Aphanomyces spp. in Swedish crops and factors affecting their occurrence

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Aphanomyces *spp. i svenska grödor och faktorer som påverkar deras förekomst*

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Abstract

Aphanomyces euteiches, *A. cochlioides*, and *A. cladogamus* are three devastating agricultural plant pathogens that respectively are causing diseases such as pea root rot, sugar beet root rot, and spinach root rot. This study aims to investigate the distribution of the three *Aphanomyces* species in parts of Sweden, Denmark, and Lithuania. It also aims to analyse macronutrient levels in the soil and explore potential connections between these soil factors and the pathogen presence. Methods of the study involve conducting biotests collected from the different regions and evaluate the presence of *Aphanomyces* spp. Additionally, a large data set from biotests conducted in commercial pea growing in Sweden was analysed.

The study revealed differences in the occurrence of *Aphanomyces* spp. between regions. *Aphanomyces euteiches* was the most widespread pathogen found in 31% of the tested samples and in 8 out of 13 totally tested regions. *Aphanomyces cochlioides* had a more limited distribution and was found in 14% of the investigated samples and in 4 out of 13 regions. *Aphanomyces cladogamus* displayed intermediate distribution in Sweden and was present in 12% of the tested samples, and in 5 out of 13 regions. *Aphanomyces euteiches* only affected pea plants. However, an overlapping host range was observed between *A. cochlioides* and *A. cladogamus*, which infected both sugar beet and spinach.

Furthermore, results showed significant differences in values of disease severity index between regions, pathogens, and for the interaction between region and pathogen. Soil analysis from all the soil samples showed that the calcium content (Ca-AL) ranged from 82 to 3600 mg/100g dry soil. When Ca-Al levels were above 250 mg/100 mg soil no species of *Aphanomyces* was detected, indicating disease suppressiveness. Similar results were observed from the commercial pea growing data set; no DSI values above 20 (with *A. euteiches*) was found in any soil sample with a Ca-AL value above 210 mg/100 g soil. Combined DSI data from regions was plotted against the combined data for soil properties and tested for correlations. Results from this analysis showed that high Ca-AL levels correlated significantly ($P \leq 0.043$) with low DSI levels in both pea, spinach, and sugar beet.

Currently, pesticides against *Aphanomyces* spp. are very limited, highlighting the need to understand the distribution patterns and the relationship between macro nutrients and the pathogens for better disease management.

Keywords: Aphanomyces euteiches, *Aphanomyces cochlioides*, *Aphanomyces cladogamus*, pea, sugar beet, spinach, calcium, distribution

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Abbreviations

DSI	Disease severity index
SLU	Swedish University of Agricultural Sciences
Spp.	Species plurimae (multiple species)

1. Introduction

Aphanomyces is a genus of oomycetes that includes several destructive pathogenic species. It is a problematic pathogen in agriculture and aquaculture where it is causing diseases in several different crop plants as well as in fish and crayfish. There are in total about 40 described species in the *Aphanomyces* genus and they range widely in lifestyle from being highly pathogenic to saprotrophic.

In aquaculture, the economically most important *Aphanomyces* species are *A. invadans*, which cause epizootic ulcerative syndrome in several fish species, and *A. astaci* that is causing a serious disease on crayfish. *Aphanomyces euteiches* and *A. cochlioides* are two of the most well-known and important plant pathogenic *Aphanomyces* species. *Aphanomyces euteiches* cause root rot in pea (*Pisum sativum*), and *A. cochlioides* cause damping-off and root rot in sugar beet (*Beta vulgaris*) (Becking et al., 2022). Another plant pathogenic *Aphanomyces* species is *A. cladogamus* that cause root rot in spinach (*Spinacia oleracea*) (Larsson & Olofsson, 1994). Both *A. euteiches* and *A. cochlioides* have been known as plant pathogens for a long time and were described for the first time in the United States in the 1920s (Jones & Drechsler, 1925; Drechsler, 1929).

General disease symptoms caused by *Aphanomyces* spp. on plants are damping off and root rot. Disease caused by *Aphanomyces* spp. are often mistaken for being caused by other plant pathogens, since the symptoms often can be diffuse and difficult to distinguish from other disease-causing agents (Moliszewska, 2017).

Crops such as peas, beets and spinach are important in many countries around the world. These crops are highly affected by *Aphanomyces* spp., responsible for causing enormous yield losses due to root rot. The yield losses can in some cases be total e.g. in pea cultivation (Papavizas & Ayers, 1974). Both sugar beets and peas are of considerable economic importance in Scandinavia and large yield losses have been reported (Persson et al., 1997) (Olsson et al., 2019).

1.1 Classification of *Aphanomyces* spp.

The *Aphanomyces* genus belongs to the class oomycetes. Oomycetes were once considered as fungi due to their similar morphology and lifestyle. Later it was discovered that oomycetes share a common ancestor with algae, and they were therefore classified as a separate group often referred to as water moulds (Gaulin et al., 2007). Further phylogenetic studies have revealed that the *Aphanomyces* genus is divided into three major groups, which separately includes aquatic animal pathogens, plant pathogens, and saprotrophic or opportunistic parasites, respectively (Fig. 1) (Becking et al., 2022).
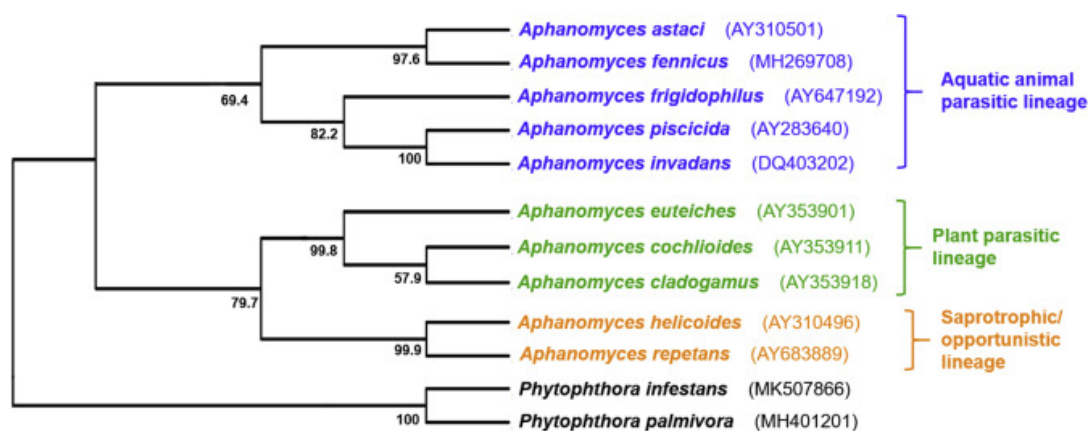
Figure 1. Phylogenetic tree showing the three major lineages of Aphanomyces *spp., and some species from each group* (Becking et al., 2022)*.

1.2 Life cycle and infection strategy of *Aphanomyces* spp.

According to Becking et al., (2022), the life cycle of plant pathogenic *Aphanomyces* spp. involves both sexual and asexual phases. Most *Aphanomyces* spp. are homothallic and can undergo self-fertilization, although outcrossing is likely to happen occasionally (Quillévéré-Hamard et al., 2018). During the sexual stage, which takes place in the plant tissue, the oogonium is fertilized, resulting in the production of oospores. These oospores, which range in size depending on the species, are thick-walled and can survive in the soil for extended periods of time. Drechsler (1929) reported that *A. euteiches* oogonia range between 25-35 µm, while *A. cochlioides* and *A. cladogamus* oogonia are slightly smaller. Larsson (1994) reported that Swedish isolates of the two latter species ranged from 20-25 µm, and the *A. euteiches* oogonia ranged from 27-30 µm. The dormant oospores in the soil serve as a potential source of inoculum for future infections. In the asexual stage,

vegetative coenocytic hyphae (without septa) generate sporangia, which produce zoospores. The zoospores are equipped with flagella, which enable them to move around and swim in the water film of the surrounding soil. Once they enter and invade the host root tissue, within a few days new oogonia are formed (Becking et al., 2022). Fig. 2 provides a detailed illustration of the life cycle.
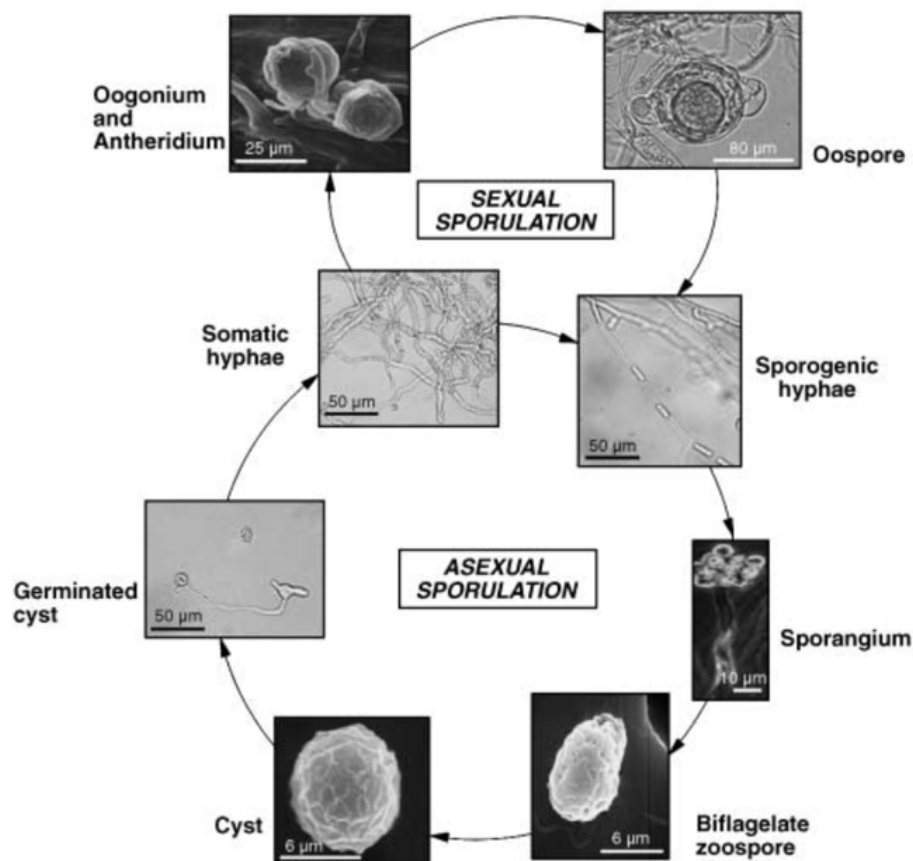
Figure 2. Illustrated life cycle of Aphanomyces euteiches (Gaulin et al., 2007)*.*

Two infection strategies of the pathogen are reported, where oospores play a central role. The oospores can either form mycelia that directly attack the root system or form sporangia producing zoospores that swim towards the roots and attack them (Bødker & Larsson, 1993). The first infection strategy suggests that oospores can germinate in response to chemical signals exuded by the roots of the host. This germination process leads to the formation of a germ tube that either can proliferate as hyphae or form a sporangium. Roots of the host plant can be directly penetrated and colonized by the hyphae (Hughes & Grau, 2007). The second infection strategy suggests that the sporangium releases zoospores that adhere to and encyst on the host plant roots. The cyst then germinates and penetrates the cortical cells, and

mycelia grow intercellularly in the root tissue. Within a few days, new oospores are formed (Gaulin et al., 2018).

