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Brassica species as a green alternative to pesticides to reduce the impact of nematodes and fungi in cereals.

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Acknowledgements

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Abstract

In cereal production plant pathogens cause yield losses, and management strategies for controlling them are important. Cover crops as a part of the cereal production have beneficial properties for the soil health, can increase the biological diversity in the fields, and the soil structure can be improved, additionally cover crops can reduce soil erosion. Therefore, this thesis argues that implementing cruciferous plants as part of the cover crops have the potential to reduce plant pathogens. Cruciferous plants used as cover crops have shown to reduce plant pathogens survival both in laboratory and field plots. The suppression is due to the volatile glucosinolate (GLS) and isothiocyanate (ITC) content cruciferous plants. As a part of a project at NIBIO Ås, an *in vitro* experiment with allyl ITC were done to look at the effect on survival of *Heterodera avenae, Fusarium graminearum* and *Microdochium nivale*, with increasing allyl ITC concentration. Also, a closed jar experiment was conducted to look at the effects of cruciferous plants on suppression of survival of *Heterodera avenae* and *Fusarium graminearum*.

The aim of the *in vitro* experiment was to see at which concentrations of allyl ITC that suppressed fungal growth of *F. graminearum* and *M. nivale* and hatching of the cereal cyst nematode *H. avenae.* The EC_{50}-values of the *F. graminearum* isolates 200 630, 201 196 and 202 058, were 6.36, 9.50 and 7.62 mg/L respectively. EC_{50}-values of *M. nivale* isolates 200 136, 200 231 and 202 786, were 8.60, 10.83 and 10.27 mg/L respectively. For *H. avenae*, the EC_{50}-value was at 5.66 mg/L. This demonstrates a differentiation of EC_{50}-values between nematodes and fungi, but also between fungal species and fungal isolates.

A closed jar experiment was conducted to see if incorporation of cruciferous plants in soil may affect the survival of *H. avenae* and *F. graminearum*. Two different time exposures in jars with either two or eight weeks of exposure in jars, were performed. After treatments in jars, the cysts were given either a diapause or no diapause treatment. The total number of eggs and J2 were significantly lower for cysts exposed for eight weeks in jars compared to two weeks, but the total number of eggs and J2 were almost equal for diapause and no diapause. The number of hatched J2 (15°C) for cysts with diapause treatment had significantly higher number of J2 than no diapause treatment. Also, the number of hatched J2 were significantly higher for two weeks exposure in jars compared to eight weeks. The mycelial growth from *F. graminearum* on oat spikelet incorporated in soil with plant material was not different from untreated.

Sammendrag:

I korndyrking er plante skadegjørere en årsak til reduserte avlinger, og derfor er metoder for å bekjempe de viktig. Fangvekster som en del av korndyrkinga har fordelaktige egenskaper for blant annet jordhelsen, kan øke det biologiske mangfoldet i åkeren og jord strukturen kan forbedres, samt at fangevokter kan redusere jorderosjon. Derfor ser denne oppgaven på mulighet til å bruke korsblomstra vekster som fangvekster, for mulig å kunne redusere plante skadegjørere. Korsblomstra vekster har vist å redusere overlevelsen av planteskadegjørere, både i laboratoriske forsøk og i frilands forsøk. Reduksjonen i overlevelse kommer av de flyktige stoffene «glucosinolate» (GLS) og «isothiocyanate» (ITC) som korsblomstravekster inneholder. Som en del av et prosjekt ved NIBIO Ås, et *in vitro* forsøk med allyl ITC ble gjort for å se på overlevelsen av *Heterodera avenae, Fusarium graminearum* og *Microdochium nivale* ved økende allyl ITC konsentrasjon. Samtidig ble et lukket glasskrukke-forsøk blitt gjort for å se på effekten av korsblomstravekster på overlevelse av *H. avenae* and *F. graminearum*.

Hensikten med *in vitro* forsøket var å se på hvilken konsentrasjon av allyl ITC som reduserte mycel vekst av *F. graminearum* and *M. nivale* og klekking av korncystenematodene H *avenae.* EC_{50}-verdien til *F. graminearum* isolatene 200 630, 201 196 og 202 058, var henholdsvis 6.36, 9.50 og 7.62 mg/L. EC_{50}-verdiene for *M. nivale* isolatene 200 136, 200 231 og 202 786, var henholdsvis 8.60, 10.83 and 10.27 mg/L. For *H. avenae* var EC_{50}-verdien på 5.66 mg/L. Dette forklarer at det er en forskjell i EC_{50}-verdi mellom nematoder og sopp, men også mellom sopp arter og sopp isolater.

Et lukket glasskrukke-forsøk ble gjort for å se på om innblanding av korsblomstra vekster kan påvirke overlevelsen av *H. avenae* and *F. graminearum.* Glasskrukkene med sopp og nematoder ble enten behandlet med to uker eller åtte uker i glasskrukker, satt i et vekstrom. Cystene fikk enten en diapause eller ingen diapause behandling etter at glasskrukke forsøket. Det totale antallet av juveniler (J2) og egg var signifikant lavere for cyster som fikk åtte uker i glasskrukker, sammenlignet med to uker i glasskrukker, men totalt antall egg og J2 var nesten likt mellom diapause og uten diapause behandling. Antall av J2 som klekte etter 21 dager (15°C) var signifikant høyere for diapause behandling sammenlignet med uten diapause. Samtidig var antallet av J2 som hadde klekt signifikant høyere for to uker i glasskrukker, sammenligna med åtte uker i glasskrukker. Overlevelsen av *F. graminearum* på havreskall inkorporert i jord med plantemateriale hadde ingen forskjell i forhold til kontrollen.

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1. Introduction

1.1 Cereal production

Cereal grains (*Cerealis*) play a major role in human food consumption. The first signs of cereal cultivation are known to be in the Middle East, about 11 000-12 000 years ago (Zohary et al., 2012). Within cultivated areas in the world, 70 % is devoted to cereal production (McDonald & Nicol, 2005). Wheat is one of the three most important cereals, followed by rice and maize (Awika, 2011), and is an important crop for daily human food consumption. As a food source, wheat accounts for about 20% of human calorie intake (Shiferaw et al., 2013). By 2050, it is estimated that the demand for cereals for human consumption will increase due to an overall growth in population globally (Dixon et al., 2009).

In Europe, cultivation of cereal crops started with wheat (*Triticum* spp.), flax (*Linum usitassium*), barley (*Hordeum* spp.) and vetch (*Vicia* spp.) (Soltvedt, 2020). In the Mediterranean countries, the agricultural history dates back 8000 years, and today wheat is the most consumed cereal crop in this area (Mefleh, 2021). Statistics from the European Commission show that wheat (46.7 %), grain maize (19.6 %) and barley (19.2 %) are the most grown cereal crops in Europe in 2022 (European commission). Climate changes are predicted influence precipitation and drought and cause higher winter temperatures, which will affect the cereal cultivation negatively with reduced yield and yield potential (Olesen et al., 2011). Especially in Southern Europe farmers may experience water shortage and enhanced extreme weather conditions which can lead to reduction in areas suitable for cultivation, resulting in yield losses (Olesen & Bindi, 2002).

In Norway, cultivation of cereal crops began approximately 7000-8000 years after the domestication in the Middle east, and examples of native cereal species are emmer and barley (Soltvedt, 2020). Norwegian cereal production is centred in the southern part of Norway, especially around the Oslofjord, the Lake Mjøsa and the Trondheimsfjord (Henriksen & Korsæth, 2013). From 1950 to 2005 the number of farms in Norway have decreased by 75 % due to economic challenges, but also due that small farms are merged to make bigger farms. During the same time period, the number of farmers were also reduced due to the emergence of larger farms with more area, and new technology which could do the work more efficiently (Ladstein & Skoglund, 2008). In these areas, cereals such as wheat, oat, rye, and barley are grown. Wheat, oats and barley are predominantly cultivated for domestic production as animal feed, but some are produced for human consumption (Kosiak et al., 2003). From 2018 to 2023

the total Norwegian cereal production increased by 5.7 %, where wheat and oats increased by 24.9 and 14.3 %, respectively (SSB).

In addition to the short growing season and cold winters, the main challenge in Norwegian cereal production is limited suitable land for optimal cultivation (Skjelvåg, 1998). In the Nordic countries, a result of climate change could increase crop productivity, expanded areas suitable for cereal cultivation and introduce new crop species due to more precipitation and higher temperatures. On the other hand, the predicted increase in frequency of extreme weather events causing plant stress, changes in the pattern of pests in addition to introduction of new pest species may increase the yield loss potential (Olesen & Bindi, 2002). Due to climate change, limited cultivar availability and monocultural cereal production in Norway, infection of cereal plant pathogens may increase. Cereal cyst nematodes (*Heterodera avenae),* and fungal pathogens (*Fusarium graminearum* and *Microdochium nivale*) are important pests who cause yield losses and affect cereal quality.

1.2 Plant Parasitic Nematodes

Nematodes are a large group of unsegmented roundworms found in all environments, such as soil, sea, freshwater, plants, and trees. Soil living nematodes including free-living nematodes, predators, fungal-, bacterial-, and plant parasitic nematodes (PPNs) (Magnusson, 2020). PPNs are well known as soil borne pests which attack the roots of a wide range of crops, including oats, wheat, barley, maize, rice, potato, vegetables and oil crops (Nicol et al., 2011). Globally, the estimation of cereal yield losses due to PPNs is 14 % (Mesa-Valle et al., 2020).

The bodies of all PPNs are cylindrical and elongated and are characterized as a "tube within a tube", with a microscopic size (Bernard et al., 2017). The size of PPNs ranges from 250 µm to 12 mm in length, and the width ranges between 15 and 35 µm (Kumar & Yadav, 2020). All PPNs have a stylet in common, which they use to penetrate the host root cells to get nutrition (Magnusson, 2020). Among the top ten PPNs having an economic and scientific interest, are the root-knot nematodes (*Meloidogyne* spp.*)*, followed by the cyst nematodes (*Heterodera* spp. and *Globodera* spp.) and root lesion nematodes (*Pratylenchus* spp.) (Jones et al., 2013). Among the cyst nematodes, cereal cyst nematodes (CCN) (*Heterodera avenae*) are the third most damaging globally, after soybean cyst nematodes (*Heterodera glycines*) and potato cyst nematodes (PCN) (*Globodera pallida* and *G. rostochiensis*) (Nicol et al., 2011). Interaction between nematodes and host plants are complex (Phani et al., 2021). Nematodes have different ways of feeding on the root. Ectoparasitic PPNs feed on the root tips, for instance *Rotylenchus*

(Jones et al., 2013). While the endoparasites either the nematodes migrate within the host roots or are sedentary. Root lesion nematodes such as *Pratylenchus* species are migratory endoparasitic. The cyst nematodes, such as PCN and CCN are sedentary endoparasites, entering the host root after which the female nematodes establish a specialised feeding site (Magnusson, 2020).

1.2.1 *Heterodera* species

The cereal cyst nematode (CCN), *Heterodera spp.,* are key PPN destroying roots of the most important cereal crops (Nicol et al., 2011). Examples of species in the *Heterodera* group are *Heterodera avenae, H. latipons, H. filipjevi, H. schachtii, H. glycines* and *H. gotland* (Smiley & Nicol, 2009) Worldwide, CCN are considered to have an economic importance in cereal production. *Heterodera avenae* (oat cyst nematode) appears to be the most damaging, causing severe yield losses in cereal production (McDonald & Nicol, 2005).

The host plant of CCN is limited to the grass family, graminaceous plants such as oat, barley, spring wheat, some grass species and grass weeds (Toumi et al., 2018). Symptoms of CCN in a highly infected field, can be seen already at the phenological development stage 13 (BBCH 13) where the cereal plants have developed three leaves (Meier, 2001; Vennatrø, 2023). Early field symptoms are poor establishment of the cereal seedlings. Later, CCN damage appears as patchy areas with stunted plants (Figure 1 A, B). Reduced competitiveness of the cereal plant will often result in more weeds in the infected patches (Figure 1 C) (Smiley & Nicol, 2009). Another symptom of *H. avenae,* is "bushy" root growth (Figure 1 D). Later in the growing season (BBCH 31-51), females may be observed as white dots on the roots (Magnusson, 2020). Symptoms of CCN look similar to other plant stresses, such as drought stress and nutrient deficiency. Therefore, in many cases, the first identification of CCN is often overlooked or the CCN problem is not identified at all (Magnusson, 2020).

Heterodera species are found mostly in temperate regions, including Western USA (Smiley, 2009), Spain (Romero et al., 1973), Turkey (İmren et al., 2016), Italy (Greco & Brandonisio, 1987), Germany (Sturhan, 2014) and China (Wu et al., 2014). In the Nordic countries, there are reported damage of *H. avenae* and *H. filipjevi* (Andersson, 2018). In Norway, *H. avenae,* pathotype Ha11 and Ha12, are the most economically important *Heterodera* species, reported in all cereal growing areas (Holgado et al., 2009). Another *Heterodera* species detected in Norway is *H. filipjevi* (rye cyst nematode), mainly in Vestfold and Østfold (Holgado et al., 2003). Nationally, CCN can result in a yield loss of €3-15 million per year, in an infected cereal field, yield loss of over 50% have been reported (Holgado et al., 2003).

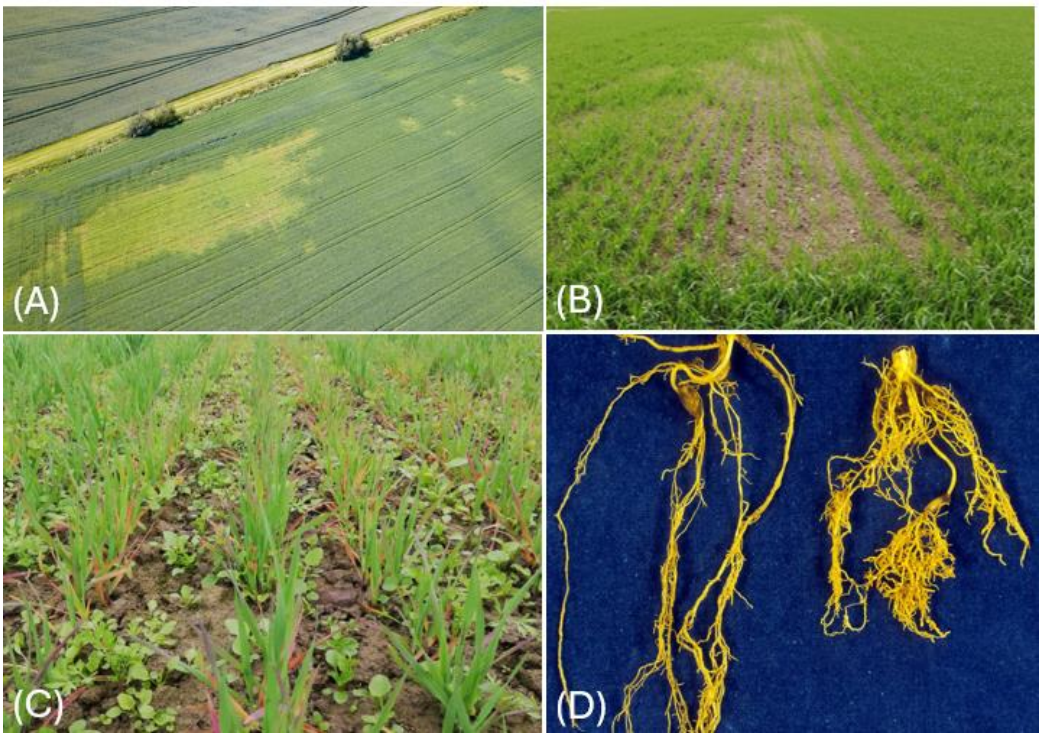


Figure 1: Symptoms of cereal cyst nematodes in field in Norway and symptoms on roots (Picture credit: (A) Erling Fløistad (NIBIO), (B, C) Marit Skuterud Vennatrø (NIBIO), and (D) Bonsak Hammeraas (NIBIO)).

1.2.2 Life cycle of CCN

The life cycle starts with a cyst containing eggs (Figure 2). The number of eggs inside one CCN cyst varies between 200 and 300 eggs (Brandsæter et al., 2009). The eggs inside the cyst will develop to a first-stage juvenile (J1), and thereafter moult to a second juvenile stage (J2) (Toumi et al., 2018). When the weather conditions are preferable for hatching, the J2 uses the stylet to cut through the eggshell and leaves the cyst. The J2, is the infective stage of the life cycle and will start the search for a host plant to infect. The J2 induce the host plant, and secrete several proteins and enzymes in the host to form a giant cell, called syncytium (Toumi et al., 2018). These secretions can lead to suppression of the plant defence system and cell wall degradation (Ali et al., 2017). Formation of syncytium in roots of the host plants, is a parasitic adaptation the cyst nematodes have evolved (Moens et al., 2018). The syncytium contains all the nutrients the cyst nematodes need to develop from J2 to third-stage juveniles (J3) (Smiley et al., 2017). The female swells and moult to fourth-stage juveniles (J4). Males and females (J4) develop together in the same root (Magnusson, 2020). The J3 females exude sex pheromones to attract the males. After mating the females continue feeding and developing of eggs. Males are non-feeding, and do not live for a long time after leaving the root. After a while, the female will die, and the dead body turn into a cyst that will encapsule and protect the eggs. The cysts are

full of eggs, and after falling off the roots they become dormant. Then they overwinter in the soil, protecting the eggs until next growing season (Moens et al., 2018).

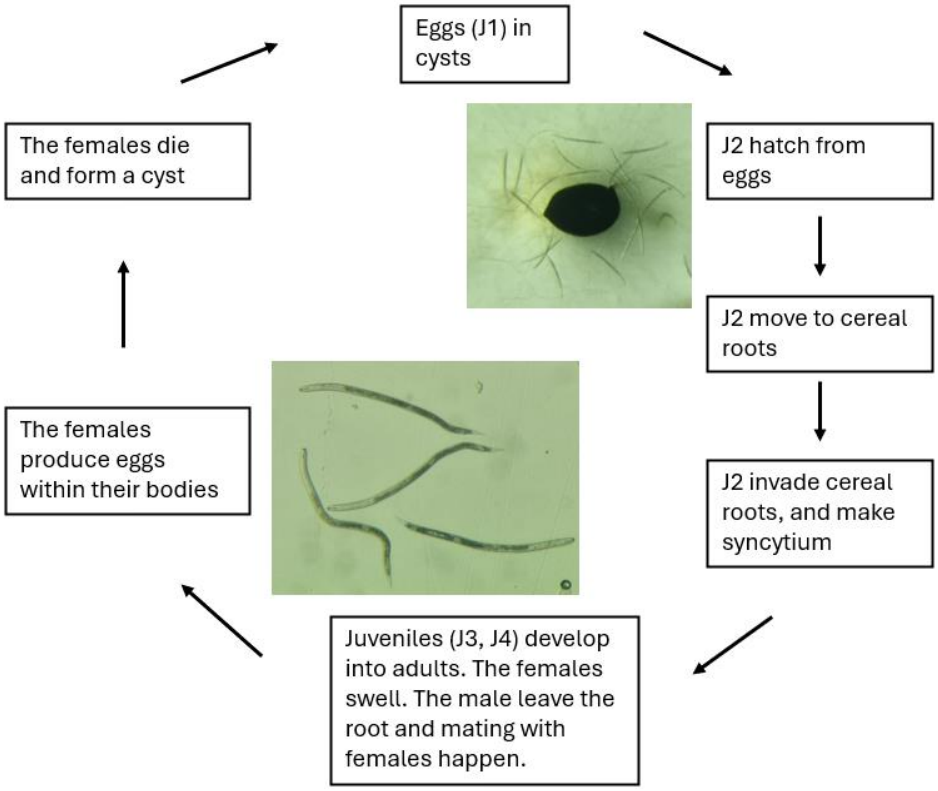


Figure 2: The life cycle of cereal cyst nematode (*Heterodera* spp.) adapted from Magnusson (2020). (Photos: Emma Skogstad).

