Swedish University of Agricultural Sciences
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Assessment of Soil Suppressiveness

– The system of fusarium foot rot on wheat

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ABSTRACT

The soil suppressiveness to fusarium foot rot caused by *Fusarium graminearum*, *Fusarium culmorum* and *Fusarium pseudograminearum* was assessed by two complementary analyses: the bioassay and the fungistasis test. The bioassay addresses suppressiveness to fusarium foot rot and the fungistasis test evaluates the pathogen suppression capacity of different soil samples. A field experiment set-up to study effects of conventional and reduced tillage and different preceding crops (wheat, oat and oilseed rape) was used for the study. In addition to establish the methodology for assessing the suppressiveness to fusarium foot rot caused by *F. graminearum* and *F. culmorum*, the different cultural practices were evaluated in their impact on the suppressiveness. Reduced tillage increased the suppressiveness to fusarium foot rot caused by *F. graminearum*, since reduced tillage decreased disease severity of the wheat plant growing on soil with wheat as preceding crop in the bioassay. In addition, reduced tillage decreased the germination rate of conidia spores of *F. graminearum* in the fungistasis test. For *F. culmorum*, no impact of the tillage treatment on the suppressiveness could be detected. Soil with oilseed rape as preceding crop showed the lowest disease incidence in the bioassay, what suggests that crop rotation with oilseed rape increases the suppressiveness to fusarium foot rot. Cultural practices which showed a significant effect on disease suppression, did not necessarily show an effect on pathogen suppression which was also influenced by the two *Fusarium* species differently due to their different ecology.

POPULAR SCIENCE SUMMARY

Soil is a very valuable and important natural resource. It provides the basis for 90% of our food, livestock feed, fibre and fuel. Especially in agriculture the soil plays a major role; it supports the plant roots and delivers water and nutrients for the plant growth. It serves as habitat for thousands of microorganisms including plant pathogens which can cause severe damages in our crop plants and lead to high yield losses. Plant pathogenic microorganisms can be found amongst fungi, bacteria, nematodes and numerous other groups within protista. The severity of the disease in the plants is influenced by several factors such as environmental conditions and soil properties. Suppressive soils are described as soils in which the pathogen is present, but the disease on the plants is reduced. Suppressiveness is a naturally occurring biological phenomenon with often an unknown origin which can persist for several decades in situations where it is caused by stable soil properties. Microorganisms living in the soil are able to outcompete plant pathogens or produce toxic substances which are harmful to pathogens, thereby leading to a reduction in the disease severity of the cropped plants.

Good agricultural practices are central for limiting outbreaks of soil borne diseases in a crop. Ideally, practices should be chosen in a way that reduces the density of the pathogen and at the same time increases the soil suppressiveness. Proper land preparation such as tillage, crop rotation, weed control and irrigation influence the soil properties and have impact on the disease severity. In this master thesis, a field experiment investigating the effects of reduced and conventional tillage and three different preceding crops (wheat, oat, oilseed rape) was used. The culture practices were evaluated in their impact on the suppressiveness to fusarium foot rot of wheat. *Fusarium* spp., which are causing fusarium foot rot, are plant pathogenic fungi living the soil and cause necrotic, brown, elongated spots with no distinctive centre or also watery-brown to dark brown discoloration of tissue along the stem of wheat plants. On small grain cereals, *Fusarium* spp. cause fusarium head blight, fusarium foot rot and fusarium seed blight. Three important species of *Fusarium* spp. causing foot rot on wheat are *F. graminearum*, *F. culmorum* and *F. pseudograminearum*; however, they differ in their ecology and their pathogenicity towards wheat plants.

As methodology a bioassay and a fungistasis test was chosen. In a bioassay, wheat seeds are sown in a soil artificially infested with fungi. The growth condition of the wheat plants is favourable towards the fungal growth. After a few weeks of growth, the plants are taken out and the disease severity is assessed by scoring the disease with a disease index scale which consists of several steps of increasing disease symptoms. In the fungistasis test, the influence of the different cultural practices on the germination rate of the fungal conidia spores was assessed.

In the bioassay, wheat plants growing in soil under reduced tillage and wheat as preceding crop showed a reduced disease severity of fusarium foot rot caused by *F. graminearum* compared to plants growing in soil under

conventional tillage. In addition, in the fungistasis test, reduced tillage reduced the germination rate of the spores of *F. graminearum*. These results suggest that reduced tillage increases soil suppressiveness, but for *F. culmorum* no distinctive impact of the tillage treatment on the soil suppressiveness could be detected. Through reduced tillage, more plant residues remain on the field after harvest and provide a habitat for *Fusarium* spp. competitive organisms which could be an explanation to a limited growth and activity of the pathogen. Soil with oilseed rape as preceding crop showed a higher suppressiveness and the growing wheat plants were less diseased compared to the plants from the soil with the other preceding crops. Oilseed rape is a bassica crop and a non-host for *Fusarium* spp. Through crop rotations with non-host plants, the density the microorganisms living in the soil and which are competitive against *Fusarium* spp. are increased. However, the different *Fusarium* species are influenced differently by the cultural practices due to their different ecology.

Cultural practices which lead to a reduced germination rate of the spores did not always necessarily lead to a reduced disease severity in the host pant. The actual outbreak of a disease in a host plant is also dependent on several other factors such as environmental conditions. A soil which shows a suppression to the disease does not inevitably reduces the growth or the activity of the pathogen. The host could be resistant or the environmental conditions are unfavourable towards the pathogen. Suppression of disease does not only take place through suppression of the pathogen.

The aim of this study was to establish a method for assessing soil suppressiveness which was achieved by the bioassay set-up. Furthermore, cultural measures should be evaluated in their impact in the soil suppressiveness in order to eventually reduce the use of fungicides in the future. Reduced tillage and crop rotations with a non-host showed an indication to increase the soil suppressiveness towards *Fusarium* spp. These results can be used as guidance in future long-term studies with a bigger scale.

OUTLINE

INTRODUCTION

Soil is a very valuable and important natural resource. It provides the basis for 90% of our food, livestock feed, fiber and fuel. Especially in agriculture the soil plays a major role; it supports the plant roots and delivers water and nutrient for the plant growth. The organisms living in the soil show a high diversity and are often linked to the functioning of the soil itself. But their activity is not necessary beneficial for our own intentions. The soil hosts also plant pathogenic nematodes, bacteria and fungi which can cause severe damage in the crop plants and therefore result in high yield losses.

In the USA, in the 18th century, farmers discovered that the wilting disease of the cotton plants was restricted to certain soil types. Due to several other observations, the disease severity was soon identified to be influenced by the soil texture. In 1970, the term of “suppressive” soils was introduced and adopted in order to describe soil which shows in a lower disease incidence although a pathogenic organism was present (Hornby, 1983). Since then the research on suppressive soils has flourished in several countries in the world, especially, in the field of biological control. The use of fungicides, which are applied to the field to restrain crop diseases, prevail their disadvantages such as the threat of food contamination and other health hazards. Through the introduction of a biological agent which restricts the pathogen and therefore prohibits the disease occurrence, fungicide use and their caused damaged could be reduced. Cultural practices such as soil preparation through tillage and crop rotation are used in order to control disease occurrence and severity. The intention is to create an unfavorable environment for the pathogenic organism which restricts its activity and spread.

The intention of my master thesis was to establish a method for an assessment of soil suppressiveness to three *Fusarium* species and fusarium foot rot of wheat. In order to address disease suppression the set-up of a bioassay was chosen. Two other smaller experiments were carried out for evaluating the suppression of the pathogen, the fungistasis test and the hyphal growth test. For the first bioassay, 3 *Fusarium* species (*Fusarium graminearum* (F.g.), *Fusarium culmorum* (F.c.) and *Fusarium pseudograminearum* (F.p.)) were chosen. For the second bioassay *F.* pseudograminearum was excluded. The soil was taken from an experimental field in Uppsala which was under two tillage treatments (conventional and reduced tillage) and the individual plots had different preceding crops (wheat, oat and oilseed rape). It was expected that the different tillage methods either increase or decrease the suppressiveness to fusarium foot rot. In a crop rotation with a non-host crop such as oilseed rape, the suppressiveness was expected to increase compared to soil without crop rotation.

