Brandsberga gård

Agro Plantarum

Slutrapport

Nya rotsjukdomar i korn och vete-
okända hot mot svensk spannmålsodling

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Del 1: Utförlig sammanfattning

Under regniga förhållanden är det vanligt att fält med stråsäd gulnar strax efter sådd och i synnerhet i blöta, låga partier. Syftet med detta projekt har varit att fokusera på de algsvampar som gynnas av vatten och som infekterar rotsystemet på stråsäd och i förlängningen minska skördeförlusterna från dessa. Målen var att identifiera de arter som orsakar skadorna och ta fram data på spridningen av dem, vidare att undersöka vilka grödor i växtföljden som de angriper och även se om det finns sortskillnader i korn i tolerans samt att undersöka om betningsmedel har någon positiv effekt. En ny art av algsvamp har beskrivits vetenskapligt i detta projekt och den har fått namnet *Aphanomyces macrosporus*. Den har visat sig angripa vår- och höstkorn, sockerbeta och spenat men inte ärt eller åkerböna. Den har hittats i fält i stora delar av Sverige och även i Danmark och i fält med låga koncentrationer av kalcium och med låga pH. Symptomen visar sig efter stora regnmängder och i fält med dålig dränering. Fältförsök med fröbetning av vårkorn på infekterad mark visar på stora skördeförluster vid regniga förhållanden. Resultaten från dessa försök visar på vissa positiva effekter på infektionen av rötterna, men inte på någon positiv effekt på skörd av vare sig de kemiska bekämpningsmedel vi har testat (hymexazol, metalaxyl), eller av biologiska betningsmedel. Däremot indikerar försök i växthus och i fält på att det finns skillnader i tolerans mellan olika sorter av vårkorn vilket kan vara av stort värde i växtföljden. I kontakter med odlare och rådgivare har det visat sig finnas en stor efterfrågan på ett diagnosverktyg för riskbedömning av fält för infektion av *A. macrosporus* i vårkorn.

Projekt har fått finansiering genom:
Stiftelsen Lantbruksforskning

Del 2: Rapporten

Background

In rainy conditions it is common that fields of cereals turn yellow, especially in wet lower parts. In this project we have focused on root rot caused by oomycetous pathogens, since we believe that they play an important role and are also less investigated. Consideration must be given to the expected climate change and in southern Sweden, spring is expected to become rainier, and this can affect soilborne diseases. Among the soilborne pathogens the stem and root-rotting Oomycetes is a large group of related fungus-like plant pathogens, including the genera *Aphanomyces*, *Pythium,* and *Phytophthora,* which cause significant yield losses and yield declines. They cause diseases that are often diffuse and very difficult to identify and control. Oomycetes can survive in plant debris and soil for decades. Symptoms of these pathogens are e.g. stunted and yellowish plants, which can be mistaken for nutrient deficiency and water logging. Observations during several years from one of the group members indicated that there was an unknown species of *Aphanomyces* infecting the root system of cereals. The pathogen was isolated, investigated and described for the first time in the doctoral thesis of M. Wikström (Larsson, 1992). This *Aphanomyces* sp. was isolated from roots of barley and spinach and caused a root rot in these crops (Larsson, 1994). Barley plants from which this *Aphanomyces* sp. was isolated, were stunted, yellowish, and grew in wet parts of several fields in the south of Sweden. We suspected that this pathogen affected the yield of mainly barley but maybe also other crops to a much greater extent than is known to date. The aim of this project was to increase productivity in Swedish cereal cultivation by paying attention and reducing the yield losses caused by early root diseases. Our specific objectives were: 1) to collect isolates of oomycete pathogens from cereals and to identify them; 2) to demonstrate differences in pathogenicity in important crops and cultivars of barley; 3) to assess the impact of seed treatments, biological and chemical, for avoiding losses.

