Population Biology of
Puccinia graminis

- Implications for the Epidemiology and Control of Stem Rust

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Cover: Cross section of telia of *P. graminis* f. sp. *avenae*
   (photo: A. Berlin)

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Abstract

Barberry has made a noticeable comeback in the agricultural landscape after the repeal in 1994 of a law requiring its eradication. It has brought with it not only biological diversity, but also stem rust caused by *Puccinia graminis* (Pers.). Rusts have been known and feared for centuries. This thesis presents the results of studies of the population structure of *P. graminis* and connects this information to the epidemiology of stem rust. The studies were done by using SSR (simple sequence repeat) markers on samples from different hosts, years and areas. The results show that *P. graminis* genetically is a very diverse pathogen and no correlation in population structure could be detected between fields or years. However, there was a clear genetic differentiation between the *forma specialis* infecting oats and the *forma specialis* infecting rye and wheat. The aecial morphology also differed between the two, and the differentiation was reflected in a phylogenetic study. It was thus shown that *P. graminis* could be divided into two phylogenetically distinct species. The grass host is the driving force in the evolution of these species. In addition, *Puccinia arrhenatheri* was identified from barberry and its grass host.

   The presence of barberry, the alternate host of the pathogen, drives stem rust epidemics in oats, and removal of the barberry bushes would not only limit development of the disease but also reduce the genetic variation in the stem rust pathogen. Even if the fungus rarely goes through a sexual cycle, such as the situation in Tajikistan, the genetic variation is high in that country. The mere presence of barberry within an area enables a large genetic variability within the pathogen. Stem rust does not seem to be common on wheat in Sweden. This may be due to the lack of virulent genotypes of the pathogen, but if they were present, stem rust could become a major problem in wheat production, since all the prerequisites for epidemics are already present.

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Det är det lilla som är stort
–Robin Sharma

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I   A. Berlin, B. Samils, A. Djurle, H. Wirsén, L. Szabo, and J. Yuen (2012). Disease development and genotypic diversity of *Puccinia graminis* f. sp. *avenae* in Swedish oat fields. *Plant Pathology* Doi:10.1111/j.1365-3059.2012.02609.x

II A. Berlin, A. Djurle, B. Samils, and J. Yuen (2012). Genetic variation in *Puccinia graminis* collected from oats, rye and barberry. *Phytopathology* 102(10), 1006-1012.

III A. Berlin, M. Rahmatov, H. Muminjanov, and J. Yuen. Clonal and sexual reproduction of *Puccinia graminis* in Tajikistan (manuscript).

IV A. Berlin, J. Kyiaschenko, A. Fejer Justesen and J. Yuen. A survey of some rust fungi forming aecia on *Berberis* spp. (submitted manuscript).

V   A. Berlin, K. Dalman and J. Yuen. Evolutionary history reveals two phylogenetically distinct species within *Puccinia graminis* (manuscript).

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The contribution of Anna Berlin to the papers included in this thesis was as follows:

I   Planned the study together with co-authors. Collected samples and carried out the laboratory work. Analysed the data. Wrote the manuscript in cooperation with co-authors.

II Planned the study, collected the field samples, carried out the laboratory work and analysis of the data. Wrote the major part of the manuscript assisted by co-authors.

III Planned the study, carried out the laboratory work and analysis of the data. Wrote the manuscript assisted by co-authors.

IV Planned the study together with co-authors. Collected the field samples, performed the statistical analysis, carried out the morphological data collection on teila and uredinia, assisted in the phylogenetic study and wrote the manuscript in cooperation with co-authors.

V   Planned the study and carried out the laboratory work. Analysis of data and writing of manuscript in cooperation with co-authors.

Abbreviations

AMOVA   Analysis of molecular variance
APR   Adult plant resistance
BSC   Biological species concept
BT   Beta tubulin
COI   Mitochondrial cytochrome oxidase subunit I
DNA   Deoxyribonucleic acid
EF1   Elongation factor 1-α
ESC   Ecological species concept
GCPSR   Genealogical concordance phylogenetic species recognition
IT   Infection type
ITS   Internal transcribed spacer
LDD   Long distance dispersal
MSC   Morphological species concept
Mya   Million years ago
PCR   Polymerase chain reaction
PSC   Phylogenetic species concept
SNP   Single nucleotide polymorphism
SSR   Simple sequence repeat
TMRCA   The most recent common ancestor

1   Introduction

Rust has been known and feared for 3000 years and stem rust, caused by *Puccinia graminis* (Pers.), remains a serious disease of cereal crops. *Puccinia graminis* is a heterogeneous species, including five spore stages and two hosts.

The Romans celebrated a special feast, Robigalia (April 25th) devoted to the cereal god Robigo, where among many things a red dog was sacrificed to please Robigo and remind him to chain up the dog star which was thought to save the crop from rust (Large, 1946; Zadoks, 1985a). Fragments of wheat infested with *P. graminis* f. sp. *tritici* were found in a storage jar from 1400 to 1200 B.C. in Israel (Kislev, 1982), confirming that it was probably that fungus which caused the wheat field to first turn red or orange, and then, if attacks were severe, the soil would be red with dust from the pustules and sometimes blow out in a reddish cloud. At the time of harvest, the dust had turned dark brown or black, as if it was the remains after the fields had been haunted by fire (Large, 1946; Zadoks, 2007).

From the seventeenth and into the nineteenth century, many tales about the rust lingered on. It was thought to be misfortunate if a red dog was in the cereal fields or to have a barberry bush in the hedge, because the bushes were thought to transfer some evil yellow-red contagion to nearby cereal fields (Large, 1946). Observations of rust were made over and over again more often in the proximity of barberry bushes than elsewhere. The first measure against rust was thus the destruction of barberry bushes, justified partly based on logic and partly on folklore. This led to the first law of barberry eradication which was passed in Rouen, France in 1660 (Large, 1946). The connection between the summer and winter spores and the spore stages on the alternate host, barberry, was confirmed by experimental studies by Schöler in 1816 and von Bönninghausen 1817-1818. Anton de Bary was the first to follow artificial infections in microscope in 1865 (Eriksson, 1896). Since then, stem rust has been one of the most well

studied plant diseases throughout the history of plant pathology (Agrios, 1997).

1.1 Background of the thesis

The last severe epidemic of stem rust reported in Sweden was in 1951 (Åkerman, 1952; Zadoks, 1965), and the yield and quality losses in wheat were recorded as one third of the total expected yield. Since then, the yield losses due to stem rust in wheat have been negligible in Sweden, and the disease lost its importance and fell into oblivion. Finally it was suggested that the Swedish law of barberry eradication, which had been in force since 1918, should be repealed (Karltorp, 1991) and this happened in 1994. The law was repealed since it was thought that fungicides and improved prediction and warning systems would be enough to manage stem rust.

In February 1999, unusually high levels of stem rust were recorded at the Kalengyere Research station in Uganda (Pretorius, 2000). The most striking with the disease outbreak was that the rusted entries were known to carry the 1BL-1RS chromosome translocation including the *Sr31*, *Lr26* and *Yr9* genes for stem- leaf- and stripe rust resistance respectively, which had been utilised and effective against rust during the last decades, particularly in the CIMMYT breeding programs (Vaidyanathan, 2011). That particular fragment was first translocated from the rye cultivar "Petkus" by Salzmunder and Weihenstephan in 1920-30 (Rabinovich, 1998) and is still common in many wheat varieties grown around the globe (Schlegel, 1997). The second remarkable observation at Kalengyere was that stem rust was known as a disease favoured by warm weather (Leonard, 2005), but this research station is situated at a high elevation and usually only stripe rust was found in that particular location. This meant that the fungus had not only overcome an important resistance gene, but also broadened its climatic adaptation.

Since the emergence of the group of races virulent to *Sr31* (Singh, 2011) in Eastern Africa, commonly known as Ug99, the threat of stem rust has regained its importance and the Ug99 group of races has been reported from South Africa, Yemen and Iran (Singh, 2011; Vaidyanathan, 2011).

After the repeal of the law of barberry eradication in 1994, the occurrence of barberry has increased in Sweden (Georgson, 1997; Rydberg, 2001; Bertilsson, 2002; Fröberg, 2006; Edqvist, 2007; Tyler, 2007; Jonsell, 2010). Concurrently, the incidence of oat stem rust has increased during the last decade and yield losses up to 30% (2 tonnes per hectare) have been reported from field trials (Mellqvist, 2010).

The overall objective of this project was to understand the population biology of *Puccinia graminis* and the epidemiology of stem rust. This should give relevant information to cereal producers on how to protect their crop against stem rust. The main questions asked to meet the objective were: Is barberry important for the stem rust population, i. e. is *P. graminis* predominantly reproducing clonally or sexually in Sweden? Is there any pattern in the distribution between genotypes, within and between fields and years, that can be used to make inferences about the spread of rust? Can rust from different grass hosts be distinguished by molecular markers, and if so, can rust samples from the alternate host be matched to rust collected from a grass host? Is there any phylogenetic differentiation within *P. graminis*?

1.2   The journey towards the answers

Samples of *Puccinia graminis* were collected during the summers of 2008–2010 in cereal growing areas in Sweden where stem rust was present. The development of disease was studied in oat fields (Paper I). Samples from the grass hosts and the alternate host, barberry, were collected. Based on this set of samples, the stem rust populations were investigated by using SSR (simple sequence repeat) markers to get a better understanding of stem rust epidemiology (Paper I and II). To investigate the population structure in another environment, samples from wheat and wild oats were collected in Tajikistan during the growing season of 2010 and analysed using the same SSR markers (Paper III). A microscopic study of rusts infecting *Berberis* spp., was performed in order to determine which species of rusts that were present on barberry in Sweden and the morphological and genetic differences between them (Paper IV). In connection to the barberry study, samples collected from oats and rye as well as the alternate hosts were studied with the same methods (Paper IV). Finally, the phylogenetic relationship between the *formae speciales* collected from grass hosts were investigated by comparing four different loci (Paper V). The evolutionary history of stem rust was also compared with the evolutionary history of the hosts.

1.3   Methods to study stem rust and *P. graminis*

The connection between barberry and stem rust was made long before de Bary followed the artificial infection in a microscope in 1865. Since de Bary's discovery, microscopy has been used to detect and characterize

morphological differences between and within *Puccinia* spp. and varieties within each species (Niks, 1986; Anikster, 2005). Also the fungal growth within the plant, such as haustorial development, resistance reactions and nutrient uptake from the host has been studied by visualizations of processes by using different types of microscopic techniques.

Since the rust fungi are biotrophic, they will only grow and multiply on living hosts. After Craigie's discovery of the function of the pycnia (Craigie, 1927), several crossing studies of the different variants within the rust species (Johnson, 1933; Johnson, 1946; Johnson, 1949) and studies of the inheritance of virulence (Loegering, 1962) were carried out.

Subdivision of *Puccinia graminis* based on ecological adaptation has been studied through inoculation of both the grass (Eriksson, 1896; Johnson, 1949) and the alternate hosts (Levine, 1932). This was followed by race analysis, which was developed after the theory of gene-for-gene resistance was presented by Flor (1946). The race differentiation is based on inoculations of a number of plantlets in a set of differentials, each having one single known resistance gene. The reaction on the hosts is then evaluated, scored, and the race is identified. The race identity shows the phenotype of the isolate and may be used for breeding purposes, population analysis or advice to farmers, since it provide information as to which resistance genes are effective (and which ones are not), with respect to that particular isolate.

The emergence of molecular techniques has enabled a new dimension in the studies of rusts. They permit studies of properties that could not be detected or seen with microscopy or various types of inoculation experiments. Since only small amounts of DNA are needed for many of the molecular based studies, environmental samples can be taken and used directly. In this way, the selection by propagation on "susceptible" hosts is avoided and also the time from sampling to analysis is reduced.