1.2.3 Environmental factors affecting hatching of CCN

Temperature is the main environmental factor influencing hatching of CCN (Baklawa et al., 2017). Temperature requirements are widely distributed among geographically regions. Rivoal (1986) found that optimal temperatures for hatching of a French population of *H. avenae* is between 10ºC and 15ºC. A temperature and storage experiment, done by Baklawa et al. (2017), with two CCN populations, one German and one Egyptian population, a difference in optimal hatching temperature was observed, the German population had higher J2 hatching between 5ºC and 10ºC, whereas the Egyptian population between 10ºC and 15ºC. CCN have an ability to enter a dormant stage, called diapause, when the environmental conditions are unfavourable for hatching. Diapause in CCN can be obligatory, programmed in the life cycle. Otherwise, the diapause can be facultative, also called quiescence, which is trigged by unfavourable environmental conditions (Smiley et al., 2017). In a Canadian population an obligatory diapause of minimum eight weeks was needed for the hatching of *H. avenae* (Fushtey & Johnson, 1966). On the other hand, for a Danish *H. avenae* population the diapause was broken when the winter

cysts were placed at suitable temperatures for hatching, and hatch in the spring when the temperature rises (Juhl, 1968). For the Danish populations, the cysts have a diapause during the winter. In this case, a Mediterranean *H. avenae* populations may hatch during the winter, whereas Northern populations are active during spring (Evans & Perry, 1976). No experiments on temperature requirements for Norwegian CCN populations have been done. In Sweden, Andersson (2018) studied a *H. avenae* population, and observed no hatching of eggs when the soil temperature was lower than 4ºC.

1.2.4 Management of CCN

To manage CCN, knowledge about the biology is important, including life cycle, susceptibility of host plants, host plant range and the damage threshold (Magnusson, 2020). Cereal monoculture is one reason for the increasing CCN problem, and a density of *H. avenae* more than three eggs per gram soil, is capable of reducing the yield of susceptible barley, and for susceptible wheat the tolerance is even lower: one egg per gram soil (Andersson, 1982). In cereal production, one important management strategy for controlling CCN is a well-planned crop rotation including resistant varieties and non-host plants (Holgado et al., 2006). One year with a non-susceptible cultivar can reduce the CCN population by 60-80% under Norwegian conditions (Fløistad & Munthe, 2007). In Norwegian farming there are few resistant cereal cultivars available, and only two-row barley cultivars are resistant to CCN (Fløistad & Munthe, 2007). Norwegian farmers, implementing non-hostplants in their crop rotation, got an increase in cereal yield of 1000 kg/ha on average (Holgado et al., 2006). CCN cysts are spread by machinery, therefore cleaning of machinery is important in avoidance of spreading CCN between field or farms (Magnusson, 2020). Chemical nematicides, like methyl bromide is not an alternative in CCN management. This nematicide was banned since 2006 due to negative impacts on the environment (Ntalli & Caboni, 2017). In Norway there are no nematicides on the market hence use of nematicides is not an alternative control strategy currently.

1.3 Fungal plant pathogens

Fungal plant pathogens are a numerous group within plant pathogens (Termorshuizen, 2017). Relationship between plants and fungi could either be endophytic, saprotrophic, parasitic or mutualistic (Jayawardena et al., 2021). The global yield loss due to pests and pathogens are estimated to be around 21.5 % (Savary et al., 2019). Fungal pathologists with an association to the journal *Molecular Plant Pathology* nominated the "Top 10" fungal pathogens based on scientific and economic importance in molecular plant pathology. Fungal genus *Fusarium* were ranked as number four (*Fusarium graminearum)* and five (*F. oxysporum)* (Dean et al., 2012).

1.3.1 *Fusarium* head blight

Fusarium head blight (FHB) is a common cereal disease. Within this disease-complex, *Fusarium graminearum* is often the most dominant species (Dweba et al., 2017). Other species which are a part of the FHB complex are for instance *F. culmorum*, *F. avenacum, F. poae* and *Microdochium nivale* (Ioos et al., 2004). Host plants of FHB are for example rice, gras, oat, maize, wheat, and barley (Goswami & Kistler, 2004). One symptom of FHB in a wheat field is bleaching of spikelets, which can develop to premature white wheat heads. Another symptom on wheat is appearance of pinkish mycelium on infected head during humid and warm weather (Figure 3 B). Symptoms of FHB on wheat and barley in the USA are low grain weight and shrunken and discoloured grains. Due to production of mycotoxins, reduced grain quality may be a consequence (McMullen et al., 2012). Other symptoms in barley and oats, may be seen as browning on the spikelets, also observations of orange spore accumulations may happen on the spikelets, typically in the case of a *Fusarium* infection (Figure 3 A, C) (Brandsæter et al., 2009).

Microdochium nivale, is also a part of the FBH disease complex, causes symptoms that are visually identical to *Fusarium*, and share some hostplants, but *M. nivale* also attack turf, and forage grasses and winter cereals (Simpson et al., 2001). In contrast to *Fusarium* species, *M. nivale* causes pink snow mould, and the fungi can survive the winter on the host plants. Due to winter survival *M. nivale* could appears as pinkish-white mycelium immediately after snowmelt (Tronsmo et al., 2001).

Yield reduction due to FHB attacks, may reach up to 50% globally (Ashiq et al., 2022b). From 1998 to 2002 the wheat yield loss, caused by FHB, was about $2,7 billion in Northern and Central America (Nganje et al., 2002). The last decades, an increase in occurrence of FBH-disease is related to a zero or reduced tillage regime in cereal production and monoculture and a higher frequency of wheat in the crop rotation (Alisaac & Mahlein, 2023). *F. graminearum* is

reducing the grain quality, more than reducing the grain yield (Dean et al., 2012). In Southern Norway, the *Fusarium*-contamination was high in 2009, and an increase in mycotoxins content were expected, especially in spring wheat, but also for oats and barley. Infection of *Fusarium* species this year, led to an elevated amount of seed lots not suitable for seeds production, due to poor germination (Kimen). In 2012, the amount of *Fusarium* in Southern Norway was highest in oats, but also for barley and spring wheat. On the other hand, in 2020 the *Fusarium* contamination was highest for barley and spring wheat (Kimen). Norwegian studies have demonstrated that deoxynivalenol (DON) is the most prevalent mycotoxin in oats and spring wheat often caused by *F. graminearum*. Likewise, detection of the mycotoxins HT-2 and T-2 were most frequent in Norwegian oats, often infected by *F. langsethiae* (Hofgaard, I. et al., 2016; Hofgaard et al., 2020; Langseth & Rundberget, 1999).

Figure 3: Symptoms of Fusarium Head blight in oat (A) and wheat (B, C). (Photo: (A, B) Jafar Razzaghian (NIBIO) and (C) Emma Skogstad).

1.3.2 Disease cycle of FHB in oat

Fusarium species survives on crop debris and in soil as saprophytic mycelium or spores (Leplat et al., 2013). Mostly, asexual spores (conidia) are produced, but *F. graminearum* are known to produce sexual spores (ascospores), and these ascospores are airborne (Brandsæter et al., 2009). Stages in the disease cycle of *Fusarium* species, are presented in figure 4 (A-G). After survival in soil or on crop debris (A), conidia spores from crops debris can lead to seedling blight (B). Then *F. graminearum* may spread airily as ascospores (C). Conidia can also be spread along the plant in humid weather conditions (D) and infect the oat spikelets (Head blight) (E). Roots can also be infected by *Fusarium* species (F). If the seeds are infected, it can lead seedling blight (G) (Brandsæter et al., 2009). Wheat is most susceptible for *F. graminearum* infection from anthesis until kernel development (McMullen et al., 2012). Rainfall with warm temperatures at

anthesis may increase the FBH-contamination (Hjelkrem et al., 2021). In seeds, *Fusarium* species can be found as mycelium or as spores, which can lead to poor germination of infected seeds (Brandsæter et al., 2009).

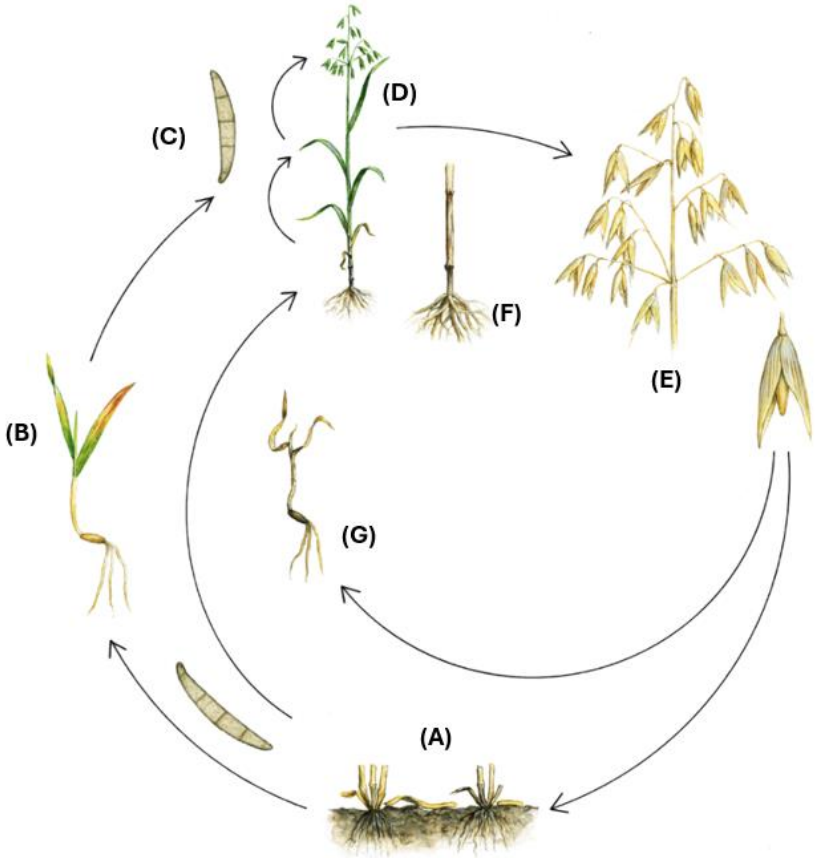


Figure 4: Stages of the disease cycle of *Fusarium* head blight species in oat. (A): fungi survive on crops debris and soil. (B): Seedling blight. (C): ascospores are spread airily. (D): conidia spores spread along the plant in humid weather. (E): Head blight. (F): Infected root. (G): seedling blight. Adapted from Brandsæter et al. (2009).

1.3.3 Mycotoxins

Several *Fusarium* species are known to produce mycotoxins in cereal grains, but also in spice crops, forage grasses, soybeans and other legumes (Munkvold, 2017). The production of mycotoxins can lead to reduced grain quality for cereals used as food and feed. Mycotoxins may have negative impacts on human and animal health (Cromey et al., 2001). *F. graminearum* is an example of fungus from the FHB-complex producing mycotoxins in grains, especially deoxynivalenol (DON), but also zearalenone (ZEA) and nivalenol (NIV). In contrast, *M. nivale* is not known to produce any toxic metabolites (Gagkaeva et al., 2020). In Norwegian cereals, *F. graminearum* seems to be the most prevalent producer of the mycotoxin DON in spring wheat and oat, and HT-2 and T-2 were mainly produced by *F. langsethiae* in oats (Hjelkrem et al., 2018; Hofgaard, I. et al., 2016). T-2 can deacetylate and form HT-2 and would therefore often

occur together (Brodal et al., 2020). In a Norway field trial, at Solør, high levels of *Fusarium* DNA and mycotoxins levels in wheat grains were correlated with the percentage of *Fusarium* infected crop debris (Hofgaard et al., 2020). Other studies from Norway, showed a higher occurrence of mycotoxin contamination, especially *F. graminearum,* in oats compared to other cereal species (I. Hofgaard et al., 2016; Langseth & Elen, 1997; Langseth & Elen, 1996). In Norway, the maximum mycotoxins levels in cereals for food are regulated by The Norwegian Food Safety Authority (Mattilsynet).

1.3.4 Management of FHB in cereal production

Integrated plant management (IPM) is one important strategy in controlling FHB. A well-planned crop rotation, using cultivars with high fungal resistance can be sufficient to reduce the impact of FHB disease (Alisaac & Mahlein, 2023). One way to reduce FHB disease, is burying the crop debris infected by *Fusarium* species deeper in the soil (Leplat et al., 2013). In Norway, reduced tillage practises have enhanced the occurrence of FHB and mycotoxin contents in cereal production (Hofgaard et al., 2020; Tørresen et al., 2012). Therefore, a management strategy against FHB is tillage. Norwegian field plots with ploughing compared to harrowing, showed that plots with ploughing reduced the inoculum potential of *Fusarium* spp. (Hofgaard, I. S. et al., 2016). Infection by *F. graminearum* is greatest at anthesis, the susceptible developmental stage, and at this stage the yield loss is highest (Dean et al., 2012). Chemical control by fungicides, mainly triazoles, have been the main strategy to control *F. graminearum* but due to reduced effectiveness and fungicide resistance other management strategies should be implemented (Klix et al., 2007; Yerkovich et al., 2020). Due to reduced effectiveness of fungicides, a combination of chemical controlling methods and other IPM strategies is therefore most efficient. Applying fungicides have shown to be most efficient at anthesis and ten days after anthesis (Alisaac & Mahlein, 2023). In a field trial, spraying with fungicide at flowering (BBCH 51+65) had best effect in controlling FHB compared to chemical treatment at BBCH 39 (Chala et al., 2003). IPM with crop rotation, resistant cultivars and applying fungicides at flowering may be efficient in controlling FHB-disease.

1.4 Biofumigation

Cruciferous plants in the family *Brassicaceae*, contain glucosinolates (GSL), which is a volatile chemical compound and have a potential in controlling plant pathogens (Kirkegaard & Matthiessen, 2004). Species from *Brassicaceae* are for instance rapeseed (*Brassica napus*), black mustard (*B. nigra*) and brown mustard (*B. juncea)* (Vaughn & Boydston, 1997). Biofumigation refers to a method where plant materials from specific plants, mainly cruciferous

plants, is incorporated into the upper part of the soil (Kirkegaard & Sarwar, 1998). Biofumigation is a strategy of controlling plant pathogens such as nematodes, weeds, and fungi (Sarwar et al., 1998). The endogenous enzyme myrosinase, located in guard cells, myrosin cells or cells along the phloem in cruciferous plants, hydrolyses GSL, for instance during tissue damage or herbivory, then glucosinolate hydrolysis products (GHPs) are produced. Examples of GHPs are isothiocyanates (ITC), thiocyanates and nitriles. The GLS sinigrin is hydrolysed to 2-propenyl, also called allyl ITC(Hanschen et al., 2015).The group of ITCs, contains several chemical compounds such as allyl ITC, 3-Butenyl ITC, 3-Methylthionpropyl ITC and Phenethyl ITC (Liu et al., 2020).

Several studies have been done to investigate the effect of biofumigation on several nematode- and fungal species. In a field trial, *Brassica juncea* reduced the population of potato cyst nematode in the UK (Ngala et al., 2015). In an *in vitro* study of *Globodera pallida,* an allyl ITC concentration of 50 ppm significantly increased the juvenile mortality and suppressed the hatching of cysts (Wood et al., 2017). Another isothiocyanate, benzyl ITC, had a strong effect on *Meloidogyne hapla,* when exposing the J2 to benzyl ITC for 24 hours, the juvenile mortality was 100 % even at det lowest concentration (0.1 µmol/mL) (Dahlin & Hallmann, 2020). In England, Ashiq et al. (2022a) conducted an experiment adding chopped *Brassica* spp. in jars with soil and inoculum bags containing *F. graminearum* infected blind oat spikelets as artificial crop debris. This experiment showed a suppression of *F. graminearum* mycelial growth, compared to jars without plant material. Another study showed that six ITCs reduced the mycelial growth of five cereal root pathogens (Sarwar et al., 1998). A study in the USA, showed that allyl-ITC and methyl-ITC had the highest inhibitory of seed germination of weeds and had a suppressive effect on weeds in a combination with reduced herbicides usage and tillage (Vaughn & Boydston, 1997).

Cruciferous plants may be valuable as a cover crop performing several important ecosystem services as well as performing a role in the biofumigation of important crop pests and diseases. Cover crops, have shown to reduce soil erosion, preservation of soil moisture, give better soil health, reduce evaporation from soil and improving soil structure by improvement the organic matter, as well as a richer biodiversity in the soil (Ntalli & Caboni, 2017).

1.5 Knowledge gaps

The global human population is increasing, which gives a higher food demand. Every year, a proportion of the cultivated cereals is attacked by pests and therefore discarded. With climate change the plant pathogen situation may be altered. Improving new strategies of pathogen management, such as biofumigation and other biological managements strategies are important. In that case, aiming more research-based knowledge about biological management is valuable, for instance for control of nematodes and fungi. Due to Norway's locations in Northern part of the world, an increase in temperature can lead to higher mean temperatures and new species can be introduced. On the other hand, this can lead to a higher demand for pesticides and knowledge. In cereals, nematodes and fungi are important plant pathogens, and cause yield losses all over the world. There are no nematicides available for Norwegian farmers, and the solution in control of nematode is a well-planned crop rotation with resistant and non-host cultivars. In control of FHB the effectiveness of pesticide may be reduced. Therefore, use of cruciferous plants in controlling nematodes and fungi has an investigative potential. The interaction between biofumigation and plant pathogens is studied to some extent in other parts of the world, but biofumigation practices in cereals are less studied in the Nordic countries. For cereal cyst nematodes (*H. avenae)*, studies with biofumigation are few, while several studies have been done on potato cyst nematodes. However, the relationship between Fusarium species and cruciferous plants are studied to a greater extent. More knowledge is needed on the relationship between biofumigation and plant pathogens in Norway.

1.6 Aim

The aim of this study was to identify cruciferous plants having a potential to suppress the survival and viability of fungi and nematodes in crop debris and soil.

1.7 Hypotheses:

1. High concentrations of allyl isothiocyanates may suppress the hatching of *Heterodera avenae*.
2. High concentrations of allyl isothiocyanates may suppress the mycelial growth of *Fusarium graminearum* and *Microdochium nivale*.
3. Incorporation of plant material from selected cultivars of cruciferous plant species into soil reduces the survival of *Fusarium graminearum* and *Heterodera avenae.*

2. Materials and methods

2.1 Plant material.

A selection of cultivars (Table 1) from plants within the family *Brassicaceae* were sown on 19th of July 2023 in a field at Øsaker, south of Ås (Norway). Each cultivar was grown in three replicate plots (1.5 x 7 m). In general, cover crops are sown during late summer and early autumn, and some of them are already grown in Norway.

Brassica carinata is a species not grown in Norway. This species has Ethiopian mustard and Ethiopian rape as common names and is known to produce sinigrin, a glucosinolate which may be converted into allyl ITC by hydrolysis (Plaszkó et al., 2021). Freudenberger market it as a catch crop (Undercover). *Brassica juncea* is also a mustard plant, and commonly known as brown mustard, Chinese mustard, Indian mustard, leaf mustard, Oriental mustard, and vegetable mustard. In our experiments we refer to this species as brown mustard. Like *B. carinata, B. juncea* also produces allyl ITC. The German seed company P.H Petersen, market a *B. juncea* cultivar called Energy, which is sold for biofumigation. Terminator marketed at Freudenberger is another *B. juncea* cultivar which is marketed as a fast-growing catch crop who suppresses weeds.

Raphanus sativus var. *oleiformis* have several common names, such as oil radish, fodder radish and oilseed radish. In our experiments we refer to this species as oil radish. The oil radish cultivars Stinger and Siletina are marketed by Norgesfôr web page, and are grown in Norway (Plantekultur). Norgesfôr has several seed mixtures containing forage tillage radish together with other cover crops such as *Vicia sativa* (common vetch) and *Phacelia tanacetifolia* Benth (Lacy phacelia). Felleskjøpet is another seed company who is marketing oil radish cultivars in their cover crop assortment (Larsen, 2024). *Raphanus sativus* var. *longipinnatus* is also called forage radish, Daikon radish, oriental radish, tillage radish and tillage radish. We refer to this species as forage radish. Norwegian Agricultural Extension Service have both forage radish as Structurator and an oil radish cultivar named Ikarus (Valand).

