Vitality of uredospores of *Puccinia spp.* depending on environmental conditions after release from leaf rust pustels

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NAME: Vitality of uredospores of *Puccinia spp.* depending on environmental conditions after release from leaf rust pustels.

ABSTRACT: The cereals are, due to their cultivation area and yield, one of the most important crops worldwide. Their high adaptability and its grains, rich in calories, do of the cereals the base of human and animal feeding. Based on the importance of the crop, there are numerous studies devoted to mitigate or reduce the damages produced by the different pathogens. Rusts caused by different *Puccinia* species, are one of the most important diseases in cereals and causes very important yield losses worldwide. Thus, the study of the evolution of the vitality of the uredospores of rust fungi, after release from leaf rust pustules subjected to different conditions, is important to understand better the fungi life cycle and can help us to prevent and reduce the damages. The study of the uredospores germination ratio has been length studied, however, there do not exist many studies which record how the conditions to which the spores are subjected during the time between the release from the pustules and the germination process affect to the vitality. The aim of this work is to analyse how the time and temperature affect the vitality of the uredospores of different species of fungi of genus *Puccinia* in wheat and rye. Spores of *Puccinia triticina Eriks*. causing brown rust in wheat of the variety "Dekan", of *Puccinia striiformis f.sp. tritici* causing yellow rust in wheat of the variety "JB Asano" and of *Puccinia disersa Erikss. Et Henn*. causing brown rust in rye of the variety "US Forsetti" have been selected. Uredospores of Yellow rust seem to be the most sensitive to the time elapsed after the release, losing entirely its vitality for any one of the studied conditions after four weeks. The brown rust uredospores in rye have an important initial vitality, which decrease progressively with time and the temperature, this kind of spores seem to lose vitality faster for high temperatures. Brown rust uredospores in wheat show a broader survival rank than the others, surviving until 3 months after the release.

KEYWORDS: Uredospores, *Puccinia spp*., vitality, cereals, environmental conditions.

TÍTULO: Vitalidad de las urediniosporas de *Puccinia spp.* en función de las condiciones ambientales tras desprenderse de pústulas de roya de la hoja

RESUMEN: Los cereales son, por su superficie y volumen de cultivo, uno de los cultivos más importantes a nivel global. Su gran adaptabilidad y sus granos, ricos en calorías, hacen de los cereales la base de la alimentación humana y animal. En base a la importancia del cultivo son numerosos los estudios dedicados a mitigar o reducir los daños producidos por los diferentes patógenos. La roya, es uno de los patógenos más importantes de los cereales y causa pérdidas muy importantes a nivel global. Por ello, el estudio de la evolución de la vitalidad de las urediniosporas de la roya tras desprenderse de las pústulas sometidas a diferentes condiciones, es importante para comprender mejor el ciclo de los hongos y poder anticiparse y reducir los daños. El estudio de la vitalidad de las urediniosporas en función de las condiciones de germinación ha sido extensamente estudiado, sin embargo, no existen muchos trabajos que documenten como afectan a la posterior vitalidad, las condiciones a las que las esporas están sometidas durante el tiempo comprendido entre el desprendimiento de las pústulas y el momento de germinación. El objetivo de este Trabajo Final de Máster es analizar cómo afectan el tiempo y la temperatura a la vitalidad de las urediniosporas de diferentes especies de hongos del genero *Puccinia* en trigo y en centeno. Para este trabajo se han seleccionado esporas de *Puccinia triticina Eriks*. o roya marrón en trigo de la variedad "Dekan", de *Puccinia striiformis f.sp. tritici* o roya amarilla en trigo de la variedad "JB Asano" y de *Puccinia disersa Erikss. Et Henn.* o roya marrón en centeno de la variedad "SU Forsetti". Las urediniosporas de la roya amarilla parecen ser las más sensibles al tiempo transcurrido tras la liberación de estas, perdiendo completamente su vitalidad para cualquiera de las condiciones estudiadas a las cuatro semanas. Las urediniosporas de la roya marrón en el centeno presentan una vitalidad inicial alta, la cual va decreciendo progresivamente con el tiempo y la temperatura, las esporas de este tipo parecen perder la vitalidad más rápido bajo las temperaturas altas. Las urediniosporas de la roya marrón del trigo, presentan un mayor rango de supervivencia que las otras, llegando a sobrevivir hasta 3 meses tras la liberación.

PALABRAS CLAVE: Uredospores, *Puccinia spp*., vitalidad, cereals, condiciones ambientales

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1. Introduction

1.1. Generalities

Rusts are plant diseases caused by pathogenic fungi of the order Pucciniales (previously known as Uredinales) of the class Basydiomycetes. An estimated 168 rust genera and approximately 7000 species, more than half of which belong to the genus *Puccinia*, are currently accepted (Mohanan, 2010). The rust fungi are one of the most widely spread of plant pathogens and causes diseases on many angiosperm and gymnosperm trees, and cereal and legume crops (Kolmer, 2013). Rust fungi are obligate parasite capable of producing infectious uredospores as long as infected leaf tissue remains alive (Bolton *et al*., 2008). Due to this parasitic character, most rust fungi are specialized parasites of certain host species. Rust fungi can be widely disseminated over large areas by wind borne basidiospores, aeciospores and uredospores and are often highly genetically diverse for races or pathotypes distinguished by virulence/avirulence to differential host genotypes (Kolmer, 2013).

The pathogen genetic modification, the dissemination capacity and how rust pathogens interact with the different host genotypes makes the rust diseases ones of the most damaging diseases for the agriculture (Kolmer, 2013).

1.2. Rust diseases in cereals

Rust fungi are among the most important pathogens in cereals and cause significant yield loses worldwide (De Wolf *et al*., 2010). Cereal rusts are heteroecious and macrocyclic requiring two taxonomically unrelated hosts to complete a five spore stage life cycle. Cereal rust fungi are highly variable for virulence and molecular polymorphism and an early and accurate detection of the pathogens would facilitate effective control of the disease (Kolmer, 2013; Miao Lui *et al*., 2013).

The fungi that cause Rust in cereals are from the genus *Puccinia* which belong to the Kingdom fungi, phylum Basidiomycota, class Urediniomycetes, order Uredinales and family Pucciniaceae (Bolton *et al*., 2008).

Cereals are vegetal species grown for their grains (very rich in calories) principally and also for straw which can be used for animal feeding and maintenance of animal beds. Mostly of the cereals belong to the grasses family; wheat, barley, rye, corn, rice, millet and oats pertain to that family (Osca, 2007).

The ancestors of the cereals were wild plants which in their maturity would grow again from their own grains. Vavilov found 8 different world "centres" from where the cereals extend to the rest of the world. These "centres" were: China, India, Central Asia, Near East, Mediterranean, Abyssinia, Central America and South America (Osca, 2007).

According to FAO in 2004 677,6 millions of hectares in the world were cultivated with cereals, being the crop that covered the most surface worldwide and the production was of 1580,8 millions of Tons. In Europe, the most cultivated cereals are wheat, barley and corn (Osca, 2007).

Wheat (*Triticum aestivum L.*) is grown on more land area than any other food crop and is the second most-produced cereal after maize with 749 million tonnes in 2016 (FAOstat 2016). Wheat production is affected by fungal diseases, being the main one rusts caused by the fungi of genus *Puccinia*. Line and Chen (1995) show that stripe rust (P*uccinia striiformis* f.sp.*tritici* W.), leaf rust (*Puccinia triticina* E.) and stem rust (*Puccinia graminis* f. sp.*tritici*) have been considered the most damaging diseases of wheat in many areas around the world and the one that has caused more yield loses (Zwer and Qualset, 1994; Singh *et al*., 2005; Roelfs *et al*., 1992).

Rye (*Secale cereale*) is a winter cereal which stands out for its cold resistance and its rusticity, therefore, it can grow in certain weather and soil conditions where wheat couldn't grow in. Its origin is between near East and the oriental Mediterranean and initially was probably a weed which later has been harnessed by humans. Rye has less importance than wheat and as previously mentioned its cultivation is limited to elevated areas or northern countries (Russia, Germany, Austria...). Although rye's rusticity prevent it from many cereal diseases, leaf rust (P*uccinia recondita* f. sp. *secalis*) has traditionally been one of the most damaging diseases that has caused high yield loses (Osca, 2007).

1.3. Symptomatology and identification

The fungi that cause these diseases are notorious for their ability to increase rapidly and overcome the varieties genetic resistance. Early detection and proper identification are critical to in-season disease management and future variety selection (De Wolf *et al*., 2010).

Rusts in cereals are characterized by the uredinal stage. The uredines cause pustules and every species has morphological differences. To identify which kind of rust is present in the plant, there are some important characteristics to consider such as: the affected plant species, the affected part of the plant and lesion size, form and colour.

The below describes the three rusts on which this work will be focus on.

Wheat leaf rust or brown rust (*Puccinia triticina* Eriks) uredinia (Figure 1a) are up to 1,5 mm in diameter, erumpent, round to ovoid, with orange to brown uredinia that are scattered on both the upper and the lower leaf surfaces of the primary host. The infection of stems and heads are rare.

Wheat stripe rust or yellow rust (*Puccinia striiformis* f. sp. *Tritici*) (Figure 1b) produces yellow uredinia stripes that grow parallel along the venations of each leaf blade. The color is characteristic of uredinia that produce yellow uredospores. Some races of yellow rust can also affect the spikelets.

Rye leaf rust or brown rust (*Puccinia recondita* Rob. Es Desm. F. sp. *Secalis*) (Figure 1c) is morphologically similar to the wheat leaf rust, but is produced by a different pathogen. The oblong-oval rust-brown uredines are scattered distributed over the whole sheet area but rarely grow on the ears.

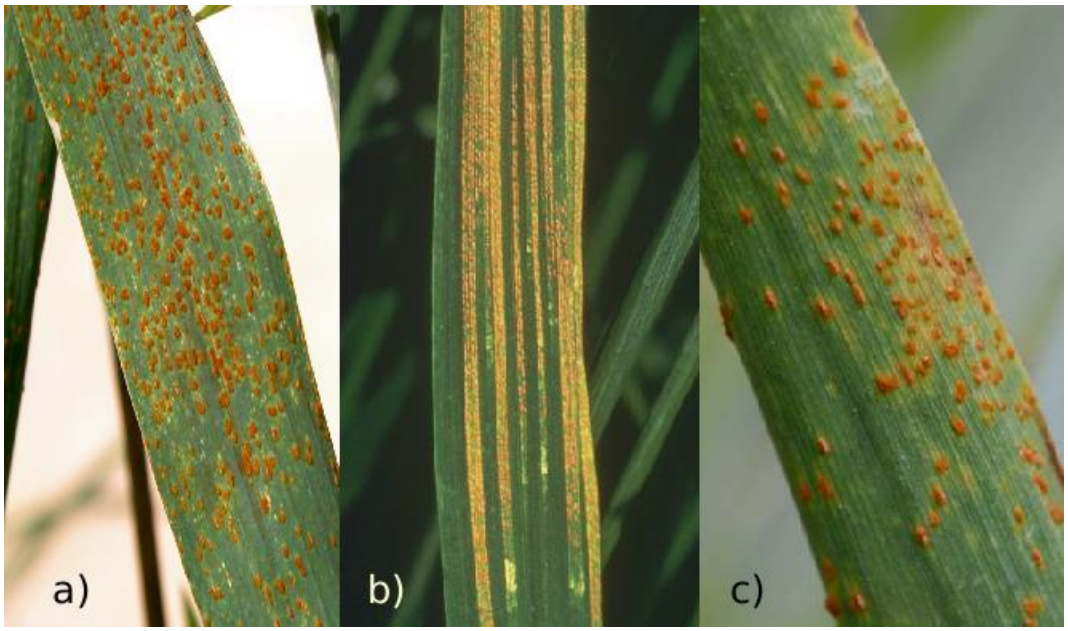

Figure1. Leaves infected by *Puccinia spp*. in uredino stage.

A. Wheat brown rust (*Puccinia triticina* Eriks): brown-orange uredinia on the upper leaf surface (TNAU Agritech Portal)
B. Wheat yellow rust (*Puccinia striiformis f. sp. Tritici*): yellow uredinia stripes parallel along the venations of leaf (Vidal L, INRA)
C. Rye brown rust (*Puccinia recondita* f.sp. *secalis*): Uredinos predominantly on the upper leaf surface (www.pflanzenkrankheiten.ch)

1.4. Economic Impact

Importance of cereal rust diseases in American agriculture is among the most damaging diseases of wheat and other small grain crops. In the Great Plains of the United States, stem rust and leaf rust epidemics often have caused yield losses in wheat far exceeding 20 million bushels. A stem rust epidemic in 1916 destroyed almost 300 million bushels of wheat in the United States and Canada. The 1935 wheat stem rust epidemic destroyed at least 135 million bushels, mostly in Dakota and Minnesota. In 1953 and 1954, stem rust caused $365,000,000 losses in wheat crop production in the U.S, including more than 75% of the durum wheat, the main source of pasta. Losses due to wheat stem rust have been abated since the 1960's by effective resistance breeding, which is not the case for wheat leaf rust. As recently as 1993, leaf rust destroyed over 40 million bushels of wheat in Kansas and Nebraska. In 1985, Texas and Oklahoma lost 95 million bushels of wheat to leaf rust. The country can ill afford such losses, especially for wheat, a major export commodity (USDA).

Yield losses due to the cereal rust have also been reported from the Indian subcontinent and the Middle East. A severe leaf rust epidemic in 1978 resulted in an estimated loss of US $86 million in Pakistan (McIntosh *et al*., 1995).

Cereal rust losses in Europe are primarily associated with stripe rust and leaf rust. The effective barberry eradication campaign in the early 20th century and the infrequent occurrence of favourable temperatures have resulted in a decline in importance of stem rust. Economic assessments in the United Kingdom by Priestley and Bayles (1988) provided estimates losses in susceptible winter wheat due to the stripe rust and leaf rust of £83 million, with the value of the disease resistance estimated at £79, 8 million.

Losses are also important in Asia, Australia and South America. Several attempts have been made to assess yield losses due to rust epidemics in Australia. Estimates of crop losses varied from 30% in leaf rust susceptible cultivars (Rees and Platz, 1975) to 55% in wheat susceptible to both stem and leaf rust (McIntosh *et al*., 1995).