Different types of molecular markers have been used to study the pathogen. Since *P. graminis* is dikaryotic in the form that infects the grass hosts (and this is the most studied spore stage), it is important to choose co-dominant markers in order to detect both alleles.

With the development of new sequencing techniques, routine sequencing has become affordable. Several studies based on sequencing of fragments of various genes and introns, up to whole genomes have now been made. Rusts and other obligate biotrophic pathogens have shown to have very diverse genomes (Duplessis, 2011; McDowell, 2011) and by being able to sequence whole genome differences in phylogeny, evolution and genomic adaptation can be detected. When phylogenetic studies are used in

combination with analyses of transcription patterns, questions about host specificity, virulence and more will be possible to answer.

2 The Suscepts

2.1 The grass hosts

Grasses have been studied for centuries due to their economic and ecological importance. Grasses are flowering monocotyledons belonging to the family Poaceae (true grasses). The family includes a wide diversity of morphological and genetic differences, and species adapted to all terrestrial habitats on earth can be found (Kellogg, 1998). The grasses include around 10,000 species (Kellogg, 2000), among them cereals, such as wheat, barley, rye, oats, maize, rice, millet, sugar cane and sorghum and an abundant number of pasture and forage grasses.

The grass hosts of *P. graminis* belong to the group Pooideae and families Triticeae, Aveneae and Poaceae, which together comprises more than 2,700 species (Kellogg, 2000). The Pooideae family is mainly distributed in temperate regions of both the southern and northern hemispheres (Clayton, 1986). *Puccinia graminis* has a very diverse host range, and the fungus has been reported to infect 365 different grasses (Leonard, 2005). The numerous grass species susceptible to the dikaryotic summer spores of *P. graminis* are divided among 58 genera (Gäumann, 1959), of which some of those found in Sweden belongs to: *Alopecurus*, *Arrhenatherum*, *Avena*, *Beckmannia*, *Brachypodium*, *Briza*, *Bromus*, *Calamagrostis*, *Chrysopogon*, *Cinna*, *Cynosurus*, *Dactylis*, *Danthonia*, *Deschampsia*, *Elytrigia*, *Festuca*, *Glyceria*, *Holcus*, *Hordeum*, *Koeleria*, *Lolium*, *Melica*, *Molinia*, *Phalaris*, *Phleum*, *Poa*, *Puccinellia*, *Sesleria*, *Stipa*, *Trisetum* and *Triticum*. The most commonly studied hosts in connection with *P. graminis* are the cereals, but studies from forage and wild grasses have also been reported (Pfender, 2004; Abbasi, 2005).

The diversity within each host family and each species varies. In general, open pollinated grasses accumulate greater variety than self-pollinated species. Breeding of commercially grown crops has of course affected the

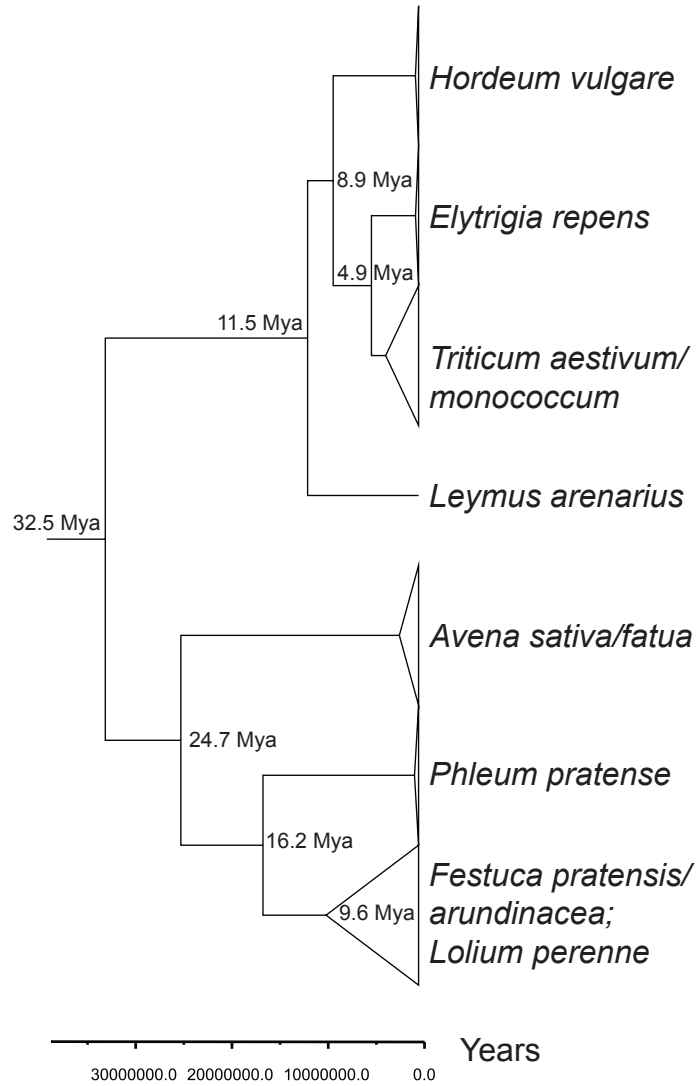*Figure 1.* Phylogenetic relationship between some of the grass hosts of *P. graminis* (Paper V). Figure by K. Dalman

diversity within each crop respectively. Another large difference between the commercially grown crops and their wild relatives is that the same or similar genotypes of the cereal crops are planted as monocultures on large areas whereas the wild relatives are growing in a natural mixed environment.

The phylogenetic relationship, based on the loci *rbcL* and *matK*, between host grasses was studied (Paper V). The hosts are divided into three groups; one consists of wheat, rye, barley and their wild relatives, one consists of oats and wild oats, and the third group includes wild and cultivated forage grasses. The oats and the wild grasses are more closely related to each other than to the wheat group (Figure 1). The calculated divergence times (Paper V) between the grasses correlate with the findings of others (Gaut, 2001; Kellogg, 2001; Chalupska, 2008).

2.2   The alternate host

The alternate hosts of *P. graminis* are *Berberis* spp. (Figure 2), a few species of *Mahonia* spp. and hybrids between the two (Gäumann, 1959; Roelfs, 1985). *Berberis* spp. (Berberidaceae) includes approximately 500–680 different species in 15 or 17 genera (Ahrendt, 1961; Landrum, 1999; Wang, 2007; Li, 2010) of which approximately 80 have been reported to be susceptible to stem rust (Gäumann, 1959). A given *Berberis* spp. is either resistant or susceptible to *P. graminis* (Roelfs, 1992).

   The group of *Berberis* with simple leaves (or true *Berberis*) includes approximately 500 species and the group with compound leaves (genus *Mahonia* Nuttal) comprises around 200 species (Ahrendt, 1961). The genus *Berberis* was divided into two main groups by Schneider in 1905; *Septentrionales* Schneider and *Australies* Schneider. The *Septentrionales* includes 300 species which are common in Eurasia, while four species are found in North Africa and two in North America (Ahrendt, 1961; Landrum, 1999). The characteristics of *Septentrionales* are yellow flowers, red berries and 1–3 (-5) fold spines. In contrast, the *Australies* occurs in South and Middle America, has black berries, deep orange flowers and foliaceous spines (Li, 2010). A phylogenetic study based on the ITS region confirmed the division between *Australies* and *Septentrionales*, but at lower levels the phylogeny did not concur with earlier findings (Kim, 2004).

   It is clear that barberry is a very diverse genus (Levine, 1932; Ahrendt, 1961), and it is extremely difficult to disentangle differences between species and distinguish species hybridization (Landrum, 1999; Kim, 2004). The debate about the species division is still on-going.

Most, but not all barberry species are susceptible to cluster cup rust caused by *P. graminis*, and only tissue two weeks old or younger is usually susceptible (Roelfs, 1985). Some specialization on the alternate hosts has been observed (Levine, 1932); the same race of *P. graminis* may give

different reactions on different barberry species. The most important susceptible species is *Berberis vulgaris* L. (Roelfs, 1985), which also is the most common barberry species in Sweden. The second most common is *Berberis thunbergii* (Krok, 2001), which is frequently cultivated in gardens and often seen in the wild. *Berberis thunbergii* is known to be resistant to *P. graminis*, but hybrids between *B. vulgaris* and *B. thunbergii* are common and they are in most cases susceptible to *P. graminis*. Since the diversity within the genus is large, it is very difficult to determine species other than the two common ones. The mode of growth also seems to vary depending on soil quality, access to sunshine and the competition from other bushes and shrubs at the place of growth. Other species that have been observed in Sweden are *B. julianae*, *B. aggregate*, *B. candidula*, *B. koreana* and *Berberis x ottawensis* (Artdatabanken, 2012). The only *Mahonia* spp. present in Sweden is *Mahonia aquifolium*.

Not only *P. graminis* has barberry as its alternate host, but *Puccinia brachypodii*, *Puccinia poa-nemoralis*, *Puccinia pygmaea*, *Puccinia montanensis* and *Puccinia brachypodii-phoenicoidis* have also been reported to form aecia on *Berberis* spp. (Cummins, 1966). Urban (1967) reported *Puccinia arrhenatheri* and *Puccinia pygmaea* on *Berberis* spp., though the former species, along with *P. poa-nemoralis* was considered to be a variety of *P. brachypodii* by Cummins and Greene (1966) based primarily on morphological criteria. It was recently found that *Puccinia striiformis*, causing yellow or stripe rust on wheat also has barberry as an alternate host (Jin, 2010). Gäumann (1959) predicted that other species, such as *Puccinia phlei-pratensis* and *Puccinia dactylis* also would have *Berberis* spp. as their alternate host due to their similarity to some *formae speciales* of *P. graminis*, although this has not been confirmed.

Rust species identified from barberry leaves were *P. graminis* (f. sp. *tritici* and f. sp. *avenae*) and *Puccinia arrhenatheri* (Klebahn) Eriksson (Paper IV). The rusts occurred both solely and in combination on the same bushes. No geographical differentiation of species could be detected. *Puccinia arrhenatheri* has a systemic appearance on the barberry bushes, creating witches brooms, whereas *P. graminis* appears in localized spots on the leaves, flowers, berries and young stem parts (Figure 2E and 2F). The species *P. arrhenatheri* was identified using sequence similarities and compared with rust infections on the grass hosts *Arrhenatherum elatius* and were thus identified as the species previously described as *Puccinia arrhenatheri* (Klebahn) Eriksson. The

Figure 2. A–E; *Puccinia graminis* and F; *P. arrhenatheri* on *Berberis* spp. Photo: A. Berlin

morphological characteristics of the fungus differed on the grass host as well (Paper IV).

Attempts to infer the phylogeny of the sampled *Berberis* spp. was made by sequencing the ITS region of the collected samples. In our case, this locus did not reveal any clear differences between the samples and when comparing our sequences to sequences deposited in GenBank, the morphology did not correspond with the species identifications of the deposited sequences. This may be expected since we are not the only ones

having difficulties to disentangle the differences within the genus using common barcoding loci (Roy, 2010).

3   Stem rust – the disease

Stem rust has a complex life cycle, including two different host plants: the shrub barberry and a grass host. The signs of the disease on the grass hosts are brown pustules predominantly on the straw, but sometimes also on the awns. When the crop matures, the pustules turn black (Figure 3). Crops infected with stem rust are prone to lodging before harvest (Roelfs, 1992).

