Swedish University of Agricultural Sciences
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Influence of Cover Crops on the Development of some Soil-borne Plant Pathogens

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May 2009
Degree project, D-level. 30 ECTS
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Course Code: EX0438
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Uppsala, 2009

Abstract

The suppressive effects of different cover crops on soil-borne plant pathogens and the diseases caused by these, were investigated in greenhouse trials. Four different cover crops and three soil borne pathogens were studied as model organisms. The cover crops were: oilseed radish (*Raphanus sativus)*, mustard (*Sinapsis alba),* rye (*Secale cereale)* and Westerwold ryegrass (*Lolium multiflorum)*. The pathogens were: *Sclerotinia sclerotiorum, Fusarium culmorum* and *Rhizoctonia solani*.

The study was carried out in 84 boxes in the greenhouse, with 28 boxes per pathogen with four replicates. The cover crops were grown in pathogen inoculated soil for eight weeks, and then cut, chopped and incorporated into the soil. Potencial pathogen inhibition was analysed by recording apothecia on the soil surface for *Sclerotinia* and by using bio-tests in barley for *Fusarium* and potato for *Rhizoctonia*.

Apothecia analyses showed a delay of ten or more days in *S. sclerotiorum* apothecia formation with ryegrass as the cover crop, whereas no effect was observed for the other three crops. This suggests that ryegrass can be a useful tool in the management of *S. sclerotiorum. Sclerotinia* infections are significant in oil-seed rape, which is particularly susceptible in the flowering stage but not later. If apothecia release can be delayed by one week or more, the susceptible stage of the plant may have passed and oil-seed rape infection can thus be avoided.

None of the tested cover crops suppressed disease development of *Fusarium* and *Rhizoctonia* in the bio-tests.

Overall, I conclude that ryegrass can be use against *S. sclerotiorum* to delay the apothecia formation. However, there was no evidence that any of the other tested cover crops species can be used to control soil-borne pathogens. More studies are required to explain the mechanisms delaying apothecia formation in that particular case and how they can be exploited for the purposes of crop protection.

Keywords: Cover crop, soil-borne pathogens, *Sclerotinia sclerotiorum, Fusarium culmorum, Rhizoctonia solani*, suppression.

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1. Introduction

Soil-borne plant diseases can severely limit plant production. Soil-borne pathogens have traditionally been controlled by the use of pesticides, among which methyl bromide has been the most widely used. Restrictions on the use of these pesticides due to the damage they cause in the environment, has prompted a search for new plant protection methods. The use of plant material from several species within the family Brassicaceae is potentially a very interesting alternative way to fight these soil-borne plant diseases. Among these brassica species, yellow mustard (*Sinapis alba*), oilseed radish (*Raphanus sativus* ssp. *oleifera*) and what is called Caliente or Indian mustard (*Brassica juncea*) have been the focus of interest and recent studies have shown that biomass or seed meal from brassicas has a suppressive effect on some soil pathogens. Fewer studies have been conducted on the use of non-brassicas as possible suppressors of soil plant pathogens, but commonly grown species within the Graminaceae such as rye (*Secale* sp.) or ryegrass (*Lolium* sp.) are possibly very interesting suppressors of plant pathogens surviving in the soil.

Species from both the Brassicaceae and Graminaceae are used as cover crops to reduce nitrogen leaching from agricultural land. In Sweden, state subsidies are available for growing cover crops in autumn after harvest of potato and cereals. The farmer can get this subsidy for growing cover crops as catch crops if they are sown before 15 August and ploughed under at the earliest two months later.

The suppressive mechanism of these plants on pathogens is being studied but is still not clear. For the Brassicaceae species, the main hypothesis is that the suppressive effect is the result of transformation of glucosinolate (GSL) into isothiocyanate (ITC), a compound that is toxic to a wide range of organisms including nematodes, bacteria and fungi. Another reason for this suppressive effect could be a change in the structure of the soil microflora. In the case of non-brassicas, the mechanisms of suppression are still more unclear but the release of allelochemicals from these plants seems to be the most likely reason for this effect.

The main objective of the present study was to evaluate the disease-suppressive effect of brassica and non-brassica cover crops of interest in Swedish agriculture. Two species were chosen from the Brassicaceae (oilseed radish, which has high GSL content, and mustard, which has low GSL content) and compared with two non-brassicas (rye and Westerwoldian ryegrass). The three soil-borne pathogens chosen as model organisms (*Sclerotinia sclerotiorum, Fusarium culmorum* and *Rhizoctonia solani*) are responsible for economically important diseases in oilseed rape, cereals and potatoes, respectively.

The starting hypothesis for the investigation was that growing the biomass from four different cover crops (two brassicas and two non-brassicas), cutting and chopping it and immediately incorporating it would suppress three important soil-borne plant pathogens.

In the case of the brassica cover crops, the mechanism behind the suppression could be either direct toxicity through the transformation of glucosinolate into isothiocyanate or an indirect result of changes in the structure of the soil microflora.

In the case of the non-brassica crops it could be due to an allelopathic effect or to other mechanisms. However, these mechanisms of suppression were not examined in the present study.

2. Background

In recent years the international use of chemical pesticides to control soil-borne pests and diseases has become a problem for environmental reasons. Chemical pesticides contain toxic and volatile compounds that can damage the environment. This has prompted a search for new plant protection methods.

Broad-spectrum pesticides have been used by many crop producers for a long time to control soil-borne plant pathogens. An example is metam sodium or sodium N-methyldithiocarbamate, which has been used since the 1950s to control pathogenic soil-borne organisms in potato and other crops. Metam sodium in contact with water generates the compound methyl isothiocyanate, which is effective against nematodes, fungi, pathogens, insects and weeds. However, since 2005 this compound has been designed a class 1 ozone-depleting substance under the Montreal Protocol. Due to restrictions on the use of chemical pesticides, many producers are seeking biological alternatives. One possibility, of great interest today, is to obtain isothiocyanates (ITCs) in a natural way through the use of crops with high levels of glucosinolates (GSLs) or other plant-derived biologically active substances for the control of soilborne diseases. The pathogen suppressive potential of ITCs released from brassica residues has been generally recognised (reviewed by Matthisen & Kirkegaard, 2006; Kirkegaard & Gimsing, 2008).

Kirkegaard *et al*. (1993) were among the first to study the ITCs produced from GSL-containing brassica species and referred to this type of pest control as 'biofumigation' . They defined biofumigation as '*the suppression of soil pests and diseases resulting from volatile hydrolysis products, principally ITCs, released in the soil after incorporation of glucosinolate-containing plant tissues*'.

Matthisen & Kirkegaard (2006) presented a review on the potential of biofumigation in soilborne pest and disease management in which they pointed out the importance but also the complexity of the mechanisms of suppression in the system. GSLs are secondary metabolites produced by the Brassicaceae and other plants from the order Capparales. GSLs are produced together with the enzyme myrosinase, which in normal conditions is separated from GSLs but, when plant tissues are disrupted, comes into contact with the GSLs and in the presence of water forms a variety of hydrolysis products (Figure 1). One of these products is the isothiocyanates.

The ITCs can be incorporated into soil as fresh plant material (green manure), seed meals or dried plant material (Matthisen & Kirkegaard, 2006; Gimsing & Kirkegaard, 2009).

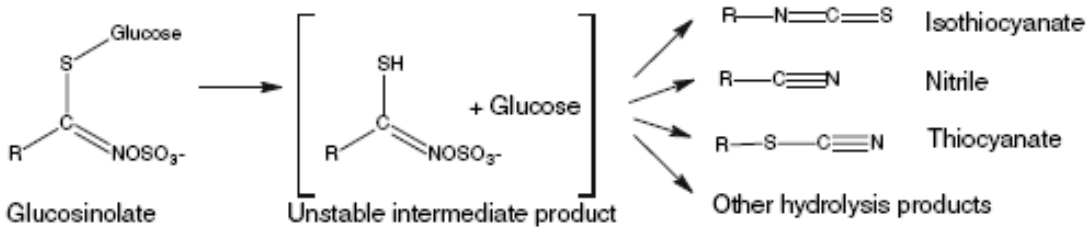

Figure 1. Hydrolysis of glucosinolates by the enzyme myrosinase. Isothiocyanate, nitrile and thiocyanate are the most common hydrolysis products.

Source: Gimsing & Kirkegaard, 2009.

To initiate the process of producing ITCs from GSLs by hydrolysis, the crop biomass must be chopped very thoroughly and quickly incorporated into the soil. The direct effect of the ITCs on the pathogens ends when the ITCs evaporate. The GSLs are contained in green plant parts and in the roots, and the maximum concentration occurs at early flowering (Plant Solutions Ltd, 2005).

One possibility to improve the biofumigation effect could be to choose varieties with a high GSL content and determine the conditions necessary to maximise the release of ITCs in soil in order to obtain the ITC concentration required to kill the soil pathogens. Among these conditions, addition of excess water and intense cellular disruption has been proposed.

Gimsing & Kirkegaard (2006) monitored the concentration of GSLs and ITCs in soil for several weeks after the incorporation of brassica biofumigants in a study in which high- and low-GSL varieties of rape (*B. napus*) and mustard (*B. juncea*) were pulverised and added to the soil. After incorporation, the soil was irrigated to investigate the importance of soil water content. The results showed that the highest levels of ITCs in soil were detected immediately after addition of high-GSL mustard into the moist soil. Several days after biomass incorporation, significant amounts of un-hydrolysed GSLs and ITCs were still detected and the addition of water to the soil after biomass incorporation did not enhance ITC release in soil. Overall, the study showed that only 30% of the ITCs potentially available in the original plant tissues were released under the conditions tested. The reason for this could be the unhydrolysed GSLs remaining in plant tissues (Gimsing & Kirkegaard, 2006).

It has also been shown that incorporation of brassica green manure and rapeseed meal, without or with very low GSL content, suppresses soil pathogenic fungi. This non-glucosinolate suppression can be due to the multiplication of antagonistic organisms in soil, to the release of toxic compounds which are not of GSL origin or to the triggering of processes in the main crop host plant, inducing disease resistance (reviewed by Matthisen & Kirkegaard, 2006; Gimsing & Kirkegaard, 2009).

