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Populationsstruktur hos* Puccinia graminis *som orsakar svartrost på vete, korn och råg i Sverige.

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Degree project/Independent project • 30 hp
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Agriculture Programme - Soil/Plant
Uppsala 2021

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Credits:	30 hp
Level:	**A2E**
Course title:	**Master thesis in Biology**
Course code:	**EX0898**
Programme/education:	**Agriculture Programme - Soil/Plant**
Course coordinating dept:	**Department of Forest Mycology and Plant Pathology**
Place of publication:	**Uppsala**
Year of publication:	**2021**
Cover picture:	Clara Kjellström

Keywords: plant pathology, plant disease, crop protection, fungi, P. graminis f.sp. tritci, P. graminis f.sp. secale, P. graminis f.sp. hordei

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Abstract

Rust fungi belong to the phylum Basidiomycetes, order Uredinales cause damage on almost all plants, including cereal crops. Some of the species within the genus *Puccinia* causes rust disease on cereal crops. One example of a disease from this genus is *Puccinia graminis,* which historically has caused severe damage in cereal crop production. During recent years, *P. graminis* have re-emerged in Sweden on barley and wheat. The aim of this work was to examine if the same population of *P. graminis* infects both wheat and barley in Sweden. In total 204 samples were collected of the crops, 123 from barley (*Hordeum vulgare)*, 10 from rye (*Secale cereale)* samples and 71 from wheat (*Triticum aestivum)* in 16 farmers' fields. The genotypic diversity was examined based on microsatellite markers and the population structure and genetic relationship within and between the collected samples were analysed.

The results indicate that the *P. graminis* population on barley and wheat seemed to be partially divided in two clusters. However, the clusters were partially overlapping, and the main branches of barley and wheat had smaller branches of barley and wheat within them. The rye samples were spread mainly in the barley cluster or branch, but more samples are needed to draw conclusions about the rye population. This could indicate that the *P. graminis* population on barley and wheat are not fully genetically separated consisting of a meta-population, but that there probably is a selection on the different cereal hosts. The result did also confirm that there is a high genetic diversity within the populations, indicating that sexual recombination can be completed on the aecial host *Berberis* spp. in Sweden. There was no correlation with geographic and genetic distance, confirming that there is mostly wind dispersal of *P. graminis.*

Keywords: **plant pathology, plant disease, crop protection, fungi, P. graminis f.sp. tritci, P. graminis f.sp. secale, P. graminis f.sp. hordei**

Sammanfattning

Växtsjukdomar orsakade av rostsvampar vilka tillhör fylumet Basidiomyceter och ordningen Urdinales orsakar skador på nästan alla växter inklusive spannmålsgrödor. Ett släkte som orsakar rostsjukdomar på våra spannmålsgrödor är *Puccinia* spp., och ett exempel en patogen i detta släkte är *Puccinia graminis* som historiskt orsakat stora skador på spannmålsgrödor. Under de senaste åren har infektioner av svartrost orsakade av *P. graminis* noterats på vete, korn och råg på flera platser i Sverige. Syftet med detta examensarbete är att undersöka om det är samma population av *P. graminis* som både infekterar vete och korn i Sverige. Totalt samlades 204 prov in a grödorna, 123 från korn (*Hordeum vulgare)*, 10 prov från råg (*Secale cereale)* och 71 från vete (*Triticum aestivum)* i 16 fält. Den genotypiska diversiteten bestämdes med hjälp av mikrosattelitmarkörer och populationsstrukturen inom och mellan de insamlade proverna analyserades.

Resultaten visar att populationer av *P. graminis* verkade vara uppdelade i två grenar eller kluster, men dessa var inte helt separerade. Huvudgrenen av korn och vete hade en mindre gren av vete eller korn inom sig. Svamppopulationen insamlad på råg var spridd främst inom kornklustret, dock behöver mer prover för att kunna dra säkra slutsatser om prover från just råg. Detta tyder på att populationerna av *P. graminis* inte är genetiskt separerade utan består av en meta-population, men att det troligtvis sker en selektion på de olika värdarna vete, korn och råg. Resultaten bekräftade även att *P. graminis* hade hög genetisk diversitet, vilket tyder på sexuell rekombination kan slutföras på den alternativa värden *Berberis* spp. Det fanns inget samband mellan geografiskt och genetiskt avstånd, vilket tyder på att *P. graminis* är huvudsakligen vindspridd.

Preface

Thanks to all the people that helped me with the sampling and the farmers which allowed us to take samples on their fields,

I also want to thank Maria for all the help in the lab.

Also especial thanks to Anna (my supervisor) and Björn (my co-supervisor)
Anna S (my friend),
for all the ideas and support during the process,

And the rest of my friends and family.

Populärvetenskaplig sammanfattning

Svartrost orsakat av svampen* Puccinia graminis *–släktskap mellan prover av svampen från vete och korn och vilka bekämpningsåtgärder kan användas mot sjukdomen?

Vad är svartrost?
Puccinia graminis är en svamp som orsakar växtsjukdomen svartrost, som historiskt har orsakat stora skador på spannmålsgrödor. Vid angrepp innebär sjukdomen lägre skörd och sämre kvalitet på skörden. Det amerikanska namnet på sjukdomen är 'stem rust', vilket beskriver symptomen då sjukdomen känns igen på att den angriper strået på spannmålsgrödor. Det svenska namnet svartrost beskriver den svarta färgen som patogenens sporer får senare på säsongen. Tidigt på säsongen känns sjukdomen igen på de rostbruna sporerna på spannmålsgrödornas strå. Svartrost var under flera årtionden inte något problem i spannmålsodlingen i världen, men under de senaste 20 åren har den åter igen blivit ett problem på flera platser i världen. I Sverige har infektioner av svartrost noterats på vete, korn, råg och havre på flera platser. Detta är ett problem som behöver uppmärksammas och undersökas för att kunna förstå varför sjukdomen har kommit tillbaka och hitta metoder att stoppa spridningen.

Komplicerad livscykel med värdväxling till berberisbusken
Puccinia graminis har en komplicerad livscykel med fem olika sporstadier. Stadierna sker på två värdar, gräsvärden (spannmålen) och berberisbusken. På gräsvärden återinfekterar sommarsporerna grödan, där de i slutet av säsongen mognar till mörka vintersporer. För att kunna slutföra sin livscykel i kalla klimat som Sverige så behöver svampen kunna värdväxla till berberisbusken när vintersporerna gror på våren. När svampen värdväxlar till berberis kan det sexuella stadiet i livscykeln slutföras, och när den sexuell reproduktionen sker så ökar den genetiska diversiteten hos svampen jämfört med klonal, asexuell förökning. I varmare klimat behöver inte svampen värdväxla för att sluta sin livscykel utan de asexuella sommarsporerna kan återinfektera grödan.

Den sexuella rekombination som slutförs på berberisbusken
innebär att det skapas nya genkombinationer. Det innebär att resistensen finns kvar hos plantan, men att den inte är effektiv mot de nya varianterna av

svampen och plantan kan då infekteras av svampen som orsakar sjukdomen svartrost.

Vad undersöktes?
Syftet med detta examensarbete var att undersöka om det är samma population av *P. graminis* som infekterar vete och korn i Sverige. Detta är intressant att veta eftersom vi behöver kunskap om svartrost kan smitta mellan fält med olika grödor under säsongen. Prover av svampen samlades därför in från vete, korn och råg på flera platser i Mellansverige. Den genotypiska diversiteten bestämdes med hjälp av mikrosattelitmarkörer. Populationsstrukturen och släktskap mellan de insamlade proverna från olika grödor analyserades.

Resultaten visar att det finns ett visst släktskap mellan olika svampprover insamlade på korn och vete. De är uppdelade i två grenar eller kluster, men dessa var inte helt separerade. Inom varje gren av proverna av svampen insamlade från korn och vete finns det ett inslag av svampindivider insamlade från det andra sädesslaget. Detta tyder på att populationerna av *P. graminis* inte är helt utan släktskap utan är en del utav en större population, men att det troligtvis sker en selektion på de olika värdarna vete, korn och råg.

Resultaten bekräftade även att *P. graminis* hade hög genetisk diversitet, vilket tyder på att det sexuella stadiet i livscykeln på den alternativa värden berberis sker ofta. Det fanns inget samband mellan geografiskt och genetiskt avstånd, vilket bekräftar att *P. graminis* huvudsakligen är en vindspridd patogen.

Vad tyder då detta på? Eftersom proverna insamlade på de olika grödorna inte är helt genetiskt separerade är det möjligt för sjukdomen att smitta mellan fält av vete och korn. Därför är det viktigt att fortsätta övervaka *P. graminis* som orsakar svartrost. Resultaten bekräftar även vikten att ta bort berberisbusken i odlingslandskapet för att minska risken för infektion.