Figure 3. Stem rust on oats; summer spores (urediniospores) of *P. graminis* (left), a combination of both summer and winter spores (teliospores) (middle), and winter spores (right). Photo: A. Berlin
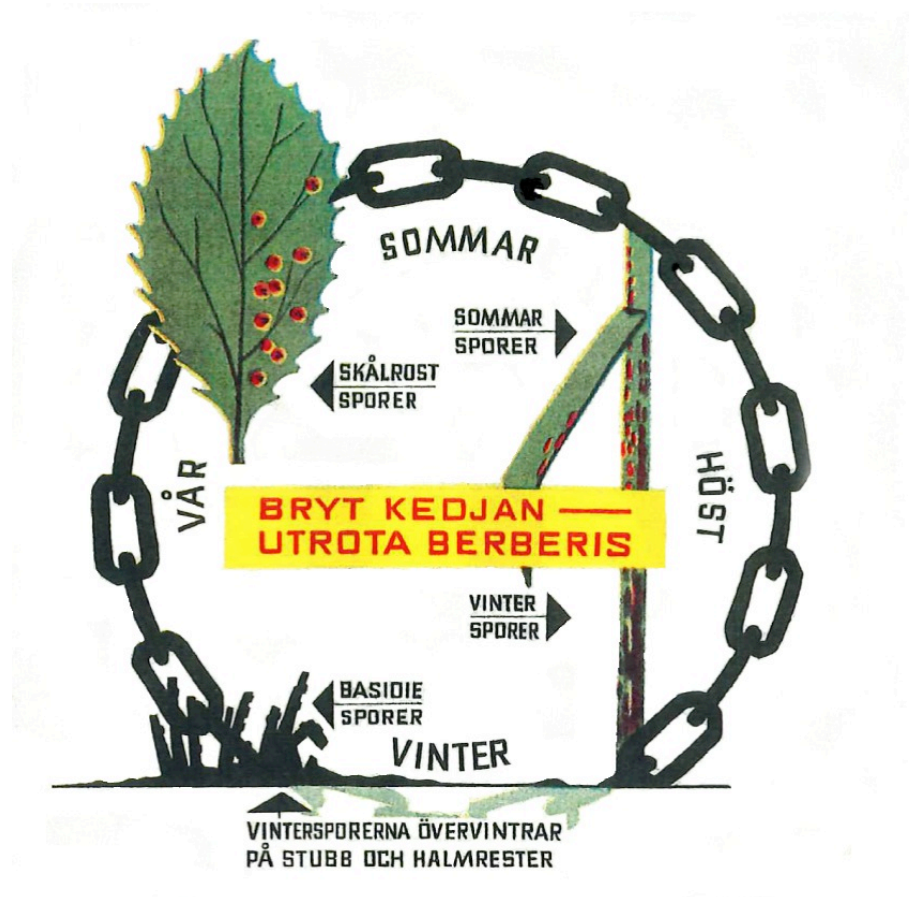
Figure 4. The disease cycle of stem rust. The illustration comes from one of the information leaflets, which was distributed to Swedish farmers during the barberry eradication program. The picture urges the reader to "break the chain – eradicate barberry". Printed with permission from Uppsala läns Hushållningssällskap.

3.1 Disease cycle

The rust overwinters on straw and plant debris in the field as black winter spores (teliospores). In springtime, these germinate into basidiospores, which can only infect barberry, where aecia (cluster cup rust) will develop (Figure 4). Spores from aecia on barberry may only infect cereals and grasses. On the infected cereals, reddish-brown summer spores (urediniospores) develop (Figure 4), mainly on the straws, and the fungus reproduces clonally in this stage of the disease cycle by reinfecting the grass host. Later when the crop matures, the black winter spores are produced in the same spore layer as the brown summer spores and the disease cycle of stem rust is completed.

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3.1.1 A yearly timetable of stem rust in Sweden – the usual suscepts

Stem rust may infect many different grass hosts, but the most agriculturally and economically important ones in Sweden are the two crops oats and rye. Oats is a spring-sown crop, planted in April or May and harvested in August or September. Rye, on the other hand is a fall-sown crop, which is planted in September, overwinters as seedlings and is harvested in August or September. The alternate host, *Berberis* spp. is a perennial woody plant. The first leaves emerge in the beginning of May and the first observations of cluster cup rust infections usually occur at the end of May or the beginning of June. The first signs of disease are observed in rye at the end of June and in oats in mid July. This pattern has been reported for a long time (Eriksson, 1896; Hermansen, 1968). Both spring and fall sown wheat is common in Sweden, but stem rust has not been observed in modern wheat cultivars. Only old land-races may occasionally show signs of disease.

3.2 Epidemiology

Plant disease epidemiology aims to understand the disease dynamics in both time and space (Milgroom, 2003). For disease to occur three factors are necessary; a favourable **environment** for disease development, a susceptible **host** and presence of **inoculum**. If one of these prerequisite is not met, there will be no or limited damage of the disease.

The temporal aspects of plant disease epidemiology are reflected by disease progress curves and investigations as to whether the pathogen studied is monocyclic or polycyclic. On the spatial scale, patterns of inoculum dispersal and disease development are studied, both quantitatively and qualitatively. In addition, analysis of dynamic changes integrating both temporal and spatial aspects can be included (Waggoner, 2000; Milgroom, 2003).

3.2.1 Environment

The stem rust has been known to be favoured by warm climatic conditions (Leonard, 2005), and the optimal growing conditions vary between different development stages (Stubbs, 1986; Roelfs, 1992). At germination, both free water and darkness is essential for successful infection and after the formation of appressoria and penetration by the fungus into the host, a period of 3 to 4 hours without free water combined with a high light intensity is needed (Table 1). With a changing climate, it is possible that the geographical distribution of *P. graminis* will alter (Roderick, 1994; Shaw, 2011).

Table 1. *Environmental conditions required for stem rust infection. Table based on Stubbs et al. (1986) and Roelfs et al. (1992).*

Process	Moisture	Temp (°C)	Light
Initiation of germination	Free water required for 2 or more hours	min >5, optimum 15-24, max 30	Strong light may inhibit stored spores, less effect on fresh spores
Germ tube growth	Free water required	Optimum ca 15-24	As above
Appressorium formation	Free water required	Optimum ca 20, 16-27	Favoured by dark conditions
Penetration	Not necessary	15-40, Optimum ca 30	Favoured by bright light
Sporulation	Adequate moisture required by host, no free water	15-40, Optimum ca 20-30	Adequate light favours host and pathogen

To understand the disease development within fields, microclimatic data was recorded during the three growing seasons in four different fields, one in each of the main cereal growing areas of Sweden. The temperature and humidity was recorded every 30 minutes from the beginning of May until just before harvest in mid August, using micro climatic loggers (Tiny-tag, Intab, Sweden). The in-field relative humidity (%) was favourable for rust infection on most days without rain. On rainy days the relative humidity was 100% for the whole day (Wirsén, 2008). The temperature in the fields agreed with the temperatures measured in nearby weather stations and could affect the variation in the rate of disease development.

In Sweden, the low temperature before the end of June prolongs the latency period, and contributes to the later appearance of uredinia on the grass hosts. The first reported incidence of stem rust on rye is usually at the end of June, and on oats in mid-July. Stem rust demands a slightly higher temperature for optimal infection than is usually recorded in June. In July, the temperature is usually more favourable for stem rust infection and that might be one reason why the incidence and severity of disease rapidly increases during July. Stem rust on rye usually is reported earlier than stem rust on oats, and this may be due to overwintering of *P. graminis* on the fall sown crop.

3.2.2 Hosts

Since *P. graminis* is an obligate biotrophic fungus, it needs a living, susceptible host to survive. Most barberry species are susceptible to stem rust (Levine, 1932), and may serve as a source of inoculum to susceptible grasses. The fungus was early divided into different *formae speciales* (Eriksson, 1896),

based on its adaptability to certain genera or groups of grass hosts, i.e. a specimen infecting oats was denominated *P. graminis* f. sp. *avenae* and a specimen infecting rye *P. graminis* f. sp. *secalis* (Eriksson, 1896). The different *formae speciales* showed lower fitness or did not infect the other grass hosts (Johnson, 1949).

Host resistance

Flor (1946) introduced the concept of gene-for-gene resistance, which is the basis for the current race nomenclature and accounts for a large proportion of the breeding efforts against cereal rusts. Races define the phenotypes of rusts, but not the genotypes. For many *Puccinia* spp. the race spectrum is used to characterize rust isolates. By inoculating a set of plantlets with known resistance (differential lines or varieties), a pattern of resistance and susceptibility can be seen, and through a nomenclature system, races are denominated (Roelfs, 1988; Fetch, 2007). Environmental conditions, like temperature, might affect certain types of resistances (Roelfs, 1986; Fetch, 2006).

The durability of resistance in a host plant towards a pathogen is a function of an evolutionary process, which depends on the pathogen fitness, possibility to recombine, and mutation rate. Durability of resistance in the host plant is negatively correlated with the evolutionary potential of targeted pathogens (McDonald, 2002; Milgroom, 2003). Approximately 50 different stem rust resistance genes have been described in wheat (McIntosh, 1995; Singh, 2011) and are being deployed in resistance breeding. Many of the resistance genes are race specific, in accordance with the gene-for-gene concept, where one resistance gene corresponds to one virulence gene in the pathogen (Singh, 2011). These are relatively easy to deploy, but the likelihood that the pathogen will overcome the resistance is high. In contrast, adult plant resistance with complex inheritance is often called slow rusting resistance. This type of resistance does not lead to a total resistance against the rusts, but rather to a moderate susceptible to susceptible reaction in the host plant (Singh, 2011). It is believed that the strategy of accumulating several minor genes will lead to more durable resistance. This resistance is more difficult to achieve, but desirable.
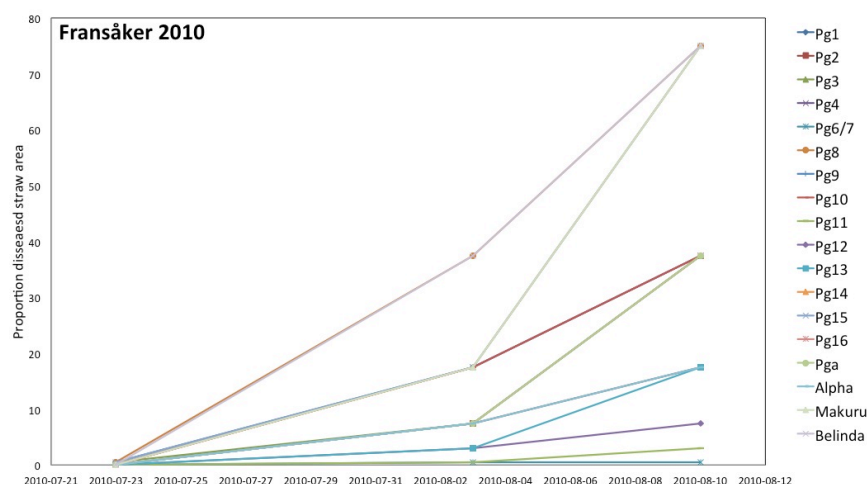
Figure 5. Disease development recorded as the proportion of diseased stem area in differential lines and oat cultivars planted at Fransåker 2010.

Race spectrum of oat stem rust in Sweden

Attempts to analyse the race differences were made using the north American differential lines (Fetch, 2007), but did not succeed. In greenhouse experiments, the differentials showed lower infection types (IT) than expected. The difficulties in the race analysis was probably due to the presence of a resistance gene (effective in Sweden) in the background of the differential set.

The differentials and susceptible host varieties were planted in a field outside Uppsala (Fransåker 2010) and the incidence of disease was evaluated at four times during the growing season (Figure 5). The lowest levels of disease were recorded for the differentials containing the resistance genes *Pg6/7*, *Pg11* and *Pg12*, at this particular location and year. The highest level of disease was recorded for the cultivar Belinda, which is the Swedish standard cultivar for oats, and the differentials containing the resistance genes *Pg8* along with *Pg14*, *Pg15* and the cultivar Makuru. The other differentials showed intermediate level of susceptibility. The observed delay of disease increase indicates that individuals with virulence to these resistance genes were present but in lower frequency or arrived later. This can be compared with the race analysis from Sweden made by MacKey and Mattsson (1972) who found a large number of races even during the years of barberry eradication.