Smolinska (2000) carried out a study on the use of cruciferous plant residues (*B. juncea* and *B. napus)* to reduce the concentration of the plant pathogenic fungi *Sclerotium cepivorum* and *Fusarium oxysporum* in soil. He observed that these residues (especially *B. juncea*) decreased the number of sclerotia of *S. cepivorum.* Furthermore, he showed that the amount of bacteria and fungi in soil one year later was greater in the soil with added plant material than in the control soil without plant residues. These changes in fungal and bacterial communities might explain the reduction of sclerotia, *e.g*. by possible parasitation of *S. cepivorum* by some organisms. Another explanation could be that the toxic compounds released during brassica decomposition may kill or weaken the pathogens.

In subsequent years, Smolinska *et al*. (2003) studied the effect of individual ITCs on *Fusarium oxysporum* in conifer seedling nursery soils. They used different ITC compounds, such as propenyl and ethyl isothiocyanates, and observed that these two compounds in particular had a large fungistatic effect. The conclusion was that brassica plants with GSLs that release high amount of propenyl isothiocyanate, such as *B. carinata, B. nigra* or *B. juncea*, can provide a solution for the control of *F. oxysporum* infections in nursery soils.

Mazzola *et al*. (2001) have carried out many studies in northern USA on disease suppression through different brassica amendments, including a study about the potential suppression of the apple root pathogens *Rhizoctonia* spp., *Pythium* spp. and the nematode *Pratylencus penetrans* by *B. napus* seed meal. Addition of the seed meal suppressed infection by *Rhizoctonia* spp. and *P. penetrans* but *Pythium* sp. infection increased at certain amounts of *B. napus*. High- and low-GSL cultivars were found to have a similar disease suppressive effect on *Rhizoctonia*, so it was concluded that there are other suppression mechanisms in addition to the ITC effect. One of the proposed mechanisms was that the nitric oxide (NO) from the N oxidation in the amendments stimulated certain plant defence pathways (Mazzola *et al*., 2001).

Cohen & Mazzola (2006) studied the effects of *B. napus* seed meal on the soil microbial community and compared them with those of added N in the suppression of *Rhizoctonia* root rot in apple trees. They found that *Streptomyces* spp. multiplied up to two-fold after addition of the rapeseed meal. Oxidation of N to NO in soil amendments may be one of the bacterial activities contributing to disease suppression, as *Streptomyces* spp. recovered from the apple roots produced a high amount of NO. The authors suggested that the high amount of bacterial NO producers found may play a major role in the plant defence against *R. solani*, since NO is known to stimulate certain plant defence pathways (Cohen *et al*., 2004; Cohen & Mazzola, 2006).

In a more recent study, Mazzola *et al.* (2007) investigated the potential of various brassica seed meals, of *B. napus, B. juncea* and *Sinapis alba*, on disease suppression. All three seed meals suppressed the development of *Rhizoctonia solani* apple root rot. When introduction of the pathogen was delayed 4 to 8 weeks after seed meal amendment, disease suppression was associated with proliferation of resident *Streptomyces* spp. and not the GSL content of the seed meal. For *B. juncea* (high-GSL) seed meal, *R. solani* suppression was associated with ITC production. *B. juncea* did not stimulate soil populations of *Pythium* sp.. Application of *B. napus* seed meal alone increased *Pythium* sp. but when it was added together with *B. juncea* no stimulation of *Pythium* sp. was observed. Only *B. juncea* seed meal suppressed soil populations of the nematode *Pratylencus penetrans*. A mixture of *B. napus* and *B .juncea* seed meal was

suggested for use, as this would give both the ITC and the soil bacteria NO-producing effect.

Larkin & Griffin (2007) performed a study on the control of soilborne potato diseases using brassica green manures with different levels of GSLs. They choose *B. juncea* with (high GSL content), *Raphanus sativus* and *Sinapis alba* (moderate GSL content) and *B. napus* (low GSL content) and evaluated their efficacy in the control of various soilborne potato pathogens. They also used non-brassica species (barley, oats and ryegrass) without GSLs. The most effective treatment in reducing disease caused by *Rhizoctonia solani* was *B. napus* green manure, while the high-GSL *B. juncea* was most effective against powdery scab (*Spongospora subterranea*) and common scab (*Streptomyces scabies*). Unexpectedly, inhibition of *Rhizoctonia* infection was observed for barley, which does not contain GSLs. The results indicate that disease reduction by brassicas is not always associated with high production of glucosinolates. Furthermore, the disease reduction brought about by non-brassica crops indicates that other mechanisms and interactions are important, particularly for control of *Rhizoctonia solani*.

Kasuya *et al.* (2006) carried out a study on the induction of soil suppressiveness to *Rhizoctonia solani* by incorporation of dried plant residues of *B. rapa* into soil. They used non-cruciferous plants such as clover and peanut as controls. Both *B. rapa* and non-GSL controls suppressed the incidence of damping-off caused by *R. solani*, but only *B. rapa subsp. rapifera* showed an inhibitory effect on mycelial growth of *R. solani* when the fungus was exposed to volatile substances produced from plant residues. When antibiotics were applied to the soil the disease suppression was reduced or nullified, so it was likely that soil bacteria were responsible for the decrease in the disease. The authors concluded that there was no evidence indicating that GSLs or ITCs were significant factors in soil suppressiveness. Furthermore, clover and peanut, without GSLs, generally suppressed the disease, so it is possible that there are another inhibitory volatile compounds released from crop residues.

The number of studies on interactions between plants and soil microbial communities has increased in recent years. Plants are the main factors determining soil microbial community structure and changes in crop management can have a high influence on soil microbial content and diversity. There appears to be a relationship between soil microbial diversity and soil health. It is known that natural ecosystems with high plant biodiversity have much fewer soil-borne plant disease epidemics than the often monoculture systems in agriculture. However, crop management practices such as varying the crop rotation, tillage or fertilisation can influence microbial communities involved in the suppression of soil-borne plant pathogens. With this in mind, it could be possible to increase soil suppressiveness through the use of appropriate agronomic practices (reviewed by Garbeva *et al*., 2006).

Garbeva *et al.* (2006) carried out a study based on the hypothesis that plant disease suppressiveness is influenced by microbial diversity present in the system, which is affected by the above-ground biodiversity. They investigated the evolution of soil microbial diversity under different agricultural regimes and observed higher levels of microbial diversity in treatments with higher aboveground biodiversity, such as permanent (species-rich) grassland and grassland newly planted with maize. There was also a positive correlation between *Rhizoctonia solani* suppression and microbial diversity. The authors concluded that appropriate agricultural management is critical for

the structure and diversity of the soil microbial community and consequently for soil suppressiveness.

Larkin (2008) evaluated various biological amendments (commercial biocontrol agents, microbial inoculants, mycorrhizae and aerobic compost tea) alone and combined with crop rotations. He studied the introduction into the soil of beneficial microorganisms from these amendments, and how they affected soil microbial community characteristics and soilborne potato diseases. Most of the amendments used altered microbial populations and activity. Some combinations of amendments and crop rotations increased crop yields through disease suppression, but the results showed that crop rotations were more effective than amendments in altering the characteristics of the soil microbial community.

Larkin (2008) also concluded that an appropriate crop rotation is important for the persistence of amendment effects in the soil.

Larkin & Honeycutt (2005) published a study about the effects of crop rotation systems on soil microbial communities. They confirmed that specific changes in the soil, especially those caused by crop rotations, can reduce pathogen activity due to three possible mechanisms: 1) by breaking the life cycle of the pathogen, 2) by changes in the soil characteristics that stimulate microbial activity, making the soil less appropriate for pathogen survival or 3) directly by inhibition of the pathogen through toxic substances released from plant residues or through stimulation of microbial antagonisms. In their study, Larkin & Honeycutt (2005) determined the effects of eight different cropping systems on soil microbial communities and *Rhizoctonia* diseases of potato. The results showed a greater concentration of fungi in barley rotations, an increase in mycorrhizal populations in maize rotations and the lowest amounts of microbial biomass and diversity in continuous potato controls.

With this study, the researchers demonstrated different effects on soil microbial communities due to specific rotation crops, and they began to evaluate the relationship between characteristics of the soil microbial community and plant health.

Some studies have been carried out on the effect of non-brassica species in the suppression of plant diseases. For example, there have been experiments with rye and ryegrass as suppressive agents of soil-borne plant diseases. Snapp *et al.* (2007) carried out a laboratory assay of some volatile compounds released from tissues of rye (*Secale cereale* L.) and Caliente mustard (*B. juncea*), and their inhibition of mycelium growth of two important potato diseases, *Rhizoctonia solani* and *Pythium ultimum*. After twenty-four hours, fungal growth was suppressed by all the compounds but 48 hours later, only the mustard residues suppressed hyphal growth. However, the authors concluded that although the suppressive effects of a rye cover crop were lower than in a mustard cover crop, rye seeds are cheaper and more resistant to the cold than mustard seeds.

Molisch (1937) defined allelopathy as '*the interaction between plants and other living organisms that is caused by specific chemicals (allelochemicals) released from plants*.'

Phenolic compounds are among these allelochemicals and they have a damaging effect on crop productivity when they appear in high concentrations in the soil. These

compounds can be found in winter rye (*Secale cereale*) and they are responsible for its phytotoxic activity (review by Wojcik-Wojtkowiak *et al.,* 1990).

The allelopathic effect of rye crops as weed suppressors has been widely studied. However, possible fungal suppression by rye cover crops has not been systematically investigated.

Wojcik-Wojtkowiak *et al.* (1990) carried out some model incubation experiments with rye seedlings, tillering plants and crop residues and found seven different phenolic acids present. Bioassays to determine the phytotoxicity level showed that this level did not correspond with the content of these acids or with the total phenolic content. The authors concluded that, in addition to phenolic acids, there must be another compound responsible for the allelopathic effects of rye. They also observed that young tissues produced high amounts of allelochemicals but crop residues did not exhibit any inhibition or toxicity.

Pathogens studied

Crop damage due to soil-borne plant pathogens is the most yield-limiting factor in the production of food, fibre and ornamental crops (Weller *et al*., 2002). In the present study the focus was on three important pathogens, *Sclerotinia sclerotiorum, Fusarium culmorum* and *Rhizoctonia solani*, which were studied as model soilborne pathogens in these experiments.