1.3 Disease symptoms and host ranges

The three different species *A. euteiches*, *A. cochlioides*, and *A. cladogamus* can all cause severe disease symptoms with similar characteristics, but they are known to be specialized in different hosts.

1.3.1 *Aphanomyces euteiches*

In 1925, Jones & Drechsler published a paper entitled "Root rot of Peas in the United States caused by *Aphanomyces euteiches*", in which they described the *A. euteiches* species for the first time. *Aphanomyces* root rot infection causes the roots of peas to turn to a yellowish, straw-like colour, which gradually darkens. The discolouration spreads throughout the root system and up towards the epicotyl. In Fig. 3, healthy and diseased plants are compared, and it shows that healthy pea roots are white.

Figure 3. Healthy pea roots to the left, in comparison with pea roots infected with A. euteiches *to the right. Photo: Mariann Wikström*
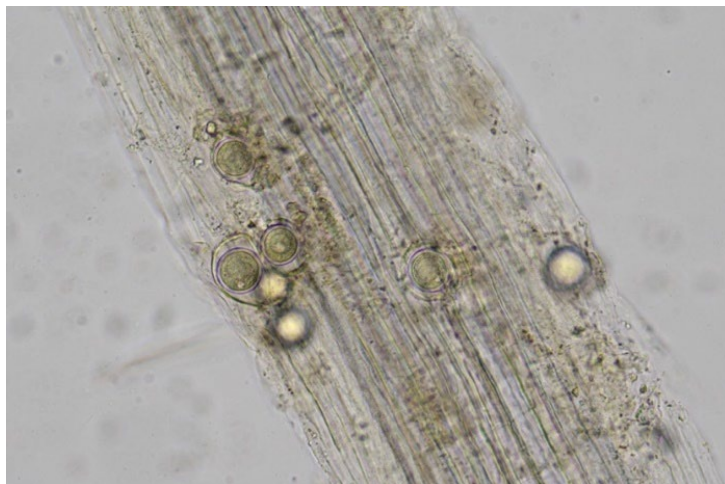
Figure 4. Oospores of A. euteiches *in infected pea roots. Photo: Josefin Wikström*

The pathogen forms mycelium that breaks down the outer layer of the root tissue. In heavily infected pea plants, the fine roots ultimately disintegrate, leaving only the vascular strands and the coarser central root. By microscopic investigation of

infected pea roots the characteristic oospores within the root tissue can be seen (Fig. 4). Pea plants affected by root rot are unable to acquire sufficient amounts of water and nutrients due to root damage, which results in premature wilting and yellowing of the leaves. Initially, the symptoms may be found in patches in the fields (Fig. 5), but they may spread across the entire field in cases of severe infection. Since the pea plant is weakened by root rot, other weaker and less aggressive fungi frequently invade the plants, causing gradual darkening of the roots (Bødker & Larsson, 1993). *Aphanomyces euteiches* was first found in Sweden in the end of the 1950th, as described in 1967 by Olofsson. The pathogen has since then been found in all pea growing areas in Sweden. It is also widespread in Denmark, where it is a very important pathogen in some areas and where it causes severe yield losses (Persson et al., 1997). Pea root rot has the potential to result in up to 80% crop loss, not only in Sweden but also globally (Gaulin et al., 2007). In certain years and circumstances, the disease can even result in a complete crop loss (Papavizas & Ayers, 1974).

Figure 3. Pea field infected with A. euteiches*, showing yellowing patches. Photo: Mariann Wikström*

Aphanomyces euteiches is spread globally and can cause disease in several species of legumes. However, infection in pea plants is deemed to be the most economically damaging. Other legumes that can be infected by *A. euteiches* under Swedish conditions include alfalfa (*Medicago sativa*), lentils (*Lens culinaris*), yellow sweet

clover (*Melilotus officinalis*), vetch (*Vicia sativa*), and green beans (*Phaseolus vulgaris*) (Jordbruksverket, 2021). In other countries, additional host plants, such as various clovers (Malvick et al., 2009) and field beans (van Leur et al., 2008), are reported.

1.3.2 *Aphanomyces cochlioides*

Aphanomyces cochlioides is widely known to cause root rot in sugar beets. It was initially identified in 1929 (Drechsler, 1929) and has since then been detected in regions where sugar beets are frequently grown, including Europe, North America, Canada, and Chile. *Aphanomyces cochlioides* is the primary pathogen responsible for root rot in sugar beets and has caused significant crop losses worldwide (Papavizas & Ayers, 1974). Also in Sweden, it is one of the most important pathogens affecting sugar beet crops (Olsson et al., 2019).

In sugar beets infected with *A. cochlioides*, the initial symptom is seedling damping off. In young plants, the disease can first be seen as browning and softening of the roots, which later extends up to the hypocotyl. This region rapidly darkens and shrivels into a thin thread (Fig. 6). In mature plants, the root rot starts at the tip of the beet and moves upward or at the junctions of lateral roots (Fig. 7). The diseased tissue becomes dark brown in colour (Windels, 2000). These symptoms can be mistaken for other soil-borne diseases, such as girth scab caused by *Streptomyces* sp. (Moliszewska, 2017).

Figure 6. Sugar beet seedlings infected with A. cochlioides*, causing the dark roots. Photo: Lars Persson*

Figure 7. Sugar beets infected with A. cochlioides *at mature stage. Photo: Lars Persson*

The presence of the pathogen can result in a patchy field, and in severe cases, it can lead to the complete destruction of the whole crop. Signs of infection can be observed above ground at the soil surface as stunted plants and yellow foliage. Infected sugar beets can either rot or remain too small for harvesting, which significantly reduces yield. Sugar beets that are infected at a later stage in the season or manage to survive the initial stages of the disease, have lower sugar content and higher impurity levels (Windels, 2000). Since sugar beets in the last decades are only grown in the southernmost part of Sweden, the pathogen has only been detected there (Persson, pers. com). However, the pathogen can also cause diseases in other crops, including table beet (*Beta vulgaris* subsp. *vulgaris conditiva* group*)*, chard (*Beta vulgaris* var. *cicla)*, spinach (*Spinacia oleracea*), and fodder beetmangel (*Beta vulgaris* subsp. *vulgaris)*. Furthermore, the pathogen has been isolated from some weeds and wild plants such as lamb's quarters (*Chenopodium album*), green carpet weed (*Mollugo verticillata*), and tumbling ted (*Saponaria ocymoides*) (Papavizas & Ayers, 1974). Together with the fact that beets for fodder and for sugar extraction have historically been grown in other parts of Sweden, this suggests that there is a possibility for an extended geographic distribution of *A. cochlioides* in Sweden.

1.3.3 *Aphanomyces cladogamus*

Aphanomyces cladogamus was first isolated in tomatoes and described by Drechsler in 1929. It is recognized as an important root rot pathogen on spinach in Sweden, (Larsson & Olofsson, 1994) and on peppers in Canada (McKeen, 1952). *Aphanomyces cladogamus* is also a problem in pansies, tomatoes, and several other crop and garden plants (UK, CAB International & Hall, 1989).

Aphanomyces cladogamus causes pre- and post-emergence damping off in spinach, with characteristic symptoms such as dark lesions on the hypocotyls that often extend to the base of the cotyledons (Fig. 8). Other symptoms such as stunted growth, chlorotic leaves, and wilting plants also occur. The pathogen has caused Swedish spinach fields to suffer yield losses of up to 35%, and low-quality harvest (Larsson & Olofsson, 1994). Similar symptoms are observed in tomatoes and peppers (McKeen, 1952).

Compared to *A. euteiches* and *A. cochlioides*, *A. cladogamus* has a broader range of hosts. Common weeds that can host the pathogen include chickweed (*Stellaria media)*, lamb's quarters (*Chenopodium album*), field pansy (*Viola arvensis*), field speedwell (*Veronica agrestis*), henbit (*Lamium amplexicaule*), groundsel (*Senecio vulgaris*), and black bindweed (*Polygonum convolvulus*) (Larsson, 1994).

Figure 4. Healthy spinach plants to the left. Spinach seedlings infected with A. cladogamus *to the right.*
Photo: Josefin Wikström

1.4 Management strategies

Today, there are no existing pesticides that effectively control plant pathogenic *Aphanomyces* spp. during the whole season of the crop (Hosseini et al., 2015). However, the fungicide Tachigaren (hymexazol), is registered for control of damping off caused by *A. cochlioides* in sugar beets as seed treatment (Kemikalieinspektionen, 2023). This is widely used in sugar beet cultivation in Sweden.