Sinapis alba is known as white mustard. and Albatros and Action are the cultivars of the species *S. alba.* Action is marketed by the seed company Freudenberger and is claimed to be beet cyst nematode resistant. Botanical powder by *S. alba* as was also shown to suppress fungal growth of *F. graminearum* in a laboratory assay (Drakopoulos et al., 2020).

Table 1: A list of the cruciferous plants grown at Øsaker, the seed producer and isothiocyanate (ITC) released if known.

Cultivar	Species (Latin)	English name	Seed company	ITC released**
Undercover*	*Brassica carinata*	Ethiopian mustard	Freudenberger	Allyl ITC
Energy*	*Brassica juncea*	Brown mustard	P.H. Petersen	Allyl ITC
Terminator	*Brassica juncea*	Brown mustard	Freudenberger	Allyl ITC
Ikarus*	*Raphanus sativus* var. *oleiformis*	Oil Radish	Strand Unikorn	
Akiro*	*Raphanus sativus* var. *oleiformis*	Oil Radish	P.H. Petersen	
Defender	*Raphanus sativus* var. *oleiformis*	Oil Radish	P.H. Petersen	
Siletina*	*Raphanus sativus* var. *oleiformis*	Oil Radish	Stand Unikorn and P.H. Petersen	
Siletta Nova	*Raphanus sativus* var. *oleiformis*	Oil Radish	P.H. Petersen	
Stinger	*Raphanus sativus* var. *oleiformis*	Oil Radish	Strand Unikorn	
Structurator*	*Raphanus sativus* var. *longipinnatus*	Forage Radish	Strand Unikorn	
Albatros	*Sinapis alba*	White mustard	Strand Unikorn	
Action*	*Sinapis alba*	White mustard	Freudenberger	

*Cultivars used in the closed jar experiment. **Confirmed by Marit Almvik and Hans-Ragnar Norli (NIBIO).

2.2 *Heterodera avenae* population

H. avenae cysts used in these experiments were collected at Ridabu, close to Hamar (Norway), and naturally *H. avenae* infected soil was collected after cereal harvest. In 2021, symptoms of *H. avenae* within the field was observed, soil samples were analysed, and *H. avenae* were detected (Personal communication Stig Rune Linde Herbrandsen). In 2023 we sampled by taking soil cores randomly down to about 15 cm and a complete sample was placed in plastic bags. The soil was taken back to the lab and stored at about 4°C for four weeks. *Heterodera avenae* cysts were extracted by using the Vye-column (Southey, 1986). Cysts of the same dark

brown colour, assuming to contain eggs, were chosen for the nematode experiments, and light-coloured likely empty cysts were discarded. Cysts from the Ridabu-population were used in all the experiments with nematodes.

2.3 Fungal isolates

Fungal isolates of *F. graminearum* (ID number 200 630, 202 058, 201 196) and *M. nivale* (ID number 202 786, 200 231, 200 136) from the isolate collection at NIBIO Ås were used in these experiments. Table 2 shows the different isolates, the year of isolation and which host plant they were isolated from. Before use the isolates were cultivated on Potato Dextrose agar (PDA) in growth cabinets at 20°C, in darkness, and for three days. All these isolates were used in the experiment of investigation on the effect of allyl ITC on fungal growth. Only *F. graminearum* isolate number 200 630 was used in the closed jar experiment.

Table 2: Description of the fungal isolates of *Fusarium graminearum* and *Microdochium nivale* used in the experiments.

Fungal species	Isolate number	Year of isolation	Isolated from
F. graminearum	200 630*	2006	Wheat
F. graminearum	201 196	2012	Wheat
F. graminearum	202 058	2013	Wheat
M. nivale	200 231	1996	Perennial ryegrass
M. nivale	202 786	2017	Wheat
M. nivale	200 136	1986	Perennial ryegrass

**F. graminearum* isolate used in the closed jar experiment.

2.4 Effect of allyl ITC on hatching of *H. avenae*

A pilot experiment was done to assess a suitable method for testing the effect of allyl ITC on hatching off eggs from *H. avenae* cysts. Three concentrations of allyl ITC were selected from an invitro experiment reported by Ashiq et al. (2022c). Water agar (6g/L) was used as the medium, where allyl ITC were added to obtain the selected concentrations: 1.2, 19.5 and 312.5 mg/L, and untreated (0 mg/L). The water agar with the various concentrations was poured into Petri dishes (3 and 9 cm diameter). CCN were added to Petri dishes; one cyst in the 3 cm Petri dishes and three cysts in the 9 cm Petri dishes. Six replications per concentration of allyl ITC were use, and three replicates per Petri dish size. The Petri dishes were placed at room temperature (~20°C) in darkness. Number of hatched J2 were counted after four, seven and nine days of exposure to allyl ITC.

To calculate the EC50 value of *H.* avenae after exposure to allyl ITC, the pilot experiment was expanded. Six concentrations of allyl ITC were prepared: 1.2, 4.9, 19.5, 78, 312.5 and 1250 mg/L, in addition to untreated (0 mg/L) poured into water agar (6 g/L) and transferred to Petri dishes (9 cm). Three cysts were added to each Petri dish. For each concentration there were six replications. The Petri dishes were sealed with Parafilm and placed in plastic bags in darkness at room temperature (~20°C). The Petri dishes with the same concentration were placed in the same plastic bag. The number of J2 hatched from the cysts were counted every third day for nine days. J2 from the previous counting were re-counted together with newly hatched J2. The experiment was repeated once.

2.5 Effect of allyl ITC on mycelial growth

To estimate the concentration of allyl ITC where fungal growth is inhibited by 50 % (EC50) for isolates of *F. graminearum* and *M. nivale*, an invitro experiment was performed. The experimental setup contained three isolates of *F.* graminearum and three isolates of *M. nivale* per concentration and three repetitions per isolate within one concentration (Table 2). Six concentrations of allyl ITC, as above, were added to potato dextrose agar (PDA) into 9 cm petri dishes, and an untreated (0 mg/L). Agar plugs (5 mm) from the periphery of the mycelial growth of *F. graminearum* and *M. nivale* were transferred to the PDA containing allyl ITC. The Petri dishes were sealed with Parafilm and incubated in a cabinet at 15°C in darkness for six days in total. Petri dishes with the same concentration, were placed in the same plastic bag. After one and six days of incubation, mycelial growth was registered by measuring the diameter of fungal colonies in two directions perpendicular to each other. The average mycelial growth (mm) and the growth rate (mm/day) were calculated. The growth rate was calculated between day one and day six. This experiment was performed three times in total.

2.6 Closed jar experiment.

To study the effect of cruciferous plants on survival of *H. avenae* and *F. graminearum,* nylon bags with *H. avenae* and *F. graminearum* were placed in jars with soil and plant material and incubated for either two or eight weeks.

2.6.1 Preparation of plant material

On the 5th of September, developmental stage (BBCH) and plant density (plants/m^2) were registered from the plants sown in Øsaker in July (Table 1). One or two representative plants of each cruciferous cultivar were taken back to the lab. Plant height and weight of root, the above ground part and three leaves (top, middle, lowest), and the height of the plant were measured. The cruciferous plants (Table 1) were harvested 13 weeks after sowing and prepared for start of closed jar experiment the same day. Nine crucierfours plants were selected (Table 3).

2.6.2 Soil medium

Two litre glass jars (34-2052-3) were filled with approximately one litre of soil containing 81% sand from Jula, 16.7 % clay from Norgo and 2.3% peat (Vekstjord fra Norgro), presented as percentage of dry weight per total weight (%). The pH of the soil was 7.40, measured by making a soil:water (1:2.5) fraction. After 24 hours of soaking the pH was measured by using a pH-meter (Sonmez et al., 2008). After another 24 hours in a drying cabinet at 105°C (O'Kelly, 2004), the percentage of dry weight was measured by using this formula mentioned by Margesin and Schinner (2005):

$$\text{Percentage dry weight (\%)} = \frac{weight\ after\ drying - weight\ of\ glass\ container}{weight\ before\ drying - weight\ of\ glass\ container}$$

2.6.3 Preparation of nylon bags with *H. avenae*

Ten cysts were placed in sealed nylon bags (7x6cm) (Amazon) (figure 5). The bags containing cysts, were stored for approximately one week at 4°C until the start of the experiment.

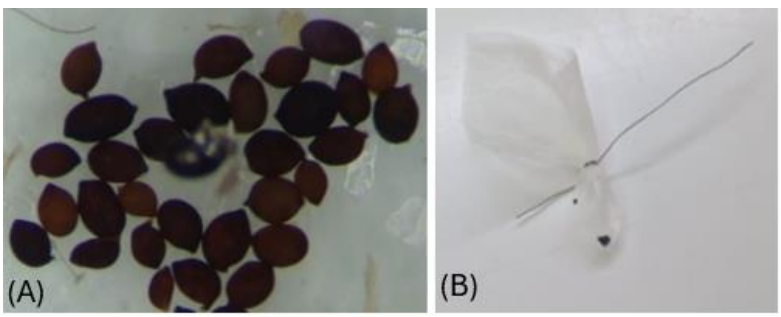


Figure 5: *Heterodera avenae* cysts from the Ridabu-population (A) and a nylon bag with ten *H. avenae* cysts inside (B) (Photo: Emma Skogstad (A, B)).

2.6.4 Preparation of nylon bags with *Fusarium graminearum*

Preparation of nylon bags with *F. graminearum* infected single-kernel oat spikelets was adapted from Ashiq et al. (2022b). The ratio between filled oats and blind oat spikelets (oat spikelets) was about 50/50. The average size of the spikelets was 3.8x12 mm. In two containers, 100 grams of oat spikelets were added to each. The oat spikelets were soaked in sterile distilled water for 18 hours and autoclaved for minimum 20 min at 121°C, and re-autoclaved after 24 hours (Ashiq et al., 2022b).

Isolate number 200 630 of *F. graminearum* was pre-cultivated on PDA in a growth cabinet at 20°C in darkness for three days. One agar plug (5 mm) from the periphery of the mycelial growth were transferred onto new Petri dishes (9 cm) containing Mung bean agar (MBA) and incubated at 20°C, 12 hours light+NUV (Leonard & Bushnell, 2003). After nine days incubation, the Petri dishes were washed with 5 mL sterile distilled water, and the spore suspension with a concentration of approximately 1 x 10^5 spores per mL was spread out on new MBA plates (9 cm) by using a sterile spreader. The MBA plates incubated for 10 days at 20°C at 12 h light + NUV, to increase the concentration of spores (Leonard & Bushnell, 2003). Into each of the two plastic bags with autoclaved oat spikelets, 10 mL of the spore suspension with a concentration of approximately 4 x10^6 spores per mL was added. The bags were incubated in darkness at 20°C for 12 days. Every second day the bags were agitated to homogenize the *F. graminearum* infection (Figure 6.A). In order to investigate the survival of *F. graminearum,* five oat spikelets were placed in each nylon bag (7x6cm) and stored at 20°C for 24 hours until the start of the experiment (Figure 6. B).

Figure 6: *Fusarium graminearum* infected oat spikelets (A). A nylon bag with five oat spikelets used in the closed jar experiment (B). (Photo: (A, B) Emma Skogstad)

2.6.5 Experimental setup.

The experiment consisted of a total of 20 different treatments including one untreated without plant materials as control, and nine with plant materials (Table 3), and two different time exposures, either two or eight weeks of exposure in the jars and five replications. A total of 200 jars were placed in five blocks, in a growth room at Kirkejordet Nord (Ås).

The cruciferous plants (Table 3) were rinsed with tap water, and the aboveground part and root were separated. Plant material was chopped into smaller pieces, to fit in the food processor (Ninja 3-i-1 blender BN800EU). Plant material was blended for approximately 15 seconds. In each jar, 15 grams of plant material was added to the soil and mixed well by hand (Figure 7.A). Nylon bags with either *H. avenae* or *F. graminearum,* were added into the middle of the jar covered with soil and the lids were closed (Figure 7.B).

Half of the jars contained two nylon bags with nematode cysts in each jar, the other half contained one nylon bag with oat spikelets infected with *F. graminearum* (Table 4). After two weeks in the growth room, half the jars with *H. avenae* and half of the jars with *F. graminearum* were taken out, and the effect of cruciferous plants were investigated. Eight weeks after the start of the experiment, the remaining half were taken out and survival registrations were similarly performed. In jars with *H. avenae* cysts, which had two nylon bags with cysts, one was placed directly at 15°C (no diapause treatment) for hatching, and the other nylon bag was placed at 4°C for 80 days (diapause treatment) before transferred to 15°C for hatching.

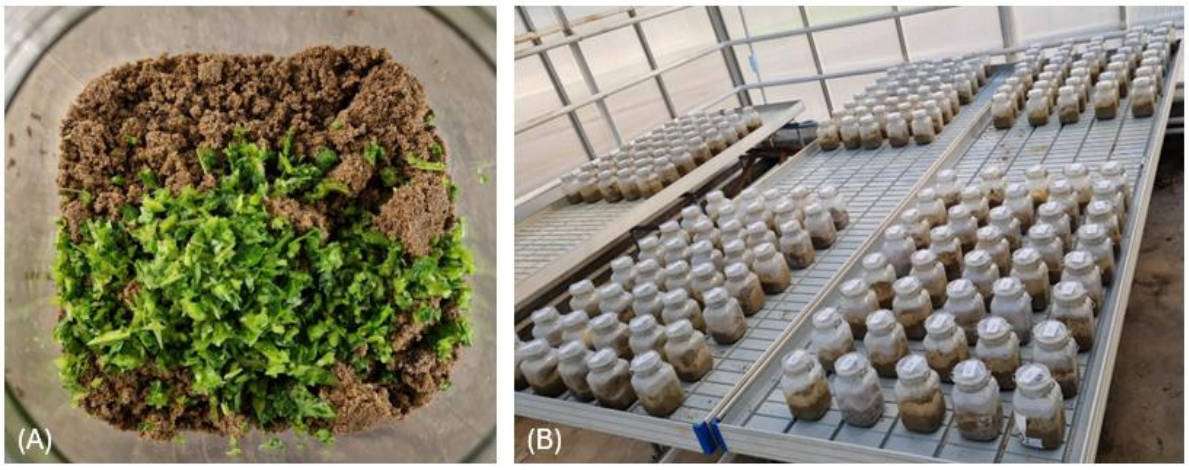


Figure 7: Chopped cruciferous plants in jars before incorporation (A), and 200 jars at the start of the closed jar experiment (B). (Photo: (A, B) Emma Skogstad).

Table 3: Cruciferous cultivars used in a closed jar experiment.

Treatment number	Treatment*	Plant part**	Species (Latin)	English name
0	Untreated			
1	Undercover	Aboveground	*Brassica carinata*	Ethiopian mustard
2	Energy	Aboveground	*Brassica juncea*	Brown mustard
3	Ikarus	Aboveground	*Raphanus sativus var. oleiformis*	Oil Radish
4	Akiro	Aboveground	*Raphanus sativus var. oleiformis*	Oil Radish
5	Ikarus	Root	*Raphanus sativus var. oleiformis*	Oil Radish
6	Siletina	Aboveground	*Raphanus sativus var. oleiformis*	Oil Radish
7	Structurator	Aboveground	*Raphanus sativus var. longipinnatus*	Forage Radish
8	Action	Aboveground	*Sinapis alba*	White mustard
9	Structurator	Root	*Raphanus sativus var. longipinnatus*	Forage Radish

*Treatment in jars containing soil and added plant material. **Chopped plant part added to the soil.

Table 4: Experimental setup for the closed jar experiment.

Pathogen species	Weeks in jars*	Post- jar treatment	Registering survival
Heterodera avenae	2 weeks	Diapause (80 days at 4°C)	21 days at 15°C (darkness)
		No diapause	21 days at 15°C (darkness)
	8 weeks	Diapause (80 days at 4°C)	21 days at 15°C (darkness)
		No diapause	21 days at 15°C (darkness)
Fusarium graminearum	2 weeks	None	Seven days at 20°C (12h light)
	8 weeks	None	Five days at 20°C (12h light)

*Duration of exposure for *H. avenae* and *F. graminearum* in jars containing soil with plant material.

2.6.6 Growth conditions in growth room

The jars were placed in a growth room at Kirkejordet Nord (Ås) with a temperature of 10°C (night) and 15°C (day) and no additional light. Six TINYTAGS, small devices measuring temperature and humidity, were placed on top of the soil in jars with eight weeks exposure, measuring the temperature and humidity inside the jar.

2.6.7 Assessment of incorporation of cruciferous plants on *H. avenae* survival

After exposure in jars, one of the two nylon bags with cysts, was placed directly into a small glass vial containing 3 mL sterile distilled water and placed at 15°C in darkness to register hatching of J2 (Figure 8). The vials were checked every third day, the water was replaced and J2 were counted. The registration of hatched J2 was terminated after 21 days as describes by Jing et al. (2014).

The second nylon bag from the same jar was prepared for diapause treatment and surface sterilised in 0,5 % chlorine (sodium hypochlorite, 4% w/w active chlorine) for 10 min, and gently shaken. Thereafter, the nylon bags were washed with sterile distilled water three times (Sahin et al., 2010). The nylon bags were placed in small glass vials, with 3 mL sterile distilled water, closed with a lid and placed for diapause treatment in a refrigerator with 2-4°C for 80 days. The vials were checked every third day for 21 days, as describes above. For all registrations the number of hatched J2 was counted, by using a stereo microscope (Leica M10). After 21 days of hatching, the cysts where crushed, and number of remaining eggs and J2 (dead and alive) were counted. This was used to calculate the percentage of nematode hatching, as described by Jing et al. (2014):

$$\text{Percentage nematode hatching (\%)} = \frac{Number\ of\ J2\ at\ the\ selected\ day - number\ of\ J2\ at\ day\ 0}{Number\ of\ eggs\ and\ J2\ remaining\ in\ the\ cyst + \ total\ J2\ emerged}$$

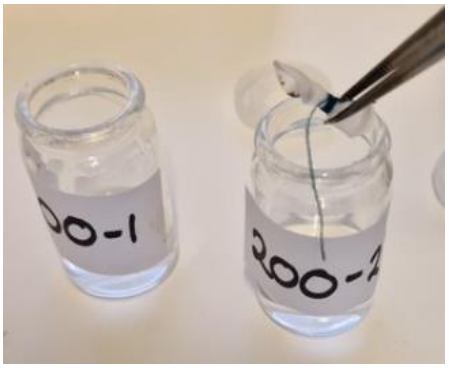


Figure 8: Changing sterile distilled water by placing the nylon bag with *Heterodera avenae* cysts into a new glass vial containing 3 mL sterile distilled water (Photo: Emma Skogstad).

2.6.8 Assessment of incorporation of cruciferous plants on *F. graminearum* survival

After exposure in jars containing soil and plant material, the five oat spikelets from one nylon bag, were placed on Petri dishes (9 cm) containing modified Czapek Dox propiconazole dichloran agar (CZPD), as described by Hofgaard, I. S. et al. (2016). CZPD medium is a modified Czapek Dox iprodione dichloran agar (CZID) (Abildgren et al., 1987). The Petri dishes were incubated at 20°C and 12 h light+NUV. Mycelial growth was observed on all oat spike from all treatments, incubated on CZPD. After five days of incubation (20°C) of oat spikelets, the diameter mycelial growth was registered by measuring the diameter of the fungal colony in two directions perpendicular to each other. During the registrations of fungal growth, bacterial growth was observed. Samples of the bacterial growth were sent to 16S sequencing at Eurofins Genomics in Germany. The bacterial colony were graded between no colony (zero) to a huge colony (four) after five days incubation.

2.6.8.1 Assessment of soil mixture on fungal growth

In order to investigate the survival of *F. graminearum* on oat spikelets, the spikelets were buried into soil with different soil mixtures and water content to observe the survival (Table 5). Two litre glass jars, the same as used in the closed jar experiment, were filled with approximately one litre of the different soil mixtures. The same oat spikelets infected with *F. graminearum* used in the closed jar experiment, were utilized in this experiment. Five oat spikelets were placed in each nylon bag (7x6cm), one bag in each jar. The glasses were placed in the growth room together with the main closed jar experiment. After two weeks in the growth chamber, half of the glasses were taken out, and the other half after eight weeks after start of the closed jar experiment. The oat spikelets from each treatment, were transferred to CZPD petri dishes, five oat spikelets on each Petri dish. The Petri dishes were incubated at 20°C with 12 h light for seven days in total. The same registrations and measurements were done as in the closed jar experiment.