❖ ***Sclerotinia sclerotiorum***

Sclerotinia sclerotiorum can affect a wide range of crops, including many herbs, celery, carrot, lettuce, potatoes, and is especially severe in oilseed rape crops. The disease caused by this pathogen is known as Sclerotinia stem rot (http://www.hgca.com/publications/documents/ cropresearch/Topic77.pdf).

There are two phases in the infection cycle of *Sclerotinia*, soil-borne and airborne (Figure 2). The survival structures of *S. sclerotiorum* are called sclerotia and can survive in the soil for ten years or more. When they germinate, they produce fruiting bodies called apothecia of approximately 1 cm in diameter. Apothecia produce ascospores, which are transported by air, via flowers or by direct germination on leaves to cause infection in host plants. When a diseased plant is wilting, sclerotia are formed in the stems and after harvest the infected tissue with sclerotia is left on the soil surface or is incorporated into the soil through subsequent tillage.

The sclerotia need high moisture and a temperature between 15 and 25 ºC to germinate and release the spores. Then, when the plant has been infected, the disease is quickly spread at 15-20 ºC (http://www.hdc.org.uk/herbs/page.asp?id=22).

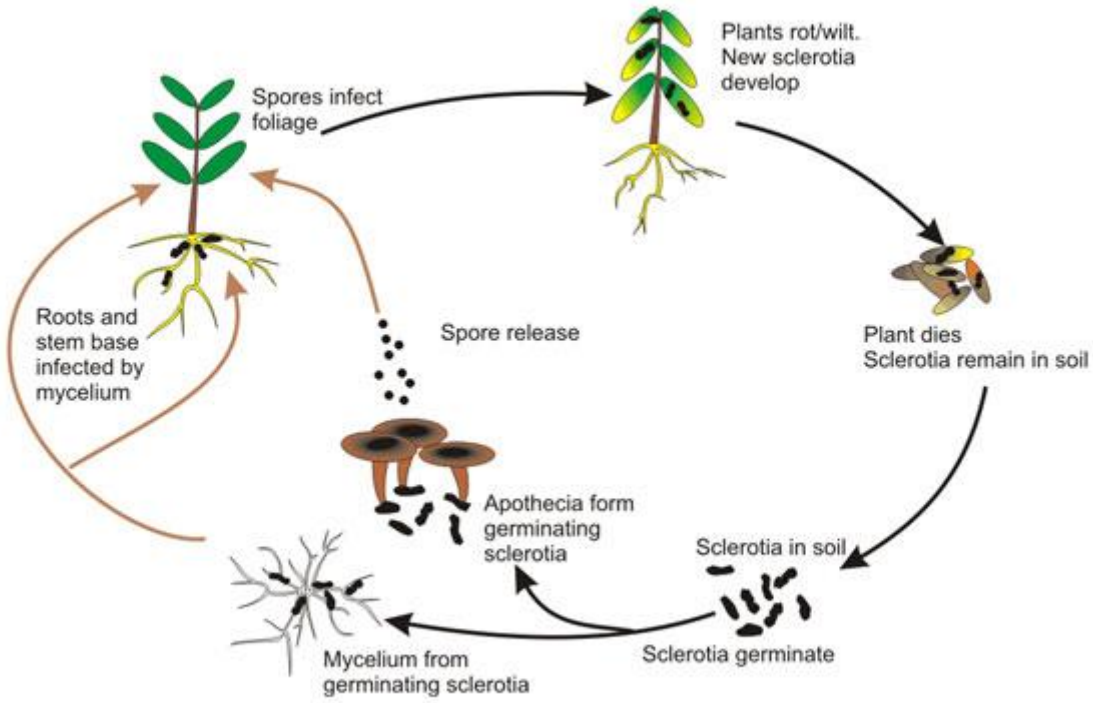

Figure 2. Infection cycle of *Sclerotinia sclerotiorum.*

Source: http://www.hdc.org.uk/herbs/page.asp?id=22.

❖ ***Fusarium culmorum***

Fusarium culmorum is one of the most frequent pathogens causing common root rot of barley. In addition to small-grained cereals, *Fusarium* spp. can also attack grass species and some dicotyledonous species. Common root rot is a very widespread disease of barley and other cereals such as wheat. The main symptoms of affected plants are the brown or red colour of the infected tissues of crown, leaf or culm. *Fusarium culmorum* produces asexual spores or conidia. The conidia are dispersed by wind or rain to the host heads (Jenkinson & Parry, 1994; Fernando *et al*., 1997).

Inoculum starts freely in the soil or in host debris or barley seeds. The fungus survives as chlamydospores for up to 8 or 9 years. The primary infection occurs on the coleoptile or on the primary roots when the conidia and chlamydospores germinate in the presence of susceptible hosts (Figure 4). Some structures or appressoria are formed on root surfaces and the fungal hyphae penetrate and grow intercellularly through the root causing a necrotic breakdown of tissues in epidermis, cortex and endodermis. Later, the stele and the vascular tissues are infected too.

Fusarium species infect the grains and ears of barley in warm and humid areas, especially if wet and rainy periods coincide with crop maturity. Temperatures above 25 ºC and moist periods of longer than 24 hours favour infection and mycotoxin production by *F. culmorum* (http://www.sciencedirect.com/science).

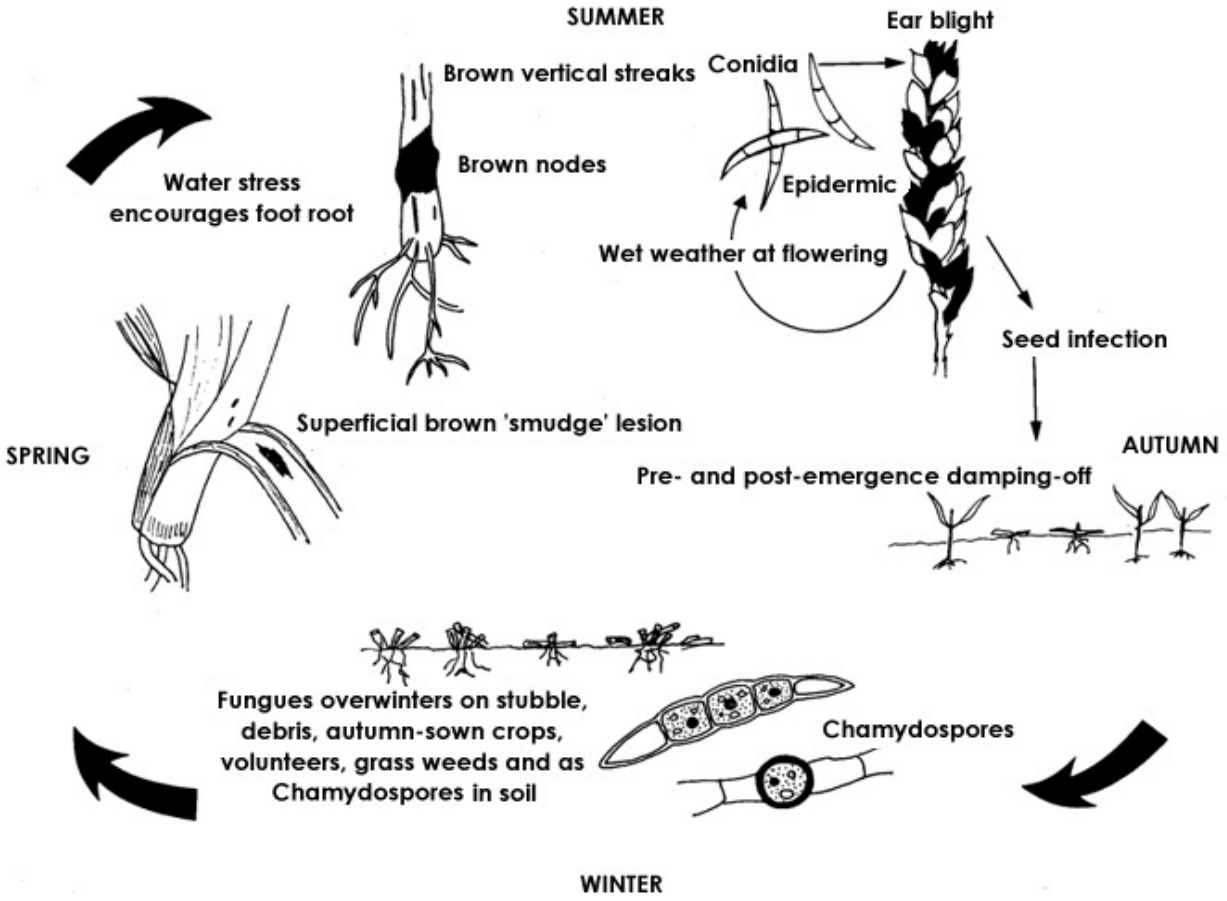

Figure 3. Life cycle of *Fusarium* species (Parry, 1990).

Source: http://www.fao.org/inpho/content/compend/text/ch31/ch31_04.htm

❖ ***Rhizoctonia solani***

Rhizoctonia solani can attack a large number of plants, both broadleaved species such as potato, tomato, beans, tobacco, and aubergine, but also grasses including cereals. However *R. solani* species are divided into so-called anastomosis groups that are linked to host pathogenicity. Damage is especially important in potato crops, where it can produce serious economic losses.
(http://www.cals.ncsu.edu/course/pp728/Rhizoctonia/Rhizoctonia.html).

The most important disease that *R. solani* produces in potatoes is black scurf on tubers (sclerotia) and stolon canker on underground stems and stolons. The symptoms of the disease can appear on aboveground or belowground parts of the plant and it is usual for the sprouts to be attacked before emergence.
(http://www.potatodiseases.org/rhizoctonia.html).

Rhizoctonia solani is a basidiomycete fungus that does not produce asexual spores and only occasionally produces sexual spores. The species exists in nature as vegetative mycelium and/or sclerotia. Rhizoctonia infections in potatoes can start either as a soilborne or seedborne inoculum (Figure 3). During the winter, the Rhizoctonia survive as black scurf on seed potatoes in storage or on plant residues in the soil, or as resting mycelium in the soil. In the spring the infected tubers are planted and the fungus colonises plant surfaces where nutrients are available, infecting root, stolon and leaf. The fungus penetrates into the tissues, causing cankers that can break the stolon or kill the growing point. The expansion of lesions can eventually be limited after emergence because plant resistance has increased by this time. Tubers and soil infected with black scurf represent the inoculum for other growing seasons.

