Cover crops for plant protection

Cover crops for control of root pathogenic *Plasmodiophora brassicae* and the root parasitic nematodes *Pratylenchus penetrans* and *Meloidogyne hapla*

Mellangrödor i syfte för växtskydd

Mellangrödor för kontroll av rot patogena *Plasmodiophora brassicae* och rot parasitära *Pratylenchus penetrans och Meloidogyne hapla*

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Master's project (30 hp)
Swedish University of Agricultural Sciences, SLU Department of Plant Protection Biology, Horticultural science
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Abstract

English

Root pathogens and parasites are an increasing problem in agricultural and horticultural production. The soil borne pathogen *Plasmodiophora brassicae* is one of the major pathogens in oilseed rape production and the plant parasitic nematodes (PPNs), *Pratylenchus penetrans* and *Meloidogyne hapla* infest important crops such as carrot, potato and strawberry. In common, both of the nematodes and *Plasmodiophora brassicae* strongly depend on host plants for reproduction, and are difficult to control. There is a need for clever integration of multi-tactic strategies to prevent their dissemination. In the present study, cover crops were examined in bioassays for their potential to decrease the population density of both nematodes and infection level of *Plasmodiophora brassicae*. The examined cover crops in the *Plasmodiophora brassicae* trial *Lolium perenne* and *Lupinus angustifolius,* revealed medium high disease severity indices (DSIs) on the indicator plant *Brassica napus.* However, no significant decrease was found in comparison to plant free soil (fallow). In the PPN trials, the cover crop species *Avena strigosa* and *Lupinus angustifolius* were investigated. Promising results were obtained for *A. strigosa* indicating high resistance to *M. hapla* and very efficient suppression of *Pratylenchus penetrans* in infested soil. *Lupinus angustifolius* was found to be susceptible to *M. hapla* and to be a poor host species for *Pratylenchus penetrans*. Potentially *A. strigosa* could be recommended in crop rotation where both nematode species are challenging the production of susceptible cultivars. *Lupinus angustifolius* should be avoided in crop rotation where *M. hapla* is present but may be used in crop rotation where *Pratylenchus penetrans* is present to prevent excessive multiplication. Further studies are needed to determine the potential of *Lupinus angustifolius,* as contradictory results have been reported from experiments with different varieties of the cover crop.

Swedish

Rot patogener och parasiter är ett ökande problem i agrikulturell och hortikulturell produktion. Den jordburna patogen *Plasmodiophora brassicae* är en av de vanligaste patogenerna i raps produktion och de växtparasitära nematoderna (PPNs), *Pratylenchus penetrans* och *Meloidogyne hapla* angriper betydelsefulla grödor som morot, potatis och jordgubbar. Gemensamt för nematoderna och *Plasmodiophora brassicae* är att de är beroende av värdväxter för reproduktion och är svåra att kontrollera. Det finns ett behov av smart integrering av multi-taktiska strategier för att förhindra spridning. I den aktuella studien undersöktes mellangrödor i biotester för deras förmåga att potentiellt reducera de utvalda nematod arterna och *Plasmodiophora brassicae*. De undersökta mellangrödorna i *Plasmodiophora brassicae* försöket, *Lolium perenne* och *Lupinus angustifolius,* visade medelhöga sjukdomsindex (DSIs) på indikatorväxten *Brassica napus*. Ingen signifikant skillnad visades dock i jämförelse med plantfri jord (träda). I experimenten med de växtprarasitära nematoderna undersöktes mellangrödorna *Avena strigosa* och *Lupinus angustifolius*. Lovande resultat erhölls för *A. strigosa* som visade hög resistens mot *M. hapla* och mycket effektivt reducering av *Pratylenchus penetrans* i infekterad jord. *Lupinus angustifolius* visade sig vara mottaglig för *M. hapla* och vara en dålig-värdväxt för *Pratylenchus penetrans*. Potentiellt kan *A. strigosa* rekommenderas i växtföljder där båda nematodarterna utmanar produktionen av mottagliga grödor. *Lupinus angustifolius* bör undvikas i växtföljd där *M. hapla* förekommer men kan användas i växtföljder där *Pratylenchus penetrans* förekommer för att motverka förökning. Ytterligare studier behövs för att fastställa potentialen för *Lupinus angustifolius*, eftersom motsägelsefulla resultat har rapporterats beroende på sort.

Keywords: Clubroot disease, plant parasitic nematodes, *Lupinus angustifolius, Avena strigosa, Phacelia tanacetifolia, Lolium perenne, Solanum lycopersicum*, *Brassica napus*

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Abbreviations

A. strigosa - Avena strigosa
B - Boron
B. napus - Brassica napus
Ca - Calcium
DSI - Disease severity index
D.w. - Dry weight
F.w. - Fresh weight
K - Potassium
L. angustifolius - Lupinus angustifolius
L. perenne - Lolium perenne
Mg - Magnesium
M. hapla - Meloidogyne hapla
N - Nitrogen
P. tanacetifolia - Phacelia tanacetifolia
Pf/Pi - Final population/Initial population
PPNs - Plant parasitic nematodes
P. brassicae - Plasmodiophora brassicae
P. penetrans - Pratylenchus penetrans
RGI - Root gall index
S. lycopersicum - Solanum lycopersicum
S - Sulfur

Introduction

Clubroot of cruciferous plants is caused by the obligate soil-borne pathogen *Plasmodiophora brassicae* (Zamani-Noor *et al.*, 2021; Wallenhammar, 1996; Friberg *et al.* 2006). The pathogen *P. brassicae* is classified within the order Plasmodiophorids of the protist supergroup Rhizaria (Hwang *et al*. 2012). The pathogen has a major impact in cultivation of crops within the Brassicaceae family as one of the major diaseases in oilseed rape production (Zamani-Noor *et al.* 2021). Clubroot outbreaks can reduce oil content and seed number significantly and result in yield loss of 30-50%, or total yield loss in fields of oilseed rape. Clubroot disease is mainly characterized by the production of tumors on plant roots (Figure 1). The formation of tumors leads to reduced water and nutrient uptake, growth inhibition and withering of the plants resulting in significant yield reduction.

Figure 1. Brassica rapa, severely infected with *Plasmodiophora brassicae.* Photo Izabella Lundborg

Plasmodiophora brassicae is a pathogen with a complex disease cycle that involves two phases: the root hair phase and the clubroot phase (Hwang *et al*. 2012). The primary infection takes place in the root hair phase, where *P. brassicae* first infects the root hairs, by motile zoospores that germinate from resting spores in the soil, infecting cortical tissue of the root. In the clubroot phase secondary plasmodia develop from the zoospores within the root cortex, the plasmodia produce cytokines that re- initiate cell division in the root and as a host defense response the expression of plant genes involved in production of growth regulators are triggered, such as auxins and cytokinin. Consequently, the plant resources get redistributed and cause hypertrophic growth of the root tissue, resulting in tumor formation. In the end of the cycle the secondary plasmodia cleave into millions of resting spores, which are released into the soil while the root decomposes. The resting spore is a long-living resistant reproductive cell containing one zoospore. The pathogen *P. brassicae* is difficult to control since resting spores can survive in the soil without hosts for up to 17.3 years (Wallenhammar *et al.* 2014). There is no available direct control of *P. brassicae* and several factors are influencing the methods used to suppress the disease (Friberg *et al.* 2006). The resting spores germinate when triggered by specific substances excreted from host plant roots, the root exudate chemical composition includes several nutrient elements, N, K, S, Ca and Mg. The composition of the nutrient elements vary as well as the pH value depending on plant species (Rashid & Strelkov, 2013). The spores also respond to abiotic cues such as increased temperature, moisture, pH and high

concentrations of Ca, Mg and B. Resting spores triggered by temperature and moisture are considered to undergo spontaneous germination.

Pathogen-resistant genotypes of oilseed rape are efficiently used to manage clubroot disease. The first resistant cultivar was released on the market in 2006 (Zamani-Noor *et al.* 2021). The resistant *Brassica napus* cv. Mendel displays a race-specific type of resistance to a number of *P. brassicae* isolates. This type of resistance is controlled by one or few resistance situated in different genes. The main step is the recognition of the pathogen from the host plant. The plant produces hypersensitive reactions (HR) or produces molecules that decrease the multiplication rate of the pathogen. It's a qualitative resistance only active against one or few homologous populations (races) (Stuthman & Miller-Garvin, 2007). The resistant cultivar stimulates germination of the resting spores in the soil, stimulating infection but stops or reduces development of disease symptoms and proliferation of the pathogen (Hwang *et al.* 2013). Many cultivars of oilseed rape have been developed in pedigree breeding programs based on the *B. napus* cv. Mendel resistance (Zamani-Noor *et al.* 2021). The management strategy using single gene resistant crops is not to rely on completely, due to possible selection of the pathogen enabling it to overcome the resistance. Research made by Hwang *et al.* (2011) has revealed that clubroot infections are affected by the amount of resting spores in the soil, and that increased inoculum density enhances clubroot disease severity and decreases yield. Infection can occur at low inoculum levels of 10^2 per g soil, but the severity of the symptoms are low. The threshold value of an inoculum level of 10^3 per g soil or above is considered to cause more severe symptoms and higher yield losses. In management strategies of the pathogen it is crucial to consider several preventive measures in combination that can influence the pathogen, the incidence and disease severity (Zamani-Noor *et al.* 2021; Dixon, 2014).