Sjukdomsangrepp kan förhindras genom följande:

- Utrotning av berberisbusken
- Tidig sådd av spannmål så grödan hinner mogna innan svartrostangrepp startar, vilket minskar risken för stora skador
- Välja spannmålssorter som är resistenta mot svartrost
- Behandling med svampmedel mot rost runt axgång. Angreppen i vete och korn har hittills varit så sena i Sverige att det inte ansetts vara ekonomiskt lönsamt, däremot kan det vara lönsamt att bekämpa vid den tidpunkten i havre.

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Abbreviations

AMOVA	Analysis of Molecular Variance
DNA	Deoxyribonucleic acid
F_{it}	Interbreeding coefficient within the individuals
F_{st}	Interbreeding coefficient within subpopulations
F or F_{is}	Inbreeding population within individuals or Fixation index
f. sp.	*formae specialis*
H	Shannon-Weiner Diversity index
H_e	Expected heterozygosity
H_o	Observed heterozygosity
MLG	Multi Locus Genotype
MSN	Minimum Spanning Network
PCoA	Principle Coordinates Analysis
p.rD	Probability value of the rbarD value
rbarD	Vector of Standardized Index of Association

1. Introduction

1.1. Rust diseases

Rust fungi is a part of the phylum Basidiomycetes in the order Uredinales. They can cause severe plant diseases and affect almost all plants, including cereal crops, vegetables, and other field crops. The fungi causing rust diseases are obligate parasites and can have up to five spore stages (Agrios, 2005).

There are several rust diseases caused by the genus *Puccinia* spp. in cereal crops. Most of these are macrocyclic with five spore stages with sexual reproduction on the aecial host plant and asexual reproduction on the gramineous host. In general, the rusts are recognized on the cereal crops by the uredinial pustules which are a part of the often complex life cycle of the rust fungi (Chaves, 2008). In our most important cereals in Sweden, barley and wheat (Statistics Sweden, 2021), there are several important rust diseases. Severe diseases on wheat are stem rust caused by *Puccinia graminis* f.sp. *tritici*, leaf rust caused by *Puccinia triticina* and stripe rust caused by *Puccinia striiformis* f. sp. *tritici*. In barley, the rust diseases are leaf rust caused by *Puccinia hordei*, crown rust caused by *Puccinia coronata var. hordei*, stem rust *Puccinia graminis* f. sp. *hordei* and stripe rust *P. striiformis* f.sp. *hordei* (Chaves, 2008). The rust diseases often lead to a lower photosynthesis rate and an increased respiration. This will result in less green mass, lower root growth, and a reduction in yield quantity and quality (Agrios, 2005).

1.2. Stem rust - the disease caused by *P. graminis*

1.2.1. Symptoms and implications

Stem rust is a disease caused by the fungus *Puccinia graminis.* The fungus is mainly infecting stems and leaf sheets of the plant, but under severe epidemics also glumes and leaves can be infected (Chaves, 2008). The first symptom after the infection of the disease is a small chlorotic mark. The mark is followed by pustules of uredinia with a red-orange appearing on stems, leaf, and leaf sheets (figure 1) which causes the epidermis to burst after approximately 8-10 days. Later in the season during the

late summer or autumn, teliospores are produced giving the stems a more blackened appearance (figure 2). The fungus overwinters in crop debris as teliospores, and in the spring basidiospores are formed, which infect *Berberis* spp. (Leonard and Szabo, 2005). Later, after the sexual reproduction have been completed, cluster cup rust on Berberis spp. forms aeciospores which infect the gramineous host (Barnes et al., 2020). On cereals the disease results in a reduction yield level, grain size and other quality parameters. The damage from the disease can vary from marginally to severe and also make the host plant more susceptible to other pathogens (Agrios, 2005). The pathogen can be spread long distances by air currents as urediniospores. One example of this is the seasonal movement of *P. graminis* across the United States (Nagarajan and Singh, 1990). The disease is often expressed late in the season, therefore late-maturing grains and especially spring-sown crops are often more affected by the disease (Roelfs, 1992).

Figure 1. Picture of the urediniospores of P. graminis, the summer spores which are revealed first in the season, breaking the epidermis of a cereal stem.

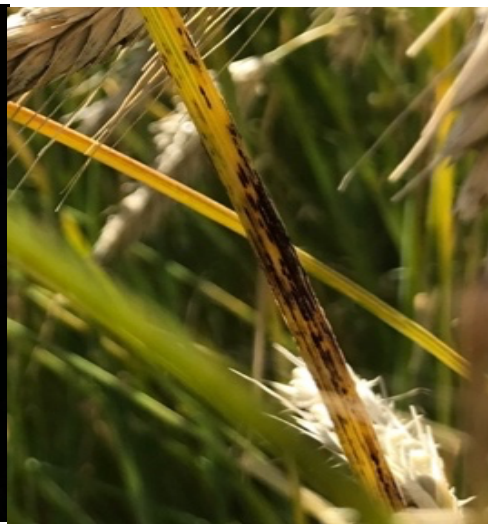

Figure 2. The urediniospores are formed into teliospores, with a more blackened appearance

1.2.2. Stem rust – historical importance

Stem rust has since ancient times been feared in cereal production. The disease have been described in ancient sources as foxes which set the cereals fields on fire, causing famine as a result (Zadoks, 2008). During the 20th century several stem rust epidemics have occurred in Europe. For example, yield losses of 20 % in winter wheat and 50 % in spring wheat was reported under the stem rust epidemic in Sweden 1951 (Zadoks, 1965). In Sweden landowners were obligated to eradicate *Berberis* spp. on their land in until 1994 (Jordbruksdepartementet, 1994). In Britain, *Berberis* spp. was limited in the landscape by eradication campaigns (Barnes et al., 2020). *Berberis* spp. was for a long time suspected to cause the stem rust disease among farmers, but the theory was not accepted among scientists before the 1900th century since the link between the two host plants was not always clear.

In wheat, the problems with the disease were limited by breeding for race-specific resistance in combination with eradication of *Berberis* spp., which suppressed the genetic diversity and the development of new races (Leonard and Szabo, 2005). However, in 1999, a new strain, called Ug99 or TTSK in the North American nomenclature system, that was able to overcome the important resistant gene *Sr31* was detected in Uganda (Pretorius et al., 2000). Ug99 has since spread to several countries in East and Southern Africa and Iran, Yemen and Egypt. (Singh et al., 2015).

1.2.3. The situation of stem rust in Europe today

During the last decade, there has been several cases of re-emergence of stem rust on wheat, *P. graminis* f. sp. *tritici* in Europe. In the summer of 2013, *P. graminis* f. sp. *tritici* was found in several locations in Germany (Olivera Firpo et al., 2017). In the same year, *P. graminis* f. sp. *tritici* was found on wheat in the United Kingdom for the first time in 60 years (Lewis et al., 2018). In 2016, a large outbreak of stem rust in wheat occurred in Sicily (Bhattacharya, 2017).

In Sweden, *P. graminis* in wheat was found in spring wheat 2017. When the genotypic diversity of the fungi in this outbreak was analysed, it could be concluded that the fungus was genotypically diverse, indicating that *Berberis* spp. was important in the epidemiology of the outbreak (Lind, 2017). Resistance test on wheat and barley showed that most wheat cultivars in Sweden lack resistance to the population of stem rust that was found in the field 2017. It was also found that a few cultivar were resistant to Ug99 (Växtskyddscentralen Skara, 2018). During the summer of 2020, stem rust was found in spring barley and oats around the area of the lake Mälaren in Sweden, however at levels that probably had a negligible impact on the yield (Växtskyddscentralen Linköping, 2020). Today, the disease is mostly found in central Sweden (A. Berlin 2021, personal communication).

1.2.4. Disease management

As already mentioned, stem rust can be managed by resistant cultivar combined with eradication of *Berberis* spp. (Agrios, 2005). Moreover, early sowing can decrease the risk of an outbreak, since a more developed crop is less susceptible to the disease (Växtskyddscentralen Linköping, 2020).

Treatment with fungicides against rust diseases at heading can have an effect against stem rust. However, according to the Swedish Boad of Agriculture (2021), fungicide treatments against stem rust are usually not profitable in wheat or barley since the disease occurs late in the season. In oat, it is recommended to treat with fungicide if the infection occurs around heading.

1.3. *Puccinia graminis* - the pathogen

As earlier mentioned*, P. graminis* has a wide host range, causing stem rust in cereal crops and grasses (Leonard and Szabo, 2005). It infects barberry (*Berberis vulgaris)* and several other related species (Cereal Disease Laboratory, 2016) causing cluster cup rust (Agrios, 2005, Barnes et al., 2020).