(http://vegetablemdonline.ppath.cornell.edu/factsheets/Potato_Rhizoctonia.htm; http://www.potatodiseases.org/rhizoctonia.html)

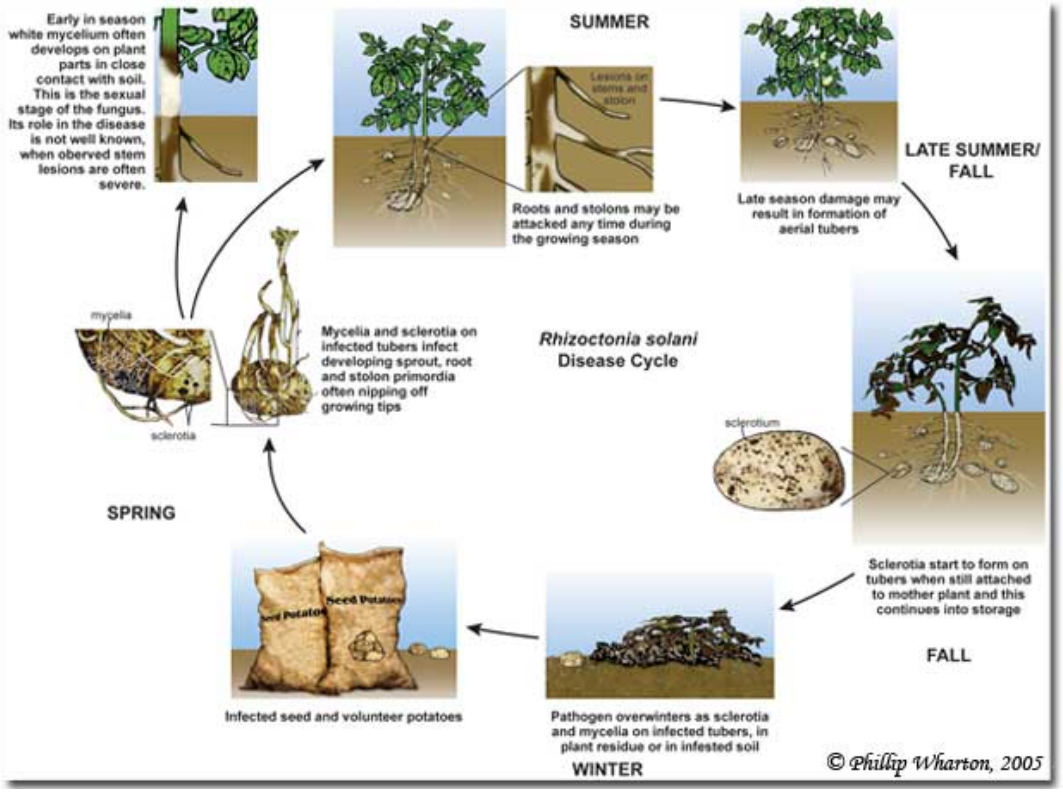

Figure 4. Disease cycle of *Rhizoctonia solani*.

Source: http://www.potatodiseases.org/rhizoctonia.html

3. Material and Methods

3.1 Experimental plan

The following experiments were carried out in the green-house at the Department of Crop Production Ecology.

The 84 boxes used for the study measured 20 cm x 30 cm x 24 cm.

The following four cover crops were studied for possible suppressive effect on three soil-borne pathogens: Oilseed radish (*Raphanus sativus),* Mustard (*Sinapis alba),* Rye (*Secale cereale)* and Westerwoldian ryegrass (*Lolium multiflorum var. westerwoldicum*). The pathogens used as model organisms were *Sclerotinia sclerotiorum, Fusarium culmorum* and *Rhizoctonia solani*.

Four replicates of each cover crop treatment were carried out for each pathogen, with three controls boxes for each replicate. These control boxes contained the pathogen but not the cover crop and each one of them simulated one effect caused by the plants in the soil. Thus one of the controls contained an amendment simulating the change in soil structure brought about by cover crop plant material, the second control contained a liquid nutrient solution corresponding to the amount of nitrogen introduced by the cover crop biomass and no material was added to the third control. The controls were established to check whether these effects in the soil had any influence on possible fungus suppression.

The replicates were divided between four tables or blocks (blocks 1-4). In total, 28 boxes were prepared per pathogen, seven for each replicate (3 controls + 4 cover crops). Thus an overall total of 28 replicates x 3 pathogens = 84 boxes were prepared in this study.

3.2 Box preparation

A. *Sclerotinia sclerotiorum* experiment:

Sclerotinia sclerotiorum inoculum was prepared by placing 20 sclerotia in 10x10 cm^2 nylon net bags. Before the experiment started, the bags were stratified in soil at +4 ºC for 10 weeks. At the time of the experiment, three sclerotinia bags were placed in each box and covered with 15 cm of soil, which was then gently compressed, followed by sowing of cover crop seeds as described below.

B. *Rhizoctonia solani* experiment:

For *R. solani*, an initial calculation of inoculum level was made to find the appropriate concentration of inoculum for the study. The pathogen was multiplied and incubated in sterile sand (100 g sand, 6 g malt and 13 ml H_2O) in glass bottles. Bottles with sand were sterilised twice at 105 ºC for one hour in an autoclave. After cooling, a fresh culture of *R. solani* (isolate 13, 12/9 isolated from potato kindly provided by U. Bång, SLU, Umeå), approx one-quarter of the Petri dish, was cut into small pieces and placed on the sand in the bottle. The bottles were incubated at room temperature in the dark for two weeks and shaken daily. Using this sand inoculum mixture, 16 small pots were prepared, four replicates of the following four levels of inoculum:

- 10 g of *R. solani* inoculum mixed with 100 g of standard sand (10%)
- 5 g of *R. solani* inoculum mixed with 100 g of sand (5%)
- 1 g of *R. solani* inoculum mixed with 100 g of sand (1%)
- 0.5 g of *R. solani* inoculum mixed with 100 g of sand (0.5%)

Five disease-free potato mini-tubers were planted in each pot. The 16 pots were covered with black plastic and incubated at +16 ºC for three weeks. After this time, the plants were taken out of the pots, cleaned with water and examined for symptoms (Figure 5).

Figure 5. Sprouted mini-tubers removed from pots

The plants had a brown discoloration caused by *R. solani*. The damage was located in the lower part of the stem, near the roots. Plants of the 0.5% inoculum pots had a very intense brown discoloration, spread along the plant. For the 1%, 5% and 10% inoculum pots the brown discoloration caused by *R. solani* was weaker than in the 0.5% treatment and it was more difficult to observe any damage. On the basis of these results, the inoculum rate chosen for the remaining experiments was 0.5%. New 0.5% *R. solani* inoculum was prepared in batches of 2000 g soil.

The boxes used in the experiment contained 2300 g soil, so the amount of *R. solani* sand inoculum added to each box was 11.5 g, to give the desired inoculation rate of 0.5%. The inoculum was incorporated into the soil at the time of sowing the cover crop.

The boxes used in the experiment contained 2300 gr of soil so the amount of *R.solani* sand inoculum to put in each box was 11,5 g (0,5 %). The inoculum was incorporated in the soil at the time for sowing the cover crop.

C. *Fusarium culmorum* experiment:

Inoculum for this experiment was prepared six weeks before the start of the greenhouse experiment. An isolate of *F. culmorum* originally isolated from barley kernels was multiplied in liquid potato dextrose broth on a shaking machine. After two weeks the culture was checked for *Fusarium* conidia. Barley kernels were sterilised by autoclaving in autoclavable bags (Figure 6). The sterile kernels in the bags were inoculated with the liquid *Fusarium* culture, which were mixed and placed at room temperature for multiplication of the fungus. The bags were thoroughly mixed daily. After four weeks the contaminated kernels were used as inoculum to be incorporated into the soil.

Figure 6. Barley kernels were in autoclavable bags

Once all the inocula of the three pathogens had been prepared, they were introduced into the respective boxes in the following way:

Each box was filled with 2300 g garden planting soil mixed with the relevant pathogen (*S. sclerotiorum*, *F. culmorum* or *R. solani*), the soil was compressed and the seeds were placed on the soil surface. The four cover crops were sown in separate boxes. For oilseed radish, mustard and rye, 20 seeds were placed in each box spread into three rows. A ruler was used for measuring the distance between seeds. For ryegrass, 30 seeds were spread randomly on the surface of the boxes. After sowing, the seeds were covered with 4 cm of soil and compressed.

For the control, the relevant pathogen-soil mixtures were added to boxes containing 2300 g soil and the soil was compressed. No cover crop seeds were used in these boxes.

All the boxes were labelled and set out in the greenhouse in four blocks.

3.3 Growing conditions in the greenhouse:

Once the boxes were prepared (Figure 7), they were left in the greenhouse for two months in the following conditions:

16 hours of light at +16 ºC and 8 hours of dark at +10 ºC.

Figure 7. Plant boxes in the greenhouse

Four days after sowing the cover crop plants began to emerge. For the first three weeks the boxes were irrigated with nutrient-free water. After the third week of growth, the boxes with plants were irrigated with water enriched with nutrients (routinely used for greenhouse crops) but the control pots were irrigated with nutrient-free water throughout to avoid adding additional nutrients to the soil. Three weeks after emergence, the number of plants in each box was counted and the number adjusted to be the same in each box (Figure 8).

Figure 8. Number of plants per box.

The blocks were divided between four tables (Figure 9). With the aim of providing the same conditions for all the boxes, the tables were rotated twice a week during the experimental period.

Figure 9. Tables or repetitions of each cover crop in the greenhouse.

3.4 Cutting

After approximately two months of growing, the plants were cut at soil level using a knife (Figure 10). Plants from each box were put in labelled bags.