An overview of global crop losses caused by rust diseases indicated varying regional significances (Saari and Prescott, 1985). Stripe rust is more prominent in west Asia, southern Africa, the Far East (China), South America and northern Europe. Leaf rust has caused more serious losses in south Asia, North Africa, south-east Asia and South America. Stem rust has traditionally been found in North America, Australasia, northern Africa, South Africa and, to some extent, Europe. Crop losses inevitably reflect the interplay between pathogen, host and environment at local, regional and global levels. Cereal rust diseases will continue to demand the attention of research and advisory personnel because of the dynamic nature of this relationship (McIntosh *et al*., 1995).

A B

Figure 2.Posters of public interest of cereal rust epidemics.
A-United States of America Barberry eradication poster. Courtesy of JJ. Burdon (McIntosh *et al*., 1955).
B-stem Rust in Australia 1973 (McIntosh *et al*., 1995).

1.5. Wheat Rust

1.5.1. Hystory

Old scriptures talk about wheat affections for mildew, wilting and smut, which nowadays are suppose to be caused, at least partway, by the rust fungi. In 1767, Italians Fontana and Tozzetti, separately carried out the first detailed and unambiguous reports about stem rust in wheat. In 1797, Persoon named *Puccinia graminis* to the organism which caused the stem rust in wheat. Chester (1946) showed in one of the first reviews of the published works about the rust (Roelfs *et al*., 1992).

In the first chronicles, there was not distinction between stem rust and leaf rust. In 1815 Candolle (1815) had shown that wheat leaf rust was caused by a different fungus, which was named as *Uredo rubigo-vera*. The name of the pathogenic agent had different names until Cummis and Caldwell (1956) proposed the name *Puccinia recondita* (Roelfs *et al*., 1992).

Morphological studies carried out by Savile (1984) and morphological and pathogen genetic studies by Anikster *et al*. (1997) showed that *P. recondita* was not the pathogen producer of wheat leaf rust. Currently *P. triticina* should be the preferred name (Roelfs *et al*., 1992).

Although Gadd described for the first time the wheat stripe rust in 1777, it wasn't till 1896 when Eriksson and Henning proved that that the stripe rust was caused by a different pathogenic agent which they named *Puccinia glumarum*. In 1953 Hylander recalled it as *Puccinia Striiformis* (Roelfs *et al*., 1992).

1.5.2. The disease

The most common wheat rust disease is called leaf or brown rust. It appears in the sheets, but it can also be found in the pods if the environmental conditions are favourable, there are high density of inoculums or are susceptible cultivars. Often, the disease doesn't shows a high teliospores production like the stem rust does at the end of the period. Furthermore it causes a brown leaf injury instead a black injury like the injury caused by the stem rust. The disease develops quickly with temperatures between 10 to 30 °C and it appears more or less wherever wheat grows. The yield loses are directly relate with the decrease of flower production. When severe epidemics occur at the same time of a drought, causes the wrinkling of the grains. In some genotypes, an early epidemic (before the flower development) can kill the flowers and the complete plant. The yield loses caused by the leaf rust are normally less than a 10 %, but sometimes, if there is a severe epidemic, yield losses can be up to 30 % or more (Roelfs *et al*., 1992).

The stem rust is also called black rust because the big amount of black teliospores formed in the uredines. The temperatures between 15 and 35 ° C and the humidity are favourable growing conditions for the stem rust. This is de most damaging form of rusts which can cause a 50 % of yield loses in one month with the right environmental conditions. In some sensitive cultivars loses can be of 100 % of the yield (Roelfs *et al*., 1992).

Stripe rust or yellow rust is a disease that attacks the wheat when there are low temperatures (2-15 °C), normally in high locations, northern latitudes or later years. This rust got its name from the uredines which produce yellow uredospores. Following an

early attack by the fungus, the plants whiter away. The yield loses can be big (50%) caused by the wrinkling of the tillers and damaged stems. In extremely situations loses can be up to 100 % (Roelfs *et al*., 1992).

In the tables 1 and 2 are indicated the pathogen, the primary and alternate hosts, symptoms and required environmental conditions for the three rust diseases.

Table 1.The rust diseases of wheat, pathogens, primary and alternate hosts and symptoms (modified from Roelfs *et al*., 1992)

Disease	**Pathogen**	**Primary hosts**	**Alternate hosts**	**Symptoms**
Leaf rust	*Puccinia recondita f.sp tritici*	hard wheat Soft wheat Triticale	*Thalictrum Anchusa Isopyrum Clematis*	Isolated Uredinos in the leafs and rarely in the pods.
stem rust	*Puccinia graminis f.sp tritici*	Hard wheat Soft wheat Barley Triticale	*Berberis vulgaris*	Isolated Uredinos in the leafs, in the stems and in the spikes
Stripe rust	*Puccinia Striiformis f.sp tritici*	Soft wheat Hard wheat Triticale Barley	*Berberis vulgaris*	Systematic Uredines in the leafs and spikes and rarely in the pods

Table 2. Environmental conditions required for wheat rusts (according to Roelfs *et al*., 1992)

Stage	Temperature (°C)			Light	Free water
	Minimum	Optimum	Maximum		
Leaf rust					
Germination	2	20	30	Low	Essential
Germling	5	15-20	30	Low	Essential
Appressorium	-	15-20	-	None	Essential
Penetration	10	20	30	No effect	Essential
Growth	2	25	35	High	None
Sporulation	10	25	35	High	None
Stem rust					
Germination	2	15-24	30	Low	Essential
Germling	-	20	-	Low	Essential
Appressorium	-	16-27	-	None	Essential
Penetration	15	29	35	High	Essential
Growth	5	30	40	High	None
Sporulation	15	30	40	High	None
Stripe rust					
Germination	0	9-13	23	Low	Essential
Germling	-	10-15	-	Low	Essential
Appressorium	-	-	(not formed)	None	Essential
Penetration	2	9-13	23	Low	Essential
Growth	3	12-15	20	High	None
Sporulation	5	12-15	20	High	None

1.5.3. Epidemiology

There are regions in the world where rust diseases are especially critical and can cause important yield loses. Table 3 provides a general summary of the current and historical importance of rust diseases worldwide. In some other regions, the conditions are not adequate and there are just serious epidemics in the years when the environmental conditions are unusually favourable, the varieties used are extremely susceptible or the cultivation methods are modified (Roelfs *et al*., 1992).

The wheat rust uredospores start germinating one to three hours after get in contact with free water under different temperatures depending of the rust type. A big amount of uredospores are produced and can be spread long distances by the wind. However, most of the uredospores are deposited close to the origin by influence of the gravity. The final velocity of the uredospores is approximately 1cm/s. One spore takes more than 8 hours and 20 minutes to fall 300 meters. For longer distances, most of the uredospores will keep suspended in the air until the rain drags them (Roelfs *et al*., 1992).

Uredospores have relative long shelf lives and can survive outside without being deposited in host plants during some weeks. They endure freezing if the water content decreases to a 20-30 %; the bioavailability decrease fast when the water content is higher than 50 % (Roelfs *et al*., 1992).

Long distance uredospores spread is influenced by the latitude and the winds characteristics. Normally, the spores move from west to east because the winds caused by the earth rotation, but there are proves of barley stripe rust uredospores moving to the south east in South America (Dubin and Stubbs, 1986). In India the spores can move to the south, probably as a consequence of the coming winds of the Himalaya. Commonly, to start the disease, it is supposed to be a long distance transport of spores, but there are not proves to differentiate the endemic inoculums from the extern source (Roelfs *et al*., 1992).

On hot days the air rises above the foliage. When the atmosphere is humid, there are less uredospores coming out from the uredines. Slow winds dry the foliage, shake the leaves and release the spores. Fast winds can cause a higher release of spores, but the concentration decrease and is more important for the long distance spread rather

than for local infection. The rain drags the spores from the air and releases them in the plants increasing the relative humidity and normally, starting the infection process. Relative Humidity decreases the movement capacity of the spores. Also, the temperature variations caused by the rain influence in the development of the disease (Roelfs *et al.*, 1992).

1.5.4. Pathogen-host interactions

Pathogen-host interactions can be classified in two categories: specific and non specific. The specific are those in which just one isolate interacts with one single genotype to cause one answer to the disease, different to another caused by another isolate in the same host. The non-specific interactions are those which every isolate cause a similar answer in a specific genotype of the host. Theoretically, non-specific resistance is the best to develop a breeding programme. However, in order to verify this, one would have to evaluate every single member of the pathogen population, which would be impossible (Roelfs *et al*., 1992).

Table 3.Current and historical importance of wheat leaf, stem and stripe rusts in different epidemiological zones (Saari and Prescott 1985)

Zone	Leaf rust		Stem rust		Stripe rust	
	Current[a]	Historical	Current	Historical	Current	Historical
Africa						
North	Major	Major	Local	Major	Local	Local
East	Local	Local	Major	Major	Major	Major
Southern	Local	Local	Local	Major	Local	Rare
Asia						
Far East	Local	Local	Local	Major	Major	Major
Central	Major	Major	Minor	Minor	Local	Local
South	Local	Major	Minor	Major	Local	Local
Southeast	Major	Major	Minor	Minor	Rare	Rare
West	Local	Local	Local	Major	Major	Major
Australia, New Zealand	Local	Local	Local	Major	Local	Rare
Europe						
East	Major	Major	Minor	Major	Local	Local
West	Local	Major	Minor	Major	Major	Major
North America	Major	Major	Minor	Major	Local	Local
South America	Major	Major	Local	Major	Local	Local

Major = important yield losses without the cultivation of resistant varieties; Minor = usual presence, but of little significance; Local = just occurs in randomly in some special areas of the region, losses in these areas may be occasionally severe if susceptible cultivars are grown; Rare = not present/important, atypical seen or recently introduced.

1.6. Wheat Leaf Rust

1.6.1. Introduction

Leaf rust, caused by *Puccinia triticina Eriks*., is the most common rust disease in wheat (*Triticum aestivum L.*). Leaf rust occurs more often and in a larger area than stem rust (*P. Graminis f. Sp. Tritici*) or stripe rust (*P.striiformis f. Sp. Tritici*). The fungus is heteroecious, therefore, requires a telial/uredinial host (usually wheat) and an alternative (pycnial/aecial) host to complete the full life cycle (Figure 3). *P. Triticina* centre of origins is the Fertile Crescent region of Middle East, where the natural range of the primary and the alternative hosts overlap. *Puccinia spp*. have afflicted wheat for thousands of years, as references to rust can be found in literature of the classical Greece and Rome and in the Bible (Chester, 1946). Based on the yield loses in wheat caused by *Puccinia Triticina,* leaf rust is now recognized as an important pathogen in wheat production worldwide. Yield losses are distributed over large geographical areas (Kolmer, 2005; Marasas *et al*, 2004; Roelfs *et al*, 1992; Saari and Prescott, 1985; Bolton *et al*, 2008). Although leaf rust is found almost everywhere wheat grows, suitable alternate hosts are rarely present for the fungus to complete the sexual cycle. The *Thalictrum* and *Isopyrum spp.* which are native to North America are relatively resistant to basidiospore infection (Saari *et al*, 1968). That suggests that the sexual cycle does not contribute epidemiologically to spread the disease and is an insignificant source of genetic variation (Kolmer, 2005). Over 70 different races of wheat leaf rust are detected each year in North America (Kolmer *et al.,* 2007) where the pathogen persists through reproduction from asexual uredospores.

1.6.2. Epidemiology

Puccinia triticina can survive the same environmental conditions that the wheat leaf survives, provided infection but no sporulation has occurred. The fungus can infect with dew periods of three hours or less at temperatures of about 20°C; however, more infections occur with longer dew periods. At cooler temperatures, longer dew periods are required, for example, at 10°C a 12-hour dew period is necessary. Few if any infections occur where dew period temperatures are above 32°C or below 2°C. Most of the severe epidemics occur when uredinia and/or latent infections overwinter at some threshold level on the wheat crop, or where spring-sown wheat is the recipient of exogenous inoculum at an early date, usually before heading. Severe epidemics and losses can occur when the flag leaf is infected before anthesis (Roelfs *et al*., 1992).

The leaf rust uredospores developed in spring from infections from last winter/autumn (endogen inoculums), use to appear in the lower leaves. The rust produced from air-disseminated uredospores (exogenous) appears commonly in the higher leaves of the plant. This is a good method to differentiate endogen from exogenous inoculums (Roelfs *et al*., 1992).

The fungus diffusion can be really fast if the environmental conditions are favourable. Spore germination to sporulation can occur within a seven- to ten-day period at optimum and constant temperatures. At low temperatures (10° to 15°C) or diurnal fluctuations, longer periods are necessary. The fungus may survive as insipid mycelia for a month or more when temperatures are near or below freezing. One uredinium can produce 3.000 spores per day. A uredinium can produce 1000 injuries in 10 days, 2000 in 11 days and 2.010.000 after 22 days (Roelfs *et al.*, 1992).

1.6.3. Life cycle

Figure 3 shows the life cycle for *P. triticina* and the disease cycle for wheat leaf rust. The time for each event and frequency of some events (sexual cycle, wheat cropping season and green-bridge) may vary among areas and regions of the world.

The alternate host currently provides little direct inoculum of *P. triticina* to wheat, but may be a mechanism for genetic exchanges between races and perhaps populations. The pathogen survives the period between wheat crops in many areas on a green-bridge of volunteer (self-sown) wheat. Inoculum in the form of uredospores can be blown by winds from one region to another (Roelfs *et al.*, 1992).

The life cycle of *P. triticina* has five development stages. The Telia formation takes place in the crop remains and they produce teleutospores, these ones, due to the meiosis in spring, produce basidiospores. The basidiospores infect the leaves of the secondary host *Thalictrum speciosissimum*, building in it picnia and aecia, which under favourable environmental conditions starts the infection of the wheat leaves, producing uredospores. The uredospores will infect the crop progressively if the humidity and temperature conditions are optimum. When the conditions are not favourable to or the plants start to senesce, teleutospores will develop under the leaves and will remain on the harvest trash during winter/autumn, closing then the fungus life cycle (Junta de Andalucía, Consejeria de Agricultura y Pesca).

Uredospores initiate germination 30 minutes after contact with free flowing water at temperatures of 15° to 25°C. The germ tube grows along the leaf surface until it reaches a stoma; an appressorium is then formed, followed immediately by the development of a penetration peg and a sub-stomatal vesicle from which primary hyphae develop. A haustorial mother cell develops against the mesophyll cell, and direct penetration occurs. The haustorium is formed inside the living host cell in a compatible host-pathogen interaction. Secondary hyphae develop resulting in additional haustorial mother cells and haustoria. In an incompatible host-pathogen response, haustoria fail to develop or develop at a slower rate. When the host cell dies, the fungus haustorium dies. Depending upon when or how many cells are involved, the host-pathogen interaction will result in a visible resistance response (Roelfs *et al*., 1992).