BACKGROUND

1. Suppressive Soils

The ability of plant pathogens such as *Fusarium* spp. to survive, grow and cause disease can vary between the different soils because of environmental conditions but also due to properties of the soil (Hornby, 1983). According to Baker and Cook (1974) suppressive soils are defined as "soils in which the pathogen does not establish or persist, establishes but causes little or no damage, or establishes and causes disease for a while but thereafter the disease is less important, although the pathogen may persist in the soil." Suppressiveness can also be described through the concept of soil receptivity which is the inability for a soil to control the activities of the microorganisms present and therefore also the pathogenic organisms. High soil receptivity results in a low capability of the soil to suppress the pathogen (Alabouvette & Steinberg, 2006). Non-suppressive soils, also called conducive soils, are the ones in which disease can develop. The shift from suppressive to conducive soil is continuous and therefore often difficult to distinguish. Another important concept of describing soil suppressiveness is the concept of soil inoculum potential. Soil inoculum potential is described as the present pathogenic energy in the soil which is dependent on the density and the pathogenic capacity of the inoculum with the influencing soil factors (Alabouvette & Steinberg, 2006). The influencing soil factors refer to the already defined soil receptivity. Soil suppressiveness in general is not an unusual phenomenon, it occurs often naturally since the soil plays a major role in the interaction between plant and potential pathogenic microorganisms.

A long standing suppressiveness can be a naturally occurring biological condition with an unknown origin which persists for several decades through its stable soil properties. A short-term suppression or induced suppression arises from a rapid alteration of the soil environment through agricultural practices such as liming, fertilization and tillage or from an introduction of an antagonistic organism against the pathogen. This suppression is lasting only for one or a few crop cycles (Höper & Alabouvette, 1995; Hornby, 1983). In general, suppressive soils are effective due to a combination of general and specific suppressiveness, but they might be influenced in different ways by the edaphic, climatic and agronomic circumstances (Weller *et al.*, 2002). General suppression is related to the global activity of the whole microbial biomass what results in the limitation of the development of the pathogenic populations. An increase in general suppression can be achieved *e.g.* through agricultural practices which increase organic matter content of the soil, build up the soil fertility and therefore raise the activity of the soil microorganisms. Since the general suppression takes into account the total microbial biomass, it is not transferable or transmittable to a conducive soil through incorporation of small amounts of suppressive soil (Höper & Alabouvette, 1995; Weller *et al.*, 2002). The specific suppression is an effect of the activity of a specific strain, a group of microorganism or a combination of several groups on the stage of a pathogen's life cycle. As specific suppression is not dependent on the total biomass, it can be transferable if the ecological

requirements of the antagonistic species are present in the other soil. These two concepts are based on the fact that suppressiveness is often a biological attribute originated by the soil microbial activities. Frequently, the effect of suppression can be eliminated by heat treatment or also by gamma radiation (Höper & Alabouvette, 1995; Mazzola, 2002; Weller *et al.*, 2002). The soil microorganisms are able to inhibit the development and growth of a pathogen through direct parasitism, production of toxins or antibiotics, competition for space and nutrients, production of enzymes and induction of defense responses in the plants (Agrios, 2005; Mazzola, 2002).

In addition to the biological factors, soil suppressiveness can also be influenced by abiotic factors such as the physical and chemical attributes of the soil (clay content, organic matter, pH) which have a direct and indirect impact on the soil microorganisms. Soils with an extreme pH-value are often suppressive against several plant diseases. The texture of the soil determines the interaction between soil particles and microorganisms, because it is the influencing factor for the soil structure, the aeration and the water potential of the soil. The organic material of the soil, which serves as substrate for many kind of microorganisms, affects also the soil structure, the moisture retention and the aeration of the soil (Höper & Alabouvette, 1995). The suppressiveness is also often influenced by a combination of biotic and abiotic factors.

2. Methodology for identification of suppressive soils

In nature suppressive soils are easily identified since the planted crops show reduced disease even with favorable conditions of the pathogen such as a susceptible host and suitable climatic conditions. This concept is transferable to the model of the bioassay in which the in the laboratory produced inoculum is introduced into the soil. The sown plant is known to be susceptible and the environmental conditions are adjusted in order to be favorable for the disease (Alabouvette & Steinberg, 2006). The system of the bioassay has been already used for many years. In 1985, a study done by Wilkinson *et al.* was identifying the relationship of type, size and concentration of inoculum source to the infection of wheat roots by *Gaeumannomyces graminis var. tritic*i in soil. In another study, bioassays were used to determine the disease suppression of soils under different cultivation practices to brown foot rot by *Fusarium culmorum* on barley (Knudsen *et al.*, 1999). After 19 days of growth, the disease severity of the single barley plants was evaluated by a disease index scale. Another method for evaluating the disease severity is presented by Alabouvette *et al.* (2005), where a disease progress curve is produced for each replicate by plotting the cumulative value of the disease index against the time. Bioassays address the disease suppression by the soil, but this does not necessarily include a suppression to the pathogen itself. The inoculum does not necessarily cause disease although it is present. In order to clarify this concept, there is an additional separation between (a) pathogen-suppressive soils in which the pathogen itself is suppressed and (b) disease-suppressive soils in which the pathogen is present but disease is limited (Alabouvette & Steinberg, 2006). In

order to assess the pathogen-suppressive soils, several approaches have been suggested *e.g.* the fungistasis test and the hyphal growth test. Fungistasis is a form of microbiostasis which describes the effect of the soil in inhibiting the germination and the growth of the fungal spores (Alabouvette *et al.*, 2005). The methods used by Wacker and Lockwood (1991) who assessed the fungistasis by counting germinated spores of *Cochliobolus sativus*, *C. victoriae* and *Fusarium graminearum* placed on a filter paper and incubated on different soil samples. These were either diluted with silica sand or mixed with a glucose-peptone nutrient solution. In certain cases the germination rate does not seem to be influenced by the soil. In order to identify a difference between the soils, the length of the germinated hyphal tubes is measured (Knudsen *et al.*, 1999). A method addressing the suppression of hyphal growth is the hyphal growth test. In this test a plug of agar with fungus is placed on water agar which covers a soil sample. The growth of the mycelium is observed and the area under the growth curve can be calculated (Ghini & Boechat Morandi, 2006).

3. *Fusarium* spp., biology and ecology

Fusarium spp. are filamentous, necrotrophic, fungi with several species which cause severe plant diseases around the world. In addition to the yield losses, mycotoxin contamination is caused by some *Fusarium* spp. produced mycotoxins for example deoxynivalenol, zearaleone, fusarin C (Agrios, 2005; Wagacha & Muthomi, 2007). On small grain cereals, *Fusarium* spp. cause fusarium head blight, fusarium foot rot and fusarium seed blight. Three important species of *Fusarium* spp. causing foot rot on wheat are *F. graminearum*, *F. culmorum* and *F. pseudograminearum*; however, they differ in their pathogenicity (Dyer *et al.*, 2009). *F. graminearum* was originally identified to be associated with warm and humid areas, whereas *F. culmorum* seemed to be present in cooler areas as north and west Europe. Nowadays, these borders seem to disappear. *F. graminearum* is also found in high amounts frequently in England, Wales and other parts of Europe including Sweden (Bateman *et al.*, 2007). According to Aoki and O´Donell (1999), *F. pseudograminearum* is favoured in regions which are characterized by dry conditions and high temperatures.

Fusarium graminearum. *F. graminearum* causes fusarium foot rot on wheat and is also an important pathogen for late season diseases such as fusarium head blight. Both diseases can appear on the same plant simultaneously. In the study of Dyer *et al.* (2007), fusarium foot rot on wheat was increased in areas where fusarium head blight was common. *F. graminearum* is able to reproduce sexually. The telemorph stage of *F. graminearum* is known as *Gibberella zeae*. For fusarium head blight, susceptible plants such as wheat and maize usually get infected during anthesis by airborne spores which enter the head through their natural openings such as the base of the lemma and the palea (Trail, 2009). After spreading in the plant tissues, the fungus starts to colonize the plant cells. On the surface of the infected plants and on plant debris, asexual spores are formed. The macroconidia are developing in sporodochia and are dispersed by rain-

splash which contributes mainly to the short distance dispersal (Trail, 2009; Bateman, 2005). The success of infection is highly dependent on climatic factors such as temperature and moisture (Wagacha & Muthomi, 2007). Through the availability of asexual and sexual spores, *F. graminearum* has a higher spread than the other assessed species in the present study. The sexual ascospores ,which are spread by wind, have a further and faster horizontal dispersal and therefore play an important role in the secondary spread as well as in the primary infection (Bateman, 2005; Booth, 1971). The over winter survival of *F. graminearum* is enabled by binucleate hyphae on plant debris in the soil from which perithecium and later the ascospores are formed.

