp. populations in the grape pomace compost were very low, which indicates differences in population size between hyperparasites present in different composts (Hoitink *et al*., 1993).

Kuter *et al*. (1983) investigated whether or not fungal populations differed in number and species in hardwood bark composts that were either conducive or suppressive to *R. solani*. Both types of composts harboured predominantly deuteromycetes, ascomycetes and zygomycetes. The total number of fungi differed between the suppressive and conducive CHB, but it did not explain the differences in suppressiveness. Large populations of *Trichoderma hamatum* and *Trichoderma harzianum* were however found in all suppressive composts while *Penicillium verrucosum* var. *cyclopium* or varieties of *Geomyces pannorum* were common in conducive composts. Relationships among species and disease suppression

were demonstrated; antagonistic activities of *Trichoderma* spp. versus *R. solani* probably helped reduce disease incidence.

Microbial Mechanisms in Disease Suppression

As already mentioned above, the composition of the compost seems to affect which microflora is established and subsequently practices for the biological control of plant pathogens. Numerous microorganisms antagonistic towards soilborne pathogens have been isolated from disease suppressive composts (Kuter *et al*., 1983; Kwok *et al*., 1987) and trials have also provided evidence that compost can benefit siderophore producing bacterial populations, which means that composts generally have the potential to create a suitable environment for proliferation of rhizosphere bacteria.
Biocontrol organisms in compost inhibit pathogens through several mechanisms commonly found among antagonists, i.e. competition, antibiosis, hyperparasitism and induced systemic resistance (ISR).

Competition, Antibiosis & Hyperparasitism

Competition for space and nutrients along with production of antibiotic substances seem to be the principal mechanisms in general disease suppression (Hoitink *et al*., 1993) and competition for nutrients alone is considered an important mechanism for suppression of e.g. *Pythium* spp. (Mandelbaum & Hadar, 1990). There is although a strain of *Pseudomonas fluorescens* Migula that inhibits growth of *Pythium ultimum* Trow by producing an antibiotic compound, which thus contributes to suppression (Elad & Chet, 1987).

Elad & Chet (1987) found that bacterial suppression of *Pythium aphanidermatum* is of competitive origin. Decrease in germination of *P. aphanidermatum* oospores was observed in the presence of antagonistic bacteria, which presumably exhibited excellent activity as a nutrient sink. Incidence of suppression was significantly correlated with competition for nutrients. A few of the antagonistic bacteria produced antibiotics *in vitro*, but never in soil. Antibiotics were therefore not believed to have any major impact. None of the bacteria were observed to produce lytic enzymes, hence hyperparasitism could be excluded. Antagonistic strains of bacteria applied to cucumber seeds were established along the roots and control was achieved in bean, pepper, melon, tomato, and cotton (Elad & Chet, 1987).

Hyperparasitism has been described as the principal mechanism in specific suppression (Hoitink *et al*., 1993) and production of hydrolytic enzymes is a common feature among biocontrol agents that hyperparasitize pathogens; parasitism of *Sclerotium rolfsii* sclerotia by *Trichoderma* spp. is for example believed to involve cell-wall hydrolytic enzymes (Benhamou & Chet, 1996). As mentioned above, *Trichoderma* spp. is not very efficient in composts that are rich in cellulose with high glucose concentrations, which might be explained by repression of hydrolytic enzyme synthesis in immature composts with fresh organic matter. The same may be true for production of antibiotics (Hoitink *et al*., 1997a).

Competition for nutrients and space, to enable colonization of an ecological niche and push the pathogen aside, has earlier been shown to be of crucial importance for suppression by *Trichoderma* spp. Production of antibiotics, chitinases and β-1,3-glucanases, are just as

important for *Trichoderma* spp. and the fungal pathogen cells presumably become more prone to invasion after exposure to antibiotics (Bélanger *et al*., 1995).

Close observations made by Bélanger *et al*. (1995) on hyperparasitism and antibiosis involved in an attack by *T. harzianum* on *Botrytis cinerea* revealed that antibiosis preceded parasitism. Within the first twelve hours, i.e. before contact, several ultrastructural changes in *B. cinerea* were observed; punctuated invaginations of the plasmalemma followed by gradual retraction of the same, disorganization of the cytoplasm, loss of turgor pressure, and finally death within 48 hours of contact between the hyphae of the interacting fungi. The ultrastructural changes are all typical manifestations of fungal cells exposed to antibiotics. Evidence of chitin degradation was not detected until several days later, though. Ten days after contact between the combatants cell walls and cytoplasm of *B. cinerea* were hardly distinguishable. *T. harzianum* seems to have worked through antibiosis until cell death, followed by degradation of the cell by means of chitinolytic enzymes. The production of antibiotics may therefore be of more importance than production of chitinolytic enzymes regarding the biocontrol superiority of *Trichoderma* spp. (Bélanger *et al*., 1995).

Induced Systemic Resistance

Several reports have provided evidence indicating that additional mechanisms other than competition, antibiosis and hyperparasitism are operating behind compost induced suppression (Zhang *et al*., 1996; 1998). Compost extracts used as sprays have been utilized for several years to control foliar diseases in plants with varying results (Zhang *et al*., 1998). Compost extracts from horse manure have reduced severity of powdery mildew in grape caused by *Unicula necator* (Boland & Kuykendall, 1998), while other compost extracts have reduced severity of downy mildew caused by *Plasmopara viticola* in grape, *B. cinerea* in strawberries, bean, tomato, pepper and grape, and *Phytophthora infestans* in potato and tomato, *Erysiphe graminis* in barley, *Erysiphe betae* in sugar beet and *Sphaerotheca fuliginea* in cucumber (Elad & Shtienberg, 1994; Zhang *et al*., 1998). The compost water extracts that actually reduced *B. cinerea* in tomato, pepper and grape by 56 to 100% were prepared from composted cattle manure, chicken-cattle manure and grape marc that had fermented for more than ten days (Elad & Shtienberg, 1994). Explanations have somewhat been focused on the possibilities of induction of plant responses similar to systemic acquired resistance (SAR) combined with direct fungal inhibition (Hoitink *et al*., 1997a; Boland & Kuykendall, 1998). Investigations regarding induction of systemic resistance by compost amendments have also been made (Zhang *et al*., 1996; Hoitink *et al*., 1997b; Zhang *et al*., 1998). Hoitink *et al*. (1997b) report that some microorganisms colonizing roots present in compost amended mixes activate biochemical pathways in plants, rendering them resistant to foliar as well as root diseases. Their conclusion was that ”substrates rich in biodegradable organic matter support microorganisms that induce systemic resistance in plants”. Elevated levels of enzyme activity were observed in healthy plants, they were better prepared to fight a pathogen.

SAR is known to be induced by chemicals, pathogens and beneficial non pathogenic microorganisms, and composts are potential sources of great populations of microorganisms that might be of importance for induction of systemic resistance (ISR). Several recent reports state that composts are capable of inducing responses reminiscent of SAR in plants. By which mechanisms composts induce this form of resistance is yet not clear. Plants grown in

substrates enriched with compost are colonized by a large number of different bacteria among which some strains capable of inducing ISR/SAR have been described. These bacteria have to be present in a certain amount in the rhizosphere in order to induce systemic resistance (Zhang *et al*., 1998).

Zhang *et al*. (1996) studied the role of the beneficial *Pseudomonas* spp., usually abundant in compost amended substrates and likewise able to provide biological control against Pythium root rot. Suppressiveness to Pythium root rot was lost as decomposition proceeded and the *Pseudomonas* spp. population declined. The mechanism by which *Pseudomonas* spp. controls Pythium root rot was identified as ISR.

It is not yet elucidated whether the induced resistance obtained through compost follows the same pathway as SAR induced by pathogens. To find out, salicylic acid-induced SAR was compared with compost-induced resistance by Zhang *et al*. (1998). Results showed that some rhizosphere bacteria that induce resistance also induce accumulation of pathogenesis related proteins, while others do not. Furthermore, peroxidase activity was reported to be higher in plants grown in compost mix compared with plants grown in peat mix (Zhang *et al*., 1996). Water extracts prepared from compost rendered an induced resistance with stronger resistance responses than when directly mixing compost material into the soil. The induced effect of water extract was retained even after autoclaving, which indicates that chemicals or alternatively heat stable proteins might be involved.
In contrast to compost mixes, peat mixes did not induce resistance, probably due to their low populations of microorganisms capable of inducing ISR. There are speculations that the induced resistance might be due to specific interactions between microorganisms and plant. An important difference between SAR and compost induced resistance (ISR) is that composts induce less strong resistance responses and at lower degrees than SAR. It can therefore be assumed that the mechanisms in resistance induced by compost differ from SAR (induced by pathogens and salicylic acid), and even water extract from compost material, since it resulted in stronger resistance responses than directly added compost material.

EXPERIMENTAL STUDY

The aim of this project was to compare eight different composts in relation to their effect on two soilborne pathogens, *Verticillium longisporum* (former *V. dahliae*) and *Pythium sylvaticum*. These pathogens have different strategies in terms of pathogenesis, growth, reproduction and survival and they both have wide host ranges. The study consisted of one *in vitro* part and one *in vivo* part.

The *in vitro* part consisted of chemical and microbial analysis of the experimental composts and isolation of their microbial residents. Attempts were made to isolate and identify microorganisms characteristic of the composts and to study their functional properties, including pathogen suppressive ability.

The *in vivo* part consisted of experiments performed in greenhouse, aiming at studies on effects of composts in terms of disease suppression. Extracts were prepared and applied to the hosts; oilseed rape and cucumber respectively. The hosts were chosen due to their susceptibility to the two test pathogens.

THE TEST PATHOGENS

Verticillium longisporum

Verticillium longisporum (earlier *V. dahliae*) is a soilborne pathogen that survives in soil predominantly as microsclerotia and infects a wide host range (oilseed crops, potato, sugar beet, flax, sunflower, lucern, tomato, cotton, certain shrubs and several weeds) mainly through root tips. Viability of microsclerotia in soil may vary from 10 to 30 years. Characteristic symptoms are yellowing of leaves and discolouration between the veins, usually on one half of the leaves. *Verticillium spp*. grow systemically in its host and longitudinal sections of hypocotyl and stem display the discoloured veins. The pathogen is best controlled by reducing inoculum in soil, for instance by organic amendments. Crop rotation is seldom efficient due to the poor host specificity of *V. longisporum*. Although not microbial, but still interesting, amendments with fresh and dry broccoli, respectively, have been shown to significantly reduce the number of *V. longisporum* microsclerotia in soil (Subbarao & Hubbard, 1996). Sweden was actually the first country from which *V. longisporum* was reported to cause damage on oil seed crops (Tjamos & Fravel, 1995; Subbarao & Hubbard, 1996; Atterwall, 1994). *Verticillium* spp. may possibly produce mycotoxins contributing to death of the plant (Atterwall, 1994).