Optimal temperatures for *P. graminis* germination are 15-24 ºC, appressoria formation 16-27 ºC, penetration 29 ºC, and growth and sporulation 30 ºC. For germination, appressorium and penetration, water is essential. The optimal condition for infection are 8-12 hours of dew at temperature of 18 ºC, followed by a light rate of 10,000 lux and a temperature increase to 30 ºC when the dew is dried slowly (Roelfs, 1992). Thus, stem rust is favoured by hot and humid weather. Stem rust in wheat is favoured by warmer weather compared to leaf and stripe rust (Roelfs, 1992).

1.3.1. The taxonomy of *P. graminis*

P. graminis are divided into *formae speciales* (*f. sp*) based on which host the fungus infects (Leonard and Szabo, 2005). Examples of *formae specialis* are *Puccinia graminis* f. sp. *hordei* causing barley stem rust, *P. graminis* f. sp. *tritici* causing wheat stem rust. The *formae specialis* is thereafter divided into different pathogenic races which are characterized by their virulence spectrum (Roelfs and Martens, 1988). Ug99 is an example of a highly virulent clonal linage (Pretorius et al., 2000).

1.3.2. Life cycle

P. graminis is a heteroecious fungus depending on both *Berberis* spp. and a grass host to complete its five spore stages in its life cycle. To be able to complete the sexual stage, several stages need to take place on *Berberis* spp. The clonal, asexual stages is completed on the grass host (Leonard and Szabo, 2005). In cold climates, *P. graminis* overwinters in a resting stage as two-celled teliospores on the grass host. The teliospores matures to basidia during the spring about the same time as *Berberis* spp. develops new leaves. Each basidium forms four basidiospores, which are transported by wind to leaves on *Berberis* spp. (Agrios, 2005). The basidiospores infect the leaves and a **pycnidium** develops. Then, the sexual stage of the life cycle is completed; haploid **pycnidiospores** (the male gamete) are formed within the pycnium and is exudated, with hyphae (the female gamete) attached to the top of the pycnium. The **pycnidiospores** are secreted together with a nectar and is transmitted to other pycnia by rain drops and insects and the two mating types are fused together to a dikaryotic stage, the cup-shaped **aecium** which is developed under the pycnidium on the leaf of *Berberis* spp. The aecium then produces **aeciospores** (single celled, dikaryotic), which are the spores that infect

the grass host. When infecting the grass-host, a hyphal mat is developed below the epidermis and produces sporophores from which **urediniospores** (single-celled, dikaryotic) are developed and are breaking the epidermis, which is the clonal, asexual stage of the life cycle. The urediniospores can then be spread by wind and reinfect the host. When the host is maturing, two-celled **teliospores** are produced and the life cycled is completed (Leonard and Szabo, 2004) (see figure 3).

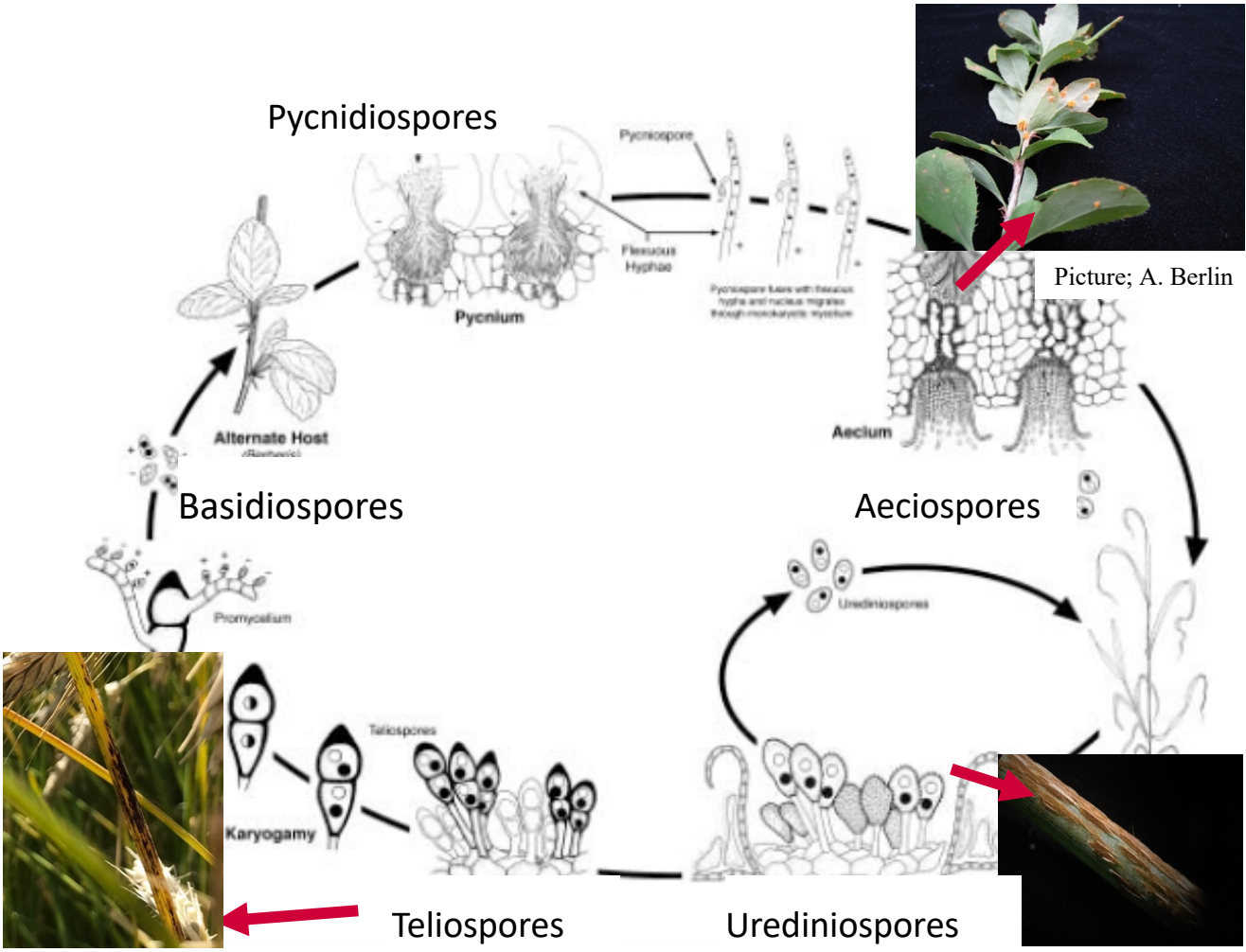

Figure 3 Picture of the life cycle of *P. graminis*, illustration of life cycle from Leonard and Szabo (2005). Photos by A. Berlin and the author

1.3.3. The role of *Berberis* spp. in the life cycle

As mentioned, *Berberis* spp. is needed to complete the sexual life cycle of *P. graminis.* Sexual recombination can lead to the emergence of new races of *P. graminis* (Berlin et al., 2012, Saunders et al., 2019). *Berberis* spp. is vital in the life cycle of *P. graminis* in temperate climate since the winter-hardy teliospores which in the spring germinates into basidiospores infects the *Berberis* spp. plant. On the *Berberis* spp., the sexual stage is performed and after this the aeciospores are transmitted from the cluster cup rust on the underside on the *Berberis* spp. leaf

which then is infecting the gracious host. Thus, the pathogen cannot complete its life cycle without *Berberis* spp., and the alternate host is crucial for winter survival since the asexual urediniospores cannot survive the cold winter season in northern climates (Barnes et al., 2020).

1.4. Population biology

Epidemiology describes development of diseases over time and space (Milgroom and Peever, 2003). Population biology aims at explaining the evolutionary and ecological dynamics and interaction between plant hosts and the pathogen populations. Population biology integrates principles in evolution, genetics and ecology together with population genetics and epidemiology (Milgroom and Peever, 2003). In order to do this, neutral genetic markers are often used (Milgroom and Peever, 2003). Microsatellite markers (synonyms Simple Sequence Repeats, SSR; Short Tandem Repeats, STR) are short sequences (1-8 base pairs) of monomer sequences that are repeated randomly in DNA sequences (Mittal Neha, 2009).

Population - definition
What is then a population? There are different definitions depending on in which field the subject is discussed. In ecology, a population can be view as a group of individuals which are living in the same area and are so isolated in such extent that immigration will not affect the dynamics of the population over a long-time span. In evolutionary terms, a population is a group of individuals which interbreed with each other and exists in the same area at the same time. However, the evolution of new, quantitate methods have found no consensus about the definition and the definition varies depending on the approach of the question discussed (Waples and Gaggiotti, 2006)

1.5. Question at issue

It is important to know how if different populations in Sweden on barley, rye and wheat are genetically the same or not to know if a wheat field infected with stem rust could spread the infection to a nearby barley field and vice versa. The aim of this work was to examine if the same population of *P. graminis* infects both wheat and barley in Sweden. This was examined by using microsatellite markers to investigate the population biology in the different population and on different levels.