There are also some preventive methods developed, which have demonstrated positive effects. One crucial preventive approach involves implementing a good crop rotation and avoid cultivating susceptible hosts in an infected field. It is advisable to wait a minimum of six to eight years between pea cultivations to avoid propagation of *A. euteiches*. In cases of high pathogen occurrence, an even longer interval is recommended (Persson et al., 1997). Additionally, cultivating crops in well-drained fields and avoiding soil compaction are also recommended essential practices (Hossain et al., 2012). By preventing the pathogen from infecting new plants, its reproduction is restricted, leading to a potential decline in the pathogen population. It is vital to determine whether a field is infected and, if so, identify the pathogen species and assess its infection severity. A bioassay can be conducted to determine the species and presence of the pathogen.

Certain *Brassica* species can be used for biofumigation, serving as sanitizing catch crops during crop rotation and effectively reducing soil-borne pathogens like *Aphanomyces* spp. These species contain glucosinolates that decompose into

volatile and toxic substances in the soil. Notably, sulphides and isothiocyanates are produced, exhibiting extreme toxicity to *A. euteiches*. Some examples of *Brassica* species known to generate these toxins include radish (*Raphanus sativus*), mustard (*Brassica juncea*), cabbage (*Brassica oleracea*), oilseed rape (*Brassica napus*), and white mustard (*Sinapis alba*) (Hossain et al., 2012).

Since *Aphanomyces* spp. thrives in acidic soils, the pathogen's growth can be suppressed through liming, which raises the pH level and increases the concentration of calcium in the soil. Studies indicate that applying lime in a pathogen-infested field significantly increases the crop yield. Therefore, it is recommended to regularly apply lime in fields to reduce diseases caused by *Aphanomyces* spp. (Olsson et al., 2019).

Evidence suggests that adding manure into the field can decrease root rot caused by soil-borne pathogens such as *Aphanomyces* spp. Pig manure is demonstrated to have the greatest effect, although similar results are observed with cattle and poultry manure. Reduced disease symptoms are reported in peas, sugar beets, and spinach following the addition of manure. Disease reduction is probably depending upon several mechanisms, e.g. production of ammonia (NH_3) and nitrous acids (HNO_2) (Ingemarsson, 2004).

Variation in susceptibility to *Aphanomyces* spp. exist among different crop cultivars with some displaying partial resistance (Desgroux et al., 2016). For example, certain sugar beet cultivars exhibit partial resistance to *A. cochlioides* (Bengtsson, 2021). Such sugar beet cultivars are commercially cultivated today. Also in peas, this knowledge is currently being utilized in breeding programmes to develop cultivars with partial resistance to *A. euteiches* at the pea growing company Findus AB (Kälin et al., 2023; Stegmark, 2015). A recent study has identified three genetically distinct groups of *A. euteiches* in Europe, which might be important when considering future management strategies (Kälin et al., 2022).

1.5 Interactions between *Aphanomyces* spp. and the environment

The occurrence of *Aphanomyces* spp. in the soil can be affected by certain soil environmental factors at varying degree. A study conducted in Sweden has revealed that *Aphanomyces* spp. exhibits varying degrees of infectivity in different types of soil. The pathogen can be inhibited and barely express any disease symptoms in certain soils, which are referred to as suppressive soils. Consequently, susceptible crops can be cultivated more frequently with a reduced risk of disease in such soils (Persson et al., 1999). Abiotic factors e.g. clay content, pH, and calcium

concentration, seem to be the cause of suppression to pea root rot in Sweden (Persson & Olsson, 2000).

Certain soil factors can also promote pathogen presence in the soil and its infectivity. One of the most important factors is soil moisture, since high moisture levels favour the production and germination of zoospores, leading to increased disease distribution. In addition, high soil moisture contributes to increased mobility of zoospores by enabling the formation of a water film in the soil, leading to increased pathogen spread and infection. According to studies conducted in Sweden, soil moisture levels below 45% of saturation level are associated with restricted pathogen spread (Hossain et al., 2012). Soil compaction is another factor that influences pathogen distribution. When the soil is compacted, it is more prone to be water saturated, leading to the production of more zoospores. This phenomenon is similarly observed in soils with a high clay content (Hossain et al., 2012), and in soils with low pH and low calcium content (Olsson et al., 2019).

1.5.1 Relationship between calcium and *Aphanomyces* spp.

Several studies have confirmed the suppressive effect of calcium on *Aphanomyces* spp. For example, Heyman et al. (2007) conducted an experiment where over 1500 soil samples from southern Sweden were tested for the potential to cause aphanomyces root rot on pea. In addition, the soil macronutrient concentrations were determined. Results showed that i) zoospore production from mycelia of *A. euteiches* was clearly inhibited by calcium concentration in the submillimolar range and ii) the mycelial growth itself was unaffected or even stimulated by the same calcium concentration range. The conclusion was that calcium is a major variable controlling the degree of soil suppressiveness against *A. euteiches*, and it was also proposed that the inhibition of zoospore production from oospores might be a potential mechanism contributing to the observed suppressiveness. In a similar study conducted by Olsson et al. (2011), soil samples from sugar beet fields were collected in different regions of south Sweden and assessed for pathogen presence and soil characteristics such as macronutrient concentrations. They found a negative correlation between calcium concentration in the soil and the presence of *A. cochlioides*, suggesting that higher calcium levels can reduce the risk of disease. Based on these findings, the authors recommend maintaining a calcium concentration above a threshold of 250 mg/100 g soil to minimize sugar beet root rot caused by *A. cochlioides*. Together these studies show a strong correlation between calcium concentration and *Aphanomyces* spp. presence.

Since pesticides against *Aphanomyces* spp. are very limited, understanding the geographic distribution of *Aphanomyces* pathogens and factors limiting disease development is important when developing future management strategies.

1.6 Objectives

The first aim of this study was to investigate the distribution of the three different *Aphanomyces* species: *A. euteiches*, *A. cochlioides*, and *A. cladogamus* in southern Sweden, and to some extent in Denmark and Lithuania. I hypothesized that presence of different *Aphanomyces* spp. follow the cultivation of the host plants regionally, but that *A. cladogamus* is found on a wider geographic scale due to its broad host range. Secondly, I hypothesized that different species of *Aphanomyces* co-exist, due to their similar ecological niche. Another aim of this study was to investigate the level of macronutrients in the soil, and to test for possible correlation with pathogen presence. More specifically, I hypothesized that high calcium levels correlate with low pathogen presence and subsequently, low disease pressure.

2. Material and methods

This study used data from biotests performed on 58 soil samples from Sweden, Denmark, and Lithuania, and data from biotests used in commercial pea cultivation in Sweden.

2.1 Biotest of disease severity

Biotests were performed in a growth chamber provided by the company Agro Plantarum AB in Åstorp in southern Sweden. The chambers provided a controlled climate with a temperature of 21°C and lighting for 15 hours per day.

In total there were 58 available soil samples (5 kg soil each) that were used in this experiment. The soil samples were taken in Sweden, Denmark, and Lithuania. Forty-two of the samples originated from Sweden, specifically from the counties Skåne, Halland, Västergötland, Östergötland, Dalsland, Värmland, Uppland, Öland and Gotland. Ten samples originated from Denmark covering areas such as Jylland, Fyn and Bornholm. The remaining six soil samples originated from Lithuania. Soil samples were taken in commercial fields. Most fields had a history of pea cultivation and was sampled with the purpose of investigating the presence of *A. euteiches*. Each soil was planted with three different plant species used as baiting plants for the three different *Aphanomyces* species. The three crops were pea, sugar beets, and spinach. Each pot was planted with ten seeds, and there were two pots (replicates) per crop and soil sample. The pots contained one litre of soil. A total of 58 soils, three different crops and two replicates resulted in a total of 348 pots. The pots were placed on individual trays to avoid contamination and spread of pathogens between the different soil samples.

After four weeks, the soil was removed and the roots were washed, and the disease severity index (DSI) was estimated for each plant. This root rot assessment was performed according to a grading method described by Persson et al. (1997). The method consists of grading plants on a DSI scale containing seven different classes (0, 5, 10, 25, 50, 75, 100). Here a DSI of 0 means symptom-free plants with white root systems and 100 means dead plants, and the intermediate classes were based

on percentage of infected root area. When all plants were graded, an average value of the DSI classes was calculated as the DSI for each pot. Since there were two replicates of each crop, an average value was calculated to represent the crop and field.

To determine if the plants from the soil samples was infected with *Aphanomyces* spp. or by other pathogens, the root assessment was combined with a microscopy assay. *Aphanomyces* spp. was identified and distinguished based on morphological characteristics and size of oogonia and oospores (e.g. Figs. 4 and 9).
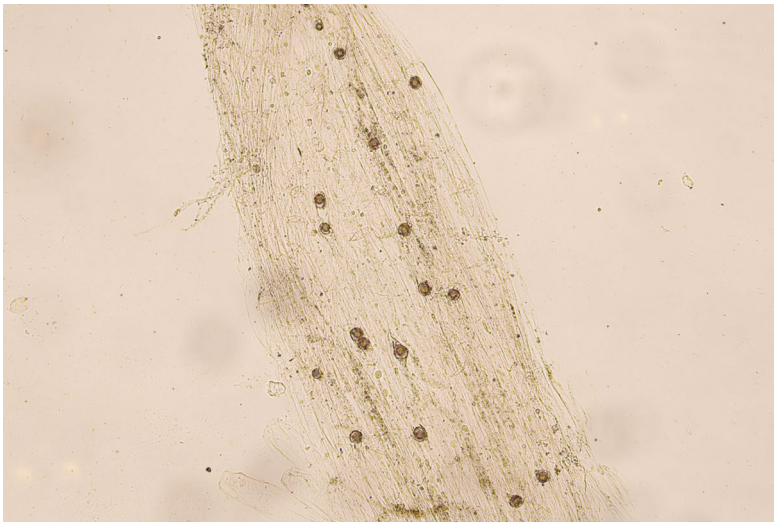
Figure 5. Aphanomyces cladogamus *in spinach roots. Photo: Josefin Wikström.*

Additionally, if oospores were detected through the microscopic assay, small root pieces were plated on selective medium for *Aphanomyces* spp. (SMA) as described by Larsson & Olofsson (1994). The SMA consists of cornmeal agar amended with metalaxyl, thiophanate methyl, and streptomycin sulphate for excluding other fungi such as *Pythium* spp., *Fusarium* spp., *Rhizoctonia* spp., and also bacteria. Hyphal tips of outgrowing colonies were transferred to cornmeal agar (CMA). Outgrowing colonies were compared with available standard isolates of *A. euteiches*, *A. cochlioides*, and *A. cladogamus,* which identity had been confirmed earlier (Larsson, 1994). There were differences in the mycelial growth between the three species, which served as a third identification strategy.