Attempts to find biological possibilities to control *V. longisporum* have been made by using a number of pure cultured antagonistic microorganisms showing capability of retarding the pathogen through various mechanisms. A well known fungal antagonist is *Talaromyces flavus* which, probably due to production of antibiotics, retards several important functions in *V. longisporum*. Germination, hyphal growth and melanization of microsclerotia may all be impeded (Madi *et al*., 1997). Microsclerotia of *Verticillium* spp. are sensitive to moist heat and a combination of heating, e.g. soil solarization, and the thermotolerant *T. flavus* has also been found to reduce their viability. The microsclerotia are weakened by heating and

hence more vulnerable to antagonism by *T. flavus* (Tjamos & Fravel, 1995). In a field trial by Marois *et al*. (1982) *T. flavus* reduced Verticillium wilt in eggplant in the field by roughly 70%. *Gliocladium roseum* is another potential biocontrol agent that has shown to be a hyperparasite of *V. longisporum* as well as many other pathogens. When *V. longisporum* propagules were buried in soil amended with *Gliocladium roseum* it resulted in strongly reduced viability of microsclerotia (Keinath *et al*., 1991). Growth ability and formation of microsclerotia by *V. longisporum* is also deteriorated by the parasitic non-pathogen *Pythium oligandrum* (Al-Rawahi & Hancock, 1998).

Rhizosphere bacteria isolated from various hosts of *V. longisporum* and their immediate environments have been screened for antagonistic abilities. It has been demonstrated that strains of *Bacillus subtilis*, *Pseudomonas fluorescens* and *Xanthomonas maltophilia* may be strong antagonists. *Pseudomonas chlororaphis*, *Erwinia herbicola* and *Pseudomonas paucimobilis* were also among those showing good antagonistic traits. *B. subtilis* performed its inhibitory effect by means of antibiotics while the others worked in terms of lytic enzymes or nutrient competition/antagonism, e.g. through production of siderophores (Berg & Ballin, 1994).

Pythium sylvaticum

Pythium sylvaticum belongs to the genus *Pythium* spp. under the Oomycota. Pathogenic species of *Pythium* spp., including *P. sylvaticum*, are known to cause diseases such as root rot, damping-off, seed rot and soft rot, which are diseases that affect practically all plant parts (Agrios, 1997). *Pythium* spp. lives as a saprophyte on dead organic matter or as parasites on living plant roots and persist in soil as oospores. The virulence of *Pythium* spp. can be increased by exogenous nutrients and nutrients supplied by root exudates (Hockenhull & Funck-Jensen, 1983). The fungus enters the hosts by direct penetration and degenerates the plant tissues using an arsenal of lytic enzymes including pectinases, proteases and cellulases. The greatest damage is usually done to the seed and seedling roots during germination either before or after emergence (Agrios, 1997). *Pythium* spp. are considered to be opportunistic pioneers but weak competitors. They are unable to colonize substrates already colonized and their saprophytic and pathogenic activities are limited under conditions of intense microbial competition (Agrios, 1997; Elad & Chet, 1987; Chen *et al*., 1988b).

Numerous reports describe the suppressive effects of composts on *Pythium* spp. (Lumsden *et al*., 1983; Chen *et al*., 1987; Chen *et al*., 1988a; Chen *et al*., 1988b; Kuter *et al*., 1988; Craft & Nelson, 1996; Zhang *et al*., 1996; Kofoed Christensen & Klamer, 2000) and practically all reports support the finding that compost from high temperature areas is at first conducive to *Pythium* spp. or even enhance its activity, but becomes suppressive with time due to increased microbial activity. Compost from low temperature areas is generally suppressive immediately. Erhardt *et al*. (1999) compared nineteen different composts (seventeen from organic household waste, one from bark and one from grape marc) and found that only one, the compost based on bark, was strongly suppressive to *P. ultimum* while the rest were mildly suppressive or conducive. A mature and recolonized compost with high microbial activity acts as a nutrient sink. It depletes nutrients and oospore germination is thus reduced.

A contributing factor may also be release of inhibitory substances to which *Pythium* spp. are known to be very sensitive (Elad & Chet, 1987).

MATERIALS & METHODS

Origin Of Experimental Composts And Preparation Of Their Extracts

Eight composts of different origin, composition and curing levels were used in this study. All composts were processed aerobically. Their origin is presented in Table 1.

Table 1. *Compost origin.*

UKWS (Uppsala Kitchen Waste with Straw)	Source separated partly decomposed organic household waste (from one household) mixed with straw in proportions (90:10, % weight) and composted in a non-hermetic insulated rotable 125 l compost container for four months.
GWHMS (Garden Waste with Horse Manure and Straw)	Garden waste mixed with horse manure rich in straw (70:30, % volume) and composted in windrows on a hard surface for about twelve weeks and thereafter cured in a pile for roughly seven months. The windrows were turned once a week, while the pile was turned once a month.
HMSD (Horse Manure with Saw Dust)	Partly decomposed horse manure mixed with horse urine and sawdust. Starting material taken from an about three months old heap of manure and composted in a non-hermetic insulated 125 l rotable compost container for three months. Fresh horse manure from the same stable was added.
RKW (Reactor Kitchen Waste)	Source separated organic household waste mixed with garden waste (70:30, % weight). Composted in concrete reactors with the dimensions 6,5×21×2 m as follows: Initially intense decomposition in closed container with forced aeration for three weeks followed by post-composting in an open container with forced aeration for four weeks and curing in pile on a hard surface for three weeks.
BCM (Bio Cow Manure)	Composted pure cow manure from a biodynamic farm located at Järna, Stockholm. No litter amendments were added. Composted for approximately three months in a closed wooden box in the cowshed.
BCMS (Bio Cow Manure with Straw)	Biodynamic cow manure and straw composted on a biodynamic farm (Järna) in windrows on grass for nine months.
BKW (Bio Kitchen Waste)	Biodynamic kitchen waste composted on a biodynamic farm (Järna) in windrows on grass for 22 months.
BGW (Bio Garden Waste)	Biodynamic plant waste and weed composted on a biodynamic farm (Järna) in windrows on grass for eight months.

All composts were stored at +2°C during the project period in order to minimize microbial activity.

The compost extracts used in the greenhouse experiment were prepared as follows. All composts were brought to room temperature (20-21°C) before extraction. Each compost á 250 g, was suspended in 750 ml de-ionized water (0,33 g compost/ml), occasionally stirred during one hour and finally filtrated through a household sieve. The extracts were prepared the day on which they were applied to the plants.

Analysis Of the Compost Extracts

Chemical Features

Samples of the eight composts were analysed at the Department of Soil Science, SLU, Uppsala, with respect to easily available phosphorus and potassium, total content of phosphorus, potassium, carbon and nitrogen. Ammonium and nitrate content was also analysed. Total nitrogen was analysed using two different methods. The analyses performed using the Kjeldahl method were conducted on moist samples.

pH and conductivity was measured in all compost extracts before adding them to plants in the greenhouse trial. pH and conductivity were hence measured twice, since extracts were applied twice, with one day in between. The conductivity is a measure of free ions in the composts, and potassium is most conducive of the nutrients analysed.

Microbial Features

To compare the microbial floras of the eight composts, extracts were spread on 50% strength potato dextrose agar (PDA, MERCK) and 50% strength tryptic soybroth agar (TSA, Difco) media. For extract preparation, a 50 g sample from each compost was kept at room temperature overnight, suspended in 50 ml de-ionized water and left for 1,5 h at room temperature. The suspensions were subsequently sieved through a household sieve and serially diluted in 0,1 M $MgSO_4$ before spreading on PDA and TSA in two replicates. The agar plates were then incubated at +18°C and microbial colonies appearing after two days were counted (no magnification used). Total population counts of the extracts were thus estimated and compared as log cfu/ml extract.

Different functional groups of microorganisms inhabiting composts were estimated in terms of proteolytic, cellulolytic and fluorescing microorganisms. Three media were prepared for this purpose; skim milk agar and cellulose agar (Arora *et al*., 2005) for proteolytic and cellulolytic activity respectively, and King´s medium B (KB) agar (King *et al*., 1954) as used for fluorescing activity. The following procedure was the same as described above.

Plant Response to Compost Extract in Absence And Presence of *Verticillium longisporum*

Oilseed rape (cv. Casino) and cucumber plants (cv. Rhensk Druv) for the study were grown in greenhouse. The inoculum (mycelium and microsclerotia) of *V. longisporum* and *P.*

sylvaticum respectively was added as described below. Controls were grown alongside with each experiment. All plants were grown in soil from Hasselfors Garden.

The compost extracts were freshly prepared as described above on the day of treatment. Inoculum of the pathogens was also prepared and pathogenicity tests were successfully performed in advance in order to confirm the pathogenic nature of the isolates used in the experiment.

The greenhouse experiments with oilseed rape and cucumber were identically designed in 10 replicates (pots) as follows:
Control pots: plant + soil (n=10)
Inoculated control pots: plant + soil + inoculum (n=10) Extract
control pots: plant + soil + compost extract (n=10×8) Inoculated
pots: plant + soil + compost extract + inoculum (n=10×8)

Extract prepared from a total of 20 g of compost was added twice (10 g + 10 g) to each pot in all cases except for BCM, where extract prepared from 10 g of compost was added on one occasion only, since the availability of the BCM sample was limited. Caution should thus be taken while comparing the effects of BCM with those of other composts. As described above, 20 replicates were arranged for each compost extract; 10 for studying the extract´s effect on plant growth in absence of the pathogen and 10 for the effect of the extract on disease development in presence of the pathogen. In total 80 compost amended pots (10 pots × 8 compost extracts) were subsequently inoculated with the pathogen.

Oilseed rape
The compost extracts were applied twice to four weeks old oilseed rape plants (2 plants/pot), i.e. when the first true leaves had just emerged, at a concentration of 1:3 (w/w). On day one 30 ml of extract was added to each plant. The procedure was repeated on day three to promote microbial colonization.

A suspension was prepared from three weeks old microsclerotia culture of *V. longisporum* on PDB (50% strength) with 3,0 g phytagel/l gently mixed with de-ionized water, and applied to oilseed rape five days after the second extract application (1 90 mm petri dish/3 pots, n=10). The suspension (20 ml/pot) was applied with pipette tips at ten different locations around the plants.

The leaves of all plants were numbered in order of appearance to facilitate observation of leaves developing wilt symptoms. The plants were harvested thirteen weeks after seeding in an identical way. To confirm identification of the pathogen, and to determine the systemic spread of infection, all fallen leaves were collected and incubated in moist chamber to stimulate formation of conidia and microsclerotia. The shoots were subsequently dried overnight at 80°C before registration of dry weights. The weights of the two plants in each pot were pooled in order to calculate the shoot dry weight per pot.

Cucumber
The compost extracts were applied twice to four days old cucumber seedlings (3 plants/pot), i.e. when the first true leaf was just about to emerge. On day one 30 ml of extract was added

to each pot of cucumber and on day four the procedure was repeated to promote microbial colonization.

P. sylvaticum was cultured on diluted PDB with 3,0g phytagel/l in petri dishes (90mm) for eight days. The mycelial mats were then harvested and carefully blended with de-ionized water (1 petri dish/80 ml water) in a Waring blender. 40 ml/pot of the suspension was poured around the stem bases of the cucumber seedlings. To further expose the seedlings to inoculum, a mycelial strip was buried in close connection to the roots and stem base of each seedling.
The plants were not watered extra on the day of treatment, neither before nor after application of the pathogens.

Unfortunately, symptoms failed to appear in the *Pythium sylvaticum* experiment despite a successful pathogenicity test, and hence no further observations were made. As in the experiment, symptoms failed to appear in the first pathogenicity test. In the second, symptoms developed, though moderately.