The zoospores of *P. brassicae* emerging at germination of the resting spores are sensitive and short-lived in the soil in absence of host plants (Ahmed *et al.* 2019; Friberg *et al.* 2006). For that reason methods that stimulate resting spore germination are of interest in management of clubroot disease to reduce the inoculum levels in the soil. Alternative cover crops have been studied for this purpose; susceptible host plants e.g., susceptible *B. napus* varieties, can be used as “trap crops”. The “trap crops” are cultivated in infected fields for a few weeks to stimulate the resting spores to germinate and infect them, while after a certain time the plants get removed or killed before *P. brassicae* completes its life cycle, hindering proliferation of resting spores. Management of *P. brassicae* by using “trap crops” is considered involving high risks, because of the difficulties in prediction of removal according to the life cycle stage (Friberg *et al.* 2006). The prediction of removal is difficult due to the distribution, spread and soil factors triggering the infection, and a too late removal of the “trap crops” entails the risk of multiplying the resting spores. Observations and research has also been focusing on the ability of non-host plants to be used for this purpose (Liu *et al.* 2020). The hosts of *P. brassicae* are proposed to be limited to cruciferous plants, despite occasionally *P. brassicae* has been reported to infect non-host species. Non-hosts of this kind have been studied in several cases in field and in vitro, showing potential to reduce the inoculum levels of *P. brassicae* in soil (Liu *et al.* 2020; Ahmed *et al*. 2019; Friberg *et al.* 2006). The function of

these non-hosts is that they are considered to act partly as hosts, producing root exudates that stimulate resting spore germination without getting infected, resulting in no new production of resting spores (Ahmed *et al.* 2019; Friberg *et al.* 2006). There are cases where non-host plants have been observed to be susceptible to the initial stage of *P. brassicae* infection. Some of the non-hosts that have been recorded to stimulate resting spore germination are velvet-grass *Holcus lanatus* L., perennial ryegrass *Lolium perenne* L., indian cress *Tropaeolum majus* L., leeks *Allium ampeloprasum var. porrum* L., rye *Secale cereale* L., field pea *Pisum sativum* and red clover *Trifolium pratense* L. The benefits of these cover crops in reducing the resting spores and disease severity in the coming susceptible main crop has been recorded in comparison to susceptible and resistant hosts as well as for fallow treatment (Friberg *et al.* 2006). The use of cover crops has been questioned, as the reduction of resting spores and disease severity appear similar to less work intensive fallow treatment (Ahmed *et al*. 2019). On the other hand, using cover crops in the crop rotation is not only useful in the management of *P. brassicae,* they provide several additional beneficial services (Udari *et al*. 2017; Finney *et al.* 2017). Cover crops provide supporting services that include prevention of soil erosion, flooding, enhancing soil and water quality and soil structure, provisioning services including green manure and cash crop yield, regulating services related to chemical sprays and water runoff, mechanical tillage, weed control, nutrient leakage, carbon sink, disease and pests and biodiversity in soil, on land and water. Accordingly, cover crops contribute to food security, functional biodiversity, sustainable agriculture, human well-being and climate friendly land use. *Lupinus angustifolius* used as cover crop in crop rotation, provides several of the beneficial services mentioned above (Petersen, 2019). In Sweden, usage of *L. angustifolius* in crop rotation is relatively rare. *Lupinus angustifolius* is recommended to be used in rapeseed crop rotations (Lindström, 2010), however other members in the fabaceae family such as field pea, *Pisum sativum* and red clover, *Trifolium pratense* L. have been observed to stimulate *P. brassicae* resting spore germination (Friberg *et al.* 2006). Regarding the influence of cover crops, research and observations have been conflicting in some cases (Ahmed *et al.* 2019; Friberg *et al.* 2006). For example, ryegrass, *Lolium perenne*, was effective in reduction of *P. brassicae* resting spores (Rod and Robak, 1994); however, in another experiment by Robak (1996) the resting spore amount did not significantly decrease. The conflicting observations and research findings are promoting usage of fallow as the influence of specific cover crops on *P. brassicae* is not fully understood (Ahmed *et al.* 2019). Therefore, one aim of my study was to assess the effect of non-host crops *L. perenne* and *L. angustifolius* on resting spore density and disease severity to possibly enhance the usage and credibility of cover crops as components in integrated clubroot management programs.

Another important threat to plant health are the plant parasitic nematodes (PPN) *Pratylenchus penetrans* and *Meloidogyne hapla*, which both are soil-borne pests of increasing concern to global agriculture (Uesugi *et al.* 2018; Hooks *et al.* 2010; Zunke, 1990). The economic losses caused by plant parasitic nematodes are presumed to exceed over US $100 billion/year worldwide, including 10-20% of agricultural yield loss (Hooks *et al.* 2010; Park *et al.* 2014). In this presumption, secondary diseases caused by the presence of parasitic nematodes are not

included which would raise substantially the severity and total costs related to nematodes. Root-knot nematodes *Meloidogyne* spp. are the most important plant parasitic nematodes, infesting over 2,000 plant species globally and are estimated to be responsible for approximately 5% of global agricultural yield loss (Park *et al.* 2014).

Plant parasitic nematodes have a piercing mouthpart called stylet which differentiates them from other non-plant parasitic species (Pettersson and Åkesson, 2011). Juvenile *M. hapla* and female *P. penetrans* are displayed in (Figure 2). Encountering a host, PPNs use the stylet to pierce the plant tissue and to feed on the plant cell nutrients. In general, the symptoms in the field are visible as small, separated areas with reduced plant growth, and commonly the yield gets reduced in the direction of the field's lane (Pettersson and Åkesson, 2011; Nilsson, 2014; Zunke, 1990). More specifically, *P. penetrans* feeding of the root cortex causes brownish wounds on the roots, leading to reduced nutrient and water uptake that simultaneously reduces growth. In root crops, symptoms involve root forking e.g. in carrots (Grabau, 2017). Secondary, the damage can provide a gateway for harmful bacteria and fungi, which in turn can aggravate the attack and promote development of other root diseases (Pettersson and Åkesson, 2011; Nilsson, 2014; Zunke, 1990). *Pratylenchus penetrans* is a migratory endoparasite (Andersson, 2018). The migratory endoparasitic species have multiple feeding sites and are neither bound to the soil nor the root and can move freely between soil and inside the root, which makes them difficult to control. *Meloidogyne hapla* is a sedentary endoparasitic nematode, which, in transit from the second juvenile stage to the third gets tied to the root (Albertson Juhlin *et al.* 2014). The females inside the root cause bile formations on host plant roots, resulting in reduced nutrient and water uptake that decreases plant growth and in severe cases results in plant death.

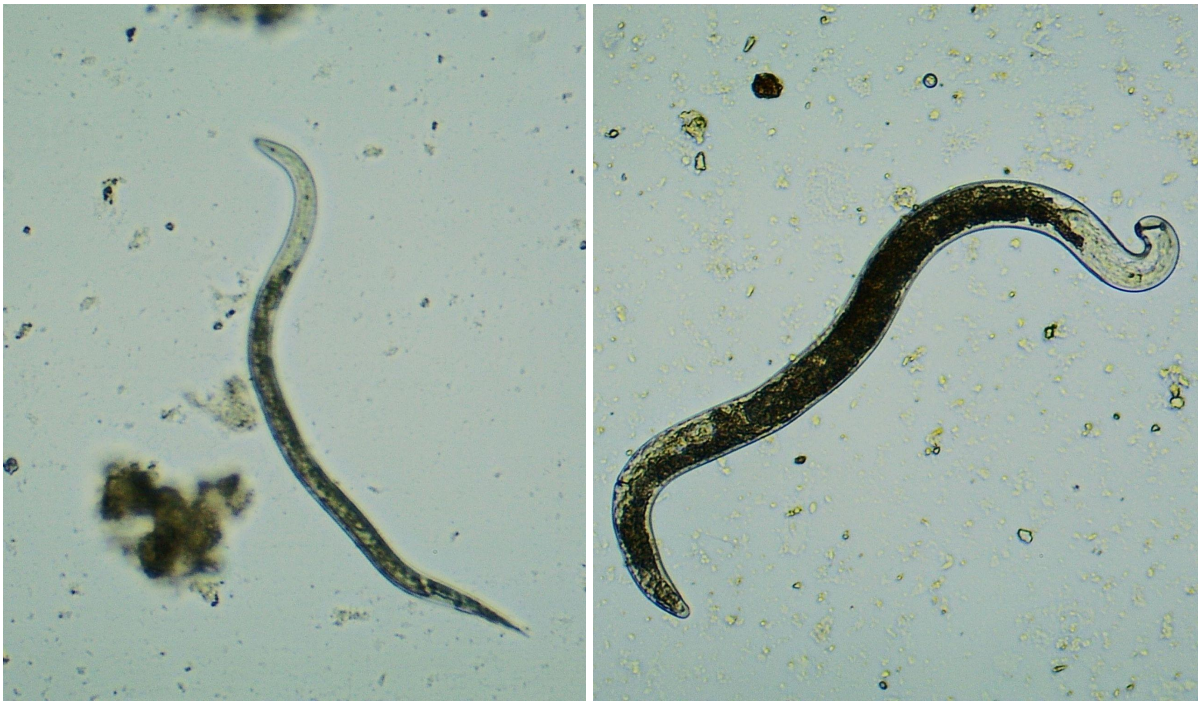

Figure 2 Left: second stage juvenile *Meloidogyne hapla* under a high resolution light microscope; right: female *Pratylenchus penetrans* under a high resolution light microscope. Photo Izabella Lundborg.