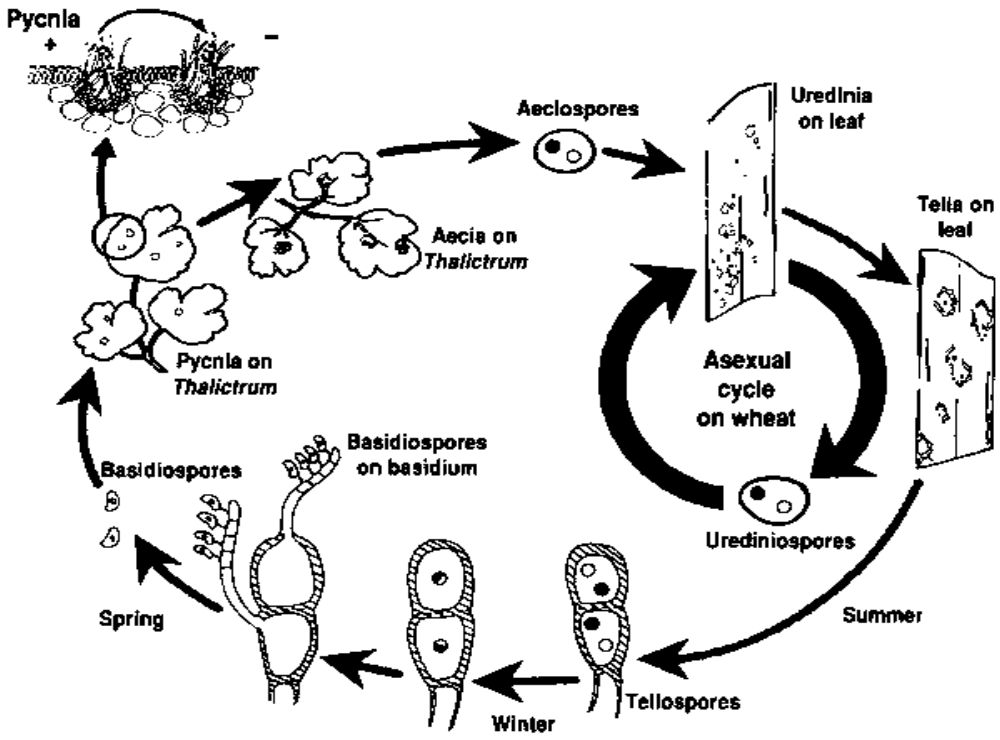

Figure 3.Life cycle of the fungus *Puccinia triticina* (V.Brewster)

1.6.4. Hosts

The *P. recondita* complex members attack a wide number of grasses, yet there seems to be a strict specialization of the host range of the different populations. *Puccinia triticina* is primarily a pathogen of wheat, its immediate ancestors and the man-made crop triticale. The *P. recondita* f. sp. *secalis* from rye (rye leaf rust) does not attack wheat. Recent evidence indicates that populations of leaf rust exist in Europe, Asia and Africa that are primarily pathogens of durum wheat. They are all distinct from the population that exists worldwide on bread wheat (Huerta-Espino and Roelfs, 1989).

1.6.4.1. Alternative hosts

Recent studies (Anikster *et al*., 1997) indicated that the primary alternate host of *P. triticina*, including the durum attacking populations, is *T. speciosissimum*. Whereas *A.agregata*, *A.undulata, Echiumglomeratum* and *Lycopsis arvensis* (Boraginaceae) were the alternate host for the leaf rust on wild wheat (*Triticum* [*Aegilops*] spp.) and rye. The alternate host is essential for the survival and spread of *P. triticiduri*.

Table 4.Alternate host of *Puccinia triticina* which have any influence in the development of the disease and their references (Roelfs et al., 1992)

Host	Reference
Anchusa italica Retz.	D'Oliveira y Samborski
Clematies mandshurica Rupr.	Azbukina
Isopyrum fumaroides L.	Brizgalova
Thalictrum flavum L.	Jackson and Mains
Thalictrum foetidum L.	Tommasi *et al*
Thalictrum japonicum Thunb.	Tommasi *et al*
Thalictrum speciosissimum Loefl.	D'Oliveira

1.6.4.2. Secondary hosts

Rust pathogens attack many grasses, but in the case of the *P. triticina* is difficult to say which hosts are naturally affected by it. In case of artificial infection, many grasses can be infected by *P. triticina,* however it seems like it can't happen in natural conditions. Some potential host for wheat rust are wild species or weeds such as, *Triticum* and *Aegilops* and the close species of *Agropyrum* and *secalis*. The most common non-crop host for wheat leaf rust is volunteer or self-sown wheat. These plants may be in fallow fields, along the edges of fields and roads, as weeds in a second crop and as a cover crop under orchards, along irrigation canals, etc. This is the major source of inoculum throughout much of the world where wheat is autumn- or winter-sown (Roelfs *et al.*, 1992).

1.6.4.3. Primary hosts

The primary host of *P. triticina* is common wheat (*Triticum aestivum*). *P. triticina* also occurs in *T. Turgidum L*, but in this one is just important in Mediterranean areas, India, Ethiopia and the Middle East, where durum wheat is most common cultivated. It is less important in *T. monococcum* L., *T. dicoccum* and *Ae. speltoides* Tausch. Wheat leaf rust would also appear to be a major threat to triticale (*Triticosecale* Wittmack), the crop derived from the man-made cross between wheat and rye (Skovmand *et al*., 1984; Roelfs *et al*, 1992).

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1.6.5. Pathogen variation and genetic resistance

The best way to achieve a real control over the leaf rust in wheat is to focus on the genetic combination in order to develop resistant varieties (Roelfs *et a.l*, 1992). The pathogen is always changing and even when a variety with a *Lr* gen is found, the isolate will most likely develop and get over the resistance gen becoming virulent for the genotype.

Variations on cereal rusts have traditionally been assessed by testing isolates on host genotypes that differ for resistance. The early differentials for wheat stem rust and leaf rust were collections of wheat cultivars and varieties that had different infection types on seedling plants to the most commonly found isolates of both rust pathogens.

These early differential sets were genetically undefined as there was no information on the resistance genes of the cultivars and varieties. Later as knowledge was gained about the genetics of rust resistance in wheat, cultivars with defined single genes for rust resistance where used as differentials. As the number of characterized resistant genes increased, resistance genes were transferred into single wheat genotype backgrounds by backcrossing (Roelfs *et al*., 1992).

The resistant genes have been mainly obtained from the varieties of *T. Aestivum*, but some have come from other species of *Triticum (Aegilops)* and from *Secalis* (rye) and *Agropyrum*. From the group of genes that determines a specific race resistance, Lr19 of *Agropyrum elongatum* still being effective in all world, but it is used commercially just in a limited area. Other resistant genes used worldwide are Lr22a, 25, 29, 32 and 33. They are effective, but there are only a few varieties with this resistance and which have been widely cultivated (Roelfs *et al.*, 1992).

1.7. Wheat Stripe Rust

1.7.1. Introduction

Yellow rust of wheat caused by the fungus *Puccinia striiformis* f.sp. *tritici* is one of the most damaging diseases of wheat in many areas around the world and severe epidemics may result in substantial losses. For example, annual losses due to yellow rust varied, on average, from 2 to 15% in Danish wheat trials between 1988 and 1990, and for the most susceptible cultivar, losses of almost 50% (equal to 4 t/ha) were recorded in 1990 (average of 236 trials). Yield losses of the same magnitude were reported for susceptible cultivars in Asia (Sharma *et al*; 1985) and in the Near East (Hovmoller, 2001; Yahyaoui, 2000; ICARDA, personal communication).

Stripe rust has a lower optimum development temperature than stem and leave rust and so the disease is not a major threat in many areas of the world. Stripe rust is principally an important disease of wheat during the winter or early spring or at high elevations. An important feature of yellow rust is the dispersal of airborne uredospores, which may travel long distances in air, either directly or via several successive shorter steps. Table 3 shows regions of the world where stripe rust has been a major or local problem (Roelfs *et al.*, 1992).

1.7.2. Epidemiology

As mentioned above, stripe rust develops in lower temperatures compared to other wheat rusts. The minimum, optimum and maxim temperatures for the infection of *Puccinia striiformis* are 0, 11 and 23 °C, respectively (Roelfs *et al.*, 1992). The pathogen can usually survive the winter in active form on the wheat planted in autumn (Figure 4).

In Europe, *P. striiformis* oversummers on wheat. The amount of over-summering rust depends on the amount of volunteer wheat, which, in turn, is a function of moisture in the off-season. The uredospores are then blown to autumn-sown wheat. In north-western Europe, overwintering is limited to urediniomycelia in living leaf tissues as temperatures of -4°C will kill exposed sporulating lesions. Latent lesions can survive if the leaf survives. In other areas of the world, snow can insulate the sporulating lesions from the cold temperatures so air temperatures below -4°C fail to eliminate the rust lesions. The latent period for stripe rust during the winter can be up to 118 days and is suspected to be as many as 150 days under a snow cover (Roelfs *et al.*, 1992).

In equatorial areas, stripe rust tends to cycle endemically from lower to higher altitudes and return following the crop phenology. In northern latitudes, the cycle becomes longer in distance with stripe rust moving from mountain areas to the foothills and plains. Due to their ultraviolet light sensitivity, yellow rust uredospores are not allowed to travel in a viable state as much as the stem and leaf rust. Zadoks (1961) inform of some stripe rust uredospores that have been transported more than 800 km. The introduction of the yellow rust in Australia was probably caused by human action with reaction airplanes, but the propagation of the pathogen from Australia to New Zeeland, 2000 km of distance, is consequence of wind transportation (Roelfs *et al.*, 1992).

In most of the studied areas it seems that volunteer wheat is an inoculums source. However, some recent experiments show the presence of inoculums in some grasses of the specie Barberis (Yue Jin *et al*., 2010). It is important to compare that the phonotype of the grass and the wheat is the same in order to confirm that this is the inoculums source (Roelfs *et al.*, 1992).

In Netherlands, for example, epidemics can start just from one overwintered uredine per hectare if the conditions are favourable. It is difficult to detect one uredine,

but when the disease develops, it is easier to find local focus of infection (Roelfs *et al.*, 1992).

1.7.3. Life cycle

Figure 4 shows the life cycle of *P. striiformis* and the disease cycle for wheat stripe rust. For many years it has been believed, that *Puccinia striiformis* was a hemiform rust which cycle consists just on the uredinial and telial stages and that stripe rust populations can exist, change in virulence and result in epidemics independent of an alternate host (Roelfs *et al*., 1992). Recently it has been discovered that the disease cycle consists in a sexual cycle in the host *Barberis spp*. and an asexual cycle in wheat, very similar to the *Puccinia triticina* cycle. The teliospores of stripe rust infect *Beberis spp*., and the aeciospores produced by the alternate host then infect the wheat, completing the live cycle of *P. striiformis* f. Sp. *tritici* (Yue –jin *et al*., 2010). However, in most of the wheat-growing regions throughout the world, urediniopores are the only inoculum for the initial and recurrent infection of wheat plants.

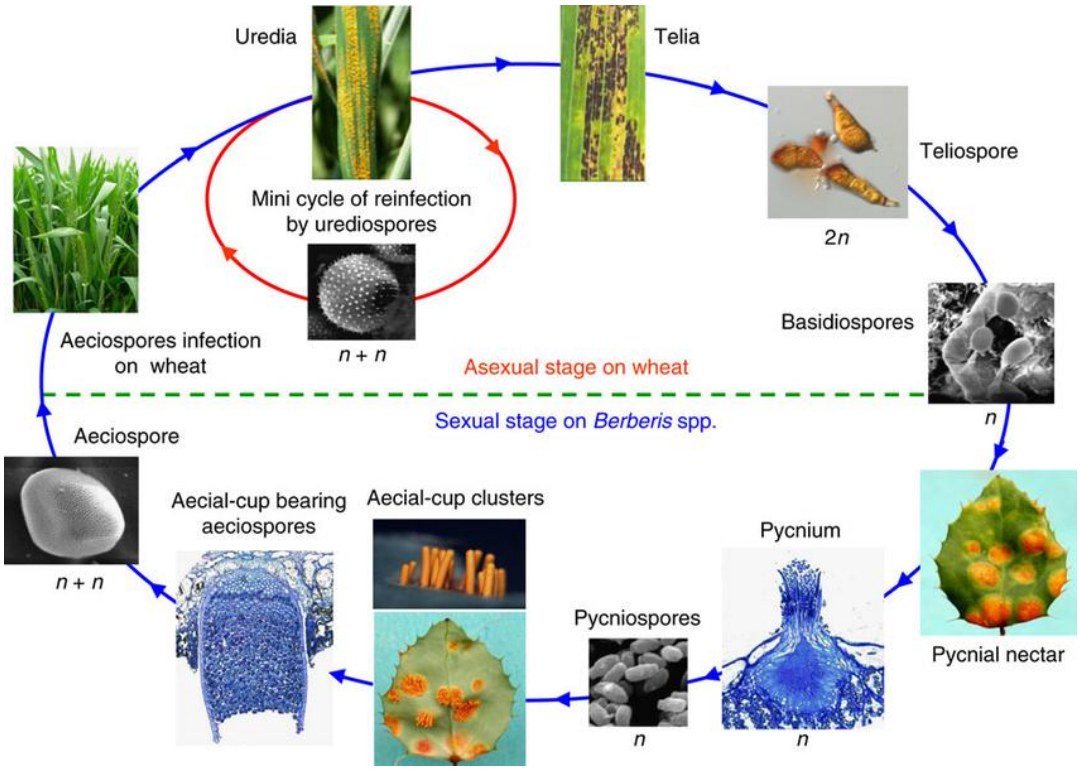

Figure 4.Live cycle of the fungus *Puccinia striiformis* f. sp. *Tritici* (Zheng *et al*., 2013)

1.7.4. Hosts

Puccinia striiformis is a common pathogen of grasses and cereals like wheat, barley, triticale and rye (Yue –jin *et al*., 2010).

1.7.4.1. Alternate hosts

For many years the live cycle of *Puccinia striiformis* has been somewhat a mystery. Part of that was because there was no certainty of an alternate host for the pathogen. Anyhow, Mains (1933) suspected *Berberis* and *Mahonia* could be alternate hosts of stripe rust due to their similarities to *P. Koeleriae*, *P. arrhenhateri*, and *P. montanensis* which are rusts of *Beberis*. Hart and Becker (1939) followed this lead but failed to infect *Berberis* or Mahonia with teliospores of *P.striiformis*. In 2010, a research carried out by the University of Minnesota, inoculated wheat with aeciospores from *Beberis spp*., which originally came from an infection made with teliospores of stripe rust in wheat, thus proving that *Berberis spp.* is an alternate host for wheat stripe rust and elucidating the complete life cycle of *P. striiformis* f. sp. *tritici* (Yue Jin *et al*., 2010).