In Vitro Effects of Compost Residents on *Verticillium longisporum*

On the basis of observations made in the greenhouse trial, four composts were selected for further study with regard to pathogen suppressive ability of compost microbes. These four composts were BCMS (no disease suppression), GWHMS and HMSD (moderate disease suppression) and BCM (disease suppression of wilt).
About twenty microorganisms apparently characteristic to each compost were isolated, pure cultured and characterized in terms of their proteolytic, cellulolytic and fluorescing activity, as described above. All three activities are considered to be of importance for disease suppression. Two replicates were consistently prepared.

To elucidate the effects of the microorganisms obtained from the four compost extracts above; BCM, HMSD, GWHMS, BCMS, they were challenged (co-inoulated) with *V. longisporum* to study their 1) direct effects and 2) indirect effects. In order to observe possible direct effects, *V. longisporum* and the test microorganism were placed equidistantly on PDA (fungal and bacterial isolates) and KBA (bacterial isolates). Two replicates were prepared for each combination. For observation of possible indirect effects, the test microbe and *V. longisporum* were placed face to face having aerial contact only. *V. longisporum* was grown on PDA and inverted on the PDA or TSA which was inoculated with the test fungus or bacterium, respectively. Two replicates were prepared for each combination except for the direct effect of bacteria which was not replicated. In both experiments, the plates were incubated at +18°C for six weeks (results were also collected after 15 days, see tables in Appendix). The diameter of *V. longisporum* cultures was measured in mm.

Statistical analysis

The statistical program SAS (SAS 9.1, SAS Institute Inc., Cary, NC, USA) was used to evaluate data obtained from the trials in greenhouse and in vitro. The ANOVA procedure was used to evaluate effects on dry weight and systemic spread of infection in vivo and

PROC GLM was used for evaluation of direct and indirect effects in vitro. All results are presented as LSD (Least Significant Difference) based on 0,05 % significance level. LSD is the least difference required between two sets in order to be significantly different. The direct effect of bacteria in vitro was not statistically evaluated since there were no replicates.

RESULTS

Analysis of the Compost Extracts

Chemical Features

The pH of the compost extracts ranged from neutral (6,99/UKWS) to highly alkaline (9,32/BCMS). The three biodynamic composts BCMS, BCM and BKW (Table 1) had a distinctly higher pH compared to the other ones. The conductivity varied widely over compost origin. pH and conductivity is shown in Table 2.

The conductivity was highest in UKWS, RKW and BCMS. These three composts also contained most potassium. BCM and BKW had the lowest conductivity, as well as the least content of potassium (Table 2).

Table 2. *pH and conductivity of eight composts based on different origins (see Table 1 for compost details).*

	pH	Conductivity (microS/cm)
UKWS	6,99	>2000
GWHMS	7,32	970
HMSD	7,52	1160
RKW	7,76	>2000
BCM	8,28	650
BCMS	9,32	>2000
BKW	8,41	660
BGW	7,3	1740

The nutrient content of the composts is shown in Table 3. The phosphorous content (P-AL) ranges from 108 mg/100g (GWHMS) to 313 mg/100g (BCMS). The potassium content (K-AL) varies from 277 mg/100g (BKW) to 1780 mg/100g (UKWS). The carbon content ranges from 9,09 % (BKW) to 37,70 % (BCM). Total nitrogen content was analysed with two methods and the results varied somewhat between the methods. UKWS and BCMS are the two most nitrogen rich composts. The Kjeldahl method also accounts for NH_4-N and NH_3-N. Overall, UKWS and BCMS were most rich in NPK, while BGW, BKW and GWHMS were poorest in NPK. The C/N ratios (Table 4) revealed that there was excess nitrogen in most composts, for them to be considered as having stabilized. HMSD and BCM seemed to have stabilized.

The dry weights of the eight composts ranged between 21% and 70% (Figure 1). The three composts containing a large share of manure (HMSD, BCM, BCMS) had the lowest percentages of dry weight. All composts but RKW have a dry weight of less than 55-65%.

Table 3. *Nutrient analysis of eight composts based on different origins.*

	mg/100 g air dried		mg/100 g air dried		% in air dried		Kjeldahl, % in air dried		
	P-AL	tot-P	K-AL	tot-K	tot-C	tot-N	NH4-N	NH3-N	tot-N
UKWS	237	472	1780	1630	23,10	3,18	0	0,33	2,86
GWHMS	108	450	334	519	11,30	0,85	0	0,00	0,79
HMSD	215	397	770	867	33,60	1,77	0	0,10	1,60
RKW	124	282	760	913	25,20	2,08	0,04	0,00	2,51
BCM	234	498	643	672	37,70	1,58	0	0,05	1,47
BCMS	313	777	1750	2110	33,50	2,81	0	0,10	3,04
BKW	163	240	277	441	9,09	0,65	0,01	0,02	0,78
BGW	116	224	463	760	9,79	0,78	0	0,24	0,79

* See "M&M" for details

Table 4. *C/N ratio of the composts.*

Compost	C/N ratio
UKWS	7,26
GWHMS	13,29
HMSD	18,98
RKW	12,12
BCM	23,86
BCMS	11,92
BKW	13,98
BGW	12,55

<15: gaseous N losses
15 – 30: stabilized ratio
>30: N deficiency

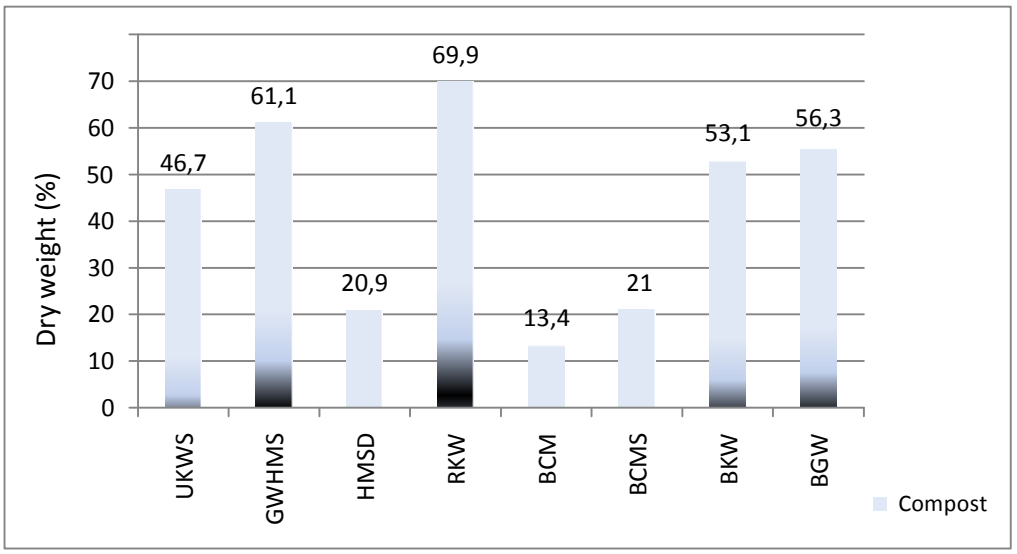

Figure 1. *Dry weights (%) of the eight composts.*

Microbial Features

All composts but one tested in this study consistently seemed to harbour more bacteria than fungi. The sizes of the fungal populations ranged from 0 to log 6,56 cfu/ml compost extract, while bacterial populations ranged from log 6,61 to log 9,04 cfu/ml compost extract depending on the compost (Figure 2). BKW harboured the least number of viable bacteria and lacked fungi when counted after two days. The fungi did not appear until after five days in BKW. BCMS and HMSD harboured practically twice as many bacteria as fungi. RKW proved to be rich in both fungi and bacteria. As for the kitchen waste composts, all seemed to show diverse fungal populations, based on ocular examination of the petri dishes. There were similarities in appearance between the populations of UKWS and RKW, when observed on the tested media.

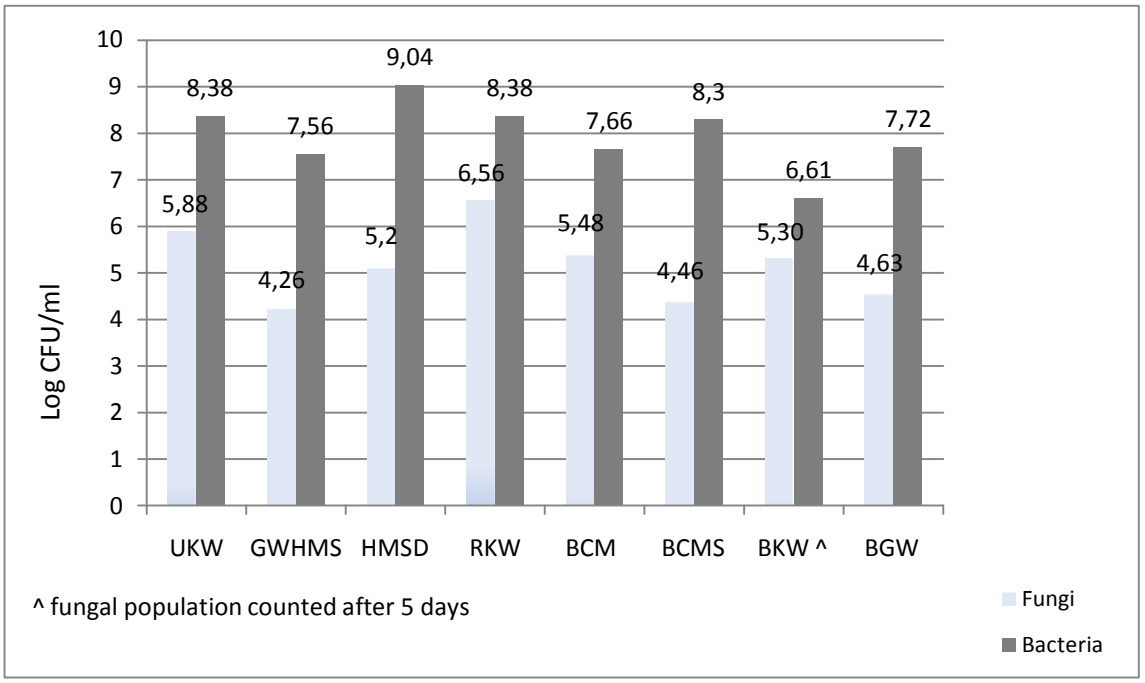

Figure 2. *Culturable fungal and bacterial populations (log cfu/ml) as estimated in the compost extracts spread on PDA and TSA, respectively. Colonies were counted after 2 days if other is not stated. N=2.*

As regards different functional groups of microorganisms, the composts differed widely depending on their origin. Table 5 presents enzymatic and fluorescent properties of microorganisms in the compost extracts. Most of the composts seemed to be colonised by microbial populations possessing at least two of the three characteristics. UKWS lacked fluorescent microorganisms and BCM seemed to lack proteolytic populations. HMSD harboured the largest population of proteolytic microbes, in number as well as in relation to total count, RKW harboured the largest number of cellulolytic microorganisms, and both these composts also contained the largest number of fluorescent microorganisms. BGW contained the largest proportion of fluorescent microorganisms.