The hypothesis in this study is: Is the same population of *P. graminis* infecting both wheat and barley in Sweden?

2. Method

2.1. Collections of data

Straws infected with *P. graminis* were collected in 16 fields of barley, wheat and rye. Each field was considered as a population. The populations came from the Swedish provinces Närke, Sörmland, Östergötland and Uppland and was collected in collaboration with the Plant Protection Centres at the Swedish board of Agriculture and the Rural Economy and Agricultural Societies in Örebro and Västerås (figure 4 and table 1). The focus was barley and wheat, but other cereals and grasses were included in the sampling but not analysed for population structure. In total 204 samples were collected of the crops, 123 from barley (*Hordeum vulgare)*, 10 from rye (*Secale cereale)* samples and 71 from wheat (*Triticum aestivum)* in 16 farmers' fields (see table 1 and figure 4). Samples collected from three grass hosts (*Phleum pratense, Alopecurus pratensis, Elymus repens)* were also included in the laboratory work, however these samples were not included in the study due to low amplification frequency.

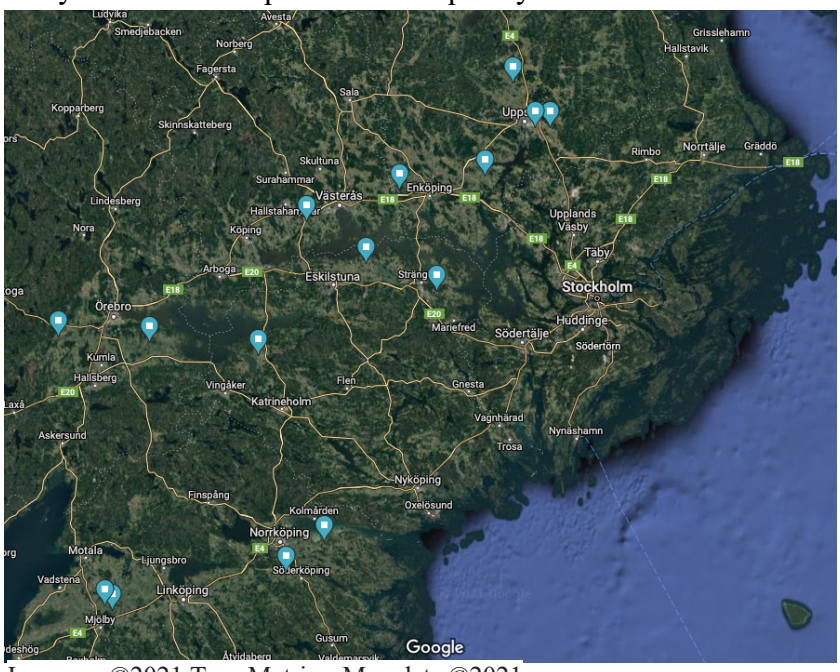

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Figure 4. Sampling locations of barley, rye and wheat. Picture source (Google maps, 2021).

*Table 1. Table of the different samples with crop, cultivar, region (based on the county letter system, C=Uppland, D=Södermanland E=Östergötland, T=Örebro U=Västmanland), farm and number of samples from each field ** Not included in the population calculations*

Population number	Crop	Farm	Region	Cultivar	Number of samples
1	Barley	Vändle	U	SW Makof	10
2	Barley	Vändle	U	RGT Planet	10
3	Barley	Lövsta Löt	C	RGT Planet	10
4	Barley	Tuna fält	C	Fairytale	10
5	Barley	Ultuna egendom	C	Laureate	10
6	Barley	Lövsta	C	Laureate	13
7	Barley	Kapellgården	D	RGT Planet	12
8	Barley	Julita	D	KWS Irina	9
9	Barley	Forsa	E	Severi	10
10	Barley	Vånga	D	RGT Planet	10
11	Barley	Evertsholm	E	Dragoon	7
12	Barley	Esplunda	C	SW Makof	12
13	Wheat	Säby	E	Happy	11
14	Wheat	Bärstad	T	Diskett	24
15	Wheat	Östra göksvalla	T	Diskett	36
16	Rye	Järstad	E	Performer	10
17	*Alopecurus pratensis* **	-	C	Björklinge	8
18	*Elymus repens* **	-	U	Västerås	2
19	*Phleum pratense***	-	C	Björklinge	6

2.2. Genotyping

Genetic markers can be used in plant pathology for several reasons including identification, diagnose and detection of pathogens. They can also be used to identify evolutionary relationships between individuals or populations of pathogens, and to find sources of outbreaks of different pathogens and to determine population structure (Milgroom, 1997). In the study reported here genetic markers were used to determine the population structure.

2.2.1. DNA extraction

For DNA extraction, at least ten pustules was collected on separate straws in each field. Each field was regarded as a population. In some locations, a smaller number of pustules were collected because of low amounts of disease in the field. A lower amount than ten samples were also analysed if there was non-successful PCR-reactions.

Single, clearly separate pustules of teliospores were carefully cut out from each

straw and placed in a tube together with glass beads and diamateous earth for homogenisation. The DNA from the fungi was extracted with *NucleoMag® Plant* (Macherey-Nagel, Germany) DNA extraction kit following the producers recommendations following the manual *Genomic DNA from plant* (Macherey-Nagel, 2018) with some adjustments (see appendix 1).

2.2.2. PCR

The extracted DNA was then diluted to a ratio of 1:100. The DNA samples was characterised with 23 microsatellite markers (table 2) commonly used for genotyping *P. graminis* f. sp. *tritici* from (Jin et al., 2009, Stoxen, 2012, Zhong et al., 2009).

Table 2. Primers used in this study. Marker group refers to the grouping of primers in the laboratory experiments.

Primer	Fluorophore	Primer sequence (5'-3')	Marker group	Reference
Pgest109	HEX	CCATCCGATCATTTCTTCGT	1	Zhong et al. (2009)
Pgest142	NED	GATGGTGAAGTCCGGTATGG	1	Stoxen (2012)
Pgest173	NED	TCCATTGAGTTCCATCGTGA	2	Zhong et al. (2009)
Pgest21	FAM	CCGAATGCAGATTACCCTTG	1	Stoxen (2012)
Pgest227	NED	CACACGTCTCGAGGAACAGA	3	Zhong et al. (2009)
Pgest24	HEX	TCATCGACCAAGAGCATCAG	2	Stoxen (2012)
Pgest293	FAM	GAACCTTGGCCTGAGTGCTA	2	Zhong et al. (2009)
Pgest318	FAM	GATGTCGGTCTTGGTCCACT	3	Stoxen (2012)
Pgest325	HEX	TTGGGTGAGTCAGAGTTTGAGA	3	Stoxen (2012)
Pgest341	NED	GGCCTTGGTACCCAATTTCT	4	Zhong et al. (2009)
Pgest353	FAM	ACGTCTTGGGTTTCTGTGGA	5	Zhong et al. (2009)
Pgest59	NED	AGGTTGATGATGAGGATGC	5	Stoxen (2012)
Pgestssr255F	FAM	CATCTGATCACCGTCACAGC	4	Berlin et al. (2012)
Pgestssr279F	HEX	ATCGAAGAGCCGTTCACTGT	4	Zhong et al. (2009)
Pgestssr368F	FAM	CATCTGATCACCGTCACAGC	7	Zhong et al. (2009)
PgtCAA39F	NED	CGTCGTCCCTCCATAGTCTTA	6	Stoxen (2012)
PgtCAA49F	HEX	TCGTCTGATCGTGAGAAACG	6	Stoxen (2012)
PgtCAA53	FAM	AGGCTCAACACCACCCATAC	8	Jin et al. (2009)
PgtCAA80	FAM	GCCTCCAGACGAATGGTTTA	6	Stoxen (2012)
PgtCAA93	HEX	CGCCTGTGATGGTTGTATTG	5	Jin et al. (2009)
PgtCAA98	HEX	ATTCGGATGGTCCGTTACTG	8	Jin et al. (2009)
PgtCAT4.2	HEX	CCGTGTCGATCCCAATAATC	7	Stoxen (2012)
PgtGAA8.1	NED	TGTCTGCCTGTCTGTCGAAC	7	Stoxen (2012)

A primer mix was used with the proportions in table 3. If several primers were used in multiplexing, the amount of water was reduced.

Table 3. Table over primer mixed used in the PCR-reaction

Ingredient	Concentration	Percentage (%)
Water		45 (reduced if several primers were added)
Dreamtaq green buffer (Thermo Scientific)	20 mM	20
dNTP	2 mM	20
Primer forward		4 (for each primer)
Primer reverse		4 (for each primer)
$MgCl_2$	25 mM	6
Dreamtaq DNA polymerase (Thermo Scientific)	5 U/µl	1.25

PCR was then conducted (see table 4) for a total volume of 15 µl per sample.