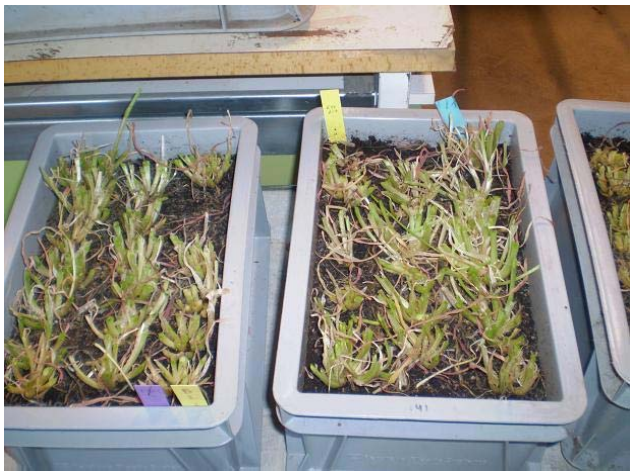

Figure 10. Box with the cut plants.

Plants from each bag were chopped mechanically into small pieces and the chopped material returned to the bag. The material in each bag was weighed and half the total weight was used for further experiments.

The root and soil material in the boxes was cut in half with a knife and crumbled, and one half was used for further experiments. The chopped biomass material and root and soil material were mixed with 2 L of new soil without any added nutrients.

In the case of the *Sclerotinia* boxes, before the root and soil material was crumbled, the net bags with sclerotia were removed. After the two months of cover crop growth it was tricky to take off the bags from root incorporation, but all root parts were removed and the net bags were placed on top of the biomass and root mixture together with 1 cm planting soil on top (Figure 11).

Figure 11. Net bags containing sclerotia of *S. sclerotiorum*

3.5 Examination of pathogen-suppressive effects

❖ ***Sclerotinia boxes:***

The boxes with net bags were incubated in the greenhouse for three weeks and were observed for the production of apothecia. In this time they were regularly irrigated to maintain the moisture level in the boxes. The *Sclerotinia* apothecia started to appear in the second week. The number of apothecia in each box was counted daily and noted on days 14, 18 and 24 after cutting . No numbers are presented for the control boxes as no apothecia formed in those boxes due to rotting of the sclerotia.

Figure 12. Apothecia formation of *S. sclerotiorum*

In the case of *Fusarium* and *Rhizoctonia* experiments, the boxes were prepared for bio testing in the following way:

❖ ***Fusarium boxes:***

Once the biomass material, the root and the soil material (including the Fusarium inoculum) had been mixed with the two litres of soil, the mixture was placed in the box and compressed. Then 30 seeds of spring barley variety Astoria were placed on the soil surface, in three rows of 10 seeds each, covered with a 2 cm layer of soil and compressed again.

The boxes were incubated in the greenhouse at +16 ºC and were irrigated with 1 L of water. Weight was noted and this weight was called the original weight. To keep a constant moisture content, the boxes were weighed and then irrigated twice a week to replace the weight lost compared with the original weight.

The barley bio-test boxes used for analysing development of *F. culmorum* after the different cover crop treatments were incubated in the greenhouse for four weeks. The barley plants were then taken out of the boxes, cleaned with water and examined. The symptoms were recorded for the part of the plant between stem and root and plants were classified according with their level of damage due to the *F. culmorum* infection into four classes:

Class 0: Plants without symptoms

Class 1: Plants with low level of infection, small brown blotches (Figure 14).

Figure 14. Barley plant from biotest showing Class 1 of infection with F.culmorum

Class 2: Infected plants with symptoms. Plants with big brown blotches or with the leaves brown or black (Figure 15)

Figure 15. Barley plant from bio-test showing Class 2 of infection with *F.culmorum*.

Class 3: Plants with an aggressive infection. Plants with the leaves completely dark and with many big blotches (Figure 16).

Figure 16. Barley plant from bio-test showing Class 3 of infection with *F.culmorum*

- ***Rhizoctonia boxes:***

Once the biomass material, the root and the soil material (including the *Rhizoctonia* inoculum) had been mixed with the two litres of soil, the mixture was placed in the box and compressed. Nine disease-free mini potato-tuber of the Early Puritan variety were placed evenly on the soil surface and covered with a 10 cm layer of soil and compressed again.

The *Rhizoctonia solani* boxes were labelled, covered with black plastic and placed in a dark and cold room (15 ºC) for two months, after which two of the blocks were analysed and no symptoms were found, so they were discarded. The remaining two blocks were left for a further four weeks and then the plants were removed from the boxes, cleaned with water and examined for symptoms. The damage was located in the lowest part of the stem and in the root. Some potato tubers also showed symptoms. The plants were classified according to their level of *Rhizoctonia solani* damage as follows:

Class 0: Plants without symptoms

Class 1: Plants with low level of infection. Plants with small brown blotches (Figure 18).

Figure 18. Potato plant from bio-test showing Class 1 of infection with *R.solani.*

Class 2: Infected plants with symptoms. Plants with brown blotches (Figure 19)

Figure 19. Potato plant from biotest showing Class 2 of infection with R.solani

Class 3: Plants with an aggressive infection. Plants with many big blotches (Figure 20)

Figure 20. Potato plant from bio-test showing Class 3 of infection with *R.solani*

- ***Control boxes:***

With the control boxes (without cover crops), a similar procedure was carried out. The soil from each box was cut in half and one half was discarded. The other half (with the corresponding pathogen) was crumbled and mixed with 2 L planting soil. One of the control boxes (Control L) was prepared with Leca (8-12 mm bentonite pellets) to simulate the change in structure made by the cover crop plant material in the soil. In Control N, a liquid nutrient solution was added with an amount nitrogen calculated to correspond to the amount introduced by the cover crop biomass. The nutrient solution was irrigated into the boxes on three occasions at one week intervals, to simulate the natural release of nutrients from the plants. No additions were made to Control 0 boxes.

As in the cover crop boxes, potatoes and barley seeds were planted in the *Rhizoctonia* and *Fusarium* control boxes, respectively, for the bio-tests.

After the experimental work in the greenhouse, all the results (number of apothecia in *Sclerotinia* experiment and number of infected and healthy plants in the *Fusarium* and *Rhizoctonia* experiments) were collated for statistical analysis using the program Minitab. Analysis of variance (ANOVA) was performed to determine whether there were any significant differences between treatments or between blocks. Where significant differences were found, a more sensitive test (Tukey test) was carried out to distinguish the mean differences that were significantly different.

The starting hypotheses were:

Ho= No one treatment suppressed fungal attack more than the others

H1= Rye suppressed fungal attack more than the other treatments

H2= Ryegrass suppressed fungal attack more than the other treatments

H3= Mustard suppressed fungal attack more than the other treatments

H4= Radish suppressed fungal attack more than the other treatments

(Ho was thus the Null hypothesis and H1, H2, H3, H4 alternative hypotheses.)

When the p-value obtained by the ANOVA test was higher than 0.05 (5% level of significance), this indicated that there were no significant differences between treatments or blocks. In this case, the null hypothesis was not rejected. When the p-value was lower than 0.05, there were significant differences between treatments and suppression of the fungal infection was not caused by chance but by a specific treatment.

4. Results

4.1 Sclerotinia sclerotiorum:

The number of apothecia in each box noted on days 14, 18 and 24 after cutting is showed below (Table 1).

Table 1. *Number of apothecia of* Sclerotinia sclerotiorum *that had emerged 14, 18 and 24 days after cutting and subsequent incorporation of the cover crop biomass into the soil*

Block	Cover crop	14 days after cutting	18 days after cutting	24 days after cutting
1	Rye	5	9	9
	Ryegrass	0	0	11
	Mustard	2	9	11
	Radish	0	0	0
2	Rye	12	30	44
	Ryegrass	0	0	50
	Mustard	25	42	49
	Radish	13	44	59
3	Rye	4	19	46
	Ryegrass	8	10	10
	Mustard	7	38	54
	Radish	0	8	12
4	Rye	18	18	20
	Ryegrass	0	0	4
	Mustard	5	9	45
	Radish	3	18	28

The means of treatments in all the blocks were calculated (Table 2) to compare the mean number of apothecia formed per treatment. The results are shown in (Figure 13).

Table 2. *Mean number of apothecia formed 14, 18 and 24 days after cutting and biomass incorporation for each treatment (variation between boxes given in brackets)*

Cover crop	14 days after cutting	18 days after cutting	24 days after cutting
Rye	9.8 (4-18)	18.8(9-30)	29.8 (20-46)
Ryegrass	2 (0-8)	2.5 (0-10)	18.5 (4-50)
Mustard	9.8 (2-25)	24.5 (9-42)	39.8 (11-54)
Radish	4 (0-13)	17.5 (0-44)	24.8 (0-59)

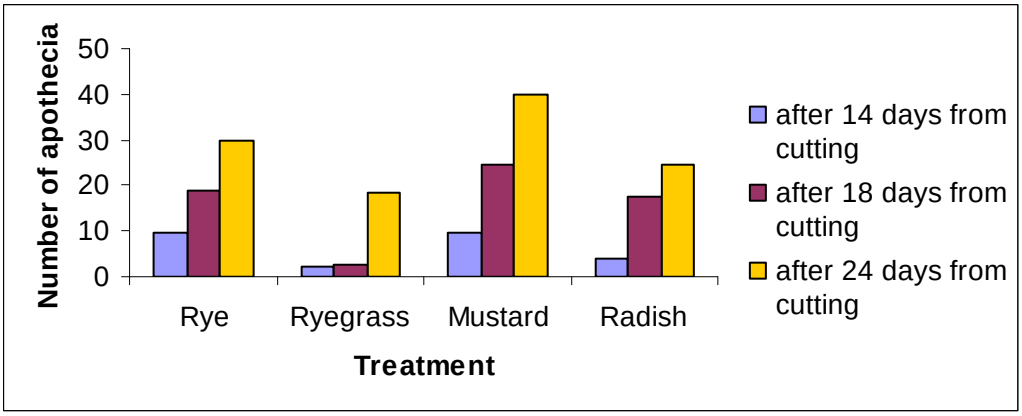

Figure 13. Number of *Sclerotinia sclerotiorum* apothecia counted in the boxes 14, 18 and 24 days after cutting the plants and subsequent incorporation of the biomass into the soil.

Separate Anova tests were performed for the time points 14, 18 and 24 days after cutting.

The results indicated that there were no significant differences between treatments or blocks 14 days after cutting ($p>0.05$). So, in this case no one treatment suppressed *Sclerotinia sclerotiorum* attack more than the others.