Table 5. *Enzymatic & fluorescent features of fungi and bacteria in compost extract.*

COMPOST	PROTEASE		CELLULASE		FLUORESCENCE	
EXTRACT	logCFU/ml	% of total	logCFU/ml	%of total	logCFU/ml	% of total
UKWS	7,4	15,3	7,7	19,0	0,0	0,0
GWHMS	5,9	6,0	6,4	20,0	5,6	4,4
HMSD	8,5	87,8	7,5	5,3	8,0	6,2
RKW	*	*	8,3	9,9	7,7	2,8
BCM	0	0	5,9	0,9	6,6	16,4
BCMS	6,4	14,3	7,2	2,1	6,4	0,2
BKW	5,8	10,0	6,0	13,4	5,8	9,0
BGW	6,1	40,5	6,1	10,6	6,3	20,5

^) Please not that "% of total" is % of total count on the specific medium, not correlated to total counts derived from culturing on PDA or TSA (figure 2).

*) Not estimated due to overgrowth of fungi.

A number of pure cultures of fungi and bacteria isolated from the four composts selected for *in vitro* experiments were also analysed with respect to their proteolytic, cellulolytic and fluorescing characteristics. Fungal isolates from each compost were labelled with letters from a-j and bacterial isolates were labelled 1-11. Proteolytic and cellulolytic activity was found to be more common among the fungi than among the bacteria, while fluorescence was seldom expressed. 14 out of the 29 fungal isolates possessed at least two characteristics, in general production of protease and cellulase. The isolated residents of both BCMS and GWHMS were largely cellulase and protease producers. As regards bacteria, 15% of the isolates possessed at least two characteristics. All three isolates from HMSD were proteolytic and HMSD did also contain the largest proteolytic population. Fluorescence was a very rare feature among fungi as well as bacteria, and only two organisms expressed fluorescence: the fungus BCMd and the bacterium HMSD2. The results are summarised in Table 6.

Table 6. *Cellulolytic, proteolytic and fluorescing characteristics of isolates from four composts.*

Fungi	Cellulase	Protease	Fluorescence
HMSDa	+	(+)	-
BCMa	-	+	-
BCMb	+	-	-
BCMc	+	-	-
BCMd	-	+	+
BCMe	-	+	-
BCMf	+	-	-
BCMg	-	-	-
BCMh	-	+	-
BCMi	-	+	-
GWHMSa	+	+	-
GWHMSb	-	+	-
GWHMSc	+	+	-
GWHMSd	+	+	-
GWHMSe	+	+	-
GWHMSf	+	+	-
GWHMSg	-	+	-
GWHMSh	+	+	-
GWHMSi	+	(+)	-
GWHMSj	+	-	-
BCMSa	-	-	-
BCMSb	+	+	-
BCMSc	+	+	-
BCMSd	+	+	-
BCMSe	+	+	-
BCMSf	+	+	-
BCMSg	-	+	-
BCMSh	+	+	-
BCMSi	+	+	-

Bacteria	Cellulase	Protease	Fluorescence
HMSD1	-	+	-
HMSD2	-	+	+
HMSD3	-	+	-
BCM1	-	(+)	-
BCM2	-	+	-
BCM3	-	+	-
BCM4	-	-	-
BCM5	-	-	-
BCM6	+	-	-
BCM7	+	-	-
BCM8	+	-	-
BCM9	+	-	-
BCM10	+	-	-
BCM11	-	-	-
GWHMS1	+	-	-
GWHMS2	-	-	-
GWHMS3	+	-	-
GWHMS4	+	+	-
GWHMS5	+	-	-
GWHMS6	+	-	-
GWHMS7	-	+	-
GWHMS8	-	-	-
GWHMS9	-	+	-
BCMS1	(+)	-	-
BCMS2	-	+	-
BCMS3	-	-	-
BCMS4	+	-	-
BCMS5	+	+	-
BCMS6	+	+	-
BCMS7	+	-	-
BCMS8	(+)	-	-
BCMS9	-	+	-
BCMS10	+	+	-

Plant Response to Compost Extracts in Absence And Presence of *Verticillium longisporum*

Dry weight

When comparing the mean shoot dry weights of all pathogen-free controls to all pathogen inoculated pots, they differed significantly (LSD: 0,2243) (Table 7).

Table 7. *Mean dry weights of controls and Verticillium longisporum inoculated oilseed rape plants. N=90.*

	Dry weight (g)	T grouping
Controls	5,9902	A
Inoculated	5,6927	B

LSD: 0,2243

Figure 3 shows the mean dry weights of pathogen inoculated sets compared to the healthy control after treatments with different compost extracts. Shoot dry weights were lower in all inoculated sets when compared to the controls, except for in BCMS and BKW. Pathogen-free controls of UKWS, GWHMS and RKW all had obviously higher dry weights than the inoculated seedlings. In the controls of HMSD, BCM and BGW the dry weights were slightly higher than in the inoculated pots. UKWS enhanced the growth of oilseed rape plants, also in presence of the pathogen. All inoculated sets except UKWS had shoot dry weights lower than the untreated control (Figure 3).

The dry weights of all shoots in compost amended pots and in inoculated pots also differed significantly between the sets. The composts may, on basis of the effect of compost extract and influence of the pathogen on the weight of oilseed rape plants, be divided into four groups labelled A, B, C and D with an LSD value of 0,4758. The grouping from A-D is based on the mean dry weight of pathogen-free and inoculated plants and each letter represents a statistically significantly different group. The group BC is significantly different from A and D, while CD is significantly different from A and B. BCM, BKW and BGW relate to two groups each while UKW constitutes a group of its own. Dry weights of plants in all individual pots are listed in Appendix, Tables A and B.

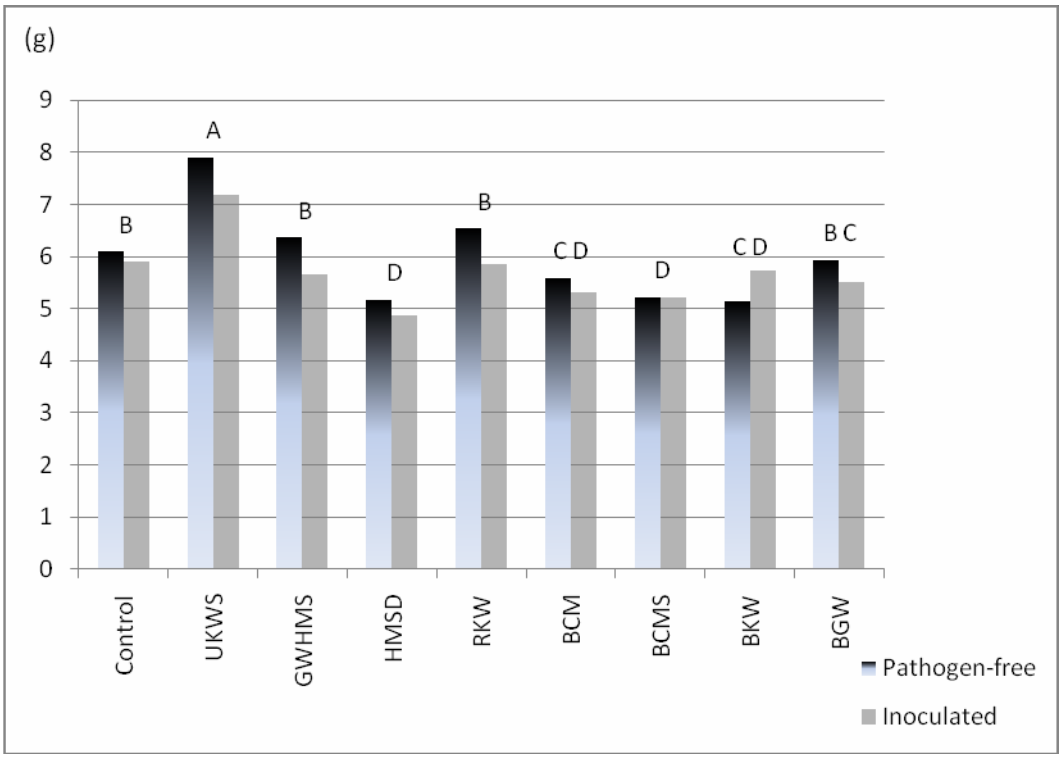

Figure 3. *Mean shoot dry weights of oilseed rape plants in pathogen-free and inoculated pots. N=10, 2 plants/pot. Bars with identical letters are not significantly different from each other and bars with two letters belong to two groups. LSD: 0,4758.*

Systemic Spread of Verticillium longisporum in Oilseed Rape Plants

Wilt symptoms were observed in all inoculated pots. The eight compost treatments and the control could be divided into two groups differing significantly in disease level with an LSD value of 1,8166 (Table 8). Table 8 shows that the systemic infection of *V. longisporum* had spread unequally high in the sets amended with different compost extracts. All plants were infected in the UKWS, BCMS and BGW pots, in which systemic infection also was most widely spread in the shoots. These three compost treatments are significantly different from the rest with respect to systemic spread of infection and seem to have had an enhancing effect. Remaining compost treatments can not be statistically separated from the control as regards infection. GWHMS had the fewest infected plants and also the least systemic spread of infection. Photos 1-4 illustrate differences between controls and treated plants and microsclerotia formation is illustrated by photo 5. Systemic spread of infection in individual pots is presented in Table C in Appendix.

Table 8. *Effect of different compost extracts on wilt caused by V. longisporum in oilseed rape.*

Compost	Mean value of youngest infected leaf*	T Grouping	
UKWS	5.8	A	
GWHMS	3.2		B
HMSD	4.4	A	B
RKW	4.8	A	B
BCM	3.5		B
BCMS	5.7	A	
BKW	4.9	A	B
BGW	5.5	A	
***V. longisporum* control**	3.4		B

LSD: 1.8166

*The values relate to the order of appearance of the leaves, indicating how high in the plant infection has spread. The composts may be divided into two groups differing significantly (A and B) and one group (AB) that belongs to both A and B.

Photo 1.
Non-compost extract amended control oilseed rape plants. The V. longisporum infected plant (left) displays typical symptoms of verticillium wilt.

Photo: Caroline Haarala

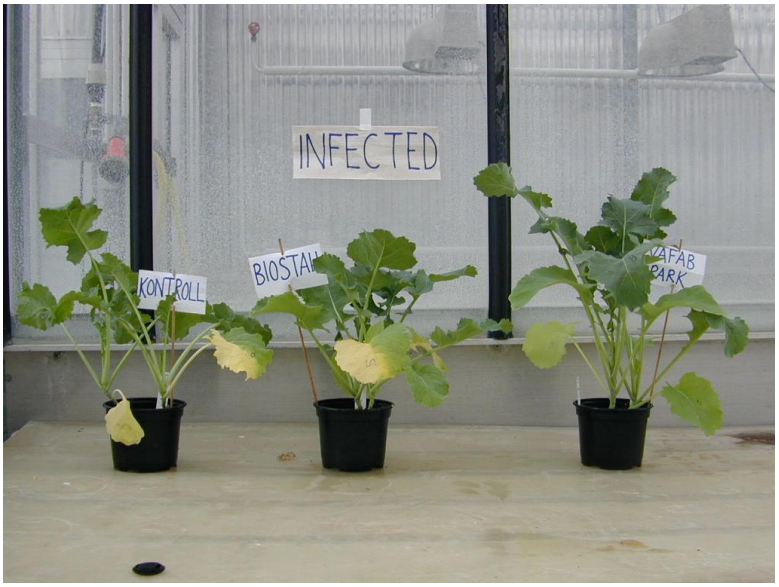

Photo 2.
Comparison of compost extract amended oilseed rape plants challenged with V. longisporum (from left to right): control, BCMS and GWHMS. No symptoms have developed in the GWHMS plant.