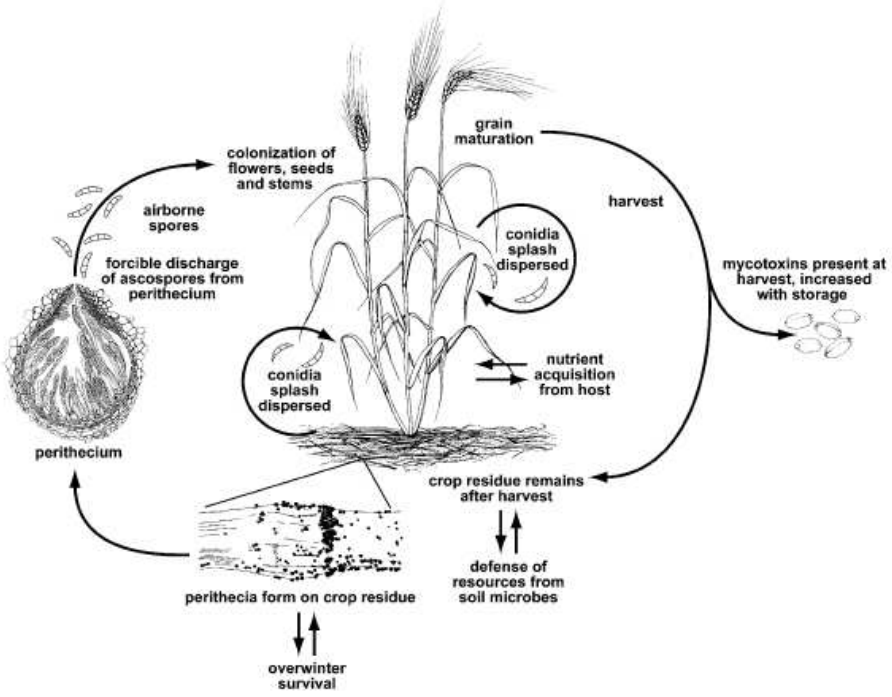

Figure 1. Life cycle of F.graminearum (Trail, 2009).

F. pseudograminarum. *F. pseudograminarum*, telemorph name *Gibberella coronicola*, was formerly considered as Group 1 of *F. graminearum*, until Aoki and O´Donell (1999) distinguished it as *F. pseudograminearum* due to morphological differences in the condia, differences in the growth rate on low nutritional culture media and the absence of perithecia production. According to Dyer *et al.* (2009), *F. pseudograminearum* causes, additionally to fusarium foot rot, late season disease like *F. graminearum*. The morphological similarity between *F. pseudograminearum* and *F. graminearum* does not reflect their phylogenetic relationship. *F. pseudograminearum* is genetically more closely related to *F. culmorum* than to *F. graminearum* (Aoki & O´Donell, 1999).

F. culmorum. Unlike *F. graminearum*, the sexual reproduction stage of *F. culmorum* has not been identified. The main inoculum source is present through macroconidia and chlamydospores. The latter serve additionally as survival

structure and are formed from hyphae or from macroconidia (Wagacha & Muthomi, 2007). Through the capability of surviving as chlamydospore, *F. culmorum* is a comparatively aggressive seedling pathogen compared to *F. graminearum* and *F. pseudograminеaum* which are dependent on plant debris for their survival (Dyer *et al.*, 2009). Host plants are, additionally to cereals and maize, grasses and several other plants such as asparagus and young sugar beet plants in which *F. culmorum* is causing foot rot and brown-patch diseases (Booth, 1971).

Fusarium foot rot symptoms and occurrence. Foot rot caused by *Fusarium* spp. is also called crown rot (Smiley *et al.*, 2005). According to Goulds and Polley (1990), the symptoms of foot root are displayed as necrotic, brown, elongated spots with no distinctive centre or as watery-brown to dark brown discolorations of tissue along the stem. The first visible symptom is often the browning of the coleoptiles and the stem base. Plants in the field which show a severe infection at a low node are easily broken and the tissue at the internodes are often softened which can, together with water stress, lead to white heads and serious crop losses (Booth, 1971). In addition to the presence of a susceptible host, the occurrence of foot rot is dependent on environmental conditions such as temperature and precipitation (Paulitz *et al.*, 2010). In the study of Smiley *et al.* (1996b) foot rot was associated with very dry and also very wet years. Water stress during the anthesis and the maturity of the wheat plants highly enhances the development of foot rot symptoms. Furthermore, the incidence of foot rot is increased by increasing soil organic nitrogen and carbon (Smiley *et al.*, 1996a).

Soil suppressiveness to *Fusarium* spp. There are some known microorganisms associated with the suppression of *Fusarium* spp. Knudsen *et al.* (1995) have shown that in organically cultivated soils, the suppression of *F. culmorum* increased through antagonistic non-pathogenic *Fusarium* species. Furthermore, some bacteria of the Family Pseudomonadacea have been associated with inhibited growth of *Fusarium spp.*, though *e.g.* production of antifungal antibiotics in the rhizosphere area of the plants (Michael & Nelson, 1972; Pal *et al.*, 2001). The selective stimulation of antagonistic rhizosphere microorganisms is part of the plant defense mechanism (Cook *et al.*, 1995). Certain strains of the bacterium *Pseudomonas fluorescens* inhibit the growth of *F. culmorum* through competition for Fe(III) (Kurek & Jaroszuk-Scisel, 2003). *Strenotrophomas maltophilia*, *Bacillus cereus* and *Trichoderma harzianum* decreased the seedling blight of wheat caused by *Fusarium graminearum* (Dal Bello *et al.*, 2002). Some soils increase their capability to inhibit the growth of *Fusarium* spp. by their physical properties such as a high clay and organic matter content (Baker & Cook, 1974; Knudsen *et al.*, 1999). Knudsen *et al.* (1999) found that the suppressiveness to *F. culmorum* was more influenced by clay content of the soil and the cultural practices used such as tillage, than by a high microbial biomass and activity. In an additional fungistasis test, the suppression appeared through reducing the hyphal growth of the fungus rather than inhibiting the germination rate of the conidia.

4. Management of fusarium foot rot

Chemical control of fusarium foot rot is practiced in a large scale in several countries within Europe. Fungicides are either applied directly on the field or used for seed treatment. The use of chemicals in the field requires exact timing of the application, otherwise the effectiveness of the fungicide decreases. Some fungicides even showed a stimulation of *Fusarium* spp. mycotoxin production when used in low doses and at sub-optimal fungal growth conditions (Wagacha & Muthomi, 2007). Biological control of foot rot has been tested in an experimental stage, in laboratory and field experiments, but is not used commercially. Bacterial isolates of the species *Pseudomonas fluorescens* show to act antagonistic to *F. culmorum* (Kurek & Jaroszuk-Scisel, 2003). Completely *Fusarium*- resistant cultivars of wheat are not known, but less susceptible cultivars which reduce the accumulation of mycotoxins are already established and used in a broad scale (Nistrup Jørgensen *et al*., 2008).

In addition to chemical control of *Fusarium spp.*, several cultural management options are practiced in order to manage foot rot, including crop rotation, irrigation, control of susceptible weeds, proper soil preparation such as tillage and well-timed harvesting. Although cultural practices are more time-consuming and more expensive than the chemical measures, the effectiveness is reasonable, especially in the long-term perspective. The most effective and important cultural practices are soil preparation by tillage and crop rotation. However, the effect of tillage appears to be inconclusive. In the case of no tillage or minimum tillage, the plant residues stay on the field and serve as an additional inoculum source for the pathogen and therefore increase the foot rot incidence (Paulitz *et al.*, 2010; Smiley *et al.*, 1996a). On the other hand, the plant debris stimulates antagonistic microorganisms and also the production of fungitoxic volatile compounds during decomposition which reduces the severity of the disease (Bateman *et al.*, 2007; Sumerell *et al.*, 1989). Although ploughing is known to reduce soil-borne diseases by burying the crop debris and therefore reducing the inoculum source on the soil surface, it has been reported to increase the disease severity of foot rot. Inoculum sources, which have been buried in the soil, can survive up to one year and are returned to the surface when ploughed up again (Bateman *et al.*, 2007).