Table 5: Soil mixtures used to investigate the effect of soil on fungal growth of *Fusarium graminearum*.

Treatment	Mixture*	Dry weight (%) **	pH**
Original	81% Sand, 16.7 % clay 2.3 % peat 1L water	87.4	7.40
0.5x water	81 % Sand 16.5 % clay 2.3% peat 0.5L water	92.2	7.30
2x peat	79 % Sand 16.3 % clay 4.4 % Peat 1L water	87.3	6.78

*Percentage of total dry weight. **Percentage dry weight and pH are measured as in closed jar experiment.

2.7 Statistical Analysis

The effective concentration of allyl ITC at 50% (EC_{50}-values) suppression of nematode hatching and mycelial growth of *F. graminearum* and *M. nivale*, were determined by nonlinear regression in Minitab® (v. 21.4.2.0; State Collage, PA, USA). Concentrations of allyl ITC (con) were converted to ln(con*100+1), and theta-values were determined (Theta 1=1, Theta 2=100, Theta 3=7, Theta 4=5). Theta1 and Theta2 were set to be zero and 100 respectively, so the graph would go through these values. For nematode hatching the EC_{50}-value was calculated from two experiments in time. For the fungal growth, the EC_{50}-values were calculated for each isolate of *F. graminearum* and *M. nivale,* based on three experiments in time.

For the closed jar experiment, statistical analyses were performed by using ANOVA and the mixed model effects (95%) in Minitab®, to study the differences between two and eight weeks of exposure in jars and in effect of incorporation of cruciferous plants in soil together with untreated jars (treatment), for *F. graminearum* and *H. avenae.* For the *H. avenae* hatching results, statistical analyses were also performed to compare diapause and no diapause treatment. The statistical analysis for *H. avenae* hatching was based on three groups of outputs: total number of hatched J2 after 21 days of exposure at 15°C (21d), the total number of eggs and J2 counted (total), and the relative number of emerged J2 (21d/total). The analysis for the whole dataset, contained “Treatment duration”, “Treatment” and “Diapause (Yes/No)” as fixed factors and “Jar ID” and “Block” were random factors, and all the possible interactions were added. A Tukey test was also performed for all outputs to compare the treatment duration, treatments in jars and diapause treatment. In Minitab there was a significant interaction between diapause treatment and treatment duration for all outputs. There was also a significant interaction between diapause and treatment for the number of hatched J2 after 21 days and the total number of eggs and J2 counted. Due to this, the dataset was separated between two and eight weeks. These two outputs also had a significant interaction between diapause and treatment. Therefore, the two weeks dataset was separated between diapause and no diapause treatment. For the fungal experimental part, the model contained “Diameter mycelial growth (mm)”, “Bacterial contamination (0-4)” and “Percentage oat spikelets with bacterial colony” as fixed factors and “Jar ID” and “Block” were random factors. No interactions were obtained. Tukey tests of all outputs were done, to compare the treatment duration and treatments in jars.

3. Results

3.1 Effect of allyl ITC on *H. avenae* hatching

The method in the pilot was successful, in such a way that hatching happen in water agar, and counting of hatched J2 in the water agar were possible. Due to the experiences in the pilot experiment, we could start the main *H. avenae* allyl ITC experiment. In the main *in* vitro experiment with allyl ITC, hatching of *H. avenae* J2 were observed after three days exposure to untreated and treated with concentrations of 1.2 and 4.9 mg/L allyl ITC, this was also the pattern after six- and nine-days exposure (Table 6). But there were a few J2 that hatched at an allyl ITC concentration of 19.5 mg/L. For the three highest concentrations, there were no hatching of J2 (Table 6). The relative nematode hatching (%) was zero after nine days exposure to an allyl ITC concentration of 78 mg/L (Figure 9). This trend was observed for both experiments. There was observed a difference between the two performed experiments, for the allyl ITC concentration 1.2 mg/L, experiment number one had a lower relative hatching compared to experiment number two (Figure 10). For *H. avenae* the EC_{50}-value was estimated to 5.66 mg/L allyl ITC.

Table 6: Average number of hatched juveniles (J2) per cyst (*Heterodera avenae*) after three, six- and nine-days exposure (room temperature (~20°C), in darkness) to concentrations of allyl isothiocyanate (allyl ITC) (mg/L), in water agar. Average is calculated from two experiments repeated in time.

Allyl ITC concentration (mg/L)	3 days	6 days	9 days
0	28	38	41
1.2	27	31	35
4.9	4	16	25
19.5	0	1	1
78	0	0	0
312.5	0	0	0
1250	0	0	0

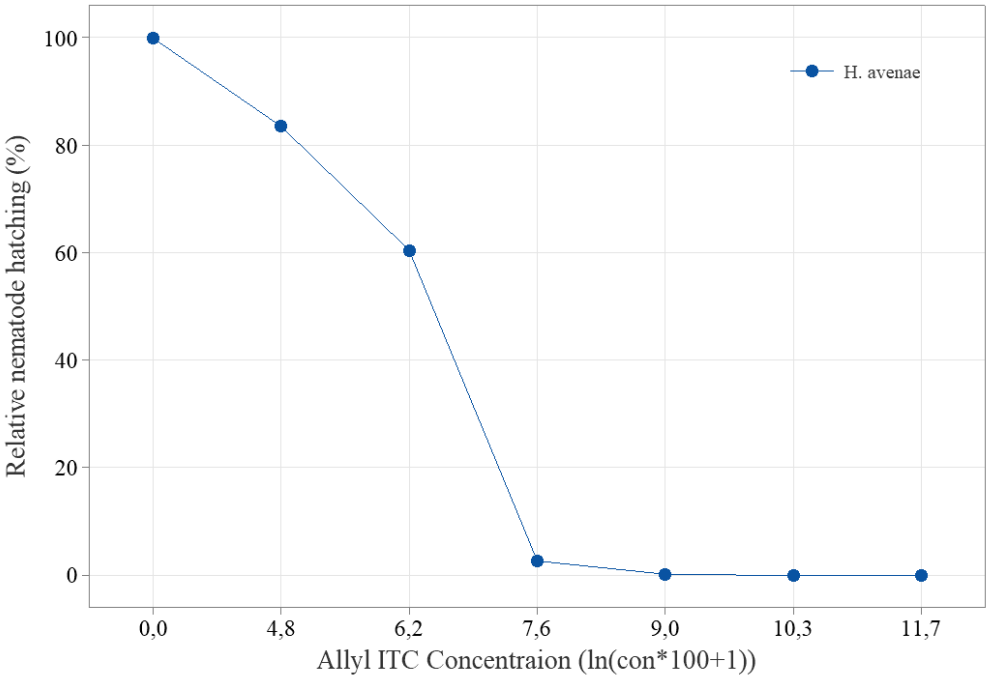


Figure 9: Relative nematode hatching (%) of *Heterodera avenae* after nine days exposure (room temperature (~20°C), darkness) to six concentrations (mg/L) of allyl isothiocyanate (allyl ITC), presented as ln(con*100+1), in addition to untreated (0mg/L). Average relative nematode hatching is calculated from two experiments.

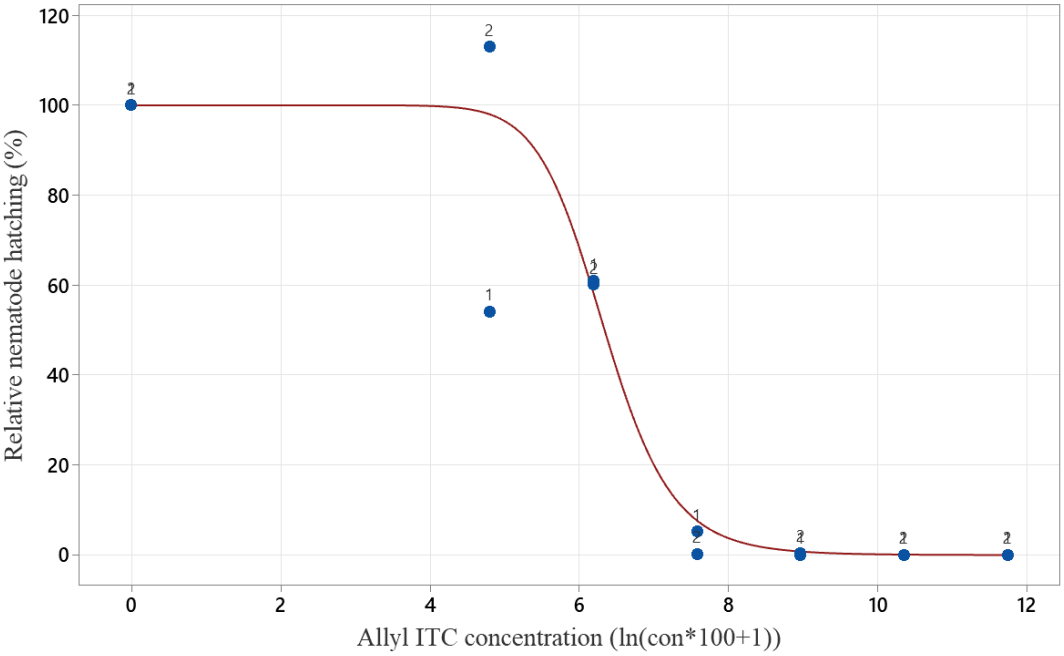


Figure 10: Estimated concentration-response curve for hatching of *Heterodera avenae* juveniles, after nine days exposure to six concentrations of allyl isothiocyanate (allyl ITC), presented as ln(con*100+1), in water agar, after nine days exposure at room temperature (~20°C), used to calculate EC_{50}-value, based on two experiments (1,2).

3.2 Effect of allyl ITC on mycelial growth

The relative mycelial growth rate (%) was reduced with increasing concentration of allyl ITC in PDA, for both *F. graminearum* and *M. nivale* and it differed slightly between the two fungal species (Figure 11). *Microdochium nivale* had a slightly higher relative growth rate than *F. graminearum* with an increasing allyl ITC concentration. For both *F. graminearum* and *M. nivale,* 100% inhibition of mycelial growth was observed at a concentration of allyl ITC of 78 mg/L, in experiment one and two. In the third experiment, a concentration of allyl ITC of 19.5 mg/L gave 100% radial mycelial inhibition for *F. graminearum*, and for *M. nivale* it was approximately 95% (Figure 11).

A concentration-response curve for allyl ITC was made for each of the six isolates, and the calculated EC_{50}-values differed between the fungal species (Figure 13 A-F). Average EC_{50}-value for *F. graminearum* was 7.83 mg/L. and *M. nivale* had 9.90 mg/L. For the three *F. graminearum* isolates a difference in EC_{50}-value was observed (Table 7). Among the *F. graminearum* isolates, number 201 196 had the highest EC_{50}-value, followed by 202 058 and 200 630 (Table 7). Difference between *M. nivale* was also observed: isolate number 200 231 had the highest EC_{50}-value, 202 786 was slightly lower, but 200 136 was much lower (Table 7)

A difference between the three fungal experiments, repeated in time, was observed. The average relative fungal growth rate of *F. graminearum* and *M. nivale* were highest in experiment number two, compared to experiment one and three (Figure 12 A-F). *M. nivale* isolate number 200 136 was not included in experiment number two, because of poor mycelial growth during pre-cultivation.

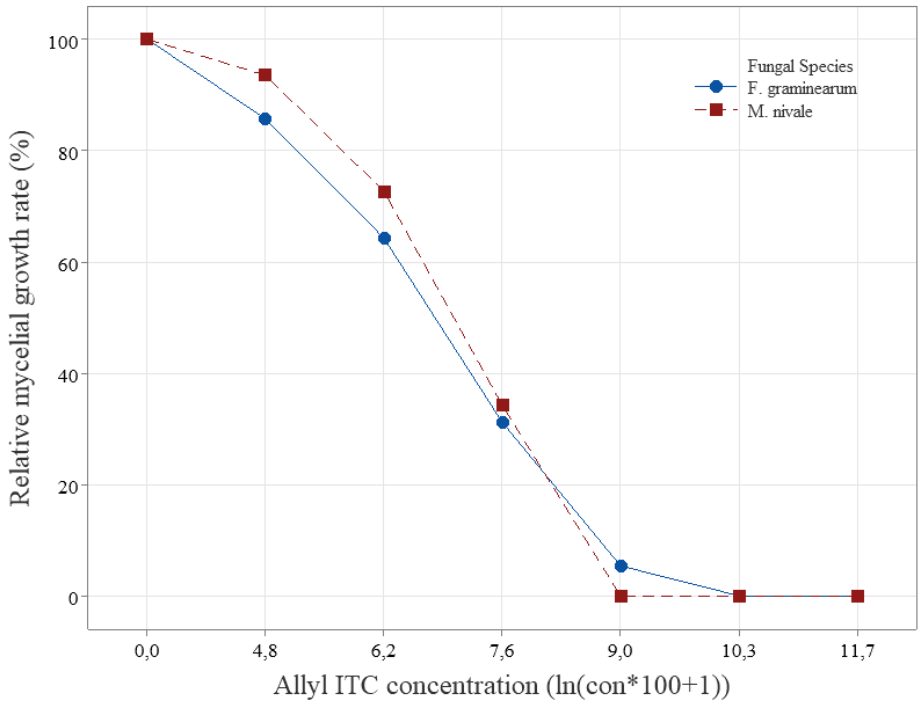
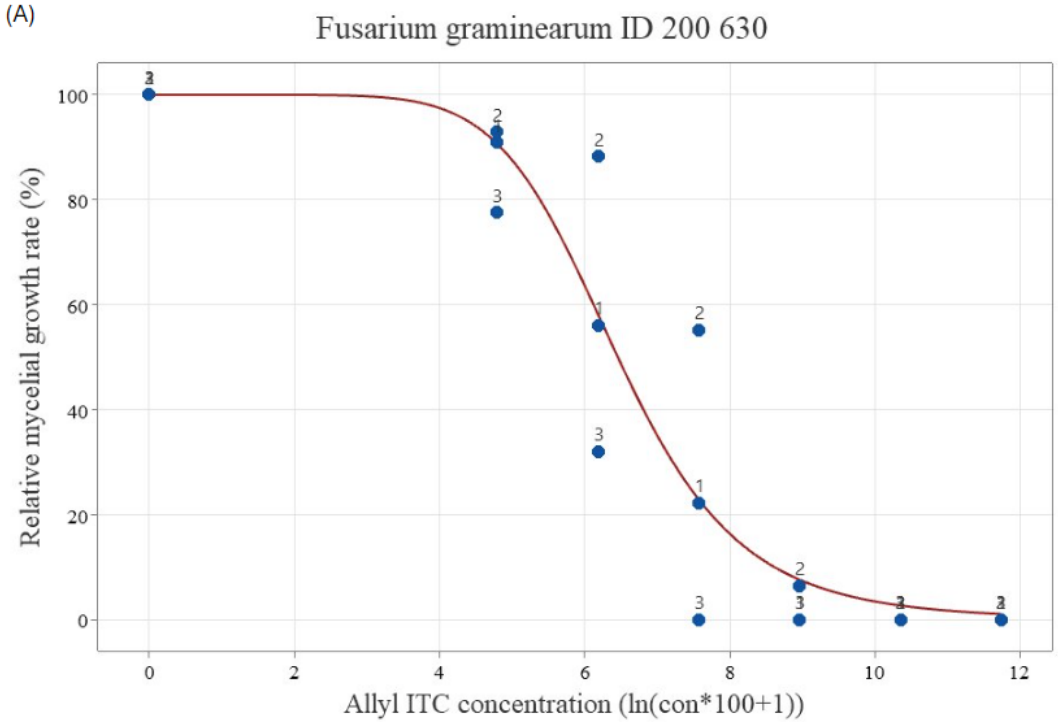
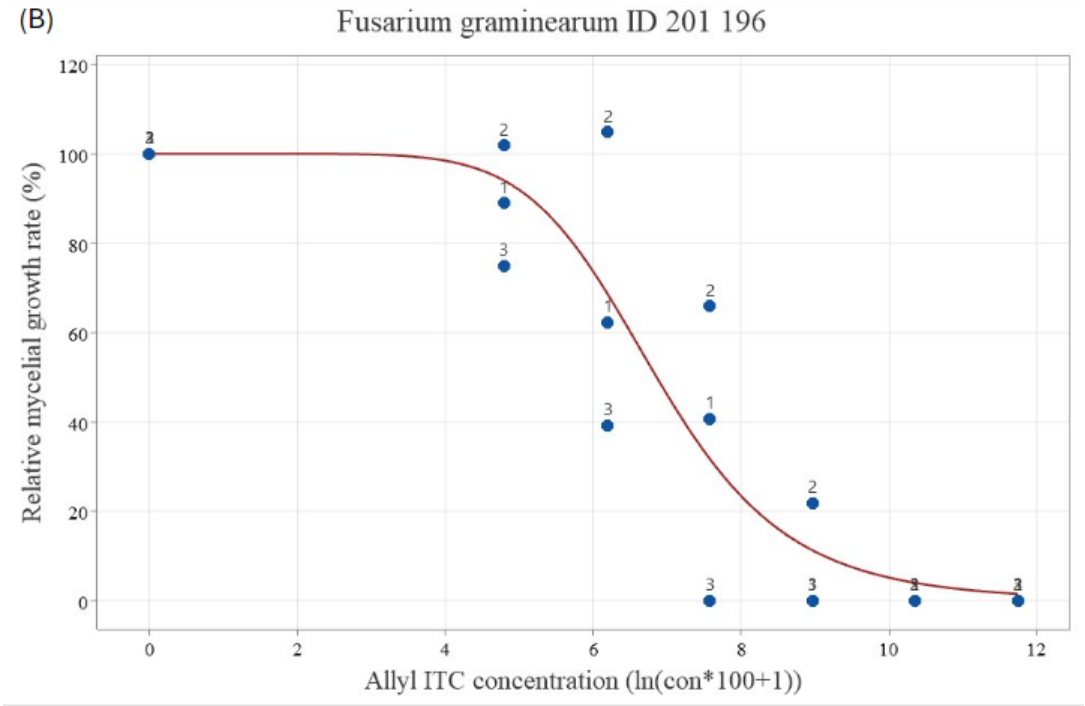


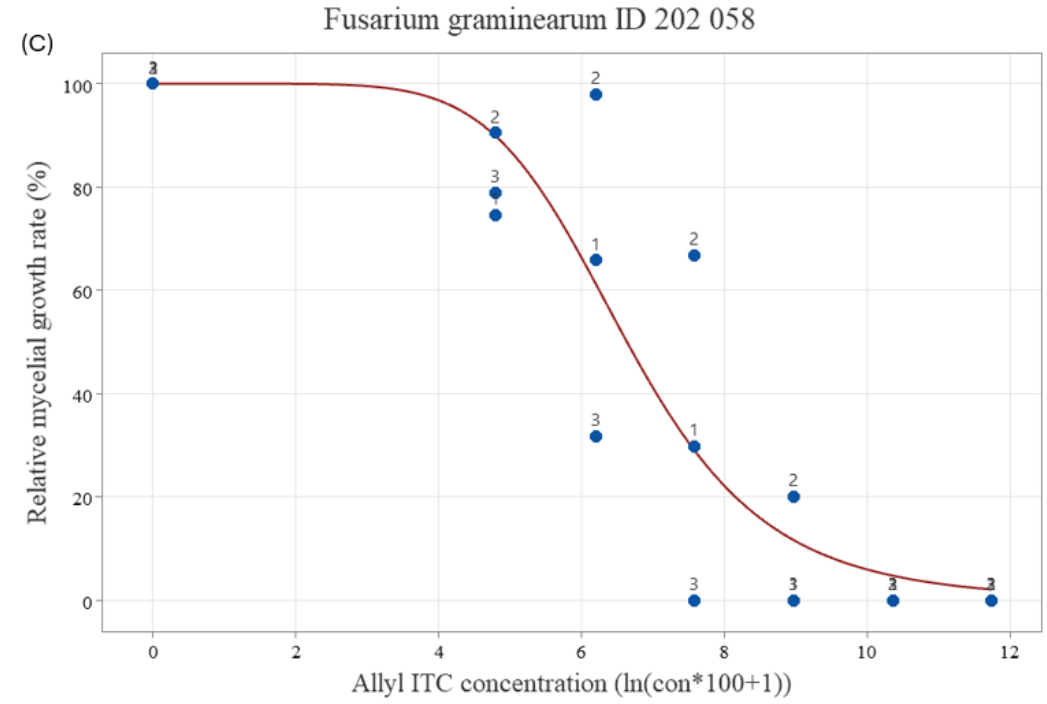
Figure 11: Relative mycelial growth rate (%) of *Fusarium graminearum* and *Microdochium nivale* at concentrations of allyl isothiocyanate (presented as ln(con*100+1)) on potato dextrose agar at 15°C (darkness). Average was calculated from three replications in time and three isolates of each fungal species.