Photo: Caroline Haarala

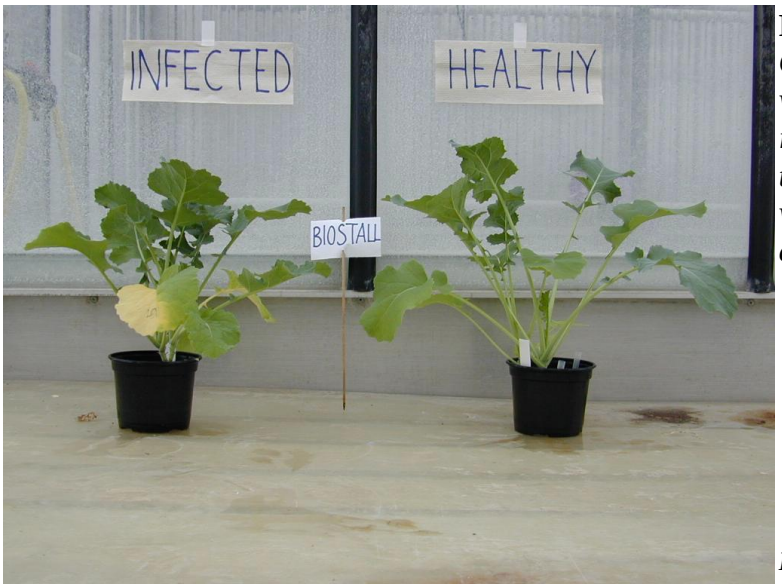

Photo 3.
Oilseed rape plants amended with BCMS. The V. longisporum infected plant (left) displays typical symptoms of verticillium wilt in relation to the healthy control.

Photo: Caroline Haarala

Photo 4.
Oilseed rape plants amended with GWHMS. The V. longisporum infected plant (left) does not display any symptoms in relation to the healthy control.

Photo: Caroline Haarala

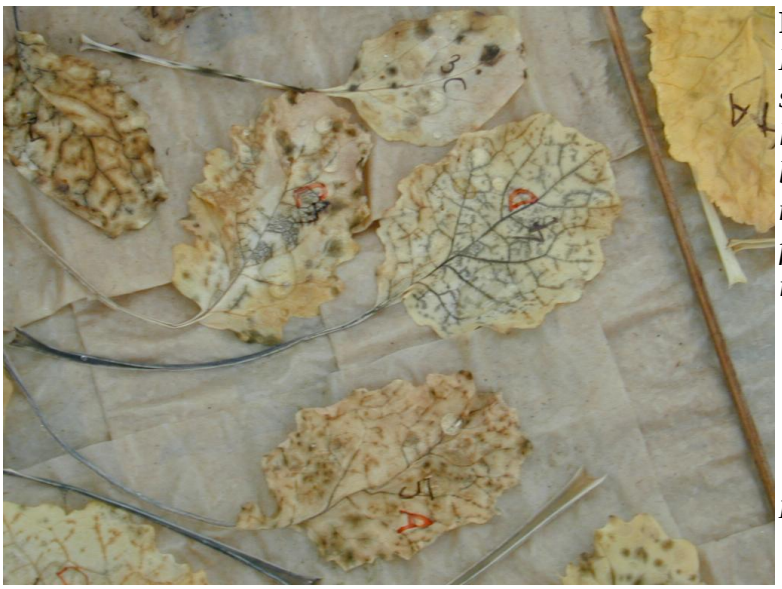

Photo 5.
Leaves from oilseed rape systemically infected with V. longisporum. The veins are blackened due to formation of microsclerotia. Microsclerotia formation was enhanced by incubation in moist chamber.

Photo: Caroline Haarala

In vitro Effects of Compost Residents on *Verticillium longisporum*

The aim of the *in vitro* test was to investigate possible antagonistic effects on *V. longisporum* in the presence of a selection of microbes isolated from the composts. All screening tests are presented as the diameter (mm) of growth of *V. longisporum* when grown on PDA in absence of other microorganisms. *In vitro* inhibition was studied as direct and indirect effects of the test organisms on the pathogen.

Direct effects of fungi

Dual culture assay with pure cultures of compost microorganisms and *V. longisporum* revealed that all fungi but one (GWHMSi) had an inhibitory effect on growth of *V. longisporum*. Not only did GWHMSi have no inhibitory effect on *V. longisporum*, rather, it seemed to stimulate growth of the pathogen. Three of the fungal isolates expressing the most evident inhibitory effect were all isolated from BCMS. When examining the effects of BCMSg after 15 days, it was evident that BCMSg induced a zone of inhibition in *V. longisporum*, which failed to develop towards the fungal compost isolate. Yet other isolates were able to inhibit the pathogen in the same way. Photo 6 illustrates how proliferation of *V. longisporum* was inhibited by inhibition zones induced by BCMa, BCMd, BCMi and BCMSg. It seemed as if BCM contained a larger share of inhibitory fungi than GWHMS. No such tendency was observed as regards bacteria in these two composts. BCMSd, BCMSf, BCMSi, GWHMSb and GWHMSc covered the petri dish and grew over *V. longisporum*. Photo 7 illustrates how BCMSd, BCMSf and BCM inhibited *V. longisporum*, in relative to the control. The majority of the fungi were clearly directly suppressive towards *V. longisporum* (Figure 4). The statistical analysis of the results proves that all isolates except GWHMSi significantly suppressed proliferation of the pathogen. 17 isolates out of 29 reduced proliferation of *V. longisporum* by more than 40%, relative to the control. The three isolates BCMSf, BCMSi and GWHMSb were strongly suppressive and reduced growth of the pathogen with more than 59%. LSD when the control (1 repetition) is compared to effects of test isolates (2 repetitions) is 9,927, and 8,106 when the effects of test isolates are compared with each other (2 repetitions).

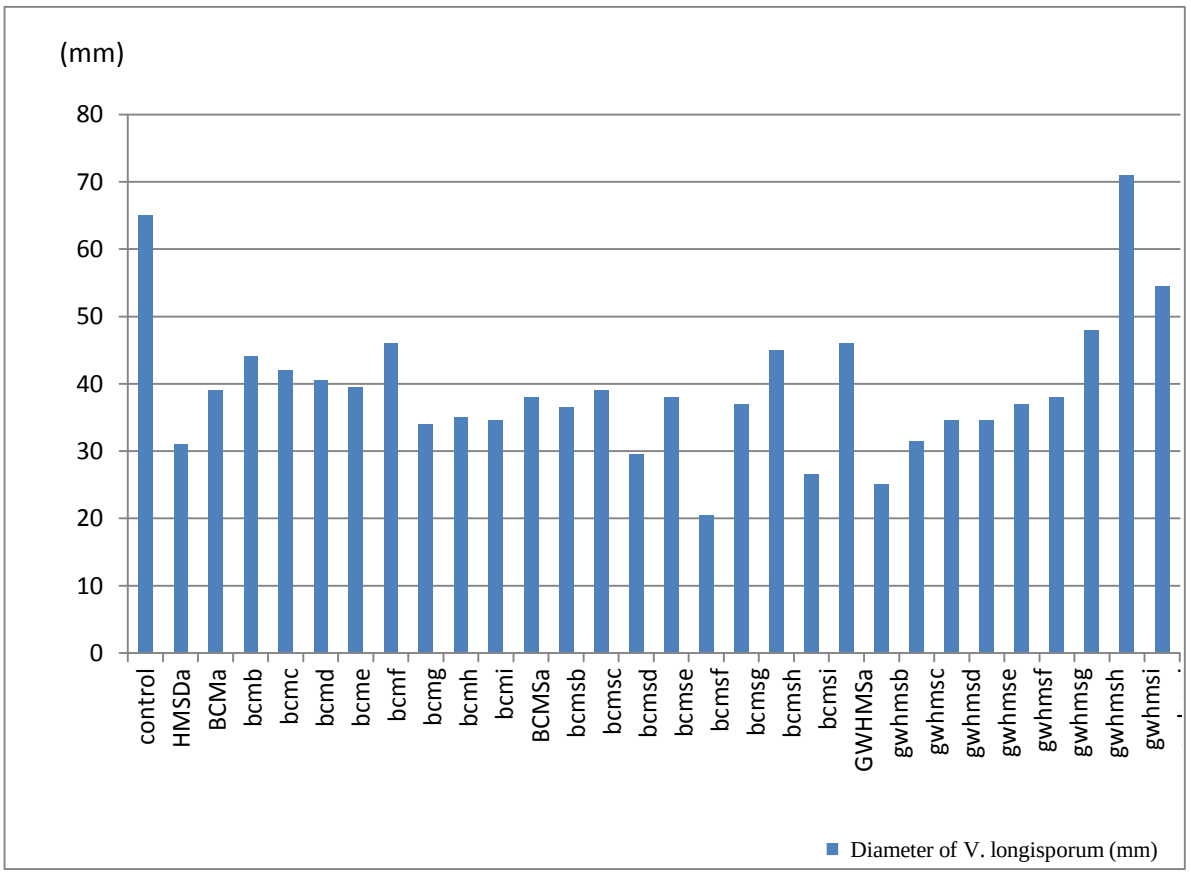

Figure 4. *Direct effects of 29 fungal isolates from composts on growth of V. longisporum on PDA. Examined after 6 weeks. N=2 except for the control where N=1.*

Photo 6.
V. longisporum (lower isolate) co-inoculated with fungal compost isolates on PDA. The compost isolates BCMa, BCMd, BCMi and BCMSg all induced inhibition zones toward V. longisporum. The proliferation of V. longisporum may be compared to the control in Photo 7.

Photo: Caroline Haarala

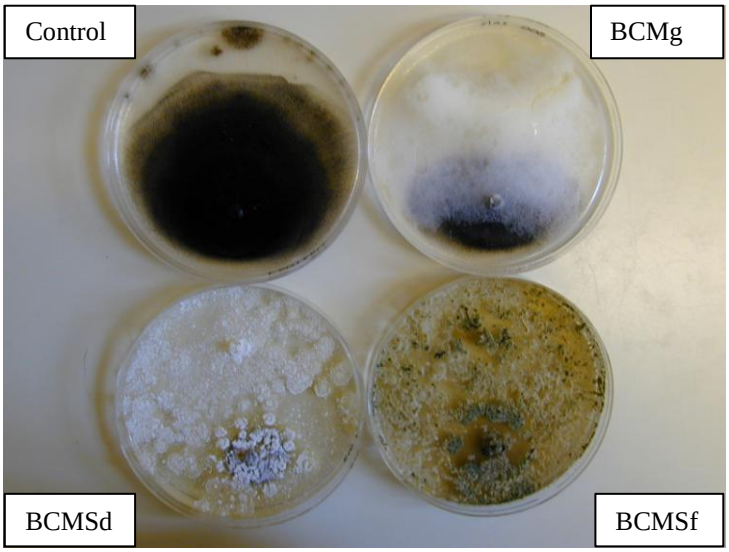

Photo 7.

V. longisporum co-inoculated with fungal compost isolates on PDA. The control in the upper left in thrives in relation to when challenged with BCMg, BCMSd and BCMSf.

Direct effects of bacteria

The pattern of inhibitory effects was different with bacterial isolates. Most bacteria tested for direct antagonism towards *V. longisporum* exhibited no or only slight inhibitory effect on proliferation of *V. longisporum*. Direct antagonism by bacteria was performed both on PDA (Figure 5 and 6) and KBA (Figure 6). The results from the KBA experiment were collected after 2 weeks and are compared to corresponding results obtained on PDA in Figure 6. After two weeks, the diameter of the *V. longisporum* control was 72 mm on PDA and 55 mm on KBA, and 72 mm respectively 48 mm (mean diameter) when co-inoculated with bacterial isolates. No compost dependent effects were observed.