Table 4. Program used for the PCR reactions.

Step	No cycles	Temperature (ºC)	Time
1	1	94	4 min
2, annealing	35	94	30 sec
		62	27 sec
		72	30 sec
3	1	72	7 min
Cooling	∞	15	

To control the amplification of the PCR, the PCR product was run on a 1,2 % agarose gel at 220 V in 45 minutes for one marker and 1 hour for multiple markers. Then, the gels were checked to see if the primers amplified (see figure 5).

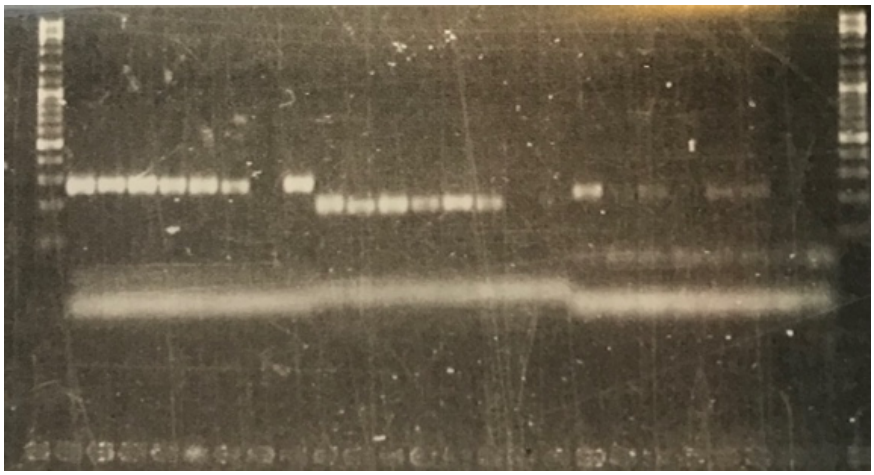


Figure 5. Picture of the result of the gel electrophoresis; band indicates a successful PCR-reaction.

In some PCR reactions, three markers were combined from the same marker group in one PCR reaction (see table 4). However, some marker groups did not amplify well when they were multiplexed. Therefore, the results of the PCR were checked and for the groups that did not amplify (see figure 5), new PCRs were performed for each marker separately and successful amplifications were pooled. Successful PCR fragments were sent to fragment analysis which was conducted on an ABI3730xl System by Macrogen (Amsterdam, Netherlands).

2.3. Data analysis

2.3.1. Scoring and identification of alleles

The result from the fragment analysis was scored in GeneMarker v. 2.6.3 (Softgenetics). The lengths of allele fragments were determined by the form of the peaks, only alleles with the right pattern of the peak were counted.

2.3.2. Control of data and markers

In the data analysis, populations from wheat, barley, and rye with at least five successfully genotyped individuals were included. The samples from grasses did not amplify by the selected markers and was therefore not included in the analysis.

The data and markers were controlled by analysing the allele frequency in Genalex6.5b2 *(Peakall, 2012, Peakall, 2006)* and Poppr (Kamvar ZN, 2014). In Poppr *(Kamvar ZN, 2014),* a genotype accumulation curve were made to control if the markers cold identify the genetic variation present within the sampled population. The missing information of the markers and the numbers of alleles were controlled in the same program. The fragment size interval and the observed heterozygosity (H_o) and expected heterozygosity (H_e) for each marker were calculated in Genalex6.5b2 (Peakall, 2012, Peakall, 2006).

2.3.3. Analysing the data in the aspect of populations

Several analyses were performed to investigate the population structure of the collected samples. All calculations described below were performed in Genalex6.5b2 (Peakall, 2012, Peakall, 2006) or Poppr (Kamvar ZN, 2014). For the population structure, only samples collected from barley, rye and wheat were included. The percent genotypic diversity was calculated as number of unique multi locus genotypes (MLGs) divided by the total number of samples and multiplied with 100.

Investigating the difference between populations

To investigate the difference between the populations, frequency- and genetic distance-based analyses were conducted. Values for H_o (observed heterozygosity) and H_e (expected heterozygosity) were calculated, as well as F-statistics. The F indicates if there is inbreeding (>0) or if there is surplus of heterozygosity (<0) or random mating (close to 0) and can thus be used as an inbreeding coefficient that measures reduction of heterozygosity. The Shannon Diversity index (H) describes the diversity in the populations and was calculated for each population. To investigate if there were any signs of random mating in the populations, rBarD, the standard index of association, was calculated, to investigate if the populations were in disequilibrium. The rbarD value was used instead of I_a, the index of association, since this measure is not dependent on the sample size (Agapow and Burt, 2001). To determine if the genetic variation was explained by geographic distance, sampling area or crop cultivar and to investigate the genetic variation on different levels, several Analysis of Molecular Variance (AMOVA) were performed. The AMOVAs were conducted with 999 permutations to analyse if there was any genetic difference between the populations and how the variation was distributed between different levels All analysis were performed on different population levels: field, cultivar, region, and host crop.

To visualize the genetic relationships between the samples a PCoA (Principal Coordinates Analyzes) based on the genetic distance as well as a minimum spanning network (MSN) based on Bruvo distance were performed.

To investigate if geographic distance correlated with the genetic distance Mantel-tests were performed. The samples were also analyzed based on cultivar and crop origin.

3. Result

3.1. Information about primers

The total number of alleles (186) were well distributed among the markers (table 5). The saturated accumulation curve indicates that the number of markers used in this study was sufficient to describe the genetic diversity (appendix 2).

Table 5. Used primers, observed and expected heterozygosity (H_o and H_e) and the standard error (SE), percent missing values and the fragment size intervals.

Primer	**Total No. Alleles**	**H_o**	**SE**	**H_e**	**SE**	**Missing value %**	**Fragment size interval**
Pgest109	5	0.401	0.059	0.528	0.046	4.9	167-224
Pgest142	13	0.698	0.055	0.725	0.023	3.3	196-238
Pgest173	8	0.255	0.050	0.527	0.043	20.9	172-193
Pgest21	6	0.728	0.062	0.708	0.025	1.6	240-255
Pgest227	15	0.585	0.072	0.702	0.059	2.2	179-230
Pgest24	8	0.546	0.072	0.620	0.047	17.6	139-160
Pgest293	6	0.542	0.063	0.556	0.057	7.1	237-252
Pgest318	9	0.767	0.059	0.672	0.048	7.1	253-277
Pgest325	9	0.376	0.046	0.604	0.030	25.8	168-189
Pgest341	5	0.374	0.074	0.451	0,042	14.3	209-221
Pgest353	7	0.501	0.081	0.587	0.026	18.1	230-248
Pgest59	8	0.321	0.067	0.570	0.049	27.5	209-233
Pgestssr255	7	0.547	0.065	0.537	0.048	9.9	230-251
Pgestssr279	5	0.525	0.071	0.487	0.039	19.8	174-189
Pgestssr368	7	0.494	0.069	0.486	0.053	15.4	220-250
PgtCAA39	29	0.831	0.046	0.807	0.034	3.8	191-284
PgtCAA53	10	0.761	0.048	0.682	0.023	0,06	184-232
PgtCAA80	9	0.762	0.049	0.653	0.035	0.038	222-246
PgtCAT4.2	10	0.719	0.056	0.696	0.034	0.033	117-144
PgtGAA8.1	10	0.766	0.043	0.719	0.024	0.115	176-211
Total mean	186	0.575	0.016	0.616	0.010	0.112	

3.2. Population information

The proportion of unique multi locus genotypes were 97.8 %, indicating a high genetic diversity. A lower genetic diversity was observed in some populations, for example two barley populations, indicating clonal reproduction. The expected heterozygosity (H_e) values were between 0.3 and 0.74 with a total heterozygosity of 0.75. The H value (Shannon Weiner diversity index) varied between 1.61-3.49 with a total H value of 5.19. The rBarD was not significant in five of the 16 samples, indicating signs of random mating in those five field populations (see table 6). The mean proportion of successfully analysed samples was 91 %.

Table 6. Population values from barley, rye and wheat. Shannon index (H), expected heterozygosity (H_e) and adjusted Index of association (rBarD) and its corresponding p-values (prD) based on clone corrected data. Missing values are from not clone corrected. Population number corresponds to populations presented in table 1.