In some cases, additional stubble management is performed in which the stubbles are either retained or removed by burning or slashing at ground level. The removal of the stubbles can reduce the foot rot disease index even under no tillage practices (Bateman *et al.*, 2007; Paulitz *et al.*, 2010; Wildermuth *et al.*, 1997). In the study of Bateman *et al.* (2007), the pathogenic activity of *F. graminearum* on wheat stems was suppressed by adding maize stalk residues to the fields. Maize stalk residues enhanced the saprophytic activity of *F. graminearum* and *F. culmorum*, and also lead to higher levels of mycotoxins in the cereal grains. However, these results varied in a certain range in the assessed years due to the highly influential environmental conditions. Since reduced tillage reduces soil erosion risk, the choice of tillage practice and stubble management is in some areas, such as Australia, relatively essential (Wildermuth *et al.*, 1997). In addition, the timing of the sowing seems to influence the severity

of foot rot. A delayed planting date decreased the incidence of foot rot together with the availability of water, since water stress of the plants enhances the disease (Paulitz *et al.*, 2002). As the inoculum of the pathogen causing fusarium foot rot partly over winters and survives in susceptible host plant tissues, crop rotation is a useful tool in suppressing the disease severity (Smiley *et al.,* 1996a). According to Smiley *et al.* (1996b), foot rot severity was directly related to soil acidity which rises through the increasing use of nitrogen fertilizer, amount of surface residues and the frequency of wheat in the crop rotation. A rotation cycle with a non-host plant, such as legume between corn and small grain crops, provides sufficient time for the residues to decompose and the *Fusarium* population to decline (Wagacha & Muthomi, 2007).

MATERIAL & METHODS

1. Soil samples, *Fusarium* isolates and wheat seeds

A field experiment at Kungsängen (Uppsala, Sweden) was chosen to study effects of conventional and reduced tillage and different preceding crops on the suppressiveness to fusarium foot rot. The field was arranged in a strip plot design with 3 replicates and a plot size of 6 x 12m. The soil type was clay (48% clay, 29,7% silt, 21,5% sand) with an organic matter content of 3,2%. As preceding crops, wheat (*Triticium aestivum*, cv. Olivin), oat (*Avena sativa*, cv. Belinda) and spring oilseed rape (*Brassica napus spp. napus*, cv. Larissa) was used. The soil was sampled in November 2009 by systematic sampling across the plot. The samples were collected from the upper 10cm at a minimum of 10 different sampling points per pot and pooled together plot-wise. The soil samples were stored at 4℃ and dry sieved (5mm) before use.

Four *Fusarium* isolates, *Fusarium graminearum* (isolate evp11), *Fusarium pseudograminearum* (evp16), *Fusarium culmorum* (evp5) and *Fusarium culmorum* (evp8) were obtained from the culture collection at the Department of Crop Production Ecology (SLU). The cultures had been stored on sterile soil at 4℃. For the experiment, the cultures were transfer red to Potato-Dextrose agar (½ dose, 19,5g PDA*L^{-1} and 7,5g agar*L^{-1}) and kept at room temperature (approx. 23℃).

The wheat seeds used in the experiment were winter wheat (*T. aestivum*, cv. Olivin), that was untreated and stored at 4℃.

2. Pathogenicity of *Fusarium* isolates

In order to test the pathogenicity of the *Fusarium* isolates used, surface sterilized wheat seeds were put on moist filter paper in a with parafilm sealed Petri dish (Ø 9cm). After germination of the seeds, an agar plug (Ø 5mm) with fungus was placed beside the germinating seed. The Petri dish was sealed with parafilm again and incubated at room temperature (approx. 23℃) for 2 weeks. The disease symptoms were assessed under a stereomicroscope (10x).

3. Bioassay to assess suppressiveness

Sources and preparation of inoculum. Barely grains were soaked in water over night and sterilized for 3 consecutive days (100ml barely seeds per 500ml Schottflask). Agar plugs (Ø 5mm) with different *Fusarium* isolates which had been growing for approximately 5 weeks were added to the sterile kernels in the flasks. The flasks were kept at room temperature (approx. 23℃) for 14 days and were shaken every second day to mix the fungal inoculum with the kernels. The contents of the flasks were dried over night in a laminar flow bench and ground with a blender.

The bioassay system. The bioassay was modified from Wilkinson *et al.* (1985). A 96 well plate with a volume of 75cm^3 per well was used. The hole in the bottom of each well was covered with paper and 30mL vermiculite was placed into each well. 15mL test soil was mixed with the appropriate inoculum in a 50mL Falcon tube. The inoculum concentrations were 0%, 1% (0,15g), 3% (0,45g) and 6% (0,9g). The concentration of 0% is also described as non-infested treatment (NI), no inoculum is added to the soil. In each well one wheat seed was placed and covered with 7,5mL calcined clay (Ikasorb® 1030). Each replicate-concentration combination was represented by 8 plants. In order to assure successful germination of the seeds, only healthy looking wheat seeds were chosen. The seed germination in soil was tested before the experiment and was found to be 97% (H. Friberg, pers. comm.). The trays were kept in a growth chamber for 5 weeks at 15℃ with a photoperiod of 16h and a light intensity of 200 µmol*m^{-2}*s^{-1}. The relative humidity of the air was kept at 70%. The plants were watered with deionised water containing 2mL*L^{-}1 complete fertilizer (Wallco(51-10-43+mikro)) every second day. Non germinated seeds were taken up after 2 weeks and a subset of them incubated on Potato-Dextrose agar with antibiotics (Streptomycine 100mg*L^{-1}, Chlorotetracycline 50mg*L^{-1}) after surface sterilization, in order to check for outgrowth of *Fusarium* spp. Disease symptoms were assessed 5 weeks after sowing, using a scale of six disease classes modified from Malalasekera and Colhoun (1968) and Knudsen *et al.* (1999) (Fig. 2). 0: healthy plant, 1: slight discolorations and lesions on stem base and roots, 2: moderate discoloration and lesions on stem base and roots, 3: severe discoloration and lesions on stem base and roots and plant visibly stunted, 4: plant emerged with heavily retarded growth and died within the 5 weeks of growth, 5: pre-emergence plant death.

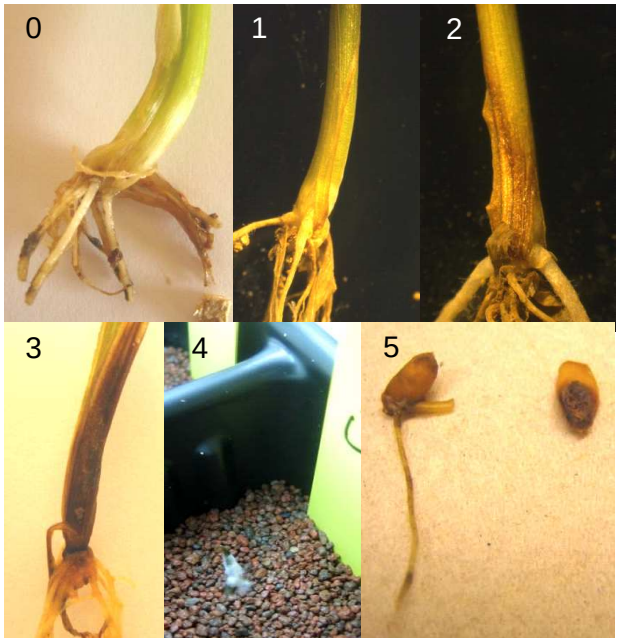

Figure 2. Disease scoring of wheat plants showing symptoms of fusarium foot rot. Step 0-5.

Plant shoot dry weights were taken after drying the shoot parts over night at 100℃. The means were calculated for the 8 test plants of each treatment. This is the disease index used. The standard deviation was formed from the mean values of the three soil replicates.

In order to establish the bioassay system, the first bioassay was carried out with the three *Fusarium* species (*F. graminearum*, *F. culmorum* and *F. pseudograminearum*) in four inoculum concentrations (0%, 1%, 3%, 6%) and the soil with both tillage treatments (conventional tillage, reduced tillage) and wheat as a preceding crop was used. In the second run only two *Fusarium* isolates (*F. graminearum*, *F. culmorum*) were used with three concentrations (0%, 1%, 3%) and soil from three preceding crops (wheat, oat, oilseed rape) were included. As control of the pathogenicity of the inoculum for the wheat plants, samples with heat sterilized soil were included. For each inoculum level 8 plants were sown.