Since no replicates were prepared in the examination of direct effects of bacterial isolates (N=1), a statistical analysis of the results was not possible. A few of the bacterial isolates showed inhibition; BCM3 (35%), BCM4 (21%) and GWHMS2 (43%). The trend is however an overall slight inhibition.

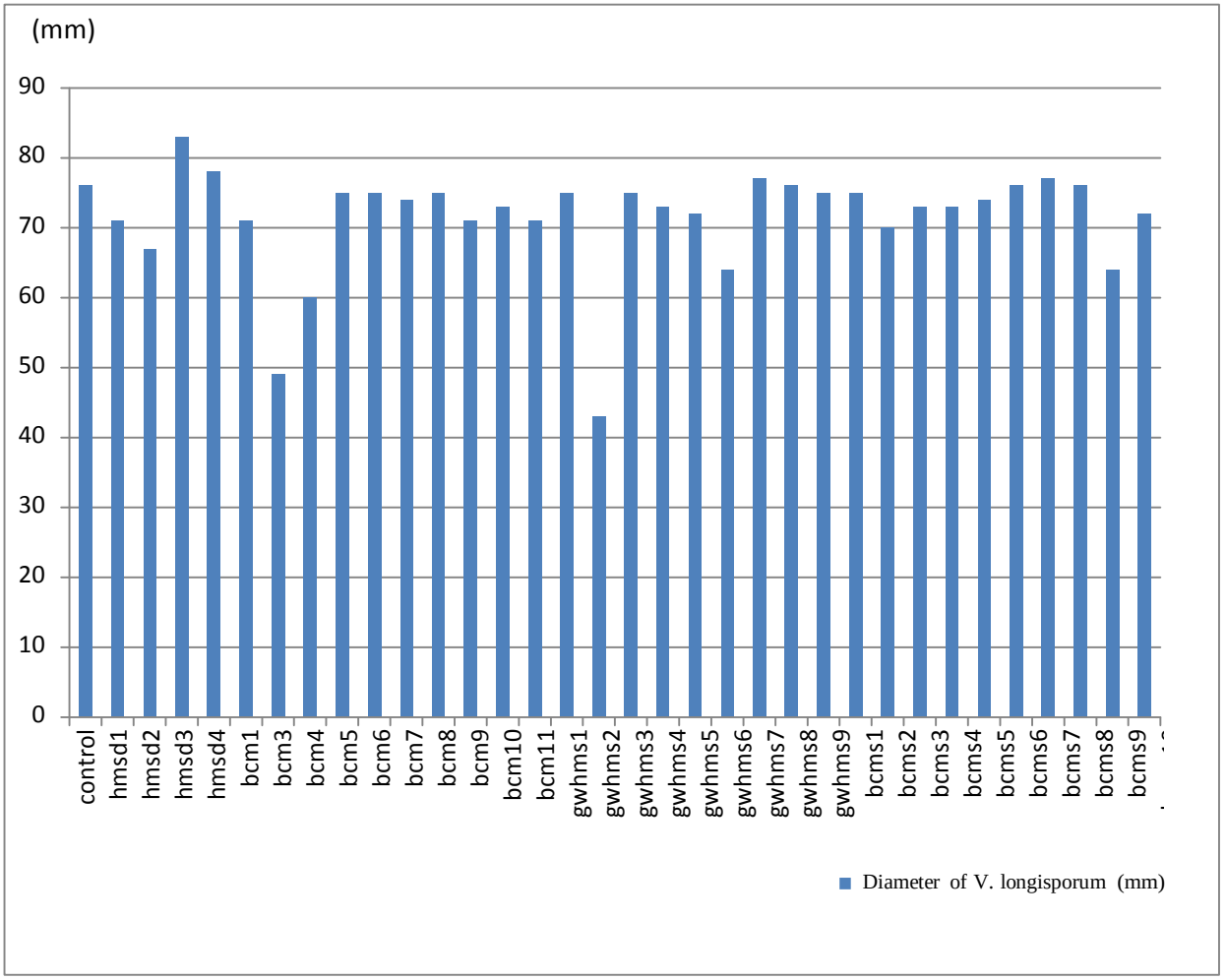

Figure 5. *Direct effects of 33 bacterial isolates from composts on growth of V. longisporum on PDA. Examined after 6 weeks. N=1.*

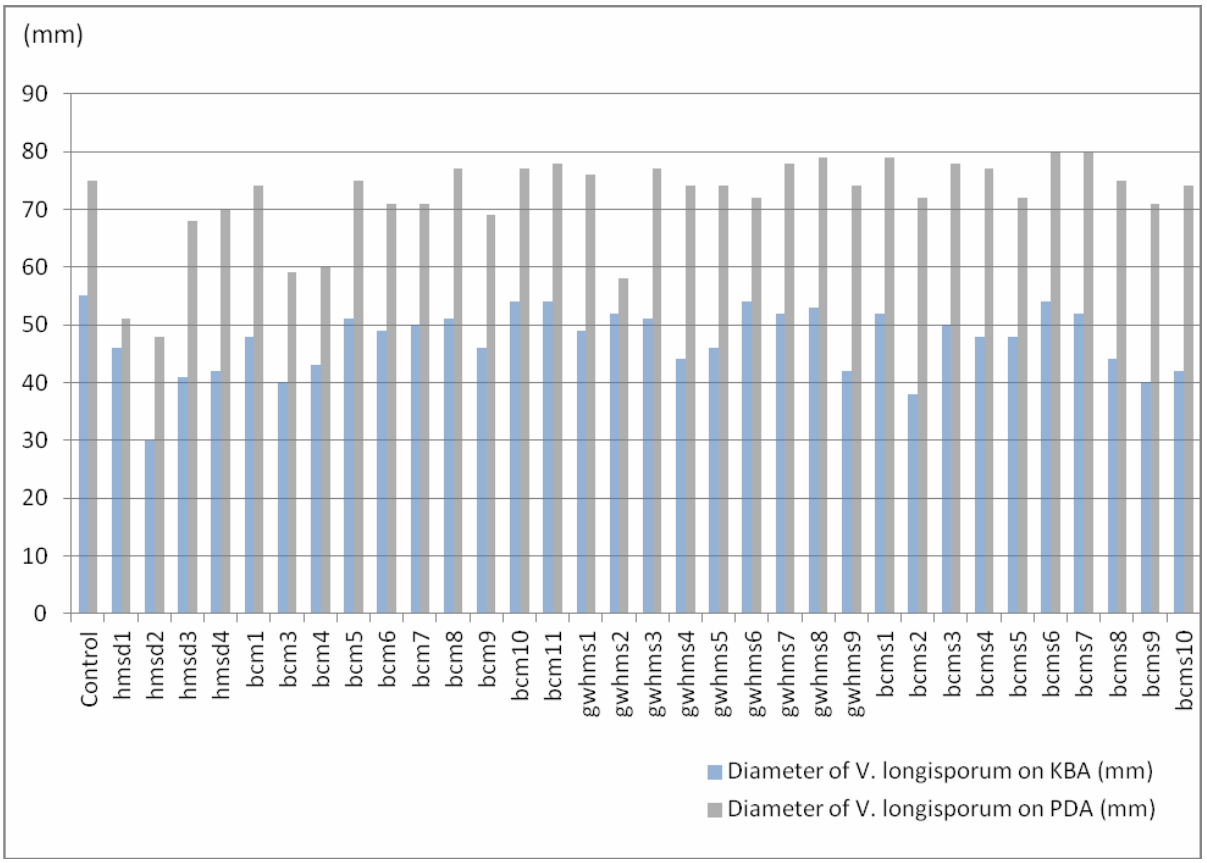

Figure 6. *Direct effects of 33 bacterial isolates from composts on growth of V. longisporum on KBA and PDA. Examined after 2 weeks. N=1.*

Indirect effects of fungi

As in the direct test, there was a general inhibition on growth of *V. longisporum*. About half of the fungal isolates exhibited some degree of pathogen suppressive effect (Figure 7).

Out of the 26 fungal compost isolates tested, 11 had a statistically significant effect on the growth of *V. longisporum*; all inhibitory except for BCMh, which seemed to have a stimulating effect. The 10 inhibitory isolates were: BCMa, BCMd, BCMg, BCMSd, BCMSi, GWHMSe, GWHMSf, GWHMSg, GWHMSh and GWHMSj.

The LSD is 7,836 when the control is compared with effects of test isolates with 2 repetitions, 6,398 when fungal isolates with 2 repetitions are compared with each other and 9,048 when fungal isolates with 1 repetition are compared with each other. The LSD of 9,048 is also applicable when comparing the control with isolates with 1 repetition.

Six isolates (BCMd, BCMSi, GWHMSd, e, f and g) induced a very reduced formation of microsclerotia in *V. longisporum*.

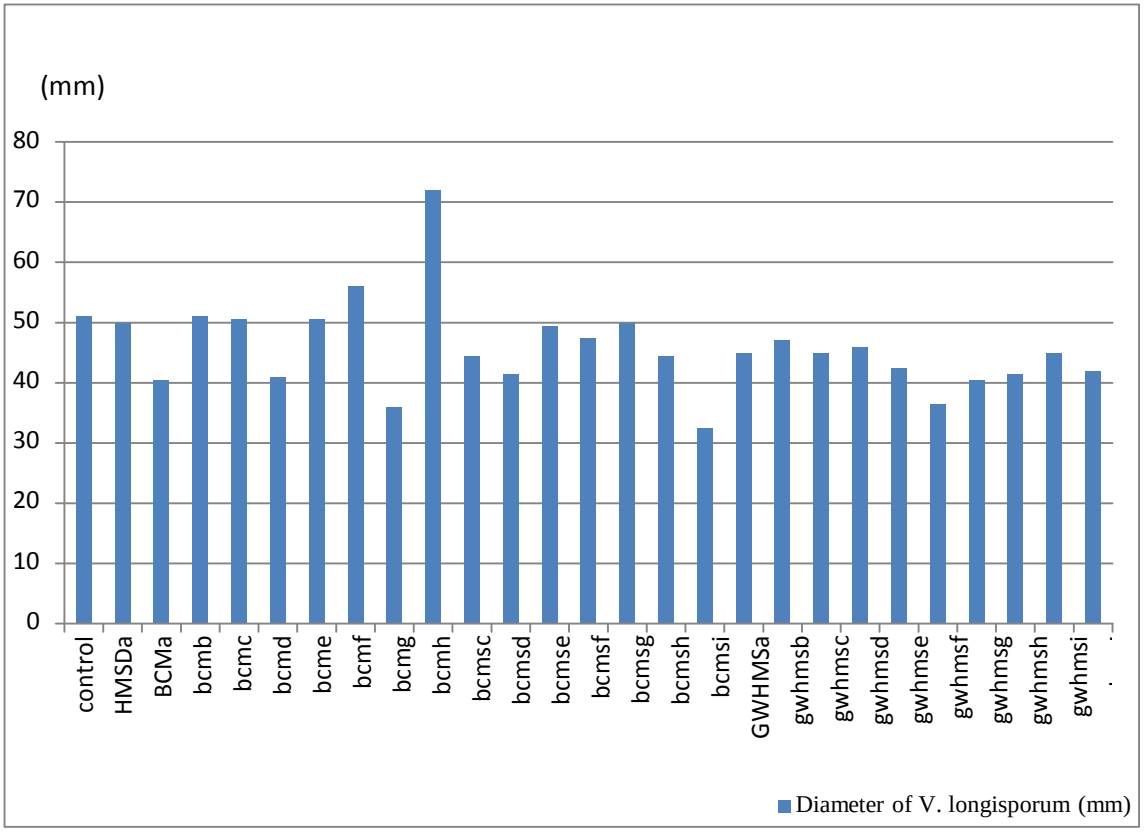

Figure 7. *Indirect effects of 26 fungal isolates from composts on growth of V. longisporum. Examined after 6 weeks. N=2 except for the control, BCMg and BCMh, where N=1.*

Indirect effects of bacteria

Practically none of the bacterial isolates showed tendencies of indirect inhibition of *V. longisporum* proliferation (Figure 8). It seemed rather to be stimulated when incubated with most of the bacterial isolates. Only one isolate (HMSD2) resulted in a *V. longisporum* diameter smaller than the control, but the effect was not statistically significant. The statistical analysis of the results showed that none of the bacterial isolates had a significant effect on the growth of *V. longisporum*. The LSD is 16,882 when the control is compared with other isolates (2 repetitions), 13,784 when bacterial isolates are compared with each other (2 repetitions) and 19,493 when bacterial isolates with 1 repetition are compared with each other. The LSD of 19,493 is also applicable when comparing the control with other isolates with 1 repetition.