Materials and methods

Identification of a new species: Aphanomyces macrosporus

A new species of *Aphanomyces* was described within the project. Morphological characteristics were evaluated and described on 10 different isolates using microscopic examinations. Molecular techniques for identification were used by sequencing the ITS-region. The PCR amplification of the region of the nuclear ribosomal DNA that included ITS1, 5.8s, and ITS 2 was carried out with ITS6 and LSU-0344R primers (Levenfors and Fatehi, 2004). The PCR products were sequenced at Macrogen Europe. Sequences were added into a sequence data set available from our previous *Aphanomyces* study (Levenfors and Fatehi, 2004) and were deposited at NCBI GenBank. ITS sequences of several other *Aphanomyces* species available from GenBank were included in this sequence alignment. Maximum parsimony (MP) analysis of the ITS data was performed and a phylogenetic tree was computed.

Field survey and collection of isolates

Fields with symptoms of yellowing after emergence were sampled and soils were tested for the presence of oomycete pathogens. Discoloured roots from yellow plants from fields were examined under the microscope for presence of oospores. Small root pieces were plated on *Aphanomyces* selective medium (SMA), as described by Larsson and Olofsson (1994). Each soil sample was divided between two 1 L pots, and 10 barley seeds were sown in each pot. The pots were placed in a greenhouse under a 16/8 h day/night photoperiod at 24/19°C. After four weeks, the barley plants and roots were washed and examined. The roots were assessed using a grading method described by Persson et al. (1997), with a disease severity index (DSI) scale containing seven different classes (0, 5, 10, 25, 50, 75, 100). A DSI of 0 means healthy roots and 100 means dead plants, and intermediate classes were based on percentage of infection of the root and hypocotyl. An average DSI value was calculated for each pot. Soils were also analysed for pH, and nutrients (ammonium lactate extractable).

Trials with limestone meal and structure lime applied in fields with alkaline soils with a crop rotation of sugar beet and cereals in Skåne, were sampled and tested in greenhouse bioassay using wheat and barley (SLF O-17-20-977: "Long-term effects of liming"). The field trials had been investigated earlier and had a presence of *Pythium arrhenomanes* (Olsson Nyström et al., 2023). The bioassay was performed as described above.

Pathogenicity tests on hosts

Two different methods for inoculation were used. The strains were cultivated on agar for 10 days; then placed as an inoculum layer in pots filled with vermiculite. Seeds were placed one centimetre above the layer. The pots were placed in a greenhouse with similar conditions to those described above. Four weeks after sowing, the roots were washed in water and the symptoms were checked. The other method was a dry inoculum which was produced by growing the isolates in oatmeal broth for four weeks, homogenizing the mycelium in a mixer and counting the concentration of oospores in a haemocytometer, adding to vermiculite and drying for one week. The inoculum could be added in a chosen concentration as oospores/ml soil. The soil was mixture of soil/sand 20/80 v/v. The sand was washed before use, and the soil was a commercial planting soil (Hasselfors garden). The inoculum and soil/sand were mixed for ten minutes in a plastic bag or in a concrete mixer. Pathogenicity tests were performed with three isolates of *A. macrosporus*, B8, B9, and B11, in several experiments. The following crops and cultivars were tested: spring barley cv. Planet, spinach cv. Kiowa, sugar beet cv. Cartoon, peas cv. Linnea, and faba beans cv. Alexia, and radish cv. Cherry Belle. The seeds were surface sterilised in a sodium hypochlorite solution and water 1:2 for 2 min and rinsed in water. All experiments were performed by sowing ten seeds per pot and four pots were used in a randomized block design, in a green house chamber and infection assessed after 4 weeks. Plants and roots were washed in water and a DSI of the root system was assessed according to above.