Both *M. hapla* and *P. penetrans* have a wide host range causing severe yield loss in several important agricultural crops such as carrot, potato, sugarbeet, tomato, strawberry and legumes (Ascard & Hansson 2013). There is a need for development and adoption of sustainable management methods against plant parasitic nematodes (Hooks *et al*. 2010; Park *et al*. 2014; Pettersson and Åkesson, 2011). Current management methods involve usage of resistant crop varieties, steaming, fallow, crop rotation, biological control, biofumigation, fumigation and chemical nematicides. Fallow treatment, steaming, fumigation and chemical nematicides entails greenhouse gas emission, harm to soil, land and water biodiversity. Moreover, the wide host range of these nematode species and the limited availability of resistant cultivars complicates the use of crop rotation in several cropping systems (Hooks *et al*. 2010). Overall, currently applied actions are in general either non efficient or non-sustainable leaving a great need and demand for alternative sustainable action methods (Park *et al*. 2014). A method aligned with an environmentally sustainable approach is the usage of resistant or antagonistic non-hosts, *e.g.,* marigold's *Tagetes* spp. have been the subject of various research projects due to their allelopathic potential against plant parasitic nematodes. Marigolds are the most studied species against plant parasitic nematodes and 29 varieties of marigold have been reported as being resistant to root-knot nematodes *Meloidogyne spp.* (Hooks *et al*. 2010).
Clearly, cultivation of resistant or antagonistic non-hosts, can reduce nematode populations in several ways, as they may act as poor hosts or non-hosts, act as trap crops, produce allelopathic compounds that inhibit plant parasitic nematodes development, and they may support development of environments that benefit nematode antagonistic flora or fauna (Wang *et al.* 2002; Hooks *et al*. 2010; Grabau *et al*. 2017). These actions may occur either simultaneously or independently reducing plant parasitic nematode densities. Therefore, another objective of this study was to investigate black oat, *Avena strigosa,* lacy phacelia, *Phacelia tanacetifolia* and blue lupine, *Lupinus angustifolius,* for their potential as resistant or antagonistic non-hosts to reduce the population densities of *M. hapla* or *P. penetrans* in soil.

Aim and research questions

The aim of this research was to assess the efficacy of cover crops as an alternative and sustainable method in plant protection. Cover crop credibility was evaluated by their ability to counteract specific nematode species or club root disease. Four main research questions guided this study:
Does the cultivation of *Lupinus angustifolius* and/or *Lolium perenne* counteract resting spores of *Plasmodiophora brassicae* in soil?

Are the cultivation of *Lupinus angustifolius* and/or *Lolium perenne* more effective in reduction of resting spores of *Plasmodiophora brassicae* in comparison to fallow treatment?

Does the density of *Pratylenchus penetrans* in the soil decrease during cultivation of *Avena strigosa, Phacelia tanacetifolia* or *Lupinus angustifolius?*

Are the cover crops *Lupinus angustifolius* and *Avena strigosa* susceptible to *Meloidogyne hapla*?

Materials and Methods

Organisms

In this thesis, I worked with the obligate soil-borne pathogen *Plasmodiophora brassicae* and the plant parasitic nematodes *Pratylenchus penetrans* and *Meloidogyne hapla.*

The isolates of *P. brassicae* were collected from infected rapeseed roots from a severely infected field situated in Ystad in Scania County, Sweden. These clubroot isolates are anticipated to have high genetic diversity (Friberg, 2005b).
The infested field soil that was used for the bioassay with *P. penetrans,* was collected from a sugar beet field situated in Löddeköpinge, Scania. This field has been intensively cropped with potato and carrots and was severely infested with *P. penetrans*. The second-stage juveniles (J2s) of *M. hapla* were recovered from a culture of tomato plants (cv. Moneymaker) kept in a climate chamber at SLU Uppsala, managed by Maria Viketoft. Juveniles to be used in the study were extracted by placing tomato roots chopped in approx. 1 cm-pieces on a net covered by tissue paper (kleenex) placed in a tray. Tap water was added to the tray so that the roots were moistened. The water, including any extracted nematodes, was poured off daily into a beaker, and new water was added to the tray. The nematode solution (500 ml) was kept in a cold storage room (5 °C), until delivered by post to SLU-Alnarp

The plant species studied in the *P. brassicae* experiments were blue lupine *Lupinus angustifolius* and English ryegrass *Lolium perenne.* In addition, a fallow treatment was kept as control treatment (bare soil inoculated with *P. brassicae* spores). For the disease assessment rapeseed *Brassica napus* plants were used as indicator plants, grown in the soil of prior cover crop and fallow treatments.

The plant species studied in the *M. hapla* experiments were blue lupine, *Lupinus angustifolius,* and black oat, *Avena strigosa.* In addition, susceptible tomato, *Solanum lycopersicum* cv.
Moneymaker was kept as control treatment.

The plant species studied in the *P. penetrans* experiments were blue lupine, *Lupinus angustifolius,* lacy phacelia, *Phacelia tanacetifolia* and black oat, *Avena strigosa.*

The seeds of *L. angustifolius, A. strigosa* and *B. napus* were provided by Agri Science Sweden AB (Scania, Sweden) and *L. perenne* from the garden laboratory at Alnap, *S. lycopersicum* cv. Moneymaker and *P. tanacetifolia* were purchased from Impecta seed trade (Katrineholm, Sweden).

Bioassay *Plasmodiophora brassicae*

General setup

The *Plasmodiophora brassicae* experiment was assessed by biological tests in a daylight chamber for a period of 13 weeks and 2 days. More precisely, cover crop treatments *Lupinus angustifolius, Lolium perenne* and the control treatment, fallow (bare soil) were kept in soil inoculated with *P. brassicae* spores for 8 weeks, followed by 5.5 weeks growth of the indicator plants *B. napus* in the soil of the previous treatments.

Background of assessment methods

The pipette method was followed as described by Voorrips (1996) and the analysis of the *P. brassicae* resting spore amount in the soil was assessed using visual grading of the diseased roots symptoms, described by Friberg *et al.* (2006), based on the work by Buczacki *et al.* (1975). The visual grading system is based on the theory that disease severity is increased depending on the number of spores attacking the root, and that an early infection of the taproot causes higher disease severity in comparison to a late infection of the lateral roots. Fresh and dry weight assessments of indicator plants roots and shoots were evaluated, as described by Friberg *et al.* (2006). Based on that, fresh shoot weights are expected to decrease, and fresh root weights to increase with stronger disease (larger club formations) (Hwang, *et al.* (2015).

Spore solution preparation

The *P. brassicaceae* infected roots were carefully washed in tap water removing remaining soil from the field. The cleaned roots were placed in a 3-liter plastic bag at room temperature 22°C for one week, to decompose. The root content of a 1.5-liter bag was then mixed with 450 ml tap water and macerated with a household blender. The blended root mass was filtered through 8 layers of cheesecloth, and the filtrate constituted the stock solution. The concentration of spores in the stock solution was analyzed using a haemocytometer under a high resolution light microscope, and the concentration of the stock solution was subsequently adjusted to 125,000 spoores per µl. Prior to inoculation the stock solution was vortexed and shaken for 40 min. The inoculation solution contained 1 liter tap water blended with 3.2 ml stock solution. A calculation sheet used for preparation of the inoculation solution of *P. brassicae* resting spores is reported in supplementary (Appendix 1).

Spore inoculation

Prior to the seeding of the cover crops the 1.5-l pots were filled with ca. 396 g d.w. flower and plant soil and were inoculated with 10^4 spores per g dry weight (d.w.) soil. The total of 990 ml of the inoculation solution were divided between 45 treatment pots, *i.e.,* 22 ml were mixed thoroughly into the soil of each of 15 pots with the treatment of *L. perenne,* 15 pots with *L. augustifolius* and 15 pots with the fallow treatment. Directly after inoculum the pots were watered from underneath with 2 dl H_2O reaching 80% field capacity.

Daylight chamber experiment

The daylight chamber experiment was performed at the SLU Biotron, and lasted for 13 weeks and 2 days. The conditions during the experiment were kept at a day temperature of 24°C and night temperature of 19°C, a relative humidity of 70% and supplementary light from sodium lamps was turned on between 06:00-22:00 when the outside light was below 400 micromol/m2/s.

Prior to seeding of the cover crops soil d.w. was estimated based on five 1.5 l pots. The pots were weighed separately without soil and with soil and the median value of the soil d.w. was determined to 396 g. The pots were filled with "flower and plant soil" (pH 7, soil temp 24°C) homogeneously and additional osmocote long term fertilizer was mixed into the soil; fertilizer amount was added according to product recommendation (3 tsp per pot). Directly before seeding the cover crops were inoculated with the spore solution. Seeds of the cover crops *L. angustifolius* and *L. perenne* were seeded in 15 1.5-l pots each. Pots with *L. angustifolius* obtained 9 seeds per pot and *L. perenne* ca. 40 seeds per pot at a depth of 2 cm. Further, 15 1.5-l pots were kept as fallow treatment without plants. Post inoculum the pots were watered from underneath regularly with 2 dl H_2O reaching 80% field capacity. Five days after emergence of the seedlings when the first true leaf was visible, the seedlings were thinned out, *L. angustifolius* to 5 plants per pot and *L. perenne* to ca. 40 plants per pot. After 8 weeks, all the cover crops above-ground parts were removed by cutting them off near to the soil surface.The remaining roots and soil in the pots were blended, the ryegrass replicates root/soil lump was massacred using a secateur due to the extensive root growth. Then, 16 seeds of rapeseed *B. napus* were seeded in all three treatments: fallow, blue lupine and English ryegrass. Subsequently, *B. napus* was grown in the daylight chamber for 5.5 weeks prior to disease assessment and plants in all treatments were classified by the development stages above ground, classification was followed according to Jordbruksverket (2021). Development stages of oilseeds: leaf development, 10, cotyledons fully developed, 11, one true leaf developed, not folded, 12, two true leaves developed, 13, three true leaves developed,14-18, 4-8 true leafs developed, 19, nine true leaves developed. Plant elongation: 30, plants start to elongate, 31, one internode is visibly extended, 32, two internodes are visibly extended, 33, tree internodes are visibly extended. In conjunction with disease assessment the *B. napus* plants were removed from the pots and were washed in water removing the remaining soil from the roots.