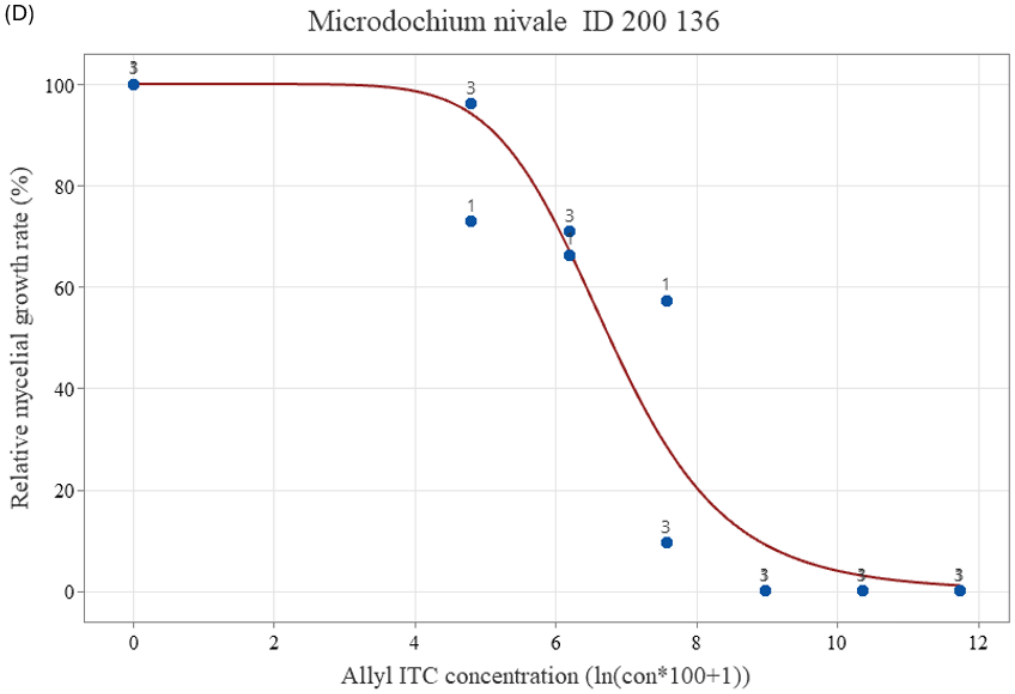
(B)


Fusarium graminearum ID 201 196
Relative mycelial growth rate (%)
Allyl ITC concentration (ln(con*100+1))

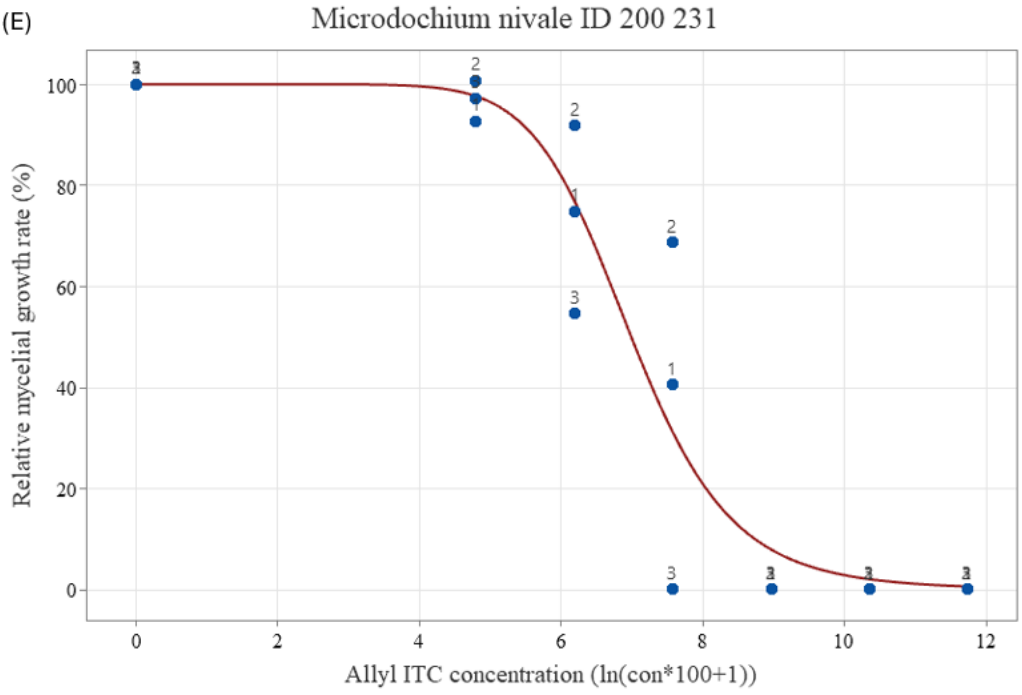
(C)


Fusarium graminearum ID 202 058
Relative mycelial growth rate (%)
Allyl ITC concentration (ln(con*100+1))

(D)


Microdochium nivale ID 200 136
Relative mycelial growth rate (%)
Allyl ITC concentration (ln(con*100+1))

(E)


Microdochium nivale ID 200 231
Relative mycelial growth rate (%)
Allyl ITC concentration (ln(con*100+1))

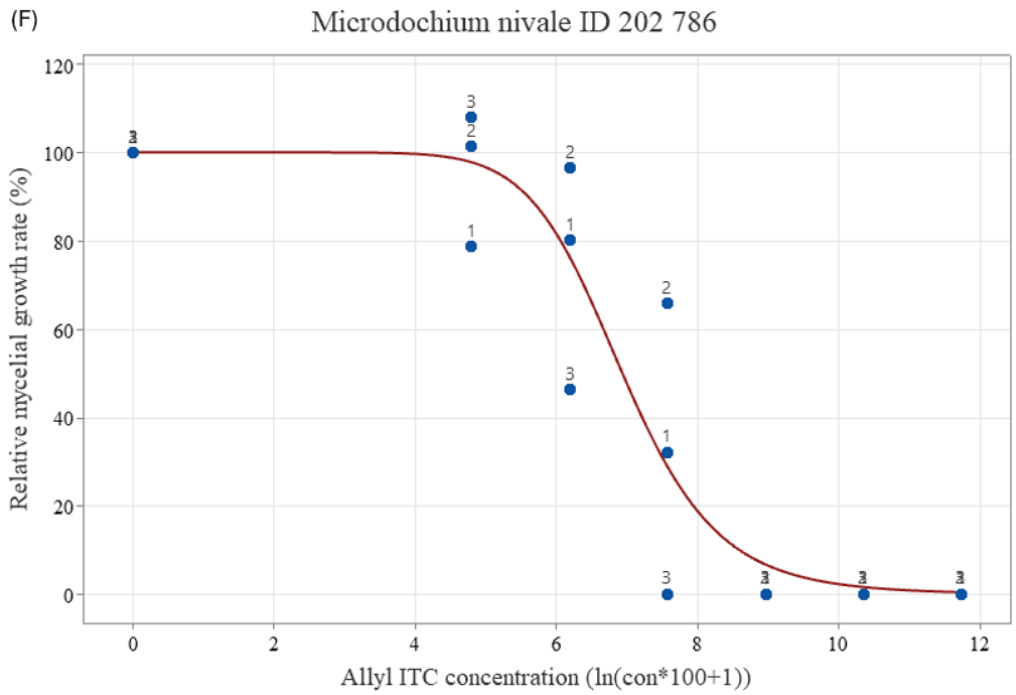


Figure 12: Estimated concentration-response curve for concentrations of allyl isothiocyanate (presented as ln(con*100+1)) in potato dextrose agar, on mycelial growth of three isolates of *Fusarium graminearum* (ID 200 630 (A), ID 201 196 (B), ID 202 058 (C)) and three isolates of *Microdochium nivale* (ID 200 136 (D), 200 231 (E), 202 786 (F)), incubated at 15°C (darkness) for six days. Data from three experiments (1,2,3) repeated in time.

Table 7: Allyl ITC concentration at 50 % inhibition of mycelial growth rate (EC_{50}-value), for three *Fusarium graminearum* isolates and three *Microdochium nivale* isolates on potato dextrose agar at 15°C (darkness) for six days.

Fungal Species	Isolate number (ID)	EC50-value (mg/L)
F. graminearum	200 630	6,36
F. graminearum	201 196	9,50
F. graminearum	202 058	7,62
M. nivale	200 136	8,60
M. nivale	200 231	10,83
M. nivale	202 786	10,27

3.3 Closed jar experiment.

The effect of cruciferous plants on the survival of *H. avenae* and *F. graminearum* in closed jars differed between the two plant pathogens. The effect of the duration in jars differed between plant pathogens and between treatment in soil added plant materials. For *H. avenae* a difference between diapause and no diapause treatment was observed.

3.3.1 Plant material

In the fields with cruciferous plants, a difference in percentage of plants in flowering stage (BBCH 60-69), and bud stage (BBCH 50-59) were observed (Table 8) (Meier, 2001). Only Undercover was in bud stage at this time. Albatross, Terminator and Energy had the highest percentage of plants in flowering stage and the lowest percentage in bud stage. Structurator, Siletta nova and defender had the highest percentage of plants in bud stage. Akiro, Defender and Siletta Nova had the highest plant density (Table 8). The height and weight differed between the cruciferous plants. The whole plant weight was highest for Akiro with 265.9 cm (Table 9). Undercover had the lowest root weight and was among the shortest plants at that time of registrations, with 3.3 g and 31 cm respectively (5thof September) (Table 9). Data from LandbruksMeteorologisk Tjeneste (LMT) showed 19.7 mm and 20.7 mm of precipitation during a short period of time, resulting in uneven establishment and growth and areas without plants (Figure 13). There were also observations of damage by insects, probably turnip sawfly (*Athalia rosae*), which caused holes in the leaves (Figure 14).

Figure 13: Weather statistics from Øsaker (Norway) downloaded from LandbruksMeteorologisk Tjeneste (LMT), show average temperature (°C) and average precipitation (mm) during the growing period of the cruciferous plants. (TM=average temperature. RR=precipitation.) (NIBIO, 2024).

Figure 14: Leaf from field at Øsaker attacked by the sawfly *Athalia rosae.* (Picture: Emma Skogstad).

Table 8: Percentage of cruciferous plants at flowering stage and bud stage, and the plant density (m^2).

Cultivar	**Flowering stage (%) BBCH 60-69***	**Bud stage (%) BBCH 50-59***	**Density (m^2) ****
Undercover	0	10	6
Energy	80	20	5
Terminator	80	20	5
Ikarus	50	50	15
Akiro	40	40	30
Defender	40	50	30
Siletina	60	40	12
Siletta nova	40	60	25
Stinger	60	40	15
Structurator	30	70	10
Albatros	90	10	12
Action	70	30	12

*BBCH stage of cultivar at 5th of September 2024 (Meier, 2001). **Observed plant density of cultivars (m^2).

Table 9: Observations of weight and height were measured for one or two representative plants per cultivate, registered 5th of September.

Cultivar	Developmental stage (BBCH)*	Aboveground plant part					Root		Whole plant	
		Height (cm)	Aboveground (g)	Top leaf (g)	Middle leaf (g)	Lowest leaf (g)	Length (cm)	Weight (g)	Length (cm)	Weight (g)
Undercover	Vegetative	31	52.9	1.3	3.8	10.6	12	3.3	43	56.2
Energy	Flowering	88	95.7	0.8	4	5.8	13	6.8	101	102.5
Terminator	Flowering	57	50.1	1.8	3.2	3.7	12	3.2	69	53.3
Ikarus	Flowering	86	83.8	1.9	7.7	11.8	10	11.2	96	94.9
Akiro	Flowering	53	210.9	1	8.4	21.8	14	55	67	265.9
Defender	Flowering	99	74.2	0.9	2.2	5.2	19	8.3	118	82.5
Defender	Bud	49	59.5	2.7	4.4	7.6	17	7.7	66	67.2
Siletina	Flowering	68	58.7	1.9	3.9	5.9	13	7.9	81	66.6
Siletta nova	Flowering	105	61.4	0.6	2	4.3	20	7.1	125	68.5
Siletta nova	Bud	23	43.2	0.7	5.2	6.9	14	9.7	37	52.9
Stinger	Flowering	88	72.4	0.8	1.5	2.8	15	12.9	103	85.3
Structurator	Flowering	60	37.9	0.6	1.1	3.6	15	4.1	75	42
Structurator	Bud	20	24.8	0.6	2	3.3	18	16.7	38	41.5
Albatros	Flowering	91	36.6	0.7	1.9	2.2	11	3.3	102	39.9
Action	Flowering	83	47.3	0.6	1.8	2.6	12	7.1	95	54.4

*BBCH stage of cultivar at 5th of September 2024 (Meier, 2001).

3.3.2 Temperature and RH registrations inside the jars and in the growth room

The relative humidity inside the jars reached 100 % the same day as the start of the experiment. Jar and growth room temperature were measured from 19th of October (00:00) to 13th of December (07:00 AM). For this period average minimum and maximum temperatures were 8.6ºC and 18.8ºC, and the average was 12.2ºC, measured inside the jars. During the same period the measured temperature in the growth room varied between 8.5ºC and 16.9ºC, and average temperature was 12.3ºC.

3.3.3 Effect of incorporation of cruciferous plants on survival of *H. avenae* in jars

Cysts exposed to soil with chopped material from various cruciferous plants, without diapause after exposure, J2 were observed already after three days at 15°C. It turned out that J2 hatched quite frequently from cysts after diapause treatments a considerable amount of J2 had already hatched after exposure to 4°C. The percentage of J2 hatching did not increase after around 18 days of exposure to 15°C, for both two and eight weeks of exposure in jars and with and without diapause treatment. Therefore, the statistical analyses were based on data for the ten cysts after 21 days. For diapause and no diapause treated cysts, the total number of eggs and J2 after two weeks in jars (790 J2+eggs), had a significantly higher total average of eggs and J2 compared to eight weeks exposure (450 J2+eggs) (Table 10). Cysts exposed to soil with plant material followed by diapause treatment, had significantly higher number of J2 and higher relative number of emerged J2 compared to no diapause treatment (Table 10). Between diapause and no diapause, the average total number of dead and alive were almost the same, the number of eggs and J2 were 617 and 624 respectively. Cysts incubated for two weeks, had significantly higher number of J2 emerged at 21 days and higher total number of eggs and J2 compared to eight weeks exposure. On the other hand, the relative number of emerged J2 was significantly higher for eight weeks (0.51) compared to two weeks (0.32). The relative number of emerged J2 and the number of hatched J2 after 21 days had significantly higher values for diapause (0.60 and 345 J2) compared to no diapause treatment (0.23 and 137 J2) (Table 10).

Tabell 10: Calculated average values of *Heterodera avenae* exposed to soil mixed with chopped material from various cruciferous plants (treatments) in a closed jar experiment, from registrations of the total number of eggs and juveniles (J2) counted from ten cysts (Total), the relative number of emerged J2 (21d/total), and number of hatched J2 from ten cysts after 21 days (21d). p-levels for duration of exposure in jars, diapause treatment after end of jar treatment, treatments in jars and the interactions between them.

	Total	**21d/total**	**21d**
Treatment duration*:			
2 weeks	790.09	0.32	271.84
8 weeks	450.49	0.51	210.12
p-level	<0.05	<0.05	<0.05
Diapause (Yes/No):**			
Diapause	617.00	0.60	344.74
No diapause	623.58	0.23	137.22
p-level	ns	<0.05	<0.05
Treatment***	620.29	0.42	240.98
p-level	<0.05	ns	<0.05
Diapause*Treatment duration	<0.05	<0.05	<0.05
Diapause*Treatment	<0.05	ns	<0,05
Treatment duration*Treatment	ns	ns	ns
Treatment duration*Treatment*Diapause	ns	ns	ns

*Two or eight weeks of exposure in jars. **Diapause treatment (yes) (80 days at 4°C) or no diapause treatment (no) after end of jar experiment. ***Treatments with incorporation of chopped cruciferous plants mixed into soil.

3.3.3.1 *Heterodera avenae* survival after two weeks exposure in jars

After incubation of *H. avenae* cysts for two weeks into soil mixed with chopped materials from different cruciferous plants, the total number of J2 and eggs differed between treatments of chopped plant material (Figure 16.A). Total number of eggs and J2 with diapause treatment (893 eggs+2), were significantly higher than no diapause treatment (687 eggs+J2) (Table 11). Ikarus-root (1100 eggs+J2) had significantly higher total number than Energy (580 eggs+J2) (Appendix 1). Untreated jars (812 eggs+J2) were not significantly different from any other treatment in jars (Appendix 1).

Cysts incubated for two weeks within soil containing chopped pant material, had approximately 50 % significantly higher relative number of hatched J2 with diapause (0.45) compared to no diapause treatment (0.20) (Table 11). On the other hand, there were no significant differences between treatments of soil with plant materials, but Untreated had the highest estimated mean value (0.39) and Energy had the lowest (0.20) (Appendix 2). Action with diapause had the highest relative number of emerged J2 and Energy without diapause had the lowest relative number (Figure 15.B).

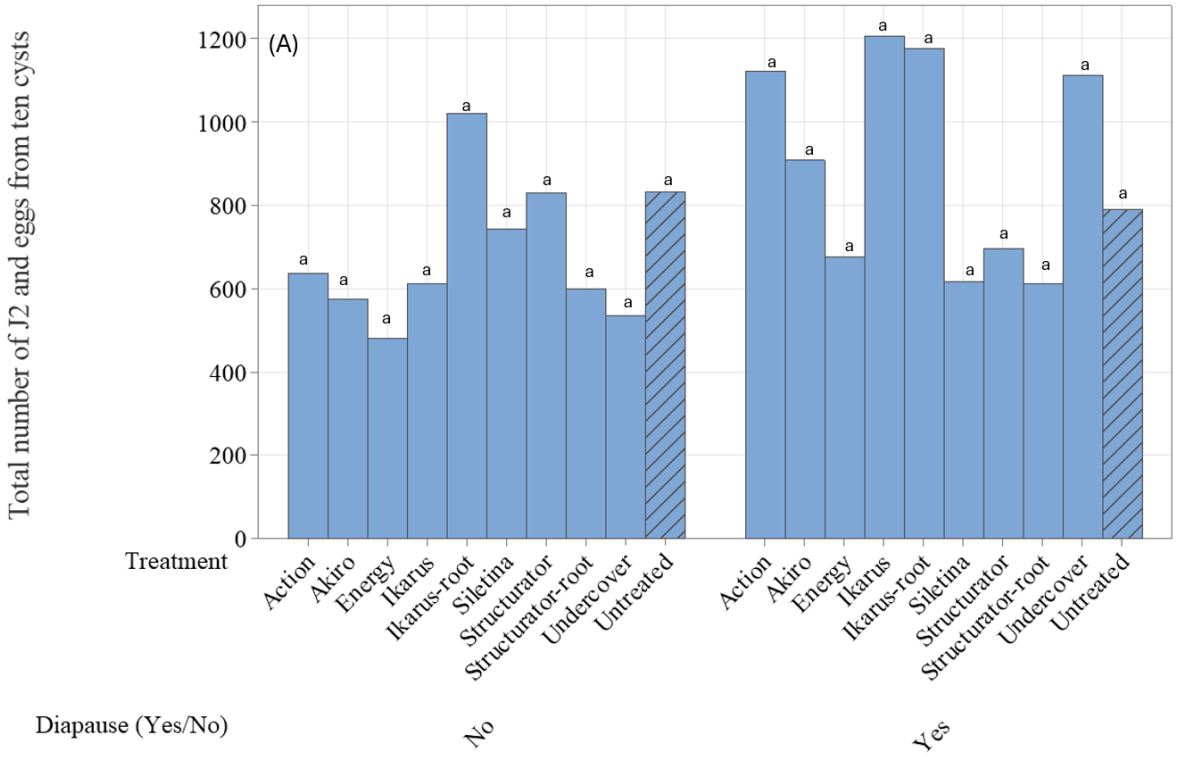
The two-week exposure showed a significantly higher hatching after 21 days for cysts with diapause (416 J2) compared to no diapause treatment (128 J2) (Table 11). Ikarus-root with 381 J2 had significantly higher number of hatched J2 compared Structurator-root and Energy, that had 168 and 124 hatched J2 respectively (Appendix 3). Energy had the lowest number of J2 for both no diapause treatment and diapause treatment (Figure 15.C). For cysts without diapause (224 J2), the untreated had a significantly higher hatching than Energy (42 J2) (Appendix 4). Cysts with diapause did not show any significant differences between treatment in jars, but Action had the highest number with 630 J2 (Appendix 5).