Five isolates (HMSD4, BCM6, BCM8, BCMS5 and BCMS11) induced a very reduced formation of microsclerotia in *V. longisporum*.

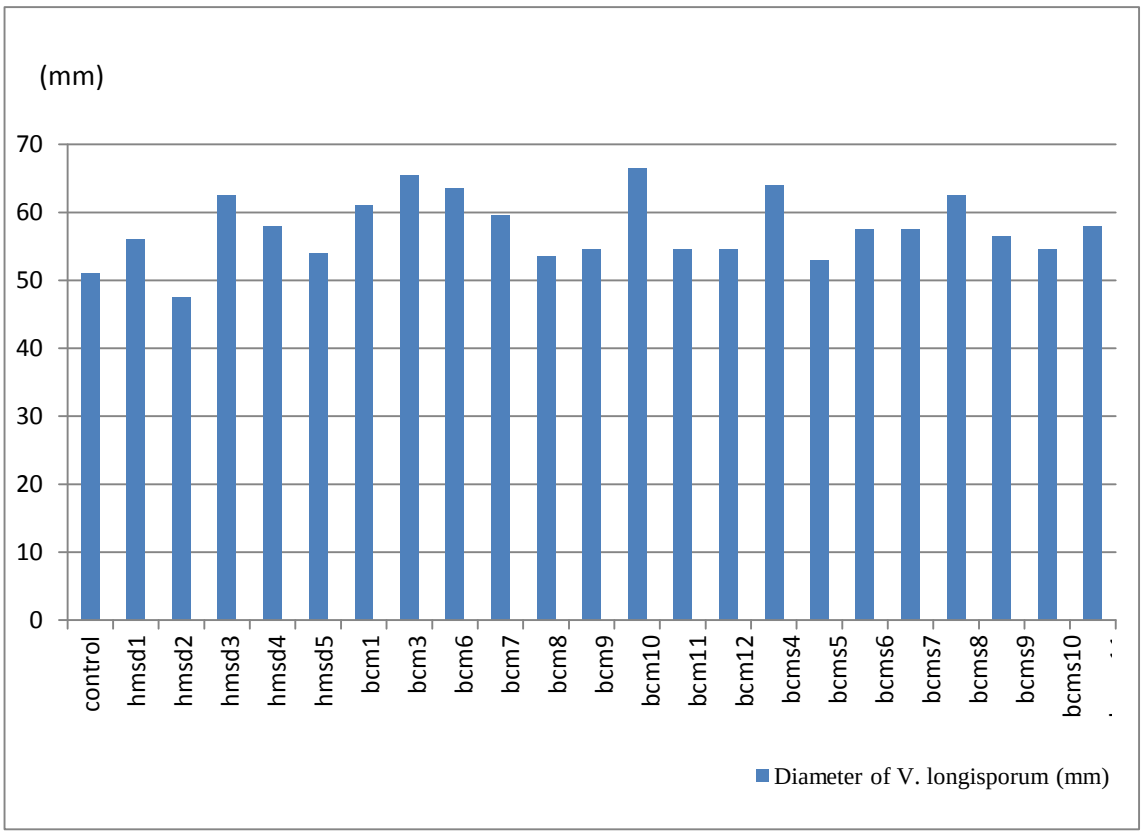

Figure 8. *Indirect effects of 22 bacterial isolates from compost on growth of V. longisporum. Examined after 6 weeks. N=2 except for the control, HMSD5 and BCMS4, where N=1.*

DISCUSSION

Chemical features of composts

The chemical properties of the composts determine the environment in which the microorganisms live. They are thus of great importance to the microbial composition and its possible pathogen inhibitory effects. The chemical properties themselves may also have inhibitory or enhancing effects on a soilborne pathogen. In addition, they may have a nutritional value to the plants in the greenhouse trial, which was reflected as controls of UKWS (the compost most rich in nitrogen and potassium) in particular thrived best, resulted in the highest dry weight and were significantly different from other compost amendments regarding dry weight.

pH ranged from neutral to alkaline in the extracts. It may be quite natural, since the decomposition rate is high at first and oxygen depletion may cause anaerobic environments, which results in the formation of organic acids. pH generally increases during the composting process due to accumulation of ammonia which gives a rise in pH and a decrease in solubility of trace elements. However, it usually stabilizes around neutral. The decrease in solubility of trace elements in alkaline conditions might suggest that BCMS, BKW and BCM contained siderophore producing populations. If so, trace elements would be available for microbial growth despite the higher pH.

Compost extracts from UKWS, RKW and BCMS all had very high conductivity, as could be expected due to their higher nutrient content and thus probably higher share of free ions, compared to the others. Based on the nutrient content of the composts, the conductivity to some extent reflects the potassium content. Since mineralization proceeds as a compost matures and salts accumulate with time, the differences may also somewhat reflect the age of the composts.
The biodynamic garden waste compost BGW had a surprisingly high conductivity, while the biodynamic kitchen waste compost (BKW) had much lower conductivity. The higher conductivity of BGW was unexpected since bark composts generally tend to have a lower conductivity compared to manure composts. The higher conductivity may indicate that the compost was prepared from more delicate plant material. The low conductivity of BKW may reflect the lower nutrient content in a biodynamic kitchen waste compost compared to a non-biodynamic kitchen waste compost. There was a large difference in conductivity when comparing the two cow manure composts BCMS and BCM. BCM had a very low conductivity compared to BCMS and possible reasons are the lower nutrient content and the lower dry weight.

In general, the composts GWHMS, BKW and BGW all had a low content of phosphorous, potassium, nitrogen and carbon, while UKWS and BCMS had a comparatively high content of NPK. BKW exhibited the lowest nutritional values and hence differed from the other two household waste composts; UKW and RKW, which is probably a consequence of its biodynamic origin. Several plant pathogens are enhanced by high nitrogen concentrations and as was presented in the literature study, nitrogen in excess in combination with other factors that are favourable for disease conduciveness may cause plant pathogen epidemics. The compost amendments most rich in nitrogen in this study did in fact cause the most severe systemic infections in the greenhouse trial, as is discussed below.
The C/N ratios were in general somewhat low to be considered as optimal for microbial growth. All except HMSD and BCM contained excess nitrogen (ratio less than 15:1), which might be either because of immaturity of the composts or an indication of declining decomposition rates (microorganisms die and nitrogen levels increase). HMSD and BCM had ratios ranging between 15:1 and 30:1 and may therefore be considered stabilized.

As regards the dry weights of the composts, composts containing manure had the lowest dry weights of about 20%. Small scale composted kitchen and garden waste had similar dry weights, around 50%, and large scale composted RKW and GWHMSD had the highest dry weights of more than 60%. The dry weights reflect the water activity and different organisms prefer different water activities. Bacteria are for example dependent on water films in order to readily colonize a surface. A moisture content of more than 35% and preferably over 45% is desired in order for beneficial microorganisms to colonize a compost. All of the studied composts but RKW did contain enough moisture to be considered favourable for microbial colonisation. Less than 35% moisture induces increased fungal colonization by moulds. The fact that moulds thrive in lower water activities is well demonstrated by RKW, which had a moisture content of 30%, and was clearly colonized by moulds when processing it into extract. RKW also was the compost to contain the most fungi (cfu/ml) in the total count after two days.

Microbial features
Fungi and bacteria were isolated from all composts. The composts differed from one another with respect to their microbial composition, both qualitatively and quantitatively. The

difference in populations reflects the composition of the composts regarding biological, physical and chemical conditions. Bacteria were generally present in larger numbers than fungi, about 2-4 log units more. Since the microorganisms were counted two days after incubation, the results for total counts can only be regarded as indicative of populations of various sizes and compositions. The more slow-growing organisms, such as many fungi, are not represented in this study, although they can be of equal importance.
There were similarities in appearance between the populations of UKWS and RKW when observed on the agar. BKW had a slightly more diverse fungal population, based on subjective ocular examination of agar plates.
It seems as though there is a greater correlation between the microbial content with respect to the nature of the compost material than whether it was of biodynamic origin or not.

In terms of functional characteristics, additional information was obtained about the microbial compost residents. The majority of the composts carried populations that were fluorescent, cellulose degrading and protein degrading. Based on the microbial isolates tested for characteristics, a larger portion of fungi than of bacteria possessed more than one quality. About half of the fungal isolates were cellulolytic as well as proteolytic, and these were most frequently isolated from GWHMS and BCMS. Of the isolates from GWHMS, more than half of the fungal isolates (60%) were cellulolytic as well as proteolytic. In isolates from BCMS, 78% were cellulolytic as well as proteolytic. The isolates of BCM were either proteolytic or cellulolytic, in terms of fungal as well as bacterial isolates. Possession of more than one characteristic was considerably less common among the bacteria; only 15% of the isolates exhibited at least two of the functional characteristics that were examined. BCMS had the highest frequency of bacterial isolates expressing at least two characteristics (30%), while GWHMS resembled the population in BCM in the sense that the microorganisms in general were either cellulolytic or proteolytic. Fluorescent fungi and bacteria were practically absent.

Easily available nutrients such as proteins are degraded in the initial phase. The fact that several of the composts contained a greater population of proteolytic microorganisms in relation to cellulolytic and fluorescent (HMSD, BCMS, BGW) might indicate that the composts were somewhat immature. The composts containing a larger share of cellulolytic microorganisms (UKWS, GWHMS) may probably have come further in the composting process. There seems to be no obvious correlation between functional groups and source. This is however difficult to tell since there is an interaction with the maturity of the material. It was not further investigated.