1.7.4.2. Secondary hosts

Puccinia striiformis attacks principally plants from the Festucoideae and Eragrostoideae family. The principal hosts are from the genus *Aegilops, Agropyrum, Bromus, Elymus, Hordeum, Secale,* and of course *Triticum*. Manner and followed by Tollenaar and Houston all explained that isn't the same virulence of the stripe rust that attacks wheat and the other secondary hosts. The fact that the pathogen shows the ability to produce some spores in a species under greenhouse conditions doesn't prove that it could happen in natural conditions (Roelfs *et al*., 1992).

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1.7.4.3. Primary host

Puccinia striiformis f. Sp. *tritici* mainly infects common wheat (*Triticum aestivum* L.), durum wheat (*T. turgidum*var. *durum* L.), cultivated emmer wheat (*T. dicoccum* Schrank), wild emmer wheat (*T. dicoccoides* Korn) and triticale (*Triticosecale*). Stripe rust can infect certain cultivated barleys (*Hordeum vulgare* L.) and rye (*Secale cereale* L.), but generally does not cause severe epidemics (Cheng *et al*., 2014)

1.7.5. Pathogen variation and genetic resistance

As well as the other rusts diseases, the best method to decrease the stripe rust incidence is to use wheat genetic resistant varieties. However, the pathogen has the ability to overcome resistances. In Mexico, for example, the varieties recommended by the INIFAP were resistant to the disease until summer of 2002, in summer 2003 it was observed a new race of the pathogen called MEX.03.37 (219MEX0), which had become resistant to many of the recommended varieties, causing important damages and yield loses (Huerta-Espino *et al*., 2009). The agronomic consequences of dispersal of yellow rust uredospores from external sources to Denmark (in a period during which large areas were planted with relatively few wheat cultivars) were demonstrated in several cases, most evidently when the *Yr9* and *Yr17* resistance genes became ineffective (Hovmoller, 2001).

Many of the resistance genes of a specific variety have been transferred from secondary hosts. Biffen (1905) did the first research studies about the yellow rust resistances. Many of these resistances were considered to be non-specific, but with time the pathogen developed and showed that it was a specific resistance (Roelfs *et al.*, 1992).

1.8. Rye Leaf Rust

1.8.1. Introduction

The brown rust of rye (*Secale cereale*) was first described in 1894 by Eriksson and Henning and as *Puccinia dispersa* Erikss. et Henn. named. Due to its similarity to wheat leaf rust, it was in 1966 that Wilson and Henderson added it in to the collective species *Puccinia recondita* Rob.ex Desm.

Rye brow rust or rye leaf rust (*Puccinia recondita* f. sp. *secalis*) is the most harmful leaf disease in rye (*Secale cereale* L.) and can be found in every region where rye is grown. After the introduction of hybrid varieties, which are poor in genetic variability and in their lack of characterized resistant genes, the disease caused big damages in rye yields. Some experiments with hybrid rye cultivars have shown a 14 % decrease in the grain weight after being infected with *Puccinia recondita*. In the case of infection of sensible varieties, loses can increase to 40 %. The use of fungicides can decrease the

damages, but ecologic factors, consumer's reserves and the costs make from the use of resistant varieties the best option (Klocke, 2004).

Puccinia recondite f. sp. *secalis* dynamics and virulence was first studied by Mains (1926), however, the characterization of different pathogens and their virulence wouldn't appear until 1995 when Leisser and Sperling studied different isolates from the species. This is important in order to create new resistance genes, because a good knowledge of the pathogen's dynamic and virulence is essential (Klocke, 2004).

1.8.2. Epidemiology

Puccinia recondite f. sp. *secalis* has a broad temperature spectrum of infection. Although, epidemics occur better under warm conditions, it does tolerate the low temperatures better than the wheat leaf rust. Daily temperatures between 20 and 26 °C improve the pathogen development. Temperatures lower than 12 °C decrease the infections rate of rye leaf rust pathogen. Spores need moisture and at least four hours to germinate. Light is also a factor which affects the disease development, solar radiation increases the spore production and infection (Obst and Gering, 2002).

The cultivation of susceptible varieties, a high nitrogen supply, mild autumn, winter weather and a hot spring promote leaf rust and allow a rapid proliferation of the disease. A leaf rust infestation in the previous year increases the risk of infestation the following year

The disease often appears relatively late and so it's unlikely there are a great financial losses (with the exception being when caused with late-ripening varieties). Mycelium and uredinia easily survive low temperatures, thus allowing the rust fungus to survive even without intermediate host in a region.

1.8.3. Life cycle

Rye brown rust is an obligate parasite pathogen and needs living tissue to develop. The fungus cycle consist in a 5 stage spore state, is heterocius and needs from two different hosts to complete the cycle (Obst und Gehring, 2002). In the secondary host, the formation of pycnium and aeciospores occurs and in the primary host takes place the formation of uredospores and teleutospores -where the basidiospores grows (Klocke, 2004).

The cycle consists in a five stage spore states which take place in two different hosts. Figure 5 shows the life cycle of the pathogen fungus of rye leaf rust. The process starts when winter ends with the germination of the basidiospores in the alternate host. The germinated spores get into the cell and infect it. In leafs top surface, the pycnium and the pycniospores develop and in leafs underside the aecia and the aeciospores appears. Wind transportation brings the aeciospores to the primary host and if there is water and the conditions are favourable, the germ tubes can develop, get into the sheets and infect the plant. After the infection takes places, the aeciospores develop in to uredospores, which have the capacity to spread de disease due to the wind-human-insect transportation. Teleutospores are present at the end of the cycle and these overwinter and have the capability to infect the alternate host again creating basidiospores which can start the process again (Roelfs *et al.,* 1992, Obst und Gehring, 2002).

The uredinia appear in the top surface of leaves. In years with severe epidemics the pustules can also appear in the underside of the leaves and the spikes. As mentioned above, uredospores are resistant to low temperatures and even can survive in snow for several months (Roelfs *et al.,* 1992; Obst und Gehring, 2002).

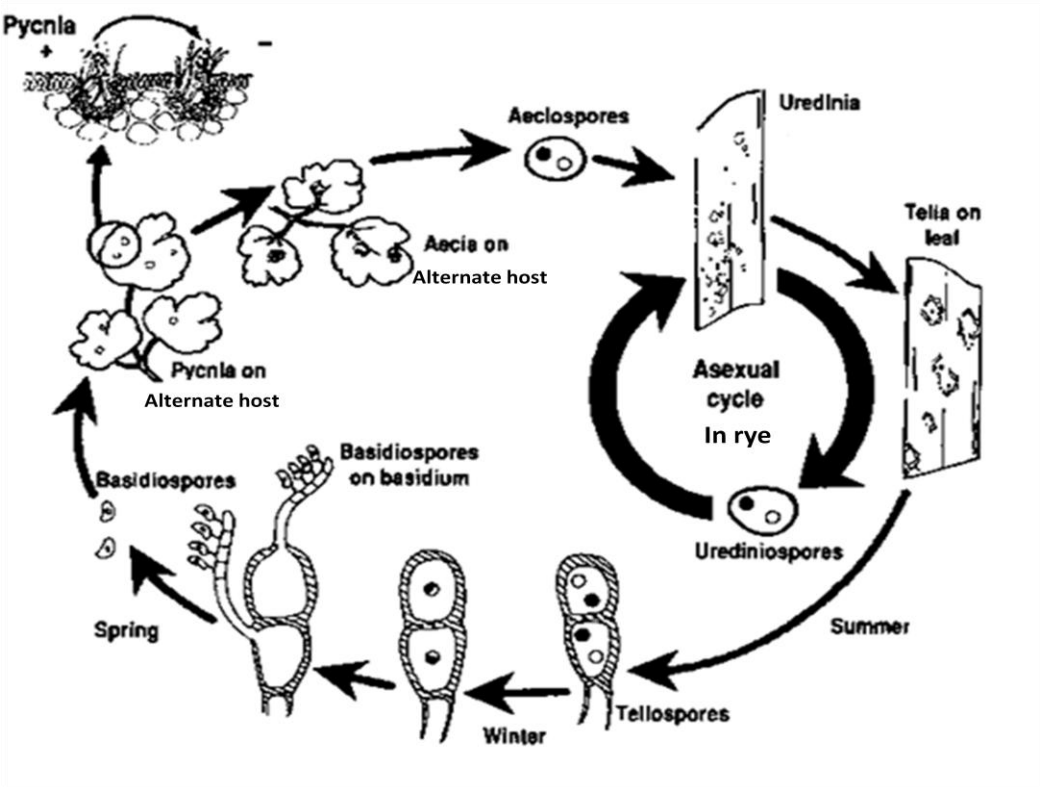

Figure 5.Life cycle of the fungus *P. recondita f. sp. secalis* (Courtesy V.Brewster).

1.8.4. Hosts

One of the differences which helped to differentiate the rye leaf rust from other rusts is their host plants (Klocke, 2004).

The brown rust of rye, *P. recondita* f. sp. *secalis*, affects only the culture of rye as a primary host. The intermediate hosts of *P. recondita* f. sp. *secalis* are ox tongue (*Anchusa* sp.), the Pride of Madeira (*Echium* sp.) and other representatives of the *Boraginaceae* Family (Klocke, 2004).

Apparently the aecial stage of this fungus is not necessary from the survival of the rust year to year. The pathogen can overwinter in the uredinia stage (Obst und Ghering 2002), which means the alternate host may not be crucial to the epidemiology and spread of the disease. However, the alternate host is important in the roll of the recombination, mutation and hybridization of the pathogen because it provides the unique possibility for the fungus to build new pathotypes (Klocke, 2004).

1.8.5. Pathogen variation and genetic resistance

Cultivars of rye are genetically resistant to the wheat leaf rust and some are resistant to rye leaf rust. Rye has been used as a source of resistance to several wheat pathogens (Klocke, 2004). Rye varieties differ in their susceptibility to leaf rust depending on if they carry one or more resistance genes.

As well as other rusts of the small grains, the discovery or development of a resistant strain of rye offers the best possibility of control of this disease. Plant breeders tried as widely as possible to combine effective resistance genes in rye varieties to make them permanently resistant to as many of rust possible. The resistance breeding suffered repeated setbacks thought as either new rust races arose or new breeds migrated in to a previously populated area. Therefore, related species, wild and primitive forms of rye will continue in local breeds, the search for new resistance genes. Some new resistance genes, however, are usually linked with genes for negative agronomic properties, so they cannot be used.

1.9. Control methods of the diseases

It is essential to know the necessary conditions for the infection and establishment of a disease before to develop a control strategy. The most effective method to control the rusts in cereals is the use of resistant varieties (Roelfs *et al*., 1992). The use of fungicides and the cultivation practices are also effective control methods. The prevention measures just temporarily stop the infection but can't stop it completely, as the spores are air-transported and it's impossible to stop them.

1.9.1. Genetic resistance

Some varieties have been resistant for many years, usually five years, as this is the approximate number of years for the agronomic life of a variety. Some of these resistant varieties are Tatcher and Hope to the stem rust; Americano 25, Americano 44d, Supreza, Frontana and fronteiza to the leaf rust; and Wilhelmina, Capelle-Desprez, Manella, Julianaand Castens VI to the stripe rust. However, some varieties become infected when they have been grown just in a small superficies of the all cultivated area. In most of the cases, the failures are related to the ignorance of the pathogen virulence. Other times it is simply down to the overcoming of the pathogen to the host's resistance. It has proven to be difficult to maintain the leaf rust resistance, but some varieties used in North America have not been infected in more than 30 years (Moricca and Ragazzi, 2008; Roelfs et al., 1992).

The majority of the resistance genes have been obtained from *T. aestivum*, but some are from other *Triticum* spp. as well as from *Triticum* (*Aegilops*), *Secale* (rye) and *Agropyron*. The usefulness or durability of resistance does not seem to be associated with the donor genera or species (Roelfs et al.,1992).

1.9.2. Chemical control

Chemical control has been successfully used in Europe, permitting high yields (6 to 7 tonnes/ha) and where prices for wheat are supported. Chemicals were also used to control a leaf rust epidemic in 1977 in the irrigated Yaqui and Mayo Valleys of Mexico. Elsewhere, chemicals have had limited use on high-yielding wheat in the Pacific Northwest of the United States for stripe and leaf rust control. Chemical control of leaf rust in the eastern and southern United States has been practised when expected yields

exceed 2 tonnes/ha. In Brazil and Paraguay, chemicals are used on wheat with expected yields of 1 tonne/ha and above to control an array of other diseases (Moricca and Ragazzi, 2008).

1.9.3. Cultural methods

Cultural methods can help to control but not to eradicate the disease. No cultural method is useful on its own but the combination of various methods helps to intensify the resistances (Roelfs *et al.*, 1992). For example the use of early maturing cultivars developed by Farrer helped to control the steam rust in Australia. In Mexico, farmers use to seed early in order to avoid the steam rust (Roelfs et al., 1992).

Zadoks and Bouwman (1985) emphasized the importance of the green-bridge in carrying the disease from one crop to the next. The green-bridge can be lengthened when some farmers plant early and others late. Removing the green-bridge with tillage or herbicides is an effective control measure for epidemics that would result from endogenous inoculums. In some areas, volunteer plants must be controlled several times during the season when wheat is not grown. Crop rotation is also a possibility to remove the green-bridge.

Some of the benefits of gene deployment can be obtained by a grower if more than one cultivar is used that differs in resistance and from those grown by immediate neighbours. In some areas, control of timing, frequency and amount of irrigation and fertilization applications can aid in disease control (Moricca and Ragazzi, 2008; Roelfs *et al.*,1992).

1.9.4. Elimination of the alternate hosts

This control method has been successfully applied in North Europe and in some states from North America for the stem rust. There are no other areas in the world where the alternate hosts take part in the epidemiology of the disease. The alternate host for leaf rust may function more as a source of sexual reproduction than a source of epidemic-generating inoculum. For southern Europe, eradication of *Thalictrum* or *Anchusa* would probably not be feasible (Roelfs *et al*., 1992).