Population number	Crop	% genotypic diversity	No private alleles	Shannon index (H)	H_e	rbarD	prD	% of amplified samples
1	Barley	100	0.05	1.95	0.719	0.0329	0.094	70
2	Barley	100	0.05	2.2	0.673	0.0060	0.558	90
3	Barley	100	0.05	2.2	0.673	0.3609	0.001	90
4	Barley	100	0.1	2.3	0.71	0.1112	0.001	100
5	Barley	100	0.1	2.3	0.721	0.0577	0.002	100
6	Barley	84.6	0.05	2.31	0.705	0.4283	0.001	100
7	Barley	91.7	0.25	2.37	0.551	0.4429	0.001	100
8	Barley	100	0	2.2	0.711	0.1760	0.001	100
9	Barley	100	0.05	2.08	0.722	0.1734	0.001	80
10	Barley	100	0.2	2.3	0.722	0.0799	0.574	100
11	Barley	100	0	1.61	0.664	0.1179	0.007	71
12	Barley	100	0.1	2.4	0.71	0.0335	0.250	92
13	Wheat	100	0	1.95	0.321	0.0180	0.423	64
14	Wheat	100	0	3.09	0.539	0.1654	0.001	92
15	Wheat	97.1	0.2	3.49	0.63	0.2373	0.001	94
16	Rye	100	0	2.3	0.739	0.1618	0.001	100
Total		97.8		5.19	0.749	0.0759		

The H_o and F value for wheat were not significant. The H_e value for barley, rye and wheat were 0.733, 0.694 and 0.626 respectively, indicating difference. The F value for barley was 0.171, indicating inbreeding (see table 7).

Table 7. Information about barley and wheat calculated as separate populations

Host	Nr of samples	H_o (SE)	H_e (SE)	F (SE)	rbarD	p.rD
barley	**113**	0.607 (0.038)	0.733 (0.023)	0.171 (0.044)	0.0536	0.003
rye	**10**	0.591 (0.062)	0.694 (0.027)	0.157 (0.082)	0.1619	0.001
wheat	**63**	0.465 (0.064)	0.626 (0.037)	0.293 (0.080)	0.1159	0.001

When divided the analysis based on cultivars, there was a change in the rBarD value. The Makof and Happy cultivar had non-significant p.rD value, indicating random mating (see table 8).

Table 8. Adjusted index of association (rBarD) and its associated p-value (p.rD) for samples collected from the different cultivars

Crop		pbarD	p.rD
Barley	Makof	0.0194	0.406
Barley	Planet	0.1114	0.001
Barley	Fairytale	0.1112	0.001
Barley	Laureate	0.1352	0.001
Barley	Irina	0.1760	0.001
Barley	Severi	0.1734	0.001
Barley	Dragoon	0.1179	0.009
Rye	Performer	0.1618	0.001
Wheat	Happy	0.0180	0.420
Wheat	Diskett	0.1279	0.001

3.3. Analyses based on genetic distance

The PCoA showed spatial differentiation between the barley and wheat clusters with some overlapping samples: The rye population was located between these two clusters but mainly within barley cluster. There were also three barley isolates clustering within the wheat cluster (figure 6).

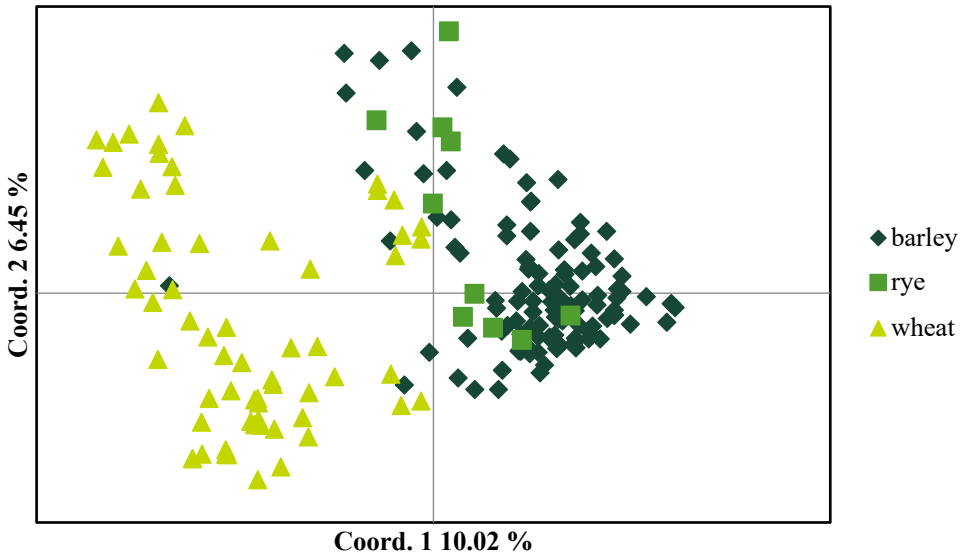


Figure 6. Principal coordinates analysis of samples collected on wheat, barley and rye based on genetic distance. Colours indicate the crop from which the sample was collected. The first axis explains 10.02 percent and the second 6.45 percent of the variation.

The Minimal Spanning Network (MSN) based on Bruvo-distance shows that the barley and wheat were grouped in two branches (figure 7). However, there was a barley branch in the wheat cluster and a wheat branch in the barley cluster. There were also four isolates of barley in the wheat branch. Thus, the two branches are not fully separated. The rye population were spread along the barley branches. The width of the lines between samples indicates the genetic differences (thin lines indicates a large genetic distance between the samples, thick lines indicate small genetic differences between samples) (figure 7). Looking at which isolate that was in the main branch with the most samples of each crop, the wheat isolates in the barley branch belong to the same population, in contrast to the wheat branch in which the barley branch belong to 8 populations. Clones were found in two barley populations and in one wheat population (population 15).

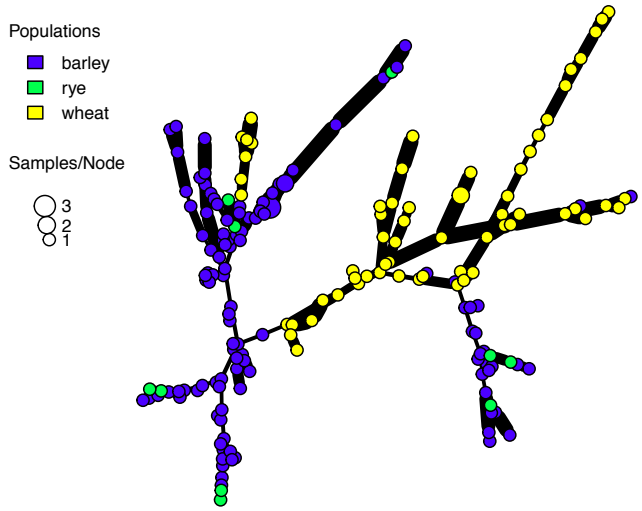


Figure 7. Minimal Spanning Network based on Bruvo-distance, the colours indicate from which cereal host the samples were collected. The size of the circles indicates the number of individuals detected for each MLG.

3.3.1. Difference in patterns between crops, cultivars and geographic distance

To determine the how the genetic variation was distributed between the different strata (regions, crops, populations, and among and within individuals), AMOVA were performed. The AMOVA conducted on regions showed a 5 % genetic difference explained by region. Between all the populations, 9 % of the genetic difference was found between the regions, 7 % between the crops and 5 % between the cultivars (see below).

AMOVA of crop and geographical regions

The AMOVA with crops (barley, rye and wheat) and regions as a stratum, resulted in a variation distribution of 5 % among crops and 7 % among cultivars. Thus, 88 % of the genetic variation could be explained among and within the individuals (see table 8). The F_{st} value between the subpopulations were 0.137. Mantel-tests showed no geographic impact on the genetic variation. For all populations on barley, wheat and rye, the P-value was 0.289.

Table 9. AMOVA of all populations of barley, rye and wheat and geographical regions

Source	df	SS	MS	Estimated variation	%
Among Regions	4	219.453	54.863	0.395	5%
Among Populations	11	265.049	24.095	0.707	9%
Among Individuals	170	1484.823	8.734	1.808	23%
Within Individuals	186	952.000	5.118	5.118	64%
Total	371	2921.325		8.028	100%

AMOVA of crops and cultivars

AMOVA based on crops and the cultivars as strata showed a variance of 7 % explained by the crop and 5 % on the cultivar (see table 9). The F_{st} value between the different cultivars was 0.124.

Table 10. AMOVA with crops and cultivars as strata

Source	df	SS	MS	Estimated variation	%
Among crops	2	177.406	88.703	0.603	7%
Among cultivars	7	149.723	21.389	0.416	5%
Among Individuals	176	1642.196	9.331	2.106	26%
Within Individuals	186	952.000	5.118	5.118	62%
Total	371	2921.325		8.244	100 %

AMOVA of crops

Another AMOVA was performed with the crops as populations, showing a genetic difference explained as 10 % explained by the different crops (table 10). The F_{st} value between the crops was 0.100.