Tests of tolerance in varieties in greenhouse and field

A test was performed in greenhouse to investigate if fludioxonil as a seed treatment (Celest) had any effect to *Aphanomyces*. Two varieties of spring barley (cultivars Planet

and Prospect) from official field variety trials treated with Celest and untreated seeds were sown in the compost/sand mixture. Isolate B11, dry inoculum with 300 oospores/ml soil was used in this test. Another test of 14 untreated varieties of spring barley was performed in greenhouse using a compost/sand mixture and a dry inoculum strain B11, 600 oospores/ml soil, with four pots in a randomised block design and four uninoculated pots in similar block design. The experiment was performed twice and lasted 8 weeks from sowing until assessment. Further, a field trial was performed in 2024 with 9 of the 14 varieties from the greenhouse tests in a randomized block design with four replicates, on a soil with a known infection of *A. macrosporus*. Each plot measuring 18 m^2 and of which 15 m^2 were harvested, 20 plants were sampled and taken to the laboratory washed in water and a disease severity index was assessed for all plants.
In early July 2024, an infected field was found close to Falkenberg, with an official variety trial of spring barley, where 20 plants from each plot were sampled from 19 varieties in 2 replicates to assess the infection. Roots were washed in water and assessed using the disease severity index grading scale described above.

Biological and chemical seed treatments
Seeds of spring barley cultivar Planet (2021-2022) and Prospect (2023) were treated with two chemical standards, Apron XL 350 ES (metalaxyl M) active against *Pythium* spp., and Tachigaren 70WP (hymexazol) active against *Aphanomyces* in sugar beet (Table 1). The biological control agents (BCA) tested for three years were Cedomon (*Pseudomonas chlororaphis*), and Polygandron STP (*Pythium oligandrum*). Mycostop (*Streptomyces griseoviridis*) was tested the first two years, and Rootfix (*Bacillus* spp.) was included the last year. Two chemical products, Vayantis (picarbutrazox) and Kick-off (phosphite and a range of micronutrients) were included in 2023.

Table 1. Seed treatments used in trials 2021-2023

Treatments	Year in trial	Active ingredient/organism	Target pathogen
Apron XL 350 ES	2021-2023	metalaxyl M	*Pythium*
Tachigaren	2021-2023	hymexazol	*Aphanomyces*
Cedomon	2021-2023	*Pseudomonas chlororaphis*	*Pythium/Aphanomyces*
Polygandron STP	2021-2023	*Pythium oligandrum*	*Pythium/Aphanomyces*
Mycostop	2021-2022	*Streptomyces griseoviridis*	*Pythium/Aphanomyces*
Rootfix	2023	*Bacillus* spp.	*Pythium/Aphanomyces*
Vayantis	2023	picarbutrazox	*Pythium/Aphanomyces*
Kick-off	2023	phosphite	*Pythium/Aphanomyces*

A pree-test was performed in 2022 in greenhouse with 3 doses of Tachigaren due to low emergence in field trials 2021: 1g, 3g and 6g/kg seed. In the test a soil was used with a natural infection of isolate B8. Spring barley cv Planet was treated with Tachigaren 1g, 3g, and 6g/kg seed and including treatments with Metalaxyl, Cedress and Polgandron.
The field trials with seed treatments of spring barley were performed on soils with a known infection of *Pythium* spp. (Skurup) and *A. macrosporus*: two trials in Varberg 2021 and 2022 and two trials in Åstorp 2022 and 2023. The trials were a randomized block design with four replicates, each plot measuring 18 m^2 and of which 15 m^2 were

harvested. Twenty plants were sampled once or twice, washed in water and assessed for DSI.

Results

Identification

The new species of *Aphanomyces* could be described by analyzing ITS nuclear rDNA sequences of 12 *Aphanomyces* isolates denoted B8-B20 originating from the roots of barley. The sequences of these isolates were identical but different from all other known *Aphanomyces* species available in GenBank. The barley isolates formed a distinct clade separated from all other *Aphanomyces* species and supported with a bootstrap value of 100%. Based on the molecular and morphological characteristics, the *Aphanomyces* sp. was considered to be a new species, and therefore the name *Aphanomyces macrosporus* sp. nov. was proposed (Wikström *et al*., 2023).