2.2 Soil analysis

From each soil sample, a small sample (350 g) were sent to Eurofins AB for soil analysis. They used extraction with ammonium lactate (AL) to determine the content of macronutrients including plant available phosphorous (P-AL), potassium

(K-AL), magnesium (Mg-AL), calcium (Ca-AL), and the soil pH. These values were later used for comparisons to the DSI.

2.3   Analysis of data from commercial pea growing

Soil samples from all fields where peas were grown by the company Foodhills AB in the south of Sweden has been analysed for pea root rot during the last seven years. The data set included a total of 1350 samples. The contents of macronutrients have also been analysed in the same soil samples. Data from these tests was kindly provided by Foodhills AB and has been analysed in this study in order to test for relationships between different macronutrients and the DSI.

2.4   Statistical analysis

To test for the factors contributing to disease severity in the dataset with samples containing only *Aphanomyces* spp. and no pathogens, analysis of variance (ANOVA) was performed using linear model. A full model accounting for region, crop and pathogen and their two-way interactions was fit. Alongside the full model, two models summarizing over crop and regions were fit. In the model summarized over crops, the effect of variables region and pathogen and their interaction was tested. Similarly, in the model summarized over regions, the effect of variables crop and pathogen and their interaction was tested. For multiple comparisons, post-hoc Tukey's tests were performed for the full model and models summarized over crops and regions.

For testing the effect of various soil properties contributing to the disease severity, a linear model with variables region, crop, pathogen, soil pH, and log10 transformed values of Ca, K, Mg, and P was tested. Alongside the linear model, Pearson's correlations were also tested for each soil variable against disease severity.

Analyses of variance for disease severity were performed in R version 4.1.1 "Kick Things" (R Core Team 2021). The linear models were fit using the package "lm". Post-hoc comparisons were performed using the packages "emmeans" (Russel 2022) and "cld" (Hothorn et al., 2008). For data processing and visualisation, "Tidyverse" suite of packages was used (Wickham et al., 2019).

3. Results

3.1 Distribution of *Aphanomyces* spp. in tested regions

Results from this study show that *A. euteiches* was found in 8 out of 13 tested regions (Table 1, Figure 10). Seven of the findings were from regions in Sweden, and one was from a region in Denmark. No *A. euteiches* was found in the Lithuanian samples. In total, *A. euteiches* was found in 31% of the investigated samples. *Aphanomyces cladogamus* was found in five of the Swedish regions, and none were found in Danish or Lithuanian regions. A total of 12% of the samples contained *A. cladogamus*. *Aphanomyces cochlioides* was found in four of the investigated regions, Skåne in Sweden, two regions in Denmark, and in Lithuania. From all the investigated samples, 14% contained *A. cochlioides*.

Table 1. Distribution[1] of different Aphanomyces spp. in the tested regions

Region	***A. euteiches***	***A. cladogamus***	***A. cochlioides***	***Aphanomyces* sp.[2]**
Skåne	+	+	+	
Halland	+	+	-	+
Västergötland	+	-	-	+
Östergötland	+	+	-	
Dalsland	+	+	-	
Värmland	+	+	-	
Uppland	+	-	-	
Gotland	-	-	-	
Öland	-	-	-	
(DK) Jylland	+	-	+	
(DK) Bornholm	-	-	-	
(DK) Fyn	-	-	+	
Lithuania	-	-	+	

[1]Minus sign (-) indicates no presence while plus sign (+) indicates pathogen presence.

[2]*Aphanomyces* sp. indicates presence of *Aphanomyces*, but they were not identified to species level.

Figure 6. Map showing the location of the analysed soil samples. Green circles represent findings of A. euteiches, *black squares represent findings of* A. cladogamus, *blue triangles represent findings of* A. cochlioides, *and locations marked with X represent no findings of any* Aphanomyces *spp.*

3.2 Composition of the root pathobiome

All data collected from the 58 soil samples, such as regions, DSI and identified pathogens, are shown in Table 2. *Aphanomyces* spp. was detected in 47% of the tested samples. Besides *Aphanomyces* spp., other plant pathogens such as *Rhizoctonia solani*, *Pythium* spp., *Phytophthora pisi*, and nematodes (mainly root lesion nematodes, *Pratylenchus* spp.) were also detected. *Rhizoctonia solani* was found in 41% of the tested samples, *Pythium* spp. in 52%, *P. pisi* in 3%, and nematodes in 9%.

Table 2. Disease severity index (DSI, 0-100) for each crop and soil sample

ID	Region	DSI pea	Identified pathogens in pea	DSI sugar beet	Identified pathogens in sugar beet	DSI spinach	Identified pathogens in spinach
100	Gotland	1	No pathogen found	18	*Pythium*	1	No pathogen found
101	Gotland	1	No pathogen found	6	No pathogen found	4	No pathogen found
102	Gotland	0	No pathogen found	17	No pathogen found	11	No pathogen found
103	Gotland	1	No pathogen found	1	No pathogen found	3	No pathogen found
104	Gotland	10	*Rhizoctonia*	1	*Pythium*	18	*Pythium*
105	Gotland	1	No pathogen found	15	*Pythium*	8	No pathogen found
106	Gotland	0	No pathogen found	3	No pathogen found	5	No pathogen found
107	Gotland	38	*Rhizoctonia*	6	*Rhizoctonia*	21	*Rhizoctonia*
108	Gotland	41	*Pythium*	25	*Rhizoctonia*	100	*Rhizoctonia + Pythium*
109	Gotland	13	*Rhizoctonia*	25	*Rhizoctonia*	20	*Rhizoctonia + Pythium*
110	Gotland	19	*Pythium*	10	*Pythium*	5	*Pythium*
200	Värmland	14	*A. euteiches*	8	*A. cladogamus*	46	*A. cladogamus*
201	Västergötland	0	No pathogen found	9	*Pythium + Aphanomyces sp.*	1	No pathogen found
202	Dalsland	71	*A. euteiches*	18	Nematodes + *Pythium*	21	*A. cladogamus*
203	Västergötland	16	*A. euteiches*	12	No pathogen found	8	No pathogen found
300	Östergötland	36	*A. euteiches + Phytophthora*	15	No pathogen found	2	No pathogen found
301	Östergötland	1	No pathogen found	10	No pathogen found	6	*Pythium*
302	Östergötland	3	No pathogen found	3	No pathogen found	5	No pathogen found
303	Östergötland	14	No pathogen found	10	No pathogen found	4	*A. cladogamus*
304	Östergötland	15	No pathogen found	26	*Rhizoctonia*	24	*Rhizoctonia*
305	Östergötland	55	*A. euteiches*	94	*A. cladogamus + Rhizoctonia*	67	*A. cladogamus + Rhizoctonia*
404	Jylland	19	*Rhizoctonia*	21	*Rhizoctonia*	45	*A. cochlioides*
405	Bornholm	2	No pathogen found	2	No pathogen found	6	*Pythium*
406	Jylland	33	*A. euteiches*	4	*No pathogen found*	9	*Rhizoctonia*
407	Jylland	78	*A. euteiches*	100	Pythium	78	*Rhizoctonia + Pythium*
408	Fyn	9	*Pythium*	8	No pathogen found	33	*A. cochlioides*
409	Jylland	65	*Rhizoctonia + Pythium*	10	*Rhizoctonia*	12	*Rhizoctonia + Pythium*
410	Fyn	17	*Pythium*	4	No pathogen found	15	*Pythium*
411	Jylland	98	*A. euteiches*	31	*Rhizoctonia + Pythium*	15	No pathogen found
412	Jylland	85	*A. euteiches*	19	*Pythium*	20	*Pythium*

413	Jylland	49	A. euteiches	7	Pythium	5	Rhizoctonia + Pythium
500	Uppland	5	No pathogen found	45	Rhizoctonia	50	Pythium
501	Uppland	9	Pythium	43	Rhizoctonia	48	Rhizoctonia
502	Uppland	5	No pathogen found	25	Pythium	24	Pythium
503	Uppland	3	No pathogen found	0	No pathogen found	4	No pathogen found
504	Uppland	61	A. euteiches + Phytophthora	24	Rhizoctonia + Pythium	38	Rhizoctonia
505	Uppland	5	Pythium	41	Pythium	36	Rhizoctonia
506	Uppland	5	No pathogen found	14	No pathogen found	5	No pathogen found
507	Uppland	7	Pythium	14	No pathogen found	13	No pathogen found
600	Öland	0	No pathogen found	16	No pathogen found	21	No pathogen found
2916	Halland	6	No pathogen found	26	Rhizoctonia + Pythium	42	Rhizoctonia
2922	Halland	98	A. euteiches	27	Pythium	24	Nematodes
2979	Skåne	5	No pathogen found	57	A. cladogamus	74	A. cladogamus
3010	Skåne	2	No pathogen found	27	Rhizoctonia	62	Nematodes
3025	Halland	41	A. euteiches	31	Pythium	47	Pythium
3031	Skåne	7	Nematodes	8	Rhizoctonia	17	Pythium + Nematodes
3049	Halland	60	A. euteiches	28	A. cladogamus	75	A. cladogamus
3102	Skåne	3	No pathogen found	66	A. cochlioides	41	A. cochlioides
3121	Skåne	11	A. euteiches	16	Rhizoctonia + Pythium	34	A. cladogamus
3131	Skåne	42	A. euteiches	67	A. cochlioides	88	A. cochlioides
3132	Skåne	78	A. euteiches	95	A. cochlioides	16	A. cochlioides
3231	Skåne	67	A. euteiches	33	No pathogen found	52	Rhizoctonia + Pythium + Nematodes
LT 1	Litauen	1	No pathogen found	18	Rhizoctonia	5	No pathogen found
LT 2	Litauen	19	Rhizoctonia	74	A. cochlioides + Rhizoctonia	28	A. cochlioides
LT 3	Litauen	3	No pathogen found	18	A. cochlioides	53	A. cochlioides
LT 4	Litauen	2	No pathogen found	45	Pythium	41	Pythium
LT 5	Litauen	1	No pathogen found	8	Pythium	9	Pythium
LT 6	Litauen	4	Rhizoctonia	48	A. cochlioides	31	A. cochlioides

In order to explore the composition of the pathobiome associated with plant roots, the data was filtered into three separate sets based on pathogen presence, each gradually more stringent.