4. Fungistasis test

The fungistasis test was modified from Wacker and Lockwood (1991). Macroconidia were produced by adding small agar plugs (Ø 5mm) to 100mL CMC (carboxymethylcellulose) medium (Wacker & Lockwood, 1991) in a 500mL Erlenmeyer flask. The flaks were incubated for 2 weeks at room temperature (approx. 23℃) on a shaker. Afterwards, the spore suspensions were washed by centrifugation at 8500 rpm for 20min. The supernatant was replaced by 5mL cold Pfeiffer's salt solution (Bristow & Lockwood, 1975). After resuspension, 1mL was taken out into a 2mL Eppendorf tube. The suspensions were washed 2 additional times by centrifugation at 12000g for 20min, replacement of the supernatant with cold Pfeiffer's salt solution and resuspension. Finally, the spores were resuspended in sterile water and kept on ice. The spore concentration was adjusted to $2x10^4$ spores per mL. A 20g soil samples was placed in a Petri dish (Ø 9cm) and the moisture content was adjusted to 450mg water*g^{-1} dry soil. The conidia were placed on membrane filters (Ø 50mm) with a pore diameter of 0,45µm by adding 0,5mL of spore suspension on the filter followed by a 15s vacuum filtration. The filters were placed on the soil and incubated over night at room temperature (approx. 23℃). One cm^2 pieces of the filters were cut out and stained with 0,05% toluidine blue as described by Knudsen *et al.* (1999). A total of 100 conidia were counted, using a microscope (100x magnification). Spores were counted as germinated, when the germ tube was at least as long as the conidia were wide. From each soil sample triplicates were prepared and as control heat sterilized soil was used. The mean values were formed from the triplicates for further use in the statistical analysis.

5. Hyphal growth test

The hyphal growth test was designed after the test for suppression of mycelial growth of *Rhizoctonia solani* (Ghini & Morandi, 2006). A thin layer of chilled water agar was placed on a 20g soil sample in a Petri dish (Ø 9cm). The moisture of

the soil was adjusted to 450mg water*g^{-1} dry soil. An agar plug (Ø 5mm) with fungal isolate studied was placed in the middle when the water agar had cooled down and solidified. The dishes were sealed with parafilm and kept at room temperature (approx. 23℃) in the dark for approxim ately one week. As control, heat sterilized soil was used. For each soil samples triplicates were prepared. The diameter of the *Fusarium* colonies were measured every second day using a stereomicroscope (10x). An Area under Growth-Curve was formed after plotting the growth of the colonies against time.

6. Statistical analysis

The software used for the statistical analysis was MINITAP Version 15 (Minitap Ltd.). The calculated mean values of each experiment were tested for normal distribution with the Anderson-Darling Normality test. Since all data turned out to be normally distributed, no further transformation was carried out. A General Linear Model (GLM) - analysis was used for identifying the significant explanatory factors. For the first bioassay, a GLM was carried out for each *Fusarium* isolate and a One-way ANOVA - analysis was done for each inoculum level separately. For the second bioassay, an additional GLM was performed for each preceding crop.

RESULTS

1. Pathogenicity

In the pathogenicity test of the four isolates of three different *Fusarium* species, the seedlings infested with *F. pseudograminearum* evp16 where overgrown by mycelium and showed the most severe disease symptoms such as retarded growth and browning of roots and coleoptiles (Fig. 3a). *F. graminearum* evp11 also caused brown discolouration on the roots and the coleoptiles, but to a lesser extent (Fig. 3b). The seedlings infested with *F. culmorum* evp5 showed hardly any symptoms (Fig. 3c); simply slight brown spots were recognizable on the roots and on the base of the plant shoot. Hence, *F. culmorum* evp8 was chosen over *F. culmorum* evp5 for the bioassays, since the seedlings showed easier distinguishable brown lesions on the coleoptiles (Fig 3d).

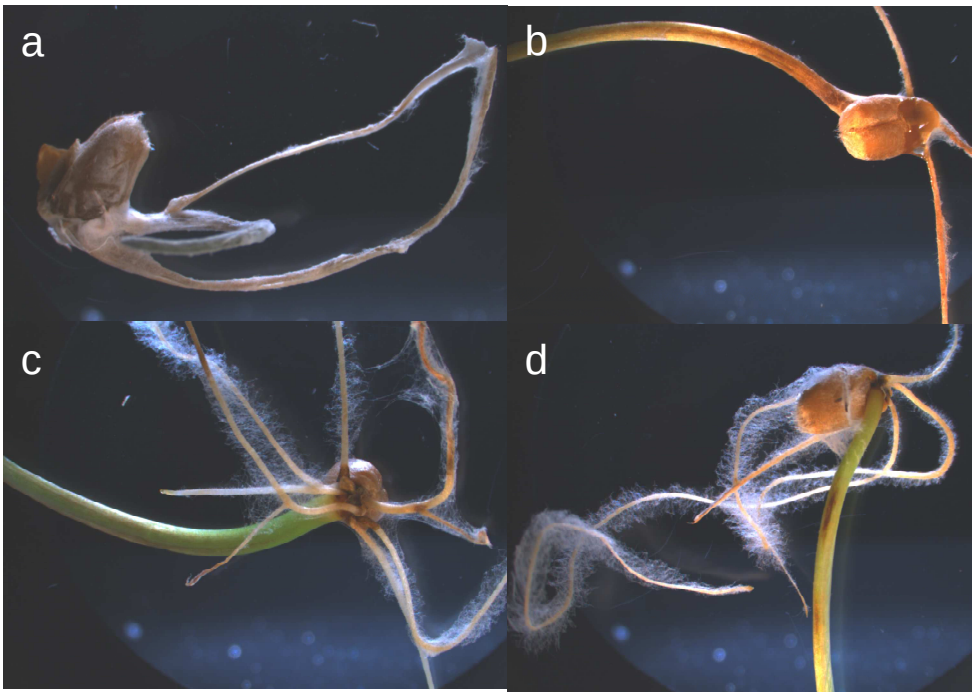

Figure 3. Disease symptoms on wheat seedlings caused by different Fusarium isolates. Symptoms caused by a) *F. pseudograminearum* evp16 b) *F. graminearum* evp11 c) *F. culmorum* evp 5. d) *F. culmorum* evp 8.

2. Bioassay

In the first bioassay, which was used to establish the bioassay system, the *Fusarium* species and the inoculum concentration were significant explainatory variables for the disease index. *F. culmorum* showed the highest disease severity and *F. pseudograminearum* the lowest. The disease severity increased with increasing inoculum concentration within each *Fusarium* species, especially

visible in the sterilized soil (Fig. 4a). For tillage, no significant effects were found for the disease index, even after assessing each *Fusarium* species separately. Although in some cases, such as the samples with 3% *F. graminearum*, a tendency was noticed, the variation remained too high and therefore no difference could be found (Fig. 4b). *F. culmorum* behaved similar to *F. graminearum* without a significant difference in the disease severity between conventional and reduced tillage (Fig. 4c). The plants growing in *F. pseudograminearum* infested soil were either healthy or scored with pre-emergence plant death; this resulted in a high variance of the data (Fig. 4d). For the subsequent bioassay, *F. graminearum* and *F. culmorum* was used. Even though no significant difference was found for tillage, the two *Fusarium* species showed less variance in disease symptoms within the different treatment than *F. pseudograminearum*. Furthermore, *F. pseudograminearum* showed difficulties in the conidia production in the CMC-Medium which was needed for the fungistasis test. In the treatment with 6% inoculum level a high pre-emergence death rate was visible for all three *Fusarium* species, especially for *F. pseudograminearum*. This led to an exclusion of the 6% inoculum treatment for the following bioassay.