Field survey, description of soils and collection of isolates

Isolates were collected from spring barley plants in infected fields and from barley plants grown in soil samples for biotests. From these surveys, the 12 isolates B8-B20 were collected from Sweden and Denmark and were analyzed for genetics. It revealed that *A. macrosporus* is widespread in the agricultural areas (Table 2).

Table 2. Findings in survey

County	Number of surveyed fields	Number of fields with *Aphanomyces macrosporus*
Skåne	176	13
Halland	12	12
Småland	1	1
Västergötland	9	4
Bohuslän	4	3
Dalsland	12	6
Värmland	1	1
Östergötland	6	1
Öland	1	0
Gotland	11	3
Uppland	8	3
Jylland	7	4
Fyn	2	1
Bornholm	1	1
Lolland/Falster	16	0
Totalt	267	53 (20%)

The pH and Ca-AL were measured in the samples from from Skåne, Halland, Bohuslän, Dalsland, Västergötland, Gotland, Värmland, Östergötland, Uppland and Denmark. The average for samples with presence of *A. macrosporus* was pH 6,7 and Ca 196 mg/100 g soil (n=25) and for soils without presence the pH was 7,0 and Ca 593 mg/100g soil (n=27).

The fields in Skåne with presence of *A. macrosporus* had a crop rotation including sugar beets, oilseed rape, pea and cereals, most common spring barley, winter wheat and oat. In Halland and Västergötland, the crop rotations were more focused on spring barley, winter wheat, pea, field bean and grass ley. *A. macrosporus* was found in both conventional and organic farms. In total, around fifty isolates were retrieved from barley fields. In early symptoms around BBCH 21, the leaves of diseased barley plants

had turned yellow, the coleoptiles were brown, and the roots were discolored. Typical *Aphanomyces* oospores were found in infected root tissues.
Bioassays and assessments of soil and plants from thirteen trials with lime in Skåne on alkaline soils indicated that the species *Pythium arrhenomanes* was very prevalent and could be isolated from both field plant roots and from biotests. No *A. macrosporus* was found or isolated from these trials. The average pH and content of calcium for these trials in untreated plots was pH 7,3 (range pH 6,8-7,9) and Ca-AL 343 mg/100 g soil (range 216-464 mg/100 g) (Olsson Nyström et al., 2023). There were no significant effects of the different treatments of lime on DSI on field plants or bioassay in wheat or barley in these trials (Olsson Nyström et al., 2023).

Pathogenicity tests
In pathogenicity tests using the inoculum layer method, disease symptoms were developed in barley, spinach, and sugar beet (Table 3). No symptoms at all were found in peas or field beans (*Vicia faba*). In the case of spinach and sugar beet, the symptoms resembled those caused by *A. cladogamus* and *A. cochlioides* infection. Isolations of root segments on selective agar media and observations of growing colonies of *Aphanomyces* verified the pathogenicity and fulfilled Koch's postulates.

Table 3. Mean disease severity index (DSI) and presence of oospores in roots in some crops inoculated with isolate B8 of *Aphanomyces macrosporus*

Crop	DSI	Oospores in roots	Reisolated
Spring barley	14	Yes	Yes
Winter barley	15	Yes	Yes
Pea	0	No	No
Field bean	9*	No	No
Common bean	34	Yes	Yes
Radish	0	No	No
Sugar beet	17	Yes	Yes
Spinach	15	Yes	Yes

*Caused by other pathogens

In a test in greenhouse with isolate B8, B9 and B11 on spring barley, sugar beet and spinach, differences in aggressiveness between isolates could be seen (Table 4). The isolate B11 was the most aggressive isolate and gave significant higher DSI and lower plant weight in spring barley and sugar beet. All three isolates gave lower plant emergence in sugar beet, and isolates B9 and B11 lower emergence in spinach.