Table 11. AMOVA with the crops as populations

Source	df	SS	MS	Estimated Variation	%
Among crops	2	177.406	88.703	0.827	10%
Among Individuals	183	1791.919	9.792	2.337	28%
Within Individuals	186	952.000	5.118	5.118	62%
Total	371	2921.325		8.282	100%

4. Discussion

4.1. Partially overlapping populations of crops and high genetic diversity

Is population of P. graminis *in wheat and barley the same?*
The distribution of the samples from the different populations in the MSN based on Bruvo-distance showed that the populations of wheat and barley were divided into two main branches, but there were some samples collected from barley in the main wheat branch and vice versa (figure 7). Also, three samples from barley clustered with the wheat samples. The branch within the wheat that clustered together with barley samples originated from different populations, but interestingly, the wheat samples in the barley branch were from only one population. The PCoA analysis showed a somewhat similar pattern (figure 6) with two partially overlapping clusters of barley and wheat. The rye samples were mainly within the barley cluster in the PCoA, and only in the barley branch in the MSN based on Bruvo-Distance, but more samples are needed to draw conclusions about the rye population. This indicates that there is a selection on the populations from the different crops, and that certain individuals probably can infect both crops.

The AMOVA performed also indicate that there is a genetic differentiation between the crops, which could indicate selection on the populations when propagated on the different crops. 10 % of the genetic variation could be explained by the crop or 7 % among the crops and 5 % among the cultivars (see table 9 and 10). However, there was an 5 % difference explained by region (see table 8), this can probably be explained by the fact that two of three wheat samples were collected in a region where no barley samples were collected.

Combining the information, it can be concluded that there is neither three distinguished populations of *P. graminis* of barley, wheat, and rye nor that samples collected from all three crops belong to the same population. This indicate that *P. graminis* from barley and *P. graminis* from wheat are not fully genetically

separated, and that they belong to the same pathogen meta-population. However, there is a level of selection driven by differences in host preference.

High genetic diversity in in Swedish populations of barley, rye and wheat
The genetic diversity within *P. graminis* in wheat and barley is revealed by a high proportion of unique genotypes, a relatively high Shannon index and mean expected heterozygosity, strongly indicating sexual reproduction. The rBarD was significant in four of 16 samples, which indicates random mating in these population and thus sexual reproduction.

In some populations of *P. graminis* collected on barley and wheat, a few clones were detected within the populations. Combined with the information of non-random mating in 11 of 16 populations, this indicate that clonal reproduction of the fungus was occurring in eleven cereal fields. The clones also imply a selection for genotypes, fit for certain hosts that indicates that clonal propagation and spread occurs within fields. Concurrently, the overall low number of clones found in the studied material could be explained by the sampling timing and technique. For example, the distance to the *Berberis* spp. could explain the number of clones, the longer distance from the *Berberis* spp. the higher chance of clones.

The Mantel-tests did not show any correlation between the genetic and physical distances, which implies that the pathogen is mainly spread by wind.

4.2. Implications for cereal crop producers

The fact that the samples collected from barley, rye, and wheat were not fully genetically separated leads to the conclusion that *P. graminis* infecting barley also can infect wheat. If *P. graminis* would evolve in a way that gives the individuals of the pathogen the ability to infect all three crops, the disease would become a more serious problem since it could be spread clonally between neighbouring fields of different crops. That could cause severe outbreaks in several crops during the summer. The high genetic diversity in *P. graminis* may increase the risk of new, virulent races. In combination with the lack of resistance toward the new *P. graminis* races in current cultivars in Sweden, this could cause problem with stem rust in barley, wheat and rye production in the future.

To be able to answer the question how the disease will develop, and if the disease will be able to switch the host during the season, more studies of the evolution of *P. graminis* in combination with resistant testing of cultivars are needed.

4.2.1. Removal of *Berberis* spp.

Because of the risks mentioned in the previous chapter, removal of *Berberis* spp. in the landscape is motivated. By removal of *Berberis* spp., the sexual life cycle of *P. graminis* cannot be completed since the pathogen cannot infect the *Berberis* spp. in the spring. By this measure, the pathogen cannot survive the winter in Swedish climate. Moreover, the risk of an outbreak in the neighbouring fields is decreased with removal of *Berberis* spp., and it also decreases the risk of the emergence of new races of *P. graminis.* Another thing to take in consideration is that the cost of controlling new races of *P. graminis* would be spread over a long period, but the cost of *Berberis* spp. eradication must be paid today. Information to farmers about the benefits of removing *Berberis* spp. could be an effective way to handle the question, which historically was a successful in the UK (Barnes et al., 2020).

If a new regulation about eradication of *Berberis* spp. should be implemented, other values than the role of *Berberis* spp. in plant disease management need to be taken in consideration. For example, the role of *Berberis* spp. regarding biological diversity. Also, *Berberis* spp. can be an appreciated plant in the landscape and have a cultural value. On the other hand, greater problems with rust diseases could lead to an increased need of use of fungicides to limit negative impact of the disease in the future. Implementing such a regulation could cause conflicts of goals and these aspects need to be further investigated.

4.3. Future scenarios of the climate change

Another factor when discussing the question of *P. graminis* is the effect of climate change on the development of rust diseases. Since *P. graminis,* who causes the most damaging rust disease on wheat, are favoured by warm weather, it could possibly be a larger problem in cereal crop production in the future. A longer growing season could make it possible for the disease to infect the crop earlier in the season, while it on the other hand may be possible to sow the crop early which could possibly allow the crop to mature before disease onset. Therefore, the effects of climate change on *P. graminis* and other rust pathogens need to be monitored and further studied.

4.4. How could the study be improved?

One thing that could be improved in the study is the sampling technique. If possible, samples from both barley, rye and wheat would be collected in all regions. Also, a more detailed sampling protocol could be sent to the samplers with a standardized distance between the samples and where in the field they should be taken.

It would be of interest to do resistance testing of current populations *of P. graminis* on cultivars of wheat and barley since it may have changed since this was latest done in 2017. It would also be interesting to investigate population structure in *P. graminis* in future seasons to determine how the population structure will change.

It would also be interesting to investigate other factors that could affect the possibility to limit the number of *Berberis* spp. in the landscape. Other things than the pathological factors as earlier mentioned should be included regarding the value of *Berberis* spp., such as biological diversity and emotional or cultural values. Therefore, the question would be interesting to investigate in a more interdisciplinary manner since these factors would have an impact on the success of eradication programs and possible legislation.

4.5. Conclusion

Do the same population of P. graminis *infect both wheat, barley and rye in Sweden?*
There is a genetic differentiation between the populations infecting barley and infecting wheat, however the populations on the different crops are not fully separated since there are overlapping individuals. The rye population mainly cluster with the barley population, but the small sample size limits the possibility to draw any solid conclusion. There is probably a selection from the host crop between *P. graminis* f. sp. *tritici* and *P. graminis* f. sp. *hordei,* but the two populations belong to the same meta-population.

Other results confirmed in the study was that *P. graminis* is dispersed by wind and a high genetic diversity in populations of barley, rye and wheat does exist.

References

AGAPOW, P.-M. & BURT, A. 2001. Indices of multilocus linkage disequilibrium. *Molecular Ecology Notes,* 1**,** 101-102.

AGRIOS, G. N. 2005. *Plant Pathology,* Elsevier Academic Press, Dana Dreibelbis.

BARNES, G., SAUNDERS, D. G. O. & WILLIAMSON, T. 2020. Banishing barberry: The history of Berberis vulgaris prevalence and wheat stem rust incidence across Britain. *Plant Pathology,* 69**,** 1193-1202.

BERLIN, A. 2021-06-11 2021. *RE: Personal communication.*

BERLIN, A., DJURLE, A., SAMILS, B. & YUEN, J. 2012. Genetic variation in Puccinia graminis collected from oats, rye, and barberry. *Phytopathology,* 102**,** 1006-12.

BHATTACHARYA, S. 2017. Deadly new wheat disease threatens Europe's crops. *Nature*.

CEREAL DISEASE LABORATORY. 2016. *Rust Susceptible Berberis, Mahoberberis, and Mahonia* [Online]. Cereal Disease Laboratory. Available: https://www.ars.usda.gov/midwest-area/stpaul/cereal-disease-lab/docs/barberry/rust-susceptible-berberis-mahoberberis-and-mahonia/ [Accessed].

CHAVES, M. A. M., JOSÉ AND GUTERRES, CAROLINE AND GRAICHEN, FELIPE 2008. The cereal rusts: an overview. *Pest Technology,* 2**,** 38-55.

GOOGLE MAPS. 2021. Sampling locations of Puccinia graminis in Sweden. Sattelite picture. [Cartographic material]. www.maps.google.com [2021-10-17]

JIN, Y., SZABO, L. J., ROUSE, M. N., FETCH, T., JR., PRETORIUS, Z. A., WANYERA, R. & NJAU, P. 2009. Detection of Virulence to Resistance Gene Sr36 Within the TTKS Race Lineage of Puccinia graminis f. sp. tritici. *Plant Dis,* 93**,** 367-370.