Plant response to compost extract in absence and presence of *V. longisporum*
The dry weights of the oilseed rape seedlings differed significantly on two levels, on the one hand between compost amended controls and pathogen inoculated seedlings, and on the other hand between the different types of amendments (including the control). It was on the whole evident that *V. longisporum* was pathogenic and decreased the dry weight of the inoculated plants.
It is difficult to evaluate the separate detrimental effects of *V. longisporum* because of plant growth effects due to interactions of e.g. nutritional values. Three of the compost amendments increased the dry weight compared to the control, and two of these were rich in nutrients (UKWS and RKW) and one was poor in nutrients (GWHMS). The nutritional effect on the dry weight of the compost amended seedlings in relation to the control was not as evident as the decrease in dry weight when the pathogen was inoculated.

It is clear that UKWS enhanced growth of oil seed rape plants, also in presence of the pathogen, which is interesting since plants in pots with UKWS at the same time was one of the most heavily infested sets. The infection seemed to be enhanced in the seedlings treated with UKWS extract, and decreased in weight although the plants grew well and were vital. The two composts that were most rich in nutrients (UKWS and BCMS) were the ones where the plants were most severely systemically infested with *V. longisporum*, based on spread of infection in the plants. Plants treated with extracts poor in nutrients, e.g. GWHMS and BCM, were in general less infested. It is also interesting to see that plants in the control, with no compost amendment, were among the least infected ones, which might support the negative synergy of nutrients and infestation. On the other hand, BGW was poor in nutrients but the seedlings still became rather severely infected with the pathogen.

In vitro inhibition of *V. longisporum* by compost residents

The *in vitro* evaluation of the inhibitory potential of the microorganisms indicates that multiple mechanisms were operating behind the pathogen suppression in this study. For example, the fungal isolates BCMg and BCMSi both had quite strong suppressive effect in the direct as well as in the indirect test, why they may be suspected to possess several mechanisms operating against *V. longisporum*. The majority of the suppressive compost isolates did however exhibit effects either directly or indirectly.

It is thus evident that some composts provided the soil/potting mixtures with beneficial microorganisms. The potential of composts is restricted due to the variability in its consistency with respect to its micro-biota. Again, the four composts selected for *in vitro* experiments were BCMS (no disease suppression), GWHMS and HMSD (moderate disease suppression) and BCM (disease suppression of wilt). All four composts proved to contain microorganisms with suppressive abilities towards *V. longisporum*, regardless of the degree of suppression in the *in vivo* experiment.

The **direct** suppressive effect of compost isolates on proliferation of *V. longisporum* would be due to non-volatile diffusates and/or space and nutrient competition and hyperparasitism. Enzymatic properties such as cellulolytic and proteolytic ones are known to be involved in hyperparasitic suppression. As discussed above, many of the **fungal** compost isolates did in fact express proteolytic as well as cellulolytic characteristics, and the positively antagonistic features in the direct test may be a result of hyperparasitism. The results and findings obtained in this study support that proteolytic and cellulolytic characteristics may matter to pathogen suppression. In contrast to the more suppressive fungi, the majority of the less inhibitory fungi possess only one out of the three functional characteristics examined. Out of the nine isolates where the diameter of *V. longisporum* exceeded 40 mm, six had only one characteristic. Those fungal isolates that proliferated largely over the petri dish and over *V. longisporum* as well were among the most suppressive. Growth of BCMSg was observed to be hindered by *V. longisporum* and would not enter the inhibition zone. A likely reason is that the pathogen produced metabolites that diffused into the medium. A small number of the **bacterial** isolates were directly antagonistic. The isolates with the greatest inhibitory effect on proliferation of *V. longisporum* possessed none or only one of the three functional characteristics tested, which is probably an indication that hyperparasitism may not be a common feature among bacteria. The inhibition pattern on KBA was to some extent similar to the pattern on PDA with respect to which isolates acted suppressive. The strength of bacterial suppression was clearly greater on KBA. *V. longisporum* proliferated better on PDA while the bacterial isolates performed better on KBA, which reflects the characteristics of the media. Though the results from the *in*

vitro experiments cannot be validated statistically, bacteria seem to be capable to suppress the fungal pathogen, although to a considerably lesser extent compared to fungi.

The method used to evaluate **indirect** effects investigated the ability of compost isolates to exercise effects from an aerial distance. The effect, if present, would be due to volatile metabolites. Six fungal and five bacterial isolates apparently induced a reduced formation of microsclerotia in *V. longisporum*, but when comparing the suppressive effect of the isolates it does not seem to be of major importance. As in the experiment on direct effects, fungi seem to be better equipped to suppress proliferation of *V. longisporum*. The traces of indirect antagonistic effects among **fungi** were not as evident as in the case with direct effects, but there actually seemed to be an indirect suppressive effect as well. Looking at Figure 6, about half of the fungi appear to have some inhibitory effect, and according to the statistical analysis, 10 out of 26 fungal isolates had a significant inhibitory effect on growth of *V. longisporum*. Most of those isolates originated from BCM or GWHMS. One isolate, BCMh, significantly stimulated growth of *V. longisporum*. Or could it be that *V. longisporum* produced volatile metabolites that had inhibitory effect on BCMh? Or is it a combination?
In general a relatively large share of the fungi isolated from composts seemed to be able to produce volatile metabolites with inhibitory effect on *V. longisporum*. None of the **bacterial** isolates had a significant inhibitory effect on growth of *V. longisporum* and they did not even reduce growth compared to the control, rather the opposite. Production of inhibitory volatile metabolites does therefore not seem to be a common trait among bacteria isolated from compost, as it is for fungi, based on this study.

Why did the Pythium experiment fail?
Had symptoms failed to appear in compost amended pots only it could have been interpreted as a consequence of general suppression, but in this case neither them nor the non-amended pathogen inoculated controls developed symptoms. Was too little inoculum used, or could inoculum have been prepared or applied differently to promote infection better? There is no obvious reason why the Pythium experiment failed with respect to inducing symptoms in cucumber seedlings. Identically, no symptoms of disease developed in the first pathogenicity test, but in the second test they did although not severe. Conditions were identical in all experiments.

CONCLUSIONS

Microorganisms with antagonistic effects were isolated from composts with suppressive as well as non-suppressive effects on *V. longisporum* in oilseed rape and the *in vitro* results confirm that composts harbour a large number of microorganisms with capacity to suppress proliferation of *V. longisporum*. Based on this study, fungi are more successful than bacteria in inhibition of *V. longisporum in vitro*, directly as well as indirectly. One explanation may be that they possess hyperparasitic and metabolic advantages over bacteria. The suppression was more effective when the antagonist and the pathogen met on a surface and when they were able to operate by means of diffusates and enzymes, than if they had aerial contact only and had to rely on volatile metabolites.
The suppressive effects of the compost microorganisms were more evident in the *in vitro* test than in the *in vivo* test, and all seedlings treated with the eight compost extracts in greenhouse were infected with *V. longisporum* to various degrees. Compost extracts with high nitrogen concentrations seemed to enhance infection in this study.

The experiment was outlined as a wide screening with results from all sub sections pointing towards antagonistic potential of composts on several levels. In retrospect, there are of course a number of perspectives and considerations that would have been desired but were left out. Chitinolytic enzymes could have been complementary to the functional characteristics. Furthermore, the total counts were estimated after two days. An estimation a few days later would have resulted in different figures including the more slowly-growing flora, bacterial as well as fungal. Especially fungi in general need more time to develop. It should also be kept in mind that PDA favours fungal growth compared to KBA. When compared after two weeks, the bacterial isolates co-inoculated with *V. longisporum* on KBA exhibited an overall greater antagonistic effect than when co-inoculated with *V. longisporum* on PDA.

The experiment could easily have become more comprising. It would for example have been interesting to examine the microbial populations of all eight composts *in vitro*, now only four of them were studied due to time restriction. However, there ought to have been more than two repetitions in the *in vitro* experiments. They were a bit too few in order to be able to make safe conclusions, especially when the control had one repetition only. After collecting the results in the *in vitro* experiment, it would have been desirable to have proceeded with a greater number of repetitions of those isolates that showed strong suppression of the pathogen. Additionally, a fraction of organic matter with a microbial flora existing in the planting soil can not be excluded. The planting soil was not extracted and examined. It would also be of interest to see whether or not the microorganisms that had a suppressive effect on *V. longisporum in vitro* exhibit suppressive effects toward other fungal soilborne plant pathogens as well?

The suppressive effect of compost is a interaction between physical, chemical and microbial parameters; the quality and quantity of the microbial flora and the chemical composition. It is an intriguing thought to design tailor-made suppressive composts using inoculum and suitable raw material in a controlled process and thus creating composts intended for soil amendment or watering extract.

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APPENDIX

Table A. *Dry weights of individual oilseed rape plants in control rows (g).*

	1	2	3	4	5	6	7	8	9	10
Control	5.14	6.62	5.40	5.81	6.55	6.27	7.14	6.45	4.97	6.50
UKW	7.24	7.68	8.06	7.69	8.72	6.11	6.88	7.91	8.07	10.56
GWHMS	6.84	6.08	6.11	6.61	6.64	5.66	6.77	6.07	6.24	6.58
HMSD	5.30	4.88	5.60	5.00	5.43	4.88	5.02	5.17	4.77	5.68
RKW	6.16	6.22	6.60	6.42	7.00	6.39	5.00	6.54	7.06	7.94
BCM	4.36	5.12	5.73	6.55	7.25	5.13	5.86	5.39	6.08	4.36
BCMS	3.52	5.09	6.27	5.03	6.33	5.09	5.18	5.83	4.50	5.25
BKW	5.33	5.60	4.52	5.79	5.12	5.13	4.31	4.78	5.13	5.73
BGW	5.99	5.37	5.97	6.75	6.11	5.97	5.61	5.64	6.12	5.80

Table B. *Dry weights of individual oilseed rape plants in inoculated (V. longisporum) rows (g).*

	A	B	C	D	E	F	G	H	I	J
V.dahliae	4.60	6.58	6.13	6.11	5.83	5.95	5.66	5.62	6.53	6.10
UKW	6.71	6.25	7.37	7.85	5.86	6.73	6.11	7.53	9.09	8.23
GWHMS	3.74	5.70	5.76	7.30	5.80	5.43	5.47	6.55	5.25	5.54
HMSD	4.34	4.86	5.21	5.13	5.69	4.08	4.55	5.16	5.39	4.32
RKW	6.37	4.76	5.14	6.60	5.01	5.35	5.80	6.37	6.91	6.24
BCM	5.47	4.02	5.81	5.76	5.57	5.98	3.16	5.74	5.02	6.70
BCMS	4.46	5.43	5.08	4.72	4.63	4.67	5.90	4.37	6.43	6.41
BKW	5.35	5.31	5.98	6.18	5.78	5.60	5.01	5.72	6.74	5.53
BGW	5.33	4.03	6.25	5.86	5.70	3.70	6.46	5.99	5.93	5.90

Table C. *Systemic spread of V. longisporum in oilseed rape leaves. The values relate to the order of appearance of the leaves, indicating how high in the plant infection has spread.*

	A	B	C	D	E	F	G	H	I	J
UKWS	7	5	6	6	7	6	6	5	6	4
GWHMS	7	5	0	0	5	6	3	0	6	0
HMSD	4	5	6	5	0	6	4	3	4	7
RKW	0	6	5	4	5	5	6	6	4	7
BCM	6	4	3	4	6	0	5	3	4	0
BCMS	6	6	5	6	6	6	6	7	4	5
BKW	6	5	3	5	6	7	8	4	0	5
BGW	7	6	5	4	8	7	2	7	5	4
V. long.	5	0	2	6	4	5	6	0	6	0

LSD: 0,2243