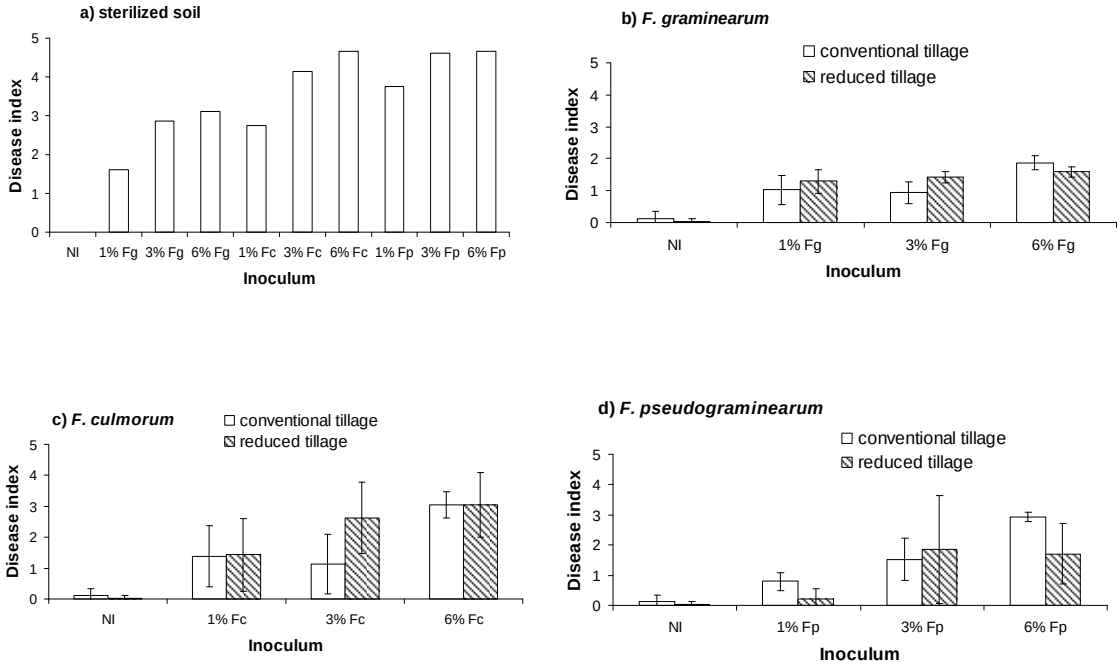

Figure 4. First bioassay. Disease index of wheat plants growing in soil infested with different levels (1%, 3%, 6%) of *F. graminearum* (Fg), *F. culmorum* (Fc), *F. pseudograminearum* (Fp) or in non-infested soil (NI), in sterilized soil (a; mean, *n=8*) and soil from the field experiment (b-d; mean±SD, *n=3*).

The shoot dry weight was significantly influenced by the *Fusarium* species and the inoculum concentration. With an increasing inoculum concentration the shoot dry weight decreased in all three species. The plants grown in *F. graminearum*

infested soil showed the highest shoot dry weights and the ones grown in *F. culmorum* the lowest. However, no difference was found between the two tillage treatments (Fig. 5).

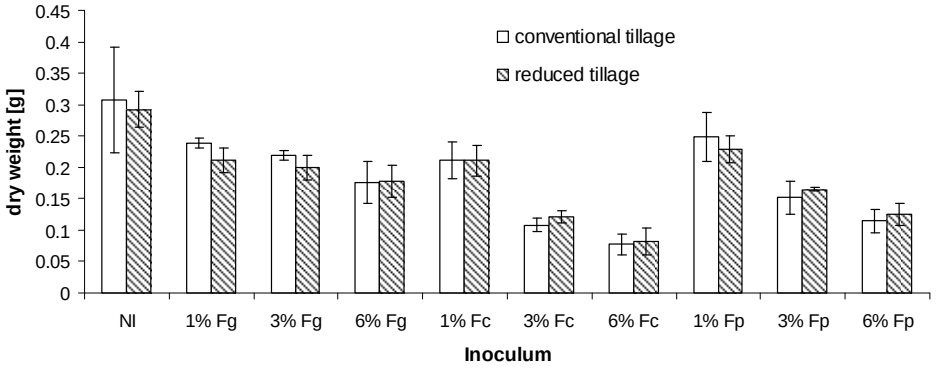

Figure 5. First bioassay. Shoot dry weight of wheat plants (mean±SD, *n=3*). In non-infested soil (NI) and soils infested at 1%, 3%, 6% with *F. graminearum* (Fg), *F. culmorum* (Fc) or *F. pseudograminearum* (Fp).

In the second bioassay, in which *Fusarium graminearum* and *Fusarium culmorum* were used in three concentrations (0%, 1%, 3%), the inoculum concentration and the preceding crop had a significant effect on the disease severity. The disease severity increased with increasing inoculum concentration. In the sterilized soil, the increasing inoculum of *F. graminearum* did not cause an increase in the disease severity (Fig 6a). However, in the case of *F. culmorum*, it increased with the inoculum. The soil samples with wheat as preceding crop showed a general higher disease severity compared to the ones with oilseed rape as preceding crop. Oat as preceding crop influenced the disease severity differently within the two *Fusarium* species. Disease symptoms caused by *F. graminearum* increased in their severity in soil samples with wheat as preceding crop as compared to the soil samples with oat as preceding crop. For the disease symptoms of *F. culmorum*, it was the opposite.

The soil without inoculum had hardly any diseased plants and no significant explanatory valuable for disease severity could be detected (Fig. 6b). For soils infested with *F. graminearum* and with wheat as preceding crop the tillage was a significant. The soil under reduced tillage had a reduced disease severity compared to the ones grown in conventional tillage (Fig. 6c, d). In the other circumstances tillage was not a significant factor.

The shoot dry weights were significantly influenced by the inoculum concentration. With an increasing concentration of the fungi, the shoot dry weight was reduced (Fig. 7). For wheat plants growing in *F. graminearum* infested soil, the preceding crop did not show a significant effect (Fig. 7a). For the samples infested with *F. culmorum*, an interaction between inoculum concentration and preceding crop was shown. The tillage did not show a significant effect, except for samples infested with 1% *F. culmorum* in which the shoot dry weight was significantly higher with reduced tillage (Fig. 7b). The shoot dry weight of the

plants growing on non-infested soil showed an average weight of 0,18g without any difference due to tillage treatment or preceding crop.

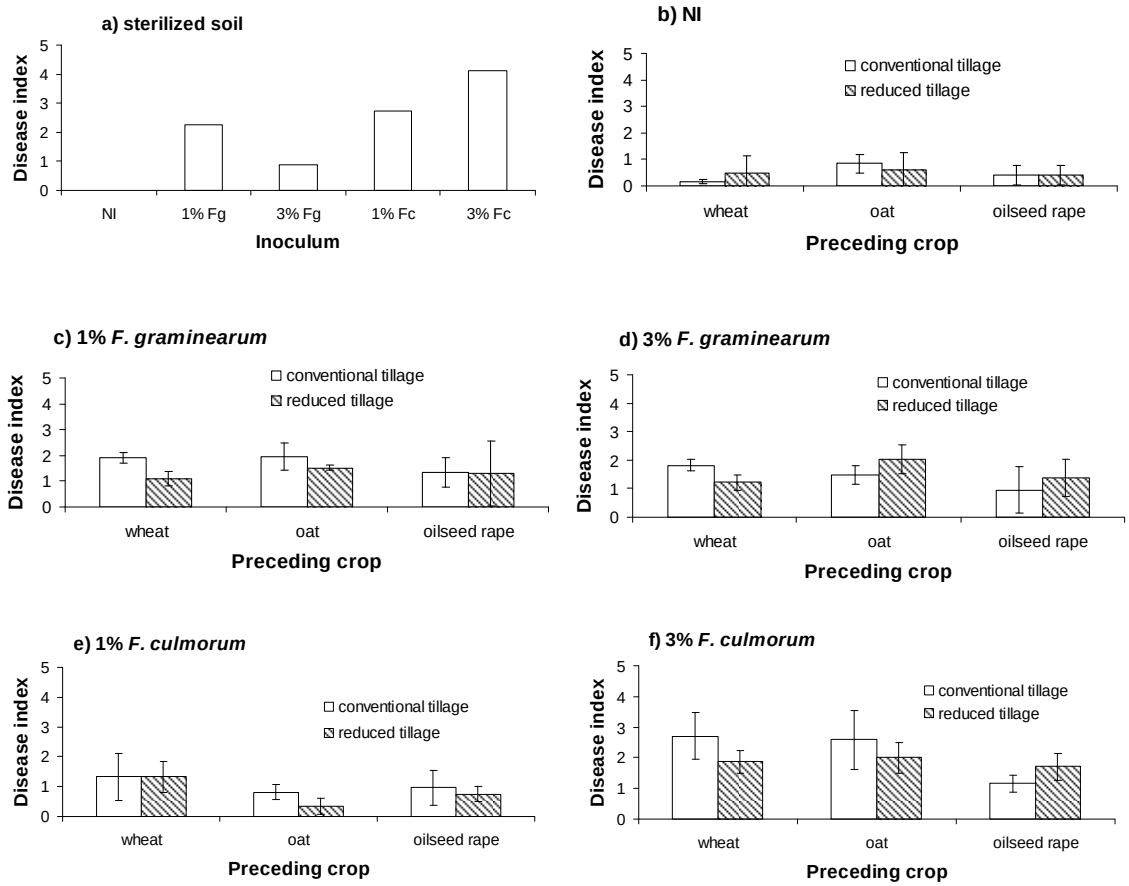

Figure 6. Second bioassay. Disease index of wheat plants growing in soil infested with different levels (1%, 3%) of *F. graminearum* (Fg), *F. culmorum* (Fc) or in non-infested soil (NI), in sterilized soil (a; mean, *n=8*) and soil from the field experiment with wheat, oat and oilseed rape as preceding crop (b-f; mean±SD, *n=3*).