JORDBRUKSDEPARTEMENTET 1994. Berberislag (1976:451);. : Jordbruksdepartementet

KAMVAR ZN, T. J., GRÜNWALD NJ. 2014. Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ,* 2:e281.

LEONARD, K. J. & SZABO, L. J. 2005. Stem rust of small grains and grasses caused by Puccinia graminis. *Mol Plant Pathol,* 6**,** 99-111.

LEWIS, C. M., PERSOONS, A., BEBBER, D., KIGATHI, R., MAINTZ, J., FINDLAY, K., BUENO-SANCHO, V., CORREDOR-MORENO, P., HARRINGTON, S., KANGARA, N., BERLIN, A., GARCIA, R., GERM·N, S., HANZALOV·, A., HODSON, D., HOVM¯LLER, M. S., HUERTA-ESPINO, J., IMTIAZ, M., MIRZA, J., JUSTESEN, A., NIKS,

R. E., OMRANI, A., PATPOUR, M., PRETORIUS, Z., ROOHPARVAR, R., SELA, H., SINGH, R., STEFFENSON, B., VISSER, B., FENWICK, P., THOMAS, J., WULFF, B. & SAUNDERS, D. 2018. Potential for re-emergence of wheat stem rust in the United Kingdom. *Communications Biology,* 1.

LIND, M. 2017. Svartrostangrepp på vete i Uppland tyder på att en besegrad fiende är tillbaka. *SLU-nyhet*.

MACHEREY-NAGEL 2018. Genomic DNA from Plant Used Manual.

MILGROOM, M. G. 1997. Genetic variation and the application of genetic markers för studying plant pathogen populations. *Journal of Plant Pathology,* 79**,** 1-13.

MILGROOM, M. G. & PEEVER, T. L. 2003. Population Biology of Plant Pathogens: The Synthesis of Plant Disease Epidemiology and Population Genetics. *Plant Disease,* 87**,** 608-617.

MITTAL NEHA, D. A. 2009. Microsatellite markers- A new practice of DNA based markers in molecular genetics. *Pharmacognosy Reviews,* 3.

NAGARAJAN, S. & SINGH, D. V. 1990. Long-Distance Dispersion of Rust Pathogens. *Annual Review of Phytopathology,* 28**,** 139-153.

OLIVERA FIRPO, P. D., NEWCOMB, M., FLATH, K., SOMMERFELDT-IMPE, N., SZABO, L. J., CARTER, M., LUSTER, D. G. & JIN, Y. 2017. Characterization of Puccinia graminis f. sp. tritici isolates derived from an unusual wheat stem rust outbreak in Germany in 2013. *Plant Pathology,* 66**,** 1258-1266.

PEAKALL, R. A. S. P. E. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6**,** 288-295.

PEAKALL, R. A. S. P. E. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics,* 28**,** 2537-2539.

PRETORIUS, Z. A., SINGH, R. P., WAGOIRE, W. W. & PAYNE, T. S. 2000. Detection of Virulence to Wheat Stem Rust Resistance Gene Sr31 in Puccinia graminis. f. sp. tritici in Uganda. *Plant Disease,* 84**,** 203-203.

ROELFS, A. P. & MARTENS, J. W. 1988. An International Systemof Nomenclature for Puccinia graminis f. sp. tritici. *Phytopathology,* 78:526-5.

ROELFS, A. P., R.P. SINGH, AND E.E. SAARI 1992. *Rust Diseases of Wheat: Concepts and methods of disease management.*

SAUNDERS, D. G. O., PRETORIUS, Z. A. & HOVMØLLER, M. S. 2019. Tackling the re-emergence of wheat stem rust in Western Europe. *Communications Biology,* 2**,** 51.

SINGH, R. P., HODSON, D. P., JIN, Y., LAGUDAH, E. S., AYLIFFE, M. A., BHAVANI, S., ROUSE, M. N., PRETORIUS, Z. A., SZABO, L. J., HUERTA-ESPINO, J., BASNET, B. R., LAN, C. X. & HOVMOLLER, M. S. 2015. Emergence and Spread of New Races of Wheat Stem Rust Fungus: Continued Threat to Food Security and Prospects of Genetic Control. *Phytopathology,* 105**,** 872-884.

STATISTICS SWEDEN. 2021. *Totalskörd, ton efter gröda och år* [Online]. Statistics Sweden. Available:

https://www.statistikdatabasen.scb.se/pxweb/sv/ssd/START__JO__JO0601/SkordarL2/table/tableViewLayout1/ [Accessed 2021-06-02 2021].
STOXEN, S. 2012. *Population structure of Puccinia graminis f. sp. tritici in the United States.* Master thesis, University of Minnesota.
SWEDISH BOAD OF AGRICULTURE 2021. Bekämpninsrekommendationer 2021. www.jordbruksverket.se/bekampningsrek: The Swedish Board of Agriculture.
VÄXTSKYDDSCENTRALEN LINKÖPING 2020. Nr 22. Är svartrost vanligare i år? *Växtskyddsbrev Växtskyddscentralen Linköping.*
VÄXTSKYDDSCENTRALEN SKARA 2018. Nr 28. Vete- och vårkornsorters mottaglighet för den nya populationen av svartrost i Sverige. *Växtskyddsbrev från Växtskyddscentralen Skara.*
WAPLES, R. S. & GAGGIOTTI, O. 2006. INVITED REVIEW: What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Molecular Ecology,* 15**,** 1419-1439.
ZADOKS, J. C. 1965. Epidemiology of Wheat Rusts in Europe. *FAO Plant Protection Bulletin*.
ZADOKS, J. C. 2008. *On the Political Economy of Plant Disease*, Wageningen Academic Publishers.
ZHONG, S., LENG, Y., FRIESEN, T., FARIS, J. & SZABO, L. 2009. Development and characterization of expressed sequence tag-derived microsatellite markers for the wheat stem rust fungus Puccinia graminis f. sp. tritici. . *Phytopathology,* 99(3)**,** 282-9.

Appendix 1

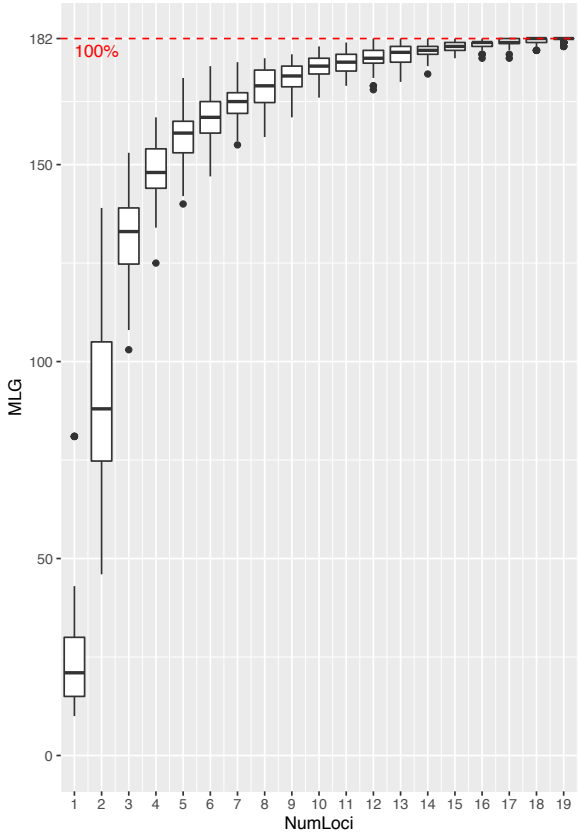
Between the steps; The solvent was mixed by pipetting up and down 15 times instead of mix by shaking.

1) Homogenize & lyse;
- Homogenizing was made with *Precyllys* tissue homogenizer (25 s, 5000 rpm).
- Centrifugation 5 sek 1100 rpm
- 200 µl MC1 was added
- Homogenizing with *Precyllys* tissue homogenizer (25 s, 5000 rpm).

The samples were then putting on a heating block.
2) The samples were then vortexed in 15 seconds followed by centrifuging in 2 min -1100 g.
100 µl cleared lysate was extracted
3) 7.5 µl NecleoMag® C-beads was added. 100 µl of the MC2 was added

Step 4, 5, 6; Instead of 600 µl of each MC2, MC3, MC4 and ethanol respectively, were 150 µl of each of the solutions added.
6, 7) Supernatant was removed by having the sampled on the magnetic bead in 2 minutes followed by 15 minutes incubation at 37 Cº.
8) 60 µl M6 solution was added.

Appendix 2



Supplementary figure 1. Genotype accumulation curve for the populations of barley, rye and wheat