Homogeneous water inputs were maintained during the 13.2 week trial of the *P. brassicae* respective cover crops and susceptible rapeseed. The soil was kept moist to meet the favorable habitat condition of *P. brassicae*. To prevent the pathogen spores from being washed out of the root area the plants were watered from underneath during the trial. Hand weeding was maintained based on needs during the 13.2 week trial. In the second week of the growth of the susceptible *B. napus* a biological control treatment against sciaridae flies was implemented in all pots using the nematode *Steinernema feltiae* (Koppert, 2021), mixed with water according to product specifications and added to the pots.

Disease assessment

Disease symptoms of the indicator plants (*B. napus)* were assessed 37 days after sowing, using a scale of six disease classes, based on the work by Friberg *et al.* (2006): 0, no symptoms; 1, small club formations on lateral roots; 2, small club formations on the main root; 3, large club formations on the main roots, lateral roots with small or few clubs only; 4, large club formations on the main root and numerous clubs on lateral roots; and 5, severe club formations on the main root leading to partial degradation, lateral roots completely destroyed, plant growth markedly affected.

Dry weight assessment

After disease assessment, the *B. napus* crops were cut below the cotyledons and separated into shoots and roots. The plant parts were then weighed separately by shoots and roots to determine the fresh weight of the crops. After weighing, the plant parts were put in plastic bags marked with replicate nr. The plant parts were then stored at 5°C for one week. Before drying, the plants were moved to paper bags marked with replicate nr. The roots were dried in an oven at 70°C for 3 days and the shoots in an oven at 60°C for 3 days, before d.w. was determined.

Plant parasitic nematodes

General setup

The PPNs experiments were assessed by biological tests in a daylight chamber, with the same chamber conditions as described above for the *P. brassicae* bioassay. The *M. hapla* experiment lasted for a period of 11 weeks and 4 days and *P. penetrans* for a period of 10 weeks. More precisely, cover crop treatments *L. angustifolius* and *A. strigosa* were kept in the soil inoculated with *M. hapla* and soil infested with *P. penetrans*. The indicator plants *Solanum lycopersicum* of *M. hapla* and *P. tanacetifolia* of *P. penetrans* were grown under the same growth conditions as the respective cover crops.

Maintenance

Homogeneous water inputs according to the crops' needs were maintained during the trial of the experiments with *P. penetrans* and *M. hapla* respective cover crops or susceptible crops. Hand weeding was maintained based on needs during the trial. To prevent the nematodes from being washed out of the root area the plants were watered from underneath on separate plates.

Bioassay *Meloidogyne hapla*

Background of assessment methods

The analysis of *M. hapla* was performed according to the bioassay method using galling indices, described by Barker *et al.* (1985). The methods were adapted to species within *Meloidogyne* spp. that cause galls or necrosis on plant roots. The galling indices are mainly

useful for the determination of the magnitude of nematode infections, the evaluation of nematicide efficiency or estimation of actual yield losses.

Daylight chamber experiment

The daylight chamber experiment lasted for 11 weeks and 4 days, the placement in the daylight chamber was randomized between the treatments. The daylight chamber conditions were kept as described above for the *P. brassicae* bioassay.

The trial started with preparation and sowing of the cover crops *L. angustifolius, A. strigosa* and the susceptible control treatment *Solanum lycopersicum* cv. Moneymaker. The substrate used was silver sand with supplemented osmocote long term fertilizer, added to each pot according to product recommendation (1 tsp per pot). The plants were seeded at a depth of 1-2 cm with 3 seeds per pot in 15 pots for each cover crop species. The seeded pots were then watered from underneath with 0.5 dl water each, reaching field water capacity. One week after seedling emergence the plants were thinned out to 1 plant/pot. Two weeks after seeding when the seedlings had developed true leaves, 12 pots of each plant were inoculated with 3.4 ml *M. hapla* suspension (see section below) containing ca 1,525 *M. hapla* juveniles. The suspension was pipetted into a ca. 2 cm deep hole in the soil, adjacent to the plant root. Three replicates from each species treatment were kept as control without nematodes.

Preparation of *Meloidogyne hapla* suspension

Melodigyne hapla diluted in H_2O were delivered in a 500-ml bottle to the laboratory. The nematodes were collected from infected tomato roots by Maria Viketoft, SLU. The bottle was left overnight to allow the nematodes to sink to the bottom. The amount of water was then reduced to 150 ml by pipetting the surface water of the sample. Then 2 ml of the suspension were pipetted to the nematode counting chamber, allowing manual counting of the nematodes under a high resolution light microscope. The *M. hapla* juveniles were counted in a downwards pattern from row to row, this was repeated two times for two samples. The mean value was evaluated for the 2 ml suspension and additionally for the amount that was going to be inoculated in the cover crop treatments (Calculation of *M. hapla* juveniles in inoculation solution; 783 + 1011 = 1794 / 2 = 897 juveniles/ 2 ml
150 ml / 45 = 3.4 ml solution/pot).

Root-knot galling classification

Galling symptoms of the three cover crops were assessed 66 days after inoculation with the *M. hapla* juveniles. The assessment was based on an 8 grade scale of the symptoms, from (Barker *et al.* 1985): 0 = without galls/ root knots,1 = < 5; 2 = 5-25; 3 = 26-100; 4 = >100; 5 = many galls/ root knots separated from each other; 6 = large gall/ root knot formations many coincides, 7 = very severe and 8 = extremely severe no root growth.

Bioassay *Pratylenchus penetrans*

Background of assessment methods

The extraction of *P. penetrans* was performed according to the Baermann funnel method, (Barker *et al.*1985). The method is useful to extract nematodes from small soil samples and root pieces. Actively moving juveniles or adults are recommended to be extracted with the Baermann funnel method which therefore is suitable for *P. penetrans,* which is a free living endoparasitic nematode that primarily occurs in the soil. The method is based on several steps and instruments enabling counting of the nematodes according to the following protocol (Barker *et al.* 1985): 1) separate soil from roots and mix the soil carefully, 2) collect and weigh 100 g soil sample, 3) prepare the funnel with a rubber hose and clamp: place a metal basket on the funnel, place a tissue in the metal basket, add water, 4) place the soil on the tissue, make sure the water is filled up making contact with the soil place a lid on top to prevent evaporation, 5) extract for 2 days allowing the nematodes to sink to the bottom, 6) tap 50 ml of the liquid and place in marked test tube, 7) place test tubes in refrigerator overnight.

Daylight chamber experiment

The daylight chamber experiment lasted for 10 weeks, the placement in the daylight chamber was randomized between the treatments. The daylight chamber conditions were kept as mentioned above for the *P. brassicae* experiments.

Soil severely infested with *P. penetrans* nematodes was collected from a sugar beet field situated in Löddeköpinge in Scania. Eight 5 l bags of field soil were collected within a small range in the field. The soil from each bag was then mixed together by hand and potted in 45 8-cm diameter pots, osmocote long term fertilizer was added to each pot, according to product recommendation (1 tsp per pot). The seeds of *L. angustifolius, A. strigosa* and *P. tanacetifolia* were then seeded at a depth of 1-2 cm in 15 pots each. In each pot 5 seeds of one cover crop were seeded. The seeded pots were then watered from underneath with 0.5 dl water, reaching field water capacity. Five days after emergence of the seedlings they were thinned out. *Lupinus angustifolius* to 3 plants per pot*, P. tanacetifolia* to 4 plants per pot and *A. strigosa* to 5 plants per pot. After 10 weeks the vegetative parts of the plants were cut off and the remaining soil and roots were collected and sorted after the replicate number into 2 l plastic bags. The badged samples were stored in a refrigerator at 4°C for about 1-14 days.

Sample extraction (Counting of the nematodes)

To extract the *P. penetrans* nematodes from the soil the Baermann funnel method was used enabling counting of the nematodes. Counting of the *P. penetrans* nematodes, 1) pipette the surface water of the sample to reduce it to 2 ml, 2) mix the solution by pipetting de liquid in and out carefully in the tube, 3) pipette the 2 ml liquid on to the nematode counting chamber, 4) read the sample under high resolution microscope.

Statistical analysis

In the clubroot experiment the disease severity index (DSI) was calculated as described by Strelkov *et al.* (2006), according to the following formula: DSI= (0xn + 1xn + 2xn + 3xn + 4xn) /N where n is the number of plants in the indicated disease score class, x is multiplied with and N is the total number of assessed plants. The disease index per pot in the tree treatments *L. angustifolius, L. perenne* and fallow was calculated according to the DSI formula, and the data were analyzed using the One-way ANOVA procedure and Tukey's test for multi comparisons ($P< 0.05$). The fresh and dry weight of leaves and roots of the rapeseed in all treatments were analyzed using One-way ANOVA procedure and Tukey's test for multi comparisons ($P< 0.05$) in Minitab version 19.