Unfortunately, few resistance genes are known for oat stem rusts (Gold Steinberg, 2005; Park, 2008), but these results show that known resistance

28

genes may be effective against the oat stem rust population in Sweden. At least one of them, *Pg11,* is an adult plant resistant gene (Fetch, 2007), which would be the most suitable since stem rust usually arrives late in the season.

3.2.3 Inoculum

Inoculum for stem rust is the spores of the fungus *P. graminis*. Cereal rusts are known to be able to spread over large distances (Kolmer, 2005) and *P. graminis* is no exception. The spores of *P. graminis* are spread by wind at various scales, from barberry bushes to fields, within fields, between fields, within a region and between regions.

The different spore types are thought to have different ranges of dispersal. Basidiospores are sensitive both to drought and solar irradiation and their dispersal is probably in the range of 100's of meters (Roelfs, 1985). On the other hand, basidiospores of the pine blister rust have been reported to be transported up to 8 km with night breezes (van Arsdel, 1967).

The barberry (*Berberis* spp.) is susceptible to infections of *P. graminis* until 12 to 16 days after the leaves unfold (Cotter, 1930). It takes approximately 5-7 days until pycnia are formed and another 5-7 days until aecia appear on the infected barberry leaves (Levine, 1932). The aecia have been reported to produce aeciospores up to 46 days after appearance (Cotter, 1930). Aeciospores are released when the wet aecia dry, and spores may be released. There is no proof that aeciospores are not transported up to hundreds of kilometers (Roelfs, 1985). Successful infections by aeciospores result in uredinia and urediniospores on the grass host.

In the uredinial stage of the life cycle, *P. graminis* is polycyclic, and the urediniospores may re-infect the grass host in close proximity or far away. The urediniospores of *P. graminis* (also known as summer spores) may travel far distances by air and stay viable since their dark surface and tough cell walls makes them withstand solar radiation (Maddison, 1972).

When the crop matures, the fungus starts to produce dark brown or black winter spores, also called teliospores, in the same sori as the urediniospores. The overwintering teliospores remain attached to the straw by a stalk in the telia. In this stage, the spread is mainly by man or water when straws are moved. The teliospores must overwinter, and will not germinate into basidiospores until they have gone through weathering by alternating drying and wetting with freezing and thawing (Roelfs, 1985).

Long distance dispersal (LDD)

Long distance dispersal (LDD) of plant pathogens refer usually to spores that are displaced over hundreds or thousands of kilometres from their origin

(Brown, 2002). Typically LDD is man mediated, spreading the infected material on clothes or by infected plant material. For rust, LDD is also possible by air.

The most common way of dispersal is a step-wise expansion, where the pathogen gradually expands within a region (Singh, 2006). One example of this is the spread of Ug99 from Uganda and Kenya to Yemen and later to Iran (Singh, 2011). A variation of this step-wise expansion is extinction and re-colonization. The classical example of this is the "Puccinia pathway" in the USA, where stem rust overwinters in the south during winter and then gradually expands northwards during the spring and summer when susceptible hosts become available (Roelfs, 1986).

Zadoks (1965) discussed the two main migration paths of rusts that lead to Scandinavia, the western and the eastern tract. The western tract follows the Atlantic coast from Morocco to Great Britain. The East European Tract, starts in Greece or even further south, then proceeds to the Danube plain where it splits into two branches (Nagarajan, 1990). One branch fades out over the west Danube plains and the other tract goes over Ukraine, Poland and ends up in Scandinavia. The later migration pathway is thought to be responsible for the stem rust epidemics in Scandinavia in 1932 and 1951 (Zadoks, 1985b).

3.2.4 Disease progress

To evaluate the level of disease within a field, the incidence and severity of disease is often recorded. The disease incidence is defined as the "number of plant units infected" and it is often reported as a percentage or proportion of the total number of assessed plant units. Disease severity is defined as "the area of plant tissue affected by disease" and is reported as the percentage or proportion of the total area affected by the disease (James, 1974; Zadoks, 1979).

When the incidence and severity of disease are known, the disease development, or the rate of disease progress can be calculated. To calculate this rate, the incidence and severity of disease must be registered on at least three occasions. The rate of disease progress may be expressed as the apparent infection rate (r) and calculated with the equation $dx_t/dt = rx_t (1-x_t)$, where x_t is the disease proportion at time t. This equation assumes that the disease increases according to logistic growth (Zadoks, 1979). The disease progress may be considered rapid when the value of r is close to 0.4 (Vanderplank, 1963).

A fully developed epidemic according to logistic growth creates an S-shaped curve, where the incidence of disease is low in the beginning and

then increases more rapidly to an inflection point (50% for a logistic curve). At this point, the available plant tissue becomes a limiting factor and the curve flattens out, approaching 100 % infected straws or tissues. The logistic growth model represents a very simplified way of looking at an on-going epidemic in the field. Even if the model is simplified, it is still usable as a tool to understand the increase of a disease within a field.

The latency period is the time from infection until the production of new inoculum. The latency period, together with the amount of sporulation and pustule development, may be used as measures of aggressiveness for a pathogen strain or isolate. Resistant varieties prolong the latency period, produce smaller pustules and fewer spores, which will delay the disease development and limit the risk for an epidemic.

Timing of the disease

The damage caused by stem rust depends on the crop's growth stage, the amount of initial inoculum, and the susceptibility of the host at the time of infection. The disease progress of stem rust is often rapid and can cause severe yield losses if *P. graminis* infects the crop at booting or heading (Vanderplank, 1963). If the infection onset is late, at the soft dough to mature stage, the losses will be none or negligible. The time of infection is therefore important in order to be able to estimate the potential damage of the disease and to decide if it is economically beneficial to treat the fields with fungicides.

Disease development in fields

The first signs of disease in the evaluated fields were reported between 10th of July and 1st of August in the years of 2008 and 2009 (Paper I). The rate of disease progress (*r*) ranged between 0.38 and 0.55 when calculated based on the incidence and between 0.17 and 0.38 when calculated based on the severity. The highest values were obtained for the field at Stora Bärby, which was infected early (10th of July). In the field at Fransåker 2009, the large differences in *r* between the values of incidence (0.46) and severity (0.17) may be because many spores infected the field concurrently and the area of each pustule might not have had time to develop between the times of scoring. The rate of disease may be seen as an average of three or more snap-shots of disease and what the values obtained tells, is that there is a potential for fast disease development.

The general theory of rust infection in a field is that one or a few spores successfully infect the field, creating one or a few focal points of disease

within the fields. The population biology studies (Paper I and II) show that it is not one or a few individuals infecting oats or rye fields in Sweden, but a great variety of genotypes initiating the disease. Spores are probably immigrating continuously into the fields, both from local barberry but probably also from more distant sources. This means that the disease does not have to go through as many clonal cycles until the level of disease reaches damaging levels. When the disease arrives in the field, the development is rapid.

The impact of immigrating spores decreases with the level of disease present in the field (O'Hara, 1996; Yuen, 2012). The genotypes of a disease agent that first infects the field have a strong selective advantage (Hovmøller, 2002; Wingen, 2012). This must be true also for stem rust, since the amount of host tissue available decreases with increasing level of disease over time. Stem rust usually arrives late and does not fully complete the S-shaped curve (Vanderplank, 1963). However, even if the disease arrives late into the fields and is reported late in the season, the disease may cause substantial yield losses.

3.3   Stem rust with sexual reproduction

Like many other fungal pathogens that have a sexual stage, *P. graminis* is sexual for only a brief period of the year and the pathogen reproduces clonally for most of the growing season. In the presence of barberry in combination with a temperate climate, such as the conditions in Sweden, the stem rust is forced to fulfil its whole life cycle to survive. The presence of barberry also makes the local populations permanent, since the life cycle is not interrupted. It has been shown, that sexual reproduction enhances the rusts ability to start to produce teliospores (Roelfs, 1982; Ali, 2010). Sexual reproduction of *P. graminis* as a result of the presence of the alternate host within an area will produce an abundance of spores with different genotypes, which then initiate disease (Paper I).

Sexual reproduction limits the importance of race-specific resistance genes within the host crop since recombination will enable the fungus to broaden its virulence diversity and the gene-for-gene resistance may potentially break down within a short time (Jin, 2011). The offspring could differ in aggressiveness and might have different ecological strategies in their mode of growth, and reproduction, as well as in competition (Newton, 1999). The sexual recombination may also "dilute" the aggressiveness of the pathogen, resulting in a large number of genotypes with less fitness than a successful, clonally reproduced genotype selected for high fitness (Lehtinen,

2009). Earlier studies have also shown that the fitness of hybrids between different *formae speciales* is lower than for a pure specimen (Johnson, 1933; Johnson, 1949). Since the genetic diversity is very high in the presence of barberry, one can only speculate as to the number of races present (Loegering, 1962).

Introduction of new virulence by LDD in a sexual population will probably not be seen in the year of introduction. If an aggressive strain will succeed depends on the access to suitable hosts, the host's resistance genes, and level of disease within the field at the time of infection, and the possibility to survive in the clonal uredinial stage. If all of these prerequisites are in favour of the pathogen, a single clonal lineage may take over the whole population. In the presence of *Berberis* spp., it is very difficult for this to happen, since the alternate host provides a possibility for sexual recombination, selecting for telia development (Ali, 2010).

Barberry is thought to enable early infection of stem rust (Roelfs, 1982), but I would argue that although barberry keeps the fungus present in Sweden, these infections do not come early. One possible early source could be asexual urediniospores from warmer regions, but this has not been observed recently. Completion of the fungus's sexual stage on barberry could delay the disease onset compared to immigrating urediniospores, because of the time needed for germination of basidiospores, infection and fusion on barberry and then release into susceptible cereal fields. A comparison can be made to the yellow rust, which has caused a lot of damage during the last years. Yellow rust is considered to reproduce more clonally than the stem rust in Scandinavia (Hovmøller, 2011), even if sexual reproduction may occur. Yellow rust may survive the winter on the fall-sown crops in southern Sweden, providing inoculum before barberry develops leaves in the beginning of May. If *P. graminis* could survive clonally in Sweden, stem rust would probably cause much more damage than it currently does. If yellow rust does reproduce sexually in Sweden, it would be very difficult to detect since the early immigrants in a field are more important than the later ones (O'Hara, 1996; Yuen, 2012). Selection has acted on clones for higher aggressiveness, and newly produced individuals would not be able to spread efficiently since the availability of susceptible host tissue would be limited and hence the new genotypes would be difficult to detect when first produced. These new individuals could eventually displace the dominating clones if they are more aggressive.

3.4 Stem rust without (obvious) sexual reproduction

Due to the complex life cycle of the pathogen and the sturdiness of the urediniospores, the epidemiology of stem rust may differ depending on the conditions in different parts of the world. Where barberry is absent or where stem rust has the possibility to survive on a grass host year round, clonal reproduction is thought to be predominant. A green crop must be present in the field throughout the year to enable a solely asexual, clonal population. Primarily clonal populations are found in Australia (Haque, 2008) where no susceptible barberry is present, in Eastern Africa where susceptible cereals are produced year round but at different elevations (Singh, 2006), or in the USA where the disease spreads northwards each growing season (Roelfs, 1986). Under such conditions, strong selection acts on the fungus and a few genotypes will dominate the population (Pariaud, 2009).