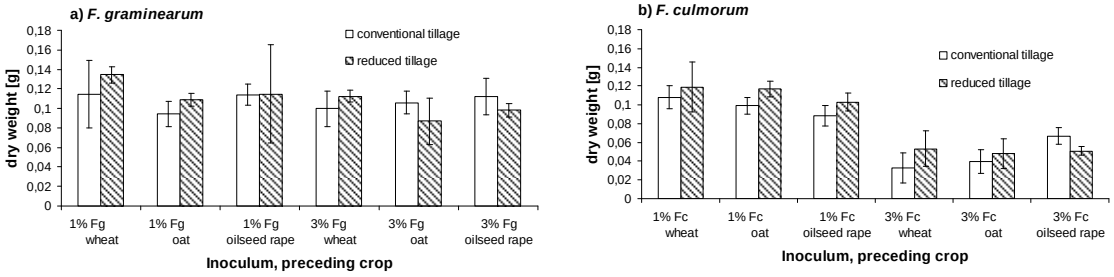

Figure 7. Second bioassay. Shoot dry weight of wheat plants (mean±SD, *n=3*) in soils with different preceding crops, infested at (a) 1%, 3% or 6% with *F. graminearum* (Fg) or (b) at 1%, 3% or 6% with *F. culmorum* (Fc).

3. Fungistasis test

The overall germination rate of the conidia differed significantly between the *Fusarium* species, the tillage and the preceding crop. For *Fusarium graminearum* the determining factor of the germination rate was the tillage treatment. Conidia placed on soil under conventional tillage had a higher germination rate than the conidia spores on reduced tillage. The preceding crops did not appear as influencing factor (Fig. 9a).

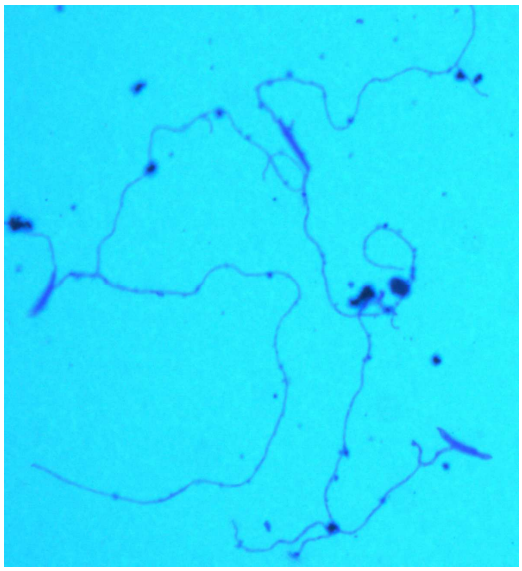

Figure 8. Germinated conidia on sterile soil.

The germination rate of the conidia of *F. culmorum* was influenced differently by the tillage among the three preceding crops. Spore germination was significantly suppressed in soil under conventional tillage and with wheat as preceding crop. However, the ones with oilseed rape as preceding crop had the opposite effect: the conventional tillage practices significantly suppressed the germination of the conidia spores. The soil which had oat as preceding crop did not show any significance in the germination rate between conventional tillage and reduced tillage treatment (Fig. 9b).

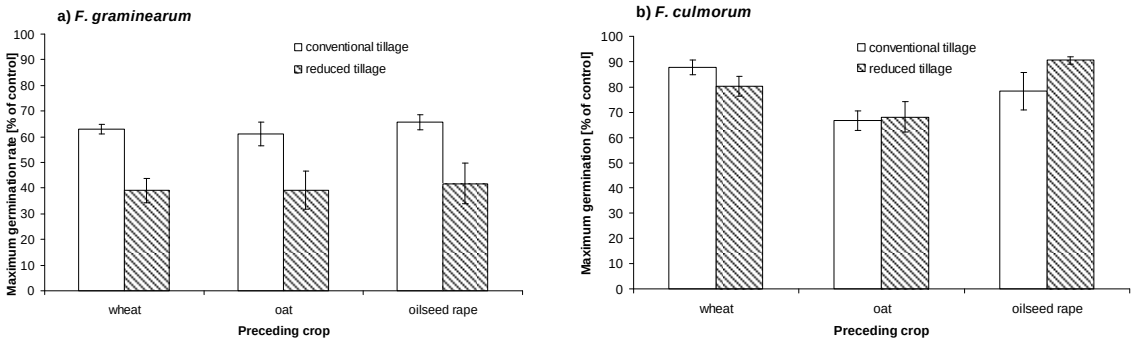

Figure 9. Germination rate of conidia spores of (a) *F. graminearum* and (b) *F. culmorum* incubated on soil samples over night (mean±SD, *n=3*).

4. Hyphal growth test

The growth of hyphae coming from the agar plug with fungi was very inconsistent. It was not possible to distinguish a well defined edge of the colonies (Fig. 10). Many samples did not show any hyphal growth. The controls with the

sterile soil were the only samples which showed an easily measured growth of the hyphae.

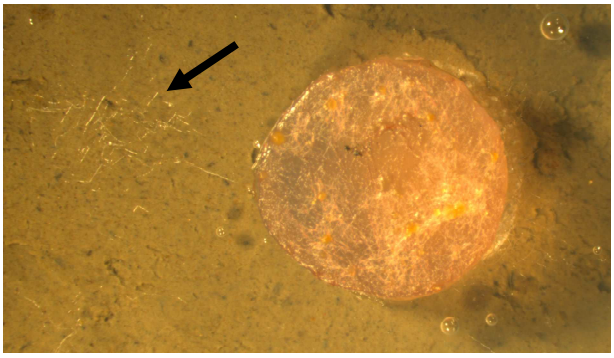

Figure 10. Inconsistent hyphal growth for *F. culmorum* on soil sample. (Size of agar plug Ø 5mm)

DISCUSSION

Assessing suppressiveness. The approach of assessing suppressiveness to *Fusarium* species through a combination of measurements of disease suppression and pathogen suppression, turned out to be valuable for evaluating the different soil treatments and their influence. Except the hyphal growth test, the experiments carried out showed no major difficulties in their set-up and their interpretation of the results. Cultural practices, which caused a reduced germination rate of the conidia in the fungistasis test, did not automatically also reduce the disease severity in the bioassays. The suppression of the germination rate does not necessarily imply suppression of disease in a host plant. Suppression of a pathogen can also take place through inhibition of the hyphal growth, instead of reduction of germination rate of the spores (Knudsen *et al.*, 1999). Through the fungistasis test the pathogen suppression capability of the soil can be evaluated and through the bioassays the disease suppressiveness is assessed. Disease suppressive soils can obtain a high level of pathogen without any or limited disease and pathogen suppressive soils are able to suppress the pathogen but not necessarily the disease (Höper & Alabouvette, 1995). The hyphal growth test would have been an additional assessment of the pathogen suppressiveness. The system of the test was taken from an approach of assessing suppression to *Rhizoctonia solani* (Ghini & Morandi, 2006), but *Fusarium* spp. grew poorly on water agar. The saprophytic growth of *Fusarium* spp. turned out to be severely suppressed in all soils which lead to the inconsistent growth of the hyphae. The fact that the control with sterile soil showed consistent and distinguishable hyphal growth can be partly explained by the process of sterilization. The sterilization took place through heat treatment (autoclavation) which leads to a degradation of soil nutrients into smaller and easier accessible nutrients and also to a liberation of nutrients in organisms which are eradicated. It is likely that the effect of the easier accessible nutrients influenced the growth of the fungi in the control. The effect of the heat sterilization and also the irregular growth of the fungus on the water agar made the experiment of the hyphal growth impossible to use for evaluating the suppressiveness of the soils. In the bioassays and the fungistasis test, the effect of the heat sterilization of the control soil seemed to not influence the hyphal growth in a way that it might have influenced the results. The control experiments with the sterile soil resulted in a stimulation of the pathogen activity in which the fungal growth was the maximum and the disease severity was the highest compared to the other soil samples. In the bioassay, an increasing inoculum concentration led to an increasing disease severity and a decreasing shoot dry weight. This shows that there was a dose response relationship within the different inoculum concentrations studied.