By 18 days after cutting, the p-value was 0.08 in Anova, *i.e*. near to the $p<0.05$ accepted as the significance level. The results indicated that the mean number of apothecia formed with ryegrass was significantly lower (ryegrass compared with mustard: $p=0.07$). However, since the p-value is higher than 0.05 in all the cases, there were not significantly different between tretaments.

After 24 days there were significance differences between blocks ($p= 0.01$) indicating that the conditions in the boxes were different for the four replicates. No differences were found between treatments, so none of the treatments suppressed the germination of *Sclerotinia sclerotiorum* sclerotia more than the others.

4.2 Fusarium culmorum:

The results of the apothecia counts are presented in Table 3.

Table 3. Fusarium culmorum *damage in barley bio-test plants grown after incorporation of four different cover crops and three controls*

Block	Cover crop	Class 0	Class 1	Class 2	Class 3	TOTAL
1	Rye	8	16	4	1	29
	Ryegrass	15	8	0	8	31
	Mustard	15	8	2	2	27
	Radish	18	9	1	1	29
	Control 0	14	6	1	1	22
	Control L	4	6	3	4	17
	Control N	14	8	3	1	26
2	Rye	12	11	4	2	29
	Ryegrass	11	16	4	1	32
	Mustard	6	16	8	0	30
	Radish	10	11	5	4	30
	Control 0	5	10	6	2	23
	Control L	4	10	2	1	17
	Control N	10	11	5	0	26
3	Rye	18	7	3	0	28
	Ryegrass	9	7	12	2	30
	Mustard	12	8	6	2	28
	Radish	19	4	7	1	31
	Control 0	7	10	4	0	21
	Control L	9	6	3	1	19
	Control N	5	5	3	0	13
4	Rye	12	14	3	0	29
	Ryegrass	10	10	6	1	27
	Mustard	11	16	2	0	29
	Radish	10	12	5	0	27
	Control 0	6	9	3	0	18
	Control L	6	9	7	4	26
	Control N	13	11	4	1	29

The results were analysed in two steps: 1) for differences between treatments and 2) for differences within treatments.

1). Analysis of differences between treatments

First, the disease index was calculated for each box as follows:

After counting the number of plants in each damage class (Table 3), an increase factor was assigned to each class. The number of plants in class 0 was multiplied by 1, the number of plants in class 1 was multiplied by 2, the number of plants in class 2 was multiplied by 3 and the number of plants in class 3 was multiplied by 4. These new numbers of plants for each treatment were added together and the total obtained was divided by the actual total number of plants in each treatment (without increase factor), giving the disease index.

For example:

The rye box in block 1 had the following number of plants in different disease classes:

Block	Cover crop	Class 0	Class 1	Class 2	Class 3	TOTAL
1	Rye	8	16	4	1	29

Applying the increase factor gave:

Block	Cover crop	Class 0	Class 1	Class 2	Class 3	TOTAL
		X1	x2	x3	x4	
1	Rye	8	32	12	4	56

The disease index was thus 56/29 = 1.93

Table 4 shows the *Fusarium culmorum* disease index for each box in the bio-tests, while Figure 17 summarises the results obtained.

Table 4. Fusarium culmorum d*isease index calculated for each box*

Block	Cover crop	Class 0	Class 1	Class 2	Class 3	Total	Disease index
		x1	x2	x3	x4		
1	Rye	8	32	12	4	56	1.93
	Ryegrass	15	16	0	32	63	2.03
	Mustard	15	16	6	8	45	1.67
	Radish	18	18	3	4	43	1.48
	Control 0	14	12	3	4	33	1.50
	Control L	4	12	9	16	41	2.41
	Control N	14	16	9	4	43	1.65
2	Rye	12	22	12	8	54	1.86
	Ryegrass	11	32	12	4	59	1.84
	Mustard	6	32	24	0	62	2.07
	Radish	10	22	15	16	63	2,10
	Control 0	5	20	18	8	51	2.22
	Control L	4	20	6	4	34	2.00
	Control N	10	22	15	0	47	1.81
3	Rye	18	14	9	0	41	1.46
	Ryegrass	9	14	36	8	67	2.23
	Mustard	12	16	18	8	54	1.93
	Radish	19	8	21	4	52	1.68
	Control 0	7	20	12	0	39	1.86
	Control L	9	12	9	4	34	1.79
	Control N	5	10	9	0	24	1.85
4	Rye	12	28	9	0	49	1.69
	Ryegrass	10	20	18	4	52	1.93
	Mustard	11	32	6	0	49	1.69
	Radish	10	24	15	0	49	1.81
	Control 0	6	18	9	0	33	1.83
	Control L	6	18	21	16	61	2.35
	Control N	13	22	12	4	51	1.76

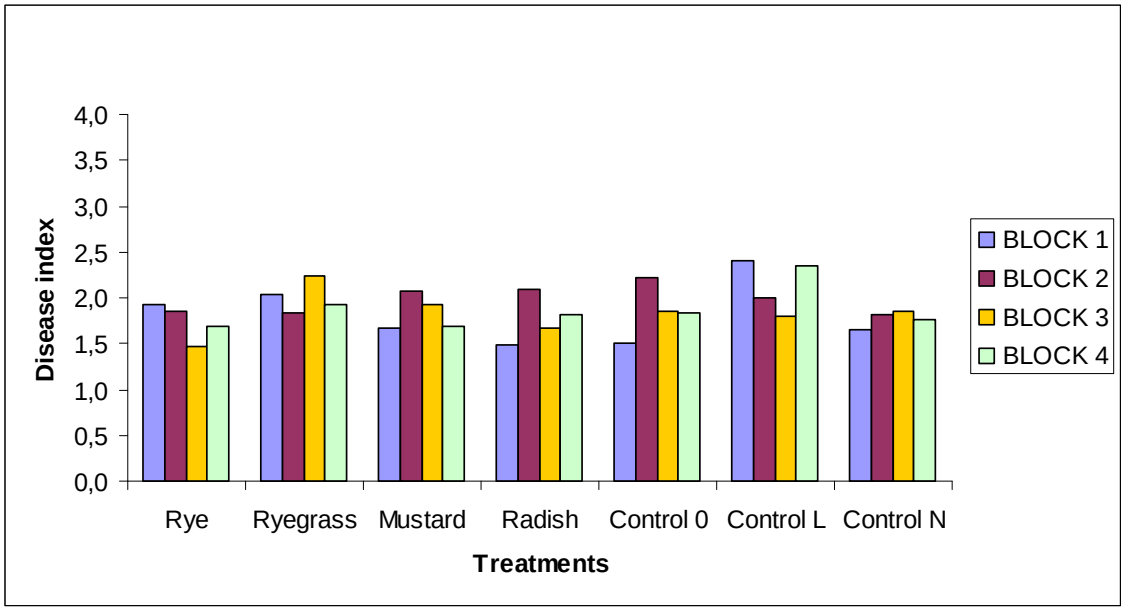

Figure 17. Effects of *Fusarium culmorum* on barley biotest plants grown in soil treated with one of four different cover crops.

An Anova test was performed on all the disease index values together in order to determine whether there were significant differences between them, and a Tukey test was carried out to distinguish any significant differences found between means.

The Anova showed a p-value of 0.2 between treatments and 0.5 between blocks. There were no significant differences between treatments or between blocks and thus no one treatment suppressed *Fusarium culmorum* fungal attack more than the others.

2). Analysis of the disease level within each treatment

Not all the 30 planted barley seeds grew, only a percentage of them. Different numbers of plants emerged in each box and a factor was calculated in order to adjust for these differences (Table 5). For example, for rye of block 1, the number of seeds sown was 30 but only 29 plants emerged, so the proportion of plants for each damage class in this box was:

Class 0: 8 x 30/29 = 8.28

Class 1: 16 x 30/29 = 16.55

Class 2: 4 x 30/29 = 4.14

Class 3: 1 x 30/29 = 1.03

Table 5. *Adjusted number of plants in each of* Fusarium culmorum *damage classes 0-3 for the different cover crops and the controls*

Block	Cover crop	Class 0	Class 1	Class 2	Class 3	TOTAL
1	Rye	8.28	16.55	4.14	1.03	30.00
	Ryegrass	14.52	7.74	0.00	7.74	30.00
	Mustard	16.67	8.89	2.22	2.22	30.00
	Radish	18.62	9.31	1.03	1.03	30.00
	Control 0	19.09	8.18	1.36	1.36	30.00
	Control L	7.06	10.59	5.29	7.06	30.00
	Control N	16.15	9.23	3.46	1.15	30.00
2	Rye	12.41	11.38	4.14	2.07	30.00
	Ryegrass	10.31	15.00	3.75	0.94	30.00
	Mustard	6.00	16.00	8.00	0.00	30.00
	Radish	10.00	11.00	5.00	4.00	30.00
	Control 0	6.52	13.04	7.83	2.61	30.00
	Control L	7.06	17.65	3.53	1.76	30.00
	Control N	11.54	12.69	5.77	0.00	30.00
3	Rye	19.29	7.50	3.21	0.00	30.00
	Ryegrass	9.00	7.00	12.00	2.00	30.00
	Mustard	12.86	8.57	6.43	2.14	30.00
	Radish	18.39	3.87	6.77	0.97	30.00
	Control 0	10.00	14.29	5.71	0.00	30.00
	Control L	14.21	9.47	4.74	1.58	30.00
	Control N	11.54	11.54	6.92	0.00	30.00
4	Rye	12.41	14.48	3.10	0.00	30.00
	Ryegrass	11.11	11.11	6.67	1.11	30.00
	Mustard	11.38	16.55	2.07	0.00	30.00
	Radish	11.11	13.33	5.56	0.00	30.00
	Control 0	10.00	15.00	5.00	0.00	30.00
	Control L	6.92	10.38	8.08	4.62	30.00
	Control N	13.45	11.38	4.14	1.03	30.00

An Anova test was performed for each class of *Fusarium culmorum* disease attack. For classes with significant differences between treatments, a Tukey test was carried out to distinguish significant differences between means.

In the Anova for class 0, the p-value was 0.4 between treatments, i.e. higher than the level of significance. This means that there were no significant differences between treatments.