The first set included samples where *Aphanomyces* spp. was present in at least one of the crops of a given soil sample. Also, samples without any detected pathogen ("No pathogen found") in all three crops of a sample were kept. Samples containing other pathogens or no *Aphanomyces* spp. in either crop was excluded. This filtering resulted in 35 soil samples (out of 58). *Aphanomyces* spp. was found in at least one bait plant in all except eight soil samples, where no pathogens were found at all (Table 3). Other pathogens such as *R. solani*, *Pythium* spp,, *P. pisi* and nematodes were commonly present in soil samples together with *Aphanomyces* spp. However,

co-infection by more than one pathogen in a single plant root was rare. Co-infection by *A. euteiches* and *P. pisi* on pea was found in two cases, co-infection by *A. cochlioides* and *R. solani* was found only once on sugar beet, while co-infection by *A. cladogamus* and *R. solani* on sugar beet was detected once.

Table 3. Disease severity index (DSI) data on samples included after the first filtering

ID	Region	DSI pea	Identified pathogens in pea	DSI sugar beet	Identified pathogens in sugar beet	DSI spinach	Identified pathogens in spinach
101	Gotland	1	No pathogen found	6	No pathogen found	4	No pathogen found
102	Gotland	0	No pathogen found	17	No pathogen found	11	No pathogen found
103	Gotland	1	No pathogen found	1	No pathogen found	3	No pathogen found
106	Gotland	0	No pathogen found	3	No pathogen found	5	No pathogen found
200	Värmland	14	*A. euteiches*	8	*A. cladogamus*	46	*A. cladogamus*
201	Västergötland	0	No pathogen found	9	*Pythium + Aphanomyces sp.*	1	No pathogen found
202	Dalsland	71	*A. euteiches*	18	Nematodes + *Pythium*	21	*A. cladogamus*
203	Västergötland	16	*A. euteiches*	12	No pathogen found	8	No pathogen found
300	Östergötland	36	*A. euteiches + Phytophthora*	15	No pathogen found	2	No pathogen found
302	Östergötland	3	No pathogen found	3	No pathogen found	5	No pathogen found
303	Östergötland	14	No pathogen found	10	No pathogen found	4	*A. cladogamus*
305	Östergötland	55	*A. euteiches*	94	*A. cladogamus + Rhizoctonia*	67	*A. cladogamus + Rhizoctonia*
404	Jylland	19	*Rhizoctonia*	21	*Rhizoctonia*	45	*A. cochlioides*
406	Jylland	33	*A. euteiches*	4	No pathogen found	9	*Rhizoctonia*
407	Jylland	78	*A. euteiches*	100	*Pythium*	78	*Rhizoctonia + Pythium*
408	Fyn	9	*Pythium*	8	No pathogen found	33	*A. cochlioides*
411	Jylland	98	*A. euteiches*	31	*Rhizoctonia + Pythium*	15	No pathogen found
412	Jylland	85	*A. euteiches*	19	*Pythium*	20	*Pythium*
413	Jylland	49	*A. euteiches*	7	*Pythium*	5	*Rhizoctonia + Pythium*
503	Uppland	3	No pathogen found	0	No pathogen found	4	No pathogen found
504	Uppland	61	*A. euteiches + Phytophthora*	24	*Rhizoctonia + Pythium*	38	*Rhizoctonia*
506	Uppland	5	No pathogen found	14	No pathogen found	5	No pathogen found
600	Öland	0	No pathogen found	16	No pathogen found	21	No pathogen found
2922	Halland	98	*A. euteiches*	27	*Pythium*	24	Nematodes
2979	Skåne	5	No pathogen found	57	*A. cladogamus*	74	*A. cladogamus*
3025	Halland	41	*A. euteiches*	31	*Pythium*	47	*Pythium*
3049	Halland	60	*A. euteiches*	28	*A. cladogamus*	75	*A. cladogamus*
3102	Skåne	3	No pathogen found	66	*A. cochlioides*	41	*A. cochlioides*
3121	Skåne	11	*A. euteiches*	16	*Rhizoctonia + Pythium*	34	*A. cladogamus*
3131	Skåne	42	*A. euteiches*	67	*A. cochlioides*	88	*A. cochlioides*
3132	Skåne	78	*A. euteiches*	95	*A. cochlioides*	16	*A. cochlioides*
3231	Skåne	67	*A. euteiches*	33	No pathogen found	52	*Rhizoctonia + Pythium + Nematodes*
LT 2	Litauen	19	*Rhizoctonia*	74	*A. cochlioides + Rhizoctonia*	28	*A. cochlioides*
LT 3	Litauen	3	No pathogen found	18	*A. cochlioides*	53	*A. cochlioides*
LT 6	Litauen	4	*Rhizoctonia*	48	*A. cochlioides*	31	*A. cochlioides*

In the second, more stringent set, only soil samples where *Aphanomyces* spp. was the only pathogen present, and samples without any pathogen, were included. Excluded samples hence included soils containing other pathogens such as *R. solani*, *Pythium* spp., or nematodes, even if it also contained *Aphanomyces* spp. A total of 17 out of 58 samples fulfilled these criteria (Table 4).

Table 4. Disease severity index (DSI) data on samples included after the second filtering

ID	Region	DSI pea	Identified pathogens in pea	DSI sugar beet	Identified pathogens in sugar beet	DSI spinach	Identified pathogens in spinach
101	Gotland	1	No pathogen found	6	No pathogen found	4	No pathogen found
102	Gotland	0	No pathogen found	17	No pathogen found	11	No pathogen found
103	Gotland	1	No pathogen found	1	No pathogen found	3	No pathogen found
106	Gotland	0	No pathogen found	3	No pathogen found	5	No pathogen found
200	Värmland	14	*A. euteiches*	8	*A. cladogamus*	46	*A. cladogamus*
203	Västergötland	16	*A. euteiches*	12	No pathogen found	8	No pathogen found
302	Östergötland	3	No pathogen found	3	No pathogen found	5	No pathogen found
303	Östergötland	14	No pathogen found	10	No pathogen found	4	*A. cladogamus*
503	Uppland	3	No pathogen found	0	No pathogen found	4	No pathogen found
506	Uppland	5	No pathogen found	14	No pathogen found	5	No pathogen found
600	Öland	0	No pathogen found	16	No pathogen found	21	No pathogen found
2979	Skåne	5	No pathogen found	57	*A. cladogamus*	74	*A. cladogamus*
3049	Halland	60	*A. euteiches*	28	*A. cladogamus*	75	*A. cladogamus*
3102	Skåne	3	No pathogen found	66	*A. cochlioides*	41	*A. cochlioides*
3131	Skåne	42	*A. euteiches*	67	*A. cochlioides*	88	*A. cochlioides*
3132	Skåne	78	*A. euteiches*	95	*A. cochlioides*	16	*A. cochlioides*
LT 3	Litauen	3	No pathogen found	18	*A. cochlioides*	53	*A. cochlioides*

In the third and most stringent filtering, only soil samples where *Aphanomyces* spp. were present in all three crops were kept. Only 4 samples fulfilled this criterion (Table 5). These samples all contained *A. euteiches* and either *A. cladogamus* or *A. cochlioides*.

Table 5. Soil samples resulting in aphanomyces *root disease on pea, spinach and sugar beet*

ID	Region	DSI pea	Identified pathogens in pea	DSI sugar beet	Identified pathogens in sugar beet	DSI spinach	Identified pathogens in spinach
200	Värmland	14	*A. euteiches*	8	*A. cladogamus*	46	*A. cladogamus*
3049	Halland	60	*A. euteiches*	28	*A. cladogamus*	75	*A. cladogamus*
3131	Skåne	42	*A. euteiches*	67	*A. cochlioides*	88	*A. cochlioides*
3132	Skåne	78	*A. euteiches*	95	*A. cochlioides*	16	*A. cochlioides*

3.3 Factors affecting disease severity

Data set number two containing samples with only *Aphanomyces* spp. and samples without any pathogens was used to explore the relationship between DSI and regions, crop, and pathogen. The ANOVA analysis revealed significant ($P < 0.001$) differences in DSI depending on region and pathogen (Table 6). There was also a significant ($P < 0.001$) interaction between region and crop. However, there was no difference in DSI between crop species.