In the *M. hapla* experiment the root gall index was calculated as described by Barker *et al.* (1985) and Strelkov *et al.* (2006), formula RGI = (0xn + 1xn + 2xn + 3xn + 4xn + 5xn + 6xn + 7xn) /N where n is the number of plants in the indicated disease score class, x is multiplied with and N is the total number of plants tested in each treatment. The values post RGI conversion were then analyzed using Kruskal- Wallis procedure and Mann-Whitney test was used to compare treatments when the analysis of variance showed significant differences among means ($P< 0.05$). In the *P. penetrans* experiment the number of nematodes in each sample within all four treatments were transformed using a variance-stabilization formula log(x+1) as described by Barker *et al.* (1985). The +1 allows for the transformation of data with zero values. The logarithm transformation produces a more symmetric, normal-appearing distribution frequency. Reproduction factor (Pf/Pi) was calculated as described by Belair *et al.* (2019) for all samples within the treatments *A. strigosa, L. angustifolius* and *P. tanacetifolia*, where Pf = final number of nematodes per 100 g in each pot and Pi = initial number of nematodes in 100 g soil (control). The initial number of nematodes in the original field soil was determined based on seven replications. After the logarithmic transformation and reproduction factor calculations the values were analyzed using One-way ANOVA and Tukey test for multi comparisons ($P< 0.05$) in Minitab version 19.

Results

Disease evaluation related to *Plasmodiophora brassicae*

The *P. brassicae* experiment showed no significant difference in disease severity in *B. napus* between the three treatments *L. angustifolius, L. perenne* and fallow. Analysis of variance revealed that the treatment based disease indices were marginally non-significant (ANOVA, $p= 0.087$, $n=40$, $df=2$), however it did show a tendency of difference. The average disease severity indices per replicate (respectively pot) are illustrated in (Figure 3). The range of DSIs per replicate (respectively pot) was from highest to lowest 0.65-0.25 for the treatment *L. perenne,* 0.75-0.3 for treatment *L. angustifolius* and 0.8-0.3 for fallow. All treatments showed medium high levels of DSI on the *B. napus* hosts according DSI evaluation by Liu (2020)

with an average DSI of 0.40 ± 0.14 for treatment *L. perenne*, 0.47 ± 0.14 for *L. angustifolius* and 0.53 ± 0.15 for the fallow treatment.

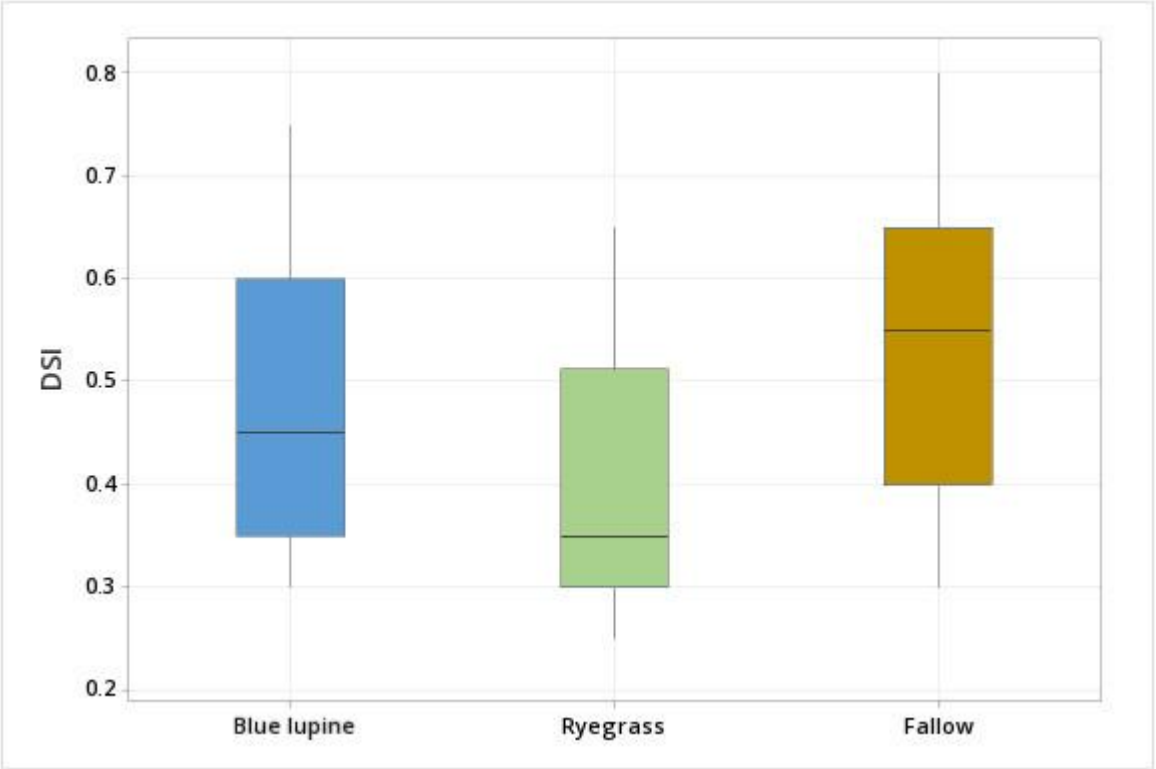

Figure 3. *Plasmodiophora brassicae* disease severity indices (DSIs) of indicator plants *Brassica napus*. Data are arithmetic means and standard deviations of each replicate (respectively pot) with five indicator plants, considering12 replicates for treatment ryegrass, *Lolium perenne*, 15 replicates for treatment blue lupine, *Lupinus angustifolius* and 15 replicates for fallow treatment.

Development stages of indicator plants related to *Plasmodiophora brassicae*

Development stages above ground were influenced by the disease among the treatments. The majority of the *B. napus* plants before DSI evaluation were in development stage 14-18, 4-8 true leaves developed and 32, two internodes are visibly extended or 33, three internodes are visibly extended.

Fresh and dry weight evaluation of indicator plants related to *Plasmodiophora brassicae*

The impact of treatments *L. angustifolius, L. perenne*, and fallow on *B. napus* growth revealed significant differences. Results of ANOVA for plant weights and statistical comparison by Tukey's test are provided in Table 1. The fresh and dry weights of roots and shoots of *B. napus* relative to the specific treatments are illustrated in (Figure 4). The fallow treatment caused a significant difference on *B. napus* root and shoot growth in comparison to *L. angustifolius* and *L. perenne.* The fallow treatment revealed higher average weights of fresh leafs, dry leafs and fresh roots in comparison to the other two treatments. Treatment with *L. angustifolius* revealed no significant difference in dry root weights in relation to treatment with fallow, however the two treatments significantly differed revealing higher

weights in relation to the treatment with *L. perenne.* In the other weighted samples the root and shoot weights of the treatment with *L. angustifolius* showed no significant difference to weights of the treatment with *L. perenne*.

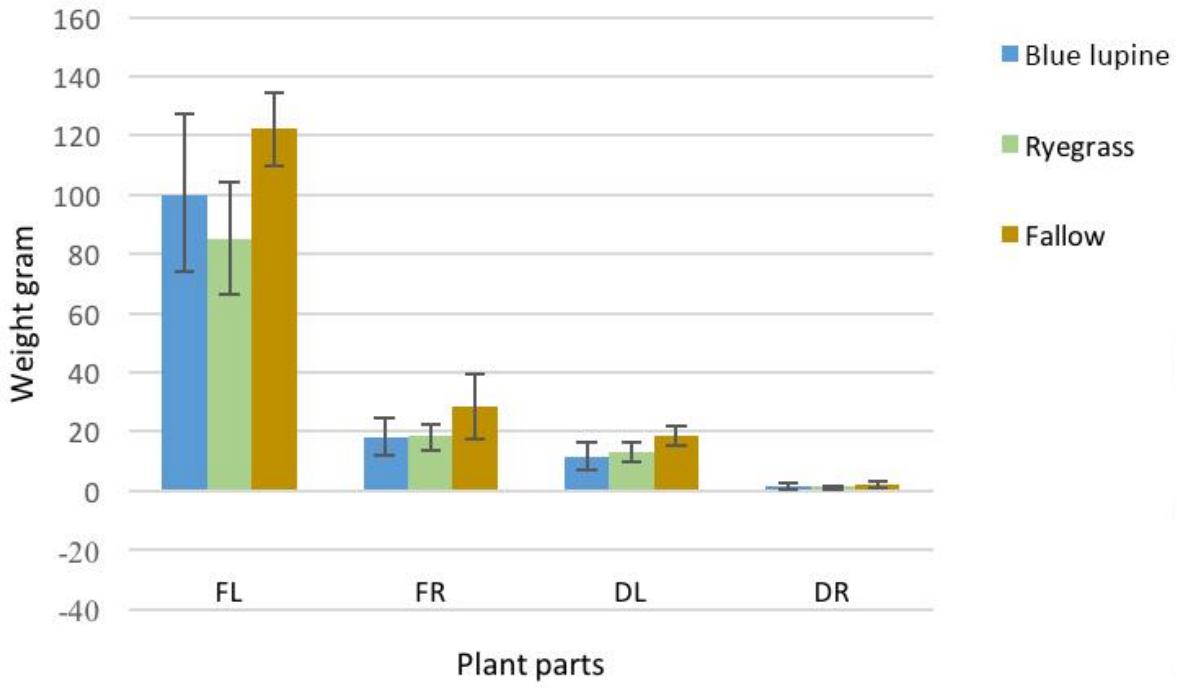

Figure 4. Fresh and dry weight [g] of roots and shoots of indicator plant *Brassica napus.* FL= fresh leaves, FR= fresh roots, DL= dry leaves, DR= dry roots. Data are arithmetic means and standard deviations of each replicate (respectively pot) with five indicator plants, considering 12 replicates for treatment ryegrass *Lolium perenne*, 15 replicates for treatment blue lupine, *Lupinus angustifolius* and 15 replicates for fallow treatment.