The relative number of dead eggs and J2 differed between treatments in jars and between diapause and no diapause treatment, but no statistical analysis was performed on this data (Figure 16). Cysts treated with Energy, untreated, Siletina and Ikarus had the highest relative numbers of dead eggs and J2 both per total counted and had the highest number of dead per total cyst content (Figure 16).

Table 11: Calculated average values of *Heterodera avenae* exposed to soil mixed with chopped material from various cruciferous plants (treatments) in a closed jar experiment for two weeks, from registrations of the total number of eggs and juveniles (J2) counted from ten cysts (Total), the relative number of emerged J2 (21d/total), and number of hatched J2 from ten cysts after 21 days (21d). p-levels for diapause (Yes/No) treatment after end of jar treatment, treatments in jars and the interactions between them.

	Total	**21d/total**	**21d**
Diapause (Yes/No)*:			
Diapause	892.82	0.45	415.98
No diapause	687.36	0.20	127.70
p-level	<0.05	<0.05	<0.05
Treatment:**	0.32	790.09	271.84
p-level	ns	ns	<0.05
Diapause (Yes/No)*Treatment	<0.05	ns	<0.05

*Diapause treatment (yes) (80 days at 4°C) or no diapause treatment (no) after end of jar experiment.
**Treatments with incorporation of chopped cruciferous plants mixed into soil.



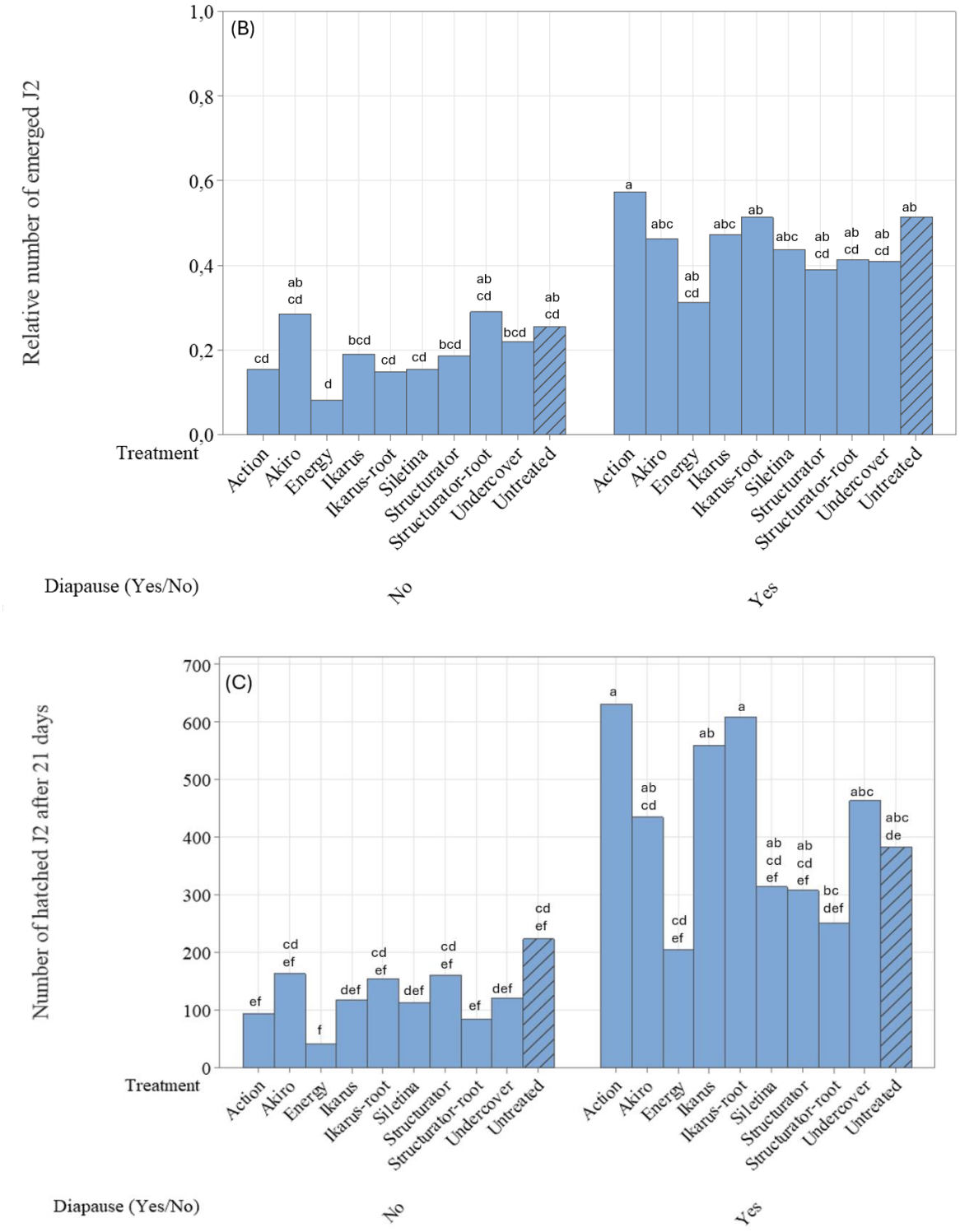


Figure 15: The effect of incorporation of chopped material from various cruciferous plants into soil (treatment) on the survival of *Heterodera avenae* after exposure for two weeks in closed jars followed by either no diapause (no) or diapause treatment (yes) (80 days at 4°C). Total number of *Heterodera avenae* eggs and J2 counted from ten cysts (Total) (A), relative number of emerged J2 (B) and the number of J2 counted from ten cysts (C), observed after 21 days at 15°C. The average numbers were calculated from five replicates per treatment after two of exposure in jars. Different letters (abcdef) over the bars indicate significant treatment effects at P<0.05 (Tukey pairwise comparison).

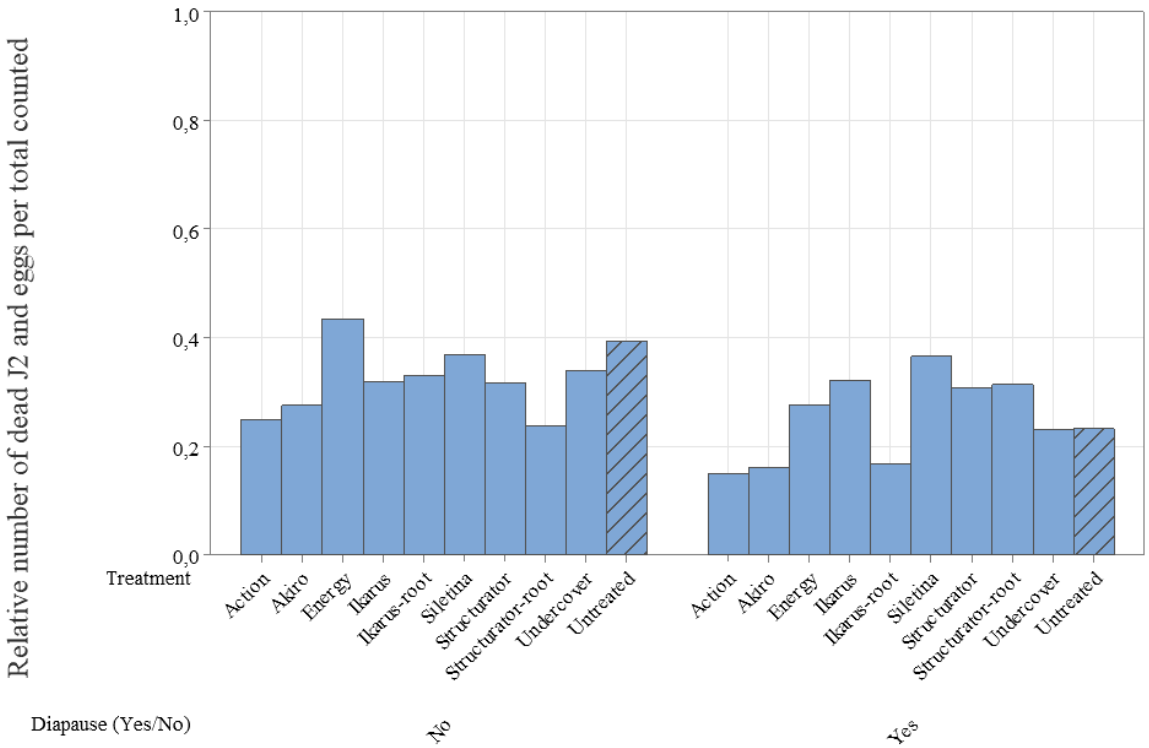


Figure 16: The effect of incorporation of chopped material from various cruciferous plants into soil (treatment) on the survival of *Heterodera avenae* after exposure for two weeks in closed jars followed by either no diapause (no) or diapause treatment (yes) (80 days at 4°C). Relative number of dead juveniles (J2) and eggs from ten *Heterodera avenae* cysts per total number of juveniles and eggs counted after 21 days at 15°C. The average numbers were calculated from five replicates per treatment after two weeks of exposure in jars.

3.3.3.2 *Heterodera avenae* survival after eight weeks exposure in jars

After eight weeks exposure of *H. avenae* cysts in soil containing chopped cruciferous plant material, the number of cysts remaining found in the nylon bags were less than ten. Less cysts at the end of the experiment led to a lower number of counted eggs and J2 from ten cysts. The total number of eggs and J2 counted after 21 days, was significantly higher for cysts without diapause with 560 J2 and eggs, compared to diapause treatment with 341 J2 and eggs (Table 12). There were no significant differences between treatments in jars, but Ikarus-root had the highest total cyst content (584 eggs+J2), and Energy had the lowest total cyst content (356 eggs+J2) (Appendix 6).

The relative number of emerged J2 differed between treatments of chopped plant material incorporated in soil in closed jars (Figure 17.B). No significant difference between treatments in jars, but Ikarus-root (0.60) had the highest relative number of emerged J2, and Structurator-root (0.39) had the lowest (Appendix 7). Untreated was not significantly different from any treatments in jars but had the third highest relative number of emerged J2 (0.55) (Appendix 7).

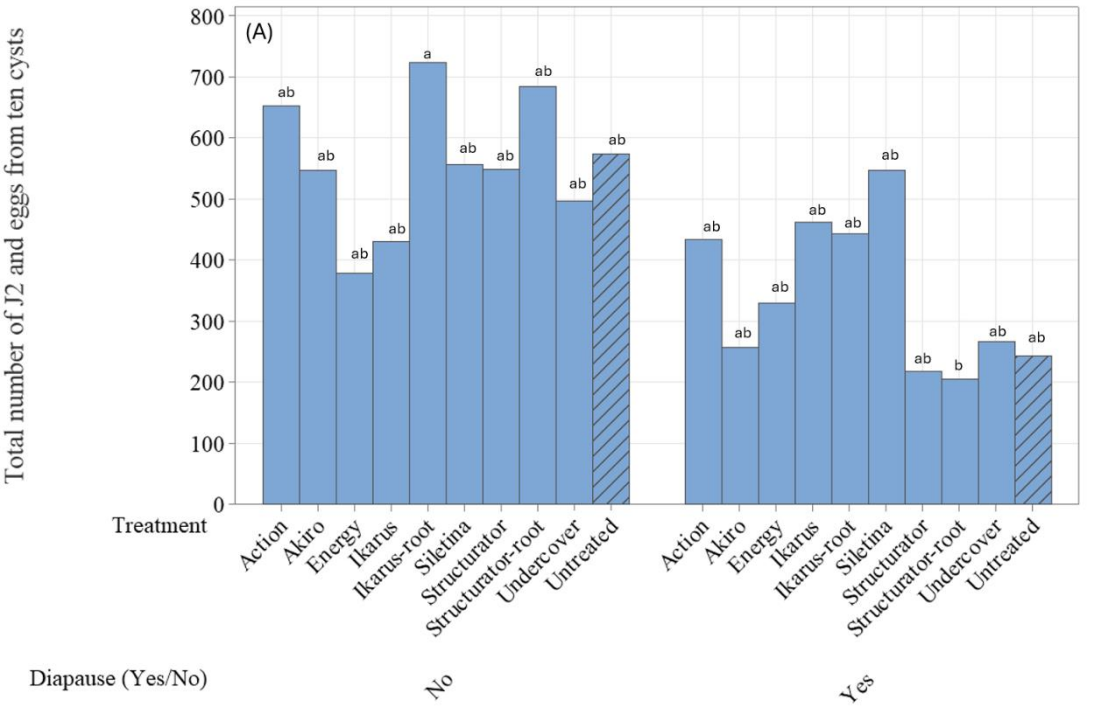
Cysts with diapause had significant higher relative number of emerged J2 (0.76) compared to cysts without diapause treatment (0.27) (Table 12).

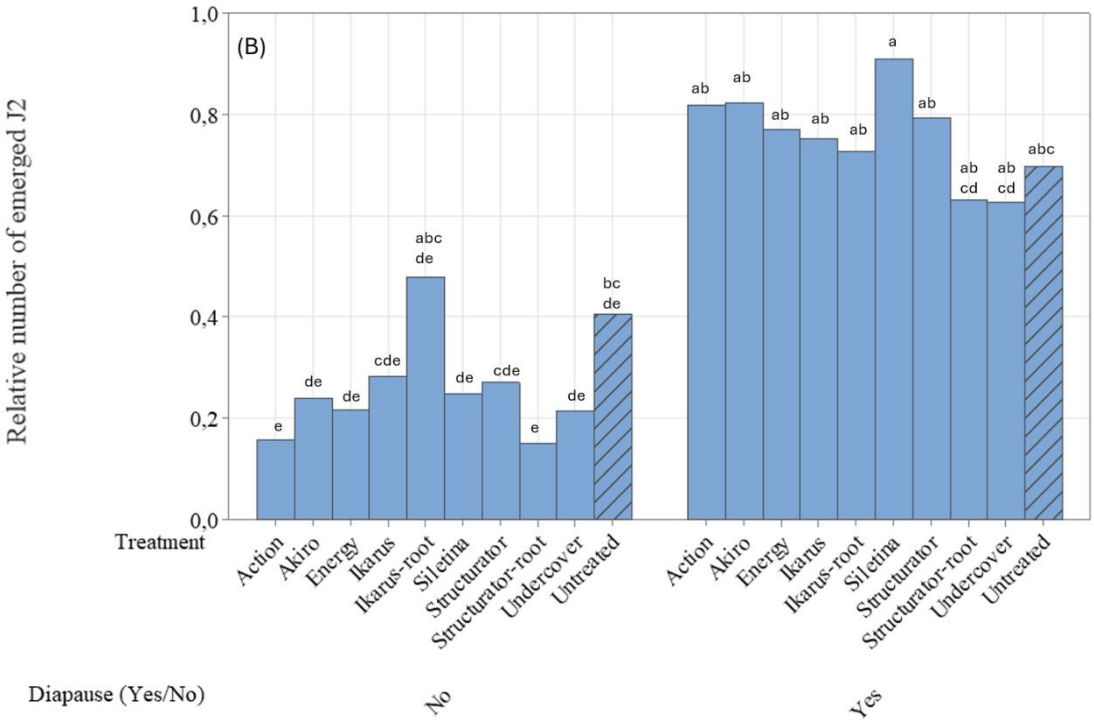
Differences in the number of J2 after 21 days for cysts incubated in jars for eight weeks, was observed between treatments in jars and differed between no diapause- and diapause (Figure 17.C). Cysts from eight weeks exposure with diapause treatment had 274 hatched J2, had significantly higher number of hatched J2 compared to no diapause treatment with 147 hatched J2 (Table 12). Between treatments in jars there were not any significant difference in numbers of J2 (Table 12). Cysts from eight weeks exposure in jars, cysts exposed to Ikarus-root (349 J2) had the highest number of hatched J2, and Structurator-root (131 J2) had the lowest number of J2 (Appendix 8).

Table 12: Calculated average values of *Heterodera avenae* exposed to soil mixed with chopped material from various cruciferous plants (treatments) in a closed jar experiment for eight weeks, from registrations of the total number of eggs and juveniles (J2) counted from ten cysts (Total), the relative number of emerged J2 (21d/total), and number of hatched J2 from ten cysts after 21 days (21d). p-levels for diapause (Yes/No) treatment after end of jar treatment, treatments in jars and the interactions between them.

	Total	**21d/total**	**21d**
Diapause (Yes/No)*:			
Diapause	341.18	0.76	273.50
No diapause	559.80	0.27	146.74
p-level	<0.05	<0.05	<0.05
Treatment:**	450.49	0.51	210.12
p-level	ns	ns	<0.05
Diapause (Yes/No)*Treatment	ns	ns	ns

*Diapause treatment (yes) (80 days at 4°C) or no diapause treatment (no) after end of jar experiment.
**Treatments with incorporation of chopped cruciferous plants mixed into soil.


(A)
Total number of J2 and eggs from ten cysts
800
700
600
500
400
300
200
100
0
ab
ab
ab
ab
a
ab
ab
ab
ab
ab
ab
ab
ab
ab
ab
ab
b
ab
ab
Treatment
Action
Akiro
Energy
Ikarus
Ikarus-root
Siletina
Structurator
Structurator-root
Undercover
Untreated
Diapause (Yes/No)
No
Yes


(B)
Relative number of emerged J2
1,0
0,8
0,6
0,4
0,2
0,0
e
de
de
cde
abc
de
de
cde
e
de
bc
de
ab
ab
ab
ab
ab
a
ab
ab
cd
ab
cd
abc
Treatment
Action
Akiro
Energy
Ikarus
Ikarus-root
Siletina
Structurator
Structurator-root
Undercover
Untreated
Diapause (Yes/No)
No
Yes

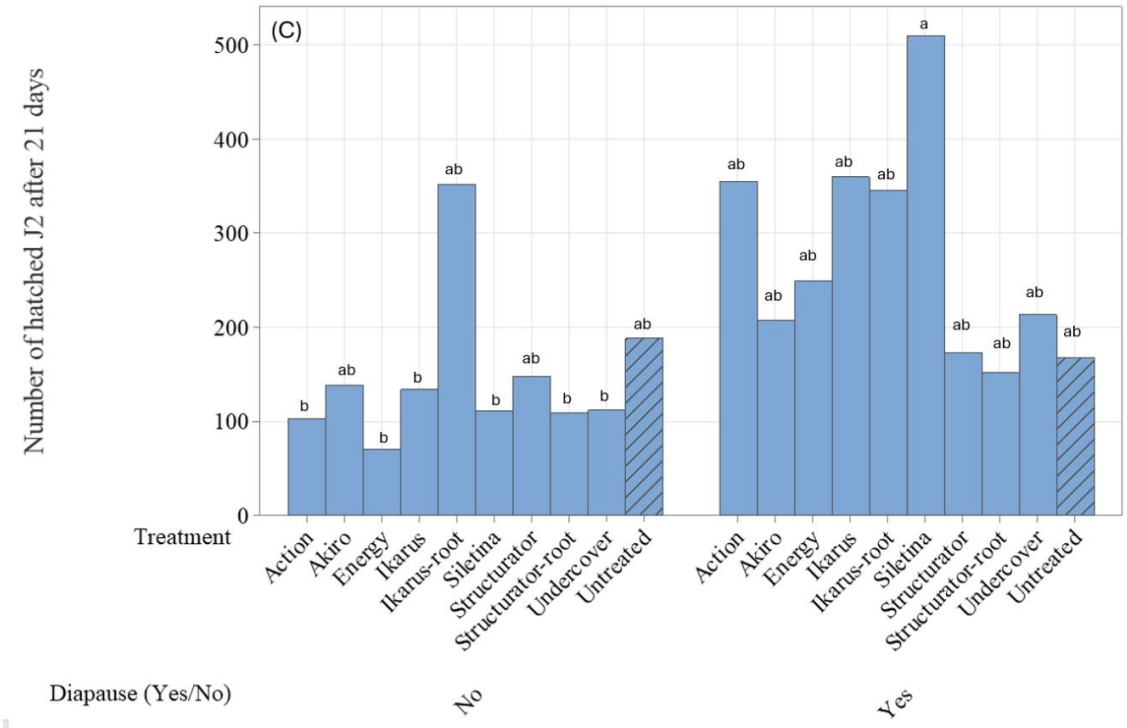


Figure 17: The effect of incorporation of chopped material from various cruciferous plants into soil (treatment) on the survival of *Heterodera avenae* after exposure for eight weeks in closed jars followed by either no diapause or diapause treatment (80 days at 4°C). Total number of *Heterodera avenae* eggs and juveniles (J2) counted from ten cysts (Total) (A), relative number of emerged juveniles (J2) (B) and the number of juveniles (J2) counted from ten cysts (C), observed after 21 days at 15°C. The average numbers were calculated from five replicates per treatment, after eight weeks of exposure in jars. Different letters (abcde) over the bars indicate significant treatment effects at P<0.05 (Tukey pairwise comparison).