Table 6. ANOVA analysis of disease severity index

Factor	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Region	12	67226	5602.2	27.3318	<2.2e-16
Crop	2	680	339.9	1.6582	0.19400
Pathogen	3	33302	11100.8	54.1586	<2.2e-16
Region:Crop	21	14682	699.2	3.4110	6.326e-06
Region:Pathogen	6	2030	338.4	1.6509	0.13713
Crop:Pathogen	1	577	576.6	2.8129	0.09562

A post-hoc test further showed that there were high and significant ($P \leq 0.05$) levels of disease, measured as DSI different from zero, in pea roots infected by *A. euteiches* in Skåne, Halland, Dalsland, Östergötland, and Jylland (Fig. 11). In spinach, significant ($P \leq 0.05$) levels of disease were caused by *A. cladogamus* in Skåne, Halland, Dalsland, and Värmland. Disease on sugar beet was geographically confined to Skåne (caused by both *A. cladogamus* and *A. cochlioides*), Halland (caused by *A. cladogamus*), and Lithuania (caused by *A. cochlioides*) (Fig. 11). Soil samples without presence of any pathogen resulted in no disease in any region or crop, with the exception of samples from Skåne on sugar beet.
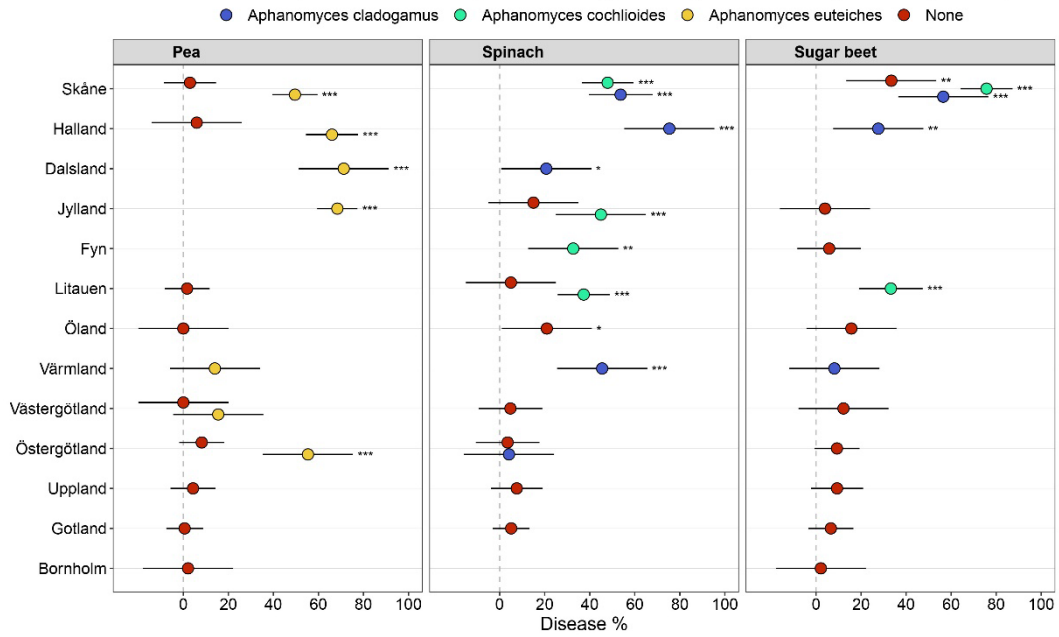
Figure 7. Disease severity index in pea, spinach, and sugar beet, caused by Aphanomyces *spp., in thirteen regions. Points represent the model mean estimate, error bars represent 95% confidence interval. Estimates that are significantly different from 0 are represented by * at p < 0.05, ** at p > 0.01, and *** at p < 0.001.*

In order to further explore the DSI effect of different regions caused by different pathogens, an ANOVA analysis was performed on data summarized over crops. The results showed significant ($P < 0.001$) differences in DSI values between regions, pathogens, and for the interaction between region and pathogen (Table 7).

Table 7. ANOVA analysis of disease severity index summarized by crops

Factor	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Region	12	67226	5602.2	24.0367	<2.2e-16
Pathogen	3	32703	10901.0	46.7718	<2.2e-16
Region:Pathogen	11	9981	907.4	3.8933	5.044e-05

A post-hoc test showed that *A. euteiches* caused higher ($P \leq 0.05$) levels of disease in Halland and Jylland, compared with Värmland (Figure 12).
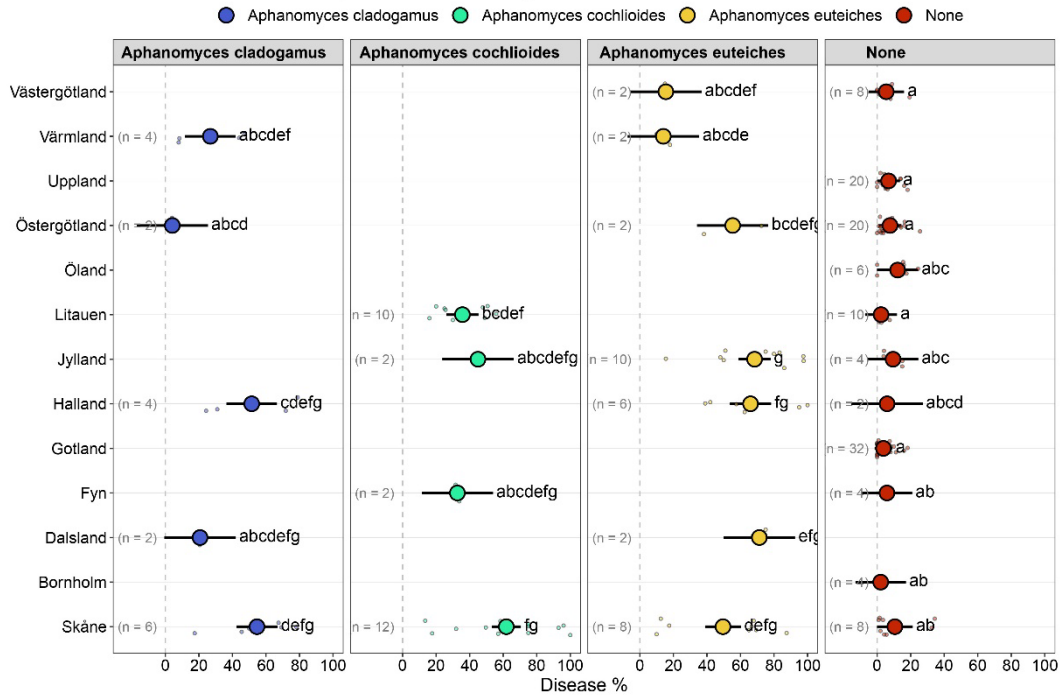
Figure 8. Disease severity index summarized over three crops in thirteen regions caused by Aphanomyces *spp. Points represent the model mean estimate, error bars represent 95% confidence interval. Treatments sharing letters are not significantly different from each other.*

Finally, the effect of pathogens on DSI was tested with an ANOVA analysis with data summarized over regions. Pathogen identity and the interaction between pathogen and crop was shown to significantly ($P \leq 0.037$) affect DSI values, but not crop identity (Table 8). More specifically, growing pea plants in soil samples containing *A. euteiches* resulted in higher ($P \leq 0.05$) DSI compared with plants grown in soil samples without any pathogen (Fig. 13). Similarly, spinach plants grown in soil containing either *A. cladogamus* or *A. cochlioides* displayed higher DSI compared with plants grown in soil without any pathogen. Finally, sugar beet plants grown in soil containing *A. cochlioides* had higher ($P \leq 0.05$) DSI than plants grown in soil without any pathogen, while there was no difference in DSI between plants grown in soil containing *A. cochoilides* or *A. cladogamus*, respectively (Fig. 13).

Table 8. ANOVA analysis of disease severity index summarized by regions

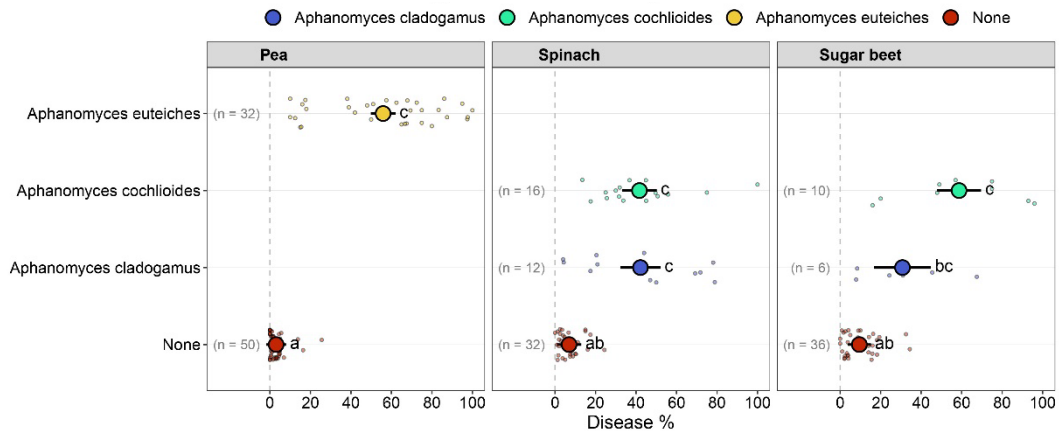| Factor	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Crop	2	174	86.8	0.2862	0.75148
Pathogen	3	90207	30069.1	99.1308	<2.2e-16
Crop:Pathogen	2	2033	1016.4	3.3507	0.03719

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Figure 9. Disease severity index summarized over thirteen regions in pea, spinach and sugar beet caused by Aphanomyces *spp. Points represent the model mean estimate, error bars represent 95% confidence interval. Treatments sharing letters are not significantly different from each other.*

3.4 Soil analyses and correlation with disease

Results from Eurofins regarding soil analyses of pH, P-Al, K-Al, Mg-Al, and Ca-Al, are shown in Table 9. Soil pH ranged from 5.1 in soil from Uppland to 8.3 in soil from Gotland. All samples with pH above 7.6 came from Gotland and Öland. Ca-AL values ranged from 82 to 3600 mg/100g dry soil, and samples with Ca-AL above 600 all came from Gotland and Öland as well. Mg-AL values ranged from 6.5 to 130, P-AL from 2.7 to 130, and K-AL from 3.1 to 210. Samples from Lithuania were not analysed.

