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The history of research into *Ramularia* leaf spot on barley

Die Geschichte der Erforschung der *Ramularia*-Blattflecken-Krankheit an Gerste

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Abstract

The historical overview of research into *Ramularia* leaf spot (Rls) starts with 1893 and ends in the presence. Research results from Europe but also New Zealand, Mexico and the Argentina are presented. The contribution focuses on the own phytopathological work at the Federal Biological Research Centre for Agriculture and Forestry. Intensive research into the disease and its pathogen, *Ramularia collo-cygni* (*Rcc*), has been done in the last 20 years. First, attention was especially paid to the occurrence, distribution, ethiology and morphology, later to taxonomy, biology, symptomatology, epidemiology and biochemistry. Although there is still more or less need to deal with these subjects, research has recently concentrated on practical aspects like importance and chemical control of the disease, host resistance and pathogen variability. Finally, the prospect for further research is given.

Key words: History, *Ramularia collo-cygni,* disease, barley, occurrence, distribution, ethiology, diagnosis, epidemiology, biochemistry, toxins, chemical control, host resistance

Zusammenfassung

Der geschichtliche Überblick über die Erforschung der *Ramularia* leaf spot disease beginnt 1893 und endet in der Gegenwart. Es werden Forschungsergebnisse aus ganz Europa, aus Neuseeland und Argentinien vorgestellt. Einen Schwerpunkt der Betrachtung bilden jedoch die eigenen phytopathologischen Arbeiten aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft. Die intensive Erforschung der Krankheit und seines Pathogens erfolgte in den vergangenen 20 Jahren. Anfangs standen Fragen über Auftreten, Verbreitung, Ätiologie und Morphologie im Mittelpunkt des Interesses, es folgten Arbeiten zu Taxonomie, Biologie, Symptomatologie, Epidemiologie und Biochemie. Obwohl es auf allen diesen Gebieten noch mehr oder weniger Forschungsbedarf gibt, sind in neuerer Zeit sehr praktische Aspekte der Erforschung in den Mittelpunkt gerückt, wie Bedeutung und chemische Bekämpfbarkeit des Erregers, weiterhin Wirtspflanzenresistenz und Erregervariabilität. Abschließend wird ein Ausblick über noch erforderliche Arbeiten gegeben.

Stichwörter: Geschichte**,** *Ramularia collo-cygni,* Krankheit, Gerste, Diagnose, Epidemiologie, chemische Bekämpfung, Resistenz

It is very peculiar that Rls was described already 100 years ago, but relatively little is known about the disease. CAVARA described the pathogen first for Northern Italy as *Ophiocladium hordei* CAVARA (1893). In the mid 20ies of the last century, JØRSTAD (1925) reported on the occurrence of the disease in Norway. This was followed by a long time without any reports. In 1980, a barley breeder in Norway found so far unknown symptoms on barley, but could not identify them (SALAMATI et al., 2002). At this time, unknown disease symptoms on barley were also found on both islands of New Zealand and false identified as physiological spots. In 1983, these spots were identified as *Ovularia hordei* (CAVARA) Sprague, a synonym of *Rcc* (CROMEY et al., 2004). In New Zealand, the disease is called *Ramularia* leaf and awn spot. In 1985, HUSS finally noted the strong occurrence of unknown leaf spots on barley in Austria. He attributed it to the fungus described by CAVARA and made a detailed description of it (HUSS et al., 1987). This was the start of intensive research into Rls in Upper Austria, which has not stopped so far. HUSS called the disease speckle disease because of the speckle-like symptoms. Shortly after, small notices on the occurrence of the same fungus on barley were also published in Switzerland (BRÖNNIMANN, 1988). Another important event was that SUTTON and WALLER identified the disease on triticale in Mexico and re-named it as *Ramularia collo-cygni* (SUTTON and WALLER, 1988). The name of the species *collo-cygni* is derived from the special swan neck shape of the conidiophores (collum = neck, cygnus = swan). In 1992, HUSS published a sensational documentation of raster electron microscopic photos on the morphology of the asexual structures of the pathogen.

A milestone in the history of *Rcc* research was the publication of the monograph of *Ramularia* and allied genera of BRAUN in 1998. He provided a detailed description, illustration, a summary of hosts and distribution and discussion of the taxonomy of this species. According to BRAUN, it has an unique morphology within the genus *Ramularia*.