The study based on samples collected in Tajikistan shows that even if the clonal propagation of stem rust is predominant, the presence of barberry creates genetic variation in the stem rust population (Paper III). In Europe, yellow rust epidemics have a similar pattern, and one or a few races dominate the population in large parts of Europe (Hovmøller, 2011).

4 *Puccinia graminis* – the pathogen

Rust fungi belong to the phylum Basidiomycota, order Uredinales, family Puccinales. They are distributed throughout the world, comprise a very diverse group of plant pathogens, and include many economically important plant pathogens (Cummins, 2003; Aime, 2006).

Puccinia graminis is a macrocyclic rust fungus with five spore stages (Figure 6). The teliospores overwinter on the grass hosts. In the teliospores, the fungus goes through karyogamy and meiosis. In spring, the teliospores germinate into haploid basidiospores, which infect young leaves of *Berberis* spp., where pycnia are formed. When a receptive hypha of one pycnium has been fertilized by pycniospores of the opposite mating type, aecia are formed from which dikyarotic aeciospores are released. The aeciospores infect a grass host where pustules with urediniospores develop. The dikaryotic urediniospores can infect the grass host over and over again, and the reproduction is asexual at this stage of the life cycle. When fall comes and the grass host senesces, the fungus prepares for overwintering by changing the spore production in the pustules from urediniospores to teliospores.

The form infecting the alternate host, barberry, was first reported by N. J. Jacquin in 1786 with the name *Lycoperdon poculiforme* (Eriksson, 1896) and five years later Gmelin gave it the name *Aecidium berberidis*. Felice Fontana was the first who described the uredinial and telial spore stages on the grass hosts in 1767, and called the fungus "ruggine rossa" and "ruggine nera" respectively. During the next century, the fungus was described several times and by different names. The first time the name *Puccinia graminis* appeared was in 1797 when Persoon gave the overwintering spores (teliospores) the name *P. graminis*.
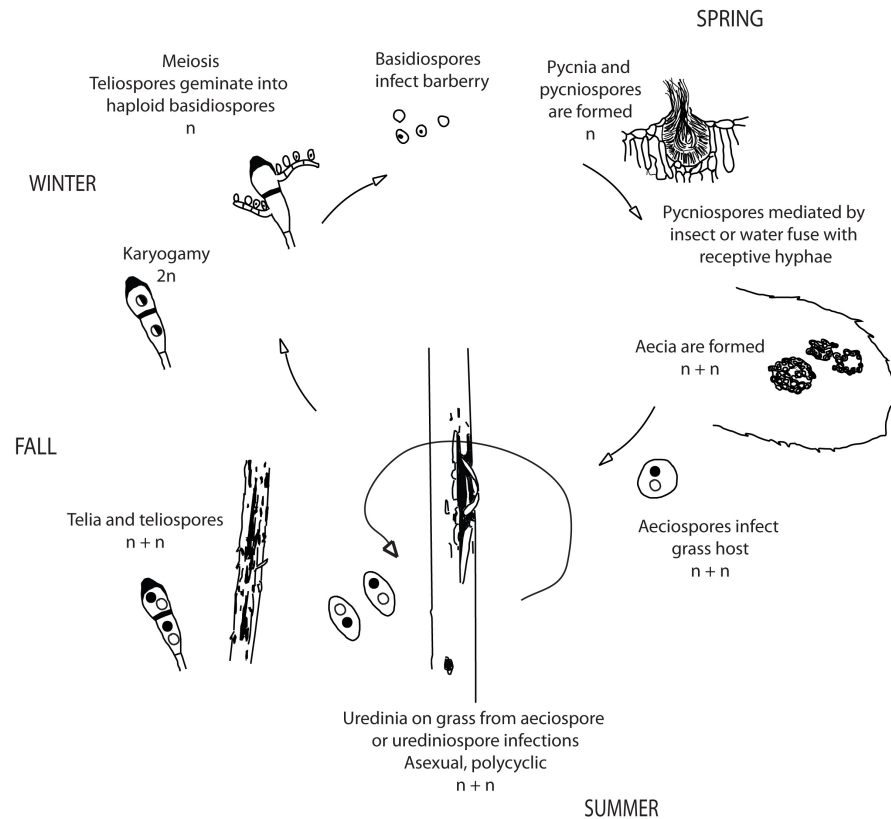
SPRING

Meiosis
Teliospores geminate into
haploid basidiospores
n

Basidiospores
infect barberry

Pycnia and
pycniospores
are formed
n

WINTER

Pycniospores mediated by
insect or water fuse with
receptive hyphae

Karyogamy
2n

Aecia are formed
n + n

FALL

Telia and teliospores
n + n

Aeciospores infect
grass host
n + n

Uredinia on grass from aeciospore
or urediniospore infections
Asexual, polycyclic
n + n

SUMMER

Figure 6. The life cycle of *P. gramnis*. Drawing by A. Berlin.

4.1 Population biology

A population can be defined as a group of individuals inhabiting a particular area, a group of inbreeding individuals, a group of individuals that are genetically isolated from others, or a group of individuals from which samples are taken for statistical measurements. The grouping may be defined by location, genetically or by sampling strategies (Xu, 2006).

The population biology of plant pathogens is often studied to gain a better understanding of the epidemiology of the pathogen of interest (Milgroom, 2003). It aims to describe a holistic perspective of the ecological, genetic and evolutionary principles within a population context to better understand the dynamics of plants and pathogens, and their interactions. More specifically, the tools of population biology may be used to track genotypes or the source of inoculum, or to investigate the predominant mode of reproduction within a pathogen population.

The disease incidence influences the mating pattern and thus the genetic structure of the pathogen. In regions with high incidence of disease, a high level of genotypic diversity is maintained and the differentiation among locations is predicted to be low (Barrett, 2008). In contrast, where incidence of disease is low, a pathogen has less possibility to recombine and the probability to encounter new genotypes is lower due to inbreeding and low genetic diversity, and this will lead to higher variation between geographic locations (Barrett, 2008).

Population studies of *P. graminis* have been carried out in different parts of the world, at different scales and with different methods (McCallum, 1999; Peterson, 2005; Keiper, 2006; Haque, 2008; Admassu, 2009; Visser, 2009; Zhong, 2009; Admassu, 2010). Most of the studies investigate the population diversity within or between regions. In general, the diversity of the pathogen is larger in the presence of barberry than in regions where no alternate host is found.

To investigate the mode of reproduction, simple sequence repeats (SSR), also called microsatellite markers, were used to infer the population structure within and between fields of the grass hosts as well as samples collected from barberry (Paper I and II). SSR markers are developed to target highly instable parts within an organism's genome. They present high levels of polymorphism due to high mutation rates e.g. caused by slippage during DNA replication. Until now, the knowledge about the function of SSRs is limited even if this type of marker is often used for various applications, including population genetic studies, breeding, studies of hypervariability and other features (Kalia, 2011). The advantage of using SSR markers in a dikaryotic organism like *P. graminis* is that they are co-dominant and alleles from both nuclei can be detected. By using SSR markers, many samples can be processed and differences within one species or closely related species may be detected.

Genetic differentiation within and between fields

The population biology studies (Papers I and II) show that it is not one or a few individuals infecting wheat or rye fields in Sweden, but it is a great variety of different genotypes initiating the disease in both oats and rye. The analysis of molecular variance (AMOVA) showed that most of the genetic variation was present within fields (Papers I). Both allelic and genotypic diversity were high, and in most fields, the number of genotypes was equal to the number of samples, indicating sexual reproduction. This applies to both oat and rye fields.

Genetically similar populations were recovered from fields that were separated by both short and long distances, and at the same time, genetically different populations were found both close to each other and far apart. The absence of correlation between geographic and genetic distances was supported by a Mantel test, and the estimated overall migration rate was fairly low (Nm was 1.7). This indicates that some migration occurs between geographical areas, but locally distinct populations have developed in some cases. Probably the barberry bushes play a role in the founding of local populations.

In Tajikistan (Paper III), the genetic variation produced by the sexual reproduction on the alternate host is important, even if the fungus is predominantely reproducing asexually during most of the year. Here, a relationship between the genetic and geographical distances was found, indicating local populations (Paper III). The analysis of the population of *P. graminis* in Tajikistan showed that both sexual and clonal reproduction of the pathogen has shaped the population structure.

The possibilities for sexual recombination do not alter the conclusion that events of long distance dispersal are important for the population structure. Within a sexual population that receives new individuals through rare events of successful immigration, the genetic diversity will increase when the genes from the new genotypes spread within the population.

Genetic differentiation between formae speciales

The study investigating the differentiation of *P. graminis* within Sweden was based on samples collected from oats, rye and barberry (Paper II) and within Tajikistan on samples collected from wheat and wild oats (Paper III). In both studies, the AMOVA showed that the majority of the variation was present between the *formae speciales* (Papers II and III), and the main result of those studies is that the samples collected from oats clearly differed genetically from samples collected from rye or wheat. Samples collected on barberry belong either to the oat group (*P. graminis* f. sp. *avenae*), the rye group (*P. graminis* f. sp. *secalis*) or an undefined group to which any grass susceptible to stem rust may be included (Figure 7). The genetic differentiation between *formae speciales* was reflected in the phylogenetic study (Paper V).
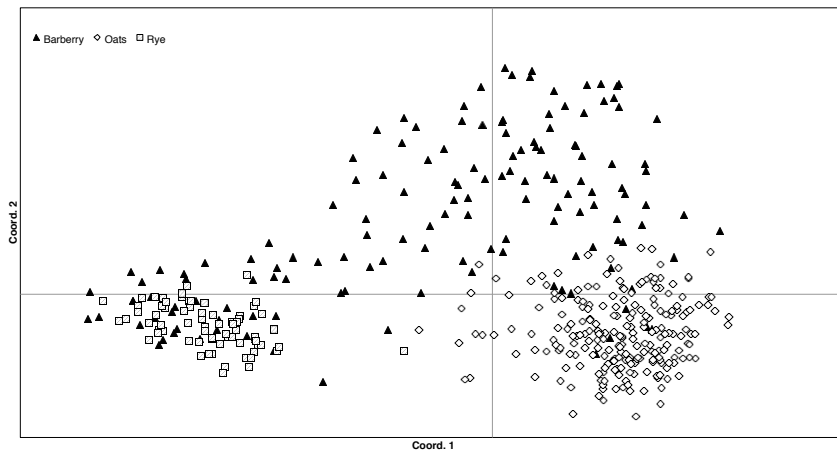
Figure 7. PCA based on the calculated genetic differences between samples, the first axis explains 41% and the second 19% of the total genetic differences (Paper II).

4.2 Morphology of fungal structures

The morphology of aecia showed clear differences between *P. graminis* f. sp. *avenae* and *P. graminis* f. sp. *tritici/secalis* (Paper IV). This was also reflected in the genetic differences, and in the morphology of uredinia and telia (Table 2, Paper IV). There was a clear correlation between the morphological and genetic differences, although the population study shows a broader spectrum of genotypes collected from barberry (Figure 7, Paper II) than what is revealed in the morphological study (Paper IV).