Table 9. Results from the soil analyses of pH and macronutrients

ID	Region	pH	P-AL [1]	K-AL [1]	Mg-AL [1]	Ca-AL [1]
100	Gotland	8.1	15	19	12	740
101	Gotland	8	14	10	7	430
102	Gotland	8.3	7.8	14	24	1200
103	Gotland	8	10	14	13	670
104	Gotland	7.3	14	3.1	6.5	270
105	Gotland	7.3	22	21	11	290
106	Gotland	7.6	9.9	7.9	87	3600
107	Gotland	7.8	13	12	15	820
108	Gotland	7.9	16	14	13	770
109	Gotland	7.7	12	14.5	12	1000
110	Gotland	8	13.5	13.8	12	980
200	Värmland	6.6	6.8	7.1	7.4	87
201	Västergötland	6.4	6.3	11	12	140
202	Dalsland	6.5	7.5	7.4	9.9	130
203	Västergötland	6.2	4.5	9.7	18	150

300	Östergötland	7	12	33	30	320
301	Östergötland	6.2	5.1	29	48	380
302	Östergötland	7	6.5	16	34	250
303	Östergötland	6.9	6.2	15	35	180
304	Östergötland	6.9	7	20	58	300
305	Östergötland	6.6	4.5	21	52	200
404	Jylland	6.6	15	17	12	150
405	Bornholm	7.4	17	15	8.2	230
406	Jylland	6.3	12	6.3	11	210
407	Jylland	6.4	8.8	13	8.7	100
408	Fyn	7.4	10	13	16	280
409	Jylland	5.6	9.2	5.6	6.7	89
410	Fyn	6.8	25	16	15	250
411	Jylland	7.3	7.2	16	8.6	210
412	Jylland	7.1	5.6	16	9	180
413	Jylland	6.1	8.2	10	7	82
500	Uppland	5.5	7.1	12	8.1	86
501	Uppland	5.2	4.7	34	38	160
502	Uppland	7.3	9.4	21	49	310
503	Uppland	6.8	130	210	130	530
504	Uppland	6	2.7	27	70	290
505	Uppland	5.1	4.4	37	12	170
506	Uppland	8.1	14	19	14	450
507	Uppland	6.3	29	30	18	260
600	Öland	8	7.8	16	31	2100
2916	Halland	6.4	10	11	7.6	160
2922	Halland	6.4	8.3	8.9	8.6	140
2979	Skåne	6.9	15	15	14	150
3010	Skåne	5.9	20	7.1	7.8	120
3025	Halland	6.4	17	8.6	14	150
3031	Skåne	6.4	4.4	16	6.5	160
3049	Halland	6.2	14	8.7	7.2	110
3102	Skåne	6.8	24	20	7.8	170
3121	Skåne	6.6	3.6	18	20	250
3131	Skåne	6.8	33	9.2	6.7	100
3132	Skåne	7.2	18	8.2	8.1	130
3231	Skåne	6.5	4.2	13	19	240

[1] mg/100 g dry soil.

Average pH and macronutrient values for each region were calculated and summarized in Table 10. Soils from Gotland and Öland displayed high (7.8 – 8.0) pH values. These regions also displayed higher Ca-AL levels compared with other regions. Average values of P-AL and K-AL was highest in Uppland, while high Mg-AL values were present in both Uppland and Östergötland.

Table 10. Mean values of macro nutrient levels and pH for each region

Region	pH	P-AL [1]	K-AL [1]	Mg-AL [1]	Ca-AL [1]
Uppland	6.3	25.2	48.8	42.4	282
Western Sweden [2]	6.4	6.3	8.8	11.8	127
Östergötland	6.8	6.9	22.3	42.8	272
Halland	6.4	12.3	9.3	9.4	140
Skåne	6.6	15.3	13.3	11.2	165
Gotland	7.8	13.4	13.0	19.3	979
Öland	8.0	7.8	16.0	31.0	2100
Denmark [3]	6.7	11.8	12.8	10.2	178

[1] mg/100g dry soil

[2] Results from Värmland, Västergötland, and Dalsland have been combined into "Western Sweden"

[3] Results from Jylland, Fyn, and Bornholm have been combined into "Denmark"

Values for pH and log10 transformed values for Ca-AL, K-AL, Mg-AL and P-AL was used in an ANOVA analysis to test for correlations with DSI, while controlling for region, crop and pathogen effects. The results showed no correlation between DSI and any soil property (Table 11). As expected, region and pathogen had a significant ($P < 0.001$) effect on DSI.

Table 11. ANOVA analysis of disease severity index and soil properties

Factor	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Region	11	31384.4	2853.1	20.6463	2.546e-16
Crop	2	8.6	4.3	0.0312	0.9693
Pathogen	3	14435.9	4812.0	34.8214	4.403e-13
Log soil Ca-AL	1	0.4	0.4	0.0030	0.9564
Log soil K-AL	1	70.7	70.7	0.5117	0.4772
Log soil Mg-AL	1	13.8	13.8	0.1000	0.7530
Log soil P-AL	1	82.3	82.3	0.5953	0.4435
Soil pH	1	31.0	31.0	0.2246	0.6373

Although no correlation between DSI and soil properties was detected in the ANOVA analysis, we acknowledge the fact that potential correlations may be nested within the significant effect by region as soil properties varied between regions (Table 9). Therefore, combined DSI data from regions was plotted against the combined data for soil properties and tested for correlations. In this analysis, high Ca-AL levels correlated significantly ($P \leq 0.043$) with low DSI levels in both pea, spinach, and sugar beet (Fig. 14). When Ca-Al levels were above 250 mg/100 mg soil, no species of *Aphanomyces* at all was detected. High K-AL levels correlated ($P = 0.020$) with low DSI in pea, while high Mg-AL levels correlated ($P \leq 0.05$) with low DSI in spinach and sugar beet. High levels of P-AL correlated ($P = 0.048$) with high DSI in sugar beet, while higher pH values correlated ($P \leq 0.041$) with lower DSI values in pea and spinach (Fig. 14).
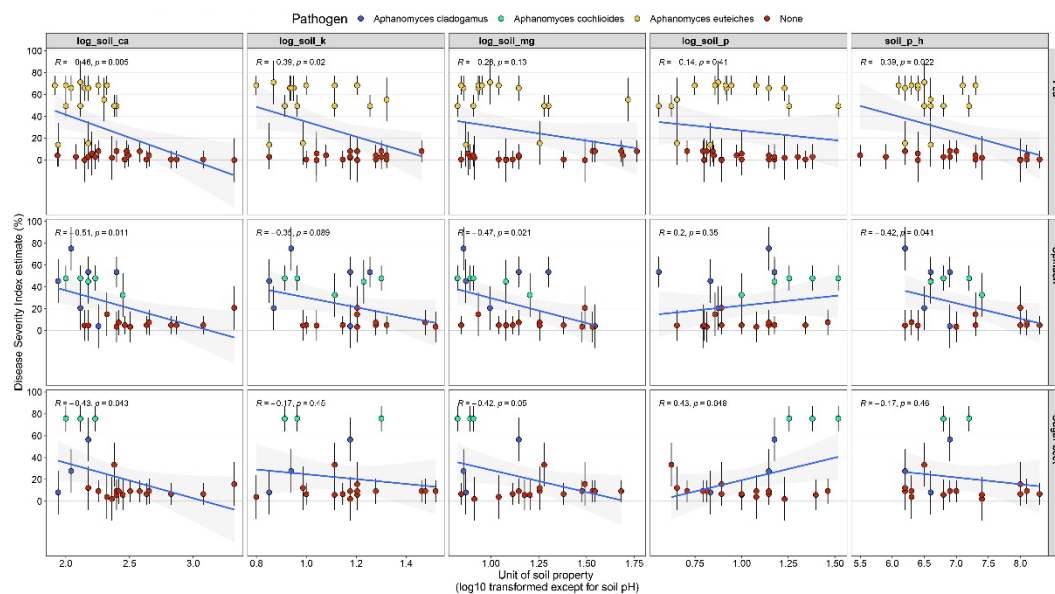

Figure 10. Correlation between disease severity index and soil properties based on a dataset where Aphanomyces *sp. was present but no other pathogen. Error bars around points represent the 95% confidence interval for the model estimates of DSI.*

3.5   Analysis of data from commercial pea growing

Data on Ca-AL and DSI in peas in 1350 soil samples from commercial pea cultivation fields provided by Foodhills AB shows a high variation in both Ca-AL (28 to 3900 mg/100 g dry soil) and DSI (0 to 92) (Fig. 15). Although no DSI values above 20 (with *A. euteiches*) was found in any soil sample with a Ca-AL value above 210 mg/100 g soil, there was no significant correlation between Ca-AL and DSI. Furthermore, *Aphanomyces* sp. was confirmed in 115 samples, all in samples with Ca-AL value below 640 mg/100 g dry soil. However, in the soil sample with

the highest Ca-AL value (640 mg/100 g dry soil) also *Pythium* spp. and *Rhizoctonia solani* occurred.
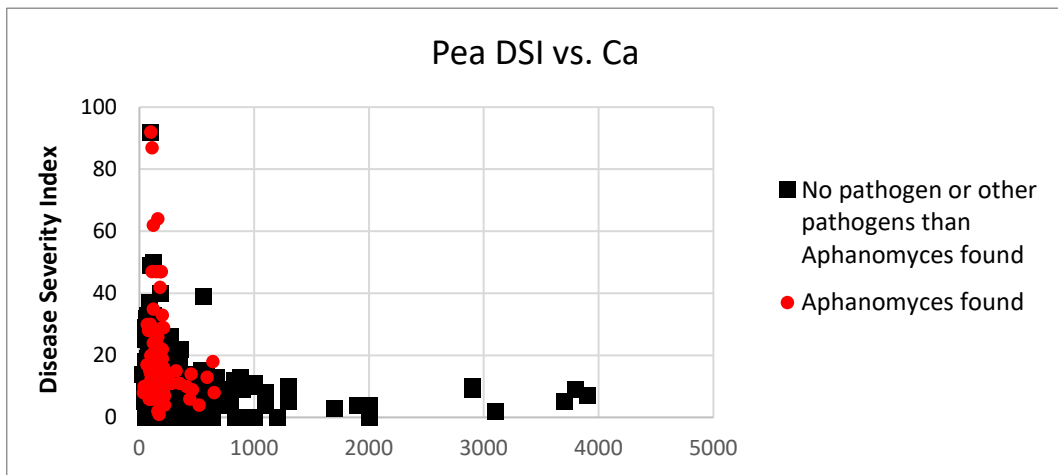

Figure 11. Disease severity index (DSI) in pea in relation to calcium content in soil samples. Data points in red indicate samples with confirmed presence of Aphanomyces euteiches. *Data from pea fields from 2017-2022, provided by Foodhills AB.*

4. Discussion

4.1 Occurrence of different *Aphanomyces* spp.

In this study, *A. euteiches* was the most frequently occurring and widespread pathogen. The presence of *A. euteiches* in all regions in Sweden, with the exception for Gotland and Öland, correlates well with the fact that pea cultivation is widespread in all the tested Swedish regions (Jordbruksverket, 2023). This may explain the wide distribution of *A. euteiches* in Sweden.