For class 1, the Anova test showed no significant differences between treatments. However there were significant differences between blocks (p=0.013).

There were p-values higher than 0.05 between treatments in the Anova test for classes 2 and 3 and thus there were no significant differences between treatments and no one treatment suppressed *Fusarium culmorum* attack more than the others.

4.3 Rhizoctonia solani:

The number of plants in each class was recorded (Table 6). Most of the plants belonged to class 0 or class 1 and only one plant was classified as class 3, in a box that contained rye.

Table 6. *Number of potato plants in each class according to their level of damage by* Rhizoctonia solani

Block	Treatment	Class 0	Class 1	Class 2	Class 3	TOTAL
3	Rye	5	1	0	1	7
	Ryegrass	10	1	0	0	11
	Mustard	7	5	0	0	12
	Radish	5	3	1	0	9
	Control 0	6	3	0	0	9
	Control L	7	2	0	0	9
	Control N	7	4	0	0	11
4	Rye	5	4	0	0	9
	Ryegrass	6	4	0	0	10
	Mustard	7	3	0	0	10
	Radish	4	3	2	0	9
	Control 0	5	4	0	0	9
	Control L	8	1	0	0	9

The results, as in the case of Fusarium, were analysed in two steps: 1) for differences between treatments and 2) for differences within treatments.

1). Analysis of the differences between treatments:

The disease index for each box was calculated (Table 7) in order to compare the level of *Rhizoctonia solani* attack in each treatment (Figure 21). The way of calculating the disease index was exactly the same as in the Fusarium boxes.

Example:

The rye box in block 3 had the following number of plants in different disease classes:

Block	Cover crop	Class 0	Class 1	Class 2	Class 3	TOTAL
3	Rye	5	1	0	1	7

Applying the increase factor gave:

Block	Treatment	Class 0	Class 1	Class 2	Class 3	TOTAL
		x1	x2	x3	x4	
3	Rye	5	2	0	4	11

Disease index was thus 11/7 = 1.57

Table 7 shows the disease index for each box in the bio-tests, while figure 21 summarises the results obtained.

Table 7. *Disease index calculated for each box*

Block	Treatment	Class 0	Class 1	Class 2	Class 3	TOTAL	Disease index
		x1	x2	x3	x4		
3	Rye	5	2	0	4	11	1.57
	Ryegrass	10	2	0	0	12	1.09
	Mustard	7	10	0	0	17	1.42
	Radish	5	6	3	0	14	1.56
	Control 0	6	6	0	0	12	1.33
	Control L	7	4	0	0	11	1.22
	Control N	7	8	0	0	15	1.36
4	Rye	5	8	0	0	13	1.44
	Ryegrass	6	8	0	0	14	1.40
	Mustard	7	6	0	0	13	1.30
	Radish	4	6	6	0	16	1.78
	Control 0	5	8	0	0	13	1.44
	Control L	8	2	0	0	10	1.11

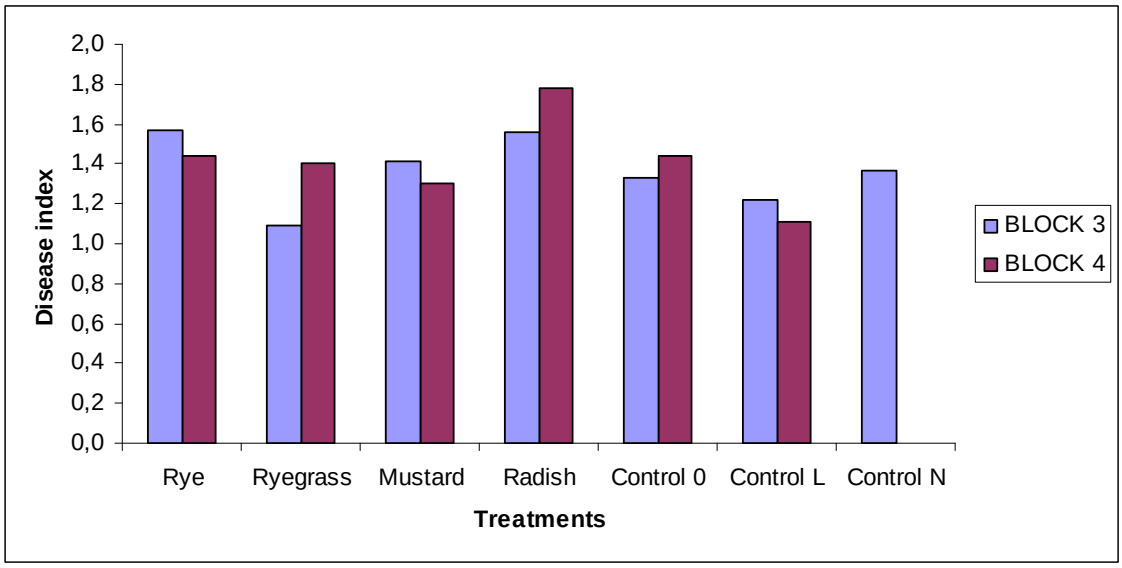

Figure 21. Effects of the four cover crops on the number of potato plants infected by *Rhizoctonia solani.*

The results from the Anova analysis showed a p-value higher than 0.05 between treatments and between blocks. These results indicate that there were no significant differences between blocks or between treatments and that no one treatment suppressed *Rhizoctonia solani* attack more than the others.

2). Analysis of the disease level within each treatment

The number of potato plants that emerged was different in each box, since although nine seed potatoes were planted in each box, not all of them emerged. Therefore a factor was calculated in order to adjust for these differences, as was done in the case of *Fusarium*.

For example, for the rye cover crop in block three, the number of potatoes planted was 9 but only 7 plants developed, so the proportion of plants for each *Rhizoctonia solani* damage class in this box was:

Class 0: 5 * 9/7 = 6.43

Class 1: 1 * 9/7 = 1.29

Class 2: 0 * 9/7 = 0

Class 3: 1 * 9/7 = 1.29

The adjusted number of plants in each damage class is shown in Table 8.

Table 8. *Adjusted fraction of potato plants in* Rhizoctonia solani *damage classes 0-3*

Block	Cover crop	Class 0	Class 1	Class 2	Class 3	TOTAL
3	Rye	6.43	1.29	0.00	1.29	9.00
	Ryegrass	8.18	0.82	0.00	0.00	9.00
	Mustard	5.25	3.75	0.00	0.00	9.00
	Radish	5.00	3.00	1.00	0.00	9.00
	Control 0	6.00	3.00	0.00	0.00	9.00
	Control L	7.00	2.00	0.00	0.00	9.00
	Control N	5.73	3.27	0.00	0.00	9.00
4	Rye	5.00	4.00	0.00	0.00	9.00
	Ryegrass	5.40	3.60	0.00	0.00	9.00
	Mustard	6.30	2.70	0.00	0.00	9.00
	Radish	4.00	3.00	2.00	0.00	9.00
	Control 0	5.00	4.00	0.00	0.00	9.00
	Control L	8.00	1.00	0.00	0.00	9.00

An Anova test was carried out on these values. The results revealed no significant differences between treatments in classes 0 and 1. However the analysis of variance for class 2 showed a p-value of 0.02 between treatments, indicating that there were significant differences between treatments. Subsequent Tukey tests revealed that radish had the lowest p-value (0.03) in most of the pair-wise comparisons with the other treatments. This fact is because radish was the only treatment that contained plants in damage class 2 (Table 8). It indicates that there was a different suppressive effect for radish than for the other cover crops.

Rhizoctonia solani damage class 3 showed no significant differences between treatments.

5. Discussion

This study examined the use of cover crop biomass for the control of some soil-borne pathogens. Material from the plant families Brassicaceae (oilseed radish and mustard) and Graminaceae (rye and ryegrass) was used as green manure in the study.

The starting hypothesis for the investigation was that cutting, chopping and immediate incorporation into the soil of two month's growth of biomass from the different cover crops (brassicas and non-brassicas) would suppress soil-borne plant pathogens.

In the case of the brassica cover crops, the possible mechanism behind this suppression is either a direct effect through the transformation of glucosinolate into isothiocyanate or a result of changes in the structure of the soil microflora. In the case of non-brassica cover crops there may be other mechanisms involved, but such mechanisms were only speculated upon and were not tested in the present study.

Many studies have been carried out using plant material with the aim of suppressing pathogens, but with very mixed results. The majority of these studies have focused on the use of brassica species containing glucosinolates as an agent to control soil-borne pathogens and there is very little information on the use of non-brassicas in crop protection.

In the present study, two species from the *Brassicaceae* and two from the *Graminaceae* were compared in terms of their pathogen control effect. The way in which the suppressive effect of these cover crops was tested differed for different pathogens. The cover crop effect on *Sclerotinia* was based on the development of ascospores formed in the apothecia, which form the basis for stem rot infection. However, in *Fusarium culmorum* and *Rhizoctonia. solani* experiments, potatoes and barley seeds respectively were planted to perform bio-tests, and the results were based on the number of plants in each class of disease attack. The different results for each pathogen are discussed below.

5.1 Sclerotinia sclerotiorum

The possible inhibition of the pathogen was analysed by counting the number of apothecia formed on the soil surface of each box. None of the cover crop treatments suppressed sclerotia germination entirely. The number of apothecia increased with time from the date of incorporation of cover crop material in all treatments.

In the statistical analysis of the results the mean level for ryegrass was significantly higher than for the other treatments (p-value= 0.07). The number of apothecia formed during the first weeks (18 days) after incorporation of ryegrass biomass was lower than for the other cover crops (rye, mustard and oilseed radish), which were not significantly different from each other. The p-value between blocks after 24 days, indicated that

there were significant differences between blocks at this time, *i.e*. that the conditions in the boxes were different for the four replicates. The plant boxes in the greenhouse had been rotated twice a week during the study in order to avoid these differences between blocks and to have the same conditions in all replicates, but some differences still arose, as the Anova test showed.