4.2.1 Aecia and aeciospores

The aecial stage was first described by de Bary (Eriksson, 1896). He examined the aecial cups under microscope and described them as "Spanish wine-flasks made of goat-skin with all the hair left on and turned inside out" (Large, 1946). Each group of aecia consists of several cups clustered together. Cross-sections of aecia and aeciospores showed morphological differences between *P. graminis* f. sp. *avenae* and *P. graminis* f. sp. *tritici* (Paper IV) when the identity of the taxa was based on *ITS* and *EF1* sequence similarities (Figure 8). The aeciospores of both *formae speciales* were obovoid, but spores identified as *P. graminis* f. sp. *avenae* were smaller than those spores identified as *P. graminis* f. sp. *tritici/secalis* (Figure 9). The aecia of *P. graminis* f. sp. *avenae* had a more oblong shape, the termination of the peridium was slightly bent towards the outside and the aecial cups were

Table 2. *Spore measurements of the formae speciales studied in Paper IV.*

	N	Length μm	SD	N	Width μm	SD	N	Area μm²	SD
Aeciospores									
f. sp. *avenae*	259	17.7	1.54	259	15.5	1.19	259	217.0	29.3
f. sp. *tritici/secalis*	349	18.7	1.65	349	16.3	1.30	349	241.0	33.9
Urediniospores									
f. sp. *avenae*	732	28.8	3.62	730	15.9	2.13	510	422.5	51.5
f. sp. *tritici/secalis*	383	27.3	2.97	383	12.9	1.67	295	348.9	40.0
Teliospores									
f. sp. *avenae*	537	44.9	7.63	580	17.2	537	–	n.a.	n.a.
f. sp. *tritici/secalis*	238	47.4	6.72	251	15.1	238	–	n.a.	n.a.

N: number of spores measured; SD: standard deviation

situated closer to each other than aecial cups of *P. graminis* f. sp. *tritici* which had a roundish shape, were deep-seated into the leaf tissue and the termination of the peridium was bent towards the inside of the aecial cup (Paper IV).

Aeciospores from individual aecial cups generally have the same genotype, whereas aeciospores from another cup in the same aecium frequently has a different genotypes (Roelfs, 1985). One question that remains to be answered is the composition of genotypes within one cluster cup. The genotypes of individual cups have been investigated in the pine blister rust (*Cronartium flaccidum*) where it was found that the genotypes in each blister (possibly resembling one cup) may differ in one set of alleles, where the other remains the same within a blister (Samils, 2011). If it is the same case in *P. graminis*, that one pycnium is fertilized by different pycniospores, it will result in different genotypes where all offspring share one set of alleles from the "mother" mycelium. Since a localized spot of aecia produces offsprings of different genotypes, the potential for genetic diversity is very large.

4.2.2 Uredinia and urediniospores

Uredinia develop on susceptible hosts after a successful infection by an aeciospore or urediniospore. Urediniospores are oblong, dikaryotic and may be transported long distances under suitable conditions. This is the asexual, clonal and most frequent spore type. It is difficult to distinguish between different rusts under a microscope in this stage, even if spore measurements show significant differences (Table 2). The urediniospores of *P. graminis* f.
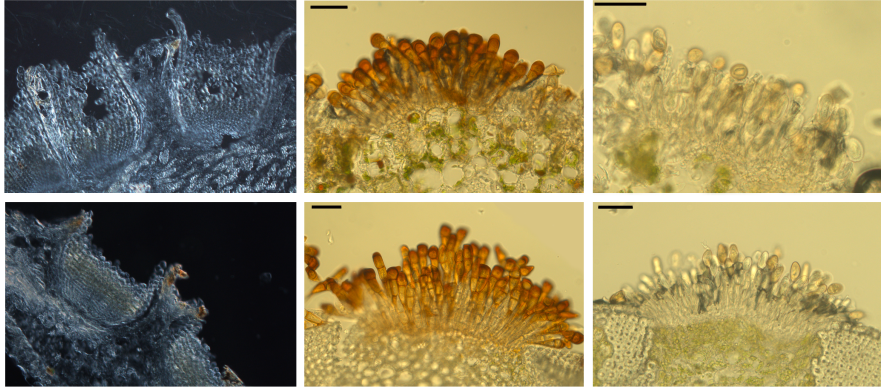
Figure 8. Cross-sections of aecia (left), telia (middle) and uredinia (right). Above, *P. graminis* f. sp. *avenae* and below *P. graminis* f. sp *tritici/secalis*. Bar = 50 μm. Photo: J. Kyiaschenko and A. Berlin.
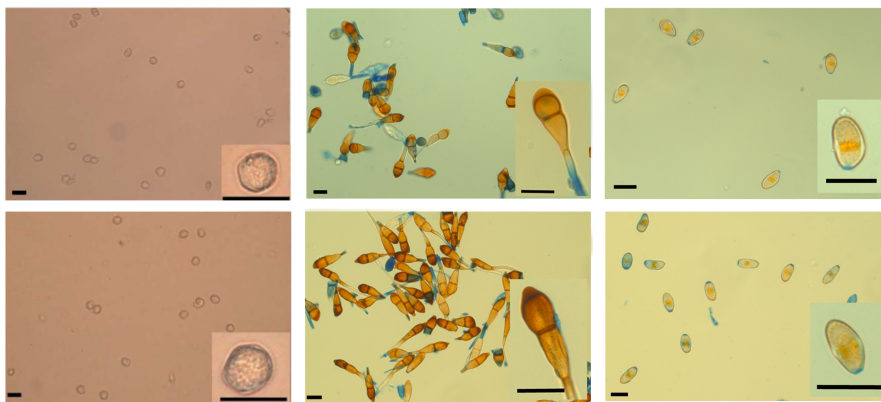

Figure 9. Aeciospores (left), teliospores (middle) and urediniospores (right). Above, *P. graminis* f. sp. *avenae*, below *P. gramnis* f. sp. *tritici/secalis*. Bar = 50 μm. Photo: J. Kyiaschenko and A. Berlin.

sp. *avenae* were short–ellipsoid and slightly larger than the urediniospores of *P. graminis* f. sp. *secalis* which were more long-ellipsoid (Figure 9). The arrangement of the spores in the uredinia differed between the two *formae speciales*. The urediniospores were adaxial for *P. graminis* f. sp. *secalis*, whereas the spores were more abaxial in the uredinia of *P. graminis* f. sp. *avenae* (Figure 8).

4.2.3 Telia and teliospores

The teliospores are produced in the same sori as the urediniospores when the crop matures. The teliospores of *P. graminis* f. sp. *avenae* were significantly shorter and obovoid compared to the teliospores of *P. graminis*

f. sp. *secalis*, which is reflected in the spore measurements. However, both *formae speciales* had dark brown and two-celled spores that were clavate at the apix. The spores of both *formae speciales* were adaxial in the sori (Figures 8 and 9).

4.3   Is *P. graminis* a single species?

Gäumann (1959) called *P. graminis* a "Mammut-species", since it contains several different morphologically and biologically distinct groups. A species may be defined in many different ways, and the most common species concepts are: Biological Species Concept (BSC) highlighting reproductive isolation, the Morphological Species concept (MSC) which highlights morphological divergence, Ecological Species Concept (ESC) which emphasizes adaptation to a particular ecological niche and the Phylogenetic Species Concept (PSC) which emphasizes nucleotide divergence. Until recently, the most commonly used criteria for species in fungi have been based on morphology (Giraud, 2009). However, cryptic species have been discovered within morphological species using BSC or PSC. An extension of the PSC is GCPSR (Genealogical Concordance Phylogenetic Species Recognition). This concept uses the phylogenetic concordance of several unlinked genes to show a lack of genetic exchange, which enables identification of species that lacks differences in morphological characters.

   Species are formed by general evolutionary processes; genetic drift, migration, natural selection, and mutations. The characters affected by those processes are very diverse. Speciation is an extended process and the different processes of divergence do not occur in any particular chronological order, and different factors act at the same time and in different places. Even if the common ground for all definitions of the species concept is that a species is a separately evolving linage (De Querioz, 2007), the definitions may vary. The problem is to describe the differences and it is in this stage, where the different species complexes are different and sometimes contradict each other.

4.3.1 Phylogeny of *P. graminis*

Based on earlier findings, the question about differentiation within *P. graminis* was asked. The phylogeny of *P. graminis* sensu lato was studied (Paper V) based on fragments of four loci; internal transcribed spacer (*ITS*) region, elongation factor 1−α (*EF1*), mitochondrial cytochrome oxidase subunit I (*COI*) and a portion of the β-tubulin (*BT*) gene. The species
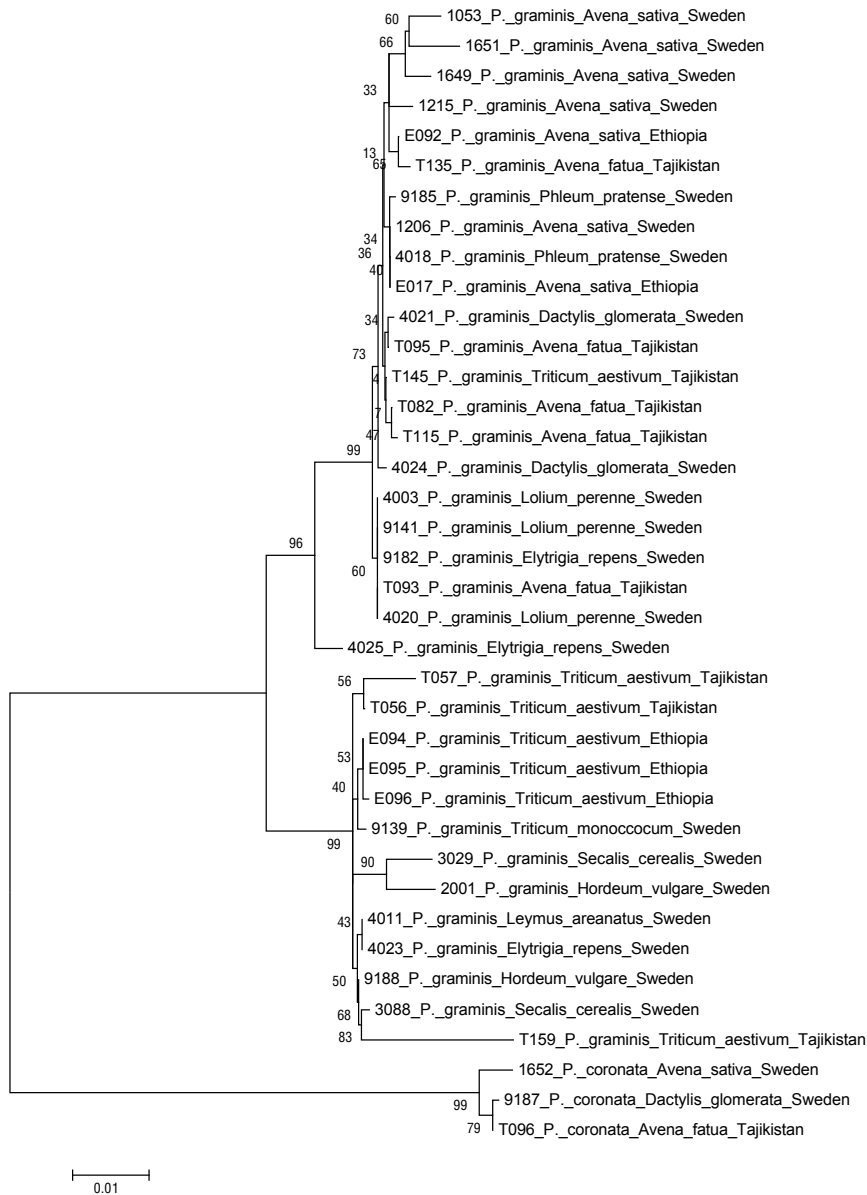
Figure 10. Neighbour-joining tree based on the *ITS* region and locus *EF1*. Samples are identified with species, host and origin.

Puccinia coronata was used as a reference group, since it is also known to be a very diverse species (Liu, 2012).

The phylogeny was inferred using both Neighbour-joining for single-locus genealogies and a Bayesian analysis for two loci. Based on the phylogenetic grouping, the nucleotide differentiation was calculated between the three phylogenetically defined species; *P. graminis* f. sp. *tritici/secalis*, *P. graminis* f. sp. *avenae* and *P. coronata*. The two groups of *P. graminis* mainly included samples infecting a certain group of grasses, but some samples seemed to have infected the "wrong" host (Figure 10).