In 1997, I obtained a sample of barley from Lower Austria. It drew my attention to the disease. The consignor informed that there were different opinions on the pathogen. Some phytopathologists thought that these spots are physiological leaf spots (pls), but HUSS was of the opinion that the symptoms were caused by *Rcc*. My microscopic investigation confirmed HUSS. Then I contacted him. This was the beginning of a fruitful co-operation. The dispute on whether the leaf spots are caused by the fungus *Rcc* or whether they are the non-parasitic leaf spots called pls had lasted for several years among phytopathologists and barley breeders and had started strong controversies. Even today not all critics are likely to be convinced of the significance of the fungus as pathogen of Rls. Since 1997, we in the Kleinmachnow Branch have tested all barley samples we obtained for an infection with *Rcc*. At first we clearly identified samples from Middle Franconia to be infected (SACHS et al., 1998), but also a herbarium barley leaf sample collected in Upper Bavaria in 1992. The identification of the disease on herbarium samples on the basis of still visible conidiophores is interesting because herbarium

samples from other regions could prove when the disease has actually been present there. In the succeeding years infection was found in Ireland (SACHS et al., 1998), Scotland (JAHN et al., 1999) and the Czech Republic (AMELUNG and SACHS, 1999), Norway (SALAMATI et al., 2004), Denmark (SACHS and HUSS, 2003), France (SACHS, 2004b) and Belgium (BALZ, pers. comm., 2005). Samples obtained from Uruguay and Argentina (SACHS, 2002) showed also strong infection.

The heavy infection with *Rcc* in Scotland, which we identified (JAHN et al., 1999), led to the 3-year Scottish research project "Development of a rationale to identify the causal agent of necrotic lesions in spring barley and identify control mechanisms". The project was headed by OXLEY from the Scottish Agricultural College, Edinburgh and integrated further British phytopathologists, but also phytopathologists from Ireland, Norway, Austria and Germany. The project activities supported the co-operation of the participating scientists and the intensive discussion of the various leaf spots on barley, especially those caused by Rls and pls (SACHS and OBST, 2000). Diagnosis and the fulfilment of the Koch's principle to identify the pathogenicity of *Rcc* (SACHS and HUSS, 1998; SALAMATI, 2004; FREI, 2004) were subject to detailed discussion, and control and susceptibility of cultivars played an important role. Another interesting subject was the isolation and cultivation of the fungus on artificial substrate of *Rcc* as basis of the future resistance tests (FREI, 2000; SACHS and HUSS, 2003; O'SULLIVAN, 2004). The establishment of a collection of strains of the fungus proved to be an important prerequisite of PCR by HAVIS (HAVIS et al., 2004).

Later we found out that the *Ramularia* toxin turns red. This knowledge has been a key to future research. It allowed to develop a diagnostic quick test (TSCHÖPE and SACHS, 2001). It uses very simple laboratory equipment to test larger quantities of samples for *Rcc* and other causes of leaf spots within 2 days. Only a short time is necessary to get familiar with the test and thus to be able to distinguish between *Rcc* and pls leaf spots and to distinguish mildew necroses and net blotch. The tests allowed to gain experience in symptomatology which is of enormous value for field work (SACHS, 2004a). BALZ and VON TIEDEMANN (2004a) developed an ELISA test to identify the disease before first symptoms are visible. The quick test we developed was the basis to conduct a monitoring throughout Germany (SACHS, 2004a). In the beginning, the South of Germany showed stronger infection, but in the following years infection increased also in the Middle and North of Germany. Even the drier East of Germany showed infections (KREYE, comm., 2005). Monitorings carried out later did not show any more a regional focus (BALZ and VON TIEDEMANN, 2004b). Monitorings were also carried out in Argentina (KIEHR et al., 2002), Austria (FORMEYER et al, 2004), Norway (SALAMATI et al., 2004), and the Czech Republic (MINARIKOVA et al., 2004).

The diagnostic quick test allowed comprehensive investigation into epidemiology (SACHS, 2004a). It was found out that first infection occurs on young winter barley on warm autumn days. The pathogen hibernates on the oldest leaves of the plants. In the following spring it starts spreading across the leaf layers with the beginning of the growing season until it reaches the awns. Mass production of conidia often starts in Germany at the flowering stage if the weather is wet at this time. Flag leaves and awns are sometimes heavily infected. From winter barley the disease spreads to spring barley, but also to other cereals, grasses and maize. In Germany, volunteer barley is of great importance as intermediate host. Dew plays a great role for infection (FORMAYER et al., 2004). According to new investigations by MINIHOFER (2003) the pathogen is able to sporulate and to infest also in winter.