Table 4. Mean disease severity (DSI), emergence and relative fresh weights of spring barley, sugar beet, and spinach inoculated with isolates B8, B9 and B11 of *Aphanomyces macrosporus*

	Barley			Sugar beet			Spinach		
Isolate	DSI	Emerg. %	Weight	DSI	Emerg. %	Weight	DSI	Emerg. %	Weight
None	0 c	95 a	100 a	0 b	88 a	100 a	0 a	88 a	100 a
B8	20 b	93 a	86 bc	0 b	60 b	86 ab	15 a	73 ab	94 a
B9	11 bc	98 a	89 ab	3 b	58 b	73 bc	15 a	63 bc	80 a
B11	55 a	98 a	76 c	28 a	40 b	58 c	37 a	55 c	67 a
P-value	<0,0001	0,64	0,01	0,004	0,03	0,008	0,071	0,017	0,13

The pathogenicity tests in greenhouse on different cultivars of spring barley was, based on previous results on aggressiveness of different isolates, performed with dry inoculum of isolate B11. A test using cultivars Planet and Prospect was performed to elucidate if seed treatment with fludioxonid had any effect on infection. The results showed no effect on infection of this treatment on either of the two cultivars Planet and Prospect. A test was performed with 14 cultivars of spring barley (coded A to N) with untreated seed in greenhouse using compost/sand mixture and 600 oospores/ml soil of isolate B11. The test was conducted in 8 weeks and the results showed a significant difference (P < 0,0001) in DSI between the cultivars (Fig. 1). There were differences in length between inoculated and uninoculated pots for some cultivars. Cultivar H with the highest DSI of 46 (0-100) also had the lowest relative length of the plants in inoculated pots, 80, compared to uninoculated pots, 100. Cultivar B with the lowest DSI also had a relative length of the plants of 112 in inoculated pots compared to uninoculated pots 100. This means no reduction in length of the infection, but instead an increase.

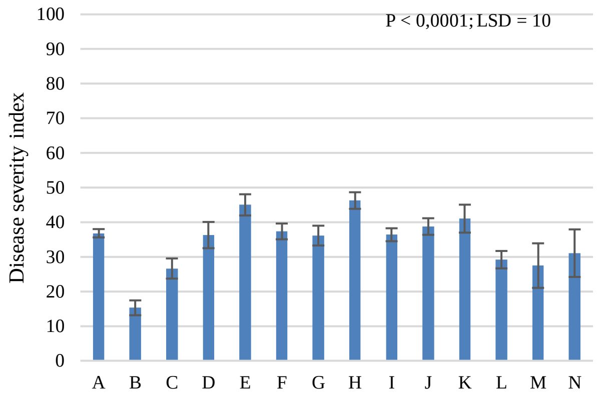

Fig. 1. Test in greenhouse with 14 spring barley cultivars and inoculation with 600 oospores/ml of isolate B11 *Aphanomyces macrosporus* in a compost/sand mixture: disease severity index.

Table 5. Yield of 9 cultivars of spring barley in a field trial, Åstorp, in a field infected with *Aphanomyces macrosporus*

Variety	Yield kg/ha
A	1650 ab
B	1962 a
C	1060 bc
D	1312 abc
E	1744 a
F	1031 bc
G	1840 a
H	692 c
I	1691 a
P-value	0,0013

The same batches of untreated seeds, cultivars A to I, were sown in a field trial on a soil infected with strain B9. The overall yield was low for all cultivars and cultivar H had significantly lower yield (692 kg/ha) compared to cultivar B (1962 kg/ha) with the highest yield (Table 5). Assessment of infection in an official variety field trial in Stafsjö, Halland indicated a high infection (DSI 63) but no significant differences between varieties.

Biological and chemical seed treatments in greenhouse and field trials

To establish a dose for Tachigaren and to perform an initial test in greenhouse, a test was done in pots with the soil with natural infection of isolate B8. The DSI was significantly lower for all chemical and biological seed treatments (Table 6). The best effect was achieved from treatment with Apron, DSI 16, compared to DSI 47 in untreated control, but also from Tachigaren. From these experiments the dose of 3 g/kg seed was chosen for Tachigaren in field trials in 2022 and 2023.