Table 1. Fresh and dry weight assessment of *Brassica napus* infected with *Plasmodiophora brassicae*

Crops (treatment)	**n**	**FL**	**DL**	**FR**	**DR**
Fallow	15	122.4 ± 12.3 A	18.5 ± 3.7 A	28.2 ±11.4 A	2.1 ± 0.6 A
Blue lupine	15	100.5 ± 26.3 B	11.7 ± 4.4 B	17.8 ± 7.3 B	1.4 ± 1.0 AB
Ryegrass	12	84.9 ± 20.1 B	13.1 ± 2.7 B	18.6 ± 3.7 B	1.5 ± 0.42 B
p- value		p<0.001	p<0.001	p=0.003	p=0.036

Data are arithmetic means and standard deviations of fresh and dry weight [g] of specific plant parts of indicator plant *Brassica napus* in all replicates (n) of *Lolium perenne*, *Lupinus angustifolius*, or fallow. FL= Fresh leafs, FR= fresh roots, DL= dry leafs, DR= dry roots. Means labeled with the same letter in the same vertical row are not significantly different; p-values assessed by ANOVA and multi comparison of means by Tukey's test.

Disease evaluation related to *Meloidogyne hapla*

The analysis of variances among the treatments in the *Meloidogyne hapla* experiment revealed a significant difference (Kruskal-Wallis, $p<0.001$, df=2) in disease severity among the three treatments, *S. lycopersicum*, *Avena strigosa* and *Lupinus angustifolius*. The root gall indices (RGI) regarding the specific treatments are reported in Table 2. The treatment of *A. strigosa* resulted in RGI 0.00 (highly resistant cultivar) in contrast to the *L. angustifolius* with RGI 0.50 ± 0.21 and *S. lycopersicum* 0.48 ± 0.04 (susceptible cultivars). Statistical analysis did not show a difference in the RGIs between the *L. angustifolius* and *S. lycopersicum* treatment (Mann-Whitney p=0.510, 95.57%).

Table 2. Disease development of *Meloidogyne hapla* in selected crop/treatment

Crops (treatment)	n	Initial inoculations	Average RGI	Susceptibility
Avena strigosa	11	136 344	0.00 ± 0.00 B	Highly resistant
Lupinus angustifolius	10	136 344	0.71 ± 0.29 A	Susceptible
Solanum lycopersicum	12	136 344	0.82 ± 0.06 A	Susceptible

Data are arithmetic means ± standard deviations of RGIs for 12 replicates (n) of *Avena strigosa*, 10 *Lupinus angustifolius*, 12 *Solanum lycopersicum*. In the same column, values labeled with the same letter are not significantly different ($p < 0.05$), as determined by Mann-Whitney and Kruskal-Wallis test. Initial inoculations are the initial amount of *Meloidogyne hapla* juveniles inoculated per replicate two weeks after seeding. Statements of susceptibility, highly resistant and susceptible were applied accordingly to the root gall index scale given by Taylor & Sasser (1978).

Disease evaluation related for *Pratylenchus penetrans*

The analysis of variance revealed that the treatments had a significant impact on the amount of *P. penetrans* in the soil (ANOVA, $p<0.001$, df= 3). Moreover, the reproduction factor Pf/Pi of *P. penetrans* differed based on the treatments (ANOVA, $p<0.001$, df= 2). Multi comparison performed by Tukey's test of nematode counts and Pf/Pi factors are illustrated in Table 3 and (Figure 5). and counts relative to treatment and replicate in Table 4. The highest nematode count was recorded in the *L. angustifolius* treatment, the reproduction factor for this crop was (0.7 ± 0.4). TheThe amount of *P. penetrans* was not significantly different from the number of nematodes initially found in the field soil (control). *Phacelia tanacetifolia* and *A. strigosa* resulted in significantly lower reproduction factors compared to the *L. angustifolius* treatment. For *P. tanacetifolia* a 10 times lower reproduction factor was recorded (0.07 ± 0.09) than for *L. angustifolius* (0.7 ± 0.4).The treatment with the lowest nematode counts and reproduction factor recorded (0.009 ± 0.007) was *A. strigosa*, which was approximately 76 times lower than for *L. angustifolius* (0.7 ± 0.4). Tukey's test revealed no significant difference of Pf/Pi between treatment *P. tanacetifolia* (0.07 ± 0.09) and *A. strigosa* (0.009 ± 0.007).

Table 3. Reduction of *Pratylenchus penetrans* in selected cover crop treatment

Crops (treatment)	n	No. of *P. penetrans* / 100 g soil Real count - Log(x+1)	Reproduction factor (Pf/Pi)
Control	7	155 ± 57 - 2.2 ± 0.2 A	
Lupinus angustifolius	15	108 ± 62 - 1.9 ± 0.4 A	0.7 ± 0.4 A
Phacelia tanacetifolia	15	11 ± 14 - 0.8 ± 0.5 B	0.07 ± 0.09 B
Avena strigosa	12	1 ± 1 - 0.3 ± 0.2 C	0.009 ± 0.007 B

Data are arithmetic means ± standard deviations of nematode counts and reproduction factors (Pf/Pi) relative to treatment of n replicates of *Lupinus angustifolius*, *Phacelia tanacetifolia, Avena strigosa* and control. In the same column, values labeled by the same letter are not significantly different ($p < 0.05$), as determined by Tukey's test. Pf = final number of nematodes per 100 g in each pot and Pi = initial number of nematodes in 100 g soil (control).

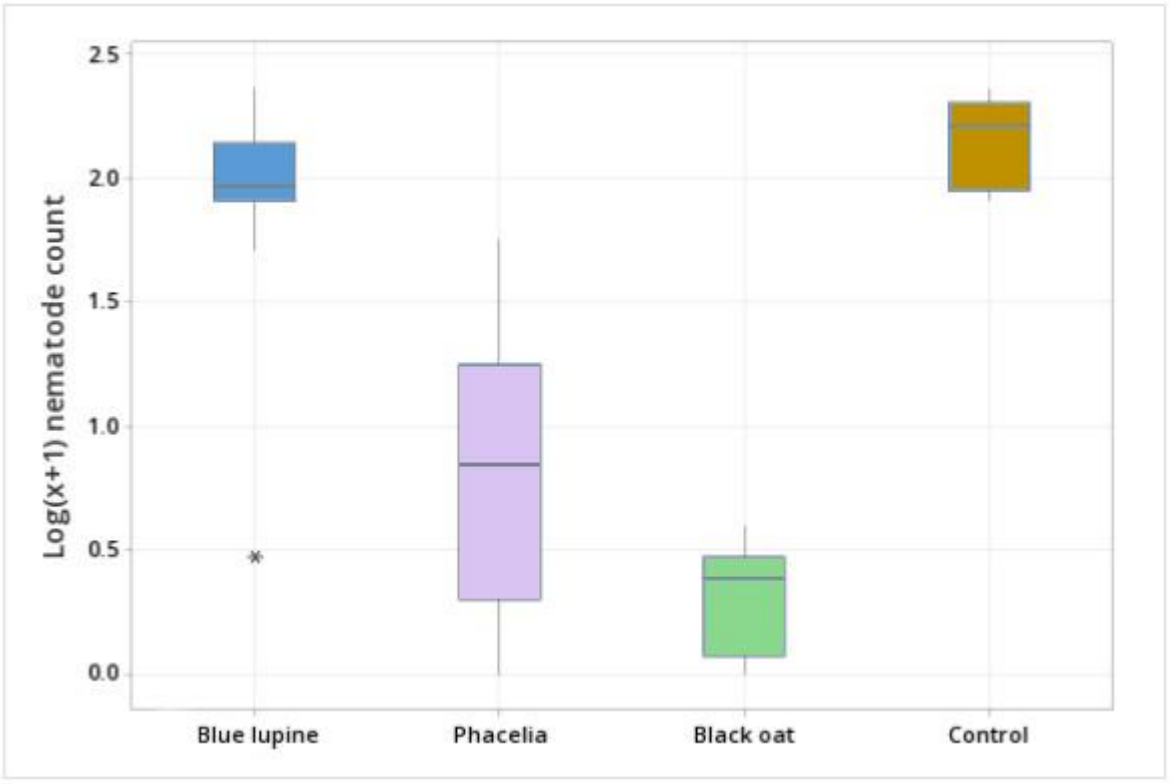

Figure 5. Average *Pratylenchus penetrans* counts in 100 g soil after growth of *Lupinus angustifolius.* (blue lupine, n=15), *Phacelia tanacetifolia* (lacy phacelia, n=15) *Avena strigosa* (black oat, n=12) or without treatment (control, n=7). Data are logarithmic (log(x+1)) values and standard deviations of nematode counts of each replicate per treatment.

Table 4. *Pratylenchus penetrans* counts in 100g soil relative to treatment and replicate

Replicates	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
Black oat	1	2	1	2	2	3	1	0	0	3	2	0	n.d.	n.d.	n.d.
Blue lupine	80	88	92	228	136	92	158	84	2	232	80	92	138	50	73
Lacy phacelia	0	2	21	5	17	2	1	1	11	9	17	15	6	0	56
Control	228	202	138	88	80	160	189	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Nematode count data relative to treatments and replicates of black oat, *Avena strigosa*, blue lupine, *Lupinus angustifolius*, lacy phacelia, *Phacelia tanacetifolia* and control. Nematode counts were documented from 100 g soil of each replicate after 10 weeks growth of the specific cover crop, and nematodes initially found in the field soil (control), n.d.= no data.