3.3.4 Survival of *F. graminearum* in jars

3.3.4.1 Effect of incorporation of cruciferous plants on survival of *F. graminearum*

After incorporation into soil mixed with chopped material from various cruciferous plants, mycelial growth of *F.* graminearum was observed from all oat spikelets, after both two and eight weeks of exposure in closed jars (Figure 18). No significant differences in diameter of mycelial colony for two and eight weeks were observed, with mycelial diameters of 21.39 mm for eight weeks incubation and 21.04 mm for two weeks (Table 13). However, no significant differences were observed between the different treatments in jars (Table 13). But diameter mycelial growth from oat spikelets exposed to Structurator-root (22.22 mm) had the largest mycelial colony, and untreated had the smallest colony (20.32 mm) (Appendix 9).

Table 13: Calculated average values of *Fusarium graminearum* infected oat spikelets, exposed to soil mixed with chopped material from various cruciferous plants (treatments) in a closed jar experiment for two or eight weeks, from registrations of percentage of oat spikelets with bacteria (%), size of bacterial colony and diameter mycelial growth from oat spikelets. p-levels for diapause (Yes/No) treatment after end of jar treatment, treatments in jars and the interactions between them are presented.

	Percentage of oat spikelets with bacteria (%)	**Size of bacterial colony (0-4)**	**Diameter of mycelial growth (mm)**
Treatment duration*:			
2 weeks	81.2	2.60	21.04
8 weeks	93.6	2.04	21.39
p-level	<0.05	<0.05	ns
Treatment:**			
p-level	<0.05	<0.05	ns
Treatment duration*Treatment	ns	ns	ns

*Two or eight weeks of exposure in jars. **Treatments with incorporation of chopped cruciferous plants mixed into soil.

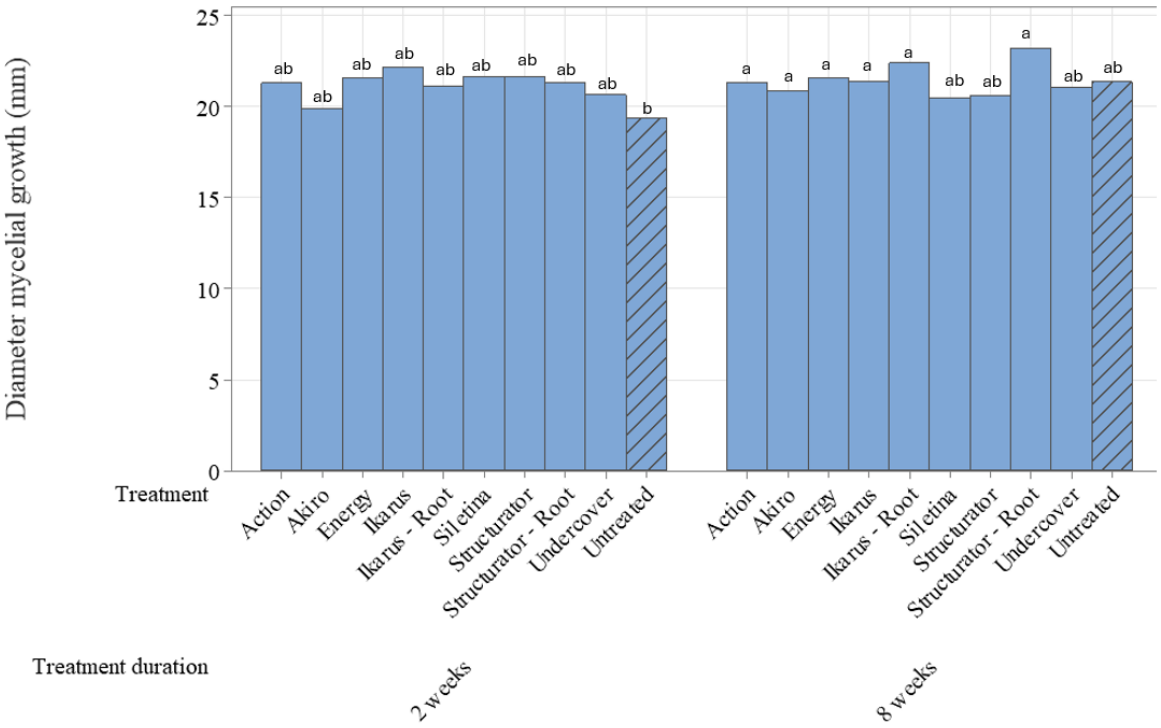


Figure 18: The effect of incorporation of chopped material from various cruciferous plants into soil (treatment) on the survival of *Fusarium graminearum* on infected oat spikelets after exposure for two or eight weeks in closed jars. Diameter of mycelial growth (mm) from *Fusarium graminearum* infected oat spikelets was observed after incubation on Czapek Dox propiconazole dichloran agar, at 20°C and 12 h light, for five days after end of exposure. The average diameters of mycelial colonies were calculated from five replication per treatment after two and eight weeks of exposure in jars. Different letters (ab) over the bars indicate significant treatment effects at P<0.05 (Tukey pairwise comparison).

3.3.4.2 Identification of bacterial colonies

The size of bacterial colony (0-4) of on *F. graminearum* infected oat spikelets, was significantly higher on spikelets incubated in soil mixed with plant material, compared to spikelets exposed to soil only (Appendix 10). Among treatments with plants, Energy had the smallest bacterial colony (2.12) (Appendix 10). A significantly higher percentage of bacterial contaminated oat spikelets was observed for spikelets exposed for eight weeks with 93.6 % bacterial contaminated oat spikelets compared to two weeks in jars with 81.2 % (Appendix 11). But the size of bacterial colony for two weeks exposure (2.60) was significantly higher than eight weeks (2.04) (Table 13). Samples of the bacterial colonies isolated from *F. graminearum* infected oat spikelets, were sent to 16S sequencing. After 16S sequencing, two bacteria species were identified: *Pseudomonas sp.* and *Stenotrophomonas sp.*

3.3.4.3 Effect of soil mixture on survival of *F. graminearum*

The effect of different soil mixtures on survival of *F. graminearum* on infected oat spikelets was observed after exposure in closed jars for two and eight weeks. A slight difference between the three soil mixtures was observed (Figure 19). After five days incubation of *F. graminearum* infected spikelets, the average diameter of the mycelial colony from spikelets that had been buried in jars containing a soil mixture containing half the amount of water, was significantly higher (23.67 mm) compared to the soil mixture used in the main closed jar experiment (21.47 mm) (Appendix 12). There was no significant difference in diameter mycelial growth between two (22.49 mm) and eight (22.51 mm) weeks of exposure in jars (Appendix 13).

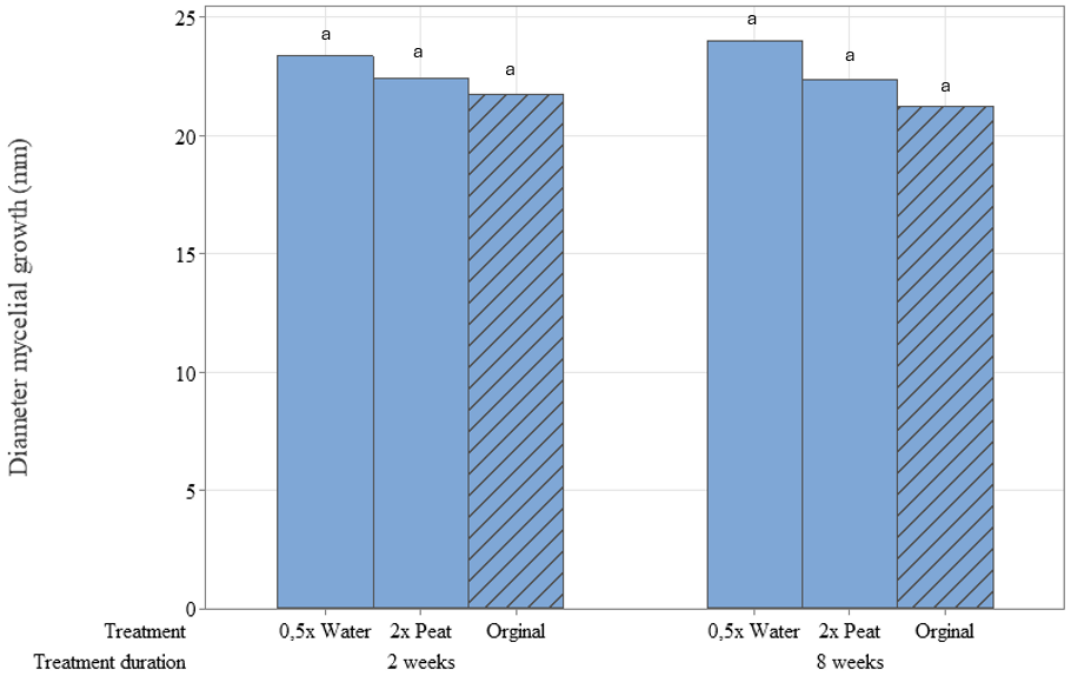


Figure 19: The effect of soil mixture (treatment) on the survival of oat spikelets infected with *Fusarium graminearum,* after exposure for two or eight weeks in closed jars. Diameter of mycelial growth (mm) from *Fusarium graminearum* infected oat spikelets was observed after incubation on Czapek Dox propiconazole dichloran agar, at 20ºC and 12 h light for five days after end of exposure. The average diameters of mycelial colonies were calculated from five replicates per treatment, after two and eight weeks of exposure in jars. Different letters (abcd) over the bars indicate significant treatment effects at $P<0.05$ (Tukey pairwise comparison).

4. Discussion

Results from the *in vitro* experiments confirmed that certain concentrations of allyl ITC influenced *H. avenae* hatching and mycelial growth of *F. graminearum* and *M. nivale*. Therefore, a further experiment was done to test the effect of GLS containing plants incorporated in soil, on survival of plant pathogens in a closed jar experiment.

In our *in vitro* hatching experiment with certain concentrations of allyl ITC into water agar, the EC_{50}-value for *H. avenae* was 5.66 mg/L. To our knowledge, there are not performed any previous studies on allyl ITC and *H. avenae*, but ITCs studies have been done for other plant parasitic nematodes. An *in vitro* study performed by Yu et al. (2005) showed that allyl ITC had a strong inhibition on hatching of two Canadian populations of *Heterodera glycines* and *H. schachtii,* with LC_{50}-values at 14.9 and 30.8 µg/mL, respectively. Mwangi et al. (2024) demonstrated in a laboratory assay, that the stubby root nematode species, *Trichodorus* and *Paratrichodorus* had a ED_{50}-value at 11 µg/mL after 24 hours exposure to allyl ITC. Compared to these studies, we got a lower EC_{50}-value in our *H. avenae* hatching experiment, which means that a lower amount of allyl ITC is needed for suppression. There are several reasons for a lower EC_{50}-value in our experiment compared to the studies mentioned, for instance, one reason may be the temperature of the PDA were around 50°C when we added the allyl ITC liquid. Allyl ITC is volatile and may evaporate if the temperature is high. (Ashiq et al., 2022c); Lim (1999) demonstrated an increasing in vapor pressure of allyl ITC when the temperature increased from 5 to 50°C, which may affect the volatility of allyl ITC. Sealing the Petri dishes with Parafilm to keep the allyl ITC inside the Petri dishes might have affected the EC_{50}-value in our experiment. Ashiq et al. (2022b) got 50 % suppression of mycelial growth of *F. graminearum* a higher concentration of allyl ITC compared to our results. However, Ashiq et al. (2022b) did not mention that they sealed the Petri dishes. A lower EC_{50}-value in our experiment could also be caused by the natural variation of the Ridabu-population. In some Petri dishes without allyl ITC, hatched J2 were not observed which may have been that the cysts were empty. The method where *H.* avenae cysts were exposed different concentrations of allyl ITC poured into water agar was practicable, even though the counting of J2 was challenging due to the recounting of hatched J2 from previous countings. Even though there can be other reasons that we got a lower EC_{50}-value, this experiment shows that allyl ITC has a potential to suppress hatching *H. avenae* by 50 % at an allyl ITC concentration of 5.66 mg/L. Due to this, suppression of *H. avenae* hatching may also happen in filed with cruciferous plants containing allyl ITC. However, no suppression of hatching may happen because in field there are many other factors that may

affect the ability of allyl ITC to suppress hatching, such as soil temperature and the release of ITC from the plants may be reduced in field compared to *in vitro*.

A difference in mycelial growth under exposure to certain allyl ITC concentrations was observed between the three isolates of *F. graminearum* with EC_{50}-values were 6.36, 9.50 and 7.62 mg/L for isolate number 200 630, 201 196 and 202 058 respectively. In an *in vitro* study from England, a ED_{50}-value at a concentration of allyl ITC 99 mg/L in PDA was observed for mycelial growth of *F. graminearum* (Ashiq et al., 2022c). Sarwar et al. (1998) demonstrated a 50 % reduction in diameter of fungal colony (SD_{50D}) of *F. graminearum* mycelium at a concentration of 2-propenyl (allyl ITC) at 4.3 µmol/L in agar. The ITC 2-propenyl is the breakdown product from sinigrin. These two studies demonstrate different results compared to our results, Ashiq et al. (2022b) that got suppression at a higher allyl ITC concentration (99 mg/L) and Sarwar et al. (1998) got suppression at a lower concentration (4.3 µmol/L). Reasons why we got 50% suppression at a lower concentration of allyl ITC compared to Ashiq et al. (2022c), could be such as volatility of allyl ITC due to temperature in agar and sealing the Petri dishes with Parafilm to reduce the emission of allyl ITC, as described for *in vitro* nematode hatching experiment above, may have reduced the EC_{50} value in our fungal growth experiment. On the other hand, Sarwar et al. (1998) got suppression of fungal growth at a lower concentration compared to our experiment. Sarwar et al. (1998) sealed the flask containing agar with vacuum grease, maybe to keep the allyl ITC in the flask, as we did with sealing the Petri dishes with Parafilm. Based on our results from *in vitro* experiment with suppressive effect on mycelial growth *F. graminearum*, allyl ITC may have a suppressive effect of *F. graminearum* mycelial growth.

There was also observed a difference in EC_{50}-values of the three M. nivale isolates 200 136, 200 231 and 202 786 where 50% suppression of mycelial growth were observed at 8.60, 10.83 and 10.27 mg/L respectively. Isolates number 200 231 and 202 786 tolerated a higher concentration of allyl ITC before 50 % suppression of mycelial growth, compared to isolate number 200 136. The differences between EC_{50}-values of mycelial growth of *M. nivale,* indicates we got a difference between isolates of *M. nivale,* which Abdelhalim et al. (2020) showed. Abdelhalim et al. (2020) observed a difference in pathogenicity of *M. nivale* between different isolates and between the host plant they were isolated from, which can explain the differences in EC_{50}-valuesof the three isolated of *M. nivale*. To our knowledge, other studies have not been performed to investigate the interaction between allyl ITC and *M. nivale.* In our *in vitro* experiment, allyl ITC suppressed the mycelial growth of *M. nivale*. Therefore, allyl ITC

may have a suppressive effect of *M. nivale* mycelial growth, but more knowledge should be obtained.

Differences were observed between the three experiments with effect of allyl ITC on mycelial growth, repeated in time. In experiment number two, all isolates had a higher relative growth rate than experiment number one and three. *M. nivale* isolate number 200 136 was not used in experiment two, because of poor mycelial growth during pre-cultivation. Lower EC_{50}-value for this *M. nivale* isolate, may be that this isolate was not used in experiment two which had higher relative growth rates for all isolates. A reason why could be that two persons performed these *in vitro* experiments. Another reason could be the period between making PDA with allyl ITC and placing the agar plug with mycelia on the PDA may have differed. If the time between these had increased more allyl ITC may have released from the petri dishes.

The closed jar experiment was implemented to see if incorporation of cruciferous plants had an effect of the hatching of *H. avenae* and mycelial growth of *F. graminearum,* and if duration of exposure in jars influenced these two plant pathogens. The *H. avenae* diapause treatments were added to see if a period of low tempera had an essential effect on hatching. Ashiq et al. (2022b) incubated jars for eight weeks and saw suppression of fungal growth from blind oat spikelets exposed in jars with two cultivars of *B. juncea, R. sativus* and *Eruca sativa*. A study that tested how long the GLSs and ITCs were detectable after incorporation were conducted by Gimsing and Kirkegaard (2006). They detected a maximum of GLSs and ITCs in soil, at 30 minutes after incorporation of pulverized GLSs and ITCs. In addition, ITCs were detectable 12 days after treatment. The duration of breakdown is important for how long the volatile GLSs, and ITCs possibly are available for suppression of plant pathogens. Based on Ashiq et al. (2022b), the jar experiment was designed to include an eight-weeks jar treatment compared with a shorter period (two weeks) of exposure to see if there was a short-term effects of the cruciferous plants. Results from two and eight weeks of exposure in jars will be discussed later.

The total number of eggs and J2 counted from ten cysts after 21 days at 15°C were lower for the eight weeks exposure compared to two weeks exposure. The total cyst content was almost equal for no diapause and diapause. Even though the Ridabu-population had a naturally variation of cyst content, the number of eggs and J2 should have been almost the same for two and eight weeks. The low egg and J2 number after eight weeks can be explained by fewer remaining cysts in the nylon bags at the end of the experiment and may related to biological degradation of cysts due to long exposure under anaerobic conditions. When opening the jars after eight weeks exposure it smelled fermented, and some degradation of the cysts may have

happened. Allyl ITC and other biofumigants are known to degrade proteins and enzymes important for cyst nematodes (Watts, 2018). Therefore, the ITCs released from the chopped cruciferous plants may have reduced the number of cysts remaining in the nylon bags. Ireholm (1996) got hatching of *H. avenae* J2 after exposure of cysts to 12°C for 26 weeks. The Ridabu-population was thought to contain cysts from more than one generation and can be assumed that some of the cysts had already got the diapause needed for hatching at the preferred temperature. The low total number of eggs and J2 could also be explained by hatching of J2 during exposure in jars. The cysts exposed for eight weeks, got six more weeks at 10-15°C preferable hatching temperatures. The low number of J2 and eggs remaining in the cysts after eight weeks exposure, could therefore also be explained by hatching of J2 during exposure. Extraction of the soil after the experiment would have been interesting to see if some J2 were remaining in the soil.