Table 6. The effect in a greenhouse test of seed treatments of spring barley on mean disease severity index (DSI), emergence and relative fresh weights in a soil with natural infection of isolate B8, *Aphanomyces macrosporus*

Treatment	DSI	Emergence %	Weight
Untreated	47 a	93 a	100 bc
Tachigaren 1g/kg seed	34 b	92 ab	104 bc
Tachigaren 3g/kg seed	32 b	83 cd	108 bc
Tachigaren 6g/kg seed	28 bc	85 abc	114 ab
Cedomon	28 b	83 bcd	110 abc
Polygandron STP	30 b	75 d	98 c
Apron XL 350 ES	16 c	88 abc	125 a
P-value	<0,05	<0,05	<0,05

Field trials with seed treatments

In total, five field trials with seed treatments of spring barley were performed in the project during 2021-2023 (Table 7). The infection on plants varied depending on the degree of soil infection of the pathogen and amount of rain and soil water.

Table 7. The effect of seed treatments on yield of spring barley in soils with natural infection of *Aphanomyces macrosporus* or *Pythium* spp.

	2021		2022		2023
Treatment	Varberg[1]	Skurup[2]	Åstorp[1]	Varberg[1]	Åstorp[1]
Untreated	1583 a	10797 a	2923 a	7985 a	1267 a
Apron XL 350 ES	1500 a	9901 a	2849 a	7697 a	1329 a
Tachigaren	1438 a	9352 a	2752 a	7909 a	1200 a
Cedomon	1590 a	10300 a	2764 a	7892 a	1196 a
Polygandron STP	1514 a	10065 a	2904 a	7677 a	1206 a
Mycostop	1535 a	9894 a	2580 a	7964 a	-
Rootfix	-	-	-	-	1162 a
Vayantis	-	-	-	-	1391 a
Kick-off	-	-	-	-	1328 a
P-value	0,18	0,059	0,46	0,67	0,62 a

[1] *Aphanomyces macrosporus*
[2] *Pythium* spp.

The trial in Varberg in 2021 had a high infection of *A. macrosporus* resulting in a yield of 1583 kg/ha in untreated control. There were no significant differences in infection of the plants or in yield. The treatment with Tachigaren gave a low plant stand due to a too high dosage (12 g/kg seed) both in Varberg and Skurup this year. The field trial in Skurup was in a field with a previous documented presence of *Pythium* spp., however the yield in untreated control was very high, 10 800 kg/ha and there were no positive effects of any treatment. The DSI and infection of *Pythium* was low. In 2022, the trial in Åstorp was in a field with a high soil infection and the yield was 2900 kg/ha in untreated control. There was a tendency of a decreased infection of the plant roots at BBCH 37 in both treatments with Apron and Tachigaren. The lower dose of Tachigaren (3 g/kg seed) used did not decrease the emergence as in 2021. There were, however, no effects on yield of any treatment. In Varberg, the weather conditions were favorable for spring barley and the yield was 7 985 kg/ha in untreated control and with no effects in either measured parameter except in infection later in the season. The biological products Cedomon, Polygandron and Mycostop gave a significant decrease in DSI compared to untreated control from DSI 27 to DSI 20, 23 and 21 respectively at BBCH 31, but no effect on plant weight or final yield. In 2023, a field trial was performed in Åstorp on a soil with a high infection of *A. macrosporus*. The yield in untreated control was 1 267 kg/ha, and with no effect on infection or final yield from treatments. The early plant weight at BBCH 21 was higher in the treatment with Apron.