MIETHBAUER et al. (2004) identified the red dye formed by *Rcc* as *Ramularia* toxin rubellin D and studied the molecular structure. They found out that the anthrachinon derivative rubellin D isolated from the cultivation substrate of the fungus causes the same symptoms on barley as the pathogen. Further investigations by HEISER et al. (2004) have recently shown that the fungus toxin contains also rubellins A, B and C which were also identified in the *Mycosphaerella* toxin. This supports the result of CROUS et al. (2000) who carried out a molecular analysis of *Rcc* and showed that the fungus belongs to the monophyletic *Mycosphaerella* claster. This means that it belongs to the ascomycetic order *Mycosphaerella* if the teleomorph of *Rcc* is found some day. Last time the biochemical work on the phytodynamic activity of rubellin D were completed (HEISER et al., 2003).

Another milestone in the history of *Ramularia* leaf spot research was the 2nd International Workshop on Barley Leaf Spots carried out in Syria in 2002. For the first time *Rcc* appeared on stage and was of great interest. Nearly all scientists working in the field presented their results. As a result SACHS and HUSS (2003) established a homepage that informs on all knowledge available up to 2002.

The last five years saw further research into the control of the disease (HUSS, 2000; BURKE et al., 2001; SALAMATI et al., 2004; CROMEY et al., 2004; GREIF, 2004; PINNSCHMIDT and HOFMØLLER, 2003; BALZ and VON TIEDEMANN, 2004b). It revealed that strobilurine-containing fungicides are most effective. There are different opinions on the application time. CROMEY (2004) recommends very early application, BALZ and VON TIEDEMANN (2004b) recommend to carry out application as late as possible. Data on additional yield as compared to the untreated control differ considerably. It might be about 10% to 15%. There is no fungicide approved for Germany, but negotiations will soon be finished. It is very likely that the combination fungicide Amistar-Opti containing azoxistrobin and chlorthalonil by the Syngenta company will be registered this year.

The importance of Rls and of its control raised the question of forecasting. CROMEY (2004) supposes that the disease can hardly be forecast due to its strong deviation in occurrence. Norway, however, has started work on forecasting together with the meteorological service (SALAMATI, oral comm.). As occurrence and leaf wetness duration are closely correlated (FORMEYER et al., 2004), forecasting has a good chance.

As the disease has obviously spread and as it has been realized as a serious threat to barley yields, several countries have made preparations for resistance breeding. So, cultivars and strains were checked for susceptibility (CROMEY et al., 2004; SACHS and HUSS, 2003; SALAMATI, 2004; BALZ and VON TIEDEMANN, 2004b; PINNSCHMIDT and HOFMØLLER, 2004). The cultivars showed differences. This is a basis for resistance breeding. Austria (BISTRICH, oral comm.) and the Skandinavian countries Denmark, Norway and Sweden (SALAMATI, oral comm.) started resistance breeding on barley against Rls.

Field trials, however, showed that it is very difficult to identify *Rcc* resistance of a cultivar under field conditions. In New Zealand, strong net blotch made it more difficult (CROMEY, 2002). In Norway, the cultivars showed a negative correlation between *Rhynchosporium* and Rls. Austria had a negative correlation between dwarf leaf rust and *Rcc* (SACHS and HUSS , 2003). Field trials carried out by HUSS (SACHS and HUSS, 2003) showed that field trials may lead to the assumption that late-ripening cultivars might be less susceptible. For this reason, tests should be carried out under controlled conditions with artificial inoculation of a defined inoculum in an environment controlled glasshouse or climatic chamber. This requires defined conidia suspensions and well identifiable disease symptoms. Our trials (SACHS and

HUSS, 2003) and those of O'SULLIVAN (2004) showed that these conditions are hardly to guarantee. Norway, however, obtained disease symptoms in glasshouse conditions similar to those in the field (SALAMATI, oral comm.). HUSS continued his work on the host range of *Rcc* and found strong infection with *Rcc* in wheat and oats in Austria in 2005 (HUSS, 2005).

Meanwhile, Rls has appeared in some German dictionaries (SCHÖBER-BUTIN et al., 1999; OBST and GEHRING, 2002). German breeders, plant protection experts and representatives of the chemical industry were trained for the identification of the disease and its pathogen in 2004 (SACHS, 2004c). This was an important contribution to general information on the disease.

This is far from answering all questions connected with Rls. We do not know why the disease is spreading so rapidly. We have insufficient knowledge of the environmental influence on the disease's spread. There is knowledge of host resistance, but the cause is not known. Furthermore, a procedure to test resistance has to be developed. We do not have enough knowledge of the influence of cultural practices on infection processes. Furthermore, we lack knowledge of pathogen variability. We guess whether the fungus might attack new host plants and which ones. As to taxonomy we still have not found the teleomorph of the fungus. But the question of all questions is: What effect does *Rcc* have on the quality of beer and whisky? And it would be ridiculous if it were not possible to raise funds for the subject.

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