2. Objectives and experimental design

The main purpose of this Master work is the study of the vitality of uredospores of *Puccinia spp.* depending on the environmental conditions after release from leaf rust pustules. This research aims to gain a better understanding of the evolution of the germination ratio of the uredospores over time after being subjected to different temperatures, which could potentially help along with other studies, to improve previous epidemiological models. This objective has been developed through the weekly determination of the germination rate of the uredospores produced for the pathogenic fungi *Puccinia recondita* in rye and *Puccinia triticina* and *Puccinia striiformis* wheat.

For carryout the experiments were used wheat plants self cultivated in a greenhouse and wheat and rye that were already cultivated in the experimental fields of the Bonn University for previous experiments. The planting and harvesting processes were staggered in order of avoid the overlapping of the subsequent inoculation and analysis of the germinations rate. The plants cultivated in the greenhouse were deliberately inoculated but the plants cultivated in the fields were inoculated spontaneously.

For analyzing the vitality of the three *Puccinia spp*. species uredospores after release from leaf rust pustules, the germination ratio for every storage temperature was recorded extensively, from one day after the harvest and every seven days until the germination rate was stable. In order to obtain some conclusions, the results were compared with each other for the different storage temperatures and time.

3. Material and methods

3.1. Fungal pathogens

This work has been developed for three different species of the fungi *Puccinia spp.* The 3 species used were: *Puccinia recondita (Roberge ex Desm)*, *Puccinia Triticina (Erikss)* and *Puccinia striiformis (Westend). Puccinia recondita* causes brown leaf rust in rye, *Puccinia triticina* causes brown leaf rust in wheat and *Puccinia striiformis* causes yellow leaf rust in wheat.

3.2. Plants and cultivation

Two different crops were used to develop the experiment: rye and wheat. Wheat plants (*Triticum spp*) were grown from seeds of the cultivar "Dekan". They were cultivated in a greenhouse at 25 ° C and relative humidity of 60-80% (Figure 6). The seeds were sowed in pots of 10x10 cm and each pot contained 10 seeds. The soil used was Torferde 1.5 "Einheitserdewerke Werkverband". After seeding, the pots were moved to the greenhouse and watered. Sodium pressure lamps were used to increase the photoperiod to 16 hours per day and an automatic watering mat system guaranteed an adequate water supply. Light, temperature and irrigation conditions did not alter and the plants grew without any kind of stress. Sulphur evaporators were used to prevent powdery mildew infections.

For the experiment, adult wheat (*Triticum spp*.) plants of the cultivar "JB Asano" already infected with yellow leaf rust and rye plants (*Secale cereale*) of the cultivar "SU Forsetti" were also used. Both groups of plants were taken from previous experiments carried out in the experimental fields of the "Institut für Nutzpflanzenwissenschaften und Ressourcenschutz" (INRES) of Bonn University.

These varieties were chosen for their sensitivity to the infection of leaf rust produced for *Puccinia spp*. and for their resistance against powdery mildew, preventing cross-contamination in the greenhouse and facilitating the inoculation.

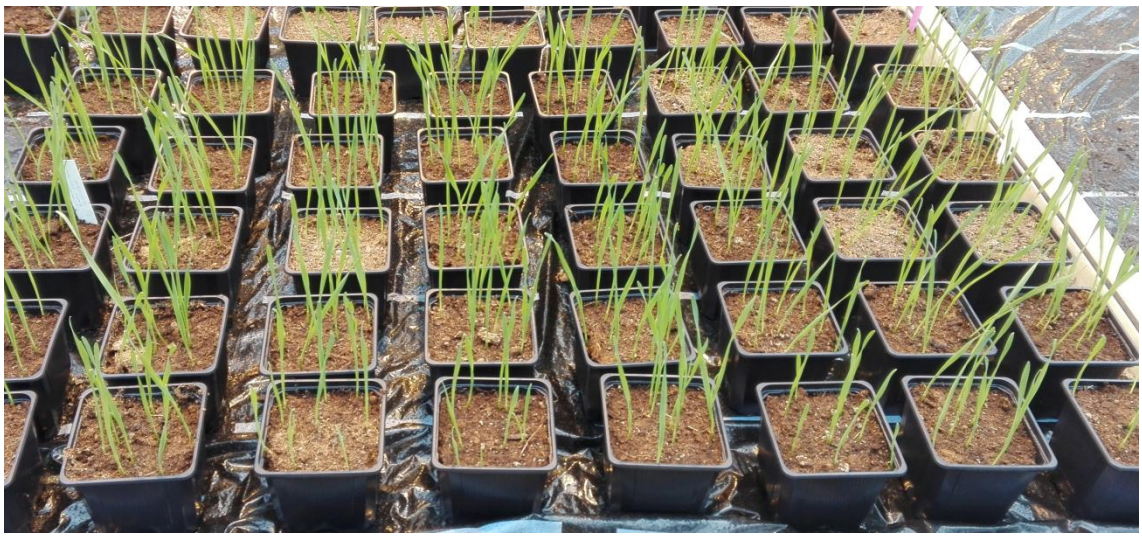

Figure 6.Wheat plants cv. Dekan in the greenhouse in 10x10cm pots used to carry out the experiment.

3.3. Inoculum and inoculation process

14 days after sowing, the wheat plants of the cultivar “Dekan” cultivated in greenhouse were inoculated with *Puccinia spp.* The inoculation was carried out by spraying a uredospores suspension on the plants. The uredospores used to prepare the suspension were from *Puccinia triticina* stored at 10 ° C of temperature and were taken from ongoing experiments. There was used 300 ml of suspension for each inoculation. The spore suspension was formed by a solution of water, a 0.01 % of Polysorbate 20 (Tween 20) and uredospores. Tween 20 is a surfactant product which is used to avoid the hydrophobic character of the spores, avoids the aggregation and helps an equal distribution of the suspension over the plants.

The concentration of the spores in the suspension was of 60.000 spores per ml. To estimate the spore concentration, a Fuchs Rosental counting chamber was used.

The suspension was filtered with gauze cheesecloth and filled up in a hand-held sprayer. The plants were sprayed with the solution and placed in a plastic box with a wet carpet at the base to maintain a 100 % humidity atmosphere (Figure 7). In order to ensure a successful inoculation, spraying was well distributed between plants and leaves. The plastic box was closed to preserve the 100 % humidity atmosphere and facilitate the inoculation. After 48 hours the plants were taken out and stored under the normal greenhouse conditions previously mentioned and fertilized with Blaukorn Classic (Compo). As plastic increases the spore’s losses by sticking, glass was the preferred material to use for the preparation of the solution.

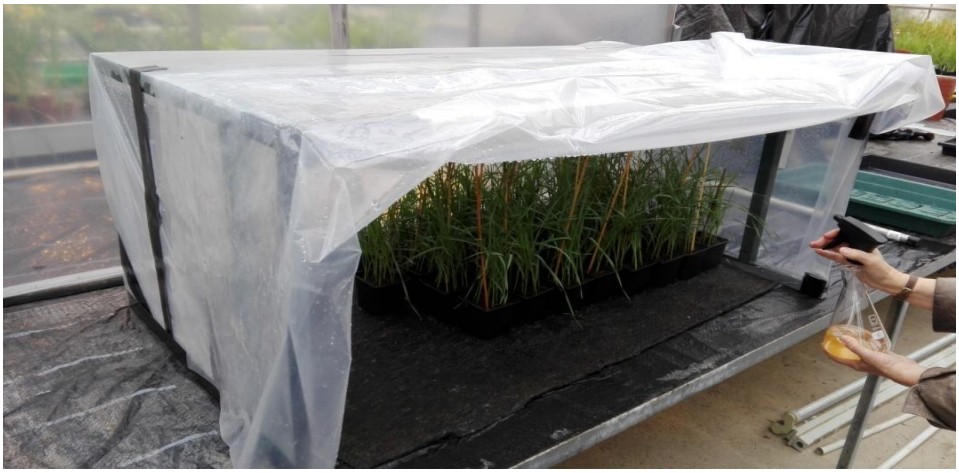

Figure 7.Plants of wheat cv.Dekan in the incubation box during the inoculation process.

The spores were collected 10-14 days after the inoculation. The spores used for the experiments in yellow leaf rust in wheat and brown leaf rust in rye were taken directly from adult field plants already infected by natural inoculation.

3.4. Harvest of spores

The spores were picked by hitting and brushing the plant's leaves with a paint brush over aluminium foil and trying to remove earth, water and remains. As the spores of the yellow rust were very firmly attached to the leaves, the spores were picked up with the original method but using much more vegetal material to collect enough quantity of spores to carry out the experiment.

3.5. Storage of spores

The collected spores of each one of the studied pathogenic fungi were stored in Eppendorf tubes under different temperature regimes. It was made in order to facilitate the storage distribution of the spores. The tubes were placed under 20, 10 and 4 °C and were analyzed the first day after the harvest and every 7 days until the germination rate was stable. Two different coolers were used for maintaining the spores at 10 and 4 °C and in order to maintain the spores at 20 ° C, an incubator was used.

3.6. Microscopic studies

3.6.1. Preparation of the samples

For analysing the germination ratio, previously we had to germinate the spores. The germination method was the same for all the spores but the germination temperature and time were different depending on the type of spores. The spores of yellow leaf rust in wheat were germinated at 10°C during 48 hours and brown leaf rust spores of rye and wheat were germinated 24 hours at room temperature (20°C).

Each sample consisted in a drop of 50 µl taken from a solution of 0,0025g of spores (weighted with a precision scale balance) and 500 µl of tap water mixed in a laboratory tube and dropped over a slide. For each one of the experiments 6 samples for each one of the spore's type and for each storage temperature were prepared. For the germination process, the slides were introduced in plastic box to prevent the air transpiration and maintain the humidity. The humidity was achieved with a piece of wet paper at the bottom of the box, covered over with a metallic grill to avoid the direct contact of the slides with the paper. These steps were made in order to prevent the

drying of the drops. As mentioned above, the boxes with the slides were stored before the microscopy analysis, 24 hours at room temperature for the brown rust whilst 48 hours at 10 ° C in a fridge for the yellow rust.

3.6.2. Determination of the germinations rate

Four of the six slides were analyzed per test. The analysis was carried out observing 100 spores per slide and counting how many of these spores were germinated. To recognise which spores were germinated, the method was to observe the presence of the germ tubes. Figure 8 shows germinated and non-germinated spores of brown and yellow rust. All light microscope observations were carried out using a Leitz DMRB light microscope (Fa.Leica Microsysteme Vetrieb GmbH, Wetzlar, Germany) with an attached Hitachi CCD HV-C20A camera (Fa. Hitachi Europe GmbH, Dusseldorf, Germany) and the Diskus software (v.4.40.1611, Fa.Hilgers, Königswinter, Germany).

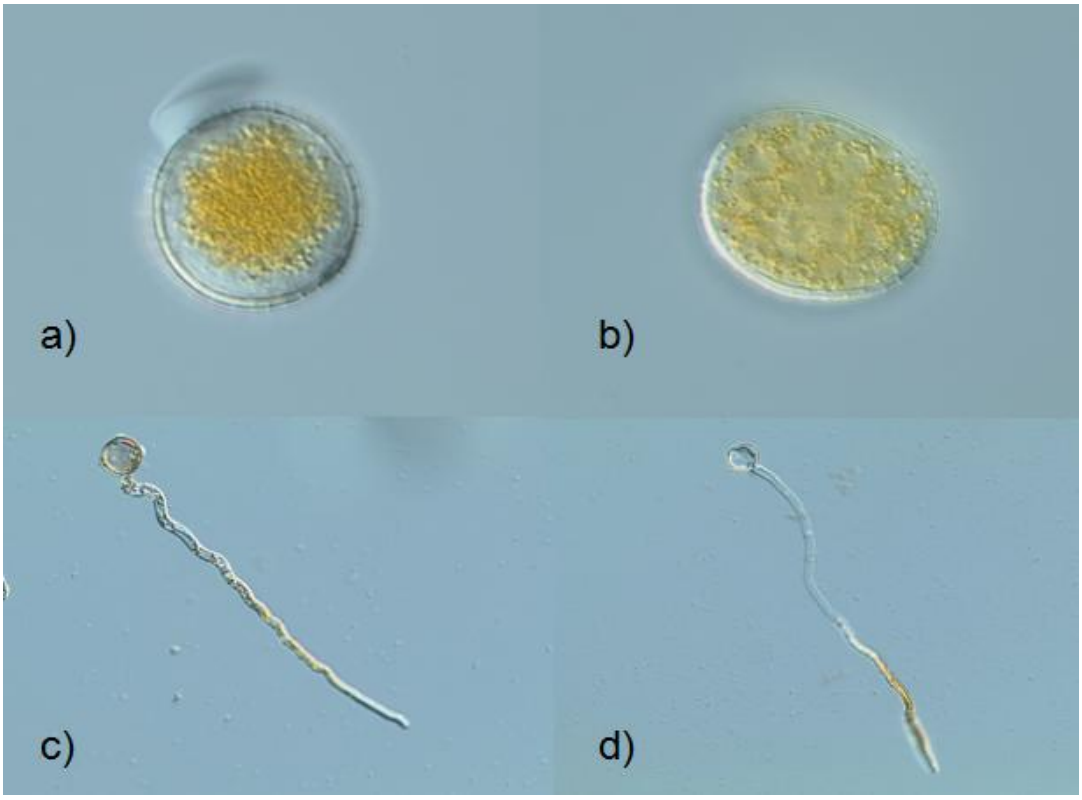

Figure 8.Leaf rust uredospores picked up from plants inoculated with *Puccinia spp*. fungi observed under microscope.
Slides A and C show a brown rust spore. Non-germinated (A) and germinated (C).
Slides B and D show a yellow rust spore. Non-germinated (B) and germinated (D).

4. Results

4.1. Morphological characteristics of uredospores of different *Puccinia* species on cereals

As previously discussed, investigations were carried out with cereal rust uredospores. The uredospores of wheat leaf rust were picked up from self cultivated and inoculated plants grew in greenhouse from the variety “Dekan”. Whereas the wheat stripe rust uredospores were picked up from wheat plants of the variety “JB Asano”, taken from ongoing experiments, grown in the fields and inoculated by natural conditions. The third studied uredospores were from rye leaf rust pustules from rye plants of the variety “SU Forsetti” taken from ongoing experiments, grown in the experimental fields of the university and inoculated by natural conditions.

Figure 9, shows the three different uredospores observed under a microscope. It can be observed in Figure 9a and 9b that the spores of *Puccinia triticina* and *Puccinia recondita* are very similar; both are spherical-ellipsoidal and rust-brown coloured. The figure 9c shows a spore of *Puccinia striiformis.* As observed in our experiments, yellow rust uredospores are morphologically very similar to the other two studied uredospores but yellow coloured. Yellow rust uredospores tend to be a bit more ovoid and elongated than the brown rust spores.