Discussion

Development of yellowing patches in barley and to some extent also in wheat fields has been observed in Sweden for a long time, particularly after heavy rainfalls during the early cropping season The first report on this observation was published almost 30 years ago by M. Wikström (Larsson, 1994). In that study, several *Aphanomyces* isolates were obtained from barley and two from spinach, and they differed from other known plant pathogenic *Aphanomyces* species in their distinct morphology, pathogenicity, and unique isozymes profile. It was suggested that these isolates could belong to a new *Aphanomyces* species; however, no name was proposed. Our new surveys during 2021–2024 from the same farms sampled 30 years ago, as well as several other new locations in Southern Sweden and Denmark, confirm the presence of this new *Aphanomyces* species associated with barley root rot. We could in this project describe it scientifically and give the name *Aphanomyces macrosporus* (Wikström *et al*., 2023).

Our findings so far have shown the relatively broad distribution of this pathogenic *Aphanomyces* in Southern Sweden and Denmark, and currently more surveys are underway to determine its geographic distribution. According to our preliminary results, *A. macrosporus* is widespread in several growing areas, which indicates that it is not a newly introduced pathogen. The isolation of *Aphanomyces* from infected plant materials can be difficult, and it requires the use of selective media and working experience with this pathogen. Therefore, it is not unlikely that *A. macrosporus* has been overlooked in many investigations on barley root rot diseases in Sweden and perhaps in other countries, at least in the Nordic region. The identification of *Aphanomyces* isolates has been greatly facilitated using ITS sequencing data, which has overcome the difficulties and challenges of using morphology (Levenfors and Fatehi, 2004; O'Rourke et al., 2010).

Soils with the presence of *A. macrosporus* had on average a lower concentration of calcium and a lower pH (Ca-AL 196 mg/100 g soil, pH 6.7) compared to soils without presence of the pathogen (Ca-AL 593 mg/100 g soil, pH 7.0). The pathogen could also not be found in the trials with lime on alkaline soils in Skåne and the soil analyses showed that these trials had an average pH of 7.3 and ammonium lactate soluble calcium of 343 mg/100 g soil in untreated plots. This may explain why it was not found in these fields. This is in line with studies on other species of *Aphanomyces* where a high pH is related to a high concentration of calcium ions in the soil, which has a suppressive effect on the pathogen (Heyman et al. 2007; Olsson et al. 2011; Persson and Olsson, 2000; Wikström et al., 2024).

Pythium may sometimes be present, together with *A. macrosporus,* or alone. Symptoms may be a root rot (Ingram and Cook, 1990), but in our areas it is more common with damping off and plant losses. The species *Pythium arrhenomanes* was very common in

the field trials with lime in Skåne, however, with no significant effect on DSI of wheat or barley from either of the two lime products (Olsson Nyström et al., 2023). The species is pathogenic on both wheat and barley (Waller, 1979) but seems to be of minor importance in terms of yield level compared to effects of *A. macrosporus* as seen in our field trials. Since the symptoms of yellow patches appeared in wet soil conditions and *A. macrosporus* was present and repeatedly isolated from the diseased plants, we conclude that *A. macrosporus* is the major cause of barley root rot in those fields.
So far, our studies have shown that *A. macrosporus* can cause a severe root rot disease in spring- and winter barley. The large impact on yield could be seen in the trials with seed treatments in Halland and Skåne with 1700 kg/ha in a heavily infested field under wet conditions compared to the expected mean value of 4700 kg/ha (2018–2022) in the area (Jordbruksverket, 2023). In 2024, there were several fields of spring barley in northwestern Skåne with heavy infestation and even larger yield losses due to heavy rains during the growing season. Oospores of *A. macrosporus* were found in these fields and confirm the presence of the pathogen.
Pathogenicity tests have shown that also spinach and sugar beet, but <u>not</u> peas, field beans, or radish are hosts for *A. macrosporus*. Our tests have also shown that there was a difference in aggressiveness for the three isolates B8, B9 and B11 in spring barley, sugar beet and spinach. Further investigations need to be done to determine the possible broader host range of *A. macrosporus*, particularly for other crops used in rotations in Sweden.
There were positive effects in trials in greenhouse from both biological and chemical seed treatments and a soil with natural infestation of *A. macrosporus* B8, but there were no positive effects in the field trial on the same soil. The product Tachigaren was included due to its effect on *Aphanomyces* in sugar beets, however no effect could be seen in our field trials. The reason for this can be that seed treatment only has an effect for 3-4 weeks and that the root system after that continues to grow into soil infected with oospores of *A. macrosporus*.
Pilot experiments in greenhouse with inoculated soil and field experiments on a soil with an infestation of *A. macrosporus* indicate differences in tolerance in cultivars of spring barley. However, these results need to be repeated in more field trials. There are commercial cultivars of sugar beet with high tolerance to *Aphanomyces* and this might also be the case in spring barley.
Thus, the discovery of the new pathogenic *Aphanomyces* species in barley, capable of causing significant yield losses, particularly in years with high rainfall, and with a potential host range in economically important crops, may have a great impact on farming systems and crop rotations in Sweden. The longevity of oospores of *Aphanomyces* also entails a long-lasting problem in crop rotations in soils with a high root rot potential, especially where barley is a common crop. Further studies are needed to broaden and fulfil our knowledge of this pathogen and to develop possible means of disease control.