The pathogen with the most restricted distribution was *A. cochlioides*. In Sweden, sugar beets are mainly cultivated in Skåne. In the year of 2022 a total of 97% of the total sugar beet yield came from Skåne, while the other 3% came from Halland and Blekinge (Jordbruksverket, 2023). Since the cultivation of sugar beets is so concentrated to one specific region, it was hypothesized that *A. cochlioides* (the sugar beet root rot causative agent) would follow this pattern and only be present in soils from this region. Our data supports this hypothesis as *A. cochlioides* was only found in Skåne in Sweden. *Aphanomyces cochlioides* was also found in Denmark in the regions Fyn and Jylland, and in samples from Lithuania. In Denmark, sugar beet and fodder beet cultivation are common and serves as an important cash crop (Danmarks statistik, 2023; Heick et al., 2020), and sugar beets are also grown in Lithuania (Romaneckas et al., 2020). This may explain the findings of *A. cochlioides* in these areas as well.

Geographically, *A. cladogamus* is more widespread in Sweden compared to *A. cochlioides*, but not as widespread as *A. euteiches*. It was earlier stated that *A. cladogamus* had the widest host range amongst the three pathogens, with several other crops and weeds as possible hosts (Larsson, 1994). However, since the most well-known host in Sweden is spinach, which is cultivated on a very small scale, the current distribution pattern may depend on other hosts. Finally, it seems that this species of *Aphanomyces* has not been investigated as much as the other two mentioned species, which makes the interpretation of the distribution pattern more difficult.

The occurrence data obtained shows several cases where more than one species of *Aphanomyces* is present in the same soil sample. This can be explained by the fact that the three species share a similar ecology and thrive under the same conditions (Papavizas & Ayers, 1974). Furthermore, the biology of the pathogens may contribute to their ability to co-exist. Oospores can lie dormant in soils for long time periods, followed by a rapid production of zoospores and root infection on separate hosts, which minimize physical interaction between pathogens.

4.2 Disease caused by *Aphanomyces* spp.

In the current study, *A. euteiches* is exclusively infecting and causing disease on pea plants, while *A. cochlioides* and *A. cladogamus* are infecting and causing disease in both sugar beet and spinach. This result indicates an overlapping host range between *A. cochlioides* and *A. cladogamus*. These findings align with results obtained by Drechsler (1929) and Larsson (1994) who found that both species are pathogenic on sugar beet and spinach. Furthermore, despite the fact that several pathogens, *Aphanomyces* spp., *R. solani*, *Pythium* spp., *P. pisi* and nematodes, are often present in the same soil, co-infection by an *Aphanomyces* sp. and other pathogens are rare. In contrast, more cases of co-infection by *R. solani* and *Pythium* spp. are detected. One hypothesis to explain this phenomenon can be the hemi-biotrophic nature of *Aphanomyces* spp., which may prevent infection of a host plant already infected by another pathogen due to the active immune response from the host. A host weakened by infection by *Aphanomyces* spp. may be more susceptible to secondary infections, but the short time of the pot bioassay experiment in the current study may further explain the lack of co-infections.

Root disease on pea caused by *A. euteiches* was detected in soil from a lower number of regions compared with the actual geographic distribution of the pathogen. For example, *A. euteiches* was present in Västergötland, Värmland, and Uppland, despite the lack of significant levels of disease. This was also true for *A. cladogamus* in Östergötland and *A. cochlioides* in Jylland and Fyn. This suggests a lower level of pathogen inoculum in soil from these regions. This may be due to the particular crop rotation history and local conditions in the sampled fields, but due to the low number of samples used in the current study it is not possible to determine if this can be extrapolated to the level of regions.

4.3 Relationship between soil macronutrients and disease

The different geological conditions between regions in Sweden results in different levels of macronutrients in the soils. For example, the bedrock in Öland and Gotland is dominated by limestone, resulting in the high calcium content in the soil. Previous data have demonstrated a disease-suppressive effect of high calcium levels for both *A. euteiches* and *A. cochlioides* (Heyman et al., 2007, Olsson et al., 2011). However, this effect was not detected in our data when correcting for region and pathogen. Still, as the Ca-AL values differ considerably between regions, we acknowledge that a potential effect from calcium may be nested within regions in the statistical analysis. This may indeed be the case as our correlation analysis, where regions were combined, showed a disease-suppressive effect by high Ca-AL levels for both pea (caused by *A. euteiches*), spinach and sugar beet (caused by *A. cladogamus* and *A. cochlioides*). However, previously mentioned correlation analysis regarding sugar beets contains too few *Aphanomyces* samples to draw any conclusions. In the investigated samples, there was no *Aphanomyces* spp. at all detected in either crop when Ca-AL levels were above 250 mg/100 mg soil. This indicates the importance of Ca-AL content in the soil.

One issue with these results is how to view samples without presence of any *Aphanomyces* species. If a host crop never or seldom has been grown on a field, there is a low probability for the pathogen being present. In such cases, the calcium content is not of importance since all DSI values will be low. On the other hand, absence of pathogens may also be due to high calcium levels, as indicated by previous work (Heyman et al., 2007, Olsson et al., 2011). The fact that no correlation between Ca-AL levels and DSI can be detected when excluding samples without *Aphanomyces* spp. does not support a connection between high calcium levels and disease-suppressiveness, although the number of available samples is low. The lack of correlation between Ca-AL and DSI in samples from commercial fields in Sweden provided by Foodhills AB may be related with the high number of samples without presence of any *Aphanomyces* pathogen. However, if the Ca-AL value was 210 mg/100 g soil or higher, there was no soil sample with detected *A. euteiches* and DSI above 20.

The correlation between high pH and low DSI levels for pea and spinach in one analysis may suggest a high pH optimum for *Aphanomyces* spp. However, pH value and calcium content are associated with each other, since liming of fields are often done with products that increase both factors. Heyman et al. (2007) stated that calcium is the influencing factor but <u>not</u> the pH. In that study, they performed experiments that included adding different types of liming products including $CaCO_3$ and $CaSO_4$ (gypsum) to soil samples. $CaSO_4$ is a material that increases the

calcium content without altering the pH level in the soil. $CaCO_3$ is a commonly used product when liming fields, which increases levels of both pH and calcium. During Heyman's study, it was concluded that free calcium ions were the major variable controlling the degree of the soil suppressiveness against *A. euteiches*. Neither magnesium nor pH levels, which to some extent follows the calcium content, had a significant impact on disease levels (Heyman et al., 2007).

As mentioned previously, it is notable that the only regions in Sweden where *Aphanomyces* spp. is not found were on the Swedish islands Gotland and Öland. When looking at the results from the soil analysis, these regions have one common factor that stands out – very high Ca-AL values. Soil samples from Gotland and Öland had on average more than five times higher Ca-AL values compared with other samples from Sweden. The high calcium content may thus be an explanatory factor for the absence of *Aphanomyces* spp., following the previous statements regarding the correlation between calcium and DSI content. Another possible reason might be lack of hosts e.g., no cultivation of susceptible hosts. However, this explanation appears less likely as peas are currently grown on both Gotland and Öland (Jordbruksverket, 2023), which means that there are hosts for *A. euteiches*. Disease suppression due to high calcium levels thus seems to be a more likely explanation.

5. Conclusions

The occurrence of *Aphanomyces* spp. in the soil depends on both host crop presence, i.e. if or how often susceptible host plants are grown, as well as the composition of the soil.

A. euteiches is a very common root rot pathogen and occurs in most tested regions. It occurs in 31% of the tested soil samples. *A. cladogamus* is sporadically found and occurs in 12% of the investigated soil samples. *A. cochlioides* is found in 14% of the investigated soil samples and is restricted to areas where sugar beets are grown.

Different *Aphanomyces* species can occur in the same soil sample, indicating no or limited competition between the different pathogens. In fact, the positive relationship between occurrences of the different species may be explained by the fact that they thrive under the same soil conditions. In addition, *A. cochlioides* and *A. cladogamus* are pathogenic on both sugar beet and spinach, while *A. euteiches* is restricted to being pathogenic on pea in the current investigation.

Initially there was no clear effect of calcium as hypothesized. However, when correcting for regional factors, there is a clear effect where calcium was found to be an important factor.

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Popular science summary

Aphanomyces spp. is a genus of oomycetes, which includes several aggressive pathogenic species. It is a problematic pathogen in agriculture, where it is causing diseases in several different crop plants. *Aphanomyces euteiches* and *Aphanomyces cochlioides* are two of the most well-known and important plant pathogenic *Aphanomyces* species. *A. euteiches* cause root rot in pea*,* and *A. cochlioides* cause damping-off and root rot in sugar beet. Another known plant pathogenic *Aphanomyces* species is *Aphanomyces cladogamus* which cause root rot in spinach. These crops, amongst others, are highly affected by *Aphanomyces* spp. which are responsible for causing enormous yield losses due to root rot. The yield losses can in some cases be total e.g. in pea cultivation. Occurrence of *Aphanomyces* spp. in the soil can be affected by some soil environmental factors at varying degree. It has been reported that calcium is a major variable controlling the degree of soil suppressiveness against *Aphanomyces* spp.

In this study, 58 soil samples from various regions in Sweden, Denmark, and additionally Lithuania was investigated. The samples were sown with peas, sugar beets, and spinach, and the presence of *Aphanomyces* spp. was evaluated. Also, the macronutrient levels of the soil samples were analysed, so that potential connections between the pathogen presence and the soil factors could be explored. Additionally, a large data set from biotests conducted in commercial pea growing in Sweden was analysed. This dataset contained 1350 soil samples and was conducted in 2017-2023. It was found that *A. euteiches* is a very common root rot pathogen and occurred in most of the tested regions. *A. cladogamus* was sporadically found, and *A. cochlioides* was only found in areas where sugar beets are grown. Sometimes different *Aphanomyces* spp. occurred in the same soil sample, and there seem to be no competition between the different species, explained by thriving in the same soil conditions. There were also some indications that the calcium content in the soil may be an important factor for *Aphanomyces* spp. presence.

Currently, there are only a few effective pesticides against plant pathogenic *Aphanomyces* spp., highlighting the need to understand the relationship between calcium and the pathogens for better disease management. Also, understanding the distribution patterns of *Aphanomyces* spp. can provide valuable insights for managing this devastating plant disease.

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