The divergence time between the two phylogenetic species of *P. graminis* (Paper V) was calculated and compared with the divergence time of the grass hosts. The molecular clock was estimated based on intron regions in the selected loci and three clock models were tested; a strict molecular clock model (assumes a global clock rate with no variation among linages in a tree), and two relaxed clock models with uncorrelated, branch-specific rates following lognormal or exponential distribution. Concurrently, the time for the most recent common ancestor (TMRCA) was calculated for each node.

The estimated effective population sizes were very large (10^6). The calculated divergence time between the two *formae speciales* was between 2.1 and 3.5 Mya (Million years ago) when assuming a mutation rate of 16.7×10^{-9} substitutions per site and year and a strict clock. The split between the *formae speciales* is not specific for the Scandinavian population of *P. graminis* but is also seen in material collected in Central Asia and Eastern Africa. This is long before the domestication of cereal crops in the fertile crescent, which was initiated approximately 13,000 years ago (Hancock, 2012), and it is in the same range as the divergence times of the grass hosts. There could be selection on the alternate host as well (Levine, 1932) but this is unlikely since both *formae speciales* can infect barberry (Paper III) and *P. graminis* as a group has been shown to coevolve with *Berberis* spp. (van der Merwe, 2008). It is thus likely that the grass host is the main evolutionary force in the divergence of the two phylogenetic species (Paper V). Both of the phylogenetic species may infect *Elytrigia repens*, since some samples collected from that particular grass clusters into one phylogenetic group but other samples cluster into the other.

Co-evolution may be expected between a plant and a biotrophic pathogen (Giraud, 2009). The speciation is likely to have occurred sympatrically, since the two phylogenetically distinct species of *P. graminis* share the alternate host, barberry. It is probable that plant – pathogen interactions have driven the speciation, and the two groups started to diverge when one had better fitness on either of the grass host groups (Johnson, 1996). Since one group of plants is resistant to the *formae speciales* adapted to the other group, this

creates a barrier, which then led to the divergence. Gene flow between the two taxa of *P. graminis* could not be ruled out (Paper V) and only a very low level of gene flow is sufficient to prevent differentiation (Slatkin, 1987). However, there would be no speciation if gene flow were present between the two groups. The large differentiation within the genome (McDowell, 2011) indicates that this is a very diverse species complex and incompatibility between the *formae speciales* has been reported (Johnson, 1949).

Possible reproduction barriers within P. graminis

The teliospore produces haploid basidiospores, which in turn infect barberry where pycnia and pycniospores are formed. The receptive hyphae of the pycnia may only be fertilized with a pycniospore from the opposite mating type. One may believe that the differences between the two studied *formae speciales* will hinder the mating in this stage, since the spores and receptive hyphae may not be compatible with each other. Johnson (1949) reported that the success of crosses between different *formae speciales* was low and that the pycnia produced little nectar and often no aecia. If mating between different *formae speciales* or subspecies does take place, the fitness of the offspring has been reported to be lower than for "pure" isolates (Johnson, 1949). Since karyogamy and meiosis take place in the teliospore, before the fungus infects barberry, the "real" fusion between the two nuclei does not take place until teliospore germination, which Johnson (1949) reports never occurred. The two nuclei may act together in the dikaryon, but the meiosis cannot take place. Thus one possible recognition event takes place when the receptive hypha is fertilized with a compatible pycniospore, and lack of proper recognition will constitute one reproductive barrier. A second possible recognition event will take place in the teliospores, where karyogamy and subsequent meiosis is required for production of the basidiospores, and if it cannot take place, this would be a second reproductive barrier.

Crossing studies and DNA sequence comparisons have supported the subdivision between some of the *formae speciales* (Leonard, 2005). Johnson (1933) found that the *P. graminis* f.sp. *tritici* and *P. graminis* f. sp. *secalis,* infecting wheat and rye respectively, were closely related since they commonly produced viable offspring, whereas *P. graminis* f. sp. *avenae*, infecting oats clearly differed from the other two and only once produced viable offspring when crossed with *P. graminis* f. sp. *tritici*.

4.3.2 Speciation within *P. graminis* – Eriksson revisited

By studying the available literature, it is clear that *P. graminis* is a very diverse species (Gäumann, 1959) and several attempts to make additional classification within the species have been made (Eriksson, 1896; Levine, 1923; Guyot, 1946; Urban, 1967). For *Puccinia* spp. on cereal crops, the spore dimensions overlap between species, making the species boundaries based on morphological features unclear (Anikster, 2005). The taxonomy within rust species is based primarily on the morphology of the teliospores. In addition to features of teliospores, other criteria and spore dimensions have been used to define grouping (such as *formae speciales*, subspecies and varieties) within the species (Guyot, 1946; Urban, 1967; Savile, 1984). The studies of the morphology and phylogeny of stem rust (Paper IV and V) clearly show the diversity in *P. graminis*. The aecia and aeciospores of samples designated as *P. graminis* f. sp. *tritici* clearly differed from those designated as *P. graminis* f. sp. *avenae*. Paper V aimed to investigate if the morphological differences, seen in Paper IV, were reflected in the phylogenetic relationship in samples collected from the grass hosts as well. In addition, the phylogenetic relationship between the samples (genotyped with SSR markers) in Paper II was compared with the phylogeny of the samples collected from grass hosts in Paper V. No clear pattern between the different grass hosts could be seen, except for the split between the two phylogenetic groups f. sp. *tritici/secalis* and f. sp. *avenae* that was already seen in Papers IV and V.

The use of *formae speciales* is an over-simplification of the classification of groups within *P. graminis*. The two main groups of *P. graminis* may be treated as two different species, since they do not only differ morphologically, but also phylogenetically. More in depth analysis of genomes from different samples would enable a more detailed phylogeny of the species. Recent results showed that the differentiation within *P. graminis* f. sp. *tritici* is very large, since 3×10^6 single nucleotide polymorphisms (SNP) were detected between samples collected in Eastern Africa and the USA (Les Szabo, personal communication). Very few obligate parasitic species possess such a large variety of hosts as *P. graminis*, and this suggests a new taxonomic division. The results presented here show that *P. graminis* sensu lato is monophyletic, and that *P. graminis* f. sp. *avenae* and *P. graminis* f. sp. *tritici/secialis* are sister taxa.

Until now, the literature describing the different types of *P. graminis* is not consistent. All of the taxonomic divisions were made before the genetic era, which means that the divisions solely depend on morphological and

ecological differences. Even if those differences most often are reflected in the phylogeny of *P. graminis*, the sub-species or variety definitions must have been muddled with some samples infecting the "wrong" host. Although the different forms may infect the "wrong" host, it is quite clear that they do not fuse on barberry (Johnson, 1933; Johnson, 1946; Johnson, 1949). Both Urban (1967) and Guyot (1946) divided stem rust into different sub-species and varieties, but their subdivisions do not match the results in this thesis since they chose to group f. sp. *hordei* in the f. sp. *avenae* group and thus their taxonomic subdivision may not be used. Instead two new species within *P. graminis* could be considered. The wheat type should be called *P. graminis* and a new name has to be found for the *forma specialis* infecting the oat-group.

5   Control of Stem rust

It is clear that stem rust meets all of the criteria for being a high-risk pathogen in Sweden by having a mixed reproduction system, large effective population size, high potential for gene flow (efficient spore dispersal and LDD) and high mutation rate (McDonald, 2002). To understand how to control stem rust, a combination of knowledge of the epidemiology of the disease and the population biology of *P. graminis* is needed.

From an agricultural point of view, the main goal is to limit the spread and development of the disease. This may be done by interfering with the disease cycle and limiting the amount of inoculum at different stages. The control measures can be divided into short and long term decisions.

5.1   Barberry

The oldest control measure against stem rust is to remove barberry from areas where cereals are produced. The bushes should not only be removed from field borders, but also in grazing areas and forests bordering agricultural fields. Common wild grass relatives may also support populations of both of the two main groups of *P. graminis*. Nowadays, the eradication of barberry might not be feasible, and an alternative way to limit the spread of early inoculum could be to spray fungicides on barberry. This would limit the diversity of the pathogen and probably require less fungicide then treating an entire field of wheat or oats. Vanderplank (1963) discusses that heavy outbreaks of stem rust in the USA made people look for barberry bushes in the areas with severe disease outbreaks. This may be the most time-efficient way to reduce the yield loss due to stem rust in Sweden, by looking for barberry bushes where the disease causes problems. In a conservation biology perspective, barberry is valued in the agricultural landscape since the berries provide good food for the wildlife.

5.2 In fields

In a short-term perspective (scale of weeks), farmers may use fungicides to limit the spread and development of a disease. Fungicide treatments are most effective when the crop is treated at an early stage of disease development (Waynera, 2009). Early detection of disease is thus a key factor for reducing the yield and quality losses. For this, frequent and timely inspections of fields are crucial. It is also important to be able to communicate the findings to the farmers who are at risk of having infested fields. The current survey system by the plant inspection service units at the Swedish board of agriculture provides such a system. Treatments with fungicides are not allowed later than at the end of flowering and beginning of the soft dough stage (DC 69-71) due to the risk of fungicide residues in the harvested crop. One problem is that the first signs of infection and the initiation of the epidemic starts when the crop can no longer be treated with fungicides and inspections are minimal. However, late onset may cause significant yield losses and a late treatment may be economically justified.

The long-term option for control of stem rust is the use of resistant cultivars. Although the law of barberry eradication was in force in 1972 and barberry bushes were removed, MacKey and Mattsson (1972) reported a high level of race diversity for oat stem rust. As with many biotrophic fungi, the diversity and adaptability of *P. graminis* is large. This means that one should expect the diversity of *P. graminis* to continue to be large also when barberry is removed. Even if some gene-for-gene resistances have been effective for a long time, like the resistance gene *Sr31* which was successfully used for decades, the preferred breeding strategy would be to focus on slow rusting genes or durable resistance (Singh, 2011). This is desirable, but difficult. Since the disease onset is late, the adult plant resistance (APR) is important.

For the farmers, one strategy could be the use of mixtures of different cultivars with different resistance genes. This may be used to limit both the risk and the spread of disease. This strategy is more important in situations where the pathogen reproduction is primarily clonal. It may also be important to grow resistant cultivars to limit the production of inoculum on a regional scale.

6 Concluding remarks

It is most likely that the stem rust infections in Sweden start from aecia every year. Thus, the stem rust populations infecting oats within Sweden must undergo sexual reproduction each year, since no grass host is available during the winter months (Paper I). It is a forced sexual population. The population on rye is less variable (Paper II), but spores originating from aecia significantly contribute to the variation within the *P. graminis* f. sp. *secalis* population in Sweden. One main conclusion from this thesis is, that if barberry were removed, the incidence of stem rust would decrease. The eradication of barberry would not only decrease the level of disease, but also the genetic diversity and thus the number of races.

If the situation is similar to that for the poplar rust (*Melampsora larici-populina*) the mere presence of the alternate host within an area will provide enough genetic variation (Gérard, 2006). This would also explain the large genetic variation among the *P. graminis* populations in Tajikistan, where clonal propagation is the predominant reproduction mode. Only a limited presence of barberry in the area (Davlatov, 2011) provides enough possibilities for sexual recombination.

Even if it is clear that *P. graminis* divides into two distinct phylogenetic species in the phylogenetic studies (Papers IV and V), the differences within each clade remain to be elucidated. However, it is clear that it must be the grass hosts that are driving the evolution within *P. graminis* sensu lato, since no differentiation was seen on barberry. The SSR study on barberry (Paper III) reveals more differentiation on barberry than what is seen in the phylogenetic studies. The relationship between *P. graminis* f.sp. *tritici* and f.sp. *secalis* is less clear and needs further investigation.