Due to variation between the total cyst content at two and eight weeks, and the uncertainty of the low total cyst content at eight weeks. I have chosen to be emphasizing the two weeks results for the further discussion.

After exposure of cysts in jars with soil and plant material for two weeks, the number of hatched J2 were significantly higher for diapause compared to no diapause treatment. In a Swedish long-term storage study, Ireholm (1996) saw the highest average cumulative emergence from *H. avenae* cysts (26 weeks at 12°C) when the cysts were pre-stored at 2°C for 6 months. In addition to (Ireholm, 1996), literature gives limited knowledge on temperature requirements for diapause and hatching requirements on CCN populations in the Nordic countries. Norwegian scientific experiences indicate a period of three months at 4°C and lower is needed to induce hatching of local *H. avenae* populations (Ricardo Holgado unpublished).

Changing the temperature from 4°C for five weeks, to 10-15°C for either two or eight weeks, and finally 21 days at 15°C seemed to be enough for the Ridabu-population to hatch to some extent even with no diapause treatment. But in accordance with Ireholm (1996) and Norwegian experiences, the diapause treated cysts who were exposed to a pre-storage for five weeks at 4°C, two weeks in jars and then 80 days diapause (a total of 115 days at 4°C) had a significant higher level of hatching than the no diapause treatment.

There was not any significant difference in the number of hatched J2 between treatments with plant materials and untreated, could be that almost all cultivars had reached the flowering stage, and some had gotten bulbs at the start of the jar experiment. Except Undercover, which only

had reached the bud stage. Sarwar and Kirkegaard (1998) described that the levels of GLS in roots and shoot tissue decreased with plant age, and the reduction was higher for the field grown plants. The content of GLS varies between *Brassica* species and plant part which was observed in a study from England. Ashiq et al. (2022a) performed a study that assumed a higher GLS content in shoots compared to the roots of *Brassica* species, and *B. juncea* had the highest amount of GLS, especially sinigrin. Allyl ITC is the breakdown product of sinigrin after enzymatic hydrolysis (Olivier et al., 1999). Due to older plants, as Sarwar et al. (1998) mentioned, the amount of GLSs and ITCs may have declined as the plants develope and therefore the cruciferous plants not gave significantly difference compared to untreated.

For cysts incubated in jars for two weeks, incorporation of *B. juncea* (Energy) tended to suppress hatching of J2 more than other treatments with cruciferous plants, but it was not significant different. Yu et al. (2007) demonstrated a potential of *B. juncea* in controlling *H. glycines, H. schachtii, Meloidogyne incognita* and *Pratylenchus* species, and showed that *B. juncea* tended to supress hatching of PPNs. Results from the jar experiment support the study by Yu et al. (2007), and indicates that *B. juncea* also will suppress hatching of *H. avenae.*

Cysts exposed to *R. sativus* (Akiro, Ikarus, Siletina and Structurator) differed between the suppressive effect. After two weeks exposure in jars and no diapause treatment, the number of hatched J2 were lower than untreated for all of them. A study from the US, demonstrated that *Raphanus sativus* had the potential to supress the number of *Meloidogyne hapla* juveniles in a greenhouse pot experiment (Melakeberhan et al., 2008). This study shows an effect on another plant parasitic nematodes, the root-knot nematode *M. hapla.* The results from the jar experiment indicate that *R. sativus* also have a potential to supress *H. avenae.* However, more knowledge should be obtained on suppression of *H. avenae* by *R. sativa*.

For cysts with two weeks exposure in jars the total number of eggs and J2 was in average 79 per cyst (790 per 10 cysts) and it differed between 110 eggs and J2 for Ikarus-root and Energy had 58 eggs and J2 per cyst. Ireholm (1996) got an average of 239 fertile eggs, from an average of ten cysts in a *H. avenae* population from Sweden. At Plantevernleksikonet, for *H. avenae* the number of eggs is described to differ between 200 and 300 within a cyst (Vennatrø, 2023). In the USA, the *H. avenae* population differed between 1 000 and 21 000 eggs plus juveniles per kg soil (Smiley et al., 2005). The number of eggs inside a female body is ranging from 100 to 400, depending on the CCN species, the host plant variety and environmental factors such as temperature and soil moisture (Smiley et al., 2017). A reason why the total number of eggs and

J2 differed a lot between treatment of chopped cruciferous plants, could be caused by not randomizing the nylon bags before placing them into jars. When making the nylon bags, different people were picking the cysts, which can lead to different choices of cysts expected to be full of eggs. Differences between treatments of plant material in jars could therefore be a natural variation in number of eggs and J2 in the cyst. Since the Ridabu-population originated from naturally infected soil, it likely represented more variation between cyst content. In general, *H. avenae* have only one generation per year (Meagher, 1977; Smiley et al., 2017). Using a lab-population of *H. avenae*, the cysts will then be of same age. Together with a lab population and randomizing the nylon bags before placing them inside the soil can reduce the variation in cyst content, to some extent, between the treatments with plant material.

From our closed jar experiment, mycelial growth was observed from all oat spikelets, and there was not any significant difference in diameter of mycelial colony between two or eight weeks of exposure in jars, or between treatments with different plant material in jars. In contrast to our results, Ashiq et al. (2022b) observed suppression of mycelial growth from *F. graminearum* infected blind oat spikelets treated with certain *Brassicaceae* species (*B. juncea, R. sativus* and *E. sativa)* in jars for eight weeks. We expected a biofumigation effect by suppression of mycelial growth from oat spikelets infected with *F. graminearum* after eight weeks of exposure in jars contain soil and chopped plant materials, like Ashiq et al. (2022b) demonstrated. Ashiq et al. (2022b) incorporated *B. juncea, R. sativus* and *E. sativa* into jars containing soil. A reason why we did not get any suppression on mycelial growth after exposure to cruciferous plants in jars could be that we harvested the plants after flowering stage and bulbs had developed, and Ashiq et al. (2022b) used shoots of the *Brassica* species at the early-bud stage. However, in a field with cruciferous plants as a cover crop, the plants would grow until they die and therefore may not release volatile ITCs before the plants starts to degrade.

A significant smaller size of bacterial colony on oat spikelets from untreated jars, compared to jars with plant material was observed. *Pseudomonas spp.* and *Stenotrophomonas spp.* were detected on the oat spikelets, and these bacteria are expected to be found in soil (Dorjey et al., 2017; Ghosh et al., 2020). This indicates that the bacteria mainly have come from the plant material incorporated in the soil. The plants got rinsed with tap water but some residuals from soil may have occurred. It could have come from the soil that we used in the jars, but if that was the case there should have been the same size of bacterial colonies in the jars without plant material as well.

The soil mixture with half the amount of water gave the significantly highest diameter of *F. graminearum* mycelial growth from oat spikelets, compared to the two other treatments. *F.* graminearum prefer humid conditions during production of inoculum (Brandsæter et al., 2009; Doohan et al., 2003). Even though this was a small experiment, a lower soil water content gave a higher mycelial growth of *F. graminearum*, does not correspond to the papers mentioned, where more humid and wet condition can give be preferable for the mycelial growth.

Results from the closed jar experiment did not show any significant difference between treatments with plant materials and untreated, both for nematode and fungi. A reason why we did not get significant suppression of *H.* avenae and *F. graminearum,* could be that the plants got attacked by the sawfly *A. rosae* in the field at Øsaker*,* and herbivory on *Brassica* species of some insects can start the hydrolysis of GLS to ITC (Ahuja et al., 2011). The sawfly *A. rosae* has the ability to feed on *Brassica* species and store them in the haemolymph, as a protecting mechanism (Müller, 2009). This review article also looked at the plant-species specific responses in *S. alba* and *B. juncea*. After feeding of *A. rosae*, the GLS levels and myrosinase activity increased in *S. abla* and decreased in *B. juncea.* A reduced amount of GLS and ITCs could reduce the cruciferous plant's ability to suppress plant pathogens. To some extent, *A. rosea* attacked plants, maybe with less GLSs and ITCs available, may have been incorporated in the soil and may have influenced the results negatively.

After GLS- and ITC chemical analyses performed at Department of pesticides and natural products chemistry (NIBIO) of the cruciferous plants, the GLS and ITC content of two *B. juncea* cultivars (Energy and Terminator), and one *B. carinata* cultivar (Undercover) were analysed. From results of these two species sinigrin and the corresponding allyl ITC were detected. The results of these chemical analysis may explain the results of low hatching of *H.* avenae after exposure to Energy *(B. juncea*) in jars. Energy was among the plant treatments with plant materials with low number of hatched J2, low relative number of emerged J2 for both two and eight weeks of incubation in jars. A biofumigation experiment with potato cyst nematode (*G. pallida*), *B. juncea* caused 95 % mortality of this cyst nematode (Lord et al., 2011). Ashiq et al. (2022b) also got suppression by *B. juncea* on mycelial growth of *F. graminearum,* when exposing *F. graminearum* infected blind oat spikes to chopped *B. juncea.* Due to the results of lower hatching of J2 when from the cysts exposed to Energy in jars, Energy can therefore have a potential to suppress *H. avenae* in field.

Results from the *in vitro* experiments demonstrated suppression on hatching of *H. avenae* and fungal growth of *F. graminearum* and *M. nivale*. In the jar experiment, only cysts incorporated in plant material from Energy tended to reduce hatching of *H. avenae,* but no treatment with plant material was significant different from untreated. However, there was no difference in reduction of mycelial growth of *F. graminearum* infected oat spikelets. Based on our results, in a field with cruciferous plant *M. nivale* may need either more plant material, or plants with higher concentration of allyl ITC compared to *F. graminearum,* to be suppressed.

5. Conclusion

Results from the experiments presented in this thesis, demonstrate that in the *in vitro* experiment allyl ITC reduced the survival of *H. avenae, F. graminearum* and *M. nivale* at a certain concentration of allyl ITC, which in turn supports the two first hypotheses. Also, *H. avenae, F. graminearum* and *M. nivale* had EC_{50}-values at 5.66, 7.83 and 9.90 mg/L, respectively, which show a variation between the plant pathogens. However, the closed jar experiment did not demonstrate that the use of cruciferous plants reduces the impact of plant pathogens, such as *H. avenae* and *F. graminearum*. The cultivar Energy (*B. juncea)* tended to be the most suppressive plant on hatching of *H. avenae.* The number of hatched J2 after 21 days was significantly higher for cysts incubated for two weeks in jars, compared to eight weeks in jars. Also, the number of hatched J2 were significantly higher for diapause treatment compared to no diapause. J2 hatched after both diapause and no diapause treatment, and the total number of eggs an J2 were almost equal in this case. On the other hand, there was not significant difference on mycelial growth from *F. graminearum* infected oat spikelets by any of the cruciferous cultivars. Due to no significant difference between plant material incorporated in soil compared to untreated, the third hypothesis is not proved. More experience and knowledge must be obtained at this subject.

Today the interest and use of other cover crops, in Norwegian farming have increased, and some radish cultivars are already used in Norway as cover crops. However, more knowledge about the use of cruciferous plants in Norwegian farming can be obtained. Together with other management strategies, cruciferous plants as cover crops, may reduce the amount of pesticide application in control of nematodes and fungi.

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7. Appendix

Appendix 1: Statistical analyses of ten *Heterodera avenae* cysts incubated for two weeks in jars containing soil with chopped plant material (Treatment). Numbers presented are the average numbers of hatched juveniles from five replications per treatment in jars, after 21 days (21d) at 15°C. Different letters indicate significant treatment effects at P<0.05 (Tukey pairwise comparison).

Treatment	N	Mean	Grouping		
Ikarus-root	10	1100,2	A		
Ikarus	10	910,0	A		B
Action	10	880,9	A		B
Undercover	10	824,3	A		B
Untreated	10	812,0	A		B
Structurator	10	764,4	A		B
Akiro	10	742,3	A		B
Siletina	10	680,4	A		B
Structurator-root	10	606,6	A		B
Energy	10	579,8			B

Appendix 2: Statistical analyses of ten *Heterodera avenae* cysts incubated for two weeks in jars containing soil with chopped plant (Treatment). Numbers presented are the average relative number of emerged J2, from five replications per treatment in jars, after 21 days (21d) at 15°C. Different letters indicate significant treatment effects at P<0.05 (Tukey pairwise comparison).

Treatment	N	Mean	Grouping
Untreated	10	0,385089	A
Akiro	10	0,374252	A
Action	10	0,364275	A
Structurator-root	10	0,351528	A
Ikarus	10	0,331736	A
Ikarus-root	10	0,330907	A
Undercover	10	0,315065	A
Siletina	10	0,296015	A
Structurator	10	0,287397	A
Energy	10	0,196763	A

Appendix 3: Statistical analyses of ten *Heterodera avenae* cysts incubated for two weeks in jars containing soil with chopped plant material (Treatment). Numbers presented are the average numbers of hatched juveniles from five replications per treatment in jars, after 21 days (21d) at 15°C. Different letters indicate significant treatment effects at P<0.05 (Tukey pairwise comparison).

Treatment	N	Mean	Grouping		
Ikarus-root	10	381,4	A		
Action	10	362,2	A	B	
Ikarus	10	339,0	A	B	
Untreated	10	303,5	A	B	C
Akiro	10	299,6	A	B	C
Undercover	10	292,6	A	B	C
Structurator	10	234,8	A	B	C
Siletina	10	213,9	A	B	C
Structurator-root	10	167,9		B	C
Energy	10	123,5			C

Appendix 4: Statistical analyses of ten *Heterodera avenae* cysts incubated for two weeks in jars containing soil with chopped plant material (Treatment), followed by no diapause treatment. Numbers presented are the average number of hatched juveniles from five replications per treatment in jars, after 21 days (21d) at 15°C. Different letters indicate significant treatment effects at P<0.05 (Tukey pairwise comparison).

Treatment	N	Mean	Grouping	
Untreated	5	223,8	A	
Akiro	5	164,0	A	B
Structurator	5	161,4	A	B
Ikarus-root	5	154,4	A	B
Undercover	5	121,4	A	B
Ikarus	5	118,2	A	B
Siletina	5	113,4	A	B
Action	5	94,0	A	B
Structurator-root	5	84,2	A	B
Energy	5	42,2		B

Appendix 5: Statistical analyses of ten *Heterodera avenae* cysts incubated for two weeks in jars containing soil with chopped plant material (Treatment), followed by diapause treatment. Numbers presented are the average number of hatched juveniles from five replications per treatment in jars, after 21 days (21d) at 15°C. Different letters indicate significant treatment effects at P<0.05 (Tukey pairwise comparison).

Treatment	N	Mean	Grouping	
Action	5	630,4	A	
Ikarus-root	5	608,4	A	
Ikarus	5	559,8	A	B
Undercover	5	463,8	A	B
Akiro	5	435,2	A	B
Untreated	5	383,2	A	B
Siletina	5	314,4	A	B
Structurator	5	308,2	A	B
Structurator-root	5	251,6	A	B
Energy	5	204,8		B

Appendix 6: Statistical analyses of ten *Heterodera avenae* cysts incubated for eight weeks in jars containing soil with chopped plant material (Treatment). Numbers presented are the average total number of eggs and juveniles from five replications per treatment in jars, after 21 days (21d) at 15°C. Different letters indicate significant treatment effects at P<0.05 (Tukey pairwise comparison).

Treatment	N	Mean	Grouping
Ikarus-root	10	584,0	A
Siletina	10	552,3	A
Action	10	544,0	A
Ikarus	10	447,2	A
Structurator-root	10	445,4	A
Untreated	10	409,2	A
Akiro	10	401,9	A
Structurator	10	383,5	A
Undercover	10	382,6	A
Energy	10	354,8	A

Appendix 7: Statistical analyses of ten *Heterodera avenae* cysts incubated for eight weeks in jars containing soil with chopped plant (Treatment). Numbers presented are the average relative number of emerged J2, from five replications per treatment in jars, after 21 days (21d) at 15°C. Different letters indicate significant treatment effects at P<0.05 (Tukey pairwise comparison).

Treatment	N	Mean	Grouping
Ikarus-root	10	0,603471	A
Siletina	10	0,579912	A
Untreated	10	0,552645	A
Structurator	10	0,533041	A
Akiro	10	0,532608	A
Ikarus	10	0,519166	A
Energy	10	0,494647	A
Action	10	0,488256	A
Undercover	10	0,421163	A
Structurator-root	10	0,392268	A

Appendix 8: Statistical analyses of ten *Heterodera avenae* cysts incubated for eight weeks in jars containing soil with chopped plant material (Treatment). Numbers presented are the average numbers of hatched juveniles from five replications per treatment in jars, after 21 days (21d) at 15°C. Different letters indicate significant treatment effects at P<0.05 (Tukey pairwise comparison).

Treatment	N	Mean	Grouping
Ikarus-root	10	348,8	A
Siletina	10	310,8	A
Ikarus	10	247,0	A
Action	10	229,0	A
Untreated	10	178,0	A
Akiro	10	173,1	A
Undercover	10	162,8	A
Structurator	10	160,9	A
Energy	10	160,1	A
Structurator-root	10	130,7	A

Appendix 9: Statistical analyses on mycelial growth from oat spikelets infected with *Fusarium graminearum,* exposed for two and eight weeks in jars containing soil with chopped plant (Treatment). Numbers presented are the estimated average diameter of mycelial growth from five replicates per treatment in jars. Different letters indicate significant treatment effects at P<0.05 (Tukey pairwise comparison).

Treatment	N	Mean	Grouping
Structurator - Root	10	22,220	A
Ikarus	10	21,750	A
Ikarus - Root	10	21,740	A
Energy	10	21,530	A
Action	10	21,270	A
Structurator	10	21,100	A
Siletina	10	21,020	A
Undercover	10	20,820	A
Akiro	10	20,350	A
Untreated	10	20,323	A

Appendix 10: Statistical analyses of the bacterial colonies (0-4) from oat spikelets infected with *Fusarium graminearum,* exposed for two and eight weeks in jars containing soil with chopped plant (Treatment). Numbers presented are the estimated average number of bacterial sizes, from five replicates per treatment in jars. Different letters indicate significant treatment effects at P<0.05 (Tukey pairwise comparison).

Treatment	N	Mean	Grouping	
Siletina	10	2,98	A	
Structurator - Root	10	2,78	A	
Structurator	10	2,64	A	
Action	10	2,62	A	
Akiro	10	2,40	A	
Ikarus	10	2,38	A	
Ikarus - Root	10	2,32	A	
Undercover	10	2,18	A	
Energy	10	2,12	A	
Untreated	10	0,78		B

Appendix 11: Statistical analyses of the percentage of oat spikelets with bacterial colony (%) from spikelets infected with *Fusarium graminearum* exposed for two and eight weeks in jars containing soil with chopped plant (Treatment). Numbers presented are the estimated average percentage of spikelets with bacterial colony from five replicates per treatment in jars. Different letters indicate significant treatment effects at P<0.05 (Tukey pairwise comparison).

Treatment	N	Mean	Grouping	
Action	10	100	A	
Structurator	10	98	A	
Ikarus - Root	10	96	A	
Ikarus	10	96	A	
Akiro	10	94	A	
Structurator - Root	10	92	A	
Undercover	10	90	A	
Siletina	10	90	A	
Energy	10	84	A	
Untreated	10	34		B

Appendix 12: Statistical analyses on mycelial growth from oat spikelets infected with *Fusarium graminearum,* exposed for two and eight weeks in jars containing different soil mixtures (0.5x water, 2x Peat and Original). Numbers presented are the estimated average diameter of mycelial growth from five replicates per soil mixture in jars. Different letters indicate significant treatment effects at P<0.05 (Tukey pairwise comparison).

Treatment	N	Mean	Grouping	
0,5x Water	10	23,67	A	
2x Peat	10	22,36	A	B
Orginal	10	21,47		B

Appendix 13: Statistical analyses on mycelial growth from oat spikelets infected with *Fusarium graminearum,* exposed for two and eight weeks in jars containing different soil mixtures (0.5x water, 2x Peat and Original). Numbers presented are the estimated average diameter of mycelial growth from soil mixture incubated for two and eight weeks. Different letters indicate significant treatment effects at P<0.05 (Tukey pairwise comparison).

Treatment duration	N	Mean	Grouping
8 weeks	15	22,5133	A
2 weeks	15	22,4867	A

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