Conclusions- including benefits for growers and recommendations
A new species is described in this project: *Aphanomyces macrosporus*. It is pathogenic on spring-, and winter barley, sugar beet, and spinach, and most probably also on some other cereals, but not on pea and field bean. It has mostly been found in soils with a low

concentration of calcium and a low pH and in areas with high rainfall, and in fields with poor drainage. It has been found in several areas both in Sweden and Denmark. Trials and observations indicate large yield losses in barley grown on infected soil. Pilot trials in field and growth chamber indicate differences between cultivars in tolerance to infection of barley root rot and further trials will be done to verify this. Trials with chemical and biological seed treatments in field, and growth chamber gave only low effects on root infection and does not seem to be a means for control of infection. There is a need for a diagnostic tool for assessing the root rot potential of a field and a further set of tools for avoiding crop losses.

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Wikström, J., Chaudhary, S., Persson, L., Wikström, M., Fatehi, J., Karlsson, M. 2020. Distribution of plant pathogenic *Aphanomyces* species in Sweden, Denmark and Lithuania and its relationship with soil factors. Submitted for publication in Journal of Plant Pathology.

Wikström, M., Persson, L., & Fatehi, J. 2023. Aphanomyces macrosporus sp. nov. Causing Root Rot in Barley and Some Other Plants. Journal of Fungi, 9(12), 1144. https://doi.org/10.3390/jof9121144

Del 3: Resultatförmedling

Vetenskapliga publiceringar	Wikström, M., Persson, L., & Fatehi, J. 2023. *Aphanomyces macrosporus* sp. nov. Causing Root Rot in Barley and Some Other Plants. Journal of Fungi, 9(12), 1144. https://doi.org/10.3390/jof9121144
Övriga publiceringar	Betodlaren 1: 2024. Ny art av rotrötesvampen *Aphanomyces*.
	Suckerroenytt 2:2024. Ny art af rodbrandsvampen *Aphanomyces*
	Arvensis 7:2024. Ny *Aphanomyces*-art angriper korn
Muntlig kommunikation	Lantbruksforskningsdagen 2024 – presentationer - Lantbruksforskning. Skara 2024-11-06
	JV Växtskyddscentralerna Webbinarium ny rotsjukdom i korn. 2024-10-18. Rådgivare vid VC.
	JV Växtskyddscentralerna Webbinarium ny rotsjukdom i korn. 2024-12-18. Rådgivare och deltagare från branschen i Sverige och Danmark, ca 80 deltagare.
	Digitalt webbinarium om ”Ny rotsjukdom i korn” i Svensk växtpatologisk förening 15 januari 2025
Studentarbete	
Övrigt	Planerat: 14th Conference of the European Foundation for Plant Pathology, Uppsala 2-5e June 2025.