Discussion

Root pathogens and parasites are causing severe loss in crop production. *P. brassicae* is one of the pathogens causing major diseases in oilseed rape production and the PPNs, *P. penetrans* and *M. hapla* infest important crops such as carrot, potato and strawberry. In common, they are highly dependent on host plants for multiplication. To prevent dissemination, growers apply crop rotation and cover crops that do not propagate the pathogen or pest, respectively.

The primary goal of the *P. brassicae* experiment was to assess the effect of non-host crops *L. perenne* and *L. angustifolius* on resting spore density and disease severity. A suppressive effect of the tested non-host crops on *P. brassicae* spores and disease would enhance the credibility of cover crops as components in integrated clubroot management programs. In the *P. brassicae* experiments, no effect of the non-host plants *L. angustifolius* and *L. perenne* were observed on the persistence of *P. brassicae* in comparison to fallow treatment, assessed by symptoms and a disease severity index of indicator plants. However, statistical analysis revealed a non-significant tendency of decreased disease severity.
Disease severity increases with a higher number of spores attacking the root, and early infection of the taproot causes higher disease severity than late infection of the lateral roots (Friberg, 2005b; Voorrips, 1996). In a previous study by Friberg *et al.* (2005a), plant induced germination of *P. brassicae* with non-host plants was studied in vitro, and *L. perenne* was found to stimulate spore germination more than host plant (*Brassica rapa* var. Pekinensis). However, in a soil environment the results were different as observed in a later study by Friberg *et al.* (2006), where growth of *L. perenne* did not lead to significant difference in DSI of indicator plants (*B. rapa* var. Pekinensis) in the field or in the greenhouse. Nevertheless, *L. perenne* at the time of decay of the roots, did significantly decrease the DSI of indicator plants in comparison to a plant-free control treatment.

In a field and greenhouse experiment assessing the effect of host and non-host crops on *P. brassicae* resting spore concentrations and clubroot of *B. rapa* made by Hwang *et al.* (2015)

non-hosts such as ryegrass and field pea were observed to reduce clubroot DSIs compared with fallow. Similar as in Hwang *et al.* (2015) our experiments suggest that non-host crops can reduce the DSI in comparison to fallow. To the best of my knowledge, blue lupine, *L. angustifolius* has not been observed before to affect clubroot disease, however other members in the fabaceae family (field pea, *Pisum sativum,* red clover, *Trifolium pratense)* have been observed by Hwang *et al.* (2015) and Friberg, (2005b) to negatively affect the persistence of *P. brassicae*. In general, *L. angustifolius* might have a similar effect as other species within the fabaceae family. For future studies, qPCR analysis could be used to complement DSI determination (Hwang *et al.* 2015) and minimize potential subjective errors of DSI grading (Friberg & Hedlund, 2021).

In Hwang *et al.* (2015), the treatments with non-hosts and fallow were observed to decrease the resting spore and DNA amount of *P. brassicae* in comparison to susceptible *B. rapa*. After growth of non-hosts, the DSI on canola roots was 16-26% lower than without crop rotation. The decrease of DSI after growth of non-host crops in the crop rotation suggests that the treatments have significant effect on the reduction of clubroot disease in *B. rapa*. In the present study the inoculum concentration was 10^4 per g soil which is considered to cause severe symptoms and high yield loss in *B. rapa.* The threshold value of mediating severe disease is 10^3 per g soil (Zamani-Noor *et al.* 2021). Inoculation values above 10^5 per g soil have been shown by Friberg & Hedlund (2021) to reach maximum values of disease score slowly. Inoculation values between 10^3- 10^4 per g soil have been observed to quickly reach high disease scores at a limited period of time. In the present study, none of the indicator plants reached the maximum disease score, which disagrees with the expected high score at the inoculum level of 10^4 per g soil. The medium high DSI score of the indicator plants therefore suggests that there has been a reduction of spore concentration in all treatments. The present study thereby supports the results of Hwang *et al.* (2015), suggesting that the use of non-hosts in the crop rotation can be useful in integrated disease management programs to reduce the DSIs of *B. rapa* together with other strategies. However, in the present study it cannot be concluded that any of the plants tested counteract, nor are more effective in reduction of, resting spores of *P. brassicae* than the other. The small changes observed between the DSI of the plant-specific treatments could have been caused by factors not related to plant species *e.g.* increased pH levels can influence changes in DSI (Dixon, 2014). High pH values in soil have been observed to prematurely interrupt development of zoospores causing malformation that prevents infection of the roots. The factors of implementation in the bioassay regarding soil mixing and decomposition of non-host plant roots may have indirectly influenced the spore survival or plant infection in these treatments. It is known that chemical and physical soil characteristics influence both the pathogen and the development of the disease, and that such changes can be affected by organic material (Friberg *et al.* 2005a). Since lower DSI was found in non-host treatments it seems likely that the mechanism was of a general nature rather than a species-specific response.

Shoot and root dry weights have previously been evaluated in response to inoculum levels of *P. brassicae,* and Friberg & Hedlund (2021) and Zamani-Noor *et al.* (2021) revealed that shoot and root weight correlates to DSIs of indicator plants (*B. rapa)*. To assess the cover crop's effect on fresh and dry weight of *B. rapa,* roots and shoots were complementary evaluated. The patterns observed for shoot and root fresh and dry weights revealed a

significant difference among treatments and the different plant parts that were weighed. Treatment of fallow consistently led to higher weights of indicator plant parts compared to other non-hosts treatments. Although only a slight difference was observed for the dry weight of roots of *L. angustifolius*. Higher root weights were expected for the fallow treatment than for the non-host treatments, as non-host were expected to reduce resting spore concentration and thereby DSIs resulting in less club formations on the roots (Hwang *et al.* 2015; Friberg *et al.* 2005a, Rod and Robak, 1994). The DSI of fallow treatment agrees with the increased root weight showing a slightly higher DSI than the other treatments, however no significant difference in DSI could be established in the present study. The relatively high fresh and dry shoot weights of the fallow treatment were not consistent with the symptoms caused by *P. brassicae.* The clubroot disease causes formation of tumors that leads to reduced water and nutrient uptake, growth inhibition and withering of the plants resulting in significant yield reduction (Zamani-Noor *et al.* 2021; Wallenhammar, 1997). In this case the tumor formation on the roots was not observed to reduce growth, which could have been caused by numerous reasons (Friberg & Hedlund, 2021). In the bioassay the DSIs were medium high and the reduced growth was expected. However, the plants had optimal conditions for growth and the soil was continuously kept moist during the trial to meet the favorable habitat condition of *P. brassicae.* The optimized growth conditions during the trial period could be one of the reasons that reduced growth was not observed, since in the field environment the conditions are more variable. Several factors affect the persistence of *P. brassicae*, such as soil characteristics, microbial composition, pathogen isolate and other environmental conditions. Environmental factors can even under highly standardized conditions, be difficult to control and may interfere with experimental studies (Friberg & Hedlund, 2021). Friberg further explains that a reduction of the soil inoculum by the treatment or by other means cannot be completely reliably predicted by a bioassay alone. Further studies are therefore needed to determine the non-host impact in integrated clubroot management programs.

In the second part of the study, we investigated plant parasitic nematodes (PPNs). The goal was to investigate black oat, *A. strigosa,* and blue lupine, *L. angustifolius,* for their potential as resistant or antagonistic non-hosts to reduce the population densities of *M. hapla* and *P. penetrans* in soil.

In the *M. hapla* experiments a significant difference was observed among the treatments. Non-host *A. strigosa* differed from the other treatments by showing no disease symptoms (highly resistant). *Avena strigosa* is currently being tested in field trials on soil infested with *Meloidogyne hapla* in Sweden (Olsson Nyström, unpublished personal communication). Results from these trials will help to elucidate the effect of *A. strigosa* on populations of *M. hapla* in the field under Swedish growing conditions. Previous findings by Andersson (2018) suggest that *A. strigosa* shows suppressive effects against *Meloidogyne spp.,* including *M. hapla*, and is considered to be a partially resistant host. The suppressive mechanisms of oat cultivars suggested by Tateishi *et al.* (2011) and Marini *et al.* (2016) are a reduced root invasion and failure of subsequent nematode development. Uesugi *et al.* (2018) suggests that the same mechanisms are likely to be involved in black oats, as they are non-hosts of *M. hapla*. Moreover, *S. lycopersicum* and *L. angustifolius* did not significantly differ in the severity of gall symptoms. Nitrogen input by growth of legumes is essential in organic crop

rotations, however legumes are highly susceptible to *M. hapla* and this may be the primary cause for high soil densities of *M. hapla* in organic farming according to Vestergård (2019). Several legumes, such as red clover (Albertsson Juhlin, 2014; Vestergård, 2019), field pea, faba bean and common vetch (Dobosz & Krawczyk, 2019), have been observed to increase proliferation and densities of *M. hapla*. However, yellow lupine, white lupine and narrow-leaved lupine have been observed to decrease *M. hapla* population densities similarly to bare fallow (Dobosz & Krawczyk, 2019). In the present study blue lupine did not show suppressive effects as the RGIs were not significantly different to the highly susceptible host *S. lycopersicum.* The analysis in the present study was restricted to visual RGI evaluation of the roots, which could inflict errors as galls caused by *M. hapla* on legumes show similarities to nitrogen fixating galls (Albertsson Juhlin, 2014). Although nitrogen fixating galls were not observed on the control plants roots without *M. hapla* present in the soil, the risk of nitrogen fixating galls being present and the confusion with galls caused by *M. hapla* can not be fully excluded. Roots of blue lupine infested and not infested (control) with *M. hapla* are displayed in (Figure 6). In further studies, analysis of the root galls should be complemented by other measures *e.g.* staining the roots with acid fuchsin in lactoglycerol to simplify the determination of origin of the galls (Albertsson Juhlin, 2014; Dobosz & Krawczyk, 2019).