The brassicas (oilseed radish and mustard) did not have a greater suppression effect on apothecia formation than the non-brassicas (rye or ryegrass). In the mustard treatment in particular, the number of apothecia after 24 days was very high (54 in one box). Smolinska (2000) studied the effect of brassica plant residues on *Sclerotium cepivorum* in the soil and observed that residues of *Brassica juncea* (Caliente mustard) decreased the number of *S. cepivorum* sclerotia. *B. juncea* contains very high levels of GSL, which could be responsible for this effect. The oilseed radish and mustard used in this study have lower amount of GSLs than the *B. juncea* used by Smolinska (2000), which could be one reason for this difference in results. In another experiment, Larkin & Griffin (2007) studied the control of soilborne potato diseases using brassica green manures with different levels of GSLs. The results indicated that disease reductions were not always associated with high production of glucosinolates. In the present study the mechanisms behind any suppressive effects of cover crops were not investigated, but since the brassicas did not have any specific suppressive effect, it can be concluded that GSLs were not a suppressive factor in this case.

For the non-brassica cover crop rye, the results were very similar to those obtained for the brassicas. The number of apothecia observed in rye boxes was very high from the first weeks (14 and 18 days after biomass incorporation), but was especially high after 24 days from biomass incorporation (44 apothecia in one of the boxes). However, ryegrass gave a different effect to the other cover crops, since almost no apothecia were observed in the first 14 and 18 days in these boxes. After the first 18 days, only a few apothecia were found in one of the boxes (that in block 3). In blocks 1, 2 and 4, no apothecia were observed until 24 days after ryegrass biomass incorporation. Ryegrass had a suppressive effect on *Sclerotinia sclerotiorum*, consisting of a delay in apothecia emergence.

The brassica crops used in this experiment did not suppress apothecia formation, so the possible mechanism for the pathogen suppression associated with production of glucosinolates was not confirmed in this study. The suppressive effects of the non-brassica ryegrass indicate that other mechanisms and interactions are important for controlling the soil-borne pathogen *Sclerotinia sclerotiorum*. This non-glucosinolate suppression can be due to the multiplication of antagonistic organisms in soil, to the release of toxic compounds which are not of GSL origin or to the triggering of processes in the main crop host plants, inducing disease resistance (Matthisen & Kirkegaard, 2006; Gimsing & Kirkegaard, 2009). However these are only speculations, and none of these possible reasons was confirmed in this study.

In other studies, researchers reached similar conclusions as regards disease suppression by non-brassicas. For example Larkin & Griffin (2007) observed inhibition of pathogenic *Rhizoctonia* infection with barley green manures that did not contain GSLs, while Kasuya *et al.* (2006) observed that non-brassicas, clover and peanut, generally suppressed *R. solani* disease, despite their lack of GSLs. They concluded that there was no evidence indicating that GSLs or ITCs are significant factors in soil suppressiveness

and it is possible that other inhibitory volatile compounds released from the crop residues are responsible.

The most interesting result from this experiment was that obtained in the ryegrass boxes. It was shown that ryegrass delayed apothecia formation by 10 or more days. The apothecia produce ascospores, which are transported by air to flowers or by direct germination on leaves and cause infection in host plants. Sclerotinia infections are important in oilseed rape and these plants are particularly susceptible in the flowering stage but not later. If ascospore release can be delayed by one week or more, the susceptible stage may have passed and plant infection can be avoided.

One unexpected problem in the present experiment was the excess humidity in the control boxes, which caused the sclerotia to rot. Due to this fact the control boxes had to be discarded and the results were based only on observations in the cover crop treatments.

In future studies better control of the humidity in the boxes would be necessary, especially in the control boxes. This would involve measuring the exact amount of water necessary in each box in order to avoid rotting of the sclerotia.

5.2 Fusarium culmorum

Possible *F. culmorum* suppression by the cover crops was analysed using barley plants in bio-tests. These plants were counted and classified into groups according to their level of damage. A disease index was calculated indicating the proportion of damage between class 1 (more disease suppression) and 4 (less disease suppression) for each box and then Anova tests were used to compare the disease index values for the boxes.

The bio-tests showed very similar behaviour for all the treatments. Most of the plants displayed few or moderate symptoms of *F. culmorum* infection (disease classes 0 and 1), with some plants in class 2 and only a few classified as a severe fungal attack (class 4). Ryegrass was the treatment that gave the highest number of infected bio-test plants in class 2 (12 in one box) and class 3 (8 in other box). Rye and mustard showed very similar behaviour, with almost the same number of plants in each class. Oilseed radish boxes had the highest number of healthy barley plants.

Anova tests revealed no significant differences in disease index between treatments or between blocks. It was therefore concluded that no one type of cover crop suppressed *Fusarium culmorum* attack more than the others.

Other studies have examined the use of brassicas to suppress Fusarium diseases. Smolinska *et al*. (2003) studied the effect of individual ITCs on *Fusarium oxysporum* in conifer seedling nursery soils. They used different ITCs compounds, *e.g.* propenyl and ethyl isothiocyanates, and observed that these two in particular had a strong fungistatic effect. They therefore concluded that the use of plants with GSLs that release high amounts of propenyl isothiocyanate, such as *Brassica carinata, B. nigra* or *B. juncea*, could be a solution for the control of *F. oxysporum* infections in nursery soils. However, no suppressive effect is observed in our study. This could be due to the species chosen not being appropriate because they did not contain sufficient amounts of

GSLs to release the propenyl isothiocyanates necessary to suppress *Fusarium culmorum* attack. Another reason for the no suppressive effect could be that the high level of organic matter contained in the soil could interfere with the ITCs and limit their potential effect. However those are only speculations since the mechanisms through which the cover crops could suppress *R. solani* infection were not analysed in this study.

An unexpected result in this experiment was that different numbers of plants emerged in each box in the bio-test although the same number of plants was sown in all boxes. This could be due to several reasons. One possible reason is that the fungal attack was so severe that the plants could not emerge, but this possibility was ruled out because no severe attack was observed in the plants that could produce this effect. Another possibility was that metabolites from the cover crop material interfered with germination of the bio-test plants, but this was ruled out since fewer plants emerged in the control boxes, which did not contain cover crops. The only possible explanation for the varying number of plants emerging was the presence of mosquito larvae, usually found in greenhouses. These could have fed on the seeds and the seedlings, stopping their growth. Pest-damaged leaves were observed in some barley boxes, which supports this fact.

In future studies, it would be interesting to compare other high-GSL brassica cover crops such as *B. juncea* with the *Raphanus sativus* and *Sinapis alba* used in this study. Another interesting option for further studies could be to wait one week between incorporation of cover crop biomass in the soil and incorporation of barley for bio-testing because in that way, when the barley was planted the GSLs would have already been transformed into ITCs and they can be faster on suppress the infection on barley.
I also recommend that some insect control must be used in future experiments.

5.3 Rhizoctonia solani

Potato plants were used in bio-tests to analyse the effects of the four different cover crops on *R. solani* damage to plants.

Only two of the four initial replicates were used in this experiment, since after one month at 15 ºC, the plants in two blocks (blocks 1 and 2) did not show any *R. solani* symptoms and had to be discarded. The other two blocks (3 and 4) were left for one further month, after which a few plants showed damage and some tubers showed symptoms too. Most of the plants had no symptoms (sort 0), only a few showed little blotches due to the infection (disease classes 1 and 2) and almost no plants suffered severe attack (class 3). In the controls too, most of the plants had no symptoms and only a few plants showed class 1 symptoms.

A disease index for damage classes between 1 and 4 was calculated for each box, relating the number of infected plants with the total number of plants. The disease index values produced were very similar for all the boxes but oilseed radish boxes had the highest index, while ryegrass had the lowest. A comparison of disease index values

of all boxes in Anova tests revealed no significant differences between treatments. *i.e*. no treatment had any effect on the *R. solani* disease index.

In this study, no treatment showed any particular suppressive effect on *Rhizoctonia solani* infection. However, other studies have shown different results. For example, Mazzola *et al*. (2007) investigated the potential of various brassica seed meals, including mustard (*S. alba*), on soilborne disease suppression and found that it suppressed the development of *R. solani* apple root rot. Furthermore, Larkin & Griffin (2007) found that brassica green manures of oilseed radish and mustard, with moderate GSL content, reduced inoculum levels of *R. solani* and potato seedling disease by a high degree. However, they did not associate these results with the production of GSLs because infection suppression was also observed for non-brassica green manures.

As for the other two pathogens studied, the mechanisms through which the cover crops could suppress *R. solani* infection were not analysed in this study. However, according to the results, and since any brassica showed any suppressive effect, I can conclude that GSL production had no influence on pathogen suppression in this case.

The number of infected plants in the experiment was lower than expected from pilot tests carried out before the experiment, where almost all the potato plants showed symptoms of *R. solani* infection three weeks after inoculation. In the pilot test, however, the inocula were directly tested on the potato plants, with the inoculum and potatoes being added to the pots at the same time. In the bio-tests, the inoculum stayed in the boxes for two months before the potatoes were introduced, which could be the reason for the much lower level of infection compared with the pilot test. Of course another explication could be that the chosen percentage of inoculum was not enough for the infection of the plants.

One recommendation for future experiments is thus to introduce the *R. solani* inoculum at the time of incorporation of biomass and to perform bio-tests with potato tubers soon after that. In nature, *Rhizoctonia* spp. survives in the soil by resting mycelium or sclerotia formation. Inoculum produced in the laboratory is probably more sensitive to the soil environment and may have more difficulties in finding substrate to live on. Another way to improve this experiment would be to increase the waiting time before looking for symptoms in the plants. One month after the bio-test was set up was not enough time in this experiment to get any symptoms in the potato plant, so for future studies, two months are recommended.

6. Conclusions and Final Remarks

The most important result of this study was that ryegrass biomass incorporation in the *S. sclerotiorum* experiment delayed apothecia formation. This result is very interesting and can be used in oilseed rape protection against the *S. sclerotiorum* infection.

For the other cover crops investigated, after two months of growth, chopping and immediate incorporation of the biomass from them in the soil, none caused any suppression of the three model soil-borne plant pathogens tested.

The pathogens did not appear to be influenced by GSL transformation, since the brassicas tested did not suppress fungal infection. The fact that ryegrass does not contain GSLs, indicates that other mechanisms were responsible for the fungal suppression. However, the mechanism by which ryegrass delayed apothecia formation was not studied in this experiment.

Further studies are required to explain the mechanisms behind the ryegrass suppressive effect and how it can be exploited in crop protection.

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