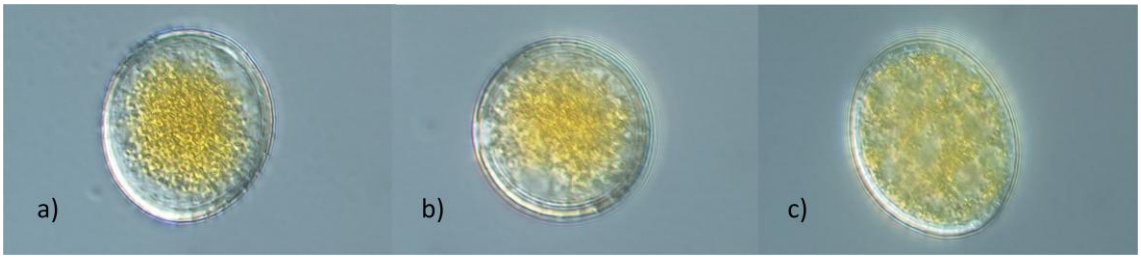

Figure 9.Uredospores of cereal rust observed under microscope.

a) Uredospore of *Puccinia triticina* in wheat
b) Uredospore of *Puccinia recondita* in rye
c) Uredospore of *Puccinia striiformis* in wheat

The size of the spores wasn't the main purpose of this study, but in order to compare with other authors some spores have been measured. Figure 10 shows a measured uredospore of *Puccinia triticina* (10a)*, Puccinia recondita* (10b) and *Puccinia striiformis* (10c), germinated in water*,* with a size within the average given by other authors for this kind of spores. All the spores measured in this experiment were after being in water at least 24 hours as the spores had to germinate to carry out the study. All measured uredospores are inside the average expected of 24-35 µm.

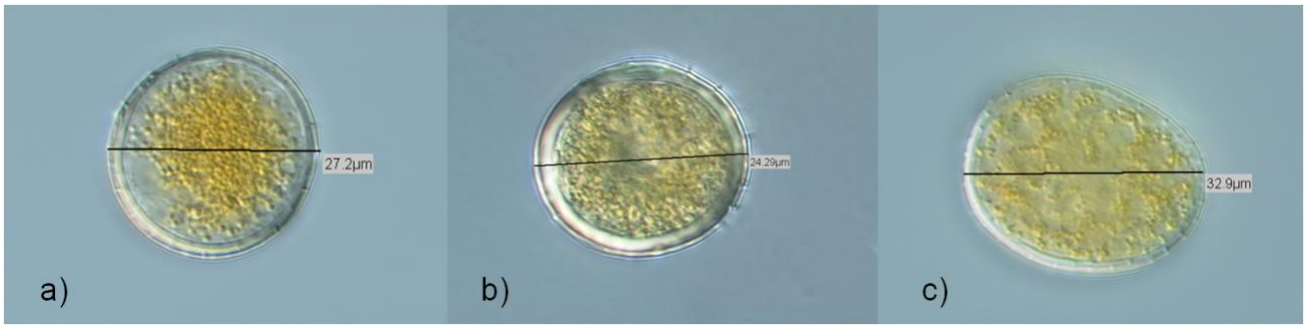

Figure 10.Measured Uredospores of *Puccinia triticina* on water

In this research, the germ tubes have also been object of interest, firstly as indicator of germination and secondly to differentiate and characterise the three kind of uredospores. Figure 11 shows the three studied uredospores germinated with presence of germ tubes. The studied germ tubes of the three different spores were wavy and undulated and they had a completely variable length.

In this work there haven't been observed differences in the germs tubes between the different races or between the different storage conditions. It is important to mention, that sometimes due to the dehydration of the samples during the germination process, the spores tended to aggregate (Figure 12) making more difficult the determination of the germinations rate of the spores and the observation of their characteristics.

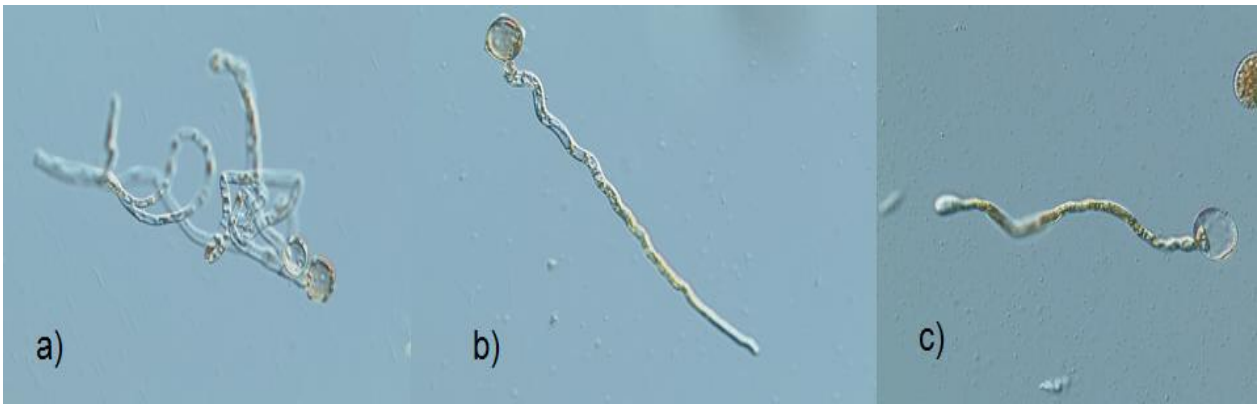

Figure 11.Uredospores of cereal rust with germ tubes observed under microscope.

a) Uredospore of *Puccinia recondita* in rye
b) Uredospore of *Puccinia striiformis* in wheat
c) Uredospore of *Puccinia triticina* in wheat

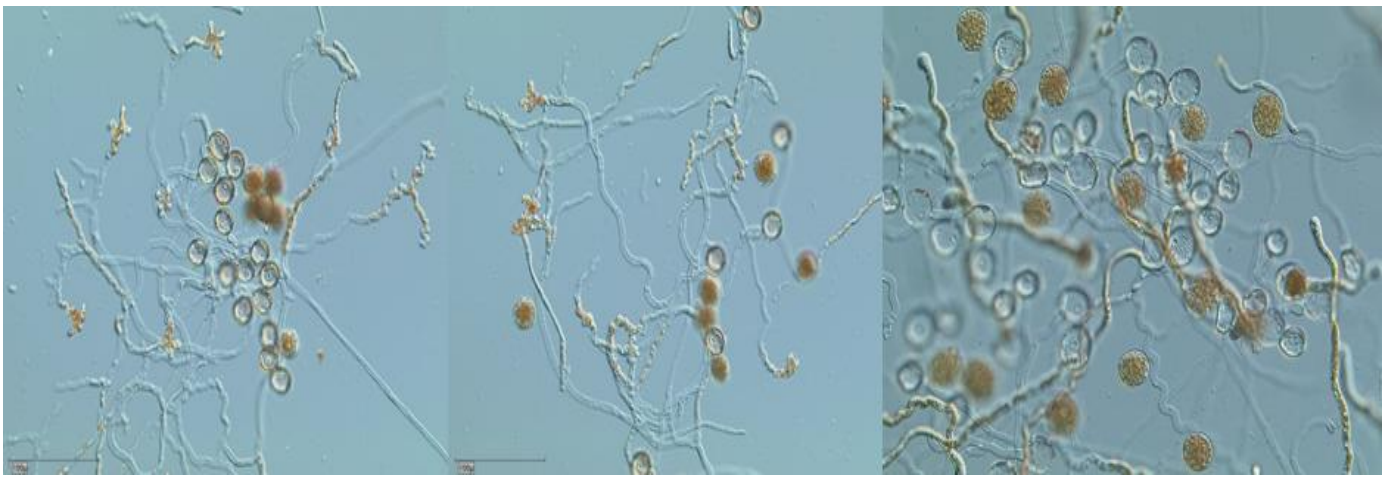

Figure 12.Aggregate brown rust uredospores observed under microscopy after the germination process.

4.2. Analysis and comparison of the germination rate of uredospores of *Puccinia spp.*

These is the main part of the research and aims to clarify how the temperature and time after release from leaf rust pustules affects the vitality of the uredospores of the fungi *Puccinia triticina* Eriks, *Puccinia striiformis f. sp. Tritici* and *Puccinia recondita* f.sp. *secalis*. With this objective, the germinations rate of each one of the spores groups one day and every week after collecting the spores until the rate was invariable was analyzed.

4.2.1. Vitality evolution of *Puccinia triticina* uredospores of wheat.

Figure 13 shows the germination rate of the uredospores of *Puccinia triticina* of the first group of plants stored at three different temperatures during thirteen weeks with weekly analysis.

The germination rate of the uredospores stored at 20 °C of temperature was recorded at 1 % the day after harvesting. The rate steadily grew and peaked at 23,5 % after six weeks of storage and decreased to a 2,5 % after thirteen weeks. The spores storage at 10 °C had a germination rate of 3 % the day after harvesting and grew up to 19,75 % in the fifth week before decreasing to values of 3,75 % at the end of thirteen weeks. The spores stored at 4° C of temperature had an initial rate of 2,75 % after one day. Between weeks 1 and 9 the rate maintained variable values between 5-15 % and decreased to 1 % after thirteen weeks.

Figure 14 shows the germination rate of the spores of *Puccinia triticina* of the second group stored under three different temperature conditions during twelve weeks with weekly analysis.

The below graphic shows a clear involution in the vitality of the spores stored under 20 °C. The spores maintain their maximum germination rate of around 40% on day one but seven days after the harvest, decrease to 27% after two weeks and reaches close to zero after three weeks. The graphic shows some anomalies for the spores stored at 10 °C. Deviations that do not follow a fixed pattern have been observed; here highlight a 70 % of germination after 7 days. It raises and falls from week two till week five, but from the fifth to the end there is a clear diminution and then by week 11, the

vitality of the spores is practically zero. As mentioned above no reduction in the germination rate of the spores stored at 4°C has been observed, we may even talk about an augmentation. This is the unique case in the *Puccinia triticina* studied spores were the rate does not went down with the time.

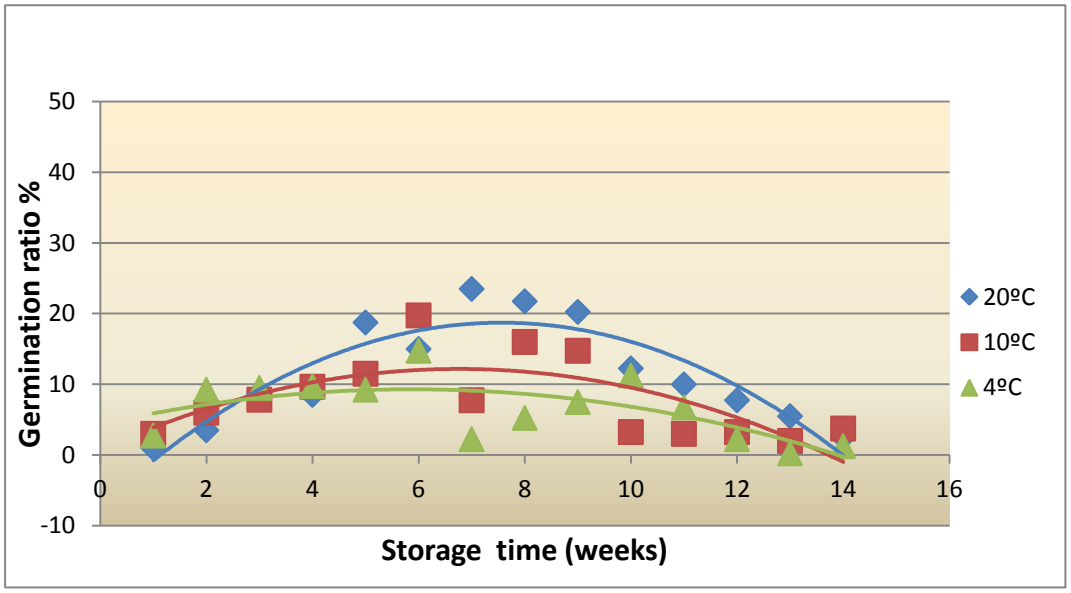

Figure 13.Evolution of the germination rate of uredospores of *Puccinia triticina* for three different storage temperatures for the duration of thirteen weeks.

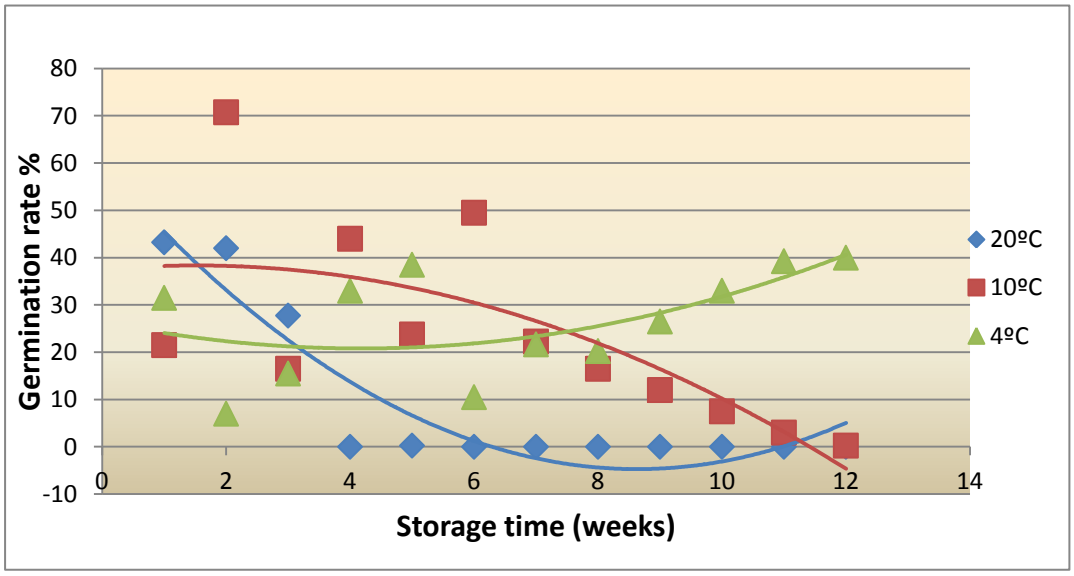

Figure 14.Evolution of the germination rate of uredospores of *Puccinia triticina* for three different storage temperatures for the duration of eleven weeks.