Figure 6. Left: roots of blue lupine symptom free (control) and right, roots of blue lupine with *Meloidogyne hapla* root galls. The black line is encircling multiple galls that coincide into a large root knot formation. Photo Izabella Lundborg.

Uniform long term PPNs management programs involving resistant varieties, soil solarization and fallow are rarely successful and alternative multi-tactics are important to integrate (Wang *et al.* 2002). Repeated usage of resistant varieties pose the risk of selection for virulence and consequently resistance breakdown (Västergård, 2019). Soil solarization is effective in

reducing *Meloidogyne* ssp. in the upper 30 cm part of the soil (Douda *et al.* 2012). However, solarization is not specific to the PPNs, other beneficial saprophagous nematodes and soil organisms are as well reduced. Therefore solarization is not suitable for field treatments but can be used for sanitation of greenhouse substrates. Starvation through fallow, may require an unacceptably long period to go below the damage thresholds of susceptible crops. Populations of *Meloidogyne* ssp. has for instance been reported to decrease only to 88% after 15 weeks of fallow. In the current study, I found that the cover crop *A. strigosa* has potential to be used in integrated management strategies of *M. hapla* supporting the previous findings of Uesugi *et al.* (2018) and Andersson, (2018). For *L. angustifolius* the revealed high susceptibility to *M. hapla* suggests that usage would propagate the nematodes in infested fields and should therefore not be integrated in management strategies targeting *M. hapla*. However, *L. angustifolius* was observed by Dobosz & Krawczyk (2019) to have suppressive effects on *M. hapla*. Further research on the topic is recommended as *L. angustifolius* possesses many beneficial qualities needed in especially organic farming and it has previously not been tested extendedly for its potential to reduce the population densities of *M. hapla.*

In the *P. penetrans* experiments, a significant difference in nematode densities and reproduction rates (Pf/Pi) was revealed between the treatments. *Avena strigosa* had the lowest nematode counts, significantly different from the other treatments, *L. angustifolius* and *P. tanacetifolia,* and was very efficient in decreasing *P. penetrans* nematode numbers. In a repeated experiment using the same naturally infested soil as in this study, several cover crops are currently being evaluated for their suppressive effect on *P. penetrans*. Among them, *A. strigosa*, *P. tanacetifolia* and two blue lupine cultivars *L. angustifolius* cv. Mirabor and *L. angustifolius* cv. Regent are studied. Preliminary results suggest that different cultivars *L. angustifolius* show different reproduction factors and that *A. strigosa* have a high suppressive effect against *P. penetrans.* Previously, *L. angustifolius* (unknown variety) has been found to provide some protection against *P. penetrans* in infested fields and is considered to be a poor host (Riley and Kelly, 2002). A high infestation in *L. angustifolius* (unknown variety) was previously reported in western Australia in 1999 (Riley and Wouts, 2001). The different results found, suggests that before considering Lupinus as a cover crop in integrated pest management programs of *P. penetrans,* it is important to consider the difference between lupine varieties to prevent proliferation.
The low reproduction rates for *A. strigosa* in both trials agree with Andersson, (2018) and LaMondia, (1999) who state that *A. strigosa* shows suppressive effects against *P. penetrans* and is considered to be a partially resistant host. *Avena strigosa* does not produce high quality grains but has many desirable traits as a cover crop (LaMondia, 1999). The root system is strong and develops and spreads quickly, which is beneficial to reduce erosion, and for nematode sanitation (Petersen, 2019). The well distributed root system increases the contact surface for the nematodes, enabling more efficient suppression. Usage of *A. strigosa* in the crop rotation also conserves soil organic matter, reduces compaction and suppresses weeds (LaMondia, 1999). In *P. penetrans* infested strawberry fields *A. strigosa* can be used for nematode suppression as well as for production of straw used to protect the plantings during winter.

The majority of replicates of *P. tanacetifolia* in the present study were infected by an unspecified root pathogen, illustrated in (Figure 7). The pathogen is possibly associated with *Pythium* spp., as the symptoms in the trial of *P. tanacetifolia* agree with the symptoms described by Krober, (1975): stem and root rot, discoloration and wilting. The pathogen influenced root development negatively, and the lack of root mass was likely to be the cause of the low nematode counts and reproduction rates, leaving the nematodes without a food source. More studies are therefore needed to evaluate the effect of *P. tanacetifolia* on *P. penetrans*. Moreover, the effect of *P. tanacetifolia* on *P. penetrans* is currently being evaluated in greenhouse and field studies (Olsson Nyström, unpublished personal communication). The studies also examine differences between root-lesion species in relation to *P. tanacetifolia,* which is important to take into account while choosing cover crops for sanitation in the field.

Figure 7. Left: symptoms of stem rot on *Phacelia tanacetifolia* plants after emergence of true leaves, right symptoms of discoloration and dieback of adult *Phacelia tanacetifolia* plants. Photo Izabella Lundborg.

The results obtained in this experiment indicate that *A. stigosa* and *L. angustifolius* have potential to be used in integrated management strategies of *P. penetrans. Avena strigosa* revealed efficient *P. penetrans* suppression qualities supporting the findings communicated by Andersson, (2018) and LaMondia, (1999). Therefore *A. strigosa* may be recommended as a cover crop in *P. penetrans* infested fields and in crop rotation of susceptible cultivars for counteracting purposes. Moreover, the low nematode densities and reproductions rates in this study are suggesting that some cultivars of *L. angustifolius* may offer some protection by maintaining the population at lower levels than crops with good host status (Riley and Kelly, 2002 ; Rahman *et al.* 2007). However, it is possible that *L. angustifolius* may become less important as other non-hosts will be further studied.

Concluding remarks

The cover crop treatments in the *P. brassicae* experiment did not reveal significant difference in DSIs to the plant free treatment (fallow), merely a non-significant tendency of decreased disease severity was revealed. However, the fresh and dry roots weights were significantly different between the fallow and the two cover crop treatments. The higher root weights of the fallow treatment suggests that the roots were more diseased than in the other treatments and agree with the tendency found of slightly higher DSIs in treatment exposed to fallow than to the cover crops. Furthermore, in other studies *L. perenne* and members within the fabaceae family have been found to decrease DSIs of indicator plants in comparison to fallow, revealing potential to be used in integrated pest management programs of *P. brassicae.* However, in the present study it cannot be concluded that any of the plants tested counteract nor are more effective in reduction of resting spores of *P. brassicae* than the other. The small changes observed between the DSI of the plant-specific treatments could have been caused by factors not directly related to plant species *e.g.* increased pH levels. Further studies on *L. angustifolius* and *L. perenne* to establish the potentially suppressive effects of *P. brassicae,* in bioassays and in fields would be an important extension of this project.

Once *P. brassicae* have reached damaging threshold values, there is no easy measure to control and reduce the infection. It is necessary to integrate suitable multi- tactic strategies to achieve satisfactory control. Non- host species can be efficient elements in *P. brassicae* integrated strategies, assuming that the suitable crop cultivars are selected and are properly managed. Beneficial ecosystem services gained by using cover crops and potentially suppressing effects of *P. brassicae* in relation to negative effects associated with fallow and of synthetic pesticides, justifies further research on non-hosts species in integrated pest management programs of *P. brassicae.*

The cover crops studied in the PPNs experiments revealed potential to be used in integrated pest management programs of *M. hapla* and *P. penetrans.* Treatments with *A. strigosa* were found to be highly resistant to *M. hapla* and to be very efficient in suppressing *P. penetrans* in infested soil. Therefore *A. strigosa* may be recommended in crop rotation where both nematode species are challenging the production of susceptible cultivars. The treatments with *L. angustifolius* revealed high susceptibility to *M. hapla* and should thereby be avoided in infested fields. Moreover, *L. angustifolius* was found to be a poor host of *P. penetrans* suppressing the nematodes to a certain degree. The potential of *L. angustifolius* seems to be dependent on variety traits, as contradictory results were reported among varieties. Further studies are needed on *L. angustifolius* variety differences in relation to *P. penetrans.*

Because of the potential of effective cover crops against PPNs and lack of safe synthetic nematicides and the negative effects associated with fallow, continued research of cover crops with non-hosts, poor hosts and resistant properties is justified. An important extension of this study would be to monitor the cover crops in infested fields.

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Appendix

Appendix 1. Calculation sheet of inoculation solution of *Plasmodiophora brassicae* resting spores.

The values are calculated according to the Fuchs-Rosenthal with 0.200 depth and one smallest square are is 0.0625 and total number of square is 16								
					x by total number of square			
Avergae number of Spore (in one square)		2500	Smallest square ares	0,0625	16	1		0,2
			Depth of Chamber	0,2				
	Number of spore (in 1 ul)	12500						
	Number of spore (in 1 ml)	12500000						
	Number of spore per ml we need	40000						40000000
	We need total volume ml	1000						
						25000		
Amount of spores required to make---- ml		3,2	ml					