Wheat is the economically most important cereal crop in Sweden, and all prerequisites for stem rust epidemics on this crop are present. However, stem rust is only found on old land races and no disease is found on modern wheat cultivars in commercial fields. The questions are; why we do not have wheat stem rust in Sweden? Is *P. graminis* f. sp. *tritici* absent because it has become extinct in Sweden? Is the stem rust on old land races of wheat actually caused by *P. graminis* f. sp. *secalis* that is infecting the "wrong" host? Is stem rust on wheat absent because existing resistance genes in the cultivars grown are effective against the *P. graminis* population within the area?

It is known that a large proportion of Swedish wheat is susceptible to the group of virulent strains called Ug99. One may also speculate about what would happen when such strains come to Sweden. What will happen depends on the aggressiveness of the genotypes, their ability to produce teliospores and their survival in a sexual population. If a virulent genotype arrives and integrates in the Swedish population of *P. graminis*, the individual must go through sexual recombination. Since the gene-for-gene virulence factors are recessive, it is likely that the virulent individual will mate with a homozygous avirulent individual. The first offspring would not be virulent, since the trait is recessive. The trait for virulence will be present in the population, however, and individuals able to infect the wheat grown could result from random mating. These individuals would have a strong selective advantage, which will only increase the frequency of virulence in the population. The resurgence of barberry in the agricultural landscape means that the potential for an epidemic caused by such virulent individuals could be devastatingly large.

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Populationsbiologi hos *Puccinia graminis* – Konsekvenser för epidemiologi och kontroll av svartrost

Svartrost, orsakad av svampen *Puccinia graminis*, är en allvarlig sjukdom i stråsädesgrödor i stora delar av världen och förr orsakade patogenen stora skördeförluster även här i Sverige. Patogenen *P. graminis* har två värdväxter, en gräsvärd på vilken den orsakar svartrost och en mellanvärd, berberis, på vilken den sexuella fasen av livscykeln avslutas. Redan i Bibeln står det skrivet om rost- och sotepidemier och romarna firade en speciell högtid, Robigalia, för att skydda grödorna mot rost.

Kopplingen mellan svartrost och mellanvärden berberis var känd redan innan man förstod sjukdomens biologi och den var tillräckligt stark för att en lag som tvingade utrotning av berberis antogs i Rouen i Frankrike 1660. I Sverige försvann svartrostproblemen i vete under 1970-talet, främst tack vare att resistenta sorter odlades, men också på grund av att berberisen var utrotad i många områden. Detta ledde till att lagen om utrotning av berberis avskaffades 1994. Sedan dess har berberisbuskarna ökat i antal i hela södra Sverige. I början av 2000-talet noterades att svartrost orsakade skördeförluster i havre och man undrade om detta kunde bero på den ökade berberisförekomsten. Samtidigt hade en ny och mycket aggressiv ras av svartrost som infekterar vete (ofta kallad Ug99) uppmärksammats i Östafrika. Efter att ha legat i glömska i ungefär fyrtio år, var svartrost återigen ett problem.

Syfte

Huvudsyftet med detta projekt var att förstå populationsbiologin hos *Puccinia graminis* och epidemiologin hos svartrost. Resultaten från projektet bör ge information till spannmålsproducenter om hur de kan skydda sina grödor mot svartrost. De viktigaste frågorna som besvarats för att möta syftet var: Är

berberis viktig för svartrostens utveckling, dvs. förökar sig *P. graminis* framförallt klonalt eller sexuellt i Sverige? Finns det något mönster i spridningen av olika genotyper, både inom och mellan fält och mellan olika år? Kan prover insamlade från mellanvärden matchas mot prover insamlade från havre- och rågfält? Finns det några fylogenetiska skillnader (skillnader i släktskap) inom arten *P. graminis*?

6.1.1 Svartrost och *Puccinia graminis*

Svartrost har rapporterats från 365 olika gräsarter från flera olika gräsfamiljer. De vanligaste är vete, råg, havre, korn, rajgräs, timotej, hundäxing och kvickrot. På berberis orsakar *P. graminis* skålrost. I hela världen finns upp till 680 olika berberisarter beskrivna och de flesta av dem är mottagliga för skålrost. *Berberis vulgaris* L. är den i Sverige vanligaste vildväxande arten och den är också mottaglig för skålrost. Även hybrider mellan *B. vulgaris* och trädgårdsvarianten *B. thunbergii* är mottagliga.

Det finns flera arter av *Puccinia* som kan infektera berberis. De olika arter som hittades i Sverige var *P. graminis* och *Puccinia arrhenatheri*. Det är enkelt att se skillnaden mellan de två arterna, eftersom *P. graminis* bildar avgränsade runda skålrost fläckar, medan *P. arrhenatheri* växer systemiskt och bildar så kallade häxkvastar. Morfologiska och genetiska skillnader mellan f. sp. *avenae* (svartrot på havre) och f. sp. *tritici/secalis* (svartrost på råg och vete) hittades också (Artikel IV).

Svartrosten har en komplex livscykel som inkluderar fem sporstadier och två värdväxter. Svartrost övervintrar i form av svarta vintersporer (teliosporer), som under våren gror till basidiosporer vilka infekterar unga berberisblad. På berberisen bildas pyknia och pykniosporer och när en pykniospor möter ett pyknium av en annan parningstyp bildas ett aecium. När aecier kan observeras på berberisbladen kallar man det skålrost. Från skålrosten sprids aeciosporer till gräsvärdar där de bruna sommarsporerna (urediniosporerna) uppträder, oftast på strået men ibland även på ax och vippa. I detta stadium av livscykeln förökas svartrosten klonalt. När grödan mognar börjar svampen bilda de svarta vintersporerna i samma sår som sommarsporerna tidigare bildats i. De svarta teliosporerna övervintrar och livscykeln är sluten.

I och med återhämtningen av berberisbeståndet har svartrosten fått större möjlighet att genomgå hela sin livscykel i Sverige. Artikel I och II visar att den genetiska variationen inom svamppopulationerna är stor, vilket tyder på att den sexuella delen av livscykeln är viktig för svartrostens överlevnad i Sverige. Berberis är även viktig för populationen i Tadzjikistan, även om förökningen till största delen sker klonat i Centralasien (Artikel III).

Populationssammansättningen varierar och det finns ingen struktur mellan och inom populationer av *P. graminis* insamlade från olika fält eller år i Sverige. Detta beror troligen på att en stor mängd sporer med olika genetisk uppsättning sprids från berberisbuskar till fälten varje år, eftersom alla provtagna individer har olika genotyper i de flesta fält.

Användningen av termen *forma specialis* (f. sp.) är en förenkling av klassificeringen inom *P. graminis*. Det finns både genetiska och morfologiska skillnader mellan *P. graminis* f. sp. *tritici/secalis*, vilken är den typ som infekterar främst råg och vete, och *P. graminis* f. sp. *avenae*, vilken främst infekterar havre och vilda grässläktingar. Detta gäller inte bara för svartrosten i Skandinavien, utan också för svartrost från Centralasien och Östafrika. Evolutionen av de två släktgrenarna korrelerar med evolutionen av gräsvärdarna, eftersom delningen av Triticeae och Avenacea/Poaceae skedde inom samma tidsram som *P. graminis* beräknats ha delats (Artikel V). Tidigare försök att dela in olika undergrupper av *P. graminis* stämmer inte överens med resultaten som framkommit i denna avhandling. Därför föreslås att *P. gramnis* endast bör användas för *tritici/secalis*–gruppen och att ett nytt namn måste ges till *avenae*-gruppen.

6.1.2 Kontroll av svartrost

Svartrost passar in i definitionen på en högrisk-patogen i Sverige, eftersom den har både sexuell och klonal förökning, stor populationsstorlek, hög potential för genflöde (långdistans-spridning av sporer) och hög mutationshastighet. För att förstå och kontrollera svartrost, behövs en kombination av kunskap från både populationsbiologi och epidemiologi. Kontrollmetoder kan delas in i både kort- och långsiktiga strategier. När det gäller berberis kan den antingen tas bort helt eller besprutas. Det skulle minska både den genetiska variationen (dvs. minska antalet raser) inom patogenen och mängden inokulum som potentiellt skulle kunna nå stråsädesfält. För trots att ett angrepp anländer sent, kan det vara ekonomiskt försvarbart att behandla fältet med fungicid.

I ett kortsiktigt perspektiv kan svartrostangrepp bekämpas med fungicider. En tidig upptäckt av sjukdomen är en nyckelfaktor för att kunna minska skörde- och kvalitetsförluster. För detta är växtskyddscentralernas prognos- och varningsverksamhet mycket viktig. I fält är det viktigt att behandla tidigt när symptom visar sig, eftersom behandling med fungicid inte är möjlig efter blomning eller begynnande mjölkmognad (DC 69-71) på grund av risk för fungicidrester i skörden. Ett problem är att de första

synliga angreppen oftast kommer när grödan inte längre kan behandlas med fungicider och varningsverksamheten trappats ner för säsongen.

I ett längre perspektiv är användningen av resistent sortmaterial mycket viktig. MacKey och Mattson (1972) hittade många raser även under åren då berberisutrotningen pågick. Som för många andra biotrofa patogener är diversiteten och anpassningsmöjligheten hos *P. graminis* stor. Det betyder att man kan förvänta sig att den genetiska variationen hos *P. graminis* kommer fortsätta att vara stor även när berberis tas bort. För att förädla mot svartrost, bör strategin vara att använda så kallade "uthålliga" gener. Det är önskvärt, men tyvärr mycket svårt. Den så kallade gen-för-gen-resistensen är den vanligaste inom resistensförädlingen mot rost. Ett exempel är resistensgenen *Sr31* som har använts inom veteförädlingen under många år och den är fortfarande effektiv mot svartrost i Sverige. För havre ser situationen annorlunda ut, där finns det inte så många kända effektiva resistensgener.

6.1.3 Slutord

Det är mycket troligt att svartrost startar från skålrost varje år. Det betyder att *P. graminis* måste genomgå sexuell förökning för att kunna överleva vintrarna (Artiklarna I och II). Det är en påtvingad sexuell förökning. En slutsats från detta arbete är att om berberis togs bort skulle svartrost minska i landet. Även om svartrost framförallt överlever klonalt, som i till exempel Tadzjikistan, upprätthåller berberisen en hög variation hos patogenen.

Även om *P. graminis* kan delas in i två fylogenetiskt distinkt skilda arter (Artikel IV och V), återstår ändå att besvara frågan om hur skillnaderna inom de två grupperna ser ut. Det är tydligt att det är gräsvärden som driver evolutionen inom *P. graminis*, eftersom ingen differentiering ses på berberis. Till exempel borde förhållandet mellan *P. graminis* f. sp. *tritici* och *P. graminis* f. sp. *secalis* undersökas ytterligare.

Vete är den ekonomiskt viktigaste stråsädesgrödan i Sverige. Svartrosten som idag finns i landet infekterar inte de vanligen odlade vetesorterna. Frågan är; Varför vi inte har svartrost på vete i Sverige?

Vi vet att en stor del av det vete som idag odlas i Sverige är mottagligt för den så kallade Ug99-rasen. Man kan bara spekulera om vad som skulle hända om den kom till Sverige. Vad som kommer att hända beror på aggressiviteten hos genotyperna, deras möjlighet att bilda vintersporer och möjligheten att överleva i en sexuell population. Om en virulent genotyp kommer, och integreras i den svenska populationen av *P. graminis*, måste den genomgå sexuell förökning. Återkomsten av berberis i odlingslandskapet betyder att en epidemi orsakad av ett sådant isolat skulle vara förödande.

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