Figures 15 and 16 show the range of temperatures and HR respectively during the formation of brown rust pustules in wheat plants, from where the spores of *Puccinia triticina* had been picked up to carry out the experiment. It may help us to analyze and discuss the different results obtained in the germination rate of both groups of brown rust uredospores in wheat. As mentioned above, the plants have been growing during the same length of time but not during the same time span. The conditions, initially presumed equal in the greenhouse for both periods, could have been different bringing us in to an erroneous result. The pustules formation process was along 14 days for both plant groups.

In Figure 15 we may observe that the range of temperatures of both groups is between 19 and 30 °C. The variations are mainly caused by the temperature changes day-night. The average temperature of Group 1 is 22,45 °C. The highest temperature registered was of 26,1°C and the lowest was 19,8°C. The average temperature of group 2 is 22,63 ° C. There are two picks of temperature one of 27,2 °C at the middle of the period and one of 27,7°C close to the end of the period. The lowest temperature registered was as in the first group 19,8. Figure 16 shows the relative humidity during the formation of the spores. The range for both groups was between 40-90 %. Normal days, the relatively humidity don't cross the barrier of the 70 %. Group one has an average of HR of 55,56 % with a pick of 87,8 % in the day 12 of formation and group 2 has an average of 53,7 % and two high points of 86,1 and 87,8 % in the days 11 and 6 respectively.

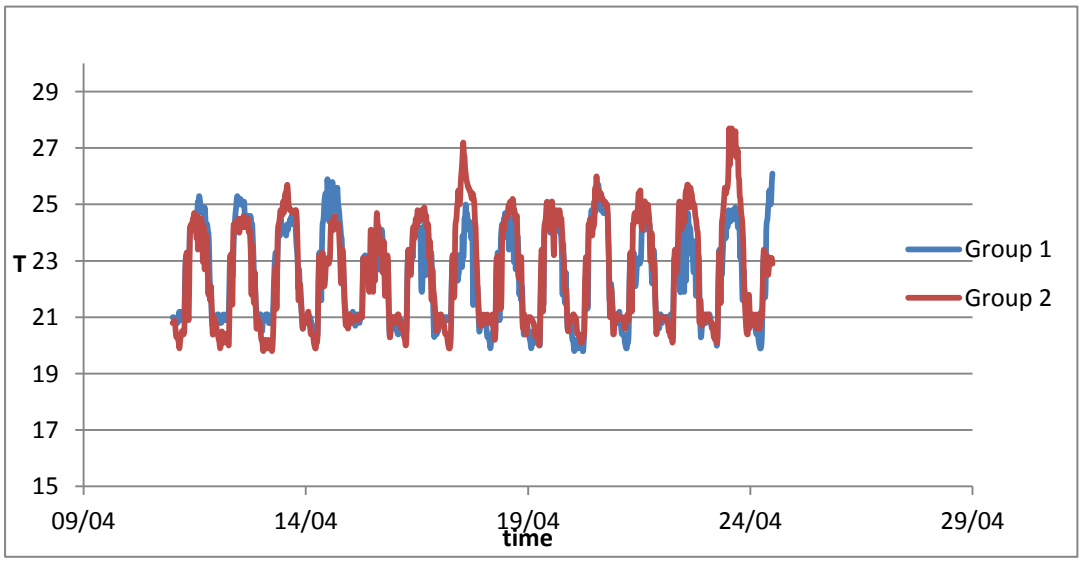

Figure 15.Range of temperatures during the development of the pustules caused by *Puccinia triticina* in two different wheat group of plants of the variety Dekan.

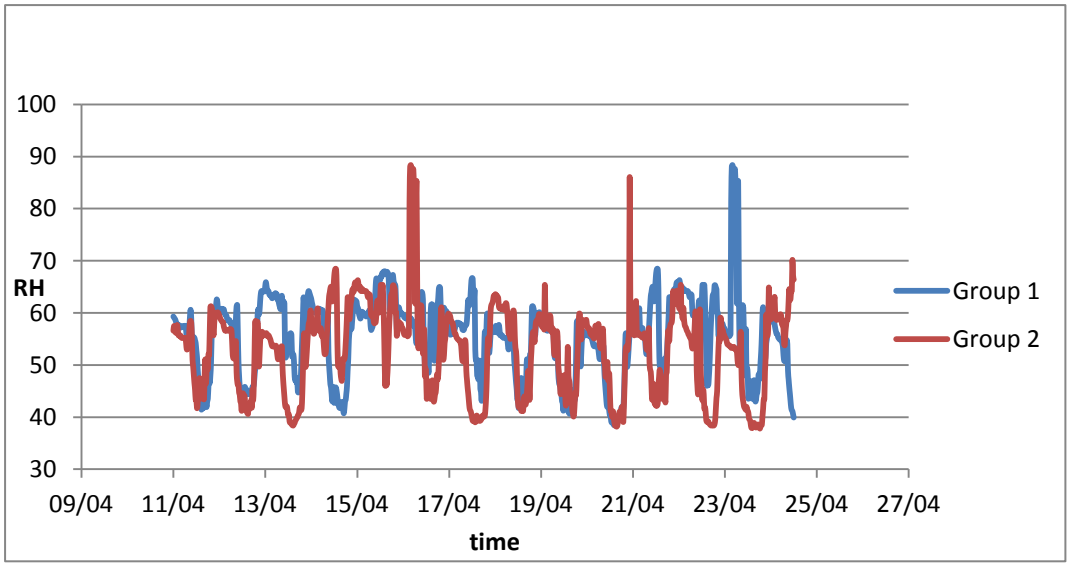

Figure 16.Range of relative humidity during the development of the pustules caused by *Puccinia triticina* in two different groups of wheat plants of the variety Dekan.

4.2.2. Vitality evolution of *Puccinia recondita* uredospores in rye

Figure 17 shows the germination rate of the spores of *Puccinia recondita* f. sp. *secalis* stored under three different temperature conditions during eight weeks with weekly analysis.

The uredospores of *Puccinia recondita* f. sp. *secalis* stored at 20 °C showed a germination rate of 58 % after seven days of being stored. The rate decreased gradually until week five when no germination was observed. The values of the weeks two, three and four are 39 %, 11,5 % and 4 % respectively. After 7 days the spores stored at 10°C of temperature had germination rate of 50%. At this temperature the rate after 8 weeks was also 50 % and so, no reduction on the germination rate was recorded. The values for the intervening weeks varied between 30 % and 60 %. For this temperature the germination augmented a 16 % for week 2 and then decreased gradually until week seven when the rate rose to 30 %. A remarkable observation was made at week 8, the last week of analysis, for there was an increment of 20 % regarding week 7. The graph shows a decreasing trend for the spores stored under 4°C, being 50% at week one and 22,5% after 8 weeks.

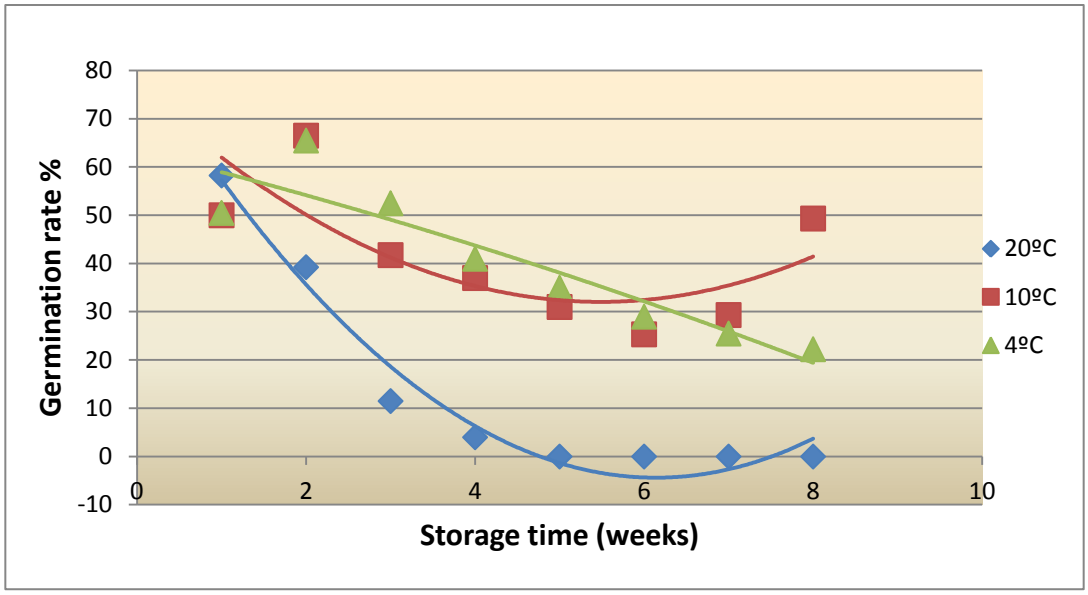

Figure 17.Evolution of the germination rate of uredospores of *Puccinia recondita* f. sp. *secalis* for three different storage temperatures for the duration of eight weeks.

4.2.3. Vitality evolution of *Puccinia striiformis* uredospores in wheat

Figure 18 shows the germination rate of the spores of *Puccinia striiformis* stored at three different temperature conditions during eight weeks with weekly analysis.

The germination rate of the uredospores stored below 20 °C remained steady the first two weeks - the germination rate after seven days was 13 % and 14 % after fourteen days. The germination decreased in the fourth week to a 0,5 %. No more germination was observed for the following weeks. This also happened for the spores stored at 10 °C with the difference being that after 14 days the germination increased slightly to a 17 %. As the spores stored at 20 °C, there wasn't more germination recorded after four weeks. The germination rate for the spores stored under 4 °C was slightly superior after seven days, 15,5 %. However, the rate decreased to a 9 % in week two and no germination was observed after four weeks of storage.

The vitality of the spores of yellow rust show that for any of the three conditions of storage, there's a clearly diminution after three weeks and no germination up to 4 weeks.

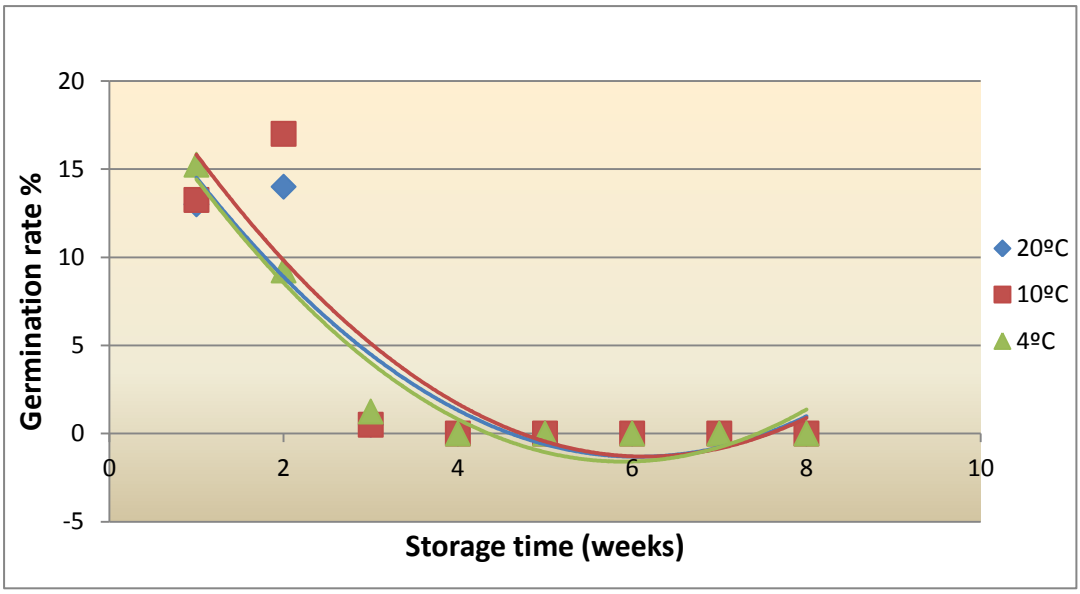

Figure 18.Evolution of the germination rate of uredospores of *Puccinia striiformis* for three different storage temperatures for the duration of eight weeks.

5. Discussion

Figure 9 of the discussion shows the three different uredospores microscopically examined. Uredospores of *Puccinia recondita* in rye are described as spherical-ellipsoidal orange-brown spores with an average size average of 23,2 µm long and 20,4 µm wide. We can observe in the figure 9b, a studied spore of *Puccinia recondita* of rye, the form and colour description contrast with the description made by Gäumann. Anikster *et al* (2005) described the uredospores of *Puccinia triticina* as spherical-ellipsoidal with an average of 23,2 µm long and 20,4 µm. The colour is also brown-orange. As we can see in the Figure 9a *Puccinia triticina* and 9b *Puccinia recondita*, there are no morphological differences between the uredospores of the two fungi. Gäumann described also in 1959 yellow wheat rust uredospores as spherical or short ellipsoidal, 14-36 µm long and 13-23 µm wide, with a colourless wall of 1-1,5 µm thick with barbed warts and orange-yellow content. In the Figure 9c we can observe the characteristic yellow colour that Gäumann was talking about. This colour make it easy to differentiate it from the others uredospores.

Germ tubes have been studied by many authors. One of the principal interests for this is the possibility to differentiate and identify different races of one pathogen as the germ tubes are characteristic of each race. Straib identified 40 races of stripe rust in wheat, barley and rye thanks to the germ tubes. In his research he differentiated two groups looking at the germ tubes. First group included wheat and rye stripe rust uredospores and the second barley uredospores. Under some conditions, the developed tubes of the first group use to be more elongated and the second one wavier and slightly undulated. Straib found that the germ tube form is hereditable, but, the environmental conditions could modify these patrons. Low germinating temperatures (2-5°C), make the differentiation more difficult as both groups develop wavy and undulated germ tubes (Schroeder 1964). In this study, there has been no observation of any important differences in length or form between the three kinds of spores. However, they all present wavy and undulated form which contrast with the findings mentioned by other authors, such as Schroeder. One cannot draw-up a theory about the germ tubes of the three different uredospores studied, as they were of random lengths and form which does not respond to any relation mentioned by another author. The colour is, in this case, a clear indicative. The germ tubes of the spores of *Puccinia*

striiformis are yellow coloured and the *Puccinia triticina* and *Puccinia recondita* are brown-rust coloured. It has been observed a tendency of the spores to aggregate; the germinated and the no germinated ones. This makes the counting of the spores much harder. This phenomenon can be observed in Figure 12. This may be related with the dehydration of the samples during the germination process and the electric charges of the spores. No literature has been found to back this theory up though. It was tryed to avoid this by applying a surfactant product (Tween 20). After some attempts it was noticed that this product had a negative effect on the structure of the spores and so could bring us erroneous results.