The results of the bioassay revealed the ecological difference of the three *Fusarium* species, but also the difference of the used *Fusarium* species isolates. Wheat plants in *F. graminearum* and *F. culmorum* infested soil appeared with disease symptoms from all steps of the disease index scale, whereas *F. pseudograminearum* hardly gave any other symptoms than pre-emergence

dying. Furthermore, the three *Fusarium* species reacted differently towards the cultural practices. One difficulty with the bioassays was the high variation in the data in the final disease evaluation. Reasons for this variation are, in addition to the inhomogeneity of the soil as a natural system, the subjectivity of the disease evaluation. In order to secure an objective evaluation and an unbiased evaluation, molecular biological methods, such as PCR- based assays, could be used in the future. Hence, the actual fungus which is responsible for the disease symptoms can be identified. Since the correlation between disease severity and number of copies per gram of plant is not known, the variation of the disease index could also be reduced through an increase of the sample size or a different wheat cultivar with a higher susceptibility. The variance in the fungistasis test was relatively low compared to the variance of the bioassay. The bioassay is more vulnerable towards influencing factors since it is an experiment over 5 weeks with a higher complexity.

The tillage treatment. As stated in the hypothesis the tillage treatment showed to have impact on the suppressiveness. In the fungistasis test, the germination rate of *F. graminearum* conidia was higher in soil under conventional tillage than in soil under reduced tillage. This was also supported by the second bioassay in which the suppressiveness to fusarium foot rot increased in soil under reduced tillage and with wheat as preceding crop. The plant residues left on the field after reduced tillage increase the organic matter in the soil and serve as habitat for microorganisms which compete for the same energy source as the fungi and additionally may excrete fungitoxic compounds (Knudsen *et al.*, 1999). Therefore, the suppressiveness of the soil to fusarium foot rot indicated to be connected with the microbial biomass and activity which is higher under reduced tillage than under conventional tillage. By contrast, studies made in the UK showed that minimum tillage increased the risk of foot rot caused by increasing *F. graminearum*, but the final severity of the disease strongly depends on environmental conditions such as weather, inoculum accumulation and mycotoxin production (Bateman *et al.*, 2007). In other recent studies, reduced tillage was also associated with a higher disease incidence since the plant residues left on the field under reduced tillage serve as additional inoculum source an increase the fungal inoculum (Wildermuth *et al.*, 1997).

In this study, for *F. culmorum*, the expected impact of tillage on suppressiveness could not be detected. There was no significant difference in disease severity of the wheat plants between the treatment with conventional tillage and that with reduced tillage. This could indicate that the two different *Fusarium* species act differently under conventional and reduced tillage. In the fungistasis test of *F. culmorum*, a difference could be detected between the germination rate of the conidia on soil under conventional and reduced tillage with wheat as preceding crop. Conidia on soil under reduced tillage showed a reduced germination. This result confirms the two concepts of disease suppressive and pathogen suppressive soil in which suppressiveness to the germination of the conidia does not necessarily include a reduction of disease severity. In the soil with oilseed rape as preceding crop, the tillage influenced the

conidial germination differently. The germination rate in the soil under reduced tillage was significantly higher than the germination of spores in conventional tillage. This result seems does not support the hypothesis, since oilseed rape is a non-host for *Fusarium* species and therefore especially under reduced tillage, where the plant residues are left on the field, the pathogen should be suppressed (Kirkegaard *et al.*, 2004).

The preceding crops. The preceding crop turned out to be a significant explanatory variable for the disease in the bioassay, whereas, in the fungistasis test the effect of preceding crop was just significant for *F. culmorum* in an interaction with tillage as mentioned above.

The effect of a non-host or a host as preceding crops affects the growth stages of the fungi through *e.g.* creating a habitat for antagonistic microorganisms which compete for the same nutrient sources or which may excrete fungitoxic compounds. Wheat is a common host plant for *Fusarium* spp. and highly susceptible to foot rot. As expected in the hypothesis, the disease incidence of the wheat plants grown on soil with wheat as preceding crop was increased compared to the ones growing on soil with crop rotations. Wheat as preceding crop decreased the soil suppressiveness to fusarium foot rot. A continuous cropping of susceptible cereal plants leads to a selection of the pathogen which is damaging for the host plant. Especially in a soil under reduced tillage this leads to an increased inoculum density (Vilich, 1993). A high inoculum density might lead to an increased disease severity depending also on other factors *e.g.* environmental conditions. In this study no *Fusarium* spp. could be detected in the non-infested soil as background value, but it might still have influenced the inoculated treatments, resulting in increased disease severity in the soil with wheat as preceding crop. Through a crop rotation and inclusion of a break-crop which is a proven non-host of the pathogen, the disease severity can be suppressed through decreased inoculum density (Knudsen *et al.*, 1999; Smiley *et al.*, 1996a). In the study of Kirkegaard *et al.* (2004), a significant reduction of foot rot was achieved by a crop rotation with non-host break crops compared to cereal hosts. Brassica crops such as the oilseed rape are considered to be a non-host and therefore decreases the inoculum density which results in a lower disease incidence in the bioassays. Cereal residues decompose faster and therefore reduce the possibilities for inoculum under brassica crops due to a greater amount of persistent stubbles which increase the moist soil surface (Kirkegaard *et al.*, 2004). In addition, the brassica crops induce a change of the soil biota which may results in increased pathogen suppression through *e.g.* antagonist *Trichoderma* spp. (Kirkegaard *et al.*, 2004). The in the hypothesis stated increase of suppressiveness by a crop rotation with oilseed rape, was detected in the bioassay. The soil with oilseed rape as preceding crop showed reduced disease severity. The crop rotation with oat, however, turned out to be inconclusive. According to Sturz and Bernier (1989), oat stubbles have been reported to increase the inoculum of *F. culmorum* and winter wheat growing in a rotation with oat, resulting in high disease occurrence. However, the disease

severity in the bioassay decreases for *F. culmorum* compared to the soil with wheat as preceding crop and increase for *F. graminearum*.

The preceding crop as a factor did not influenced the shoot dry weight, although, according to Sturz and Bernier (1989), crop rotations with brassica crops have shown to result in a higher winter wheat survival and in a greater plant height. Crop rotations increase the yield through increasing the soil organic matter and improving the soil structure (Kirkegaard *et al.*, 2008). In general, not much is known about the effects of different crops *e.g.* in the crop rotation on the suppressiveness itself, when is comes to the species of *Fusarium*. For fusarium wilt on water melon caused by *F. oxysporum f. sp. melons*, it has been shown that monoculture with water melon for several years induced suppressiveness without reducing pathogen growth or inhibition of chlamydospore germination by induced systematic resistance by the plants and a non-pathogenic strain of *Fusarium* spp. (Weller et al., 2002).

CONCLUSION

Since disease suppression does not necessarily include pathogen suppression, it is necessary to measure the pathogen suppression separately from the disease suppression for a full assessment of the suppressiveness of soil. The fungistasis test and the bioassay set-up were appropriate for assessment of suppressiveness to *F. graminearum* and *F. culmorum* and to foot rot of wheat caused by theses two species. Inoculum concentrations of 1% and 3% were sufficient to cause disease in a broad scale of symptoms.

The effect of tillage varied with the pathogen studied and with the preceding crop. For *F. graminearum*, the expected difference between conventional and reduced tillage could be proven. Reduced tillage treatment caused a reduced germination rate of the conidia and increased disease suppressiveness in the bioassay. However, the increased suppressiveness could only be detected for the soil with wheat as preceding crop. This leads to the conclusion that reduced treatment increases the suppressiveness of the soil to *F. graminearum*, possibly by increasing organic matter content and favouring conditions for competing organisms. For *F. culmorum* this effect could not be proven in the bioassay. For the fungistasis test, however, the soil under reduced tillage and with wheat as preceding crop showed reduced germination rate. The effects of the preceding crops turned out to be significant, the estimated increase in suppressiveness in the soil with crop rotation occurred. Oilseed rape as preceding crop increased the disease suppressiveness to foot rot caused by *F. graminearum* and *F. culmorum*.

For future studies, the bioassay and the fungistasis test are a useful method to assess soil suppressiveness to foot rot caused by *F. graminearum* and *F. culmorum*, whereas, it is advisable to choose a big enough scale in order to avoid high variances. The results could be used as indication for further studies on the effect of cultural practices on the suppressiveness. Furthermore, also other aspects could be included such as different wheat cultivars or different environmental conditions *e.g.* temperature.

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