Rust fungi have a big adaptation, dispersion and evolution capacity. However, they depend on the environmental conditions and the presence of alternate hosts to complete their cycle and survive. The fungi can travel large distances - more than 2,000 km - to find more favorable conditions. In Figure 19 it can be seen the dynamic of rust spores, for e.g. the rust uredospores travel from Mexico to Canada at the beginning of the year before returning to Mexico in the autumn in order to find the conditions to complete its five spore stage cycle.

The fungi have an explosive infection capacity under certain conditions of temperature and relative humidity. The evolution strategy of the fungi is based in the high quantity spore production, wide range dispersion (directional and distance) and the combination between areas where during the different periods the conditions are optimum and there is presence of host plants. Observing the evolution and reproductive tendency of the fungi, it's important to anticipate our reactions to its movements in order to avoid epidemics and control the disease. Knowing that the uredospores are the main source for the rust fungi to spread and infect, this experiment has been raised as an opportunity to gain better understanding of how different conditions affects vitality of the uredospores in order to apply this results to the conditions that the spores are affected by in the nature; like wind, temperature, light or relative humidity and try to take the right preventive measures against the disease. For example, when the wind carries the spores, they reach heights of hundreds of meters. The temperatures at these heights are not the same as in the earth surface and the spores could have been travelling for days. If we know the temperatures that the spores have been submitted to during the spread and how long have they been travelling and we have a previous

knowledge about how react the spores to these conditions, it would be to work out the best strategy to tackle the disease.

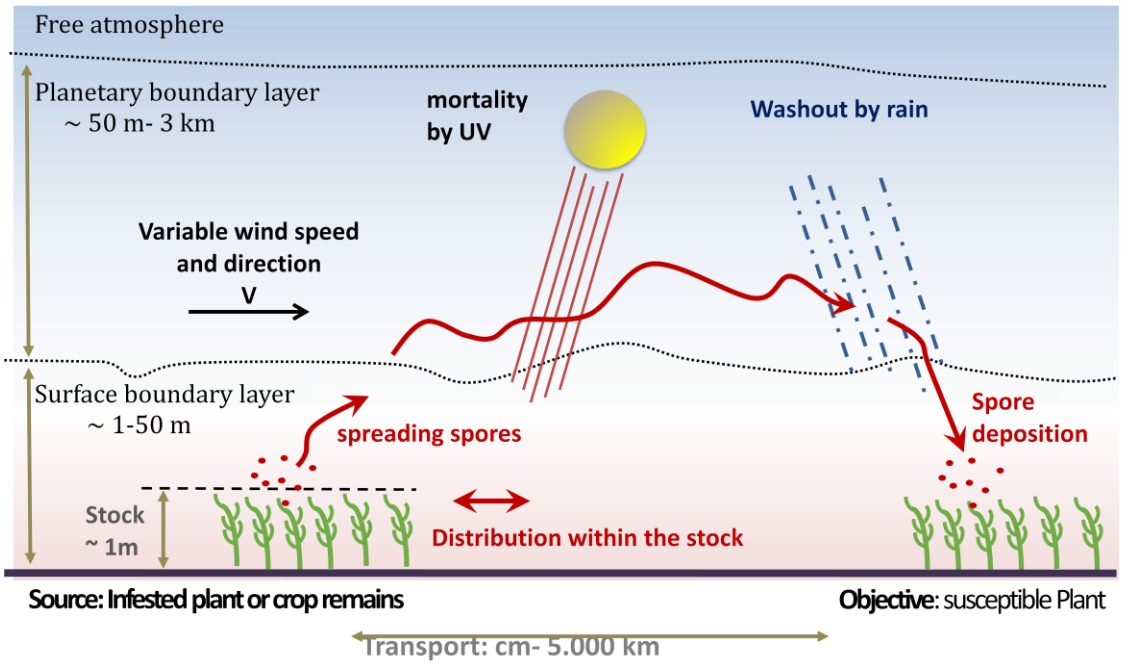

Figure 19.Dynamic and spread of rust uredospores. Diagram of uredospores spread and the different conditions which affect it (Schmale and Ross, 2015).

The development of the germination rate of the uredospores may be influenced by various outside factors which have an effect as early as during the fructification of the fungi. It was thought that the differences in the results obtained in the germination rate of the uredospores of *P. triticina* of both groups of wheat plants could be caused by differences in the environmental conditions during the fructification of the pustules. In our experiments, we have used the variety "Dekan" for both groups and so this eliminates the variety to be an influential factor in the germination process. Anyhow, Straib and Schroeder also coincided that the variety is not a factor of influence in the germination capacity. On the other hand, many authors have written about how the temperature during the development of the pustules is an important factor for the later germination capacity. Straib established that very low cultivation temperatures (aprox. 2°C) compared to the usual cultivation temperature of approximately 15 °C strongly stimulated the subsequent germination of the stripe rust spores. Straib also mentioned

that high cultivation temperatures (higher than 20°C) could have a favourable effect on the germination capacity. In our experiments with wheat leaf rust, higher germinations rates on the spores of the second group, at least in the initial analyse after harvesting the spores were observed. Nevertheless, in the experiments with the first group of plants, very low germinations rates were recorded. The average temperature is similar for both groups of spores, being a 0,18 °C higher for the group 2. Based on this, the differences on the vitality of the spores are not related to the variety of wheat, isolate or plant growth temperature.

Another important factor during the formation of the spores which can affect the vitality is the relative humidity relative humidity. Some authors studied this but their experiments led to very different results. It has been recorded with a relative humidity of 85 % the germinations rates were lower (15%) than for 55-70 % HR with more than a 90 % of germinations rate. However, Straib observed that the lower was the relative humidity, lower the germinations capacity was. The relative humidity during the fructification time for both groups of uredospores of *Puccinia triticina* was 55,6 % for the first group and 53,7 % for the second group. Based on these data given, it can be said that the relative humidity hasn't had any impact on the different results obtained for both groups of spores.

Based in the theory of that the uredospores vitality can vary for plants grown under different conditions and once analyzed the condition under which the two groups of plants were grown, which are practically the same, we can conclude that the differences in the vitality in both groups are not related with the environmental conditions. There are also other factors which could have also affected the germination rate. There could be contaminants in the water or in the atmosphere which could inhibit the spore's germination or a different fertilizer dose. In the harvesting of the spores, any insect, drop of water or crop remain would have had the potential to modify the bioavailability of the spores.

One of the two important factor studied in this master work is how the time elapsed after the uredospores were released from the rust pustules affects their later germination capacity. It is not necessary special methods to keep uredospores vital for a short term. Storage at room temperature and humidity are enough if the spores are going to be germinated in a couple of days. El-Fiki *et al* (1998) observed that the vitality of rust spores of the different tested spore rust collections (isolates) was decreased to different extents as storing period of these spore collections increased from 60 to 210

days comparing with the fresh spores with controlled conditions. This matches this study's findings for it was observed that each type of spore either lost or decreased its vitality as the storage time increased. The rye brown rust spores stored under 4 °C and 10 °C and the wheat brown rust spores stored at 4 °C, still proved to be vital come the end of the experiment but this is most likely probably to drop after a longer period.

The rust uredospores are vegetative spores without true dormancy. It remains in resting state only in the absence of conditions favourable for germination. At room temperatures and moderate relative humidity, spores of *P.graminis* remain viable for about 4 to 6 weeks (Rowell, 1984). If we take 20°C as a room temperature, it contrasts with our results where the spores stored at 20 °C maintain the vitality between 3 and 6 weeks and then the vitality started to decrease, except for the uredospores of wheat leaf rust of group one which keep vital for 11 weeks, but with a very low rate.

Many studies about how time after release from rust pustules affects the vitality of the spores show that the spores lose vitality over the time. A study carried out by Fromme about the duration of the vitality of the uredospores of *Puccinia conifera* showed that the vitality 12 hours after picking up was high, close to the 100 %. The analysis was repeated after 6, 10, 15, 29, and 48 days and the germination rate was always between 9 and 16 %. The last analyse had been made after 3,5 months and the spores were colourless and no germination was observed. The graphics show that the germinations rate after 24 hours weren't as high as what Fromme observed, but it does not have to be the same as every species and every isolate have a different vitality evolution. In this research, wheat leaf rust uredospores of group one didn't show any vitality after 14 weeks of storage. The spores of brown leaf rust of the second group showed a higher germination rate after 24 hours, being 43 % for the spores stored at 20 °C, 21 % at 10 °C and 32 % for the spores stored at 4 °C but they didn't show vitality after eleven weeks of storage except for the spores stored at 4°C. The uredospores of *Pucccinia recondita* stored at 20 °C didn't show vitality after 5 weeks, but the uredospores of this type stored at 4°C and 10°C keep vital after 8 weeks. Spores of *Puccinia striiformis* didn't show any vitality after 4 weeks. The results obtained for Fromme, Bary and Gibson about how long the uredospores keep vital contrast with this work's results. They estimated the point where the spores lost the vitality between two and three and a half months after harvesting. In these experiments germinations after three and a half months were not observed, except, as mentioned before, for the uredospores of wheat leaf rust of group two stored at 4 °C.

The second important factor studied in this research is how temperatures after release from leaf rust pustules until the germination takes place affect the vitality of the uredospores. As mentioned above, the literature shows that germination temperatures near to 20 °C are the optimum germination temperature for most of the cereal rusts spores. However, talking about the temperatures during the time between the release and the germination, we found that fresh temperatures helped to maintain better and longer the vitality of the uredospores. A study evaluated the viability and infectivity of uredospores stored in liquid nitrogen (-196°C), deep-freezer (-80°C), modified-refrigerator (5°C), biochemical oxygen demand (BOD 25°C) and herbarium specimens (25°C), for 150 days. Every 30 days, the germination and infectivity were evaluated, the first *in vitro* and the second on *Eucalyptus grandis* plants. The maximum germination (24.9%) and infectivity level (162 pustules/leaf) occurring in modified-refrigerator environment were higher at 17.6 and 30 days, respectively. Uredospores germination was highest when preserved in deep-freezer (34.3%) and liquid nitrogen (36.3%), at 45 and 40 days, respectively. The highest infectivity occurred at 60 days for the deep-freezer (77 pustules/leaf) and 90 days for the liquid nitrogen (67 pustules/leaf). Uredospores kept in deep-freezer, liquid nitrogen, and modified-refrigerator maintained their viability and infectivity for 150 days (Pozza *et al*., 2008). Extreme low temperatures were not used in this experiment as in the experiment mentioned below, but the results show that the uredospores stored at 4°C maintained the vitality of the spores better than temperatures of 10 and 20°C. In case of the wheat leaf rust of the group two is really significant, being the rate after 12 weeks 0 for the spores stored by 10 and 20 °C and 40 % for the ones stored at 4°C. Olso the spores of *Puccinia recondita* (Figure 20) maintained the vitality better for temperatures of 10° C and 4°C than for temperatures of 20°C. One can conclude that the brown rust studied fungi maintain the vitality better for lower temperatures, which contrast with the previous literature. There have not been observed any difference in the evolution of the vitality of the uredospores of *Puccinia striiformis* based in the different storage temperatures.

Most authors that studied how the temperature affects to the vitality of the uredospores of *Puccinia striiformis* agree in the preference of the yellow rust for lower germination temperatures. There are of course differences between races and biotypes. Schroeder (1964) studied also the direct effect of environmental conditions on the germination. Schroeder studied the vitality of the uredospores of yellow rust of different

races in order to compare results with Straib. He observed that most of the races germinate better at 10 °C (9-12) with a substrate of 1 % water agar and have a minimum germination temperature of 1°C. Stripe rust germinates rarely at 20 °C and nothing at 24°C and 26°C (Schroeder, 1964). In our experiments, based in the results obtained for other authors previously, the germination process has been also carried out at 10 °C. For our three temperatures of storage, 4, 10 and 20 °C, it has been observed that the highest germination rate was for the uredospores stored at 10 °C after 2 weeks with a rate of 17 %, but this is not a remarkable fact, as the results obtained for the other two storage temperatures were very similar. The results obtained by Schroeder where there have been observed germinations of the 90 % are, compared with ours, very high. These differences may be related with the fact that there are many different races with different vitality responses. For example, race 20 A (T) and (Gr) have at 5°C a germination of less than 3 % but other races like 7, 23, 24, 26 have a germination rate of 70-80 % at 2°C after 24 hours. There is yet to be a study about the reaction of the uredospores of *P. striiformis* to the length of storage, but after analyzing these results one may conclude that they don't maintain the vitality as long as others spores do.

In this study's results we can see a large variability of the results. There are many factors which can affect the vitality of the spores, not just during the germination process (light, temperature), but also during the storage (time and temperature) and during the growth and development of the plants (light, T, host plant and humidity). It is because of that that many results can vary despite the attempt to maintain the same conditions for the three species studied.

After analysing the results and draw conclusions, it is important to apply the knowledge gained to along other studies try to improve the response to the disease.

When the wind raises the spores hundreds of meters for long distance spread, the temperatures decrease. In this experiment it has been shown that lower temperatures are not good for the germination process, but are better for maintaining the vitality of the spores for longer. This is optimum for the disease spread, as it avoids the germination during the transport, but maintains the spore vital for when the rain drags them in to a new host. High temperatures, however, have the opposite effect; improve the germination rate but decrease the period during which the uredospores maintain the vitality, killing them at temperatures up 30 °C.

6. Conclusions

- The uredospores of *Puccinia triticina*, *Puccinia recondita* and *Puccinia striiformis* lose vitality with the increase of the temperature and time elapsed after release from the leaf rust pustules.

- The highest germination rates have been recorded for the uredospores stored at 10°C of *Puccinia triticina* (70%) one week after release from the leaf rust pustules and *Puccinia recondite* (66,5%) two weeks after release from the leaf rust pustules.

- Uredospores of *Puccinia triticina* do not show germination after fourteen weeks for any of the studied conditions except for the uredospores from the second group of plants stored at 4°C, which increased the germination rate from 30% one day after harvesting to 40% after twelve weeks.

- Uredospores of *Puccinia striiformis* show no difference in the vitality evolution between the different storage temperatures.

- Uredospores of *Puccinia striiformis* are the most sensitive to the time elapsed after release from leaf rust pustules, losing completely their vitality after four weeks for any of the conditions studied.

- Uredospores of *Puccinia recondita* lose their vitality faster after being exposed to temperatures of 20°C than after being exposed to temperatures of 10 and 4°C after release from leaf rust pustule.

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